



# Can natural pigments in different emulgel phases stabilize each other against UV radiation? Anthocyanin and $\beta$ -carotene co-loaded in an emulgel based on soy protein isolate-gellan gum conjugates

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## ABSTRACT

Despite poor stability of natural pigments against degradation, using these colorants have attracted great interest due to their various beneficial effect on human health. Accordingly, in the present study, an emulgel based on soy protein isolate-gellan gum conjugate was fabricated via Millard reaction. Then, the effectiveness of emulgel on improving the stability of anthocyanin (ACN) and  $\beta$ -carotene (BC) with different loading concentration (5, 10, and 15 mg/mL) against UV-C irradiation was investigated. Degradation kinetic results exhibited the higher stability of ACN upon co-loading with BC, as the half-life of ACN in free aqueous solution, loaded and co-loaded in emulgel was found to be 0.698, 2.648 and 3.164 days, respectively. The emulgel effectively improved the stability of BC, as well, and no degradation was observed during storage time. The release studies of the pigments showed Fickian diffusion mechanism. Furthermore, their release patterns were found to be independent and differences among the release from individual or simultaneous loaded system were rather small. Overall, our findings elucidated the promising potential of co-loading within emulgel as a safe delivery system in stability enhancement of natural pigments.

## 1. Introduction

Color is one of the most important features of human food, which has a significant impact on the perception of flavor. Changes in natural color of food due to food processing along with increasing demand for variety of food colorants cause overuse of synthetic colorants, which are known for their adverse effects on human health such as allergenicity issues and cancer risks. Consequently, consumer demand for alternative natural colorants has extensively increased during the last decades (Downham & Collins, 2000).

Anthocyanins are the largest and most important class of natural water-soluble pigments based on the flavylium nucleus, which are responsible for blue, purple, and red colors of many flowers and fruits in nature. They belong to a large group of polyphenolic compounds called flavonoids, whose variation in glycosylation and acylation has led to numerous anthocyanins (He & Giusti, 2010). Anthocyanin-derived colorants serve as food colorants and in the European Union, they are recognized with the code of E-163 (Wrolstad & Culver, 2012).

Among lipophilic pigments, carotenoids are the most studied natural pigments, which have a C40 skeleton consisting of eight isoprene units

(C<sub>5</sub>H<sub>8</sub>) and show colors ranging from yellow to red. These compounds have a lot of structural diversity, being  $\beta$ -carotene as one of the most common and important one among more than 750 carotenoids found in nature (Solymosi et al., 2015).

Unlike synthetic colorants, natural pigments possess various potential health-promoting functions that make them suitable alternatives to synthetic colorants. Nonetheless, their low stability against environmental factors such as heat and ultraviolet (UV) radiation makes them highly prone to oxidation and degradation, which hinder their applications (Wrolstad & Culver, 2012). As a nonthermal decontamination technique, the effect of UV treatments including UV-A (320–400 nm), UV-B (280–320 nm), and UV-C (200–280 nm) on natural pigment has been reported in literatures (Ma et al., 2012). Results revealed that photodegradation of anthocyanins upon UV exposure occurs as a result of absorbing radiation energy or being attacked by UV-induced free radicals. In this regard, relevant research shows the most destructive effect of UV-C on anthocyanin (Ma et al., 2012) and  $\beta$ -carotene (Cvetković & Marković, 2008), due to its highest energy and ability to induce free radicals.

Extensive studies have been carried out to improve the stability of

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natural pigments against UV radiation, including pretreatments, optimization of extraction methods (Ngamwonglumlert et al., 2017), copigmentation (Cortez et al., 2017), as well as encapsulation via various approaches such as freeze drying, spray drying, and emulsification (Jurić et al., 2022). Among encapsulation techniques, emulsion gels (emulgels) are biphasic systems with both emulsion and gel structures, in which the dispersed phase is trapped within a gel network. It is evident that the structural properties of emulgels are highly dependent on the type of biopolymers (proteins, polysaccharides) and the gelation techniques (Mao et al., 2020).

Emulgels are mostly fabricated from soy protein, whey protein, starch granules, pectin, gellan, etc., or a mixture of them (Mao et al., 2020, Liang et al., 2024). Among these, considerable attention has been paid to soy protein isolate (SPI) as a plant protein due to its high nutritional properties, water solubility, and numerous functional applications such as gelling, foaming, and emulsifying agents (Shen et al., 2015). However, from a functional perspective, the weak structure and poor stability of emulgels stabilized only by SPI are the most important obstacles to their application in different food matrices (Zhao et al., 2023).

Numerous scientific reports have demonstrated that Maillard reactions, as a green modification strategy, can regulate the functional characteristics of biopolymers and resulted emulgels via formation of protein-polysaccharide conjugates (Liang et al., 2024). For instance, Zhao et al. (2023) found that emulgel prepared from heat-denatured soy protein isolate-soy oligosaccharide conjugate (HSPI-SOS) had higher hardness and water-holding capacity, along with a more compact microstructure.

Considering the coexistence of oil and water phases in emulgels, they are known as a suitable protection and delivery system for both hydrophilic and lipophilic compounds. In this regard, some studies reported the simultaneous incorporation of  $\beta$ -carotene and riboflavin (Zhang et al., 2022) or epigallocatechin gallate and  $\beta$ -carotene (Chen et al., 2021) within the emulgel matrices. On the other hand, the interaction of natural antioxidants with different solubilities is mentioned as one of the synergistic mechanisms by Becker et al. (2004). Also, the mutual effects of anthocyanins and lycopene (Phan et al., 2019) as well as anthocyanins and  $\beta$ -carotene (Phan et al., 2018) were investigated in terms of their bioaccessibility and bioactivities, and better health outcomes were reported for co-digestion of these bioactive compounds.

To the best of our knowledge, the simultaneous incorporation of natural food pigments in emulgels for enhanced protection against UV degradation has not been explored so far. Therefore, this study aims to fabricate an emulgel with soy protein isolate- gellan gum (SPI-GG) conjugate using the Maillard reaction to synergistically stabilize and preserve anthocyanins and  $\beta$ -carotene against UV radiation. Then, the possible synergistic effect and the release kinetics of the aforementioned pigments were investigated in a model system.

