

Encapsulation of Betalains Extracted from Red Dragon Fruit Peels by Freeze-drying using Microcrystalline Cellulose and Dragon Fruit Peel Pectin as Wall Materials

Uyen P.N. Tran^{a,*}, Trung Dang-Bao^{b,d}, Phung Thi Kim Le^{c,d}, Uyen D.H. Huynh^a, Thanh T.H. Nguyen^a, Tan M. Le^{c,d}

^aFaculty of Engineering and Technology, Van Hien University (VHU), Ho Chi Minh City, Viet Nam

^bFaculty of Chemical Engineering, Ho Chi Minh City University of Technology (HCMUT), Ho Chi Minh City, Vietnam

^cRefinery and Petrochemicals Technology Research Center (RPTC), Ho Chi Minh City University of Technology (HCMUT), Ho Chi Minh City, Vietnam

^dVietnam National University Ho Chi Minh City (VNU-HCM), Linh Trung ward, Thu Duc City, Ho Chi Minh City, Vietnam
 uyenpth@vhu.edu.vn

Red dragon fruit (*Hylocereus polyrhizus*) has good potential to be processed into many kinds of food products. The processing of dragon fruit always discarded large volumes of peel waste. The residual peel of red dragon fruit is rich of betalains and pectin, which are alternative food additive sources. This study utilized red dragon fruit peels to produce betalains and pectin. Because betalain pigment is unstable in processing and storage, it was encapsulated in carbohydrate matrices of pectin (recovered from red dragon fruit peel - DFP) and microcrystalline cellulose (MCC). An appropriate freeze-drying procedure was employed to form pigment powder with 60 to 93 % encapsulation yield. The stability of the encapsulated betalains was investigated under various conditions such as pH, water activity, temperatures, and light after 30-day storage. Betalains encapsulated in a combination of MCC and DFP showed stability improvement compared to non-encapsulated betalains, which is potentially beneficial in food processing.

1. Introduction

Red dragon fruit (*Hylocereus polyrhizus*) is a tropical fruit grown in various tropical and subtropical regions for consuming fresh fruits, and food products from them are also favored. The consumption of red dragon fruits in processed form creates a large amount of peel waste, containing some beneficial components, especially pectin as a food thickener, emulsifier, gelling agent, and stabilizer. The peel also has a high content of betalains, which play the role of red-purple pigments in different foods. This natural pigment also exhibits antioxidants, anti-cancer, anti-lipidemic, and antimicrobial properties (Calva-Estrada et al., 2020). Betalains have poor stability, limiting their application in the food industry because of their rapid degradation in oxygen, light, pH < 3 or > 7, water activity, or high temperature (Castro-Enríquez et al., 2020).

One of the efficient and well-known methods to protect betalains from rapid degradation is encapsulation. The encapsulation of natural pigments is considered an emerging window for the food industry as this method provides excellent stability from processing to final products and superior storage stability. The main factors contributing to the betalains encapsulation were matrix type, encapsulation technique, and matrix porosity (Castro-Enríquez et al., 2020). The matrix should be a physical barrier protecting betalains from being exposed to physicochemical factors. Polysaccharides, proteins, or their combination were employed as matrices to shield the influence of external factors and maintain their structure and biological activity (Castro-Enríquez et al., 2020). The mixture of polysaccharides with different matrix characteristics contributed to more excellent protection for the betalains (Castro-Enríquez et al., 2020). Spray-drying, freeze-drying, ionic gelation, and emulsions have been reported to be suitable encapsulation technologies for betalains stabilization. The freeze-drying method is usually preferred for encapsulating water-soluble materials and aromatic and bioactive substances and promoting an easy-to-scale-up process from many aspects (Jafari et al., 2016). In recent years, researchers have focused on improving the stability of betalains by evaluating the effect of different wall materials

accompanying the freeze-drying technique on encapsulation efficiency and stability of betalains. Rodriguez et al. (2016) reported that the freeze-drying encapsulation of betalains in maltodextrin-gum Arabic and maltodextrin-pectin matrices stabilize them in preserving and improving their biological activities. A combination of maltodextrin and xanthan gum to wrap betalains of red beetroot (*Beta vulgaris L.*) reported by Atigo et al. (2018) showed the enhanced stability of freeze-dried betalains for 7 d at different pH (Castro-Enríguez et al., 2020). Also, using freeze drying, according to Mohamed et al. (2018), only 25.87 % of the betalains (from *S. fruticose*) were lost after the 8-week storage at 60 °C as encapsulated by the combined matrix of gum Arabic and maltodextrin.

