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Sauerkraut juice fermented with different symbiotic starter cultures: comprehensive assessment of physicochemical, rheological, antioxidant, and microbiological characteristics

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The current study investigated the fermentation of sauerkraut juice (SKJ) utilizing various symbiotic starter cultures (specifically kombucha and water kefir starter cultures, respectively). It aimed to assess the physicochemical, rheological, antioxidant, and microbiological properties of the resulting beverages. Black tea kombucha and apple juice water kefir fermented beverages were also analyzed for comparative purposes. Key parameters such as pH values, total acidity, total soluble solids, and total dissolved solids were measured. The initial pH exhibited significant variation, decreasing over the course of fermentation due to organic acid production. As fermentation progressed, total acidity increased, a phenomenon attributed to the activities of acetic and lactic acid bacteria. The flow behavior of the fermented beverages was characterized using the Power-law model, revealing that most samples displayed non-Newtonian behavior, indicating that their viscosity and shear stress changed with shear rate. Specifically, the consistency index declined while the flow behavior index rose. Additionally, seven biogenic amines were detected in the fermented samples, with their low concentrations posing minimal risk to consumer safety, resulting from microbial activity during fermentation. Antioxidant activity was assessed using DPPH and ABTS radical scavenging assays, revealing that black tea kombucha showed the highest levels of antioxidant activity. The total phenolic content varied between samples and decreased over time, particularly in the water kefir-like beverages. The microbiological analysis indicated a gradual increase in beneficial microorganisms, such as lactic acid bacteria and yeasts, throughout the fermentation process. These findings underscore the potential of SKJ as a promising base for developing functional beverages, providing valuable insights into how different fermentation starter cultures influence the quality and health-promoting properties of fermented beverages. In light of the growing consumer interest in functional, particularly plant-based foods, the fermentation of SKJ presents an opportunity to create probiotic-rich beverages.

KEYWORDS

sauerkraut juice, black tea, apple juice, kombucha, water kefir, rheology, biogenic amines, antioxidants

1 Introduction

In recent years, there has been a considerable increase in consumer awareness of diseases and foods or beverages. As a result, there has been much interest in the development of new types of functional food. Additionally, many consumers are very interested in functional foods that are not dairy-derived due to the allergic effect of dairy products. Furthermore, due to the increase in the vegan and vegetarian population, veganism and vegetarianism have become a significant diet (or preferred dietary habits) (Granato et al., 2010). Fermented products have become more and more popular as a result of modern consumers setting a greater value on well-being. Furthermore, fermentation is a metabolic process wherein bacteria, yeast, and their enzymes metabolize carbohydrates mainly into alcohols or organic acids and other secondary sensory-active metabolites (Nkhata et al., 2018; Ciska et al., 2021). Fermentation is a valuable way of modifying the organoleptic characteristics of foods by intensifying flavor and taste, and increasing their shelf life. Additionally, food fermentation can offer health advantages such as better vitamin and mineral bioavailability, faster digestion of proteins and carbohydrates, and probiotic benefits (Şanlier et al., 2019; Drabińska and Ogrodowczyk, 2021). In general, fruit or vegetable juices have all been thought of as potential food systems in recent years for the production of novel products with probiotics and, antioxidant and health-promoting effects. Therefore, such a probiotic-rich product should be considered kombucha and water kefir (Latorre-García et al., 2007; Randazzo et al., 2016).

Kombucha is a fermented beverage made from black or green tea that also contains sugar and a symbiotic culture of bacteria and yeast (SCOBY). Additionally, acetobacteria and the yeasts *Brettanomyces* and *Saccharomyces*, which primarily produce acetic and lactic acids, ethanol, and CO_2 as part of their metabolism, make up most of the starter culture known as SCOBY (Frias et al., 2019). Kombucha SCOBY is associated with the production of bacterial cellulose that floats on the surface of the resulting beverage, sometimes incorrectly referred to as a "tea fungus," harbouring bacteria and yeast that function as a unified fermentation unit. The producer of this polymer is predominantly *Komagataeibacter xylinus* (Han et al., 2024; Prajapati et al. 2024). Water kefir is an ancient and artisanal fermented beverage that is low in alcohol (usually less than 1% v/v), sometimes fruity, acidic, sweet, and softly carbonated with a high concentration of lactic acid (up to 2% w/w). Additionally, water kefir is manufactured by fermenting a water solution containing sucrose with water kefir grains, to which dried fruits or other sources of fermentable saccharides can be added. Furthermore, other names for water kefir include "sugary kefir" and "acquakefir," which are more localized terms (Tu et al., 2019; Moretti et al., 2022). The microorganisms are embedded in a polysaccharide-formed matrix found in water kefir grains, which is mainly composed of dextran and slightly less levan (Moretti et al., 2022; Coma et al., 2019). The grains are irregularly shaped and range in size (from a few mm to a few cm), appearing jelly-like and translucent in color from yellow to brown (Moretti et al., 2022). Water kefir grain contain yeasts (*Kluyveromyces*, *Candida*, and *Saccharomyces*), lactic acid bacteria (LAB), acetic acid bacteria (AAB), and sporadically bifidobacteria (Verce et al., 2019; Pendón et al., 2021). In addition, some of these bacteria can transit through the liquid phase and co-exist symbiotically in the grains.

Large quantities of by-products or agro-waste are generated in the agrotech and food industries, and these materials can become serious environmental pollutants. According to Ishangulyyev et al. (2019), approximately 1.3 billion tons of food is wasted annually, corresponding to nearly one-third of all food produced for human consumption. A considerable portion of this waste occurs during agricultural production and post-harvest handling, comprising plant-based residues such as leaves, stems, peels, and surplus produce. Effective strategies for the valorization of these by-products are essential to promote sustainable food systems, reduce environmental burdens, and enhance resource efficiency. Hence, a possible solution to the above-mentioned problem, could be found in fermentation (an approach to produce bioactive value-added and health-promoting products using microorganisms). Particularly, SKJ is regarded as an industrial by-product of the fermentation process of cabbage (*Brassica oleracea* convar. *Capitata* var. *alba*) (Jansone et al., 2023; Liu et al., 2023). In central and eastern Europe, sauerkraut is one of the most popular and frequently consumed fermented vegetables (Ciska et al., 2021). Furthermore, SKJ is a significant source of bioactive substances such as phenolic compounds, glucosinolates, minerals, organic acids, sugars, and vitamin C. Consumption of SKJ was linked to improved gut health, immune function, and anti-inflammatory effects (Marco et al., 2016; Martău et al., 2023). Thus, exploring innovative applications for sauerkraut juice not only promotes sustainability but also can offer tangible health benefits. However, due to its short shelf life, bioactive components, and distinct scent, SKJ's uses in the food sector are restricted because it is regarded as an agro-waste or by-product (Jansone et al., 2023). Information on the properties of SKJ is scarce in the scientific literature.

To our knowledge, no study has described the application of kombucha and water kefir starters in the manufacture of fermented SKJ. The latter approach in the fermentation of SKJ could be a strategy to develop products with enhanced functional value, microbial diversity and organoleptic characteristics. Additionally, the use of the above-mentioned symbiotic starter cultures in vegetable-based matrices (such as SKJ) aligns with the growing demand for non-dairy probiotic beverages and facilitates the development of novel functional beverages with potential health-promoting benefits. Therefore, the aim of the current study was to evaluate the fermentation process of SKJ with the use of kombucha starter and water kefir grains and to describe the resulting changes in the physical, chemical, rheological, antioxidant, microbiological, and sensory properties of SKJ. For comparative purposes, black tea kombucha and apple juice water kefir fermented beverages were also analyzed.

2 Materials and methods

2.1 Materials

The following raw materials were used in this study: SKJ [pasteurized; DM drogerie markt s.r.o., Prague, Czech Republic; nutrition information declared on the packaging (per 100 mL): fat 0.5 g, carbohydrate 1.5 g, of which sugars 1.0 g, protein 1.0 g, fiber 0.5 g, sodium chloride 0.6 g], apple juice [pasteurized; Linea Nivnice, a.s., Nivnice, Czech Republic; nutrition information declared on the packaging (per 100 mL): fat 0.1 g, carbohydrate 11.0 g, of which sugars 9.6 g, protein 0.1 g, sodium chloride 0.05 g],

black tea (Assam Orangajuli SFTGFOP—India; Oxalis, s.r.o.; Slušovice; Czech Republic), kombucha starter culture (fermentarium.cz; Beroun, Czech Republic), water kefir grains (fermentarium.cz; Beroun, Czech Republic) and sucrose (brown sugar; PRO-BIO s.r.o., Šumperk, Czech Republic). All chemicals and reagents, unless otherwise stated, were purchased from Merck (Merck Life Science spol. s r.o., Prague, Czech Republic) and were of analytical grade.

2.2 Activation of the kombucha and water kefir starter cultures

Black tea (2.6% w/v) with sucrose (5% w/v) were brewed for 15 min and utilized to activate the kombucha starter culture (purchased from a commercial supplier; Stevikom s.r.o., Prague, Czech Republic). To activate kombucha, 300 mL of the culture liquid was added to the black tea infusion together with the kombucha starter culture. After that, the mixture was allowed to ferment at 25 ± 1°C for 21 days (under aerobic conditions). The water kefir grains (UNIBIOM s.r.o., Břeclav, Czech Republic) had been previously frozen, and then they were activated for a period of 7 days in an incubator at 25 ± 1°C without stirring in an aqueous solution (filtered water) of sucrose (soluble solids concentrations of 5% w/v). With a constant exchange of nutrients (sucrose solution) every 24 h, the water kefir grains were maintained viable and active for fermentation. A 1 L Erlenmeyer with a 500 mL substrate volume was used for the fermentation procedure.

2.3 Preparation of the model fermented beverages

A total of four model fermented beverages, two samples fermented with kombucha starter and two samples fermented with water kefir grains were manufactured. For each type of fermented beverage model, one of the two substrates (environments) examined was the typical substrate to which the symbiotic cultures are most commonly applied. In particular, for kombucha-like beverages, black tea extract and SKJ were used, whereas for water kefir-like beverages, apple juice and SKJ were applied. The black tea extract and the apple juice were utilized because they are the typical media to which the tested symbiotic cultures are most commonly applied.

Following infusion (10 g/L of dry leaves in 1 L of water at 85 ± 1°C for 10 min), the leaves were filtered through a nylon filter to produce the extract of black tea (*Camellia sinensis*). Subsequently, the black tea extract was poured into sterile glass containers and the concentration of sucrose was adjusted to a target value of 10–11 °Brix. The black tea infusion used to make the fermented beverage was chilled to about 25°C (at room temperature for 2 h) and then divided among 1.5 L glass containers, one for each fermentation day that would be evaluated. The glass containers for the cultivation of SCOBY or for kombucha-like beverages production were sterilized (at 121°C for 15 min). Thereafter, symbiotic kombucha culture (5%, w/v;) and 10% (v/v) of kombucha liquid previously fermented were applied. Subsequently, the glass containers were covered with clean cotton cloths under aerobic conditions (Figure 1: part A—black tea and part B—SKJ). Additionally, fermented SKJ beverages with kombucha starter were prepared in the same way as it was described above, with

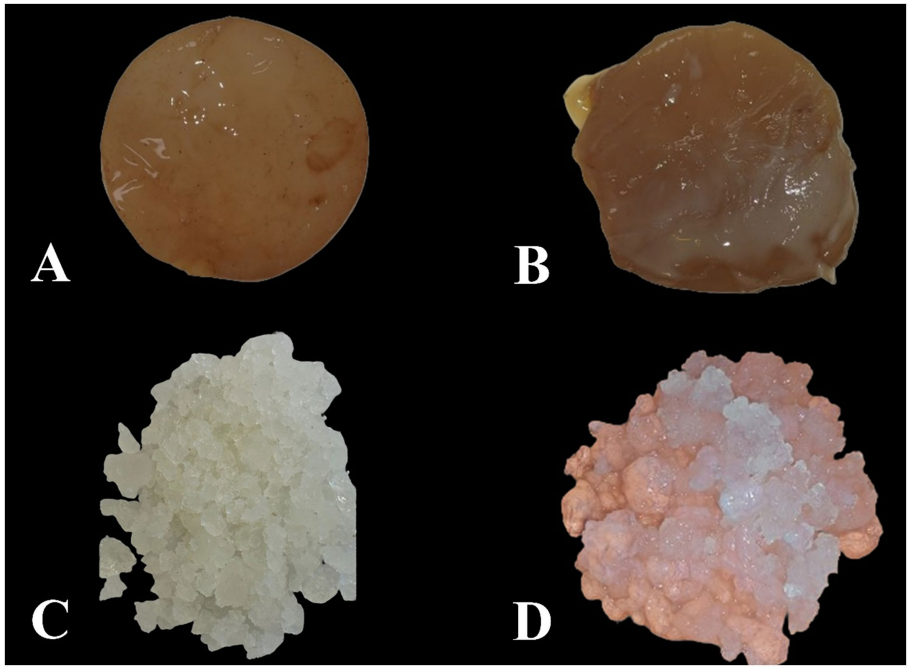

FIGURE 1
Kombucha-like beverages fermentation in glass containers (part **A**—black tea; part **B**—sauerkraut juice) and water kefir-like beverages fermentation in glass containers (part **C**—apple juice; part **D**—sauerkraut juice). Kombucha-like beverages bottled after 216 h of fermentation (at 25 ± 2°C; part **E**—black tea; part **F**—sauerkraut juice) and water kefir-like beverages after 72 h of fermentation (at 25 ± 2°C; part **G**—apple juice; part **H**—sauerkraut juice). Red arrows—cellulose film (SCOBY; symbiotic culture of bacteria and yeast); blue arrows—water kefir grains.

the main difference that SKJ was used instead of black tea extract. The kombucha-like beverages were incubated at 25 ± 3°C and fermentation was carried out in a dark chamber (at 25 ± 2°C) and evaluated at times of 0, 24, 48, 72, 96, 168 and 216 h, respectively. After fermentation, SCOBY (Figure 2: part A—black tea and part B—SKJ) were removed from the model kombucha liquids, and the remaining liquids were stated as kombucha-like beverages (Figure 2: part E—black tea and part F—SKJ).

