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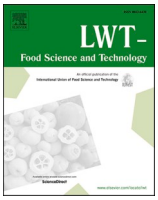

Functional properties and bioaccessibility of alginate based phycocyanin-honey hydrogels

Oya Irmak Sahin [a], Kubra Uzuner [b], Ayse Neslihan Dundar [b], Mahmud Ekrem Parlak [b], Latife Betul Gul [c], Adnan Fatih Dagdelen [b], Furkan Turker Saricaoglu [b], Senay Simsek [d,*]

[a] Department of Chemical Engineering, Faculty of Engineering, Yalova University, 77200, Yalova, Turkey
[b] Department of Food Engineering, Faculty of Engineering and Natural Science, Bursa Technical University, 16310, Bursa, Turkey
[c] Department of Food Engineering, Faculty of Engineering, Giresun University, 28200, Giresun, Turkey
[d] Whistler Center for Carbohydrate Research, Department of Food Science, Purdue University, West Lafayette, IN, 47907, USA

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ABSTRACT

Food gels have become attractive due to their biocompatibility, environment-friendly characteristics, and wide array of medical and food applications. One of the main design principles of a functional food matrix is the encapsulation, protection, and controlled release of nutraceuticals. The present study utilized two products having great interest recently, phycocyanin and honey, which were embedded in a gel-type delivery system composed of different concentrations of alginate (0.5, 1.0, 1.5, and 2.0 g/100 mL) and gelatin (7 g/100 mL). The phycocyanin-honey gel balls (PHB) were characterized in terms of physical, rheological, textural, morphological, and sensory properties, as well as *in vitro* digestion, bio-accessibility, and total phenolic content release kinetics. The increasing alginate concentration significantly increased ($p < 0.05$) total phenolic content. Also, increasing alginate ratios caused sheet-like inner layers observed in scanning electron microscopy (SEM) images. *In vitro* digestibility of phenolic content derived from both honey and phycocyanin was significantly improved ($p < 0.05$) and protected from the mouth and gastric medium by hydrogel structures of alginate and gelatin. PHB showed high release (>≈85 %) and bio-accessibility (>≈84 %) of phenolic content in the intestinal medium. Consequently, alginate could be successfully used at 1.5 g/100 mL concentration with gelatin to enhance the functionality and bio-accessibility of functional ingredients without affecting sensory properties.

1. Introduction

"Food gels" can be defined as three-dimensional polymeric structures that are insoluble, resistant under pressure, and more or less mechanically rigid. Simply they are "a form of matter intermediate between a solid and a liquid with both elastic/solid and viscous/liquid characteristics" (Cao & Mezzenga, 2020; Nazir et al., 2017). Many gel forms have been used since the 1900s, both for biomedical and food applications. Generally, biopolymers like alginate, gelatin, pectin, etc., are used to transform the gel structure. Also, different gelation agents like agar, carrageenan, alginate, or glucomannan (polysaccharide based), and gelatin, casein, whey protein, or zein (protein based) are used for thickening properties (McClements, 2017; Renard et al., 2006). In addition to the recently demonstrated compatibility with natural tissues and industrial products, *hydrogels* have been evaluated as suitable systems for the stabilization of active substances with technical and biological activity due to the high controllability of sample texture and active substance release in these systems (Coşkun & Gülseren, 2018).

Bioactive compounds have drawn attention in recent years for applications in food, health, disease prevention, and other areas. Even so, there has been a profound interest in the identification, extraction, and designation of food matrixes with enhanced bioactivity. The effectiveness of these bioactive products depends on the preservation of the bioavailability. Factors limiting the activity and potential health benefits of nutraceutical ingredients after oral administration are insufficient gastric residence time, low permeability and/or solubility in the gut and instability under conditions encountered in food processing and storage (temperature, oxygen, light) (Bell, 2000). Therefore, protective mechanisms that can preserve the active molecular form until consumption and delivery to the physiological target inside the organism need to be designed and fabricated (Mozafari et al., 2008; Subramani & Ganapathyswamy, 2020).

* Corresponding author.
E-mail address: ssimsek@purdue.edu (S. Simsek).

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McClements (2017) stated that effectiveness of nutraceuticals can be improved with hydrogels, constructed with proteins and/or polysaccharides by protecting them from chemical degradation. Commercial examples of these types of hydrogels are gel balls, tapioca pearls, and bubble teas. These products have been common in Asian regions since the 1990s, with a great increasing demand since 2000s for both America and Europe (Liu et al., 2021).

Many studies evaluated valuable bioactive compounds of bee products as they have been used since ancient times for both their nutraceutical and therapeutic effects. Several studies on honey have revealed various therapeutic properties for wound healing applications. These include antioxidant, antibacterial, anti-inflammatory, and moisturizing properties related to wound healing (El-Kased et al., 2017; Pereira et al., 2013; Samraj.S et al., 2021). They are a potential source of natural antioxidants that can promote health and reduce many diseases. Honey contains about 200 compounds that contribute to its bioactivity, mainly sugars (fructose 25–45 g/100 mL and glucose 20–40 g/100 mL), amino acids, enzymes, protein, vitamins, minerals, ash, organic acids, and phenolic and flavonoid compounds (Martinello & Mutinelli, 2021).

Another prominent source of bioactive substances is algae. Many metabolites of micro- and macroalgae have been studied for many years. Phycocyanin, an extracted pigment mainly from blue-green microalgae, has been widely used as a natural blue pigment in many industrial applications. Bioactive substances from algae have been determined as useful compounds for the treatment of many diseases, such as Alzheimer's, Parkinson's, and Huntington's diseases (Fratelli et al., 2021; Gabr et al., 2020). Despite high bioactive properties, algal biomass and other algal metabolites are not preferable functional food individually, due to their taste and aroma. There is a need to mask these undesirable sensorial properties, and gel structures can be used as an excellent material for palatability. A very well-known gel structure in the pharmaceutical industry is soft-gel capsules. These structures or vesicles prepared with gelatin or gelatin and carbohydrate polymers, are being used for their improved oral bioavailability, content uniformity and stability (Damian et al., 2021).

This study intends is to fabricate functionally-pigmented one-time chewable pearls with phycocyanin and honey that will attract the attention of consumers. As one of the main design principles of a functional food matrix is the encapsulation, protection, and controlled release of nutraceuticals, in the present study, phycocyanin and honey, were embedded in a gel type delivery system, composed of different concentrations of alginate and gelatin. To the best of our understanding, this is the first study of a functional food gel matrix enhanced with phycocyanin and honey which examines the interactions of sodium alginate of different ratios with a gelatin-based gel matrix on sensorial, textural, and rheological properties. The encapsulation rate, *in vitro* bioaccessibility of antioxidant activity and total phenolic content with release kinetics of these gel balls, were also discussed.

