REVIEW PAPER

An update on the new achievements in the nanocapsulation of anthocyanins

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ABSTRACT

Natural food pigments are commonly utilized for the improvement of the qualitative properties of foods and/or inhibit the development of chronic and degenerative diseases. Several studies have documented the beneficial health effects of natural food pigments, such as anthocyanins, chlorophylls, and carotenoids. These effects mainly depend on the stability, bioactivity, and bioavailability of these pigments. Various techniques have been used to encapsulate natural pigments. Anthocyanins are a member of flavonoid groups, which are responsible for attractive food colors. Due to the positive surface charge of anthocyanin molecules, they absorb light and gain color. The micro- and nano-encapsulation of ingredients using natural polymers are important techniques to improve their stability, solubility, and bioavailability. This review study aimed to elaborate on the recent advancement in the encapsulation of anthocyanin as an attractive natural pigment using five techniques, including coacervation, spray drying, liposomal system, electrospraying, and microwave-assisted encapsulation methods.

Keywords: *Anthocyanins, Bioavailability, Bioactivity, Nano-encapsulation, Polymers*

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INTRODUCTION

Recently, the potential health benefits of natural food pigments have attracted the attention of researchers. Evidence suggests that natural pigments such as anthocyanins, carotenoids, and betalains have antimicrobial and antioxidant properties [1-5]. Furthermore, natural pigments are extensively used as coloring agents in various foods, while there are some limitations to their application. For instance, natural pigments might be lost during the storage period and food processing. Moreover, some of these agents (e.g., anthocyanins) are extremely sensitive to pH and high temperature [6]. It is also notable that the efficiency of active compounds in disease prevention depends on their bioaccessibility [7].

Micro- and nano-encapsulation are broadly employed as efficient techniques to enhance the stability and bioactivity of food ingredients. Various techniques are used to encapsulate natural pigments, such as spray drying, spray coacervation (simple and complex), fluidized bed coating, liposome, extrusion, and inclusion complexation [8]. Several reports have been focused on the encapsulation of various natural pigments using biopolymers [9-13]. However, no comprehensive studies have reviewed the recent advances in micro- and nano-encapsulation and controlling the release of these natural pigments. This review study aimed to elaborate on the updated data on the nano-encapsulation of anthocyanins, as well as the recent advancement in the encapsulation of anthocyanins.

ANTHOCYANINS

Anthocyanins are a member of flavonoid groups, which are responsible for attractive food

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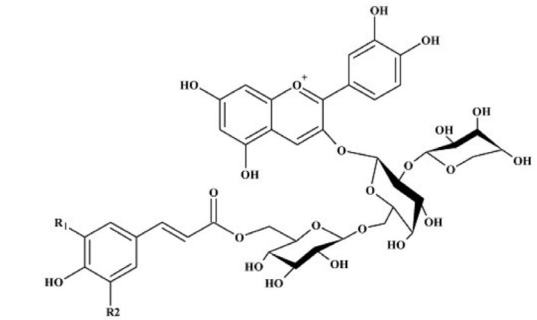
colors. Due to the positive surface charge of anthocyanin molecules, they absorb light and gain color. From a chemical perspective, the structure of anthocyanins is composed of a sugar esterified at three positions, which make anthocyanins water-soluble [14]. The general chemical structure of anthocyanins is depicted in Fig 1.



R_1	R_2
OCH3	OCH3
OCH3	H
H	H

Fig 1. General Chemical Structure of Anthocyanins (26)

According to the literature, anthocyanins are widely used owing to their beneficial health effects on the human body. For instance, anthocyanins have been reported to inhibit digestive enzymes and the DNA damage in cancer cells [15, 16], while reducing inflammatory responses [17] and improving the tightness of blood vessels [18].

However, the stability of anthocyanins is affected by various factors, such as pH, light, and temperature. Furthermore, the low bioaccessibility of anthocyanins has cast doubts on their beneficial health effects. Therefore, it is essential to explore new and effective strategies to protect these pigments against deteriorative factors. A broad range of techniques have been employed in the literature for the encapsulation of bioactive compounds [19, 20]. In the following section, we will discuss the recent advances in the improvement of the stability of anthocyanins against environmental conditions using various encapsulation methods (Table 1).

ENCAPSULATION OF ANTHOCYANINS

Coacervation technique

Coacervation is considered to be an important method for the encapsulation of phytochemicals. The electrostatic interaction between oppositely charged molecules is the main driving force in this technique. In simple coacervation, the electrostatic interaction between the charged bioactive component and the oppositely charged polymer leads to the fabrication of particles, while complex coacervation occurs with the electrostatic interaction of the positively and negatively charged polymers [21-23]. In general, the coacervation technique involves five steps:

Table 1. Recent Advances in Encapsulation of Anthocyanins Using Various Methods

Encapsulation Technique	Encapsulator	Reference
Coacervation	Chitosan	Ko Lee (25)
Coacervation	Whey Protein Isolate (WPI) and Beet Pectin (BP)	Arroyo-Maya and McClements (26)
Coacervation	*Hibiscus sabdariffa* L. Polysaccharide	De Moura Berling (29)
Coacervation	Pectin	Ge Yue (31)
Coacervation	Cress Seed Gum Arabic Gum Maltodextrin	Jafari Mahdavi-Khazaei (30)
Coacervation	Chitosan/Chondroitin Sulfate	Tan Selig (33)
Coacervation	Chitosan/Chondroitin Sulfate	Tan Celli (34)
Coacervation	Chitosan/Chondroitin Sulfate	Tan Celli (35)
Coacervation	Chitosan Hydrochloride and Carboxymethyl Chitosan	Ge Yue (31)
Spray Drying	Maltodextrin, Gum Acacia, and Tricalcium Phosphate	Nayak and Rastogi (37)
Spray Drying	Maltodextrin	Osorio Acevedo (39)
Spray Drying	Maltodextrin or Soybean Protein Isolate	Robert Gorena (42)
Spray Drying	Maltodextrin/Arabic gum, Maltodextrin/Gelatin and Maltodextrin	Mahdavi Jafari (43)
Spray Drying	Maltodextrin/Arabic Gum, Soluble Starch	Idham Muhamad (46)
Spray Drying	Maltodextrin/Arabic Gum, Maltodextrin/Gelatin and Maltodextrin Maltodextrin/Arabic Gum, Soluble Starch	Idham Muhamad (44)
Spray Drying	Maltodextrin/Arabic Gum, Soluble Starch maltodextrin/Cyclodextrin/Arabic Gum	Burin Rossa (47)
Spray Drying	Fenugreek Gum/ Maltodextrin/Microcrystalline Cellulose	Yousefi Emam-Djomeh (50)
Spray Drying	β-glucan and β-cyclodextrin	Ahmad Ashraf (53)
Spray Drying	Citrus Pectin	Mueller Jung (54)
Spray Drying	Maltodextrin/Arabic Gum/Sodium Caseinate	Cai (55)
Spray Drying	Arabic Gum and Maltodextrin	Xue Su (56)
Spray Drying	Polyvinylpyrrolidone	Da Fonseca Machado Rezende (57)
Electrospray	Chitosan, Gelatrin	Atay Fabra (60)
Liposomal Systems	-	Guldiken Gibis (63)
Liposomal Systems	Lecithin, Cholesterol	Hwang Kuo (62)
Liposomal Systems		Zhao Temelli (64)
Microwave-assisted	Maltodextrin/Arabic Gum	Mohd Nawi Muhamad (66)

