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Innovative approaches to enhancing kombucha through flavour additives: a phytochemical and antioxidant analysis†

Karolina Jakubczyk, [a] Klaudia Melkis, [a] Dominika Maciejewska-Markiewicz,[a] Anna Muzykiewicz-Szymańska, *[b] Anna Nowak[b] and Karolina Skonieczna-Żydecka[c]

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This study aimed to determine the phytochemical profile (flavonoids, phenolic acids, caffeine, vitamin C, and acetic acid), antioxidant potential (DPPH, ABTS, and FRAP method), total polyphenol (TPC) and flavonoid (TFC) content, as well as pH of eight commercial green tea-based kombuchas. The beverages were enriched with lemongrass; lavender; liquorice and mint; turmeric and lemon; mango; reishi and chaga; mint, rose, and pomegranate. The highest tested properties were found for kombucha with reishi and chaga (FRAP), with mint, rose, and pomegranate (ABTS), as well as with turmeric and lemon (DPPH, TPC, TFC). Among the identified phenolic acids, *p*-coumaric acid was found in the highest concentration (kombucha with reishi and chaga), while among the flavonoids – rutin (kombucha with liquorice and mint). Kombucha with reishi and chaga was the richest source of vitamin C, caffeine, and acetic acid. The addition of certain plant materials significantly affects the phytonutrient content of green tea-based kombucha.

1. Introduction

Kombucha is a non-alcoholic effervescent tea beverage obtained by fermenting sweetened leaf tea infusion with symbiotic cultures of bacteria and yeast (SCOBY).[1,2] The key substrates in the production of kombucha are tea and a sweetener that provides the energy source needed for the fermentation process. According to the traditional recipe, black tea is often substituted with both unfermented green tea and fermented teas such as red, black, yellow, or white, while white sugar (sucrose) is interchangeably used with coconut sugar or cane sugar.[3,4] In addition, other plant materials, such as coffee or herbs, are increasingly being used instead of tea leaves. Honey, maple syrup, sweeteners such as stevia, xylitol, and erythritol, and by-products of the food industry such as molasses are used instead of sugar.[5]

The consortium to produce fermented tea includes aerobic and anaerobic microbial strains that form a dense cellulosic biofilm called tea fungus or Japanese fungus.[6,7] The most common are acetic acid bacteria (AAB) of the genus *Gluconacetobacter* and *Acetobacter*, lactic acid bacteria (LAB), mainly of the genus *Lactobacillus*, and yeasts of the genus *Zygosaccharomyces*, *Saccharomyces* or *Brettanomyces*.[8] These organisms initiate the fermentation process and play a significant role in enriching the chemical composition of the final product.[2] Their synergistic action leads to the transformation of organic compounds derived from the base raw materials (such as *e.g.* sugars and tea-derived polyphenols) into a wide range of bioactive metabolites including organic acids, vitamins, and modified phenolic compounds that give kombucha distinctive organoleptic characteristics and numerous health-promoting properties, thanks to which it is classified as a functional food.[1,9–11]

Previously published analyses have shown that kombucha contains a number of nutrients, which include organic acids: acetic acid, glucuronic acid, gluconic acid, lactic acid, and sometimes tartaric acid, malic acid, citric acid, succinic acid, oxalic acid, pyruvic acid, usnic acid, minerals: manganese (Mn), copper (Cu), iron (Fe), chromium (Cr), zinc (Zn), vitamins: C, K, E, and B group, including B_1, B_2, B_6, B_{12}, polyphenols, ethanol, fibre, carbon dioxide, sugars, amino acids and derivatives (especially tannin, a glutamine derivative), biogenic amines, purines, lipids, proteins, and dyes.[4,6,9,12–15] Moreover, it is suspected that this beverage may be a source of microor-

[a]*Department of Human Nutrition and Metabolomics, Pomeranian Medical University in Szczecin, 24 Broniewskiego St., 71-460 Szczecin, Poland.*
E-mail: karolina.jakubczyk@pum.edu.pl, 58147@student.pum.edu.pl, dominika.maciejewska.markiewicz@pum.edu.pl
[b]*Department of Cosmetic and Pharmaceutical Chemistry, Pomeranian Medical University in Szczecin, 72 Powstańców Wielkopolskich Ave., 70-111 Szczecin, Poland.*
E-mail: anna.muzykiewicz@pum.edu.pl, anna.nowak@pum.edu.pl
[c]*Department of Biochemical Science, Pomeranian Medical University in Szczecin, 24 Broniewskiego St., 71-460 Szczecin, Poland.*
E-mail: karolina.skonieczna.zydecka@pum.edu.pl

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ganisms with probiotic properties that positively influence the state of the intestinal microbiome.[1,16]

Among the components of kombucha, polyphenolic compounds that exhibit strong antioxidant properties, such as flavonoids, catechins, and their derivatives, constitute a numerous group.[2] Up to 127 phenolic compounds have been found in it thus far.[17,18] However, depending on the tea used, the presence and amount of each group of phytochemicals may vary.[9,11,17] This is due to the fact that the chemical composition of tea, the primary ingredient in kombucha, differs due to several factors. These include the type of tea, its region of origin (encompassing the place of growth, prevailing climate, presence of pollutants, and the type of soil in which it is cultivated), as well as processing methods. For example, Chinese teas, such as Longjing or Pu-erh, are characterized by a unique phytochemical profile that influences the properties of the beverage.[19,20] Variations in technological processes, such as fermentation or oxidation of tea leaves, result in different classes of teas with distinct biological properties, which can significantly impact the final composition of kombucha. Unlike the production of black tea, where the leaves undergo full oxidation, the production of green tea involves minimal oxidation, allowing *Camellia sinensis* leaves to retain higher levels of phytonutrients.[1,17,21] As a result, kombucha prepared from green tea has the highest total polyphenol content, potentially offering greater health benefits to consumers.[9]

The results of the studies conducted so far prove that this beverage exhibits antioxidant, antimicrobial, anti-inflammatory, antihypertensive, hypoglycaemic, cholesterol-lowering, immune and digestive system-supporting, and liver detoxification-stimulating properties.[9,22,23] Although these properties have been confirmed *in vitro* and *in vivo* studies, there is still a lack of clinical studies documenting the health-promoting effects of kombucha on human health. Despite the growing popularity of kombucha, there are significant gaps in the literature regarding the detailed composition and properties of this beverage. In particular, there is a lack of clinical studies confirming its health properties. In addition, the mechanisms of biotransformation of basic ingredients and the effect of various fermentation parameters and additives on the content of bioactive compounds remain incompletely understood. Recent studies indicate the need for more detailed analyses that would consider the diversity of raw materials and technological processes in the context of the potential health benefits of kombucha.[24,25]

Due to the wide range of health-promoting properties of kombucha, especially in recent years, it has been a frequent subject of research by scientists from various fields. Despite the popularity of this drink, it is difficult to find in the available literature an extensive analysis of kombucha with various flavour additives such as fruit, herbs, and spices.

To address gaps in the literature, this study conducted an extensive phytochemical analysis of various kombucha variants, examining the content of selected phenolic acids, flavonoids, caffeine, vitamin C, and acetic acid. The antioxidant activity of all tested kombucha samples was also evaluated using methods based on different mechanisms of action, alongside the determination of total polyphenol and flavonoid content. Additionally, the pH of the beverages was measured. The diversity of kombucha samples tested, combined with the comprehensive scope of analyses, highlights the novelty of this study.

The aim of this study was to determine whether flavour additives affect the content of bioactive compounds, such as polyphenols, vitamin C, and the antioxidant potential of kombucha. To clearly define the objectives and focus of the study, guiding the research process and helping to structure the manuscript, the following research questions were formulated:

1. How do different flavour additives affect the phytochemical profile of kombucha?

2. How does the antioxidant potential vary depending on the additives used?

3. Are there significant differences between different kombucha variants that may be appealing to different consumer groups?

HYPOTHESIS: Flavour additives enrich kombucha with bioactive compounds and increase its antioxidant properties compared to the basic variant.

2. Materials and methods

2.1 Materials

The study material consisted of 8 commercial green tea-based kombucha variants with the addition of the following raw materials: lemongrass; lavender; liquorice and mint; turmeric and lemon; mango; reishi and chaga; mint, rose, and pomegranate as well as without additives. The chosen additives were carefully selected to represent various groups of bioactive compounds, including herbs (lavender, mint), fruits (mango, pomegranate), and medicinal mushrooms (reishi, chaga).

All products were from an organic preserve manufacturer specializing in fermented foods, who is one of the largest kombucha manufacturers in Poland, providing the most diverse range of products (delikatna.bio, Poland). The composition and nutritional value included on the commercial labels of all kombucha are given in ESI Table 1.†

Chemical reagents (all of analytical grade): ethanol 96%, hydrochloric acid 35–38%, sodium hydroxide, methanol, Folin–Ciocalteu reagent, aluminum chloride, potassium persulfate, iron(III) chloride, sodium carbonate, sodium nitrite, glacial acetic acid, iron(II) sulfate were purchased from Chempur (Piekary Śląskie, Poland), 2,6-dichlorophenolindophenol sodium salt hydrate (2,6-DCPIP), *o*-xylene, 2,4,6-Tris(2-pyridyl)-*s*-triazine (TPTZ), 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2′-azobis(3-ethylbenzothiazolin-6-sulfonate) (ABTS), gallic acid, rutin, oxalic acid, ascorbic acid, 2-hydroxycinnamic acid, 4-hydroxybenzoic acid, sinapic acid, caffeic acid, caffeine, ferulic acid, 3,4-dihydroxybenzoic acid, ellagic acid, *p*-coumaric acid, apigenin, epicatechin gallate, kaempferol, myricetin, quercetin, resveratrol were purchased from Sigma-Aldrich (Darmstadt, Germany).