Additionally, the model water kefir-like beverages were prepared using SKJ and commercial pasteurized apple juice. The juices were supplemented with water kefir grains (10 g/L) that had been activated (in each medium) for 48 h at 25 ± 1°C at a level of 5% (w/w). Fermentation (static) was then conducted in a dark chamber at 25 ± 1°C for 72 h. Fermentation of the model samples was realized in sealed sterile glass containers (1.5 L of volume; Figure 2: part C—apple juice and part D—SKJ) and the concentration of sucrose (only in the SKJ sample) was adjusted to a target value of 10–11 °Brix. After fermentation, the water kefir grains (Figure 1: part C—apple juice and part D—SKJ) were drained from the water kefir liquids, and the remaining liquids were stated as water kefir beverages (Figure 2: part G -apple juice and part H—SKJ). Analyses were carried out at the following time intervals: 0, 24, 48 and 72 h, respectively.

The purpose of the coding system for the kombucha-like samples was as follows: K—kombucha, X—fermentation time, C—black tea; K—kombucha, X—fermentation time, Z—SKJ. In particular, the samples were coded as K_X_C for the black tea infusion kombucha-like samples and K_X_Z for the SKJ kombucha-like samples [(where X represents their respective fermentation times (black tea infusion kombucha samples—K_0_C: time 0 h; K_24_C: time 24 h; K_48_C: time 48 h; K_72_C: time 72 h; K_96_C: time 96 h; K_168_C: time 168 h; K_216_C: time 216 h), SKJ kombucha samples—K_0_Z: time 0 h; K_24_Z: time 24 h; K_48_Z: time 48 h; K_72_Z: time 72 h; K_96_Z: time 96 h; K_168_Z: time 168 h; K_216_Z: time 216 h)]. Furthermore, the coding system for the water kefir-like samples was as follows: V—water kefir, X—fermentation time, J—apple juice; V—water kefir, X—fermentation time, Z—SKJ. Particularly, the samples were coded as V_X_J for the apple juice water kefir samples and V_X_Z for the SKJ water kefir samples [(where X represents their respective fermentation times (apple juice water kefir samples—V_0_J: time 0 h; V_24_J: time 24 h; V_48_J: time 48 h; V_72_J: time 72 h), SKJ water kefir samples—V_0_Z: time 0 h; V_24_Z: time 24 h; V_48_Z: time 48 h; V_72_Z: time 72 h)]. Each fermented beverage was manufactured in triplicate (kombucha-like samples—2 substrates × 7 fermentation times × 3 repetitions = 42 lots); water kefir-like samples—(2 substrates × 4 fermentation times × 3 repetitions = 24 lots) resulting in 66 lots of model fermented samples in total. Table 1 is summarizing the fermentation conditions utilized in the manufacturing of the model fermented samples.

2.4 Basic physicochemical analysis

A glass tip electrode of a calibrated pH-meter (Foodcare HI 99161, Hanna Instruments Czech s.r.o., Prague, Czech Republic) was used to determine the pH values of the tested samples. The alkaline titration method (AOAC, 2003) was used to measure total acidity, which was expressed as lactic acid for samples that resembled water kefir and acetic acid for samples that resembled kombucha. The total soluble solids (TSS; % Brix) values were determined at 20 ± 1°C using a portable digital refractometer (Kern ORF 45BE; Kern & Sohn

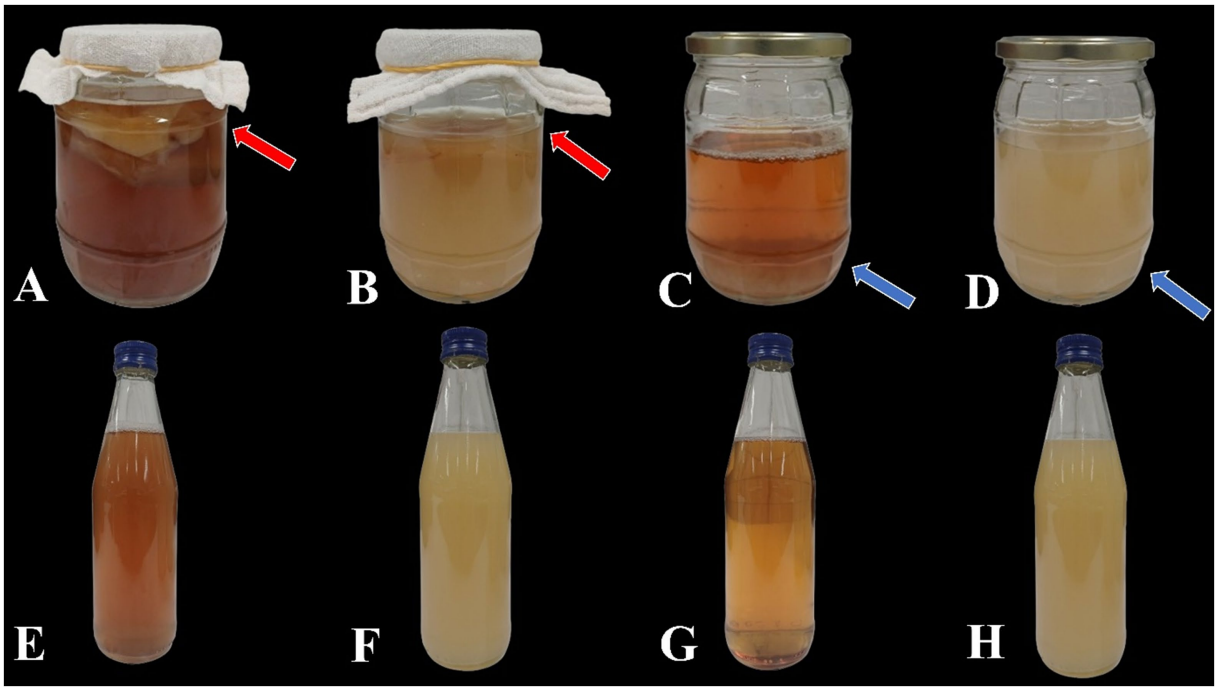

FIGURE 2
Cellulose film (SCOBY; symbiotic culture of bacteria and yeast; part **A**—black tea; part **B**—sauerkraut juice) formed after 216 h of fermentation (at 25 ± 2°C) and water kefir grains (part **A**—apple juice; part **B**—sauerkraut juice) after 72 h of fermentation (at 25 ± 2°C).

TABLE 1 A brief summary presenting fermentation conditions utilized in the manufacture of the model fermented beverages.

Beverage type	Fermentation conditions						Lighting regime
	Substrate	Symbiotic starter culture	Time (h; maximum)	Temperature (°C)	Oxygen requirement	Technique	
Kombucha-like	Black tea	Kombucha	216	25 ± 3	Aerobic	Static	Dark
	Sauerkraut juice	Kombucha	216	25 ± 3	Aerobic	Static	Dark
Water kefir-like	Apple juice	Water kefir	72	25 ± 1	Anaerobic	Static	Dark
	Sauerkraut juice	Water kefir	72	25 ± 1	Anaerobic	Static	Dark

GmbH, Balingen, Germany). The determination of total dissolved solids (TDS) values was performed using a conductivity electrode (CyberScan CON 110, Eutech Instruments, Oakton, Malaysia). Using the Anton Paar Density Meter DMA 4,500 M with Alcolyzer Beer ME module (Anton Paar GmbH, Austria), the extract content (apparent and actual), degree of fermentation (apparent and real), concentration of ethanol, and density of the fermented beverages were assessed. The water activity (a_w) of the samples was determined at 25.0 ± 0.1°C using the AquaLab 4TE apparatus (Qi Analytical, s.r.o., Prague, Czech Republic, Decagon). To ensure the accuracy of the results, a standard solution (a_w = 0.92 NaCl 2.33 mol/L in H_2O; Qi Analytical, s.r.o., Prague, Czech Republic) was used both before and during the analyses.

2.5 Rheological analysis

A HAAKE RheoStress 1 stress-controlled rheometer (Haake, Bremen, Germany) equipped with a coaxial concentric cylinder double gap measurement system at 20°C, managed by the Rheowin Job Manager (version 2.5), was used to test the rheological characteristics of the fermented model beverages. To maintain the temperature, water was circulated through the jacket encircling the rotor and cup assembly in a controlled-temperature bath. Forty mL of the tested sample was deposited in the rheometer cup. To obtain the flow curves, two rheological measurements were conducted: one with a rising shear rate (0–100 s^{-1}) within 250 s, and another with a descending shear rate (100–0 s^{-1}) within 250 s. Data from the first curve were fitted to the Ostwald-de Waele (Power law) model (Equation 1) using the Rheowin Data Manager software (version 2.5) (Kocabaş et al., 2022).

$$\tau = \mathrm{K}\gamma^{n} \quad (1)$$

The parameters, K (mPa·s^n) the consistency index and n as the flow index (unitless) were used to characterize the flow properties of the samples. All the tests conducted in triplicates.

2.6 Determination of biogenic amine content

The samples of fermented beverages that had been decarbonized (using an ultrasonic bath) were diluted 1:1 (v/v) with perchloric acid (1.2 mol/L). After being derivatized with dansyl-chloride, eight biogenic amines (BA; histamine, tyramine, phenylethylamine, tryptamine, putrescine, cadaverine, spermidine, and spermine) were determined using high performance liquid chromatography (LabAlliance, State College, USA; Agilent Technologies, Agilent, Paolo Alto, California, USA). The procedure previously outlined by Dadáková et al. (2009) and Buňka et al. (2012) was followed for derivatization, chromatographic separation (column: ZORBAX Eclipse Plus C18, 50 mm × 3.0 mm, 1.8 µm, Agilent Technologies, Santa Clara, California, USA), and detection [spectrophotometrically at a wavelength of 254 nm]. Every sample of fermented beverage was examined from two different containers (both having the same fermentation time). Each derivatized mixture was placed onto the chromatographic column three times after the samples from each container had been derivatized three times (3 derivatizations × 3 repetitions × 2 samples from each fermentation time × 4 batches = 72).

Considering sample preparation involves several steps, the internal (1,7-diaminoheptane) method was used to modify the concentration of BAs in the sample in accordance with Komprda et al. (2007). The validation phase of the approach also included determining the detection and quantification limits, recovery, and repeatability. To evaluate the reproducibility of the analytical procedure, five extracts of the selected sample with a low BA content and a combination of the BA standards following derivatization were injected 10 times each (shown as a relative standard deviation, or RSD). For the instrument and method repeatability, the RSD values were 0.1–0.7% and 1.3–3.8%, respectively. A real fermented beverage sample with a variety of BA standards added at a concentration level of 2 mg/L was used five times to assess recoveries (Komprda et al., 2007). Individual BA recovered between 89.5 to 97.9%. The individual BA's limits of detection (LOD) and quantification (LOQ) fell between 0.02 and 0.11 mg/L and 0.13 and 0.52 mg/L, respectively. Standard chromatographic methods (Wenzl et al., 2016) and ISO 17025 (ISO17025, 2017) were followed in determining the LOD and LOQ (Table 2).

2.7 Determination of total antioxidant activity and total polyphenol content

2.7.1 Antioxidant activity measured using DPPH (2, 2-diphenyl-1-picrylhydrazyl) radicals

According to Sumczynski et al. (2015), samples were used in antioxidant activity tests that relied on the quenching of synthetic radicals DPPH. In short, 8.55 mL of newly prepared DPPH radical solution in methanol (0.17 M) was mixed with 450 µL of the sample extract. The absorbance at 515 nm was determined after standing in the dark for 60 min. As a reference standard, Trolox at concentrations

TABLE 2 Values (mg/kg)* of limits of detection (LOD) and limits of quantification (LOQ) for the monitored biogenic amines in the model fermented beverages.

Biogenic amine	LOD	LOQ	R^2
Tryptamine	3.11 ± 0.19	9.14 ± 0.28	0.9996
Phenylethylamine	3.08 ± 0.12	9.01 ± 0.35	0.9995
Putrescine	3.28 ± 0.08	10.15 ± 0.28	0.9997
Cadaverine	1.42 ± 0.07	4.68 ± 0.32	0.9997
Histamine	1.72 ± 0.04	5.51 ± 0.21	0.9998
Tyramine	3.52 ± 0.19	10.47 ± 0.32	0.9995
Spermidine	3.29 ± 0.14	10.12 ± 0.25	0.9997
Spermine	3.48 ± 0.05	10.74 ± 0.33	0.9997

*The results are expressed as the mean ± standard deviation ($n = 6$).

ranging from roughly 0 to 60 mg/L was used. The antioxidant activity values were reported as milligrams of Trolox equivalent antioxidant capacity per liter of the sample (mg TE/L sample).