## 2. Materials and methods

### 2.1. Materials

The powder of anthocyanins (ACN) from purple carrot and the liquid solution of  $\beta$ -carotene (BC) were obtained from Diana-Food Co. (France). Gellan gum (GG) (low acyl,  $\geq 85$  % gellan content) and soy protein isolate (SPI) (90 % protein content) were supplied from Amstel Products BV Co. (Netherlands) and Yuwang Co. (China), respectively. Oila® sunflower oil was purchased from a local store. All other chemicals were of analytical grade and purchased from Merck Co. (Darmstadt, Germany) and Sigma-Aldrich Co. (St. Louis, MO, USA).

### 2.2. Preparation of emulgel

A SPI-GG conjugate-based emulgel was prepared according to our

previous study (unpublished data). In brief, 0.1 g SPI and 0.3 g GG were fully dissolved in 100 mL of phosphate buffer with pH adjusted to 7, under continuous stirring at room temperature. The desired conjugates were prepared through the Maillard reaction by heating the GG-SPI solution in a water bath at 90 °C for 90 min. After cooling down to 25–30 °C, sunflower oil was added to the conjugate solution at a ratio of 3:1, and homogenization was done using Ultra-Turrax (T25 IKA-Labortechnik, Staufen, Germany) at 10,000 rpm for 3 min. Calcium chloride as a gelling agent was added to the emulsion at a final concentration of 0.2 mol/L. The resulting solution was stored in the refrigerator for 24 h to complete the gelation and form the emulgel. The physical properties of emulsion and emulgel based on SPI-GG conjugate are given in supplementary document (Table S1).

### 2.3. Investigating the effect of co-loading of anthocyanins (ACNs) and $\beta$ -carotene (BC) on their UV stability

#### 2.3.1. Co-loading of pigments

For this purpose, during emulgel preparation and before homogenization, ACN was dissolved in the conjugate solution (Section 2.2) at concentrations of 5, 10, and 15 mg/100 mL (denoted respectively as A5, A10, and A15) and BC at similar concentration levels (denoted respectively as B5, B10, and B15), was dissolved in the sunflower oil. Then, the pigment-containing phases were used to prepare emulgels with different ACN/BC ratios (9 samples denoted as A5B5 to A15B15) in the same way as mentioned in the Section 2.2.

#### 2.3.2. Exposure to UV radiation

To study the stability of pigments against UV radiation, a 25 × 25 × 50 cm box with 2 UV lamps (UV-C Osram 4 W) was designed in order to provide a power of 6.4 mW/cm<sup>2</sup> at a distance of 20 cm from the surface of the samples. Inside of the box was covered with aluminum foil, and the temperature was kept at 25 °C using a small fan. Emulgels were placed in the box, and sampling was done at predetermined intervals.

#### 2.3.3. Pigments extraction and measurement

The extraction of pigments from emulgels was conducted according to Lu et al. (2022) with slight changes. Regarding BC, 2 g of the emulgel was extracted using 7 mL of *n*-hexane and vigorously mixed on vortex. The solution was centrifuged at 4000g for 5 min and the supernatant was collected. This extraction procedure was repeated another two times. The content of BC in combined *n*-hexane phases was determined using a spectrophotometer (Rayleigh, UV-2601, Beijing, China) at 450 nm according to Wimalasiri et al. (2017).

To extract ACN, 2 g of emulgel was dispersed in 7 mL of deionized water and vortexed well. The resulting mixture was then centrifuged at 4000 xg for 5 min and the lower aqueous phase containing ACN was separated. This process was repeated once more time and the combined aqueous phases were analyzed for ACN content using pH differential method (AOAC 2005.2) (Lee et al., 2005) according to the following equation (Eq. 1):

$$\text{Total monomeric anthocyanins (mg/L)} = \frac{A \times MW \times DF \times 10^3}{\epsilon \times l} \quad (1)$$

where A is  $(A_{526nm} - A_{700nm})_{pH1.0} - (A_{526nm} - A_{700nm})_{pH4.5}$ , MW is the cyanidin-3-glucoside molar weight (449.2 g/mol), DF is the dilution factor, l is the light path length (1 cm) and  $\epsilon$  is the molar extinction coefficient (26,900 L mol<sup>-1</sup> cm<sup>-1</sup>).

#### 2.3.4. Kinetics studies of pigments degradation

The degradation kinetics of pigments were studied using first-order (Eq. 2) and Weibull (Eq. 3) models.

$$C_t = C_0 \exp(-k \times t) \quad (2)$$

$$C_t = C_0 \exp \left[ - (k \times t)^\beta \right] \quad (3)$$

where  $C_t$  is the pigments concentration at time  $t$  (mg/100 mL),  $C_0$  is the initial pigment concentration (mg/100 mL),  $t$  is time (days), and  $k$  is the rate constant ( $\text{day}^{-1}$ ), and  $\beta$  is the shape parameter which indicates concavity or convexity of the fitted curve when it takes values below and above 1, respectively. Furthermore, the half-life of pigments was calculated using Eq. 4 for the first-order model and Eq. 5 for the Weibull model (Lavelli & Sereikaitė, 2022).

$$t_{1/2} = \frac{-\ln(0.5)}{k} \quad (4)$$

$$t_{1/2} = \frac{\ln(2)^{1/\beta}}{k} \quad (5)$$

### 2.3.5. Color determination of emulgels

Color analysis of the emulgels was conducted using a colorimeter (Chroma meter CR-410, Konica Minolta, Japan) in the CIELab coordinates, and the color parameters including  $L^*$  (lightness),  $a^*$  (red/green),  $b^*$  (yellow/blue), and  $\Delta E^*$  (total color difference, Eq. 6), were obtained.

$$\Delta E^* = \sqrt{\Delta a^{*2} + \Delta b^{*2} + \Delta L^{*2}} \quad (6)$$

## 2.4. Effect of matrix on UV stability of pigments

In order to investigate the effect of matrix on the UV stability of pigments, four treatments including ACN loaded emulgel (A-LE), BC loaded emulgel (B-LE), free ACN solution in water (FAS), and free BC solution in oil (FBS) were prepared at a concentration of 10 mg/100 mL, and then the stability kinetics of pigments in different matrices were compared. In addition, (for more investigation of co-loading effect) ACN and BC co-loaded emulgel (each with a concentration of 10 mg/100 mL, A10B10) was used to compare with these samples. UV exposure, extractions, measurements, and the degradation kinetics of pigment, were carried out according to procedures as mentioned in previous sections.

