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Edible blue flowers (butterfly pea): Extraction methods and application in flavored sweet water spheres by basic spherification technique

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ABSTRACT

This study investigates the extraction and application of butterfly pea (*Clitoria ternatea* Linn.) flowers in developing flavored sweet water spheres using basic spherification. Hot and cold extraction methods were compared, with cold extraction yielding a brighter blue extract containing higher phenolic content (43.05 mg GAE) and stronger antioxidant activity. This optimized extract was used to create edible spheres with sodium alginate and calcium chloride. Four formulations were prepared with 0–5 g sugar per 100 g extract to represent sweetness levels of 0–100 %, and were evaluated by 110 panelists. The 100 % sweetness formulation received the highest sensory scores across all attributes. Physicochemical analyses showed the spheres had desirable elasticity and bursting texture while retaining significant antioxidant activity (FRAP assay). The spheres maintained acceptable appearance and structure for up to 7 days at 5 °C. These results demonstrate the potential of butterfly pea extract in molecular gastronomy, providing both visual appeal and antioxidant properties in innovative food products. However, further research is needed to assess long-term stability, bioavailability, and potential health benefits of the developed spheres.

1. Introduction

Butterfly pea (*Clitoria ternatea* Linn.) is a Southeast Asian flower widely recognized for its vibrant blue petals, attributed to its rich anthocyanin content. Traditionally used in regional cuisine and herbal remedies, the flower has gained scientific interest for its health-promoting properties, particularly its antioxidant potential linked to high levels of phenolics, flavonoids, and anthocyanins (Sakdiah et al., 2022; Junpatiw et al., 2017). These water-soluble pigments not only impart a distinctive blue color but also contribute to health-related functions such as anti-inflammatory and anti-diabetic effects. However, the yield and stability of anthocyanins during extraction are highly influenced by the method used. While advanced techniques—such as solvent extraction, ultrasonic-assisted extraction (UAE), microwave-assisted extraction (MAE), and enzyme-assisted extraction (EAE)—have demonstrated high efficiency in recovering anthocyanins from plant materials (Chandrasekhar et al., 2012), they often require specialized equipment and are less accessible for small-scale or food-grade applications.

In contrast, water-based extractions using hot or cold temperatures are commonly adopted in food and herbal contexts due to their simplicity, low cost, and suitability for clean-label formulations (Vidana Gamage et al., 2021; Voss et al., 2020). Hot water extraction generally yields more compounds but may degrade heat-sensitive constituents like anthocyanins, whereas cold extraction better preserves these compounds, albeit with lower overall recovery. Given the thermal sensitivity

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of anthocyanins, this study specifically tested whether cold extraction could retain higher levels of phenolics and antioxidant activity for use in innovative spherified food systems. These two thermally contrasting methods were selected to evaluate their effectiveness in preserving bioactive and visual qualities of butterfly pea flower extract for food-grade application. Although several studies have investigated anthocyanin extraction from butterfly pea flowers, their use in spherified edible formats—particularly those retaining functional and aesthetic qualities—remains insufficiently addressed.

Molecular gastronomy techniques like spherification offer valuable opportunities to enhance both the visual appeal and nutritional value of culinary products. Spherification transforms flavored liquids into gel-like spheres through ionic interactions between sodium alginate and calcium ions (Gaikwad et al., 2019). Beyond its aesthetic appeal, this technique allows encapsulation of functional ingredients, improving sensory qualities and stabilizing bioactive compounds. Incorporating butterfly pea extract into spherified products provides multiple benefits: a natural blue color, antioxidant properties, and pH-sensitive anthocyanins that change hue with acidity—creating visually interactive, health-focused food components. Additionally, the basic spherification method is cost-effective and easily adaptable for other natural functional colorants, making it a versatile tool for chefs and researchers.

This study connects the development of natural, anthocyanin-rich extracts with modern culinary techniques by integrating butterfly pea flower extract into a spherified edible system. It provides novel insight into the behavior and stability of plant-derived functional compounds within a gel matrix, contributing to the advancement of visually engaging and nutritionally enriched food innovations.

Therefore, the objective of this study was to compare cold and hot extraction methods for butterfly pea flower and apply the selected extract in developing flavored sweet edible spheres through basic spherification, evaluating their physicochemical, sensory, and antioxidant properties.

2. Materials and methods

2.1. Materials

Fresh edible blue flowers of butterfly pea (*Clitoria ternatea* Linn.) were sourced from the Thailand Institute of Scientific and Technological Research (TISTR), Khlong Luang, Pathum Thani, Thailand. The double-petaled butterfly pea (*Clitoria* spp.), belonging to the family *Leguminosae–Papilionoideae*, was used in this study. The specific cultivar, known in Thai as *'Anchan Dok Son'*, is characterized by five uniformly arranged petals per flower, resulting in consistent yields and deep blue pigmentation with a high anthocyanin content (>70 mg per 100 g fresh petals). Flowers were harvested daily in the morning at full bloom, with a short harvesting cycle averaging six days.

