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Investigation of the Bioactive Properties and *In vitro* Bioaccessibility of Functional Butterfly Pea Flower (*Clitoria ternatea* L.) Beverages Produced by Ultrasound-Assisted Extraction and Infusion Methods

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ABSTRACT

The butterfly pea flower (*Clitoria ternatea* L.) and purple basil (*Ocimum basilicum* L.) are tropical plants rich in phenolic compounds, including anthocyanins, flavonols, polyphenols, and flavonoids. In this study, butterfly pea flower beverage and butterfly pea flower beverage containing purple basil were obtained using the infusion method (IM) and the ultrasound-assisted extraction (UAE) method. Both extraction process were carried out at two different durations: 3 and 7 minutes. Total phenolic content (TPC), total antioxidant capacity (TAC) and total monomeric anthocyanin (TMA) values increased in all beverage samples prepared by addition of purple basil extract. The color parameters of the samples were determined and the sensory characteristics were evaluated. This study will provide a new perspective on the extraction methods of butterfly pea flower plant, which has a characteristic blue color and contains functional biocomponents; its processing into a functional beverage; and its enrichment with purple basil, another plant rich in phytochemicals.

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Introduction

Plant-based bioactive beverages are gaining more and more attention for their positive effects on health thanks to their functional components. Plant extracts used in such beverages are rich in bioactive compounds such as phenolic compounds, flavonoids and anthocyanins, which exhibit potent antioxidant properties and may contribute to the reduction of oxidative stress (Silva et al., 2020; Males et al., 2022; Popova et al., 2023) . Functional beverages containing ingredients such as butterfly pea flower (*Clitoria ternatea* L.) and purple basil (*Ocimum basilicum*) are particularly notable for their color change properties and high phenolic content. Butterfly pea flower provides its characteristic blue color thanks to its ternatins, while purple basil is rich in anthocyanins and other phenolic compounds, increasing the bioactive capacity of the beverage (Filip, 2017; Escher et al., 2020). The development of such beverages represents an approach that addresses both consumer demand and functional health benefits. Moreover, the extraction methods used in the production of these beverages are critical to optimizing the levels of total phenolic content (TPC), total antioxidant capacity (TAC) and total monomeric anthocyanin (TMA). In this context, the application of techniques such as the infusion method (IM) and ultrasound-assisted extraction (UAE) allows for the improvement of the functional and sensory properties of the beverage.

Clitoria ternatea L., commonly known as butterfly pea flower, is predominantly found in tropical and subtropical regions. Various components of this plant, including its roots, flowers, leaves, and seeds, have been employed in traditional Indian folk medicine due to their medicinal properties (Escher et al., 2020). The blue petals of *C. ternatea* are rich in phenolic compounds, particularly anthocyanins such as ternatins (A1–A3, B1–B4, C1–C5, D1–D3), along with flavonols including kaempferol, quercetin, and myricetin. Scientific investigations have demonstrated that extracts derived from *C. ternatea* flowers possess significant antioxidant, antihyperglycemic, antihyperlipidemic, antimicrobial, and hepatoprotective effects, as evidenced by both *in vitro* and *in vivo* studies (Mehmood et al., 2019).

The distinct deep blue hue of the leaves has made the plant a widely utilized dye for centuries. An interesting characteristic of the beverage is its ability to exhibit color changes based on the pH level of substances introduced to it; for instance, the addition of lemon juice results in a purple hue (Chakraborthy et al., 2018).

The butterfly pea flower flower contains polyacylated derivatives of delphinidin 3,3',5'-triglucoside, known as "ternatins", which are the major anthocyanins present. These polyacylated anthocyanins found in the blue pea flower offer a valuable natural food coloring option due to their greater stability compared to unacylated anthocyanins. The color in ternatins is derived from anthocyanins, as stated (Zussiva et al., 2012).

One of the variations of butterfly pea flower, which is a rich source of phytochemicals, was obtained by adding purple basil (*Ocimum basilicum*) in our study. The purpose of adding purple basil is primarily to enrich the aroma, but it is also aimed at improving the functional properties of the beverage with another source rich in anthocyanin.

Purple basil is an important agricultural species recognized for its high content of essential oils, polyphenols, phenolics, flavonoids, and phenolic acids. This herb, which is a member of the Lamiaceae family, originates from tropical regions (Filip, 2017). Traditional uses of purple basil include treating kidney problems, aiding in childbirth, relieving earaches, regulating menstrual cycles, alleviating arthritis symptoms, stimulating appetite, combating colds, and treating malaria (Sonmezdag et al., 2018). Research has shown that purple basil possesses antiviral, antifungal and antibacterial properties, making it effective against a range of infections. Additionally, purple basil leaves have been utilized in treating fevers, coughs, influenza, asthma, bronchitis, and diarrhea (Chenni et al., 2016).

In addition to the production process and components of herb, the levels of antioxidants in beverage can also be influenced by brewing conditions. Research shows that phenolic compounds are relatively stable at various temperatures, but prolonged processing at high temperatures can lead to degradation of phenolic compounds (Volf et al., 2014). For example, it has been observed that black tea brewed at lower temperatures contains fewer tannins compared to those brewed at higher temperatures (Dmowski et al., 2011). Other studies have indicated that brewing time and temperature can impact the polyphenol content of both black and green tea. While most research focuses on green and black tea, there is limited information available on the effects of brewing time on the antioxidant content of other types of tea, such as herbal and fruit tea (Winiarska-Mieczan & Baranowska-Wójcik, 2024). Therefore, it is important to investigate the influence of extraction time on the antioxidant activity of butterfly pea flower beverage to determine the optimum extraction time for maximizing the health benefits of beverage consumption. The extraction method as well as the extraction time affect the properties of the beverage. In addition to the traditional IM, different extraction methods are important parameters for beverage composition.

