Research Article

pISSN: 2345-5357
eISSN: 2423-4214

APPLIED FOOD BIOTECHNOLOGY, 2022, 9 (3): 227-237
Journal homepage: www.journals.sbmu.ac.ir/afb

Effects of Copigmentation on the Stability of Phycocyanin Pigments Extracted from *Spirulina platensis* Using Spray Dryer

Reza Karazhyan[1*], Maryam Ameri[1], Najme Gord Noshahri[1], Ahmad Ehtiati[2]

1. Department of Industrial Microbial Biotechnology, Research Institute for Industrial Biotechnology Academic Center for Education Culture and Research (ACECR), Mashhad, Iran
2. Department of Food Quality and Safety, Research Institute of Food Sciene and Technology, Iranian Academic Center for Education Culture and Research (ACECR), Mashhad, Iran

Abstract

Background and Objective: Phycocyanin is a blue pigment extracted from *Spirulina platensis* algae as an excellent alternative for the comparison of synthetic dyes in various industries, including food industries. The aim of the present study was to assess effects of copigmentation on the stability of phycocyanin pigments using spray drying method.

Material and Methods: An aqueous solution of phycocyanin (500 mg l^{-1}) was prepared at three pH values of 3, 5 and 7. Then, polyphenolic compounds containing rosmarinic acid, tannic acid and digallic acid (0, 75, 150, 225 and 300 mgl^{-1}) were separately added to the solution as copolymers. Pigment solutions were transferred into cylindrical containers with similar sizes under a light source at an intensity of 7000 l mm^{-2} and ambient temperature. Color changes of the solutions were assessed for 14 d. Phycocyanin pigment solution was copigmented with tannic acid (the best copolymer) and mixed with a combination of maltodextrin and Arabic gum (100:0, 75:25, 50:50, 25:75 and 0:100). Ratio of the core to the wall was 1:10. Spray dryer was used for drying and stability of the dried coated pigment powder was assessed for 14 d by investigating the absorption reduction ratio at the maximum absorption wavelength of phycocyanin (620 nm) using spectrophotometer.

Results and Conclusion: Based on the results, using tannic acid (300 mgl^{-1}) as the best copigmenting compound induced higher resistance to phycocyanin. In addition, the most stable pigment treatment was seen with maltodextrin and Arabic gum coating (ratio: 100:0). In particle size, findings showed that the powder samples containing maltodextrin were larger than the samples with Arabic gum (350.2 and 40.1 nm, respectively). Moreover, results showed that phycocyanin copigmented with tannic acid included higher resistance to environmental changes and encapsulation using spray dryer was further effective in increasing stability of phycocyanin.

Conflict of interest: The authors declare no conflict of interest.

How to cite this article

Karazhyan R, Ameri M, Gord Noshahri N, Ahmad Ehtiati. Effects of Copigmentation on the Stability of Phycocyanin Pigments Extracted from Spirulina platensis Using Spray Dryer. Appl Food Biotechnol. 2022; 9 (3): 227-237.
http://dx.doi.org/10.22037/afb.v9i3.38309

Article Information

Article history:
- Received 1 May 2022
- Revised 5 May 2022
- Accepted 9 May 2022

Keywords:
- Blue Pigment
- Copigmentation
- Stability
- Phycocyanin

***Corresponding author:**

Reza Karazhyan*
Department of Industrial Biotechnology on Microorganisms, Iranian Academic Center for Education Culture and Research (ACECR), Mashhad, Iran

Tel: +98-51-31997442

E-mail: reza_karazhyan2002@yahoo.com

1. Introduction

Recently, use of natural pigments has been popular in food and medicine industries owing to their safety and health-promoting characteristics, compared to synthetic dyes. Synthetic dyes are widely used in food industries to create colors in various food products, such as beverages. Synthetic dyes are generally resistant to temperature, light and pH [1]. Use of natural dyes is further interested by the producers and consumers of food products due to the negative effects of synthetic dyes on general human health, especially in children. Therefore, interests in food dyes with natural origins are increasing [2]. Of these natural dyes, the blue pigment has greatly been interested, while its limited sources make it difficult to recover and extract this pigment. The C-phycocyanin pigment is a protein that is currently used as a natural dye in foods and medicines and includes multiple health and nutritional characteristics owing to its antioxidant activities. Phycocyanin is approved by the FDA (Food and Drug Administration) and EFSA (European Food Safety

Authority) [2,3]. Phycocyanin includes good resistance at temperatures below 47 °C, while the pigment stability decreases significantly at high temperatures [4]. With changing temperature, pH of the environment changes as well. At high temperatures, pH of the environment increases too. Up-to-date, several methods have been used to prevent thermal degradation of phycocyanin; some of these methods include addition of citric acid, glucose, sorbic acid, sodium azide, pH adjustment, sucrose and sodium chloride [4]. According to Martelli, high concentrations of sugar can increase thermal resistance of phycobiliproteins. These substances cannot protect the phycocyanin molecule and its chemical structure from structural changes [5]. Furthermore, cross-linking reactions between the protein molecules and the silver nanoparticles, methylglyoxal and formaldehyde increase stability of phycoerythrin as well as preventing protein aggregation, thermal processes, high pressure and copigmentation processes [6].

Farhadi reported that the copigmentation of anthocyanin compounds effectively stabilized these compounds, thereby linking polyphenolic compounds, flavonoids and anthocyanins through hydrophobic forces or hydrogen bonds. Copigmentation causes hyperchromic effects on the pigment (e.g., increased absorption), leading to a bathochromic phenomenon or shifting the maximum wavelength of absorption to other wavelengths [7]. Polyphenolic compounds, flavonoids, amino acids and organic acids are copigmenting compounds. Numerous studies have shown that polyphenolic compounds and phenolic acids can stabilize anthocyanin solutions extracted from fruits and berries against significant changes in the matrices of food products such as juices, purees, jams and syrups [8]. This color stability can occur due to the chromophore interactions of phycocyanin with hydroxyl functional groups in the copolymer compounds, thus protecting phycocyanin from the addition of water [8]. According to Heras-Roger et al., addition of copigments increases and stabilizes color of wine, thereby preventing formation of colorless compounds and intensifying color compounds. Anthocyanin may increase by addition of copigments. Color of saffron can be stabilized by tannic acid and gallic acid and formation of complexes and copigments in saffron (e.g., anthocyanins) increases light absorption and shifts in length with higher waves [8]. Encapsulation and microencapsulation are effective methods for the pigment stabilization. Microencapsulation has been introduced as a method for stabilizing color compounds and is used in food and pharmaceutical industries to coat dyes, fragrances and other effective substances. This method is widely used in food industries since it decreases decompositions or reactions with other food compounds during food safety improveements and controlled releases of effective compounds [9].