## 2.5. Pigments release study

### 2.5.1. Pigments loading, sampling and measurements

In order to study the mutual effect of ACN and BC on their release, three different emulgels were prepared and compared as follows: ACN-BC co-loaded emulgels (AB-CLE), ACN-loaded emulgel (A-LE) and BC-loaded emulgel (B-LE). The concentration was set at 10 mg/100 mL for each pigment. A cylindrical emulgel with 2 cm diameter, 1 cm height and 3 g weight was separated from the middle part of the emulgel. Afterwards, samples were immersed in 20 mL of release media at 25 °C. Deionized water as hydrophilic (based on EU regulation Council directive 82/711EEC) and n-hexane (according to Chinese National Standard (GB 31604.1–2015)) as lipophilic food simulants were chosen for ACNs and BC release, respectively (Wang et al., 2019).

For ACN, 2 mL of media were taken from the aqueous phase (water) at regular time intervals during 1 day and the total ACN was quantified using the pH differential method as mentioned before. For BC, sampling was conducted over 10 days by taking 1 mL of organic phase (hexan) at specified time intervals, and the released BC was measured using a spectrophotometer as mentioned previously.

### 2.5.2. Release kinetics

Some models including zero order (Eq. 7), first order (Eq. 8), Korsmeyer-peppas (Eq. 9), Weibull release model (Eq. 10) and Lonsdale-baker model (Eq. 11) were used to determine the kinetics of pigments release from emulgels. The release data were fitted via the KinetDS3 program.

**Table 1**

GoF and kinetic parameters of ACN UV degradation in co-loaded emulgels.

Emulgels	Weibull model					
	GoF			Kinetic parameters		
	R <sup>2</sup>	SER	AIC	k (day <sup>-1</sup> )	T <sub>1/2</sub> (day)	β
A5B5	0.992	0.151	-14.039	0.363 ± 0.002 <sup>a</sup>	2.564 ± 0.395 <sup>b</sup>	2.156 ± 0.166 <sup>ab</sup>
A5B10	0.984	0.197	-12.063	0.317 ± 0.020 <sup>abc</sup>	2.657 ± 0.161 <sup>b</sup>	2.119 ± 0.040 <sup>abc</sup>
A5B15	0.991	0.191	-11.382	0.316 ± 0.019 <sup>abc</sup>	2.703 ± 0.220 <sup>b</sup>	2.559 ± 0.055 <sup>a</sup>
A10B5	0.985	0.361	-5.113	0.2434 ± 0.014 <sup>bcd</sup>	3.128 ± 0.368 <sup>ab</sup>	2.042 ± 0.123 <sup>abc</sup>
A10B10	0.985	0.388	-9.152	0.255 ± 0.028 <sup>bcd</sup>	3.164 ± 0.593 <sup>ab</sup>	2.219 ± 0.056 <sup>ab</sup>
A10B15	0.985	0.457	-5.876	0.322 ± 0.007 <sup>ab</sup>	2.616 ± 0.053 <sup>b</sup>	1.923 ± 0.123 <sup>bc</sup>
A15B5	0.990	0.436	-6.829	0.223 ± 0.039 <sup>d</sup>	4.232 ± 0.009 <sup>a</sup>	1.825 ± 0.163 <sup>bc</sup>
A15B10	0.991	0.412	-7.192	0.225 ± 0.038 <sup>cd</sup>	3.185 ± 0.054 <sup>ab</sup>	1.837 ± 0.345 <sup>bc</sup>
A15B15	0.993	0.301	-8.551	0.196 ± 0.011 <sup>d</sup>	4.093 ± 0.329 <sup>a</sup>	1.515 ± 0.034 <sup>c</sup>

Means with different letters are significantly different (based on Tukey test) ( $p < 0.05$ ).

A (5 to 15) and B (5 to 15) refers to ACN (5 to 15 mg/100 mL) and BC (5 to 15 mg/100 mL) respectively.

$$Q = (k \times t) + Q_0 \quad (7)$$

$$\frac{1}{Q} = (k \times t) + \frac{1}{Q_0} \quad (8)$$

$$Q = k \times t^n \quad (9)$$

$$Q = 100 \left( 1 - \exp \left[ \frac{-(t)^\beta}{a} \right] \right) \quad (10)$$

$$\frac{3}{2} \left[ 1 - (1 - Q)^{\frac{2}{3}} \right] - Q = k \times t \quad (11)$$

where  $t$  is time (min),  $Q_0$  is the released concentration of pigment at  $t = 0$  (%),  $Q$  is the released concentration of pigment at time  $t$  (%),  $k$  is the equation rate constant,  $n$  is the release exponent,  $\beta$  is the shape parameter, and  $a$  is the scale parameter.