2.2 The determination of the ferric ion reducing antioxidant power (FRAP)

The methodology described by Benzie and Strain[26,27] was used to assess the ferric ion reducing antioxidant power using the FRAP method. FRAP solution was prepared by mixing (10 : 1 : 1 v/v/v) 300 mM acetate buffer (pH = 3.6), 10 mM 2,4,6-Tris(2-pyridyl)-*s*-triazine (TPTZ) solution in 40 mM HCl, and 20 mM $FeCl_3$ aqueous solution. 3 mL of the FRAP reagent, 0.1 mL the test sample and 0.3 mL distilled water were added into the vial. The vial contents were thoroughly mixed and placed for 5 min at 37 °C. The measurements at 593 nm were performed using a spectrophotometer Agilent Technologies 8453UV (Santa Clara, USA). All assays were performed in nine replicates. The ferric ion reducing antioxidant power was determined from the calibration curve using μM Fe(II) per L as the reference standard (0–5000 μM Fe(II) per L). The results were expressed as μM Fe(II) per L (Table 1).

2.3 Antioxidant activity assessed using the DPPH and ABTS methods

The methodology described by Brand-Williams *et al.* and Pekkarinen *et al.*[28,29] was used to assess antioxidant activity using the DPPH method and methodology described by Jakubczyk *et al.*[30] was used to assess antioxidant activity using the ABTS method. In order to measure antioxidant activity using synthetic radical DPPH 2.9 mL of a 96% ethanol, 1.0 mL of 0.3 mM DPPH ethanolic solution, and 0.1 mL the test sample were added into the vial. The vial contents were thoroughly mixed and incubated in the dark for 30 min at room temperature. The reference solution was prepared in the same way but instead of the tested sample 96% ethanol was added. To measure antioxidant activity using ABTS reagent was prepared ABTS stock solution by mixing 5 mL 7 mM ABTS aqueous solution and 5 mL 2.45 mM potassium persulfate ($K_2S_2O_8$) aqueous solution and incubated in the dark for 12–16 h. The ABTS stock solution was diluted with ethanol to obtain an absorbance of 1.0 ± 0.02 at 750 nm. Then, 0.1 mL of the tested sample and 2.9 mL of ABTS reagent were added into the vial. The vial contents were thoroughly mixed and incubated in the dark for 6 min at room temperature. The absorbance measurements (518 nm and 750 nm, for the DPPH and ABTS methods, respectively) were made using a spectrophotometer Agilent Technologies 8453UV (Santa Clara, USA). All assays were performed in nine replicates. Antioxidant potential of tested solutions was expressed by the percent of DPPH/ABTS inhibition (Table 1), using the following formula:

$$\%\,\text{inhibition} = \frac{A_0 - A_s}{A_0} \times 100$$

where: A_0 – absorbance of DPPH or ABTS solution without tested sample; A_s – absorbance of the tested sample.

Table 1 Antioxidant potential of kombuchas tested by the FRAP, ABTS, and DPPH method. Data represent the minimum, 25th percentile, median, 75th percentile, and maximum values of three replicates. Statistically significant differences ($p \leq 0.05$)

Flavour additive	Minimum	25th percentile	Median	75th percentile	Maximum	K–W *p* value
FRAP (μM Fe(II) per L)						
Lavender[a]	4459.70	4467.00	4521.20*[b–h]	4562.25	4578.20	<0.000001
Lemongrass[b]	2995.20	3000.25	3033.50*[a,c–h]	3049.38	3062.90	
Liquorice and mint[c]	4929.50	4932.40	4954.20*[a,b,d–h]	4976.50	5008.10	
Mango[d]	4075.10	4116.22	4289.70*[a–c,e–g]	4520.45	4573.20	
Mint, rose, pomegranate[e]	5230.60	5235.48	5273.90*[a–d,f–h]	5315.93	5346.80	
Reishi and chaga[f]	5425.30	5519.08	5545.10*[a–e,g,h]	5605.03	5635.90	
Turmeric and lemon[g]	4623.90	4630.53	4649.50*[a–f,h]	4689.78	4724.60	
Without additives[h]	4250.70	4267.93	4345.20*[a–c,e–g]	4367.95	4375.10	
ABTS (%)						
Lavender[a]	96.43	96.48	97.07*[e,f]	97.30	97.50	0.0003
Lemongrass[b]	89.94	95.50	97.72*[d,e]	98.06	98.09	
Liquorice and mint[c]	96.91	96.97	97.38*[d,e]	97.65	97.69	
Mango[d]	95.37	95.54	96.03*[b,c,e–g]	96.91	97.07	
Mint, rose, pomegranate[e]	97.38	97.48	98.26*[a–d,g,h]	98.48	98.53	
Reishi and chaga[f]	97.12	97.18	98.05*[a,d,h]	98.27	98.29	
Turmeric and lemon[g]	97.10	97.15	97.31*[d,e]	97.97	97.99	
Without additives[h]	94.45	94.47	95.52*[e,f]	98.86	98.87	
DPPH (%)						
Lavender[a]	84.14	84.43	87.51*[b–d,f–h]	88.75	88.99	<0.000001
Lemongrass[b]	90.32	90.40	92.37*[a,e–g]	92.58	92.80	
Liquorice and mint[c]	89.40	89.55	90.96*[a,b,d,e,g]	91.76	92.05	
mango[d]	88.97	89.00	89.19*[a–c,e–h]	89.74	89.80	
Mint, rose, pomegranate[e]	88.06	88.11	88.27*[b–d,f–h]	89.11	89.22	
Reishi and chaga[f]	90.65	90.73	91.38*[a,b,d,e,g]	91.73	92.00	
Turmeric and lemon[g]	92.35	92.40	94.99*[a–f,h]	95.11	95.14	
Without additives[h]	89.56	89.75	91.78*[a,d,e,g]	92.31	92.39	

Different letters (a–h) in the superscript represent statistically significant differences *$p < 0.05$ between particular type of kombucha: [a]kombucha with lavender, [b]kombucha with lemongrass, [c]kombucha with liquorice and mint, [d]kombucha with mango, [e]kombucha with mint, rose, and pomegranate, [f]kombucha with reishi and chaga, [g]kombucha with turmeric and lemon, [h]kombucha without additives; K–W – Kruskal–Wallis.

2.4 The determination of the total polyphenol content (TPC) and total flavonoid content (TFC)

The determination of total polyphenol content using the Folin–Ciocalteu method was performed according to the previously described methodology Singleton V. L., Rossi J. A.[31] 5.0 mL of a 10% Folin–Ciocalteu aqueous solution and 1.0 mL of test sample were added into the vial. The vial contents were thoroughly mixed and after 5 min. 4.0 mL of 7.5% Na_2CO_3 solution was added and incubated for 60 min at room temperature. The reference solution was prepared the same way but instead of tested sample distilled water was added. The total flavonoid content was assessed according to the method described by Hu *et al.* and Pękal and Pyrzynska.[32,33] Firstly, 0.6 mL of a 5% $NaNO_2$ aqueous solution and 2.0 mL of test sample were added into the vial. The vial contents were thoroughly mixed and incubated for 6 min, then 0.5 mL of 10% $AlCl_3$ aqueous solution was added. Incubation was repeated under the same conditions. Then 3.0 mL of NaOH aqueous solution (4.3%) and 3.9 mL of distilled water were added to the vial. The contents of the vial were again mixed thoroughly and incubated for 15 minutes. The absorbance measurements (765 nm and 510 nm, for the TPC and TFC methods, respectively) were made with a spectrophotometer Agilent Technologies 8453UV (Santa Clara, USA). All assays were performed in nine replicates. The content of polyphenols was determined from the calibration curve using gallic acid as the reference standard (0–200 mg L^{-1} of gallic acid). The results were expressed as gallic acid equivalents (GAE) – mg GAE per L. The content of flavonoids was determined from the calibration curve using rutin as the reference standard (0–120 mg RE per L). The results were expressed as rutin equivalents (RE) – mg RE per L (Table 2).

2.5 The determination of vitamin C content

The determination of vitamin C content was carried out according to ISO 6557-2:1984. Absorbance measurements were taken at 500 nm (Agilent Technologies 8453UV (Santa Clara, USA)) in 1 cm quartz cuvette with xylene as a reference. All assays were performed in nine replicates. The concentration of vitamin C was expressed in mg of vitamin C per 100 mL of kombucha (mg per 100 mL) (Table 3).

2.6 The determination of the phenolic acids, flavonoids, and caffeine content

Liquid chromatography (Agilent Technologies 1260 HPLC System, USA) was used to determine the concentration of polyphenol compounds. The column used was Hypersil Gold (150 × 4.6). The temperature was maintained at 25 °C. The detection of phenolic compounds was performed by UV absorption at λ = 278 nm. Each compound was identified based on its retention time and by comparison with standards under the same conditions. The mobile phase consisted of 1% aqueous acetic acid solution (A) and 100% MeOH (B). The samples were eluted with the following gradient: 90% A and 10% B from 0 to 6 min, 84% A and 16% B from 7 to 25 min, 72% A and 28% B from 26 to 37 min, 65% A and 35% B from 38 to 47 min, 50% A and 50% B from 48 to 64 min, and 90% A and 10% B from 65 to 70 min, to restore the initial conditions, before injection of a new sample. The flow rate was 0.8 mL min^{-1}, and the injection volume was 30 μL.