Hydrocolloids such as maltodextrin, Arabic gum and sodium gelatin caseinate have been used as coating materials for effective compounds. Several studies have verified protective effects of whey protein compounds, Arabic gum and maltodextrin on microencapsulation of various bioactive compounds such as pigments, antioxidants, enzymes and drugs [10]. Maltodextrin is addressed as a coating material in various microcoating methods owing to its ability to form networks. High efficiency of malt coating by maltodextrin, low viscosity of the solutions produced from this compound, availability in various molecular weights and its relatively low price are key advantages of maltodextrin in coating of effective materials. Arabic gum is also a commonly used coating in microencapsulation of susceptible compounds (e.g., carotenoids) due to its emulsifying characteristics. Each compound includes unique characteristics in aqueous solutions and special behaviors during microcoating by spray dryer. Akhavan Mahdavi et al. used a microencapsulation method to coat anthocyanins with maltodextrin, Arabic gum and gelatin. The major reason included that these compounds enclosed their cavities for the protection from environmental conditions such as light, heat, moisture and oxygen, which could increase shelf lives of the products and control releases of the encapsulated materials [10]. Pournamayati et al. investigated stability of phycocyanin microcapsules using maltodextrin and kappa carrageenan as coating materials. In the highlighted study, spray drying method was used at various inlet temperatures [11]. Novelty of the current study included use of copigments to increase stability of the phycocyanin pigments against environmental conditions (temperature, light and pH). The major aims of this study were to assess stability of phycocyanin in various treatments with copigments, to achieve appropriate compositions and concentrations of these compounds, to coat the compounds with maltodextrin and Arabic gum and to assess other characteristics (e.g., moisture, microscopic structure, particle size) of the powdered pigments in various treatments.

2. Materials and Methods

2.1. Preparation of the Phycocyanin Pigment

Food grade phycocyanin pigments extracted from *Spirulina platensis* microalgae were perchased as freeze-dried powder (A620/280=0.8) from the Industrial Biotechnology Research Institute, Academic Center for Education, Culture and Research (ACECR) Mashhad, Iran.

2.2. Copigmentation of Phycocyanin with Polyphenolic Compounds

Phycocyanin at the concentration of 500 mgl^{-1} was separately dissolved in buffer solutions at pH 3, 5 and 7. Tannic acid, rosmarinic acid and gallic acid (0, 75, 150, 225 and 300 mgl^{-1}) were separately added to the phycocyanin solution at each pH and mixed well to form complexex. Then, vials were tightly closed and stored in rows under a completely controlled environment with distances of 30 cm from the light source; therefore, samples were exposed to

7,000 lmm^{-2} light. Changes in color intensity during the incubation were measured through maximum absorption using spectrophotometer (Biotech, Germany) [12].

2.3. Pigment Stability of the Copigmented Phycocyanin

Changes in color intensity in each treatment were assessed for 14 d (2-d intervals). Pigment stability was assessed by calculating the absorption:reduction ratio at the maximum absorption wavelength of phycocyanin (620 nm) using spectrophotometer (Biotech, Germany).

2.4. Phycocyanin Encapsulation in various Treatments with Tannic Acid and Wall Compounds

2.4.1. Phycocyanin Copigmentation with Tannic Acid

As shown in Fig. 1, treatment containing tannic acid included the lowest decrease in phycocyanin absorption over the time. Therefore, this was selected for further experiments. At this stage, phycocyanin was dissolved in buffer solution at pH 3 with the concentration of 500 mgl^{-1}. Tannic acid (75, 100 and 300 mg l^{-1}) was added to the phycocyanin solution to form a complex.

2.4. 2. Phycocyanin Encapsulation in various Treatments

Copigmented phycocyanin treatments were coated with maltodextrin and Arabic gum solution (0.1% w v^{-1}) at various ratios of maltodextrin to Arabic gum (100:0, 75:25, 50:50, 25:75 and 0:100) and a core-to-wall ratio of 1:10 in five treatments; hence, proportion of the total solid matter was set to approximately 10%. To prepare the solution, samples were homogenized using homogenizer (IKA T25, Germany) to achieve particles of similar sizes. Prepared solution was dried using spray dryer (Model 191 B, Buchi, Switzerland) with a nozzle diameter of 0.5 mm, an inlet temperature of 150 °C and an outlet temperature of 90 °C [13,14].

2.4.3. Stability of the Spray-dried Phycocyanin Pigments

Changes in pigment uptake intensity in each treatment were assessed for 14 d (2-d intervals). A phycocyanin solution was prepared from each treatment (total five) at pH 3 at the concentration of 500 mgl^{-1} and sealed tightly using special glass vials. Then, vials were stored under a completely controlled environment at 37 °C in straight rows with distances of 30 cm from the light source; hence, samples were exposed to 7,000 lmm^{-2} light. Pigment stability was investigated by decreasing ratio of the prepared solution at the maximum absorption wavelength of phycocyanin (620 nm) according to Helgason (2016) using BioQuest CE2502 Spectrophotometer (Progen Scientific, UK).

2.4.4. Moisture and Water Activity (aw) Assessments

Moisture content of the particles was assessed at 105 °C using oven. In addition, water activity of the microcapsules was assessed using aw-Sprint TH500 System (Novasina, Switzerland) [15].

2.4.5. Microstructure Morphology Assessment Using SEM

Scanning electron microscope (SEM; Oxford Model 360-S, UK) was used to assess external microstructure of the microcapsules and structure of the dried powders [14].

2.4.6. Particle size and Zeta Potential

The mean particle diameter and zeta potential of the coated phycocyanin pigment and light scattering index were calculated using special particle measuring device (Model 2000, Malvern Instruments, UK) [14,15].

2.5. Statistical Analysis

Data analysis was carried out using SAS Software v.3 and v.9 (SAS, USA) and one-way analysis of variance (ANOVA). Differences between the means were compared at Tokay's levels using Tokay Software (Tokay, USA) (p<0.01).

3. Results and Discussion

3.1. Assessment of the Most Appropriate Compound for Copigmentation

As seen in Fig. 1, optical absorption of the phycocyanin solution containing the three copigments decreased within 14 d of storage. Sample containing tannic acid included the highest pigment optical absorption and sample with no copigment combination (control) included the lowest pigment optical absorption. Therefore, it could be concluded that phycocyanin solutions containing tannic acid included the highest resistance to deteriorative environmental factors.

