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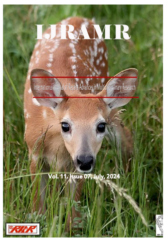

RESEARCH ARTICLE

FUNCTIONAL BEVERAGES CO-PIGMENTED WITH LEGUME EXTRACT AND ITS EFFECT ON ANTIOXIDANT PROFILE AND ANTHOCYANIN STABILITY AND ITS MINERAL AND VITAMIN CONTENT

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ABSTRACT

Anthocyanins are antioxidant-rich pigments found in red, purple, and blue fruits and vegetables, offering cardiovascular protection, anti-inflammatory, antimicrobial, and vision-enhancing benefits. They also support gut health and reduce the risk of chronic diseases. Copigmentation, the formation of molecular complexes between pigments and non-colored organic substances, leads to increased absorbance and potential shifts in pigment absorption wavelengths, playing a crucial role in color stabilization and modulation in various natural and food products. A study focused on developing functional beverage examined antioxidant properties using diverse assays, including Folin-Ciocalteu for Total Phenolic Content, Aluminum Chloride for Total Flavonoids Content, pH differential technique for Total Anthocyanin Content, and Bisulfite bleaching for Monomeric and Polymeric Color. Antioxidant activities were evaluated through DPPH IC50 and FRAP content assays. Among four formulated beverages, FB4 (40:42:12:4:2) exhibited significantly higher levels of total anthocyanin (9070.57 mg Cy-3-G/L), Cyanidin-3-Rutinoside (2458 mg/L), Pelargonidin-3-Glucoside (1080.6 mg/L), and total flavonoids (10.6 mgQCE/ml) compared to FB1 (40:54:0:4:2). FB4 also demonstrated superior antioxidant activity with an IC50 value of 22.1 ± 0.16 and FRAP content of 48.4 mgAscE/ml. Additional parameters such as Browning Index, Hyperchromic shift, and Bathochromic shift were calculated, with FB4 showing significantly higher values than FB1. FB 4 has higher sodium (309.1mg/100ml), zinc (40.0mg/100ml), vitamin E (18.0mg/100ml), and vitamin K (0.133mg/100ml) content. FB 1 had higher calcium (269.7mg/100ml), magnesium (737.3mg/100ml), potassium (2047.8mg/100ml), iron (14.2mg/100ml), manganese (37.8mg/100ml), vitamin C (26.0) content. These findings suggest that a beverage composed of black soybeans, corn silk, and black mulberries offers a potent functional drink with therapeutic potential, particularly suitable for consumers with metabolic ailments. This research highlights the importance of anthocyanin-rich beverages in providing health benefits, including antioxidant, anti-inflammatory, and antimicrobial properties, while emphasizing the role of copigmentation in enhancing the stability and visual appeal of these beverages.

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INTRODUCTION

Anthocyanins are natural plant pigments with a well-known reputation for their diverse range of bioactive characteristics. These compounds are responsible for the striking red, blue, and purple colors found in various plants, fruits, and extracts like berry juices and red wines (Mattioli *et al*., 2020). They are frequently employed as natural food colorants to enhance the visual appeal of products (Chen *et al*., 2020).