2.7 The determination of the acetic acid content

Gas chromatographic analyses were conducted using the Agilent Technologies 7890 A GC system with a flame ionization detector (FID). A fused-silica capillary column with a free fatty

Table 2 The total polyphenols content (TPC) and the total flavonoids content (TFC) in kombuchas. Data represent the minimum, 25th percentile, median, 75th percentile, and maximum values of three replicates. Statistically significant differences ($p \leq 0.05$)

Flavour additive	Minimum	25th percentile	Median	75th percentile	Maximum	K–W p value
TPC (mg GAE per L)						
Lavender[a]	245.80	245.97	247.38*[b–h]	252.20	254.29	<0.000001
Lemongrass[b]	182.11	182.22	182.25*[a,c–h]	185.93	186.16	
Liquorice and mint[c]	262.41	263.79	264.56*[a,b,d–h]	264.98	266.83	
Mango[d]	71.12	71.17	71.42*[a–c,e–h]	77.18	77.21	
Mint, rose, pomegranate[e]	87.49	87.51	88.41*[a–d,f–h]	88.78	88.78	
Reishi and chaga[f]	99.46	99.48	100.13*[a–e,g,h]	100.33	100.34	
Turmeric and lemon[g]	262.76	264.88	265.82*[a–f,h]	266.68	267.92	
Without additives[h]	234.98	235.68	239.45*[a–g]	240.67	240.91	
TFC (mg RE per L)						
Lavender[a]	220.43	220.73	231.62*[b–h]	241.48	241.66	<0.000001
Lemongrass[b]	90.30	90.37	100.63*[a,c–g]	112.02	112.11	
Liquorice and mint[c]	155.75	156.29	162.31*[a,b,d–f,h]	167.94	168.34	
mango[d]	26.44	26.73	32.91*[a–c,e–h]	32.95	33.22	
Mint, rose, pomegranate[e]	22.90	22.97	27.03*[a–d,f–h]	27.64	28.20	
Reishi and chaga[f]	49.85	50.57	51.41*[a–e,g,h]	55.12	55.82	
Turmeric and lemon[g]	156.28	156.63	164.54*[a,b,d–f,h]	227.68	228.59	
Without additives[h]	86.52	86.62	93.21*[a,c–g]	114.73	114.94	

Different letters (a–h) in the superscript represent statistically significant differences *$p < 0.05$ between particular type of kombucha: [a]kombucha with lavender, [b]kombucha with lemongrass, [c]kombucha with liquorice, and mint, [d]kombucha with mango, [e]kombucha with mint, rose, and pomegranate, [f]kombucha with reishi, and chaga, [g]kombucha with turmeric and lemon, [h]kombucha without additives; K–W – Kruskal–Wallis; GAE – gallic acid equivalent; RE – rutin equivalent.

Table 3 The total vitamin C content in kombuchas. Data represent the minimum, 25th percentile, median, 75th percentile and maximum values of three replicates. Statistically significant differences ($p \leq 0.05$)

Flavour additive	Minimum	25th percentile	Median	75th percentile	Maximum	K–W p value
Lavender[a]	32.14	32.20	36.08*[b,c,e,g,h]	36.34	36.42	0.000008
Lemongrass[b]	27.01	27.03	27.19*[a,c–g]	27.33	27.38	
Liquorice and mint[c]	29.98	30.04	30.61*[a,b,h]	31.08	31.18	
Mango[d]	29.91	30.01	30.11*[b,h]	38.24	38.32	
Mint, rose, pomegranate[e]	29.50	29.56	30.27*[a,b,h]	31.46	31.60	
Reishi and chaga[f]	23.72	23.76	37.53*[b,h]	39.58	39.62	
Turmeric and lemon[g]	27.46	27.73	30.28*[a,b,h]	34.42	34.48	
Without additives[h]	24.57	24.61	27.20*[a,c–g]	28.19	28.32	

Different letters (a–h) in the superscript represent statistically significant differences *$p < 0.05$ between particular type of kombucha: [a]kombucha with lavender, [b]kombucha with lemongrass, [c]kombucha with liquorice, and mint, [d]kombucha with mango, [e]kombucha with mint, rose, and pomegranate, [f]kombucha with reishi, and chaga, [g]kombucha with turmeric and lemon, [h]kombucha without additives; K–W – Kruskal–Wallis.

acid phase (DB-FFAP, 30 m × 0.53 mm × 0.5 μm) was used. The carrier gas was hydrogen at a flow rate equal to 14.4 mL min^{-1}. The initial temperature (100 °C) was maintained for 0.5 min, then raised to 180 °C with ramping of 8 °C min^{-1} to be constant for 1 min. Subsequently, the temperature was increased to 200 °C (ramping 20 °C min^{-1}) and sustained for 5 min. The injection volume was 5 μL, and the run time of a single analysis was 17.5 min. Results were presented as a percentage of acetic acid content, according to the surface area. Moreover, the amount of acetic acid was evaluated using the calibration curve method (mM of acetic acid per L).[34]

2.8 The determination of pH

The pH of kombucha was determined by a pH meter (SCHOTT Instruments; SI Analytics Mainz, Mainz, Germany).

2.9 Statistical analysis

Statistical analysis was performed using MedCalc® Statistical Software version 20.218 (MedCalc Software Ltd, Ostend, Belgium; https://www.medcalc.org; 2023) and Microsoft Excel 2017. Results were expressed by median, upper and lower quartile, minimum, and maximum values. The non-parametric Kruskal–Wallis test with Conover's *post hoc* test was used to assess differences between the study parameters. Differences were considered significant at $p \leq 0.05$.

Principal component analysis (PCA) was performed to reduce the dimensionality of the dataset and capture the variability among kombucha products. The data were standardized before PCA, and the first two principal components (PC1 and PC2) were selected for visualization, explaining the most variance in the data. A scatter plot was created to visualize the products as dots in the reduced dimensional space, allowing for the identification of product clusters based on chemical profiles. A Euclidean distance matrix was calculated based on the PCA scores (PC1 and PC2), and hierarchical clustering using Ward's method was applied. The resulting dendrogram revealed the relationships and clusters between products, with merging heights indicating the degree of similarity. Additionally, a one-way ANOVA was performed on the PC1 scores to assess significant differences between products, followed by Tukey's HSD *post hoc* test for pairwise comparisons. These tests demonstrated significant differences between certain product groups. ll statistical analyses, including PCA, hierarchical clustering, and ANOVA, were performed using Python (version 3.8) with the libraries scikit-learn for PCA and clustering, scipy for ANOVA, and statsmodels for Tukey's HSD *post hoc* test. Data visualization was conducted using matplotlib. The hierarchical clustering dendrogram was generated using the scipy. cluster.hierarchy module.

3. Results

3.1 The analysis of the antioxidant properties and phytochemical composition of kombuchas

The antioxidant activity of the tested kombuchas was assessed by its ability to neutralize radicals (DPPH, ABTS) and reduce iron ions (FRAP).

Kombuchas antioxidant potential expressed as the ability to reduce iron ions, ranged from 3033.5 to 5545.1 μM Fe(II) per L. Kombucha with reishi and chaga had the highest activity, while kombucha with lemongrass had the lowest. Statistically significant differences were observed between all kombuchas, except kombucha with mango *vs.* kombucha without additives (Table 1).

The antioxidant potential of the tested kombuchas, expressed as a percentage of ABTS radical inhibition, ranged from 95.52 to 98.26%. Kombucha with mint, rose, and pomegranate had the highest radical scavenging ability, while kombucha without additives as well as kombucha with mango had the lowest. Statistically significant differences between the tested kombuchas are presented in Table 1.

The antioxidant potential expressed as a percentage of DPPH radical inhibition, ranged from 87.51 to 94.99%. The highest result was found for kombucha with turmeric and lemon, while the lowest for kombucha with lavender. No statistically significant differences were observed between kombucha with lavender *vs.* kombucha with mint, rose, and pomegranate; kombucha with lemongrass *vs.* kombucha without additives; kombucha with liquorice and mint *vs.* kombucha without additives; kombucha with liquorice and mint *vs.* kom-

bucha with reishi and chaga, as well as kombucha with reishi and chaga *vs.* kombucha without additives (Table 1).

The total polyphenol content (TPC) of the tested kombuchas ranged from 71.42 to 265.82 mg GAE per L. Kombucha with turmeric and lemon had the highest polyphenol content, while kombucha with mango had the lowest. Statistically significant differences were observed between all kombuchas (Table 2).

The total flavonoid content (TFC) of the tested kombuchas ranged from 27.03 to 231.62 mg RE per L. Kombucha with lavender had the highest total flavonoid content, while kombucha with mint, rose, and pomegranate had the lowest. Statistically significant differences were observed between all kombuchas except kombucha with lemongrass *vs.* kombucha without additives as well as kombucha with liquorice and mint *vs.* kombucha with turmeric and lemon (Table 2). Kombuchas also appears to be a good source of vitamin C (Table 3). The highest concentration of it was identified in kombucha with reishi and chaga – 37.53 mg per 100 mL, while the lowest concentration was identified in kombucha with lemongrass – 27.19 mg per 100 mL as well as in kombucha without additives – 27.2 mg per 100 mL. The juxtaposition of the obtained results, it is not possible to identify a single product that is the best in terms of all the analysed parameters (Fig. 1) kombucha with turmeric and lemon showed the highest content of polyphenols and the highest antioxidant potential tested by the DPPH method. Kombucha with lavender contained the highest content of flavonoids. Kombucha with reishi and chaga proved to be the best source of vitamin C and showed the highest potential assessed by the FRAP method. The highest antioxidant potential evaluated using the ABTS technique was shown by kombucha with mint, rose, and pomegranate.

