

Functional beverages based on herbal and tea extracts: evaluation of bioactive phytochemical properties, nutritional value and effects on α -amylase and glucosidase inhibition

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Abstract

Functional beverages are a rich source of vitamins and minerals, but also support the body. The aim of the study was to develop functional beverages based on clean-label herbal and tea extracts. The beverages were tested for, among other things, water content using the drying method, fat content using the Soxhlet method, mineral compounds in the form of ash by combustion, tannin content using the titration method, color, pH, antioxidant activity using the DPPH method, and inhibition of α -amylase and α -glucosidase activity, and an organoleptic assessment was also performed. The designed beverages showed a stronger effect on α -glucosidase than on α -amylase. Variant C2 (drink properties: calming and sleep-promoting properties; composition: black tea, lemon balm, chamomile, water, orange juice) inhibited α -glucosidase the most strongly – by 85.13% – and α -amylase by 59.82%. The designed drinks are characterized by a high content of vitamins and fiber, but also hydrate the body well.

Highlights

- Variant C2 inhibited α -glucosidase most strongly, by 85.13%.
- Variant C2 inhibited α -amylase most strongly, by 59.82%.
- The highest antioxidant potential was shown by drink S2 (91.16%).
- Variant C2 has a high content of vitamin B9 (45.35 $\mu\text{g}/100\text{ ml}$).

1. Introduction

Consumers are increasingly choosing functional beverages, especially in the USA [Siró, Kápolna, Kápolna & Lugasi, 2008]. This is due to many reasons, including the fact that they quench thirst, nourish the body, and offer health benefits. On the other hand, they are looking for drinks that have effects similar to dietary supplements, reducing the need to swallow often very large and un-pleasant-tasting tablets. Functional beverages are a significant group of functional foods [Siró et al., 2008; Sugajski, Buszewska-Forajta & Buszewski, 2023; Gupta, 2023]. These include beverages enriched with dietary fiber, minerals, vitamins, plant ex-tracts, amino acids, probiotics, and oligosaccharides. Furthermore, functional beverages can be divided into: supplementary (with energizing and isotonic properties), health (supporting the body with vitamins and minerals), and therapeutic [Gayathry & John, 2021; Panou & Karabagias, 2025; Sa'aïd & Tan, 2025]. Depending on the effect a drink has on the body, beverages can also be divided into: adding energy and vitality with natural ingredients – the main bioactive ingredients are caffeine, ginsenosides, guarana, theanine and taurine. These ingredients support concentration, reduce fatigue, increase the body's stimulation and improve performance [Rath, 2012; Childs, 2014; Gualberto, Benvindo, Waclawovsky & Deresz, 2024; Tamamoto, Schmidt & Lee, 2010]. Isotonic drinks – the main ingredients are potassium, sodium and magnesium, enriched with vitamins and glucose. These drinks provide electrolytes during intense exercise and hydrate the body [Gironés-Vilaplana, Villaño, Moreno & García-Viguera, 2013; Gonçalves et al., 2018; Wyspiańska, Kucharska Sokół-Łętowska & Kolniak-Ostek, 2019; Bendaali, Vaquero, Escott, González & Morata, 2023]. Probiotics – contain *Lactobacillus*, *Bifidobacterium* and prebiotics (inulin), for example, with the right composition, they can be used to prevent osteoporosis [Angelov, Gotcheva, Kuncheva & Hristozova 2006; Benton Williams & Brown, 2007]. Immunity – ingredients in these drinks include vitamin C, D3, ginger, zinc, selenium and turmeric. These ingredients support the immune system and have anti-inflammatory and antiviral properties [Riso et al., 2006; Lee, Lee, Kim & Kim, 2021; Lubis & Rasmaliah, 2022]. Collagen - the main ingredient is hydrolyzed collagen, vita-min C, and hyaluronic acid. This kind supports hair growth and improves the condition of the skin and nails [Bilek & Bayram, 2015; Al Hajj et al., 2024]. Relaxing drinks - containing lemon balm, ashwagandha, and L-theanine. These ingredients promote good sleep, calmness, relaxation and have a calming effect [Stacy, 2011; Pinto & Vilela, 2021; Routray, Valappil & Du, 2024]. One of the basic ingredients of functional drinks is tea (*Camellia sinensis* or *Camellia assamica*).

Tea is very rich in chemical composition, containing approximately 300 bioactive substances. These include polyphenols, carbohydrates, proteins, lignins, amino acids, chlorophyll, caffeine, theobromine, theophylline, saponins, linalool, geraniol, vitamins (B vitamins, K and C), and minerals (calcium, iron, manganese, potassium, iodine, silicon, fluorine, and phosphorus) [Sun et al., 2022; Luo, Luo, Zhao, Wang & Luo, 2024; Toniolo, Patriarca, De Vita, Santi & Sciubba, 2026]. Tea black and green owes many of its beneficial properties to the presence of polyphenols in the leaves (green tea is particularly rich in them). Their content varies depending on the growing climate, sunlight and humidity. The growth phase in which the leaves are harvested is also crucial. Young leaves have the highest polyphenol content, while older leaves have the lowest [Khan & Mukhtar, 2007; Wang, Han, Pu & Wang, 2022; Radeva-Ilieva, Stoeva, Hvarchanova & Georgiev, 2025]. Green tea is a source of catechins (catechin gallate and epigallocatechin gallate, epicatechin and epigallocatechin), which are responsible for the astringent and bitter taste of the infusion [Singh, Shankar & Srivastava, 2011; Xu et al., 2018; Xu et al., 2024] These compounds also have antioxidant properties, protecting the body's cells against oxidative stress, inhibiting the formation of free nitrogen and oxygen radicals and chelating metal ions [Yan, Zhong, Duan, Chen & Li, 2020; Chaudhary et al., 2023]. Catechins have the ability to destroy cancer cells and reduce the risk of skin cancer, while EGCG, or epigallocatechin gallate, reduces the risk of developing cancer. Antioxidants also inhibit and delay the development of cardiovascular diseases [Singh, Shankar & Srivastava, 2011; Capasso et al., 2025]. Green tea extract may inhibit the oxidation of LDL

cholesterol, reducing coronary atherosclerosis. Green tea contains caffeine, a purine alkaloid, often referred to as theine in this raw material, which stimulates the central nervous system and the heart, improving psychophysical performance and reducing drowsiness and fatigue. The caffeine content of a green tea infusion may vary depending on the brewing time and water temperature [Tinahones et al., 2008; Suzuki-Sugihara et al., 2016]. According to EFSA, a 3–5 minute infusion of green tea provides 40–45 mg/100g of caffeine. In addition to caffeine, tea infusions also contain theophylline and theobromine, which dilate blood vessels, accelerate heart rate, and have a diuretic effect. Longer brewing increases the content of tannins and polyphenols, which have a calming and soothing effect [Alok et al., 2014; Bolognesi et al., 2017; Ayakdaş & Ağagündüz, 2025].

Herbs are another ingredient are often added to functional beverages. Herbs are rich in biologically active substances such as flavones, flavans, glycosides, anthocyanins, tannins, essential oils, alkaloids, bitter substances, mucilage, phenolic acids, phenols, diterpenes, and others [López-Romero et al., 2018; Parham et al., 2020; Mandal, Sarkar & Chakraborty, 2023; Skrovankova & Mlcek, 2025]. For example: flavones and flavans have diuretic, anti-inflammatory, anticancer, antiallergic, antiulcer, and antioxidant properties. Polyphenolic glycosides have antithrombotic, anti-inflammatory, analgesic, and antipyretic effects. Tannins have anti-inflammatory, astringent, antibacterial, and antidiarrheal properties [Ullah et al., 2020; Chagas et al., 2022; Mahmud et al., 2023.]. Essential oils exhibit calming, antibacterial, and diuretic properties, and stimulate intestinal peristalsis [Rezaie, Bitarafan, Horowitz & Feinle-Bisset, 2021; Matera, Lucchi & Valgimigli, 2023; Budriesi et al., 2024; Zuluaga, 2024]. Therefore, this study used teas (green, black and matcha) and herbs to create drinks that have calming properties, promote good sleep, stimulate and improve memory and concentration, and provide energy, thanks to the addition of protein, which increases glycogen stores. The aim of the study was to develop functional beverages based on herbal and tea extracts, as well as to evaluate their bioactive properties, nutritional value and effect on α -amylase and glucosidase inhibition. The research hypothesis assumes that by selecting the appropriate composition of a functional beverage, it is possible to obtain a drink with acceptable sensory characteristics, determining the minimum shelf life after storage under refrigerated conditions and specific health-promoting effects.

2. Materials and Methods

2.1. Chemicals

TPTZ, methanol, ethanol, iron(III) chloride, Folin-Ciocalteu reagent, tannic acid, water, potassium permanganate, sodium chloride, aluminum silicate hydroxide, formic acid, cyanide-3-glucoside chloride, 2,2-diphenyl-1-picrylhydrazyl, Folin-Ciocalteu reagent were purchased from Merck (Darmstadt, Germany).

2.2. Materials

Research materials used in this study included Lipton black tea (tea leaves, *Camellia sinensis*, Unilever, country of origin: Indonesia), Yunnan green tea (tea leaves, *Camellia sinensis*, Yunnan, country of origin: China), Biofix lemon balm (leaves, *Mentha*, country of origin: Poland), Biofix chamomile (chamomile flower, *Chamomillae anthodium*, country of origin: Poland), Kawon peppermint (leaves, *Mentha x piperita* L., country of origin: Poland), Astron Siberian ginseng (root, *Eleutherooccus senticosus*, country of origin: Siberia), Taheebo matcha (leaves, *Camellia sinensis pulvis*, country of origin: Japan), Farmvit ginkgo biloba (leaves, *Ginkgonis folium*, country of origin: Japan), oranges (*Citrus sinensis*, country of origin: Spain), apples (*Champion* apples, Poland), and whey protein isolate (per 100g fat 1g, carbohydrates 4g, fiber 0g, proteins 92g, salt 0g). The products were purchased from local stores and herbalists in Poland in 2025. A commercial product, Fuze Tea, a blueberry and lavender-flavored beverage with sugar-free green tea extract, was purchased as a store-bought beverage. This drink is sold commercially as an energy and calming drink, supporting memory and concentration.

