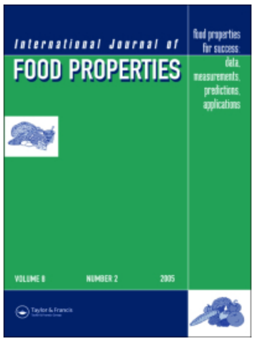

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Exploitation of red beet peel powder as a natural food ingredient in whey-fruit based beverage

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ABSTRACT

Scientists and food producers are studying the potential of utilizing different by-products as highly nutritious food components to meet consumers' growing demand for healthier and natural goods. Beetroot belongs to the *Amaranthaceae* family and is highly rich in antioxidants, specifically phenolics and betalains with excellent health properties. The peel of red beetroot, which is generally discarded, has a high concentration of bioactive phytochemicals (betalains, dietary fibers). These compounds are well-known for their strong antioxidant effects, improving cardiovascular health, reducing oxidative stress, and enhancing immunological function. Incorporating red beetroot peel (RBP) powder into whey-fruit-based beverages is a promising method to improve nutritional content and sensory attractiveness. This study investigated the influence of adding RBP to whey-fruit-based beverages, specifically examining its effects on physicochemical and phytochemical characteristics, antioxidant capacity, color, microbiological, and rheological attributes, and consumer preference. The RBP extract exhibited high amounts of total polyphenols (1239.35 ± 0.51 mg GAE/100 g dw) and antioxidant activity (90.02 ± 0.22%). The findings indicated that RBP had a notable impact on the antioxidant capacity (16.38 ± 0.37 μmol TE/g dw for BRBP1, 26.69 ± 0.10 μmol TE/g dw for BRBP3, 36.75 ± 0.31 μmol TE/g dw for BRBP6) of the beverages and enhanced their color without negatively impacting their sensory characteristics. The dynamic oscillatory rheological measurements revealed that beverage supplementation with increasing amounts of RBP obtained stronger networks with solid-like viscoelastic behavior. Using RBP powder as a natural food ingredient in whey-fruit-based beverages enhances their nutritional value and promotes sustainable food processing by making use of by-products from the food industry into innovative food options.

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Introduction

The functional beverages industry is experiencing steady growth, making it crucial to prioritize research on developing new products that are abundant in bioactive components and specifically

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target lifestyle disorders. The bioactive potential of agro-industrial waste is a promising sector that should be thoroughly investigated.[1]

Red beetroot (*Beta vulgaris L.*) is a member of the *Chenopodiaceae* family and is a widely recognized vegetable appreciated for its vivid hue and its abundant nutritional content. The plant is grown extensively in America, India, and Europe, and is rich in bioactive substances, such as betalains, phenolics, vitamins (A, B1, B2, B3, B5, B6, B9, and C), minerals (Na, Ca, Fe, P, K, Mg, Zn), and dietary fibers.[2,3] Red beetroot is highly effective in terms of antioxidant activity since it contains several active components, including carotenoids, glycine betaines, saponins, betalains, polyphenols, and flavonoids.[4]

Red beetroot is consumed by humans in various forms, including fresh and dried. It can be found in chips, tea, and powder used in baking. Additionally, it is commonly used as a natural red food coloring agent to enhance the color of tomato paste, sauces, desserts, ice creams, and cereals.[2] Global beetroot production reached approximately 275 million tons in 2018,[5] and 85% of red beetroot is utilized for processing, while around 30% of the whole crop is lost or destroyed during processing due to poor root quality.[6] The food processing sector utilizes large amounts of beetroots, resulting in the production of huge by-products, including beetroot peels. These peels are frequently thrown away, even though they contain abundant beneficial substances. Using these residual materials can help reduce waste and enhance the value of food products, in line with sustainable practices in food processing. Beetroot peel contains antioxidant properties, making it valuable in the food and nutritional supplements sectors. Also, have a variety of biological actions, including antibacterial, antioxidant, antihypertensive, anti-inflammatory, anti-anxiety, anticancer, and antidiabetic characteristics.[7–9] It is important to emphasize that approximately 50% of the total phenolics (p-coumaric and ferulic acids), which contribute to the antioxidant effect, are found in the peel. Approximately 15%–30% of the raw material produced by the juice enterprise, which includes the red beet peel and its pulp, is considered a waste and is consequently thrown as animal feed.[10,11]

Betalains are nitrogenous chemicals that are soluble in water and can be classified into two subclasses: betacyanins, which are red, and betaxanthins, which are yellow-orange pigments. Delgado-Vargas et al.,[12] reported that betacyanins account for around 75–95% of the pigments found in beetroot, whereas the remaining 5–25% consists of betaxanthins.

Betalains, which are powerful antioxidants, are found in beetroot peels and are responsible for their vivid red and yellow colors. Studies have demonstrated that betalains possess the ability to decrease oxidative stress and inflammation, thereby providing a preventive impact against cardiovascular illnesses and some forms of cancer.[13,14] Given beetroot bioactive potential and health-related benefits, it is important to further exploit the by-products as functional components or integrate them into various food products or supplements.[10]

Prior research has attempted to utilize beetroot as a preservative to prolong the storage duration of several food items, including mayonnaise and dairy products,[15,16] and has the potential to serve as an alternative to artificial colorants.[17] The European Union has approved the inclusion of betalains in food products, namely under the food code E162. This addition is derived from mechanically processed beetroot juice and comprises several betacyanins that have been purified.[9]

Beetroot powder or extracted pigments can be utilized to improve the hue and nutritional composition of products. The utilization of red beetroot peel (RBP) powder in different food products has attracted interest because of its ability to enhance nutritional content and offer functional advantages. Yadav & Masih[18] developed beverage using dehydrated beetroot powder, which received favorable sensory ratings. The nutritional value of beverage improved as the concentration of beetroot powder increased, resulting in higher levels of protein, carbohydrates, fats, and ash. Beverages, specifically, are an appropriate medium for integrating RBP because they provide a convenient means for consumers to get the health advantages of beetroot phytochemicals.

Whey, a by-product of cheese production, makes up 85–90% of the total amount of milk utilized.[19] About 20% of milk proteins and 55% of milk minerals are found in cheese whey. Whereas whey is valorized in affluent nations into value-added products like ricotta cheese, whey protein isolates, concentrates, and whey-based fermented beverages, most whey is still dumped untreated into water sources.[20]

This study addressed the gap in prior research concerning the insufficient investigation of incorporating red beetroot powder (RBP) as a bioactive component in whey-fruit-based beverages, particularly emphasizing the optimization of RBP concentration to improve nutritional value and sensory attributes while maintaining product quality. Although research has examined the addition of fruits and vegetables in whey beverages and their nutritional benefits, the specific incorporation of beetroot peel powder to enhance the antioxidant profile, flavor, and texture of these beverages remains underexplored.

Our objective in this study was to develop a blend of beverages that are made using sustainable food waste (whey and RBP) and are flavored with banana and melon. Therefore, different concentrations of RBP powder were incorporated into the whey-fruit beverage to investigate the impact of RBP on the physico-chemical and phytochemical characteristics, antioxidant capacity, color, microbiological, rheological characteristics, and consumer preference of whey-fruit-based drinks. This will offer significant knowledge on the potential use of RBP powder as a bioactive ingredient in foods.

Materials and methods

Reagents and chemicals

Folin-Ciocâlteu reagent, 2,2-diphenyl-1-picrylhydrazyl (DPPH), 6-hydroxy2,5,7,8 tetramethylchromane-2-carboxylic acid (Trolox), Gallic acid, sodium carbonate (Na2CO3) 20%, sodium nitrite (NaNO2) 5%, aluminum chloride (AlCl3) 10%, sodium hydroxide (NaOH) 1 M, 1% citric acid (C6H8O7), ethanol(C2H5OH) and methanol (CH3OH) which were purchased from Sigma Aldrich (Germany). Whey protein isolate was brought from Kemin Industries (Belgium). Banana, melon, erythritol, almond milk was provided from the local market in Iasi, Romania.

RBP powder preparation

Red beetroot (*Beta vulgaris ssp. vulgaris var. conditiva Rubiniu*) were purchased from a supermarket (Iasi, Romania) in June 2023. The beets were sorted, washed with distilled water, and with the help of a knife the peels were removed, taking care not to cut much of the pulp. The peels were sliced into little fragments and stored at −25°C until they were frozen. Afterwards, the beetroot peels were freeze-dried (BIOBASE BK-FD10T, Jinan, China) at a temperature of −42°C and a pressure of 0.10 mBar, the experiment was conducted for 48 h. Ultimately, the dehydrated peels were pulverized using a grinding mill into a fine powder and stored in a plastic container with a lid. Before adding to the beverage, the RBP powder was decontaminated by a UV laser procedure.[21,22]

Proximate composition of RBP powder

The RBP powder's moisture, ash, protein, fat, fiber, and carbohydrate (by difference) content was determined using the established techniques indicated in the AOAC[23] standard methodology. The samples were subjected to proximate analysis in duplicates, and the findings were expressed as percentages. The protein content was quantified by the Kjeldahl technique, with nitrogen (N) content multiplied by 5.7.[24] The lipid content was quantified gravimetrically with the Soxhlet method.[25] The ash content was assessed by weighing 5 g of the sample in a pre-weighed crucible and subjecting it to a furnace at 600°C for 4 hours. Samples had been cooled in a desiccator, and their weight was measured to determine the ash level.[26] For the moisture content of the sample the oven drying method (105 °C) was used. The crude fibers (an enzymatic-gravimetric procedure) of the powder were determined according to the method described by Chantaro et al.[27] The total amount of carbs was calculated by difference [100 - (moisture + protein + fat + ash + fiber) %].

