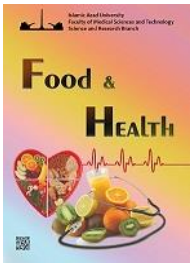

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Physical Properties of Microencapsulation of *Echium amoenum*. L and *Altaea rosea* var nigra Extract Prepared by Freeze Dryer Method

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ABSTRACT

Phenolic compounds are secondary metabolites in plants that also have health effects. Due to the effect of synthetic antioxidants on human health, there is a growing tendency to replace them with natural antioxidants. This research aims to investigate the beneficial properties of borage extract (BE) and black hollyhock extract (BHE). First, the BE and BHE were encapsulated with whey protein and maltodextrin at 90:10, 50:50, 10:90 ratio by freeze drying method. Then, the analytical characteristics of BE and BHE were investigated. The amount of encapsulation of anthocyanin increased when the capsule had a higher whey protein content. In the encapsulation powder extract of BE and BHE with a whey protein ratio of 90 to 10 maltodextrin, the lowest amount of moisture and solubility was observed. When comparing the bulk density of the encapsulated extract of BE and BHE, no significant difference was observed, and the type of extract had no effect on the powder density. The combination of coating materials with a ratio of 10 maltodextrin to 90 whey protein has the highest encapsulation efficiency. Capsules with higher levels of whey protein were thicker walled capsules. Therefore, the optimal treatment was the capsule with a concentration of 90% whey protein and 10% maltodextrin.

1. Introduction

Phenolic compounds have displayed antioxidant, antimicrobial, anti-inflammatory and anticancer activities (1) containing phenolic compounds from Herbs. Despite this fact, their demand and use in food is limited due to their capability to bitter or acrid taste and low bioavailability (2). The microencapsulation approach boosts the release of phenolic substances in variant food matrices, leading to made better stability and solubility of bioactive compounds during processing, storage or gastric digestion of the product (3). Microencapsulation encompasses of the protection of diverse food components or functional ingredients against external factors such as temperature, oxygen, light, moisture or interactions with food components such as proteins (3, 4). Variation of coating materials are used depending on their rheological properties and ability to disperse and stabilize the active ingredient. Maltodextrin (MD) is a kind of hydrolyzed starch with suitable solubility and low viscosity. Whey protein isolate (WPI) is a good carrier of bioactive compounds due to its good emulsifying, gelling and thickening attributes (5). Borage (*Echium amoenum*) belongs to the family Boraginaceae, which is considered a valuable and medicinal plant in traditional medicine, especially in Iran, whose flowers are rich in polyphenols and anthocyanins (6). This plant is used in traditional medicine to relieve pain, pulmonary and cardiovascular diseases, antidepressant, anti-inflammatory and antifebrile, for influenza and infectious diseases and cancer (7). Borage also exhibits various functional activities such as antioxidant (8), antiviral (9), and antimicrobial (10). The functional activities of borage are due to the presence of remarkable amounts of phenolic compounds, flavonoids, anthocyanins, and fatty acids (8,7). Althaea rosea or hollyhock is another valuable medicinal plant that belongs to the

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Malvaceae family and is known for the distinctive color of its flowers (11). Research has shown that black hollyhock is a rich source of polyphenols, anthocyanins, flavonols, carotenoids, and phenolic acids. The flowers of this herb have remarkable enzyme inhibitory and anti-aging activity (12). The good antioxidant and antimicrobial activity of this herb has also been reported by previous researchers (11, 13-15) showed that citrus flavone microcapsules prepared by whey protein concentrate and acacia gum complex had better retention efficiency after 3 months of storage than those prepared by acacia gum alone. The objective of this study was to investigate the properties of encapsulating extracts from two plants (BE, BHE) and to examine the encapsulation efficiency of encapsulated plant extracts. The study was designed to investigate the possibility of preserving functional phenolic compounds with encapsulated by freeze drying method.

2. Materials and Methods

2.1. Materials

Maltodextrin with DE of 4-7 and whey protein isolate were purchased from Merck Co. (Germany) and Sigma Aldrich Co. (America), respectively. Borage flowers were collected from the mountainous areas of Mazandaran province and approved by the herbarium organization. Black hollyhock was purchased from Zarband Pharmaceutical Co. (Iran). Chemicals used in this research were prepared by Merck Co. (Germany).

2.2. Preparation of BE and BHE

The borage flower and black hollyhock were dried completely in an oven at 50 °C, and then powdered. The extracts were extraction using ultrasound device. In such a way that, 10 g of each of the powders was homogenized with 100 ml of water and then subjected to an ultrasound device (AMMM-M.P. Interconsulting, Switzerland) at power of 240 W and temperature of 20 °C for 10 min. The tubes containing solutions were covered by the aluminum foil and then placed in a shaker for 24 h. Finally, the filtered extracts were kept at 4°C until further use (16).