Food-grade sodium alginate ($C_6H_7O_6Na$, M.W. ~85,000) and calcium chloride dihydrate ($CaCl_2{\cdot}2H_2O$) were purchased from Chemrich Intertrade Co., Ltd. (Bangkok, Thailand). Granulated white sugar was obtained from Mitr Phol Sugar Corp., Ltd. (Bangkok, Thailand), and 100 % coconut water was sourced from Malee Coco, Malee Group Public Co., Ltd. (Samut Sakhon, Thailand). All other chemicals and reagents used in the experiments were of analytical grade and purchased from standard commercial suppliers.

2.2. Sample preparation and extraction methods

Fresh, fully bloomed butterfly pea (*Clitoria ternatea*) flowers were cleaned by removing calyx and stamens, rinsed with tap and distilled water, and gently blotted dry. No drying or dehydration was applied. A total of 120 g of cleaned petals was used per extraction.

Cold Extraction (modified from Sakdiah et al., 2022): Petals were soaked in 200 g distilled water in opaque, sealed containers and refrigerated at 5 ± 1 °C for 7 h. This condition, modified from earlier studies, was chosen based on preliminary trials to preserve anthocyanin color while improving processing efficiency. Extracts were filtered through muslin and Whatman No.1 paper, packed in foil pouches, and stored at −18 °C.

Hot Extraction (adapted from Voss et al., 2020; Vidana Gamage et al., 2021):

Petals were boiled in 200 g of distilled water for 5 min in a covered pot, then filtered, cooled to room temperature, packed, and stored in the same manner.

These methods were selected to assess their effects on physical (color, pH, TSS), chemical (phenolics), and bioactive (FRAP, DPPH) properties. Each extraction was performed in triplicate using fresh materials to ensure reproducibility and minimize variability.

2.3. Analysis of physical, chemical, and bioactive properties of butterfly pea flower extracts

The extracts obtained from both cold and hot extraction methods were subjected to physical and chemical analyses, as well as evaluation of their bioactive compound content. Color parameters were measured using a colorimeter. The pH was measured using digital pH meter (Orion 2 Star benchtop, Thermo Scientific, U.S.A.). Total soluble solids (TSS) were analyzed according to the method of AOAC (2000) using a Hand-Held Refractometer 0–33°Brix (Master-M, ATAGO, Japan). The total phenolic content (TPC) was determined using the method described by Lu et al. (2007). Antioxidant capacity was assessed using two different assays: the Ferric Reducing Antioxidant Power (FRAP) assay, with results expressed as μmol Trolox equivalents (TE), was conducted according to the method of Benzie and Strain (1996), and the DPPH radical scavenging assay, with results expressed as mmol Trolox equivalents (TE), was performed following the method of Katsube et al. (2004).

2.4. Development of butterfly pea beads (spheres) using basic spherification technique in molecular gastronomy

Basic spherification transforms liquids into gel-like spheres surrounded by a thin membrane, offering novel textures while preserving flavor. In this study, butterfly pea extract—prepared as described in Section 1—was diluted (1:3 with water) and used as the base liquid. The mixture was divided into two components:

Part 1: Spheres Preparation, Butterfly pea extract (100 g) was mixed with sugar at 0 %, 1.25 %, 2.5 %, or 5 % (w/w), representing relative sweetness levels of 0 %–100 %. Sodium alginate (1.5 % w/w) was added as the gelling agent (see Table 1). The mixture was blended, defoamed, and rested at 5 °C for 1 h before being transferred to a squeeze bottle (6 mm nozzle).

Part 2: Calcium Bath, A 1.5 % calcium chloride solution was prepared as the setting bath. The mixture was dropped into the bath, forming spherical beads. To prevent clumping, gentle stirring was applied. Spheres were left in the bath for 3 min to allow membrane formation, following Gaikwad et al. (2019).

Table 1
Ingredients used in the preparation of butterfly pea beads (spheres).

Sweetness Level (%)	Content (g)		
	Butterfly Pea Extract	Sodium Alginate	Granulated Sugar
0	100	1.5	0
25	100	1.5	1.25
50	100	1.5	2.5
100	100	1.5	5.0

2.5. Sensory evaluation of butterfly pea beads (spheres)

To evaluate consumer acceptance, four spherified butterfly pea extract samples—prepared using hot or cold extraction with varying sugar levels—were assessed through sensory evaluation. A total of 110 untrained panelists (aged 18–50, both genders) from the university community participated, consistent with ISO 11136:2014 guidelines (International Organization for Standardization, 2014). Testing was conducted in a controlled sensory lab (25 ± 2 °C) under uniform white lighting, with panelists seated individually to avoid external influence. Verbal and written instructions were provided. Samples were served in Malee-brand coconut water and presented in randomized order using computer-generated permutations. Each sample was coded with a unique three-digit number to ensure blinding. Panelists evaluated six attributes: appearance, color, aroma, taste, texture (crispness/bursting), and overall liking, using a 9-point hedonic scale (1 = dislike extremely, 9 = like extremely). Water was provided for palate cleansing between samples. The sensory data offered insights into consumer preferences and helped assess the suitability of extraction methods for functional food applications.