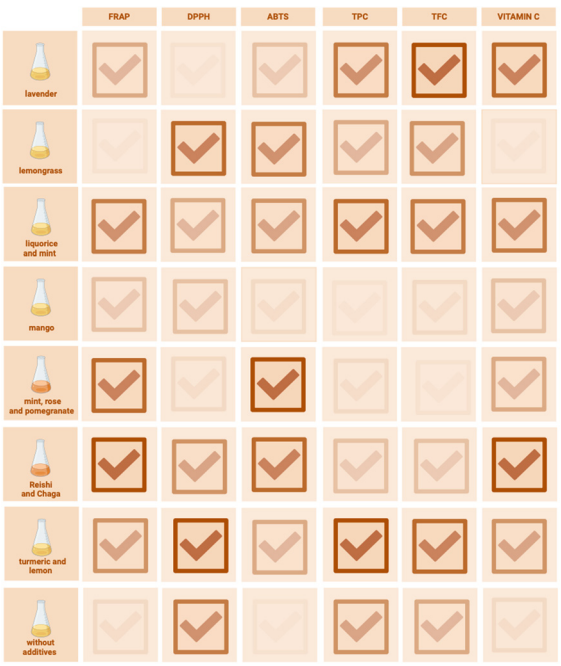

Fig. 1 The comparison of the antioxidant properties and phytochemical composition of tested kombuchas. The value of the tested parameters was determined by changing the color intensity.

Analysis of the quantitative and qualitative composition of polyphenolic compounds in kombucha revealed the presence of 17 compounds, including caffeine; phenolic acids: gallic acid, 4-hydroxybenzoic acid, 2-hydroxycinnamic acid, *p*-coumaric acid, ferulic acid, sinapic acid, ellagic acid, 3,4-dihydroxybenzoic acid, caffeic acid; flavonoids: epicatechin gallate, rutin, resveratrol, myricetin, quercetin, kaempferol, and apigenin. Of the phenolic acids, the tested beverages contained the highest amounts of *p*-coumaric acid, 4-hydroxybenzoic acid, and gallic acid, while among the flavonoids, myricetin and epicatechin gallate (Tables 4 and 5).

The content of the identified compounds was statistically significantly different between the flavour variants tested. The highest apigenin content (3.30 mg L^{-1}) was detected in kombucha with mango, while the highest ellagic acid content (6.75 mg L^{-1}) was identified in kombucha without additives. Kombucha with reishi and chaga had the highest content of *p*-coumaric acid (236.98 mg L^{-1}), 3,4-dihydroxybenzoic acid (46.94 mg L^{-1}), 4-hydroxybenzoic acid (180.72 mg L^{-1}), quercetin (11.25 mg L^{-1}), resveratrol (8.22 mg L^{-1}), epicatechin gallate (203.53 mg L^{-1}), caffeine (1457.29 mg L^{-1}) and together with kombucha with liquorice and mint showed the highest content of 2-hydroxycinnamic acid. Kombucha with liquorice and mint contained the highest amounts of rutin (227.69 mg L^{-1}), sinapic acid (28.62 mg L^{-1}) and kaempferol (6.76 mg L^{-1}). Kombucha with lavender had the highest content of ferulic acid (20.17 mg L^{-1}) and gallic acid (105.84 mg L^{-1}). Kombucha with mint, rose, and pomegranate showed the highest content of caffeic acid (5.67 mg L^{-1}) and, together with kombucha with reishi, and chaga, contained significantly higher amounts of myricetin than the other kombuchas.

3.2 The analysis of pH and acetic acid content of kombuchas

The pH values of the tested kombuchas ranged from 2.72 to 3.72 (Table 6). Statistically significant differences were observed between all kombuchas, except kombucha with lavender *vs.* kombucha without additives (Table 6).

The acetic acid content of the kombucha tested ranged from 28.281 to 37.029 mM L^{-1}. Statistically significant differences are shown in Table 7.

3.3 Principal component analysis (PCA)

PCA reduced the dataset to two principal components, with PC1 and PC2 explaining a substantial portion of the total variance. In the PCA plot (Fig. 2), products were clearly separated into distinct clusters, indicating differences in their chemical profiles. Products such as “GREEN” and “LEMONGRASS” clustered closely within their respective groups, while products like “REISHI_CHAGA” and “MANGO” appeared further apart, reflecting their distinct profiles.

Table 4 Content of phenolic acids and caffeine in kombuchas. Data represent the minimum, 25th percentile, median, 75th percentile and maximum values of three replicates. Statistically significant differences ($p \leq 0.05$)

Compound	Flavour additive	Minimum [mg L^{-1}]	25th percentile [mg L^{-1}]	Median [mg L^{-1}]	75th percentile [mg L^{-1}]	Maximum [mg L^{-1}]	K–W p value
2-Hydroxycinnamic acid	Lavender[a]	3.72	3.73	3.75*[b–h]	3.78	3.79	0.0022
	Lemongrass[b]	3.54	3.55	3.58*[a,c–f,h]	3.60	3.61	
	Liquorice and mint[c]	16.12	16.16	16.27*[a,b,d,e,g,h]	16.39	16.42	
	Mango[d]	5.67	5.68	5.72*[a–c,e–h]	5.76	5.77	
	Mint, rose, pomegranate[e]	2.99	3.00	3.02*[a–d,f–h]	3.27	3.36	
	Reishi and chaga[f]	15.95	15.99	16.10*[a,b,d,e,g,h]	16.21	16.25	
	Turmeric and lemon[g]	3.54	3.55	3.57*[a,c–f,h]	3.60	3.61	
	Without additives[h]	7.864	7.88	7.94*[a–g]	7.99	8.01	
4-Hydroxybenzoic acid	Lavender[a]	28.50	28.57	28.77*[b–h]	28.97	29.03	0.0019
	Lemongrass[b]	38.27	38.36	38.63*[a,c–h]	38.90	38.99	
	Liquorice and mint[c]	69.94	70.10	70.59*[a,b,d–h]	71.08	71.24	
	Mango[d]	67.82	67.98	68.46*[a–c,e–h]	68.93	69.09	
	Mint, rose, pomegranate[e]	59.81	59.95	60.37*[a–d,f–h]	60.79	60.93	
	Reishi and chaga[f]	179.05	179.47	180.72*[a–e,g,h]	181.98	182.40	
	Turmeric and lemon[g]	26.03	26.09	26.27*[a–f,h]	26.45	26.52	
	Without additives[h]	47.44	47.55	47.88*[a–g]	48.22	48.33	
Sinapic acid	Lavender[a]	6.00	6.02	6.06*[b–h]	6.11	6.13	0.0020
	Lemongrass[b]	9.51	9.53	9.60*[a,c–h]	9.68	9.70	
	Liquorice and mint[c]	28.36	28.43	28.62*[a,b,d–h]	28.86	28.94	
	Mango[d]	8.13	8.15	8.20*[a–c,e–h]	8.27	8.29	
	Mint, rose, pomegranate[e]	10.24	10.27	10.34*[a–d,f–h]	10.42	10.45	
	Reishi and chaga[f]	24.89	24.95	25.12*[a–e,g,h]	25.33	25.40	
	Turmeric and lemon[g]	9.69	9.71	9.78*[a–f,h]	9.86	9.89	
	Without additives[h]	0.00	0.00	0.00*[a–g]	0.00	0.00	
Caffeic acid	Lavender[a]	3.18	3.19	3.21*[b–h]	3.23	3.24	0.0019
	Lemongrass[b]	0.27	0.27	0.27*[a,c–h]	0.27	0.27	
	Liquorice and mint[c]	4.66	4.70	4.80*[a,b,d–h]	4.84	4.85	
	Mango[d]	1.21	1.21	1.22*[a–c,e–h]	1.23	1.23	
	Mint, rose, pomegranate[e]	5.62	5.63	5.67*[a–d,f–h]	5.71	5.72	
	Reishi and chaga[f]	3.31	3.32	3.34*[a–e,g,h]	3.57	3.64	
	Turmeric and lemon[g]	1.89	1.90	1.91*[a–f,h]	1.92	1.93	
	Without additives[h]	2.05	2.06	2.07*[a–g]	2.09	2.09	
Ferulic acid	Lavender[a]	19.987	20.03	20.17*[b–h]	20.34	20.40	0.0019
	Lemongrass[b]	4.93	5.06	5.43*[a,c–h]	5.46	5.48	
	Liquorice and mint[c]	16.97	17.10	17.48*[a,b,d–h]	17.60	17.64	
	Mango[d]	6.10	6.11	6.15*[a–c,e–h]	6.20	6.22	
	Mint, rose, pomegranate[e]	6.81	6.82	6.87*[a–d,f–h]	6.93	6.94	
	Reishi and chaga[f]	13.58	13.65	13.86*[a–e,g,h]	14.57	14.80	
	Turmeric and lemon[g]	7.715	7.73	7.79*[a–f,h]	7.85	7.87	
	Without additives[h]	10.26	10.28	10.36*[a–g]	10.44	10.47	
3,4-Dihydroxybenzoic acid	Lavender[a]	20.97	21.02	21.16*[b–h]	21.31	21.36	0.0021
	Lemongrass[b]	12.54	12.57	12.66*[a,c–h]	12.75	12.78	
	Liquorice and mint[c]	28.76	28.83	29.03*[a,b,d–g]	29.23	29.30	
	Mango[d]	16.76	16.80	16.92*[a–c,e–h]	17.04	17.08	
	Mint, rose, pomegranate[e]	37.73	37.81	38.08*[a–d,f–h]	38.34	38.43	
	Reishi and chaga[f]	46.50	46.61	46.94*[a–e,g,h]	47.27	47.37	
	Turmeric and lemon[g]	15.03	15.07	15.17*[a–f,h]	15.28	15.31	
	Without additives[h]	28.95	29.02	29.22*[a,b,d–g]	29.43	29.50	
Ellagic acid	Lavender[a]	4.53	4.54	4.57*[b–h]	4.60	4.61	0.0019
	Lemongrass[b]	1.53	1.54	1.545[a,c–h]	1.56	1.56	
	Liquorice and mint[c]	0.00	0.00	0.00*[a,b,d–h]	0.00	0.00	
	Mango[d]	2.10	2.10	2.12*[a–c,e–h]	2.13	2.13	
	Mint, rose, pomegranate[e]	5.07	5.08	5.12*[a–d,f–h]	5.55	5.70	
	Reishi and chaga[f]	6.31	6.33	6.37*[a–e,g,h]	6.42	6.43	
	Turmeric and lemon[g]	1.47	1.48	1.49*[a–f,h]	1.50	1.50	
	Without additives[h]	6.69	6.70	6.75*[a–g]	6.80	6.81	
***p*-Coumaric acid**	Lavender[a]	11.83	11.85	11.94*[b–h]	12.04	12.07	0.0019
	Lemongrass[b]	70.09	71.84	77.10*[a,c–h]	77.63	77.81	
	Liquorice and mint[c]	37.80	38.08	38.93*[a,b,d–h]	39.20	39.29	
	Mango[d]	50.10	50.22	50.57*[a–c,e–h]	50.98	51.12	
	Mint, rose, pomegranate[e]	21.87	21.92	22.08*[a–d,f–h]	22.26	22.32	

