

Development and Characterization of a Functional Whey-Based Beverage Enriched with Encapsulated Elderberry (*Sambucus nigra*) Anthocyanins, *Bacillus coagulans* GBI-30, and Inulin

Elif Büşra Özgür

Burdur Mehmet Akif Ersoy University

Ahmet Hulusi Dinçoğlu

adincoglu@mehmetakif.edu.tr

Burdur Mehmet Akif Ersoy University

Mustafa Özgür

Burdur Mehmet Akif Ersoy University

Article

Keywords: Anthocyanin, *Bacillus coagulans* GBI-30 6086, Elderberry, Inulin, Whey

Posted Date: November 22nd, 2025

DOI: <https://doi.org/10.21203/rs.3.rs-8026920/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Additional Declarations: No competing interests reported.

Version of Record: A version of this preprint was published at Scientific Reports on June 26th, 2026. See the published version at <https://doi.org/10.1038/s41598-026-58405-1>.

Development and Characterization of a Functional Whey-Based Beverage Enriched with Encapsulated Elderberry (*Sambucus nigra*) Anthocyanins, *Bacillus coagulans* GBI-30, and Inulin

Elif Büşra Özgür¹, Ahmet Hulusi Dinçoğlu^{2*}, Mustafa Özgür²

¹ Department of Food Hygiene and Technology, Institute of Health Sciences, Burdur Mehmet Akif Ersoy University, Burdur 15100, TURKEY

² Department of Nutrition and Dietetics, Faculty of Health Sciences, Burdur Mehmet Akif Ersoy University, Burdur 15100, TURKEY

Corresponding author: Ahmet Hulusi Dinçoğlu, adincoglu@mehmetakif.edu.tr

Abstract

This study aims to examine the use of encapsulating elderberry fruit anthocyanins with whey protein isolates in *B. coagulans* (BC30) and whey beverages with additional inulin. Anthocyanins were extracted from elderberry fruit and combined with whey protein isolate to create 6 unique whey beverages that contained BC30 and inulin. Control group K, anthocyanin-containing group A, BC30-containing group B, BC30 and inulin-containing group C, BC30 and anthocyanin-containing group D, BC30, anthocyanin, and inulin-containing group E were determined. These beverages were stored at 4°C for 28 days, and the determination of anthocyanin content in the beverages, determination of anthocyanin stability, microbiological, physicochemical, and sensory analyses were performed. The total amount of anthocyanin was found to be 174.8 mg L⁻¹ after anthocyanin extraction, and the encapsulation efficiency was 50.46%. The group B had the most TMAB on 28th day of analysis. The TPAB counts of their beverages were discovered to be 4.52-4.63 log₁₀ CFU mL⁻¹ on the first day of analysis. The group with the lowest BC30 count on the first day of analysis of the beverage groups (B, C, D, and E) with BC30 added is group D, with 7.1 log₁₀ CFU mL⁻¹. The group with the highest number of BC30 is group B with 7.83 log₁₀ CFU mL⁻¹. The anthocyanin-containing groups (A, D, and E) had radical scavenging activity values of 51.35% ± 19.4%, 60.21% ± 15.7%, and 60.63% ± 15.2%, respectively. Consequently, it is anticipated that the created whey beverages will provide the food sector with crucial market data.

Key words: Anthocyanin, *Bacillus coagulans* GBI-30 6086, Elderberry, Inulin, Whey

Introduction

Sambucus nigra L., sometimes known as the European elderberry, is one of the little fruits that grows wild or is cultivated for production in the Northern Hemisphere with the broadest application area (Ferreira et al., 2022). Due to its expanded usage regions and simplicity of production, elderberry (*Sambucus nigra* L.), which has been used as a medicinal plant for hundreds of years, has started to find wider cultivation sites (Charlebois et al., 2010). Elderberry production has increased, particularly

in the last few years, largely because of the COVID-19 pandemic, owing to the strong demand for goods based on elderberries and dietary supplements (Uhl et al., 2022). The global elderberry market is experiencing a period of substantial growth, with projections indicating a surge of \$389.8 million on a global scale from 2023 to 2028, at a compound annual growth rate of 8.8% (Cai et al., 2024). This will lead to the processing of elderberries, releasing a significant amount of waste (pulp), which will present a variety of opportunities for use. Elderberries are mostly composed of anthocyanins, a polyphenolic composition with a strong antioxidant potential. Elderberry juice and polar extracts contain the primary anthocyanins from elderberries, cyanidin-3-glucoside and cyanidin-3-sambubioside (Vlachojannis et al., 2015). Elderberries are also a great source of procyanidins, phenolic acids, and flavanols. Flavonoids, including kaempferol, astragalin, quercetin, quercetin-3-O-glucoside, routine, isoquercitrin, and hyperoside, as well as phenolic acids, gallic acid, and gentisic acid, are particularly abundant in elderberry blooms (Domínguez et al. 2020). Given the absence of any substantiated reports of deleterious effects resulting from the consumption of *S. nigra* ripe fruit or fruit extract, and the absence of any known potentially harmful compounds at any reasonable dosage, it is considered safe for use as a temporary or continuous dietary supplement (Porter and Bode, 2017).

B. coagulans is a gram-positive, facultative anaerobic, nonpathogenic, spore-forming, lactic acid-producing bacterium. The optimum growth temperature of *B. coagulans*, a heat-resistant bacterium, is 35-50°C, and the optimum growth pH is 5.5-6.5 (Majeed et al., 2016). Some strains of *B. coagulans* have been reported as facultative anaerobic, thermophilic bacteria that can grow at pH 6.2 and 60–65°C (Benson et al., 2012). *B. coagulans* improves the preservation of food when provided particularly because it produces lactic acid. Some strains not only produce lactic acid but also thermostable α -amylase (De Clerck et al., 2004). *B. coagulans* is therefore a significant industrial bacterium. The US Food and Drug Administration (FDA) has recognized *B. coagulans* as Generally Recognized as Safe (GRAS) (FDA, 2016). A dose as high as 9.52×10^{11} CFU was demonstrated to be well tolerated and safe for a 70 kg individual in an *in vivo* research assessing the safety of *B. coagulans* (Endres et al., 2009). Furthermore, *B. coagulans* genome study has demonstrated that no other genes with possible safety concerns were found, and genes linked to antibiotic resistance in this species cannot be readily transmitted to other bacteria (Salveti et al., 2016). Today, there are many types of functional foods on the market that contain *B. coagulans*, such as pasta, chocolate, and ice cream. This is mainly because *B. coagulans* is a spore-forming bacterium that can remain stable and viable in functional foods, unlike other probiotic bacterial strains (Majeed et al., 2016). Spores of *B. coagulans*, for instance, resist high-temperature food preparation, including boiling and heating. Because of this, *B. coagulans* is the perfect option for creating beneficial goods (Konuray and Erginkaya, 2018). The

preferability of the products is increased by the fact that the inclusion of *B. coagulans* does not adversely affect their sensory or nutritional qualities (Kobus-Cisowska et al., 2019).

Because of the prebiotics' technological applications and health benefits, the dairy industry is using increasing quantities of prebiotic compounds in the development of its products. One of the most investigated prebiotics is inulin. A class of carbohydrates called fructans, inulin is attached to glucose and fructose molecules through β (2-1) bonds and, α (1-2) and, respectively. The richest sources of inulin include chicory root, onion, garlic, leek, bananas, immature wheat, rye, and barley, as well as Jerusalem artichokes (Scheid et al., 2013). Inulin is used in the food industry as a prebiotic in dairy products, a low-energy sweetener, and an indigestible fiber that helps form gels, raise viscosity, and improve organoleptic properties (Meyer et al., 2011). A study showed that an emulsion based on whey protein could offer additional protection for the inulin encapsulation. Inulin encapsulated with whey has been shown to enhance probiotic bacterial stability during storage. According to Ha et al. (2016), the synbiotic formulation of this probiotic and prebiotic has focused on its potential to improve the proliferation of probiotic bacteria in the intestine and thus change the intestinal microflora (Özer et al., 2005). One of the most important by-products of the dairy industry is whey. Approximately 180 million tons of whey were produced in 2012. However, approximately 30-50% of the total production is not used (Sitanggang et al., 2016). Despite its environmental pollutant potential, whey is considered a valuable source of numerous nutritional, functional, and bioactive compounds. Whey offers a high lactose and protein content that can be used to produce versatile, beneficial ingredients (Kareb and Aïder, 2019). The interactions between phenolic compounds (such as anthocyanins) and proteins (such as whey) have been studied by many scientists. The excess of hydroxyl groups on phenolic compounds provides opportunities for interaction with proteins (Buitimea-Cantúa et al., 2018). The formation of their complexes leads to structural, functional, and nutritional changes of both proteins and phenolic compounds (Zhang et al., 2020). The stability of anthocyanins poses a major challenge for their application in the food industry. Whey protein can be used as a wall material by encapsulation to keep anthocyanins stable in the digestive tract and improve bioavailability (Sharif et al., 2020). Anthocyanin-loaded whey protein microgels can rapidly dissolve in the gastrointestinal tract, and the resulting liquid microparticles inhibit the release and degradation of anthocyanins (Liao et al., 2021).

This study was carried out to determine the anthocyanin level and some quality characteristics of elderberry (*Sambucus nigra* L.) fruit grown in Burdur province;

- To measure the effectiveness of anthocyanin components by encapsulating the obtained anthocyanins with whey protein isolate (WPI),

- To determine the effect of anthocyanins obtained from elderberry and encapsulated with WPI on whey,
- Determining the stability of the whey in the beverage, revealing the effect of encapsulated anthocyanin and inulin on *B. coagulans* during the storage of whey beverages,
- Examining the shelf life of the encapsulated whey beverages with elderberry anthocyanin, inulin, and *B. coagulans* and the changes that will occur in the quality parameters during the storage process.

Materials and Methods

The study was carried out in two stages. The first step involved analyzing the parts and structure of the elderberry fruits that were used for production and preparing the fruits for use in the final product. The second step involved producing whey using BC30 and/or inulin with encapsulated elderberry anthocyanins added, and then preserving it according to the method's stated analyses. Ripe and fresh black elderberry fruits were supplied from a specific farm in the Altinyayla district of Burdur province. Until they were used, fruits were stored frozen at $-18 \pm 1^{\circ}\text{C}$.

Analyses Conducted before Whey Beverage Production

Elderberry Fruit Moisture and Dry Matter Content Measurement

Using the gravimetric method, the amount of moisture and dry matter in elderberry fruit was calculated (The AOAC, 2019). The following procedure was used to compute the dry matter and moisture content:

$$\text{Moisture\%} = \frac{m_2 - m_3}{m_2 - m_1} \times 100$$

m_1 : The dried, empty drying container's weight (g)

m_2 : Before the drying procedure, the test sample's weight in the drying container (g)

m_3 : The test sample inside is the weight of the drying container after the drying process (g)

$$\text{Dry matter \%} = (m_3 - m_1) / (m_2 - m_1) \times 100$$

$$\text{Total dry matter \% (g/100g)} = 100 - \% \text{ moisture}$$

Anthocyanin Extraction from Elderberry

A supercritical CO_2 system was used for the extraction procedure. An extractor with a 1500 mL capacity, 400 bar pressure resistance, and three phases divided by specialized filters was utilized for the extraction process. Liquid CO_2 was pumped at high pressure using an ISCO pump. According to Seabra et al. (2010), extraction was carried out with minor adjustments to the given protocol. 1/3 of

the volume of elderberry fruits was added to the extractor. A heat jacket with digital control was used to heat the extraction unit. Ethanol, ethanol-water (5:5, v/v), and water were used as co-solvents, and the co-solvents were transferred to the extractor using a peristaltic pump. The extractor's gas intake and outflow components are equipped with specialized filters. A constant 50°C temperature was maintained, and CO₂ flow was ensured. In the first step, low-polarity carbon dioxide-soluble components were removed during a 15-minute static and 40-minute dynamic period. In the second step, extraction was carried out using a co-solvent for the extraction of polar components for 45 minutes. Liquid carbon dioxide pressure was reached in the range of 200-350 bar. When the extraction was complete, the CO₂ and extracts were transported to the collection unit by opening the output valve. Here, the CO₂ pressure was lowered to yield extracts.

Calculating the Total Anthocyanin Content of the Elderberry Extract

The pH differential method was employed in accordance with a widely accepted protocol to determine the total anthocyanins in the resultant extract (Zbik et al., 2023). The examined extract was divided into two dilutions, one with potassium chloride buffer (pH 1.0) and the other with sodium acetate buffer (pH 4.5), and they were allowed to equilibrate for 15 min. At 520 and 700 nm, the absorbance of the dilutions was measured. The diluted sample (A)'s absorbance was computed using the following formula:

$$A = (A_{520} - A_{700})_{\text{pH } 1.0} - (A_{520} - A_{700})_{\text{pH } 4.5}$$

Next, the concentration of monomeric anthocyanin pigment (MAPC) was computed using the following formula:

$$\text{MAPC (mg/L)} = \frac{A \times \text{MW} \times \text{DF} \times 1000}{e \times l}$$

MW: molecular weight, DF: dilution factor, e: molar absorptivity, l: optical path length.

Encapsulation of Anthocyanins and Determination of Encapsulation Efficiency

The carrier material was whey protein isolate (WPI) that was purchased from a local company. The anthocyanin extract and carrier suspension were combined at a ratio of 1:9 (v/v) and homogenized at 20,000 rpm for 20 min. after the WPI was suspended at a concentration of 20% (g/v) in 1% citric acid solution (CLS Scientific/ CLPM- 400). The produced samples were freeze-dried and kept for 96 h. (-54°C, 230-300 Hg) following a 24-h. cooling period to -40°C. Using a mortar and pestle, dried materials were ground and sieved before being kept in closed plastic bags in desiccators at room temperature until needed (Souza et al., 2017).

The encapsulation efficiency was calculated based on the amount of anthocyanin before and after encapsulation (Murali et al., 2015).

$$\text{Encapsulation efficiency} = \frac{\text{Total amount of anthocyanin in the powder}}{\text{Total amount of anthocyanin in the extract}} \times 100$$

Scanning Electron Microscope (SEM) Examination of Capsule Morphological Characteristics

The surface morphology of capsules has been studied using SEM analysis of beverages (Rosenberg et al., 1985). Samples were fixed on aluminum stubs with double-sided carbon tape and sputter-coated with gold–palladium for 90 s at a thickness of approximately 10 nm (JEOL JSM-7100-F). To detect varying magnification powers, the scale was calibrated at three different levels: 1, 10, and 100 μm . The SEM was used for observation at a temperature of less than -140°C and 10.0 kV. Images were obtained at accelerating voltages of 10.0 kV, with a working distance of 10 mm. At magnifications of 200, 500, 1000, 2000, 5000, and 15000 x, respectively, the particle size of $n \geq 200$ microcapsules was measured using ImageJ software.

Preparation of Inoculation Culture

Ganeden BC30, also known as *B. coagulans* GBI-30, 6086, was created by modifying the procedure described by Abhari et al. (2016). Using the boot plate method, *B. coagulans* was grown on Nutrient yeast extract salt agar (NYSM) and incubated for 24 h. at 37°C in an aerobic environment. From the agar in issue, a colony was chosen, moved to Nutrient Yeast Extract Salt Broth, and cultured for 48 h. at 37°C with 250 rpm. Following incubation, the pellets were mixed with 0.9% sterile physiological saline, the supernatant was discarded, and the mixture was centrifuged at $3000 \times g$ for 20 min. using an Electro-Mag M 815 A. The last pellet was put back into a sterile normal saline suspension. In order to calculate the number of spores per milliliter in suspension, serial dilutions were prepared, heated to 80°C for 15 min., and then NYSM medium was cultivated using the pour plate method. Following the procedures, the concentration in sterile physiological saline was measured using a cell densitometer (Biosan DEN-1) at 0.5 McFarland ($10^8/\text{mL}$).

Preparation of Whey Beverage and Addition of Ingredients

To make an experimental whey beverage, 20% whey powder (Mirel®) was dissolved in sterile water and pasteurized for 15 min. at 65°C . The whey beverage in groups A, D, and E was supplemented with 0.1% of elderberry anthocyanins encapsulated with WPI, following the instructions listed in Table 1. Groups B, C, D, and E were each given 10^8 spores/mL of *B. coagulans*. To promote *B. coagulans*, 1% inulin was added to groups C and E.