2. Materials and methods

2.1. Materials

Sodium alginate, gelatin, ethanol, Folin- Ciocalteu phenol reagent, all antioxidant analysis reagents, and chemicals for simulating the mouth, stomach, and gastrointestinal mediums were purchased from Merck (Darmstadt, Germany). Honey (Balparmak, Turkiye) was purchased from local markets. Phycocyanin was extracted from the biomass acquired from the cultivation of *Arthrospira (Spirulina) platensis* (UTEX LB 2340). The cultivation was conducted in *Spirulina* medium at 25 ± 2 °C with a 12:12 lightning period at the Food Biotechnology and Biochemistry Laboratory, Chemical Engineering Department, Yalova University. The extraction procedure was carried out with some modifications as described by Li et al. (2020). Spirulina biomass was hydrated with a ratio of 1:40 (v:v). The pH was fixed to 6.5 with phosphate buffer (100 mmol/L) and ionic strength was adjusted with NaCl, whose concentration was 15 g/100 mL in the final mixture, for 4 h, to prevent the co-extraction of chlorophyll. The crude extract of samples was purified by centrifugation at 12,290×*g* for 5 min and freeze-dried (Teknosem, TRS-2 model, Turkey) for 72 h.

2.2. Sample preparation

For the determination of the effects of sodium alginate concentration on the gel-forming structure and therefore encapsulation efficiency, different proportions of sodium alginate (0.5, 1.0, 1.5, 2.0 g) were dissolved in 40 mL of water at 70 °C. In a second solution, 7 g of gelatin and 0.5 g of phycocyanin were dissolved in 60 mL of water at 40 °C and each solution was mixed for 5 min in a water bath (Nuve, ST-30, Turkey). The solutions were cooled for 1 h to room temperature (25 °C) and mixed. Then, 50 g of honey was added to this mixture and the mixed solution was extruded into an 8 mm-20 mL syringe, which was then placed into the syringe pump (NE-400, NY, USA). Next, the solution was pumped dropwise from a 15 cm distance into a bath of cooled corn oil (−9 °C) at a 10 cm height. A stainless-steel filter spoon was used to collect the gel balls, which were then placed in a 10 g/100 mL $CaCl_2$ solution for 30 min. Then, the samples were filtered out of the $CaCl_2$ solution and stored at 4 °C prior to analysis. The gel balls (PHB) were coded as PHB1 for Phycocyanin-Honey-Balls prepared with 0.5 g/100 mL alginate, PHB2 for Phycocyanin-Honey-Balls prepared with 1.0 g/100 mL alginate, PHB3 for Phycocyanin-Honey-Balls prepared with 1.5 g/100 mL alginate and PHB4 for Phycocyanin-Honey-Balls prepared with 2.0 g/100 mL alginate.

2.3. Rheological measurement and textural analysis

The gelation properties of PHBs were evaluated with a controlled stress rheometer (Anton Paar, MCR302, Austria). The samples were loaded on a flat plate with a cone-plate geometry (25 mm diameter and 2° cone angle) and held at 20 °C for 120 s for temperature equilibrium. The storage modulus (G') and loss modulus (G'') were determined during the heating process from 20 to 50 °C and cooling process from 50 to 0 °C with heating ramp and cooling ramp rates of 2 °C/min. Prior to all measurements, the linear viscoelastic range (LVR) was determined as 1 % strain at 1 Hz frequency.

Texture profile analysis of PHBs was made by using a texture analyzer (Stable Micro Systems, TA HD Plus model, Godalming, UK) with a P 36R probe. Samples were directly placed on the center of heavy-duty platform, and the probe was compressed to 50 % of samples at 5 mm/s test speed (Zazzali et al., 2019). The waiting time between the two cycles of the compression was 5 s and data were averaged after ten measurements.

2.4. Fourier-transform infrared spectroscopy

Fourier-transform infrared (FT-IR) spectroscopy (Nicolet IS50, Thermo Fisher, USA) was used to investigate the possible interactions of different sodium alginate ratios and gelatin, phycocyanin and honey. Lyophilized PHBs were converted into powder form with the help of a mortar and powdered samples (~20 mg) were analyzed. FT-IR spectra were taken directly with the help of ATR diamond crystal at a 2 cm^{-1} scan resolution between 4000 and 750 cm^{-1} wavenumber range.

2.5. Scanning electron microscope analysis

Scanning electron microscope (SEM) (Carl Zeis, Gemini 300, Germany) was used for visualization of the microstructure of PHBs, that was cracked after being immersed in liquid nitrogen for 1 min, then fixed on the aluminum stubs by double-sided tape, followed by gold-palladium coating of samples in a low vacuum chamber (Leica, EM ACE200, Germany). Images were captured at an accelerating voltage of 20 kV. The surface microstructural images were captured at 50x and 500x

magnifications.

2.6. Visual appearance and color measurement

PHBs were visually observed with a stereo zoom microscope (Leica EZ4E, Switzerland) at 8x magnification, and the images were captured with an integrated 5-megapixel camera.

The color L*, a^*, and b^* values were recorded at 10 different locations with a colorimeter (PCE, CSM3 model, UK). Prior to color measurement of the samples, calibration of the instrument was done using a white standard plate ($L^* = 96.11$, $a^* = -0.25$, $b^* = 1.06$).

2.7. Entrapment efficiency, determination of total phenolic content and antioxidant capacity

The entrapment efficiency (EE %) was calculated by measuring the total phenolic compounds (TPC) before and after the formation of the gel balls (Saikia et al., 2015). For this purpose, 1 g of PHB and samples were mixed with 10 mL of ethanol:water (70:30 v:v) in a shaking water bath at 25 °C for 2 h. The mixtures were then centrifuged, and the supernatant was used for TPC analysis. The EE was determined with the following equation:

$$EE\ (g\ /\ 100\ mL) = \frac{(TPC\ of\ Phy\ before\ loading - TPC\ of\ boba\ balls)}{TPC\ of\ Phy\ before\ loading} \times 100 \quad (1)$$

Extractable fractions of PHBs were prepared for the determination of TPC and antioxidant capacity (Singleton et al., 1999). To obtain extraction fractions, 1 g of PHB was weighed and 20 mL of methanol: water (70:30) solution was added. The mixture was shaken at 20 °C for 2 h on an orbital shaker, then centrifugated at 2,500×*g* for 10 min. The supernatant (extractable fraction) was separated. An aliquot (× μL) of diluted fractions, or a standard solution of gallic acid, (2- ×) mL of distilled water, 2.5 mL of mixed alkaline copper solution [solution C; the mix of 50 mL of solution A (2 g/100 mL $NaCO_3$ in 0.1 mol/L NaOH) and 1 mL of solution B (1 g/100 mL $NaKC_4H_4O_6$ in 0.5 g/100 mL $CuSO_4$)] were mixed within volumetric flasks. After 10 min, 0.25 mL of Folin–Ciocalteu reagent (Sigma-Aldrich) diluted 1:3 w:v with distilled water was added and mixed thoroughly. The solution was kept in the dark for 30 min at room temperature. Then, the absorbance of the solution was measured with a spectrophotometer (Rigol/3660UV, China) at 750 nm. Distilled water was used as the blank and gallic acid (5–500 mg L^{-1}) was used as the standard. Calculation of TPC was done using gallic acid to construct the calibration curve, and the results were specified as mg equivalents (GAE)/g of dry matter.