Table 1
Recipes of the beverages.

Calming properties and ensuring good sleep							
Beverage variant	Black tea	Lemon balm	Chamomile	Apple juice	Orange Juice		
	[g]	[g]	[g]	[mL]	[mL]		
C1	2	2	2	50	-		
C2	2	2	2	-	50		
Stimulating/energizing properties							
Beverage variant	Green tea	Mint	Ginseng	Matcha	Apple juice	Orange Juice	
	[g]	[g]	[g]	[g]	[mL]	[mL]	
S1	2	1	2	2	50	-	
S2	2	1	2	2	-	50	
Improves memory and concentration							
Beverage variant	Green tea	Ginseng	Ginkgo boloba	Apple juice	Orange Juice		
	[g]	[g]	[g]	[mL]	[mL]		
I1	2	2	2	50	-		
I2	2	2	2	-	50		
Energizing with added proteic							
Beverage variant	Green tea	Mint	Ginseng	Matcha	Whey protein	Apple juice	Orange Juice
	[g]	[g]	[g]	[g]	[g]	[mL]	[mL]
P1	2	1	2	2	3	-	50

2.2.1. Beverages Preparation

Appropriate amounts of tea and herbs (Table 1) were mixed together and then poured with water (100 ml) at a temperature of 90°C for variants C1 and C2 (the temperature at which black tea is brewed) and 85°C for the other variants (the temperature at which green tea is brewed). After 30 minutes, the extracts were filtered through a sieve (size of the sieve – 53 mm). Appropriate amounts of cold water (100 ml) and juices (50 ml) were then added (water total 200 ml + 50 ml juice, total 250 ml). No sugar was added. The sugar they contain is of natural origin. The juice was made from apples and oranges using a juicer. 200g of apples yielded 100ml of juice, while 300g of oranges yielded 100ml of juice. Figure 1 shows sample drinks with orange juice.

2.2.2. Storing and preserving beverages

Beverages preserved by ultrafiltration (Synthware™ 47 mm filtration apparatus with fritted support and vacuum pump) were stored in a closed glass bottle (250 mL) in a refrigerator at 4°C in a dark place. Beverages that were pasteurized were stored in a closed bottle under refrigeration at 4°C in a dark place. Pasteurization was performed in a water bath at 85°C, and the closed beverage bottles were heated for 20 s after reaching 85°C. Ultrafiltration (Synthware™ 47 mm filtration apparatus with fritted) was performed using a flask, a ceramic filter and a vacuum pump – vacuum filtration. The beverages were stored until they became sensorially unsuitable for consumption. The beverages were stored in a refrigerator at 4°C and assessed organoleptically. A negative organoleptic evaluation indicated spoilage. Ultrafiltration and pasteurization of beverages were chosen as standard methods for extending the shelf life of clean label products.

2.3. Physicochemical parameters

2.3.1. pH

The pH of functional beverages was determined using a digital pH meter (Denver In-strument - Model 15 Meter, Denver, Colorado). The method is AOAC 973.41 compliant. The measurement was made by immersing the pH meter electrode in a beaker containing 50 mL of functional drink. Before testing, the meter was calibrated using standard calibration buffers (pH = 4 and 7) [Jia et al., 2024].

2.3.2. Determination of water content

Water content was determined using a moisture analyzer (MA 50R Moisture Analyzer, Radom, Poland)). 3 g of the drink was added to the device plate, it was dried automatically at 110°C and the water content was measured.

2.3.3. Determination of total fat content

A extraction thimbles (cellulose) containing approximately 1 g of freeze-dried (lyophilized 250 mL of the prepared drink, lyophilization was carried out for 24 hours, lyophilizer Christ Delta 1–24 LSC, (GmbH, Osterode am Harz, Germany) functional drink was prepared. The extraction thimbles was weighed and extracted (hexane) using an automatic Soxhlet extractor for 3 h (Buchi B-811 extraction system, Raypa, Barcelona, Spain). After fat extraction was completed, the sachets were placed in a desiccator for 30 min and then weighed. The result was converted to 100 g d.m. [Rayhan et al., 2019].

2.3.4. Determination of ash content

1 g of the lyophilized beverage was weighed into a crucible (ceramic, capacity 20 mL), and the crucible was weighed. The sample was fired in a muffle furnace, gradually increasing the temperature to 525°C, for 5 h. The sample was then placed in a desiccator for 1 h, after which the crucible was weighed [56].

2.3.5. Color parameters

Color parameters were measured using a Konica Minolta CR-400 colorimeter (Tokyo, Japan). The device measures color components in the CIE L*a*b* system. SpectraMagic™ NX software was used for the analysis. The color of the beverages was tested in five replicates [Lee, 2000].

2.3.6. Total dietary fiber

Total dietary fiber was determined using an enzymatic–gravimetric method according to AOAC procedure 991.43 and AACC 32–07 (Foss, Fibertec E – 1023 system, Hillerød, Denmark).

2.3.7. Protein content

Protein content analysis of the beverages was performed according to the standard Kjeldahl method AOAC 928.08 (Kjeldahl distillation system - KjelMaster K-375, BUCHI, Switzerland). The nitrogen conversion factor was 6.25.

2.3.8. Reducing sugar content according to Negrulescu

The beverages were diluted 100-fold and filtered. 1.5 ml of the filtrate was removed and 1.5 ml of 3,5-dinitrisalicylic acid was added. The samples were incubated for 10 min at 90°C and then cooled to room temperature. Absorbance was measured at 540 nm (absorbance was measured spectrophotometrically, SPECORD M40, Germany), and a blank sample was used to zero the instrument. Glucose (0.1–1.95 mg/ml) was used to prepare a calibration curve, and the result was expressed in g/100 ml of beverage [Tomczyk, Olesiuk & Dżugan, 2019].

2.3.9. Sensory evaluation

An organoleptic evaluation of the beverages was carried out to select the best variant of the tea and herb-based beverage. The beverages were evaluated freshly prepared, after ultrafiltration every two days of storage and after pasteurization after every week of storage. The beverages were stored in bottles in the refrigerator. The beverages were assessed for consumer acceptability by six sensory research specialists (six women, 35–56 years). The panelists were academic staff from the Faculty of Biotechnology and Food Sciences who were trained for 6 h using commercially available products. A five-point (hedonic) scale was adopted, where: 5 = Like extremely, 4 = Like moderately, 3 = Neither like nor dislike, 2 = Dislike moderately and 1 = Dislike extremely. Clarity, color, aroma, and taste were assessed. The raters' scores were summed and averaged. Informed consent was obtained from all subjects involved in the study.

2.4. Phytochemical analysis

2.4.1. Total tannin content

Beverage samples were filtered through filter paper (filter paper is made of cellulose, weight: 75 g/m²) before analysis. Indigo carmine (12.5 mL, 1 g indigo carmine in 100 ml distilled water, Chemland) and distilled water (375 mL) were added to 5 mL of the extract and 0.01 mg tannic acid. The mixture was stirred and titrated with KMnO₄ solution (0.02 N solution) until the color changed from blue to slightly pink. The control sample was prepared in the same way as the tested one, without the addition of the extract. The control sample was subtracted from the actual sample. A blank test was then performed to determine the content of non-tannin substances. For this purpose, 50 mL of the beverage was mixed with a solution of gelatin in saturated NaCl solution (25 mL), acidic NaCl solution (50 mL, 25 ml of concentrated sulfuric acid and 975 ml of saturated NaCl solution), and 5 g of kaolin. The mixture was mixed and shaken (15 min) and filtered. The procedure was then repeated as for the main test [Khasnabis, Rai & Roy, 2015].

2.4.2. Total anthocyanin content

Anthocyanin analysis was performed according to the method described by Kabilan et al. [Kabilan, Baskar & Poorami, 2019]. 1 mL of filtered extract and 8 mL of formic acid were added to the test tube (0.1-1 mg/mL). The contents of the test tube were mixed, and the absorbance was read at 530 nm (absorbance was measured spectrophotometrically, SPECORD M40, Germany). The anthocyanin content was calculated according to the formula.

$$\text{Anthocyanin content} = \frac{\text{Absorbance} \times \text{molecular weight of cyanide} - 3 - \text{glucoside chloride} \times \text{dilution factor} \times 1000}{\text{Molar absorbance} \times 1}$$

2.4.3. Total phenolic content

Total phenolic content (TPC) was determined by the Folin-Ciocalteu method as de-scribed by Xu et al. [Xu, Chen & Wang, 2019]. Distilled water (5 mL) was added to the test tube. Then 1 mL of 95% ethanol and 0.5 mL of Folin-Ciocalteu reagent (diluted with water in a 1:1, v:v ratio) were added. The contents of the tube were mixed and then 1 mL of filtered extract was added. The tube was closed, mixed and after 5 min 1 mL of 5% Na₂CO₃ was added and the contents of the tube were mixed again. Samples were incubated for 1 h in the dark. The tubes were mixed before measuring the absorbance at 760 nm (absorbance was measured spectrophotometrically, SPECORD M40, Germany). The results were expressed as gallic acid equivalents (GAE) (mg/100 g d.m.). The measuring cuvette was 1 cm.

2.5. Antioxidant potential

The antioxidant potential of the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay was checked using a modified method described by Chan et al. [Chan, Soh, Tie & Law, 2011]. The beverage extract 5 mL was diluted 20 times with 10% methanol and then filtered through filter paper. 3.9 mL of 70% methanol, 0.1 mL of the diluted and filtered beverage, and 1 mL of DPPH solution were added to the test tube. The tube was capped and mixed and the sample was poured into a cuvette. After mixing, the absorbance was measured at 517 nm every 5 min for 30 min (absorbance was measured spectrophotometrically, SPECORD M40, Germany). Measurements were also performed for a control sample containing 4 mL of methanol and 1 mL of DPPH solution (9.8 mg/ 50 mL). Antiradical activity was expressed as the percentage of quenched DPPH radicals.