2.6. Physical, chemical, and bioactive properties analysis of butterfly pea beads (spheres)

The highest-rated formulation based on sensory scores was selected for further analysis. Color parameters (L*, a*, b*) were measured using a HunterLab ColorFlex EZ colorimeter (reflectance mode, D65 illuminant, 8 mm aperture), calibrated with a standard white tile. Beads were placed in a quartz cup to ensure uniform thickness and avoid background interference. Bead diameter and texture were assessed following Gaikwad et al. (2019), using a TA. XT Plus Texture Analyzer under standard conditions for gel-like samples at 25 ± 2 °C. For each replicate, 20–25 beads were tested (n = 3).

Total phenolic content was determined using the Folin–Ciocalteu method (Lu et al., 2007), expressed as mg GAE/g. Antioxidant activity was evaluated by FRAP (Benzie and Strain, 1996) and DPPH (Katsube et al., 2004) assays. FRAP absorbance was read at 593 nm after 10 min incubation at 37 °C, with Trolox calibration (100–1000 μmol/L). DPPH absorbance was measured at 517 nm after 30 min in the dark, with Trolox calibration (50–500 μmol/L). All results are presented as mean ± SD from triplicate measurements.

2.7. Physical changes in butterfly pea beads during storage

The control (0 % sugar) and selected formulations of butterfly pea beads (spheres) were stored in glass bottles containing butterfly pea extract solution prepared using the same extraction method as the beads. The storage solutions matched the sugar concentrations of their respective formulations—0 % sugar for the control and the corresponding sugar level (e.g., 1.25 %, 2.5 %, or 5 %) for the selected samples. These solutions maintained the original extract concentration, with a pH of approximately 6 and no additional preservatives. The storage medium matched the hot or cold extraction method applied to the beads for consistency. Samples were kept at 5 °C for 7 days without replacement of the storage solution. Physical observations were performed regularly to monitor changes in bead appearance, including size, shrinkage, and color alterations, to assess physical stability and quality retention over time.

2.8. Experimental design and statistical analysis

The study consisted of two phases: (1) Extraction Phase – Hot and cold water extracts of butterfly pea flowers were prepared using a Completely Randomized Design (CRD, n = 3). Measured parameters included yield, pH, color (L*, a*, b*), and total anthocyanins. (2) Bead Development and Sensory Evaluation – Beads were formulated from each extract and evaluated in triplicate. Sensory evaluation followed a Randomized Complete Block Design (RCBD) with 110 untrained panelists (aged 18–50) assessing coded samples in randomized order. Data were analyzed using SPSS v19. Independent t-tests compared extraction methods, while one-way ANOVA with DMRT was used for sweetness levels. Normality and variance were assessed via Shapiro–Wilk and Levene's tests; non-parametric tests or data transformation were applied as needed. Significance was set at $p < 0.05$, and exact p-values or effect sizes were reported where appropriate. Results are expressed as mean ± SD (n = 3). For texture, 20–25 beads per replicate were analyzed from three independent batches. Each sensory panelist served as an independent replicate.

3. Result and DISCUSSION

3.1. Effects of extraction methods on the physical, chemical, and bioactive properties of butterfly pea extracts

3.1.1. Extraction yield and color

The visual appearance of butterfly pea extracts obtained through hot and cold extraction methods is shown in Fig. 1, which illustrates their distinct color differences. To evaluate the impact of extraction method on the physical, chemical, and bioactive properties of the extracts, Table 2 summarises key analytical results, including pH, yield, color values (L*, a*, b*), and antioxidant activity. In general, both conventional (hot) and non-conventional (cold) extraction methods have specific advantages, and the choice depends on the sample type and intended application. Prior to extraction, plant materials are usually reduced in size to increase the surface area for solvent interaction. According to previous studies, *Clitoria ternatea* flowers have commonly been used in dried, powdered, or freeze-dried forms (Srichaikul, 2018; Phrueksanan et al., 2014; Kamkaen and Wilkinson, 2009; Lakshan et al., 2019; López Prado et al., 2019). Some researchers have used fresh flowers that were cut, washed, and freeze-dried before extraction (Shen et al., 2016).

In this study, fresh butterfly pea flowers were used without size reduction—only washed and cleaned—prior to extraction. The resulting extracts from both hot and cold methods displayed a deep blue color and a natural scent reminiscent of beans and grass. The pH of both extracts was approximately 6.2, indicating slightly alkaline characteristics. Table 2 shows that the extract yield differed slightly between methods. From 120 g of fresh flowers and 200 g of distilled water, the hot extraction yielded 280 ± 3 g of liquid extract, while cold extraction produced 270 ± 3 g. The slightly lower yield in cold extraction was likely due to increased water absorption during prolonged soaking. Notably, the extract obtained from cold extraction was more concentrated and visually more vibrant, with a brighter deep blue appearance. This was supported by higher L* values (indicating greater lightness) and b × values (indicating more intense blue) in colorimetric analysis. The L*, a*, b × system represents the lightness and chromaticity of color: L* indicates brightness (0 = black, 100 = white), a × represents the green–red axis, and b × the blue–yellow axis.