Beyond their role as colorants, anthocyanins offer potential health advantages, including their ability to help regulate blood sugar and cholesterol levels, as well as their anti-microbial and anti-inflammatory properties (Song *et al*., 2021). Nevertheless, anthocyanins are vulnerable to a range of factors, including pH, temperature, oxygen, light, ascorbic acid, sugar, and interactions with other substances in the matrix. These factors can lead to a loss of color and reduced stability over time (Martynenko & Chen, 2016). Copigmentation enhances color stability and results in more vibrant colors than what would be expected based solely on the concentration of anthocyanins and the pH of the medium (Cai *et al*., 2022). Consequently, ensuring the preservation of anthocyanin strength is of paramount importance during the preparation and storage of foods rich in anthocyanins (Balandrao *et al*., 2021).To extend the shelf life of perishable fruits and vegetables, they are often transformed into ready-to-serve (RTS) beverages (Morales-de la Pena *et al*. 2016). RTS beverages are prized for their medicinal properties, nutritional value, and appealing attributes (Rathinasamy et.al., 2022). These beverages can be enriched with bioactive compounds such as vitamins C, E, and β-carotene, which act as natural antioxidants to combat oxidative stress-related illnesses (Chambial *et al*., 2013). These antioxidants are particularly beneficial during stressful situations as they help reduce the formation of free radicals, which are associated with tissue damage and inflammation, hallmarks of oxidative stress (Papaccio *et al*., 2022).Herbs have played a significant role in the development of modern medicine, with many traditional plants containing medicinal properties due to their natural antioxidant content, especially phenolic components (Wan *et al*., 2010; Patel *et al*., 2018). Corn Silk (*Stigma Maydis*), for instance, is a delicate thread with a yellowish/brownish appearance(Fazilatun *et al*., 2021), known for its therapeutic properties in treating various conditions like cystitis, fluid retention, kidney stones, diuretics, prostate issues, urinary infections, bedwetting, and obesity(George and Khan, 2021). Black Mulberry (*Morus nigra*) is another noteworthy plant due to its dark color resulting from a high concentration of anthocyanins, which offer antioxidant benefits and various health advantages, including traditional uses for hepatitis, analgesia, diuresis, anemia, and arthritis (Mehta and Kumar, 2021). Black Soybean (*Glycine max.* L) is an East Asian legume rich in anthocyanins with antioxidant, anti-obesity, anti-hyperglycemic, and anti-hyperlipidemic effects, making it a valuable addition to the realm of functional foods (Ganesan and Xu, 2017).The aim of the current study was to develop anthocyanin rich ready-to-serve beverage co-pigmented with black soybean extract and its effect on, antioxidant properties and anthocyanin stability toimprove the potential advantages and promote the development of novel food products.

MATERIALS AND METHODS

Raw Materials: Mulberries used in this research were sourced from TRIKAYA, a company located in Bangalore, and were transported to the CFTRI Mysore campus under freeze-packed conditions. Black soybean and corn silk were obtained from a local store in Mysore, while lemon and ginger Extracts were prepared in the laboratory. The mulberries were consistent in size, fully ripe, and showed no signs of bruising. Sample preparation was conducted in the Fruits and Vegetable Technology Department, and subsequent analysis took place in various departments of the Institute, depending on instrument availability. Sterilized plastic bottles were employed for packing and storing the ready-to-serve (RTS) beverages within the institute's laboratory. These beverages were maintained at refrigerated temperatures for further testing.

Formulation of Ready toServe beverage: The corn silk was separated from selected corn for preparation of extract. Black soybeans were handpicked, washed off dirt and kept for drying. As previously stated, completely ripened, bruise- free, consistent mulberries were chosen for beverage preparation. To remove the adherent dirt, the corn silk, black mulberry and black soybean was rinsed under running tap water. The corn silk was weighed and then subjected to boiling treatment (temperature 100-150 °C) for 30-45 minutes and then cooled in normal water, afterwards the extract was filtered through double muslin cloth to eliminate contaminants and big particles, and same was done for black soybean. The mulberries were put in a blender / pulper and pulpy juice was extracted. In the variants FB1, FB2, FB3, and FB4, the corn silk extract was combined with mulberry juice and black-soybean extracts in the following ratios: FB1 (40:60:0), FB2 (40:56:4), FB3 (40:52:8) and FB4 (40:48:12) initially. The extracts of ginger and lemon were mixed to the juice treatments for palatability and enhancement of nutritional properties, thus modifying the ratios of the treatments to FB1 (40:54:0:4:2), FB2 (40:50:4:4:2), FB3 (40:46:8:4:2) and FB4 (40:42:12:4:2). The similar method was used in all treatments. The final juices were homogenized with a juicer for overall uniform proportion. The Produced juices were pasteurized for 10 minutes at 80°C temperature. The pasteurized juice was poured into 200 ml clean, pre- sterilized dried plastic bottles with a 2.0 cm headspace and sealed airtight with a crown corking machine. All four formulated beverages were then kept at refrigerated condition for further estimation and sensory analysis.