Table 1 Experimental whey beverage groups

Whey beverages	Anthocyanin	<i>B. coagulans</i>	Inulin
Control (K)	-	-	-

A	+	-	-
B	-	+	-
C	-	+	+
D	+	+	-
E	+	+	+

Analysis Conducted on Whey Beverages after Production

Determination of Anthocyanin Amount in Experimental Whey Beverage

Using the pH difference method and a widely accepted protocol, the total anthocyanins in the experimental whey beverage were determined (Zbik et al., 2023).

Determination of Stability of Encapsulated Anthocyanins in Experimental Whey Beverage

The stability of anthocyanins depending on storage time was determined according to the method specified by Da Rosa et al. (2019). The decay constant (k) and half-life ($t_{1/2}$) were calculated using equations 1 and 2, respectively.

$$\ln(C) = \ln(C_0) - k(t) \text{ (Equation 1)}$$

C: anthocyanin concentration at time t (mg/mL)

C_0 : Initial concentration of anthocyanins (mg/mL)

t: Storage period (days)

$$t_{1/2} = \ln(2) / kt \text{ (Equation 2)}$$

$t_{1/2}$: Half-life time (days)

k: Kinetic decay constant

t: time (day)

Microbiological Analyses

Six distinct product groups were established in total (Table 2). During the storage period, the products were kept in a refrigerator at 4°C. During the first, 7th, 14th, 21st, and 28th day of the preservation procedure, laboratory analyses were conducted on each set of products that were generated experimentally. On the scheduled analysis days, samples were obtained from every experimental whey beverage group and subjected to the following microbiological tests. Using the pour plate method, plantings were done in two series, and plates with between 30-300 colonies were assessed after the incubation period. The values of measurement are log₁₀ CFU mL⁻¹.

For total mesophilic aerobic bacteria (TMAB) and total psychrophilic aerobic bacteria (TPAB) count, the prepared dilutions were inoculated onto Plate Count Agar (PCA) (Merck 1.05463) using the cast plate method and incubated at 30 ± 1°C for 48 h. (ISO, 2008) and 10°C for 5 days, respectively.

In determining coliform bacteria, the medium was diluted to yield 1 mL of Violet Red Bile Agar (VRBA) (Merck 1.01406), which was then cultivated using the double-layer pouring method. After the petri dishes were incubated at $37 \pm 1^\circ\text{C}$ for 24 h., typical colonies developed and counted (ISO, 2008). By applying the pour plate method to the prepared dilutions, they were inoculated into Potato Dextrose Agar (PDA) (Merck 1.10130) medium and counted as yeast and molds after 5 days of incubation at $22 \pm 1^\circ\text{C}$ (ISO, 2008). *Bacillus coagulans* count was determined according to the method described by Sekhavatizadeh et al. (2019). Tryptone Glucose Yeast Extract Agar (Condalab, 1190.00) medium was used. The prepared dilutions were first kept in a water bath (Mettler model WB 14, Germany) for 10 min. at 80°C , then immediately cooled to 45°C and then inoculated using the cast plate method. Petri dishes were incubated at 37°C for 48 h. under anaerobic conditions (Soares et al., 2019).

Physicochemical Analyses

Using the Kjeldahl method, the levels of crude protein in the whey beverage were calculated (Lynch and Barbano, 1999). The correction factor of 6.38 was used to get total protein from total nitrogen. The whey beverage's titratable acidity was assessed using the alkaline titration method. The results were represented as a percentage of lactic acid. The pH of samples was determined using a digital pH meter (704 pH Meter, Metrohm). The gravimetric method was used to calculate the dry matter content of beverage samples (AOAC, 2019). The "100-dry matter ratio" is used to compute the moisture. Viscosity measurements were performed by modifying the method defined by Nooshkam et al. (2022). The rheological characterization of the samples was performed using a Brookfield DV-II+ Pro model rotary viscometer with an RV2 spindle (Spindle No. 2). The rheometer setup consisted of a measuring chamber (with a diameter of 36 mm and a height of 70 mm) and a cylinder spindle (with a diameter of 25 mm and a height of 45 mm) for rheological analysis. Rheological characterization of the samples was carried out under constant temperature conditions without any heat treatment. All samples were held at the laboratory temperature of $22 \pm 1^\circ\text{C}$ for approximately 3 hours prior to measurement, allowing them to reach thermal equilibrium. The shear rate varied between 160 s^{-1} and 200 s^{-1} during the 10-second test period. Viscosity results were reported in mPa.s. Color parameters were measured using a tristimulus colorimeter (Minolta CR-400, Osaka, Japan), which was equipped with a sample holder for 10 mm plastic cells suited for liquid analysis. It was calibrated with a white reference plate. The standard illuminant used was D65, and a 2° observer was employed in the L^* , a^* , b^* color space. The reflectance (R) spectrum was acquired from 360 to 740 nm at 10 nm intervals. The colorimeter yielded CIE L^* values, which indicate lightness and range from 0 (for black) to 100 (for white), a^* [$-a^*$ (for

greenness) + The color system "varies between a* (for redness)] was used to express it, b* [b* (yellowness) + b* (blueness)].

Determination of Antioxidant Capacity

The method used by Brand-Williams et al. (1995) was developed with small modifications to determine the amount of DPPH antioxidant capacity. A UV-VIS spectrophotometer (PGI brand T60U series) was used to measure the absorbance at 517 nm using ascorbic acid as the standard and methanol as a blank solution. The following equation was used to calculate the percent inhibition (%I) of DPPH radical scavenging activity.

$$I (\%) = \frac{A_0 - A_1}{A_0} \times 100$$

A0: Absorbance of control group

A1: Absorbance of sample

Sensory Analysis

A sensory assessment was conducted in compliance with ISO (2016) standards. Ten expert panelists were selected from the Department of Food Hygiene and Technology, Faculty of Veterinary Medicine at Burdur Mehmet Akif Ersoy University, comprising five women and five men are consistent with ISO standards. Panelists were informed of the study, and experiments were carried out with those who provided informed consent. Panelists with allergies to the beverage ingredients, impaired taste sensations due to conditions such as viral infections, and those unable to adequately taste or smell were excluded from the study. The assessment took place in a clean, well-ventilated room with an ideal room temperature of 20°C. Each single-blind randomized sample used in a panel session was placed in a plastic cup labelled with the corresponding group letter. The 50 mL beverage samples were left at room temperature (around 20°C) for 30 min. before analysis. Panelists were provided with drinking water and unsalted crackers, which they were instructed to consume following each sampling. Evaluation was made using a 9-point hedonic scale (9 = fairly good; 1 = extremely poor). The panelists assessed the beverage samples for appearance, texture, odor, aroma, taste, consistency, color, homogeneity, and general acceptability.

Statistical Analysis

Analyses were made using the Statistical Package for the Social Sciences (SPSS) program version 26. Descriptive data in tables are presented as mean ($\bar{x}$) $\pm$ standard deviation (SD), whereas figures display mean ($\bar{x}$) $\pm$ standard error (SE) to illustrate variability and precision, respectively. The Shapiro-Wilk test was used to check if the data were normally disturbance. In comparing two datasets, a paired samples t-test was used for normally distributed data, and a Wilcoxon test was used for non-normally

distributed data. Data were analyzed using a one-way ANOVA (or Kruskal–Wallis test, where non-normally distributed data) to compare groups at each storage day. When statistically significant differences were found, the Tukey post-hoc test was used for multiple comparisons. $p < 0.05$ was accepted as the significance level. Each experimental condition was produced in triplicate ($n=3$), and all analyses were performed in duplicate to ensure analytical reliability.

Results

Analyses Conducted before Whey Beverage Production

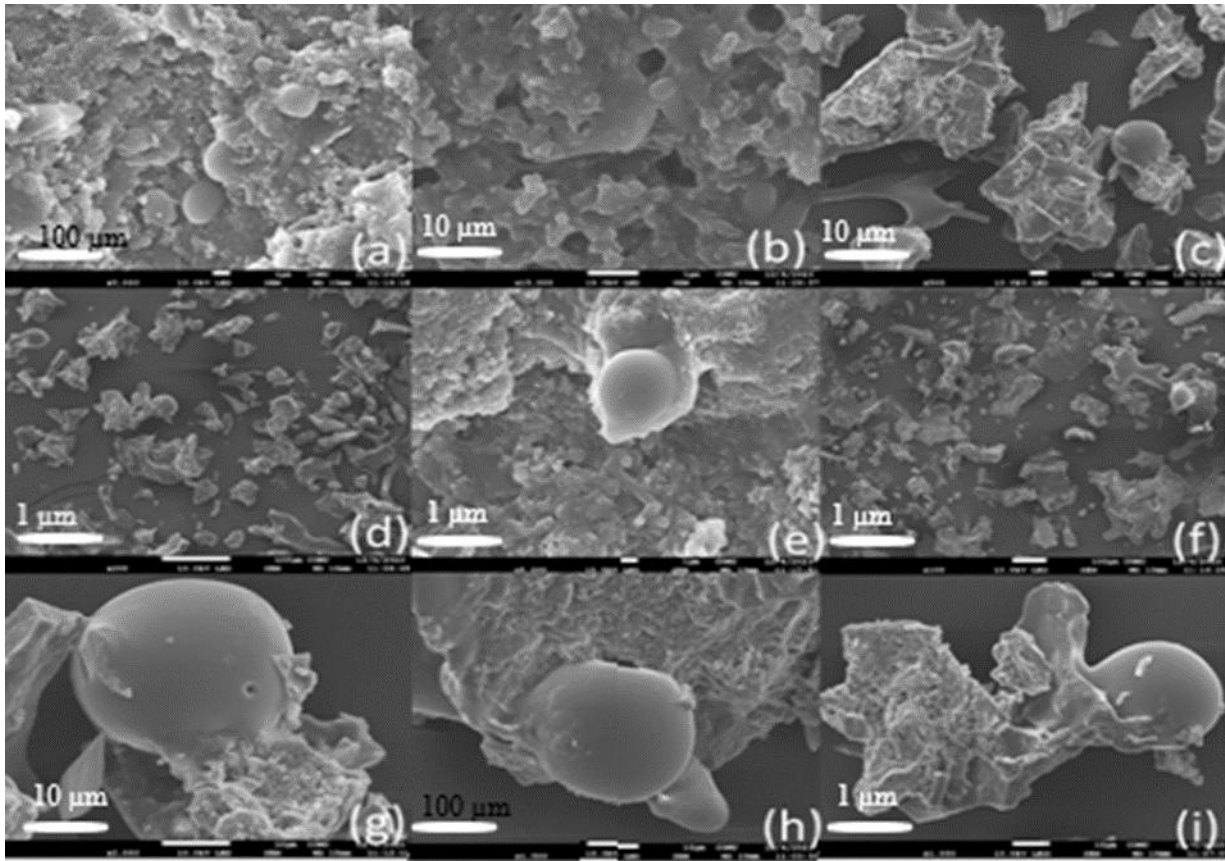
Fresh elderberry fruits had a moisture content of $78.9\% \pm 0.1\%$ and a dry matter ratio of $21.1\% \pm 0.1\%$. Although the results are consistent with those of other studies (Costica et al., 2019; Diviš, Ferreira et al., 2022; Pořízka et al., 2015) found a lower dry matter ratio in their study. The storage duration and conditions following fruit harvest, in addition to the region and environmental elements where elderberries are found, might alter the product's dry matter content.

Anthocyanins, which constitute 25–40% of the total weight of elderberry fruit (Brønnum-Hansen and Hansen, 1983), especially cyanidin 3-sambubioside-5-glucoside, cyanidin 3,5-glucoside, cyanidin 3-sambubioside, and cyanidin 3-glucoside, provide significant protection against oxidative damage and help prevent endothelial cell dysfunction (Youdim et al., 2000). In this study, the total anthocyanin amount of elderberry extracts extracted by the Supercritical CO₂ method and turned into powder was found to be 174.8 mg L^{-1} , which is consistent with previously reported ranges for *S. nigra* extracts. According to studies, the fractionated high-pressure extraction method (Seabra et al., 2010) and HPLC yield a total anthocyanin content of $39\text{--}153 \text{ mg g}^{-1}$ and $28.8\text{--}64.6 \text{ mg L}^{-1}$, respectively, when using the conventional extraction method (Domínguez et al., 2020). Using the method, it was found to be between 73.8 and 282.0 mg L^{-1} (Kaack et al., 2008). Various methods can yield different effects since they alter the anthocyanin content directly. The best anthocyanin ratios under the ideal pressure and temperature can be achieved with the use of optimization studies, even though the supercritical CO₂ approach is highly effective. Furthermore, the number of anthocyanins in the extracted fruit can be directly impacted by every environmental component it comes into contact with, from the harvest to the laboratory setting.

The encapsulated powder's anthocyanin content was found to be 88.2 mg L^{-1} , and its encapsulation efficiency was 50.46%. Because anthocyanins are unstable and are impacted by a variety of factors, including pH, temperature, light, metal ions, oxygen enzymes, ascorbic acid, sugars, and the products of their breakdown, proteins, and sulfur dioxide, their bioavailability is limited (Fernandez-Lopez et al., 2013). The use of anthocyanin encapsulation technology increases bioavailability and protects against adverse effects. One of the most crucial factors defining the microencapsulation process is encapsulation efficiency. According to Ren et al. (2021), WPI is an

excellent wall material for encapsulating anthocyanins and can enhance their stability, color, and antioxidant capability. The encapsulation effectiveness of the anthocyanins obtained in our investigation, which were encapsulated with WPI utilizing the freeze-dryer method, was determined to be 50.46%. In a study using β -glucan as the encapsulation agent, the group containing 0.5% β -glucan had the best encapsulation efficiency, 93.9 % (Sobieralska and Kurek, 2019). Elderberry extract was encapsulated into nanoparticles using varying phospholipids by Bryła et al. (2015), who found that the encapsulation effectiveness ranged from 25 to 69%. The encapsulation effectiveness of anthocyanins with liposomes, achieved by the supercritical CO₂ method, was determined to be 50.6% by Zhao et al. (2017). The anthocyanins isolated from cranberry fruits were freeze-dried by Enache et al. (2020) and then encapsulated at varying rates using a mixture of WPI, inulin, and chitosan. The encapsulation efficiency was found to be $89.16\% \pm 1.23\%$. Tao et al. (2017) reported that the ratios of WPI, maltodextrin, β -cyclodextrin, and gum Arabic used to encapsulate blueberry anthocyanins ranged from 47.0% to 96.3%. All of the findings indicate that the encapsulation efficiency is directly impacted by variations in the encapsulation techniques and wall materials used.

