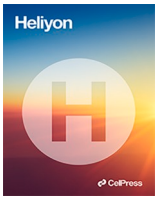
Heliyon 10 (2024) e31780

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon



Research article

Unravelling the effect of extraction on anthocyanin functionality and prebiotic potential

Muzaffar Hasan [a,b], Kailashpati Tripathi [c], Mohd Harun [d], Veda Krishnan [a], Rajeev Kaushik [e], Gautam Chawla [f], Najam A. Shakil [d], M.K. Verma [g], Anil Dahuja [a,**], Archana Sachdev [a,***], Jose M. Lorezo [h,i], Manoj Kumar [j,*]

[a] *Division of Biochemistry, ICAR-Indian Agricultural Research Institute, New Delhi-110012, India*
[b] *Centre of Excellence for Soybean Processing and Utilisation, ICAR-Central Institute of Agricultural Engineering, Bhopal-462038, India*
[c] *Division of Agricultural Chemicals, ICAR-Indian Agricultural Research Institute, New Delhi-110012, India*
[d] *Division of Design of Experiments, ICAR-Indian Agricultural Statistics Research Institute, New Delhi-110012, India*
[e] *Division of Microbiology, ICAR-Indian Agricultural Research Institute, New Delhi-110012, India*
[f] *Division of Nematology, ICAR-Indian Agricultural Research Institute, New Delhi-110012, India*
[g] *Division of Fruits and Horticulture Technology, ICAR-Indian Agricultural Research Institute, New Delhi-110012, India*
[h] *Centro Tecnológico de la Carne de Galicia, Parque Tecnológico de Galicia, Avd. Galicia n° 4, San Cibrao das Viñas, 32900, Ourense, Spain*
[i] *Área de Tecnología de los Alimentos, Facultad de Ciencias de Ourense, Universidad de Vigo, 32004 Ouren-se, Spain*
[j] *Chemical and Biochemical Processing Division, ICAR—Central Institute for Research on Cotton Technology, Mumbai, 400019, India*

ARTICLE INFO

Keywords:
Soymilk
Prebiotic
Probiotic
Fermentation
Functional food

ABSTRACT

Anthocyanins, considered as prebiotic ingredients for functional foods, were extracted from black soybean (BS), black grape (BG), black carrot (BCPm), and black rice (BR) using conventional solvent extraction (CSE) and microwave-assisted extraction (MAE). The study employed a split-plot design with CSE and MAE as main plot factors and anthocyanin extracts (AEs) as subplot factors. Anthocyanins were evaluated for stability (polymeric color, degradation index) and functionality (antioxidant capacity). Prebiotic potential on *Lactobacillus rhamnosus*, *Lactobacillus acidophilus*, *Weissella confusa* was assessed in fermented soymilk. MAE showed higher extraction yield than CSE in BG (3-fold), BS (2-fold), BCPm (1.2-fold), and BR (1.6-fold). Black grape (1255.76 mg/L) and black soybean (976.5 mg/L) had highest anthocyanin with better stability, functionality, and prebiotic potential. The SCFA concentration (propionic acid and butyric acid) increased significantly in BG fortified-fermented soymilk. Overall, anthocyanin-enriched soymilk exhibited higher prebiotic potential, with MAE as the superior extraction method for anthocyanin functionality and stability.

Abbreviations

BCPm	Black carrot pomace
BCPw	Black carrot slices

(*continued on next page*)

* Corresponding author.
** Corresponding author.
*** Corresponding author.
E-mail addresses: ad_bio@yahoo.com (A. Dahuja), arcs_bio@yahoo.com (A. Sachdev), manoj.kumar13@icar.gov.in (M. Kumar).

https://doi.org/10.1016/j.heliyon.2024.e31780
Received 24 August 2023; Received in revised form 1 May 2024; Accepted 22 May 2024
Available online 27 May 2024

(*continued*)

BG	Black grape
BI	Browning index
BR	Black rice
BS	Black soybean
C3G	Cyandin-3-glucoside
CD	Color Density
CSE	Conventional solvent extraction
D3G	Delphinidin-3-glucoside
DI	Degradation Index
LA	*Lactobacillus acidophilus* 1132
LR	*Lactobacillus rhamnosus* 1136
M3G	Malvidin-3-glucoside
MAE	Microwave-assisted extraction
P3G	Peonidin-3-glucoside
PC	Polymeric color
Pt3G	Petunidin-3-glucoside
SCFAs	Short-chain fatty acids
TMAC	Total monomeric anthocyanin content
WC	*Weissella confusa* 30082b

1. Introduction

Cereals, vegetables, and fruits are highly consumed as a primary source of daily diets in many countries and provide high nutritional and functional compounds like vitamins, phenolics, minerals, and dietary fibers to the consumer. The ever-increasing global population has fuelled an increase in demand for food products, resulting in a plethora of by-products. It is generally thought that these by-products would be discarded as food waste, further damaging the environment and the economy [1]. The fruits and vegetable juice and beverage industries scrap a large quantity of fruit and vegetable waste. Such waste contains many high-value substances that need to be considered. Many reports have been published in the last decades to reutilize these plant-derived by-products as food ingredients in developing bakery products, extruded snacks, functional foods, and the food packaging sector. Many researchers are investigating possible ways of extracting beneficial bioactive components from plant-derived by-products.

Anthocyanins and phenolics are the most sought-after food additives in the food business due to their colourant and therapeutic characteristics [2]. Anthocyanins are derived from 2-phenyl-benzopyrylium either as polyhydroxy or poly ethoxy, and mostly in plants found as sugar derivatives in mono, di, or tri-glycosides form linked by α- or β-linkages [3]. Glucose (6C), galactose (6C), rhamnose (6C), arabinose (5C), and xylose (5C) are the most common sugar moieties attached to the base structure of anthocyanins in monomeric form. Colour determines the behaviour of anthocyanins, which is governed by their chemical structure and the pH of the surrounding environment. The anthocyanin samples -are red at low pH (1–3) due to flavylium cation, show colourless carbinol formation at pH (5), and exhibit a blue-purple colour due to quinoidal at pH 7–8 [4]. This high sensitivity of anthocyanin toward pH indicates that anthocyanin are highly vulnerable molecules and easy to degrade in the presence of light, oxygen, enzymes, or metal ions. Anthocyanin, like other flavonoids, has been shown to have significant antioxidant properties as a free radical scavenger in various *in vitro* experiments [4,5]. Anthocyanins also show numerous health-beneficial properties. Several studies showed anti-diabetic properties of anthocyanin extract, like insulin secretion, lipid-lowering, and vaso-protective activity [6]. Other health-promoting properties of the anthocyanin extracts, for example, improved vision acuteness and chemo-protective properties, are also reported [4]. Therefore, anthocyanin extract from the plant by-product would be of great value and can be exerted as assets from the plant waste. It is possible to add anthocyanins to foods like tomato puree, kefir, and milk in order to boost polyphenol levels, boost antioxidant activity, and enhance consumer acceptance [7–9].

Aside from the therapeutic potential reported above, anthocyanins can enhance beneficial bacteria proliferation. *In vitro* studies revealed that when grape and purple sweet potato anthocyanins were ingested, the proliferation of health-beneficial bacteria (Lactobacilli and Bifidobacteria species) increased [10,11]. It has been reported that gut microbes convert anthocyanin into small phenolic acids through methylation, ring cleavage, and dehydroxylation process [12]. Polyphenols' metabolites have shown that they can selectively promote the growth of helpful bacteria while inhibiting the proliferation of dangerous bacteria [13–15]. These findings showed a prebiotic tendency of anthocyanins. Prebiotics are utilized preferentially by host bacteria to produce health advantages. The supplementation of prebiotics in food items enhanced the nutritional and health-beneficial quality of the foods [16]. Prebiotic carbohydrates, especially raffinose, have received the greatest attention and have the most commercial presence. The probiotics utilize oligosaccharides as prebiotics to propagate and produce short-chain fatty acids (SCFAs) such as acetate (2C), propionate (3C), and butyrate (4C), widely reported as essential metabolites for intestinal well-being as well as other parts of the body.

Due to the high vulnerability of the anthocyanins, the extraction procedure plays a critical role in determining the anthocyanins' stability and functionality. The selection of extraction procedures has been shown to affect anthocyanin recovery and stability substantially [17]. The yield and quality of extracted compounds could be increased by using an appropriate extraction process. The extraction procedure, on the other hand, should be environmentally benign. In the case of anthocyanins, organic solvent-based extraction procedures (methanol, ethanol, acetone, etc.) are the most prevalent. In addition to conventional solvent extraction (CSE) methods, several novel techniques, including microwave-assisted extraction (MAE), enzyme-assisted extraction (EAA),

pressurized liquid extraction (PLE), ultrasound-assisted extraction (UAE), pulse electric field extraction (PEF), supercritical CO_2 extraction (SCE), and high hydrostatic pressure (HHP) or high-pressure processing (HPP), have been used to efficiently and securely extract anthocyanins from plant by-products [18].

In this research, two different methods of anthocyanins extraction, CSE and MAE, were studied from four plant-waste sources such as the seed coat of black soybean, peels of the black grape, pomace of black carrot, and hull of black rice. Moreover, this study sought to understand how anthocyanins affect the development of probiotics. Several probiotic strains were used to ferment soymilk while adding anthocyanin extracts from diverse sources. To our knowledge, there is no research on the impact of anthocyanin supplementation on microbial development and modifications in the concentration of SCFAs during soymilk fermentation. Because of the beneficial impact of plant by-products as prebiotics on the growth of probiotics, we further reported the results on the anthocyanin extraction techniques and the prebiotic potential of anthocyanin extracts.

2. Materials and methods

2.1. Sample preparation

Four different sources, such as black soybean (variety- VL Bhat 65), black grapes (variety- Pusa Navrang), black carrot (variety- Pusa Asita), and black rice (variety- Chakhao amubi), were procured from ICAR-VPKAS, Almora, Uttarakhand, India; Division of fruit science, ICAR-IARI, New Delhi; Division of Vegetable Science, ICAR-IARI, New Delhi; and Division of Biochemistry, ICAR-IARI, New Delhi, respectively. Black soybean seed coat (BS), black grape peels (BG), black carrot slices (BCPw), black carrot pomace (BCPm), and black rice hull (BR) were dried at 45 °C (Meta lab Hot air oven zenex96) overnight. The seed coat, peels, and hulls were manually extracted from soaked soybean seeds, black grapes, and black rice. Similarly, slices and pomace from the black carrot were obtained using a sharp stainless steel knife and a juicer grinder, respectively. 5g samples from each source were grounded uniformly with pestle and mortars after being frozen with liquid nitrogen. The ground samples were stored at 4 °C for further use.