Based on the results (Fig. 2), increasing quantities of various copigments (tannic acid, rosmarinic acid and gallic acid) in solutions containing phycocyanin increased absorption of the solutions due to the increased resistance of the phycocyanin solution. Moreover, resistance was higher in tannic acid than rosmarinic acid and higher in rosmarinic acid than gallic acid. At various concentrations of these compounds, the least significant decrease in pigment uptake was seen at the concentration of 300 mgl^{-1} and the most significant decrease in pigment uptake was observed at the concentration of 75 mgl^{-1} with polyphenolic compounds (each of the three compounds). Findings demonstrated that the phycocyanin pigment solutions containing higher quantities of copigments were further stable (Fig. 3).

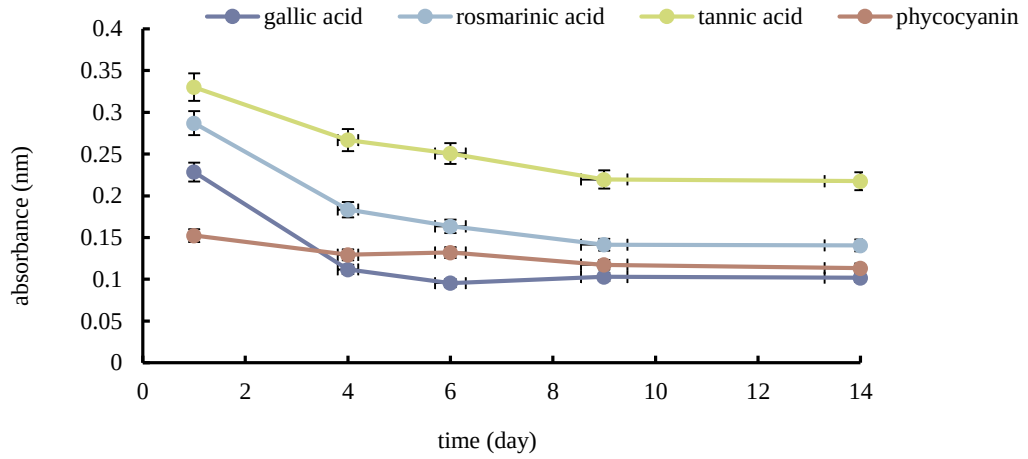

Figure 1. Absorption curves of the phycocyanin solution with various copigments during storage

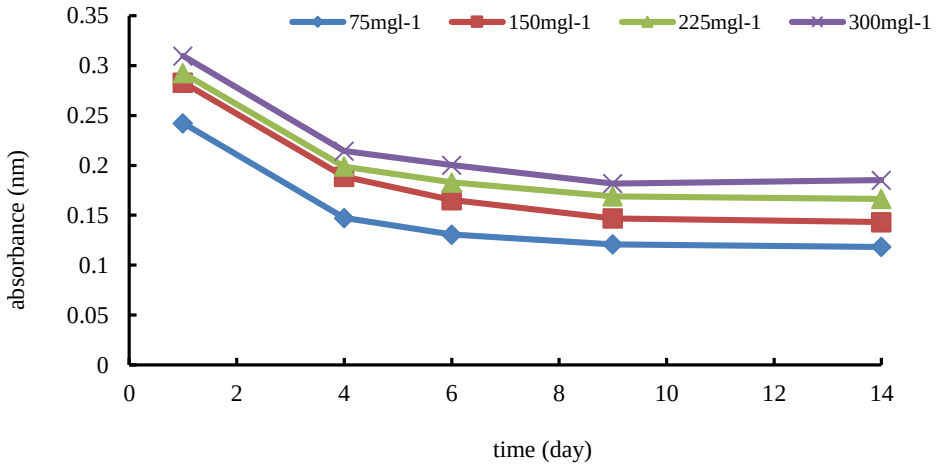

Figure 2. Effects of copigment concentrations on absorption of phycocyanin solutions during storage

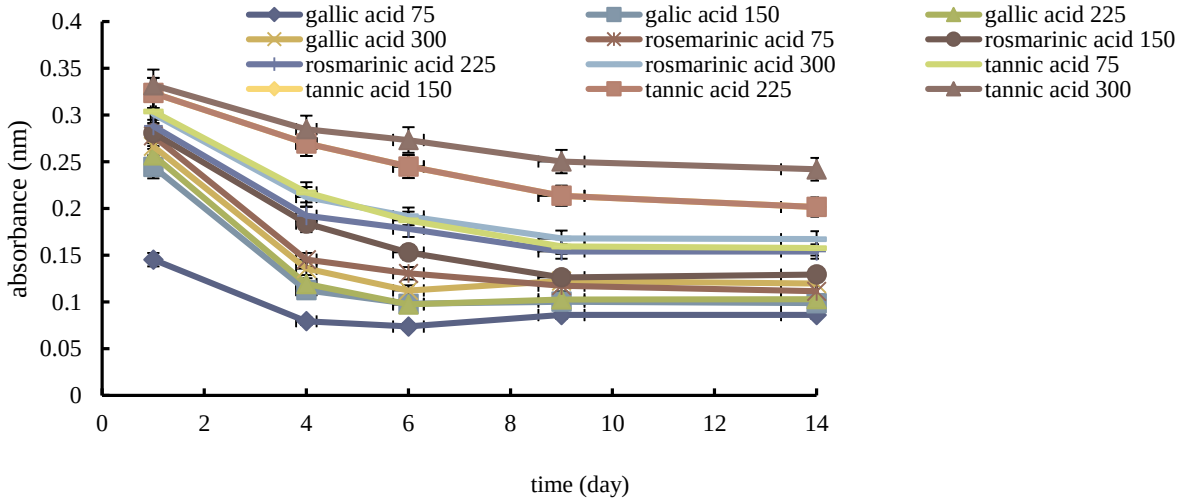

Figure 3. Absorption curves of phycocyanin solutions containing copigments at various concentrations (mg l^{1}) during storage

Significantly, solutions containing copigments were further resistant with higher concentrations of these compounds. Concentration of the copigments affected color stability and increased concentrations of copigments improved stability of the pigment solutions. Results of the present study revealed that intensity of pigmentation depended on molar concentrations of the compounds [16].

As seen in Fig. 4, results of phycocyanin copigmentation with three polyphenolic compounds at various pH showed the highest adsorption of the copigmented phycocyanin solution in tannic acid treatment at various pH, while the gallic acid treatment represented the lowest adsorption. At various pH, adsorption was significantly different between the three copigments, while adsorption decreased during storage.

Based on Table 1, the maximum absorption (600-605 nm) in the rosrmarinic acid and tannic acid treatments did not change significantly during the shelf life of the samples, while the maximum absorption changed in solutions containing gallic acid to 565 and 450 nm on Days 4 and 10, respectively (the bathochromic effect).