2.7.2 Antioxidant activity measured using ABTS radicals

Sumczynski et al. (2015) conducted the antioxidant activity utilizing the ABTS radical. In order to produce the radical ABTS$^{\bullet+}$, the ABTS stock solution was made with 7 M ABTS and 60 mM potassium persulfate in a volume ratio of 1:50. It was then allowed to sit at room temperature for 16 h in the dark. 2.5 mL of the ABTS stock solution and 97.5 mL of a pH 4.3 acetic buffer (0.2 M CH_3COONa and 0.2 M CH_3COOH in a 1:2 ratio) were combined to create the ABTS working solution. 12.0 mL of ABTS working solution was combined with 150 µL of the sample extract. After the mixture was left to rest at room temperature for a period of 30 min, absorbance was measured at 734 nm. The antioxidant activity values were represented as milligrams of TE per liter of the sample (mg TE/L sample), with trolox serving as the reference standard.

2.7.3 Ferric antioxidant power assay (FRAP)

The analysis was conducted out using a Lambda 25 spectrophotometer (Perkin Elmer, Waltham, MA, USA) in accordance with Esposto et al. (2021). The process relies on reducing Fe^{3+}-TPTZ complexes to their ferrous state in acetic buffer at 3.6 pH. By analyzing the changes in absorption at 593 nm, the decline was tracked. In short, 20–400 µL of the sample were combined with 2 mL of FRAP working solution ($FeCl_3$: TPTZ:acetic buffer = 1:1:10), and following a 15-min incubation period, the absorbance was measured at 37°C. The absorbance values in the test mixture were subtracted from the values produced from the increased concentrations of Fe^{3+} to determine the FRAP values. The final results were reported as mg TE/L, or milligrams of Trolox equivalent per gram.

A ferrous complex and redistilled water were combined with a diluted sample in a cuvette. Absorbance was measured at a wavelength of 593 nm following a 10-min incubation period (Esposto et al., 2021). Trolox equivalents per 1 L of sample (mmol TE/L) were used to express the results. Three replications of the analyses were conducted.

2.7.4 Total polyphenol content

Folin-Coicalteu reagent was used to colorimetrically assess total phenolic contents (TPC) (Singleton et al., 1999). In brief, 0.5 mL of Folin-Ciocaulteu reagent and 5 mL of redistilled water were combined with 10–100 µL of the sample. The material was properly mixed and neutralized with 1.5 mL of 20% $NaCO_3$ following a 5-min equilibration period. After 30 min of rest, a UV/VIS spectrophotometer (Lambda 25; Perkin Elmer, MA, USA) was used to measure the absorbance at 765 nm. Gallic acid equivalent (GAE) mg per liter of the sample were used to express TPC values.

2.8 Microbiological analysis

Ninety mL of 0.1% peptone water was used to homogenize 10 mL of the model fermented beverage samples under aseptic conditions. The serial dilutions that were evaluated as suitable were cultivated. For every analysis, the spread plate method was employed. Mannitol salt agar (MSA) was used for the determination of pathogenic representatives of the genus *Staphylococcus*, by incubating the samples at 37°C for 24 h under aerobic conditions. *Enterococcus* spp. count was determined by incubating at 37°C for 24 h under aerobic conditions on Slanetz-Barley (SB) medium. Moreover, anaerobic bacteria of the genus *Clostridium* were counted by incubating the samples on Reinforced Clostridial Broth (RCB) at 30°C for 24 h. *Lactococcus* spp. count was determined by incubating at

30°C for 48 h on M17 agar. *Lactobacillus* spp. count was determined on MRS agar by incubating for 3 days at 30°C. Total Acetic Acid Bacteria (AAB) counts were enumerated on AAB culture medium at 30°C for 48 h. Yeast count was performed on Sabouraud Dextrose Agar (SAB) by incubating for 48 h at 25°C (Coton et al., 2017; Witthuhn et al., 2005).

2.9 Instrumental analysis of color

An UltraScan PRO spectrophotometer (Hunter Associates Laboratory, Inc., Reston, VA, USA) was used to do the instrumental analysis of the fermented beverages' color. The evaluation was conducted using the CIE Lab color scale ($L^*a^*b^*$) at a 10° angle and with the illuminant D65 (standard daylight). Using white (A41 1,014–635 Rev. B; Hunterlab ColorFlex CZ; Hunter Associates Laboratory, Inc.) and black (A41-1017-037 Rev. A; Hunterlab ColorFlex CZ; Hunter Associates Laboratory, Inc.) reference tiles, the reflectance mode was set for the spectrophotometer's calibration, excluding specular reflection. Furthermore, parameter L^*, which reflects lightness, correlated with values between 0 and 100 (0—black,

100—white). According to Vincová et al. (2023), parameter a^* represented the red to green spectrum (from green $-a^*$ to red $+a^*$), whereas parameter b^* represented the yellow to blue spectrum (from blue $-b^*$ to yellow $+b^*$).

2.10 Sensory analysis

The sensory evaluation of the model fermented beverages (at the end of their fermentation period; 216 h for kombucha-like samples and 72 h for water kefir-like samples) was conducted based on parameters that encompassed appearance, taste, aroma, carbonation level, off-flavours, and overall rating. Sixteen expert assessors, comprising 10 women and 6 men aged between 19 and 62 years, participated in the sensory analysis assessment. The samples were presented in 50 mL glass containers, each labelled with a three-digit code, and were distributed in randomly designated order at a controlled temperature of 22 ± 2°C. The sensory analysis was conducted in sensory analysis laboratory, employing individual sensory booths for each panelist, in compliance with ISO standards (ISO 8589:2007, 2007). To avoid carryover effects, water and toast bread were provided during the testing and evaluation of the model fermented beverages. A 10-min interval was observed following each sample to prevent palate fatigue. The parameters of appearance, taste, aroma, carbonation level, and off-flavour were assessed using a 5-point scale (1 representing excellent, 3 representing good, 5 representing unacceptable; each point on the scale was objectively defined with specific quality characteristics). The overall rating assessment was also conducted on a 5-point scale, where 1 denoted extraordinarily good and 5 denoted extraordinarily bad. Additionally, ethical approval was not necessary for this study. Participants were apprised of the study's objective and the fact that their participation was entirely voluntary, allowing them to cease their involvement at any time, with responses remaining anonymous.

2.11 Statistical analysis

All analyses were repeated at least three times. The results were expressed as mean ± standard deviation. The results obtained were processed using the non-parametric analysis of variance through the Kruskal–Wallis and Wilcoxon tests (Minitab® 16 software; Minitab Ltd.; Coventry, UK), with the significance level set at 0.05.

3 Results and discussion

3.1 Basic physicochemical analysis

The results of the pH values, titratable acidity, total soluble solids, and total dissolved solids for the tested model fermented beverages are presented in Table 3.

The initial pH of the model fermented beverages ranged between 3.75 ± 0.01 and 6.85 ± 0.01, with black tea infusion kombucha samples reporting the highest pH values (6.85 ± 0.01), while apple juice water kefir and SKJ water kefir samples reported the lowest starting pH values. Moreover, the initial pH value of the SKJ kombucha sample was 4.06 ± 0.07. This pH-value variability could be due to the different composition of the raw materials, particularly because of the organic acid content (Morales et al., 2023). In general, for all tested samples the pH values decreased with the increased fermentation time ($p < 0.05$). The above-mentioned decrease in the samples pH, could be attributed due to the fact that during fermentation organic acids are produced (Rejdlová et al., 2024). The tested beverages showed a very similar pattern in decreasing pH during fermentation. In particular, a slight and slow reduction during the first 216 h (for the kombucha-like beverages) or 72 h (for the water kefir-like beverages), finally reaching pH values in the range of 3.33 ± 0.02–3.61 ± 0.01 (for the kombucha-like beverages) and 3.41 ± 0.02–3.45 ± 0.01 (for water kefir-like beverages), respectively. The results are in accordance with that previously reported by Arapović et al. (2024), da Silva et al. (2024), Rejdlová et al. (2023), and Ozcelik et al. (2021). The pH range considered safe for human consumption is in the range of 2.5–4.2, as lower values can indicate excessive organic acid (acetic/lactic) levels, and higher values can be correlated with a significant risk of undesired microorganism growth (Cardoso et al., 2020). Thus, the upper or lower limits were not exceeded by any kombucha-like or water kefir-like beverage during the whole fermentation time.

The results revealed significant changes in the titratable acidity in response to increasing fermentation time and model fermented beverages substrates ($p < 0.05$). For kombucha-like samples, the initial titratable acidity values increased steadily from 0.25 ± 0.02 to 2.82 ± 0.02 for black tea infusion kombucha samples and from 0.89 ± 0.03 to 2.85 ± 0.05% for SKJ kombucha samples, respectively ($p < 0.05$). Additionally, for water kefir-like beverages, the starting titratable acidity values were in the range of 1.64 ± 0.34–3.12 ± 0.08 for apple juice water kefir-like samples and 1.62 ± 0.32–3.04 ± 0.25% for SKJ water kefir-like samples. This acidity increase is due to the activity of AAB or LAB, because these bacteria produce organic acids, such as acetic, lactic, gluconic and glucuronic acids. In general, producing organic acids through microbial metabolism contributes to the increased acidity of the model fermented beverages, reducing the pH values (Arapović et al., 2024; da Silva et al., 2024). The production of organic acids like lactic acid can indicate the possible metabolic heterogeneity of LAB population, in which homofermentative and heterofermentative pathways are simultaneously active. The great majority of LAB during fermentation utilize glucose via the Embden–Meyerhof pathway for lactic acid production. However, some heterofermentative LAB species utilize glucose via the hexose monophosphate pathway producing lactic acid, ethanol, acetic acid, glycerol, mannitol, and CO_2. Furthermore, another organic acid, the acetic acid, was formed probably by heterolactic bacteria and *Acetobacter*, and the fermentation temperature may have contributed to the population increase of acetic acid producing microorganisms. It is generally accepted that lactic acid and acetic acid can provide a pleasant taste and inhibit the development of undesirable or pathogenic microorganisms, due to the increasing acidity (Puerari et al., 2012).

According to Tavares et al. (2023), the metabolism of microorganisms, which can utilize the sugars in the substrate for converting metabolites like acetic and lactic acids, is the reason why the total soluble solids (TSS) generally decreased in value over the fermentation period. In particular, the TSS decreased in black tea infusion kombucha-like samples from 9.12 ± 0.08 to 8.73 ± 0.05 °Brix and in SKJ kombucha-like samples from 11.62 ± 0.02 to 11.02 ± 0.04 °Brix, respectively. In addition, the TSS decreased in apple juice water

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TABLE 3 Values of pH, total acidity (TA), total soluble solids (TSS) and total dissolved solids (TDS) of the model fermented beverages during fermentation.

Sample	Time (h)	Parameters			
		pH	TA (% of acetic acid, % of lactic acid)	TSS (°Brix)	TDS (ppm)
K_1_C	0	6.85 ± 0.01[a]	0.25 ± 0.02[a]	9.12 ± 0.08[a]	45.20 ± 1.16[a]
K_2_C	24	3.95 ± 0.02[b]	0.94 ± 0.34[b]	9.01 ± 0.01[b]	66.27 ± 0.31[b]
K_3_C	48	3.91 ± 0.02[b]	1.06 ± 0.34[b]	9.03 ± 0.09[b]	64.37 ± 0.40[b]
K_4_C	72	3.89 ± 0.01[c]	1.24 ± 0.34[b]	9.02 ± 0.01[b]	65.90 ± 0.50[b]
K_5_C	96	3.75 ± 0.04[d]	1.64 ± 0.34[b]	8.73 ± 0.05[c]	76.53 ± 0.39[c]
K_6_C	168	3.52 ± 0.01[e]	2.49 ± 0.13[c]	8.53 ± 0.05[d]	77.00 ± 0.36[c]
K_7_C	216	3.33 ± 0.02[f]	2.82 ± 0.02[d]	8.73 ± 0.05[c]	78.33 ± 0.39[d]
K_1_Z	0	4.06 ± 0.07[a]	0.89 ± 0.03[a]	11.62 ± 0.02[a]	1550.04 ± 49.67[a]
K_2_Z	24	3.95 ± 0.01[b]	0.94 ± 0.34[a]	11.57 ± 0.05[b]	1416.67 ± 4.71[b]
K_3_Z	48	3.91 ± 0.02[b]	1.06 ± 0.34[a]	11.53 ± 0.05[b]	1536.67 ± 60.18[a]
K_4_Z	72	3.82 ± 0.05[c]	1.54 ± 0.24[a]	11.43 ± 0.05[b]	1446.67 ± 26.25[b]
K_5_Z	96	3.75 ± 0.01[d]	1.64 ± 0.34[a]	11.30 ± 0.22[c]	1523.33 ± 20.55[a]
K_6_Z	168	3.65 ± 0.04[e]	2.66 ± 0.19[b]	11.13 ± 0.05[c]	1586.67 ± 4.71[a]
K_7_Z	216	3.61 ± 0.01[f]	2.85 ± 0.05[b]	11.02 ± 0.04[c]	1640.00 ± 8.16[c]
V_1_J	0	3.75 ± 0.01[a]	1.64 ± 0.34[a]	10.93 ± 0.05[a]	223.67 ± 1.25[a]
V_2_J	24	3.64 ± 0.04[b]	2.66 ± 0.19[b]	9.07 ± 0.21[b]	235.33 ± 11.91[a]
V_3_J	48	3.53 ± 0.01[c]	2.49 ± 0.13[b]	7.70 ± 0.05[c]	213.33 ± 1.55[b]
V_4_J	72	3.45 ± 0.02[d]	3.12 ± 0.08[c]	5.71 ± 0.05[d]	267.00 ± 2.45[d]
V_1_Z	0	3.76 ± 0.01[a]	1.62 ± 0.32[a]	9.77 ± 0.05[a]	1346.67 ± 81.79[a]
V_2_Z	24	3.64 ± 0.03[b]	2.66 ± 0.16[b]	9.73 ± 0.09[a]	1470.00 ± 35.59[b]
V_3_Z	48	3.52 ± 0.02[c]	2.49 ± 0.13[b]	5.80 ± 0.08[b]	1636.67 ± 17.01[c]
V_4_Z	72	3.41 ± 0.02[d]	3.04 ± 0.25[c]	5.73 ± 0.02[b]	1763.33 ± 12.47[d]