## 2.6. Statistical analysis

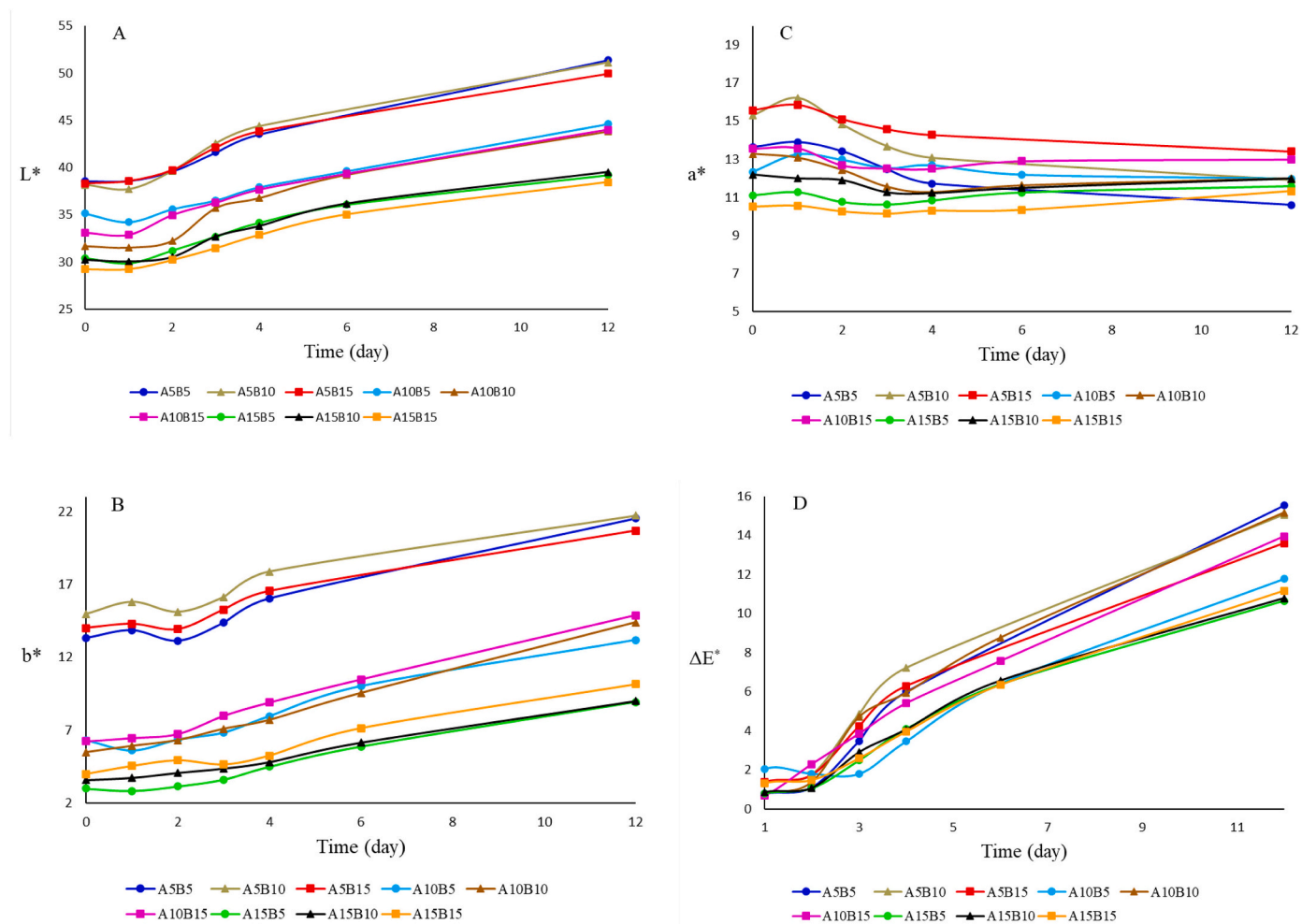
All the experiments were performed at two replicates and results were reported as mean ± standard deviation. Data were analyzed using Minitab version 20. A completely randomized design in factorial arrangement was used to study the mutual effect of pigments on their stability. The matrix effect and the release kinetics were also analyzed using a completely randomized design, and t-Student test, respectively. The statistical difference ( $p < 0.05$ ) was determined by computing the F-value at  $p < 0.05$  using Tukey test.s.

## 3. Results and discussion

### 3.1. Co-loading effect on UV-stability of pigments

#### 3.1.1. Anthocyanins

Retention of co-loaded pigments was monitored during 6 days of exposure to UV radiation (Fig. S1 A), and the results were fitted to the first-order and the Weibull models. The coefficient of determination ( $R^2$ ), standard error of regression (SER), and Akaike information



**Fig. 1.** Color parameters changes during UV exposure in co-loaded emulgels: A:  $L^*$  (lightness/darkness) B:  $b^*$  (yellowish/blueness) C:  $a^*$  (redness/greenness) D:  $\Delta E^*$  (total color changes).

criterion (AIC) were used as the goodness of fit (GoF) criteria to compare the fitted models. Although based on GoF parameters, both mathematical models had a good fit to the ACN degradation data, the Weibull model was selected considering higher  $R^2$  and lower AIC and SER values. Accordingly, statistical analysis was performed on the kinetic parameters obtained from the Weibull model (Table 1).

As could be seen from Table 1, the ACN degraded quite fast under UV irradiation. However, over the concentration range studied (5 to 15 mg/100 mL), higher concentration was accompanied by notable decrease in the degradation rate constant ( $k$ ), and enhancement in the half-life. This means that the stability of ACN improved at higher concentrations. This could be due to intramolecular and intermolecular hydrogen bonding with other ACN or phenolic compounds (molecular stacking). Probably, by forming networks of phenolic compounds and hydrogen bonds, the adverse effect of losing hydrogen and/or the formation of radicals is spread throughout the network. Also, these interactions can cause spatial reorientation and the formation of more stable forms (Barry, 2013). The positive effect of these interactions on ACN stability has been confirmed by Azman et al. (2022) and Malien-Aubert et al. (2001), as well.

As shown in Table 1, the change in BC concentration from 5 to 15 mg/100 mL in co-loaded emulgels had no significant effect on the kinetic parameters of ACN degradation. However, the results of statistical analysis showed that the simultaneous presence of ACN and BC (with mutual effects) had significant effects on all kinetic parameters of the ACN degradation. Additionally, in all emulgels, whenever the

concentration of ACN exceeded BC, lower rate constant and higher half-life was obtained, which proves the meliorate stability of ACN.

This mutual effect of pigments may be the result of antioxidant synergy through being in different phases (solubility) and having different antioxidant mechanisms, as Becker et al. (2004) pointed out in their research on synergy mechanisms of food bioactives. In another study, Chen et al. (2021) prepared emulgels containing only BC or epigallocatechin gallate (EGCG) and emulgels co-loaded with BC and EGCG. Assessing the stability of the aforementioned compounds in emulgels confirmed the mutual effect of BC and EGCG on each other. In addition to antioxidant synergistic effect, this result could be ascribed to changes in the polarity of the oil/water phases and interface, which leads to the change in the distribution and movement of compounds and free radicals.

It is also worthwhile to note that when both pigments were simultaneously loaded in emulgel, the shape parameter ( $\beta$ ) of ACN loss was greater than one (from 1.515 to 2.559), which means that the curve of ACN degradation follows a convex shape. Therefore, it can be concluded that the rate of ACN degradation in the early stages of UV treatment is less than that of in the final stages. The same result has been reported in previous study (Odriozola-Serrano et al., 2009).