Color analysis of RBP powder

The color of the RBP powder was measured using a MINOLTA Chroma Meter CR-410 (Konica Minolta, Osaka, Japan) after standardization with a white calibration plate according to the equipment specifications. The determined parameters were L* (lightness/darkness), a* (red/green) and b* (yellow/blue). The hue angle (arctan(b*/a*) for quadrant I(+a*, +b*), visual color appearance, and chroma ($\sqrt{(a^*)^2 + (b^*)^2}$), color intensity, were also calculated.[28]

Mineral analysis of RBP powder

One gram of RBP powder was digested with nitric acid (HNO3) and hydrochloric acid (HCl) in a ratio of 8:2, at 180°C for 40 minutes. The sample was dissolved in 25 mL deionized water in a standard flask. Ca, P, K, Mg, Zn, Fe, and Na, contents were determined according to[29] using Atomic Absorption Spectrophotometry.

Ultrasound-assisted extraction of RBP powder

The slightly modified method presented by Šeremet et al.,[30] was used to obtain the RBP extract. 1 g of RBP with 9 mL of 70% ethanol and 1 mL of 1% citric acid was mixed. The samples were subjected to ultrasound treatment (Elmasonic S 180 h, Elma, Singen, Germany) at 40 kHz for 30 minutes at 25 ± 5°C, and then centrifuged for 10 minutes at 5500 rpm and 4°C. Afterward, the supernatant was stored under refrigeration(±8°C) until further use.

RBP extract characterization

Total Betalain Contents: The betaxanthins and betacyanins in the extracts were quantified using spectrophotometry at 538 nm and 480 nm using a UV-Vis spectrophotometer (Analytik Jena Specord 210 Plus, Jena, Germany).[31] The findings were presented as milligrams per gram of dry weight (dw). The betalain contents was calculated as follows (1):

$$\text{Betalains}\,(\text{mg/g dw}) = \frac{\text{A} \times \text{MW} \times \text{DF}}{\varepsilon \times \text{l} \times \text{m}} \tag{1}$$

where A- the absorption at 538 and 480 nm for betacyanins and betaxanthins, respectively; MW- molecular weight; l- cuvette pathlength; DF- the dilution factor; m-the amount of sample; ε- molar extinction coefficients. For betacyanins ε = 60,000 L/mol cm in H2O; (MW = 550 g/mol) and betaxanthins (ε = 48,000 L/mol cm in H2O (MW = 308 g/mol) were applied. The total betalain content (in mg per 100 g of sample) was determined by summing the values for betacyanin and betaxanthin.

Total Polyphenol Contents: The total polyphenol content was determined using the Folin – Ciocâlteu colorimetric method, as outlined by.[31] In a test tube, 0.20 mL of sample was added, followed by 15.8 mL of distilled water and 1 mL of Folin-Ciocâlteu reagent. They were left to stand for 6 minutes, after which 3 mL of 20% sodium carbonate were added and left to stand for one hour at room temperature in the dark. The absorbance was read at a wavelength of 765 nm. In addition, a standard curve was generated using various doses of Gallic acid, and the findings were expressed as milligrams of Gallic acid equivalents (GAE) per gram of dry weight (dw).

Total Flavonoid Contents: The method described by Dewanto et al.,[32] was used to assess the total flavonoid content. A volume of 250 µL sample is mixed with 1.25 mL of distilled water and 0.075 mL of 5% sodium nitrite. The mixture is left to act for 5 minutes, after which 0.15 mL of 10% aluminum chloride is added and left to act again for 6 minutes. Then add 0.5

mL of 1 M sodium hydroxide and 0.775 mL of distilled water. The absorbance of the resulting mixture is immediately read at a wavelength of 510 nm. The total flavonoid content was determined by generating a calibration curve using catechin and the results were reported as milligrams of catechin equivalents (CE) per gram of dry weight (dw).

Antioxidant Activity: The technique for measuring the antioxidant activity by neutralizing the DPPH radical was conducted following the approach outlined by Castro-Vargas et al.[33] DPPH stock solution was obtained by homogenizing 3.8 mg of DPPH with methanol, made up to the mark, in a 100 mL volumetric flask. Afterward, 100 µL of the sample to be analyzed was added to a test tube, along with 3.9 mL of DPPH. For the control sample, 100 µL of methanol were used, along with 3.9 mL of DPPH. The absorbance was read using a UV – visible spectrophotometer (Analytik Jena Specord 210 Plus, Jena, Germany), after 90 minutes of rest in the dark at a wavelength of 515 nm. In addition, a standard curve was generated by utilizing various Trolox concentrations. The results were reported as µmol of Trolox equivalent (TE) per gram of dry weight (dw). The radical scavenging activity was expressed also as the percentage of inhibition based on equation (2):

$$\text{DPPH scavenging activity (\%)} = \frac{\text{Abs Control} - \text{Abs Sample}}{\text{Abs Control}} \times 100 \quad (2)$$

where Abs Sample- the absorbance value of the DPPH solution combined with RBP extract; Abs Control- the absorbance value of the DPPH solution only.

Preparation and characterization of supplemented beverage

To produce the enhanced beverage and evaluate its functionality, three distinct ratios of RBP powder were utilized (1%-BRBP1, 3%- BRBP3 and 6%- BRBP6). These concentrations were chosen based on previous research and nutritional equilibrium, with the objective of improving both the sensory and functional attributes of the obtained beverage. The concentrations of RBP powder at 1%, 3%, and 6% were selected to investigate their effects on the physicochemical properties and sensory attributes of the obtained beverage.

The beverage in the control sample (BC) had no powder added. The whey-fruit based beverage consisted of a combination of 13% unsweetened almond milk, 38% liquid sweet whey, 2% whey protein isolate, 23% banana pulp, 23% melon pulp, and 2.5% erythritol. The RBP powder was completely blended with the prior mixture using ratios of 1%, 3%, and 6%. All ingredients were mixed with a blender (PHILIPS Core HR357390, China) until were properly homogenized. The combination of banana pulp, melon pulp, whey, and almond milk have been designed to balance flavor, texture, and nutritional value.

Unsweetened almond milk offers a plant-based substitute for conventional dairy, enhancing its attractiveness to consumers pursuing lactose-free or vegan alternatives. Almond milk enhances creaminess and improves the texture of the beverage. The incorporation of almond milk may equilibrate the texture of whey and improve overall palatability. Banana pulp and melon pulp were important ingredients for boosting the sweetness and nutrient content of the beverage and adding a refreshing flavor profile. Pulp fruits can also improve the viscosity of the drink, contributing to a thicker, more satisfying texture. The natural sweetness of banana pulp can complement erythritol, diminishing the necessity for more artificial sweeteners Erythritol facilitates the development of a reduced-calorie beverage; it enhances the sweetness without overshadowing the natural flavors of the fruits or the RBP powder. The prepared beverages were thereafter filled into sterilized glass bottles (100 mL) with a 2.5 cm gap at the top and sealed tightly with a crown cork to prevent air from entering. Subsequently, were subjected to pasteurization at 60–65°C for 20–30 minutes. Afterward, they were cooled to an ambient temperature. The samples were stored for a period of 14 days in glass bottles in a refrigerator (4 ± 1°C) for further analysis, and the characterization was conducted on days 1 and 14.

Characterization of physicochemical and phytochemicals of supplemented beverage

The following parameters were measured for the supplemented beverage (AOAC[23]): humidity, dry matter, ash, protein, fiber, carbs, and fat. Using the method described by Gavril et al.,[34] the energetic value of the samples was calculated based on the findings of the proximate analysis.

Energy value = (% crude protein x 4) + (% crude fat x 9) + (% carbohydrates x 4)+ (% crude fiber x 2)

The total levels of betalawas flavonoids, polyphenols, and the antioxidant activity in both the control and the RBP-enriched beverage were determined using the methods outlined in section 2.4.

Storage stability test

The supplemented beverages were stored for a period of 14 days in glass bottles in a refrigerator (8 ± 1ºC). They were analyzed for their bioactive contents and antioxidant activities, color parameters, and microbial analyses during 14 days of storage.

Color analysis of supplemented beverage samples

Determination of color parameters of the RBP-enriched and control beverage was performed using the portable colorimeter with illuminator C (MINOLTA Chroma Meter model CR-410, Osaka, Japan). The color parameters determined were L*(lightness/darkness), a*(red/green) and b*(yellow/blue). The mathematical calculation of the color parameters (Hue angle, ΔE, and Chroma) was carried out in accordance with the equations presented by Mocanu et al.[28]

a_0 Hue angle = arctan(b*/a*) for quadrant I(+a*, +b*), which describes visual color appearance;

$\Delta E = \sqrt{(L^* - L_0)^2 + (a^* - a_0)^2 + (b^* - b_0)^2}$, the total color difference (L_0, a_0, and b_0 represent control values);

Chroma $= \sqrt{(a^*)^2 + (b^*)^2}$, which describes color intensity.

Mineral analysis of supplemented beverage samples

The mineral compositions of the samples were analyzed using atomic absorption spectrometry (ContrAA 700, Analytik Jena, Jena, Germany) with a flame atomizer system. The results were reported in milligrams per 100 g of dw. This analysis was performed utilizing a MiniWAVE Microwave (SCP Science, Baie-d'Urfé, Canada) digestion system.[29] The mineral contents measured in raw plant material and beverage samples included Ca, P, K, Mg, Zn, Fe, Mn, Cu, and Na.

Rheological measurements on the supplemented beverages

The AR2000ex rheometer (TA Instruments, Ltd, New Castle, USA) was utilized to test the influence of RBP addition on the rheological properties of the beverage. A cone with 20 mm base diameter and 2° angles was selected to run the rheological measurements on a plate-cone geometry, while using a gap size of 1 mm. The samples tempered at 5°C, were subjected to small amplitude oscillatory strain sweep (strain of 0.1–100% and frequency of 0.5 hz) to identify the linear viscoelastic region (LVR), which was further considered for the frequency sweep test (strain of 0.5% and frequency of 0.1–10 hz). The storage and loss moduli (G' and G") were recorded, and the strain value associated to the G'-G" crossover (γG′-G″) was associated to the beginning of flow. Rotational stepped flow tests at increasing the shear rate from 0.1 to 100 s^{-1} were also performed, followed by peak hold consisting of maintaining the samples at high shear rate of 100 s^{-1} for 3 min, such as to evaluate the time dependency of the viscosity.