[a]The values are expressed as means ± standard deviation (n = 18; each batch of fermented beverage was analyzed from two bottles, the samples from each bottle were derivatized three times, and each derivatized mixture was loaded onto the chromatographic column three times). The means within a column (the difference between BA amount in different fermentation times) followed by different superscript letters differ ($p < 0.05$); each fermented beverage type was evaluated separately. *Black tea infusion kombucha samples—K_0_C: time 0 h; K_24_C: time 24 h; K_48_C: time 48 h; K_72_C: time 72 h; K_96_C: time 96 h; K_168_C: time 168 h; K_216_C: time 216 h, Sauerkraut kombucha samples—K_0_Z: time 0 h; K_24_Z: time 24 h; K_48_Z: time 48 h; K_72_Z: time 72 h; K_96_Z: time 96 h; K_168_Z: time 168 h; K_216_Z: time 216 h, apple juice water kefir samples—V_0_J: time 0 h; V_24_J: time 24 h; V_48_J: time 48 h; V_72_J: time 72 h, sauerkraut water kefir samples—V_0_Z: time 0 h; V_24_Z: time 24 h; V_48_Z: time 48 h; V_72_Z: time 72 h.

kefir-like beverages from 10.93 ± 0.05 to 5.71 ± 0.05 and in SKJ water kefir-like samples from 9.77 ± 0.05 to 5.73 ± 0.02 °Brix. This reduction can be attributed to the use of sucrose or saccharides from the SKJ (mainly maltose, glucose, and fructose) and apple juice (mainly fructose, glucose, and sucrose), as a carbon source and depositing various compounds, including pigments, minerals, proteins, and pectin (Karadeniz and Ekşi, 2002; Yashasvi Waisundara, 2019; Jansone et al., 2023).

Model fermented samples showed (regardless of the applied substrate) an increase in the TDS value during the fermentation time in all examined cases ($p < 0.05$). The conductivity of the kombucha-like or water kefir-like beverages could be explained by the recorded TDS values. Thus, higher TDS values indicated more electrolytes, minerals or dissolved solids, which were present in the tested beverages (Rejdlová et al., 2023). The results are in accordance to that of Dwiloka et al. (2020).

The results of the ethanol content, density, real degree of fermentation (Rdf), real extract (ER), and water activity (a_w) of the model fermented beverages are shown in Table 4. In the case of the black tea infusion kombucha samples, the determined ethanol content values ranged from 0.21 (24 h) to 0.57% v/v (216 h). Generally, the increase in ethanol content was typical for this type of beverage. In the study of Ihsani et al. (2021), the variation of ethanol content was observed as a function of different lengths of fermentation time. The total fermentation time was 8 days, and the range of ethanol content values was 0.02–0.32% (v/v). Thus, there was also a significant increase in ethanol content. However, the final concentration depends on the composition of the substrate, especially the fermentable carbohydrate and inoculum content (Ihsani et al., 2021). For the SKJ kombucha samples, the ethanol values were in the range of 0.45 (24 h)–0.86 (216 h) % v/v over the whole fermentation time. Generally, the ethanol content in the model beverages fermented with water kefir starter culture increased over the fermentation time. In particular, for the apple juice water kefir, the highest measured value was 4.21% v/v (72 h), and for the SKJ water kefir samples, the highest measured value was 5.56% v/v (72 h; at the end of the fermentation time). In all samples, the increase in ethanol content corresponded to a decrease in total soluble solids, and a decrease in fermentable carbohydrate

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TABLE 4 Values of density, ethanol content, real degree of fermentation (Rdf), real extract (ER), and water activity (a_w) of the model fermented beverages during fermentation.[a]

Sample*	Time (h)	Parameters				
		Density	Ethanol (v/v)	Rdf (% w/w)	ER	a_w
K_1_C	0	1.036 ± 0.01[a]	ND**	2.75 ± 0.01[a]	9.45 ± 0.01[a]	0.994 ± 0.001[a]
K_2_C	24	1.034 ± 0.01[a]	0.21 ± 0.01[a]	3.06 ± 0.01[b]	9.08 ± 0.01[b]	0.993 ± 0.001[a]
K_3_C	48	1.034 ± 0.01[v]	0.22 ± 0.01[a]	3.15 ± 0.01[c]	9.02 ± 0.01[c]	0.994 ± 0.001[a]
K_4_C	72	1.034 ± 0.01[a]	0.23 ± 0.01[a]	3.42 ± 0.01[d]	9.04 ± 0.01[c]	0.992 ± 0.001[a]
K_5_C	96	1.034 ± 0.01[a]	0.31 ± 0.02[b]	3.36 ± 0.01[e]	9.08 ± 0.02[b]	0.991 ± 0.001[a]
K_6_C	168	1.031 ± 0.01[b]	0.49 ± 0.02[c]	9.31 ± 0.03[f]	8.47 ± 0.01[d]	0.990 ± 0.001[a]
K_7_C	216	1.031 ± 0.01[b]	0.57 ± 0.02[d]	9.51 ± 0.05[g]	8.58 ± 0.02[e]	0.991 ± 0.001[a]
K_1_Z	0	1.052 ± 0.01[a]	ND	7.42 ± 0.01[a]	13.63 ± 0.04[a]	0.986 ± 0.001[a]
K_2_Z	24	1.045 ± 0.01[b]	0.45 ± 0.02[a]	9.39 ± 0.01[b]	12.04 ± 0.02[b]	0.985 ± 0.001[a]
K_3_Z	48	1.046 ± 0.02[b]	0.62 ± 0.02[b]	9.56 ± 0.01[c]	12.10 ± 0.04[b]	0.985 ± 0.002[a]
K_4_Z	72	1.045 ± 0.01[b]	0.71 ± 0.01[c]	12.42 ± 0.01[d]	11.96 ± 0.03[c]	0.987 ± 0.002[a]
K_5_Z	96	1.046 ± 0.01[b]	0.76 ± 0.02[d]	14.67 ± 0.01[e]	12.10 ± 0.04[b]	0.989 ± 0.001[a]
K_6_Z	168	1.045 ± 0.01[b]	0.82 ± 0.01[e]	16.93 ± 0.01[f]	11.15 ± 0.02[d]	0.983 ± 0.001[a]
K_7_Z	216	1.042 ± 0.01[c]	0.86 ± 0.01[f]	16.94 ± 0.01[f]	11.75 ± 0.01[e]	0.984 ± 0.001[a]
V_1_J	0	1.043 ± 0.01[a]	ND	2.70 ± 0.01[a]	11.28 ± 0.01[a]	0.984 ± 0.001[a]
V_2_J	24	1.038 ± 0.02[b]	1.12 ± 0.01[a]	13.13 ± 0.01[b]	10.39 ± 0.04[b]	0.993 ± 0.002[b]
V_3_J	48	1.023 ± 0.01[c]	2.78 ± 0.02[b]	37.05 ± 0.01[c]	7.13 ± 0.06[c]	0.991 ± 0.001[b]
V_4_J	72	1.009 ± 0.02[d]	4.21 ± 0.02[c]	60.81 ± 0.01[d]	4.38 ± 0.08[d]	0.991 ± 0.001[b]
V_1_Z	0	1.034 ± 0.01[a]	ND	11.60 ± 0.01[a]	11.90 ± 0.03[a]	0.982 ± 0.001[a]
V_2_Z	24	1.029 ± 0.02[b]	1.98 ± 0.01[a]	25.11 ± 0.01[b]	8.51 ± 0.04[b]	0.983 ± 0.001[a]
V_3_Z	48	1.007 ± 0.01[c]	4.58 ± 0.01[b]	67.57 ± 0.01[c]	4.35 ± 0.02[c]	0.985 ± 0.001[b]
V_4_Z	72	1.006 ± 0.01[c]	5.56 ± 0.02[c]	68.00 ± 0.02[c]	4.23 ± 0.03[d]	0.984 ± 0.001[b]

*Black tea infusion kombucha samples—K_0_C: time 0 h; K_24_C: time 24 h; K_48_C: time 48 h; K_72_C: time 72 h; K_96_C: time 96 h; K_168_C: time 168 h; K_216_C: time 216 h, Sauerkraut kombucha samples—K_0_Z: time 0 h; K_24_Z: time 24 h; K_48_Z: time 48 h; K_72_Z: time 72 h; K_96_Z: time 96 h; K_168_Z: time 168 h; K_216_Z: time 216 h, apple juice water kefir samples—V_0_J: time 0 h; V_24_J: time 24 h; V_48_J: time 48 h; V_72_J: time 72 h, sauerkraut water kefir samples—V_0_Z: time 0 h; V_24_Z: time 24 h; V_48_Z: time 48 h; V_72_Z: time 72 h.

**ND—not detected.

[a]The values are expressed as means ± standard deviation (n = 18; each batch of fermented beverage was analyzed from two bottles, the samples from each bottle were derivatized three times, and each derivatized mixture was loaded onto the chromatographic column three times). The means within a column (the difference between BA amount in different fermentation times) followed by different superscript letters differ ($p < 0.05$); each fermented beverage type was evaluated separately.

content. It is known that *Saccharomyces cerevisiae* exhibits strong fermentative metabolism and tolerance to ethanol, and is the primarily responsible microorganism for ethanol production. In addition, some bacteria from the genus *Lactobacillus* also possess the ability of producing ethanol, since they have alcohol dehydrogenase activity, an enzyme able to convert acetaldehyde to ethanol (Puerari et al., 2012). The results are in accordance to that previously reported by Randazzo et al. (2016). Furthermore, the limit of 0.5% (v/v) for non-alcoholic beverages is applied in most European countries (Rejdlová et al., 2023). According to the obtained data, it can be concluded that six samples of the black tea kombucha-like group (K_1_C; K_2_C; K_3_C; K_4_C; K_5_C; K_6_C) and two samples of the SKJ kombucha-like group (K_1_Z; K_2_Z) can be classified as non-alcoholic [the ethanol content was lower than 0.5% (v/v)]. Additionally, in the case of water kefir-like samples none of the model fermented beverages could be characterized as non-alcoholic.

The density of the of the kombucha-like and water kefir-like beverages decreased during the fermentation time, regardless of the applied substrate. However, a more intensive density decrease pattern was identified for the water kefir-like beverages. The decrease in density for all water kefir samples was probably due to the relatively high ethanol production and a decrease in extract during fermentation. Additionally, the above-mentioned trend was also observed for the real extract samples values. The real extract value provides information about the content of unfermented carbohydrates of the tested sample. On the other hand, the real degree fermentation values increased with the increasing fermentation time. Real extract and real fermentation degree are parameters that are almost always monitored during the manufacturing of fermented beverages such as beer, wine or cider.

Water activity is an important parameter for food and beverages, especially in terms of shelf life, quality and safety. In addition, water activity is a measure of the available water for microorganisms, and is defined as the ratio of the water vapor pressure of the food/beverage to the water vapor pressure of pure water at the same temperature. The values of water activity of the kombucha-like and water kefir-like samples (Table 4) were high ($\geq$ 0.982) and, presented a slight decrease during the fermentation period. In particular, during fermentation, microbial metabolism (primarily by yeasts) led to ethanol and organic

acids production and sugar depletion, which could slightly reduce the water activity values. All food/beverages products with a water activity lower than 0.85 are stable at room temperature. Thus, storage of the model fermented beverages at a refrigeration temperature is essential (Kenyon et al., 1986; Sołowiej et al., 2023).