### 3.1.2. $\beta$ -Carotene

During 12 days of storage under UV radiation, no significant loss was detected for BC content in any of the co-loaded emulgels (Fig. S1 B). These results show that the emulgel based on SPI and GG conjugate has

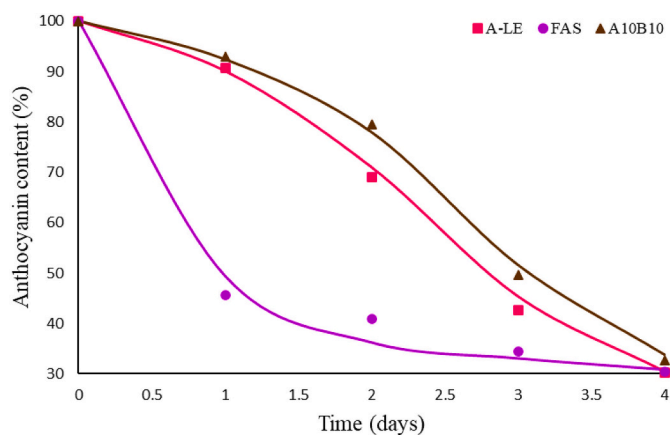


Fig. 2. UV degradation of ACN over time in A10B10, A-LE and FAS samples.

Table 2

GoF and kinetic parameters of ACN UV degradation in A10B10, A-LE and FAS samples.

Samples	Weibull					
	GoF			Kinetic parameters		
	R <sup>2</sup>	SER	AIC	k (day <sup>-1</sup> )	T <sub>1/2</sub> (day)	β
A10B10	0.985	0.388	-9.152	0.255 ± 0.028 <sup>a</sup>	3.164 ± 0.593 <sup>a</sup>	2.219 ± 0.056 <sup>a</sup>
A-LE	0.988	0.327	-13.245	0.329 ± 0.008 <sup>b</sup>	2.648 ± 0.072 <sup>a</sup>	2.703 ± 0.051 <sup>b</sup>
FAS	0.997	0.198	-10.956	0.408 ± 0.007 <sup>c</sup>	0.698 ± 0.346 <sup>b</sup>	0.302 ± 0.122 <sup>c</sup>

A10B10: ACN and BC co-loaded emulgel (10 mg/mL), A-LE: ACN loaded emulgel, FAS: Free ACN solution.

Means with different letters are significantly different (based on Tukey test) ( $p < 0.05$ ).

high potential and ability to protect and elevate the stability of BC as a fat-soluble pigment.

### 3.1.3. Colorimetry

Fig. 1 shows variation in color parameters of the emulgels in *CIE L\*a\*b\** color space as a function of pigment fractions and their nature during UV radiation. As can be seen in Fig. 1, the  $L^*$  of all samples decreased with the increase in ACN concentration from 5 to 15 mg/100 mL, while the effect of BC concentration on this parameter was negligible (Fig. 1A). This finding implies that ACN content was the most relevant variable for color of co-loaded emulgels and have had greater impact on the final color, as following the degradation of ACN over time,  $L^*$  was increased and the samples became brighter.

A reduction in  $b^*$  was observed along with increasing concentration of ACN. However, it enhanced in all samples over time and following the ACN degradation upon UV radiation, which can be attributed to the strengthening the effect of BC color (yellow color) (Fig. 1B). In a study, Bhat and Stamminger (2015) showed that the exposure of strawberry juice to UV radiation led to an increase in the  $L^*$  and  $b^*$  values as a result of rising the color effect of carotenoid compounds.

All co-loaded emulgels had positive  $a^*$  values, which did not change considerably over time (Fig. 1C). As mentioned earlier, with the degradation of ACNs and the preservation of BC, the color changed toward the yellow axis.

Fig. 1D presents  $\Delta E^*$  compared to the first measurement during the UV exposure. It is also worth mentioning that the slope of  $\Delta E^*$  diagrams changed slowly during the first 3 days of UV exposure. Thereby, the value of this change until the third day was less than 5 units in all samples, which means that the color changes are not perceivable by the

human eye (Hutchings, 1999). Considering the greater effect of ACN on the final color of the samples, the low rate of  $\Delta E^*$  change exhibited a good correlation with the shape parameter of the Weibull model, which indicates better preservation of ACN in the initial days.

## 3.2. Effect of matrix on UV stability of pigments

### 3.2.1. Anthocyanins

The role of emulgel in preserving ACN was assessed by measuring its content in three samples, including ACN and BC co-loaded emulgel (each with a concentration of 10 mg/100 mL, A10B10), ACN loaded emulgel (10 mg/100 mL, A-LE), and a free ACN aqueous solution (10 mg/100 mL, FAS), upon exposure to UV light and the results were displayed in Fig. 2 and Table 2. As shown in Fig. 2, the Weibull model fitted well and described the loss of ACN under UV radiation in all samples. Besides, Fig. 2 presents clear evidence of a dramatic reduction of ACN content in free form, which demonstrates the protective effect of emulgel on analyzed pigments. This data can be confirmed by statistical analysis of kinetic parameters of the Weibull model (Table 2), as FAS had the highest rate constant and the lowest half-life, followed by A-LE and A10B10, respectively. The shape parameter for the degradation of ACN loaded in emulgels (A10B10 and A-LE) was always greater than 1, while it was estimated to be 0.302 for FAS (free form). In this regard, Modesto Junior et al. (2023) also reported shape factor values of  $<1$  (concave curves) for ACN in non-capsulated extract solution.

These results could be justified by steric barrier of biopolymers in absorbing, reflecting, and scattering UV radiation (Guo et al., 2018), disturbing the movement of free radicals and prooxidants due to the reduction of free water in the gel network (Betz & Kulozik, 2011), and hydrogen and electrostatic interaction between ACN and SPI-GG conjugate (Zang et al., 2022). Also, the better stability of ACN in co-loaded emulgel (A10B10) compared to ACN loaded emulgel (A-LE) can be related to the synergistic effect of these pigments during the simultaneous loading, as explained in previous sections.

### 3.2.2. $\beta$ -Carotene

Regarding the overall effect of emulgel on the UV stability of BC, three samples including ACN and BC co-loaded emulgel (each with a concentration of 10 mg/100 mL, A10B10), BC loaded emulgel (10 mg/100 mL, B-LE), and free BC solution in oil (10 mg/100 mL, FBS) were prepared and exposed to UV light. As expected, the free BC (FBS sample) underwent more UV degradation over time and presenting better GoF in first-order model than the Weibull model and the average rate constant was found to be  $0.0557 \pm 0.0034$  (Fig. S2).

