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# Protein-binding approaches for improving bioaccessibility and bioavailability of anthocyanins

(article starts on next page)

**COMPREHENSIVE REVIEW**

# Protein-binding approaches for improving bioaccessibility and bioavailability of anthocyanins

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**Abstract**

Color is an important characteristic of food. Over the last 15 years, more attention has been paid to natural colorants because of the rising demand for clean-label food products. Anthocyanins, which are a group of phytochemicals responsible for the purple, blue or red hues of many plants, offer a market advantage. In addition, anthocyanin-rich foods are associated with protection against cardiovascular disease, thrombosis, diabetes, cancer, microbial-based disorders, neurological disorders, and vision ailments. However, the real health value of anthocyanins, whether as a natural colorant or a functional ingredient, is dependent on the ultimate bioaccessibility and bioavailability in the human body. Many animal and human clinical studies revealed that, after intake of anthocyanin-rich foods or anthocyanin extracts, only trace amounts (< 1% of ingested content) of anthocyanins or their predicted metabolites were detected in plasma after a standard blood draw, which was indicative of low bioavailability of anthocyanins. Protein binding to anthocyanins is a strategy that has recently been reported to enhance the ultimate bioactivity, bioaccessibility, and bioavailability of anthocyanins as compared to anthocyanins delivered without a protein carrier. Therefore, in this review, we address anthocyanin properties in food processing and digestion, anthocyanin-protein complexes used in food matrices, and changes in the bioaccessibility and bioavailability of anthocyanins when bound into anthocyanin-protein complexes in foods. Finally, we summarize the challenges and prospects of this delivery system for anthocyanin pigments.

**KEYWORDS**
anthocyanin–protein complexes, bioavailability, digestibility, gastrointestinal, pigments

## 1 | INTRODUCTION

Color is arguably one of the most important organoleptic features of food, as it contributes to visual appeal to the consumer, and serves as a gauge of ripeness, freshness, and potential for full flavor (M. Shen et al., 2018). In the current clean label-focused marketplace, where discerning consumers try to avoid artificial or synthetic ingredients, the presence of visually-attractive natural anthocyanins in foods offers a market advantage. By using functional fruit- or vegetable-derived anthocyanins extracted from traceable natural sources as ingredients to naturally offset

color lost in processed food products (including beverages, confections, dairy, and bakery goods), potential consumer backlash against artificial colorants is mitigated (de Mejia et al., 2020; Ge et al., 2019). The multiple shades of orange–red–blue–purple contributed by naturally-sourced anthocyanin extracts from fruits or vegetables are appropriate and attractive shades in many food products.

An additional advantage to anthocyanins in foods and food products is the "health halo" associated with this pigment, as evidenced over multiple experimental platforms and disease targets including in vitro, pre-clinical, clinical, and epidemiological research (de Mejia et al., 2020; Fallah et al., 2020; Kimble et al., 2019; Lila et al., 2016). Anthocyanin-rich food is associated with protection against cardiovascular disease, thrombosis, diabetes, cancer, microbial-based disorders, neurological disorders, and vision ailments (Kimble et al., 2019; Krga & Milenkovic, 2019; Y. Shen et al., 2022). The anthocyanins are well recognized as anti-inflammatory mediators, beneficial modulators of the gut microbiome, free-radical scavengers, and protectors against ROS-mediated oxidative damage. They contribute to the prevention of DNA damage, estrogenic activity, and enzymatic inhibition (Amararathna et al., 2020; Lila et al., 2012; Peng et al., 2019; Skates et al., 2018).

The real health value of anthocyanin pigments, whether as natural colorants or functional ingredients, is dependent on the ultimate bioaccessibility and bioavailability in the human body. Current evidence suggests that anthocyanin bioavailability may be far greater than initially assumed (Eran Nagar et al., 2020; Kay et al., 2017). In light of new analytical evidence, in particular, the role of the gut microbiome in anthocyanin catabolism and release of active phenolic metabolites, innovative food processing/encapsulation technologies have attempted to improve stabilization of anthocyanin molecules in the gastrointestinal tract (GIT) to allow intact delivery for colonic uptake. Protein binding to anthocyanins is a strategy that has recently been reported to enhance the ultimate bioactivity, bioaccessibility, and bioavailability of anthocyanins as compared to anthocyanins delivered without a protein carrier (Stübler, 2022; Wu et al., 2020; Xiong et al., 2020; Zang et al., 2021). To our knowledge, no comprehensive review of this topic has previously been compiled. Therefore, in this review, we address anthocyanin properties in food processing and digestion, anthocyanin–protein complexes used in food matrices, and changes in the bioaccessibility and bioavailability of anthocyanins when bound with various plant or animal protein carriers to create anthocyanin–protein complexes in foods. Finally, we summarize challenges and prospects of this delivery system for anthocyanin pigments.

## 2 | ANTHOCYANIN PROPERTIES DURING FOOD PROCESSING AND DIGESTION

### 2.1 | Anthocyanins in food processing

Generally, analysis of anthocyanin-rich foods has focused on raw foods rather than the cooked and processed forms consumed by most people (Loannou et al., 2012). In fact, processing, storage, and cooking processes can work to degrade the structure of these pigments and blunt both color and bioactive properties (Loannou et al., 2012; Patras et al., 2010). Anthocyanins (ANC) are water-soluble flavonoids in plants that give the red, purple, and blue coloration of many fruits, flowers, and leaves (Ongkowijoyo et al., 2018). The basic structure of anthocyanins is the flavylium cation (2-phenylbenzopyrylium), a C6–C3–C6 structure which consists of an aromatic ring bound to a heterocyclic pyran ring that is also bound by a carbon–carbon bond to a second aromatic ring.

Like many natural pigments, anthocyanins tend to fade in food processing. Food processing challenges from heat, drying/dehydration, enzymes, metal ions, light and oxygen reduce the hue, stability, and shelf life of natural anthocyanin pigments, and the colors are muted particularly at neutral and high pH values (Mansour et al., 2020). Especially once isolated, anthocyanins are quite unstable, sensitive to heat, and susceptible to degradation reactions and color loss (Loannou et al., 2012; Oancea, 2021). The food matrix can act as a barrier to protect against thermal degradation, and crude extracts tend to deliver more thermally stable anthocyanins than purified pigments (Loannou et al., 2012; Oancea, 2021).

Anthocyanins are glycosidic forms of anthocyanidins which vary in the number of methoxy and/or hydroxyl groups as well as in the type and number of the sugar moieties attached to the aglycon structure (Dangles & Fenger, 2018). The most common sugar moieties (glucose, galactose, arabinose, rutinose, rhamnose, and xylose) are bound to the anthocyanidins as mono-, di-, or trisacchararides. The most common anthocyanidins in plants are pelargonidin, cyanidin, peonidin, delphinidin, petunidin, and malvidin. Acylation improves the stability of the anthocyanin molecules, and glycosylation confers stability and water solubility to the parental anthocyanidins. Most anthocyanins in nature are glycosylated in the 3-OH position and also to a lesser extent in both the 3-OH and the 5-OH positions (I. Fernandes et al., 2014). Glycosyl acylation (which can be induced enzymatically) not only enhances the hue of anthocyanins but will also help maintain the chemical stability both in a food product,

and during the digestive process, consequently improving bioavailability and bioefficacy (Oliveira et al., 2019; Yang et al., 2018, 2019). Copigmentation (with metals, organic acids, phenolic acids, proteins, etc.) can also be used to stabilize anthocyanins via complex associations (Zhu et al., 2020) and is particularly relevant to the wine industry.

Recent innovations in extraction technologies have greatly improved the efficiency of anthocyanin recovery from plant tissues (including fruit and vegetable waste streams, such as pomaces). Sub/supercritical fluid extraction (a current industry standard), enzyme-assisted extraction, continuous multifrequency microwave-assisted extraction, ultrasonication, pressurized liquid extraction, and pulsed electric field extraction methodologies have enhanced extraction yields and reduced thermal exposure, energy consumption, and processing time as compared to conventional solvent extractions. These advanced techniques require up to 15 times less energy demand, are more efficient, and contribute to enhanced stability (Oancea, 2021; Sridhar et al., 2021). Recent innovations in drying anthocyanin extracts, including microencapsulation and copigmentation strategies, have proven effective for maintaining or even intensifying anthocyanin color in a stored food product (de Moura et al., 2018; Tan et al., 2021; R. Zhang et al., 2020; Zhu et al., 2020) and have subsequently been found to also prolong gastrointestinal release in vivo (Tan et al., 2018).