3.1.2. Phenolic and antioxidant properties

Cold extraction has been associated with better preservation of heat-sensitive phenolic compounds compared to hot extraction, as supported by previous studies on thermally sensitive pigments such as anthocyanins (Patras et al., 2010; Castañeda-Ovando et al., 2009). However, since total anthocyanin content was not specifically analyzed in this study, this observation is limited to TPC. The results of this study demonstrate that the extraction method significantly influences the physical, chemical, and bioactive properties of butterfly pea flower extracts. Cold extraction preserved more phenolic compounds and antioxidant activity, in line with earlier research showing that anthocyanins are highly susceptible to thermal degradation (Patras et al., 2010; Castañeda-Ovando et al., 2009). Heat-induced degradation can lead to color

(a) Hot extraction

(b) Cold extraction

Fig. 1. Appearance of butterfly pea extracts obtained by (a) Hot and (b) Cold extraction methods.

loss and a reduction in antioxidant capacity, primarily due to the breakdown of anthocyanin molecular structures (Castañeda-Ovando et al., 2009). When the butterfly pea extract was analyzed for TPC, the extract obtained through the cold extraction method was found to contain 43.05 mg eq GA, which was twice as high as the TPC found in the extract obtained through the hot extraction method (21.01 mg eq GA). Additionally, the antioxidant capacity of the cold-extracted butterfly pea extract was found to be 2 to 3 times higher than that of the hot-extracted sample, as determined by both FRAP and DPPH assays. Butterfly pea (*Clitoria ternatea* Linn) is known for its high content of bioactive compounds such as anthocyanins, flavonoids, and phenolic acids, contributing to its antioxidant, anti-inflammatory, and neuroprotective effects (Akter et al., 2021; Kamkaen and Wilkinson, 2009). In this study, cold extraction resulted in a twofold higher phenolic content and stronger antioxidant activity measured by FRAP and DPPH assays, highlighting its potential as a functional food ingredient. These results were found to be consistent with the findings of Sakdiah et al. (2022), who reported that butterfly pea extract obtained via cold extraction exhibited a bluish-purple color due to the presence of high levels of anthocyanins, which are water-soluble pigments. The heat applied during the boiling process in the hot extraction method was suggested to be responsible for the reduction in anthocyanin content, as compared to cold extraction. This finding was also in agreement with the study conducted by Junpatiw et al. (2017), which investigated the effect of heat on the anthocyanin content in purple-colored vegetables such as purple eggplant and purple cabbage. The vegetables were subjected to cooking by boiling and steaming at temperatures of 98–100 °C, and the anthocyanin content was compared to that of fresh vegetables. It was found that cooking by boiling and steaming at 98–100 °C caused a reduction in anthocyanin content, though the reduction was lower compared to that observed in fresh samples.

Table 2
Physical, chemical, and bioactive properties of butterfly pea extracts.

Physical, Chemical, and Bioactive Properties	Extraction Method	
	Hot extraction	Cold extraction
Color		
L × ^{ns}	1.41 ± 0.03	1.69 ± 0.11
a*	+6.04a±0.05	+3.22 b ± 0.01
b*	−3.89a±0.02	+0.10 b ± 0.02
pH^{ns}	6.2 ± 0.02	6.2 ± 0.02
Total Soluble Solid (TSS) (Brix°)^{ns}	0	0
Total Phenolic Content (mg eq GA)	21.01^{b} ± 0.10	43.05^{a}±0.16
Antioxidant Capacity		
FRAP Method (μmol TE)	44.70^{b} ± 1.57	180.00^{a}±2.16
DPPH Method (mmol TE)	88.16^{b} ± 1.67	208.70^{a}±1.13

Remarks:.
Values are expressed as mean ± standard deviation (n = 3). Different superscript letters (a, b, c) within the same row indicate statistically significant differences at $p < 0.05$, as determined by Duncan's Multiple Range Test (DMRT).
Ns indicates values that were not significantly different.

3.2. *Visual and textural properties of butterfly pea spheres produced by basic spherification*

The appearance of the butterfly pea spheres, developed using the basic spherification technique, is shown in Fig. 2. Spherification—based on the ionic interaction between sodium alginate and calcium ions—produces gel-like beads that encapsulate flavor and bioactive compounds, offering a burst-in-the-mouth texture (Barham et al., 2010; O'Mahony, 2013). While earlier studies have explored the use of spherification for flavor encapsulation and visual presentation in molecular gastronomy (e.g., Gaikwad et al., 2019), the application of natural anthocyanin-rich flower extracts such as butterfly pea in this format remains limited. This study advances previous work by demonstrating that cold-extracted butterfly pea flower can be successfully incorporated into spherified edible spheres while retaining functional antioxidant properties and delivering high sensory appeal. The resulting beads were transparent with a deep blue color, and those with higher sugar content exhibited a bluish-purple hue, likely due to the pH sensitivity of anthocyanins. The spheres were small (~4 mm diameter), elastic, and