3.2 Rheological analysis

Understanding the rheological characteristics of different food and beverage products is crucial for material handling as well as for the construction and functionality of processing equipment used in the food industry (Penna et al., 2001). Furthermore, rheology is a fundamental design tool that may be used to examine the overall structure and the interactions between specific colloidal components. It is also significant for processing, shelf stability, and sensory perception, including texture and mouthfeel (Stokes et al., 2013). Furthermore, to evaluate the flow curves of the kombucha-type and water kefir-type fermented beverages, the obtained experimental data were fitted into the Power-law model, from which the rheological parameters [consistency coefficient (K), and flow behavior index (n)] were obtained (Table 5). In general, the model fitting was verified using the R^2 values (coefficient of determination), as a value close to the unity indicating higher accuracy fit of the model for calculating the correlation between shear stress and shear rate in the model samples (Punoo et al., 2023; Rejdlová et al., 2024). In particular, the Power-law model described the rheological properties with a high determination coefficient (R^2) with values in the range of 0.9931 to 0.9996. The flow behavior index is a parameter indicating how fluids tend to behave in Newtonian flow (Comak Gocer and Koptagel, 2023). Based on the power-law model, all samples showed a non-Newtonian, slightly dilatant (shear thickening) flow behavior (n ˃1) and the value of n increased with the increasing fermentation time. Hence, probably the applied stress lead to the disruption in the samples structure, resulting in particle interactions and resistance to flow (Wagner and Brady, 2009). On the contrary, the consistency index (K) exhibited oppositional behavior compared to the flow behavior index (n). In particular, the values of K decreased with the increasing fermentation time for all tested samples. Table 5 shows that the black tea infusion kombucha, apple juice water kefir, and SKJ water kefir samples presented higher flow behavior index values. The water kefir-like samples (regardless of the substrate

TABLE 5 Rheological parameters estimated by the Power-law model for flow curves of the model fermented beverages manufactured with kombucha and water kefir starter as affected by fermentation time.*

Sample**	Time (h)	Parameters		
		K (Pa.s)*	*n**	R^2
K_1_C	0	$0.3590^{a} \times 10^{-3}$	1.226^{a}	0.9953
K_2_C	24	$0.3395^{b} \times 10^{-3}$	1.244^{b}	0.9961
K_3_C	48	$0.3392^{b} \times 10^{-3}$	1.238^{c}	0.9956
K_4_C	72	$0.3343^{c} \times 10^{-3}$	1.243^{d}	0.9957
K_5_C	96	$0.3309^{d} \times 10^{-3}$	1.241^{d}	0.9958
K_6_C	168	$0.3303^{d} \times 10^{-3}$	1.246^{e}	0.9954
K_7_C	216	$0.3114^{e} \times 10^{-3}$	1.271^{f}	0.9953
K_1_Z	0	$0.4323^{a} \times 10^{-3}$	1.204^{a}	0.9965
K_2_Z	24	$0.4213^{b} \times 10^{-3}$	1.206^{a}	0.9965
K_3_Z	48	$0.4201^{c} \times 10^{-3}$	1.213^{b}	0.9964
K_4_Z	72	$0.4066^{d} \times 10^{-3}$	1.204^{a}	0.9974
K_5_Z	96	$0.4018^{e} \times 10^{-3}$	1.212^{b}	0.9965
K_6_Z	168	$0.3858^{f} \times 10^{-3}$	1.219^{c}	0.9962
K_7_Z	216	$0.3841^{g} \times 10^{-3}$	1.219^{c}	0.9974
V_1_J	0	$0.6569^{a} \times 10^{-3}$	1.183^{a}	0.9962
V_2_J	24	$0.3912^{b} \times 10^{-3}$	1.219^{b}	0.9994
V_3_J	48	$0.3822^{c} \times 10^{-3}$	1.221^{b}	0.9961
V_4_J	72	$0.2586^{d} \times 10^{-3}$	1.292^{c}	0.9931
V_1_Z	0	$0.4084a \times 10^{-3}$	1.231^{a}	0.9951
V_2_Z	24	$0.3744b \times 10^{-3}$	1.255^{b}	0.9996
V_3_Z	48	$0.3305c \times 10^{-3}$	1.262^{c}	0.9931
V_4_Z	72	$0.3141d \times 10^{-3}$	1.275^{d}	0.9965

*Values are presented as the median.

**Black tea infusion kombucha samples—K_0_C: time 0 h; K_24_C: time 24 h; K_48_C: time 48 h; K_72_C: time 72 h; K_96_C: time 96 h; K_168_C: time 168 h; K_216_C: time 216 h, Sauerkraut kombucha samples samples—K_0_Z: time 0 h; K_24_Z: time 24 h; K_48_Z: time 48 h; K_72_Z: time 72 h; K_96_Z: time 96 h; K_168_Z: time 168 h; K_216_Z: time 216 h, apple juice water kefir samples—V_0_J: time 0 h; V_24_J: time 24 h; V_48_J: time 48 h; V_72_J: time 72 h, sauerkrauet water kefir samples—V_0_Z: time 0 h; V_24_Z: time 24 h; V_48_Z: time 48 h; V_72_Z: time 72 h.

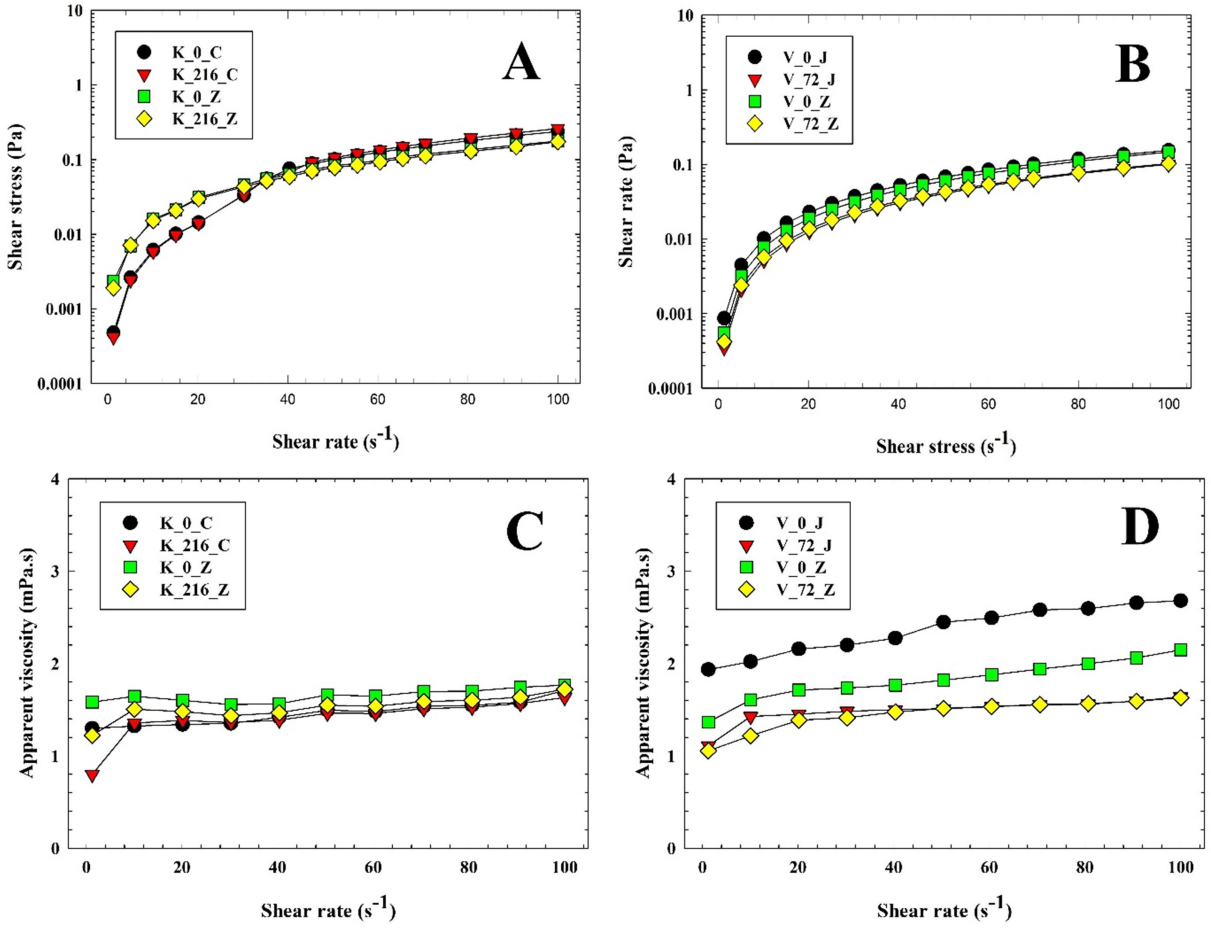

FIGURE 3
Shear stress (parts **A, B**) and apparent viscosity (parts **C, D**) curves for the fermented beverages (kombucha like samples: part **A**—black tea and **C**—sauerkraut juice); water kefir-like samples: part **B**—apple juice and **D**—sauerkraut juice) at the beginning (and at the end of the fermentation period. Parts **A, C**: black circle, K_0_C—black tea kombucha beverage at 0 h; red triangle, K_216_C—black tea kombucha beverage at 216 h; green square, K_0_Z—sauerkraut juice kombucha-like beverage at 0 h; yellow rhombus, K_216_Z—sauerkraut juice kombucha-like beverage at 216 h. Parts **B, D**: black circle, V_0_J—apple juice water kefir beverage at 0 h; red triangle, V_72_J—apple juice water kefir beverage at 72 h; green square, V_0_Z—sauerkraut juice water kefir-like beverage at 0 h; yellow rhombus, V_72_Z—sauerkraut juice water kefir-like beverage at 72 h.

applied; SKJ or apple juice) presented the highest values of flow behavior index (at the end of the fermentation period). On the contrary, SKJ kombucha-like samples had the lowest flow behavior index values, indicating a higher tendency toward Newtonian behavior than other samples (Comak Gocer and Koptagel, 2023). In Figure 3 are presented the flow curves (shear stress and apparent viscosity) of the fermented beverages (at the beginning and at the end of the fermentation period). An increase in shear stress as a function of shear rate was observed (Figure 3) in all tested samples. In general, for all tested samples.

The latter phenomena may be linked to lactobacilli, which change the internal structure of beverages, increasing the system's inner layer's resistance and, as a result, its viscosity (Paredes et al., 2022). Viscosity and intermolecular attractions are associated, according to Cui et al. (2022). As the rotation speed increases, the intermolecular energies in the beverage samples vary, which alters the molecular binding of the beverages and further changes their viscosity. In general, changes in the rheological properties of fermented beverages can influence not only mouthfeel but also flavor release, aftertaste, and the perception of carbonation and acidity, being all critical to consumer acceptance (Gi Chun et al., 2024). Thus, modifying flow properties by adjusting fermentation parameters (starter culture selection, and fermentation time) can offer a practical way to optimize sensory quality and consumer perception.

3.3 Biogenic amine content

The results of the determination of the BA content of the tested fermented samples are given in Table 6. PHE and SPM were detected in all examined samples, but their concentrations were below 5 mg/L at the end of the fermentation time, whereas SPD was not detected in any of the fermented beverages. The presence of SPM can be explained by its role in nucleic acid metabolism and its presence in fermented beverages could be expected (Lorencová et al., 2020). Additionally, in Table 6 is presented that low CAD levels were also detected in the samples; however, the concentrations were < 15 mg/L. In general, from a food safety point of view, the above-mentioned BA concentrations can be considered to be of low risk for the consumers safety. Furthermore,

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TABLE 6 Contents of biogenic amines (BA; mg/L) in the tested fermented beverages as affected by storage time.[a]