2.2. Chemicals and microbial strains

All the chemicals used to extract and evaluate anthocyanins were procured from Sigma Aldrich, USA. HPLC grade trifluoroacetic acid (TFA) and water were procured from MERCK, USA. Standards of anthocyanins like cyanidin-3-glucoside (C3G) and peonidin-3-glucoside (P3G) were procured from Sigma Aldrich, USA.

The probiotic bacterial isolates, *Lactobacillus rhamnosus* 1136 (LR), *Lactobacillus acidophilus* 1132 (LA), and *Weissella confusa* 30082b (WC) were obtained from National Centre for Microbial Resource, Pune, India, Division of Microbiology, ICAR-Indian Agricultural Research Institute, New Delhi, India, and RIKEN Bioresource Research Centre, Japan Collection of Microorganisms, Japan, respectively. The procured bacterial cultures were maintained in MRS broth at 37 °C for LR and LA and 33 °C for WC.

2.3. Isolation and composition analysis of anthocyanins

Two types of extraction methods have been used for anthocyanin extraction, conventional solvent extraction (CSE) and microwave-assisted extraction (MAE) method, and further analysis of extracted anthocyanins by High-pressure liquid chromatography with a diode array detector (HPLC-DAD).

2.3.1. Conventional solvent extraction (CSE) method

For CSE, 500 mg ground samples were extracted thrice with 20 mL acidified ethanol (85 %) with 1 % HCl as described by Ref. [19]. Before shaking, the final pH of the combination was set to pH 3.0, and after 30 min of shaking (MaxQ 600 thermo scientific incubated/refrigerated stackable shaker, New Delhi, India), it was examined and re-adjusted if necessary. The mixture was kept in a shaker at 200 rpm for 1h at room temperature. Further, the extracts were centrifuged at 10,000×*g* at 4 °C for 10 min (Megafuge 16R thermo scientific centrifuge, New Delhi, India), and the supernatant was collected. The collected supernatant was filtered through Whatman no. 1 (Whatman International Ltd., Kent, UK). All the filtered supernatant were pooled and kept at 4 °C until the next step.

2.3.2. Microwave-assisted extraction (MAE) method

For MAE, followed the [20], protocols with slight modifications. 500 mg of a ground sample of BS, BCPm, BCPw, BG, and BR were dispersed in 85 % acidified ethanol (with 1 % HCl) in a 40:1 (v/w) solvent-to-solute ratio separately and kept in an ordinary household microwave (CQ 1570L Samsung model, New Delhi, India) with 450 W for 5 min (30 s. Pulse with 10 s. Interval). After microwave treatment, the suspensions in the beaker cooled to room temperature before being centrifuged at 4193×g for 10 min at 4 °C. Afterward, the collected supernatant was filtered through the Whatman number 1 filter paper, repeated thrice with the remaining residues, pooled all the filtered supernatant, and kept at 4 °C until the next step.

The pooled extracts from CSE and MAE methods were concentrated separately through the rotary evaporator (Heidolph) at a constant vacuum at a low temperature (40 °C) to avoid anthocyanin degradation. The concentrated extracts were collected to dark color vials. The partial purification of the anthocyanin extracts was done by using Amberlite XAD7 in column chromatography. The stability, functionality, and HPLC profiling of the extracts were analyzed prior to freeze drying. Anthocyanin extracts were subjected to freeze drying in a Lypholizer (Alpha 2–4 LSCbasic) and stored at −20 °C until further use [20].

2.3.3. Composition analysis of anthocyanins extracts by HPLC-DAD

Identification of the anthocyanins was performed using reverse phase (RP)-HPLC (Shimadzu UFLC equipped with LC-20 AD pump) as described by Ref. [19]. BS, BG, BCPm, BCPw, and BR extracts obtained by CSE and MAE were filtered through a 0.45 μm PVDF (polyvinylidene fluoride) filter to amber-colored vials (2 mL). The anthocyanins were separated and identified on silica-based C_{18} RP-column (Waters Spherisorb, 250 × 4.6 mm, 5 μm; core-shell technology). Solvent A (0.4 % TFA in distilled water) and 18 % solvent B (0.45 % TFA in acetonitrile) were used as the mobile phase at 1.0 mL $min.^{-1}$ in isocratic mode. A 20 μL sample injected into the column was set at 40 °C, and the program was nominated for 15 min. The diode array detector (DAD) monitored the analytes at 520 nm. The retention time of pure external standards was used to tentatively identify anthocyanin peaks. Cyanidin 3 glucoside, peonidin 3 glucoside, and malvidin 3 glucoside were used as external standards.

2.4. Stability and functional analysis of extracted anthocyanins

2.4.1. Total monomeric anthocyanin content (TMAC)

Total monomeric anthocyanin content (TMAC) was measured using the pH-differential method described by Ref. [21], and expressed as mgL^{-1} of the extract. The absorption reading took at λ_{max}.520 nm and 700 nm, and the total monomeric anthocyanin content was determined using the following equation (1).

$$\text{Total monomeric anthocyanin content (mg / L)} = \frac{\text{A} \times \text{Mw} \times \text{DF} \times 1000}{\varepsilon \times 1} \quad (1)$$

Where: A is absorbance $= (A_{\lambda\ max.} - A_{700})_{pH\ 1.0} - (A_{\lambda\ max.} - A_{700})_{pH\ 4.5}$, Mw represents the molecular weight of cyanidin-3-glucoside which is 449.2 g/mol, DF indicates the dilution factor in this study DF was 10, and ε expresses molar absorptivity, and for cyanidin-3-glucoside the value of ε is 26,900 $L.mol^{-1}cm^{-1}$. For the unknown sample, the determined anthocyanin content was described as cyanidin-3-glucoside.

2.4.2. Determination of polymeric color (PC), color density (CD), % tannin contribution, degradation index (DI), and browning index (BI)

The extract's polymeric color (PC) was determined by following the potassium meta bisulfite bleaching method described by Ref. [22], with slight modification. 50 μL sample made up to 3 mL with distilled water and further equally divided; one was bleached with 100 μL, 20 % potassium metabisulfite, while the other control was treated with distilled water in a similar volume. Visible absorption reading of the control and bleached sample was taken on 420 nm, 520 nm, and 700 nm using a dual beam spectrophotometer. PC, CD, % tannin contribution, DI, and BI were calculated by using the following equations (2)–(6), respectively [21]. The turbidity of the samples was corrected by subtracting any absorbance at 700 nm. In the following equations, DF indicates the dilution factor.

$$\textit{Polymeric colour (bleached sample)} = \{(A_{520} - A_{700}) + (A_{420} - A_{700})\} \times DF \quad (2)$$

$$\textit{Colour density (unbleached sample)} = \{(A_{520} - A_{700}) + (A_{420} - A_{700})\} \times DF \quad (3)$$

$$\%\ \textit{contribution of tannin} = \frac{\textit{polymeric colour}}{\textit{colour density}} \quad (4)$$

$$\text{Degradation index} = \frac{\text{Anthocyanin content by single pH(1.0) method}}{\text{Anthocyanin content by pH (1.0 \&4.5)differential method}} \quad (5)$$

$$\text{Browning index} = \frac{A_{520}}{A_{420}} \quad (6)$$

2.4.3. Determination of total antioxidant activity by DPPH

2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals was used to measure the antioxidant activity of anthocyanin extracts by following the [23] protocol with slight modification. 0.5 mL of alcoholic DPPH solution (0.2 mM) was added to anthocyanin extract (100 μL), and the mixture was shaken and kept in the dark for 30 min. The absorbance of the mixture was recorded at 517 nm, and the DPPH radicals scavenging potential of anthocyanin extracts was calculated using the following equation (7).

$$\%\ \text{DPPH scavenging} = \frac{A_0 - A_1}{A_0} \times 100 \quad (7)$$

Where A_0 = absorbance of the control, A_1 = absorbance of a sample.

2.4.4. Determination of reducing power by FRAP

The reducing power of the anthocyanin extracts was examined using the FRAP assay [24]. The ferric ion reducing antioxidant power (FRAP) reagent was prepared with 0.3 M acetate buffer (pH 3.6), 0.01 M TPTZ (2,4,6-trispyridyl-S-triazine), and 0.02 M $FeCl_3 \bullet 6H_2O$ in a ratio of 10:1:1 and kept in the dark at 37 °C for 30 min 75 μL of the sample was mixed with FRAP reagent (2.25 mL) and incubated in the dark for 10 min at room temperature. The absorbance was measured at 595 nm using a 96-well microplate reader. The calibration curve was prepared using solutions of Trolox. The FRAP value was calculated and expressed in μmol of Trolox

equivalent (TE)/100 g dry weight.

2.5. Prebiotic potential of anthocyanin

2.5.1. Soymilk preparation

Soymilk (containing 1.83 % protein) from food-grade soybean (variety Krune) was prepared through the Hot-water extraction method. Soybean seeds were cleaned and soaked in distilled water overnight. The swollen soybean seeds were manually dehulled, ground with hot water (80 °C) in a 1:10 ratio, and filtered through a double layer of cheesecloth to remove insoluble residues [25]. The extracted soymilk was transferred to a glass bottle and sterilized through autoclaving at 121 °C for 15 min, then cooled at room temperature and stored at 4 °C for 48 h till further use.

2.5.2. Fortification and fermentation of soymilk

All three probiotic strains, LR, LA, and WC, were inoculated in 2 % (v/v) MRS broth and incubated at 37 °C (LR and LA) and 30 °C (WC) for 24 h. For the fortification of soymilk, 100 mL stored soymilk was supplemented with 0.3 % (w/v) lyophilized anthocyanin extracts and inoculated with 1 % (v/v) three bacterial strains separately (Supplementary Fig. 1). The number of the LR, LA, and WC were 1584.89 cfu/mL (3.2 log cfu/ml), 1558.83 cfu/mL (3.19 log cfu/mL), and 1597.32 cfu/mL (3.202log cfu/mL) respectively. The fortified and inoculated samples were fermented at 37 °C (LR and LA) and 30 °C (WC) for 24 h. The non-fortified but inoculated samples incubated in the same experimental conditions were used as a control to determine the prebiotic potential of anthocyanin extracts.