## 2.2 | Anthocyanins during digestion

The general pathway and metabolic machinery for uptake and translocation of anthocyanins and their metabolites through the human body have been reviewed in detail previously (de Aguiar Cipriano et al., 2022; de Ferrars et al., 2014; H. Han et al., 2021). In brief, anthocyanins consumed in foods move through the oral cavity, the stomach, and through the gastrointestinal tract in response to smooth muscle contraction. Figure 1 illustrates the pathways of anthocyanin digestion, absorption, distribution, and metabolism in humans.

In the mouth, foods are chewed into small pieces and coated and partly digested with saliva, which facilitates swallowing. Anthocyanins (up to 50%, depending on the anthocyanin structure) can be degraded in the mouth due to interaction with human enzymes, microbiota in the oral cavity, and to some extent, spontaneous binding to salivary proteins (Kamonpatana et al., 2012, 2014). Smooth muscle contraction moves the food into the stomach by way of the esophagus. From the stomach, some fraction of anthocyanin glycoside content may be transported, for acute effect, directly into circulation via the bilitranslocase. Both the small and large intestines offer large surface areas

for anthocyanin contact, sites for enterohepatic recycling, and significant exposure to microbiota for catabolism into phenolic metabolites (Gui et al., 2022). Peristalsis, mixing, and the general forms and stability of the ingested anthocyanins during transport are conditioned by the metabolic activity of the gastrointestinal tract (Lila et al., 2016). The pH, water content, dissolved gas composition, and metabolic activity vary along the GIT (I. Fernandes et al., 2014). These varying conditions are expected to influence anthocyanin form and stability, including the propensity for adsorption and absorption. Anthocyanins are transported via the portal vein into the liver and distributed to hepatocytes. After metabolism in the liver, anthocyanins may return to the enteric system through bile or enter the circulation before removal by the kidneys and excretion in urine (Lila et al., 2016).

Circulating anthocyanins are in contact with absorptive surfaces of the GIT, hepatic, and renal systems in the body, where they may be modified by mammalian enzymes (e.g., deglycosylation), whereas the fraction of anthocyanins in the lumen of the GIT is subject to microbial catabolism. Conjugation (glucuronidation, sulfation, and methylation) can occur in the small intestine, and bioconversion of anthocyanins into phenolic catabolites by gut microbiota can occur in both small intestine and colon, followed by transport via the portal vein into liver for subsequent distribution to hepatocytes. In this highly dynamic environment of anthocyanin metabolism, the original molecular structure of anthocyanins can be constantly degraded or biotransformed by biological and chemical interactions (Lila et al., 2016). Anthocyanins in circulation largely appear in the form of phenolic metabolites, following catabolism in the microbiome, although aglycones, sulfate and methyl conjugates, glycosides, and glucuronide may also be detected in the blood. Anthocyanins are detected in nearly all tissues of animals who have consumed them, including brain, and especially liver and kidney, which suggests that mammalian tissues may be chronically exposed to various molecular forms or metabolites of anthocyanins. Anthocyanins can be recirculated in bile, via enterohepatic recycling (Gui et al., 2022; Lila et al., 2016). Behavior and transport during digestion is conditioned by the molecular structure and aglycone moiety of individual anthocyanins, which may parallel different anthocyanin's efficacy for human health interventions.

## 2.3 | Models to study anthocyanin bioavailability

In nutrition research, bioaccessibility is the amount of a substance released from the food matrix and passed across
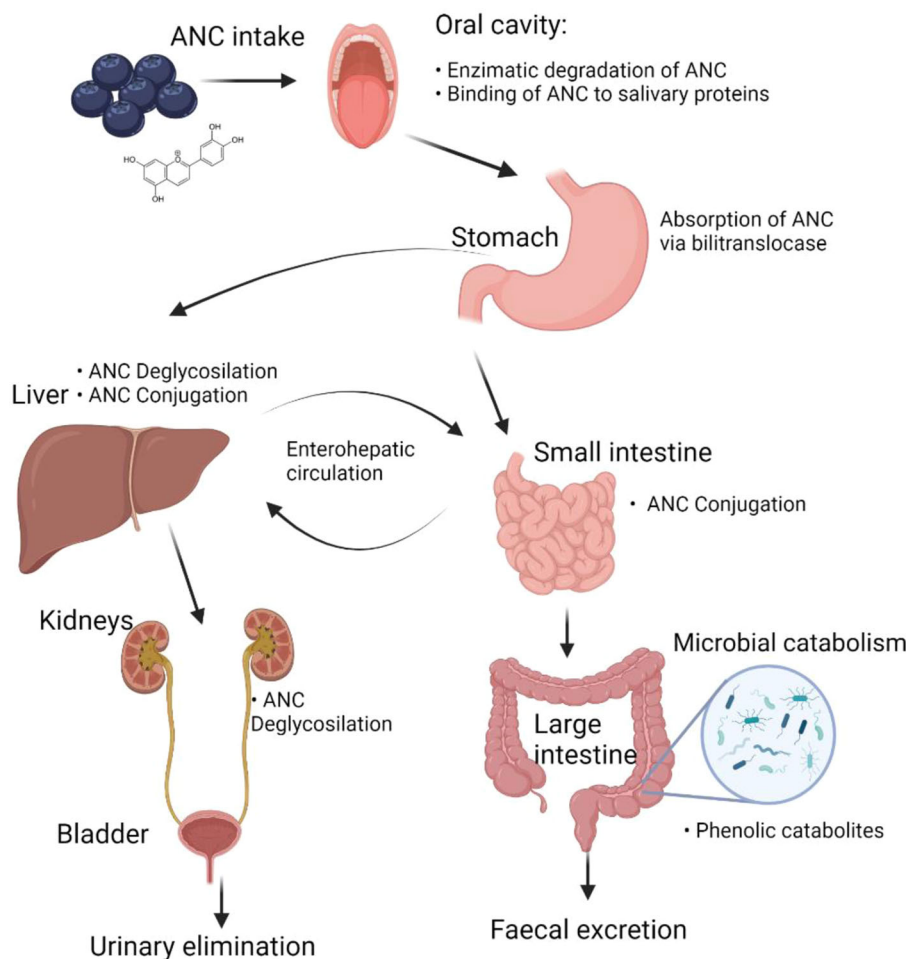
**FIGURE 1** Digestion and absorption of anthocyanin in human body

membranes during transit through the stomach and small intestine (Shahidi & Peng, 2018). Bioavailability refers to absorption, transport and utilization of those substances (Herrera-Balandrano et al. 2021). According to the US Food and Drug Administration, bioavailability is defined as the "rate and extent to which an active ingredient or moiety is absorbed and becomes available at the site of action" (FDA, 2021).

The multiplicity of anthocyanin-associated health benefits accounts for rising commercial interest in incorporating anthocyanin-rich extracts or ingredients into products. However, like any medicinally-active agent, the effectiveness of anthocyanins at preventing or treating disease ultimately depends on bioavailability (Alvarez-Suarez et al., 2021). Anthocyanin bioavailability is determined by a complex set of mechanisms (Sandoval-Ramírez et al., 2018). To elicit health-relevant bioactivities, anthocyanins must be released during digestion of foods, pass through membranes to be absorbed, enter circulation, and be available to target tissues and organs (de Aguiar Cipriano et al., 2022; de Ferrars et al., 2014; Lila et al., 2016). Many animal and human clinical studies revealed that, after intake of

anthocyanin-rich foods or anthocyanin extracts, only trace amounts (< 1% of ingested content) of anthocyanins or their predicated metabolites were detected in plasma after a standard blood draw (Kuntz et al., 2015; Lila et al., 2012). Therefore, researchers previously assumed that despite the ample evidence for health-protective benefits after consumption, anthocyanins must be poorly bioavailable.

However, recent evidence of anthocyanins' extensive degradation and metabolism after ingestion, and more robust understanding of absorption, distribution, metabolism and elimination (ADME) have greatly revised these earlier assumptions. New and evolving evidence indicates anthocyanin bioavailability was previously underestimated (Eran Nagar et al., 2020; Kay et al., 2017). The tenacity of anthocyanins throughout the digestive process, their multiple molecular structures (including the C6–C3–C6 flavonoid backbone as well as multiple phenolic acid metabolites) and complicated traffic patterns taken within the body suggest that enterohepatic recycling may be responsible for prolonged residence time and slow clearance (de Ferrars et al., 2014; Kalt, 2019; Lila et al., 2016). Colonic catabolism is now recognized as a major

contributor to anthocyanin metabolism, with metabolites present in circulation at much higher levels and for longer duration than parent anthocyanin structures (de Ferrars et al., 2014).