Antioxidant Content and Activity: To assess the total flavonoidscontent (mgQE/g) in the beverages, an aluminum chloride colorimetric assay was employed, following the procedure outlined by Nirmala *et al*. 2020. The determination of the total phenolic content (mgGAE/g) was conducted using the Folin-Ciocalteu test, following the methodology described by Ramirez *et al*. in 2021.Each measurement was conducted in triplicate.The assessment of DPPH Radical Scavenging Activity (IC_{50} g/ml) followed the protocol established by Baliyan *et al*. in 2022. The IC_{50} value was determined by plotting the percentage of inhibition against the sample concentration. Ascorbic acid (Vitamin C) was used as the positive control, and the results are presented in terms of IC_{50}values.For the Ferric Reducing Antioxidant Assay (mgAscE/g), Ascorbic Acid served as the standard, while pure water was used as the blank control. A standard curve was constructed using Ascorbic Acid concentrations ranging from 5 to 100 mg/g, following the procedure outlined by Xiao *et al*. in 2020.

Determination of Monomeric, Polymeric, Color Density, Browning Index and Total Anthocyanin Content: The determination of both Total Anthocyanin Content (TAC) and Monomeric Anthocyanin Content (MAC) was conducted using the pH differential method (Le *et al*., 2019). The dilution factor was determined to be 20, which was calculated by dividing the final sample volume by the initial volume. Subsequently, two separate dilutions of the aqueousextract, each approximately 1 ml in volume, were prepared. One dilution was created using a 0.025 M Potassium Chloride buffer of pH 1 while the other was prepared using a 0.4 M sodium acetate buffer of pH 4.5. Both dilutions were adjusted using the previously determined dilution factor. Absorbance measurements were performed using a UV/VIS Spectrophotometer at their respective maximum wavelengths, which were 520 nm and 700 nm, following a 20-minute equilibrium period. The calculation of both total anthocyanin content and MAC was conducted using the equation. (1).

$$\text{Total Anthocyanin} = \frac{A \times MW \times DF \times 1000}{E \times L} \qquad (1)$$

In the provided equation, where A represents the calculation involving absorbance values at pH 1.0 and pH 4.5 with specific wavelengths (A520 - A700), it is important to consider the following parameters:

- MW (molecular weight) is equal to 449.2 g/mol for cyanidin-3-glucoside.

- DF stands for the dilution factor.
- 1 represents the path length in centimeters.
- ε represents the molar extinction coefficient, which is 26,900 L/(mol · cm) for cyanidin-3-glucoside.
- The factor 1000 is applied for converting from grams to milligrams.

The determination of Polymeric Color (PC), Percent Polymeric Color (PPC), Color Density (CD) followed the method outlined by described by Aamer *et al.* (2021) To achieve this, the beverages were first diluted with distilled water until their absorbance readings fell within the range of 0.5 to 1.0 at an unspecified wavelength.Next, 0.2 mL of a potassium metabisulfite solution (0.90M) was introduced into 2.8 mL of the diluted sample, resulting in the creation of a bisulfite-bleached sample. Simultaneously, 0.2 mL of distilled water was combined with 2.8 mL of the diluted sample to form a non-bleached control sample. These samples were allowed to equilibrate for 30 minutes.Subsequently, the samples were assessed at wavelengths of λ = 700 nm, 510 nm, and 420 nm. The calculation of CD was performed utilizing the non-bleached control sample and the following Equation (1). Equation 4 was used to compute the browning index (BI), which represents the ratio of total anthocyanin pigment loss to the development of brown color. After diluting the beverages with distilled water (1:1), absorbance was measured.

$$CD = [(A_{420}nm - A_{700}nm) + (A_{510}nm - A_{700}nm)] \text{ x dilution factor} \qquad (1)$$

$$PC = [(A_{420}nm - A_{700}nm) + (A_{510}nm - A_{700}nm)] \text{ x dilution factor} \qquad (2)$$

$$PPC = (PC/CD) \text{ x } 100 \qquad (3)$$

$$BI = (A\ 420\ nm)/(A\ 520\ nm) \qquad (4)$$

Determination of Co-pigmentation Effects: The hyperchromic shift, which measures the increase in absorbance when a co-pigment is present compared to when it is absent, was calculated using the following Equation (1). The bathochromic shift, which quantifies the difference between the initial maximum wavelength (λ0) and the increased maximum wavelength (λ) after the addition of the co-pigment, was determined using the following Equation (2)(Klisurova *et al.*, 2019).