2.5.3. Determination of prebiotic potential of anthocyanins

The viability of the probiotics cells was measured using the plate dilution method with MRS agar [54] (De Man et al., 1960). Each fermented soymilk sample was serially diluted and plated in duplicate, with the plates incubated at 37 °C (LR and LA) and 30 °C (WC) for 48 h. The results were expressed as colony-forming units per milliliter (cfu/mL). The pH of the samples was measured at different fermentation time intervals (0, 6, 24, 48, and 72 h) with a pH meter (Oakton pH 550 benchtop). Titratable acidity was examined using 0.1 N NaOH and represented as percent lactic acid (%TA) [26,27]. Soymilk diluted with distilled water (1:1) titrated against 0.1 N NaOH by using 3–4 drops of phenolphthalein as an indicator. Titratable acidity (%TA) was measured by using the following formula.

$$\%Titratable\ acidity\ (\%TA) = \frac{Volume\ of\ 0.1N\ NaOH\ consumed\ for\ neutralization \times 0.09 \times 100}{Volume\ of\ soymilk \times Specific\ gravity}$$

2.5.4. Short-chain fatty acids analysis through gas chromatography

Fermented soymilk (50 mL) was dissolved in hexane (1:2) and left overnight at room temperature with frequent shaking after fat extraction and organic phase was collected. Hexane was removed through a rotary evaporator and collected in a double-necked round bottom flask. Methanol with 1 % H_2SO_4 was used for the esterification reaction for the formation of fatty acids methyl esters (FAMEs), performed at a reflux water bath (100 °C for 15 h). Excess of H_2SO_4 was removed by a saturation solution of Na_2CO_3 in order to reach a pH of 7.0. Distilled water (10–15 mL) and chilled diethyl ether (50 mL) were used in the separating funnel to separate the FAMEs. A pinch of anhydrous sodium sulfate helped remove the water from the diethyl ether layer; further air-dried diethyl ether to obtain FAMEs.

FAMEs were examined by Gas chromatography [Shimadzu GC2010 equipped with flame ionisation detector (FID)]. Each sample (50 min per run) was carried out with an on-column injector and using a GC column (Rtx-5, 10,223, by RESTEK) (30 m × 0.25 mm i. d. × 0.25 μm film thickness) with a split ratio (20:0). The initial GC column temperature was 60 °C, increased at rate 3 °C/min till 220 °C. The flow rate of the injected samples was 1 mL/min. FID temperature was 260 °C, and the nitrogen (inert gas), hydrogen (carrier gas), and airflow rates were 30 mL/min, 40 mL/min, and 400 mL/min, respectively. Individual peaks were identified by comparing their retention times (RTs) with standards of FAMEs.

2.5.5. Microstructure analysis by scanning electron microscope (SEM)

Fermented soybean was freeze-dried and spread on double side adhesive tape mounted on SEM stubs. Stubs were placed in SC7620 sputter coater from Quorum and were coated with gold palladium. The material was examined under TESCON (Tescan Orsay Holding, Czech Republic) Vega 3 LMU scanning electron microscope. The images were recorded at 500x and 4.0kx magnification.

2.6. Statistical design and analysis

The experiment was conducted under a split-plot design with CSE and MAE methods as the main plot factor and different anthocyanin extracts as subplot factors. The solvent extraction methods (CSE and MAE) are taken as the two levels of the main plot factor as they are hard-to-change factors and need to be compared with higher precision in comparison to anthocyanin extracts considered as levels of sub-plot factor. The analysis of variations (ANOVA) were performed for the data obtained through the experimental design for various biochemical parameters (Supplementary Table 1). SPSS 17.0 was used to analyze the data (SPSS Inc., India). The $p < 0.05$ level of statistical significance was used in this investigation. The heatmap mentioned in this paper is drawn with the help of Python 3.4 using the seaborn package.

3. Results

Extracted anthocyanins from the four mentioned sources by using CSE, and MAE was evaluated based on yield (Total Monomeric Anthocyanin Content- TMAC), stability (color density (CD), polymeric color (PC), degradation index (DI), Browning Index (BI), and % tannin), and functionality (antioxidant potential) parameters. The prebiotic potential of the anthocyanins extracts was also analyzed by the cell's viability, titratable acidity, and change in pH that occurred during the fermentation.

3.1. Total monomeric anthocyanin content (TMAC)

MAE showed high anthocyanin yields compared to CSE. For MAE, we used 50 % ethanol with a 40:1 ratio and 450 W microwave power for 5 min [2]. TMAC obtained from all the samples is presented in Fig. 1A (Supplementary Table 2). TMAC from BG, BS, BCPm, BR, and BCPw obtained in MAE were 1255.76 mg/l, 976.5 mg/L, 604 mg/L, 369.88 mg/L, and 356.52 mg/L, respectively. While TMAC from BG, BS, BCPm, BR, and BCPw obtained in CSE were 418.31 mg/L, 473.41 mg/L, 502 mg/L, 226.27 mg/L, and 742.71 mg/L, respectively.

3.2. Anthocyanin profiling

Chromatograms of anthocyanin obtained through HPLC-DAD from anthocyanin extracts of BG, BS, BCPw, and BR are shown in Fig. 3. Anthocyanin identification in these extracts was determined by running known anthocyanins through the same instrument under the same conditions. Cyanidin-3-glycoside (C3G) was identified and quantified in BG (0.926 mg/g), BS (0.813 mg/g), BCPw (0.308 mg/g), and BR (0.190 mg/g) anthocyanin extracts while Peonidin-3-glycoside (P3G) was identified in BR (0.179 mg/g) and Malvidin 3 glucoside (M3G) was identified in BG (0.295 mg/g) anthocyanin extracts. Delphinidin-3-glucoside (D3G) and petunidin-3-glucoside (Pt3G) were detected in BS anthocyanin extract at concentrations of 0.031 mg/g and 0.058 mg/g, respectively.

3.3. Stability and degradation analysis of anthocyanin extracts

The experimental values of PC, BI, and DI of all the anthocyanin extracts from different sources obtained through CSE and MAE are presented in Fig. 1B (Supplementary Table 2). PC was lowest in BS (0.21) and BG (0.37) extract to MAE, while CSE in BCPm (0.36) extract was lowest. Similarly, DI was also lowest in BG (0.33) and BS (0.96) in MAE, while DI for BCPm (0.89) was lowest in CSE. According to PC, DI, and BI values, the anthocyanin extract from BS and BG is more stable than BR, BCPw, and BCPm. Additionally, it was shown that anthocyanin extracts from MAE had higher stability (lower values of PC, BI, and DI) than anthocyanin extracts from CSE.

Anthocyanins can be found in monomeric and polymeric forms and combined with other phenolic compounds to generate colored pigments. Bisulfite-bleaching is resistant to these colorful polymeric anthocyanin-tannin complexes, but monomeric anthocyanins bleach quickly. %Tannin contribution determined the polymeric anthocyanin-tannin complexes formation in the extracts. The value of the % tannin contribution of all the anthocyanin extracts is presented in Fig. 2A (Supplementary Table 2). The % tannin contribution obtained was lowest in the BG (31.09) of MAE, while for CSE, it was lowest in BCPm (21.18).

3.4. Functionality analysis of anthocyanin extracts

The experimental values of % DPPH scavenging and FRAP are presented in Fig. 2B (Supplementary Table 2). The % DPPH scavenging was maximum in BG (28.15 %) and BS (27.5 %) obtained through MAE.

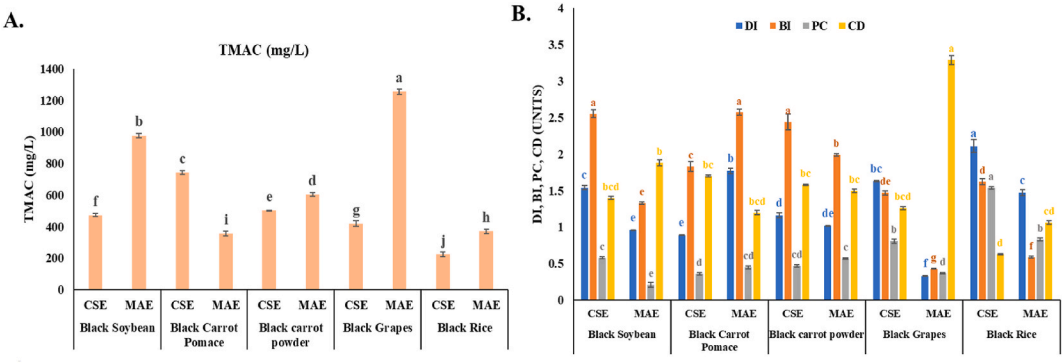


Fig. 1. Anthocyanin content and stability parameters of anthocyanin extracts. (A.) Showing the Total monomeric anthocyanin content (TMAC- mg/L), and (B.) Showing polymeric color (PC), color density (CD), degradation index (DI), and browning index (BI). Data are presented as mean ± SD. Different letters indicate that values are statistically significant ($P < 0.05$). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

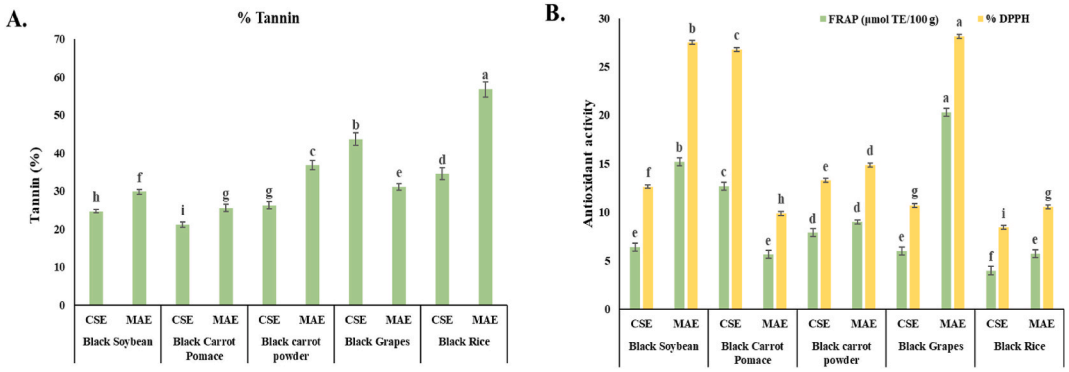


Fig. 2. Tannin contribution and functionality parameters of anthocyanin extracts. (A.) Showing the % Tannin contribution and (B.) representing the antioxidant activity using DPPH scavenging activity (%) and ferric ion reducing antioxidant potential (FRAP- µmol TE/100g). Data are presented as mean ± SD. Different letters indicate that values are statistically significant ($P < 0.05$).