2.6. Inhibition of α-amylase activities

Inhibition of α-amylase activities was performed according to the method described by Xu et al. [Xu et al., 2019]. The tested beverages were filtered and then 0.5 mL and 0.25 mL of 0.02 M sodium phosphate buffer at pH 6.9 were taken from the filtrate (the buffer contained α-amylase solution (13 U/mL) and NaCl (6 mM)). Samples were mixed and incubated (24°C, 10 min). Then, 2 mL of dinitrosalicylic acid was added to the samples and incubated for 5 min at 25°C. 14 mL of distilled water was added and the absorbance of the sample at 540 nm was measured against a blank. Blank and control tests were performed; in the blank test, the buffer was added instead of the enzyme, while in the control test, the buffer was added instead of the test sample extract. α-amylase inhibition was calculated using the formula: α-amylase (%) = ((absorbance of the control sample - absorbance of the actual sample)/absorbance of the control sample) * 100.

2.7. Inhibition of α-glucosidase activities

Inhibition of α-glucosidase activities was performed according to the method described by Xu et al. [Xu et al., 2019]. The spectrophotometric method using p-nitrophenyl-α-D-glucopyranoside (PNPG) is used to determine alpha-glucosidase activity. The enzyme breaks down PNPG into glucose and p-nitrophenol (PNP), which has an intense yellow color in an alkaline environment. The reaction was carried out in a buffer (e.g. phosphate) with a pH of approximately 6.8–7.0. 0.5 mL of filtered functional drink was mixed with 1 mL of phosphate buffer at pH 6.9 containing 100 U/mL of α-glucosidase. The mixture was mixed and incubated (25°C, 10 min), and then 1 mL of 0.1 mol/L NaOH was added to stop the reaction. The absorbance of the sample and control was measured at a wavelength of 405 nm. The α-glucosidase inhibitory activity was calculated from the formula: α-glucosidase (%) = (1 – (absorbance of the actual sample/absorbance of the control sample)) * 100.

2.8. Analysis of vitamin B₂ and B₉

The beverages were lyophilized before analysis. 2 mg of the lyophilizate was dissolved in 10 mL of 0.1% (v/v) formic acid in water and filtered through a 0.2 µm, 17 mm nylon filter. Analysis was performed by UHPLC-ESI-MS using a UHPLC chromatograph with an ESI-MS detector (Shimadzu, Kyoto, Japan). 2 µL samples were then injected onto a Kinetex C18 column (2.1 × 150 mm, 5 µm, Thermo Scientific). The ESI source was operated in positive ion mode. The mobile phases were eluent A, acetonitrile/formic acid (99.9/0.1, v/v), and eluent B plus water. The flow rate was 0.2 mL/min. The gradient was 0–5.00 min, 0–20% B, 5.00–5.20 min, 20–100%; 20–6.20 min, 100–100% B; 6.20–6.40 min, 100–0% B; 6.40–10.00 min, 0%. The column temperature was 35°C, the drying gas temperature was 250°C, and the capillary temperature was 400°C. Full mass spectra were acquired in the mass range from m/z 50 to 600 in positive ion mode. The obtained MS spectrum signal data were acquired and processed using LabSolutions software. A standard curve of the vitamins riboflavin (B₂) and folic acid (B₉) was prepared

simultaneously. Calibration curves were constructed for standard compounds using six different concentration levels in the range of 0.01–1.0 mg/ml [Grzelczyk, Gałązka-Czarnecka, Drożdżyński & Oracz, 2025].

2.9. Analysis of vitamin C

The beverages were lyophilized before analysis. 2 mg of the lyophilizate was dissolved in 10 ml of 0.1% (v/v) formic acid in water and filtered through a 0.2 µm, 17 mm nylon filter. Analysis was performed by UHPLC-ESI-MS using a UHPLC chromatograph with an ESI-MS detector (Shimadzu, Kyoto, Japan). 2 µL samples were then injected onto a Kinetex C18 column (2.1 × 150 mm, 5 µM, Thermo Scientific). The mobile phases were eluent A, methanol/formic acid (99.9/0.1, v/v), and eluent B, water/formic acid (99.9/0.1, v/v). The flow rate was 0.2 mL/min and the gradient was 0–5.00 min, 30–100% B, 5.00–5.01 min, 100% B, 5.01–7.00 min, 100–30% B. ESI source operated in negative ion mode, drying gas temperature—250°C, capillary temperature—350°C. The column temperature was 30°C. Full mass spectra were obtained in the m/z range from 50 to 350 in positive ion mode. Calibration curves were made using standard compound standards using six different concentration levels ranging from 0.03–1.00 mg/mL [Grzelczyk et al., 2025].

3.0. Analysis of vitamin E

Prepare samples as in the point above. 10 µL samples were injected. The mobile phases were eluent A, water/formic acid (99.9/0.1, v/v), and eluent B, methanol/formic acid (99.9/0.1, v/v) The flow rate was 0.2 mL/min and the gradient was 0–1.00 min, 0% B, 1.00–6.01 min, 0–100% B, 6.01–25.00 min, 100–0% B. ESI source operated in negative ion mode, capillary temperature—350°C, column temperature was 30°C. Full mass spectra were obtained in the m/z range from 50 to 500 in positive ion mode. Calibration curves were made using standard compound standards using six different concentration levels ranging from 0.05–1.00 mg/mL. [Grzelczyk et al., 2025].

3.1. Statistical Analysis

The analyzed beverages were prepared four times. Parametric data were analyzed using one-way analysis of variance (ANOVA) and correlation analysis. Data are expressed as mean ± SD. Tukey's HSD post-hoc test was performed to identify differences between means. Significant differences were identified at $p < 0.05$. Correlation was calculated as Pearson's correlation coefficient. All tests were performed using Statistica 10.0 (StatSoft, Inc., Tulsa, USA).

4. Result

4.1. Physicochemical parameters

4.1.1. Nutritional value

Functional beverages also serve to hydrate the body, so the beverages developed consisted mainly of water. This is confirmed by their high water content (Table 2).

Table 2
Water content, total fat, ash, and pH of formulated beverages.

Beverage variant	Water content [%]	Fat content [g/100g]	Ash content [g/100g]	pH	Protein [g/100 ml]	Fiber [g/100 ml]	Reducing sugars [g/100 ml]
Store-bought beverage	97.20 ± 0.55 ^a	0.01 ± 0.00 ^a	0.34 ± 0.05 ^a	5.69 ± 0.05 ^a	0.00 ± 0.00 ^a	0.00 ± 0.00 ^a	3.36 ± 0.15 ^a
C1	90.21 ± 1.18 ^b	0.01 ± 0.00 ^a	1.54 ± 0.45 ^b	4.26 ± 0.02 ^b	0.50 ± 0.01 ^b	0.03 ± 0.01 ^b	2.52 ± 0.01 ^b
C2	92.22 ± 2.58 ^c	0.02 ± 0.00 ^b	1.31 ± 0.05 ^c	4.96 ± 0.02 ^c	0.45 ± 0.01 ^c	0.05 ± 0.01 ^c	1.13 ± 0.01 ^c
S1	90.95 ± 2.55 ^b	0.01 ± 0.00 ^a	1.58 ± 0.19 ^b	4.24 ± 0.03 ^b	0.48 ± 0.01 ^c	0.08 ± 0.01 ^d	2.59 ± 0.01 ^b
S2	90.55 ± 3.54 ^b	0.01 ± 0.00 ^a	1.52 ± 0.45 ^b	5.03 ± 0.02 ^d	0.43 ± 0.03 ^c	0.10 ± 0.03 ^e	1.09 ± 0.03 ^c
I1	91.18 ± 1.15 ^d	0.02 ± 0.00 ^b	1.25 ± 0.25 ^d	4.16 ± 0.03 ^b	0.48 ± 0.01 ^c	0.05 ± 0.01 ^c	2.32 ± 0.01 ^d
I2	89.21 ± 2.98 ^e	0.01 ± 0.00 ^a	1.74 ± 0.49 ^e	4.81 ± 0.01 ^c	0.42 ± 0.01 ^c	0.03 ± 0.01 ^b	1.08 ± 0.01 ^c
P1	88.34 ± 1.15 ^f	0.08 ± 0.01 ^c	1.87 ± 0.55 ^f	4.79 ± 0.01 ^c	1.59 ± 0.02 ^d	0.08 ± 0.02 ^d	2.89 ± 0.02 ^e

Different letters ^{a-f} within columns indicate statistically significant differences between variants ($p < 0.05$). Values are expressed as mean ± SD; $n = 4$.

The store-bought beverage had the highest water content (97.20%), followed by drink C2 (92.22%). The lowest water content was found in drink P1 (88.34%), which is related to the addition of whey protein. The higher the water content in the product, the lower the ash content ($r = -0.98$, $p \leq 0.36$). Ash content ranged from 0.34 to 1.87 g/100g. Variant P1 had the highest ash content, while the store-bought beverage had the lowest (Table 2). Variant P1 contained whey protein, ginseng, green tea, orange juice, matcha, and mint. It was also observed that the apple juice variant of the drink had a higher ash content than the orange juice variant, which may indicate a higher mineral content in apple juice in the tested varieties (Table 2). The store-bought beverage contained 3 to 5 times less ash than the designed beverages. This suggests that the developed recipes contain a higher content of mineral compounds than in the commercial drink.

The fat content in most beverages was low (0.01–0.02 g/100 g). Only variant P1 showed a slightly higher fat content (0.08 g/100 g), which may be related to the addition of whey protein (Table 2). However, this is not a high fat content; P1 drink still belongs to the group of low-fat products. In the case of the store-bought beverage, the fat content was the same as in the designed variants of drinks C1, S1, S2 and I2.

The pH values of teas and herbal beverages ranged from 4.16 to 5.03. The lowest values were recorded in variants C1, S1 and P1 due to the addition of orange juice, which lowered the pH of the drink. The highest pH was found in beverage S2, which contained apple juice (Table 2).