1-Preparation of oil/water emulsion for the lipophilic phytochemicals in the oil phase;
2-Mixing of the prepared emulsion under extreme mechanical stirring;
3-Induction of three immiscible phases;
4-Cooling;
5-Cross-linking (optional)
This method could be employed for the encapsulation of both non-polar and polar molecules [24]. Since these processes are carried out at room temperature, they are able to encapsulate sensitive phytochemicals, such as anthocyanins. However, this technique has some limitations, as follows [8]:
1-The fabricated coacervate is often unstable;
2-The particles have a broad size range;
3-The steps of this method are complex;
4-The particles obtained by this technique are not perfectly spherical;
5-This method is relatively costly.

Recent advances in the encapsulation of anthocyanin using the coacervation technique

In a recent study, a nano-encapsulation system based on chitosan was employed to improve the stability of black soybean anthocyanin [25]. Moreover, the ionic gelation method was used to encapsulate the pigment, the stability of which was evaluated in terms of color and antioxidant activity. According to the findings, the encapsulation of anthocyanin resulted in the significant improvement of the color and antioxidant activity of the pigment. Furthermore, the authors assessed the effects of copigmentation as the loose combination of anthocyanins with other substrates (mostly flavonoids) on the stability of anthocyanin. To this end, they copigmented anthocyanin with sinapic acid, and the maximum stability of anthocyanin was obtained for the copigmented anthocyanin that was loaded into the nanoparticle-based sodium tripolyphosphate and chitosan, demonstrating that the combination of both techniques could be effective in enhancing the stability of the pigment. Therefore, it was concluded that since the combination of copigmentation and nanoencapsulation could enhance the color and antioxidant activity of anthocyanin, it could be an efficient strategy to preserve anthocyanin against deteriorative factors. Due to diverse health-promoting features, the combination of copigmented and nanoencapsulated anthocyanin of black soybean could be practically used in food and nutrition industry.

In another study, an encapsulation system was fabricated from whey protein isolate (WPI) and beet pectin (BP) using the coacervation method [26]. As mentioned earlier, complex coacervation is a specific type of polyelectrolyte complexation, which is achieved by the cross-linking of two biopolymers with opposite charges. For instance, when pH reduced to 6.3, the amino groups on the backbone of chitosan were ionized, thereby causing a positive charge. The ionization led to an electrostatic interaction between negatively and positively charged polymers [27]. In the mentioned study, the authors observed that at the pH of 4, the diameter of the developed nanoparticles was less than 200 nanometers, while the size of the particles increased at both lower and higher pH, which was attributed to the particle flocculation [26].

According to the literature, particle adsorption depends on the size of particles, and decreased particle size is associated with higher adsorption. From a pharmaceutical perspective, particles with submicron size are desirable for such purposes [28]. Therefore, the developed nanoparticle-based WPI and BP have proper adsorption in the body. Anthocyanins are highly sensitive to heating. As expected, the heat stability of anthocyanins improved after encapsulation using the coacervation method. Furthermore, the authors observed that the antioxidant capacity of the encapsulated anthocyanins was lower compared to pure pigment, which may be due to the thermal degradation of the pigments during particle development and the interaction of the pigments with the biopolymers. Additionally, it was denoted that the applied encapsulation system did not inhibit the degradation of anthocyanins, and an alternative strategy should be developed to preserve the antioxidant activity and color of anthocyanins against deteriorative environmental conditions.

In another research, the thermal stability and antioxidant activity of pure anthocyanin and anthocyanin encapsulated by the coacervation technique were compared (De Moura, Berling) [29]. In the mentioned study, pectin was used as the carrier, and the encapsulated pigment was observed to have higher thermal stability compared to the free pigment. When the storage temperature increased from 5ºC to 25ºC, the values

of lightness and yellowness increased; however, the redness degree decreased. Furthermore, the antioxidant capacity of the encapsulated pigments was higher than the pure pigment. Therefore, it was concluded that the fabricated encapsulation system could be used as an excellent vehicle to preserve anthocyanin against oxidative reactions.

Recently, Jafari and Mahdavi-Khazaei [30] compared the encapsulation efficiency of encapsulated anthocyanins using cress seed gum as a conventional source of biopolymer, along with two encapsulation systems composed of commercial biopolymers, including Arabic gum and maltodextrin. The stability of the encapsulated anthocyanins in terms of color attributes was evaluated over the storage period (10 weeks at 35°C), and no significant difference was observed between the anthocyanin content of the tested formulation before and after the storage time ($P>0.01$), indicating the acceptable preservative effects on the qualitative properties of anthocyanin.

In a similar study, Ge Yue [31] attempted to encapsulate antocyanins into wall material composed of chitosan hydrochloride (CHC) and carboxymethyl chitosan (CMC) using electrostatic interactions. According to the findings, the combination of CHC and CMC with the respective ratio of 1.2:1 led to the formation of a nanocomplex with small particle sizes (178 nm), high encapsulation efficiency, high zeta potential, and acceptable polydispersity index. In addition, an absolute value of surface charge of higher than 30 mV caused the suspension to become stable. The results of transmission electron microscopy (TEM) also indicated a spheroidal shape for the nanocomplex. Recognition of the release mechanism of ingredients is a key factor to predicting the release of the bioactive compound and optimizing the release phenomena [32]. Several factors may alter the release behavior of ingredients from nanoparticles, such as molecular weight, surface charge, concentration of the ingredient, and morphological properties of the nanoparticles; the last factor largely influences the release properties of bioactive agents.