$$\text{Hyperchromic effect} = ((A - A0) / A0) \times 100 \qquad (1)$$

Where A represents the absorbance in the presence of the co-pigment,

A0 represents the absorbance in the absence of the co-pigment

$$\text{Bathochromic effect} = ((\lambda - \lambda 0) / \lambda 0) \times 100 \qquad (2)$$

Where λ represents the maximum wavelength after the addition of the co-pigment

λ0 represents the initial maximum wavelength in the absence of the co-pigment

Mineral and Vitamin estimation: The mineral analysis was done using Atomic Absorption Spectroscopy method for zinc, iron, calcium, magnesium, sodium, potassium and manganese as per standard procedures (AOAC, 2016). Vitamins were determined by HPLC-DAD method(AOAC, 2016)

Statistical analysis: Data was submitted to one-way analysis of variance (ANOVA)and Tukey's Test considering 5% probability ($p \leq 0.05$), along with Mean ± SEM. Statistical analysis were performed using Statistical software, version 10 (StaSoft Inc., Tulsa, USA.

RESULTS AND DISCUSSION

Antioxidant Content and Activity: It is advisable for individuals to incorporate appropriate dietary habits that involvetheconsumption of bioactive antioxidant compounds, as free radicals can have detrimental effects on health (Forni *et al.*, 2021; Khan *et al.*, 2021). Natural antioxidants with non-enzymatic properties can be found in fruits and vegetables, and they primarily fall into four major categories: vitamins, carotenoids, polyphenols, and minerals. Among polyphenols, there are two significant subgroups to consider: flavonoids and phenolic acids (Arias *et al.*, 2022). Table 1 illustrates the application of the Folin-Ciocalteu test, which quantifies total phenols in natural products through an oxidation/reduction reaction, with a challenging endpoint after 120 minutes. Phenolic compounds, known for their antioxidant and redox characteristics, include gallic acid, which is the second most prevalent chemical in this context. Gallic acid has the potential to inhibit the overproduction of reactive oxygen species, possibly reducing the risk of neurological diseases such as Parkinson's and Alzheimer's (Bhuia *et al.*, 2023). The study identified significant differences in Total Phenolic Content among four formulated beverages, with FB1 having the highest content at 47.6±0.23 mg GAE/g.Flavonoids, a subgroup of naturally occurring polyphenols found in vegetables, fruits, grains, and tea, play pivotal roles in various biological processes and plant responses to environmental stimuli. They are particularly well-known for their ability to modulate the activity of key cellular enzymes and possess properties such as antioxidative, anti-inflammatory, anti-mutagenic, and anti-carcinogenic effects (Panche *et al.*, 2016). The study also detected notable differences in the Total Flavonoid Content among the four formulated beverages, with FB4 having the highest content (10.6±0.31 mg QCE/g), while FB1 had the lowest at 7.9± 0.38. All these values showed significant differences at a significance level of $p \leq 0.05$. The antioxidant activity of fruits plays a crucial role in combating degenerative diseases like mutagenesis, carcinogenesis, cardiovascular issues, and aging. These diseases are often triggered by free radicals produced within biological systems or encountered from external sources.

Therefore, understanding the concentration and activity of antioxidants in foods is essential for preventing oxidative damage and maintaining both economic and nutritional value (Singh and Kedare, 2011). The assay measures the ability of biological samples to reduce DPPH, a deep purple free radical, to 1,1-diphenyl-2-picryl hydrazine. The color change observed depends on the ability of antioxidants to donate hydrogen atoms, with higher antioxidant activity indicating greater radical scavenging capacity (Baliyan, *et al*., 2022). In the study, DPPH levels (Table 1) were analyzed in four formulated beverages, with beverage 1 having an IC50 (µg/ml) of (25.7±0.30) and formulated beverage 4 having an IC50 (µg/ml) of (16.8±0.16).The FRAP test involves electron transport to reduce Fe3+ to Fe2+ and operates primarily at an acidic pH of 3.6. The reaction, in the presence of 2,4,6-trypyridyl-s-triazine, produces a colorful complex with Fe2+, and the degree of hydroxylation and conjugation influences the FRAP value (Cerretani and Bendini, 2010). The study identified significant differences in FRAP content among the four formulated beverages, with beverage 4 having the highest content at 48.4±0.25 mg AscE/g, suggesting potential health benefits.