Plant-based antioxidants, derived from various fruits, vegetables, herbs, and medicinal plants, play a crucial role in protecting against oxidative stress due to their high phenolic, flavonoid, and carotenoid content. The extraction of these bioactive compounds requires effective techniques to maximize yield and preserve their functional properties. Conventional extraction methods, such as maceration, Soxhlet extraction, and hydrodistillation, rely on solvent-based processes where the choice of solvent—ranging from polar (water, methanol, acetone) to non-polar—significantly influences the extraction efficiency. However, these methods often suffer from drawbacks such as long processing times, high solvent consumption, and thermal degradation of sensitive compounds. To overcome these limitations, non-conventional extraction techniques, including UAE, microwave-assisted extraction (MAE), enzyme-assisted extraction (EAE), and supercritical fluid extraction (SFE), have been introduced. These modern methods offer enhanced selectivity, improved extraction yields, and reduced environmental impact by minimizing solvent use and energy consumption. The selection of an appropriate extraction technique depends on factors such as compound polarity, solvent affinity, economic feasibility, and sustainability, ensuring the optimal recovery of plant-based antioxidants for applications in food and pharmaceutical industries (Harun et al., 2021; Kumar et al., 2021; Abeyrathne et al., 2022).

In the present study, we conducted a comparative analysis of UAE and IM. UAE is an innovative technique that utilizes high-frequency sound waves to optimize the extraction of bioactive compounds from plant matrices. The enhanced extraction efficiency observed with ultrasound is primarily due to the phenomenon of acoustic cavitation, which occurs when ultrasonic waves induce the formation, expansion, and subsequent collapse of gas bubbles within the solvent. This process generates high pressure and temperature at the surface of the plant material, producing shock waves that facilitate the extraction of compounds. Furthermore, ultrasound promotes the infiltration of the solvent into the sample matrix, thereby increasing the interaction between the solid and liquid phases. Collectively, these effects contribute to improved mass transfer and significant disruption of cellular structures during the extraction process (Ramić et al., 2015). The UAE has been shown to increase the efficiency and yield of extraction compared to conventional methods, leading to higher concentrations of antioxidants and other bioactive compounds in the final product. However, UAE has some drawbacks, such as the potential degradation of heat-sensitive compounds and changes in the sensory properties of the beverage (Kumar et al., 2021; Ramić et al., 2015). Overall, UAE could be used to improve the extraction rate of bioactive compounds, but further research is needed to optimize the process and minimize its negative effects.

In this study, variations were made in the extraction conditions of traditionally consumed butterfly pea flower beverage, which is known for its bioactive components. IM and UAE methods were also compared in beverage samples with extraction times as 3 and 7 minutes. The same variables were taken into consideration and extraction processes were compared in the samples to which purple basil was added. The pH, total soluble solid content (brix) and color values (L^*, a^*, b^*, C^* and h^o) determined in all samples. Also, TAC (DPPH and FRAP methods), TPC, TMA and their *in vitro* bioaccessibility were evaluated. Sensory analyses were also performed on the beverages.

Thanks to the findings obtained in this study, the interactions between different extraction processes, plant combinations and more functional alternatives for herbal beverages, which are widely consumed, will be revealed.

Material and Methods

Material

Dried butterfly pea flower and purple basil leaves were purchased from a local herbalist in Bursa, Türkiye. Apple juice concentrate used as a sweetener was purchased from a local market in Bursa, Türkiye.

Methods

In the study, two different extraction methods (IM and UAE), two different extraction times (3 min and 7 min), and two different beverage types (butterfly pea flower beverage and butterfly pea flower beverage containing purple basil) were investigated. Potable water at 90±5 ºC was used for extraction. For 1 L beverage production 10 g of butterfly pea flower leaves was used. In the IM method, butterfly pea flower petals were infused for 3 min and 7 min and then filtered through a paper filter. Apple juice concentrate was used as a sweetener and added at 10% w/v after the beverage had cooled.

In the UAE method, potable water at 90±5 ºC containing butterfly pea flower (1%, w/v) was held in an ultrasonic bath [Bandelin (Germany) 140/560 W, 35 kHz] at 50% power setting for 3 min and 7 min, respectively. After the time had elapsed, the straining process was carried out through a paper filter. To sweeten the samples, apple juice concentrate (10% w/v) was added after the beverage had cooled.

For the samples containing purple basil, the same processes were followed with both butterfly pea flower (1%, w/v) and purple basil (0.35%, w/v) leaves. Apple juice concentrate (10% w/v) was added to sweeten the strained samples after the beverage had cooled.

pH and Brix

The assessment of brix and pH values was conducted utilizing a refractometer (RA-500 KEM) and a pH meter (Sartorius, Germany). Analyses were performed with 3 replications.

TPC and TAC Analyses

The assessment of TAC was performed using both the DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) and FRAP (Ferric Reducing Antioxidant Power) assays. In the DPPH assay, a 0.1 mL aliquot of the sample extract was combined with 2 mL of DPPH solution and mixed thoroughly using a Vortex Mixer Classic (Velp Scientifica, Italy) for 30 seconds. The mixture was then incubated in a dark environment at room temperature for 30 minutes. The absorbance of the DPPH solution was measured at 517 nm across a range of trolox concentrations (10-100 µmol/L), which facilitated the construction of a trolox calibration curve exhibiting a high coefficient of determination (R^2 = 0.9972). The results were subsequently expressed in terms of micromoles of trolox equivalents (TE) per gram of dry matter, in accordance with the methodology described by Kumaran and Karunakaran (2006). In the FRAP assay, the sample extract was combined with a FRAP reagent and allowed to incubate before measuring absorbance at 593 nm. This measurement provided an assessment of antioxidant capacity expressed as micromoles of trolox equivalents (TE) per gram of dry matter, with a high coefficient of determination (R^2 = 0.9992), as outlined by Benzie and Strain (1996). TPC and TAC of the beverage samples were evaluated utilizing a UV-visible spectrophotometer (Shimadzu UV-1800, Japan). The determination of TPC was conducted using the Folin-Ciocalteu method. In this procedure, 100 µL of the beverage sample was mixed with 0.75 mL of Folin-Ciocalteu reagent and incubated for a duration of 5 minutes. Following this incubation, 0.75 mL of a 6% sodium carbonate solution was added to the mixture. The test tubes were subsequently maintained at room temperature for 90 minutes, after which the TPC was quantified as milligrams of gallic acid equivalents (GAE) per 100 grams of dry matter, following the methodology established by Velioglu et al. (1998). Also, analyses were performed with 3 replications.