Table 4 *(Contd.)*

Compound	Flavour additive	Minimum [mg L^{-1}]	25th percentile [mg L^{-1}]	Median [mg L^{-1}]	75th percentile [mg L^{-1}]	Maximum [mg L^{-1}]	K–W *p* value
	Reishi and chaga[f]	232.24	233.42	236.98*[a–e,g,h]	249.10	253.14	
	Turmeric and lemon[g]	23.93	23.99	24.15*[a–f,h]	24.35	24.42	
	Without additives[h]	65.05	65.20	65.66*[a–g]	66.20	66.38	
Gallic acid	Lavender[a]	104.87	105.11	105.84*[b–h]	106.58	106.83	0.0019
	Lemongrass[b]	30.31	30.38	30.59*[a,c–h]	30.80	30.88	
	liquorice and mint[c]	69.61	69.77	70.25*[a,b,d–h]	70.74	70.91	
	Mango[d]	40.44	40.53	40.82*[a–c,e–h]	41.10	41.19	
	Mint, rose, pomegranate[e]	98.09	98.32	99.00*[a–d,f–h]	99.69	99.92	
	Reishi and chaga[f]	100.47	100.70	101.40*[a–e,g,h]	102.12	102.35	
	Turmeric and lemon[g]	33.07	33.15	33.38*[a–f,h]	33.61	33.69	
	Without additives[h]	78.92	79.10	79.65*[a–g]	80.21	80.39	
Caffeine	Lavender[a]	521.63	524.29	532.27*[b–h]	540.42	543.13	0.0022
	Lemongrass[b]	393.26	400.92	423.93*[a,c–f,h]	430.42	432.58	
	Liquorice and mint[c]	740.59	742.33	747.55*[a,b,d–g]	758.99	762.81	
	Mango[d]	440.44	442.69	449.43*[a–c,e–h]	456.31	458.60	
	Mint, rose, pomegranate[e]	594.00	597.03	606.12*[a–d,f–h]	615.40	618.49	
	Reishi and chaga[f]	1428.15	1435.43	1457.29*[a–e,g,h]	1531.83	1556.68	
	Turmeric and lemon[g]	392.38	394.38	400.39*[a,c–f,h]	406.52	408.56	
	Without additives[h]	754.69	758.54	770.09*[a,b,d–g]	781.88	785.80	

Different letters (a–h) in the superscript represent statistically significant differences $*p < 0.05$ between particular type of kombucha: [a]kombucha with lavender, [b]kombucha with lemongrass, [c]kombucha with liquorice, and mint, [d]kombucha with mango, [e]kombucha with mint, rose, and pomegranate, [f]kombucha with reishi, and chaga, [g]kombucha with turmeric and lemon, [h]kombucha without additives; K–W – Kruskal–Wallis; nd – not detected.

3.4 Hierarchical cluster analysis

Hierarchical clustering (Fig. 3) based on Euclidean distances between products further supported the grouping observed in the PCA plot. The dendrogram revealed well-defined clusters, with products merging at different heights. Products like "GREEN" and "LEMONGRASS" clustered together at lower heights, indicating higher similarity, while others merged at higher levels, suggesting greater dissimilarity.

4. Discussion

4.1 Impact of flavour additives on phytochemical composition on antioxidant efficacy in kombucha

The popularity of fermented beverages has been rapidly growing in recent years, which has resulted in strong growth of the kombucha market. In 2018, its value was $1.5 billion. It is estimated that it could grow to around $5 billion by 2025, with an assumed average annual growth rate (CAGR) of 23%.[10,35] Food manufacturers, recognizing the huge interest in this segment of the food industry and the growing consumption of kombucha, are offering a wide range of commercial products. What's more, to meet consumer expectations, both in terms of nutritional and sensory qualities, producers will introduce new variants that are a modification of the traditional recipe. Although a few scientific publications indicate that additions of a plant's raw materials can favourably affect the composition and properties of kombucha, these data are still incomplete. Promising additions, it seems, are fruits, herbs, and spices due to the proven health benefits of their consumption, thanks to which they are counted as traditional functional foods.[36] They positively influence on the body condition and reduce the risk of certain chronic diseases.[37–39] Numerous health-promoting properties are due to the presence of many bioactive substances, including vitamins, minerals, and antioxidants.[37,40,41] It is believed that the addition of spices to fermented substrates affects the microbial environment of the process, which in turn significantly affects the chemical profile and antioxidant properties. The result is a product with better taste, higher content of bioactive compounds, and higher antioxidant activity.[42,43] Shahbazi *et al.* studied the effect of the addition of cinnamon, cardamom, and thyme (Shirazi) extracts on physiochemical, antioxidant, and sensory properties of kombucha. They considered the cinnamon-flavoured kombucha to be the best quality product, which had the highest content of polyphenols (0.582 mg GAE per mL) and flavonoids (0.312 mg CTE per 100 mL) and showed the best inhibitory activity against *E. coli*, *S. typhimurium*, and *S. aureus*. In addition, it received the highest ratings in terms of taste, aroma, pleasantness, acidity, and colour. Their results confirmed that the enrichment of the base tea with selected plant materials can cause the enhancement of antimicrobial and antioxidant activity while improving the taste and aroma of the product.[44,45] Researchers investigating kombuchas prepared from blends of oolong tea and peppermint came to similar conclusions. Analysis of the antioxidant potential after 14 days of fermentation, showed that kombucha based on a blend of oolong tea and peppermint in a ratio of 9 g: 3 g showed the highest antioxidant potential as measured by DPPH (96.70 ± 0.11%) and ABTS (100.02 ± 0.04%). The highest flavonoid

Table 5 Content of flavonoids in kombuchas. Data represent the minimum, 25th percentile, median, 75th percentile, and maximum values of three replicates. Statistically significant differences ($p \leq 0.05$)