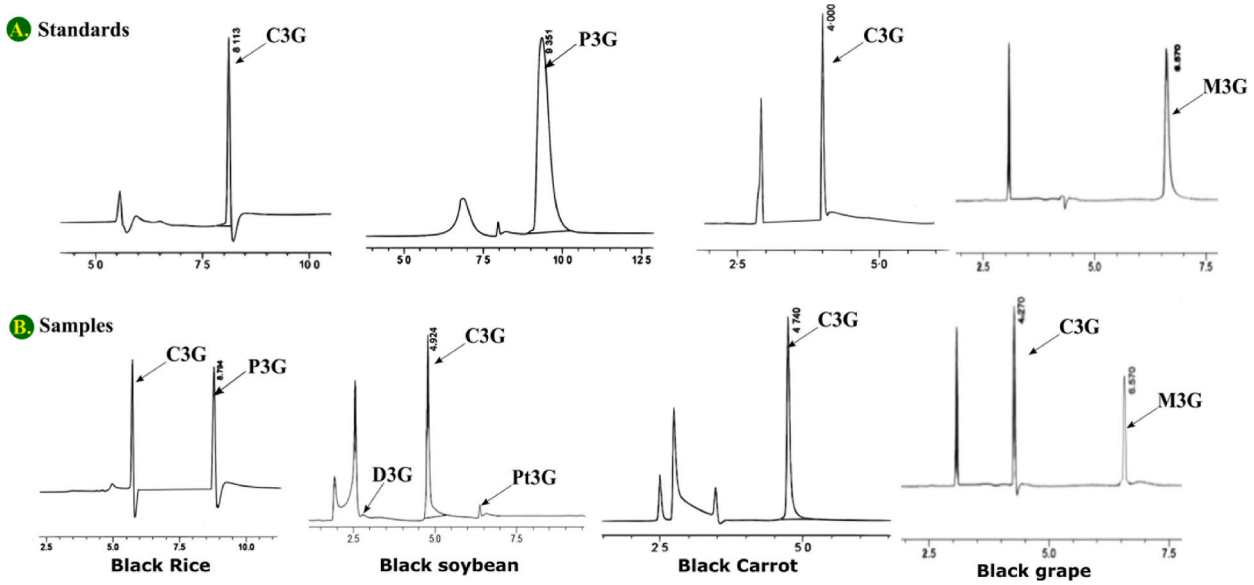


Fig. 3. HPLC profiling of anthocyanins extracts obtained from MAE. (A) showing the chromatograms from left to right of C3G (at 0.5 mL/min flow rate), P3G (at 0.5 mL/min flow rate), C3G (at 1.0 mL/min flow rate), and M3G (at 1.0 mL/min flow rate) (B) showing the chromatograms of identified anthocyanins in black rice (C3G and P3G), black soybean (C3G, D3G, and Pt3G), black carrot (C3G), and black grape (C3G and M3G). Here; C3G mean Cyanidin-3-glucoside, P3G mean Peonidin-3-glucoside, M3G mean Malvidin-3-glucoside, D3G mean Delphinidin-3-glucoside, and Pt3G mean Petunidin-3-glucoside.

This study assessed the antioxidant's reducing potential. The reaction of an antioxidant with the TPTZ-Fe^{3+} complex to produce TPTZ-Fe^{2+} is the basis for this test. The absorbance of TPTZ-Fe^{2+} at 595 nm was measured to ascertain the reducing power of the samples. The FRAP value was maximum in BG (20.31 µmol TE/100 g) and BS (15.21 µmol TE/100 g) obtained by MAE.

3.5. *Prebiotic potential of anthocyanins*

The viability cell count in log cfu/mL indicated that anthocyanin supplementation enhanced the growth and proliferation of LR, LA, and WC in fermented soymilk (Fig. 4). Anthocyanins extracted by MAE showed significantly ($p < 0.05$) high growth of LR, LA, and WC in fermented soymilk at 37 °C after 48 h compared to anthocyanins extracted by the CSE method. BCPm anthocyanin extracts from the CSE method also showed better LR, LA, and WC growth, which may be due to the presence of high TMAC in BCPm. Among the MAE-isolated anthocyanins, BG and BS-supplemented soymilk showed high viability of all three bacterial strains. LA showed more growth in all the samples among all three bacterial strains.

The changes in pH during the fermentation of soymilk supplemented with MAE-based anthocyanins by all three bacterial strains showed declined trend in pH at different time intervals. For instance, Fig. 5 shows the changes in pH during the fermentation of

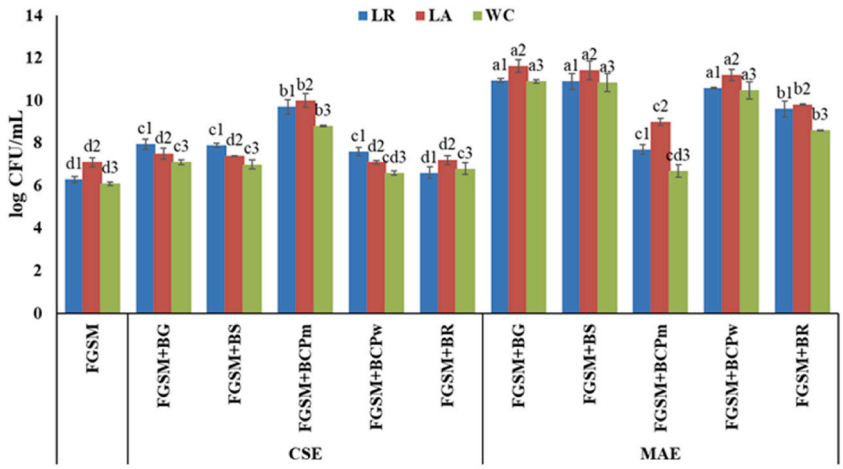


Fig. 4. Growth and proliferation of *L. rhamnosus* 1136, *L. acidophilus* 1132, and *Weissella confusa* 30082b at 37 °C (LR and LA) and 33 °C (WC) for 48h. Data are presented as mean ± SD. LR, LA, and WC indicate *Lactobacillus rhamnosus* 1136, *Lactobacillus acidophilus* 1132, and *Weissella confusa* 30082b, respectively. For a given probiotic bacterial strain, different letters indicate that the values are statistically significant ($P < 0.05$).

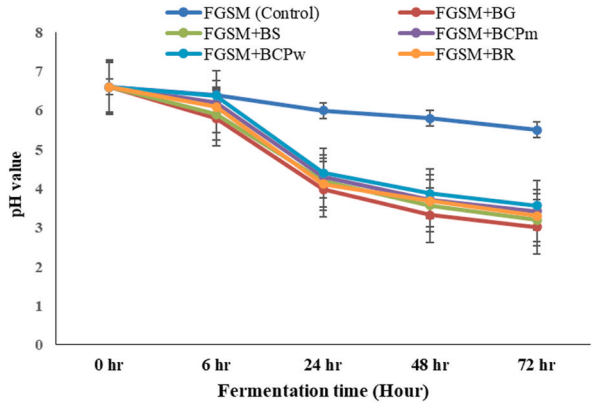


Fig. 5. Changes in pH during soymilk fermentation supplemented with anthocyanin extract (MAE) and fermented by *L. acidophilus* 1132. Non-fermented Food grade soymilk (FGSM) control, Black soybean anthocyanin fortified-fermented food grade soymilk (FGSM + BS), Black carrot powder anthocyanin fortified-fermented food grade soymilk (FGSM + BCPw), Black grape anthocyanin fortified-fermented food grade soymilk (FGSM + BG), Black carrot pomace anthocyanin fortified-fermented food grade soymilk (FGSM + BCPm), and Black rice anthocyanin fortified-fermented food grade soymilk (FGSM + BR) samples are shown here.

anthocyanin-supplemented soymilk fermented by *L. acidophilus*. Soymilk supplemented with BG (MAE) anthocyanins extracts showed a more decreasing pattern of pH (6.6 at 0 h to 3.2 after 72 h) compared to other samples shown in Fig. 5.

The production of organic acids in fermented soymilk is presented in terms of the percentage of titratable acidity (% TA) (Fig. 6). Soymilk supplemented with anthocyanins (MAE) and fermented with all three bacterial strains showed an enhanced level of % TA than control except for BCPw. Soymilk fermented through LA shows an elevated level of % TA compared to LR and WC-fermented soymilk.

3.6. Short-chain fatty acids in fortified-fermented soymilk samples

Fortified-fermented soymilk enhanced the amount of SCFAs in soymilk. Chromatograms obtained through GC (Fig. 7 and Supplementary Fig. 3) indicate that anthocyanin-fortified fermented soymilk enhanced the propionate and butyrate concentration in the sample (Fig. 8). Fortified soymilk with black grape anthocyanin extracts showed a high amount of propionate and butyrate from the control (FGSM) value of 0.048 mg/mL to 1.138 mg/mL (24-fold) and 0.085 mg/mL to 3.867 mg/mL (45-fold), respectively after 48 h of fermentation by *L. acidophilus*. In BS fortification, propionate, and butyrate were enhanced 9-fold and 13-fold, respectively, compared to control food-grade soybean milk (FGSM).

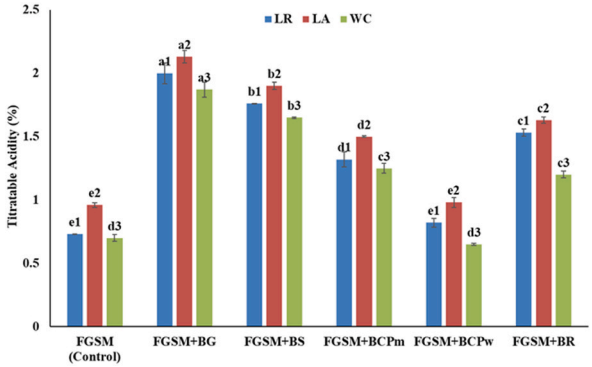


Fig. 6. Titratable acidity (% TA) of anthocyanin extract supplemented (MAE) soymilk fermented by *L. rhamnosus* 1136, *L. acidophilus* 1132, and *Weissella confusa* 30082b at 37 °C (LR and LA) and 33 °C (WC) after 48 h. Data are presented as mean ± SD. For a given probiotic bacterial strain, different letters indicate that the values are statistically significant ($P < 0.05$). Non-fermented Food grade soymilk (FGSM) control, Black soybean anthocyanin fortified-fermented food grade soymilk (FGSM + BS), Black carrot powder anthocyanin fortified-fermented food grade soymilk (FGSM + BCPw), Black grape anthocyanin fortified-fermented food grade soymilk (FGSM + BG), Black carrot pomace anthocyanin fortified-fermented food grade soymilk (FGSM + BCPm), and Black rice anthocyanin fortified-fermented food grade soymilk (FGSM + BR) samples are shown here.