Color Analysis

The chromatic characteristics of beverage samples were assessed utilizing a CR-5 Konica Minolta Chroma Meter manufactured in Osaka, Japan. This analysis was performed in triplicate, with measurements for *L*, a*, b*, C*,* and *h°* being documented. The *L** parameter denotes the lightness or darkness of the sample, while the *a** parameter reflects the balance between greenness and redness. The *b** parameter indicates the spectrum of blueness to yellowness. Furthermore, *C** and *h°* correspond to color intensity and hue angle, respectively, with the hue angle spanning from 0° to 360°. Analyses were performed with 3 replications.

TMA Analysis

The quantification of TMA was conducted utilizing the pH differential method. In this procedure, 0.1 mL of the sample was mixed with 0.025 M potassium chloride solution (pH 1) in one test tube, while in a separate test tube, it was combined with 4.5 M sodium acetate (pH 4.5). Absorbance measurements were recorded at wavelengths of 512 nm and 700 nm, and the data were subsequently analyzed according to Equation 1. Analyses were performed with 3 replications.

$$TMA\ (mg/kg) = A.\ MW.\ DF.(1000/€.l) \quad (1)$$

$A = (A\lambda_{512}\text{-}\lambda_{700})pH_{1.0} - (A\lambda_{512}\text{-}\lambda_{700})pH_{4.5}$

MW = Dominant anthocyanin in the sample molecular weight (for cyanindin-3-glucoside: 449.2 g/mol)

DF = Dilution factor

€ = Absorbance coefficient (for cyanindin-3-glucoside: 26.900)

L = Layer thickness of spectrophotometer cuvettes (cm) (Lee et al., 2005)

In vitro Gastrointestinal Digestion

The beverage samples were subjected to in vitro gastrointestinal digestion in accordance with the methodology established by Minekus et al. (2014), with certain modifications. This digestion process emulated both gastric and intestinal phases. During the gastric phase, a mixture was

prepared consisting of simulated gastric fluid, porcine pepsin solution, and calcium chloride. The pH of this solution was adjusted to 3 through the addition of hydrochloric acid, and the mixture was subsequently incubated at 37 °C for a duration of 2 hours in a shaking water bath. Aliquots of 4 mL were extracted from each test tube at this stage. Following the gastric phase, the resulting residue was combined with simulated intestinal fluid, pancreatin solution, and bile solution for the intestinal phase. The pH was then adjusted to 7.0 using sodium hydroxide, and this mixture was also incubated at 37 °C for an additional 2 hours in the shaking water bath. After incubation, the mixture underwent centrifugation and filtration, and the supernatant was collected. The samples derived from both gastric and intestinal digestion were stored at -20 °C until further analysis. Subsequently, these extracts were evaluated for their TAC, TPC and TMA values.

Sensory Analysis

Seven sensory trained panelists from Bursa Uludağ University, Department of Food Engineering participated in the sensory evaluation. Three-digit numbers were used to code each sample. A 5-point scale was applied. Panelists evaluated the color, clarity, odor, taste and overall acceptability of the beverage.

Statistical Analysis

Statistical analyses were conducted utilizing IBM SPSS Statistics version 23. To ascertain significant differences among means at a significance threshold of 5%, both Analysis of Variance (ANOVA) and Duncan's multiple range test were applied. Each assessment was performed in triplicate, and the findings are presented as mean values along with their corresponding standard deviations.

Results and Discussion

pH and Total Soluble Solid Content (Brix) Values

The brix values of the butterfly pea flower beverages developed in our study varied between 6.20 and 7.00 depending on the formulation and extraction conditions among the samples (Table 1). The samples with the highest brix values were B7IM, P7IM and P7UAE with 7.00. Similarly, Sofiah et al. (2021) reported that the brix value of butterfly pea flower beverages extracted with IM using water ranged between 5.5-7.2 and the extraction of dissolved components increased with brewing time.

In the food industry, brix value is critical for developing an effective quality control correlation, especially during production. Brix values of beverage can affect the acceptance of the product by influencing the sugar content or sweetness (Shaik et al., 2023).

In our study, pH values of 8 different beverage samples were measured as between 5.10 and 5.69 (Table 1). The highest pH value was found in the B7IM while the lowest pH value was found in the P7UAE. When the IM and UAE methods were compared, the pH values of the same sample extracted with IM were found to be higher. This indicates that the extraction method causes variations in the pH values of the beverage. Moreover, purple basil added butterfly pea flower beverage samples had lower pH values than butterfly pea flower beverage samples.

pH values of butterfly pea flower beverage are a very important criterion in terms of color. At different pH values, the characteristic blue color can be replaced by a red-purple or green color. In a study investigating the relationship between the color of butterfly pea flower extract and pH; magenta color was observed between pH 0.5-3, purple color between pH 3-4, blue color between pH 5-8, and yellow color between pH 8-13 (Fu et al., 2021). In parallel with this study, the pH values in our study were between 5.10-5.69 and the color observed in the beverage samples was blue. Since the color of the beverage is affected by the pH value, the storage conditions of the beverage also require attention to avoid pH changes (Song et al., 2018).

TPC

The TPC values of undigested beverage samples varied between 9.30 mg/100 g GAE and 70.78 mg/100 g GAE (Table 2). The highest TPC value was obtained from P3IM.There was no statistically significant difference between the TPC values of B3IM, B7IM and B7UAE samples ($p > 0.05$) and these samples had lower TPC results than the other samples. In a study in which butterfly pea flower extraction was carried out by ultrasound (temperature 50 °C, time 150 min, solid to liquid ratio 1 g:15 mL, 70% amplitude and 240 W, 20 kHz frequency, 3 s on and 3 s off) and shaking infusion (water bath for 150 min, 50 °C at 150 rpm) methods, the TPC values of the samples were 87 mg of gallic acid/g of dm and 72 mg of gallic acid/g of dm, respectively (Mehmood et al., 2019). Also, Soedirga et al. (2023) extracted butterfly pea flower at 60 ºC for 15, 30 and 45 minutes. TPC values were determined between 123.29 and 138.42 mg GAE/g dm. The differences between the TPC values in our study and those in the literature may be closely related to the extraction time. In addition, the type of plant used, harvest time, origin, etc. can also affect the amount of TPC (Lakshan et al., 2020).