Food waste or agricultural residue disposal leads to global issues. Valorizing industrial fruit by-products is essential to decrease the volume of food waste accumulated in landfills and add economic value to the production when these by-products represent a rich source of valuable compounds, which can be useful for future industrial applications (Benucci et al., 2022). Towards developing fruit waste valorization strategies to obtain new value-added products, the aim of this study is to evaluate the stability of betalains from red dragon fruit peels as affected by carbohydrate encapsulation from a combination of pectin (recovered from red dragon fruit peels - DFP) and microcrystalline cellulose (MCC).

2. Materials and methods

2.1 Extraction of betalains and pectin from red dragon fruit peel

Fresh red dragon fruit peels were collected from HG Food Joint Stock Company, Long An, Viet Nam. The green parts of the peel were removed. The red parts were blanched in water for 1 min to deactivate pectic enzymes. The peels were cut into 2-3 cm pieces. Betalains and pectin extraction from red dragon fruit peel were modified from a conventional method (Stintzing et al., 2002). Red dragon fruit peels were blended in a homogenizer with the same weight of 95 % ethanol to obtain a pulp. The extraction process of the pulp was then carried out at 40-50 °C for 60-90 mins. The mixture was filtered and washed by minimum absolute ethanol volume to obtain a colored solution and solid waste. The colored solution was concentrated by a rotavap (B-100HB, BUCHI) to obtain betalains extract. The concentrated extract was stored at 4 °C for further use. The solid was dried at 60 °C in a conventional oven (UM500, Memmert GmbH) and utilized for pectin extraction. The extraction process of pectin was carried out using acidified water (0.1–0.5 M citric acid) at 85 °C for 120 min. A solid-to-liquid weight ratio of 1:40 was applied. The hot acid extract was then filtered to remove the pulp. The filtrate was precipitated using a double volume of 95 % ethanol. The precipitates were filtered and washed with a solution of 70 % ethanol. The resulting pectin was dried in the oven at 65 °C until a constant weight was reached.

2.2 Quantification of betalains

The absorbance of betalain extract was measured at 537 nm by a UV-vis spectrophotometer (Biochrom Libra S22). The concentration was calculated by Eq(1) (Stintzing et al., 2002):

$$\text{Total betalain content (mg/L)} = \frac{A \cdot M_w \cdot D_f \cdot 1000}{E \cdot l} \quad (1)$$

where A: absorbance at 537 nm, M_w : molecular weight of betacyanin (535), D_f : dilution factor, E: molar extinction coefficient of betacyanin (60,000), l: path length (1 cm).

2.3 Structural analysis of pectin

The structures of pectin samples were evaluated using Fourier-transform infrared spectroscopy (Frontier FT-IR/NIR) with a wavelength range of 4,000–400 cm^{-1} . The data were then compared to commercial pectin (Pectin Classic CS 502, Corporate Group Herbstreith and Fox). Degree of esterification of Pectin (DE) was determined by the titrimetric method of Food Chemical Codex (FCC, 1981), and compared to commercial pectin.

2.4 Encapsulation of Betalains using a freeze-drying technique

Microcrystalline Cellulose (MCC) (from Maple Biotech Pvt. Ltd., India), pectin (DFP) (recovered from red dragon fruit peels), and maltodextrin (MD) (from Safeking Co. Ltd., Vietnam) were used as wall materials to encapsulate betalains. Three kinds of matrices applied to encapsulation were: DFP, a mixture of MCC and DFP, and a mixture of MD and DFP. Wall materials were dissolved in 50 g of distilled water to form a 10 wt% solution. The wall material solutions were cooled and stored at 4 °C for 24 h to achieve proper hydration. For the combined matrix, DFP or MD was mixed with MCC in 50 g of water with a weight ratio of 3:2 under high-speed stirring to form a homogenous 10 wt% solution of wall material. Betalains encapsulation was performed based on previous works such as Rodriguez et al. (2015) and Li et al. (2022) with modifications. Betalains extract was mixed with wall materials for a core-wall weight ratio of 1:5 or 1:10 and followed by sonication. The solution was pre-frozen at –60 °C and dried with a vacuum freeze dryer (TPV-50f Freeze Dryer) for 48 h to obtain freeze-dried powders.