Antioxidant capacity of samples was determined by radical cation decolorization assay (2,2′-azino-bis 3-ethylbenzothiazoline-6-sulphonic acid, ABTS) (Apak et al., 2008), and cupric ion reducing antioxidant capacity (CUPRAC) (Apak et al., 2006). The calibration curve of the Trolox (6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid) was prepared and the results were given as μmol Trolox equivalents (TE)/g dry weight.

For ABTS radical cation solution, 7 mmol/L ABTS in water and 2.45 mmol/L potassium persulfate, stored in the dark at room temperature for 12–16 h before use were mixed to obtain an absorbance at 734 nm. ABTS•+ radical solution of blue-green color was diluted with ethanol (96g/100 mL) at a ratio of 1:10. 1 mL of the ABTS solution was added to (x) mL of extract and (4.0–x) mL of ethanol, and then the absorbance measured at 734 nm after 6 min by using UV–Vis spectrometer (Rigol/3660UV, China).

For CUPRAC assay briefly, one mL of 0.01 mol/L $CuCl_2$, 1 mL 75 μmol/L neocuproine, 1 mL ammonium acetate buffer solutions, x mL extract of samples and (4–x) mL of water were added and mixed. The final mixture at 4.0 mL total volume was left to stand at room temperature and after 30 min, the absorbance at 450 nm (Rigol/3660UV, China) was recorded against a reagent blank.

2.8. In vitro gastrointestinal digestion, bioaccessibility and release properties

In vitro digestion of gel balls was carried out with the method according to Brodkorb et al. (2019). Firstly, 1 g samples were mixed with simulated salivary fluid (SSF) and kept at 37 °C for 2 min in a water bath. Then, the solutions were transferred into the simulated gastric fluid (SGF) at pH 3, before being held at 37 °C for 2 h while shaking at 100 rpm in a shaking water bath. Following this period, the solutions (10 mL) were transferred into the simulated intestinal fluid (SIF) and held in a shaking water bath for 2 h at 37 °C while shaking at 100 rpm. At the completion of the hydrolysis procedures, samples were centrifuged at 4 °C for 30 min at 9,000×*g*, and then supernatants were stored at −18 °C.

In vitro digestion of samples was determined with the TPC and antioxidant capacity analyses as mentioned above. The bioaccessibility (%) was calculated by dividing the results obtained from the bioaccessible fraction by the sum of the extractable fraction and multiplying by 100.

Samples were taken from the gastric and intestinal mediums once every 15 min for 120 min and then, once every 30 min for another 120 min. The release property was determined by measuring the total phenolic content according to the method mentioned above.

The release kinetics of phenolic compounds from PHBs in stomach and intestine medium was evaluated by the Korsmeyer-Peppas (Eq. (2)), Peppas-Sahlin (Eq. (3)), and Higuchi (Eq. (4)) kinetic models, which are widely used in release kinetic studies:

$$\frac{M_t}{M_\infty} = kt^n \quad (2)$$

$$\frac{M_t}{M_\infty} = k_1 t^m + k_2 t^{2m} \quad (3)$$

$$\frac{M_t}{M_\infty} = k_h t^{1/2} \quad (4)$$

where M_t and M_∞ denote to the liberation of TPC at t and infinite times, respectively. k and n denote the kinetic constant depending on the geometrical and structural properties of particles and the release exponent, which shows the release mechanism, respectively. k_1, k_2 and m are kinetic constants, diffusional exponent for Peppas-Sahlin model, and k_h is the Higuchi constant.

2.9. Sensory analysis

The samples were organoleptically assessed by 35 panelists using 5-point hedonic scales for appearance, elasticity, taste and aroma, consistency, odor, general acceptability, and affordability. The sensory panel consisted of the students and academicians of the Food Engineering Department at Bursa Technical University, Turkey. Panelists included 20 women and 15 men aged between 19 and 42 years old who were semi-trained about the product and evaluation criteria before starting the evaluation.

2.10. Statistical analysis

All analyses were conducted at least in triplicate, while texture and color analysis were measured for 10 samples per batch of PHB. The results of these tests are given as mean ± standard deviation. A one-way analysis of variance was performed to determine any significant differences between treatments, followed by Duncan's multiple range test at a 95 % confidence interval with SPSS v21 (IBM, NY, USA). OriginPro v8.5 (OriginLab Corp., MA, USA) was used to visualize the results, and MS Excel with DDSolver was used for the experimental data of release

kinetic mathematical models.

3. Results

3.1. Rheological, textural, and optical properties of gel-balls (PHBs)

The rheological, textural, and optical properties were characterized due to the concentration of the alginate. The visual appearance of PHBs captured by a stereo microscope is shown in Fig. S1. Spherical shaped gel balls were obtained for all concentrations of alginate. The temperature of sample preparation (40 °C) caused the blue color of phycocyanin to turn green. Gelation is increased by increasing the alginate concentration [19]. As can be seen in Fig. S1.d, PHB4 balls and surfaces showed a viscous appearance.

Color attributes of PHBs are given in Table 1, and the influence of alginate concentration was found to be statistically insignificant ($p>0.05$) for brightness (L^*) and greenness (a^*) values. The amounts of honey and phycocyanin used as core material in the study are constant; only the alginate concentration changes. However, a statistically significant ($p<0.05$) increase in the yellowness (b^*) values of the samples was observed when the alginate concentration increased above 1g/100 mL.