Scanning electron microscopy of supplemented beverage samples

The morphology of the RBP powder and RBP-supplemented beverages and their elemental composition were analyzed using a scanning electron microscope (Quanta 450, FEI, Thermo Fisher Scientific, Hillsboro, USA) and an energy dispersive X-ray detector (EDX) (EDAX, AMETEK Inc., Berwyn, PA, USA). Each sample was thinly applied to aluminum stubs in a layer of about 2 mm, and the aluminum stubs were then put inside the analysis chamber. The EDX spectra analysis was carried out by EDAX Inc.'s TEAM version V4.1 system. The samples were subjected to low vacuum conditions, with a magnification of 500× (10 μm), an electron acceleration voltage of 15 kV, and a pressure of approximately 6.1×10^{-4} Pa.

Microbiological analyses of supplemented beverage samples

The analytical procedures for assessing the microbiological load of tested samples were performed under sterile conditions. In order to conduct microbial analyses, 10 mL of beverage were homogenized with 90 mL of buffered peptone water in a laboratory blender (Seward, UK) at 250 rpm for 5 minutes. The bacteria, yeast, and molds were isolated from the serial dilutions using the spread and pour plate techniques. These dilutions were prepared by combining 1 mL of the previous dilution with 9 mL of buffered peptone water in test tubes.

The nonselective Plate Count Agar (PCA) supplemented with 1 g/L skimmed milk powder and Potato Dextrose Agar (PDA) along with the selective chromogenic agar Rapid E. coli 2 (RE) and Rapid Staph (RS; Bio-Rad, France) were the microbiological media used for plating following inoculation with 1 mL of laboratory sample. After a 72-hour incubation period at 30°C, the total aerobic bacteria (TAB) on PCA plates were counted. After five days of cultivation on PDA plates at 28°C, yeast and molds were enumerated. The number of *Escherichia coli* and other coliform colonies was determined using the ISO 16,140 method on RE medium after incubating at 37°C for 24-hours. The samples were grown on RS medium, which ensures the identification and counting of coagulase-positive staphylococci (*Staphylococcus aureus*) within 24-h at 37°C.

Following the incubation period, the microorganisms present in the beverage samples were enumerated using the automated colony counter Scan 1200(Interscience, France). The results were reported as the logarithmic value of colony forming units per milliliter (log CFU mL^{-1}).

Sensory analysis of supplemented beverage samples

The sensory characteristics of the beverage samples were evaluated by a group of 22 panelists including students and staff members from the Faculty of Food Science and Engineering (Dunarea de Jos University of Galati). The panelists were chosen for their knowledge of food products and their willingness to participate. The panelists participated in two training sessions to analyze and discuss various sensory descriptors that were evaluated, including the changes in color, texture, and flavor of the beverages. The panelists were told to assess each sensory attribute of nine scores using the Hedonic scale, which ranges from 1 (indicating extreme dislike) to 9 (indicating extreme liking). The RBP-enriched beverages were randomly coded (three-digit codes) and were served in transparent glasses following the control beverage. The panelists were asked to evaluate the differences between the following attributes: appearance, color, odor, taste, flavor, aftertaste, consistency, and overall impression.[29]

Statistical analysis

The data are displayed as the average values for three repeated measurements with the standard deviation given. The statistical analysis was performed using Minitab Software for Windows, Version 19.1. A comparative analysis using the variance analysis (ANOVA) method assessed the disparities among the average values. Tukey's test was used with a 95% confidence interval if there were notable differences in this aspect. Principal components analysis was applied on the descriptive sensory data using XLSTAT, an add-in software for Microsoft Office Excel (Trial Version 2024, Addinsoft, Paris, France).

Results and discussion

Phytochemical profile of RBP powder

Using a solid-liquid solvent ultrasound-assisted extraction procedure, the RBP powder was extracted for phytochemical analysis (Table 1). The extract had a total betalains of 221.17 ± 0.11 mg/100 g dw and total polyphenol of 239.35 ± 0.51 mg GAE/100 g dw. The antioxidant activity of the RBP extract was 25.27 ± 0.05 μmol TE/g dw with an inhibition of 90.02 ± 0.22%.

The betalain content varied from 0.29 to 1.44 mg/g DW, while the polyphenolic yield varied from 1.64 to 2.74 mg/g DW in an etanolic extract from beetroot peel. The optimal parameters were 1.5% citric acid concentration, 50% ethanol concentration, 52.5°C temperature, and 49.9 minutes extraction time for the highest recovery of betalains and phenols.[35] In a study by Zin & Bánvölgyi[36] the maximum amounts of total betalains (202.08 ± 2.23 mg/100 g FW), betacyanin (115.89 ± 1.08 mg/100 g FW), and betaxanthin (86.21 ± 1.16 mg/100 g FW) were obtained by pure water solvent after 150 s of microwave-assisted extraction at 800 W.

Alshehry[37] reported a higher total polyphenolic content of 255±10.48 mg GAE/100g and antioxidant activity of 137±8.29 mg TE/100 g sample on a beetroot pulp extract. Šeremet et al.,[30] used an ultrasound-assisted technique to recover bioactive compounds from the peel of beetroot therefore the betacyanin content was 3.87±0.03 mg/g, the polyphenols content was 44.07±0.22 mg GAE/g and the antioxidant activity was 0.042±0.00 mmol Trolox g/g dw. The extraction process and kind of solvent used have a discernible impact on the extraction efficiency of phenolics from RBP, according to Kujala et al.[10] The polyphenol content in a methanolic extract was reported 24.1 mg GAE/g.

Table 1: Phytochemical profile and color coordinates of RBP powder.

Parameter	RBP Powder
Betacyanin, mg/100g dw	124.50 ± 0.11
Betaxanthin, mg/100g dw	97.58 ± 0.12
Total Betalains, mg/100g dw	222.08 ± 0.11
Total flavonoids, mg CE/100g dw	130.52 ± 0.47
Total polyphenols, mg GAE/100g dw	239.35 ± 0.51
Antioxidant activity,μmol TE/g dw	25.27 ± 0.05
Inhibition, %	90.02 ± 0.22
L*	36.89 ± 0.18
a*	31.71 ± 0.39
b*	5.16 ± 0.09
Chroma	32.13 ± 0.38
Hue angle	0.16 ± 0.01
Humidity, %	8.03 ± 0.71
Total carbohydrates, %	67.72 ± 1.14
Crude fibre, %	31.67 ± 1.05
Proteins, %	17.10 ± 0.85
Lipids, %	0.57 ± 0.04
Ash, %	6.58 ± 0.52
Calcium (Ca, mg/100 g)	36.15 ± 0.09
Sodium (Na, mg/100 g)	97.58 ± 0.33
Phosphorus (P, mg/100 g)	86.82 ± 0.32
Potassium (K, mg/100 g)	258.42 ± 1.02
Magnesium (Mg, mg/100 g)	85.74 ± 0.53
Zinc (Zn, mg/100 g)	0.36 ± 0.01
Iron (Fe, mg/100 g)	0.61 ± 0.01
Manganese (Mn, mg/100 g)	0.57 ± 0.01
Copper (Cu, mg/100 g)	0.64 ± 0.01

The L* parameter had a value of 36.89±0.18. A greater value of the L* corresponds to a lighter color. The parameter a* value was 31.71±0.39, and a positive number indicates colors toward red. The vibrant purple color of beetroot is attributed to the presence of betalains, which are pigments water-soluble pigments classified into two main types: betacyanins, (responsible for the red to violet hues) and betaxanthins, which contribute to yellow to orange colors.[14] The positive value of b*(5.16±0.09) suggests a color toward yellow. The other color properties were assessed and the values for Chroma, and Hue angle were determined to be 32.13, and 0.16, respectively. The powder was situated in quadrant I (+a*, +b*) based on the color indices findings. Chroma that shows the strength and degree of saturation of the color were 32.13±0.38. The hue angle value was proportional to the received color indicating the redness of RBP powder since hue angles were lower than 10º.[38] In general, an angle of 0° or 360° signifies a red hue, whereas angles of 90°, 180°, and 270° correspond to yellow, green, and blue hues, respectively.[39] A low hue angle (0.16) corresponds to the characteristic red or purple color, which is expected for betalain-rich sources such as beetroot peels.

Table 1 also presents the proximate composition of RBP powder. Beetroot peel is rich in carbs, protein, and fiber source, making it a valuable nutritional powder. Upon comparing the research findings to the published literature, it becomes evident that there are notable disparities in the physicochemical composition. Šeremet et al.,[30] found similar results for RBP powder therefore, the crude protein level was 18.3 ± 0.2%, the crude fat content was 0.6 ± 0.0%, the dry matter was 90.8 ± 0.0%, the total dietary fiber was 33.6 ± 1.0%, and the soluble sugar content was 12.5 ± 0.2%. The study conducted by Shuaibu et al.,[40] discovered variations in the proximate analysis of beetroot peel. The examination revealed the following composition: moisture content (30.88%), ash content (10.58%), crude fat (3.29%), crude fiber (6.98%), crude protein (4.10%), and carbohydrate (44.17%).

RBP is abundant in minerals such as manganese, sodium, potassium, magnesium, iron, and copper.[41] Alshehry[37] reported that potassium (26.0 mg/g) is the major compound in beetroot powder, followed by sodium (6.26 mg/g) and phosphorus (3.50 mg/g). The observed variations in results could be attributed to differences in geographical origins or genotypes investigated, extraction techniques, and analytical methodologies.[42] Research has demonstrated that RBP is a highly beneficial source of phytochemicals possessing antioxidant properties. These powders can be incorporated into foods as a natural ingredient to effectively reduce the presence of agro-industrial residues.

Characterization of bioactive potential of supplemented beverages

Phytochemical compounds contribute to the development of appealing hues, increased antioxidant capacity, and ultimately improved overall acceptance. Table 2 summarizes the analysis of the phytochemical components, including betalains, flavonoids, and polyphenols, as well as the samples' antioxidant activity and stability throughout a 14-day storage period.