In the current study, use of organic acids and the subsequent changes in pH caused changes in the half-life of anthocyanins from cabernet sauvignon (*Vitis vinifera* L.) grape crude extracts. Increased half-life ($t_{1/2}$) of anthocyanins was seen with red color appearance after addition of tannic acid and gallic acid in assessment of pH. However, the exact mechanism of copigmentation is unknown. In some pigments, copigmentation can preserve C-2 chromophore in the pyranic ring from a hydrophilic attack, often resulting in discoloration. Moreover, addition of tannic acid resulted in a longest half-life of the anthocyanin solutions (2,585 h) and therefore increased their stability [17]. Copigmentation becomes more efficient with increasing concentration of the copigments or anthocyanins, which increases exposure of anthocyanin and copigment structures. In a study, Clement et al. investigated effects of increased concentration of caffeic acid copigment on copigmentation of anthocyanins in grape wastes, reporting that increased concentration of the copigments linked to the crude extract led to higher absorption rates [18]. In fact, increasing concentration of anthocyanins was associated to increased possibility of accumulations between the anthocyanin molecules, which increased their stability and absorption. In contrast, increasing concentration of the copigment created a complex between the copigment and anthocyanin, which further increased their stability and absorption.

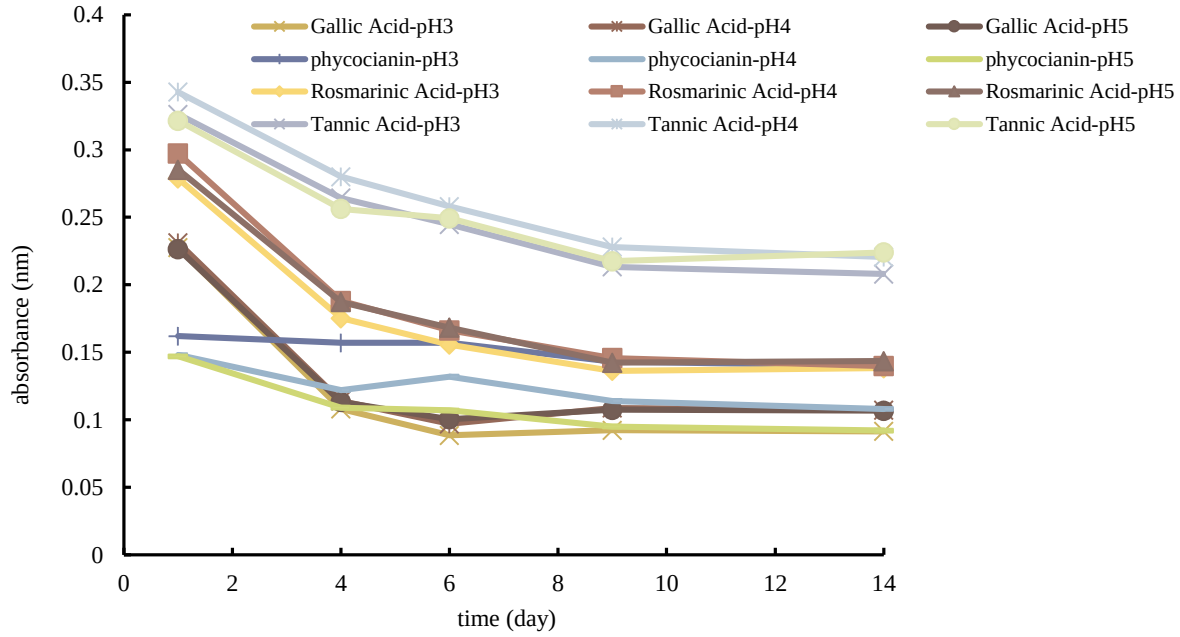

Figure 4. Effects of various pH on absorption of phycocyanin solutions containing copigments during storage

Table 1. Maximum absorption (nm) in copigments at various pH during storage

		Days				
Phycocyanin	pH	1	4	7	10	14
(control)	3	620	450	450	450	450
	5	620	450	450	450	450
	7	620	450	450	450	450
Gallic Acid	3	600	565	565	450	450
	5	600	565	565	450	450
	7	600	565	565	450	450
Rosmarinic	3	605	605	605	605	605
Acid	5	605	605	605	605	605
	7	605	605	605	605	605
Tannic Acid	3	605	605	605	600	600
	5	605	605	605	600	600
	7	605	605	605	600	600

Copigmented anthocyanins are degraded to lesser degrees than anthocyanins alone. Through the formed complex, copigmentation prevents breakdown of the anthocyanin bonds that are exposed to light and stability of anthocyanins increases against light radiation by addition of the copigment. Davies and Mazza (1993) suggested that copigmentation of anthocyanins with chlorogenic acid, caffeic acid and rutin was higher at pH 3.3-3.6, where anthocyanins majorly included colorless hemiacetals formed due to the hydration of flavylium cations [16,18]. In beverages with no copigments, findings showed that the chroma for beverages colored with purple carrots was significantly higher, compared to *Sambucus peruviana* (SP) and *S. nigra* after 8 w [19]. In absence of copigments, half-lives of the chroma of the model beverages colored with *S. peruviana* and *S. nigra* were similar (23 w); however, they were less than the half-lives of beverages colored with acylated anthocyanin from phycocyanins (26 w). Copigmentation of non-acylated antocyanins with ferulic acid has been reported to significantly decrease rates of the chroma destruction (p<0.01), thereby increasing their half-life from 23 to 49 w with *S. peruviana* and from 23 to 55 w with *S. nigra* [20].

3.2. Assessment of Stability of the Spray-dried Phycocyanin

Table 2 shows comparison of the mean proportions of adsorption losses after 14 d of storage. Accordingly, Sample 11 included the highest strength and stability under storage conditions, which decreased by 11.35% of the initial absorption of the sample after 14 d of storage.

In tortal, three types of changes were observed in the current study. In the first group, storage time increased, thereby leading to steady increases in the proportion of absorption losses at constant rates. The second group showed relatively sharp absorption losses on Day 1. Nevertheless, absorption decreased further at constant lower rates, compared to other samples. In the third group, absorption losses increased within the first few days of storage at constant rates followed by a steady state; in which, absorption of the samples was relatively constant.