3.7. Microstructure analysis of soymilk

Probiotic-fermented soymilk offers a method for enhancing flavor and aroma. Additionally, the water holding capacity (WHC), macrostructure, and perceived viscosity of soymilk are regulated and enhanced. Fortified-fermented soymilk has more micro-pores than fermented (FGSM + LA) (Fig. 9). Fermented soymilk fortified with BG anthocyanin extracts has more pores with a lesser size (~3 μm). In comparison, fermented soymilk fortified with BS anthocyanin extracts has lesser pores with a bigger pore size (~10 μm). In the case of fermented soymilk without fortification, have a compact structure.

4. Discussion

The anthocyanin owes its natural color to highly resonating a fully conjugated ten-electron A-C ring system, with a small contribution from the B-ring, mean disturbance in the resonance, or any structural damage responsible for the lost color of anthocyanin. The fundamental issue in using anthocyanins as natural food colorants is their low stability after extraction. PC, BI, and DI are all considered indicators of anthocyanin or related colored phenolic degradation or low stability parameters. As a result, the durability of colored phenolics, such as anthocyanins, is directly proportional to the low value of these parameters. Plant polyphenols can be extracted rapidly and cheaply with MAE, but process parameters must be tuned. In our experiments, MAE showed high anthocyanin yield in BG (3-fold), BS (2-fold), BR (1.6-fold), BCPm (1.2-fold), and BCPw (0.5-fold) to CSE. The high yield of anthocyanins from all the studied sources indicated that MAE extracted more anthocyanins than CSE. These results align with similar observations stated in the literature, which found that using a microwave technique to extract plant secondary metabolites improves extraction yield considerably compared to traditional extraction methods [2,28,29]. As a result of microwave irradiation, MAE often results in excellent extraction yields of anthocyanins. In the presence of microwaves, localized temperature increases cause cell ruptures and the migration of phenolics into the surrounding solvent [30]. The ethanol (50 %) concentration and solvent to a solute ratio (40:1) determined the yield of extracted anthocyanins in MAE. Ethanol may improve the contact surface area between the plant matrix and the solvent and improve extraction yields [31]. An increase in solvent concentration leads to an increase in the number of soluble phenolic compounds in the extraction solvent ([32,33], reported that a shorter time (6–9 min) with a 400 W power level was more efficient for extracting anthocyanins from Marasca sour cherry.

Similarly, for *Myrtus communis* L. leaves, the best MAE conditions were 500 W microwave power for 1-min irradiation, with 42 % ethanol concentration, and 32 mL/g solvents to the material ratio [31]. These findings show that extracting phenolics from plant materials using MAE may be most effective when employing greater microwave power for a short time [34]. The phenols may be thermally degraded if the irradiation time is extended with increased microwave power. Consequently, MAE is more appropriate for extracting phenolics from dry plant sources, such as peels and seed coats [34,35].

Further, the extracted anthocyanins were evaluated on the basis of yield (Total Monomeric Anthocyanin Content- TMAC), stability (color density- CD, polymeric color- PC, degradation index- DI, Browning Index- BI, and % tannin), and functionality (antioxidant potential) parameters. Many studies have demonstrated that MAE is a highly effective approach for extracting antioxidants from waste horse tail and waste coffee (Pavlović et al., 2013; Ranic et al., 2014; [36]). Because of the type and content of individual anthocyanins, a greater link between individual anthocyanins and biological activities has been discovered by many researchers [37]. Several cyanidin-based anthocyanins have been studied *in vitro* antioxidant properties based on structural differences [38]. A well-established idea proposed that di-acylated anthocyanins exhibited more antioxidant activity than mono- or non-acylated anthocyanins, which may be used to explain the scavenging action of anthocyanins [39]. The presence of hydroxyl groups in acylated anthocyanins enhanced the O^{2-} scavenging effects [40]. The capacity of an antioxidant to scavenge DPPH radicals is usually attributed to its hydrogen-donating

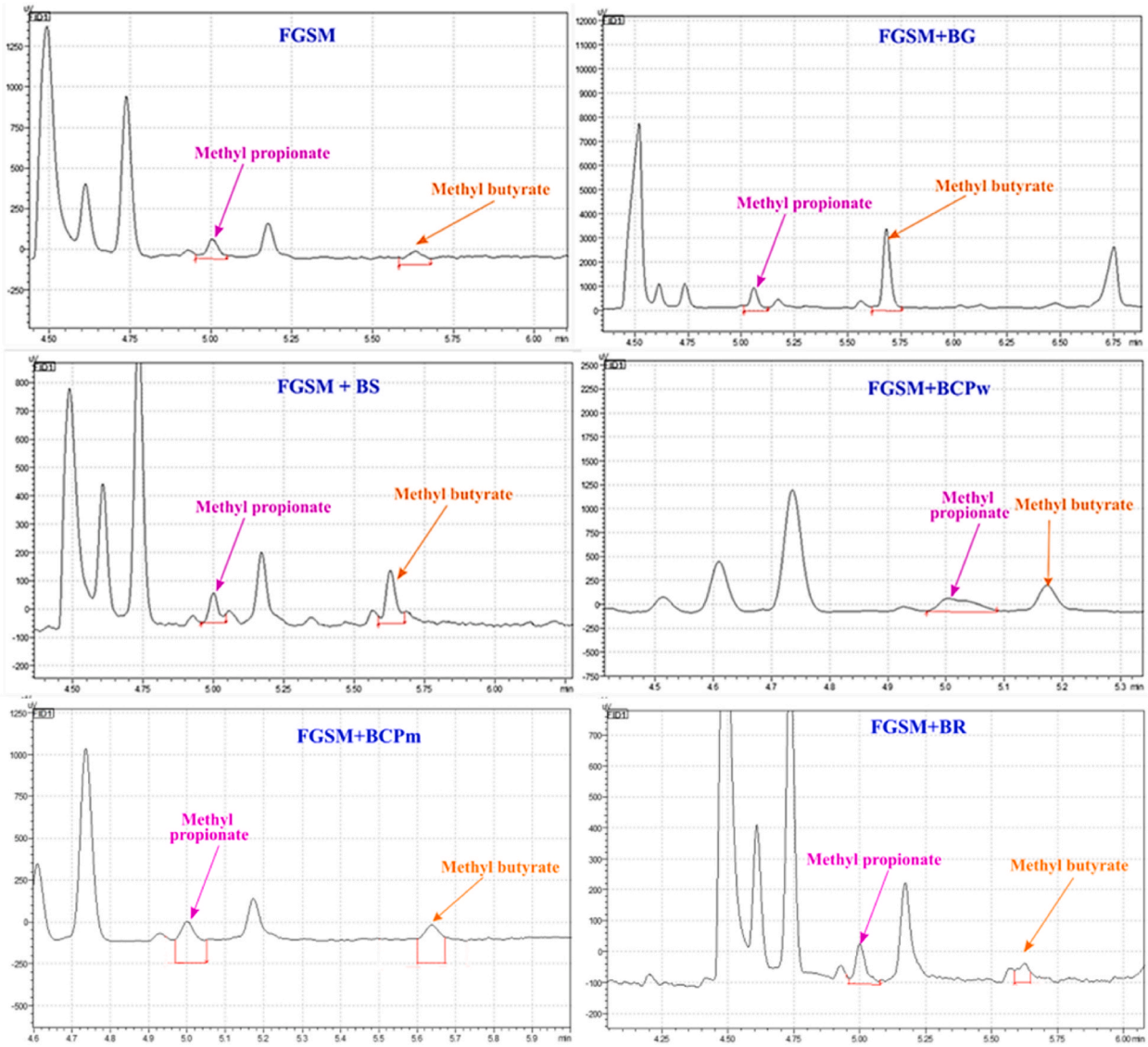


Fig. 7. Chromatograms of fatty acid methyl esters (FAMEs) obtained through gas chromatography (GC) of anthocyanin-fortified soymilk fermented by *L. acidophilus* after 48 h. Non-fermented Food grade soymilk (FGSM) control, Black soybean anthocyanin fortified-fermented food grade soymilk (FGSM + BS), Black carrot powder anthocyanin fortified-fermented food grade soymilk (FGSM + BCPw), Black grape anthocyanin fortified-fermented food grade soymilk (FGSM + BG), Black carrot pomace anthocyanin fortified-fermented food grade soymilk (FGSM + BCPm), and Black rice anthocyanin fortified-fermented food grade soymilk (FGSM + BR) samples are shown here.

ability. DPPH scavenging activities of the anthocyanin extracts are increased with the obtained TMAC yield, which suggested that the % DPPH quenched values were dependent on the concentration of anthocyanins and their stability at a certain reaction time. Recent studies have focused a lot of emphasis on the potential scavenging activity of anthocyanins against reactive oxygen and nitrogen species, which may prevent cell damage [41–43].

The degree of anthocyanin polymerization and browning is measured by polymeric color. Low PC, BI, and DI values indicate excellent anthocyanin stability and minimal degradation, making them extremely desirable [44]. The low value of the anthocyanin extracts' PC, BI, and DI are attributed to the presence of ethanol, which improves the stability of the anthocyanins [45]. By forming chalcone, an intermediate of anthocyanin degradation, PC synthesis was accelerated with temperature and polyphenol oxidase activity [46]. The chalcone is unstable and can easily break down into brown-colored compounds, causing a large rise in PC. The PC also contributes to color changes from reddish to yellowish or brownish, which BI represents. These PC, DI, and BI values showed that the anthocyanin extract from BS and BG is more stable than BR, BCPw, and BCPm. In addition, it also indicated that anthocyanin extracts obtained from MAE governed better stability (low values of PC, BI, DI) compared to anthocyanin extracts obtained from CSE (Fig. 10). The maximum value of CD was for BG (3.29) and BS (1.88) extracted by MAE. Heatmap analysis of all the parameters (TMAC, CD, PC,

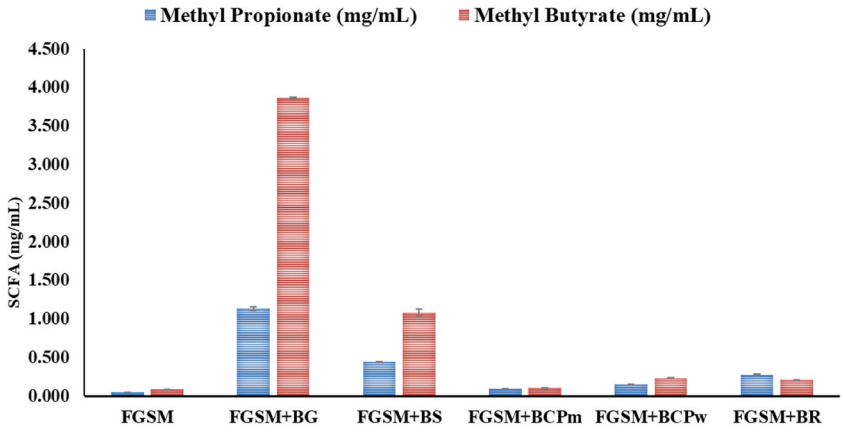


Fig. 8. Concentration of short-chain fatty acids (SCFA) formed in anthocyanin-fortified soymilk fermented by *L. acidophilus*. Non-fermented Food grade soymilk (FGSM) control, Black soybean anthocyanin fortified-fermented food grade soymilk (FGSM + BS), Black carrot powder anthocyanin fortified-fermented food grade soymilk (FGSM + BCPw), Black grape anthocyanin fortified-fermented food grade soymilk (FGSM + BG), Black carrot pomace anthocyanin fortified-fermented food grade soymilk (FGSM + BCPm), and Black rice anthocyanin fortified-fermented food grade soymilk (FGSM + BR) samples are shown here.