Emulgel based on SPI-GG conjugate was an effective system for protecting BC against UV radiation even without the presence of ACN. Despite the monitoring of loaded samples over 12 days, BC loaded in emulgel (B-LE) did not degrade significantly, so the kinetic parameters of degradation was not calculated. In accordance with our findings, Chen et al. (2016), concluded that compared to its free form, the incorporation of BC in emulgel caused a significant increase in its stability against UV radiation. This phenomenon can be explained by the presence of polymers at the interface and the continuous phase of the emulgel as a barrier against UV radiation and prooxidants (Chen et al., 2010), the antioxidant properties of the Maillard reaction products produced during the preparation of the SPI-GG conjugates (Augustin et al., 2006), and the possibility of hydrophobic interactions between BC and proteins leading to the creation of more stable forms of carotenoids (Wackerbarth et al., 2009), as well as the protective role of the strong gel network against physical stress and penetration of prooxidants into the oil phase (Chen et al., 2016).

## 3.3. Release kinetics

Considering the importance of release in delivery systems, the release kinetics of ACN and BC were studied. For this purpose,

**Table 3**

Kinetic parameters of ACN and BC release in water and hexane as food simulants.

	Sample	Korsmeyer-Peppas		Weibull
		k	n	$\beta$
ACN Release in water	A-LE	17.357 ± 1.07 <sup>a</sup>	0.186 ± 0.013 <sup>a</sup>	0.255 ± 0.022 <sup>a</sup>
	AB-CLE	15.481 ± 0.707 <sup>a</sup>	0.185 ± 0.006 <sup>a</sup>	0.245 ± 0.002 <sup>a</sup>
	B-LE	0.306 ± 0.016 <sup>b</sup>	0.316 ± 0.012 <sup>b</sup>	0.327 ± 0.013 <sup>a</sup>
BC Release in hexane	AB-CLE	0.384 ± 0.105 <sup>b</sup>	0.280 ± 0.043 <sup>ab</sup>	0.284 ± 0.044 <sup>a</sup>

AB-CLE: ACN and BC co-loaded emulgel (10 mg/mL), A-LE: ACN loaded emulgel, B-LE: BC loaded emulgel.

Means with different letters are significantly different (based on Tukey test) ( $p < 0.05$ ).

cylindrical samples from ACN and BC co-loaded emulgels (AB-CLE), ACN loaded emulgel (A-LE) and BC loaded emulgel (B-LE), were taken and placed in water or hexane to monitor the pigments release. Table S2 represents the release data from different system fitted to different kinetic models, both for the ACN and BC.

According to the GoF parameters (Table S2), the Korsmeyer-Peppas and Weibull release models were the most appropriate ones for describing the kinetic behavior of pigments release.

Although, the GoF parameters of the Weibull model were slightly better than those of Korsmeyer-Peppas, Weibull model was not chosen due to some drawbacks over Korsmeyer-Peppas model, such as the lack of parameter that is directly related to intrinsic dissolution rate (Malekjani & Jafari, 2021).

Regarding the dominant release mechanism, research has shown that the  $\beta$  parameter of the Weibull release model corresponds to the  $n$  parameter in the Korsmeyer-Peppas model, so that  $\beta < 0.75$  and  $n < 0.45$  indicate that the release obeys Fick's laws, while  $\beta > 0.75$  and  $n > 0.45$  indicate non-Fickian release and express more than one release mechanism (Malekjani & Jafari, 2021; Papadopoulou et al., 2006). Accordingly, the values of  $n$  and  $\beta$  obtained for the release of ACN and BC in water and hexane (Table 3) suggest that the predominant release mechanism in both water and hexane was Fickian diffusion.

From the graphs of Fig. 3, and the Korsmeyer-Peppas rate constant ( $k$ ) of Table 3, it was clearly shown that ACN was released significantly faster than BC. The release of ACN in water within 24 h reached around 60 %, while after 10 days, less than 7 % of BC was released in the hexane. Compare to the release of ACN in aqueous solution (Atay et al., 2018) and BC in lipophilic solution (Barbosa et al., 2021), the emulgel prepared based on SPI-GG conjugate shows a strong potential for delayed and sustained release of these compounds. This delayed release of

pigments might be due to getting trapped within the gel structure. Basically, gel-like emulsions are known as structures with a good capacity to control and delay the release of loaded bioactive compounds (Chang et al., 2022), especially for BC in this study, which is surrounded by biopolymers within oil droplets trapped in the gelled matrix.

Besides, although ACN and BC released slower when incorporated simultaneously than individually within emulgel, their mutual effect was rather small ( $p > 0.05$ ). Therefore, it can be concluded that the release of pigments is mainly affected by the structure of the emulgel. In consistent with our observation, Kamlow et al. (2022) also reported that the co-loading of hydrophilic and hydrophobic compounds in an emulgel had a negligible effect on their release kinetics.

#### 4. Conclusion

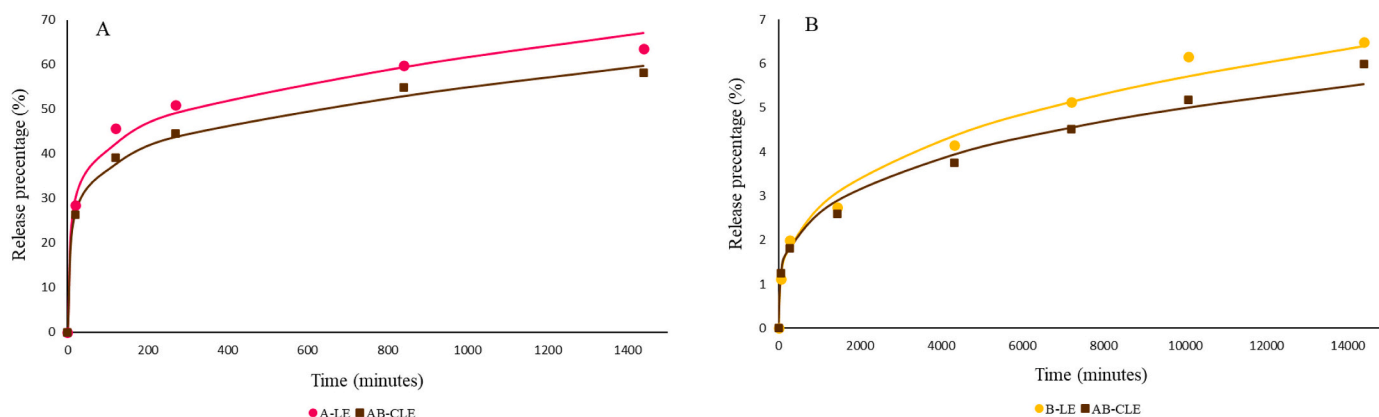
The present study intended to explore the role of emulgels prepared using SPI-GG conjugate in enhancement of the ACN and BC stability against UV radiation. Afterwards, the delivery of ACN and BC, individually or simultaneously loaded within emulgels, was investigated. Free ACN in aqueous solution was degraded too fast when it was exposed to UV light. Nonetheless, the kinetic results obtained by fitting the Weibull model showed that emulgel significantly raised the stability of ACN, particularly in the initial days. Unlike ACN, the BC was fully preserved over the time. Besides, the simultaneous loading of these pigments within emulgels showed synergistic effect on ACN stability as it was more preserved in the systems upon exposure to UV light. From the release study, it was found that BC could be more retained within emulgels than ACN. Moreover, both pigments had Fickian diffusion mechanism based on Korsmeyer-Peppas model and the simultaneous loading of compounds had no remarkable effect on their release kinetics.