Food gels can be classified as hydrogels and are very popular in the industry and among consumers due to their good elasticity and hardness, which affect swallowing physiology. The textural properties of hardness, springiness, cohesiveness, gumminess, chewiness, and resilience were determined and given in Table 1. The textural properties of the gel structures were affected by the gel formation polymers and their concentration. Increasing the alginate concentration from 0.5 g/100 mL (PHB1) to 2.0 g/100 mL (PHB4) caused a significant ($p<0.05$) increase in hardness. Lower springiness means a breakable structure of gel, and springiness significantly ($p<0.05$) increased in PHB2 (1.0 g/100 mL alginate) and then decreased as the concentration increased. These findings were also compatible with a previous study based on anthocyanin-fortified konjac glucomannan/sodium alginate boba balls (Liu et al., 2022). Presented results indicate that chewiness and gumminess increased significantly ($p<0.05$). Cohesiveness, the strength of the internal bonds of gel structure or the quality of the core-wall materials sticking together, showed an irregular decreasing ($p<0.05$) trend as the alginate concentration was increased, where the lowest value of cohesiveness was obtained for PHB2. Emami et al. (2018) stated that the mechanical properties of hydrogels are directly affected by the cross-link density, molecular weight distribution (MWD), and functional groups of the base polymers. Here, gel bonding may be restricted, as a result, a gel ball structure was formed by establishing a bond not only between alginate and gelatin, but also between alginate-gelatin-phycocyanin-honey.

Rheology is used for determining the mechanical properties in terms of storage (G') and loss (G'') modulus. As the mechanical properties of hydrogels are the most important parameters, heating-swelling and cooling-molding of the polymer mix were evaluated. Fig. 2 illustrates the storage (G') modulus and loss (G'') modulus from the temperature sweep test in the linear viscoelastic region (LVR).

The storage modulus, G', shows the elastic response and the loss modulus, G'', shows the viscous behavior of gel structures. As shown in Fig. 1, all PHBs show a similar attribute to the temperature shift for melting and gelation except for PHB3. Increased storage modulus values indicate the great extent of cross-linking in the resultant gel structure. On the other hand, decreasing in the G' values mean an increase in syneresis for food gels. G' and G'' values increased sharply as the temperature decreased, implying gel formation. The increasing concentration of alginate slightly decreased the melting and gelation temperature ($p > 0.05$).

Table 1
Color attributes and textural properties of "Phycocyanin-Honey Gel Balls" (PHBs).

	PHB1	PHB2	PHB3	PHB4
T_m	33.45 ± 1.25	32.53 ± 0.98	32.93 ± 1.57	33.10 ± 1.18
T_g	22.09 ± 0.32	21.56 ± 0.45	21.50 ± 0.57	21.88 ± 0.66
L^*	49.77 ± 0.48^a	47.69 ± 2.09^a	53.73 ± 3.27^a	50.76 ± 1.20^a
a^*	−26.41 ± 0.98^a	−25.43 ± 0.54^a	−26.14 ± 0.75^a	−26.52 ± 0.25^a
b^*	4.88 ± 0.57^b	4.07 ± 1.03^b	7.18 ± 0.60^a	7.22 ± 0.19^a
Hardness (g)	101.94 ± 22.55^c	157.121 ± 32.72^c	251.423 ± 43.29^b	338.56 ± 41.38^a
Springiness	0.98 ± 0.020^b	1.01 ± 0.02^a	0.99 ± 0.01^{ab}	0.99 ± 0.01^{ab}
Cohesiveness	0.83 ± 0.03^a	0.60 ± 0.10^c	0.72 ± 0.01^b	0.72 ± 0.02^b
Gumminess (g)	84.45 ± 11.12^c	96.55 ± 18.73^c	181.20 ± 22.97^b	245.45 ± 23.48^a
Chewiness (g)	88.14 ± 5.94^c	102.93 ± 27.88^c	179.40 ± 22.16^b	243.39 ± 36.38^a
Resilience	0.98 ± 0.05^a	0.78 ± 0.024^b	0.76 ± 0.04^b	0.76 ± 0.05^b

Values are means ± standard deviation. a-c Means within the same row with different letters are significantly different ($p < 0.05$). PHB1: Phycocyanin-Honey Gel Ball prepared with 0.5 g/100 mL alginate, PHB2: Phycocyanin-Honey Gel Ball prepared with 1.0 g/100 mL alginate, PHB3: Phycocyanin-Honey Gel Ball prepared with 1.5 g/100 mL alginate, PHB4: Phycocyanin-Honey Gel Ball prepared with 2.0 g/100 mL alginate.

3.2. FT-IR spectrums of gel-balls (PHBs)

FT-IR was performed to detect the interactions between the wall (alginate and gelatin) and core (phycocyanin and honey) and FT-IR spectrums between 4000 and 750 cm^{-1} are presented in Fig. 2. Characteristic FT-IR spectrum bands for PHBs was detected at 3275.37, 2924.02, 2361.11, 1744.40 and 1025.55 cm^{-1}. It has been concluded that two characteristic absorption bands at 1627 cm^{-1} and 1409 cm^{-1} can be detected for pure alginate, and the characteristic absorption bands of gelatin were at 1653 cm^{-1}, 1546 cm^{-1} and 1236 cm^{-1} (Dong et al., 2006). A previous study has presented 1800-1600 and 3600-3000 cm^{-1} as characteristic bands for honey (Başar, 2016), which were used in the presented study. Phycocyanin has characteristic peaks at 1056, 1395, 1541, 1649, and 2300 cm^{-1}. The band of 2361.11 cm^{-1} is caused by the presence of CO_2 in the FT-IR analysis or can be recorded from a modified structure of phycocyanin due to differences in extraction and purification processes (Sahin et al., 2022). As seen in Fig. 2, the slight shift or decrease of this peak substantiates that the phycocyanin and hydrogel structures in PHBs are chemically well-bonded to each other.

All these results showed that the phycocyanin and honey used in this work had strong intermolecular interactions with the matrixes of the alginate and gelatin. The absence of a new characteristic band permits the conclusion that there was no obvious chemical reaction between alginate-gelatin-phycocyanin-honey. This result is an important explanation for the conservation of the bioactivity of core material. These findings were compatible with Dong et al. (2006). At the same time, Rosellini et al. (2009) stated that as the amount of alginate increased, the gelatin adsorption band at 1538.8 cm^{-1} shifted towards higher wavelengths. This change shows evidence of strong intermolecular interactions between alginate and gelatin.