Human studies are a trusted method to assess the bioavailability of anthocyanins (Braga et al., 2018), which is usually measured in blood/plasma and urine (Table 1). For instance, Schon et al. (2018) conducted a human trial to study the bioavailability of maqui berry (*Aristotelia chilensis*) extract. Healthy subjects under fasting conditions received a single standardized dose of 1000 mg (35% of total anthocyanins) of berry extract powder, and their blood was sampled at different time points from 0.5 h to 8 h after the intake of the extract. The bioavailability of anthocyanins was investigated based on two selected anthocyanins (delphinidin-3-O-glucoside and cyanidin-3-O-sambubioside). The maximum plasma concentration was observed for delphinidin-3-O-glucoside (21.39–63.55 nmol/L) between 0.5 h and 1.5 h after ingestion, while for cyanidin-3-O-sambubioside, $C_{max}$ levels (3.46–12.09 nmol/L) were found between 1 h and 4 h (Schon et al., 2018).

Despite its advantage of being more reliable, human studies are inconvenient, expensive, and a time-intensive method for monitoring the entire digestion process, absorption, and metabolism of anthocyanins. Human studies also mandate use of nonintrusive techniques and adherence to ethical standards (Brodkorb et al., 2019; Hur et al., 2011). Thus, investigators have devised multiple surrogate methods that simulate human digestion and absorption. Table 1 lists the most common methods and models utilized over the last decade for study of anthocyanin digestion, bioaccessibility, and bioavailability.

Animals (e.g., rat, pig, and rabbit) are frequently used as preclinical models to simulate the digestion and bioavailability of anthocyanins in humans. For example, recently, Xu et al. (2021) investigated the bioavailability, absorption, and metabolism of pelargonidin-based anthocyanins using rats. Pelargonidin-3-O-glucoside and pelargonidin-3-O-rutinoside were administered to rats separately by stomach intubation (50 mg/Kg body weight) or injected (2 mg/kg body weight) via the caudal vein tail. The blood samples were collected at different time points from 0 min to 480 min after administration of the doses. The results suggested that the bioavailability of anthocyanins was associated with the type of sugar molecule linked to the anthocyanidin (Xu et al., 2021). The bioavailability of pelargonidin-3-O-rutinoside (1.13%) was fourfold higher than that of pelargonidin-3-O-glucoside (0.28%). The concentration of pelargonidin-3-O-rutinoside in the plasma reached a maximum plasma concentration of 175.38 nM ± 55.95 nM at 60 min after oral administration, while the maximum plasma concentration of

pelargonidin-3-O-glucoside was 91.65 ± 4.24 nM after 30 min. Platosz et al. (2021) conducted a study where sheep were given a 1 L suspension of powdered dry chokeberry (10 mg cyanidin/kg body weight) via intraruminal administration. Blood and cerebrospinal fluid samples were collected every 30 min, and urine samples were collected every 60 min after the administration within 10 h of experiment (Platosz et al., 2021). The authors found that cyanidin compounds permeate across the blood–cerebrospinal fluid barrier, and they hypothesized that the bilitranslocase membrane plays an important role in this process. Although animal studies are valuable, caution is necessary when extrapolating findings from model systems to humans because digestive enzymes and microbiota differ between animals and humans (Heinritz et al., 2013; Tullberg et al., 2016).

Simulated in vitro GIT digestion methods are another type of model to study anthocyanin assimilation. Simulated GIT digestion models usually consist of sequential steps that mimic the physiological conditions of the upper gastrointestinal tract (oral, gastric, and small intestinal stages of digestion). The concentrations of digestive enzymes, pH, digestion time, temperature, and ions are manipulated to establish the different steps (Minekus et al., 2014; Ribnicky et al., 2014). Simulated GIT digestion models can be static or dynamic. In static in vitro digestion models, food is mixed with fluids that simulate the electrolytes and enzymes associated with each step of digestion; the different mixtures are incubated at 37°C with agitation. Static models have advantages over dynamic models in terms of simplicity, significant reproducibility intra- and interlaboratory, relativity low cost, and simple assessment of each stage of digestion. There are limitations, however, since static models use a constant ratio of food to enzymes and electrolytes, and a constant pH for each digestive step and, are not able to mimic the complex dynamics of the digestion process or the physiological interactions within the host (Brodkorb et al., 2019). After digestion in simulated GIT models, anthocyanin absorption and bioavailability are usually assessed with cell models, particularly the Caco-2 cell line. Caco-2 cells express the morphological and functional features of small intestinal cells (Kamiloglu et al., 2015). Because of the early appearance of anthocyanins in plasma, demonstrated in some in vivo studies (Gui et al., 2022; Mueller et al., 2017, 2018), the use of gastric cell lines, such as MKN-28, have also been considered in studies of absorption and bioavailability of anthocyanins (F. Han et al., 2020). For instance, Oliveira et al. (2019) conducted a study to evaluate the bioavailability of acylated anthocyanins from purple-fleshed sweet potato using a static in vitro GIT model. They assessed the kinetics of absorption and the effect of food matrix using two cell

**TABLE 1** Model systems to study the bioaccessibility and bioavailability of anthocyanins

| Model | Anthocyanin source | Analysis | References |
|---|---|---|---|
| **Human studies** | | | |
| A total number of 20 volunteers were included in the study. The basal characteristics of the volunteers are described as mean ± standard deviation (SD) for the following variables: age (43 ± 8 years), sex (16 men and 4 women), weight (85.4 ± 10.4 kg), height (1.77 ± 0.09 m), body mass index (BMI) (27.25 ± 2.43 kg/m$^2$), and fat mass percentage (29.6 ± 8.1). | Anthocyanin-rich juices were prepared by three separate sweeteners (stevia, sucralose, and sucrose), the anthocyanin composition of the juices was characterized. Thus, the presence of eight anthocyanins was observed, with the most abundant being Dp 3-*O*-sambubioside, Dp 3-glucoside, Dp 3,5-*O*-diglucoside, and Dp 3-*O*-glucoside, with average concentrations of 3.15, 3.49, and 2.93 mg/100 ml, on average, and respectively. | The identification and quantification of anthocyanin metabolites from urine samples was analyzed by ultraperformance liquid chromatography tandem mass spectrometry method with electrospray ionization. | Agulló et al. (2020) |
| Ten healthy participants (five females and five males, mean age 37.3 ± 8.4 years) with a BMI of 18.5−24.9 kg/m$^2$. | Freeze-dried apple cubes. The concentration of cyanidin-3-*O*-galactoside and cyanidin arabinoside are 39.7 and 2.60 mg/80 g portion, respectively. | The anthocyanins and their metabolites in plasma and urine samples were detected by ultraperformance liquid chromatography coupled to tandem mass spectrometry. | Yuste et al. (2019) |
| Twelve nonsmoking female and male participants aged 18–50 years with a BMI ≥ 19 or ≤30 kg/m$^2$ | Commercial and standardized anthocyanin extracts from *Aristotelia chilensis* (maqui berry). | Delphinidin-3-*O*-glucoside and cyanidin-3-*O*-sambubioside in the plasma were monitored by liquid chromatography–mass spectrometry in electrospray ionization multiple reaction monitoring mode. | Schon et al. (2018) |
| Five male volunteers aged between 24 and 32, with BMI values between 22.9 kg/m$^2$ and 25.3 kg/m$^2$ | Blackcurrant extract (*Ribes nigrum* fruit extract, 20%) | Delphinidin-3-*O*-rutinoside and cyanidin-3-*O*-rutinoside in plasma and urine Samples were measured by high-performance liquid chromatography/electrospray ionization tandem mass spectrometry. | Röhrig et al. (2019) |
| Twelve healthy individuals between 20 and 60 years old | The blackcurrant anthocyanin-rich extract (BAE) consisted of 34% anthocyanins | Plasma anthocyanins were measured by liquid chromatography-mass spectrometry. | Hurst et al. (2019) |

(Continues)