Table 2. Proportions of the adsorption decreses in phycocyanin pigment samples spray-dried with various ratios of tannic acid, maltodextrin and Arabic gum

Sample	Tannic Acid (ppm)	Maltodextrin Arabic gum	Pigment Absorption Percentage of Loss		
			Day 1	Day 7	Day 14
1	75	100:00	3.1 ± 0.02^{Ch}	$7.2^{i}\pm0.24^{Bi}$	14.1 ± 0.27^{Af}
2	75	75:25	12.1 ± 0.22^{Ca}	18.3 ± 0.32^{Be}	22.1 ± 0.41^{Ad}
3	75	50:50	4.7 ± 0.26^{Cf}	12.2 ± 0.24^{Bg}	14.2 ± 0.32^{Af}
4	75	25:75	10.3 ± 0.16^{Cb}	30.1 ± 0.40^{Bc}	49.8 ± 1.03^{Ac}
5	75	0:100	10.5 ± 0.32^{Bb}	68.2 ± 0.32^{Aa}	70.4 ± 0.32^{Aa}
6	100	100:00	2.2 ± 0.20^{Ch}	$6.2^{i}\pm0.36^{Bi}$	12.5 ± 0.36^{Ag}
7	100	75:25	10.1 ± 0.31^{Cb}	16.2 ± 0.24^{Bef}	20.1 ± 0.64^{Ad}
8	100	50:50	3.6 ± 0.24^{Cg}	10.2 ± 0.57^{Bh}	12.5 ± 0.27^{Ag}
9	100	25:75	7.2 ± 0.24^{Cd}	26.1 ± 0.14^{Bd}	47.2 ± 0.43^{Ac}
10	100	0:100	7.4 ± 0.16^{Cd}	61.1 ± 1.52^{Bb}	66.2 ± 0.06^{Ab}
11	300	100:00	1.49 ± 0.06^{Cj}	5.5 ± 0.40^{Bi}	11.35 ± 0.25^{Ag}
12	300	75:25	8.3 ± 0.24^{Cc}	14.2 ± 0.48^{Bf}	18.1 ± 0.23^{Ae}
13	300	50:50	2.4 ± 0.24^{Ci}	9.2 ± 0.16^{Bh}	11.6 ± 0.32^{Ag}
14	300	25:75	6.3 ± 0.32^{Ce}	24.2 ± 0.24^{Bd}	45.1 ± 2.24^{Ac}
15	300	0:100	7.9 ± 0.40^{Bd}	60.1 ± 0.35^{Ab}	64.6 ± 2.82^{Ab}

Different lower-case letters in each column indicate a significant difference between data. Different uppercase letters in each row indicate a significant difference between treatments (p < 0.01).

Accordingly, these samples were expected to include the highest stability in the final products during the storage. Due to the lower proportion losses, Sample 11 could be a viable option for use in beverage formulation.

3.3. Moisture and aw Assessments of the Spray-dried Phycocyanin Pigments

Technically, type of the wall materials affects the final moisture contents of powdered pigments. Table 3 shows that moisture contents of the samples with Arabic gum were higher since Arabic gum included several heteropolysaccharide components with branched structures as well as hydrophilic groups, which led to its binding to water molecules and prevented exit of these molecules [21].

Moreover, findings showed that types of the wall materials affected physicochemical characteristics of the produced microcapsules [9]. Moisture contents of the microcapsules prepared with maltodextrin and a mixture of maltodextrin and Arabic gum were estimated as 4.4 and 3.52%, respectively. This difference might be due to the number of water-binding groups in maltodextrin and Arabic gum molecules, resulting in differences in moisture contents of the powdered samples.

3.5. Morphology Assessment of the Spray-dried Coated Pigments Using SEM

Comparison of the results showed that types of the wall materials affected structure morphology of the microcapsules. Samples included spherical shapes of maltodextrin, smoother surfaces and fewer wrinkles, compared to microcappsules composed of Arabic gum. In addition, microcapsules prepared with a mixture of maltodextrin and Arabic gum included smooth surfaces that were not uniform, as well as minimum density and depression on the surfaces. Furthermore, these microcapsules included more pores and were larger and better distributed. Wrinkles were due to the mechanical stresses caused by the movement of moisture on surfacse of the droplets during non-uniform drying. Reason for the serrated surfaces of microcapsules is their contractions when drying in the spray chamber was due to the rapid evaporation of water. Numbers of this shrinkage varied depending on compositions of the walls [13,22].

In a study, Hojjati reported that microcapsules of Arabic gum included serrated surfaces. These microcapsules were almost spherical, including small cracks and fissures on their surfaces. In the highlighted study, structure of the microcapsules showed that the samples prepared with maltodextrin included larger particle sizes [13]. Characteristics of similar shapes were also reported by Santana et al., who carried out microencapsulation of jussara fruit pulps with Arabic gum, modified starch and whey protein concentrates [22]. In another study, Pang et al. reported that increasd maltodextrin concentration resulted in production of particles with further softer regular surfaces [23]. Figure 5 demonstrates SEM images of the phycocyanin microcapsules. As seen in the figure, most of the phycocyanin microcapsules included irregular cracked shapes.

3.6. Particle Size and Poly Dispersibility Index

Based on the results of particle size assessment (Table 4), types and concentrations of the wall materials significantly affected sizes and specific surface areas of the produced powders (p<0.01).

Table 3. Moisture and a_w of the spray-dried pigments

Sample	Moisture Content (%)	aw
1	4.4 ± 0.16^{a}	75 ± 0.16^{a}
2	3.9 ± 0.20^{c}	71 ± 0.65^{b}
3	3.6 ± 0.20^{d}	68 ± 0.81^{c}
4	4.2 ± 0.12^{ab}	70 ± 0.16^{b}
5	4.3 ± 0.20^{a}	75 ± 0.40^{a}
6	4.35 ± 0.24^{a}	75 ± 0.40^{a}
7	3.8 ± 0.24^{c}	70 ± 0.40^{b}
8	3.55 ± 0.24^{d}	67 ± 0.81^{c}
9	4.1 ± 0.24^{b}	70 ± 0.16^{b}
10	4.2 ± 0.44^{ab}	71 ± 0.65^{b}
11	4.32 ± 0.24^{a}	73.5 ± 0.40^{ab}
12	3.76 ± 0.17^{cd}	68.3 ± 0.16^{bc}
13	3.52 ± 0.28^{d}	66 ± 0.81^{c}
14	4.05 ± 0.07^{b}	69 ± 0.81^{b}
15	4.12 ± 0.18^{b}	71.8 ± 0.47^{b}

Different lower-case letters in each column indicate a significant difference between data (p<0.01).

Measurement of microcapsule particles is critical as these particles affect texture of the foods. Shabanpour reported that wall materials and microcoating methods significantly affected sizes, shapes and overall structures of the microcappsules [24].