Protein content was in the range of 0–1.59 [g/100 mL], for the store-bought beverage and P1 (added protein content), respectively. Apple juice drinks had higher protein content. However, the dietary fiber content was higher in products containing orange juice. Fibre content was 0–0.10 g/100 ml for store-bought drink and S2. The highest content of reducing sugars was found in the drink purchased in the store – 3.36 g/100 ml, and the lowest in the I2 version – 1.08 g/100 ml. The lowest content of reducing sugars was found in orange juice drinks.

Fresh beverages without the use of preservatives are suitable for consumption within one day. Therefore, one of the preservation methods used was ultrafiltration [Hu & Wu, 2023]. After ultrafiltration, the beverages were stored in a refrigerator at 4°C. A study was conducted to determine whether ultrafiltration would positively impact the quality and storage stability of the developed beverages. The results are presented in Table 3.

Table 3
Water content, total fat content and pH for ultrafiltered beverages.

Beverage variant	Water content [%]	Fat content [g/100g]	Ash content [g/100g]	pH	Protein [g/100 ml]	Fiber [g/100 ml]	Reducing sugars [g/100 ml]
Ultrafiltered – “0” day							
C1	98.91 ± 0.02 ^a	0.01 ± 0.00 ^a	0.83 ± 0.11 ^a	4.94 ± 0.01 ^a	0.05 ± 0.01 ^a	0.00 ± 0.00 ^a	2.44 ± 0.09 ^a
C2	97.39 ± 0.01 ^b	0.02 ± 0.00 ^b	0.58 ± 0.04 ^b	5.27 ± 0.03 ^b	0.01 ± 0.01 ^b	0.01 ± 0.01 ^b	1.10 ± 0.05 ^b
S1	98.85 ± 0.03 ^a	0.01 ± 0.00 ^a	0.91 ± 0.15 ^c	4.79 ± 0.02 ^a	0.05 ± 0.01 ^a	0.02 ± 0.01 ^c	2.52 ± 0.08 ^c
S2	97.39 ± 0.01 ^b	0.02 ± 0.00 ^b	0.58 ± 0.04 ^b	5.27 ± 0.03 ^b	0.01 ± 0.01 ^b	0.03 ± 0.01 ^d	1.05 ± 0.10 ^d
I1	97.77 ± 0.02 ^b	0.02 ± 0.00 ^b	0.56 ± 0.09 ^b	3.98 ± 0.02 ^c	0.05 ± 0.01 ^a	0.01 ± 0.01 ^b	2.28 ± 0.15 ^e
I2	98.41 ± 0.02 ^a	0.01 ± 0.00 ^a	0.63 ± 0.03 ^d	5.01 ± 0.02 ^b	0.01 ± 0.01 ^b	0.00 ± 0.00 ^a	1.03 ± 0.09 ^d
P1	99.22 ± 0.02 ^c	0.08 ± 0.01 ^c	0.78 ± 0.10 ^e	6.50 ± 0.02 ^d	0.78 ± 0.05 ^c	0.00 ± 0.00 ^a	2.55 ± 0.24 ^c
Ultrafiltered – 2 days							
C1	98.90 ± 0.02 ^a	0.01 ± 0.00 ^a	0.82 ± 0.03 ^a	4.32 ± 0.03 ^a	0.05 ± 0.01 ^a	0.05 ± 0.01 ^a	2.42 ± 0.05 ^a
C2	97.34 ± 0.03 ^b	0.02 ± 0.00 ^b	0.57 ± 0.05 ^b	4.89 ± 0.02 ^a	0.01 ± 0.01 ^b	0.01 ± 0.01 ^b	1.08 ± 0.08 ^b
S1	97.75 ± 0.02 ^b	0.02 ± 0.00 ^b	0.55 ± 0.02 ^b	4.49 ± 0.01 ^a	0.05 ± 0.01 ^a	0.02 ± 0.01 ^c	2.51 ± 0.01 ^c
S2	98.41 ± 0.02 ^a	0.01 ± 0.00 ^a	0.63 ± 0.03 ^d	5.01 ± 0.02 ^b	0.01 ± 0.01 ^b	0.03 ± 0.01 ^d	1.04 ± 0.05 ^d
I1	97.75 ± 0.02 ^b	0.02 ± 0.00 ^b	0.55 ± 0.02 ^b	4.49 ± 0.01 ^a	0.05 ± 0.01 ^a	0.01 ± 0.01 ^b	2.25 ± 0.03 ^e
I2	98.31 ± 0.02 ^a	0.01 ± 0.00 ^a	0.69 ± 0.04 ^d	4.82 ± 0.02 ^a	0.01 ± 0.01 ^b	0.00 ± 0.00 ^a	1.01 ± 0.05 ^d
P1	99.20 ± 0.02 ^c	0.08 ± 0.02 ^c	0.78 ± 0.09 ^e	5.05 ± 0.01 ^b	0.78 ± 0.04 ^c	0.00 ± 0.00 ^a	2.53 ± 0.01 ^c
Ultrafiltered – 4 days							
C1	91.42 ± 0.05 ^d	0.01 ± 0.00 ^a	0.81 ± 0.05 ^a	4.62 ± 0.01 ^a	0.03 ± 0.01 ^d	0.05 ± 0.01 ^a	2.41 ± 0.25 ^a
C2	97.02 ± 0.01 ^b	0.02 ± 0.00 ^b	0.56 ± 0.09 ^b	4.69 ± 0.02 ^a	0.00 ± 0.01 ^e	0.01 ± 0.01 ^b	1.07 ± 0.18 ^b
S1	97.98 ± 0.15 ^b	0.01 ± 0.00 ^a	0.90 ± 0.03 ^c	4.62 ± 0.03 ^a	0.03 ± 0.01 ^d	0.02 ± 0.01 ^c	2.50 ± 0.02 ^c
S2	90.11 ± 0.02 ^e	0.01 ± 0.00 ^a	0.61 ± 0.15 ^d	5.04 ± 0.03 ^b	0.00 ± 0.01 ^e	0.03 ± 0.01 ^d	1.03 ± 0.09 ^d
I1	93.75 ± 0.10 ^f	0.02 ± 0.00 ^b	0.55 ± 0.04 ^b	4.57 ± 0.02 ^a	0.03 ± 0.01 ^d	0.01 ± 0.01 ^b	2.24 ± 0.12 ^e
I2	97.99 ± 0.05 ^b	0.01 ± 0.00 ^a	0.67 ± 0.15 ^d	5.01 ± 0.01 ^b	0.00 ± 0.01 ^e	0.00 ± 0.00 ^a	1.00 ± 0.09 ^d

Beverage variant	Water content [%]	Fat content [g/100g]	Ash content [g/100g]	pH	Protein [g/100 ml]	Fiber [g/100 ml]	Reducing sugars [g/100 ml]
P1	93.45 ± 0.07 ^f	0.07 ± 0.01 ^d	0.75 ± 0.10 ^e	5.08 ± 0.02 ^b	0.72 ± 0.05 ^c	0.00 ± 0.00 ^a	2.53 ± 0.07 ^c
Ultrafiltered – 7 days							
C2	96.85 ± 0.01 ^g	0.02 ± 0.00 ^b	0.54 ± 0.02 ^b	4.92 ± 0.01 ^a	0.00 ± 0.01 ^e	0.01 ± 0.01 ^b	1.03 ± 0.18 ^d
S1	96.85 ± 0.01 ^g	0.02 ± 0.00 ^b	0.54 ± 0.02 ^b	4.92 ± 0.01 ^a	0.03 ± 0.01 ^d	0.02 ± 0.01 ^c	2.48 ± 0.02 ^a
S2	96.11 ± 0.05 ^g	0.01 ± 0.00 ^a	0.61 ± 0.03 ^d	4.99 ± 0.02 ^a	0.00 ± 0.01 ^e	0.03 ± 0.01 ^d	1.00 ± 0.09 ^d
Ultrafiltered – 9 days							
C2	96.03 ± 0.09 ^g	0.02 ± 0.00 ^b	0.52 ± 0.01 ^b	5.25 ± 0.02 ^b	0.00 ± 0.01 ^e	0.01 ± 0.01 ^b	1.02 ± 0.05 ^d

Different letters a-j within columns indicate statistically significant differences between variants ($p < 0.05$). Values are expressed as mean ± SD; $n = 4$.

The ultrafiltration process increased the water content by separating tea sediment, fruit particles and protein from the beverage. The water content remained similar during storage due to storage in tightly closed bottles. The water content ranged from 90.11% (S2, stored for 4 days) to 99.22% (P1, fresh drink after ultrafiltration) (Table 3). However, ultra-filtration did not affect the fat content of the samples. All ultrafiltrated variants showed lower ash content compared to non-ultrafiltrated samples. It indicates that a part of minerals was linked to the material separated under ultrafiltration. No changes in ash content were observed during storage. The fat content did not change after ultrafiltration. During storage it ranged from 0.01 to 0.07 g/100 g. Ultrafiltration increased the pH in variants C1, S1, C2, I2 and P1, while it decreased the pH in variants S2 and I1. An increase in pH may be caused, for example, by coagulation of infusion ingredients such as protein or pectin. An increase in pH was observed in apple juice drinks (Table 3). The greatest increase in pH was observed for variant P1, which included orange juice and whey protein. The increase or decrease in pH of the developed beverages with storage affected the taste and aroma of the beverages. Lowering the pH of the beverages resulted in a more sour taste. The ultrafiltration process reduced the content of fiber and protein, but had a small effect on reducing sugars (Table 3).

The second treatment used to preserve beverages was pasteurization. The pasteurized products were stored under refrigerated conditions after cooling. The analysis results are presented in Table 4.

Table 4
Water content, total fat content and pH for pasteurization beverages.