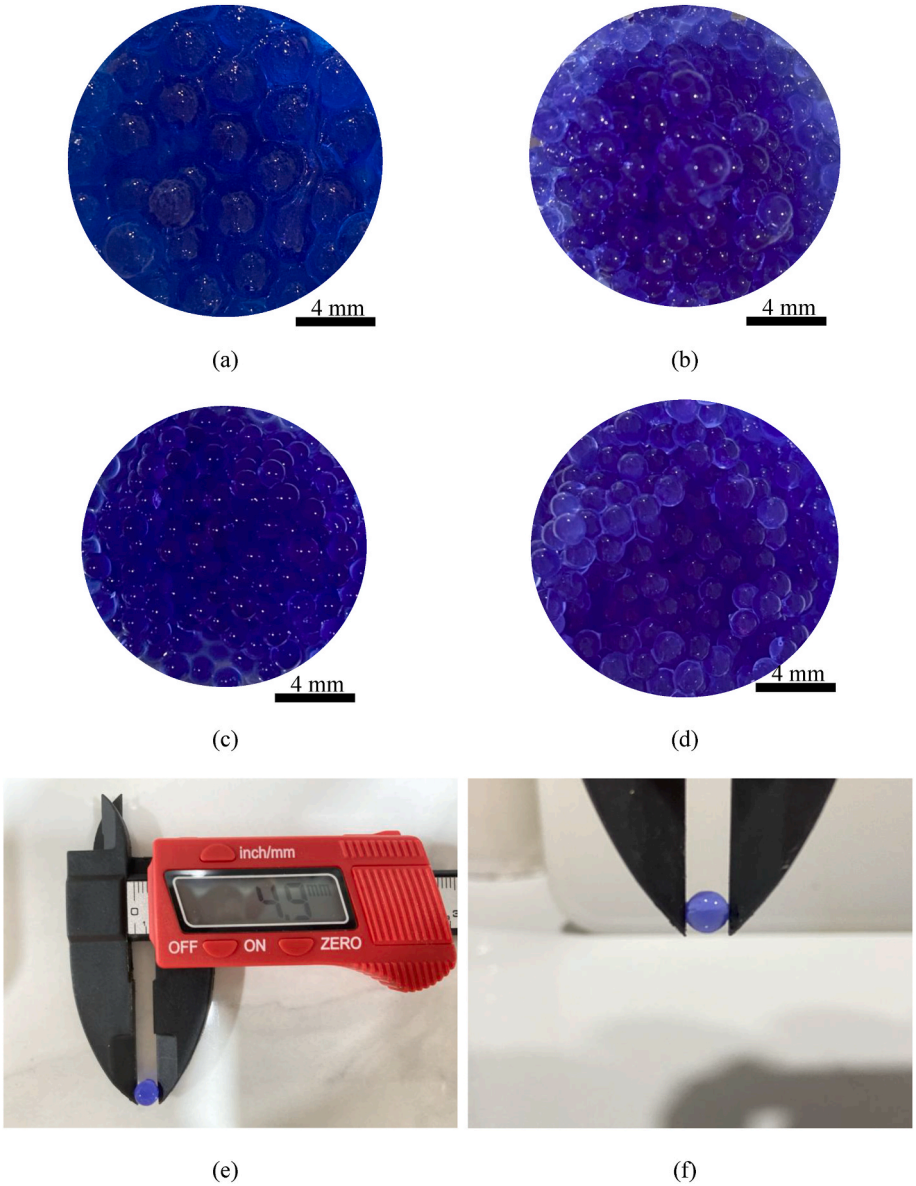

Fig. 2. Appearance of butterfly pea spheres prepared with varying sweetness levels using basic spherification: (a) 0 %, (b) 25 %, (c) 50 %, (d) 100 % sugar; (e) diameter measurement of the spheres; and (f) close-up view of sphere diameter.

burst upon chewing—consistent with the desired texture for this application. Notably, at higher sugar concentrations, the formation of "tails" was observed during dripping into the calcium chloride bath, attributed to increased viscosity. This highlights formulation considerations for optimizing texture and shape in future applications. However, a visual or flavor-neutral control formulation was not included, which limits the ability to isolate the specific contribution of the butterfly pea extract to color, texture, and antioxidant function. Future work should incorporate such controls to further clarify these effects.