In a research in this regard, the stability of the control and encapsulated anthocyanins was compared at different temperatures, ascorbic acid concentrations, pH values, and fluorescent light intensities. According to the obtained results, the encapsulation pigments had higher stability at all the tested conditions. Overall, it could be suggested that the anthocyanins capsulated at the developed capsules had great potential for application in functional foods.

In another study, Tan Selig [33] attempted to encapsulate anthocyanin using a polyelectrolyte complexation between chitosan (positively charged biopolymer) and chondroitin sulfate (CS) (negatively charged polymer). Considering the acid association constant of these polymers, the pH of 3 was used, where both biopolymers produced charged polyelectrolyte complexes (PECs). The copigmented PECs had the highest encapsulation efficiency, and copigmentation led to the increased temperature stability of anthocyanin. Surprisingly, the color retaining of the copigmented samples was thrice the non-encapsulated samples. Accordingly, the coacervate was formed through the interaction between chitosan and chondroitin sulfate, which protected anthocyanin against adverse conditions.

In another research, Tan Celli [34] reported that copigmentation led to the intensified red color of both pure and chitosan/CS encapsulated anthocyanins. Furthermore, it increased the anthocyanin encapsulation efficiency due to the formation of a dense network through hydrogen bonding. Tan Celli [35] also investigated the influence of various pH (3.3, 4.0, and 5.0) and addition of various copigments (catechol, gallic acid, gallic acid ethyl ester, and (-)-epigallocatechin gallate) on the encapsulation of anthocyanin by chitosan and chondroitin sulfate. At the pH of 3.3 and 4.0, the addition of copigments enhanced the encapsulation efficiency of the developed nanoparticles. On the other hand, no significant effect was observed after copigmentation at the pH of 5.0. Use of copigmentation and encapsulation together had a synergistic effect on the preservation of anthocyanins against ascorbic acid attack at the pH of 3.3. The efficiency of the tested copigments to improve the emcapsulation efficiency was as follows: gallic acid ethyl ester > catechol > gallic acid > (-)-epigallocatechin gallate.

In another study, Ge Yue [31] improved the stability of anthocyanins through encapsulation. They developed nanocomplexes based on CHC and CMC. The optimal condition in terms of particle size, surface charge, PDI, and encapsulation efficiency was 1.2 grams of CHC to 1.0 gram of CMC (w/w, 8 mg of ACNs). In this condition, the nanoparticles had the highest encapsulation

efficiency (44.0%) with a small particle size (178.1 nm), high surface charge (+25.6 mV), and acceptable PDI value (0.315). In addition, the stability of the encapsulated anthocyanins against various temperatures, L-ascorbic acid (AA) content, and pH in the presence of white fluorescent light were compared with the control samples. In conclusion, it was suggested that the fabricated nanoparticles had great potential for use in foods and nutraceutical systems.

Spray drying technique

The spray drying method is commonly used for the encapsulation of ingredients, which has numerous advantages, such as cost-efficiency and reproductivity [36]. In the spray drying encapsulation method, the ingredient is dissolved or dispersed in the biopolymer solution, and the resulting solution/dispersion is atomized in a heated air chamber. The atomization process leads to the removal of the solvent and production of a dried particle containing the active ingredient entrapped into a capsule. Several studies have been focused on the encapsulation of anthocyanins using the spray drying technique.

Recent advances in the encapsulation of anthocyanin using the spray drying technique

The influence of maltodextrin, gum acacia, and tricalcium phosphate on the encapsulation of anthocyanin using the spry drying method has been evaluated by Nayak and Rastogi [37]. According to their findings, maltodextrin is an effective drying aid to encapsulate anthocyanin. Additionally, they reported that gum acacia and tricalcium phosphate improved the stability of the capsules. The optimal formulation for the encapsulation system was 5% maltodextrin, 0.25% gum acacia, and 0.25% tricalcium phosphate. In this formulation, the fabricated encapsulation system had the lowest hygroscopic moisture content, the highest amount of anthocyanin, and the maximum antioxidant activity. According to the SEM analysis, the formed capsules had a smooth shape with the particle size of 2-50 micrometers. The nanoparticles used in a drug delivery system should be large enough to prevent their rapid leakage into blood capillaries, while small enough to escape capture by the fixed macrophages that are lodged in the reticuloendothelial system (e.g., liver and spleen) [38]. The stability of the developed capsules was tested over the storage period at 4ºC and ambient temperature. According to the obtained results, the shelf-life of the samples stored at 4ºC was considerably higher compared to those stored at an ambient temperature. In another study [39], anthocyanin was isolated from the corozo fruit and encapsulated with maltodextrin using the spray drying method. The SEM images exhibited that the fabricated capsules had a spherical shape with the particle size of smaller than 50 micrometers. The thermal stability of the capsules was analyzed using differential scanning calorimetry (DSC) and thermogravimetric analysis (TGA). According to the obtained results, the capsules were stable until the temperature reached 100ºC. Polysaccharides such as maltodextrin have excellent thermal stability, and it is expected that encapsulated anthocyanins have higher thermal stability compared to pure anthocyanins. Mathematical modeling of the release of ingredients from micro-nanocapsules could be utilized in systems with the desired release profiles. Various models are used to predict the release of ingredients, such as the zero-order diffusion, first-order diffusion, Higuchi's diffusion, Korsmeyer-Peppas, and Hixson-Crowell models. The Zero-order or pseudo-zero-order diffusion model is as follows [8]:

$$M_t/M_\infty = k \cdot t \quad \text{Eq.1}$$

where M_t/M_∞ and k are the fractions of the ingredient released in time t and zero-order constant, respectively.

The first-order diffusion model is as follows [8]:

$$\ln (1 - M_t/M_\infty) = - k_1 \cdot t \quad \text{Eq. 2}$$

where k_1 is the first-order rate constant ($1.s^{-1}$) Higuchi's diffusion model is as follows:

$$Q_t = k \cdot (t)^{0.5} \quad \text{Eq. 3}$$

where M_t/M_∞ and k are the ingredients released in time t and release constant, respectively. Higuchi's diffusion model is commonly employed to describe the release of ingredients from the insoluble matrix [40].