SEM analysis can be used to look at the external structures of the capsules during the encapsulation process. Fig. 1 shows the results of the SEM imaging of anthocyanins encapsulated with WPI. Upon closer inspection, it was discovered that certain encapsulated anthocyanins had smooth surfaces while others had rough surfaces (Fig. 1g). In addition to irregular particles, multilayer structures and irregularly shaped particles are also observed (Fig. 1a-b). The particles' size distribution is not regular (Fig. 1d). The cohesive quality of the encapsulating agent used is responsible for this creation (Rather et al., 2017). The analysis yielded the average size of the capsules, which was found to be $147 \pm 65.5 \mu\text{m}$. There were visible splits or fissures in the microparticles. The quick sublimation of ice from particles and the creation of pores in place of ice crystals result in the spongy structure that is clearly seen in Figure 1a (Amine et al., 2014). The limited quantity of free molecules in Figure 1c-d indicates that the anthocyanins have been properly encapsulated. All of the samples showed inconsistent shrinkage and rough surfaces in the SEM investigation of capsules made with various types and ratios of wall materials, including WPI (Tao et al., 2017). Furthermore, all particles seem to have glassy forms, which is consistent with our research. The anthocyanins within the capsules can be shielded from heat and oxidation by means of these glassy structures. Additionally, it was determined that certain areas of the capsules included porous features, indicating that the encapsulating process was successful but not entirely successful. According to Sobieralska and Kurek (2019), microcapsules with 0.5% β -glucan had an aggregated sphere and a smooth surface. According to Deng et al. (2023), the anthocyanin capsules containing WPI had smooth, crack-free surfaces.



a: 5.000 x; **b:** 15.000 x; **c:** 500 x; **d:** 200 x; **e:** 5.000 x; **f:** 100 x; **g:** 2.000 x; **h:** 1.500 x; **i:** 1.000 x
Scale bars: 100 μm (a,h), 10 μm (b,c,g), 1 μm (d,e,f,i)

Fig. 1 Microstructure by scanning electron microscopy of anthocyanins encapsulated by freeze drying
Analysis Conducted on Whey Beverages after Production

Amount of Anthocyanin in Experimental Whey Beverages

Anthocyanins are unstable chemicals with little stability. Therefore, their quantities in products might decrease depending on the storage process. In the present study, anthocyanins were encapsulated with whey protein isolate (WPI) and incorporated into the product in order to reduce the loss. The measurements in the anthocyanin-containing groups (A, D, and E) showed that the anthocyanin contents of whey beverages on the first day were 6.5 ± 0.7 , 5.8 ± 0.5 , and $4.0 \pm 0.7 \text{ mg L}^{-1}$, respectively. Group E showed the lowest anthocyanin concentration among the beverage groups that had been stored for a day; there was a statistically significant difference ($p < 0.05$) between it and groups A and D. Groups A, D, and E were found to have anthocyanin contents of 5.6 ± 0.1 , 5.0 ± 0.1 , and $2.7 \pm 0.1 \text{ mg L}^{-1}$ on the 28th day of storage ($p < 0.05$). Only in the E group did it turn out that there was a statistically significant difference ($p < 0.05$) in the anthocyanin contents of the whey beverage groups between days. Anthocyanins may have been used by the probiotic BC30 in group D and transformed into other metabolites. Probiotics metabolize anthocyanins because they give them a carbon source

(Wang et al., 2022). According to Oliveira et al. (2021), the dried strawberry group without *B. coagulans* BC4 has higher anthocyanins than the group with BC4. In contrast to groups A and D, group E's inulin content raised the beverage's density and may have reduced its overall anthocyanin content. In their study, Tomas et al. (2020) discovered that, in comparison to the control group, the total amount of monomeric anthocyanins in blackberry puree samples containing inulin and pectin dropped significantly ($p < 0.05$). According to Tomas et al. (2018), the total phenolic material content of tomato sauce was dramatically decreased by the combination of inulin and tomato sauce. Dietary fibers and phenolic compounds can interact in ways that are either beneficial or detrimental to the bioactivities of polyphenols (Suharoschi et al., 2019). According to reports, storage below 4°C has a more beneficial impact on anthocyanin stability (Palencia-Argel et al., 2022; Urbano da Silva et al., 2019). Similar studies on different products containing BC30 revealed that anthocyanin levels steadily dropped throughout the course of the next few storage days (Cheng et al., 2023; Miranda et al., 2020). According to Jing and Giusti (2005) and Ren et al. (2021), these reductions could be caused by the way anthocyanins interact with other elements in the products and by the way they establish intricate hydrogen bonds with environmental proteins.

Stability of Encapsulated Anthocyanins in Experimental Whey Beverage

Anthocyanins' storage stability is enhanced by the encapsulation process (Rezvankhah et al., 2020), but over time, their structures degrade due to high molecular mobility and easy oxygen diffusion, particularly in environments with high water activity (Baeza et al., 2021). This investigation determined the whey beverage's anthocyanin degradation constant, half-life, and loss rates over the first and last days of storage. Group E had the highest degradation constant (k) (0.0147). Group E was found to have a shorter half-life (50.2 days) than groups A and D ($p < 0.05$). The rates of anthocyanin loss were determined to be $12.7\% \pm 8.2\%$, $12.1\% \pm 8.8\%$, and $32.5\% \pm 8.0\%$ for groups A, D, and E, respectively. The anthocyanin loss in group E is statistically higher than that in groups A and D ($p < 0.05$), although there is no statistically significant difference between groups A and D when the loss rates between the groups are compared. Whey protein microgels are a type of anthocyanin encapsulation material that dissolves quickly in the digestive system to create liquid particles that inhibit the release and degradation of anthocyanins (Ren et al., 2021). According to Chung et al. (2015), heat-denatured WPI and natural WPI would exhibit the best anthocyanin stability. In studies where the encapsulation of elderberry anthocyanins was carried out with different materials, it was reported that the stability of anthocyanins changed depending on factors such as storage conditions, coating materials, different environmental conditions, and water activity (Baeza and Chirife, 2021; Casati et al., 2019; Ribeiro et al., 2019). Zhao et al. (2021) determined the anthocyanin degradation

constant in 4 different beverage groups with encapsulated anthocyanin addition as 0.474, 0.172, 0.134, and 0.085, respectively. This is explained by the complex formation of anthocyanin with the other components used, and also by the chemical protection of the anthocyanin chromophore by preserving the highly electrophilic C2-position of the flavylum cation. There should be a comparable association between anthocyanin and inulin based on the closeness between the degradation constants in this study and those found in group E (0.0147) in our investigation. These findings collectively demonstrate the beneficial effects of whey protein isolate on the storage and bioavailability of anthocyanins.

Microbiological Analysis

Microbiological analysis results of whey beverage groups on days 1, 7, 14, 21, and 28th are shown in Table 2. The most often used metric to assess a product's microbiological quality is its total mean absorbance bond (TMAB) content (ISO 4833:2003(E), 2003). According to Samarižija et al. (2012), there is a strong link between increased bacterial load caused by storage and beverage deterioration during shelf life. The amount of TMAB in the product reduced in the anthocyanin-containing groups (A, D, and E) during storage, while it rose in the other groups. This difference was determined to be statistically significant on the seventh day of storage ($p < 0.05$). These findings imply that the total bacterial load in the beverage is decreased when anthocyanin is added to whey. In contrast to our study, Maity et al. (2008) observed that during 15 days of storage, the total bacterial count decreased. In another study, after 15 days of storage, it was found that the quantity of TMAB in a whey beverage made with goat milk increased ($p > 0.05$) (Vieira et al., 2020). According to a study where rose oil was added to a whey beverage at varying rates, after 28 days of storage, the TMAB number increased from 6.44 log₁₀ CFU mL⁻¹, which was the starting point, to 7.88 log₁₀ CFU mL⁻¹ (Dinçoğlu and Rugji, 2021). Rugji et al. (2022), the amount of TMAB in whey beverages with prebiotic added was 8.69 log₁₀ CFU mL⁻¹ on the first day, but it dropped until the 28th day, when it was 8.58 log₁₀ CFU mL⁻¹.

In all groups, the quantities of TPAB generally rose with the length of storage. This increase differed in the other groups that had components, but it happened consistently in the K group, which has no additives in its composition. It is normal for beverages kept at 4°C to produce psychrophilic bacteria. Certain groups' structural components, including BC30, inulin, and anthocyanins, demonstrated a suppressive effect on the regular growth of these bacteria. It is hypothesized that the psychrophilic group bacteria's inability to adjust to the production environment on the first day may be the cause of the statistically significant increase in TPAB levels ($p < 0.05$) in all groups on the seventh day. The TPAB count in a trial utilizing blueberry extract increased from 4.5 log₁₀ CFU mL⁻¹ on day 1 to 5.5 log₁₀ CFU mL⁻¹ on day 9. However, the amount of anthocyanin in the extracts made without the use of an enzyme was lower than that made using other methods, and this circumstance is

claimed to lessen the bacterial inhibitory effect of polyphenols (Dinkova et al., 2014). According to De Jesus et al. (2020), the bacteria belonging to the psychrophilic group increased from 5.21 log₁₀ CFU mL⁻¹ to 6.30 log₁₀ CFU mL⁻¹ after 28 days of storage of açai pulp.

Studies show that coliforms have limited ability to survive in low pH fermented milk products, and their presence would indicate contamination (Hervet et al., 2017). None of the whey beverages made for our investigation contained bacteria from the coliform category (Table 2). This instance demonstrates that the product was stored at the proper temperature, contamination did not occur during storage, and the samples were prepared hygienically. Coliform group bacteria were not found in whey beverages in the majority of comparable research (Ahmed et al., 2023; Larionov et al., 2020).

Although fermented milk products are generally considered microbiologically stable, due to the ability of many types of yeasts and molds to grow in low pH and low temperature conditions, product quality deterioration and shortening of shelf life may occur (Nielsen et al., 2021). In the current study, mold and yeast were found in group K from the outset of storage, whereas isolation was carried out in the other groups starting on the fourteenth day. This could be because of the components other than group K, which are believed to inhibit the growth of molds and yeasts. It is possible to interpret the notable rise in yeast and mold counts on the 21st day of storage in all groups as a sign that the product has started to lose its quality as a result of storage. On the other hand, Dinçoğlu and Rugji (2021) observed that the number of yeast molds was 6.08–6.43 log₁₀ CFU mL⁻¹ on the first day and increased on the 28th day (indicating a statistically significant difference). According to research by Ismail et al. (2011), during the course of the 30-day storage period, there was a steady decline in the quantity of mold and yeast in whey beverages containing mango. The same study found that beverages' acidity rates rose when they were kept in storage.

Probiotic viability in food is directly impacted by storage conditions, temperature, oxygenation, acidity, moisture content, and ingredients added to the product's structure (Shah, 2000). The fact that both inulin and anthocyanins promote BC30 reproduction may be the reason why the amount of BC30 in group B samples was at its highest level at the start of storage and decreased on the 7th day of storage, while the highest number of BC30 was reached in group E, which contains both anthocyanin and inulin. The fact that the increased presence of other microbes generated a competitive environment that hindered BC30 reproduction can be used to explain why the quantity of BC30 fell in all groups on the 14th day of storage. On the final day of storage, the BC30 count rose in all groups except group E ($p < 0.05$). According to Tomas et al. (2020), there is a possibility that this is because group E has a larger concentration of other microbes or that the anthocyanin and inulin in this group interact negatively with BC 30. Similar to our findings, Rugji and Dinçoğlu (2022) found that the greatest BC30 number was inulin (6.82 log₁₀ CFU mL⁻¹) on the last day of storage, and that the number of

components in white brine cheeses to which BC30 was added declined from the first day to the 90th day of storage ($p < 0.05$). They claimed to have seen it in the group that included. In the study they conducted using yogurt samples that had additional BC30, Cao et al. (2022) reported that the BC30 levels of yoghurts kept for 14 days stayed stable ($p > 0.05$) during the course of the storage period. In their investigation using BC30-containing kashar cheese, Sekhavatizadeh et al. (2019) reported that the overall number of BC30 dropped during storage. In a study that was comparable to this one, Ehsannia and Sanjabi (2016) assessed the characteristics of cheeses that had *B. coagulans* added and found that as storage times rose, the amount of *B. coagulans* dropped.

Table 2 Microbiological analysis results of whey beverage groups (log10 CFU mL⁻¹)

Parameters	Days	Beverages groups ($\bar{x} \pm SD$)					
		K	A	B	C	D	E
TMAB	1	6.82 $\pm$ 0.1 Aa	6.86 $\pm$ 0.1 Ac	7.39 $\pm$ 0.2 Bb	7.62 $\pm$ 0.1 BCb	7.62 $\pm$ 0.1 BCb	7.74 $\pm$ 0.1 Cb
	7	6.43 $\pm$ 0.1 Ba	5.52 $\pm$ 0.4 Aa	7.57 $\pm$ 0.1 CDbc	7.95 $\pm$ 0.1 Dc	6.96 $\pm$ 0.1 BCab	7.30 $\pm$ 0.6 CDab
	14	7.91 $\pm$ 0.1 Ebc	6.52 $\pm$ 0.1 Ac	6.96 $\pm$ 0.1 Da	6.90 $\pm$ 0.1 CDa	6.70 $\pm$ 0.1 Ba	6.77 $\pm$ 0.1 BCa
	21	7.68 $\pm$ 0.3 Bb	6.67 $\pm$ 0.1 Ac	7.88 $\pm$ 0.0 Bc	7.72 $\pm$ 0.2 Bb	6.73 $\pm$ 0.1 Aab	6.84 $\pm$ 0.1 Aa
	28	8.20 $\pm$ 0.2 Bc	6.03 $\pm$ 0.3 Ab	9.06 $\pm$ 0.0 Bd	8.87 $\pm$ 0.1 Bd	6.38 $\pm$ 0.7 Aa	6.89 $\pm$ 0.1 Aa
TPAB	1	4.58 $\pm$ 0.1 ABa	4.52 $\pm$ 0.1 Aa	4.54 $\pm$ 0.1 ABa	4.52 $\pm$ 0.1 Aa	4.60 $\pm$ 0.1 ABa	4.63 $\pm$ 0.1 Ba
	7	7.16 $\pm$ 0.1 ABb	6.89 $\pm$ 0.1 Ac	7.59 $\pm$ 0.4 Bb	7.08 $\pm$ 0.1 ABb	6.84 $\pm$ 0.1 Ab	7.00 $\pm$ 0.1 Ab
	14	7.43 $\pm$ 0.8 b	6.70 $\pm$ 0.1 b	7.11 $\pm$ 0.1 b	7.37 $\pm$ 0.1 b	7.35 $\pm$ 0.1 c	6.99 $\pm$ 0.1 b
	21	8.81 $\pm$ 0.1 Bc	9.11 $\pm$ 0.1 Ce	8.89 $\pm$ 0.1 BCc	8.68 $\pm$ 0.2 Bc	9.13 $\pm$ 0.1 Ce	8.16 $\pm$ 0.1 Ac
	28	8.91 $\pm$ 0.1 Cc	8.54 $\pm$ 0.1 Ad	8.80 $\pm$ 0.1 BCc	8.53 $\pm$ 0.2 Ac	8.63 $\pm$ 0.1 ABd	8.41 $\pm$ 0.1 Ad
Coliform	1	ND	ND	ND	ND	ND	ND
	7	ND	ND	ND	ND	ND	ND
	14	ND	ND	ND	ND	ND	ND
	21	ND	ND	ND	ND	ND	ND
	28	ND	ND	ND	ND	ND	ND
Yeast-Mold	1	3.90 $\pm$ 0.1 a	ND	ND	ND	ND	ND
	7	6.76 $\pm$ 0.1 b	ND	ND	ND	ND	ND
	14	7.13 $\pm$ 0.3 Bb	6.43 $\pm$ 0.3 Aa	6.98 $\pm$ 0.1 Ba	6.88 $\pm$ 0.1 ABa	6.76 $\pm$ 0.1 ABa	6.69 $\pm$ 0.1 ABa
	21	8.30 $\pm$ 0.1 Cc	8.11 $\pm$ 0.1 Bb	7.83 $\pm$ 0.1 Ac	7.69 $\pm$ 0.1 Ab	8.11 $\pm$ 0.1 Bc	7.70 $\pm$ 0.1 Ab
	28	8.07 $\pm$ 0.5 Bc	7.30 $\pm$ 0.4 ABb	7.17 $\pm$ 0.1 Ab	7.58 $\pm$ 0.2 ABb	7.87 $\pm$ 0.1 ABb	7.85 $\pm$ 0.1 ABc
<i>B.</i>	1	-	-	7.83 $\pm$ 1.2	7.43 $\pm$ 0.1 b	7.09 $\pm$ 0.1 c	7.51 $\pm$ 0.1 c

<i>coagulans</i>	7	-	-	7.25 ± 0.2 A	8.20 ± 0.3 Bc	7.89 ± 0.1 Bd	9.14 ± 0.1 Cd
	14	-	-	6.84 ± 0.3	6.77 ± 0.1 a	6.76 ± 0.1 a	6.71 ± 0.1 b
	21	-	-	6.70 ± 0.1 A	6.76 ± 0.1 Ba	6.81 ± 0.1 Cab	6.89 ± 0.1 Db
	28	-	-	7.80 ± 0.1 C	8.10 ± 0.1 Dc	6.97 ± 0.1 Bbc	5.97 ± 0.1 Aa

* **K:** Whey, **A:** Whey + Anthocyanin, **B:** Whey + *B. coagulans*, **C:** Whey + *B. coagulans* + Inulin, **D:** Whey + Anthocyanin + *B. coagulans*, **E:** Whey + Anthocyanin + *B. coagulans* + Inulin. $\bar{x} \pm \text{SD}$: Mean and standard deviation. **TMAB:** Total mesophilic aerobic bacteria, **TPAB:** Total psychrophilic aerobic bacteria, **ND:** Not detected (<1 log₁₀ CFU g⁻¹). Differences between groups are shown in uppercase letters, and differences between days are shown in lowercase letters.