Table 1. pH values of samples

Sample*	pH	Brix
B3IM	5.62±0.01^{b}	6.20±0.01^{e}
B3UAE	5.48±0.00^{d}	6.60±0.00^{d}
B7IM	**5.69±0.00^{a}****	**7.00±0.00^{a}**
B7UAE	5.36±0.01^{e}	6.80±0.01^{c}
P3IM	5.49±0.01^{d}	6.90±0.01^{b}
P3UAE	5.33±0.01^{f}	6.80±0.00^{c}
P7IM	5.55±0.01^{c}	**7.00±0.01^{a}**
P7UAE	5.10±0.01^{g}	**7.00±0.01^{a}**

*B3IM: BP beverage, 3 minute IM; B3UAE: BP beverage, 3 minute UAE; B7IM: BP beverage, 7 minute IM; B7UAE: BP beverage, 7 minute UAE; P3IM: PB added BP beverage, 3 minute IM; P3UAE: PB added BP beverage, 3 minute UAE; P7IM: PB added BP beverage, 7 minute IM; P7UAE: PB added BP beverage, 7 minute UAE; ** The highest values in each column are shown in bold font.

Table 2. TPC values of samples

Sample*	Undigested (mg/100 g GAE)	In vitro gastric digested (mg/100 g GAE)	In vitro intestinal digested (mg/100 g GAE)
B3IM	14.38±0.35^{dC**}	286.83±1.71^{eB}	903.97±2.54^{bA}
B3UAE	10.33±0.00^{eC}	288.32±1.82^{eB}	884.73±0.51^{bA}
B7IM	10.72±0.06^{eC}	304.40±1.98^{dB}	855.12±0.89^{bA}
B7UAE	9.30±0.42^{eC}	327.01±1.70^{cB}	874.11±0.95^{bA}
P3IM	**70.78±1.00^{aC***}**	439.48±1.23^{bB}	**993.20±0.81^{aA}**
P3UAE	63.07±0.24^{bC}	**463.55±1.98^{aB}**	**1039.22±2.05^{aA}**
P7IM	**69.93±0.20^{aC}**	437.42±0.76^{bB}	**1021.50±0.69^{aA}**
P7UAE	60.25±0.45^{cC}	436.25±0.55^{bB}	**1004.74±2.07^{aA}**

*B3IM: BP beverage, 3 minute IM; B3UAE: BP beverage, 3 minute UAE; B7IM: BP beverage, 7 minute IM; B7UAE: BP beverage, 7 minute UAE; P3IM: PB added BP beverage, 3 minute IM; P3UAE: PB added BP beverage, 3 minute UAE; P7IM: PB added BP beverage, 7 minute IM; P7UAE: PB added BP beverage, 7 minute UAE; ** Values followed by different lowercase letters within the same column are significantly different ($p < 0.05$) Values followed by different capital letters within the same row are significantly different ($p < 0.05$); *** The highest values in each column are shown in bold font.

In purple basil added butterfly pea flower beverage samples, it was observed that the samples extracted by the IM had higher TPC values than the samples extracted by UAE method. In our study, the lower TPC values of the samples extracted with UAE compared to IM extraction can be explained by cavitation. Cavitation causes localized heating, leading to the destruction of the plant cell wall (Poyraz et al., 2021). This may damage the phenolic compounds in the UAE-treated samples and cause their quantitative decrease. When butterfly pea flower beverage and purple basil added beverages were compared, the latter had higher TPC values. This revealed that the addition of purple basil significantly increased the TPC values of butterfly pea flower beverage.

Purple basil is a significant source of polyphenolic compounds, including anthocyanins, cinnamic acid, caffeic acid, sinapic acid, and ferulic acid. Research done by Kwee & Niemeyer (2011) documented TPC in purple basil ranging from 3.47 to 17.58 mg of gallic acid equivalents per g of dry weight. For this reason, an increase in the TPC value of butterfly pea flower beverage enriched with purple basil, is expected.

When the effect of process time on TPC values in butterfly pea flower beverage samples without purple basil was examined, it was observed that the extraction time increased in the IM method, 25.45% decrease in TPC value was detected. The decrease in TPC values with increasing extraction time can be explained by the thermal degradation of phenolic compounds with low thermal resistance (Nollet & Gutierrez-Uribe, 2018). When the correlation between processing time and TPC values was evaluated in purple basil added samples, no statistically significant difference ($p > 0.05$) was detected in the IM method. In purple basil added UAE samples 4.47% decrease in the TPC value was observed as the extraction time increased.

When the undigested samples were compared with the samples after *in vitro* digestion, an increase in TPC values was observed in the samples after *in vitro* digestion. Especially the TPC values of *in vitro* intestinal digested samples were much higher than the others. The observed elevations in the *in vitro* gastric phase indicate that phenolic compounds continue to be extracted during gastric digestion, with the released compounds demonstrating stability in the stomach's acidic milieu. Prior studies have also noted an increase in TPC during intestinal digestion, suggesting that prolonged contact between food and intestinal fluids, as well as the action of intestinal enzymes, contribute to the release of phenolic compounds (Bouayed et al., 2011; Kamiloglu, 2019; Özdemirli & Beştepe, 2022).

After *in vitro* gastric digestion, the highest TPC value was detected in the P3UAE.The lowest TPC value was detected in B3IM and B3UAE and there was no statistically significant difference ($p > 0.05$) between them. When the TPC results of the samples after *in vitro* intestinal digestion were evaluated, the TPC values of all samples to which purple basil was added were found to be higher compared to butterfly pea flower beverage samples. The extraction time and extraction method of these samples did not cause a statistically significant difference in TPC values ($p > 0.05$).

TAC

The TAC values of the samples determined by the DPPH method varied between 2.57 and 55.93 µmol trolox/g dm in the undigested phase. The lowest TAC value was determined in the B3IM, while the highest TAC value was determined in the P3IM. DPPH values of butterfly pea flower beverage samples extracted by UAE method were found to be higher (Table 3).