#### CRediT authorship contribution statement

**Seyed Mahdi Tabatabaei:** Writing – original draft, Visualization, Validation, Software, Investigation, Formal analysis, Data curation. **Mehdi Varidi:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Methodology, Funding acquisition, Conceptualization. **Marzieh Moeenfar:** Writing – review & editing, Methodology, Conceptualization.

#### 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.



**Fig. 3.** Release percentage of pigments in food simulant over time: A: ACN release from A-LE and AB-CLE in water. B: BC release from B-LE and AB-CLE in hexane.

## 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.fochx.2024.101781>.

## References

- Atay, E., Fabra, M. J., Martínez-Sanz, M., Gomez-Mascaraque, L. G., Altan, A., & Lopez-Rubio, A. (2018). Development and characterization of chitosan/gelatin electrospayed microparticles as food grade delivery vehicles for anthocyanin extracts. *Food Hydrocolloids*, 77, 699–710.
- Augustin, M. A., Sanguansri, L., & Bode, O. (2006). Maillard reaction products as encapsulants for fish oil powders. *Journal of Food Science*, 71(2), E25–E32.
- Azman, E. M., Yusof, N., Chatzifragkou, A., & Charalampopoulos, D. (2022). Stability enhancement of anthocyanins from blackcurrant (*Ribes nigrum* L.) pomace through intermolecular copigmentation. *Molecules*, 27(17), 5489.
- Barbosa, A. E. G., Bastos, L. P. H., & Garcia-Rojas, E. E. (2021). Complex coacervates formed between whey protein isolate and carboxymethylcellulose for encapsulation of  $\beta$ -carotene from sachu inchi oil: Stability, in vitro digestion and release kinetics. *Food Biophysics*, 1–13.
- Barry, A. M. (2013). *Encapsulation, color stability, and distribution of anthocyanins from purple corn (Zea mays L.), blueberry (Vaccinium sp.), and red radish (Raphanus sativus) in a cold-setting pectin-alginate gel*. The Ohio State University.
- Becker, E. M., Nissen, L. R., & Skibsted, L. H. (2004). Antioxidant evaluation protocols: Food quality or health effects. *European Food Research and Technology*, 219, 561–571.
- Betz, M., & Kulozik, U. (2011). Whey protein gels for the entrapment of bioactive anthocyanins from bilberry extract. *International Dairy Journal*, 21(9), 703–710.
- Bhat, R., & Stammering, R. (2015). Impact of ultraviolet radiation treatments on the physicochemical properties, antioxidants, enzyme activity and microbial load in freshly prepared hand pressed strawberry juice. *Food Science and Technology International*, 21(5), 354–363.
- Chang, C., Li, J., Su, Y., Gu, L., Yang, Y., & Zhai, J. (2022). Protein particle-based vehicles for encapsulation and delivery of nutrients: Fabrication, digestion, and release properties. *Food Hydrocolloids*, 123, Article 106963.
- Chen, B., McClements, D. J., & Decker, E. A. (2010). Role of continuous phase anionic polysaccharides on the oxidative stability of menhaden oil-in-water emulsions. *Journal of Agricultural and Food Chemistry*, 58(6), 3779–3784.
- Chen, H., Lu, Y., Yuan, F., Gao, Y., & Mao, L. (2021). Effect of interfacial compositions on the physical properties of alginate-based emulsion gels and chemical stability of co-encapsulated bioactives. *Food Hydrocolloids*, 111, Article 106389.
- Chen, X.-W., Fu, S.-Y., Hou, J.-J., Guo, J., Wang, J.-M., & Yang, X.-Q. (2016). Zein based oil-in-glycerol emulgels enriched with  $\beta$ -carotene as margarine alternatives. *Food Chemistry*, 211, 836–844.
- Cortez, R., Luna-Vital, D. A., Margulis, D., & Gonzalez de Mejia, E. (2017). Natural pigments: Stabilization methods of anthocyanins for food applications. *Comprehensive Reviews in Food Science and Food Safety*, 16(1), 180–198.
- Cvetković, D., & Marković, D. (2008). Stability of carotenoids toward UV-irradiation in hexane solution. *Journal of the Serbian Chemical Society*, 73(1), 15–27.
- Downham, A., & Collins, P. (2000). Colouring our foods in the last and next millennium. *International Journal of Food Science & Technology*, 35(1), 5–22.
- Guo, J., Giusti, M. M., & Kaletunç, G. (2018). Encapsulation of purple corn and blueberry extracts in alginate-pectin hydrogel particles: Impact of processing and storage parameters on encapsulation efficiency. *Food Research International*, 107, 414–422.
- He, J., & Giusti, M. M. (2010). Anthocyanins: Natural colorants with health-promoting properties. *Annual Review of Food Science and Technology*, 1(1), 163–187.
- Hutchings, J. B. (1999). *Food color and appearance*. Gaithersburg, Md.: Aspen Publishers, Inc.
- Jurić, S., Jurić, M., Król-Kilińska, Ž., Vlahoviček-Kahlina, K., Vinceković, M., Dragović-Uzelac, V., & Donsi, F. (2022). Sources, stability, encapsulation and application of natural pigments in foods. *Food Reviews International*, 38(8), 1735–1790.
- Kamlow, M.-A., Holt, T., Spyropoulos, F., & Mills, T. (2022). Release and co-release of model hydrophobic and hydrophilic actives from 3D printed kappa-carrageenan emulsion gels. *Food Hydrocolloids*, 132, Article 107852.
- Lavelli, V., & Sereikaitė, J. (2022). Kinetic study of encapsulated  $\beta$ -carotene degradation in dried systems: A review. *Foods*, 11(3), 437.