The Korsmeyer-Peppas model is as follows:

$$M_t/M_\infty = k \cdot t^n \quad \text{Eq. 4}$$

where M_t/M_∞, n, and k are the fractions of the ingredient released in time t, release exponent, and release constant, respectively. The value of the release exponent indicates the release mechanism and is system geometry-dependent. The n value of equal to 0.43 shows the Fickian release mechanism, and the n values within the range of 0.43-0.85 reveal the anomalous or non-Fickian transport, and the n values of >0.85 represent case II transport [41].

The Hixson-Crowell model is as follows:

$$(1 - Mt/M\infty)^{\frac{1}{3}} = - k. t^{n} \quad \text{Eq. 5}$$

According to the findings of Osorio Acevedo [39], the pseudo-first-order model was optimal for describing the release of anthocyanin pigments. Therefore, it was concluded that the encapsulation of anthocyanin isolated from the corozo fruit with maltodextrin using the spray drying process could effectively preserve the physicochemical properties of this sensitive pigment.

In another study, Robert Gorena [42] encapsulated the anthocyanins obtained from pomegranate with maltodextrin or soybean protein isolate (SPI) using the spray drying method. The stability of the developed capsules was tested at the temperature of 60ºCover 56 days. Comparatively, the encapsulation efficiency of the formed capsules by the SPI was significantly better compared to those fabricated by maltodextrin. On the other hand, the authors observed that the protective effects of the capsules based on maltodextrin were more prominent compared to maltodextrin.In another study [43], anthocyanin extracted from bearberry was encapsulated by different wall materials (maltodextrin/Arabic gum, maltodextrin/gelatin and maltodextrin) using the spray drying method. The storage stability of the developed systems was analyzed at different temperatures and humidity over a storage period of 90 days. As was expected, the stability of the encapsulated anthocyanins was higher than the pure anthocyanins. Furthermore, the encapsulation system composed of maltodextrin/ Arabic gum showed the highest encapsulation efficiency and lowest degradation rate at all the tested temperatures and humidity; therefore, it was selected as the optimal formulation for the encapsulation of the extracted anthocyanins. In the mentioned study, the encapsulated pigment was also utilized as coloring agents in jelly. From a sensory perspective, the jelly containing 7% encapsulated anthocyanins had better sensory scores compared to those containing synthetic coloring agents. In addition, the jelly containing the encapsulated pigment had lower syneresis compared to commercial samples.

Idham Muhamad [44] produced microcapsules to extend the shelf life and bioavailability of Roselle anthocyanins using soluble starch, Arabic gum, maltodextrin, and a combination of Arabic gum and maltodextrin with the respective ratio of 40:60 as the wall materials. The temperature-dependency of the capsules at three temperatures was evaluated during the storage period (105 days), and the Arrhenius model was employed to evaluate the temperature stability of the samples [45]:

$$k = e^{(\frac{Ea}{R})/T} \quad \text{Eq.1}$$

In the equation above, *Ea* (kJ/kg mol), *R* (8.314 kJ/kg mol K), and *T* (K) are the activation energy, universal gas constant, and absolute temperature, respectively. Higher activation energy indicated increased temperature sensitivity. According to the obtained results, the shelf life of the encapsulated extract was significantly higher compared to the control samples. However, storage temperature had no significant effects on the color attributes of the fabricated capsules, while the type of the carrier had significant effects on these properties. Overall, the authors reported that the combination of Arabic gum and maltodextrin as the encapsulator provided the highest encapsulation efficiency, as well as the lowest degradation rate and color change.

In another study, Idham Muhamad [46] investigated the influence of higher temperatures (60ºC, 80ºC, and 98ºC) on the stability of Roselle anthocyanins. Similarly, they used soluble starch, Arabic gum, maltodextrin, and a combination of Arabic gum and maltodextrin with the respective ratio of 40:60 as the wall materials. As was expected, the authors observed that the thermal stability of the capsulated extract was significantly higher compared to the control samples. Furthermore, it was reported that the anthocyanins capsulated by the combination of gum Arabic and maltodextrin had the highest temperature stability, while those capsulated by soluble starch had less stability against heat treatment. In another research, Burin Rossa [47] attempted to encapsulate anthocyanins extracted from Cabernet Sauvignon grapes using different encapsulating agents, including maltodextrin (MA), cyclodextrin (C), and Arabic gum (AG). After the encapsulation of the ingredient, they evaluated the stability of encapsulated anthocyanin at various temperatures, as well as in the presence/ absence of a fluorescence lamp. For this purpose, they plotted the log anthocyanin content against time (40 days), and the following equations were employed to fit the obtained data:

$$\text{The slope of the straight line} = \frac{-k}{2.303} \quad \text{Eq. 2}$$

$$t_{\frac{1}{2}} = -\ln 0.5 \times k^{-1} \quad \text{Eq. 3}$$

In Equation 3, k and show the first-order kinetic constant and half-life time, respectively.

$$R\ (\%) = \left(\frac{A_t}{A_0}\right) \times 100 \quad \text{Eq. 4}$$

In Equation 4, A_t and A_0 are the absorbance read at time t and initial time, respectively.

According to the findings of the mentioned study, the encapsulator and tested treatments changed the k and values of anthocyanins, while they had no effect on the order of reaction. Therefore, degradation of anthocyanin as a function of time at the tested treatments followed the first-order kinetic. This observation is consistent with those reported by Wang and Xu [48], who claimed that the degradation of anthocyanin from different sources follows the mentioned model. In the mentioned research, when anthocyanin was encapsulated in the combination of MA+C or MA+AG, the k value was significantly less than the individual encapsulants, demonstrating that these combinations had higher efficiency in anthocyanin protection. Since the combination of MA+AG had the lowest k value, the authors considered it to be the optimal encapsulator for the protection of anthocyanin. This observation is probably due to the branched structure of Arabic gum, which could effectively entrap the encapsulated compounds. Furthermore, the presence of the proteins that covalently linked to polysaccharide chains improved the ability of Arabic gum to act as an encapsulator [49].