3.3. Chemical interactions in spherification

Spherification relies on the ionic interaction between sodium alginate (a linear polysaccharide derived from brown seaweed) and calcium ions (Ca^{2+}) to form a gel network. In basic spherification, sodium alginate is dissolved in the flavored liquid (in this case, butterfly pea extract), and the solution is then dropped into a calcium chloride ($CaCl_2$) bath. Upon contact, calcium ions cross-link with the guluronic acid residues of alginate, creating a three-dimensional hydrogel matrix around each droplet (Draget et al., 1994; Lee and Mooney, 2012). The butterfly pea extract contains anthocyanins, predominantly ternatins, which are polyacylated delphinidin derivatives. These pigments are sensitive to pH and ionic strength, and their stability can be influenced by interactions with metal ions, temperature, and exposure to oxygen (Kong et al., 2003). In mildly acidic to neutral pH (pH ~6–7), butterfly pea extract appears blue to violet due to the quinonoidal base form of delphinidin derivatives. However, at lower pH (e.g., from $CaCl_2$ interaction or acidification), anthocyanins may shift to a reddish hue due to conversion to the flavylium cation form. Additionally, Ca^{2+} ions may affect anthocyanin stability through two mechanisms: (1) by altering the pH microenvironment during gelling, and (2) by forming complexes with anthocyanins or phenolic compounds, potentially destabilizing or changing their color (Giusti and Wrolstad, 2003). Thermal degradation is another key concern during extract preparation and storage. Anthocyanins degrade via hydrolysis of glycosidic bonds and cleavage of the chromophore, especially at elevated temperatures or under oxidative stress. Non-thermal extraction methods like cold infusion are thus preferred when preserving anthocyanin integrity is desired (Patras et al., 2010). To ensure color retention in the final spheres, pH and ion concentrations must be carefully controlled throughout the process. The encapsulation itself also offers some protection against environmental degradation, forming a semipermeable gel barrier that reduces oxygen exposure and stabilizes color.

3.4. Sensory evaluation of the butterfly pea beads (spheres)

The sensory evaluation of butterfly pea beads (spheres) was conducted with 110 panelists, primarily aged 20–30 years, single, and holding a bachelor's degree. Most participants were students or working professionals without food or edible flower allergies. The majority had prior experience with edible flower products, typically consumed once per month. The results of the preference test are presented in Table 3. Among the three formulations, the 100 % sweetness level received the highest scores for all sensory attributes (appearance, aroma, flavor, texture, and overall liking). One-way ANOVA revealed significant differences ($p < 0.05$) across sweetness levels for flavor, texture, and overall liking, while appearance and aroma did not differ significantly ($p > 0.05$). Post hoc analysis using DMRT confirmed that the 100 % sweetness formulation was significantly preferred over the 0 % and 50 % formulations in the significantly differing attributes. These findings suggest that sweetness plays a critical role in enhancing flavor perception and overall acceptance, consistent with previous work showing that sweetness can intensify flavor and aroma perception (Lawless and Heymann, 2010).

Beyond sweetness, the instrumental texture properties also appeared to influence sensory acceptance. The 100 % sweetness beads exhibited higher springiness and structural integrity, contributing to a denser and more cohesive matrix. This textural profile may have enhanced the "bursting sensation" and mouthfeel, leading to favorable texture ratings. The increased sugar concentration likely contributed to a firmer gel network, which supports elasticity and improved oral sensation.

Additionally, while antioxidant activity is not directly perceivable by consumers, the retention of phenolics and anthocyanins in the 100 % sweetness sample may enhance the product's functional and health appeal, particularly among health-conscious consumers. This highlights the importance of aligning sensory desirability with nutritional quality in developing functional food innovations using natural ingredients like butterfly pea extract.

Table 3
Sensory evaluation of the butterfly pea beads (spheres).

Attributes	Sweetness Level (%)			
	0	25	50	100
Appearance	5.76^{a}±1.88	5.87^{a}±1.81	5.49^{b} ± 1.78	5.40^{b} ± 1.88
Color	5.98^{a}±1.85	6.09^{a}±1.76	5.60^{b} ± 1.77	5.33^{b} ± 1.87
Flavor	4.35^{b} ± 1.89	4.60^{a}±1.96	4.65^{a}±1.97	4.65^{a}±2.06
Taste	4.20^{b} ± 1.74	4.47^{ab} ± 1.81	4.56^{a}±1.84	4.51^{a}±2.03
Softness	4.87^{c}±1.77	5.20^{b} ± 1.72	5.56^{a}±1.66	5.73^{a}±1.99
Brittleness	4.67^{c}±1.94	5.16^{b} ± 1.91	5.58^{a}±1.97	5.64^{a}±2.09
Overall liking	4.89^{c}±1.69	5.02^{bc}±1.60	5.18^{ab} ± 1.78	5.38^{a}±1.93

Remarks.
Values are expressed as mean ± standard deviation (n = 3). Different superscript letters (a, b, c) within the same row indicate statistically significant differences at $p < 0.05$, as determined by Duncan's Multiple Range Test (DMRT).
Ns indicates values that were not significantly different.

3.5. Physical, chemical, and bioactive properties analysis of butterfly pea beads (spheres)

3.5.1. Physical characteristics

The results of the physical and chemical quality analysis of butterfly pea beads (spheres) are presented in Table 4. A decrease in brightness was observed based on the L* value, while a deep blue coloration was indicated by the negative b × value, which represents blueness. Chroma values indicated that the 0 % sugar formulation (32.54 ± 0.35) exhibited a more intense color compared to the 100 % sugar sample (24.13 ± 1.07). This suggests a higher concentration of visibly active anthocyanins in the unsweetened formulation, despite its lower TPC. This

Table 4
Physical, Chemical, and Bioactive Properties of the butterfly pea beads (spheres).