Lakshan et al. (2020) performed DPPH analysis on different butterfly pea flower varieties. Authors reported DPPH results as between 15.66 and 47.82 µmol trolox/g dm (Lakshan et al., 2020). In another study, 3 g of powdered blue pea flowers were extracted in 1 L of distilled water at 59.6 °C for 37 minutes. At the end of the time, the result of DPPH analysis of the sample was reported as 45.98 (Lakshan et al., 2019). Tüfekçi et al. (2023) determined the TAC value determined by DPPH method in purple basil between 296.12 and 338.80 µmol trolox/g dm. Since purple basil has higher antioxidant capacity than butterfly pea flower, DPPH values of purple basil added were higher. Also, when the effect of extraction time on DPPH values was examined, no statistically significant difference was found between B3IM and B7IM ($p > 0.05$). Except these samples, DPPH value decreased due to the increase in extraction time. A similar result was obtained in the extraction of *Hypericum perforatum* L. (Seyrekoğlu & Temiz, 2020). In this study, ultrasonically assisted extraction was used for extraction and extraction processes were carried out at different temperatures (30-

70°C) and extraction times (30-70 min.). The highest DPPH values were detected in the sample extracted at 40 °C and it was reported that the increase in time decreased the DPPH value. Ghiasvand et al., (2016), also reported that DPPH values were negatively affected by the increase in extraction time. This can be attributed to the antioxidant components that may be oxidized with the extraction time (Shojaee-Aliabadi et al., 2013).

In purple basil added samples, extraction method did not cause a statistical significant difference in the DPPH values ($p > 0.05$). The difference in herb variety caused significant variations in DPPH values of the samples after *in vitro* digestion. DPPH values of *in vitro* intestinal digested samples were higher in purple basil added beverage samples.

In P3IM and P3UAE samples with added purple basil extracted for 3 min, the DPPH values after *in vitro* intestinal digestion were lower than the DPPH values of the same samples after undigested and *in vitro* gastric digestion.

FRAP values of beverage samples were determined between 10.10 and 100.69 µmol trolox/g dm in pre-digested samples (Table 3). When TAC values were measured by two different methods, the results showed differences. The reason for these differences in DPPH and FRAP values may be due to the fact that only hydrophobic antioxidants can be detected by DPPH method and only hydrophilic antioxidants can be detected by FRAP method (Kamiloglu, 2019).

There was no statistically significant difference between the FRAP values of all butterfly pea flower beverage samples ($p > 0.05$). In addition, FRAP values of these samples were found to be lower compared to purple basil added samples. A similar result was revealed in a study by Abidoye et al. (2022). In this study, a functional beverage was prepared from dried roselle calyces and dried sweet basil leaves. It was found that the TAC values of the beverage increased with the addition of dried purple basil leaves. Lakshan et al. (2020) performed FRAP analysis on different butterfly pea flower varieties. FRAP values varied between 42.59-58.17 µmol trolox/g dm. Mehmood et al. (2019) extracted butterfly pea flower plant by ultrasonic and infusion method for 150 minutes in their study. FRAP results of the samples after extraction ranged between 16.76 and 23.17 µmol trolox/g dm.

The highest FRAP value was determined in the P3UAE and FRAP values decreased as the extraction time increased. Also, FRAP values of both samples (P3IM and P3UAE) with 3 minutes of extraction were higher compared to the samples extracted for 7 minutes (P7IM and P7UAE).

There was no statistically significant difference between the FRAP values of undigested butterfly pea flower samples ($p > 0.05$). In addition, FRAP values of the *in vitro* intestinal digested samples were found to be higher. The significant difference determined between *in vitro* gastric digestion and *in vitro* intestinal digestion in butterfly pea flower samples ($p < 0.05$). Addition of purple basil to butterfly pea flower beverage also increased FRAP values in samples after *in vitro* digestion. However, there was no significant difference between *in vitro* gastric digestion and *in vitro* intestinal digestion in purple basil added butterfly pea flower beverages ($p > 0.05$).

The increase in TAC values after *in vitro* digestion can be explained by the fact that new phenolic compounds with antioxidant properties may be formed after *in vitro* digestion. In addition, the antioxidant capacity of new phenolic compounds formed after *in vitro* digestion may not be as high as the antioxidant capacity of phenolic compounds present before digestion. This phenomenon may explain the decrease in TAC values after *in vitro* digestion (Rodríguez-Roque et al., 2013).

Table 3. TAC values of samples

Sample*	Undigested (µmol trolox/g dm)	*In vitro* gastric digested (µmol trolox/g dm)	*In vitro* intestinal digested (µmol trolox/g dm)
	DPPH		
B3IM	$2.57\pm0.00^{eB**}$	11.59 ± 0.00^{eAB}	37.36 ± 0.65^{bcA}
B3UAE	8.75 ± 0.71^{eC}	10.56 ± 0.00^{eB}	18.00 ± 0.71^{dA}
B7IM	2.79 ± 0.52^{eC}	12.42 ± 0.61^{dB}	23.15 ± 0.97^{dA}
B7UAE	8.41 ± 0.01^{dC}	17.11 ± 0.66^{cB}	39.76 ± 0.25^{abcA}
P3IM	**$55.93\pm0.66^{aB***}$**	**79.04 ± 0.62^{aA}**	**$46.58\pm0,95^{aC}$**
P3UAE	**50.97 ± 0.23^{aB}**	**73.12 ± 0.89^{aA}**	44.02 ± 0.01^{abC}
P7IM	44.51 ± 0.47^{bA}	40.18 ± 0.50^{bB}	44.38 ± 0.79^{abAB}
P7UAE	$44.96\pm0,80^{bA}$	35.18 ± 0.10^{bB}	34.10 ± 0.51^{cC}
	FRAP		
B3IM	10.10 ± 0.08^{dB}	9.04 ± 0.11^{eB}	27.19 ± 0.38^{cA}
B3UAE	11.54 ± 0.05^{dB}	10.35 ± 0.10^{eB}	23.36 ± 0.80^{dA}
B7IM	12.71 ± 0.33^{dB}	12.28 ± 0.28^{dB}	28.31 ± 0.49^{cA}
B7UAE	13.55 ± 0.63^{dB}	13.16 ± 0.25^{dB}	28.35 ± 0.23^{cA}
P3IM	74.95 ± 0.11^{bA}	43.21 ± 0.64^{abB}	41.65 ± 0.04^{abB}
P3UAE	**100.69 ± 0.8^{aA}**	**43.74 ± 0.11^{aB}**	39.69 ± 0.50^{abB}
P7IM	55.89 ± 0.60^{cA}	41.64 ± 0.24^{bB}	**42.00 ± 0.04^{aB}**
P7UAE	49.09 ± 0.11^{cA}	37.91 ± 0.39^{cB}	39.09 ± 0.10^{bB}