Beverage variant	Water content [%]	Fat content [g/100g]	Ash content [g/100g]	pH	Protein [g/100 ml]	Fiber [g/100 ml]	Reducing sugars [g/100 ml]
Pasteurization – “0” day							
C1	90.02 ± 0.03 ^a	0.01 ± 0.00 ^a	1.54 ± 0.45 ^a	4.77 ± 0.01 ^a	0.50 ± 0.15 ^a	0.03 ± 0.01 ^a	2.51 ± 0.05 ^a
C2	92.02 ± 0.03 ^b	0.02 ± 0.00 ^b	1.33 ± 0.15 ^b	5.08 ± 0.01 ^b	0.45 ± 0.05 ^b	0.05 ± 0.01 ^b	1.12 ± 0.05 ^b
S1	90.83 ± 0.02 ^a	0.01 ± 0.00 ^a	1.58 ± 0.25 ^a	5.04 ± 0.02 ^b	0.48 ± 0.05 ^b	0.08 ± 0.02 ^c	2.59 ± 0.03 ^a
S2	90.15 ± 0.06 ^a	0.01 ± 0.00 ^a	1.52 ± 0.10 ^a	4.76 ± 0.02 ^a	0.43 ± 0.06 ^b	0.10 ± 0.05 ^d	1.08 ± 0.05 ^b
I1	91.02 ± 0.04 ^c	0.02 ± 0.01 ^b	1.25 ± 0.28 ^c	4.74 ± 0.01 ^a	0.48 ± 0.02 ^b	0.05 ± 0.02 ^b	2.31 ± 0.03 ^c
I2	89.07 ± 0.04 ^d	0.01 ± 0.00 ^a	1.73 ± 0.39 ^d	4.88 ± 0.01 ^c	0.42 ± 0.02 ^b	0.03 ± 0.02 ^a	1.07 ± 0.05 ^b
P1	88.05 ± 0.03 ^e	0.07 ± 0.02 ^c	1.86 ± 0.34 ^e	5.25 ± 0.01 ^d	1.59 ± 0.05 ^c	0.08 ± 0.03 ^c	2.85 ± 0.15 ^d
Pasteurization – 1 week							
C1	90.02 ± 0.02 ^a	0.01 ± 0.00 ^a	1.52 ± 0.33 ^a	4.76 ± 0.02 ^a	0.50 ± 0.05 ^a	0.03 ± 0.01 ^a	2.49 ± 0.09 ^a
C2	92.02 ± 0.02 ^b	0.02 ± 0.01 ^b	1.30 ± 0.10 ^b	5.08 ± 0.02 ^b	0.45 ± 0.07 ^b	0.05 ± 0.01 ^b	1.10 ± 0.15 ^b
S1	90.81 ± 0.03 ^a	0.01 ± 0.00 ^a	1.57 ± 0.18 ^a	5.03 ± 0.02 ^b	0.48 ± 0.01 ^b	0.08 ± 0.03 ^c	2.54 ± 0.10 ^a
S2	92.02 ± 0.02 ^b	0.02 ± 0.01 ^b	1.30 ± 0.10 ^b	5.08 ± 0.02 ^b	0.43 ± 0.02 ^b	0.10 ± 0.03 ^d	1.05 ± 0.05 ^b
I1	90.14 ± 0.03 ^a	0.01 ± 0.00 ^a	1.50 ± 0.09 ^a	4.77 ± 0.02 ^a	0.48 ± 0.03 ^b	0.05 ± 0.01 ^b	2.30 ± 0.04 ^c
I2	89.06 ± 0.03 ^d	0.01 ± 0.00 ^a	1.71 ± 0.15 ^d	4.88 ± 0.02 ^c	0.42 ± 0.03 ^b	0.03 ± 0.01 ^a	1.05 ± 0.05 ^b
P1	88.01 ± 0.05 ^e	0.08 ± 0.02 ^d	1.85 ± 0.18 ^e	5.25 ± 0.01 ^d	1.59 ± 0.15 ^c	0.08 ± 0.02 ^c	2.80 ± 0.09 ^d
Pasteurization – 2 week							
C2	90.85 ± 0.02 ^a	0.02 ± 0.01 ^b	1.22 ± 0.39 ^c	5.11 ± 0.01 ^b	0.44 ± 0.05 ^b	0.04 ± 0.01 ^b	1.05 ± 0.09 ^b
S1	89.99 ± 0.02 ^d	0.01 ± 0.00 ^a	1.55 ± 0.44 ^a	5.07 ± 0.02 ^b	0.46 ± 0.03 ^b	0.07 ± 0.03 ^c	2.51 ± 0.03 ^a
S2	88.35 ± 0.05 ^e	0.01 ± 0.00 ^a	1.45 ± 0.25 ^f	4.58 ± 0.01 ^e	0.41 ± 0.05 ^b	0.09 ± 0.03 ^c	1.03 ± 0.02 ^b
I2	88.46 ± 0.03 ^e	0.01 ± 0.00 ^a	1.66 ± 0.05 ^g	4.79 ± 0.01 ^f	0.41 ± 0.09 ^b	0.02 ± 0.01 ^a	1.02 ± 0.07 ^b
Pasteurization – 3 week							
S1	88.15 ± 0.13 ^e	0.01 ± 0.00 ^a	1.49 ± 0.55 ^f	4.97 ± 0.01 ^g	0.40 ± 0.05 ^b	0.06 ± 0.01 ^c	2.12 ± 0.05 ^e

Different letters a-g within columns indicate statistically significant differences between variants ($p < 0.05$). Values are expressed as mean $\pm$ SD; n = 4.

The pasteurization process did not change the water content compared to unprocessed, freshly prepared beverages. It also did not affect the fat content (Tables 2, 3, and 4). However, the ash content decreased slightly during storage. The pasteurization process caused an initial increase in the pH of the resulting beverages, which may have been related to the increased temperature of the beverages. Only in variant S2 the pasteurization process caused a decrease in pH, which may be due to the addition of matcha and apple juice. The beverages soured during storage, except for variant C2. The pasteurization process reduces the fiber, protein and sugar content.

4.1.2. Color parameters

The beverages varied in color, which resulted from the addition of different base ingredients. Beverages C1, S1 and I1 contained orange juice, which lowered the solution pH below 5 and made it more acidic (Table 1). Anthocyanins in an acidic environment take on a pink-red color, which confirms the higher proportion of yellow-red color in these drinks and their greater brightness. In turn, beverages C2, S2 and I2 had a pH above 5 due to the addition of apple juice, tending towards a greenish-yellow color. Drink no. P1 was characterized by medium brightness and a greenish-yellow color. Even though it contained orange juice, the addition of whey protein increased the product's acidity and greener tones (Table 5).

Table 5
Color parameters.

Beverage variant	L*	a*	b*
Fresh beverage			
C1	52.33 ± 4.89 ^a	7.68 ± 1.06 ^a	43.30 ± 2.79 ^a
C2	78.39 ± 5.19 ^b	0.41 ± 1.83 ^b	40.46 ± 5.29 ^a
S1	46.63 ± 10.59 ^c	-2.55 ± 0.85 ^c	33.40 ± 5.57 ^b
S2	44.99 ± 4.55 ^c	-3.30 ± 0.58 ^d	35.04 ± 2.61 ^b
I1	66.55 ± 3.62 ^d	0.68 ± 0.58 ^b	35.01 ± 1.50 ^b
I2	81.54 ± 2.22 ^e	-1.72 ± 0.05 ^e	15.21 ± 1.30 ^c
P1	40.15 ± 2.56 ^c	-3.36 ± 0.53 ^d	26.03 ± 3.63 ^d
Ultrafiltered – “0” day			
C1	71.76 ± 1.58 ^b	-3.45 ± 0.23 ^d	7.03 ± 2.18 ^e
C2	81.03 ± 4.38 ^e	-0.49 ± 0.88 ^f	31.02 ± 5.44 ^b
S1	70.28 ± 0.17 ^b	-3.63 ± 0.01 ^d	8.04 ± 0.13 ^e
S2	72.44 ± 0.57 ^b	-3.71 ± 0.12 ^d	8.83 ± 0.33 ^e
I1	91.43 ± 1.56 ^f	-0.62 ± 0.12 ^f	5.69 ± 0.59 ^e
I2	72.70 ± 1.03 ^b	-3.18 ± 0.04 ^d	7.60 ± 1.37 ^e
P1	86.65 ± 1.09 ^e	-1.56 ± 0.09 ^e	11.36 ± 1.03 ^c
Ultrafiltered – 2 days			
C1	69.72 ± 2.43 ^d	-3.74 ± 0.28 ^d	9.65 ± 2.06 ^e
C2	82.07 ± 5.42 ^e	-3.82 ± 0.01 ^d	20.09 ± 3.54 ^d
S1	86.56 ± 2.46 ^e	-3.56 ± 0.18 ^d	14.77 ± 1.55 ^c
S2	75.19 ± 1.02 ^b	-0.58 ± 0.31 ^f	38.71 ± 0.95 ^b
I1	84.26 ± 5.15 ^e	-3.28 ± 0.15 ^d	15.38 ± 2.98 ^c
I2	83.67 ± 2.08 ^e	-3.19 ± 0.07 ^d	16.01 ± 1.10 ^c
P1	73.82 ± 2.41 ^b	-3.36 ± 0.08 ^d	19.39 ± 1.95 ^c
Ultrafiltered – 4 days			
C1	82.59 ± 1.55 ^e	-2.55 ± 0.63 ^c	14.84 ± 1.07 ^c
C2	82.29 ± 3.68 ^e	-1.86 ± 0.08 ^e	13.63 ± 2.02 ^c
S1	80.44 ± 4.24 ^e	-2.38 ± 0.13 ^c	15.07 ± 2.04 ^c
S2	76.62 ± 0.88 ^b	-2.33 ± 0.11 ^c	23.05 ± 0.66 ^d
I1	82.29 ± 3.68 ^e	-1.86 ± 0.08 ^e	13.63 ± 2.02 ^c
I2	81.92 ± 3.15 ^e	-1.72 ± 0.04 ^e	15.18 ± 1.87 ^c
P1	81.96 ± 0.11 ^e	-1.96 ± 0.31 ^e	13.08 ± 3.01 ^c