3.3. Morphology of gel-balls (PHBs)

The interactions between the wall (alginate and gelatin) and core (phycocyanin and honey) can be further explained by morphological characterization. For this purpose, PHBs were investigated in terms of surface (50 x) and cross-section (500 x) imaging by SEM. Unlike PHB1, PHB2 and PHB4, where the surface structure gives a tighter appearance, PHB3 has higher encapsulation efficiency (Table 2). The visible porous

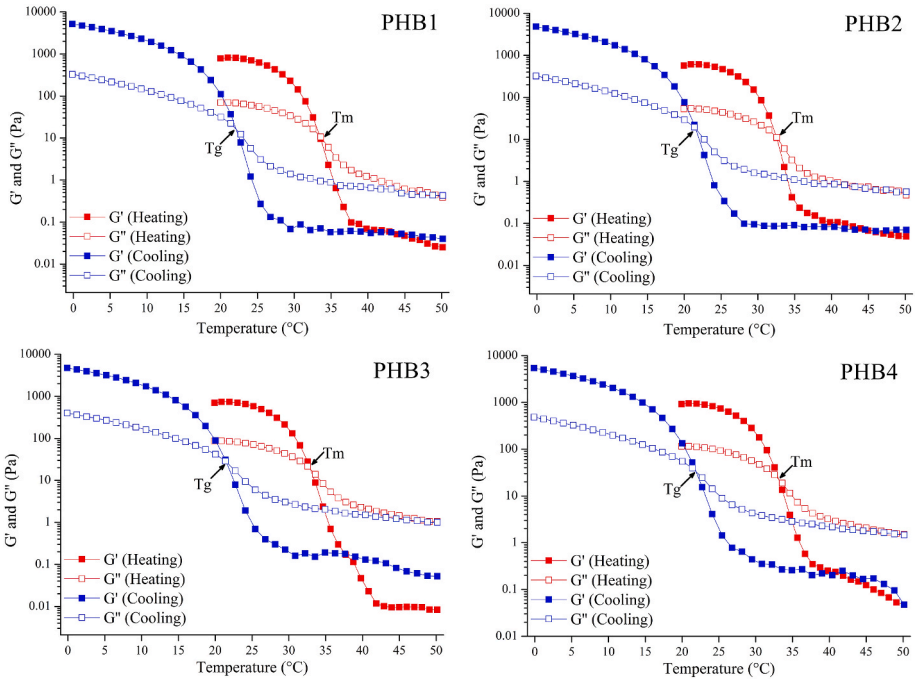

Fig. 1. Effects of alginate concentration on the storage (G′) and loss (G′′) modulus of PHBs during heating and cooling process. Tm: Melting temperature (°C); Tg: Gelation temperature (°C), PHB1: Phycocyanin-Honey Gel Ball prepared with 0.5g/100 mL alginate, PHB2: Phycocyanin-Honey Gel Ball prepared with 1.0 g/100 mL alginate, PHB3: Phycocyanin-Honey Gel Ball prepared with 1.5 g/100 mL alginate, PHB4: Phycocyanin-Honey Gel Ball prepared with 2.0 g/100 mL alginate.

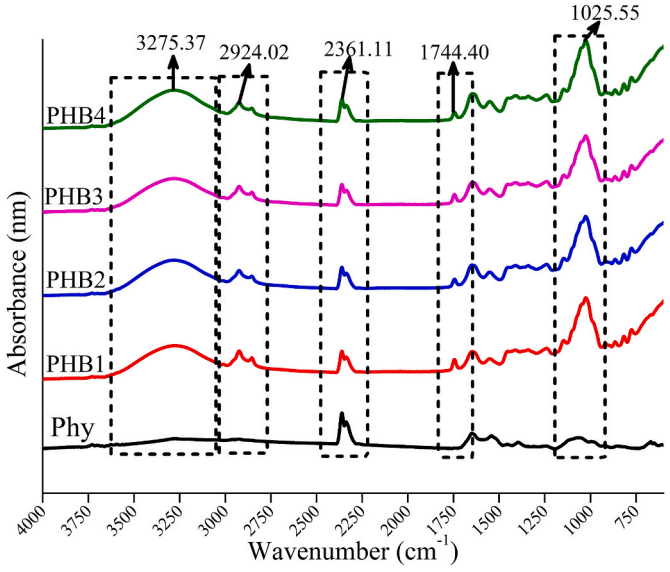

Fig. 2. The effects of alginate concentration on the Fourier-transform infrared (FT-IR) spectrums. Phy: Phycocyanin, PHB1: Phycocyanin-Honey Gel Ball prepared with 0.5 g/100 mL alginate, PHB2: Phycocyanin-Honey Gel Ball prepared with 1.0 g/100 mL alginate, PHB3: Phycocyanin-Honey Gel Ball prepared with 1.5 g/100 mL alginate, PHB4: Phycocyanin-Honey Gel Ball prepared with 2.0 g/100 mL alginate.

surfaces for all PHBs, which might positively affect the controlled release of scattering bioactive compounds, honey and phycocyanin for this study, were compatible with previous studies (Abdin et al., 2021; Saarai et al., 2013) but also in contrast to another one (S. Liu et al., 2022). These differences can be explained by the chemistry of the core materials.

An increase in the alginate concentration conducted a significant change in the structure as can be seen in the cross-section (a_c, b_c, c_c and d_c) images of PHBs (Fig. 3). Emami et al. (2018) concluded that strong gelation resulted in a denser structure with smaller pores, as demonstrated by the cross-section images of PHBs in Fig. 3.c and 3.d. In addition, for PHB3 and PHB4, sheet-like internal layers might increase the penetration during controlled release during *in vitro* digestion (Abdin et al., 2021).

Table 2
Bioactive properties and encapsulation efficiency of PHBs.

Properties	Phycocyanin	Honey	PHB1	PHB2	PHB3	PHB4
EE (%)	-	-	71.05 ± 0.19	72.52 ± 0.24	73.46 ± 2.93	71.23 ± 0.10
TPC mg GAE/g	26.40 ± 0.18	0.25 ± 1.40	37.50 ± 0.57^{d}	38.08 ± 0.25^{c}	38.87 ± 0.10^{b}	39.09 ± 0.49^{a}
ABTS μmol TE/g	100.79 ± 1.16	0.47 ± 0.53	1.43 ± 0.12	1.49 ± 0.15	1.53 ± 0.23	1.55 ± 0.06
CUPRAC μmol TE/g	286.37 ± 0.44	2.01 ± 0.85	1.94 ± 0.30	1.94 ± 0.27	1.88 ± 0.1	1.78 ± 0.14

Values are means ± standard deviation. A-d Means within the same row with different letters are significantly different ($p < 0.05$). EE: Encapsulation efficiency (%); TPC: Total phenolic content (mg gallic acid equivalent (GAE)/g of extract); ABTS and CUPRAC are antioxidant capacity measurement methods as μmol trolox equivalent (TE)/g; PHB1: Phycocyanin-Honey Gel Ball prepared with 0.5 g/100 mL alginate, PHB2: Phycocyanin-Honey Gel Ball prepared with 1.0 g/100 mL alginate, PHB3: Phycocyanin-Honey Gel Ball prepared with 1.5 g/100 mL alginate, PHB4: Phycocyanin-Honey Gel Ball prepared with 2.0 g/100 mL alginate.