In the current study, sizes of the particles with various ratios of wall compositions were in the range of 40.1-350.2 nm (p<0.01). Based on the particle size distribution curves, capsules prepared with various treatments (except Samples 3 and 4) included single peaks, which indicated uniform particle-size distributions. In addition, poly dispersibility indices (PDI) of the microcapsules were in the range of 0.314-1.329, which indicated appropriate dispersions of the particles and particle size homogeneity. Based on the current findings, higher concentrations of maltodextrin led to the production of larger particles. This might be associated to the viscosity of feeds, which increased logarithmically with higher maltodextrin concentrations. Moreover, the mean sizes of the liquid droplets distinctly differed from the viscosity of liquids in the device, which converted elements into the fine particles at a constant rate. These findings were similar to those by Renata et al. [25]. Based on a study by Swetank et al., wall materials and the microcoating techniques significantly affect sizes, shapes and overall structures of microcapsules [26].

In the present study, all experiments included only one distinct peak with particle diameters of 40.1-350.2 nm. Furthermore, the mean particle diameters of the encapsulated phycocyanin powders changed with various wall materials. Encapsulated phyco-cyanin powder with a higher maltodextrin fraction in the wall material mixture included a larger particle size, while a higher Arabic gum fraction decreased the particle size (p<0.05).

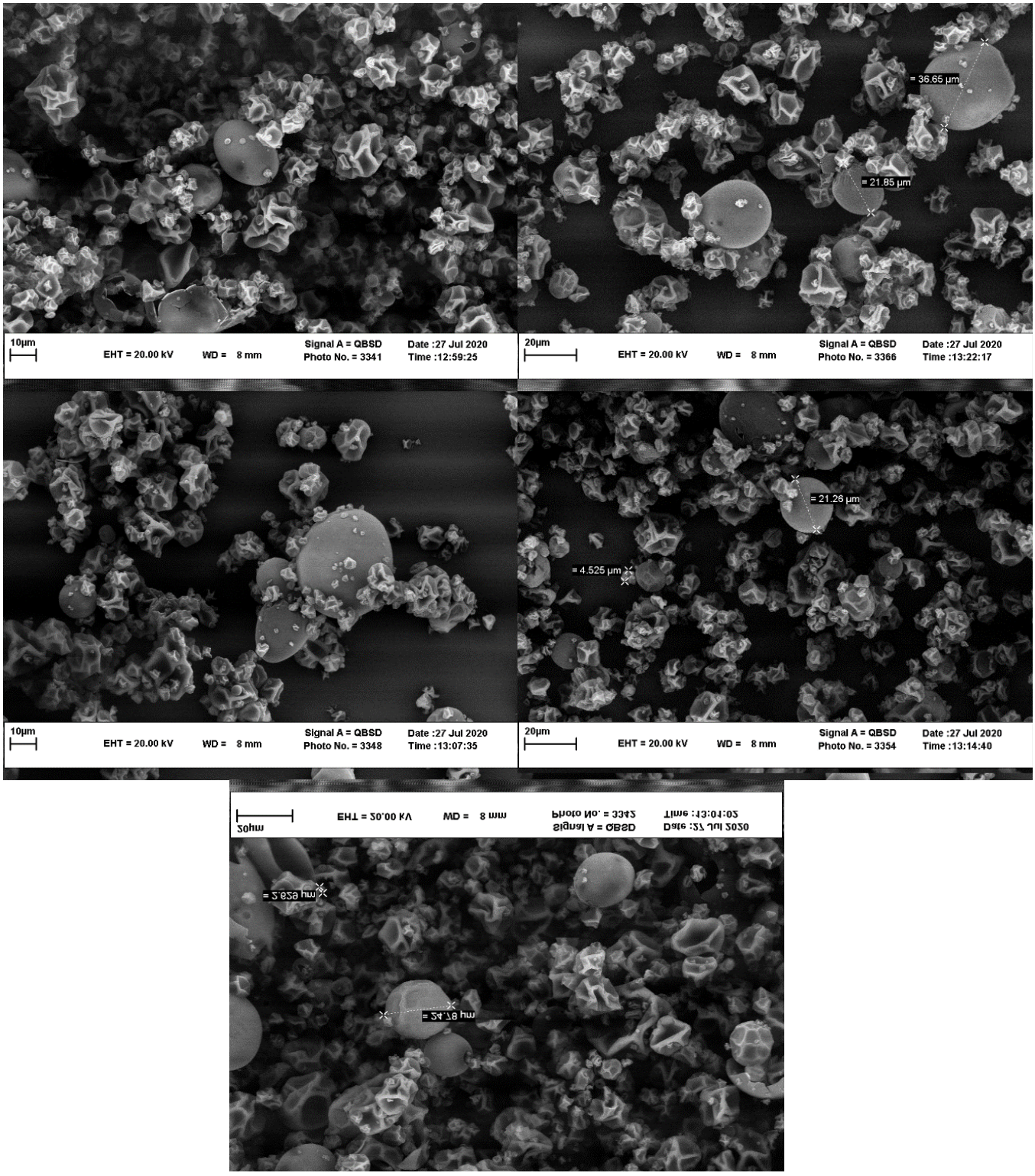

Figure 5. Microstructure of microcapsules at 2000× magnification. 1) Sample 6, maltodextrin and Arabic gum (100:0); 2) Sample 7, maltodextrin and Arabic gum (75:25); 3) Sample 8, maltodextrin and Arabic gum (50:50); 4) Sample 9, maltodextrin and Arabic gum (25:75); and 5) Sample 10, maltodextrin and Arabic gum (0:100)

Table 4. Particle size and poly dispersibility index in spray-dried phycocyanin samples

Sample	Particle Size (nm)	PDI
1	49± 0.2^{b}	0.369± 0.01^{b}
2	328.8± 2.19^{a}	1.316± 0.02^{a}
3	41.8± 0.2^{d}	0.359± 0.00^{b}
4	44.9± 0.5^{c}	0.333± 0.01^{c}
5	40.1± 0.6^{e}	0.314± 0.00^{d}
6	49.85± 0.31^{b}	0.372± 0.00^{b}
7	335.5± 0.5^{a}	1.312± 0.00^{a}
8	41.6± 0.2^{d}	0.365± 0.00^{b}
9	43.7± 0.35^{c}	0.337± 0.01^{c}
10	40.5± 0.5^{e}	0.318± 0.01^{d}
11	50.35± 0.39^{b}	0.384± 0.00^{b}
12	350.23± 3.05^{a}	1.329± 0.00^{a}
13	42.26± 0.7^{d}	0.377± 0.00^{b}
14	45.2±0.85^{c}	0.344± 0.00^{c}
15	41.3±0.35^{e}	0.328± 0.00^{d}

4. Conclusion

One of the problems with use of phycocyanin pigments includes their sensitivity to temperature and pH due to their protein nature. In this study, these pigments were stabilized via copigmentation, especially in combination with tannic acid. Results revealed that phycocyanin copigmented with tannic acid included higher resistances to environmental changes. To increase resistance of the phycocyanin pigments to pH, pigments were covered by polymers using spray-drying method. In this study, maltodextrin and Arabic gum of various proportions were used to coat the pigments using spray dryer. Furthermore, the current findings demonstrated that coated pigments with higher ratios of maltodextrin included the highest resistance to light and ambient temperature with larger particle sizes.