Compound	Flavour additive	Minimum [mg L^{-1}]	25th percentile [mg L^{-1}]	Median [mg L^{-1}]	75th percentile [mg L^{-1}]	Maximum [mg L^{-1}]	K–W p value
Apigenin	Lavender[a]	0.00	0.00	0.00*[c–g]	0.00	0.00	0.0019
	Lemongrass[b]	0.00	0.00	0.00*[c–g]	0.00	0.00	
	Liquorice and mint[c]	1.45	1.4549	1.46*[a,b,d–h]	1.47	1.48	
	Mango[d]	3.27	3.28	3.30*[a–c,e–h]	3.32	3.33	
	Mint, rose, pomegranate[e]	2.67	2.68	2.70*[a–d,f–h]	2.93	3.00	
	Reishi and chaga[f]	1.47	1.48	1.49*[a–e,g,h]	1.50	1.50	
	Turmeric and lemon[g]	0.96	0.96	0.97 *[a–f,h]	0.98	0.98	
	Without additives[h]	0.00	0.00	0.00*[c–g]	0.00	0.00	
Epicatechin gallate	Lavender[a]	30.07	30.14	30.35*[b–h]	30.60	30.68	0.0019
	Lemongrass[b]	45.77	46.91	50.34*[a,c–h]	50.70	50.81	
	Liquorice and mint[c]	38.00	38.09	38.35*[a,b,d–h]	38.67	38.78	
	Mango[d]	61.05	61.20	61.62*[a–c,e–h]	62.13	62.30	
	Mint, rose, pomegranate[e]	31.12	31.19	31.41*[a–d,f–h]	31.67	31.76	
	Reishi and chaga[f]	199.46	200.48	203.53*[a–e,g,h]	213.94	217.41	
	Turmeric and lemon[g]	28.64	28.71	28.91*[a–f,h]	29.15	29.23	
	Without additives[h]	21.99	22.04	22.19*[a–g]	22.38	22.44	
Kaempferol	Lavender[a]	1.65	1.65	1.66*[b–d,f–h]	1.67	1.68	0.0021
	Lemongrass[b]	1.14	1.14	1.15*[a,c–h]	1.16	1.16	
	Liquorice and mint[c]	6.69	6.71	6.76*[a,b,d–h]	6.80	6.82	
	Mango[d]	1.11	1.12	1.13*[a–c,e–h]	1.13	1.14	
	Mint, rose, pomegranate[e]	1.53	1.54	1.55*[b–d,f–h]	1.68	1.72	
	Reishi and chaga[f]	2.63	2.64	2.65*[a–e,g,h]	2.67	2.68	
	Turmeric and lemon[g]	3.58	3.59	3.61*[a–f,h]	3.64	3.65	
	Without additives[h]	1.92	1.92	1.94*[a–g]	1.95	1.95	
Myricetin	Lavender[a]	39.80	39.90	40.17*[b–h]	40.45	40.55	0.0021
	Lemongrass[b]	8.71	8.74	8.80*[a,c–h]	8.86	8.88	
	Liquorice and mint[c]	41.84	41.94	42.23*[a–b,d–h]	42.52	42.62	
	Mango[d]	20.63	20.68	20.82*[a–c,e–h]	20.97	21.02	
	Mint, rose pomegranate[e]	89.99	90.20	90.83*[a–d,g,h]	98.55	101.12	
	Reishi and chaga[f]	93.05	93.27	93.92*[a–d,g,h]	94.57	94.79	
	Turmeric and lemon[g]	5.02	5.03	5.07*[a–f,h]	5.10	5.12	
	Without additives[h]	15.73	15.77	15.88*[a–g]	15.99	16.02	
Quercetin	Lavender[a]	0.73	0.73	0.74*[b–h]	0.74	0.74	0.0021
	Lemongrass[b]	0.59	0.59	0.60*[a,c–h]	0.60	0.60	
	Liquorice and mint[c]	2.20	2.20	2.22*[a,b,d–h]	2.23	2.24	
	Mango[d]	2.76	2.77	2.78*[a–c,f–h]	2.80	2.81	
	Mint, rose, pomegranate[e]	2.71	2.72	2.74*[a–c,f–h]	2.97	3.05	
	Reishi and chaga[f]	11.15	11.18	11.25*[a–e,g,h]	11.33	11.36	
	Turmeric and lemon[g]	0.00	0.00	0.00*[a–f,h]	0.00	0.00	
	Without additives[h]	3.62	3.63	3.65*[a–g]	3.68	3.68	
Resveratrol	Lavender[a]	0.00	0.00	0.00*[b–g]	0.00	0.00	0.0019
	Lemongrass[b]	2.01	2.01	2.02*[a,c–h]	2.04	2.04	
	Liquorice and mint[c]	2.60	2.61	2.63*[a,b,d–h]	2.64	2.65	
	Mango[d]	2.96	2.97	2.99*[a–c,e–h]	3.01	3.02	
	Mint, rose, pomegranate[e]	2.19	2.20	2.21*[a–d,f–h]	2.40	2.46	
	Reishi and chaga[f]	8.14	8.16	8.22*[a–e,g,h]	8.27	8.29	
	Turmeric and lemon[g]	1.14	1.14	1.15*[a–f,h]	1.15	1.16	
	Without additives[h]	0.00	0.00	0.00*[b–g]	0.00	0.00	
Rutin	Lavender[a]	0.00	0.00	0.00*[c,f,h]	0.00	0.00	0.0018
	Lemongrass[b]	0.00	0.00	0.00*[c,f,h]	0.00	0.00	
	Liquorice and mint[c]	225.59	226.11	227.69*[a,b,d–h]	229.27	229.80	
	Mango[d]	0.00	0.00	0.00*[c,f,h]	0.00	0.00	
	Mint, rose, pomegranate[e]	0.00	0.00	0.00*[c,f,h]	0.00	0.00	
	Reishi and chaga[f]	34.37	34.45	34.69*[a–e,g,h]	34.93	35.01	
	Turmeric and lemon[g]	0.00	0.00	0.00*[c,f,h]	0.00	0.00	
	Without additives[h]	45.66	45.77	46.09*[a–g]	46.41	46.52	

Different letters (a–h) in the superscript represent statistically significant differences *$p < 0.05$ between particular type of kombucha: [a]kombucha with lavender, [b]kombucha with lemongrass, [c]kombucha with liquorice, and mint, [d]kombucha with mango, [e]kombucha with mint, rose, and pomegranate, [f]kombucha with reishi, and chaga, [g]kombucha with turmeric and lemon, [h]kombucha without additives; K–W – Kruskal–Wallis.

Table 6 The pH of kombuchas. Data represent the minimum, 25th percentile, median, 75th percentile and maximum values of three replicates. Statistically significant differences ($p \leq 0.05$)

Flavour additive	Minimum	25th percentile	Median	75th percentile	Maximum	K–W p value
Lavender[a]	2.98	2.98	2.99*[b,c–g]	2.99	2.99	0.0028
Lemongrass[b]	2.94	2.94	2.94*[a,c–h]	2.95	2.95	
Liquorice and mint[c]	3.07	3.07	3.08*[a,b,d–h]	3.09	3.09	
Mango[d]	3.72	3.72	3.72*[a–c,e–h]	3.74	3.74	
Mint, rose, pomegranate[e]	3.56	3.56	3.56*[a–d,f–h]	3.58	3.58	
Reishi and chaga[f]	3.69	3.69	3.69*[b–g]	3.69	3.69	
Turmeric and lemon[g]	2.69	2.70	2.72*[a–f,h]	2.74	2.74	
Without additives[h]	2.99	2.99	2.99*[b–g]	2.99	2.99	

Different letters (a–h) in the superscript represent statistically significant differences *$p < 0.05$ between particular type of kombucha: [a]kombucha with lavender, [b]kombucha with lemongrass, [c]kombucha with liquorice and mint, [d]kombucha with mango, [e]kombucha with mint, rose, and pomegranate, [f]kombucha with reishi and chaga, [g]kombucha with turmeric and lemon, [h]kombucha without additives; K–W – Kruskal–Wallis.

Table 7 The acetic acid content in kombuchas. Data represent the minimum, 25th percentile, median, 75th percentile and maximum values of three replicates. Statistically significant differences ($p \leq 0.05$)

Flavour additive	Minimum	25th percentile	Median	75th percentile	Maximum	K–W p value
Lavender[a]	31.08	31.08	31.09*[d–h]	31.11	31.11	0.0029
Lemongrass[b]	30.93	30.95	31.03*[d–h]	31.31	31.40	
Liquorice and mint[c]	30.97	31.01	31.11*[d–h]	31.11	31.11	
Mango[d]	32.03	32.03	32.04*[a–c,e,f,h]	32.21	32.27	
Mint, rose, pomegranate[e]	30.02	30.02	30.03*[a–d,f,g]	30.16	30.20	
Reishi and chaga[f]	36.90	36.93	37.03*[a–e,h]	37.10	37.13	
Turmeric and lemon[g]	32.30	32.30	32.31*[a–c,e,h]	32.37	32.39	
Without additives[h]	28.28	28.28	28.28*[a–d,f,g]	28.29	28.29	

Different letters (a–h) in the superscript represent statistically significant differences *$p < 0.05$ between particular type of kombucha: [a]kombucha with lavender, [b]kombucha with lemongrass, [c]kombucha with liquorice and mint, [d]kombucha with mango, [e]kombucha with mint, rose, and pomegranate, [f]kombucha with reishi and chaga, [g]kombucha with turmeric and lemon, [h]kombucha without additives; K–W – Kruskal–Wallis.

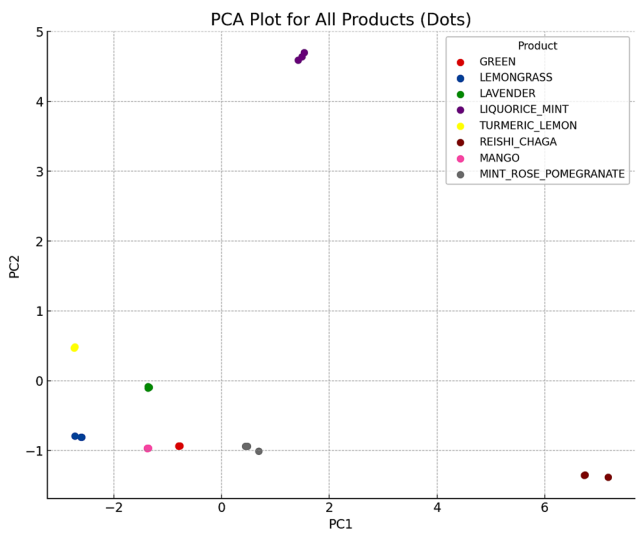

Fig. 2 Principal component analysis (PCA) of different kombucha variants.

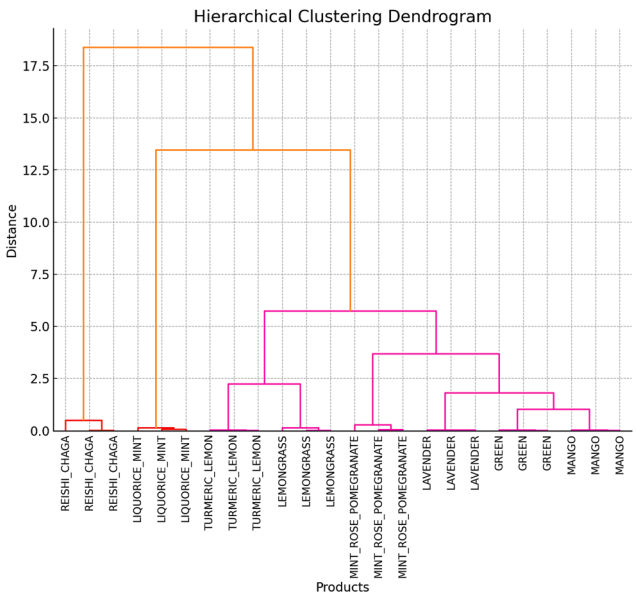

Fig. 3 Dendrogram of the hierarchical clustering.

content was identified in kombucha made with 2 g of oolong tea and 10 g of peppermint (1678.67 ± 12.49 μg QE per mL), while in kombucha based on oolong tea without peppermint, the value was 1301.47 ± 4.16 μg QE per mL.[46] However, more research is needed to clarify the mechanism of influence and the effects obtained through flavour additives. In the present study, the tested material consisted of eight commercial green

tea-based kombucha variants with lemongrass, lavender, liquorice and mint, turmeric and lemon, mango, reishi and chaga, as well as mint, rose, and pomegranate. To the best of our knowledge, the phytochemical composition and antioxidant potential of most of them have been assessed for the first time.