2.5 Encapsulation efficiency

The method to determine encapsulation efficiency was based on Feng et al. (2018). 50 mg of powders were washed in 5 mL of acetone solution and centrifuged at 4,000 rpm for 10 min. The betalain content of the supernatant (C_1) was calculated by Eq(1). Total betalains in 50 mg of powders were extracted entirely by crushing and mixing the particles in ethanol 50 %, then vortexed thoroughly, followed by 20-min centrifugation to release betalains completely into the ethanol solution. The total betalains content of 50 mg capsules (C_o) is calculated by Eq(1). Encapsulation yields (EE) were estimated as in Eq(2).

$$EE\% = \frac{C_o - C_1}{C_o} \quad (2)$$

where C_o : total betalains content of 50 mg capsules (mg/L); C_1 : betalains content of the supernatant (mg/L). The total phenolic content was determined by Folin Ciocalteu's method (following the standard procedure - ISO 14502-1:2005) for encapsulated and non-encapsulated samples.

2.6 Evaluation of the stability of betalains

The effects of temperature, pH, water activity, and storage conditions on the stability of encapsulated betalains and betalain extract were tested as a previous procedure reported by Rodriguez et al. (2015). All experiments were performed in triplicate, and data are expressed as the mean of three samples with standard deviation.

The pH stability of the non-encapsulated and encapsulated betalains was evaluated using different buffers at different pH: 0.1 M HCl and 0.2 % NaCl buffer solution (pH 1.2) and citrate-phosphate buffer solutions (pH 3-7). Betalain retention was calculated through the UV absorbance of the solutions at 537 nm for 3 h.

The heat stability of the aqueous solutions of encapsulated and non-encapsulated betalains (0.1 % w/v) was measured by heating them at 80 and 100 °C. The solution was quickly cooled in an ice bath to stop degradation, and the betalains content was calculated at time intervals.

The effect of water activity of the non-encapsulated and encapsulated betalains was investigated by storing these samples in desiccators at two different water activities (a_w). One was attained using saturated NaOH solution with $a_w = 0.089$, and another was saturated $BaCl_2$ solution with $a_w = 0.898$. Betalains retention was monitored at 537 nm over 7 d.

The storage stability of the non-encapsulated and encapsulated betalains was investigated by storing these samples at four storage conditions: cool and dark (black ziplock bags, amber bottles for BE and kept at 4 °C refrigerator); room temperature, dry and dark (black ziplock bags for powder, amber bottles for BE extract and kept at a desiccator), room temperature, dry and daylight (transparent ziplock bags, transparent bottle for BE extract and kept at a desiccator); atmosphere and daylight (transparent ziplock bags of powders and a transparent bottle of BE extract were at free space in the lab with humidity > 90 %). Total betalains content was monitored for 30 d.

3. Results and discussion

3.1 Betalains and pectin extraction from red dragon fruit peels

In this study, the content of betalains in the extract was 2.09 ± 0.04 mg g^{-1} dry peel weight, corresponding with 95.01 % of moisture content of peel. This value was lower than those reported by Rodriguez et al. (2015) but higher than the values of Li et al. (2022) and Wu et al. (2006). These differences can be attributed to the growing environment, varieties, and extraction methods of the red dragon fruit (Le, 2022). Pectin was recovered from the solid residue of betalains extraction in 23.5 % yield and 35.5 % DE, corresponding with 11.20 % moisture content. The structure of pectin obtained was determined using FT-IR as shown in Figure 1, and compared with commercial pectin.