3.4. Encapsulation efficiency and bioactive properties of gel-balls (PHBs)

Table 2 shows the encapsulation efficiency (EE), total phenolic content (TPC) and antioxidant capacity (ABTS and CUPRAC) of PHBs, and also, the bioactive properties of phycocyanin and honey. The EE of gel balls were determined based on TPC content and increasing the

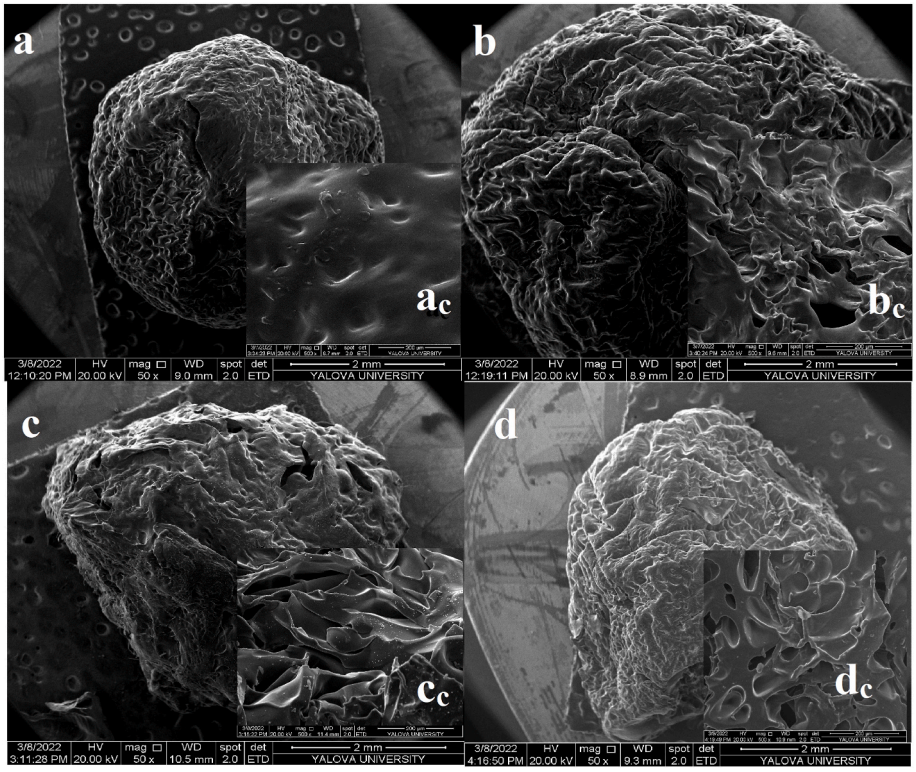

Fig. 3. Scanning electron microscope (SEM) images of surface (50x) and cross-section (500x) of PHBs. (a: (PHB1) Phycocyanin-Honey Gel Ball prepared with 0.5 g/100 mL alginate, b: (PHB2) Phycocyanin-Honey Gel Ball prepared with 1.0 g/100 mL alginate, c: (PHB3) Phycocyanin-Honey Gel Ball prepared with 1.5 g/100 mL alginate, d: (PHB4) Phycocyanin-Honey Gel Ball prepared with 2.0 g/100 mL alginate.).

alginate concentration did not significantly affect the EE ($p>0.05$). The higher alginate concentration resulted in the production of a penetrated and strong gel network structure, as can be reinforced by the SEM images and EE values of PHB3, 73.46 ± 2.93 % of EE. In previous investigations, it was stated that alginate and gelatin concentrations with a suitable ratio are responsible for a high value of EE (Chan et al., 2010; Nooeaid et al., 2017; Saarai et al., 2012).

The lowest CUPRAC and the highest ABTS values were detected for PHB4 (2.0 g/100 mL alginate). While alginate concentration increased, the CUPRAC values of PHBs decreased, and the ABTS values increased. However, all these changes are found to be statistically insignificant ($p>0.05$). This might be explained by a previous study on alginate hydrogels, which stated that the guluronic acid content of Ca-alginate structures could play a role in the absorption, encapsulation, or accumulating antioxidants within the hydrogel matrix (Chan et al., 2010). Additionally, Kelishomi et al. (2016) observed higher free radical scavenging potentials at higher concentrations of alginate. This increasing ABTS data could be associated with the increase of functional groups with scavenging potentials as the alginate concentration increases.

On the other hand, while the alginate concentration increased from 0.5 g/100 mL to 2.0 g/100 mL, TPC values also significantly increased ($p<0.05$) from 37.50 ± 0.57 to 39.09 ± 0.49, depending on the cooperation of the high total phenolic contents of phycocyanin and also alginate. Generally, previous investigations did not focus on total phenolic content or antioxidant capacity (Hadiyanto et al., 2017; Mun et al., 2016; Saarai et al., 2013; Wu & McClements, 2015) except some studies on *in vitro* release of nutraceuticals and pharmaceuticals (Abdin et al., 2021; Liu et al., 2022; Mun et al., 2015a; Nooeaid et al., 2017).

3.5. *In vitro digestion of gel balls (PHBs)*

Encapsulation of nutraceuticals and/or pharmaceuticals is important to protect and control the release of bioactive compounds of digested fractions. This is crucial for bioaccessibility, namely the availability of bioactive components for absorption in the intestines (Mun et al., 2015b). The bioaccessibility of gel balls was evaluated by the assessment of TPC and antioxidant capacity, as shown in Fig. 4-a. Previous studies showed that only 50 % of phycocyanin could be detected (Sahin et al., 2022), and for honey, it was impossible to detect total phenolics in the digested fraction (Cianciosi et al., 2020). This study intended to increase the bioaccessibility of phycocyanin and especially honey with the food gel structures fabricated for the gastro-intestinal environment.

Oral bioaccessibility was determined to be below 5 %, which means the structure of alginate/pectin and phycocyanin/honey is successful for protection in the oral medium. Similar protection for the gastric medium was also noted for all PHBs, having a gastric bioaccessibility lower than 20 %. As can be seen in Fig. 4-a, high levels of intestinal bioaccessibility for TPC and antioxidant activity could be achieved in all PHBs. TPC bioaccessibility ranged between 84.76 and 85.13 %, which means alginate/pectin gel balls remained intact throughout the oral and gastric digestions. Alginate concentration did not significantly affect ($p>0.05$) the TPC bioaccessibility. However, alginate concentration had a significant positive effect ($p<0.05$) on the protection of antioxidants in the gastric medium).

Despite being no profound changes between prepared PHBs, it was demonstrated that alginate and pectin form a good digestion system barrier for the protection of phenolic substances (Abdin et al., 2021; Nooeaid et al., 2017). Also, hydrogel structures are known to be important carriers for bioactive compounds and provide release within the gastrointestinal tract (Zhang et al., 2016). The protective nature of the hydrogels allowed the sustainability of the positive effect of pectin on the bioaccessibility of polyphenolic structures such as epigallocatechin gallate (ECGG). In a previous study, 97 % of the antioxidant activity of epigallocatechin gallate (ECGG) was detected after the digestion of gelatin micro-hydrogels (Gómez-Mascaraque et al., 2016).