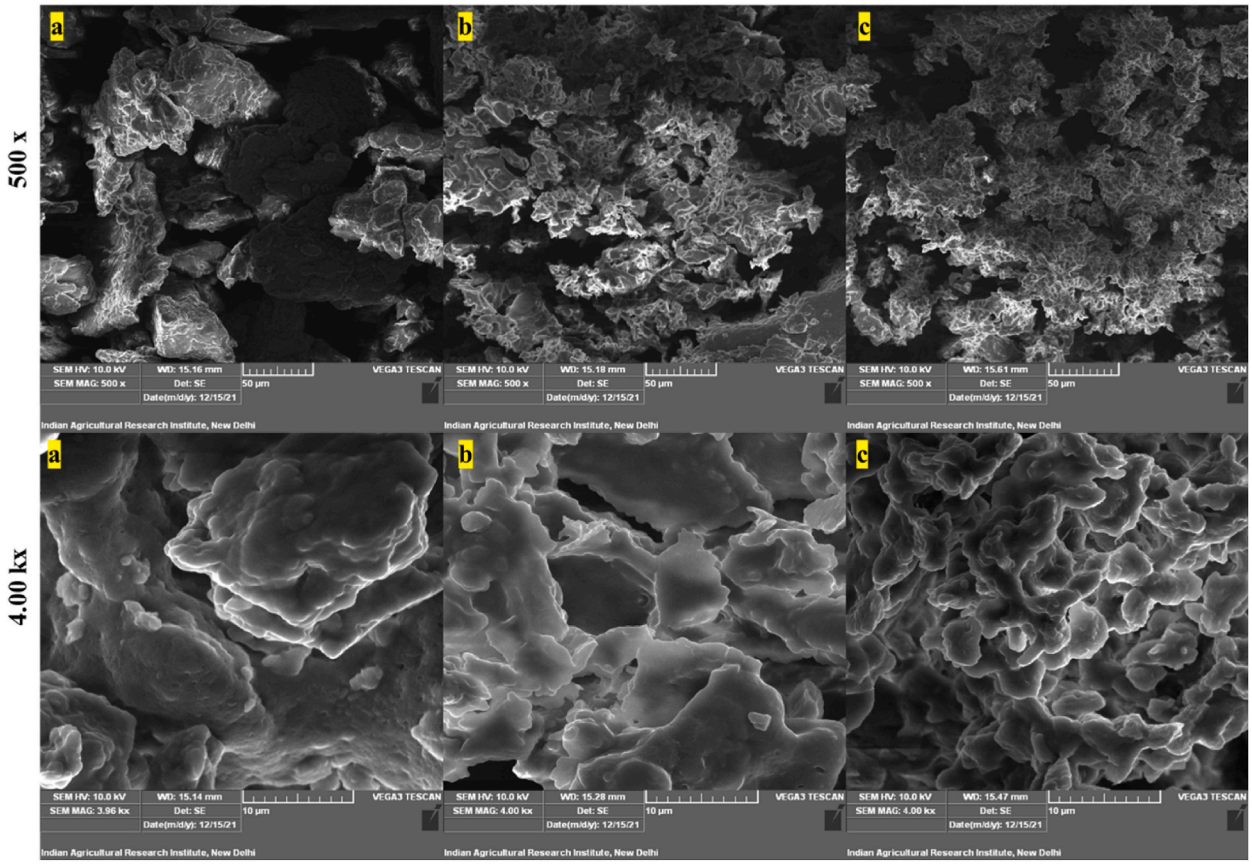


Fig. 9. Scanning electron micrographs of lyophilized fortified-fermented soymilk samples after 48 h of fermentation at 500 x and 4.00 kx magnificence. Here, a: food-grade soymilk fermented by L. acidophilus (FGSM + LA); b: black soymilk anthocyanin-fortified soymilk fermented by *L. acidophilus* (FGSM + BS + LA); c: black grape anthocyanin-fortified soymilk fermented by *L. acidophilus* (FGSM + BG + LA).

DI, BI, %DPPH, FRAP, and %Tannin) of the anthocyanin extracts indicates that BG extracts and BS extracts of MAE fall in light color boxes in comparison to other extracts.

As shown in the results, the increase in the bacterial population and decreased pH of fermented soymilk reflected the modulatory

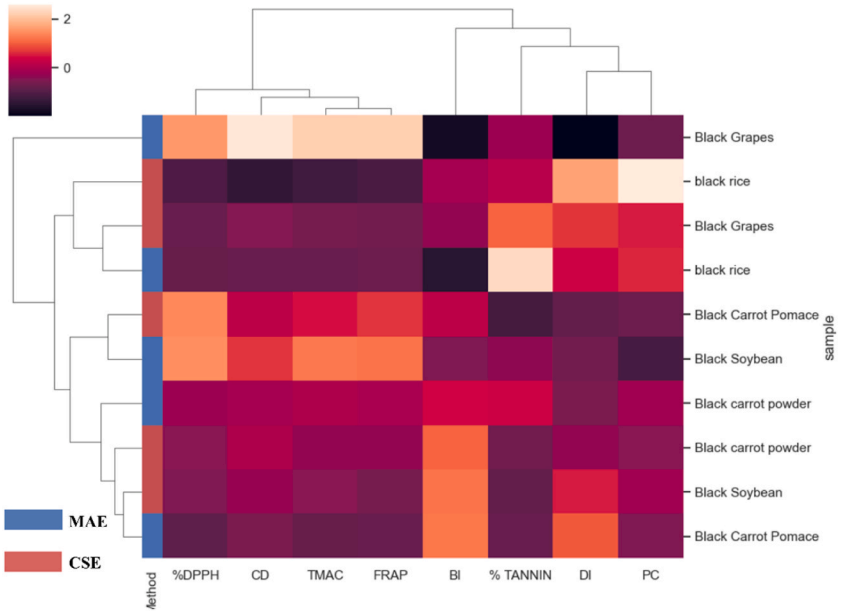


Fig. 10. Distinct patterns of anthocyanin extracts by heatmap analysis using Python 3.4. Different color boxes denote conventional solvent extraction (CSE) and microwave-assisted extraction (MAE). The color brightness is directly proportional to the content ratio, according to the color scale given. Light color boxes indicate higher values while dark color boxes indicate lower values of the corresponding parameter like total monomeric anthocyanin content (TMAC), color density (CD), polymeric color (PC), degradation index (DI), browning index (BI), DPPH radicals scavenging activity (% DPPH), ferric ion reducing antioxidant power (FRAP in μmol TE/100g), and %Tannin. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

effects of anthocyanin extracts on LR, LA, and WC. It has been hypothesized that during the probiotic fermentation, the breakdown of β-glucoside linkages and degradation of 2-phenylbenzopyrylium of anthocyanin, leads to the production of lactic acid, phenolic acids, and short chain fatty acids which led to a decrease in medium pH and created favourable pH for probiotic growth ([47]; [55] (Sasi et al., 2022); [48]). All the soymilk samples supplemented with anthocyanin extracts showed the growth and proliferation of LR, LA, and WC compared to control FGSM. Fermentation by LA showed more growth and a high %TA in soymilk after 48 h at 37 °C compared to LR and WC-based fermentation in all the samples. The growth and titratable acidity of the fermented soymilk were high in the soymilk, which was supplemented with anthocyanins extracted by MAE. FGSM supplemented with BG, and BS showed high growth of LR, LA, and WC, possibly due to the increased stability and functionality of the BG and BS anthocyanin extracts. Anthocyanin from BCPm extracted by CSE showed high functionality and low degradation, helping the growth and proliferation of LR, LA, and WC compared to MAE extracts of BCPm, which was low in functionality and high in degradation parameters. These findings indicated that anthocyanins extracts have significant prebiotic potential. Overall, the anthocyanins extracted by MAE showed the high prebiotic potential of anthocyanin in anthocyanin-supplemented soymilk, which stated the stability and functionality of the anthocyanins determined the prebiotic potential of the anthocyanins.

An HPLC analysis was performed to identify the anthocyanins present in the extracts. C3G was identified in all the extracts, while P3G was found in black rice extracts and M3G was observed in black grape extract. In addition, D3G and Pt3G were also found in the black soybean anthocyanin extarcts. These findings align with the previous result [2,19]. The C3G is the most common anthocyanin derivative that profoundly occurs in all the extracts.

SCFAs are the main source of energy for probiotics and help to enhance the nutritional property of soymilk. Humans do not digest oligosaccharides in soybean due to a lack of hydrolysis enzyme as α-galactosidase, but probiotics can digest and produce SCFAs through α-galactosidase enzyme [49]. Similarly, probiotics convert anthocyanins into small phenolic acids and SCFAs by various chemical processes, including ring breakage, methylation, and dehydroxylation [50]. Fortification of soymilk through anthocyanin enhanced the viability of the cell, responsible for the increment in the SCFAs concentration in fortified-fermented soymilk. An increment in the amount of SCFAs after fortification and fermentation indicates the enhancement in the conversion of oligosaccharides and anthocyanin to SCFAs through probiotics viability which showed the prebiotic potential of anthocyanins. Zhou et al. [51] reported that 0.5 % of soybean oligosaccharides supplementation increments the SCFAs primarily acetate (6.61 mg/g to 12.05 mg/g), propionate (5.16 mg/g to 7.53 mg/g), and butyrate (2.37 mg/g to 6.67 mg/g) during *in vitro* fermentation studies in comparision to 0.5 % corn starch supplementation. These results suggest that anthocyanin fortification may encourage the growth of lactate-producing bacteria.