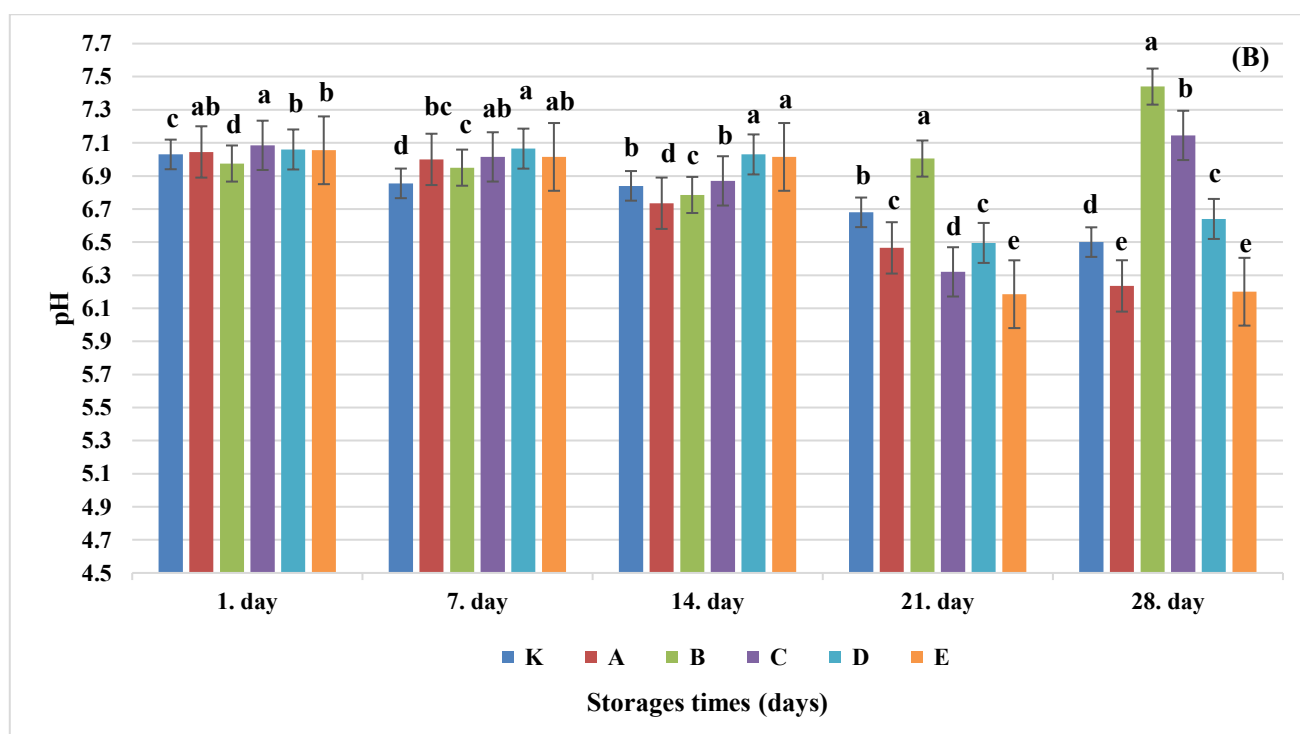
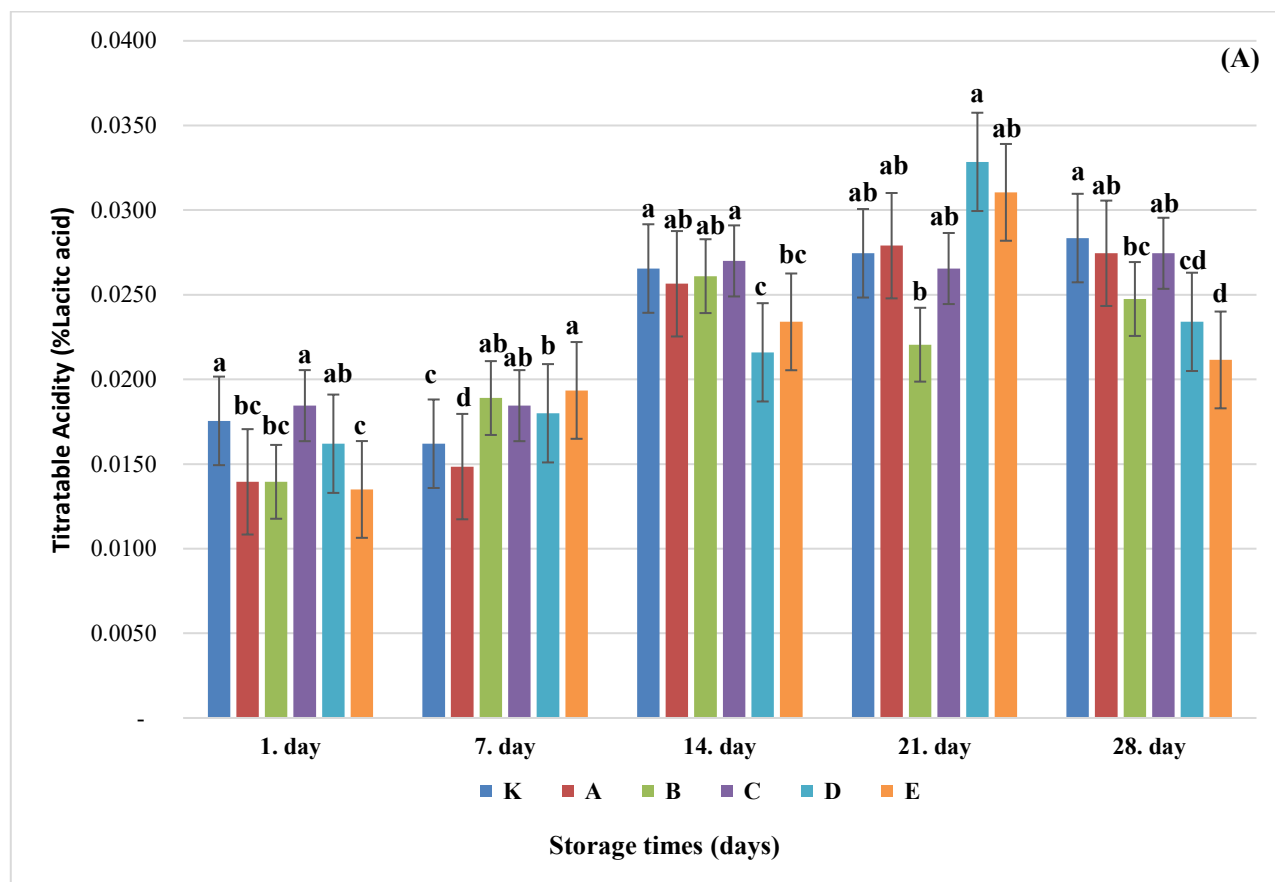
Physicochemical Analysis

The total nitrogen content, expressed as crude protein equivalent using the Kjeldahl method (factor 6.38), ranged from 7.5% to 10.6% on day 14. These values reflect not only true whey proteins but also non-protein nitrogen (NPN) sources, including bacterial metabolites, cellular nitrogen, and anthocyanin-protein complexes formed during storage. In the experimental whey beverages, group D had the highest protein amount (10.6% ± 0.1%), whereas group K had the lowest protein level (7.5% ± 0.1%). A study found that the protein values of a probiotic fermented whey beverage with rose oil ranged from 0.15 ± 0.15 to 0.68 ± 0.02 (Dinçoğlu and Rugji, 2021). In a study on whey and mango-based functional beverage products, it was determined that the crude protein content of beverages containing 70% whey was 7.66% on the first day of storage, decreasing to 7.58% on the 25th day. The rise in the crude protein ratio in whey protein isolates used in beverage production accounts for the high crude protein ratio found in whey beverages (Ahmed et al., 2023). A physicochemical assessment of various probiotic whey-pineapple beverages was conducted by Islam et al. (2021), who found that the beverages' protein ratios ranged from 0.61 ± 0.22 to 1.48 ± 0.24.

Fig. 2 shows the titratable acidity and pH values obtained for each beverage group on days 1, 7, 14, 21, and 28 of storage. The control of pH and acidity in food products has an impact on the food's microbiological and physicochemical stability. The stability of the proteins in dairy products is also affected by the acidic environment, which can prevent the formation of numerous bacterial species. However, because of shelf life, increased acidity in food can potentially be a sign of microbial deterioration (Awuah et al., 2007). On the first day of titratable acidity measurements, groups K, C, and D were found to have statistically significantly higher acidity than the other groups, based on the data gathered as a result of acidity measurements taken on each analysis day for each beverage group. Groups B and D had higher acidity levels on the 7th day, and all groups had higher acidity levels on day 14th. It is thought that this can be because the product starts to degrade in the days that follow storage. In research investigating the prebiotic properties of whey beverages with soursop (*Annona muricata*), Guimaraes et al. (2019) observed that the beverages' acidity ranged from 0.50 ± 0.10 to 0.56 ± 0.03 in terms of citric acid. Similarly, a study on functional beverages made with whey and

pineapple found that their acidity values, in terms of lactic acid, increased between 0.65 ± 0.02 and 0.74 ± 0.02 depending on their shelf life (Islam et al., 2021). The explanation for this is assumed to be that bacteria digest the beverages' simple sugars, increasing the product's acidity. Additionally, this study found a negative association between protein, dry matter, and acidity. Nevertheless, our investigation did not find this kind of effect. Naik et al. (2023) created a functional beverage with whey and carrots and investigated its physicochemical characteristics. According to the study, the beverage groups' acidity values ranged from 0.018 to 0.036; however, there was no discernible difference between the products' acidity and pH variations over storage. The transformation of lactose and protein into lactic acid and amino acids, however, has reportedly been suggested as a potential source of the acidity shift. Similar to this, during the course of the 21st storage period, Oliveira et al. (2022) noticed an increase in the acidity of the orange juice whey beverage. Dinçoğlu and Rugji (2021) observed that on the first day of production, the titratable acidity values of the functional whey beverages, which they made with the inclusion of probiotics and rose oil, ranged from 0.31 ± 0.01 to 0.38 ± 0.01 . Group B samples had the greatest pH value on the 28th day of storage, while having the lowest pH value on the first day of storage, according to an analysis of the pH values of the beverage groups in the current study. The pH levels of every group, except groups B and C, dropped with increasing storage duration. In their research, Guimaraes et al. (2009) discovered that the pH range of whey beverages was 5.41 ± 0.1 to 5.4 ± 0.1 . In this research, the prebiotic food utilized explained the low pH. According to Islam et al. (2021), whey beverages had a pH of 4.3 ± 0.1 on day one and 3.5 ± 0.1 on day 35th. Naik et al. (2023) reported that the pH values of whey-carrot-based beverages varied between 5.5 and 6.5. Dinçoğlu and Rugji (2021) stated that the pH values of whey beverages ranged between 5.07 ± 0.02 and 5.25 ± 0.02 on the first day of production, and between 4.47 ± 0.03 and 5.14 ± 0.04 on the 28th day. They stated that the pH values of the groups containing rose oil were lower than the other groups. It has been reported that the low pH values detected in this study can be associated with the presence of rose oil as well as the activity of probiotic bacteria. It is observed that different functional beverage products have different titratable acidity and pH values. The main reason for this is said to be the components that add functionality to the product, the shelf life of the products, and the microbiological activities in the products. In this study, the lower acidity value of the products was reflected in the sensory evaluation, and while all beverages received high scores in the sensory evaluation on the first day of production, relatively lower scores appeared from the 21st day of production. The relatively low titratable acidity values (0.014-0.032%) compared to *Lactobacillus*-fermented products reflect the lower acid-producing capacity of *B. coagulans* GBI-30, 6086. This strain is specifically selected for probiotic applications requiring minimal pH reduction (Cao et al., 2022). Our values are consistent with those of Naik et al. (2023), who reported 0.018-0.036% in similar whey beverages. The gradual

increase from day 1 to 28, coupled with corresponding pH decline (7.03 to 6.64 in Group D), confirms expected fermentation patterns.



* Changes in titratable acidity (A) and pH (B) of whey beverages during storage. **K:** Whey, **A:** Whey + Anthocyanin, **B:** Whey + *B. coagulans*, **C:** Whey + *B. coagulans* + Inulin, **D:** Whey + Anthocyanin + *B. coagulans*, **E:** Whey + Anthocyanin

+ *B. coagulans* + Inulin. Mean $\pm$ SE with distinct lowercase letters on the bar indicating significant differences at $p < 0.05$ using the Tukey test. Differences between groups are shown in lowercase letters. Error bars represent the standard error (SE) of triplicate measurements (n=3)

Fig. 2 Changes in titratable acidity and pH of whey beverages during storage

On the first, 14th, and 28th day of storage, dry matter and moisture content were measured in the whey beverage groups (Table 3). Dry matter determination is an analysis generally performed on foods with high moisture content to determine whether the food complies with the standards and to determine its stability or preservation period. When the dry matter ratios of the whey beverages produced in the study were examined, it was seen that the dry matter contents increased in the following storage days in groups A, C, and E. The group with the highest dry matter content was group E with 18.96% on the 28th day, and the group with the least dry matter content was group K with 15.39% on the first day of storage. It is thought that this may be due to the presence of both anthocyanin and inulin in group E and the increase in dry matter ratio as a result of microbiological activities. Rugji et al. (2022) found the dry matter ratios of whey beverages prepared at 20% and analyzed for 28 days at 4°C to be between 17.67-17.70%. Kassem et al. (2025) stated that storage time did not change the total solids in milk and whey-based beverages enriched with Mango and *Lactobacillus plantarum*, and the only significant difference ($p < 0.001$) was between the total solids of fermented milk and the total solids of fermented whey-based beverages. In a study conducted, it was found that total solids content was highest in probiotic-added whey beverages combined with condensed milk, and storage time affected the total solids content (Skryplonek et al., 2019). Dry matter and moisture content in foods are among the parameters that provide information about the metabolic activities occurring in the product during the preservation process. A decrease in the dry matter ratio may occur as a result of the lipolytic and proteolytic activities of the bacteria present in the food. In our study, there is a positive relationship between storage time and dry matter in all groups. It was determined that the group with the highest dry matter ratio was group E. This is thought to be due to the antibacterial effects of encapsulated anthocyanin and *B. coagulans* in group E.