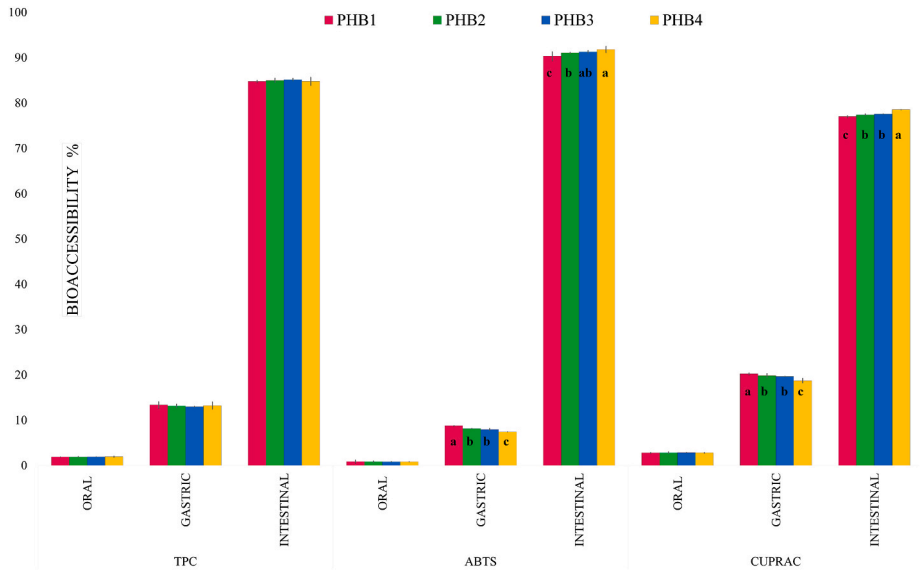

Fig. 4. (a) The *in vitro* bio-accessibility of PHBs in simulated digestion mediums. **(b)** Cumulative release of PHBs in simulated digestion mediums. PHB1: Phycocyanin-Honey Gel Ball prepared with 0.5 g/100 mL alginate, PHB2: Phycocyanin-Honey Gel Ball prepared with 1.0 g/100 mL alginate, PHB3: Phycocyanin-Honey Gel Ball prepared with 1.5 g/100 mL alginate, PHB4: Phycocyanin-Honey Gel Ball prepared with 2.0 g/100 mL alginate.

Although this study data is compatible with the presented findings, effective bioaccessibility of lipid-based bioactive compounds was also reported, for an oil-in-water emulsion with polysaccharide based hydrogels (Mun et al., 2015b). It can be concluded that alginate has a statistically significant effect ($p<0.05$) on the bioaccessibility of phenolic compounds.

The cumulative release rate (%) of TPC of phycocyanin and honey from alginate-gelatin gels (PHBs) was studied in gastric and intestine mediums (Fig. 4-b). As shown in Fig. 4-b, only PHB1 reached over 15 % of TPC release, while the least release was detected for PHB4. Release rates were determined as PHB1<PHB2<PHB3<PHB4. These results can be explained by the increasing viscosity caused by alginate, providing a stable gel membrane, as mentioned by Elnashar et al. (2010). In contrast to a previous study by Yan et al. (2019), the gel structures of the present study were protected in acidic conditions. From this point of view, it can be concluded that increasing alginate concentration positively affected the phenolic release in gastric conditions. Alginate added double emulsion systems were previously used for encapsulation and controlled release of phycocyanin (Teixé-Roig et al., 2022), and the lowest phycocyanin release in the gastric phase was observed for emulsions containing 2 g/100 mL and 1.5 g/100 mL alginate.

Studies showing rapid release from alginate hydrogels in alkaline conditions due to the chelating of calcium and phosphate have been reported (Aslani & Kennedy, 1996). As expected, after gastric digestion, an increase in the TPC release of PHBs was recorded. Results showed protection in the stomach and a controlled release in the intestinal environment. The cumulative release of PHBs was recorded after gastric digestion as 44.22, 33.20, 32.01 and 27.70 % and after intestinal digestion as 85.52, 85.85, 86.85 and 88.50 g%, respectively, for PHB1, PHB2, PHB3, and PHB4. The diffusion of honey and phycocyanin seems to be alginate concentration-dependent, as it can be concluded from Fig. 4-b where the higher cumulative release was recorded for PHB4 with 2g/100 mL alginate concentration. Increasing the alginate concentration from 1.5 g/100 mL to 2.0 g/100 mL caused a significant ($p<0.05$) change in gastric digestion so the release decreased from 32.01 % to 27.70 % and, consequently, release percentage increased in the intestine to 88.50 %.

A previous investigation based on vitamin D and C encapsulation and controlled release determined strong protection and high release (>80 %) in gastro-intestinal digestion (Gautam & Santhiya, 2019) compatible with our findings. In another investigation on gel systems in which alginate was supported on chitosan, the cumulative release of palm olein was reported as >90 % for the intestinal environment (Lim et al., 2020). For honey-based hydrogels prepared for wound healing applications, a release ratio higher than 70 % has been reported as well (Abraham et al., 2022; El-Kased et al., 2017; Nezhad-Mokhtari et al., 2021; Saarai et al., 2012). The results of this work showed the strong cross-linking forces between alginate-gelatin and honey-phycocyanin. From the practical point of view, gelatin hydrogels incorporated with 2.0 g/100 mL sodium alginate is suitable for functional food applications because of their slow release (approximately only 28 % of total phenolic content was released as a maximum in the gastric medium in 120 min). On the other hand, the rapid and high-rate release of total phenolics can be utilized advantageously for the bioaccessibility of polyphenol substances.

The *in vitro* release kinetic of phenolic compounds in PHBs in the gastric and intestinal mediums was evaluated with widely used release models Korsmeyer-Peppas, Peppas-Sahlin, and Higuchi. Table 3 indicates the kinetic mechanism scenarios for PHBs. As seen in Table 3, Peppas-Sahlin release model showed high R^2 values (>0.950) for the stomach medium and Korsmeyer-Peppas release model showed high R^2 values (>0.950) for the intestine medium. Simulated gastric medium showed a good fit with Peppas-Sahlin model with regression coefficients 0.9876, 0.9831, 0.9774 and 0.9629, respectively. In Peppas–Sahlin model k_1 and k_2 are diffusion constant and erosion constant, respectively. The ratio of k_1/k_2 in all media was far greater than 1 which indicated the vanillin release was mainly controlled by diffusion. Also, when $0.43 < n < 0.85$, release mechanism is governed by a non-Fickian transport. In the Korsmeyer-Peppas model, the exponent n describes the release mechanism and highly depends on the geometry of the particles. As it can be determined from Table 3, $n< 0.5$ is observed for all PHBs in intestinal medium. It means that the mechanism of release is controlled diffusionally and called Fickian. The increase in alginate concentration from 0.5 to 2.0 g/100 mL (w/v) tended to increase the n values from 0.054 to 0.121 in intestinal medium, but did not increase to a non-Fiskian diffusion model.