Monomeric, Polymeric, Color Density, Browning Index and Total Anthocyanin Content: Anthocyanins are natural pigments present in various parts of plants, such as fruits, flowers, seeds, and vegetative tissues, imparting colors ranging from orange, red, purple, to blue. These compounds have the ability to absorb UV light and possess antioxidant properties, which can contribute to improved health by reducing the risk of chronic diseases (Mattioli *et al*., 2020). Table 2 illustrates the Mean±SEM values of Total Anthocyanin Content (mgCy-3-G/L) for four formulated beverages (FB), namely, FB 1, 2, 3, and 4. Notably, among these beverages, FB 4 displayed the highest total anthocyanin content (mgCy-3-G/L) at 9070, while FB1 had the lowest total anthocyanin content (mgCy-3-G/L) at 5143.1 Importantly, all beverages exhibited significant differences at the significance level of p≤0.05. In a liposomal system, certain anthocyanins, such as pelargonidin-3-glucoside, cyanidin-3-glucoside, and delphinidin-3-glucoside, exhibit strong antioxidant properties and can inhibit the production of malondialdehyde (Khoo *et al*., 2019). The study further analyzed the chemical composition of the four formulated beverages, with a particular focus on Cyanidin-3-Rutinoside (Cy-3-R) and Pelargonidin-3-Glucoside (Pg-3-G). It was observed that the highest concentrations of Cy-3-R (2458±0.57 mg/L) and Pg-3-G (1086.6±0.66 mg/L) were found in FB4, whereas the lowest concentrations were observed in FB1, with Cy-3-R at 2101±0.57 mg/L and Pg-3-G at 728.4±0.26 mg/L. Once again, all four beverages exhibited significant differences at the p≤0.05 level.Polymeric color, color density and % polymeric color content amplified significantly with increase in the concentration of black soybean extract. The highest concentration of PC (1.363 ± 0.11), CD (11.9 ± 0.02), %PC (11.4±0.01) was found in formulated beverage 4, while the lowest was in FB 1 PC (0.153 ± 0.03), CD (5.0± 0.01), %PC (3.0±0.11). All four beverages showed significant differences at the p≤0.05 level. Bronwing index for formulated beverage 4 (4.58 ± 0.09) was significantly higher as compared to FB1 (1.05 ± 0.02). The increasing BI implies a buildup or addition of darker pigment at the expense of diminished red anthocyanin pigments. The BI indicates change in overall color of formulated beverages

Determination of Copigmentation effect: The findings regarding the copigmentation effect in all the four formulated beverages assessed reactions at 20°C have been consolidated in Table 1. It was observed that an increase in the molar ratio also led to a greater hyperchromic shift ($p<0.05$), signifying that the copigmentation process is influenced by the concentration of the copigment under investigation. In the instance of the complexation reaction, a molar ratio of 48:12 (2.44) yielded the most significant hyperchromic effect compared to the other three formulated beverages. The most substantial enhancement of color intensity, denoted by the hyperchromic shift, during the copigmentation of C3G at 20°C was observed in FB 4. Among these copigments, the most pronounced hyperchromic shift indicated a notably stronger intermolecular interaction with C3G ($p<0.05$).Furthermore, in addition to the hyperchromic shift, the bathochromic shift in all reactions also increased with a higher copigment concentration at 20°C, at 564 nm, the formulated beverage 4 had the greatest λ max as detailed in Table 1.Copigmentation was definitely visible in all of the formulated beverages copigmented by BSE. Both hyperchromic and bathochromic shifts were evident in the observations. The hyperchromic shift was characterized by elevated absorption values at λ max, which escalated from 0.346 to 2.444. Notably, concentrations exceeding 4% led to a further rise in absorbance at λ max. Table 3 provides a comprehensive overview of the impact of incrementally increasing the copigment percentage while keeping the volume of the prepared beverages constant. The bathochromic shift was evident in the displacement of λ max towards longer wavelengths. This shift caused the wavelength to move from 498 nm to 564 nm.Values are Mean ± SEM of triplicate determinations. Values sharing different superscript letters between columns are significantly different at $p \leq 0.05$