There was a substantial increase($p < .05$) in the amounts of the bioactive components when RBP powder (1, 3, and 6%) was added to the beverage composition. Based on the results, total betalains ranged from 68.22 ± 0.44 to 105.08 ± 0.42 mg/100 g dw, total flavonoids ranged from 154.21 ± 5.15 to 35 l.40 ± 9.09 mg CE/100 g dw and total polyphenols went from 216.41 ± 6.56 to 636.54 ± 10.11 mg

Table 2: Phytochemical characteristics and the antioxidant activity of beverages and stability during 14 days of storage.

Phytochemical Characteristics	Storage Time, (Days)	BC	BRBP1(1%)	BRBP3(3%)	BRBP6(6%)
Total betalains, mg/100 g dw	0	–	68.2 ± 0.4^{aC}	84.5 ± 0.6^{aB}	105.1 ± 0.4^{aA}
	14	–	64.9 ± 0.3^{bC}	80.9 ± 0.5^{bB}	101.1 ± 0.4^{bA}
Total polyphenols mg GAE/100g dw	0	106 ± 7^{aD}	216 ± 7^{aC}	428 ± 8^{aB}	637 ± 10^{aA}
	14	58 ± 6^{bD}	161 ± 5^{bC}	369 ± 7^{bB}	572 ± 9^{bA}
Total flavonoids mg CE/100g dw	0	49 ± 3^{aD}	154 ± 5^{aC}	244 ± 7^{aB}	35l ± 9^{aA}
	14	26 ± 3^{bD}	109 ± 5^{bC}	178 ± 7^{bB}	287 ± 7^{bA}
Antioxidant activity, µmol TE/g dw	0	7.3 ± 0.5^{aD}	16.3 ± 0.4^{aC}	26.7 ± 0.1^{aB}	36.8 ± 0.3^{aA}
	14	5.5 ± 0.3^{bD}	14.2 ± 0.2^{bC}	24.3 ± 0.2^{bB}	34.4 ± 0.3^{bA}

Mean values followed by different superscript lowercase letters in the same column are significantly different ($p < .05$). Mean values followed by different superscript uppercase letters in the same raw are significantly different.

GAE/100 g dw for RBP supplemented beverages. Furthermore, compared to the control sample, all of the samples (BRBP1, BRBP3, and BRBP6) exhibited significantly greater quantities of bioactive compounds($p < .05$). Additionally, their antioxidant activity increased because the RBP-enhanced beverage samples included more bioactive components than the control. According to the findings, the BRBP6 sample had the highest antioxidant activity (36.75 ± 0.31 μmol TE/g dw). The BC control sample had the lowest level of antioxidant activity (7.29 ± 0.53 μmol TE/g dw).

Similarly, Panghal et al.,[43] obtained a probiotic beetroot drink using beetroot juice fermented with *L. rhamnnosus, L. plantarum and L. delbrueckii sb*. The authors reported that total phenols, flavonoids and antioxidant activity increased in the probiotic beetroot drink. Also, Abdo et al.,[44] reported that the beverages enriched with RBP extract and whey proteins had increased bioactive components in a concentration-dependent manner, with higher phenolics and flavonoids compared to the control. The overall levels of betalains, flavonoids, and polyphenols in the samples decreased over time($p < .05$). Expectedly, the DPPH scavenging activity exhibited a decline in all samples after 14 days. However, the activity of BRBP, BRBP3, and BRBP6 remained higher than that of the control. El-Dardiry et al.,[45] also observed that antioxidants levels of functional beverages supplemented with pumpkin pulp decreased somewhat during cold storage.

The antioxidant capacity is also influenced, either positively or adversely, by the interaction between phenolic compounds and whey proteins.[46–48] The reasons for these opposite outcomes include variations in antioxidant tests, protein, and phenolic compound types, and protein-to-phenolic compound ratios.[49] Table 2 shows that RBP powder improves the quality of beverages, as evidenced by the good amounts of betalains and polyphenols. Due to their ability to scavenge free radicals and promote health, antioxidants make the beverage highly beneficial.

The presence of polyphenols and betalains in beetroot peels not only enhances the beverage's health benefits and color but also increases its marketability as a functional, visually appealing, and nutritionally rich food. The polyphenols and betalains enhance the beverage's nutritional profile by incorporating bioactive substances with anti-inflammatory, anticancer, and antidiabetic attributes.[3]

Physico-chemical characterization of supplemented beverages

A physico-chemical analysis was conducted on the nutritional features of the RBP-supplemented beverages, as well as the control sample (Table 3). The incorporation of RBP powder into whey-fruit based beverage significantly enhanced its chemical composition when compared to the control sample. There was a notable variation($p < .05$) in the proximate composition of the beverage samples with and without RBP. The inclusion of RBP induces substantial fluctuations in the levels of total dry matter, fat, carbohydrates, protein, fiber, ash, moisture and energetic value contents.

Furthermore, the addition of RBP to beverage resulted in a reduction in fat and moisture content, while simultaneously leading to an increase in protein, fiber, carbohydrates, ash, and total dry matter, in proportion to the amount of RBP added. The moisture content of formulated beverages ranged from 86.48 ± 0.07 to 81.34 ± 0.03%. The moisture content significantly declines as the quantity of RBP powder increased. This can be attributable to the varying quantities of RBP added to the prepared

Table 3: Physico-chemical characteristics of control and supplemented beverages.

Physical-Chemical Characteristics	BC	BRBP1(1%)	BRBP3(3%)	BRBP6(6%)
Total dry matter, %	13.52 ± 0.03^{d}	15.46 ± 0.02^{c}	16.54 ± 0.03^{b}	18.66 ± 0.04^{a}
Fat, %	0.38 ± 0.04^{a}	0.36 ± 0.02^{a}	0.34 ± 0.03^{a}	0.32 ± 0.05^{a}
Protein, %	2.12 ± 0.06^{d}	2.28 ± 0.04^{c}	2.41 ± 0.03^{b}	2.50 ± 0.08^{a}
Carbohydrates, %	10.47 ± 0.02^{d}	12.12 ± 0.03^{c}	12.98 ± 0.06^{b}	14.89 ± 0.08^{a}
Fiber, %	8.01 ± 0.02^{d}	10.69 ± 0.04^{c}	11.12 ± 0.05^{b}	13.37 ± 0.06^{a}
Humidity, %	86.48 ± 0.07^{d}	84.54 ± 0.06^{c}	83.46 ± 0.05^{b}	81.34 ± 0.03^{a}
Ash, %	0.55 ± 0.04^{d}	0.70 ± 0.02^{c}	0.81 ± 0.03^{b}	0.95 ± 0.02^{a}
Energetic value, Kcal/100 g Kj/100 g	55.15 ± 0.03^{d}	62.39 ± 0.04^{c}	66.26 ± 0.06^{b}	74.28 ± 0.05^{a}
	230.75 ± 0.03^{d}	261.04 ± 0.04^{c}	277.23 ± 0.06^{b}	310.79 ± 0.05^{a}

Values of each physicochemical parameter and sample that do not have the same superscript lowercase letter are substantially different at $p < .05$.

beverages. Our findings were consistent with Bhavsagar et al.,[50] and David and Kumar,[51] who reported moisture content in whey-fruit based drinks from 75.60 to 86.20%.

The protein content of control beverage was 2.12 ± 0.06% and that of beverages containing incremental levels of beetroot peel powder rose from 2.28 ± 0.04 to 2.50 ± 0.05%. The inclusion of the powder leads to a rise in protein levels in the beverages, as demonstrated by a direct correlation between the amount of RBP used and the increase in protein content. BRBP6 had the highest protein content. The results on fiber content show significant differences($p < .05$) among the four analyzed samples. Specifically, the addition of RBP increases the fiber contents of beverages so, BRBP6 had the highest content of 13.37 ± 0.06%. The presence of a significant quantity of dietary fiber and crude protein in beetroot peel powder may be the cause of these results.[30,52]

The mean ash contents in the formulated beverages were found to be 0.70 ± 0.02%, 0.81 ± 0.03%, and 0.95 ± 0.02% for BRBP1, BRBP3, and BRBP6, respectively. The ash contents of the beverages were affected by the inclusion of different quantities of RBP powder. Each of the samples exhibited statistically significant differences from one another($p < .05$). This could be attributed to the inclusion of whey, banana, melon, and almond milk. Several researchers, including Bhavsagar et al.,[50] Hiralal and Raj[53] (2014), and Kamate,[54] have conducted studies on the utilization of fruits and vegetables in the development of whey beverages. Their unified conclusion is that adding fruits and vegetables to whey beverages increases the ash levels.

In the formulated beverages, fat levels decreased with increased RBP powder addition but with no significant differences. The beverages with the highest fat concentration were found in BC (0.38 ± 0.04%) whereas the lowest ash content was observed in BRBP6(0.32 ± 0.05%). In contrast, in terms of the total dry matter, the differences were significant($p < .05$) between the enriched samples (BRBP1, BRBP3, BRBP6). As a result, as the concentration of RBP increases, so does the dry matter. The formulated beverages had varying amounts of carbs (10.47 ± 0.22 to 14.89 ± 0.03%). The quantity of RBP powder increased along with a significant increase in the amounts of carbs. The average energy value for BC was 55.15 ± 0.03 kcal/100 g, whereas for BRBP6 it was 74.28 ± 0.05 kcal/100 g. Researchers such as Dhadage, Shinde, and Gadhave[55] conducted a study where they developed and examined a multigrain snack that was enriched with beetroot pulp (30%). They evaluated both the sensory attributes and chemical characteristics of the snack. The multigrain snacks were found to have elevated levels of proteins, fibers, and minerals, while still maintaining satisfactory sensory qualities. The results of the proximate chemical composition study showed that there were discernible changes when powdered bioactive substances were added to the beverages. The nutritional composition of the beverage samples showed an improvement in quality as compared to the control sample as the RBP concentration increased.