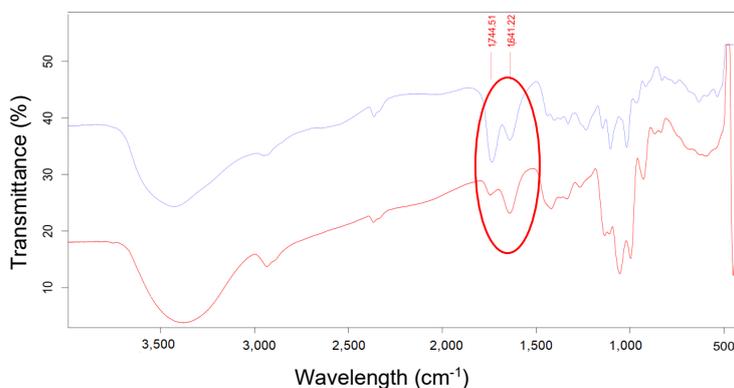


Figure 1: FT-IR spectra of red dragon fruit peel pectin (red) and commercial pectin (blue)

The red dragon fruit pectin is classified as low-methoxy pectin (DE < 50 %) at such DE value, while commercial pectin gets high DE up to 88.8 %. Other studies also reported the hot citric acid extraction of low DE pectin from red dragon fruit peel (Zaidel et al., 2017). A broader absorption band at 3,400 cm^{-1} was attributed to O-H stretching. An absorption band at 2,900 cm^{-1} was due to the C-H stretching of CH₂ groups. The absorption bands of the fingerprint regions of the typical pectin polymers are in the wavelengths 800 – 1,200 cm^{-1} . The absorption of the free and esterified carboxyl groups was at 1,641 cm^{-1} and 1,744 cm^{-1} (Zaidel et al., 2017).

3.2 Biopolymeric encapsulation of betalains

Freeze-drying technique was chosen in betalains encapsulation to obtain a pure and easy-to-use pigment in powder form due to its favorable mild process parameters (low temperature) to avoid degradation of bioactive compounds. Using different mixtures of wall materials impacts encapsulation yield. Encapsulation in DFP alone afforded the highest encapsulation yields of 92.9 %. Pectin has ideal properties as a wall material for the microencapsulation process because it can form emulsions at a very low concentration, which is crucial to covering water-repellant components (Sun et al., 2020). Rodriguez et al. (2015) discussed that adding pectin (with carboxyl groups in the glucuronic acid and galacturonic acid units) makes the wall matrix polyanionic and low-methoxy pectin may promote strong electrostatic interactions with cationic betalains, which led a high encapsulation efficiency of up to 95.7 % in the combined matrix of MD and DFP. In this study, a freeze-dried MD-DFP coated powder was prepared with the same 3:2 ratio of MD and DFP, which yielded 85.87 %. The combination of DFP and MCC in the ratio of 3:2 in the core-wall ratio of 1:5 and 1:10 resulted in lower encapsulation yields of 60.54 and 81.14 %. This difference may come from the structural and water-soluble differences between the β -(1,4)-glycosidic linkages of MCC and α -(1,4)-glucosidic linkages of MD. Maltodextrins are derived from starch with Degree of Polymerization (DP) values in the range of 2–20 and are soluble in water, while cellulose has an average DP > 500, making it insoluble in water (Siccama et al., 2021), might result in a lower dispersion of wall solution. In table 1, total phenolic content changed insignificantly after freeze-dried, and the values are pretty similar. Total phenolic content of powders was slightly lower than BE extract.

Table 1: Physicochemical characteristics of betalains (BE) and their encapsulates

| | BE extract | DFP (core:wall=1:5) | DFP-MCC (core:wall=1:5 DFP:MCC=3:2) | DFP-MCC (core:wall=1:10 DFP:MCC=3:2) | MD-DFP (core:wall=1:10 MD:DFP=3:2) |
|--|--------------|------------------------|---|--|--|
| Encapsulation yield (%) - | | 92.87 ± 0.34 | 60.54 ± 1.13 | 81.14 ± 0.78 | 85.87 ± 1.47 |
| Betalains content (mg/100 g dm) | 208.8 ± 0.03 | 169.68 ± 1.14 | 115.223 ± 1.01 | 124.90 ± 1.70 | 125.22 ± 1.88 |
| Total phenolics content (mg GAE/100 g dm) | 307.90 | 279.33 | 218.62 | 288.03 | 270.71 |