Sample	Time (h)	Biogenic amines (mg/L)							
		TRY**	PHE**	PUT**	CAD**	HIS**	TYR**	SPD**	SPM**
K_1_C	0	ND***	0.6 ± 0.5[a]	1.3 ± 0.1[a]	1.1 ± 0.1[a]	ND	0.9 ± 0.1[a]	ND	1.2 ± 0.8[a]
K_2_C	24	ND	0.7 ± 0.3[a]	1.4 ± 0.2[a]	1.2 ± 0.5[a]	ND	1.1 ± 0.5[a]	ND	1.3 ± 0.2[a]
K_3_C	48	ND	0.7 ± 0.2[a]	1.4 ± 0.5[a]	1.2 ± 0.6[a]	ND	1.1 ± 0.2[a]	ND	1.4 ± 0.4[a]
K_4_C	72	ND	0.8 ± 0.5[a]	1.5 ± 0.1[a]	1.3 ± 0.6[a]	ND	1.2 ± 0.2[a]	ND	1.4 ± 0.5[a]
K_5_C	96	ND	0.9 ± 0.1[a]	1.5 ± 0.3[a]	1.4 ± 0.5[a]	1.3 ± 0.5	1.5 ± 0.4[a]	ND	1.5 ± 0.1[a]
K_6_C	168	1.7 ± 0.1[a]	1.1 ± 0.5[a]	1.5 ± 0.2[a]	1.6 ± 0.7[a]	1.4 ± 0.2	1.5 ± 0.6[a]	ND	1.5 ± 0.2[a]
K_7_C	216	1.8 ± 0.2[a]	1.2 ± 0.8[a]	1.6 ± 0.5[a]	1.6 ± 0.4[a]	1.5 ± 0.2	1.6 ± 0.8[a]	ND	1.7 ± 0.5[a]
K_1_Z	0	ND	0.8 ± 0.1[a]	33.1 ± 1.2[a]	6.9 ± 1.2[a]	18.6 ± 2.1[a]	14.1 ± 1.1[a]	ND	1.1 ± 0.2[a]
K_2_Z	24	4.1 ± 0.5[a]	0.9 ± 0.2[a]	33.4 ± 2.5[a]	7.3 ± 0.8[a]	19.2 ± 3.5[a]	14.2 ± 1.8[a]	ND	1.1 ± 0.2[a]
K_3_Z	48	4.2 ± 1.1[a]	1.1 ± 0.5[a]	35.7 ± 1.5[a]	7.9 ± 1.5[a]	20.1 ± 1.8[a]	14.7 ± 2.2[a]	ND	1.2 ± 0.1[a]
K_4_Z	72	4.5 ± 1.2[a]	1.1 ± 0.2[a]	36.8 ± 1.5[a]	7.9 ± 1.1[a]	21.5 ± 1.5[a]	16.4 ± 1.5[a]	ND	1.3 ± 0.1[a]
K_5_Z	96	4.8 ± 0.5[a]	1.2 ± 0.1[a]	38.3 ± 2.7[a]	8.2 ± 0.9[a]	21.5 ± 1.6[a]	16.6 ± 0.8[a]	ND	1.4 ± 0.2[a]
K_6_Z	168	5.1 ± 0.3[a]	1.3 ± 0.2[a]	40.1 ± 2.8[a]	8.9 ± 1.5[a]	22.5 ± 2.2[a]	18.2 ± 1.9[a]	ND	1.5 ± 0.1[a]
K_7_Z	216	5.2 ± 0.1[a]	1.4 ± 0.4[a]	46.2 ± 3.4[a]	10.1 ± 0.7[a]	28.4 ± 2.8[a]	21.5 ± 0.5[a]	ND	1.5 ± 0.1[a]
V_1_J	0	3.8 ± 1.2[a]	0.87 ± 0.1[a]	1.1 ± 0.1[a]	1.5 ± 0.1[a]	0.9 ± 0.4[a]	1.1 ± 0.1[a]	ND	1.1 ± 0.1[a]
V_2_J	24	18.4 ± 0.5[b]	0.92 ± 0.6[b]	1.4 ± 0.2[a]	1.6 ± 0.2[a]	0.9 ± 0.1[a]	1.3 ± 0.1[a]	ND	1.1 ± 0.1[a]
V_3_J	48	69.4 ± 0.5[c]	1.04 ± 0.3[b]	1.6 ± 0.2[a]	1.9 ± 0.3[a]	0.9 ± 0.2[a]	1.3 ± 0.2[a]	ND	1.1 ± 0.2[a]
V_4_J	72	98.5 ± 3.1[d]	1.06 ± 0.3[b]	1.6 ± 0.2[a]	2.3 ± 0.3[a]	1.1 ± 0.1[a]	1.7 ± 0.[1a]	ND	1.2 ± 0.3[a]
V_1_Z	0	5.6 ± 0.2[a]	0.96 ± 0.1[a]	32.9 ± 3.3[a]	6.2 ± 1.1[a]	15.2 ± 1.5[a]	11.2 ± 0.1[a]	ND	1.1 ± 0.1[a]
V_2_Z	24	10.8 ± 0.2[b]	1.09 ± 0.5[b]	45.3 ± 4.7[b]	9.1 ± 2.4[b]	23.2 ± 2.7[b]	18.1 ± 0.8[b]	ND	1.2 ± 0.1[a]
V_3_Z	48	70.2 ± 2.6[c]	1.99 ± 0.6[c]	57.1 ± 3.6[c]	8.8 ± 2.5[c]	25.1 ± 3.2[c]	19.9 ± 1.2[c]	ND	1.9 ± 0.2[b]
V_4_Z	72	79.6 ± 1.2[d]	3.36 ± 0.6[d]	63.7 ± 0.5[d]	12.3 ± 0.9[d]	28.2 ± 1.8[d]	32.1 ± 2.4[d]	ND	2.3 ± 0.4[c]

*Black tea infusion kombucha samples—K_0_C: time 0 h; K_24_C: time 24 h; K_48_C: time 48 h; K_72_C: time 72 h; K_96_C: time 96 h; K_168_C: time 168 h; K_216_C: time 216 h, Sauerkraut kombucha samples samples—K_0_Z: time 0 h; K_24_Z: time 24 h; K_48_Z: time 48 h; K_72_Z: time 72 h; K_96_Z: time 96 h; K_168_Z: time 168 h; K_216_Z: time 216 h, apple juice water kefir samples—V_0_J: time 0 h; V_24_J: time 24 h; V_48_J: time 48 h; V_72_J: time 72 h), sauerkrauet water kefir samples—V_0_Z: time 0 h; V_24_Z: time 24 h; V_48_Z: time 48 h; V_72_Z: time 72 h.

**TRY, tryptamine; PHE, phenylethylamine; PUT, putrescine; CAD, cadaverine; HIS, histamine; TYR, tyramine; SPD, spermidine; SPM, spermine.

***ND—not detected.

[a]The values are expressed as means ± standard deviation (n = 18; each batch of fermented beverage was analyzed from two bottles, the samples from each bottle were derivatized three times, and each derivatized mixture was loaded onto the chromatographic column three times). The means within a column (the difference between BA amount in different fermentation times) followed by different superscript letters differ ($p < 0.05$); each fermented beverage type was evaluated separately.

in the analyzed black tea infusion kombucha samples, were detected almost all BAs, whereas their concentrations were < 2 mg/L. A variety of amino acids are present in the fermentation broth of kombucha, mainly derived from free amino acids (such as lysine, cysteine, tryptophan, leucine) in tea leaves and from microbial fermentation. Although amino acids play an important role in the metabolism of human body, they can be converted into biogenic amines. In general, BAs are the consequence of microbial decarboxylation during fermentation. However, limited information is available from studies focusing on BA concentrations in kombucha beverages. Additionally, kombucha SCOBY contains elevated concentrations of lysine, isoleucine, and leucine, while other amino acids (e.g., tryptophan, phenylalanine, and proline) are lower. Concerning the amino acids identified in SCOBY, only lysine, phenylalanine, and tryptophan are precursors of potentially detrimental BAs (Diez-Ozaeta and Astiazaran, 2022; Bishop et al., 2022; Huang, 2024).

In the SKJ kombucha-like samples, the most abundant BAs were PUT, and HIS, followed TYR. The latter BA concentrations are in accordance with that reported by Jastrzębska et al. (2023). TYR, HIS and CAD could be probably formed during the sauerkraut fermentation process by lactic acid bacteria (mainly lactobacilli) (Lorencová et al., 2020). The latter mentioned BAs were detected in concentrations < 50 mg/L. Furthermore, in the SKJ water kefir-like samples, TRY and PUT, were the most abundant BAs detected. The above-mentioned BAs were detected in concentrations > 50 mg/L after 48 h of fermentation. Additionally, TRY was detected in the higher concentration also for the water-kefir like apple juice samples. According to Jaguey-Hernández et al. (2021), the European Food Safety Authority (EFSA) identifies HIS and TYR as the most crucial BAs in terms of toxicology. Ingesting 25–50 mg of HIS and 600 mg of TYR per meal does not cause health issues to a healthy person (Majcherczyk and Surówka, 2019). With respect to the obtained results, none of the samples did not exceed the above-mentioned limits and could be characterized as safe. Additionally, concentrations of BAs up to 100 mg/L (or 100 mg/Kg) are considered to be safe for the consumer. However, substances such as ethanol and various drugs can significantly reduce the effectiveness of the detoxification mechanism (Lorencová et al., 2020). Nowadays, there is a rise in the use of

antidepressants like monoamine oxidase inhibitors or diamine oxidase inhibitors, making even small amounts of BAs in food hazardous to individuals, thus leading to an increased focus on BAs analysis in the food industry (Givanoudi et al., 2023). On the whole, with the prolonging fermentation time the concentrations of all detected BAs increased ($p < 0.05$). However, the sensory quality of foods/beverages with high BA content is often unacceptable and BAs are used as a freshness indicator in many food matrices (Naila et al., 2010). Although BAs are typically found naturally in a variety of meals and drinks, the amounts of these compounds can range greatly between and within different food and beverage kinds. The fermentation process used to produce food and drinks can have a significant impact on and even improve the synthesis of these molecules in the finished product. Additionally, these variations can be explained by a number of factors, including the way raw materials are treated, the variety and availability of amino acids, the presence of decarboxylase-positive microorganisms, redox potential, pH, temperature, NaCl concentration, water activity, oxygen supply, manufacturing/processing methods, storage time, and temperature (Okici et al., 2020). Lactic acid bacteria (LAB) are known to form BAs in fermented vegetables in order to survive during fermentation on an acidic pH. The ideal temperature range for bacteria to produce them is 20–37°C. Further, in acidic settings with a pH between 4.0 and 5.5, amino acid decarboxylation is more active (Visciano and Schirone, 2022).

3.4 Total antioxidant activity and total polyphenol content

Antioxidant activity, such as the DPPH and the ABTS assays, and TPC determination represent convenient methods for identification of potential sources of antioxidant compounds in foods and beverages (Sumczynski et al., 2015). DPPH scavenging activity test measures the activity of the sample against DPPH, which is a stable organic nitrogen radical, induced oxidation (Esatbeyoglu et al., 2023). Regarding the DPPH test, DPPH radical scavenging activity values of the model fermented beverages obtained from black tea, SKJ and apple juice are depicted in Table 7. The DPPH of non-fermented samples (0 h) was 1,678 ± 42 mg Trolox equivalents (TE)/L for black tea kombucha sample, 80 ± 2 mg TE/L for SKJ kombucha-like samples, 179 ± 4 mg TE/L for apple juice water kefir-like samples and 80 ± 3 mg TE/L for SKJ water kefir-like samples. After fermentation

TABLE 7 FRAP, ABTS and DPPH radical scavenging activity of phenolic fractions and total phenolic content (TPC) of the model fermented beverages during fermentation.[a]

Sample*	Time (h)	FRAP (mg TE/L)	ABTS (mg TE/L)	DPPH (mg TE/L)	TPC (mg GAE/L)
K_1_C	0	561±8^{a}	1895±130^{a}	1,680±42^{a}	918±43^{a}
K_2_C	24	497 ± 20^{b}	2,250 ± 184^{b}	1,400 ± 118^{b}	606 ± 2^{b}
K_3_C	48	515 ± 2^{c}	1,650±78^{a}	1,300 ± 61^{b}	533 ± 67^{c}
K_4_C	72	570 ± 32^{d}	2,140 ± 14^{b}	1,485 ± 67^{b}	736 ± 36^{d}
K_5_C	96	546 ± 48^{d}	2070 ± 74^{b}	1,490 ± 22^{b}	689 ± 35^{e}
K_6_C	168	462 ± 32^{b}	1,570±130^{a}	1,190 ± 68^{c}	539 ± 28^{c}
K_7_C	216	585 ± 21^{d}	1960±142^{a}	1,490 ± 57^{b}	725 ± 12^{f}
K_1_Z	0	166±1^{a}	215±10^{a}	80±2^{a}	309±3^{a}
K_2_Z	24	232 ± 12^{b}	236 ± 7^{b}	91 ± 7^{b}	397 ± 15^{b}
K_3_Z	48	214 ± 18^{b}	224 ± 6^{b}	87±4^{a}	377 ± 10^{b}
K_4_Z	72	187 ± 14^{c}	172 ± 5^{c}	79±6^{a}	311±9^{a}
K_5_Z	96	171 ± 6^{c}	169 ± 10^{c}	65 ± 3^{c}	227 ± 19^{c}
K_6_Z	168	225 ± 19^{b}	215±4^{a}	85±7^{a}	373 ± 9^{b}
K_7_Z	216	168±1^{a}	193 ± 10^{d}	78±3^{a}	344 ± 10^{d}
V_1_J	0	198±2^{a}	385±8^{a}	179±4^{a}	345±2^{a}
V_2_J	24	200±6^{a}	392±13^{a}	176±5^{a}	338 ± 1^{b}
V_3_J	48	212±6^{a}	391±35^{a}	182±6^{a}	337 ± 5^{b}
V_4_J	72	195±10^{a}	351±25^{a}	169±6^{a}	331 ± 4^{b}
V_1_Z	0	202±11^{a}	226±20^{a}	80±3^{a}	338±6^{a}
V_2_Z	24	209±4^{a}	229±14^{a}	81±5^{a}	330±14^{a}
V_3_Z	48	212±2^{a}	207±20^{a}	82±3^{a}	334±6^{a}
V_4_Z	72	169 ± 15^{b}	183±5^{a}	65 ± 1^{b}	262 ± 11^{b}

*Black tea infusion kombucha samples—K_0_C: time 0 h; K_24_C: time 24 h; K_48_C: time 48 h; K_72_C: time 72 h; K_96_C: time 96 h; K_168_C: time 168 h; K_216_C: time 216 h, Sauerkraut kombucha samples samples—K_0_Z: time 0 h; K_24_Z: time 24 h; K_48_Z: time 48 h; K_72_Z: time 72 h; K_96_Z: time 96 h; K_168_Z: time 168 h; K_216_Z: time 216 h, apple juice water kefir samples—V_0_J: time 0 h; V_24_J: time 24 h; V_48_J: time 48 h; V_72_J: time 72 h, sauerkrauet water kefir samples—V_0_Z: time 0 h; V_24_Z: time 24 h; V_48_Z: time 48 h; V_72_Z: time 72 h.