Color evaluation of supplemented beverages

The color significantly impacts consumer approval of dairy products, this attribute is equally important as their shelf life.[56] The color properties of beverage samples were determined by testing their L*, a*, and b* values for first control day and after 14 days of storage (Table 4). Beetroot peel is a plant that is abundant in pigments, including betalains (betacyanin and betaxanthin), and compounds that are phenolics and flavonoids.[2] Figure 7 shows the color change of the beverage samples.

Table 4: Colorimetric attributes of control and RBP-enriched beverages during storage for 14 days.

Samples	Storage Time, (Day)	L*	a*	b*	Chroma	Hue Angle	ΔE
BC	0	81.88 ± 0.36^{aA}	3.03 ± 0.15^{aD}	8.19 ± 0.17^{aA}	8.73 ± 0.10^{aD}	1.22 ± 0.04^{aA}	-
	14	83.57 ± 0.38^{bA}	2.72 ± 0.14^{bD}	10.34 ± 0.19^{bA}	10.69 ± 0.11^{bD}	1.31 ± 0.02^{bA}	-
BRBP1 (1%)	0	38.50 ± 0.15^{aB}	19.04 ± 0.13^{aC}	4.98 ± 0.16^{aB}	19.68 ± 0.14^{aC}	0.26 ± 0.01^{aC}	46.35 ± 0.28^{aA}
	14	40.13 ± 0.17^{bB}	17.11 ± 0.12^{bC}	7.28 ± 0.17^{bB}	18.59 ± 0.13^{bC}	0.40 ± 0.03^{bB}	45.86 ± 0.29^{bA}
BRBP3 (3%)	0	31.24 ± 0.25^{aC}	19.29 ± 0.15^{aB}	3.42 ± 0.12^{aC}	19.59 ± 0.15^{aB}	0.18 ± 0.02^{aC}	53.40 ± 0.25^{aB}
	14	33.92 ± 0.27^{bC}	17.23 ± 0.14^{bB}	5.64 ± 0.15^{bC}	18.13 ± 0.13^{bB}	0.32 ± 0.02^{bC}	51.94 ± 0.27^{bB}
BRBP6 (6%)	0	28.01 ± 0.14^{aD}	20.52 ± 0.18^{aA}	2.51 ± 0.10^{aD}	20.67 ± 0.11^{aA}	0.12 ± 0.02^{aD}	56.92 ± 0.24^{aC}
	14	30.11 ± 0.22^{bD}	17.78 ± 017^{bA}	4.64 ± 0.13^{bD}	18.38 ± 0.12^{bA}	0.26 ± 0.03^{bD}	55.83 ± 0.25^{bC}

Color parameter variation over time is highlighted by small letters. The color differences between the samples are highlighted by capital letters. Values that share a lower/uppercase letter are not significantly different(p>.05).

The data analysis of the color parameters revealed that the addition of RBP had a significant (p<.05) impact on the beverages' color. More precisely, the experimental beverages exhibited the lowest levels of lightness(L*), and yellowness(b*) and the highest levels of redness(a*).

When beetroot peel powder concentrations increased, the L*values of the enriched samples declined. The maximum L* value was discovered in control samples (81.88 ± 0.36), while in enhanced samples (BRBP6) had the lowest value (28.01 ± 0.14). A progressive decrease in lightness value was observed with an increase in the level of beetroot peel powder. The decrease in L* values of beverages indicated a diminished luminosity, brightness, and consequently a darker appearance of the beverages. These findings are in line with those of Alshehry,[37] on cupcake with 7.5–10% of beetroot powder. Meanwhile, redness was gradually increased significantly(p<.05) in beverage from 19.04 ± 0.13 at level 1% substituted RBP powder to 20.52 ± 0.18 at substituted level 6% compared with control sample was 3.03 ± 0.15, respectively.

The yellowness value(b*) of control beverage was 8.19 ± 0.17 and beverage enhanced with different levels of beetroot peel powder decreased from 4.98 ± 0.16 to 2.51 ± 0.10 for BRBP1 and BRBP6 respectively. The higher degree of addition of beetroot peel powder led to a considerable decrease in the hue value. Chroma parameter, the measure of color saturation followed the trend of parameter a*. Beverages with 6% beetroot peel powder presented higher total color difference values due to the larger amount of beetroot added (34.81 ± 0.24 for BRBP6) while lower values were observed for the control sample (82.35 ± 0.30).

When compared to the BC sample, the RBP-enriched beverages showed noticeably higher a* and b* values at the beginning and end of storage. Changes in the L*, a*, and b* values of the beverage samples were observed to be statistically significant after 14 days of storage(p<.05). All of the samples' redness dropped while their lightness and yellowness increased after storage. The beverage containing 1% RBP had the highest L* and b* values (BRBP1) after storage, and, aside from the control sample, sample BRBP1 also had the lowest a* values. Similar to the values in this study, Abdo et al.,[44] has found that the redness of the beverages enriched with beetroot peel was reduced during storage. The authors conclude that the treatments displayed a reasonable level of redness stability throughout storage.

ΔE is the feature of total color change ranged from 46.35 to 56.92 for the sample at initial moment and 45.86 to 55.83 for the sample at 14 days of storage. The addition of RBP powder had significant effect ($p < .05$) on ΔE values of control and RBP-enriched beverages. However, increase in the ratio of RBP from 1 to 6% significantly increase the ΔE. One key reason for estimating total color difference (ΔE) is to determine whether the color difference between samples with added RBP powder and the control was perceptible to the human eye. Visual color differences can be categorized by total color difference as follows: imperceptible (0–0.5), moderately perceptible (0.5–1.5), perceptible (1.5–3.0), distinctly visible (3.0–6.0), and significantly different (6.0–12.0).[57,58] Chroma that shows the strength and degree of saturation of the color were at highest in BRBP6 sample and lowest in control sample. The hue angle value was proportional to the received color indicating the redness of RBP powder and RBP-enriched beverage since hue angles were lower than 10º.[38]

Mineral profile of supplemented beverages

Table 5 displays the mineral contents analysis for the prevailed samples from beverage without RBP and supplemented beverage with RBP used in three different ratios (1%,3% and 6%). The amounts of mineral contents increased with the increased amount of RBP powder. The differences between the beverage samples in terms of the quantity of K, Mg, Na, P, Ca, Fe, Mn, Cu and Zn were found to be statistically significant($p < .05$). The RBP-enriched beverage is a good source of minerals, particularly potassium, magnesium, sodium, phosphorus, and calcium. The potassium (K) content was higher compared to other minerals, ranging from 127.06 ± 0.30 to 143.18 ± 0.51 mg/100 g, followed by Mg, Na, P, Ca, Fe, Mn, Cu and Zn.

Table 5: Mineral composition of analyzed beverage samples.

Parameter	BC	BRBP1(1%)	BRBP3(3%)	BRBP6(6%)
Calcium (Ca, mg/100 g)	29.03 ± 0.02^{c}	29.32 ± 0.04^{c}	30.32 ± 0.11^{b}	31.66 ± 0.36^{a}
Sodium (Na, mg/100 g)	42.63 ± 0.11^{d}	43.75 ± 0.32^{c}	44.78 ± 0.07^{b}	48.02 ± 0.13^{a}
Phosphorus (P, mg/100 g)	30.40 ± 0.05^{d}	31.50 ± 0.44^{c}	32.82 ± 0.25^{b}	35.40 ± 0.38^{a}
Potassium (K, mg/100 g)	127.06 ± 0.30^{d}	129.22 ± 0.42^{c}	133.50 ± 0.41^{b}	143.18 ± 0.51^{a}
Magnesium (Mg, mg/100 g)	66.28 ± 0.66^{c}	67.06 ± 0.10^{bc}	68.40 ± 0.05^{b}	70.87 ± 1.09^{a}
Zinc (Zn, mg/100 g)	0.07 ± 0.01^{b}	0.08 ± 0.01^{ab}	0.09 ± 0.01^{ab}	0.10 ± 0.03^{a}
Iron (Fe, mg/100 g)	1.42 ± 0.01^{c}	1.45 ± 0.01^{c}	1.61 ± 0.02^{b}	1.79 ± 0.03^{a}
Manganese (Mn, mg/100g)	1.13 ± 0.01^{c}	1.19 ± 0.02^{c}	1.33 ± 0.04^{b}	1.50 ± 0.05^{a}
Copper (Cu, mg/100 g)	0.41 ± 0.01^{b}	0.42 ± 0.01^{b}	0.43 ± 0.01^{b}	0.47 ± 0.02^{a}

Superscripts with different letters within a row are significantly(p<.05) different.

RBP-supplemented beverage samples contain additional essential elements including calcium and phosphorus. Notably, the highest concentration of Ca was discovered in the BRBP6 (31.66 ± 0.36 mg/100 g) sample with 6% RBP powder. The results of the statistical analysis showed that there were statistically significant differences in the amount of P in each beverage sample. The maximum amount of P was found for the beverage with 6% RBP powder, followed by the beverage with 3% and 1% RBP powder. The control sample showed the lowest P amount (30.40 ± 0.05 mg/100 g). Furthermore, Mg and Na concentrations were highest in the beverage enhanced with 6% RBP powder, followed by the beverage with 3% and 1% RBP powder, and lowest in the control.

Significant changes ($p < .05$) were observed in the iron, manganese, copper, and zinc contents between the samples containing RBP powder and the control sample. Although the values of these elements are lower compared to the previously mentioned elements, the incorporation of 1%, 3% and 6% of RBP (BRBP1, BRBP3 and BRBP6) led to a higher content of Fe, Mn, Cu, and Zn as compared to BC. The study's findings showed that adding powdered RBP raw material to whey-fruit based beverage increased the quantity of minerals in the final product from 6.92 to 42.86%. As can be seen in Table 1, freeze-dried beetroot peel powder is rich in K, Na, P, Mg, and Ca. Hence, RBP powder is suitable for incorporation as a bioactive ingredient in foods.