*B3IM: BP beverage, 3 minute IM; B3UAE: BP beverage, 3 minute UAE; B7IM: BP beverage, 7 minute IM; B7UAE: BP beverage, 7 minute UAE; P3IM: PB added BP beverage, 3 minute IM; P3UAE: PB added BP beverage, 3 minute UAE; P7IM: PB added BP beverage, 7 minute IM; P7UAE: PB added BP beverage, 7 minute UAE; ** Values followed by different lowercase letters within the same column are significantly different ($p < 0.05$) Values followed by different capital letters within the same row are significantly different ($p < 0.05$); *** The highest values in each column are shown in bold font.

TMA

The TMA values of undigested samples were determined to between 7.29 and 27.22 mg/kg (Table 4). Gomez et al. (2022) applied five different techniques [aqueous (distilled water) extraction; acidified aqueous (1% citric acid) extraction; solvent extraction (50% ethanol); acidified solvent extraction (50% ethanol with 1% citric acid) and microwave assisted extraction with aqueous solvent] for butterfly pea flower extraction. The aqueous extraction method showed the highest efficiency, resulting in a higher TMA concentration of 7925.29 mg/L. In another research, the level of anthocyanin compounds present in butterfly pea flower plants was quantified as 2.88 mg/g following a 2-hour extraction process at 60 ºC (Tuan Putra et al., 2021). Comparable results were observed in a study conducted by Jaafar et al. (2020), where TMA value of freeze-dried *Clitorea ternatea* flower was measured as 2.86 mg/g. Furtherrmore, Siti Azima et al. (2014) documented a TMA content of 2.98 mg/g in the *Clitoria ternatea* extract. Similarly, Al-Snafi (2016) reported TMA content of water-extracted *C. ternatea* flowers as 1.46 mg/g. There are various factors that may impact the levels of anthocyanins extracted during the steeping process, including variables such as temperature and duration. The extraction conditions were found to have a substantial impact on the overall levels of monomeric anthocyanins. For example, Soedirga et al. (2023) reported that the total amount of TMA in the butterfly pea flower flower petals extract increased with increasing temperature.

Also, studies have demonstrated that a lower pH is optimal for extracting anthocyanins (Mauludifia et al., 2019). This suggests that a decrease in pH enhances the extraction process by converting anthocyanins into the more stable flavium cation form, which may promote the breaking of vacuole cell walls and increase pigment solubility, leading to a higher concentration of extracted anthocyanins. However, it has also been suggested that the inhibition of enzymatic oxidation of phenolics and the preservation of the stability of extracted anthocyanins at low pH could be additional contributing factors (Jeyaraj et al., 2021; Ruenroengklin et al., 2008). Similarly, in our study, the highest TMA value was detected in sample P7UAE, which was found to have the lowest pH value. This situation reveals that in addition to the interaction of color characteristics with pH, anthocyanin content are also closely related to pH values.

In general, the addition of purple basil increased the TMA values of the undigested samples. Gülhan et al. (2022) determined the TMA values of dried purple basil. The results varied between 1.74 mg/g and 10.91 mg/g. According to the literature, purple basil is reported to have a higher anthocyanin content than butterfly pea flower, for this reason, it is expected that TMA values of purple basil added beverages increased.

Purple basil extraction was performed by different methods (classical solvent extraction, ultrasound assisted extraction and microwave assisted extraction) and optimization was performed (Kocamaz Özcan, 2018). In the study, 30, 45 and 60 ºC temperature, extraction times of 10, 20 and 30 minutes were determined as variables. In this study, the total anthocyanin amounts of the samples varied between 172.83-379.58 mg Cy-3-glu/kg as a result of ultrasound assisted extraction. When the classical solvent method was compared with the ultrasound-assisted extraction method, it was determined that ultrasound was a more effective extraction method on the total anthocyanin amount. Similar results were also observed in our study by comparing B3IM (7.29 mg/kg) and B3UAE (10.74 mg/kg) samples and P7IM (25.38 mg/kg) and P7UAE (27.22 mg/kg) samples among themselves. In both cases, higher results in terms of total monomeric anthocyanins were obtained in samples with ultrasound-assisted extraction. Similar results were also reported by Wang et al. (2023) and Bonfigli et al. (2017) that ultrasound assisted extraction increased total monomeric anthocyanin values.

Extraction time had different effects on TMA values. When undigested samples of B3IM and B7IM were compared, there was 110.01% increase in TMA values. However, there was no statistically significant difference between the TMA values of undigested samples of B3UAE and B7UAE ($p > 0.05$). Similar to our study, Soedirga et al. (2023) extracted butterfly pea flower plants in water at 60 ºC for 15, 30 and 45 minutes. They found an increase in TMA values depending on the increase in time. TMA values were higher in purple basil added samples after *in vitro* intestinal digestion. The TMA values of the *in vitro* intestinal digested samples extracted with UAE were higher in purple basil added samples. However, this situation was different in *in vitro* intestinal digested butterfly pea flower beverages. In the extraction process applied for 3 minutes, a higher TMA value was obtained with the UAE process, while the IM method showed better performance in the samples after 7 minutes of treatment.

TMA values of all samples increased after *in vitro* intestinal digestion. The highest TMA value after *in vitro* intestinal digestion was obtained from P7UAE. This sample was followed by P3UAE.