**TABLE 1**  (Continued)

| Model | Anthocyanin source | Analysis | References |
|---|---|---|---|
| Recruited participants were 20−45 years of age and had a BMI ranging from 25 kg/m$^2$ to 33 kg/m$^2$. | Anthocyanin-rich beverage was prepared by wild blueberries. | Cyanidin-3-O-glucoside, delphinidin-3-O-glucoside, petunidin-3-O-glucoside, peonidin-3-O-glucoside, and malvidin-3-O-glucoside in plasma were measured by ultraperformance liquid chromatography tandem mass spectrometry method with electrospray ionization. | Zhong et al. (2017) |
| Seventeen healthy men aged between 18 years and 45 years. | Purple potatoes (*Solanum tuberosum* L. "Synkeä Sakari") extract. | Five anthocyanin degradants (Cyanidin-3-O-glucoside, as confirmed with the standard compound, and tentatively identified, malvidin-rutinoside, petunidin-glucoside, peonidin-glucoside, and peonidin-glucuronide) in plasma and urine Samples were measured by ultra-performance liquid chromatography tandem mass spectrometry method with electrospray ionization. | Jokioja et al. (2021) |
| **Animal studies** | | | |
| Adult Polish Lowland sheep (ewes, 9 months old, 36.5−55.5 kg body weight [BW]) | Freeze dried powder of 100% natural chokeberry (*Aronia melanocarpa*). | The anthocyanins and their phase II metabolites in blood, urine, and cerebrospinal fluid were analyzed by micro-HPLC-MS/MS. | Platosz et al. (2021) |
| Male Sprague−Dawley rats weighing 230 ± 20 g (specific pathogen free) | Pelargonidin-3-O-rutinoside and pelargonidin-3-O-glucoside were isolated from strawberries (*Fragaria ananasa*). | The metabolites of pelargonidin-3-O-rutinoside and pelargonidin-3-O-glucoside in plasma were identified by UPLC-Q-TOF-MS/MS. | Y. Xu et al. (2021) |
| Male 7-week-old Sprague–Dawley (SD) rats | Anthocyanin powders were extracted by 90% ethanol from Bilberry (*Vaccinium myrtillus L.*). | Anthocyanins in plasma and gastrointestinal extracts were analyzed by HPLC in conjunction with a diode-array detector system. | Nohara et al. (2018) |
| Sixteen female Polish Lowland sheep of the same age (9 months), 36.5−55.5 kg of BW. | The red cabbage (10 mg cyanidin/kg bw) | The anthocyanins and their phase II metabolites in blood, urine, and cerebrospinal fluid were analyzed by micro-HPLC-MS/MS. | Platosz et al. (2020) |
| Male Wistar rats (640 ± 43 g, 30 weeks of age) | Anthocyanin phenolic compounds were extracted from red Grenache grapes (*Vitis vinifera*). | The anthocyanins and their metabolites in serum were detected by high-performance liquid chromatography/electrospray ionization tandem mass spectrometry. | Iglesias-Carres et al. (2019) |

(Continues)

**TABLE 1** (Continued)

| Model | Anthocyanin source | Analysis | References |
|---|---|---|---|
| Specific pathogen-free male Wistar rats (aged 5 weeks and BW of 160 g) | Polyacylated anthocyanins were derived from butterfly pea (*Clitoria ternatea L.*) petals. | Nine ternatins were detected, together with preternatin A3, in rat blood plasma at 15 min after oral administration. | Ichiyanagi et al. (2021) |
| Male Wistar rats (*Rattus norvegicus*; age, 12 weeks; weight: 423 ± 42 g). | Red and white wine pomace-derived products from the vinification of *Vitis vinifera L. cv. Tempranillo* and *Verdejo*. | The anthocyanins in plasma and urine samples were measured using high-performance liquid chromatography-diode-array detection. | Gerardi et al. (2020) |
| Six Sprague–Dawley rats for each group (three males and three females, 6 weeks in age, average BW of 220 g). | Anthocyanin extracts from *Aronia melanocarpa* | Four anthocyanins (cyanidin-3-*O*-galactoside; cyanidin-3-*O*-glucoside; cyanidin-3-*O*-arabinoside; cyanidin-3-*O*-xyloside) in plasma, urine, and feces were analyzed by high-performance liquid chromatography-tandem mass spectrometry. | Tong et al. (2021) |
| Commercial, newly weaned piglets (6.4 ± 0.2 kg) | Apple (*Malcus domestica*) extract, grape seed (*Vitis vinifera*) extract, and bilberry (*Vaccinium myrtillus L.*) extract | Anthocyanins of plasma, urine, and brain tissue extracts were measured by tandem mass spectrometry-combination with liquid chromatography. | Chen et al. (2015) |
| **In vitro digestion + cell studies** | | | |
| Static in vitro digestion model + Caco-2 cells | Anthocyanin extract from ripe berries of the species *Gaultheria* phillyreifolia (*Gp*) and G. poeppigii *pink fruits* (*Gpp*) and G. poeppigii *white fruits* (*Gpw*) | The concentrations of 3-O-caffeoylquinic acid, quercetin-3-O-glucuronide, and cyanidin-3-O-arabinoside from those samples before and after in vitro oral, gastric, and intestinal digestion by high-performance liquid chromatography-diode-array detection. | Mieres-Castro et al. (2022) |
| Static in vitro digestion model + INT-407 cells | The aqueous extract from black soybean, grape, and purple sweet potato. | The mixture obtained from the in vitro digestion were used to culture with INT-407 cells. Anthocyanins were identified and quantified in cell lysates using ultraperformance liquid chromatography-tandem mass spectrometry. | Ryu et al. (2021) |
| Static in vitro digestion model + MKN-28 or Caco-2 cells | Extraction of anthocyanins from purple-fleshed sweet potato. | The content of total anthocyanins during the in vitro digestion were measured and the transepithelial transport of anthocyanins of digests were tested in MKN-28 or Caco-2 cells. | Oliveira et al. (2019) |

(Continues)

**TABLE 1** (Continued)

| Model | Anthocyanin source | Analysis | References |
|---|---|---|---|
| Static in vitro digestion model + Caco-2 cells | Anthocyanins extracts from purple sweet potatoes. | The content of total anthocyanins at different states of an in vitro gastrointestinal digestion were monitored. The percent transport efficiency from the apical to the basolateral side of a Caco-2 monolayer were measured. | de Aguiar Cipriano et al. (2022) |
| Static in vitro digestion model + Caco-2 cells | Anthocyanins extracts from Artichoke (*Cynara cardunculus L.* subsp. *scolymus Hayek*). | A clear modification of the phytochemical profiles was highlighted comparing raw to in vitro digested and fermented samples. The bioavailability of anthocyanins from artichoke was evaluated using Caco-2 cells monolayers as a model of absorption in the large intestine. | Rocchetti et al. (2020) |
| Static in vitro digestion model + Caco-2 cells | Fresh red cabbage (var. capitata f. rubra), carrot (carota subsp. sativus), baby spinach (*Spinacia oleracea*), and cherry tomato (*Solanum* lycopersicum var. cerasiforme). | Three anthocyanins cyanidin-3-(p-coumaroyl)-diglucoside-5-glucoside, cyanidin-3-(feruloyl)-diglucoside-5-glucoside, and cyanidin-3-(sinapoyl)-diglucoside-5-glucoside were identified and quantified at different states of an in vitro gastrointestinal digestion. The absorption of anthocyanins from digests were analyzed by liquid chromatography system coupled with a hybrid ion trap-Orbitrap mass spectrometer. | Phan et al. (2019) |

models from the gastrointestinal tract, MNK-28 (stomach) and Caco-2 (intestine) cells (Oliveira et al., 2019). The researchers found no significant effect of saliva and simulated gastric digestion on the stability of anthocyanins. However, at the intestinal level, they observed a significant degradation of anthocyanins (decrease of 27–43%). Overall, for both cell models, the authors have found that a time-dependent transport of anthocyanins and food components such as glucose and proteins affect the absorption efficiency of these pigments (Oliveira et al., 2019). In dynamic models, conditions are changed continuously as occurs in the gastrointestinal tract, including pH changes, changes in enzymes and their concentrations, peristaltic forces, and transport (Hur et al., 2011; Lila et al., 2012). For example, Kubow et al. (2016) described the use of a computer-controlled human dynamic gastrointestinal and colonic digestion system to study the bioaccessibility and biotransformation of anthocyanins from purple-fleshed

sweet potato (Kubow et al., 2016). In this study, samples were assessed for anthocyanins profile from all the compartments of the GIT model (stomach, small intestine, ascending, transversal, and descending colon). The authors reported stability of some anthocyanins under simulated upper intestinal tract. On the other hand, several anthocyanins were unstable in the intestinal vessel (Kubow et al., 2016), which agrees with other studies describing the chemical degradation of anthocyanins at neutral pH before their subsequent exposure to colonic microbial metabolism (David et al., 2019; Kim et al., 2020; Zannou et al., 2021).