[a]The values are expressed as means ± standard deviation. The means within a column (the difference between FRAP, ABTS, DPPH, and TPC values in different fermentation times) followed by different superscript letters differ ($p < 0.05$); each fermented beverage type was evaluated separately.

(72 h), DPPH values decreased significantly in all tested samples, regardless of the applied substrate ($p < 0.05$). According to Esatbeyoglu et al. (2023) the above-mentioned decrease after fermentation could be due to the structure of phenolic compounds which can be affected by activity of microbial enzymes that convert them into other molecules, thus affecting the antioxidant activity of the beverage. Furthermore, microbial transformation could generate antioxidant compounds undetectable by DPPH/ABTS assays, which may still retain biological activity. Black tea kombucha samples demonstrated the highest DPPH radical-scavenging activity than the other samples (SKJ and apple juice) ($p < 0.05$). One of main reasons of the antioxidant activity of water kefir is due to the lactic acid bacteria in the water kefir grain as well as bioactive compounds in exopolysaccharide structure formed during fermentation (Ozcelik et al., 2021). It is established that the antioxidant activity might be affected by factors, such as extraction solvent and tested system. Therefore, it is necessary to perform different antioxidant activity assessments to consider various mechanisms of action (Sumczynski et al., 2015). Due to this reason, another TEAC assay based on the ability of antioxidant to scavenge ABTS was used to determine antioxidant activity. Results of the ability to scavenging ABTS are presented in Table 7. The TE values varied from 1,570 ± 130 to 2,250 ± 184 mg/L for the black tea kombucha samples, from 169 ± 10 to 236 ± 7 mg/L for the SKJ kombucha-like samples, from 392 ± 13 to 351 ± 25 mg/L for the apple juice water kefir-like beverages and from 183 ± 5 to 229 ± 14 mg/L for the SKJ water kefir-like samples. The highest values were monitored for the black tea kombucha samples. In general, for almost all tested samples, the TE values followed a decreasing trend with the prolonging of the fermentation time, with exception being the black tea kombucha samples. The results obtained using ABTS and DPPH tests corresponded with each other. The FRAP test estimates the ability to reduce ferric (III) ions to ferrous (II) ions. The FRAP values for all evaluated tested samples are presented in Table 7. The highest content of reductive compounds labelled by the FRAP method was observed in black tea kombucha samples, followed by SKJ kombucha-like samples. With the progress of the fermentation time the FRAP values for the kombucha-like samples slightly increased, whereas a decrease in the water kefir-like samples was observed. When analyzing the type of the tested substrate, black tea kombucha was characterized by the highest reductive potential.

The Folin–Ciocalteu method is used to determine TPC and relies on the transfer of electrons from phenolic compounds to the Folin–Ciocalteu reagent in alkaline media. It is a simple and reproducible method and has been used in many studies (Zhou et al., 2022). In black tea infusion kombucha samples, the TPC ranged between 918 ± 43 and 533 ± 67 mg GAE/L. The SKJ kombucha-like beverages presented a TPC in the range of 397 ± 15 to 227 ± 19 mg GAE/L (Table 7). Higher contents of TPC were monitored before fermentation (0 h) than those after fermentation (216 h) for the black tea infusion kombucha samples. The TPC values development in relation to fermentation time was different from that reported in the literature, where the TPC values increased with fermentation time. A possible explanation of this difference could be related to the differences of the used black tea and kombucha starter culture (microbial diversity) or environmental conditions (pH, oxygen availability, enzymes), leading to decreased polyphenol stability (Chu and Chen, 2006; Jakubczyk et al., 2020; Pasquet et al., 2024). Furthermore, according to Esatbeyoglu et al. (2023), and Septembre-Malaterre et al. (2018) the TPC decrease may be explained by the metabolic degradation of phenolic compounds by different strains of lactic acid bacteria involved in the fermentation pattern. On the other hand, the SKJ kombucha-like samples presented an increase of the TPC content with the progress of the fermentation time. Furthermore, the water kefir-like samples (regardless of the substrate used) reported a decrease in the TPC values with the prolonging of the fermentation time. Additionally, a more intensive decrease was monitored for the SKJ samples. These decreases to the metabolic degradation of phenolic compounds could be due by different strains of lactic acid bacteria involved in the fermentation (Septembre-Malaterre et al., 2018). In particular, the latter decrease could be due the degradation of phenolic compounds as possible mechanisms of antimicrobial detoxification of yeasts and bacteria (Paredes et al., 2022). The results are in accordance to that previously reported by Randazzo et al. (2016), and Ozcelik et al. (2021). The highest TPC was assessed in samples from apple juice (345 ± 2 mg GAE/L) followed by SKJ samples (338 ± 6 mg GAE/L) ($p < 0.05$). On the contrary, the lowest TPC was found in SKJ samples at the end of the fermentation period (262 ± 11 mg GAE/L) ($p < 0.05$).

3.5 Microbiological analysis

The microbiological properties of the tested fermented beverages are shown in Figure 4. Microbiological analysis was performed to detect microorganisms that were expected to increase in the model fermented beverages. The latter microorganisms were mainly yeasts and lactic acid bacteria (lactobacilli, lactococci) and acetic acid bacteria. According to the available literature, an increase in lactic acid bacteria (mainly species of the genera *Lactobacillus*, *Lactococcus* and), acetic acid bacteria (mainly species of the genera *Acetobacter*, and *Gluconobacter*) and yeasts (mainly species of *Saccharomyces cerevisiae*, *Kloeckera apiculata*, *Saccharomyces ludwigii*, *Torulopsis delbrueckii*, *Brettanomyces bruxellensis*) can be expected (Chakravorty et al., 2016; Villarreal-Soto et al., 2018; Şafak et al., 2023).

Substrate inoculations were also carried out, essentially serving as a control in case of contamination with undesirable microorganisms such as staphylococci, enterococci, clostridia and coliforms, which are characterized as (potentially) hazardous to human health, especially at higher concentrations. In Figure 4 are presented the microbiological results only for the microorganisms for which a continuous count increase has been recorded. Hence, no increase was observed on the media, for the following groups of microorganisms, thus were not detected: staphylococci, enterococci, clostridia, and, coliforms.

Thus, for kombucha-like samples, a gradual increase of microorganism counts was observed, while for water kefir-like samples, relatively high CFU/ml values were already detected at fermentation time 0 h (5.20–5.48 CFU/mL for the apple juice water kefir-like samples; 4.73–5.74 CFU/mL for the SKJ water kefir-like samples). However, considering the ratio of the mother culture to the total amount of substrate, this could be an expected phenomenon. The microbiological results for the black tea kombucha-like samples are depicted in part A (Figure 4). During fermentation, the most significant increases in colonies of lactobacilli (up to 7.38 CFU/mL) and yeasts (up to 7.14 CFU/mL) were observed. Also, acetobacteria and lactococci were detected after 24 h of fermentation. The increase in these groups of microorganisms (acetobacteria and lactococci) was gradual.

Furthermore, the microbiological results for the SKJ kombucha-like samples are shown in part B (Figure 4). From the obtained results it can be seen that at fermentation time 0 h, acetobacter (1.62 CFU/mL), yeasts (1.22 CFU/mL), lactococci (1.11 CFU/mL) and lactobacilli (1.61 CFU/mL) were detected. The counts of acetobacter and lactobacilli were the most abundant. This is probably related to the nature of SKJ applied, which is product from the lactic fermentation of cabbage. During fermentation, the most significant increase was monitored in the case of yeasts (up to a 6.58 CFU/mL). Furthermore, a slight increase in the counts of acetobacteria, lactococci and lactobacilli was also observed. Similar results were reported by Chakravorty et al. (2016), and Grassi et al. (2022).

The microbiological results for the apple juice water kefir-like samples are depicted in part C (Figure 4). From the obtained results it can be stated that a significant increase of yeasts was monitored during the first 24 h of fermentation (to 7.08 CFU/mL). Furthermore, a similar increase in lactobacilli and acetobacteria was detected, with the higher increase occurring after 48 h of fermentation. The increase in lactic cocci was gradual. The detected lactococci slightly increased with the progress of the fermentation period (from 5.20 to 6.21 CFU/mL). Additionally, the SKJ water kefir-like microbiological results are presented in part D (Figure 4). In the case of the water kefir sample from SKJ, a similar pattern, as in the case of SKJ kombucha-like samples, in acetobacteria (from 5.74 to 7.28 CFU/mL), lactobacilli (from 4.73 to 6.68 CFU/mL) and yeast (from 5.36 to 7.36 CFU/mL) counts were observed. This could be probably related to the nature of the original raw material, i.e., SKJ. The increase in lactococci count was the less intensive in the case of this sample. The results are in accordance to that previously reported by Fiorda et al. (2017), Ozcelik et al. (2021), and Limbad et al. (2023).

During the fermentation of kombucha and kombucha-like products, yeast cells hydrolyze sucrose into fructose and glucose, which yeast then metabolizes to produce ethanol and CO_2. The high osmotic pressure also allows yeast to produce glycerol, which AAB can then further oxidize to dihydroxyacetone. In addition, some esters are formed during this process, which aids in the development of kombucha products flavor profile. The ethanol produced is subsequently metabolized by AAB to generate acetic acid, lowering the ethanol concentration in the kombucha. Additionally, the low ethanol concentration aids in the production of the cellulosic pellicle.

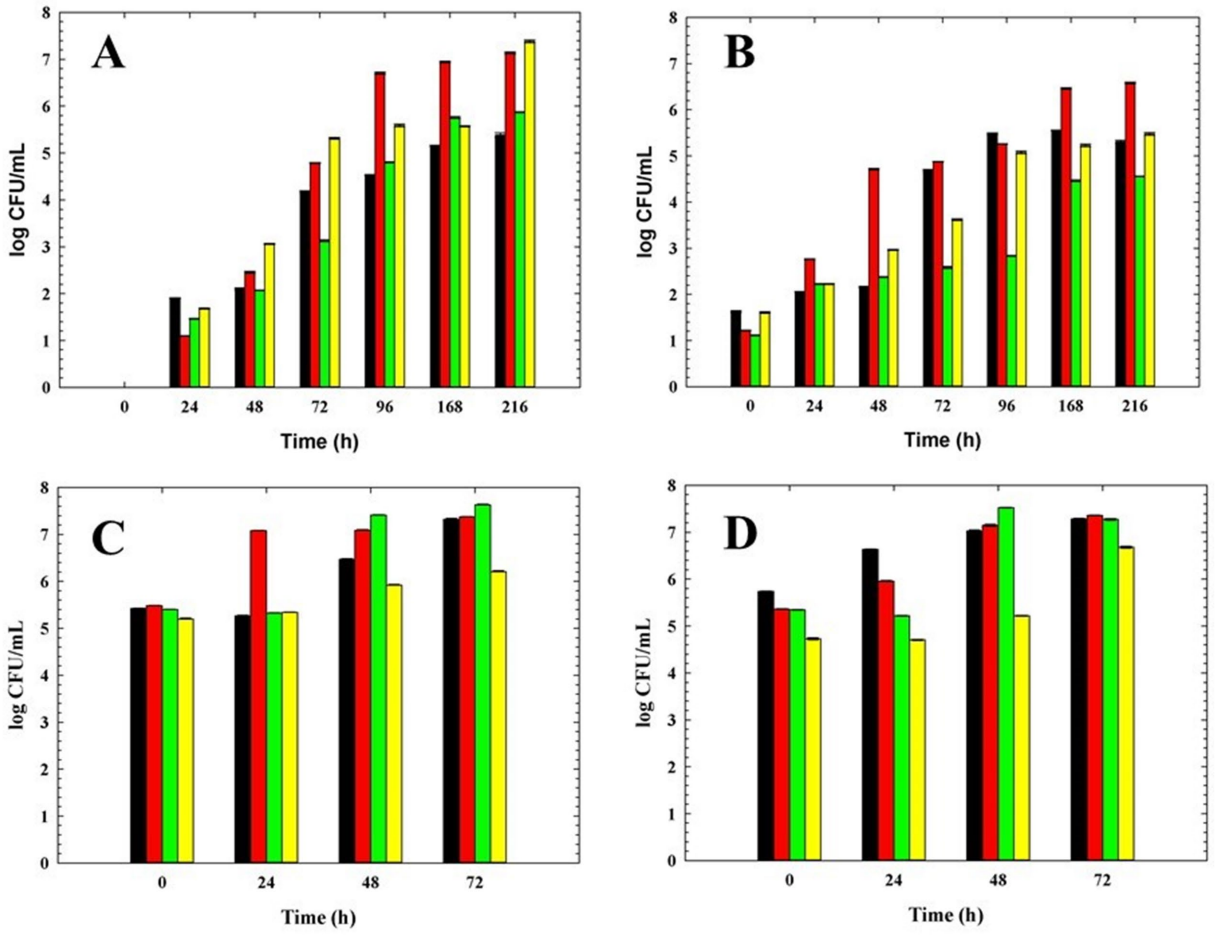

FIGURE 4
Microbiological characteristics (log CFU/mL) of fermented beverages with kombucha starter culture (part **A**—black tea kombucha beverages and part **B**—sauerkraut kombucha-like beverages) and with water kefir started culture (part **C**—apple juice eater kefir-like beverage and part **D**—sauerkraut water kefir-like beverage) in relation to fermentation time. Black columns—acetobacteria; red columns—yeasts; green columns—lactococci; yellow columns—lactobacilli.