Beverage variant	L*	a*	b*
Ultrafiltered – 7 days			
C2	71.40 ± 1.35 ^b	1.95 ± 0.09 ^g	38.83 ± 0.03 ^b
S1	85.40 ± 0.87 ^e	-2.23 ± 0.04 ^c	12.89 ± 0.78 ^c
I2	81.43 ± 3.25 ^e	-1.74 ± 0.01 ^e	16.43 ± 1.80 ^c
Ultrafiltered – 9 days			
C2	74.32 ± 2.96 ^b	2.26 ± 0.90 ^h	38.53 ± 1.99 ^b
Pasteurization – “0” day			
C1	40.44 ± 2.05 ^c	3.15 ± 0.54 ⁱ	26.35 ± 5.22 ^d
C2	33.12 ± 3.33 ^g	-0.82 ± 0.45 ^f	18.06 ± 1.94 ^c
S1	52.01 ± 1.08 ^a	-1.05 ± 0.23 ^e	23.61 ± 5.75 ^d
S2	56.11 ± 2.12 ^a	-4.65 ± 0.85 ^j	20.01 ± 2.05 ^d
I1	36.02 ± 1.26 ^g	-2.45 ± 0.36 ^c	18.55 ± 1.44 ^c
I2	60.23 ± 2.35 ^d	-2.31 ± 0.46 ^c	17.42 ± 2.21 ^c
P1	60.03 ± 1.66 ^d	-4.64 ± 0.13 ^j	29.42 ± 4.25 ^d
Pasteurization – 1 week			
C1	40.76 ± 3.09 ^c	3.01 ± 1.49 ⁱ	26.67 ± 7.93 ^d
C2	33.42 ± 5.03 ^g	-0.71 ± 0.85 ^f	18.04 ± 6.80 ^c
S1	52.06 ± 4.07 ^a	-0.98 ± 0.52 ^f	23.68 ± 6.78 ^d
S2	56.52 ± 1.25 ^a	-4.54 ± 0.19 ^j	20.00 ± 1.05 ^d
I1	36.11 ± 3.96 ^g	-2.36 ± 0.06 ^c	18.54 ± 2.09 ^c
I2	60.37 ± 1.09 ^d	-2.23 ± 0.47 ^c	17.47 ± 2.21 ^c
P1	60.84 ± 10.78 ^d	-4.53 ± 0.44 ^j	29.49 ± 4.81 ^d
Pasteurization – 2 week			
C2	31.80 ± 0.52 ^g	-0.29 ± 0.14 ^f	21.69 ± 0.49 ^d
S1	58.33 ± 5.22 ^a	9.88 ± 2.46 ^k	46.87 ± 0.26 ^a
S2	58.61 ± 9.35 ^a	1.50 ± 1.41 ^g	34.16 ± 0.75 ^b
I2	87.05 ± 1.21 ^e	-1.34 ± 0.06 ^e	15.96 ± 1.56 ^c
Pasteurization – 3 week			
S1	51.56 ± 1.09 ^a	13.05 ± 0.64 ^l	47.48 ± 0.46 ^a

Different letters a-l within columns indicate statistically significant differences between variants ($p < 0.05$). Values are expressed as mean ± SD; n = 5.

Among all samples, variant P1 was characterized by the lowest brightness (40.15) and variant I2 was the highest (81.54). The highest value of red color was shown by variant C1 (7.68 ± 1.06), the highest value of green color – variant P1 (-3.36 ± 0.53), and the highest value of yellow color – variant C1 (43.30 ± 2.79). None of the samples showed blue color contribution (Table 5).

The ultrafiltration process resulted in an increase in the brightness of the beverages and an increase in the share of green-yellow colors. Among all samples subjected to ultrafiltration, variant S1 was characterized by the lowest brightness (70.28) and variant I1 was the highest (91.43). Variant S2 was characterized by the highest green color value (-3.71), and variant C2 by the highest yellow color value (31.02). None of the samples showed any blue or red color (Table 5). After 2 days of storage of ultrafiltered beverages, variant C1 showed the lowest brightness (69.72), while variant S1 showed the highest brightness (86.56). Variant S2 showed the highest green content (-3.82), while variant C2 showed the highest yellow content (38.71). After 4 days of storage of ultrafiltered beverages, variant C2 showed the lowest brightness (70.06), while variant C1 showed the highest brightness (82.59). Variant C1 showed the highest green content (-2.55), while variant C2 showed the highest yellow content (41.32). Sample C2 showed a red colorants content (2.88). After 7 days of storage of ultrafiltered beverages, only variants 2, 4 and 6 remained (the remaining variants showed signs of spoilage). Variant C2 continued to have the low-brightness (71.40), while variant S1 showed the highest brightness (85.40). Sample C2 showed a red color share (1.95). Variant S1 showed the highest green color share (-2.23), while variant C2 showed the highest yellow color share (38.83). After 9 days of storing the ultrafiltered beverages, only variant no. 4 remained, with high brightness and a red-yellow color share.

After 1 week of storage, beverage C1 showed moderate brightness and reddish-yellow color (Table 5). Beverages S1, S2, C1, I1, I2 and P1 were characterized by moderate brightness and greenish-yellow color. Of all pasteurized samples, after 1 week of storage, variant C2 had the lowest brightness (33.42) and variant P1 had the highest (60.84). Only variant C1 showed red color (3.01), variant S2 had the highest green color value (-4.54) and variant P1 had the highest yellow color value (29.49). After 2 weeks of storage, variant C2 still showed the lowest brightness (31.80) and variant I2 had the highest brightness (87.05). Variant S1 showed the highest red dyes content (9.88), while variant I2 showed the highest green dyes content (1.34) and variant S1 showed the highest yellow colorants content (46.87). After 3 weeks of storage, only the S1 variety remained, with medium brightness and red-yellow color dyes content.

4.1.3. Sensory evaluation

A sensory evaluation of the beverages was carried out to check their acceptability. The results of the fresh beverage evaluation are presented in supplementary materials S1.

Freshly prepared drinks received high organoleptic scores. Variant C2 received the maximum score of 20 points. Variant P1 received the lowest score (13.1 points). Beverages C2, S2, and I2 were sweeter than the others due to the natural addition of apple juice. Variant P1 had the lowest clarity due to the addition of protein, which caused sediment in the drink, which also negatively affected its taste. Beverages S1 and I1 also received slightly lower scores due to matcha tea residue. The aroma and taste depended on the added herbs. Variants C1 and C2 were characterized by an intense chamomile flavor and aroma, while variants S1, I1 and P1 were dominated by mint aroma and flavor, and variants S2 and I2 had a very delicate aroma and flavor due to the lack of intense herbs. The beverages were then subjected to organoleptic evaluation after ultrafiltration and during storage. The results are shown in supplementary materials S2.

After ultrafiltration, the C2 drink received the highest ratings throughout the entire storage period and was suitable for consumption for the longest period. Signs of spoilage appeared after 9 days. Beverage P1 received the lowest ratings, and began to sour on the 4th day of storage, indicating the beginning of spoilage. Variant C1, I1, and S2 also showed signs of spoilage – an unpleasant sour taste after the 4th day of storage, while variants S2 and I2 showed signs of spoilage on the 7th day of storage. The ultrafiltration process removed solid particles and sediment from the beverages, improving their clarity. However, the color of ultrafiltered beverages was less intense compared to freshly prepared beverages. The beverages had a more delicate taste and aroma than the freshly prepared ones, but consumer acceptance was still high. All ultrafiltered beverages received higher ratings in the apple juice flavor. Figure S3 shows the consumer acceptability results after beverage pasteurization and during storage.

In the first week of storage, variant 4 received the highest score (20 points) and variant 7 the lowest (8.3 points). In the second week of storage, variant 2 received the highest score (17.5 points) and variant 5 the lowest (12.3 points). Only variant 2 was evaluated in the third week of storage, as the others deteriorated after the first or second week. Pasteurization did not affect the clarity or color of the beverages. The pasteurization process has reduced consumer acceptance of beverages. All types of beverages received higher scores after pasteurization in the apple juice variant.

4.2. Phytochemical analysis

4.2.1. Total phenolic compounds, tannins and anthocyanin content

Phytochemicals are among the most important compounds produced by plants. They include polyphenols and among them tannins and anthocyanins. Table 6 shows the content of total polyphenols, tannins and anthocyanins in the developed beverages.

Table 6
Total phenolic compounds, tannins and anthocyanins content in fresh beverages.

Beverage variant	Total phenolic compounds [mg GAE/100 mL]	Tannins [g TAE/100 mL]	Total anthocyanins [g C3GE/100 mL]
Store-bought beverage	10.34 ± 0.05 ^a	0.22 ± 0.07 ^a	nd
C1	115.45 ± 1.15 ^b	0.12 ± 0.02 ^b	0.18 ± 0.03 ^a
C2	147.34 ± 1.61 ^c	0.16 ± 0.08 ^c	0.20 ± 0.05 ^b
S1	164.77 ± 1.33 ^d	0.11 ± 0.05 ^b	0.20 ± 0.02 ^b
S2	197.64 ± 1.64 ^e	0.15 ± 0.02 ^d	0.22 ± 0.04 ^c
I1	177.34 ± 1.12 ^d	0.15 ± 0.03 ^d	0.12 ± 0.05 ^d
I2	189.15 ± 1.15 ^{d,f}	0.14 ± 0.04 ^e	0.16 ± 0.05 ^a
P1	59.51 ± 1.82 ^g	0.10 ± 0.05 ^e	0.09 ± 0.03 ^d

Different letters a-g within columns indicate statistically significant differences between variants ($p < 0.05$). Values are expressed as mean ± SD; $n = 4$.

Freshly prepared beverages contained 0.10–0.16 g of tannins/100 mL (unhydrolyzed tannins, which are formed from flavan-3-ols, characteristic of tea). These values are typical for teas and indicate that the tea has been brewed correctly. The highest tannin content was 0.16 g/100 mL for variant C2, and the lowest 0.10 g/100 mL for variant P1 (Table 6). However, the commercial beverage contained a higher tannin content than the prescribed beverages.