The structural characteristics of the anthocyanidin backbone, the presence or absence of glycosylation and hydroxylation, and the number, type, and acylation of attached sugar molecules are closely tied to specific plant species and result in distinct patterns of anthocyanins. Therefore, the type of plant and its composition play a crucial role in determining the quantity and accessibility, and consequently the bioavailability, of anthocyanins. Additionally, factors such as food pH and temperature can also impact the bioavailability of anthocyanins (Cavalcanti et al., 2011). Furthermore, the presence of other compounds formed or added during food processing that can interact with and stabilize anthocyanins may alter their bioavailability. Food processing, including household cooking methods, is a key factor in determining the stability of anthocyanins and their interaction with the food matrix (Eker et al., 2019). Various research studies have investigated the impact of both thermal and nonthermal processing conditions on the levels of anthocyanins, as well as their bioaccessibility and bioavailability. Generally, thermal processing leads to a reduction in anthocyanin content, but an increase in their bioaccessibility and bioavailability. This is due to the fact that thermal treatments disrupt cell walls, releasing the contents within the cells and enhancing the absorption of anthocyanins (Celli & Brooks, 2017).

Table 4. TMA values of samples

Sample*	Undigested	In vitro gastric digested	In vitro intestinal digested
B3IM	7.29±0.24^{fB**}	1.75±0.00^{eC}	77.71±0.55^{eA}
B3UAE	10.74±0.37^{eB}	0.72±0.53^{dC}	79.65±0.76^{dA}
B7IM	15.31±0.40^{dB}	2.12±0.14^{bC}	81.55±0.91^{cA}
B7UAE	11.58±0.10^{eB}	1.73±0.38^{cC}	75.26±0.82^{fA}
P3IM	25.22±0.38^{bB}	**4.79±0.22^{aC}**	81.10±0.46^{cA}
P3UAE	21.93±0.22^{cB}	**4.73±0.47^{aC}**	82.77±0.34^{bA}
P7IM	25.38±0.29^{bB}	**3.79±0.45^{aC}**	81.60±0.31^{cA}
P7UAE	**27.22±0.32^{aB}**	**4.45±0.47^{aC}**	**84.66±0.74^{aA}**

*B3IM: BP beverage, 3 minute IM; B3UAE: BP beverage, 3 minute UAE; B7IM: BP beverage, 7 minute IM; B7UAE: BP beverage, 7 minute UAE; P3IM: PB added BP beverage, 3 minute IM; P3UAE: PB added BP beverage, 3 minute UAE; P7IM: PB added BP beverage, 7 minute IM; P7UAE: PB added BP beverage, 7 minute UAE; ** Values followed by different lowercase letters within the same column are significantly different ($p < 0.05$)
*** The highest values in each column are shown in bold font.

Table 5. Color values of samples

Sample*	*L**	*a**	*b**	*C**	*h°*
B3IM	**68.10±0.01^{a**}**	-18.49±0.07^{h}	-28.97±0.05^{d}	34.38±0.01^{d}	237.55±0.01^{h}
B3UAE	66.51±0.01^{b}	-15.46±0.01^{g}	-32.7±0.00^{f}	36.21±0.01^{c}	244.72±0.02^{e}
B7IM	51.33±0.01^{f}	-11.75±0.01^{e}	-41.58±0.01^{h}	**43.20±0.01^{a}**	254.22±0.02^{c}
B7UAE	56.54±0.01^{d}	-11.17±0.01^{d}	-40.51±0.01^{g}	42.02±0.00^{b}	254.58±0.02^{b}
P3IM	55.90±0.01^{e}	-11.87±0.01^{f}	-18.94±0.01^{b}	22.36±0.02^{g}	237.91±0.00^{g}
P3UAE	63.24±0.00^{c}	-9.73±0.01^{c}	**-16.53±0.00^{a}**	19.18±0.00^{h}	239.50±0.01^{f}
P7IM	45.72±0.00^{g}	-8.80±0.01^{b}	-25.31±0.00^{c}	26.80±0.00^{f}	250.81±0.03^{d}
P7UAE	44.50±0.01^{h}	**-6.23±0.02^{a}**	-31.41±0.02^{e}	32.03±0.01^{e}	**258.78±0.04^{a}**

*B3IM: BP beverage, 3 minute IM; B3UAE: BP beverage, 3 minute UAE; B7IM: BP beverage, 7 minute IM; B7UAE: BP beverage, 7 minute UAE; P3IM: PB added BP beverage, 3 minute IM; P3UAE: PB added BP beverage, 3 minute UAE; P7IM: PB added BP beverage, 7 minute IM; P7UAE: PB added BP beverage, 7 minute UAE; ** The highest values in each column are shown in bold font.

Color Analysis

Color plays a significant role in determining the primary visual characteristics of beverages (Vázquez-Cabral et al., 2014). The color parameters of beverage samples are outlined in Table 5, showing a range of values for *L**, *a**, *b**, Chroma (*C**), and hue angle (*h*°).

Specifically, *L** values ranged from 44.50±0.01 to 68.10±0.01, *a** value varied between -6.23±0.02 and -18.49±0.07, *b** value were between -16.53±0.00 and -41.58±0.01, *C** value measured between 19.18±0.00 and 43.20±0.01, and *h*° value ranged from 237.55±0.01 to 258.78±0.04.

The *L** value characterizes the brightness of beverage samples. In butterfly pea flower beverages (B3IM and B3UAE) the *L** value was higher than the other samples. When the extraction time was increased to 7 minutes in these samples (B7IM and B7UAE), *L** values decreased by 24.62% and 14.99%, respectively.

The *a** value characterizes the greenness/redness property and when this value is less than 0, it means that the sample is green and when it is greater than 0, it means that the sample is red. In our study, all samples had *a** values less than 0. This indicates the tendency of the samples toward green rather than red. However, especially the *a** values of P3UAE, P7IM and P7UAE samples are closer to 0 compared to other samples. This shows that the red/purple color, which is the characteristic color of purple basil, affects the sample color.

The *b** value represents the blue color, which is the characteristic color of butterfly pea flower beverage. *b** value less than 0 reveals the blueness of the sample. In particular, the *b** values of samples B7IM and B7UAE revealed the highest blueness. Although this is an expected result, it shows that the extraction time of 7 minutes is more effective in the emergence of the characteristic blue color in butterfly pea flower beverage.

In a similar study (Gomez et al., 2022), color analysis was performed on butterfly pea flower extract. As a result of this study, *L**, *a**, *b**, *C** and *h*° values of the extracts were determined in the range of 18.09 to 49.24; 36.78 to 46.90; -38.02 to -63.25; 52.90 to 78.74; and 306.56 to 319.44, respectively. Also, in another study (Hariadi et al., 2018), the color values of butterfly pea flower extracts were *L**: 51.72, *a**: 23.50 and *b**: 8.42 were found.