Considerable debate exists concerning the usefulness or validity of GIT digestion models to predict human responses, especially for in vitro simulations. However, in vitro results are frequently well-correlated for a given compound evaluated using in vivo and in vitro systems (Brown et al., 2014). The great advantage of in vitro methods is their

ease of operation, lower costs and fewer administrative requirements. In addition, experiments can be replicated many more times compared with in vivo tests. Nevertheless, investigators have begun to contemplate the influence of microbiota in transformation of anthocyanins (Kubow et al., 2016).

# 3 | ANTHOCYANIN-PROTEIN FORMATION IN FOOD MATRICES

## 3.1 | Molecular interactions between anthocyanins and proteins

Anthocyanins can spontaneously bind to proteins in a food matrix, due to the natural attraction between medium polarity anthocyanin molecules and proline-rich proteins. Recently, the food industry has further exploited this stable binding phenomenon in order to deliberately formulate functional foods and nutraceutical ingredients that feature concentrated health-relevant anthocyanins and healthy proteins (Attaribo et al., 2020; Wang et al., 2021). Binding to a protein carrier provides a means of stabilizing and protecting anthocyanin pigments in processed foods (Ge et al., 2019; Wang et al., 2021).

Anthocyanins bound to dietary proteins exhibit enhanced oxidative, color, and thermal stability and shelf life, and they tend to be better protected from degradation during the process of intestinal digestion (Ren et al., 2021). The physicochemical properties acquired by anthocyanin-bound proteins include improved structural features (foamability, emulsification properties) and modified digestibility. The overall size of anthocyanin–protein particles may increase (relative to native protein particles) (Ren et al., 2021); however, they may can decrease at higher concentrations of anthocyanins, due to tightening of molecular structures (R. Zhang et al., 2020).

The biomolecular interactions between anthocyanins and edible proteins (plant or animal based) are primarily noncovalent, and the type of noncovalent binding depends on the nature of the anthocyanin molecule and the side chain groups and amide (peptide) bonds of the proteins (Figure 2). Hydroxyl groups of flavonoids attracted to polar peptides interact through hydrogen bonding, whereas anthocyanins with hydrophobic attachments exhibit hydrophobic interactions with proteins. Electrostatic interactions and van der Waals forces are also at work in noncovalent anthocyanin-protein complexes (Wang et al., 2021). Covalent bonding (sharing of electrons) between anthocyanins and nucleophilic groups of amino acid residues of various plant- or animal-derived proteins can also be involved in complex formation, and the strength of the binding definitively alters the functionality of the anthocyanin–protein

aggregate particles (Jiang et al., 2019). Anthocyanin coloration may be diminished in covalently-bound complexes due to oxidation reactions and the formation of quinones (R. Zhang et al., 2020). Anthocyanin–protein interactions in neutral or acidic environments tend to be noncovalent interactions, whereas more covalent linkages occur in alkaline environments (Wang et al., 2021).

## 3.2 | Complexation to improve the color and stability of anthocyanins

Increasing consumer awareness of food safety and potential health risks of some artificial colorants have escalated industry's interest in adding plant-derived anthocyanin pigments as color enhancers and functional ingredients in ready-to-drink beverages, fillings, snacks, and dairy products (Attaribo et al., 2020, Ge et al., 2019). Natural anthocyanin pigments are inherently unstable once they are extracted from plant tissues (fruits, roots, foliage, etc.), so the utilization of these pigments as a functional food ingredient or natural food colorant has been a serious industry challenge. However, binding anthocyanins into stable protein–anthocyanin aggregate matrices has proven to be an effective color stabilization strategy, with added benefits in terms of phytoactive content (functional food value, including antioxidant activity) and structural functionality of the food product (Ge et al., 2019; Neuenfeldt et al., 2022; R. Zhang et al., 2020).

When blueberry anthocyanins bound to protein blend (whey protein isolate [WPI] and bovine serum albumin [BSA]) were deliberately subjected to environmental stressors (thermal, light, and osmotic) to accelerate anthocyanin degradation, the pigments were visibly protected in the anthocyanin-protein matrix (Zang et al., 2021). Not only was anthocyanin stability significantly improved, but the functionality of the proteins were enhanced in terms of foamability, solubility, and emulsifying properties. In these experiments, the WPI proved to be more protective to the anthocyanins than the BSA. In other work, nanocomplexes were fabricated using a combination of chitosan derivatives and $\beta$-lactoglobulin as carriers in order to improve the stability and sustained release of anthocyanins (Ge et al., 2019). The anthocyanins proved to be more stable in simulated gastric and intestinal fluids. Similarly, Xiong et al. (2020) demonstrated using an in vitro gastrointestinal digestion model that binding berry anthocyanins in a protein matrix stabilized the anthocyanins in vitro. Compared with protein-free polyphenol, the protein-complexed polyphenol was delivered more efficiently in the digest, and the recovered polyphenols post digestion demonstrated higher antioxidant and anti-inflammatory capacities.

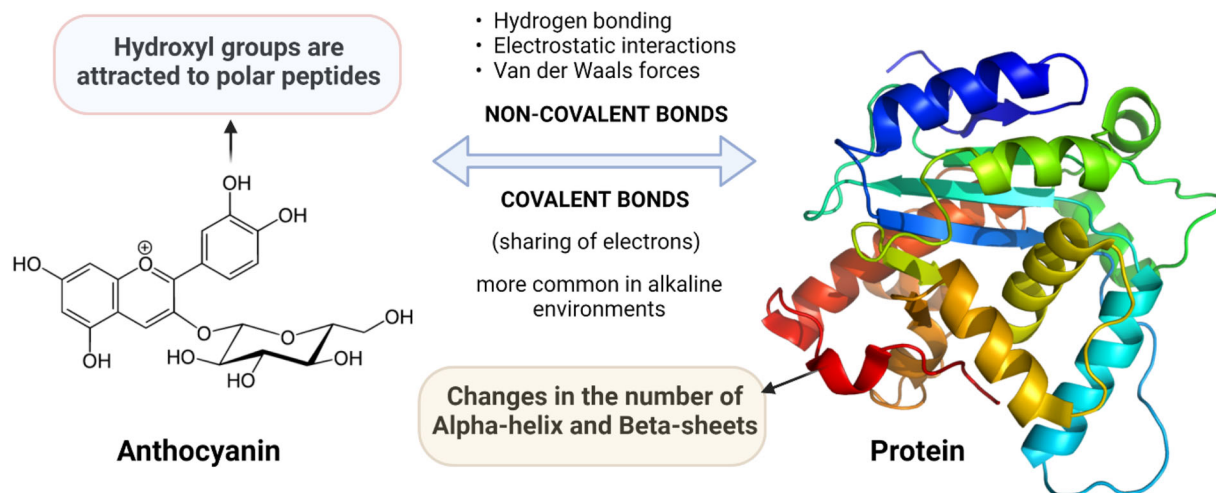## ANC-P complex formation - Types of interactions



**FIGURE 2** Molecular mechanisms of the interactions between anthocyanin and protein
Abbreviation: ANC, anthocyanins; P, protein.

Recent experiments on anthocyanins bound with milk proteins (casein and $\beta$-lactoglobulin) revealed that the predominant type of anthocyanin-to-protein binding (non-covalent vs. covalent) had a significant impact on the physicochemical properties, functionality, and nutritive value of the resultant aggregate complexes (Q. Zhang et al., 2021). Multiple spectroscopic techniques illustrated higher stability, a greater change in protein conformation and slower digestibility for the covalently-bound treatments. Interestingly, the covalently-bound complexes, but not noncovalently-bound, were able to attenuate milk protein allergenicity symptoms (reduced IgE-binding levels) ex vivo, using sera from milk-sensitive patients (Q. Zhang et al., 2021).