The total polyphenols content in the commercial drink was at a low level of 10 mg GAE/100 mL. However, in the developed TPC beverages it was in the range of 59.51–197.64 mg GAE/100 mL. The lowest polyphenol content was found in the P1 drink, which may be related to the addition of whey protein, which binds polyphenols engaging the phenolic moiety and making it unavailable for reaction with F-C solution and causing the protein to precipitate [Jauregi, Guo & Adeloje, 2021]. The highest amount of polyphenols was found in the S2 drink, which may be due to the addition of matcha. It was observed that the juice used also influenced the polyphenol content. Apple juice has a higher polyphenol content than orange juice. Therefore, apple juice-based beverages contained higher levels of TPC than orange juice drinks (Table 6).

The commercial drink showed anthocyanin content below the device's detection threshold. In the developed beverages, the anthocyanin content was in the range of 0.09–0.21 g C3gE/100 mL for P1 and S2, respectively. As with polyphenols, the anthocyanin content was different in drinks depending on the kind of juice added, i.e. apple vs. orange, where the positive correlation with the dosage of apple juice in the formulation was observed ($r = 0.57$; $p \leq 0.05$).

The ultrafiltration process reduced the tannin content in the beverages by a factor of two. The highest tannin content after ultrafiltration was 0.09 g/100 mL for variant S2, and the lowest 0.05 g/100 mL for variant C1, after four days of storage (Table 7).

Table 7
Total phenolic compounds, tannins and anthocyanin content in ultrafiltrated beverages.

Beverage variant	Total Phenols [mg GAE/100 mL]	Tannin content [g TAE/100 mL]	Total anthocyanins [g C3GE/100 mL]
Ultrafiltered – “0” day			
C1	111.25 ± 1.15 ^a	0.05 ± 0.01 ^a	0.17 ± 0.02 ^a
C2	142.31 ± 1.21 ^b	0.07 ± 0.02 ^b	0.20 ± 0.01 ^b
S1	159.64 ± 1.25 ^b	0.06 ± 0.02 ^c	0.19 ± 0.05 ^b
S2	192.20 ± 1.20 ^c	0.09 ± 0.03 ^d	0.21 ± 0.08 ^b
I1	173.44 ± 1.24 ^d	0.07 ± 0.01 ^b	0.10 ± 0.02 ^c
I2	184.35 ± 1.33 ^c	0.08 ± 0.02 ^e	0.15 ± 0.06 ^d
P1	55.11 ± 1.05 ^e	0.03 ± 0.01 ^c	0.07 ± 0.05 ^e
Ultrafiltered – 2 days			
C1	111.23 ± 1.15 ^a	0.05 ± 0.01 ^a	0.17 ± 0.04 ^a
C2	142.40 ± 1.18 ^b	0.07 ± 0.01 ^b	0.19 ± 0.03 ^b
S1	159.81 ± 1.26 ^b	0.06 ± 0.01 ^c	0.19 ± 0.01 ^b
S2	192.39 ± 1.23 ^c	0.09 ± 0.02 ^f	0.20 ± 0.02 ^b
I1	173.16 ± 1.35 ^d	0.07 ± 0.02 ^b	0.10 ± 0.03 ^c
I2	184.20 ± 1.41 ^c	0.08 ± 0.01 ^e	0.13 ± 0.05 ^d
P1	55.10 ± 1.18 ^e	0.03 ± 0.01 ^c	0.04 ± 0.01 ^f
Ultrafiltered – 4 days			
C1	106.18 ± 0.55 ^f	0.05 ± 0.01 ^a	0.14 ± 0.02 ^d
C2	139.24 ± 0.45 ^g	0.07 ± 0.01 ^b	0.17 ± 0.03 ^a
S1	144.95 ± 0.32 ^b	0.06 ± 0.02 ^c	0.18 ± 0.05 ^a
S2	188.53 ± 0.22 ^d	0.09 ± 0.02 ^d	0.19 ± 0.04 ^b
I1	98.32 ± 0.31 ^h	0.07 ± 0.01 ^b	0.08 ± 0.03 ^e
I2	124.01 ± 0.15 ⁱ	0.08 ± 0.01 ^e	0.11 ± 0.05 ^c
P1	35.55 ± 1.25 ^j	0.03 ± 0.01 ^c	0.02 ± 0.01 ^f
Ultrafiltered – 7 days			
C2	136.55 ± 1.22 ^g	0.06 ± 0.02 ^c	0.17 ± 0.05 ^a
S1	132.30 ± 1.01 ^b	0.05 ± 0.01 ^a	0.15 ± 0.03 ^d
S2	159.41 ± 1.12 ^d	0.03 ± 0.01 ^b	0.14 ± 0.06 ^d
Ultrafiltered – 9 days			
C2	131.77 ± 0.55 ^g	0.04 ± 0.01 ^a	0.16 ± 0.03 ^a

Different letters a-j within columns indicate statistically significant differences between variants ($p < 0.05$). Values are expressed as mean ± SD; n = 4.

Similarly, the content of total polyphenols and anthocyanins decreased after ultrafiltration. The content of these compounds also decreased with storage time. However, this treatment was more beneficial than pasteurization. The pasteurization process resulted in greater losses of polyphenols and anthocyanins

However, pasteurization allowed the tannin content to be maintained at a level similar to that found in fresh beverages. That was due to the fact that a part of tannins was separated under ultrafiltration what does not occur under pasteurization. The tannin content decreased during storage, except for sample I2 (0.02 g/100 mL) (Table S1).

4.2.2. Antioxidant potential

An analysis of the antioxidant potential of the designed beverages was carried out. The store-bought beverage showed no antioxidant potential under applied typical methodology. However, the developed beverages showed high antioxidant potential ranging from 55.97% to 91.16% for P1 and S2, respectively (Figure S4).

The higher the antioxidant potential, the higher the polyphenol content in beverages (Table 5). The addition of apple juice also increased the antioxidant potential. The beverages were tested again after ultrafiltration (Figure S5).

Ultrafiltration, by removing particles larger than 1 μm from the drink, resulted in a reduction in the antioxidant potential. The highest antioxidation potential was shown by beverages S2 (83.97%) and S1 (80.97%). On the first day, all drinks showed an antioxidant potential above 50%. After 2 days, the P1 drink reduced its oxidative potential by 40%. The remaining beverages slightly reduced their ability for free radical scavenging. After 4 days, the I1 and I1 drinks dropped below 50% of their antioxidant potential. Furthermore, C1 and S2 beverages reduced their antioxidant potential by up to 50%. However, drinks S1 and C2 continued to demonstrate high antioxidant potential of approximately 70% until day 7. Drink S1 demonstrated a decrease in antioxidant potential to 62% on day 9. Figure S6 shows the results of the analysis of antioxidant potential after pasteurization.

The pasteurization process resulted in a reduction of the oxidation potential in beverages I1, I2 and P1 below 21%. However, the remaining beverages showed high antioxidant potential after pasteurization, exceeding 65% (C1, C2, S1, S2). After one week, only beverages C2 (69.12%), S1 (74.94%) and S2 (75.96%) showed high levels of free radical scavenging. After two weeks, the S2 drink showed low antioxidant potential, below 50%. However, beverages S1 (62.12%) and S2 (71.15%) still showed high antioxidant potential. After three weeks, only the S2 drink was suitable for consumption, with an antioxidant potential of 62.19%.

4.3. Inhibition of α -amylase and α -glucosidase activity.

The developed beverages did not contain added sugar, but also sweeteners were not added. Fruit juice was added to the beverages as a source of natural sugars and a substitute for "sweeteners." Since the addition of orange or apple juice was not the main ingredient of the drinks, they have a low glycemic load and it is reasonable to analyze their potential to reduce the activity of enzymes contributing to the increase in glucose levels. Table S2 presents the results of α -glucosidase and α -amylase inhibition.

The beverages showed a stronger effect on α -glucosidase than α -amylase. The C2 variant inhibited α -glucosidase the most strongly (85.13%), while P1 inhibited it the least (65.45%). Similarly, the C2 variant showed the strongest inhibition of α -amylase (59.82%), and the P1 variant the weakest (33.12%) (Table S2).

It was observed that ultrafiltration had a positive effect on α -amylase inhibition, and only the P1 variant did not show 50% enzyme inhibition. During refrigerated storage of beverages, variants C1, S1 and I1 showed enzyme inhibition below 50% at day 4 (Table S2). Only C2 and I2 beverages showed the best inhibition of both enzymes throughout the storage period. Beverages C1, I1, I2 and P1 were not organoleptically acceptable after 7 days of storage. However, the C2 drink was fit for consumption after 9 days of refrigerated storage and showed inhibition of the α -amylase and α -glucosidase enzymes. In terms of inhibition of α -amylase and α -glucosidase, the pasteurization process was less effective than ultrafiltration. Inhibition of the enzymes α -amylase and α -glucosidase was reduced. Only variants I1, C2, S2 and I2 showed α -amylase inhibition of slightly above 50%. After 3 days of storage, only drink 2 was suitable for consumption, but it only inhibited α -glucosidase.

4.4. Vitamin content

Table S3 shows the vitamin content of beverages before and after ultrafiltration and pasteurization.

The store-bought beverage contained no vitamins. The vitamin C content for fresh beverages ranged from 2.12–6.56 mg/100 ml, for P1 and C2, respectively. The pasteurization process reduced the vitamin C content by almost half of the initial value. Vitamin C degraded with storage time. The ultrafiltration process reduced vitamin C slightly. The highest content was observed for the freshly filtered beverage, at 6.23 mg/100 ml. The lowest content was observed for the P1 beverage after 4 days of storage (subject to ultrafiltration), at 0.45 mg/100 ml. Wiatmina E w świeżych napojach wynosiła od 0,01 do 0.55 mg/100 mlm odpowiednio dla C1 i S1. However, vitamin E was not detected in beverage C2. The

ultrafiltration process reduced vitamin E levels in beverage C1 to undetectable levels. In the remaining beverages, vitamin E levels were stable during storage. The pasteurization process also reduced vitamin E levels to levels below those detected in beverage C1. In the other beverages, vitamin E levels were reduced by almost half. Vitamin B₂ was 0.01 mg/100 ml, with the exception of the C2 drink, which was 0.02 mg/100 ml. Ultrafiltration and pasteurization did not significantly alter the vitamin content. However, vitamin B₉ is less stable to heat, and the pasteurization process reduced its content by approximately 50%.