In a similar study, Yousefi Emam-Djomeh [50] used response surface methodology (RSM) to optimize the fabrication of capsules for maximum efficiency in the protection of anthocyanin extracted from black raspberry juice. In addition, they used three saccharides (fenugreek gum, maltodextrin, and microcrystalline cellulose) as the encapsulating agents. The results of RSM demonstrated that the optimal conditions to achieve the highest total anthocyanin content, total phenolic content, solubility, and powder recovery were the maltodextrin concentration of 20.0%, fenugreek concentration of 0.93%, and microcrystalline cellulose content of 1.74%. The highest amount of anthocyanin and phenolic compound was obtained in the samples capsulated by 20.0% maltodextrin, 1.0% fenugreek, and 1.50% microcrystalline cellulose. The hydrophilic functional groups (e.g., carboxyl and hydroxyl) in the structure of maltodextrin and fenugreek interacted with the hydrophilic groups of phenolic compounds, thereby providing a protectant for phenols [51]. Furthermore, the glass-like matrix formed by maltodextrin below the glass transition temperature led to the enclosing of anthocyanins and their protection against environmental conditions [52].

Ahmad Ashraf [53] used the spray drying technique to encapsulate saffron anthocyanins, with β-glucan and β-cyclodextrin utilized as the encapsulators. According to the findings, β-glucan could effectively improve the stability of anthocyanin during passage through the simulated conditions of the gastrointestinal tract. The developed encapsulation system also increased the bioavailability of anthocyanins in the intestinal section. Since both anthocyanin and β-glucan have diverse health effects, the use of β-glucan as a carrier for anthocyanins could provide additional health benefits.

Mueller Jung [54] investigated the potential application of citrus pectin to encapsulate anthocyanins from bilberries using the spray drying technique. After encapsulation, the authors analyzed the degradation rate of the tested anthocyanins in the urine, plasma, and ileal effluent of healthy volunteers and ileostomies. It was observed that with the use of citrus pectin for anthocyanin encapsulation, intestinal availability increased during passage through the small intestine.

In a recent study, Cai [55] used three encapsulating agents (40% maltodextrin, 34% maltodextrin with 6% Arabic gum, and 50% sodium caseinate) in order to improve the stability of anthocyanins from purple corn. To this end, the spray drying method was utilized, and the resulting microcapsules were tested in terms of morphology, encapsulation efficiency, and stability against light. Unexpectedly, the encapsulated samples were reported to have lower light stability compared to pure anthocyanin. Further analyses are required to explain the mechanism behind this unexpected phenomenon.

Xue Su [56] reported that the use of copigmentation and spray drying technique to encapsulate red-fleshed apple anthocyanins had profound effects on the enhancement of anthocyanin stability. They used caffeic acid and a mixture of Arabic gum and maltodextrin for copigmentation and encapsulation, respectively. The encapsulation efficiencies of the developed nanoparticles were within the range of 93.84-96.85%, and the particle size of the capsules was

smaller than 350 nanometers. In addition, the light stability analysis demonstrated that the half-life of anthocyanins increased considerably after treatment with copigmentation and encapsulation.

In a similar research, Da Fonseca Machado Rezende [57] compared the efficiency of the spray drying method with the freeze drying and supercritical antisolvent techniques. According to the findings, the particles with good anthocyanin yields and high antioxidant activity were obtained in all the tested methods.

From a morphological perspective, those obtained by the spry drying technique had spherical shape, while those fabricated by the other techniques had irregular agglomerates.

Selection of proper wall materials to preserve the quality of anthocyanin is essential. Finally, the results of the aforementioned studies indicated that maltodextrin could be used as an effective wall material for the encapsulation of anthocyanins. Furthermore, there is a synergistic effect between Arabic gum and maltodextrin, and their combination may be an appropriate candidate for use as an encapsulating agent.

Electrospray technique

Electrospray is another technique used for the encapsulation of ingredients. In this technique, nanoparticles are formed by the electrostatic force induced by high voltage.

One of the most prominent advantages of this method is the high encapsulation efficiency [58, 59].

Recent advances in the encapsulation of anthocyanin using the electrospray technique

In a recent study, electrosprayed particles based on chitosan/gelatin were fabricated as food-grade delivery vehicles for anthocyanin extracts (Fig 2) [60].

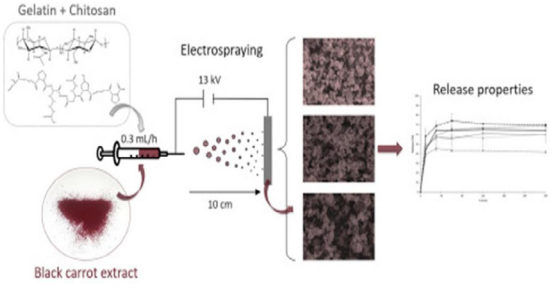


Fig 2. Schematic of Development and Characterization of Electrosprayed Particles Based on Chitosan/Gelatin (60)

The authors initially evaluated the effects of the molecular weight of chitosan, polymers, and solvent composition on the formation of the particles. As was expected, the electrostatic interaction between gelatin and chitosan led to the formation of clustered networks. In the next step, an anthocyanin-rich black carrot extract was incorporated into the fabricated chitosan/gelatin electrosprayed nanoparticles. In addition, evaluation of the encapsulation efficiency and release behavior of the loaded pigment into 10% ethanol and 3% acetic acid as food simulant demonstrated that the release of the pigment into the acetic acid medium was faster and more significant (Fig 2).

Liposomal system

Liposomes could be employed in the encapsulation, targeted delivery, and control release of water-soluble, lipid-soluble, and amphiphilic components. The formation of liposomes and nanoliposomes is based on the hydrophilic-hydrophobic interaction between phospholipids and water molecules. A major advantage of their use is the ability to control the release rate of the loaded components and their delivery to the right place at the right time [61].

Recent advances in the encapsulation of anthocyanin using the liposomal system

The inhibitory effects of liposomal-capsulated anthocyanin (LCA) obtained from *Hibiscus sabdariffa* Linn have been investigated by Hwang Kuo [62] through the assessment of several factors, such as melanin content, tyrosinase expression, and cell viability. The DPPH scavenging activity of anthocyanins was reported to improve significantly, and melanogenesis was observed to be inhibited when encapsulated through the LCA method. Furthermore, the encapsulated samples showed higher stability. In general, the authors suggested that LCA could be an appropriate encapsulation technique due to its protective effects on the skin.