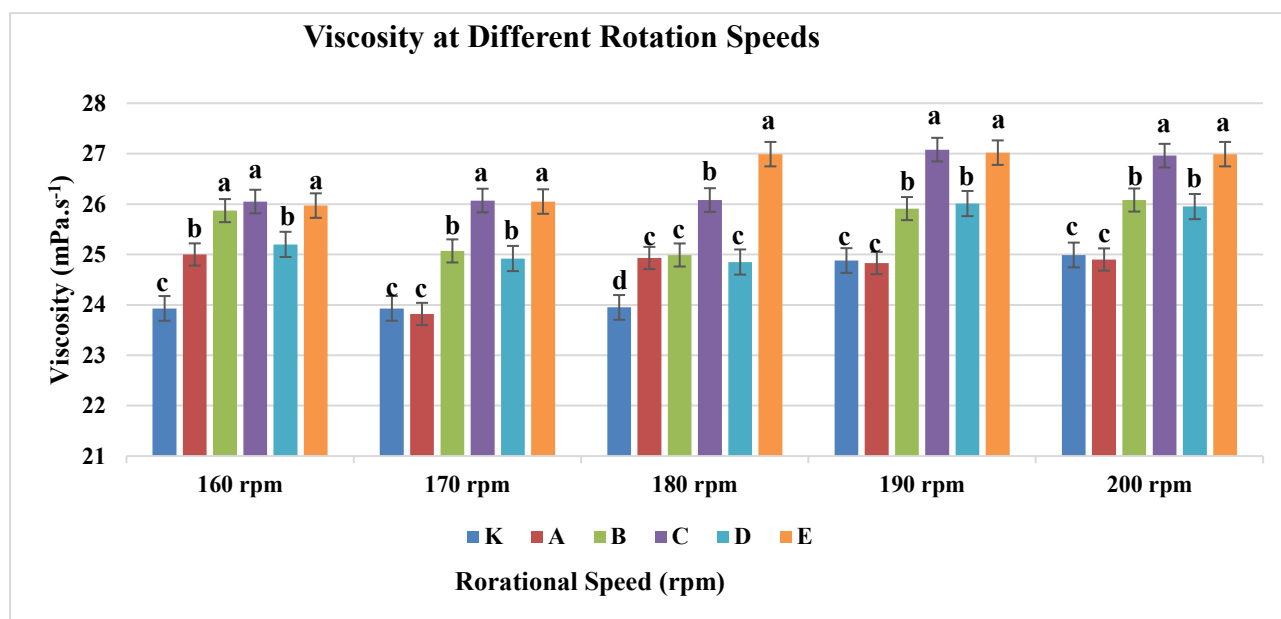
Table 3 Dry matter and moisture amount of whey beverage groups (%)

Days	K ($\bar{x} \pm SD$)	A ($\bar{x} \pm SD$)	B ($\bar{x} \pm SD$)	C ($\bar{x} \pm SD$)	D ($\bar{x} \pm SD$)	E ($\bar{x} \pm SD$)
Dry matter						
1	15.39 $\pm$ 0.1 A	15.83 $\pm$ 0.1 Ca	15.58 $\pm$ 0.1 B	16.41 $\pm$ 0.1 Da	15.56 $\pm$ 0.1 AB	16.27 $\pm$ 0.1 Da
14	15.48 $\pm$ 0.1 AB	16.33 $\pm$ 0.1 Cb	15.59 $\pm$ 0.1 B	16.51 $\pm$ 0.1 Dab	15.39 $\pm$ 0.1 A	16.37 $\pm$ 0.1 Ca
28	15.76 $\pm$ 0.1 A	16.46 $\pm$ 0.1 Bb	15.69 $\pm$ 0.1 A	16.97 $\pm$ 0.1 Cb	15.65 $\pm$ 0.1 A	18.96 $\pm$ 0.1 Db

Moisture						
1	84.61 ± 0.1 D	84.17 ± 0.1 B	84.42 ± 0.1 C	83.59 ± 0.1 A	84.44 ± 0.1 CD	83.73 ± 0.1 Ab
14	84.52 ± 0.1 CD	83.67 ± 0.1 B	84.41 ± 0.1 C	83.49 ± 0.1 A	84.61 ± 0.1 D	83.63 ± 0.1 Bb
28	84.34 ± 0.1 D	83.64 ± 0.1 C	84.41 ± 0.1 D	83.13 ± 0.1 B	84.45 ± 0.1 D	81.14 ± 0.1 Aa

* **K:** Whey, **A:** Whey + Anthocyanin, **B:** Whey + *B. coagulans*, **C:** Whey + *B. coagulans* + Inulin, **D:** Whey + Anthocyanin + *B. coagulans*, **E:** Whey + Anthocyanin + *B. coagulans* + Inulin. $\bar{x} \pm \text{SD}$: Mean and standard deviation. Differences between groups are shown in uppercase letters, and differences between days are shown in lowercase letters.

The viscosity results of beverage samples at different rotation speeds (rpm) are shown in Fig. 3. It is seen that the beverage groups show similar viscosities at different speeds. The lowest viscosity at 160 and 180 rpm was detected in group K (24 mPas⁻¹), while group E beverages had the highest viscosity (27 mPas⁻¹) at 180 and 190 rpm. Natural and artificial stabilizing agents are employed in the manufacturing of milk and dairy-based beverages to give rheological qualities such as texture, viscosity, and hardness (Oliveira et al., 2018; Silveira et al., 2015). In addition, one of the elements influencing viscosities is the beverage's entire solid and fibrous composition (Zaman et al., 2023). The beverage groups in this analysis exhibit comparable viscosities at various rates. Yanes et al. (2002) observed the rheological behavior of chocolate milk beverages and reported that the viscosity varied between 2.7-18.7 mPas⁻¹ at 25°C and increased in viscosity at 5°C. Rocha et al. (2018), in a study on whey beverages containing jabuticaba anthocyanins, 0.5%; In beverages containing 2%, 4% and 6% whey, viscosity values are <20.0, respectively; <20.0; They found it to be 21.0 and 62.0 mPas⁻¹. Şen and Yüceer (2019) detected viscosity values of 1.37 ± 0.0, 1.38 ± 0.1, 1.64 ± 0.1, and 1.61 ± 0.1 cP on the first day in whey beverage samples they developed using kefir. They reported that there was an increase in viscosity depending on shelf life. It was shown that the viscosity of a functional whey beverage with sugar cane increased with time (12.86 ± 0.21 to 13.95 ± 0.05 mPas⁻¹) until the end of storage (day 28), and the storage process had a highly significant effect on the viscosity of the beverage (Zaman et al., 2023). Rugji et al. (2022) determined the viscosity value as 14.5 mPas⁻¹ at 200 rpm in the group containing only whey in the functional whey beverages they prepared as mixtures with two different prebiotics and probiotics. Viscosity values of 5.8 mPa and 7.9 mPa were found by Vargas-Díaz et al. (2023) in their work, where they generated a fermented beverage from whey sweetened with tagatose. Thus, the same viscosity values of whey beverages can be explained by their identical total dry matter content, pH, and titratable acidity values. These findings may have a direct impact on the sensory enjoyment of prepared beverages.



* **K:** Whey, **A:** Whey + Anthocyanin, **B:** Whey + *B. coagulans*, **C:** Whey + *B. coagulans* + Inulin, **D:** Whey + Anthocyanin + *B. coagulans*, **E:** Whey + Anthocyanin + *B. coagulans* + Inulin. Mean $\pm$ SE with distinct lowercase letters on the bar indicating significant differences at $p < 0.05$ using the Tukey test. Differences between groups are shown in lowercase letters. Error bars represent the standard error (SE) of triplicate measurements ($n=3$)

Fig. 3 Viscosity chart of whey beverages at different rotation speeds (rpm)

On the 14th day of storage, the beverages' colors were measured, and the results are shown in Table 4. The groups' L^* values were between 53.3-56.9, and there was no statistically significant difference ($p > 0.05$) observed between these values. Groups A, D, and E that contained anthocyanins showed higher a^* values than the other groups, and this difference was statistically significant ($p < 0.05$). Group A has the highest value in comparison to the other groups, and this difference is also statistically significant ($p < 0.05$) when looking at the b^* value. Since whey is a yellow-green colored beverage due to its high riboflavin content, color analysis results for a pure whey beverage should show -a and +b values. The groups having anthocyanin in the whey beverages utilized in the study should have greater -a and +b values, as expected. Jing and Giusti (2005) discovered that the L^* value of anthocyanin-rich extracts from purple maize cobs was 56.2; the a^* and b^* values were 3.2 and -2.3, respectively. The color may have been redder and the a^* value higher due to the higher amount of anthocyanin extract applied in this investigation. As can be observed, the L^* value was discovered to be comparable to our research. L^* , a^* , and b^* values dropped with increasing whey content, according to Wang et al. (2023) investigation using whey containing rose anthocyanins extract. L^* , a^* , and b^* values in another study showing favorable values when 2% jabuticaba anthocyanins were added to whey beverages were noted, and the L^* value tended to rise with increasing whey concentration (Rocha et al., 2018).

Table 4. Color analysis of whey beverages during storage

Color value	Beverage samples					
	K ($\bar{x} \pm SD$)	A ($\bar{x} \pm SD$)	B ($\bar{x} \pm SD$)	C ($\bar{x} \pm SD$)	D ($\bar{x} \pm SD$)	E ($\bar{x} \pm SD$)
L*	56.9 $\pm$ 2.8	53.6 $\pm$ 2.8	53.3 $\pm$ 0.8	55.9 $\pm$ 3.6	53.9 $\pm$ 2.1	53.3 $\pm$ 2.8
a*	-3.98 $\pm$ 0.3 A	-1.54 $\pm$ 0.1 B	-3.52 $\pm$ 0.4 A	-3.29 $\pm$ 0.5 A	-1.71 $\pm$ 0.3 B	-1.3 $\pm$ 0.3 B
b*	4.58 $\pm$ 0.2 AB	4.80 $\pm$ 0.1 B	3.51 $\pm$ 0.3 A	4.53 $\pm$ 0.1 AB	4.49 $\pm$ 0.4 AB	4.51 $\pm$ 0.5 AB

* **K:** Whey, **A:** Whey + Anthocyanin, **B:** Whey + *B. coagulans*, **C:** Whey + *B. coagulans* + Inulin, **D:** Whey + Anthocyanin + *B. coagulans*, **E:** Whey + Anthocyanin + *B. coagulans* + Inulin. $\bar{x} \pm SD$: Mean and standard deviation. Differences between groups are shown in uppercase letters.

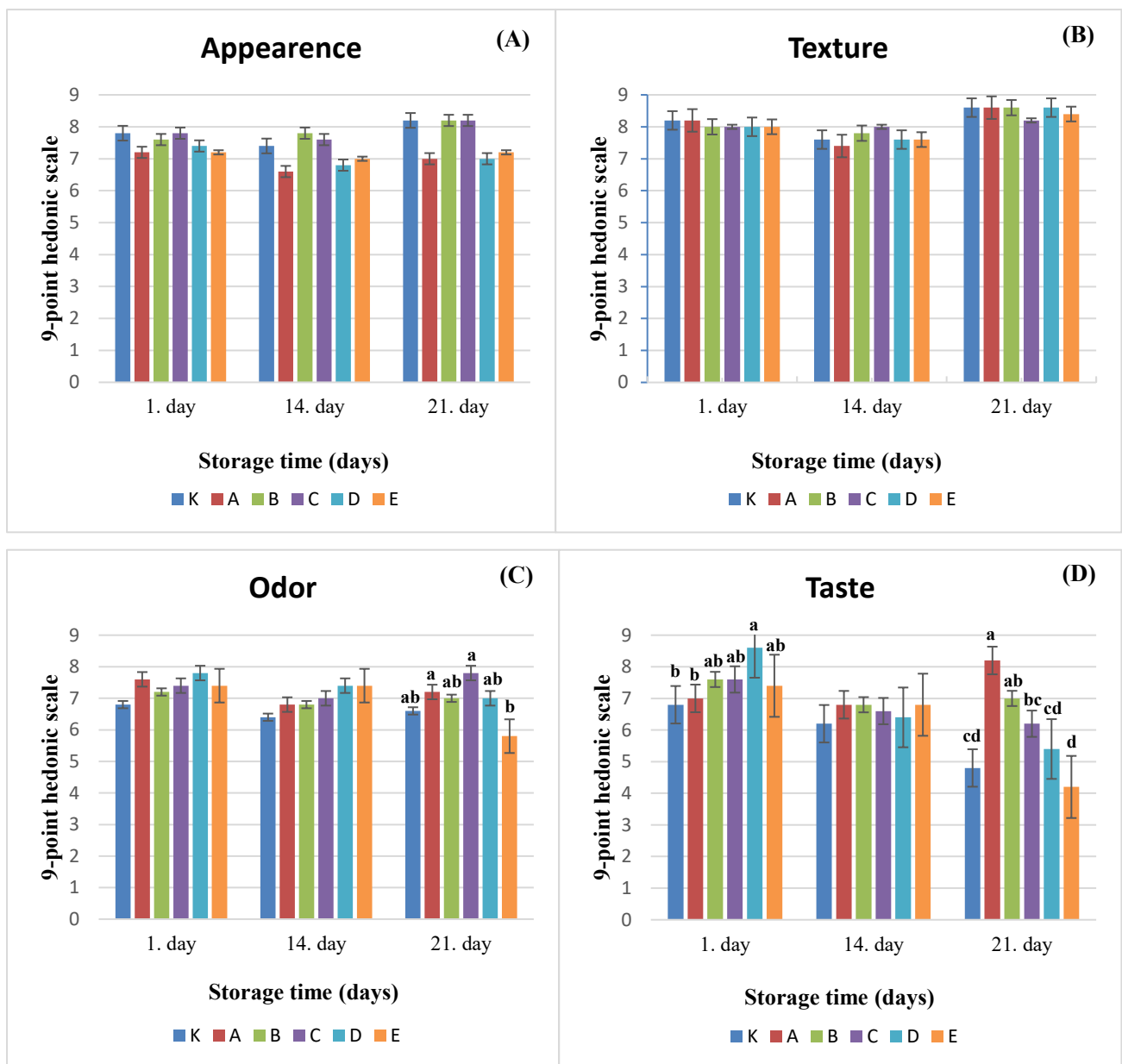
Antioxidant Capacity

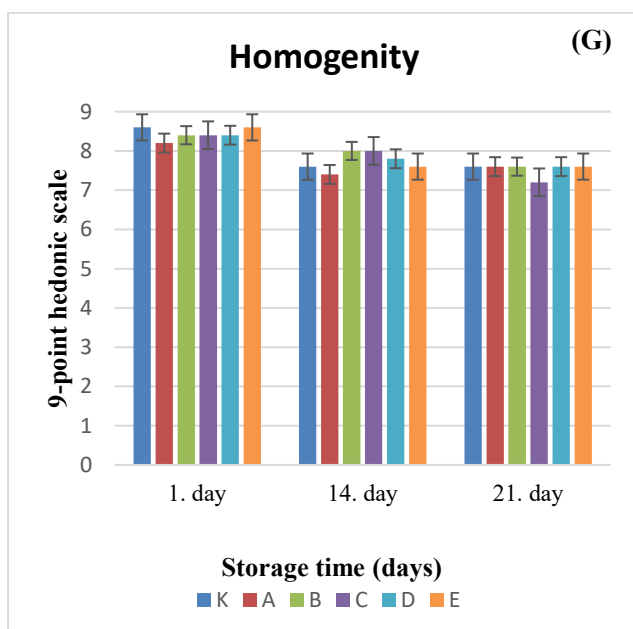
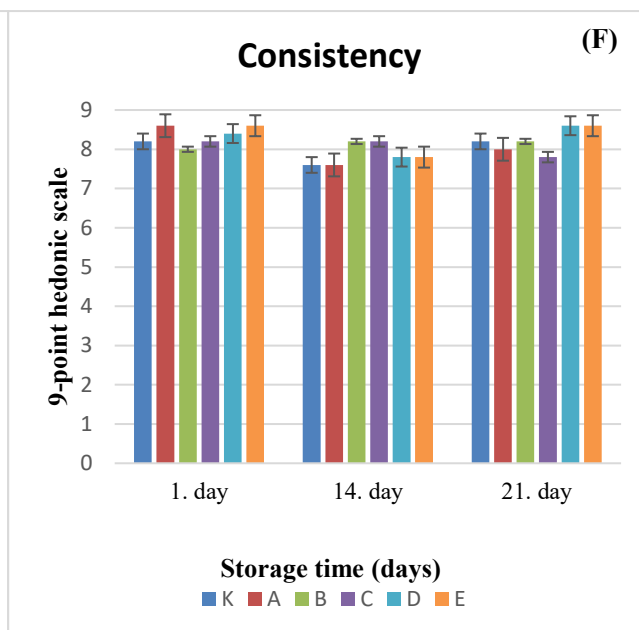
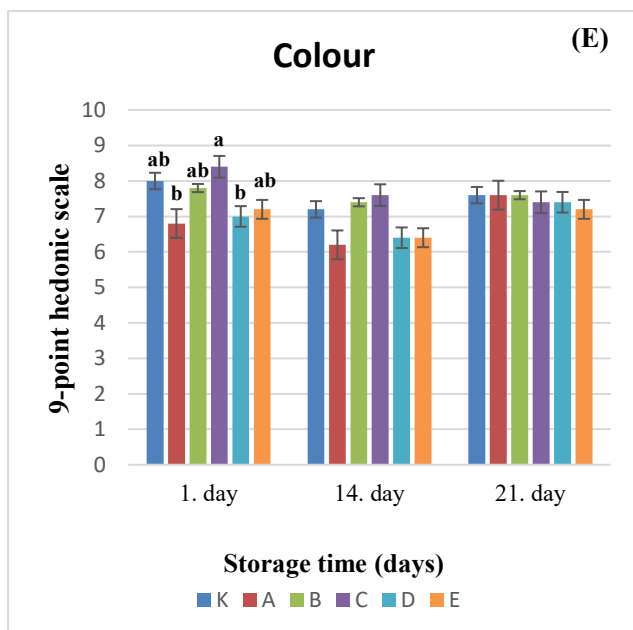
The whey beverages' DPPH-radical scavenging activities (RSA) were measured on the first day of storage. There was no statistically significant difference ($p > 0.05$) between the groups. However, the groups containing anthocyanin (E, D, and A) had the highest RSA values, with 60.63%, 60.21%, and 51.35%, respectively. The lowest value was found in group K, with 44.19%. The results of our study indicate that anthocyanins, even when employed in extremely low levels in beverages, can considerably boost antioxidant capacity since groups containing them demonstrated better antioxidant capacity than other groups. In a study conducted by Zaman et al. (2023), it was found that the antioxidant activity of a functional whey beverage containing sugar cane remained constant during storage, and storage days had an insignificant effect on antioxidants ($p > 0.05$). As the amount of sugar cane juice increased, antioxidants also increased. Oliveira et al. (2019) reported that there was a decrease in antioxidant activity in fermented whey beverages with *Kluyveromyces lactis* and beet juice between the beginning and the last days of shelf life (0 to 21 days), and the inhibition of DPPH varied between 38.69% and 81.02% after 21 days of storage. They say that antioxidant activity also increased due to the increase in the amount of beet juice. Purkiewicz and Pietrzak-Fiećko (2021) conducted a study on the antioxidant capacity of whey beverages and discovered that the highest antioxidant capacity was found in homemade whey beverages containing green fruits and vegetables (79.32%), while supermarket whey beverages containing red fruits and vegetables (7.35%) had the highest antioxidant capacity. They concluded that its antioxidant capacity was the lowest. In their investigation using fermented milk and yoghurt containing acai berries, Campos et al. (2017) reported that antioxidant activity had a strong association with anthocyanins and declined as these components decreased.