Physical, Chemical, and Bioactive Properties	Sweetness Level (%)	
	0	100
Color		
L*	12.57^{a}±0.18	6.12^{b} ± 0.84
a^{ns}	14.74 ± 0.00	12.59 ± 0.28
b*	-29.01^{a}±0.28	-20.58^{b} ± 0.71
C*	32.54^{a}±0.28	24.13^{b} ± 1.07
Diameter (mm)^{ns}	4.3	4.3
Texture		
Hardness (g force) ^{ns}	168 ± 5.00	174 ± 2.00
Springiness (%)	78.50^{b} ± 0.10	83.99^{a}±0.01
pH^{ns}	6.2 ± 0.02	6.2 ± 0.02
Total soluble solid (TSS) (Brix°)	0^{b}	5^{a}
Total Phenolic Content (mg eq GA)^{ns}	9.05 ± 0.05	9.35 ± 0.25
Antioxidant Capacity		
FRAP Method (μmol TE)^{ns}	16.01 ± 0.41	14.09 ± 0.60
DPPH Method (mmol TE)	Not detected	Not detected

Remarks.
Values are expressed as mean ± standard deviation (n = 3). Different superscript letters (a, b, c) within the same row indicate statistically significant differences at $p < 0.05$, as determined by Duncan's Multiple Range Test (DMRT).
Ns indicates values that were not significantly different.

apparent contradiction may be due to sugar-induced structural changes or co-pigmentation effects that impact pigment stability and visibility (Castañeda-Ovando et al., 2009). Moreover, the FRAP value was also higher in the 0 % sugar sample, which aligns with studies indicating that antioxidant activity, especially via electron donation, may not directly correlate with total phenolic concentration alone (Benzie and Strain, 1996). These results underscore the importance of considering compound-specific effects and the influence of formulation components such as sugar on pigment behavior and antioxidant function. Both formulations exhibited a uniform spherical shape with a diameter of approximately 4 mm.

The texture analysis revealed no significant difference ($p > 0.05$) in hardness between the two formulations. A slight increase in hardness was observed, which may have resulted from the thicker gel membrane caused by increased sweetness levels. As a result, the beads became slightly more viscous and denser, while still maintaining elasticity and the ability to rupture upon biting. Various parts of the butterfly pea plant contain several types of secondary metabolites, particularly phenolic compounds such as anthocyanins, which are found in the flowers. Anthocyanins are responsible for the red, blue, or purple color of the flower petals, fruits, and stems of the plant. Additionally, they possess antioxidant properties. Other phenolic compounds, which also exhibit antioxidant activities, are present as well. In molecular gastronomy, spherification allows encapsulation of natural extracts, enhancing both functionality and visual appeal (Barham et al., 2010; O'Mahony, 2013).

3.5.2. Chemical and bioactive properties

Chemical property analysis, specifically the determination of total phenolic content and antioxidant capacity, revealed a reduction in total phenolic content when compared to the anthocyanin extracts from the butterfly pea flowers. A decrease in antioxidant capacity was observed with the DPPH method, whereas measurable activity was still detected using the FRAP assay. This difference may be attributed to the distinct reaction mechanisms of each assay: DPPH involves radical scavenging via electron or hydrogen atom donation, while FRAP is based on single electron transfer to reduce ferric (Fe^{3+}) to ferrous (Fe^{2+}) ions. Therefore, compounds that may no longer scavenge DPPH radicals can still contribute to antioxidant capacity via iron-reducing mechanisms detected by FRAP (Benzie and Strain, 1996). This is a characteristic feature of anthocyanins. The total phenolic compounds in butterfly pea extracts are predominantly anthocyanins, whose stability is highly sensitive to pH variations. Anthocyanins remain stable under acidic conditions but undergo structural changes and degradation as the pH shifts toward neutrality or alkalinity. One limitation of the present study is the use of TPC as the sole indicator for comparing the efficacy of cold and hot extraction methods. While TPC provides a general measure of phenolic compounds, it does not specifically quantify anthocyanins—the major bioactive and pigment compounds in butterfly pea flowers. Given that anthocyanins are highly sensitive to temperature and pH and directly contribute to both the functional and aesthetic qualities of the extract, a more targeted analysis such as total anthocyanin content using the pH differential method would have provided deeper insight into pigment preservation and extraction efficiency. Future studies should incorporate both TPC and anthocyanin-specific analyses to better differentiate the performance of extraction methods, particularly in relation to color intensity, antioxidant properties, and product appeal. Expanding the analytical scope in this way would help strengthen the theoretical contribution of the study and enhance its relevance for developing anthocyanin-rich functional food applications.

3.5.3. Antioxidant retention after spherification

Although the butterfly pea spheres in this study were stored in an acidic solution to help stabilize anthocyanins, degradation over time is generally unavoidable. Prior research confirms that anthocyanins degrade during storage, especially under suboptimal pH or temperature conditions, resulting in color fading and reduced antioxidant activity due to structural changes (Lu et al., 2007; Giusti and Wrolstad, 2003). Similar trends are observed in encapsulated systems, where pigment stability depends on maintaining low pH and minimizing oxygen exposure (Santos-Buelga and Mateus, 2011).