Mineral and Vitamin Content: Table 4 shows the mineral content and vitamin content FB (FB 1, FB 2, FB 3 and FB 4). FB 4 due to higher proportion of black soybean extract (12%) has the higher sodium (309.1mg/100ml), zinc (40.0mg/100ml), vitamin E (18.0mg/100ml), and vitamin K (0.133mg/100ml) content. Black soybeans include a special combination of high sodium, potassium, zinc and vitamin E that is rarely found in a single food source, making them advantageous for those with low blood pressure. Although black soybean is grown in the nation, its usage in beverages is limited due to a lack of knowledge about cultivars that are suited for the manufacture of soy milk and beverages (Patel and Pandya, 2015). Since black soybean includes a multi-meric iron store protein called ferritin, which is easily absorbed and accessible, it is advised that it be included in the diet of persons who suffer from anaemia. Black soybean extract has platelet aggregation inhibition action (in vitro) produced by collagen (Kim *et al*., 2011). In Formulated Beverage 1 due to larger percent of black mulberry pulp (54%), FB 1 had higher calcium (269.7mg/100ml), magnesium (737.3mg/100ml), potassium (2047.8mg/100ml), iron (14.2mg/100ml), manganese (37.8mg/100ml), vitamin C (26.0) content, Black Mulberries are high in calcium, magnesium and iron which is necessary for bone and muscular formation.

Table 1. Antioxidant properties of corn silk-mulberry formulated beverages incorporated with black-soybean extract at different concentrations

Antioxidant properties	FB 1	FB 2	FB 3	FB 4
Total Phenolic Content (mg GAE/ml)	47.6^{a}± 0.23	46.3^{b}± 0.20	45.3^{c}± 0.20	44.4^{d}± 0.26
Total Flavonoids Content(mg QCE/ml)	7.9^{a}± 0.38	8.6^{b}± 0.23	9.5^{c}± 0.11	10.6^{d}± 0.31
DPPH IC$_{50}$(µg/ml)	25.7^{a}± 0.30	21.6^{b}± 0.06	19.5^{c}± 0.05	16.8^{d}± 0.16
FRAP (mg AscE/ml)	37.4^{a}± 0.30	43.3^{b}± 0.6	45.3^{c}± 0.20	48.4^{d}± 0.25

Values are Mean ± SEM of triplicate determinations. Values sharing different superscript letter between rows are significantly different at $p \leq 0.05$

Table 2. Monomeric, Polymeric Color, Percent Polymeric Color, Color density, Browning Index and Total Anthocyanin Content of corn silk-mulberry formulated beverages incorporated with black-soybean extract at different concentrations

Formulated Beverages	Anthocyanins						
	Monomeric		Polymeric				Total
	Cy-3-R (mg/L)	Pg-3-G (mg/L)	Polymeric Color	Color Density	%Polymeric Color	Browning Index	TAC (mg Cy-3-G/L)
FB 1	2101^{a}±0.57	728^{a}±0.26	0.153^{a}±0.03	5.0^{a}±0.01	3.0^{a}±0.11	1.05 ± 0.02	5143.1^{a}±0.52
FB 2	2307^{b}±0.88	933^{b}±0.27	0.383^{b}±0.01	6.6^{b}±0.22	5.7^{b}±0.40	2.25 ± 0.02	7048.3^{b}±0.58
FB 3	2431^{c}±0.88	1059^{c}±0.87	0.793^{c}±0.20	8.9^{c}±0.02	8.8^{c}±0.01	3.44 ± 0.02	8375.6^{c}±0.88
FB 4	2458^{d}±0.57	1087^{d}±0.66	1.363^{d}±0.11	11.9^{d}±0.02	11.4^{d}±0.01	4.58 ± 0.09	9070^{d}±0.57

Values are Mean ± SEM of triplicate determinations. Values sharing different superscript letters between columns are significantly different at $p \leq 0.05$.