In a study in this regard, Guldiken Gibis [63] investigated the effects of various liposomal systems on the color attributes and stability of anthocyanin at different ascorbic acid concentrations (Fig 3). After increasing lecithin concentration, the encapsulation efficiency of the system improved up to 50%. Additionally, the authors reported that the encapsulation of

anthocyanins led to the improvement of the color properties and their stability during the storage period. Overall, it was concluded that the use of the developed liposomal system could decrease the anthocyanin degradation caused by ascorbic acid (Fig 3).

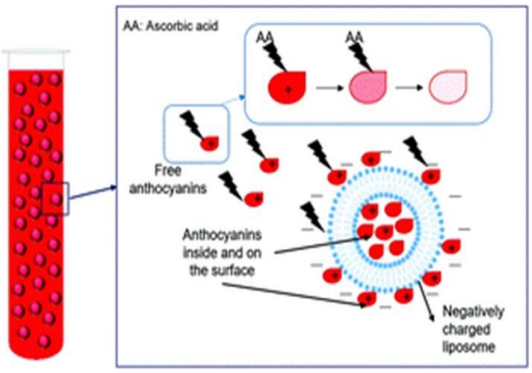


Fig 3. Liposomal System for Encapsulation of Anthocyanins

The effects of anthocyanin and sterol concentration on the physicochemical properties of liposomal-encapsulated anthocyanin have been investigated by Zhao Temelli [64]. For this purpose, various features of the fabricated capsules (particle size, zeta potential, encapsulation efficiency, loading capacity, and *in-vitro* release profile) were evaluated. The particle size, zeta potential, polydispersity index, and encapsulation efficiency of the liposomal-encapsulated anthocyanin were determined to be 159 nanometers, -40.2 mV, 0.244, and 50.6%, respectively. Following an increase in anthocyanin concentration, the particle size of the capsules increased, and their uniformity decreased. This effect may be associated with the reinforcement of the electrostatic interaction and hydrogen bonding between anthocyanin and phospholipids. The release profile of anthocyanin in the simulated gastric fluid was observed to be low, while it was rapid in the simulated intestinal fluid. The authors suggested that the developed particles have great potential to use in the formulation of functional foods.

Microwave-assisted encapsulation

Microwave-assisted encapsulation is a novel and economical encapsulation technique, which has high efficiency in preserving food colorants (e.g., anthocyanins). This method produces particles with low water activity, extending the shelf life of bioactive agents [65]. Since microwave has high potential capacity, use of microwave processing has attracted the attention of researchers in recent years. However, there have been limited studies on the encapsulation of anthocyanins using microwave-assisted encapsulation [66].

The main advantages of microwave-assisted encapsulation are as follow [67]:

1- Short drying time;
2- Cost-efficiency;
3- High-quality products;
4- High flexibility to produce various dried products

Recent advances in the encapsulation of anthocyanins using microwave-assisted encapsulation

As mentioned earlier, there has been limited research on the encapsulation of anthocyanins using microwave-assisted encapsulation. In a recent study, Mohd Nawi Muhamad [66] used this technique for the encapsulation of purple sweet potato anthocyanin with various wall materials, such as maltodextrin, Arabic gum, and the combination of maltodextrin and Arabic gum with the ratio of 1:1. According to the findings, the particles that were encapsulated by the combination of biopolymers were better in terms of water activity and moisture content. The fabricated capsules had a flake-like structure with a smooth surface. This structure is expectable for the capsules fabricated by this technique, and their particle size was approximately 100 micrometers.

FUTURE TREND

According to the literature, encapsulation could be used as an appropriate technique to improve the stability and thermal properties of natural pigments. Various studies have been focused on the encapsulation of anthocyanins. Using combined strategies (e.g., copigmentation) will improve the potential of natural pigments for application in food, pharmaceutical, and other industries.

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REFERENCES

1.Gengatharan A, Dykes GA, Choo WS. Betalains: Natural plant pigments with potential application in functional foods. LWT-Food Sci and Technol. 2015; 64(2): 645-649.

2.Tang Y, Li X, Zhang B, Chen PX, Liu R, Tsao R. Characterisation of phenolics, betanins and antioxidant activities in seeds of three Chenopodium quinoa Willd. genotypes. Food Chem. 2015; 166: 380-388.

3.Azeredo HM. Betalains: properties, sources, applications, and stability–a review. Int J Food Sci & Technol. 2009; 44(12): 2365-2376.

4.Mohana D, Thippeswamy S, Abhishe R. Antioxidant, antibacterial, and ultraviolet-protective properties of carotenoids isolated from Micrococcus spp. Radiation Protection and Environment. 2013; 36(4): 168-174.

5.Butera D, Tesoriere L, Di Gaudio F, Bongiorno A, Allegra M, Pintaudi AM, Kohen R, Livrea M. Antioxidant activities of Sicilian prickly pear (Opuntia ficus indica) fruit extracts and reducing properties of its betalains: betanin and indicaxanthin. J Agr Food Chem. 2002; 50(23): 6895-6901.

6.Ravanfar R, Tamaddon AM, Niakousari M, Moein MR. Preservation of anthocyanins in solid lipid nanoparticles: Optimization of a microemulsion dilution method using the Placket–Burman and Box–Behnken designs. Food Chem. 2016; 199: 573-580.

7.Bell LN. Stability testing of nutraceuticals and functional foods. Handbook of nutraceuticals and functional foods: CRC Press; 2002. p. 504-519.

8.Gaonkar AG, Vasisht N, Khare AR, Sobel R. Microencapsulation in the food industry: a practical implementation guide: Elsevier; 2014.

9.Gutiérrez FJ, Albillos SM, Casas-Sanz E, Cruz Z, García-Estrada C, García-Guerra A, García-Reverter J, García-Suárez M, Gatón P, González-Ferrero C, Olabarrieta I, Olasagasti M, Rainieri S, Rivera-Patiño D, Rojo R, Romo-Hualde A, JoséSáiz-Abajo M, Musson M. Methods for the nanoencapsulation of β-carotene in the food sector. Trends Food Sci & Tech. 2013; 32(2): 73-83.

10.Gul K, Tak A, Singh A, Singh P, Yousuf B, Wani AA. Chemistry, encapsulation, and health benefits of β-carotene-A review. Cogent Food & Agriculture. 2015; 1(1): 1018696.