Goosen et al.,[59] found that the fruit juice of the indigenous African sour plum had higher levels of K (525 mg/100 g), Fe (170 mg/100 g), and lower levels of Ca (3.04 mg/100 g), Mg (15.9 mg/100 g), P (24.6 mg/100 g), Na (0.82 mg/100 g). The mineral contents of whey-mango-based mixed beverages indicated higher amounts of calcium (41.37 ± 2.72 mg/100 mL), and phosphorus (49.86 ± 1.25 mg/100 mL) and lower levels of iron 0.15 ± 0.02 mg/100 mL).[60]

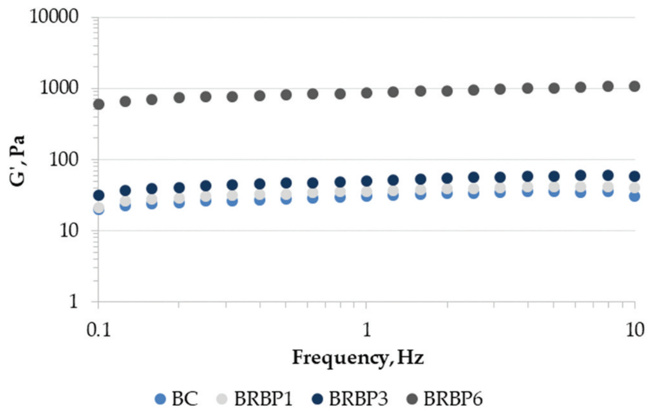
Rheological properties of the supplemented beverages

Low-amplitude oscillatory sweeps and rotational shear tests were performed to assess the impact of RBP powder addition on the rheological properties of the beverage. The results of the dynamic sweep test, as shown in Table 6 and Figure 1. The upper limit of the LVR was identified at 1–3% strain, suggesting rather good resistance of the beverages to the applied strain. The RBP concentration increase from 0 (BC) to 3% (BRBP3) resulted in significant increase of the $\gamma G'$-G'' (p < .05), suggesting the delayed beginning of the flow (flow point), as the result of the destruction of internal structure of the beverages.

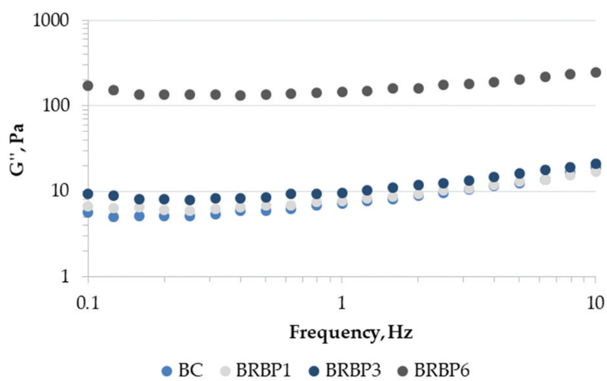
Table 6: Rheological parameters of the beverages supplemented with different amounts of RBP.

	Beverages			
Rheological parameter	BC	BRBP1	BRBP3	BRBP6
Strain sweep test				
$\gamma_{G'-G''}$, %	24.38 ± 3.86^{c}	42.48 ± 0.81^{b}	70.50 ± 0.05^{a}	-
Peak hold test				
η_{100}, Pa·s	0.052 ± 0.01^{c}	0.053 ± 0.01^{c}	0.116 ± 0.01^{b}	0.398 ± 0.03^{a}

$\gamma G'$-G'' - strain corresponding to G'–G'' crossover, where the flow begins. η100 – viscosity measured at shear rate of 100 s-1. Different superscript letters assigned to the mean values on the same row denote significant differences with ANOVA and Tuckey post-hoc test (p<.05).



(a)



(b)

Figure 1. Evolution of the (a) storage modulus (G′) and (b) loss modulus (G″) under frequency sweep test.

No G'-G" crossover point was observed for the BRBP6 sample. The improved stability of the beverages including higher amounts of RBP might be explained by the higher fiber content (Table 3).

The evolution of the G' and G" as a function of frequency is reported in Figure 1. Regardless of the RBP supplementation level, G' values prevailed over the G',' suggesting the dominant elastic behavior of the samples.[61] As expected, the addition of increasing amounts of RBP powder led to the rise of the G' values, within the entire frequency range.

In case of all tested samples the shear stress values increased with the shear rate, indicating the non-Newtonian rheological behavior. Our results comply with de de Oliveira Ribeiro et al.,[62] who tested the rheological behavior of the smoothy beverages prepared with strawberry and banana. The pseudoplastic shear-thinning behavior of the investigated beverages can be observed in Figure 2. Because of the molecules and particles alignment and molecules structure alteration induced by the hydrodynamic forces,[62] the gradual destruction of the samples' network occurred while shearing at increasing rates, resulting in the apparent viscosity decrease (Figure 2).

The influence of the composition on the viscosity of the beverages was checked while running peak hold test at shear rate of 100 s^{-1}, such as to comply with the conditions applied in industrial juice processing.[62] No significant differences were noticed between BC and BRBP1 samples, but the RBP increase from 1% to 6% caused the significant raise of the viscosity values ($p < .05$), from 0.053 ± 0.01 Pa·s to 0.398 ± 0.03 Pa·s (Table 6). A time independent pseudoplastic behavior was noticed in case of all tested beverages.

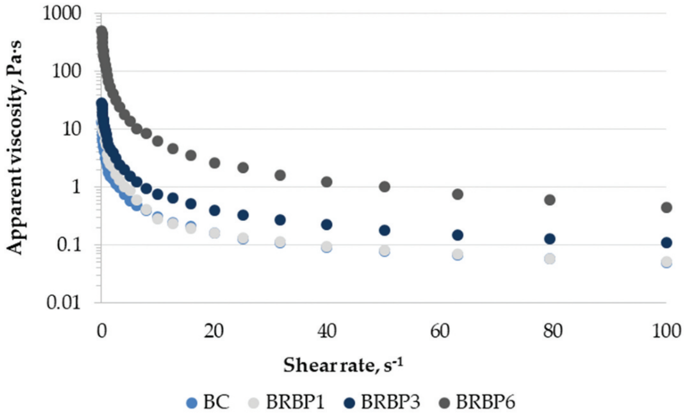


Figure 2. Evolution of the apparent viscosity of the beverages under forced flow conditions.

Microstructure analysis of supplemented beverages

The microstructure and surface characteristics of the RBP powder and the designed beverages was observed by scanning electron microscopy (Figure 3). The RBP powder (Figure 3a,b) exhibits a stratified fibrous structure, characterized by distinct gaps between the fibers and pores inside them. Based on the assessment of the visual fields, amorphous areas are prevalent in the RBP powder. The RBP sample

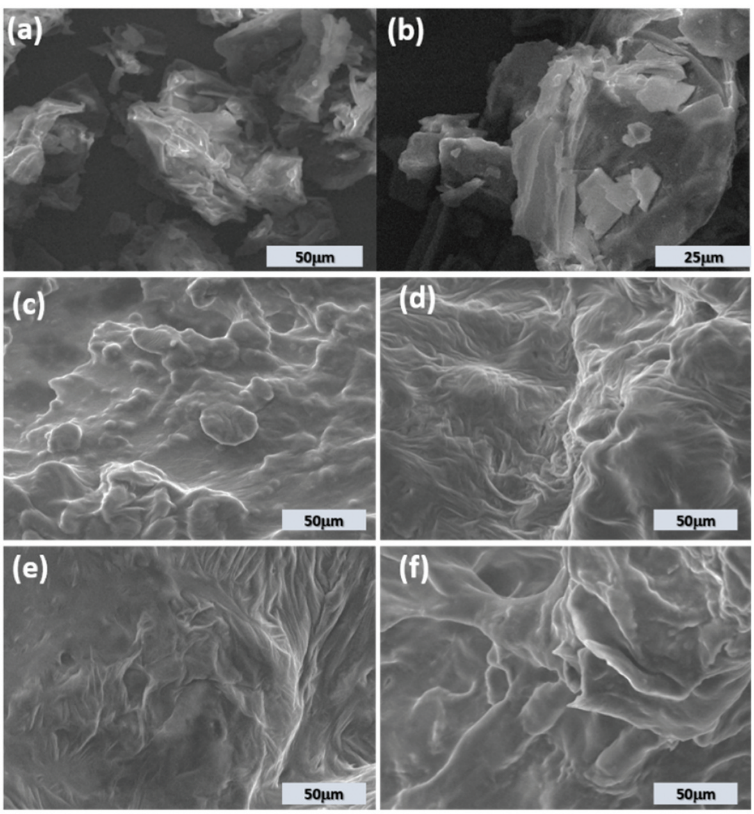


Figure 3. SEM micrographs of beverage enriched with RBP powder: (a,b) RBP powder, (c) BC beverage without powder addition; (d) BRBP1, (e) BRBP3 and (f) BRBP6 beverage with 1,3% and 6% of RBP powder, respectively.

consisted of granular aggregated structures composed of shaped medium-sized granules. The images of the peel of beetroot show that the by-product resemble a dry, compact powder. The results correspond with the observations of Šoštaric et al.,[63] on microstructure of beetroots by-products.

The rheological and functional features of dietary fibers are influenced by its microstructure, as documented Gu et al.[64] These qualities include viscoelasticity, and water-holding capacity, which are directly linked to the density of its network structure. The beverages incorporated with RBP powder had a distinct microstructure from the control beverage (Figure 3c). Observations of the beverages microstructure using scanning electron microscopes showed the arrangement of the beetroot peel fibers and the porosity of the materials.