Sensory Analysis

When the sensory analysis results of the beverage samples were evaluated (Figure 1), B3IM and P3UAE were the most appreciated samples in terms of color (Table 6). In contrast, the lowest-rated samples were the purple basil-added samples extracted for 7 minutes (P7IM and P7UAE), indicating that prolonged extraction time negatively affected color perception. A similar trend was observed in beverage clarity, where B3IM and P3UAE received the highest scores. Studies by Barani et al. (2022) also reported that extended extraction times could lead to the degradation of anthocyanins and phenolic compounds, causing a decline in visual appeal.

Regarding odor, B3UAE was found to be the most favorable, whereas B7UAE received the lowest score. This suggests that extraction time causes notable sensory differences not only in color but also in odor perception.

In the taste evaluation, B3IM, P3IM, P7IM, and P7UAE received the highest scores, with no statistically significant difference between them ($p>0.05$). Interestingly, despite their lower color and clarity scores, P7IM and P7UAE were well-received in taste. This suggests that although color influences the initial perception of a beverage, taste plays a more critical role in consumer preference (Spence et al., 2010).

Table 6. Sensory analysis results of samples

Sample*	Color	Clarity	Odor	Taste	General acceptibility
B3IM	**4.85±0.37^{a**}**	**4.85±0.37^{a}**	3.42±0.97^{ab}	**3.71±0.05^{a}**	3.71±0.05^{ab}
B3UAE	4.14±0.69^{ab}	4.14±0.69^{ab}	**4.42±0.53^{a}**	3.00±0.01^{ab}	3.57±0.07^{b}
B7IM	4.00±0.81^{ab}	4.14±0.69^{ab}	3.42±0.27^{ab}	3.28±0.11^{ab}	3.57±0.07^{b}
B7UAE	4.14±0.69^{ab}	4.14±0.69^{ab}	3.14±0.46^{b}	2.85±0.06^{b}	3.57±0.07^{b}
P3IM	4.00±0.57^{ab}	4.00±0.57^{b}	4.14±0.69^{ab}	**3.71±0.01^{a}**	**4.14±0.08^{a}**
P3UAE	**4.85±0.37^{a}**	**4.85±0.37^{a}**	3.71±0.11^{ab}	3.14±0.21^{ab}	3.71±0.07^{ab}
P7IM	3.57±0.97^{b}	3.57±0.97^{b}	3.85±0.37^{ab}	**3.71±0.07^{a}**	3.71±0.07^{ab}
P7UAE	3.57±0.97^{b}	3.71±0.95^{b}	3.85±0.06^{ab}	**3.71±0.01^{a}**	**4.00±0.01^{a}**

*B3IM: BP beverage, 3 minute IM; B3UAE: BP beverage, 3 minute UAE; B7IM: BP beverage, 7 minute IM; B7UAE: BP beverage, 7 minute UAE; P3IM: PB added BP beverage, 3 minute IM; P3UAE: PB added BP beverage, 3 minute UAE; P7IM: PB added BP beverage, 7 minute IM; P7UAE: PB added BP beverage, 7 minute UAE; ** The highest values in each column are shown in bold font.



Figure 1. Beverage samples
(Sample codes in order from left to right: B7IM, B7UAE, P7IM, P7UAE, B3IM, B3UAE, P3IM, P3UAE)

The highest scores in the general acceptability parameter were obtained by P3IM and P7UAE, both of which also scored high in taste evaluation.

The results of this study confirm that extraction parameters significantly influence sensory characteristics, and optimizing these conditions is crucial for achieving the best consumer acceptance. Further comparisons with previous studies suggest that adjusting extraction time and method can enhance the sensory attributes of functional beverages while maintaining their bioactive potential.

Conclusion

In this investigation, butterfly pea flower beverage and purple basil added butterfly pea flower beverage were produced by using IM and UAE for two different duration (3 and 7 minutes). The addition of purple basil to butterfly pea flower plant led to an increase in TPC, TAC, and TMA values in all beverage samples. The TPC values of the beverages ranged between 9.30 mg/100 g GAE and 70.78 mg/100 g GAE in the undigested phase and showed a general increasing trend after *in vitro* digestion. The TAC values of the samples were evaluated using DPPH and FRAP methods. When the DPPH values of butterfly pea flower beverages (B3IM and B3UAE) treated with IM and UAE for 3 minutes were compared, it was determined that the DPPH value of the butterfly pea flower beverage produced with UAE was 240.46% higher than the beverage produced with IM. Similarly, in butterfly pea flower beverages (B7IM and B7UAE) extracted for 7 minutes, the DPPH value of the B7UAE sample was found to be 201.43% higher than the B7IM sample. This shows that ultrasound-assisted extraction increases the DPPH value of butterfly pea flower beverage. This result is in line with the results showing the superiority of ultrasound in studies comparing different extraction methods (Caldas et al., 2018; Kocamaz Özcan, 2018). While TAC values fluctuated after digestion, TMA levels peaked after in vitro intestinal digestion. The L^* value, which expresses the brightness of the beverages, varied between 68.10 and 44.50 in the samples, and when the $h°$ values were evaluated, it was determined that the values ranging between 258.27 and 237.55 revealed the typical blue color of the butterfly pea flower plant. According to the sensory analysis results, P3IM and P7UAE were the most appreciated samples and it was observed that the addition of purple basil also improved the sensory characteristics of the beverage. This study showed that the addition of purple basil was effective in improving the functional and sensory properties of butterfly pea flower beverage.

In addition, further optimization studies are required to determine the ideal formulation that maximizes the retention and bioaccessibility of bioactive compounds. By refining extraction parameters such as time, temperature, and solvent ratios, future research can establish an optimized recipe rich in bioactive components.

Furthermore, this study demonstrates that process modifications significantly impact the bioactive composition of the beverage, underscoring the importance of extraction conditions. Future research should focus on developing predictive models to optimize these conditions systematically. By addressing these aspects, upcoming studies will contribute to the development of a scientifically validated, functionally enriched, and consumer-preferred plant-based beverage.

Declarations

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.

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