Antioxidant activity was assessed by the ability to reduce iron ions (FRAP) and neutralize free radicals (DPPH, ABTS). Measurements were made using three methods, as each has different selectivity and limitations. For example, the ABTS and FRAP methods allow the determination of the antioxidant capacity of both hydrophilic and lipophilic compounds.[47] In contrast, DPPH is soluble only in organic solvents, making it possible to determine the activity of only lipophilic compounds.[48] The following results were obtained from the measurements. The values of antioxidant potential, expressed as a percentage of ABTS radical inhibition, ranged from 95.52 to 98.26%. The highest potential was recorded for kombucha with mint, rose and pomegranate, as well as kombucha with reishi and chaga, while the lowest was beverage without additives as well as kombucha with mango. The antioxidant potential of kombucha measured by the FRAP method ranged from 3033.50 to 5545.10 μM Fe(II) per L. Kombucha with reishi and chaga had the highest recorded value, while kombucha with lemongrass had the lowest. The results obtained by the DPPH method ranged from 87.51 to 94.99%. The highest recorded value was kombucha with turmeric and lemon, while the lowest was kombucha with lavender as well as mint, rose, and pomegranate.

The obtained results indicate that depending on the type of additive, it may cause an increase or decrease in the antioxidant potential of kombucha compared to the basic variant. The addition of flavouring agents, such as herbs and fruits, may introduce secondary metabolites or substrates that alter the metabolic pathways of SCOBY microorganisms. For example, the high polyphenol content in reishi and chaga kombucha could promote the activity of *Acetobacter* species, enhancing the production of acetic acid and bioactive polyphenols.

The reduction in antioxidant potential observed in certain flavoured variants of kombucha can be attributed to chemical interactions and the microbiological metabolism of bioactive compounds. Some additives may introduce components that accelerate the degradation of polyphenols or other antioxidant compounds during fermentation. For example, enzymes present in certain fruits (such as polyphenol oxidase in apples) can cause the oxidation of polyphenols, reducing their antioxidant activity. Chemical compounds in flavour additives can react with polyphenols, flavonoids, or other antioxidants, leading to the formation of less active forms. For instance, tannins can form insoluble complexes with proteins, decreasing their availability as antioxidants. Microorganisms from the SCOBY consortium may preferentially metabolize antioxidants introduced with flavour additives, resulting in their reduced levels. For example, flavonoids in fruits can be broken down into less active metabolites by bacteria or yeast. Some flavour additives may contain compounds that act as inhibitors of antioxidants. For instance, minerals like iron or copper can catalyse pro-oxidative reactions, reducing the overall antioxidant potential of the beverage. The addition of new ingredients alters the chemical environment of kombucha (*e.g.*, pH, viscosity, ion concentration), which can affect the effectiveness of antioxidants. For example, a more alkaline environment may decrease the stability of polyphenols, leading to a reduction in their activity. This phenomenon can be observed in the case of kombucha with mango, which recorded the highest pH (3.72) and simultaneously the lowest total polyphenols content, as well as one of the lowest antioxidant potential values measured using the ABTS method.

The present study analysed the content of polyphenols (TPC), flavonoids (TFC), and vitamin C. It turned out that the studied kombuchas, depending on the type of additive, differed statistically significantly in the content of polyphenols. The values obtained ranged from 71.42 to 265.82 mg GAE per L. Kombucha with turmeric and lemon showed the highest polyphenol content. The results obtained are justified by the valuable composition of the raw materials used. Lemon is characterized by a high content of biologically active components, especially antioxidants such as flavonoids and phenolic acids, as well as coumarin compounds and vitamins (C, A, E).[49–52] To our knowledge, the effect of lemon on kombucha properties has not been studied yet. However, Kim and Wang investigated the effect of the addition of another citrus fruit, which is tangerine. As a result of the experiment, they noted the beneficial effect of tangerine juice on the nutritional values and sensory qualities of black tea-based kombucha.[53] More than 235 compounds belonging to polyphenols and terpenoids were identified in the rhizome of *Curcuma longa* (turmeric), from which the popular spice with its characteristic intense yellow colour is obtained. Among polyphenols, the main group of biologically active constituents are curcuminoids, which include curcumin, demethoxycurcumin and bisdemethoxycurcumin.[54–56] These compounds exhibit anti-inflammatory, immune-boosting, antioxidant, and anticancer activities.[43,57] The Yong *et al.* study showed that *Lactobacillus* fermentation of turmeric enhances its antioxidant and anti-inflammatory properties. An *in vivo* study of Zubaidah *et al.* showed that kombucha based on turmeric infusion was more effective than kombucha made from black tea in improving both acquired and innate immune responses.[58,59] Khazi *et al.* studied and compared black tea-based kombuchas enriched with different concentrations of turmeric juice with kombucha prepared without flavouring. As a result, they found that the phenolic content of the fermented beverage increased as the concentration of turmeric juice increased, so the highest phenolic content was identified in kombucha with the addition of a concentration of 1% (0.8 mg GAE per mL). The addition of turmeric also caused a concentration-dependent increase in the antioxidant activity of the tested kombuchas. Kombucha containing 0.8% turmeric showed the highest antioxidant activity measured by the DPPH method (89%), while the value for kombucha without turmeric was 42%. Measured by the

ABTS method, kombucha with 1% turmeric had the highest antioxidant activity (91.8%), while kombucha without turmeric showed an antioxidant activity of 41.5%.[60] The conclusions of the cited studies are consistent with the results we obtained. Thus, it seems that the addition of both turmeric and lemon to the fermentation process of kombucha can be an effective method to obtain a healthier alternative to fermented tea obtained from traditional ingredients.

Total flavonoid and vitamin C content also varied from product to product. Kombucha with lavender contained the most flavonoids (231.62 mg RE per L), while kombucha with reishi and chaga proved to be the best source of vitamin C (37.53 mg per 100 mL). In comparison, 27.19 mg of vitamin C was detected in 100 mL of green tea-based kombucha without additives. Other researchers detected vitamin C in traditional kombucha at 1.61 mg L^{-1}.[61] The identified phytonutrients can positively affect the body's functioning, as they exhibit antioxidant, anti-inflammatory, anti-diabetic, and cardioprotective effects, as well as have beneficial effects on cognitive function, helping to reduce the incidence of neurodegenerative diseases.[62–65] Moreover, ascorbic acid enhances iron absorption, participates in the synthesis of neurotransmitters, promotes wound healing, participates in collagen synthesis, and prevents infections.[66–68] Since the human body cannot the ability to synthesize and store vitamin C, it is necessary to supply it in adequate amounts with food. Considering that the recommended daily allowance (RDA) is 75 mg for women and 90 mg for men, drinking 100 mL of kombucha with reishi and chaga can cover as much as 50.04% of the requirement for women and 41.70% for men.[69]

A similar experiment was conducted by the team of Yang *et al.* They tested nine commercial kombuchas with different flavours. The best in terms of polyphenol content (380 mg GAE per L) and antioxidant potential (842 mg L^{-1} TEAC) was a product with the following composition: black tea, cane sugar, ginger, lemongrass, orange peel, green mint, peppermint, SCOBY.[70] The ingredients considered the best, differ from those highlighted in our study. However, Yang *et al.* experiment has a limitation, as the products compared differed in the base ingredient, with some using green tea, others using black tea, or both, which may interfere with assessing the impact of specific additives. As mentioned in the introduction, the total polyphenol content and the concentrations of their respective groups in kombucha vary depending on the type of tea used, so the choice of tea is extremely important.[9] According to the literature, it is the tea leaves used to make the tea decoction that is the main source of components with antioxidant properties in traditional kombucha.[12] It is reported that the concentration of catechins in green tea is 70%, while in black tea it is about 30%. The most abundant catechin in green tea is epigallocatechin gallate (EGCG). Up to 200 mg of EGCG is found in 200 mL of green tea infusion. In addition, tea is also abundant in polyphenolic compounds such as epicatechin, epicatechin gallate, gallocatechin, and epigallocatechin, as well as caffeic acid, caffeine, chlorogenic acid, coumaric acid, ellagic acid, gallic acid, kaempferol, myricetin, quercetin, quinic acid, and rutin.[71] Moreover, the fermentation process, due to the metabolic activity of the organisms contained in SCOBY, can potentiate the content of these components. Some bacteria and yeasts included in the starter cultures show the ability to produce and release enzymes, *i.e.* invertase, cellulase, glucanase, and glucosidase which break down complex molecules, into smaller monomers with higher biological activity, thus increasing the overall content of polyphenolic compounds.[10,35,72] These compounds exhibit enhanced antioxidant properties, which may explain the observed activity in FRAP and DPPH assays. Furthermore, the production of organic acids, such as acetic and gluconic acids, contributes to the antioxidant capacity by maintaining the acidic environment and stabilizing phenolic compounds. This is reflected in the final chemical composition of the resulting product, hence even in kombucha not enriched with any additives, as many as 127 phenolic compounds have been identified.[1,17,18]