Figure 4a shows EDX spectrum of the EDX spectrum of beetroot powder (RBP) revealed the presence of alkali elements (potassium, sodium) and alkaline earth ions (magnesium) on surface which is accordance with Table 1. In the EDX spectrum of the supplemented beverages the presence of calcium and phosphorus is noted (Figure 4 b-e). At a 500× magnification, notable variations in surface features were detected amongst analyzed samples. The BC exhibited a different appearance, and with a magnification of 50 μm, the characteristic aggregates of proteins were visible. Fiber particles can be noticed in the experimental beverages BRBP1, BRBP3 and BRBP6. The structure of RBP-supplemented beverages had dense and compact surfaces with no obvious porous structure. The samples' electron microscopy images of enriched beverages showed loose and agglomerated components in a heterogeneous composition.

The protein aggregates network in the samples with fiber addition appears to be distinct from that of BC beverage, since it exhibits an open structure with larger aggregates. In other studies, scanning electron microscopy of cellular material from mango and passion fruit smoothies revealed large aggregations of cells.[65] The dietary fiber of RBP has the ability to bind water and raise the water-holding capacity of beverage samples, which improves protein network connections and the structure.[66]

Microbial quality of supplemented beverages

Table 7 display a summary of the microbiological analysis for the prevailed samples from beverage without RBP and enriched beverage with RBP used in three different ratios (1%, 3% and 6%) and indicate that the product was safe for consumption for up to 14 days when stored at 4°C. The microbial population of bacteria, yeasts, and molds in the beverages showed an increasing trend over time, regardless of the kind of beverage, as indicated in Table 7. On the initial day, right after the heat treatment of the beverages, the bacterial count was minimal, with average concentration values ranging from 1.23 to 1.92 log CFU/mL.

All of the microorganism groups' population growth followed a linear trend throughout the storage period. Conversely, the RBP-containing beverages had lower levels. The beverages had a small increase in the number of bacteria after storage, even with their low pH levels (4.89 ± 0.02) and sterile manufacture and storage at 4°C. The average concentration values in the beverage that had RBP added in three different amounts (1%, 3%, and 6%) went down from 3.34 log CFU/mL to 2.63 log CFU/mL as the percentage of RBP increased. After 14 days of storage, the mean concentration value for the control sample was 3.39 log CFU/mL.

According to Silveira et al.,[67] beverages with a maximum value of 4.87–5.17 log CFU/mL of aerobic mesophilic bacteria and no coliform growth are considered safe to drink. All beverage samples showed pathogen absence for coliforms, *Escherichia coli*, and coagulase-positive staphylococci (*Staphylococcus aureus*). The absence of Enterobacteriaceae indicate that no fecal contamination of the beverages was noticed.

The increase in yeast and mold counts in our study with storage time was not surprising as the beverages were produced by mixing ingredients and with pasteurization at a low temperature (65 °C), which aligns with the studies reported by Nedanovska et al.[68] (2022) and Sakhale et al.[69] After being stored for 14 days at 4°C, similar to bacteria, yeasts also showed lower average concentration values as

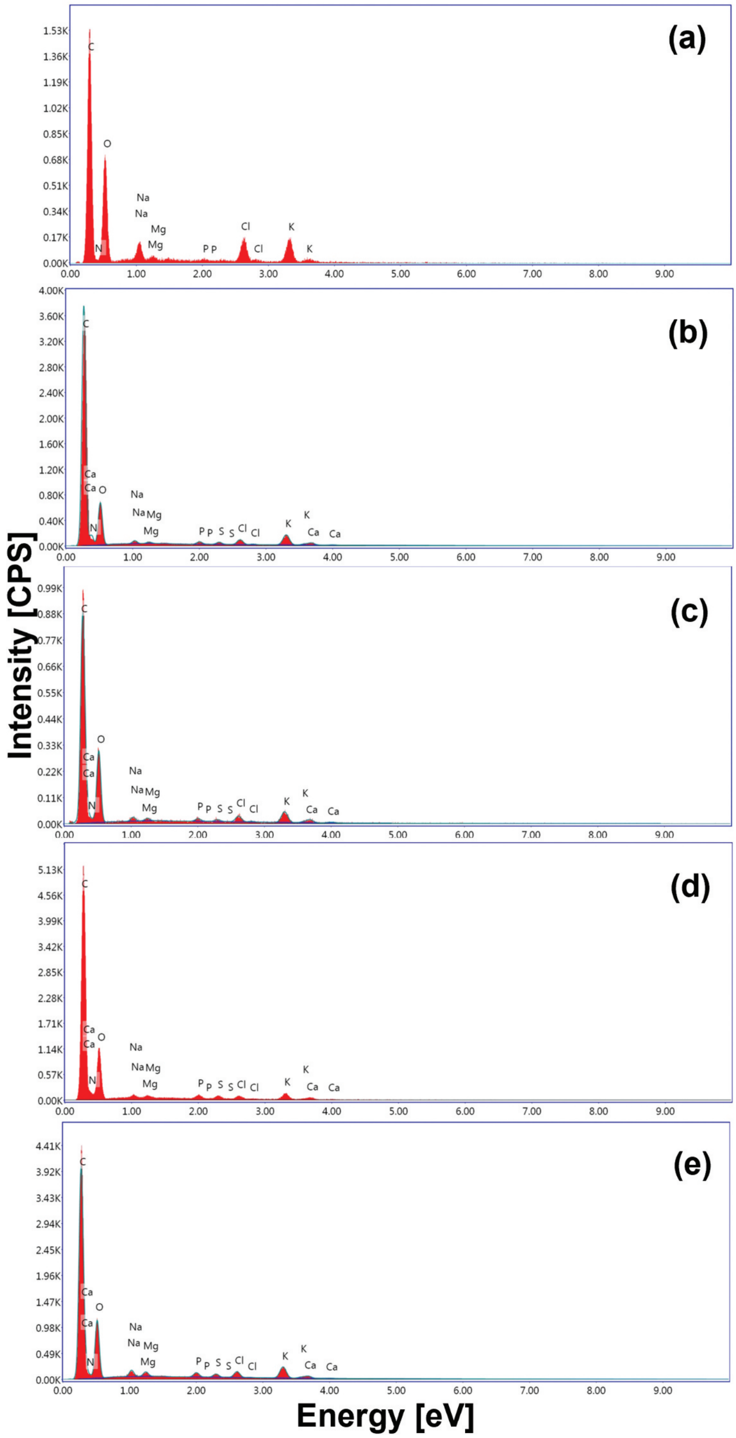


Figure 4. EDX analysis of RBP (a), BC(b), BRBP1 (c), BRBP3 (d) and BRBP6 (e)

Table 7: Microbial quality of the analyzed beverage samples.

Parameter	Storage Time, (Day)	BC	BRBP1(1%)	BRBP3(3%)	BRBP6(6%)
TAB, log CFU/mL	0	1.23 ± 0.39^{aB}	1.71 ± 0.17^{aB}	1.87 ± 0.33^{aB}	1.92 ± 0.16^{aB}
	14	3.39 ± 0.27^{aA}	3.34 ± 0.11^{aA}	2.81 ± 0.15^{bA}	2.63 ± 0.18^{bA}
Yeast, log CFU/mL	0	0.93 ± 0.20^{aA}	1.22 ± 0.13^{aB}	1.29 ± 0.31^{aA}	1.35 ± 0.19^{aA}
	14	3.09 ± 0.13^{aB}	2.49 ± 0.23^{aA}	1.69 ± 0.41^{bA}	1.48 ± 0.14^{bA}
Moulds, log CFU/mL	0	0.43 ± 0.10^{cB}	0.90 ± 0.16^{bB}	1.18 ± 0.21^{abB}	1.34 ± 0.09^{aB}
	14	1.94 ± 0.10^{aA}	2.08 ± 0.27^{aA}	2.25 ± 0.19^{aA}	2.18 ± 0.20^{aA}
Coliforms, log CFU/mL	0	Not detected	Not detected	Not detected	Not detected
	14	Not detected	Not detected	Not detected	Not detected
Escherichia coli	0	Not detected	Not detected	Not detected	Not detected
	14	Not detected	Not detected	Not detected	Not detected
Coagulase-positive staphylococci	0	Not detected	Not detected	Not detected	Not detected
	14	Not detected	Not detected	Not detected	Not detected

Within a row, mean values with different superscript lowercase letters show a significant difference (p<.05). Within a single column, the mean values denoted by superscript uppercase letters exhibit significant variation.

the RBP concentrations in beverages increased (3.09 log CFU/mL in the control sample vs. 1.48 log CFU/mL in the BRBP6 sample). The historical utilization of red beet as a medicinal plant for treating contagious illnesses, as well as the research linking red beet extracts to antioxidant and antimicrobial properties, establish a foundation for investigating its potential as an antimicrobial agent.[70] Therefore, RBP powder has potential as an effective antibacterial agent and requires additional research.

Due to the linear growth of microorganisms, pasteurization of the enriched beverage by conventional or novel methods is required to achieve better preservation for the beverages.

UV treatment is commonly used to reduce microbial loads on various food ingredients,[71] ensuring the RBP powder is free from harmful bacteria or other microorganisms before being added to the beverage. UV treatment may indeed have a positive impact on the decontamination of RBP powder, which can subsequently enhance the microbial safety of formulated beverages and lower the risk of introducing unwanted microorganisms that could spoil the beverage or pose health risks.

The use of UV laser decontamination can pose challenges for small and medium-sized enterprises (SMEs), many of which may not have access to such advanced and expensive technologies. However, there are more affordable alternatives for RBP decontamination such as heat treatment, steam sterilization, or other methods (microwave treatment, high-pressure processing) that are more feasible for SMEs. SMEs could also explore partnerships with larger suppliers to determine if the microbial load on the RBP could be managed by them, reducing the need for in-house decontamination by smaller manufacturers.

Sensorial analysis of supplemented beverages

Figure 5 shows the average results that were acquired from the sensory examination. Various sensory properties, including color, odor, appearance, consistency, taste, flavor, aftertaste and overall impression, were analyzed.