3.6. Sensory properties of gel-balls (PHBs)

Sensorial analysis of PHBs was applied according to the properties of appearance, affordability, elasticity, smell, consistency, taste and

Table 3
The release model parameters of total phenolic content from PHBs in gastric and intestinal mediums.

Medium	Model	Coefficients	PHB1	PHB2	PHB3	PHB4
Gastric	Korsmeyer-Peppas	k	9.336	5.716	5.847	5.090
		n	0.123	0.169	0.167	0.172
		R^2	0.9754	0.9517	0.9383	0.8787
	Peppas-Sahlin	k_1	3.779	2.586	2.577	2.310
		k_2	−0.200	−0.120	−0.117	−0.106
		m	0.450	0.450	0.450	0.450
		R^2	0.9876	0.9831	0.9774	0.9629
	Higuchi	k	1.504	1.151	1.168	1.037
		R^2	0.8489	0.9279	0.9364	0.9280
Intestinal	Korsmeyer-Peppas	k	18.732	15.268	15.998	12.642
		n	0.112	0.161	0.159	0.210
		R^2	0.9790	0.9925	0.9949	0.9543
	Peppas-Sahlin	k_1	7.660	7.840	8.318	7.969
		k_2	−0.433	−0.444	−0.482	−0.441
		m	0.450	0.450	0.450	0.450
		R^2	0.9420	0.9531	0.9353	0.9681
	Higuchi	k	2.838	2.904	2.996	3.026
		R^2	0.8480	0.8081	0.7517	0.8215

aroma, and acceptability (Fig. 5). The increasing concentration of alginate had no significant effects ($p>0.05$) on the sensory properties and acceptability of PHBs. However, consistency, elasticity, and appearance scores were increased with the alginate concentration up to 1.5 g/100 mL. As the concentration increased to 2 g/100 mL, which is coded as PHB4, the elasticity, taste and aroma, smell, affordability, and total acceptability scores were drastically decreased. As a general evaluation of sensory scores, there was no significant effect ($p>0.05$) of alginate concentration on the acceptability and affordability of final products. PHB3 (1.5 g/100 mL alginate concentration) has the highest acceptability and affordability, and it can be concluded that alginate-gelatin gel balls could be used as supplementary material for "phycocyanin and honey" to enhance antioxidant and bio-accessibility properties with an alginate concentration up to 1.5–2 g/100 mL.

4. Conclusions

In the present study, honey was embedded into alginate/gelatin hydrogel ball structures, and phycocyanin was used to enhance the total phenolic and antioxidant properties. Prepared PHBs were found to be a successful vesicle for bioaccessibility with strong protection in acidic conditions and an elevated release in alkaline conditions.

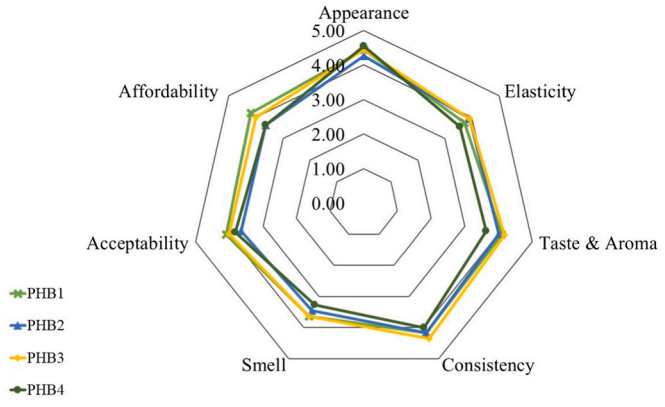

Fig. 5. Sensory evaluation of PHBs. PHB1: Phycocyanin-Honey Gel Ball prepared with 0.5 g/100 mL alginate, PHB2: Phycocyanin-Honey Gel Ball prepared with 1.0 g/100 mL alginate, PHB3: Phycocyanin-Honey Gel Ball prepared with 1.5 g/100 mL alginate, PHB4: Phycocyanin-Honey Gel Ball prepared with 2.0 g/100 mL alginate.

The effect of alginate concentration on gel ball physical, chemical, and morphological properties, bioactivity and bioaccessibility were observed. Although it did not significantly affect ($p>0.05$) these properties, the best EE, highest TPC, and antioxidant capacity values were reached in PHB3. FTIR spectrums clearly revealed the chemical bonds occurring between alginate/gelatin and honey/phycocyanin. As the alginate concentration increased, based on morphological properties observed with SEM images, it can be concluded that the strong gelation with denser structure and smaller pores on the surface, also the increased penetration with sheet-like internal layers.

The food industry has focused on developing food products that are rich or enriched in natural antioxidants, as they may have a potential role in the prevention of cardiovascular disease, cancer, and similar diseases. As a consequence of the study, it has been shown that alginate hydrogels used as drug encapsulation and wound healing material can be used for functional food or food components. The results show that alginate, at no more than 1.5 g/100 mL, is the best option to produce phycocyanin-rich honey beads or boba balls. In these structures, resistance in the gastric environment and release in the intestinal environment, which is a desirable feature, could be achieved at high rates. Compared to the directly incorporated honey capsules, the gel balls enhanced with natural and functional colorant phycocyanin demonstrated higher phenolic content. The limitation of the gel ball structure with alginate and gelatin is the color loss of phycocyanin. Hence, further studies recommended <formulation with another co-polymer instead of gelatin in the structure for the protection of the natural blue color of phycocyanin.

CRediT authorship contribution statement

Oya Irmak Sahin: Conceptualization, Investigation, Formal analysis, Writing – original draft. **Kubra Uzuner:** Methodology, Formal analysis. **Ayse Neslihan Dundar:** Conceptualization, Formal analysis, Investigation. **Mahmud Ekrem Parlak:** Methodology, Formal analysis. **Latife Betul Gul:** Conceptualization, Formal analysis, Investigation. **Adnan Fatih Dagdelen:** Formal analysis, Investigation. **Furkan Turker Saricaoglu:** Formal analysis, Data curation, Writing – original draft, Visualization. **Senay Simsek:** Data curation, Writing – review & editing, Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial

interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.lwt.2023.115099.

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