Li *et al.* determined the content of polyphenolic compounds in traditional green tea-based kombucha using the HPLC method. The analysis showed the presence of, among others, gallic acid (48.13 μg mL^{-1}), *p*-coumaric acid (9.63 μg mL^{-1}), isoferulic acid (5.24 μg mL^{-1}), caffeine (92.32 μg mL^{-1}), epicatechin gallate (17.5 μg mL^{-1}), epigallocatechin gallate (40.39 μg mL^{-1}), epigallocatechin (13.61 μg mL^{-1}), rutin (2.68 μg mL^{-1}), and quercetin was not detected.[73] In this study, the analysis of the content of polyphenolic compounds in 8 commercial green tea-based kombucha revealed the presence of 17 polyphenolic compounds, including caffeine and phenolic acids (among other gallic acid, 4-hydroxybenzoic acid, 2-hydroxycinnamic acid, *p*-coumaric acid, ferulic acid, sinapic acid, ellagic acid, 3,4-dihydroxybenzoic acid, caffeic acid), and flavonoids (for example, epicatechin gallate, rutin, resveratrol, myricetin, quercetin, kaempferol, and apigenin). Among the phenolic acids, the tested drinks contained in the highest concentration *p*-coumaric acid, 4-hydroxybenzoic acid and gallic acid, and among the flavonoids myricetin and epicatechin gallate. However, the content of the identified compounds differed statistically significantly between the tested products. Sinapic acid was not detected in kombucha without additives, ellagic acid was not identified in kombucha with liquorice and mint, and kombucha with turmeric and lemon did not contain quercetin. These compounds were present in other variants. Rutin considered one of the best therapeutically active phytochemicals, was detected only in kombucha with liquorice and mint, reishi and chaga, and in the drink without additives. The highest apigenin content was detected in kombucha with mango (3.30 mg L^{-1}), while the highest ellagic acid content was identified in kombucha without additives (6.75 mg L^{-1}). Kombucha with reishi, and chaga had the highest contents of *p*-coumaric acid (236.98 mg L^{-1}), 3,4-dihydroxybenzoic acid (46.94 mg L^{-1}), 4-hydroxybenzoic acid (180.72 mg L^{-1}), quercetin (11.25 mg L^{-1}), resveratrol (8.22 mg L^{-1}), epicatechin gallate (203.53 mg L^{-1}), caffeine (1457.29 mg L^{-1}) and together with kombucha with liquorice, and mint showed the highest content of 2-hydroxycinnamic acid.

Kombucha with liquorice and mint contained the highest amounts of rutin (227.69 mg L^{-1}), sinapic acid (28.62 mg L^{-1}) and kaempferol (6.76 mg L^{-1}). Kombucha with lavender had the highest contents of ferulic acid (20.17 mg L^{-1}) and gallic acid (105.84 mg L^{-1}). Kombucha with mint, rose, and pomegranate showed the highest content of caffeic acid (5.67 mg L^{-1}) and together with kombucha with reishi, and chaga, contained significantly higher amounts of myricetin than the other kombuchas. The obtained results indicate a significant impact of flavour additives in the form of herbs, spices, or fruits on both the quantitative and qualitative composition of green tea-based kombucha, which may translate into the final properties of the product.

4.2 Differentiation of kombucha variants using PCA and hierarchical clustering analysis

The application of principal component analysis (PCA) enabled dimensionality reduction and identification of key differentiating features among the studied kombucha variants. Statistical analyses, including PCA and hierarchical analysis, provided significant insights into the differences in the chemical profile of the tested kombucha variants. PCA results showed that the first two principal components (PC1 and PC2) explain a substantial portion of the total variance, highlighting their importance in differentiating the studied samples. Distinct clusters visible in the PCA plot (Fig. 2) reflected differences in chemical composition, especially between variants such as 'GREEN' and 'LEMONGRASS', which showed high similarity, and 'REISHI_CHAGA' and 'MANGO', which were more distinct.

Antioxidant potential assessed by FRAP, ABTS, and DPPH methods is related to presence of polyphenols and flavonoids. The DPPH assay, which measures radical scavenging ability, demonstrated higher activity in kombucha variants enriched with turmeric and lemon, suggesting the presence of curcuminoids and flavonoids with potent scavenging properties. Similarly, the ABTS assay, sensitive to hydrophilic and lipophilic antioxidants, showed elevated activity in samples with fruit and herbal additives, aligning with the diverse polyphenol profiles observed in these variants. Principal component analysis (PCA) revealed distinct clustering of kombucha samples based on their antioxidant activity and polyphenolic content. Variants with higher flavonoid levels, such as kombucha enriched with liquorice and mint, demonstrated a stronger correlation with DPPH and ABTS activities.

The hierarchical analysis (Fig. 3) further confirmed the PCA results. The dendrogram clearly displayed defined clusters, with products like 'GREEN' and 'LEMONGRASS' grouping at lower hierarchical levels, indicating high chemical similarity. In contrast, products such as 'REISHI_CHAGA' and 'MANGO' were clustered at higher levels, suggesting greater differences in their phytochemical composition. These results underscore that the chemical profiles of kombucha are strongly differentiated depending on the flavor additives used. The close clustering of 'GREEN' and 'LEMONGRASS' across both analyses can be attributed to similarities in polyphenol content and lower diversity of added ingredients. Meanwhile, the greater separation of 'REISHI_CHAGA' from other products corresponds with the unique bioactive composition of reishi and chaga mushrooms, which are rich in specific polysaccharides and antioxidants. Similarly, 'MANGO', due to the presence of fruit secondary metabolites such as carotenoids, exhibits a distinct chemical profile.

These clustering differences are significant in the context of potential health benefits and consumer preferences. For example, products with clear profiles, like 'REISHI_CHAGA', may be seen as more functional and targeted towards specific consumer groups seeking health benefits related to mushroom additives. Conversely, more similar variants, such as 'GREEN' and 'LEMONGRASS', may appeal to consumers who value a delicate taste and versatile health benefits.

In conclusion, the use of PCA and hierarchical analysis as analytical tools allows for precise identification of differences between kombucha products, which is crucial for further research on recipe optimization and understanding the impact of additives on the functional properties of beverages.

4.3 Evaluation of pH and acetic acid in kombucha

It is worth noting, however, that the fermentation process causes not only an increase in nutritional value but also a lower pH of the resulting product, which is also a consequence of the metabolic activity of microorganisms, which results in the formation of many organic acids, such as gluconic acid or glucuronic acid.[8,9,74] In our study, the pH values of selected kombuchas ranged from 2.69 to 3.74. Statistically significant differences were observed between all kombuchas, except kombucha with lavender *vs.* kombucha without additives. From a technological perspective, low pH provides microbiological safety, while, from a medical perspective, regular consumption of a low pH beverage can adversely affect the digestive system and even cause metabolic acidosis.[7,9]

The main organic acid found in kombucha is acetic acid. As Shahbazi *et al.* found, the content of this chemical changes during the fermentation process.[45] Gaggìa *et al.* showed that at day 7 of fermentation, the highest concentration of acetic acid is found in kombucha based on white tea (9.18 mg mL^{-1}), green tea (7.65 mg mL^{-1}), and the least in kombucha prepared from rooibos tea (4.89 mg mL^{-1}).[72] Other researchers observed that green tea-based kombucha at day 15 of fermentation had the highest acetic acid content (9500 mg L^{-1}).[75] Our tested kombuchas contained between 28.28 and 37.03 mM L^{-1} of acetic acid. Kombucha with reishi and chaga as well as kombucha with turmeric and lemon having the highest value, while kombucha with mint, rose, and pomegranate as well as kombucha without additives had the lowest value.

4.4 Strengths and limitations of the study

The study demonstrates that flavour additives significantly influence the content of bioactive compounds and the antioxidant properties of kombucha. These findings contribute to expanding knowledge on the potential for enriching functional beverages with health-promoting substances.

The results indicate that flavour additives can significantly enhance kombucha's content of compounds with high antioxidant potential, making it an attractive functional product. The growing consumer interest in health-oriented beverages opens up new opportunities for manufacturers, particularly in the context of personalizing products to meet specific health needs, such as supporting the immune system or protecting against oxidative stress. This approach could contribute to the development of products tailored for specific consumer groups, such as athletes or individuals exposed to high stress levels.

The findings of this work can also be implemented in the cosmetic industry, where ingredients with high antioxidant activity could be used to develop innovative cosmetic formulations. These products could help protect the skin from oxidative stress, slow down the aging process, and support the regeneration of skin exposed to environmental factors.

This research also encourages the exploration of new potential additives, such as superfoods (*e.g.*, spirulina, matcha, chlorella), exotic fruits (*e.g.*, pitaya, passion fruit, guava), and spices (*e.g.*, cardamom, cinnamon, turmeric), which can enhance both the health benefits and sensory appeal of the product. Moreover, the promotion of plant-based food additives supporting functional properties can be a step towards more sustainable use of resources. This could help reduce waste of plant raw materials and expand their potential applications across various industries.

However, further research is needed in this area. Future studies should include clinical trials to confirm the health-promoting properties and explore new flavour additives and their impact on the phytochemical profile and functional properties of kombucha.

Unfortunately, this work has some limitations. One of the key limitations is the variability in the quality of raw materials, such as tea and flavor additives, which can affect the results of the analysis. Factors such as the location of cultivation, climatic conditions, processing methods, and storage practices can introduce significant differences in the chemical composition and biological properties of these materials. Additionally, the lack of standardization in the fermentation process at an industrial scale may lead to significant differences in the chemical composition of kombucha between different production batches.

5. Conclusions

The results of the present study show that the addition of certain of plant-based raw materials (and in particular turmeric and lemon, lavender, reishi and chaga, mint, rose and pomegranate) to the green tea-based kombucha fermentation process may have a positive effect on the phytonutrient content, thus can be an effective method to achieve a healthier alternative to fermented tea derived from traditional ingredients. Therefore, producers of functional beverages are advised to use additives such as medicinal mushrooms, herbs, spices, flowers or fruits rich in vitamin C or polyphenols to increase the health and market attractiveness of kombucha.

Author contributions

Karolina Jakubczyk: conceptualization, methodology, investigation, writing – review & editing, funding acquisition, supervision, project administration, Klaudia Melkis: writing – original draft, Dominika Maciejewska-Markiewicz: methodology, investigation, Anna Muzykiewicz-Szymańska: writing – review & editing, Anna Nowak: writing – review & editing, Karolina Skonieczna-Żydecka: formal analysis.

Data availability

The data supporting this article have been included as part of the ESI.†

Conflicts 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.

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