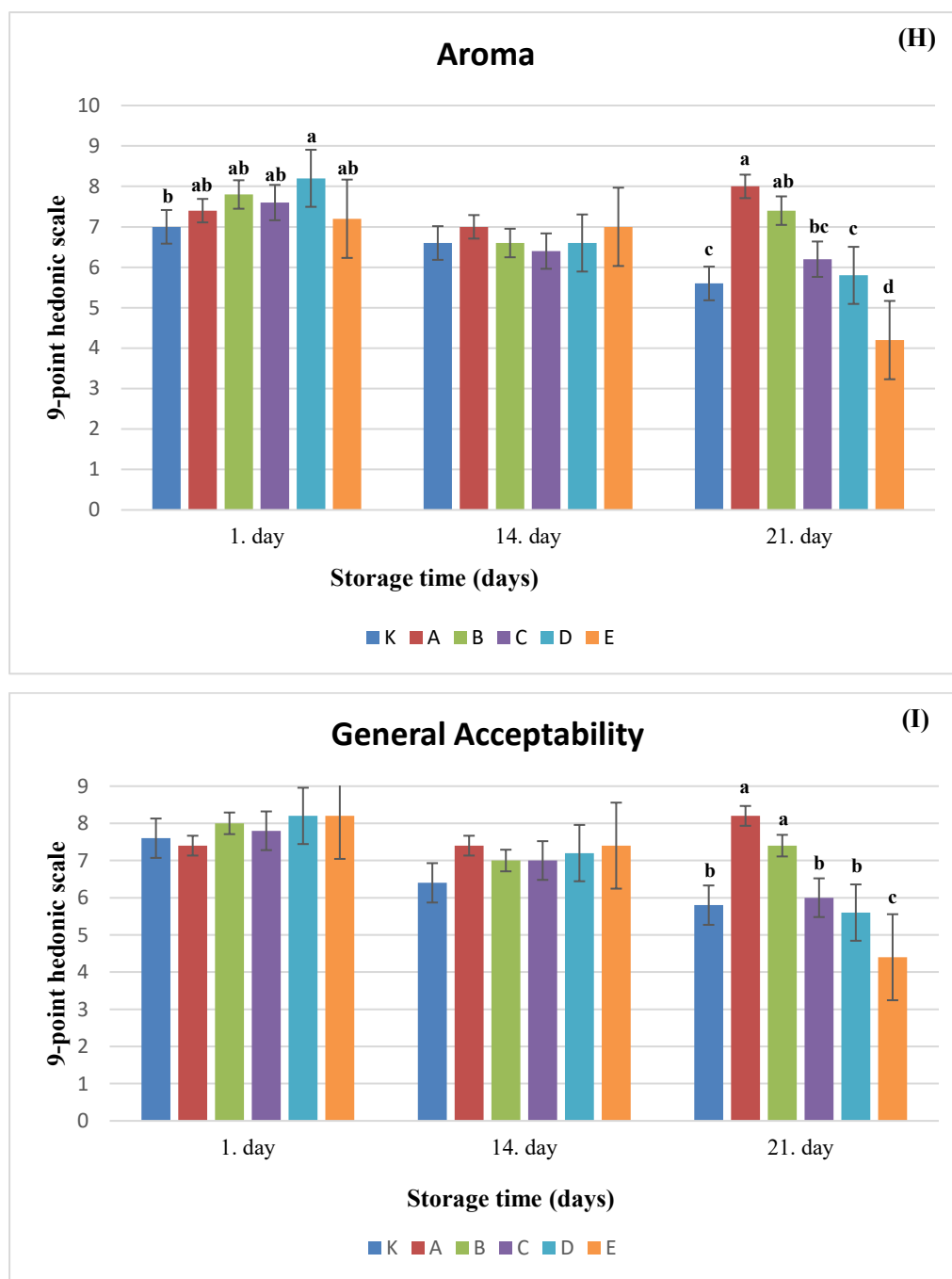
Sensory Analysis

Sensory analyses of whey beverages were carried out on the first, 14th, and 21st days after storage (Fig. 4). Regarding the 'odor' parameter, only the score provided by the panelists on the 21st day in group E was found to be statistically lower than the scores on the other days ($p < 0.05$). On the 14th day, there is no statistically significant difference between the groups when examining the 'aroma' scores of whey beverages ($p > 0.05$). When the panelists evaluated the beverages according to 'taste' ($p > 0.05$), there was no statistically significant difference between the groups on the 14th day. Group A achieved the highest scores, while group E obtained the lowest scores on the 21st day, similar to the 'aroma' parameter. It is obvious that the group was given the anthocyanin-containing groups (A, D, and E) scored lower than the other groups on the first and 14th day, according to the analysis of the 'Color' parameter; however, this difference was not statistically significant ($p > 0.05$). There were no significant differences between the groups in terms of 'general acceptability' assessments on the first and 14th day ($p > 0.05$). In the analysis conducted on the 21st day, group A scored the highest with 8.2, while group E scored the lowest with 4.4. There is still much to be learned about how to raise the amount of whey consumed in the beverage sector, even though whey has been extensively researched and used in the composition of many dairy products. A large number of patents about the process of preparing whey beverages through the addition of different components have been registered recently. For instance, whey beverages have been made with a variety of citrus fruits, tropical fruits, and other fruits like strawberries, apples, cherries, pears, apricots, or melons (Djurić et al., 2004). Nevertheless, whey lacks pleasing color, flavor, and aroma when consumed alone. As a result, different substances are added to food to enhance its sensory qualities as well as its nutritional value. The finished product must be consumable or acceptable to customers, even though functional features are also extremely important. Protein concentrations utilized in combination with anthocyanins still require optimization, according to Ren et al. (2021), because they have an impact on the rheological and sensory qualities of anthocyanin-protein complexes, two crucial characteristics for food and beverage products. Panelists assessed whey beverages according to homogeneity, homogeneity, appearance, texture, odor, flavor, taste, consistency, and color in the current study. It was concluded that there was no statistically significant variation in the panelists' ratings for appearance, texture, consistency, and homogeneity among groups and days. Islam et al. (2021) found statistically significant variations in the average scores of whey-pineapple beverages for color and appearance, flavor, mouthfeel, sweetness, and overall acceptability. On the other hand, a 0-5 scoring system was used, and graphical results were displayed. Upon closer inspection, the graph reveals that whey-pineapple beverages gradually lose points while they are stored. It was stressed at the study's conclusion that the beverages made were a

significant development for food technology. Based on the sensory analysis results of the current study, it is thought that whey beverages have a high potential to be a new functional beverage. Dinçoğlu and Rugji (2021), in their study on the use of rose oil as a functional food in probiotic fermented whey, found that the control group received the lowest score (2.38 ± 0.54) on the first day of production in the scores the panelists gave to whey beverages for the 'generally acceptable' parameter. Similar results were determined on other analysis days and were reported as 2.00 ± 0.47 , 1.81 ± 0.36 , 1.75 ± 0.39 , and 1.63 ± 0.45 , respectively. In our study, it is seen that the highest score given by the panelists to whey beverages on the 21st day is 8.2 ± 0.4 (group A), and the lowest score is 4.4 ± 0.5 (group E). For this reason, although whey beverages received lower scores in our study due to the progress of preservation, it can be said that they received higher scores compared to the study of Dinçoğlu and Rugji (2021). Skryplonek et al. (2019) assessed the physicochemical and sensory characteristics of fermented beverages containing probiotic bacteria based on acid whey combined with milk, condensed milk, or skim milk powder. It was reported that the hardness of the samples did not change during storage, and the beverage made from whey, milk, condensed milk, and *L. acidophilus* had the best sensory properties. In their study, Ahmed et al. (2023) asked the panelists to rate the whey-mango beverages on a scale of 1 to 9 for color, flavor, taste, appearance, and overall acceptability. It was found that the panelists' ratings of the beverages declined in direct proportion to the duration of storage. Thus, it can be concluded that one of the most crucial factors in determining whether or not the newly produced functional beverage is accepted is the sensory evaluation. Even if whey beverages don't have appealing sensory qualities, it can be said that the more ingredients added to the beverage, the more consumable it becomes. In addition to the use of different food components and hedonic scales in the studies, sharing graphical presentations rather than numerical data prevents an effective discussion. However, it can still be said that the consumption desires of different whey beverages decrease due to the advancement of their shelf life. The existence of microbiological activities related to the preservation of beverages affects the results, such as pH, acidity, color, odor, aroma, and viscosity. These parameters are directly reflected in sensory evaluations.







Sensory evaluation shows Appearance (A), Texture (B), Odor (C), Taste (D), Colour (E), Consistency (F), Homogeneity (G), Aroma (H), and General Acceptability (I) parameters. K: Whey, A: Whey + Anthocyanin, B: Whey + *B. coagulans*, C: Whey + *B. coagulans* + Inulin, D: Whey + Anthocyanin + *B. coagulans*, E: Whey + Anthocyanin + *B. coagulans* + Inulin. Mean $\pm$ SE with distinct lowercase letters on the bar indicating significant differences at $p < 0.05$ using the Tukey test. Differences between groups are shown in lowercase letters. Error bars represent the standard error (SE) of panelist scores (n=10)

Fig. 4 Sensory analysis chart of whey beverages

Conclusion

Some of the 6 different whey beverage groups produced in the study were fortified with WPI-encapsulated elderberry anthocyanins, *B. coagulans*, and inulin. In the anthocyanin-containing groups

(A, D, and E), there were reductions in both the stability and quantity of anthocyanin. The panelists evaluated the beverages and gave very positive feedback on the first day of storage, but the scores tended to decrease on the 21st day. Sensory analyses could not be carried out on the 28th day due to the physicochemical and microbiological changes in the beverages. According to the available literature, no research has been conducted on the creation of a functional whey beverage with probiotic and prebiotic mixes, elderberry fruit extracts, and encapsulated anthocyanins. This is a noteworthy area of study. Promising results from analyses on whey beverages that were enhanced with encapsulated anthocyanins, BC30, and inulin suggest that the resulting beverage may have beneficial characteristics. The beverage produced demonstrates promising in vitro antioxidant activity and sustains probiotic viability at acceptable levels. Nonetheless, any prospective health advantages necessitate confirmation via in vivo and clinical investigations. The results shown here describe the beverage's functional properties, but they don't make any health claims. The sensory analysis findings suggest that the product is readily consumable. Conducting research and development in conjunction with facilities that have more standardized and closed system production conditions can help to obtain optimal results and facilitate the product's integration into the industrial field, which is one of the study's objectives. The information gathered and provided in this study holds promise for helping researchers and scientists understand the potential health impacts of the product. To aid the integration of this functional beverage into the food industry, interventional clinical studies will be carried out using the available data.

Acknowledgements

The authors gratefully acknowledge financial support by Burdur Mehmet Akif Ersoy University Scientific Research Projects Coordinator (grant number 0503-YL-18).

Author Contribution

EBÖ: Writing - original draft, Visualization, Project administration, Methodology, Investigation, Formal analysis, Conceptualization. AHD: Writing - review and editing, Visualization, Supervision, Formal analysis, Conceptualization, Resources, Project administration, Funding acquisition. MÖ: Writing - review and editing, Visualization, Formal analysis.

Funding

This research was funded by Burdur Mehmet Akif Ersoy University Scientific Research Projects Coordinator (grant number 0503-YL-18).

Data availability

All data generated or analyzed during this study are included in this published article.

Declarations

Competing interests

The authors declare no competing interests.