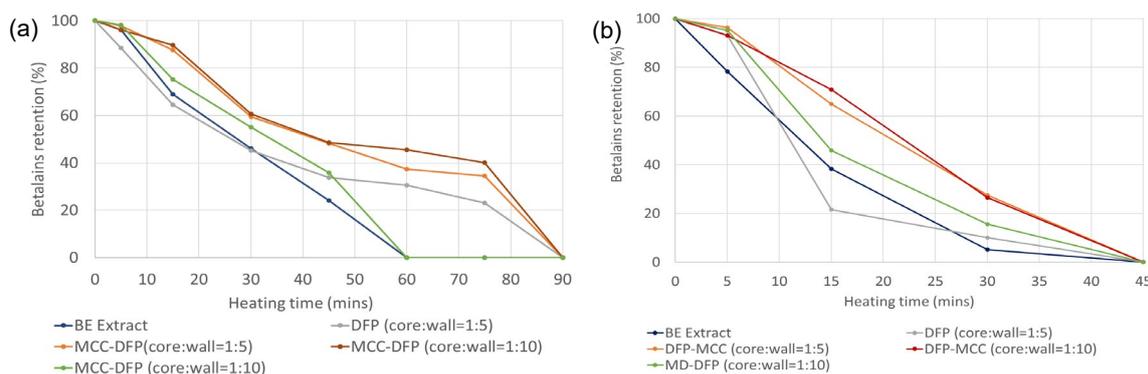


Figure 2: Degradation plots of encapsulated and non-encapsulated betalains at (a) 80 °C, and (b) 100 °C

3.3 Thermal stability of encapsulated betalains

All food processing factors affect the stability of betalains, limiting their application in food. Temperature is one factor that has the most significant effect on the structure of betalains. Figure 2 shows a reduction of betalains content in the aqueous solution after prolonged heating time at 80 °C and 100 °C, a typical cooking temperature range. In general, increased temperature resulted in a rise in the degradation rate and the structural modification of betalains through a range of reactions, leading to color changes and degradation (Calva-Estrada et al., 2022). The loss of color was faster when the temperature increased to 100 °C, particularly in the presence of oxygen, irreversible betalains degradation is promoted (Figure 2b). In a heating condition of 80 °C (Figure 2a), except

DFP powder, two-layer encapsulated powders improved the degradation rate of betalains compared to BE extract, in which the combination of MCC and DFP at two ratios showed superior heating stability compared to the other two at 100 °C, even though their betalain encapsulation did not reach a high trapping efficiency. Freeze-dried powders coated with MCC and DFP can be capable colorants in food products.

3.4 Effect of water activity on the stability of encapsulated betalains

Encapsulated betalains showed more excellent betalain retention after 7 d at two water activity levels (Table 2). A higher rate of betalain degradation of the extract was caused by exposure to a higher water activity environment. The reason might be that the mobilization of betalains at high a_w caused aldimine bond cleavage, leading to degradation and loss of color (Rodriguez et al., 2015). There are not many differences in the degradation rates of the four capsules in both high and low water activity conditions. With low encapsulation yields, freeze-dried two-layer powders exhibited similar betalain retention but slightly lower than DFP powder at both water activities. The performance of MD-DFP powder under both conditions is higher than the ones reported by Rodriguez et al. (2015).

Table 2: Degradation plot of betalains in the betalain extract and their encapsulates

| Water activity | Betalains Retention after a week (%) | | | | |
|----------------|--------------------------------------|------------------------|---|--|--|
| | BE extract | DFP (core:wall=1:5) | DFP-MCC (core:wall=1:5 DFP:MCC=3:2) | DFP-MCC (core:wall=1:10 DFP:MCC=3:2) | MD-DFP (core:wall=1:10 MD:DFP=3:2) |
| $a_w = 0.089$ | 50.32 | 96.06 | 91.82 | 94.18 | 89.78 |
| $a_w = 0.898$ | 42.32 | 88.11 | 80.99 | 84.05 | 78.25 |

3.5 Effect of pH on the stability of encapsulated betalains

Figure 3a shows betalain retention plots under different pH levels. Betalains in both encapsulated and non-encapsulated forms exhibited high and similar retention values at pH 3–7, which is compatible with Rodriguez et al. (2016) report. On the other hand, freeze-dried powders and BE extract showed lower stability at pH 1.2, in which the betalains retention of DFP-coated powder is slightly higher than two-layer blended ones (> 75 % of retention). The polyanionic matrix of low methoxy DFP might make the capsule more stable at low pH. The BE encapsulation in DFP alone or in the combination of DFP and MCC with the core-wall weight ratio of 1:10 improved BE stability at pH 1.2. As a result, they can be a stable colorant in food applications with pH 3.6–7.4 as they might protect bioactive betalains from the stomach's acidic environment (pH 1.2) and can be released and absorbed entirely in the intestines.