Furthermore, glucose is transformed into gluconic and glucuronic acids by AAB. Vitamins and other nutrients from yeast autolysis aid in the development of the AAB (Villarreal-Soto et al., 2018; Wang et al., 2022). Moreover, in the fermentation process of water kefir and water kefir-like beverages, yeast and bacteria produce compounds contributing to the aroma and functional properties of the final product. Initially aerobic, the fermentation process becomes anaerobic as oxygen is depleted and carbon dioxide is produced by yeasts. Furthermore, sucrose is converted into ethanol, CO2, lactic acid, acetic acid, mannitol, vitamins, amino acids, glycerol, esters, and other organic compounds, reducing sucrose concentration by up to 98% within the first 24 h. Yeasts such as *Saccharomyces*, *Zygotorulaspora*, and *Dekkera* use an enzyme to break down sucrose into glucose and fructose, which are metabolized to produce ethanol, later converted to AAB. Despite a higher abundance of LAB, yeast-driven processes dominate, with organic acid production by LAB providing energy for yeasts early in fermentation (Lynch et al., 2021; Spizzirri et al., 2023).

In general, it could be reported that microbial species' diversity of fermented kombucha-like and water kefir-like beverages consists of a stable consortium of microorganisms composed mainly by lactic acid bacteria, acetic acid bacteria, and yeasts.

3.6 Instrumental analysis of color

The values of the instrumental analysis of color are depicted in Table 8. In the case of black tea infusion kombucha samples, a slight increase in the L^* values was monitored during the fermentation period ($p < 0.05$). The values of a^* were in the red region ($+a^*$) and slightly decreased during fermentation ($p < 0.05$). Moreover, b^* values were in the yellow region ($+b^*$) and slightly decreased with the increasing fermentation time ($p < 0.05$). The typical dark brown color observed in kombucha beverages is indicating the presence of active microorganisms using soluble solids as energy sources. Moreover, with the progress of the fermentation time the soluble solids are consumed, and the initially dark brown liquid becomes lighter (da Silva et al., 2024). Additionally, the trends mentioned above could be explained by the production of organic acids by the microbiota present (Yıkmış and Tuğgüm, 2019). In the case of the SKJ kombucha-type samples, a slight increase in the values of L^* during the fermentation time was also observed ($p < 0.05$). The $^*a$ values were in the negative, i.e., green, region, which is consistent with the nature of the product. The b^* values, which correspond to the yellow region,

TABLE 8 Values of lightness (L^*), chromaticity on a green-to-red axis (a^*), chromaticity on a blue-to-yellow axis (b^*), chroma (C^*), hue angle (h °), of the model beverage samples manufactured with kombucha and water kefir consortiums in relation to fermentation time.

Sample**	Time (h)	Parameter				
		L^*	a^*	b^*	h^*	C^*
K_1_C	0	26.94 ± 0.01^a	2.62 ± 0.01^a	6.23 ± 0.01^a	67.20 ± 0.02^a	6.77 ± 0.02^a
K_2_C	24	27.38 ± 0.02^b	2.54 ± 0.01^b	5.12 ± 0.01^b	65.67 ± 0.08^b	6.22 ± 0.01^b
K_3_C	48	26.92 ± 0.01^a	2.61 ± 0.01^a	5.48 ± 0.01^c	64.47 ± 0.03^c	6.07 ± 0.03^c
K_4_C	72	27.67 ± 0.01^b	2.60 ± 0.01^a	5.27 ± 0.01^d	63.72 ± 0.01^d	5.88 ± 0.02^d
K_5_C	96	27.11 ± 0.02^c	2.51 ± 0.02^b	4.93 ± 0.01^e	63.02 ± 0.12^e	5.54 ± 0.01^e
K_6_C	168	29.39 ± 0.01^d	1.64 ± 0.01^c	3.89 ± 0.01^f	67.05 ± 0.06^f	4.22 ± 0.02^f
K_7_C	216	28.52 ± 0.01^d	2.32 ± 0.01^d	5.33 ± 0.01^g	66.45 ± 0.02^g	5.82 ± 0.01^g
K_1_Z	0	29.69 ± 0.02^a	−0.11 ± 0.01^a	3.98 ± 0.01^a	88.35 ± 0.02^a	3.99 ± 0.01^a
K_2_Z	24	29.51 ± 0.03^b	−0.11 ± 0.01^a	3.83 ± 0.02^b	88.21 ± 0.02^b	3.84 ± 0.01^b
K_3_Z	48	29.89 ± 0.01^c	−0.11 ± 0.02^a	3.91 ± 0.02^c	88.32 ± 0.02^c	3.92 ± 0.02^c
K_4_Z	72	30.51 ± 0.02^d	−0.22 ± 0.01^b	3.55 ± 0.02^d	86.53 ± 0.03^d	3.55 ± 0.01^d
K_5_Z	96	30.17 ± 0.02^e	−0.24 ± 0.02^b	4.04 ± 0.01^e	86.68 ± 0.03^e	4.05 ± 0.02^e
K_6_Z	168	30.27 ± 0.02^f	−0.30 ± 0.01^c	3.88 ± 0.02^f	85.51 ± 0.02^f	3.90 ± 0.02^f
K_7_Z	216	31.25 ± 0.01^g	−0.20 ± 0.02^b	2.91 ± 0.01^g	85.98 ± 0.02^g	2.92 ± 0.03^g
V_1_J	0	28.68 ± 0.02^a	0.63 ± 0.01^a	5.20 ± 0.01^a	79.13 ± 0.01^a	4.03 ± 0.02^a
V_2_J	24	29.48 ± 0.03^b	0.23 ± 0.01^b	3.04 ± 0.01^b	85.59 ± 0.02^b	3.05 ± 0.02^b
V_3_J	48	31.84 ± 0.03^c	0.24 ± 0.01^c	6.11 ± 0.01^c	85.50 ± 0.02^c	3.12 ± 0.01^c
V_4_J	72	30.44 ± 0.04^d	0.03 ± 0.01^d	3.35 ± 0.01^d	89.32 ± 0.03^d	3.35 ± 0.02^d
V_1_Z	0	29.86 ± 0.02^a	−0.19 ± 0.02^a	3.86 ± 0.02^a	79.13 ± 0.03^a	3.87 ± 0.02^a
V_2_Z	24	29.17 ± 0.02^b	−0.56 ± 0.01^b	2.68 ± 0.01^b	78.12 ± 0.02^b	2.74 ± 0.01^b
V_3_Z	48	32.66 ± 0.02^c	−1.14 ± 0.02^c	2.89 ± 0.02^c	68.36 ± 0.01^c	3.10 ± 0.02^c
V_4_Z	72	30.22 ± 0.03^d	−0.30 ± 0.02^d	3.31 ± 0.01^d	84.57 ± 0.04^d	3.33 ± 0.01^d

*Black tea infusion kombucha samples—K_0_C: time 0 h; K_24_C: time 24 h; K_48_C: time 48 h; K_72_C: time 72 h; K_96_C: time 96 h; K_168_C: time 168 h; K_216_C: time 216 h, Sauerkraut kombucha samples—K_0_Z: time 0 h; K_24_Z: time 24 h; K_48_Z: time 48 h; K_72_Z: time 72 h; K_96_Z: time 96 h; K_168_Z: time 168 h; K_216_Z: time 216 h, apple juice water kefir samples—V_0_J: time 0 h; V_24_J: time 24 h; V_48_J: time 48 h; V_72_J: time 72 h, sauerkraut water kefir samples—V_0_Z: time 0 h; V_24_Z: time 24 h; V_48_Z: time 48 h; V_72_Z: time 72 h. The means within a column (the difference between colorimetric parameters values in different fermentation times) followed by different superscript letters differ ($p < 0.05$); each fermented beverage type was evaluated separately.

TABLE 9 Results of the sensory analysis of the model fermented beverages (appearance, aroma, taste, carbonation level, off-flavour, and overall rating).*, **, ***

Sample	Time(h)	[1]Appearance	Taste	Aroma	Carbonation level	Off-flavour	Overall rating
K_7_C	216	1 [a]	1 [a]	2 [a]	3 [a]	1 [a]	2 [a]
K_7_Z	216	1 [a]	2 [b]	2 [b]	2 [b]	1 [a]	2 [a]
V_4_J	72	1 [a]	1 [a]	1 [a]	1 [a]	1 [a]	2 [a]
V_4_Z	72	1 [a]	1 [a]	2 [b]	1 [a]	1 [a]	2 [a]

*Black tea infusion kombucha samples—K_216_C: time 216 h, Sauerkraut kombucha samples—K_216_Z: time 216 h, apple juice water kefir samples—V_72_J: time 72 h, sauerkraut water kefir samples—V_72_Z: time 72 h.
**Median values within a column (difference between substrate type; comparing the same starter culture) followed by different superscript letters statistically differ ($p < 0.05$); the samples manufactured with different starter culture were evaluated independently.
***Appearance: 1—excellent, 3—good, 5—unacceptable; taste: 1—excellent, 3—good, 5—unacceptable; aroma: 1—excellent, 3—good, 5—unacceptable; overall rating: 1—extraordinarily good; 5—extremely bad.

were not affected by the progress of the fermentation. The common brownish-yellow color of apple juice results from the enzymatic browning process (due to polyphenol oxidase activity) in basic raw material (da Silva et al., 2024). Furthermore, the apple juice water kefir samples presented a slight increase in L^* values concerning the fermentation time ($p < 0.05$). Moreover, a slight decrease in the values of $a^*$and b^* was observed as the fermentation time increased ($p < 0.05$). In the case of SKJ water kefir samples, an increase in the L^* values during fermentation was observed ($p < 0.05$). The value of a^* for the SKJ water kefir samples corresponded to the green area, and a decreasing trend can be observed during fermentation ($p < 0.05$). The b^* values for SKJ water kefir were in the yellow region, and there were no significant changes during fermentation ($p > 0.05$). In general, it could be stated that the resulting values of the color parameters correspond to the nature of the substrate applied.

3.7 Sensory analysis

The results of the sensory analysis are presented in Table 9. In general, the sensory evaluation revealed significant differences among the tested samples ($p < 0.05$). In particular, the black tea kombucha-like sample (K_216_C) showed generally favorable sensory attributes, receiving top scores (1 = excellent) for appearance, taste, aroma, carbonation, and overall rating. In contrast, the SKJ kombucha-like sample (K_72_J) and SKJ water kefir-like sample (V_72_Z) scored slightly lower, especially in aroma and taste, though their appearance and carbonation levels remained acceptable. Additionally, the scores for the attribute of off-flavour were low (indicating minimal off-flavours) across all tested samples, supporting their sensory viability. Statistical differences ($p < 0.05$) between samples suggest that the type of starter culture and substrate type influenced the sensory properties. While black tea kombucha-like sample performed the best overall rating, the SKJ fermented beverages maintained acceptable sensory profiles, suggesting potential for consumer acceptance with formulation optimization. In summary, SKJ kombucha-like and SKJ water kefir-like beverages could be noted as viable probiotic alternatives to kombucha and water kefir, owing to its favorable sensory acceptance, the presence of bioactive substances, and its demonstrated antioxidant activity. However, it should be noted that the elevated levels of acidity and alcohol content might have an important influence on their organoleptic profile and evaluation.

4 Conclusion

The study evaluated the physicochemical, rheological, antioxidant, and microbiological properties of sauerkraut juice (SKJ), black tea infusion, and apple juice fermentation using kombucha, and water kefir starter cultures. Results showed initial pH fluctuated, and overall acidity increased due to lactic and acetic acid bacteria. The Power-law model was used to analyze the flow behavior of fermented beverages, revealing non-Newtonian behavior and increased viscosity with longer fermentation times. Biogenic amines were present, but low levels pose little risk. The study found that black tea kombucha has the highest antioxidant activity, with varying total phenolic concentrations in water kefir-like beverages. In general, based on the obtained results, fermentation of SKJ can create functional beverages with health-promoting qualities, and the fermentation of SKJ offers a chance to produce plant-based, probiotic-rich beverages. Hence, the use of SKJ in the development of fermented beverages could be a promising way to utilize a by-product (agro-waste) from the technological process of fermented cabbage, through an efficient and sustainable process supporting circular bioeconomy frameworks, resulting in a final bioactive value-added and health-promoting beverage. However, further studies are needed to confirm the functional and health effects in clinical or consumer settings.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

Author contributions

RS: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. PP: Methodology, Writing – review & editing. DS: Data curation, Investigation, Methodology, Writing – review & editing. ŠV: Methodology, Validation, Writing – review & editing. JK: Investigation, Writing – review & editing. AR: Investigation, Methodology, Project administration, Writing – review

& editing. EL: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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