The whey-fruit-based beverages that contained 1%, 3%, and 6% RBP powder showed higher ratings for sensory characteristics in comparison to the control. For the product to be preferred by consumers, the color and appearance must be uniform. In evaluating the appearance and color of the whey-based beverage supplemented with RBP powder (at 1%, 3%, and 6% ratios), the panelists focused on the color intensity and brightness, uniformity of color, clarity, or opacity, as well as the comparison between samples with different RBP concentrations. Each sample had a slightly varied color and appearance, but the most appreciated was the whey-based beverage with 3% beetroot peel powder added. BRBP3 gained the highest scores for color and all the other attributes and was the most acceptable whey-based beverage.

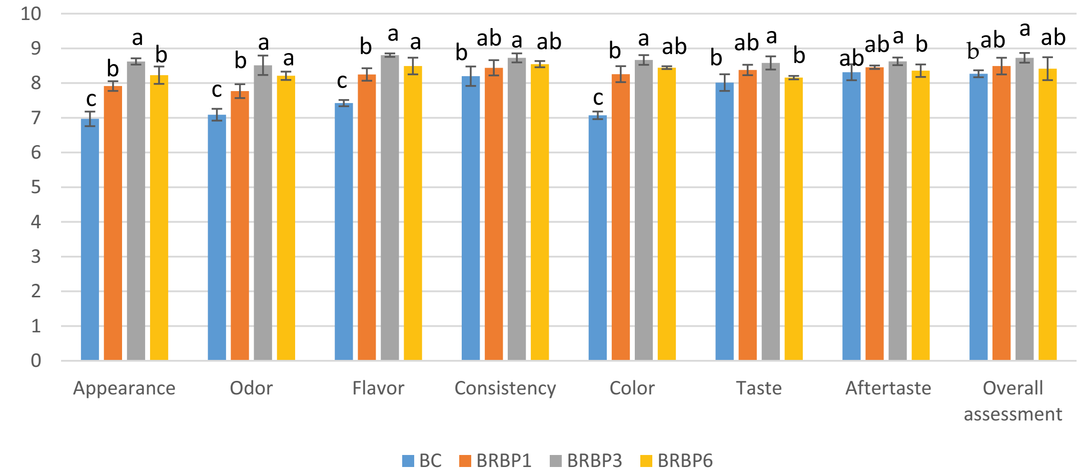


Figure 5. Comparative chart of the sensory attributes specific to supplemented beverages (averages with different letters "a," "b," and "c" in the columns signify statistically significant differences (p < .05)). BC—beverage without powder addition; BRBP1, BRBP3 and BRBP6—beverage with 1,3 and 6% powder of RBP.

Texture is a crucial factor in food products that significantly influences consumer approval.[28] The panelists rated RBP powder-containing beverages higher due to their superior consistency compared to BC. The taste sensory evaluation results indicated that the enhanced beverages exhibited a sweet, distinct taste compared to the control. The inclusion of 6% RBP had a significant impact on the odor and flavor of the beverage ($p < .05$). Overall, the flavor and odor scores remained higher than the acceptable limit, with a score of > 7.0 being achieved. Similarly, there was a noticeable variation in the taste and aftertaste attribute ratings among the beverages. The beverages containing beetroot peel powder were assessed to have a well-balanced taste, odor, and flavor. Consequently, since the panelists did not notice any detrimental effects on the attributes of appearance, color, odor, texture, taste, or overall impression, RBP may be employed as a color-natural additive in whey-based beverages. Similar data dealing with the development of beetroot and whey-fruit-based beverages and their quality evaluation were reported by Njoya et al.;[72] Abdo et al.,[44] Nedanovska et al.,[68] and Ashraf et al.[73]

The Principal Component Analysis (PCA) analysis biplot (Figure 6) provides a distinct visualization of the variations and connections between the assessed sensory attributes and the batches of beverages. The characteristics located in the upper-right quadrant are represented by aftertaste, taste, and overall assessment were positively presented in the same quadrant in the first axis (F1), indicating a positive correlation. Also, color, odor, appearance, consistency, and flavor have positive contributions on axis F1. The positioning of these sensory characteristics indicates that BRBP3 (closest to these characteristics and positioned on the positive side of the Biplot) is perceived favorably by consumers in terms of taste and overall assessment. The two axes explained 84.51% of the total variance. The BC was neutral since all the sensory attributes were correlated only to the same axis(F1). By analyzing the PCA findings, we can observe that BRBP3 emerged as the frontrunner in terms of consumer preferences. It was characterized by a sweet taste, fruity flavor, and an attractive appearance.

In general, it was noted that all the samples supplemented with beetroot peel powder received positive sensory assessments. The addition of RBP powder resulted in a reddish-purple hue (Figure 7) in the experimental beverages, which was well favored by the panelists. The panelists concluded that incorporating 3% RBP into beverages was the most enjoyable enhancement and yielded superior outcomes compared to other beverage treatments. Our result suggests that RBP can be added at a 3% concentration; higher concentrations (>3%) may affect flavor and taste.

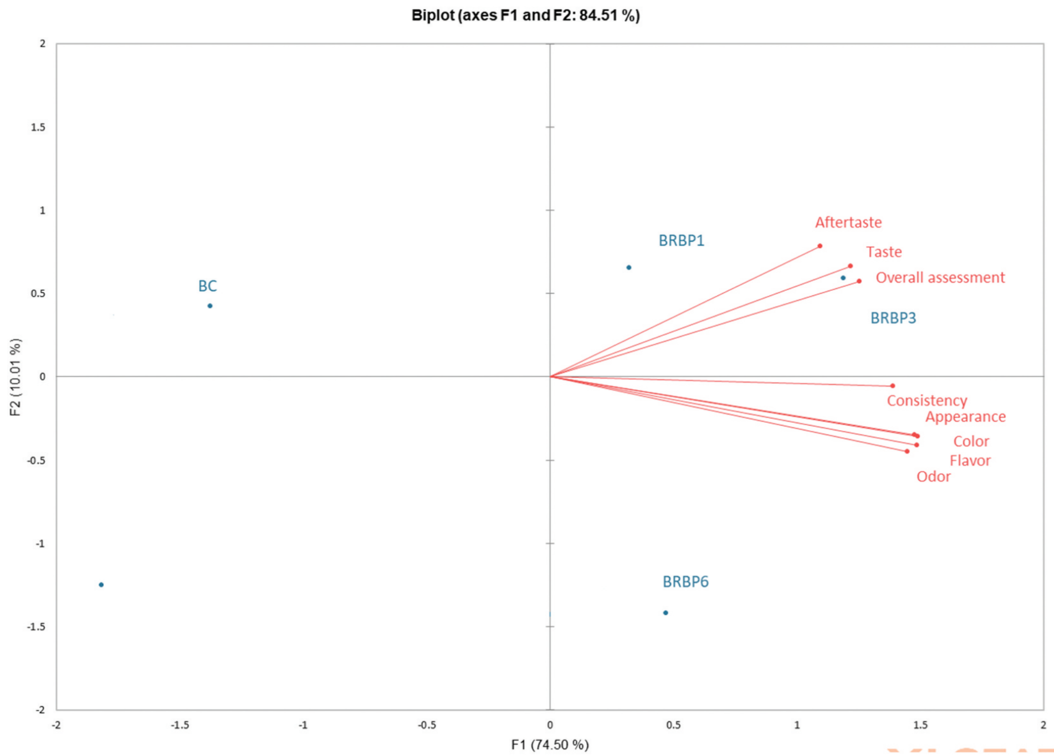


Figure 6. Representation of correlations between sensory attributes using Principal Component Analysis (PCA).

Figure 7. Beverage samples with different percentages of RBP powder: BC (control), 1% (BRBP1), 3% (BRBP3), and 6% (BRBP6).

Conclusion

The findings of this study highlight the capacity of RBP to be utilized in the development of whey-fruit -based beverages. Adding vegetable by-products to whey could be a promising choice for creating nutritious beverages based on whey that has optimal sensory qualities. The potential of by-products obtained from the industrial processing of beetroots can be explored as an alternative to synthetic colors and as a source of antioxidants. The findings indicated that the RBP extract serves as a significant source of bioactive compounds possessing potent antioxidant properties. The inclusion of RBP powder enhanced the nutritional content and physical qualities of the final product by enhancing antioxidants, color, and sensory characteristics among other benefits. Supplementation with increasing amounts of RBP resulted in beverages with higher viscosity and better resistance to flow. After sensorial analysis, it was concluded that the BRBP3 whey-fruit-based beverage sample with 3% RBP powder had the best formulation. Overall, incorporating RBP powder as a natural component in whey-fruit-based beverages offers a favorable prospect for enhancing nutritional value and advocating for environmentally friendly food processing methods. The results highlight the capacity of RBP to be employed in diverse food items, enhancing health advantages and aiding in the development of novel and eco-friendly food solutions. Before industrial-scale production, further research is required to determine the digestion-related bioavailability of bioactives, regarding shelf life enhancing quality by utilizing stabilizers and opaque packaging, improving process conditions, and conducting a clinical trial to guarantee safety and assess the functional advantages of the beverage for human health.

Additionally, the potential limitations of the study are: short storage stability evaluation, lack of nitrate content measurement and the Implications for the RBP-enriched beverage, explore the interactions between whey proteins and polyphenols and bioavailability of polyphenols and betalains during digestion. Addressing these gaps in future research would provide a more comprehensive understanding of the potential and bioavailability of RBP-enriched whey beverages.

Disclosure statement

No potential conflict of interest was reported by the author(s).

CRediT authorship contribution statement

The concept for the manuscript was developed by all authors, and they all helped with topic and subsection structure, proofreading, and editing. Gabriela Râpeanu, Corresponding author: Conceptualization, Investigation, Supervision, Validation, Writing–original draft. Florina Stoica: Conceptualization, Investigation, Writing–original draft, Writing–review & editing. Iuliana Aprodu, Roxana Nicoleta Rațu: Writing–original draft, Resources, Visualization. Florin Daniel Lipșa; Iuliana Motrescu; Irina Gabriela Cara: Supervision, Writing–original draft, Visualization. Denis Țopa, Gerard Jităreanu: Supervision, Methodology, Investigation.

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