While the calcium alginate gel formed during spherification may offer some protection, it is semi-permeable to oxygen and ions, which can contribute to gradual degradation of bioactive compounds (O'Mahony, 2013). In this study, antioxidant activity and total phenolic content were not tracked during storage, but initial analysis showed a decline post-spherification—especially in DPPH assay results—while FRAP activity remained relatively stable. This divergence reflects differences in assay sensitivity: DPPH measures radical scavenging, which is more susceptible to phenolic degradation, while FRAP assesses ferric ion reduction, which may persist despite partial compound breakdown.

Anthocyanin degradation during encapsulation may result from near-neutral pH during gelation, oxygen exposure, or partitioning of phenolics into the alginate matrix, reducing their availability in solution. The gel's permeability may further influence oxygen diffusion and retention of specific compounds, thereby impacting antioxidant pathways differently.

To improve anthocyanin stability in future applications, strategies such as using denser or multilayered encapsulation systems and incorporating stabilizing agents like ascorbic acid or rosemary extract have been suggested (Butz and Tauscher, 2002). Future studies should monitor bioactive retention during storage and employ advanced analyses (e.g., HPLC) to track individual anthocyanins. Moreover, this study relied on total phenolic content as a broad indicator of extraction efficiency and did not quantify specific anthocyanins or include detailed sensory profiling beyond hedonic evaluation—gaps that should be addressed in future research to optimize both stability and consumer acceptance.

Finally, only water-based hot and cold extraction methods were evaluated. More selective techniques such as ultrasound-assisted or enzyme-assisted extraction may improve anthocyanin yield and stability and should be explored in future work.

4. Conclusion

This study highlights the potential of cold-extracted butterfly pea flower anthocyanins in spherified edible formats, combining visual appeal with functional benefits. Cold extraction yielded higher phenolics (43.05 vs. 21.01 mg GAE) and greater antioxidant activity than hot extraction. The optimized extract was used to develop soft, elastic beads via basic spherification (1.5 % sodium alginate, 5 % sugar), which retained notable antioxidant capacity and phenolics despite minor losses during processing. The product maintained quality over 7 days at 5 °C, though longer-term stability warrants further investigation. Key strengths include the innovative use of butterfly pea extract in a molecular gastronomy context, an integrated evaluation combining chemical, physical, and sensory data, and the identification of a formulation with strong consumer acceptance. The partial antioxidant retention further supports the potential of this approach for functional, visually engaging culinary applications. Further studies are needed to assess the bioavailability, long-term stability, and economic feasibility—including cost and scalability—of the extraction and spherification processes to enable commercial development.

Implications for gastronomy

Innovative Presentation: The use of butterfly pea extract in molecular gastronomy offers a visually striking and novel way to enhance dishes with vibrant colors and appealing textures. The natural blue hue of the extract adds an aesthetic element to food and beverages.

Health Benefits: The high antioxidant content of butterfly pea extract provides potential health benefits, which could be marketed as functional ingredients in gourmet cuisine. This aligns with the growing

trend of incorporating health-conscious ingredients into culinary experiences.

Versatility: The versatility of butterfly pea extract in creating edible spheres can inspire chefs to experiment with different textures, flavors, and presentations in a variety of dishes, from appetizers to desserts and beverages.

Sustainability and Natural Ingredients: As consumers increasingly seek natural and sustainable food options, using butterfly pea extract can appeal to those interested in plant-based and eco-friendly ingredients, reducing reliance on artificial colors and flavors.

Potential for Customization: The ability to control sweetness levels and other variables in the spherification process allows chefs to tailor the product to specific tastes and dietary preferences, making it adaptable to different culinary contexts.

CRediT authorship contribution statement

Pornyupan Pornsuksawat: Writing – original draft. **Teeranuch Chysirichote:** Writing – original draft, Data curation, Conceptualization. **Peerada Pongtong:** Writing – original draft, Formal analysis, Data curation, Conceptualization. **Anan Piriyaphattarakit:** Writing – original draft, Formal analysis, Data curation, Conceptualization. **Panyapathk Pinkaew:** Writing – review & editing, Methodology, Investigation, Formal analysis, Data curation, Conceptualization.

Consent to participate

Informed consent was obtained from all individual participants included in the study. All participants were aged 18 years or older.

Consent to publish

The authors affirm that human research participants provided informed consent for publication of the anonymized data.

Declarations

The collection of the plant materials used in this study complied with all applicable local and national regulations.

Ethics approval

The aforementioned project has been reviewed and approved according to the Declaration of Helsinki by Ethical Review Subcommittee for Human Research in Health Science, Research and Development Institute, Suan Dusit University.

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This protocol complies with a “Research with exemption” category.

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Code availability

Not Applicable.

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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 manuscript.

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Data availability

The raw data and materials for this study are available from the corresponding author on reasonable request.

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