5. Discussion

Functional beverages are becoming increasingly popular in the daily diet. Their main task is to nourish the body, improve mood, combat fatigue and stress, and supplement the diet with vitamins and minerals. However, most of these drinks contain artificial colour-ants and flavours, and have added sweeteners, which is increasingly being diagnosed as a food intolerance [Orrù et al., 2018; Kalogerakou & Antoniado 2024]. That's why we've developed seven natural drink variants, without artificial dyes, flavors, sweeteners or added sugar. The drinks contained only natural sugars from orange juice and apple juice. Both juices have a low glycemic index, making them suitable for people with diabetes [Pepin, Stanhope & Imbeault, 2019; Visuthranukul, Sampatanukul, Aroonparkmongkol, Sirimongkol & Chomtho, 2022]. Although apple juice generally has a higher fructose-to-glucose ratio (often $\geq 2:1$) than orange juice (around 1:1). It is known that fructose metabolism differs from glucose, potentially impacting liver fat production and insulin resistance, which are factors in diabetes development. From that reason the variants with added orange juice may be a better choice for people with a risk of developing type 2 diabetes [Gonzalez, 2024].

Thanks to their high water content of over 88% (without preservation processes), these drinks can help balance our daily diet and provide our bodies with adequate hydration level. This allows them to replace beverages other than water consumed throughout the day. Everyone should consume about 2.5 L of water, of which about 1 L should come from products such as fruits, vegetables and meat. The rest should come from un-sweetened beverages, although it's important that the beverages are rich in minerals and vitamins [Sugajski, Buszewska-Forajta & Buszewski, 2023]. These drinks, thanks to their composition, enrich the diet with vitamins and minerals. The developed in the studymemory- and concentration-enhancing drink contained: green tea, ginkgo biloba, ginseng, water, and orange or apple juice. Green tea is rich in flavanols and catechins. The main compounds are epicatechin, epicatechin-3-gallate, epigallocatechin, and epigallocatechin-3-gallate. They have antioxidant, anticancer and antineurodegenerative properties [Kaur, Pathak, Pandhi & Khanduja, 2008]. Ginkgo biloba contains, among others, minerals, quercetin, proanthocyanins, glycosides, kaempferol, vitamins C and E. It is characterized by supporting brain function and regenerating the mind after exercise [oor-E-Tabassum et al., 2022]. Ginseng contains iron, iodine, zinc, selenium, magnesium, vitamins E, C, B7, and B12, flavonoids, and sesquiterpenes. Ginseng helps relieve stress and protects the nervous system [Oliynyk & Oh, 2013; Kumar, Kumar, Nayal, Shukla & Kailkhura, 2023]. Whey protein is rich in minerals and can provide calcium, magnesium, aluminum, and sodium, among others [González-Weller et al., 2023]. Ginseng contains minerals such as zinc, phosphorus, calcium, iron, and potassium [Choi et al., 2007]. In the study by Najman et al. [Najman, Sadowska, Wolińska, Starczewska & Buczak, 2023], the matcha beverage contained less than 0.1 g of fat per 100 mL, while the addition of milk increased the fat content to 0.7 g/100 mL [Najman et al., 2023]. A'yuni et al. [A'yuni, Wijaya & Mastuti, 2022] evaluated a functional beverage based on JAVA tea; the resulting beverage contained 0.6 g/100 mL of fat [A'yuni et al., 2022]. The functional beverages designed have a significantly lower fat content than the examples shown above. It can be said that the drink will be suitable for people who care about a low-calorie diet, especially for those on a low-fat diet.

According to Taib et al. [Taib, Hazmi, Rhim & Alias, 2024], black tea leaves can contain approximately 41 mg/g d.m. of tannins, while green tea contains approximately 101 mg/g [Taib et al., 2024]. Compared to teas, the resulting beverage provided a lower tannin content and it did not exceed the recommended daily intake of 0.1 to 0.5 g [Serrano, Puupponen-Pimiä, Dauer, Aura & Saura-Calixto, 2009]. Tannins are substances with health-promoting properties when consumed in small amounts. They are antioxidants that positively affect inflammation, accelerate wound healing, support the immune system, and aid the digestive system. However, excessive consumption of tannins limits the absorption of other health-promoting substances and nutrients, particularly iron absorption (non-hydrolyzing (from tea)). They also cause problems with stomach diseases [Pleszczyńska & Szczodrak, 2005; Hoque et al., 2025]

α -Glucosidase is responsible for regulating blood glucose levels after consuming a meal containing oligosugars. High activity of the enzyme after a meal can cause hyperglycemia. α -Amylase, on the other hand, is responsible for starch digestion. This enzyme breaks down starch into glucose subunits, which can temporarily accumulate and cause hyperglycemia [Yang et al., 2019]. It can be concluded that the developed beverages will be a good product for people struggling with diabetes in the case of variants: I1, C2, S2, and I2. Variant C1, S1, and P1 showed too low levels of α -amylase inhibition, below 50%. Research by Li et al. [Li, Qian & Li, 2010] indicates that pu-erh tea is a good α -glucosidase inhibitor, with $IC_{50} = 3.55$ mmol/L. This effect was explained by the content of bioactive compounds such as epigallocatechin gallate and epicatechin gallate [Li et al., 2010]. Compared to the store-bought beverage, the developed beverages showed higher α -amylase and α -glucosidase inhibition activity, whereas the commercial beverages showed low enzyme-inhibition levels, below 50% for α -amylase and slightly above 50% for α -glucosidase.

The beverages were characterized by a high vitamin content in the freshly prepared versions. While the pasteurization process increased the beverages' stability, it reduced the vitamin content, while ultrafiltration preserved the higher vitamin content. Vitamin C should be consumed between 75 and 90 mg/day [Alberts, Moldoveanu, Niculescu & Grumezescu, 2025]. Consumption of fresh C2 drink would allow the utilization of over 50% of vitamin C, drinking one liter per day (65 mg/L of vitamin C). Vitamin E recommended intake is 7.5–10 mg [Szewczyk & Górnicka, 2023]. A liter of fresh S1 and S2 drink provides approximately 70% of the daily requirement for this vitamin. We should consume about 1.6 mg/day of vitamin B2 [Shinagawa, Serizawa, Yamazaki, Minematsu, Miyakoshi, Yano & Ota, 2022]. A liter of drinks will use up a small portion of your energy during the day if you consume one liter. We should consume vitamin B9 in the amount 300 µg/100 ml [Pravst, et al., 2021]. Fresh C1 and C2 drink in the amount of 1 liter provides vitamin B9 as a dose for the whole day. Fresh drinks contain a high vitamin content thanks to apple and orange juices. Additionally, the use of these juices creates a sweeter taste, more acceptable without added sugar.

6. Conclusions

The study showed that the developed beverage recipes, particularly the apple juice variants, contained a higher mineral content than the commercial beverage. The highest ash content was found in the beverage with added protein, which is rich in minerals, primarily calcium and phosphorus. Ultrafiltration increased water content and reduced ash content by removing sediments from teas, herbs, and fruits. Pasteurization, however, did not change the water or ash content. The pasteurization process extends the shelf life of beverages. The tannin content was typical for teas, but lower than the tannin content in the commercial beverage. However, it is worth noting the herb content in the developed beverages, which may reduce the tannin content in the overall beverage. Among the beverages produced, variant C2 (properties of the drink: calming properties and ensuring good sleep; composition: black tea, lemon balm, chamomile, water, juice orange) showed the highest tannin content. Ultrafiltration reduced the tannin content in the beverages by a factor of two. Pasteurization, on the other hand, allowed tannin values to remain similar to those of fresh beverages, although the tannin values decreased during storage. The α -amylase and α -glucosidase inhibition assay showed a stronger effect on α -glucosidase than on α -amylase, indicating the potential of tea- and herbal-based beverages to inhibit the final digestion of carbohydrates, thereby preventing rapid increases in plasma glucose. The α -amylase and α -glucosidase inhibition study showed that the C2 drink has the potential to slow down glucose absorption from the gastrointestinal tract the most. It can be believed that the drink will help prevent rapid fluctuations in blood sugar levels, which is important for people with type 2 diabetes. Future studies should focus on microbiological studies, *in vitro* digestion and bioavailability and cell lines analysis of glucose metabolism together with antioxidant and anti-inflammatory activities.

Abbreviations

The following abbreviations are used in this manuscript:

DPPH 2,2-diphenyl-1-picrylhydrazyl

C1 Calming properties and ensuring good sleep – functional beverages with orange juice

C2 Calming properties and ensuring good sleep – functional beverages with apple juice

S1 Stimulating/energizing properties – functional beverages with orange juice

S2 Stimulating/energizing properties – functional beverages with apple juice

I1 Improves memory and concentration – functional beverages with orange juice

I2 Improves memory and concentration – functional beverages with apple juice

P1 Energizing with added protein – functional beverages with orange juice

Declarations

Supplementary Materials: Not applicable.

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Figures



Figure 1

Sample drinks with orange juice. From the left, variants C1 (beverages: calming properties and ensuring good sleep with apple juice), S1 (beverages: Stimulating/ energizing properties with apple juice), I1 (beverages: Improves memory and concentration with apple juice), and P1 (beverages: Energizing with added protein with orange juice).



Figure 1

Sample drinks with orange juice. From the left, variants C1 (beverages: calming properties and ensuring good sleep with apple juice), S1 (beverages: Stimulating/ energizing properties with apple juice), I1 (beverages: Improves memory and concentration with apple juice), and P1 (beverages: Energizing with added protein with orange juice).

(beverages: Energizing with added protein with orange juice).

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