Microstructure analysis of fortified-fermented soymilk indicates the effect of fortification on fermented soymilk. Typically, soymilk is employed as an appropriate culture medium for the development and biochemical operations of various probiotics. The water extract of soybeans, known as soymilk, is regarded as a colloid that includes minerals, water, soluble protein, and particulate protein.

These ingredients were used in the probiotic fermentation of soymilk, reducing the sample's compactness [52]. Probiotic fermentation of soymilk disrupted the existing protein network and created interactions between polysaccharides and protein, which led to the distortion of the protein network and generated pores in SEM micrographs [52,53]. Fortification of soymilk enhanced probiotics' viability and created more pores with lesser size. These results suggest that anthocyanin-fortified and fermented soymilk enhanced the viability of probiotics which shows the prebiotic potential of anthocyanin. An overall snapshot of the results obtained in the current research study is shown in Fig. 11.

5. Conclusion

In conclusion, microwave-assisted extraction (MAE) was an effective approach for extracting anthocyanins with high stability, high functionality, and low degradation parameters. MAE outperformed the conventional solvent extraction (CSE) approach. Anthocyanin extracts from MAE showed good prebiotic potential in comparison to anthocyanin extracts by CSE. The stability and functionality of the anthocyanins determined the prebiotic potential of the anthocyanins. As a result, the MAE can surely be a option for the cost-effective and environmentally friendly extraction of anthocyanins from peels of black grapes, seed coats of black soybean, pomace of black carrot, and bran of black rice and could also be applied as prebiotics for the food industry. SCFAs production and micro-structures analysis of the fortified-fermented soymilk samples reveal that apart from the prebiotic potential of anthocyanin, anthocyanin fortification might be helpful to improve the textural and health-beneficial properties of fermented soymilk.

Declarations

The authors declare that there is no conflict of interest.

CRediT authorship contribution statement

Muzaffar Hasan: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis. **Kailashpati Tripathi:** Investigation, Formal analysis. **Mohd Harun:** Investigation, Formal analysis. **Veda Krishnan:** Methodology. **Rajeev Kaushik:** Resources, Methodology. **Gautam Chawla:** Resources, Methodology. **Najam A. Shakil:** Resources. **M.K. Verma:** Resources. **Anil Dahuja:** Writing – review & editing, Writing – original draft, Resources, Conceptualization. **Archana Sachdev:** Writing – review & editing, Writing – original draft, Resources, Conceptualization. **Jose M. Lorezo:** Writing – review & editing, Writing – original draft.

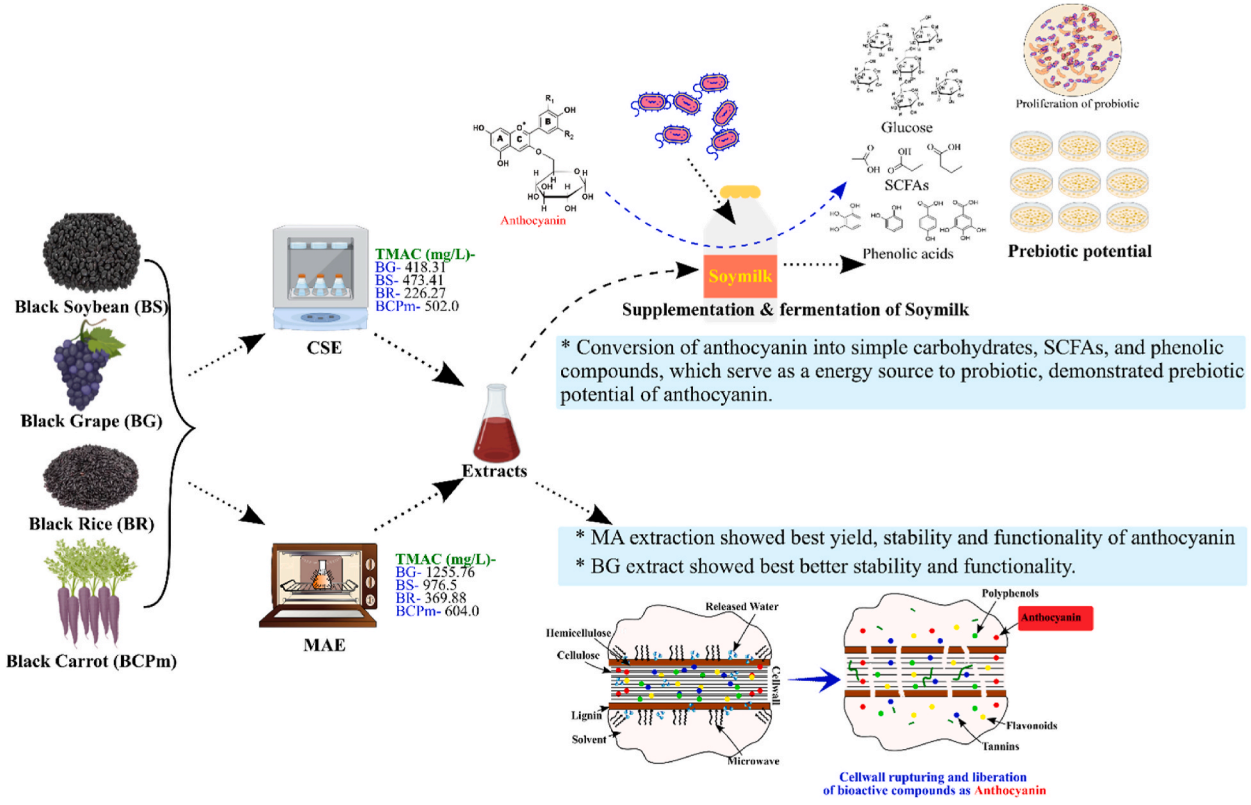


Fig. 11. An overall snapshot of the results obtained in the current research study.

Manoj Kumar: Writing – review & editing, Formal analysis.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

The ICAR-Indian Agricultural Research Institute, New Delhi, Government of India, is thankfully acknowledged for providing the facility to carry out this research.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e31780.

References

[1] H. Jiang, W. Zhang, Y. Xu, Y. Zhang, Y. Pu, J. Cao, W. Jiang, Applications of plant-derived food by-products to maintain quality of postharvest fruits and vegetables, Trends Food Sci. Technol. 116 (2021) 1105–1119.
[2] M. Kumar, A. Dahuja, A. Sachdev, C. Kaur, E. Varghese, S. Saha, K.V.S.S. Sairam, Evaluation of enzyme and microwave-assisted conditions on extraction of anthocyanins and total phenolics from black soybean (*Glycine max* L.) seed coat, Int. J. Biol. Macromol. 135 (2019) 1070–1081.
[3] Ø.M. Andersen, M. Jordheim, Basic anthocyanin chemistry and dietary sources, ANTHOCYANINS IN HEALTH AND DISEASE 1 (2013) 13–89.
[4] N. Tena, J. Martín, A.G. Asuero, State of the art of anthocyanins: antioxidant activity, sources, bioavailability, and therapeutic effect in human health, Antioxidants 9 (5) (2020) 451.
[5] F. Blando, N. Calabriso, H. Berland, G. Maiorano, C. Gerardi, M.A. Carluccio, Ø.M. Andersen, Radical scavenging and anti-inflammatory activities of representative anthocyanin groupings from pigment-rich fruits and vegetables, Int. J. Mol. Sci. 19 (1) (2018) 169.
[6] J. Shipp, E.S.M. Abdel-Aal, Food applications and physiological effects of anthocyanins as functional food ingredients, Open Food Sci. J. 4 (1) (2010).
[7] C. Gerardi, C. Albano, N. Calabriso, M.A. Carluccio, M. Durante, G. Mita, F. Blando, Techno-functional properties of tomato puree fortified with anthocyanin pigments, Food Chem. 240 (2018) 1184–1192.
[8] X.Z. Tian, Q. Lu, P. Paengkoum, S. Paengkoum, Effect of purple corn pigment on change of anthocyanin composition and unsaturated fatty acids during milk storage, J. Dairy Sci. 103 (9) (2020) 7808–7812.
[9] S.A. Kabakcı, M. Türkyılmaz, M. Özkan, Changes in the quality of kefir fortified with anthocyanin-rich juices during storage, Food Chem. 326 (2020) 126977.
[10] X. Zhang, Y. Yang, Z. Wu, P. Weng, The modulatory effect of anthocyanins from purple sweet potato on human intestinal microbiota in vitro, J. Agric. Food Chem. 64 (12) (2016) 2582–2590.
[11] R. Tabasco, F. Sánchez-Patán, M. Monagas, B. Bartolomé, M.V. Moreno-Arribas, C. Peláez, T. Requena, Effect of grape polyphenols on lactic acid bacteria and bifidobacteria growth: resistance and metabolism, Food Microbiol. 28 (7) (2011) 1345–1352.
[12] Y. Zhu, H. Sun, S. He, Q. Lou, M. Yu, M. Tang, L. Tu, Metabolism and prebiotics activity of anthocyanins from black rice (*Oryza sativa* L.) in vitro, PLoS One 13 (4) (2018) e0195754.
[13] H.C. Lee, A.M. Jenner, C.S. Low, Y.K. Lee, Effect of tea phenolics and their aromatic fecal bacterial metabolites on intestinal microbiota, Res. Microbiol. 157 (9) (2006) 876–884.
[14] C. Cueva, M.V. Moreno-Arribas, P.J. Martín-Álvarez, G. Bills, M.F. Vicente, A. Basilio, B. Bartolomé, Antimicrobial activity of phenolic acids against commensal, probiotic and pathogenic bacteria, Res. Microbiol. 161 (5) (2010) 372–382.
[15] M. Koudoufio, Y. Desjardins, F. Feldman, S. Spahis, E. Delvin, E. Levy, Insight into polyphenol and gut microbiota crosstalk: are their metabolites the key to understand protective effects against metabolic disorders? Antioxidants 9 (10) (2020) 982.
[16] M.A.C. Albuquerque, R. Bedani, J.G. LeBlanc, S.M.I. Saad, Passion fruit by-product and fructo-oligosaccharides stimulate the growth and folate production by starter and probiotic cultures in fermented soymilk, Int. J. Food Microbiol. 261 (2017) 35–41.
[17] S. Silva, E.M. Costa, C. Calhau, R.M. Morais, M.E. Pintado, Anthocyanin extraction from plant tissues: a review, Crit. Rev. Food Sci. Nutr. 57 (14) (2017) 3072–3083.
[18] S. Farooq, M.A. Shah, M.W. Siddiqui, B.N. Dar, S.A. Mir, A. Ali, Recent trends in extraction techniques of anthocyanins from plant materials, J. Food Meas. Char. 14 (6) (2020) 3508–3519.
[19] V. Krishnan, R. Rani, S. Pushkar, S.K. Lal, S. Srivastava, S. Kumari, A. Sachdev, Anthocyanin fingerprinting and dynamics in differentially pigmented exotic soybean genotypes using modified HPLC–DAD method, J. Food Meas. Char. 14 (4) (2020) 1966–1975.
[20] V. Farzaneh, I.S. Carvalho, Modelling of microwave assisted extraction (MAE) of anthocyanins (TMA), JOURNAL OF APPLIED RESEARCH ON MEDICINAL AND AROMATIC PLANTS 6 (2017) 92–100.
[21] M.M. Giusti, R.E. Wrolstad, Characterization and measurement of anthocyanins by UV-visible spectroscopy, CURRENT PROTOCOLS IN FOOD ANALYTICAL CHEMISTRY (1) (2001) F1–F2.
[22] T.C. Somers, M.E. Evans, Wine quality: Correlations with colour density and anthocyanin equilibria in a group of young red wines, J. Sci. Food Agric. 25 (11) (1974) 1369–1379.
[23] L.L. Sun, W. Gao, M.M. Zhang, C. Li, A.G. Wang, Y.L. Su, T.F. Ji, Composition and antioxidant activity of the anthocyanins of the fruit of *Berberis heteropoda Schrenk*, Molecules 19 (11) (2014) 19078–19096.
[24] I.F. Benzie, Y.T. Szeto, Total antioxidant capacity of teas by the ferric reducing/antioxidant power assay, J. Agric. Food Chem. 47 (2) (1999) 633–636.
[25] O.N. Donkor, A. Henriksson, T. Vasiljevic, N.P. Shah, Rheological properties and sensory characteristics of set-type soy yogurt, J. Agric. Food Chem. 55 (24) (2007) 9868–9876.
[26] J. Wang, Z. Guo, Q. Zhang, L. Yan, W. Chen, X.M. Liu, H.P. Zhang, Fermentation characteristics and transit tolerance of probiotic *Lactobacillus casei* Zhang in soymilk and bovine milk during storage, J. Dairy Sci. 92 (6) (2009) 2468–2476.
[27] dos D.C. Santos, J.G. de Oliveira Filho, A.C.A. Santana, B.S.M. de Freitas, F.G. Silva, K.P. Takeuchi, M.B. Egea, Optimization of soymilk fermentation with kefir and the addition of inulin: physicochemical, sensory and technological characteristics, LWT 104 (2019) 30–37.
[28] H.F. Zhang, X. Zhang, X.H. Yang, N.X. Qiu, Y. Wang, Z.Z. Wang, Microwave assisted extraction of flavonoids from cultivated *Epimedium sagittatum*: extraction yield and mechanism, antioxidant activity and chemical composition, Ind. Crop. Prod. 50 (2013) 857–865.