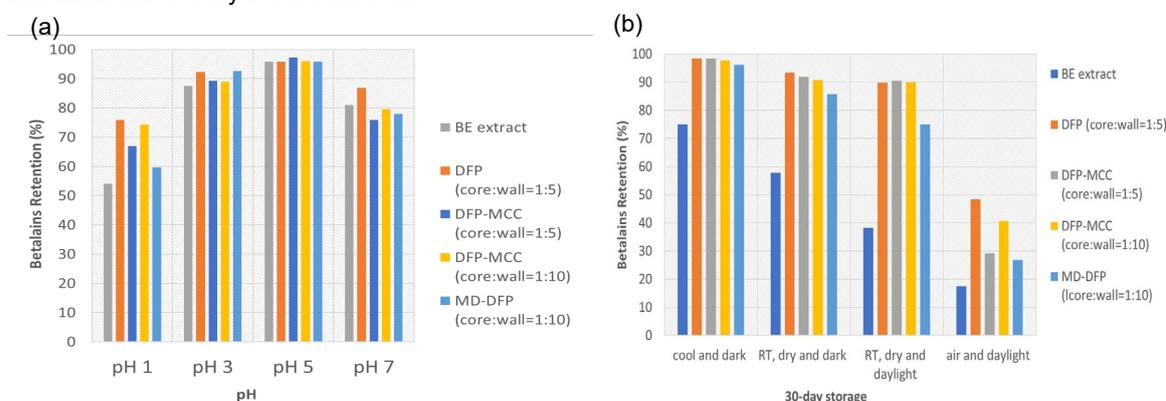


Figure 3: Betalains retention (%) of BE extract and Freeze-dried powders (a) after 3 h at different pH levels; and (b) after 30 d under different storage conditions

3.6 Storage stability evaluation

Storage factors, including temperature, light, moisture, and oxygen, affect the stability of betalains and have to be considered to ensure their properties. Betalain retention over 30 d of freeze-dried betalains and its extract stored under various conditions is shown in Figure 3b. In four storage conditions, the retention of three betalains powders indicated improved stability. Betalains retention was most excellent at 4 °C, then at room temperature without light in the desiccator, and lowest at room temperature with light in the desiccator. All samples exposed to air without protection indicated a considerable decrease in betalain content after one month. These results were consistent with previous works (Castro-Enríquez et al., 2020). Using MCC and DFF as a mixture of wall material remained the stability of betalains samples stored in refrigerator and desiccator. DFP wall material was

more stable than the others when exposed to air. Encapsulation in two carbohydrates contains about 15-40 % of free betalains, which were degraded easily by moisture, light, and oxygen in the lab space. There is not much difference between the remaining percentage of BE powders encapsulated by MCC and DFP with light exposure and ones without light exposure. As a result, betalains coated by DFP and a mixture MCC and DFP can be potentially commercialized with packaging in a dry condition.

4. Conclusions

The study prepared three betalains capsules with betalains extracted from red dragon fruit peels in support of the freeze-drying technique with 60–93 % of encapsulation yields. The stability of betalains capsules was investigated with full of processing and storage factors. By encapsulation in carbohydrate biopolymers, betalains from red dragon fruit peels can be protected and stabilized under a variety of storage conditions, high temperatures, pH levels, and water activity levels. More particularly, microcrystalline cellulose (MCC) combined with dragon fruit peel pectin (DFP) proved to be a stable heating carrier for betalains encapsulation. The BE encapsulation in DFP and MCC matrices with the core-wall weight ratio of 1:10 enhanced betalains stability at pH 1.2, contributing to intensifying the sufficient take-up of bioactive compounds in the food system with pH 3.6 – 7.4. Adequate conservation and packaging avoiding moisture should be recommended for these freeze-dried powders, making them more useful as commercialized food colorants. The study promoted fruit waste valorization to produce add-value products such as pectin and betalains.