11.Rodriguez-Amaya DB. Update on natural food pigments-A mini-review on carotenoids, anthocyanins, and betalains. Food Res Int. 2018.

12.Horuz Tİ, Belibağlı KB. Nanoencapsulation by electrospinning to improve stability and water solubility of carotenoids extracted from tomato peels. Food Chem. 2018; 268: 86-93.

13.İnanç Horuz T, Belibağli KB. Nanoencapsulation of Carotenoids Extracted from Tomato Peels into Zein fibers by Electrospinning. J Sci Food and Agr. 2018. 99 (2): 759-766.

14.Pervaiz T, Songtao J, Faghihi F, Haider MS, Fang J. Naturally occurring anthocyanin structure, functions and biosynthetic pathway in fruit plants. J Plant Biochem Physiol. 2017; 5(2): 187.

15.Hou D-X. Potential mechanisms of cancer chemoprevention by anthocyanins. Curr Mol Med. 2003; 3(2): 149-159.

16.McDougall GJ, Fyffe S, Dobson P, Stewart D. Anthocyanins from red cabbage–stability to simulated gastrointestinal digestion. Phytochemistry. 2007; 68(9): 1285-1294.

17.Tall JM, Seeram NP, Zhao C, Nair MG, Meyer RA, Raja SN. Tart cherry anthocyanins suppress inflammation-induced pain behavior in rat. Behav Brain Res. 2004; 153(1): 181-188.

18.Schwarz M, Hillebrand S, Habben S, Degenhardt A, Winterhalter P. Application of high-speed countercurrent chromatography to the large-scale isolation of anthocyanins. Biochem Eng J. 2003; 14(3): 179-189.

19.Jones OG, McClements DJ. Functional biopolymer particles: design, fabrication, and applications. Comprehensive Reviews in Food Science and Food Safety. 2010; 9(4): 374-397.

20.Matalanis A, Jones OG, McClements DJ. Structured biopolymer-based delivery systems for encapsulation, protection, and release of lipophilic compounds. Food Hydr. 2011; 25(8): 1865-1880.

21.Fathi M, Emam-Djomeh Z, Sadeghi-Varkani A. Extraction, characterization and rheological study of the purified polysaccharide from Lallemantia ibrica seeds. Int J Biol Macromol. 2018; 120: 1265-1274.

22.Salehi E, Emam-Djomeh Z, Askari G, Fathi M. Opuntia ficus indica fruit gum: Extraction, characterization, antioxidant activity and functional properties. Carbohyd Polym. 2019; 206: 565-572.

23.Salehi E, Emam-Djomeh Z, Fathi M, Askari G. Opuntia ficus-indica Mucilage. Emerging Natural Hydrocolloids: Rheology and Functions. 2019.

24.de Vos P, Faas MM, Spasojevic M, Sikkema J. Encapsulation for preservation of functionality and targeted delivery of bioactive food components. Int dairy J. 2010; 20(4): 292-302.

25.Ko A, Lee JS, Sop Nam H, Gyu Lee H. Stabilization of black soybean anthocyanin by chitosan nanoencapsulation and copigmentation. J Food Biochem. 2017; 41(2): e12316.

26.Arroyo-Maya IJ, McClements DJ. Biopolymer nanoparticles as potential delivery systems for anthocyanins: Fabrication and properties. Food Res Int. 2015; 69: 1-8.

27.Sherahi MH, Fathi M, Zhandari F, Hashemi SMB, Rashidi A. Structural characterization and physicochemical properties of Descurainia sophia seed gum. Food Hydr. 2017; 66: 82-89.

28.Tiwari R, Takhistov P. Nanotechnology-enabled delivery systems for food functionalization and fortification. Nanotechnology research methods for foods and bioproducts Wiley-Blackwell, Oxford. 2012: 55-101.

29.de Moura SC, Berling CL, Germer SP, Alvim ID, Hubinger MD. Encapsulating anthocyanins from Hibiscus sabdariffa L. calyces by ionic gelation: Pigment stability during storage of microparticles. Food Chem. 2018; 241: 317-327.

30.Jafari S-M, Mahdavi-Khazaei K, Hemmati-Kakhki A. Microencapsulation of saffron petal anthocyanins with cress seed gum compared with Arabic gum through freeze drying. Carbohyd Polym. 2016; 140: 20-25.

31.Ge J, Yue P, Chi J, Liang J, Gao X. Formation and stability of anthocyanins-loaded nanocomplexes prepared with chitosan hydrochloride and carboxymethyl chitosan. Food Hydr. 2018; 74: 23-31.

32.Wise DL. Handbook of pharmaceutical controlled release technology: CRC Press; 2000.

33.Tan C, Selig MJ, Abbaspourrad A. Anthocyanin stabilization by chitosan-chondroitin sulfate polyelectrolyte complexation integrating catechin co-pigmentation. Carbohyd Polym. 2018; 181: 124-131.

34.Tan C, Celli GB, Selig MJ, Abbaspourrad A. Catechin modulates the copigmentation and encapsulation of anthocyanins in polyelectrolyte complexes (PECs) for