## 4 | ANTHOCYANIN BIOACCESSIBILITY AND BIOAVAILABILITY IN ANTHOCYANIN–PROTEIN COMPLEXES

The unique anthocyanin chemistry (four different molecular forms in dynamic equilibrium; flavylium cation, blue quinoidal structure, colorless hemiketal form and yellow chalcone forms depending on pH, temperature, and time) affects absorption kinetics, digestion, overall bioavailability, and metabolism, including the efficacy for human health protection after ingestion (Lila et al., 2016). In addition, as discussed in section 2, the role of colonic microbiota has emerged as a major factor in anthocyanin metabolism, facilitating the release of bioactive phenolic catabolites into circulation (Mattioli et al., 2020; Wang et al., 2021; Zang et al., 2021). Given that anthocyanin stability and integrity is highly dependent on environmental conditions, copigments, ambient enzymes, and molecular structures, various research teams have worked to devise novel delivery or encapsulation systems to prolong both the shelf life (in a food) and the digestive stability/bioaccessibility of these pigments (Mansour et al., 2020; Mueller et al., 2018; Wang et al., 2021; Zang et al., 2021). Several groups have recently reported that delivery as protein-based anthocyanin complexes can improve stability and bioaccessibility of anthocyanin, thus potentially enhancing bioavailability (Milea et al., 2020; Salah et al., 2020; Xiong et al., 2020). Therefore, it is important to review these different scientific studies to have a more holistic comprehension of how anthocyanin–protein aggregate complexes behave in the GIT, with the result of enhanced anthocyanin bioavailability. Table 2 summarized the effect of ANC-P interaction on the bioaccessibility or bioavailability of anthocyanins.

Plant proteins (e.g., soy, rice, and pea) and milk proteins (whey or casein) are commonly used to prepare anthocyanin–protein complexes (Y. Shen et al., 2022; Zang et al., 2021). For instance, Ribnicky et al. (2014) compared the bioaccessibility of blueberry (*Vaccinium angustifolium*) juice (control) and complexed soy-blueberry particles, which each delivered the same 156 mg of total monomeric anthocyanins to the TNO (TIM-1) gastrointestinal model of the upper human GIT. The anthocyanin molecules delivered in the complexed particles had greater bioaccessibility than anthocyanins from the blueberry juice in jejunal samples (22.7% vs. 18.4%, respectively) and ileal samples

**TABLE 2** The effect of ANC-P interaction on the bioaccessibility or bioavailability of anthocyanins

| Anthocyanins | Protein | Preparation the ANC-P complex | Design of study | Key findings | References |
|---|---|---|---|---|---|
| Blueberry ANC extract | α-casein | Blueberry anthocyanins and α-casein were dissolved in citric acid buffer solutions with pH 3.0 and pH 6.6, respectively. Then, anthocyanins were mixed with an equal volume of citric acid buffer (pH 6.6) and α-casein solution, respectively. | Each rat was intragastrically administered with 22 ml of anthocyanins/kg BW of the rat. The blood samples (500 μl) were obtained in EDTA tubes from the orbital vein of the rats at 5, 10, 15, 20, and 30 min and 1, 1.5, 2, 4, 6, 8, 12, and 24 h after ingestion. The plasma from blood were further analyzed to monitor the absorption of anthocyanins. | Rats administered α-casein-ANC showed 1.5–10.1-fold greater ANC in plasma compared with animals that received free ANC. | Lang, Li, et al. (2021) |
| Pelargonidin-3-O-glucoside (P3G) | β-lactoglobulin (BLG) | Both P3G and BLG were dissolved in acetate buffer (0.1 M, pH 4) at room temperature under constant mixing (400 rpm). After that, the pH of all solutions was adjusted to 4.0 using 1 M HCl prior to prepare model solutions. The concentrations were as follows: 0.33 mg/ml for P3G and 3 mg/ml for BLG. | Pelargonidin-3-O-glucoside and its complex with β-lactoglobulin were conducted to in vitro gastrointestinal digestion model. Bioaccessibility of pelargonidin-3-O-glucoside was measured by high-performance liquid chromatography. | Stable complexes (P3G-BLG) protected P3G from gastrointestinal degradation by promoting progressive release from the food complex. | Gowd et al. (2020) |
| Blueberry ANC extract | Gelatin and soy protein isolate (SPI) | The soy protein isolate was dissolved in distilled water at a ratio of 1:4 (g:ml) at 35°C. After that, the suspensions were equilibrated at 4°C for 24 h. The anthocyanin extracts were added into the SPI solution at a ratio of 1:4 and then the pH was adjust to 2.0. | The anthocyanin extracts and their protein complex were conducted to in vitro-simulated gastrointestinal digestion and colonic fermentation trials. The total anthocyanins and monomeric anthocyanin concentration were measured during in vitro gastrointestinal digestion and colonic fermentation. | ANC–SPI complex delayed release of ANC and promoted the biosynthesis of short-chain fatty acids by microbiota. | Wu et al. (2020) |
| Anthocyanin extract from red raspberry pomace | β-lactoglobulin (BLG) | BLG (10 mg/mL) was dissolved in 10 mM NaCl solution, and pH was adjusted to 7.0 using NaOH (1 M) under stirring at 1200 rpm for 2 h. Different concentrations from 0 to 13 × 10⁻⁴ M of anthocyanin were then added to BLG solution. | The bioavailability of anthocyanin after three stages of digestion experiment including mouse (pH 6.8), gastric (SG, pH 2), and simulated intestine digestion (SI, pH 6.9) were detected. | ANC–BLG nanoparticles were more stable in mouth (pH 6.8), simulated gastric (pH2) and unencapsulated ANC. Moreover, the addition of simulated intestinal (pH 6.9) fluids than ANC increased the antioxidant activity of BLG nanoparticles. | Salah et al. (2020) |

**TABLE 2**  (Continued)

| Anthocyanins | Protein | Preparation the ANC-P complex | Design of study | Key findings | References |
|---|---|---|---|---|---|
| Anthocyanin extract from wild blueberry and muscadine grape Noble variety pomace (seeds, skins, and residual pulp) | Pea protein (82.8% protein) and rice protein (87.6% protein) | The lyophilized ground pomaces were stirred in the solvent at a ratio of 1:10 w/v for 2 h in a water bath at 80°C, then filtered and centrifuged. Complexation with protein was accomplished by the addition of 10% (w/v) protein mixtures (pea:rice, 1:1 w/w). | The unmodified extracts and protein–anthocyanins particles were digested in the simulated gastrointestinal digestion model. The recovery index (RI) for TPC, total anthocyanins (ANC), and proanthocyanidin content (PAC), after the simulated gastrointestinal digestion. | Pomace polyphenols were protected after complexation (binding) into protein–polyphenol aggregate particles, affording them a significantly higher level of RI, antioxidant, and anti-inflammatory bioactivity post-digestion. | Xiong et al. (2020) |
| Blueberry ANC extract | Casein | Casein aqueous stock solution was prepared by dispersing it in a 0.1 M NaOH solution and stirring continuously at 50°C for 2 h and then the pH was adjusted into 7.0. ANC were solubilized in citric acid buffer solution (pH 3.0). The ANC solution was slowly added to the CA solution dropwise and stirred for 30 min at 25°C. | Simulated in vitro digestion of ANC solution and ANC–casein was evaluated. A cellulose dialysis membrane was used to analyze the bioaccessibility of ANC and ANC-casein after digestion. The contents of ANC monomers at different digestion stages were analyzed using an LC-MS/MS system. | The casein based nanocomplexes improved the retention of total ANC and their monomers during in vitro-simulated digestion, and further enhanced bioaccessibility. | Cui et al. (2022) |
| ANC extracted from purple rice | Whey protein loaded in pectin and coated with zein protein | Pectin–whey protein isolate complexes were prepared at pH 7.0. The mixture was then adjusted to pH 4.75 and heated at 83°C for 15 min in order to form pectin–WP complexes and then ANC was mixed. | In order to examine the potential of the pectin-based capsules as a colon targeted delivery system, swelling, and release characteristics of pectin-based capsules loaded with ANC were investigated under simulated gastric and intestinal fluids. | Both whey and zein protein added to pectin capsules had significantly lower ANC release in simulated intestinal fluids. | Chotiko and Sathivel (2017) |
| Bilberry ANC extracts | Whey protein | Protein Isolate (94% (w/w)) and distilled water, after which BE powder was slowly added and allowed to dissolve. The pH was adjusted to 1.5 with aqueous hydrochloric acid | Intervention study: Volunteers (healthy and ileostomists) received a diet of ANC extract encapsulated with whey protein. Urine, plasma, and ileal effluents were analyzed. | Whey protein encapsulation led to higher concentrations of ANC and their metabolites in the urine. | Mueller et al. (2018) |

Abbreviation: ANC, anthocyanins.