Table 3. Hyperchromic and Bathochromic shift of corn silk-mulberry formulated beverages incorporated with black-soybean extract at different concentrations

Formulated Beverages	Hyperchromic Shift (Abs)	Bathochromic shift (λ max)
FB 1	0.35^{a}±0.01	498^{a}±0.02
FB 2	0.96^{b}±0.02	520^{b}±0.03
FB 3	1.79^{c}±0.03	543^{c}±0.13
FB 4	2.44^{d}±0.02	564^{d}±0.30

Values are Mean ± SEM of triplicate determinations. Values sharing different superscript letters between columns are significantly different at $p \leq 0.05$

Table 4. Nutritional Characteristics of Corn Silk-Mulberry Formulated Beverages Incorporated with Black-Soybean Extract at Different Concentrations

PARAMETERS	FB 1	FB 2	FB 3	FB 4
Calcium (mg/ 100 ml)	269.7±0.34 ^{a}	253.7 ± 0.07 ^{b}	235.5 ± 0.59 ^{c}	221.4 ± 0.88 ^{d}
Magnesium (mg/ 100 ml)	73.7 ± 0.25 ^{a}	70.0± 0.37 ^{b}	66.3 ± 0.44 ^{c}	62.4 ± 1.67 ^{d}
Sodium (mg/ 100 ml)	22.1 ± 0.88 ^{a}	25.2 ± 0.57 ^{b}	28.1 ± 0.57 ^{c}	30.9 ± 0.6 ^{d}
Potassium(mg/ 100 ml)	2047.8 ± 0.57 ^{a}	2005.5 ± 0.29 ^{b}	1962.8 ± 0.57 ^{c}	1920.1 ± 0.57 ^{d}
Zinc (mg/ 100 ml)	2.9 ± 0.92 ^{a}	3.4 ± 0.28 ^{b}	4.1± 0.37 ^{c}	4.9± 0.57 ^{d}
Iron(mg/ 100 ml)	1.4 ± 0.11 ^{a}	1.3 ± 0.28 ^{b}	1.2± 0.05 ^{c}	1.1± 0.41 ^{d}
Manganese (mg/ 100 ml)	3.7± 0.16 ^{a}	3.6± 0.05 ^{b}	3.5± 0.30 ^{c}	3.4± 0.12 ^{d}
Vitamin C (mg/ 100 ml)	26.0 ± 0.14^{a}	24.8± 0.53^{b}	22.4±0.07^{c}	19.7± 0.57^{d}
Vitamin E (mg/ 100 ml)	0.3 ± 0.08^{a}	0.6 ± 0.35^{b}	0.8±0.25^{c}	1.4± 0.12^{d}
Vitamin K (mg/ 100 ml)	0.06 ± 0.01^{a}	0.09 ± 0.06^{b}	0.13±0.00^{c}	0.15± 0.12^{d}

Values are Mean ± SEM of triplicate determinations. Values sharing different superscript letters between columns are significantly different at $p \leq 0.05$

Iron is required for the production of hemoglobin. Iron was also detected in all the four beverages in significant amount. Iron is an essential metal for the human body that aids in oxygen and electron transmission. These minerals are very good for pregnant women and are used to relieve constipation (Ercisli *et al*., 2010).

CONCLUSION

There is an ongoing interest in developing safe and efficient methods for creating colorful and stable food products that can withstand the various conditions involved in food preparation and preservation while remaining safe for consumption. Co-pigmentation has recently garnered significant attention within the food industry. This heightened interest is a result of recent advancements in the field that allow for the creation of new combinations of natural pigments and co-pigments (colorant and enhancer) with stable and precisely controlled colors. By combining effective extraction techniques with readily available and cost-effective raw materials, this approach offers an environmentally responsible and budget-friendly alternative to traditional extraction methods, which typically involve large quantities of organic solvents and extended extraction times. Furthermore, this study has revealed that co-pigmentation enhances the antioxidant activity of a beverage. This, in turn, helps neutralize reactive oxygen species (ROS) and free radicals, reducing cellular damage and supporting overall health and cellular function.

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Conflict of interest both authors declare that they do not have conflict of interest

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