natural colorant stabilization. Food Chem. 2018; 264: 342-349.
35.Tan C, Celli GB, Abbaspourrad A. Copigment-polyelectrolyte complexes (PECs) composite systems for anthocyanin stabilization. Food Hydr. 2018; 81: 371-9.
36.Yeo Y, Baek N, Park K. Microencapsulation methods for delivery of protein drugs. Biotechnol Bioproc Eng. 2001; 6(4): 213-230.
37.Nayak CA, Rastogi NK. Effect of selected additives on microencapsulation of anthocyanin by spray drying. Dry Technol. 2010; 28(12): 1396-1404.
38.Cho K, Wang X, Nie S, Shin DM. Therapeutic nanoparticles for drug delivery in cancer. Clin Cancer res. 2008; 14(5): 1310-1316.
39.Osorio C, Acevedo B, Hillebrand S, Carriazo J, Winterhalter P, Morales ALa. Microencapsulation by spray-drying of anthocyanin pigments from corozo (Bactris guineensis) fruit. J Agri Food Chem. 2010; 58(11): 6977-3685.
40.Huang X, Brazel CS. On the importance and mechanisms of burst release in matrix-controlled drug delivery systems. J ControlledRelease. 2001; 73(2-3): 121-136.
41.Siepmann J, Peppas N. Modeling of drug release from delivery systems based on hydroxypropyl methylcellulose (HPMC). Adv Drug Deliver Re. 2012; 64: 163-174.
42.Robert P, Gorena T, Romero N, Sepulveda E, Chavez J, Saenz C. Encapsulation of polyphenols and anthocyanins from pomegranate (Punica granatum) by spray drying. Int J Food SciTechnol. 2010; 45(7): 1386-1394.
43.Mahdavi SA, Jafari SM, Assadpour E, Ghorbani M. Storage stability of encapsulated barberry's anthocyanin and its application in jelly formulation. J Food Eng. 2016; 181: 59-66.
44.Idham Z, Muhamad II, Sarmidi MR. Degradation kinetics and color stability of spray-dried encapsulated anthocyanins from hibiscus sabdariffa l. J Food Proc Eng. 2012; 35(4): 522-542.
45.Reyes LF, Cisneros-Zevallos L. Degradation kinetics and colour of anthocyanins in aqueous extracts of purple-and red-flesh potatoes (Solanum tuberosum L.). Food Chem. 2007; 100(3): 885-894.
46.Idham Z, Muhamad II, MOHD SETAPAR SH, Sarmidi MR. Effect of thermal processes on roselle anthocyanins encapsulated in different polymer matrices. J Food Proc Pres. 2012; 36(2): 176-184.
47.Burin VM, Rossa PN, Ferreira-Lima NE, Hillmann MC, Boirdignon-Luiz MT. Anthocyanins: optimisation of extraction from Cabernet Sauvignon grapes, microcapsulation and stability in soft drink. Int J Food Sci Technol. 2011; 46(1): 186-193.
48.Wang W-D, Xu S-Y. Degradation kinetics of anthocyanins in blackberry juice and concentrate. J Food Eng. 2007; 82(3): 271-275.
49.Dangles O, Brouillard R. A spectroscopic method based on the anthocyanin copigmentation interaction and applied to the quantitative study of molecular complexes. Journal of the Chemical Society, Perkin Transactions 2. 1992 (2): 247-257.
50.Yousefi S, Emam-Djomeh Z, Mousavi M, Kobarfard F, Zbicinski I. Developing spray-dried powders containing anthocyanins of black raspberry juice encapsulated based on fenugreek gum. Adv Powder Technol. 2015; 26(2): 462-269.
51.Yousefi S, Emam-Djomeh Z, Mousavi S. Effect of carrier type and spray drying on the physicochemical properties of powdered and reconstituted pomegranate juice (Punica Granatum L.). J Food Sci Technol. 2011; 48(6): 677-684.
52.Chegini G, Ghobadian B. Spray dryer parameters for fruit juice drying. World J Agri Scie. 2007; 3(2): 230-236.
53.Ahmad M, Ashraf B, Gani A, Gani A. Microencapsulation of saffron anthocyanins using β glucan and β cyclodextrin: Microcapsule characterization, release behaviour & antioxidant potential during in-vitro digestion. Int J Biol Macromol. 2018; 109: 435-442.
54.Mueller D, Jung K, Winter M, Rogoll D, Melcher R, Kulozik U, Schwarz K, Richling E. Encapsulation of anthocyanins from bilberries–Effects on bioavailability and intestinal accessibility in humans. Food Chem. 2018; 248: 217-224.
55.Cai Y. Light stability of purple corn anthocyanins microencapsulated with different wall materials. 2018.
56.Xue J, Su F, Meng Y, Yurong G. Enhanced Stability of Red-Fleshed Apple Anthocyanins by Copigmentation and Encapsulation. J the Sci Food and Agri. 2018.
57.da Fonseca Machado AP, Rezende CA, Rodrigues RA, Barbero GF, e Rosa PdTV, Martínez J. Encapsulation of anthocyanin-rich extract from blackberry residues by spray-drying, freeze-drying and supercritical antisolvent. Powder Technol. 2018; 340: 553-562.
58.Hao S, Wang Y, Wang B, Deng J, Liu X, Liu J. Rapid preparation of pH-sensitive polymeric nanoparticle with high loading capacity using electrospray for oral drug delivery. Mater Sci Eng: C. 2013; 33(8): 4562-4567.
59.Zhang S, Kawakami K. One-step preparation of chitosan solid nanoparticles by electrospray deposition. Int J Pharm. 2010; 397(1-2): 211-217.
60.Atay E, Fabra MJ, Martínez-Sanz M, Gomez-Mascaraque LG, Altan A, Lopez-Rubio A. Development and characterization of chitosan/gelatin electrosprayed microparticles as food grade delivery vehicles for anthocyanin extracts. Food Hydr. 2018; 77: 699-710.
61.Schafer V, von Briesen H, Andreesen R, Steffan A-M, Royer C, Troster S, Jorg K, Helga R. Phagocytosis of nanoparticles by human immunodeficiency virus (HlV)-infected macrophages: a possibility for antiviral drug targeting. Pharm Rese. 1992; 9(4): 541-546.
62.Hwang JM, Kuo HC, Lin CT, Kao ES. Inhibitory effect of liposome-encapsulated anthocyanin on melanogenesis in human melanocytes. Pharml Biol. 2013 ;51(8): 941-947.
63. Guldiken B, Gibis M, Boyacioglu D, Capanoglu E, Weiss J. Impact of liposomal encapsulation on degradation of anthocyanins of black carrot extract by adding ascorbic acid. Food & Funct. 2017;8(3):1085-1093.
64.Zhao L, Temelli F, Chen L. Encapsulation of anthocyanin in liposomes using supercritical carbon dioxide: Effects of anthocyanin and sterol concentrations. J Funct Food. 2017; 34:159-167.
65.Desai KGH, Jin Park H. Recent developments in microencapsulation of food ingredients. Dry Technol. 2005; 23(7): 1361-1394.
66.Mohd Nawi N, Muhamad II, Mohd Marsin A. The physicochemical properties of microwave-assisted encapsulated anthocyanins from Ipomoea batatas as affected by different wall materials. Food Sci Nut. 2015; 3(2): 91-99.
67.Haghi A, Amanifard N. Analysis of heat and mass transfer during microwave drying of food products. Braz J Chem Eng. 2008; 25(3): 491-501.