5. Acknowledgements

The authors express their gratitudes to Iranian National Science Foundation (INSF) for the financial support of this study. The authors also thank colleagues at INSF for their helps.

6. Conflict of Interest

The authors report no conflicts of interest.

7. Authors' Contributions

R.K., conceptualization and project administration; M.A. and N.G.N., methodology; A.E., software and data curation; and R.K., drafting of the preliminary manuscript.

References

1- Zhang S, Zhang Z, Dadmohammadi Y, Li Y, Jaiswal A, Abbaspourrad A. Whey protein improves the stability of C-phycocyanin in acidified conditions during light storage. Food Chem. 2020;344: 128642- 128650 https://doi.org/10.1016/j.foodchem.2020.128642

2- Hadiyanto H, Christwardana M, Sutanto H, Suzery M, Amelia D, Febrina Aritonang R. Kinetic study on the effects of sugar addition on the thermal degradation of phycocyanin from *Spirulina* sp. Food Biosci. 2018; 22: 85-90. https://doi.org/10.1016/j.fbio.2018.01.007

3- Rahman DY, Sarian FD, Van Wijk A, Martinez-Garcia M, Van der Maarel MJEC. Thermostable phycocyanin from the red microalga *Cyanidioschyzon merolae*, a new natural blue food colorant. J Appl Phycol. 2016; 29: 1233-1239. https://doi.org/10.1007/s10811-016-1007-0

4- Chaiklahan R, Chirasuwan N, Bunnag B. Stability of phycocyanin extracted from *Spirulina* sp: Influence of temperature, pH and preservatives. Process Biochemistry. 2012; 47: 659-664. https://doi.org/10.1016/j.procbio.2012.01.010

5- Martelli G, Folli C, Visai L, Daglia M, Ferrari D. Thermal stability improvement of blue colorant C-Phycocyanin from *Spirulina platensis* for food industry applications. Process Biochem. 2014, 49, 154-159. https://doi.org/10.1016/j.procbio.2013.10.008

6- Ming H, Gustavo C, Luis M. Black bean anthocyanin-rich extract from supercritical and pressurized extraction increased *In Vitro* antidiabetic potential, while having similar storage stability. Food. 2020; 9(655): 1-17. https://doi.org/10.3390/foods9050655

7- Farhadi Chitgar M, Aalami M, Kadkhodaee R, Maghsoudlou Y, Milani E. Effect of thermosonication and thermal treatments on phytochemical stability of barberry juice copigmented with ferulic acid and licorice extract. Innov Food Sci Emerg Technol. 2018; 50: 102-111. https://doi.org/10.1016/j.ifset.2018.09.004

8- Heras-Roger J, Alonso-Alonso O, Gallo-Montesdeoca A, Diaz-Romero C, Darias-Martin J. Influence of copigmentation and phenolic composition on wine color. J Food Sci Technol. 2016, 53(6): 2540-2547. https://doi.org/10.1007/s13197-016-2210-3

9- Hojjati M, Razavi SH, Rezaei K, Gilani K. Stabilization of canthaxanthin produced by *Dietzia natronolimnaea* HS-1 with spray drying microencapsulation. J Food Sci Technol. 2014; 51(9): 2134-2140. https://doi.org/10.1007/s13197-012-0713-0

10- Akhavan Mahdavi S, Jafari SM, Assadpoor E, Dehnadaa D. Microencapsulation optimization of natural anthocyanins withmaltodextrin, gum Arabic and gelatin. Int J Biol Macromolecules. 2016; 85: 379-385. https://doi.org/10.1016/j.ijbiomac.2016.01.011

11- Purnamayati L, Dewi E, Kurniasih RA. Phycocyanin stability in microcapsules processed by spray drying method using different inlet temperature. IOP Conf. Ser: Earth Environ Sci. 2018; 116: 1-6. https://doi.org/10.1088/1755-1315/116/1/012076

12- Helgason T, Bohn H, Weiland A, Sowa C, Gottschalk T. 2016. Stabilized phycocyanin for blue color. US20160324745A1.

13- Hojjati M, Razavi H, Rezaei K, Gilani K. Effect of wall components on characteristics of natural canthaxanthin microencapsulated using spray-drying. Iran J Nutr Sci Food Technol. 2013; 8 (3): 45-54.

14- Najafi NM, Mortazavi A, Kadkhodaee R, Tabatabaee F. Influence of Hi-Cap 100 and Tween 80 Interaction on the Properties of Cardamom Oil-in-water Emulsion and its Microcapsules. Iran Food Sci Technol Res J. 2011: 6(4): 254-262.
https://doi.org/10.22067/ifstrj.v6i4.9282

15- Vatankhah Lotfabadi S; Mortazavi A; Yeganehzad S; Sadeghian A. Evaluation of type and concentration of wall materials in D-Limonene microencapsulation to determination of optimum condition for flavored rock candy production. 2018; 5(2): 159-176.
https://doi.org/10.22104/JIFT.2017.512

16- Davies AJ, Mazza G. Copigmentation of simple and acylated anthocyanins with colorless phenolic compounds. J Agric Food Chem. 1993; 41(5): 716-720.
http://doi.org/10.1021/jf00029a007

17- Gauche C; Malagoli E; Bordignon Luiz M. Effect of pH on the copigmentation of anthocyanins from Cabernet Sauvignon grape extracts with organic acids.. Sci Agric. (Piracicaba, Braz.). 2010; 67, 1.41-46.
http://doi.org/10.1590/S0103-90162010000100006

18- Clemente E, Galli D. Stability of the anthocyanins extracted from residues of the wine industry. Cienc. Tecnol Aliment Campinas. 2011; 31(3): 765-768.
https://doi.org/10.1590/S0101-20612011000300033

19- Nuryati P, Miyagusuku-Cruzado G, Giusti M. Copigmentation with chlorogenic and ferulic acid affected color and anthocyanin stability in model beverages colored with sambucus peruviana, sambucus nigra and daucus carota during storage. Foods 2020; 9: 1476.
https://doi.org/10.3390/foods9101476

20- Wanida P, Siriluck I. Enhanced microencapsulation of C-phycocyanin from *Arthrospira* by freeze-drying with different wall materials. Food Technol Biotechnol. 2020; 58(4): 423-432.
https://doi.org/10.17113/ftb.58.04.20.6622

21- Ghazali E, Gharekhani M, Hamishekar H. Study physical and antioxidant properties of the microcapsules of citrus urantium extract prepared by spray drying method. J Innovative Food Technol. 2019; 6: 441-453.
https://doi.org/10.22104/jift.2018.2603.1614