References

- Abhari, K., Shekarforous, S. S., Hosseinzadeh, S., Nazifi, S., Sajedianfard, J., & Eskandari, M. H. (2016). The effects of orally administered *Bacillus coagulans* and inulin on prevention and progression of rheumatoid arthritis in rats. *Food & Nutrition Research*, 60(1), 1–9. <https://doi.org/10.3402/fnr.v60.30876>
- Ahmed, T., Sabuz, A. A., Mohaldar, A., Fardows, H. S., Inbaraj, B. S., Sharma, M., et al. (2023). Development of novel whey–mango-based mixed beverage: Effect of storage on physicochemical, microbiological, and sensory analysis. *Foods*, 12(2), 237. <https://doi.org/10.3390/foods12020237>
- Amine, K. M., Champagne, C. P., Salmieri, S., Britten, M., St-Gelais, D., Fustier, P., et al. (2014). Effect of palmitoylated alginate microencapsulation on viability of *Bifidobacterium longum* during freeze-drying. *LWT – Food Science and Technology*, 56(1), 111–117. <https://doi.org/10.1016/j.lwt.2013.11.003>
- AOAC International. (2019). *Official methods of analysis of AOAC International* (21st ed.). Washington, DC: AOAC International.
- Awuah, G. B., Ramaswamy, H. S., & Economides, A. (2007). Thermal processing and quality: Principles and overview. *Chemical Engineering and Processing: Process Intensification*, 46(6), 584–602. <https://doi.org/10.1016/j.cep.2006.08.004>
- Baeza, R., & Chirife, J. (2021). Anthocyanin content and storage stability of spray/freeze-drying microencapsulated anthocyanins from berries: A review. *International Journal of Food Engineering*, 17(12), 927–944. <https://doi.org/10.1515/ijfe-2021-0184>
- Baeza, R., Sánchez, V., Salierno, G., Molinari, F., López, P., & Chirife, J. (2021). Storage stability of anthocyanins in freeze-dried elderberry pulp using low proportions of encapsulating agents. *Food Science and Technology International*, 27(2), 135–144. <https://doi.org/10.1177/1082013220937867>
- Benson, K. F., Redman, K. A., Carter, S. G., Keller, D., Farmer, S., Endres, J. R., et al. (2012). Probiotic metabolites from *Bacillus coagulans* GanedenBC30 support maturation of antigen-presenting cells *in vitro*. *World Journal of Gastroenterology*, 18(16), 1875–1883. <https://doi.org/10.3748/wjg.v18.i16.1875>

- Brand-Williams, W., Cuvelier, M. E., & Berset, C. L. W. T. (1995). Use of a free radical method to evaluate antioxidant activity. *LWT – Food Science and Technology*, 28(1), 25–30. [https://doi.org/10.1016/S0023-6438\(95\)80008-5](https://doi.org/10.1016/S0023-6438(95)80008-5)
- Brønnum-Hansen, K., & Hansen, S. H. (1983). High-performance liquid chromatographic separation of *Sambucus nigra* L. *Journal of Chromatography A*, 262, 385–392. [https://doi.org/10.1016/S0021-9673\(01\)88125-5](https://doi.org/10.1016/S0021-9673(01)88125-5)
- Bryła, A., Lewandowicz, G., & Juzwa, W. (2015). Encapsulation of elderberry extract into phospholipid nanoparticles. *Journal of Food Engineering*, 167, 189–195. <https://doi.org/10.1016/j.jfoodeng.2015.07.025>
- Buitimea-Cantúa, N. E., Gutiérrez-Urbe, J. A., & Serna-Saldívar, S. O. (2018). Phenolic–protein interactions: Effects on food properties and health benefits. *Journal of Medicinal Food*, 21(2), 188–198. <https://doi.org/10.1089/jmf.2017.0057>
- Campos, D. C. D. S., Neves, L. T. B. C., Flach, A., Costa, L. A. M. A., & Sousa, B. O. D. (2017). Post-acidification and evaluation of anthocyanins stability and antioxidant activity in açai fermented milk and yogurts (*Euterpe oleracea* Mart.). *Revista Brasileira de Fruticultura*, 39, e-871. <https://doi.org/10.1590/0100-29452017871>
- Cai, Z., Stubblefield, K., Thomas, A. L., & Aguilar, F. X. (2024). From niche to mainstream: U.S. consumer trends and preferences for elderberry products. *HortScience*, 59(12), 1723–1729. <https://doi.org/10.21273/HORTSCI18180-24>
- Cao, J., Yu, Z., Zhang, Q., Yu, L., Zhao, J., Zhang, H., et al. (2022). Effects of *Bacillus coagulans* GBI-30, 6086 as an adjunct starter culture on the production of yogurt. *Food Research International*, 160, 111398. <https://doi.org/10.1016/j.foodres.2022.111398>
- Casati, C. B., Baeza, R., & Sánchez, V. (2019). Physicochemical properties and bioactive compounds content in encapsulated freeze-dried powders obtained from blueberry, elderberry, blackcurrant, and maqui berry. *Journal of Berry Research*, 9(3), 431–447. <https://doi.org/10.3233/JBR-190409>
- Charlebois, D., Byers, P. L., Finn, C. E., & Thomas, A. L. (2010). Elderberry: Botany, horticulture, potential. *Horticultural Reviews*, 37, 213–280. <https://doi.org/10.1002/9780470543672.ch4>
- Cheng, X., Zhu, J., Chen, Z., Wu, Z., Zhang, F., Wu, C., et al. (2023). Color stability and degradation kinetics of anthocyanins in mulberry stirred yoghurt fermented by different starter cultures. *Food Science and Biotechnology*, 32, 1351–1359. <https://doi.org/10.1007/s10068-023-01271-8>
- Chung, C., Rojanasasithara, T., Mutilangi, W., & McClements, D. J. (2015). Enhanced stability of anthocyanin-based color in model beverage systems through whey protein isolate complexation. *Food Research International*, 76, 761–768. <https://doi.org/10.1016/j.foodres.2015.07.003>

- Costica, N., Stratu, A., Boz, I., & Gille, E. (2019). Characteristics of elderberry (*Sambucus nigra* L.) fruit. *Agriculturae Conspectus Scientificus*, 84(1), 115–122. <https://hrcak.srce.hr/218529>
- Da Rosa, J. R., Nunes, G. L., Motta, M. H., Fortes, J. P., Weis, G. C. C., Hecktheuer, L. H. R., et al. (2019). Microencapsulation of anthocyanin compounds extracted from blueberry (*Vaccinium spp.*) by spray drying: Characterization, stability and simulated gastrointestinal conditions. *Food Hydrocolloids*, 89, 742–748. <https://doi.org/10.1016/j.foodhyd.2018.11.042>
- De Clerck, E., Rodriguez-Diaz, M., Forsyth, G., Lebbe, L., Logan, N. A., & De Vos, P. (2004). Polyphasic characterization of *Bacillus coagulans* strains, illustrating heterogeneity within this species, and emended description of the species. *Systematic and Applied Microbiology*, 27(1), 50–60. <https://doi.org/10.1078/0723-2020-00250>
- De Jesus, A. L. T., Cristianini, M., Dos Santos, N. M., & Júnior, M. R. M. (2020). Effects of high hydrostatic pressure on the microbial inactivation and extraction of bioactive compounds from açai (*Euterpe oleracea* Martius) pulp. *Food Research International*, 130, 108856. <https://doi.org/10.1016/j.foodres.2019.108856>
- Dinçoğlu, A. H., & Rugji, J. (2021). Use of rose oil in probiotic fermented whey as a functional food. *Journal of Food Science and Technology*, 58, 2705–2713. <https://doi.org/10.1007/s13197-020-04778-8>
- Deng, W., Li, X., Ren, G., Bu, Q., Ruan, Y., Feng, Y., et al. (2023). Stability of purple corn anthocyanin encapsulated by maltodextrin and its combinations with gum arabic and whey protein isolate. *Foods*, 12(12), 2393. <https://doi.org/10.3390/foods12122393>
- Dinkova, R., Heffels, P., Shikov, V., Weber, F., Schieber, A., & Mihalev, K. (2014). Effect of enzyme-assisted extraction on the chilled storage stability of bilberry (*Vaccinium myrtillus* L.) anthocyanins in skin extracts and freshly pressed juices. *Food Research International*, 65, 35–41. <https://doi.org/10.1016/j.foodres.2014.05.066>
- Diviš, P., Pořízka, J., Vespalcová, M., Matějček, A., & Kaplan, J. (2015). Elemental composition of fruits from different black elder (*Sambucus nigra* L.) cultivars grown in the Czech Republic. *Journal of Elementology*, 20(3), 549–557. <https://doi.org/10.5601/jelem.2015.20.1.758>
- Djurić, M., Carić, M., Milanović, S., Tekić, M., & Panić, M. (2004). Development of whey-based beverages. *European Food Research and Technology*, 219, 321–328. <https://doi.org/10.1007/s00217-004-0950-1>
- Domínguez, R., Zhang, L., Rocchetti, G., Lucini, L., Pateiro, M., Munekata, P. E. S., et al. (2020). Elderberry (*Sambucus nigra* L.) as potential source of antioxidants: Characterization, optimization of extraction parameters, and bioactive properties. *Food Chemistry*, 330, 127266. <https://doi.org/10.1016/j.foodchem.2020.127266>

- Ehsannia, S., & Sanjabi, M. R. (2016). Quality characterization of processed cheese inoculated by *Bacillus coagulans* during cold storage: Compositional and sensorial attributes and probiotic microorganism viability. *Journal of Food Processing and Preservation*, 40, 667–674. <https://doi.org/10.1111/jfpp.12646>
- Enache, I. M., Vasile, A. M., Enachi, E., Barbu, V., Stănciuc, N., & Vizireanu, C. (2020). Co-microencapsulation of anthocyanins from cornelian cherry fruits and lactic acid bacteria in biopolymeric matrices by freeze-drying: Evidence on functional properties and applications in food. *Polymers*, 12(4), 906. <https://doi.org/10.3390/polym12040906>
- Endres, J. R., Clewell, A., Jade, K. A., Farber, T., Hauswirth, J., & Schauss, A. G. (2009). Safety assessment of a proprietary preparation of a novel probiotic, *Bacillus coagulans*, as a food ingredient. *Food and Chemical Toxicology*, 47(6), 1231–1238. <https://doi.org/10.1016/j.fct.2009.02.018>
- U.S. Food and Drug Administration (FDA). (2016). *Notice to the U.S. Food and Drug Administration that Bacillus coagulans GBI-30, 6086 is generally recognized as safe for use in non-exempt term infant formula*. Seattle, WA, United States.
- FAO/WHO. (2001). *Health and nutritional properties of probiotics in food including powder milk with live lactic acid bacteria*. Report of a Joint FAO/WHO Expert Consultation, Córdoba, Argentina.
- Fernandez-Lopez, J. A., Angosto, J., Gimenez, P., & Leon, G. (2013). Thermal stability of selected natural red extracts used as food colorants. *Plant Foods for Human Nutrition*, 68, 11–17. <https://doi.org/10.1007/s11130-013-0337-1>
- Ferreira, S. S., Silva, A. M., & Nunes, F. M. (2022). *Sambucus nigra* L. fruits and flowers: Chemical composition and related bioactivities. *Food Reviews International*, 38(6), 1237–1265. <https://doi.org/10.1080/87559129.2020.1788578>
- Guimaraes, J. T., Silva, E. K., Ranadheera, C. S., Moraes, J., Raices, R. S., Silva, M. C., et al. (2019). Effect of high-intensity ultrasound on the nutritional profile and volatile compounds of a prebiotic soursop whey beverage. *Ultrasonics Sonochemistry*, 55, 157–164. <https://doi.org/10.1016/j.ultsonch.2019.02.025>
- Ha, H. K., Jeon, N. E., Kim, J. W., Han, K. S., Yun, S. S., Lee, M. R., et al. (2016). Physicochemical characterization and potential prebiotic effect of whey protein isolate/inulin nanocomplex. *Food Science of Animal Resources*, 36(2), 267–274. <https://doi.org/10.5851/kosfa.2016.36.2.267>
- Hervet, C. J., Martin, N. H., Boor, K. J., & Wiedmann, M. (2017). Survival and detection of coliforms, *Enterobacteriaceae*, and gram-negative bacteria in Greek yogurt. *Journal of Dairy Science*, 100(2), 950–960. <https://doi.org/10.3168/jds.2016-11553>

- Islam, M. Z., Tabassum, S., Harun-ur-Rashid, M., Vegarud, G. E., Alam, M. S., & Islam, M. A. (2021). Development of probiotic beverage using whey and pineapple (*Ananas comosus*) juice: Sensory and physicochemical properties and probiotic survivability during *in-vitro* gastrointestinal digestion. *Journal of Agriculture and Food Research*, 4, 100144. <https://doi.org/10.1016/j.jafr.2021.100144>
- Ismail, A. E., Abdelgader, M. O., & Ali, A. A. (2011). Microbial and chemical evaluation of whey-based mango beverage. *Advance Journal of Food Science and Technology*, 3(4), 250–253.
- ISO. (2016). *Sensory analysis — Methodology — General guidance for establishing a sensory profile* (ISO Standard No. 13299:2016). Geneva, Switzerland: International Organization for Standardization.
- ISO. (2008). *Microbiology of food and animal feeding stuffs — Horizontal method for the enumeration of yeasts and molds — Part 2: Colony count technique in products with water activity less than or equal to 0.95* (ISO Standard No. 21527-2:2008). Geneva, Switzerland: International Organization for Standardization.
- Jing, P., & Giusti, M. M. (2005). Characterization of anthocyanin-rich waste from purple corn cobs (*Zea mays* L.) and its application to color milk. *Journal of Agricultural and Food Chemistry*, 53, 8775–8781. <https://doi.org/10.1021/jf051247o>
- Kaack, K., Frette, X. C., Christensen, L. P., Landbo, A. K., & Meyer, A. S. (2008). Selection of elderberry (*Sambucus nigra* L.) genotypes best suited for the preparation of juice. *European Food Research and Technology*, 226, 843–855. <https://doi.org/10.1007/s00217-007-0605-0>
- Kareb, O., & Aïder, M. (2019). Whey and its derivatives for probiotics, prebiotics, synbiotics, and functional foods: A critical review. *Probiotics and Antimicrobial Proteins*, 11(2), 348–369. <https://doi.org/10.1007/s12602-018-9427-6>
- Kassem, J. M., Tawfik, N. F., El-Shafei, K., Assem, F. M., Effat, B. A., Dabiza, N., et al. (2025). Polyphenols, physicochemical, and microbiological properties of functional carbonated milk and whey-based beverages enriched with mango and *Lactobacillus plantarum*. *Egyptian Journal of Chemistry*, 68(4), 479–490. <https://doi.org/10.21608/ejchem.2024.283560.9606>
- Kobus-Cisowska, J., Szymanowska, D., Maciejewska, P., Szczepaniak, O., Kmiecik, D., Gramza-Michałowska, A., et al. (2019). Enriching novel dark chocolate with *Bacillus coagulans* as a way to provide beneficial nutrients. *Food & Function*, 10(2), 997–1006. <https://doi.org/10.1039/C8FO02099J>
- Konuray, G., & Erginkaya, Z. (2018). Potential use of *Bacillus coagulans* in the food industry. *Foods*, 7(6), 92. <https://doi.org/10.3390/foods7060092>

- Larionov, G., Semenov, V., Lavrentyev, A., Sherne, V., Kayukova, O., Mardaryeva, N., et al. (2020). Production of mint whey drink at private and collective farms and agricultural holdings. *IOP Conference Series: Earth and Environmental Science*, 604(1), 012042. <https://doi.org/10.1088/1755-1315/604/1/012042>
- Liao, M., Ma, L., Miao, S., Hu, X., Liao, X., Chen, F., & Ji, J. (2021). The *in-vitro* digestion behaviors of milk proteins acting as wall materials in spray-dried microparticles: Effects on the release of loaded blueberry anthocyanins. *Food Hydrocolloids*, 115, 106620. <https://doi.org/10.1016/j.foodhyd.2021.106620>
- Lynch, J. M., & Barbano, D. M. (1999). Kjeldahl nitrogen analysis as a reference method for protein determination in dairy products. *Journal of AOAC International*, 82(6), 1389–1398. <https://doi.org/10.1093/jaoac/82.6.1389>
- Maity, T. K., Kumar, R., & Misra, A. K. (2008). Development of healthy whey drink with *Lactobacillus rhamnosus*, *Bifidobacterium bifidum*, and *Propionibacterium freudenreichii* subsp. *shermanii*. *Mljekarstvo*, 58(4), 315–325. <https://hrcak.srce.hr/30635>
- Majeed, M., Nagabhushanam, K., Natarajan, S., Sivakumar, A., Eshuis-de Ruiter, T., Booij-Veurink, J., et al. (2016). Evaluation of genetic and phenotypic consistency of *Bacillus coagulans* MTCC 5856: A commercial probiotic strain. *World Journal of Microbiology and Biotechnology*, 32, 1–12. <https://doi.org/10.1007/s11274-016-2027-2>
- Meyer, D., Bayarri, S., Tárrega, A., & Costell, E. (2011). Inulin as a texture modifier in dairy products. *Food Hydrocolloids*, 25(8), 1881–1890. <https://doi.org/10.1016/j.foodhyd.2011.04.012>
- Miranda, J. S., Costa, B. V., de Oliveira, I. V., de Lima, D. C. N., Martins, E. M. F., Júnior, B. R. D. C. L., et al. (2020). Probiotic jelly candies enriched with native Atlantic Forest fruits and *Bacillus coagulans* GBI-30 6086. *LWT – Food Science and Technology*, 126, 109275. <https://doi.org/10.1016/j.lwt.2020.109275>
- Murali, S., Kar, A., Mohapatra, D., & Kalia, P. (2015). Encapsulation of black carrot juice using spray and freeze drying. *Food Science and Technology International*, 21(8), 604–612. <https://doi.org/10.1177/1082013214557843>
- Naik, B., Kohli, D., Walter, N., Gupta, A. K., Mishra, S., Khan, J. M., et al. (2023). Whey–carrot-based functional beverage: Development and storage study. *Journal of King Saud University – Science*, 35, 102775. <https://doi.org/10.1016/j.jksus.2023.102775>
- Nielsen, L., Rolighed, M., Buehler, A., Knöchel, S., Wiedmann, M., & Marvig, C. (2021). Development of predictive models evaluating the spoilage-delaying effect of a bioprotective culture on different yeast species in yogurt. *Journal of Dairy Science*, 104(9), 9570–9582. <https://doi.org/10.3168/jds.2020-20076>