[29] W. Liu, C. Yang, C. Zhou, Z. Wen, X. Dong, An improved microwave-assisted extraction of anthocyanins from purple sweet potato in favor of subsequent comprehensive utilization of pomace, Food Bioprod. Process. 115 (2019) 1–9.
[30] A. Álvarez, J. Poejo, A.A. Matias, C.M. Duarte, M.J. Cocero, R.B. Mato, Microwave pretreatment to improve extraction efficiency and polyphenol extract richness from grape pomace. Effect on antioxidant bioactivity, Food Bioprod. Process. 106 (2017) 162–170.
[31] F. Dahmoune, B. Nayak, K. Moussi, H. Remini, K. Madani, Optimization of microwave-assisted extraction of polyphenols from *Myrtus communis* L. leaves, Food Chem. 166 (2015) 585–595.
[32] A.E. Ince, S. Şahin, S.G. Şümnü, Extraction of phenolic compounds from melissa using microwave and ultrasound, Turk. J. Agric. For. 37 (1) (2013) 69–75.
[33] I.E. Garofulić, V. Dragović-Uzelac, A.R. Jambrak, M. Jukić, The effect of microwave assisted extraction on the isolation of anthocyanins and phenolic acids from sour cherry Marasca (*Prunus cerasus* var. Marasca), J. Food Eng. 117 (4) (2013) 437–442.
[34] T.S. Ballard, P. Mallikarjunan, K. Zhou, S. O'Keefe, Microwave-assisted extraction of phenolic antioxidant compounds from peanut skins, Food Chem. 120 (4) (2010) 1185–1192.
[35] N. Sutivisedsak, H.N. Cheng, J.L. Willett, W.C. Lesch, R.R. Tangsrud, A. Biswas, Microwave-assisted extraction of phenolics from bean (*Phaseolus vulgaris* L.), Food Res. Int. 43 (2) (2010) 516–519.
[36] M. Milutinović, N. Radovanović, M. Ćorović, S. Šiler-Marinković, M. Rajilić-Stojanović, S. Dimitrijević-Branković, Optimisation of microwave-assisted extraction parameters for antioxidants from waste Achillea millefolium dust, Ind. Crop. Prod. 77 (2015) 333–341.
[37] H.W. Kim, J.B. Kim, S.M. Cho, M.N. Chung, Y.M. Lee, S.M. Chu, D.J. Lee, Anthocyanin changes in the Korean purple-fleshed sweet potato, Shinzami, as affected by steaming and baking, Food Chem. 130 (4) (2012) 966–972.
[38] F.C. Stintzing, A.S. Stintzing, R. Carle, B. Frei, R.E. Wrolstad, Color and antioxidant properties of cyanidin-based anthocyanin pigments, J. Agric. Food Chem. 50 (21) (2002) 6172–6181.
[39] H.M. Ali, W. Almagribi, M.N. Al-Rashidi, Antiradical and reductant activities of anthocyanidins and anthocyanins, structure–activity relationship and synthesis, Food Chem. 194 (2016) 1275–1282.
[40] M.M. Rahman, T. Ichiyanagi, T. Komiyama, Y. Hatano, T. Konishi, Superoxide radical-and peroxynitrite-scavenging activity of anthocyanins; structure-activity relationship and their synergism, Free Radic. Res. 40 (9) (2006) 993–1002.
[41] W. Li, P. Gong, H. Ma, R. Xie, J. Wei, M. Xu, Ultrasound treatment degrades, changes the color, and improves the antioxidant activity of the anthocyanins in red radish, LWT 165 (2022) 113761.
[42] M.B. Perez, M.J.D.P. Hamparsomian, R.E. Gonzalez, G.I. Denoya, D.L. Dominguez, K. Barboza, P.F. Cavagnaro, Physicochemical properties, degradation kinetics, and antioxidant capacity of aqueous anthocyanin-based extracts from purple carrots compared to synthetic and natural food colorants, Food Chem. 387 (2022) 132893.
[43] R. Romano, A. Aiello, L. De Luca, F. Pizzolongo, A. Durazzo, M. Lucarini, A. Santini, Deep-frying purple potato Purple Majesty using sunflower oil: effect on the polyphenols, anthocyanins and antioxidant activity, Heliyon 8 (5) (2022) e09337.
[44] M. Türkyılmaz, O. Yemiş, M. Özkan, Clarification and pasteurisation effects on monomeric anthocyanins and percent polymeric colour of black carrot (*Daucus carota* L.) juice, Food Chem. 134 (2) (2012) 1052–1058.
[45] E.A. Hayouni, M. Abedrabba, M. Bouix, M. Hamdi, The effects of solvents and extraction method on the phenolic contents and biological activities in vitro of Tunisian *Quercus coccifera* L. and *Juniperus phoenicea* L. fruit extracts, Food Chem. 105 (3) (2007) 1126–1134.
[46] B.K. Tiwari, A. Patras, N. Brunton, P.J. Cullen, C.P. O'Donnell, Effect of ultrasound processing on anthocyanins and color of red grape juice, Ultrason. Sonochem. 17 (3) (2010) 598–604.
[47] A. Faria, I. Fernandes, S. Norberto, N. Mateus, C. Calhau, Interplay between anthocyanins and gut microbiota, J. Agric. Food Chem. 62 (29) (2014) 6898–6902.
[48] M. Hasan, S.R. Arpitha, C. Das, R. Laishram, M. Sasi, S. Kumar, A. Dahuja, Research trends and approaches for the nutritional and bio-functionality enhancement of fermented soymilk, J. Funct.Foods 107 (2023) 105698.
[49] C. Amorim, S.C. Silvério, B.B. Cardoso, J.I. Alves, M.A. Pereira, L.R. Rodrigues, *In vitro* fermentation of raffinose to unravel its potential as prebiotic ingredient, LWT 126 (2020) 109322.
[50] T.A. Verediano, H. Stampini Duarte Martino, M.C. Dias Paes, E. Tako, Effects of anthocyanin on intestinal health: a systematic review, Nutrients 13 (4) (2021) 1331.
[51] X.L. Zhou, X.F. Kong, G.Q. Lian, F. Blachier, M.M. Geng, Y.L. Yin, Dietary supplementation with soybean oligosaccharides increases short-chain fatty acids but decreases protein-derived catabolites in the intestinal luminal content of weaned Huanjiang mini-piglets, Nutr. Res. 34 (9) (2014) 780–788.
[52] C. Li, W. Li, X. Chen, M. Feng, X. Rui, M. Jiang, M. Dong, Microbiological, physicochemical and rheological properties of fermented soymilk produced with exopolysaccharide (EPS) producing lactic acid bacteria strains, LWT–Food Sci. Technol. 57 (2) (2014) 477–485.
[53] A.N. Hassan, R. Ipsen, T. Janzen, K.B. Qvist, Microstructure and rheology of yogurt made with cultures differing only in their ability to produce exopolysaccharides, J. Dairy Sci. 86 (5) (2003) 1632–1638.
[54] J.C. De Man, D. Rogosa, M.E. Sharpe, A medium for the cultivation of lactobacilli, J. Appl. Bacteriol. 23 (1) (1960) 130–135.
[55] M. Sasi, S. Kumar, M. Hasan, E. Garcia-Gutierrez, S. Kumari, O. Prakash, A. Dahuja, Current trends in the development of soy-based foods containing probiotics and paving the path for soy-synbiotics, Crit. Rev. Food Sci. Nutr. (2022) 1–19.