- Lee, J., Durst, R. W., & Wrolstad, R. E. Collaborators: Eisele T Giusti MM Hach J Hofsommer H Koswig S Krueger DA Kupina, S Martin SK Martinsen BK Miller TC Paquette F Ryabkova A Skrede G Trenn U Wightman JD. (2005). Determination of total monomeric anthocyanin pigment content of fruit juices, beverages, natural colorants, and wines by the pH differential method: Collaborative study. *Journal of AOAC International*, 88(5), 1269–1278.
- Liang, P., Chen, S., Fang, X., & Wu, J. (2024). Recent advance in modification strategies and applications of soy protein gel properties. *Comprehensive Reviews in Food Science and Food Safety*, 23(1), 1–21.
- Lu, Y., Zhong, Y., Guo, X., Zhang, J., Gao, Y., & Mao, L. (2022). Structural modification of O/W bigels by glycerol monostearate for improved co-delivery of curcumin and epigallocatechin gallate. *ACS Food Science & Technology*, 2(6), 975–983.
- Ma, C., Yang, L., Yang, F., Wang, W., Zhao, C., & Zu, Y. (2012). Content and color stability of anthocyanins isolated from Schisandra chinensis fruit. *International Journal of Molecular Sciences*, 13(11), 14294–14310.
- Malekjani, N., & Jafari, S. M. (2021). Modeling the release of food bioactive ingredients from carriers/nanocarriers by the empirical, semiempirical, and mechanistic models. *Comprehensive Reviews in Food Science and Food Safety*, 20(1), 3–47.
- Malien-Aubert, C., Dangles, O., & Amiot, M. J. (2001). Color stability of commercial anthocyanin-based extracts in relation to the phenolic composition. Protective effects by intra- and intermolecular copigmentation. *Journal of Agricultural and Food Chemistry*, 49(1), 170–176.
- Mao, L., Lu, Y., Cui, M., Miao, S., & Gao, Y. (2020). Design of gel structures in water and oil phases for improved delivery of bioactive food ingredients. *Critical Reviews in Food Science and Nutrition*, 60(10), 1651–1666.
- Modesto Junior, E. N., Martins, M. G., Pereira, G. A., Chisté, R. C., Pena, R., & d. S. (2023). Stability kinetics of anthocyanins of Grumixama berries (*Eugenia brasiliensis* lam.) during thermal and light treatments. *Foods*, 12(3), 565.
- Ngamwonglumlert, L., Devahastin, S., & Chiewchan, N. (2017). Natural colorants: Pigment stability and extraction yield enhancement via utilization of appropriate pretreatment and extraction methods. *Critical Reviews in Food Science and Nutrition*, 57(15), 3243–3259.
- Odrizola-Serrano, I., Soliva-Fortuny, R., & Martín-Belloso, O. (2009). Influence of storage temperature on the kinetics of the changes in anthocyanins, vitamin C, and antioxidant capacity in fresh-cut strawberries stored under high-oxygen atmospheres. *Journal of Food Science*, 74(2), C184–C191.
- Papadopoulou, V., Kosmidis, K., Vlachou, M., & Macheras, P. (2006). On the use of the Weibull function for the discernment of drug release mechanisms. *International Journal of Pharmaceutics*, 309(1–2), 44–50.
- Phan, M. A. T., Bucknall, M., & Arcot, J. (2018). Interactive effects of  $\beta$ -carotene and anthocyanins on cellular uptake, antioxidant activity and anti-inflammatory activity in vitro and ex vivo. *Journal of Functional Foods*, 45, 129–137.
- Phan, M. A. T., Bucknall, M. P., & Arcot, J. (2019). Interferences of anthocyanins with the uptake of lycopene in Caco-2 cells, and their interactive effects on anti-oxidation and anti-inflammation in vitro and ex vivo. *Food Chemistry*, 276, 402–409.
- Shen, R.-L., Liu, X.-Y., Dong, J.-L., Si, J.-L., & Li, H. (2015). The gel properties and microstructure of the mixture of oat  $\beta$ -glucan/soy protein isolates. *Food Hydrocolloids*, 47, 108–114.
- Solymosi, K., Latruffe, N., Morant-Manceau, A., & Schoefs, B. (2015). Food colour additives of natural origin. In *Colour additives for foods and beverages* (pp. 3–34). Elsevier.
- Wackerbarth, H., Stoll, T., Gebken, S., Pelters, C., & Bindrich, U. (2009). Carotenoid–protein interaction as an approach for the formulation of functional food emulsions. *Food Research International*, 42(9), 1254–1258.
- Wang, C., Gao, W., Liang, Y., Jiang, Y., Wang, Y., Zhang, Q., & Jiang, G. (2019). Migration of chlorinated paraffins from plastic food packaging into food simulants: Concentrations and differences in congener profiles. *Chemosphere*, 225, 557–564.
- Wimalasiri, D., Brkljača, R., Piva, T. J., Urban, S., & Huynh, T. (2017). Comparative analysis of carotenoid content in *Momordica cochinchinensis* (Cucurbitaceae) collected from Australia, Thailand and Vietnam. *Journal of Food Science and Technology*, 54, 2814–2824.
- Wrolstad, R. E., & Culver, C. A. (2012). Alternatives to those artificial FD&C food colorants. *Annual Review of Food Science and Technology*, 3, 59–77.
- Zang, Z., Tang, S., Li, Z., Chou, S., Shu, C., Chen, Y., Chen, W., Yang, S., Yang, Y., & Tian, J. (2022). An updated review on the stability of anthocyanins regarding the interaction with food proteins and polysaccharides. *Comprehensive Reviews in Food Science and Food Safety*, 21(5), 4378–4401.
- Zhang, M., Yin, L., Yan, W., Gao, C., & Jia, X. (2022). Preparation and characterization of a novel soy protein isolate-sugar beet pectin emulsion gel and its application as a multi-phased nutrient carrier. *Foods*, 11(3), 469.
- Zhao, C., Chu, Z., Mao, Y., Xu, Y., Fei, P., Zhang, H., ... Liu, J. (2023). Structural characteristics and acid-induced emulsion gel properties of heated soy protein isolate-*soy oligosaccharide glycation conjugates*. *Food Hydrocolloids*, 137, Article 108408.