- Nooshkam, M., Varidi, M., & Alkobeisi, F. (2022). Bioactive food foams stabilized by licorice extract/whey protein isolate/sodium alginate ternary complexes. *Food Hydrocolloids*, 126, 107488. <https://doi.org/10.1016/j.foodhyd.2022.107488>
- Oliveira, A. S., Niro, C. M., Bresolin, J. D., Soares, V. F., Ferreira, M. D., Sivieri, K., et al. (2021). Dehydrated strawberries for probiotic delivery: Influence of dehydration and probiotic incorporation methods. *LWT – Food Science and Technology*, 144, 111105. <https://doi.org/10.1016/j.lwt.2021.111105>
- Oliveira, D. F., Granato, D., & Barana, A. C. (2018). Development and optimization of a mixed beverage made of whey and water-soluble soybean extract flavored with chocolate using a simplex-centroid design. *Food Science and Technology*, 60, 413–420. <https://doi.org/10.1590/1678-457X.07017>
- Oliveira, D. R., Lopes, A. C. A., Pereira, R. A., Cardoso, P. G., & Duarte, W. F. (2019). Selection of potentially probiotic *Kluyveromyces lactis* for the fermentation of cheese whey-based beverage. *Annals of Microbiology*, 69, 1361–1372. <https://doi.org/10.1007/s13213-019-01518-y>
- Oliveira, G. A., Guimarães, J. T., Ramos, G. L. P., Esmerino, E. A., Pimentel, T. C., Neto, R. P., et al. (2022). Benefits of thermosonication in orange juice whey drink processing. *Innovative Food Science and Emerging Technologies*, 75, 102876. <https://doi.org/10.1016/j.ifset.2021.102876>
- Özer, D., Akin, S., & Özer, B. (2005). Effect of inulin and lactulose on survival of *Lactobacillus acidophilus* LA-5 and *Bifidobacterium bifidum* BB-02 in acidophilus-bifidus yoghurt. *Food Science and Technology International*, 11(1), 19–24. <https://doi.org/10.1177/1082013205051275>
- Palencia-Argel, M., Rodríguez-Villamil, H., Bernal-Castro, C., Díaz-Moreno, C., & Fuenmayor, C. A. (2022). Probiotics in anthocyanin-rich fruit beverages: Research and development for novel synbiotic products. *Critical Reviews in Food Science and Nutrition*, 1–17. <https://doi.org/10.1080/10408398.2022.2104806>
- Porter, R. S., & Bode, R. F. (2017). A review of the antiviral properties of black elder (*Sambucus nigra* L.) products. *Phytotherapy Research*, 31(4), 533–554. <https://doi.org/10.1002/ptr.5782>
- Purkiewicz, A., & Pietrzak-Fiećko, R. (2021). Antioxidant properties of fruit and vegetable whey beverages and fruit and vegetable mousses. *Molecules*, 26(11), 3126. <https://doi.org/10.3390/molecules26113126>
- Rather, S. A., Akhter, R., Masoodi, F. A., Gani, A., & Wani, S. M. (2017). Effect of double alginate microencapsulation on *in vitro* digestibility and thermal tolerance of *Lactobacillus plantarum* NCDC201 and *L. casei* NCDC297. *LWT – Food Science and Technology*, 83, 50–58. <https://doi.org/10.1016/j.lwt.2017.04.036>

- Ren, S., Jiménez-Flores, R., & Giusti, M. M. (2021). The interactions between anthocyanin and whey protein: A review. *Comprehensive Reviews in Food Science and Food Safety*, 20(6), 5992–6011. <https://doi.org/10.1111/1541-4337.12854>
- Rezvankhah, A., Emam-Djomeh, Z., & Askari, G. (2020). Encapsulation and delivery of bioactive compounds using spray and freeze-drying techniques: A review. *Drying Technology*, 38(1–2), 235–258. <https://doi.org/10.1080/07373937.2019.1653906>
- Ribeiro, A. M., Estevinho, B. N., & Rocha, F. (2019). Spray-drying encapsulation of elderberry extract and evaluating the release and stability of phenolic compounds in encapsulated powders. *Food and Bioprocess Technology*, 12, 1381–1394. <https://doi.org/10.1007/s11947-019-02304-z>
- Rocha, J. D. C. G., Viana, K. W. C., Mendonca, A. C., Neves, N. D. A., Carvalho, A. F. D., & Minim, V. P. R., et al. (2018). Protein beverages containing anthocyanins of jabuticaba. *Food Science and Technology*, 39, 112–119. <https://doi.org/10.1590/fst.27917>
- Rosenberg, M., Kopelman, I. J., & Talmon, Y. (1985). A scanning electron microscopy study of microencapsulation. *Journal of Food Science*, 50(1), 139–144. <https://doi.org/10.1111/j.1365-2621.1985.tb13295.x>
- Rugji, J., Çalışkan, Z., Dinçoğlu, A. H., Özgür, M., Erol, Z., & Özgür, E. B. (2022). Prebiotic effect of D-allulose and β -glucan on whey beverage with *Bifidobacterium animalis* and investigation of some health effects of this functional beverage on rats. *Food Science and Technology*, 42, e07022. <https://doi.org/10.1590/fst.07022>
- Rugji, J., & Dinçoğlu, A. H. (2022). Biocontrol of *Listeria monocytogenes* by *Bacillus coagulans* GBI-30, 6086 in a synbiotic white brined cheese: An *in vitro* model study. *LWT – Food Science and Technology*, 169, 113982. <https://doi.org/10.1016/j.lwt.2022.113982>
- Salveti, E., Orrù, L., Capozzi, V., Martina, A., Lamontanara, A., & Keller, D., et al. (2016). Integrated genome-based assessment of safety for probiotic strains: *Bacillus coagulans* GBI-30, 6086 as a case study. *Applied Microbiology and Biotechnology*, 100, 4595–4605. <https://doi.org/10.1007/s00253-016-7416-9>
- Samaržija, D., Zamberlin, Š., & Pogačić, T. (2012). Psychrotrophic bacteria and their negative effects on milk and dairy products quality. *Mljekarstvo*, 62(2), 77–95. <https://hrcak.srce.hr/83325>
- Scheid, M. M. A., Moreno, Y. M. F., Junior, M. R. M., & Pastore, G. M. (2013). Effect of prebiotics on the health of the elderly. *Food Research International*, 53(1), 426–432. <https://doi.org/10.1016/j.foodres.2013.04.003>
- Seabra, I. J., Braga, M. E., Batista, M. T., & de Sousa, H. C. (2010). Fractioned high-pressure extraction of anthocyanins from elderberry (*Sambucus nigra* L.) pomace. *Food and Bioprocess Technology*, 3(5), 674–683. <https://doi.org/10.1007/s11947-008-0134-2>

- Sekhavatizadeh, S. S., Aminlari, M., Gheisari, H. R., Shekarforoush, S. S., & Golmakani, M. T. (2019). Physicochemical and microbiological properties of probiotic *kashk* containing *Bacillus coagulans*. *Journal of Consumer Protection and Food Safety*, 14, 377–387. <https://doi.org/10.1007/s00003-019-01226-4>
- Shah, N. P. (2000). Probiotic bacteria: Selective enumeration and survival in dairy foods. *Journal of Dairy Science*, 83, 894–907. [https://doi.org/10.3168/jds.S0022-0302\(00\)74953-8](https://doi.org/10.3168/jds.S0022-0302(00)74953-8)
- Sharif, N., Khoshnoudi-Nia, S., & Jafari, S. M. (2020). Nano/microencapsulation of anthocyanins: A systematic review and meta-analysis. *Food Research International*, 132, 109077. <https://doi.org/10.1016/j.foodres.2020.109077>
- Silveira, E. O., Neto, J. H. L., da Silva, L. A., Raposo, A. E. S., Magnani, M., & Cardarelli, H. R. (2015). The effects of inulin combined with oligofructose and goat cheese whey on the physicochemical properties and sensory acceptance of a probiotic chocolate goat dairy beverage. *LWT – Food Science and Technology*, 62, 445–451. <https://doi.org/10.1016/j.lwt.2014.09.056>
- Sitanggang, A. B., Drews, A., & Kraume, M. (2016). Recent advances on prebiotic lactulose production. *World Journal of Microbiology and Biotechnology*, 32(9), 1–10. <https://doi.org/10.1007/s11274-016-2103-7>
- Skryplonek, K., Dmytrów, I., & Mituniewicz-Malek, A. (2019). Probiotic fermented beverages based on acid whey. *Journal of Dairy Science*, 102(9), 7773–7780. <https://doi.org/10.3168/jds.2019-16385>
- Soares, M. B., Martinez, R. C., Pereira, E. P., Balthazar, C. F., Cruz, A. G., & Ranadheera, C. S., et al. (2019). The resistance of *Bacillus*, *Bifidobacterium*, and *Lactobacillus* strains with claimed probiotic properties in different food matrices exposed to simulated gastrointestinal tract conditions. *Food Research International*, 125, 108542. <https://doi.org/10.1016/j.foodres.2019.108542>
- Sobieralska, M., & Kurek, M. A. (2019). Beta-glucan as wall material in encapsulation of elderberry (*Sambucus nigra*) extract. *Plant Foods for Human Nutrition*, 74, 334–341. <https://doi.org/10.1007/s11130-019-00741-x>
- Souza, A. C. P., Gurak, P. D., & Marczak, L. D. F. (2017). Maltodextrin, pectin and soy protein isolate as carrier agents in the encapsulation of anthocyanins-rich extract from jaboticaba pomace. *Food and Bioprocess Processing*, 102, 186–194. <https://doi.org/10.1016/j.fbp.2016.12.012>
- Suharoschi, R., Pop, O. L., Vlaic, R. A., Muresan, C. I., Muresan, C. C., & Cozma, A., et al. (2019). Dietary fiber and metabolism. In *Dietary fiber: Properties, recovery, and applications* (pp. 59–77). Academic Press. <https://doi.org/10.1016/B978-0-12-816495-2.00003-4>

- Şen, İ., & Yüceer, Y. K. (2019). Production and characterization of whey beverage by using grain or lyophilized kefir cultures. *Akademik Gıda*, 17(3), 362–370. <https://doi.org/10.24323/akademik-gida.647719>
- Tao, Y., Wang, P., Wang, J., Wu, Y., Han, Y., & Zhou, J. (2017). Combining various wall materials for encapsulation of blueberry anthocyanin extracts: Optimization by artificial neural network and genetic algorithm and a comprehensive analysis of anthocyanin powder properties. *Powder Technology*, 311, 77–87. <https://doi.org/10.1016/j.powtec.2017.01.078>
- Tomas, M., Beekwilder, J., Hall, R. D., Simon, C. D., Sagdic, O., & Capanoglu, E. (2018). Effect of dietary fiber (inulin) addition on phenolics and *in vitro* bioaccessibility of tomato sauce. *Food Research International*, 106, 129–135. <https://doi.org/10.1016/j.foodres.2017.12.050>
- Tomas, M., Rocchetti, G., Ghisoni, S., Giuberti, G., Capanoglu, E., & Lucini, L. (2020). Effect of different soluble dietary fibres on the phenolic profile of blackberry puree subjected to *in vitro* gastrointestinal digestion and large intestine fermentation. *Food Research International*, 130, 108954. <https://doi.org/10.1016/j.foodres.2019.108954>
- Uhl, K. R., Fyhrie, K. J., Brodt, S. B., & Mitchell, A. E. (2022). Blue elderberry (*Sambucus nigra* ssp. *cerulea*): A robust and underutilized fruit for value-added products. *ACS Food Science & Technology*, 2, 347–358. <https://doi.org/10.1021/acsfoodscitech.1c00436>
- Urbano, J., da Silva, M., Mazzocato, M., Tulini, F., & Favaro-Trindade, C. (2019). Probiotic and synbiotic sorbets produced with jussara (*Euterpe edulis*) pulp: Evaluation throughout the storage period and effect of the matrix on probiotics exposed to simulated gastrointestinal fluids. *Probiotics and Antimicrobial Proteins*, 11(1), 264–272. <https://doi.org/10.1007/s12602-017-9346-y>
- Vargas-Díaz, S., Ciro-Velasquez, H. J., & Sepúlveda-Valencia, J. U. (2023). Development and characterization of a fermented dairy beverage from permeated and concentrated sweet whey sweetened with tagatose. *Revista Facultad Nacional de Agronomía Medellín*, 76(1), 10201–10212. <https://doi.org/10.15446/rfnam.v76n1.100958>
- Vieira, A. H., Balthazar, C. F., Guimaraes, J. T., Rocha, R. S., Pagani, M. M., Esmerino, E. A., et al. (2020). Advantages of microfiltration processing of goat whey–orange juice beverage. *Food Research International*, 132, 109060. <https://doi.org/10.1016/j.foodres.2020.109060>
- Vlachojannis, C., Zimmermann, B. F., & Chrubasik-Hausmann, S. (2015). Quantification of anthocyanins in elderberry and chokeberry dietary supplements. *Phytotherapy Research*, 29, 561–565. <https://doi.org/10.1002/ptr.5284>

- Wang, M., Zhang, Z., Sun, H., He, S., Liu, S., Zhang, T., et al. (2022). Research progress of anthocyanin prebiotic activity: A review. *Phytomedicine*, 102, 154145. <https://doi.org/10.1016/j.phymed.2022.154145>
- Wang, Y., Yang, C., Zhang, J., & Zhang, L. (2023). Interaction of preheated whey protein isolate with rose anthocyanin extracts in beverage model system: Influence on color stability, astringency, and mechanism. *Food Chemistry*, 412, 135507. <https://doi.org/10.1016/j.foodchem.2023.135507>
- Yanes, M., Durán, L., & Costell, E. (2002). Rheological and optical properties of commercial chocolate milk beverages. *Journal of Food Engineering*, 51(3), 229–234. [https://doi.org/10.1016/S0260-8774\(01\)00061-9](https://doi.org/10.1016/S0260-8774(01)00061-9)
- Youdim, K. A., Martin, A., & Joseph, J. A. (2000). Incorporation of the elderberry anthocyanins by endothelial cells increases protection against oxidative stress. *Free Radical Biology and Medicine*, 29(1), 51–60. [https://doi.org/10.1016/S0891-5849\(00\)00329-4](https://doi.org/10.1016/S0891-5849(00)00329-4)
- Zaman, Q. U., Sahar, A., Sameen, A., Ahmad, M. H., Muhammad, N., Huma, N., et al. (2023). Development and storage stability of whey–sugarcane-based functional beverage. *International Journal of Food Properties*, 26(1), 752–763. <https://doi.org/10.1080/10942912.2023.2183170>
- Żbik, K., Onopiuk, A., Szpicer, A., & Kurek, M. (2023). Comparison of the effects of extraction method and solvents on biological activities of phytochemicals from selected violet and blue pigmented flowers. *Journal of Food Measurement and Characterization*, 17(6), 6600–6608. <https://doi.org/10.1007/s11694-023-02158-2>
- Zhang, Q., Cheng, Z., Wang, Y., & Fu, L. (2020). Dietary protein–phenolic interactions: Characterization, biochemical–physiological consequences, and potential food applications. *Critical Reviews in Food Science and Nutrition*, 61(21), 1–27. <https://doi.org/10.1080/10408398.2020.1803199>
- Zhao, L., Pan, F., Mehmood, A., Zhang, H., Rehman, A. U., Li, J., et al. (2021). Improved color stability of anthocyanins in the presence of ascorbic acid with the combination of rosmarinic acid and xanthan gum. *Food Chemistry*, 351, 129317. <https://doi.org/10.1016/j.foodchem.2021.129317>
- Zhao, L., Temelli, F., & Chen, L. (2017). Encapsulation of anthocyanin in liposomes using supercritical carbon dioxide: Effects of anthocyanin and sterol concentrations. *Journal of Functional Foods*, 34, 159–167. <https://doi.org/10.1016/j.jff.2017.04.021>