22- Santana A, Cano-Higuita D, De Oliveira R, Telis V. Influence of different combinations of wall materials on the microencapsulation of jussara pulp (Euterpe edulis) by spray drying. Food Chem. 2016; 212: 1-9.
https://doi.org/10.1016/j.foodchem.2016.05.148

23- Pang SF, Yusoff MM, Gimbun J. Assessment of phenolic compounds stability and retention during spray drying of *Orthosiphon stamineus* extracts. Food Hydrocoll. 2014; 37:159-165.
https://doi.org/10.1016/j.foodhyd.2013.10.022

24- Shabanpour B, Mehrad B, Pourashouri P, Jafari SM. The effect of wall material and encapsulation method on physicochemical properties micro-encapsulated fish oil. Q J Res Innov Food Sci Technol. 2018; 7(1): 13-28.
https://doi.org/10.22101/jrifst.2018.05.19.712

25- Renata T, Catherine B, Miriam H. Influence of process conditions on the physicochemical properties of acai (*Euterpe oleraceae* Mart.) powder produced by spray drying. J Food Engin. 2008; 88: 411-418.
htpp://doi.org/10.1016/j.jfoodeng.2008.02.029

26- Swetank Y, Hundre P, Anaharamakrishnan C. Effect of whey protein isolate and bcyclodextrin wall systems on stability of microencapsulated vanillin by spray-freeze drying method. Food Chem. 2015; 174: 16-24.
https://doi.org/10.1016/j.foodchem.2014.11.016

Research Article
pISSN: 2345-5357
eISSN: 2423-4214
OPEN ACCESS
APPLIED FOOD BIOTECHNOLOGY, 2022, 9 (3):227-237
Journal homepage: www.journals.sbmu.ac.ir/afb

بررسی تاثیر کوپیگمانتاسیون بر پایداری رنگدانه فیکوسیانین استخراج شده از *اسپیرولینا پلاتنسیس* توسط خشک کن پاششی

رضا کاراژیان[1*]، مریم عامری[1]، نجمه گرد نوشهری[1]، احمد احتیاطی[2]

۱- گروه بیوتکنولوژی صنعتی میکروارگانیسم‌ها، پژوهشکده بیوتکنولوژی صنعتی، جهاد دانشگاهی خراسان رضوی، مشهد، ایران
۲- گروه پژوهشی کیفیت و ایمنی مواد غذایی، پژوهشکده علوم و فناوری مواد غذایی، جهاد دانشگاهی خراسان رضوی، مشهد، ایران

تاریخچه مقاله
دریافت ۱ می ۲۰۲۲
داوری ۵ می ۲۰۲۲
پذیرش۹ می ۲۰۲۲

واژگان کلیدی
- فیکوسیانین
- کوپیگمانتاسیون
- پایداری
- رنگدانه آبی

***نویسنده مسئول**
رضا کاراژیان
گروه بیوتکنولوژی صنعتی میکروارگانیسم‌ها، پژوهشکده بیوتکنولوژی صنعتی، جهاد دانشگاهی خراسان رضوی، مشهد، ایران

تلفن: ۳۱۹۹۷۴۴۲-۵۱-۹۸+

پست الکترونیک:
reza_karazhyan2002@yahoo.com

چکیده

سابقه و هدف: فیکوسیانین رنگدانه‌ای آبی رنگی است که از جلبک اسپیرولینا پلاتنسیس استخراج می‌شود و می‌تواند جایگزین بسیار خوبی برای رنگ‌های سنتزی در صنایع گوناگون، از جمله صنایع غذایی، باشد. هدف از این تحقیق ارزیابی اثرات روش کوپیگمانتاسیون بر پایداری رنگدانه‌های فیکوسیانین به روش خشک‌کن پاششی می باشد.

مواد و روش ها: محلول‌های آبی فیکوسیانین ($500 mgl^{-1}$) در سه pH (۳، ۵ و ۷) تهیه شدند. سپس، ترکیبات پلی‌فنولی رزمارینیک اسید، تانیک اسید، دیگالیک اسید با نسبت‌های گوناگون (۰، ۷۵، ۱۵۰، ۲۲۵ و ۳۰۰ mgl^{-1}) به‌صورت جداگانه به‌عنوان کوپلیمر به محلول آبی اضافه شد. محلول‌های حاوی رنگدانه به ظروف استوانه‌ای با ابعاد یکسان منتقل و در برابر منبع نوری با شدت $7000\ lmm^{-2}$ و دمای محیط. قرار داده شدند تغییرات رنگ محلول‌ها طی بازهٔ ۱۴ روز بررسی شد. محلول رنگدانه فیکوسیانین توسط تانیک اسید (بهترین کوپلیمر) کوپیگمنت شد و با مالتودکسترین و صمغ عربی در نسبت‌های (صمغ عربی: مالتودکسترین ۰:۱۰۰، ۲۵:۷۵، ۵۰:۵۰، ۷۵:۲۵ و ۱۰۰:۰) مخلوط شدند. ریزپوشانی با نسبت هسته به دیواره ۱:۱۰ انجام شد. از خشک کن پاششی برای خشک کردن استفاده و پایداری پودر رنگدانه پوشش دار و خشک شده به مدت ۱۴ روز با ارزیابی نسبت کاهش جذب در حداکثر طول موج جذب فیکوسیانین (۶۲۰ nm) توسط طیف سنجی ماورای بنفش و مریی انجام شد.

یافته‌ها و نتیجه‌گیری: براساس نتایج به‌دست آمده، استفاده از تانیک اسید ($300 mgl^{-1}$) به‌عنوان بهترین ترکیب کوپیگمنت‌کننده، مقاومت بیشتری در فیکوسیانین را موجب می‌شود. به‌علاوه، پایدارترین تیمار رنگدانه مربوط به پوشش مالتودکسترین و صمغ عربی (۰:۱۰۰) بود. در ارتباط با اندازه ذرات، یافته‌ها نشان داد که نمونه های پودر حاوی مالتودکسترین در مقایسه با نمونه‌های حاوی صمغ عربی از اندازه درشت تری برخوردار هستند (به‌ترتیب ۳۵۰/۲ و ۴۰/۱ نانومتر). همچنین نتایج نشان داد که فیکوسیانین کوپیگمنت شده با تانیک اسیدمقاومت بیشتری در برابر تغییرات محیطی دارد و ریزپوشانی با خشک کن پاششی در افزایش پایداری فیکوسیانین موثرتر بود.

تعارض منافع: نویسندگان اعلام می‌کنند که هیچ نوع تعارض منافعی مرتبط با انتشار این مقاله ندارند.