Acknowledgments

The study was supported by The Youth Incubator for Science and Technology Program, managed by Youth Development Science and Technology Center - Ho Chi Minh Communist Youth Union and Department of Science and Technology of Ho Chi Minh City, the contract number is “21/2021/HĐ-KHCNT-VU”.

References

- Atigo J.L.D., Bergamasco R.D.C., Madrona G.S., 2018, Effect of pH on the stability of red beet extract (*Beta vulgaris* L) microcapsules produced by spray drying or freeze drying, *Food Science Technology*, 38, 72–77.
- Benucci I., Lombardelli C., Mazzocchi C., Esti M., 2022, Natural colorants from vegetable food waste: Recovery, regulatory aspects, and stability—A review, *Comprehensive Reviews in Food Science and Food Safety*, 21, 2715–2737.
- Calva-Estrada S.J., Jiménez-Fernández M., Lugo-Cervantes E., 2022, Betalains and their applications in food: The current state of processing, stability and future opportunities in the industry, *Food Chemistry: Molecular Sciences*, 4, 100089–100100.
- Castro-Enríquez D.D., Montaña-Leyva B., Del Toro-Sánchez C.L., 2020, Stabilization of betalains by encapsulation—A review, *Journal of Food Science and Technology*, 57, 1587–1600.
- Feng Z., Li M., Wang Z., Zhu M., 2018, Astaxanthin from *Phaffia rhodozyma*: Microencapsulation with carboxymethyl cellulose sodium and microcrystalline cellulose and effects of microencapsulated astaxanthin on yogurt properties, *LWT*, 96, 152–160.
- Jafari S.M., Mahdavi-Khazaei K., Hemmati-Kakhki A., 2016, Microencapsulation of saffron petal anthocyanins with cress seed gum compared with Arabic gum through freeze drying, *Carbohydrate Polymers*, 140, 20–25.
- Li X., Zhang Z.-H., Qiao J., Qu W., Wang M.-S., Gao X., Zhang C., Brennan C. S., Qi X., 2022, Improvement of betalains stability extracted from red dragon fruit peel by ultrasound-assisted microencapsulation with Maltodextrin, *Ultrasonics Sonochemistry*, 82 (2), 105897–105905.
- Le N.L., 2022, Functional compounds in dragon fruit peels and their potential health benefits: A review, *International Journal of Food Science & Technology*, 57, 2571–2580.
- Mohamed E., Iwamoto S., Yamauchi R., 2018, Optimization of betalain extraction from *Salicornia fruticosa* and its encapsulation, *Journal of Agroalimentary Processes and Technologies*, 24, 1–8.
- Rodriguez E., Vidallon M., Mendoza D., Dalisay K., Reyes C., 2015, Stabilization of Betalains from the Peel of Red Dragon Fruit [*Hylocereus polyrhizus* (Weber) Britton & Rose] through Biopolymeric Encapsulation, *The Philippine Agriculturist*, 98, 382–391.
- Stintzing F., Schieber A., Carle R., 2002, Betacyanins in fruits from red-purple pitaya, *Hylocereus polyrhizus* (Weber) Britton & Rose, *Food Chemistry*, 77(1), 101–106.
- Siccama J.W., Pegiou E., Eijkelboom N.M., Zhang L., Mumm R., Hall R.D., Schutyser M.A.I., 2021, The effect of partial replacement of Maltodextrin with vegetable fibres in spray-dried white asparagus powder on its physical and aroma properties, *Food Chemistry*, 356, 129567–129578.
- Sun X., Cameron R.G., Manthey J.A., Hunter W.B., Bai J., 2020, Microencapsulation of tangeretin in a citrus pectin mixture matrix, *Foods*, 9, 1200–1212.
- Zaidel D., Rashid J., Hamidon N., Salleh L., Kassim A., 2017, Extraction and characterisation of pectin from dragon fruit (*Hylocereus Polyrhizus*) peels, *Chemical Engineering Transactions*, 56, 805–810.