(13.3% vs. 7.9%, respectively); total bioaccessibility was 36.0% for complexed anthocyanins and 26.3% for blueberry juice. These data indicated that the protein–anthocyanin complex protected anthocyanins during transit through the upper GI tract and enabled anthocyanins bound to the protein matrix to be more bioaccessible, as the ileal efflux after digestion of blueberry–soy complexes contained three times more anthocyanins than recovered after digestion of blueberry juice (Ribnicky et al., 2014). Anthocyanins may be more likely to form covalent instead of noncovalent interactions with soy protein (Sui et al., 2018), and they are stronger at alkaline conditions, such as in the ileal section of the small intestine, where quinonoid ionic forms are predominant. Furthermore, hydroxyl (OH) groups, which generally are abundant in anthocyanins, can interact with the sulfhydryl (SH) groups of soy protein. Also, quinones may interact with SH groups to form C–S bonds (Sui et al., 2018). These factors may explain the higher ileal efflux bioaccessibility of anthocyanins when complexed with soy protein. The TIM-1 model does not simulate any influence from microorganisms in the in vivo human upper intestine, but the model does imply that a significantly greater amount of intact anthocyanins from protein–anthocyanin complexed particles would be available for biotransformation by colonic microbiota, permitting subsequent uptake of more bioactive phenolic metabolites into circulation. Similarly, Ju et al. (2020) found improved stability and resistance to in vitro digestion from soy protein–anthocyanin nanoparticles, which were used to prepare a Pickering emulsion. The breakdown of the Pickering emulsion was reflected by the amount of free fatty acids released from emulsion. The Pickering emulsion fabricated with soy protein–anthocyanins nanoparticles retarded the free fatty acids release rate by up to 6.9% during GI digestion (Ju et al., 2020). The ANC–soy protein complex nanoparticles present in the Pickering emulsion worked as a barrier which reduced lipase accessibility to lipid substrate and thus retarded lipid digestion. In addition, the complexes may also prevent lipid oxidation. Therefore, this approach suggests an interesting strategy to delivering nutrients since the ANC–soy proteins are stable nanoparticles with improved antioxidant capacity.

Another example of soy protein applied as carrier to protect anthocyanins was conducted by Zheng et al. (2021) who used a static in vitro GIT digestion model to assess the effect of soybean 7S and 11S globulins on the bioaccessibility of cyanidin-3-$O$-glucoside (C3G). Both soybean 7S and 11S globulins forming protein–anthocyanin complexes exerted a protective effect on the stability of C3G during simulated digestion. Soybean 11S protein provides better protection, possibly due to its different hydrogen bonding and hydrophobic interactions (Zheng et al., 2021). Among other soybean protein categories, 11S (glycinin) and

7S ($\beta$-conglycinin) make up more than 80% of total proteins and their structural differences lead to different physicochemical functions (Y. Yang et al., 2021). Physicochemical properties of 11S and 7S proteins are affected by interactions with phenolic compounds and ionic strength, with 11S more sensitive than 7S (Y. Yang et al., 2021), which might explain the higher stability for complexes made from ANC and 11S soy protein.

Soy protein, widely used in the food industry, is commonly obtained by removing oil from soybean at low temperature (Nishinari et al., 2014). On an average dry matter basis, soybean contains approximately 40% protein and 20% oil. An increasing body of evidence (Dumitrascu et al., 2020; Ju et al., 2020; Patel et al., 2020) has indicated that soy proteins are potential building materials to produce a variety of nanostructured delivery system because their chemical properties allow the development of nanoparticles that can be used as carriers for bioactive compounds, especially those, such as anthocyanins, with low bioavailability (Roopchand et al., 2012; Tang, 2019).

Wu et al. (2020) showed that the use of soy protein isolate as wall material for blueberry anthocyanins delayed the release of anthocyanins during in vitro colonic fermentation, providing more phenolic substrate to gut microorganisms. Most anthocyanins can be biotransformed by microbiota before absorption producing other phenolic compounds, such as syringic and protocatechuic acids (H. Song et al., 2021), which are related to health benefits. Furthermore, Wu et al. (2020) also reported that the presence of anthocyanins encapsulated with soy protein promoted the biosynthesis of short-chain fatty acids by gut microbiota and modulated the microbial community. Therefore, the association of ANC with soy protein may contribute to a favorable microenvironment in the gut, where healthy metabolites can be produced due to higher anthocyanin availability, and microbiota can be modulated to maximize human positive benefits. In another work, Ribnicky et al. (2014) showed, in an in vivo animal study, that polyphenols extracted from *Artemisia dracunculus* L. (commonly named "Russian tarragon") complexed to soy protein enhanced the bioavailability of polyphenols and increased hypoglycemic activity in C57BL/6 mice (Ribnicky et al., 2014). The main polyphenols from *Artemisia* have low water solubility; therefore, they likely interacted with nonpolar surfaces of soy protein through reversible electrostatic interactions such as van der Waals forces. This is important to demonstrate that noncovalent interactions are also important to increase ANC stability during digestion.

Xiong et al. (2020) complexed polyphenols extracted from blueberry (*Vaccinium angustifolium*) pomace with 1:1 w/w pea:rice protein and assessed the stability of the polyphenols in a static in vitro GIT digestion model.

The protein–polyphenol complex was more stable and resulted in significantly higher polyphenol concentrations surviving the artificial GIT digestion process compared with extract from blueberry pomace alone. The formation of stable covalent C–N and C–S bonds between proteins and phenolic rings may account for the enhanced stability (Xu et al., 2022). Storage proteins from cereals, such as prolamins which are chemically characterized as containing a large amount of proline and glutamine amino acids (J. Song et al., 2021; Tapia-Hernández et al., 2019) also can be used as delivery systems for different bioactive compound especially because their amphiphilic properties facilitate the formation of micro/nanoparticles (J. Song et al., 2021). Some examples of prolamins and their sources are: zein (corn), gliadin (wheat), hordein (barley), secalin (rye), and kafirin (sorghum).

Caseins correspond to approximately 80% of the total proteins in bovine milk and the main types are $\alpha_{S1}$-, $\alpha_{S2}$-, $\beta$-, and K-casein (Casanova et al., 2021; Lucey & Horne, 2018). Due to their specific structures and physicochemical properties, these abundant milk proteins can also interact with polyphenols (Gong et al., 2021; He et al., 2016; Lang et al., 2019; Wei et al., 2018) and thus have been used as efficient carriers for bioactive compounds such as anthocyanins (Cui et al., 2022; Lang, Gao, et al., 2021; Liao et al., 2021). Recently, a study showed that the interactions between $\alpha$-casein or $\beta$-casein with blueberry anthocyanins, such as cyanidin-3-$O$-glucoside (C3G), can protect these pigments against degradation during simulated digestion (Lang, Gao, et al., 2021). The recovery of anthocyanins bound to $\alpha$-casein (0.042 mg C3G/ml) or $\beta$-casein (0.032 mg C3G/ml) in the simulated intestinal system was at least 3× greater compared with free anthocyanin (0.011 mg C3G/ml).

Hydrogen bonds and intermolecular forces are the major driving forces in the formation of casein–anthocyanin complexes (Gong et al., 2021). The active groups of casein amino acid residues can interact with the hydroxyl groups of the three rings (A, B, and C) of anthocyanins, which might help to protect it from cleavage and, consequently, improve its stability. Later, Lang, Li, et al. (2021) evaluated the effects of $\alpha$-casein on the absorption of blueberry anthocyanins and metabolites in plasma based on pharmacokinetic analysis. Two groups of rats were intragastrically administered blueberry anthocyanin with and without $\alpha$-casein complexation; blood samples were taken within 24 h after ingestion. Animals administered aggregate complex had 1.5–10.1-fold greater anthocyanins and metabolites in plasma compared with rats that received free blueberry anthocyanins (Lang, Li, et al., 2021). These results from in vivo experiments confirm the protective effects of $\alpha$-casein on anthocyanins and indicate that complexation with casein can facilitate flavonoids and their metabolites to enter blood circulation.

In another recent study, Cui et al. (2022) utilized casein combined with carboxymethyl cellulose to encapsulate anthocyanins into complexes by adding different raw materials sequentially during the complexation reaction. The complexes showed high encapsulation efficiency and effectively attenuated the degradation of anthocyanins during simulated intestinal digestion, especially when the particles were built with carboxymethyl cellulose added prior to anthocyanin and subsequently casein. The authors found that these results were due to the smaller particle size of the structure compared with the other sequence combinations, and its highly dense structure with better protection for anthocyanins (Cui et al., 2022). The carboxyl groups are anionic polysaccharides and can interact with the flavylium cations (A. Fernandes et al., 2020). Together with the surface charging effect of casein, which has a sponge-like structure, it may strengthen the complexation of anthocyanins. In the same study, the authors used a cellulose dialysis membrane to determine the bioaccessibility of anthocyanins and anthocyanin-loaded nanocomplexes after digestion. They found that the content of anthocyanins from noncomplexed particles that penetrated the dialysis membrane (which corresponded to the bioaccessible part) was 6.00 $\mu$g/ml. On the other hand, the concentration of anthocyanins passing through the dialysis membrane coming from the complexes I, II, and III were 10.22, 15.44, and 9.26 $\mu$g/ml, respectively. The authors hypothesized that the anthocyanins were protected from breakdown by digestive enzymes because they were encapsulated in the protein matrix (Cui et al., 2022).

Whey protein, which represents about 20% of the total milk protein, is comprised mainly by soluble proteins such as $\beta$-lactoglobulin (50%), $\alpha$-lactoalbumin (20%), immunoglobulins (10%), bovine serum albumin (10%), lactoferrin (3%), and lactoperoxidase (0.3%) (Ramos et al., 2016; Ren et al., 2021). Whey proteins are widely used as ingredients and additives in the food industry because of their nutritional and functional characteristics. Recently, these dairy proteins have been studied due to their capacity to form complexes with polyphenols, such as anthocyanins, leading to changes in structure, functionality, and nutritional value for both protein and anthocyanins (Condurache et al., 2021; Gowd et al., 2020; Khalifa et al., 2021). For instance, nanoparticles of $\beta$-lactoglobulin and anthocyanins extracted from red raspberry pomace were produced using a desolvation method combined with ultrasonication process leading to an encapsulation efficiency of approximately 77% (Salah et al., 2020). The anthocyanin–protein complexes were evaluated during simulated in vitro digestion and the results showed a significant protection of anthocyanin throughout transit in the whole digestive tract. Similar results have been recently found also for blueberry (Zang et al., 2021) and

mulberry anthocyanins (Khalifa et al., 2021) complexed with whey protein. The $\beta$-lactoglobulin has the ability to covalently and noncovalently bind together with anthocyanins. It has been shown that hydrophobic interactions (a type of noncovalent interaction) play the dominant role in the binding of anthocyanins and $\beta$-lactoglobulin, and that the binding process may occur spontaneously producing stable complexes (Meng et al., 2021). This may explain the resistance of anthocyanins through digestive tract when complexed with whey protein.

Oancea et al. (2017) used sour cherry (*Prunus ceresus* L) anthocyanins with heat-treated bovine $\beta$-lactoglobulins to prepare complexes. The complexed particles and free anthocyanins were subjected to a static in vitro GIT digestion model. The anthocyanins were protected within the protein core during the digestive process. $\beta$-lactoglobulin is used extensively as encapsulating material in functional foods because of its high nutritional value, biodegradability, biocompatibility, and pepsin resistance. Gowd et al. (2020) evaluated the effect of interactions between $\beta$-lactoglobulin and pelargonidin-3-*O*-glucoside in improving the bioavailability of this anthocyanin. The content of free anthocyanin after GI digestion was 73.59 $\mu$g/ml for noncomplexed anthocyanin, and 66.59 $\mu$g/ml for anthocyanin complexed with $\beta$-lactoglobulin, which was indicative of the greater complexation of anthocyanin with polysaccharides or proteins during the digestive process. The complexes resulted in protection and progressive subsequent release of pelargonidin-3-*O*-glucoside (Gowd et al., 2020).

In an interesting human study, Mueller et al. (2018) assessed in vivo pharmacokinetics of whey protein complexed with anthocyanins from bilberries. The investigators measured the content of intact anthocyanins and metabolites in intestinal effluent, plasma, and urine to determine whether the whey–anthocyanin complex affected accessibility and bioavailability of anthocyanins. The administration of complexed particles led to higher concentrations of anthocyanins and their metabolites in the urine when compared with non-complexed anthocyanins (Mueller et al., 2018). However, the whey protein capsules did not stabilize the anthocyanins in the bowel milieu. Because anthocyanins bound to whey protein were subjected to an extended duration of stomach occupancy, the authors proposed that absorption via the stomach might explain the high urinary concentrations of anthocyanins and their metabolites (Mueller et al., 2018).

In general, the delivery of anthocyanins in a protein complex can prevent the degradation of anthocyanins during GIT transit and improve anthocyanin bioaccessibility in the small intestine. The complexed particles can also prevent premature release of anthocyanins during gastric digestion, which may limit absorption in the stomach

but permit more delivery of intact molecules to the colon for catabolism at the gut microbiota level. Regarding the main mechanisms of interaction, anthocyanins can be conjugated with proteins by means of covalent and noncovalent binding, which can alter the secondary structure of proteins and entrap the flavonoids (Khalifa et al., 2022). Therefore, these protein-based complexes may control release rate of anthocyanins and consequently reduce its degradation leading to improvements in bioaccessibility and bioavailability (Y. Shen et al., 2022). Furthermore, anthocyanins might be oxidized to the equivalent quinones at physiological pH instead of undergoing full degradation. The quinone can react with protein's nucleophilic assembles preserving the phenolic structure until they reach the colon for catabolism and subsequent absorption.

# 5 | CHALLENGES AND FUTURE PROSPECTS

This review provided an overview of the interactions between anthocyanins and proteins, which can lead to improved anthocyanin bioaccessibility and bioavailability. In general, protein complexation seems to be an interesting alternative strategy for protection of these sensitive pigments during digestion, by acting as a carrier through the gastrointestinal tract, allowing a higher proportion of intact anthocyanins to successfully reach the colonic microbiota. The resulting catabolism of anthocyanins at the gut microbiome level and subsequent entry of bioactive phenolic metabolites into circulation benefits both the food industry and consumers by enhancing overall anthocyanin bioavailability and transport to human therapeutic targets. However, there are still many unanswered questions and aspects that need to be further elucidated. While generally the health relevance of anthocyanin pigments is enhanced by delivery on a protein carrier, the effects of complexation of the proteins has been underreported. Anthocyanins can be absorbed from the stomach and from the small intestine, but most anthocyanins arguably are catabolized at the gut microbiome level, and later enter circulation as phenolic metabolites. Therefore, delivery of more intact anthocyanins to the microbiome in the form of protein–anthocyanin complexes is clearly advantageous. On the other hand, proteins are typically broken into peptides and absorbed earlier (small intestine). So, if the components are consumed as anthocyanin–protein complexes and the complexes remain stable during early digestion in the GIT, how is protein digestion affected ultimately? The interaction between proteins and anthocyanins changes the functional and structural properties of proteins. There has been relatively little investigation on the metabolic fate of proteins delivered in the form

of protein–anthocyanin complexes. Besides that, the optimum dosage of protein to be used in combination with anthocyanins still needs to be determined as it influences the sensory and rheological properties of food and beverage products.

Also, different anthocyanin structures will have different binding forces and affinities to plant or animal derived proteins, and pretreatment/processing of native proteins will alter binding affinities. These factors need to be elucidated on a case-by-case basis. Another key challenge consists of understanding properly how other polyphenols, small molecules like fatty acids, glucose, various metabolites, and metal ions interfere with the interactions between proteins and anthocyanins, since competitive binding forces of polyphenols (or other molecules/minerals) with a particular protein may affect the complex formation between anthocyanins and proteins. Because in vitro experiments are not able to exactly mimic in vivo conditions, it is essential to evaluate more human clinical studies on anthocyanin bioavailability from anthocyanin–protein complexes, using larger numbers of participants. The evidence on anthocyanin bioaccessibility and bioavailability to date strongly suggest that targeted clinical trials are warranted to confirm the health benefits associated with delivery as from anthocyanin–protein complexes.

## AUTHOR CONTRIBUTIONS

**Haizhou Wu**: writing original draft; writing review editing; conceptualization; supervision. **Gabriel Oliveira**: writing original draft; writing review editing. **Mary Ann Lila**: writing original draft; writing review editing; supervision.

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## CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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