2.3. Microencapsulation of BE and BHE

Carrier materials including the mixture of maltodextrin and whey protein in the ratio of 10 to 90, 50 to 50 and 10 to 90 were used in this research. The carrier materials and extract (BE or BHE) were mixed together and dissolved in distilled water containing 0.02% preservative (w/w; sodium azide) using a magnetic shaker at 1000 rpm and 45 °C for 30 min. After that, the solutions were homogenized for 2 min at 13000 rpm and for 10 min at 22000 rpm and then immediately used to prepare the capsules powder. Two-flowing nozzle dryer (Counter-current, Iran) was used to dry the microencapsulated extracts. The solutions were injected into the freezer dryer by a pump (400 kPa air pressure). The inlet air temperature was 180 °C and the outlet temperature was 80 °C. The produced powders were collected in the sub circumference chamber, and immediately transferred to the desiccator to cool down. Finally, BE and BHE microcapsule powders were poured into glass jars covered with aluminum foil and kept at 4 °C (17).

2.4. Determination of BE and BHE microcapsules characterization

2.4.1. Measurement of moisture content

Use the method described in the AOAC Standard to determine the moisture content of the powder.

2.4.2. Solubility Measurement

One gram of powder was dissolved in 100 ml of water and the solution was centrifuged (Rotofix 32 A, German) at 7500 rpm for 10 minutes to separate the insoluble parts. Then, 25 ml of the transparent part of the test tube was removed and placed in a binder (BINDER, Germany) for 5 hours at 105°C. The solubility value was calculated as a percentage according to the following equation: $S= (M1-M2)/0.25\times100$

In this equation, S, M2, M1 were the weight of the container after removal from the oven, the volume of the empty container and the solubility after 5 hours (18).

2.4.3. Measuring the water activity

The water activity of the powder was determined by means of a water activity meter (20°C) (19).

2.4.5. Determine the density of the mass

A quantity of 5 ml of powder prepared in a graduated cylinder was measured and weighed. The measurement was repeated three times and the mean value was noted. The following equation was used to calculate the bulk density:

$$pb = m/Vb$$

Where, in this formula, pb is the mass density, M is the sample mass and Vb is the sample mass volume (20)

2.4.6. Determine the amount of anthocyanins

Potassium chloride solution 0.025 M was adjusted to pH = 1 with hydrochloric acid. And then 3/6 cc of this solution was mixed with 0.4 cc sample of the extract and its absorbance was measured at two wavelengths of 510 and 700 nm after obtaining a stable state by spectrophotometer (Photonix Ar 2015, Iran). Then, the amount of adsorption (A) and the total amount of anthocyanins were obtained from the following equation: $TA\ (mg / L) = \Delta A / \varepsilon \times 1\times M \times 103 \times D$

In this equation, TA is the total anthocyanin concentration based on anthocyanin mg in 100 ml, M is the dominant molecular weight of anthocyanins, D is the dilution factor, ε is the molar ratio of cyanidine, and ΔA is the difference between the adsorption at pH = 1 and pH = 4.5 (21).

2.4.7. Measurement of encapsulation efficiency of BE and BHE

After obtaining the total and surface anthocyanin in the above manner, we obtained the following encapsulation efficiency according to the following equation:

$$\%EE = (TA\text{-}TS)/TA \times 100$$

Where, EE is the encapsulation efficiency, TA is the total anthocyanin content, and TS is the anthocyanin content (22).

2.5. Statistical analysis of data

Statistical analysis was done in triplicate for all the samples and experiments, and the obtained data are demonstrated as mean ± SD. The one- way ANOVA analysis, Duncan multi-range post hoc test, and IBM SPSS Statistics 22.0 (Chicago, USA) were used to analyze the data at significance level of $p<0.05$.

3. Results and Discussion

3.1. Effect of wall material on the moisture content of powders

As shown in Fig. 1, the type and concentration of the wall material effectively overshadowed the final moisture content of the produced powders, such that the highest moisture content was observed in the wall of the sample that contained the highest amount of maltodextrin (90 maltodextrin to 10 whey protein ratio). BE extract had a higher moisture content compared to BHE. This issue is probably related to the nature of BE extract that it has less solids and higher solubility. (23) showed that maltodextrin with DE = 25 affects the reduction of moisture content due to the difference in chemical structure between the two carriers. In addition, (24) suggested that the increase in moisture content associated with the use of maltodextrin coatings is due to the fact that diffusion of water from large maltodextrin molecules is difficult.

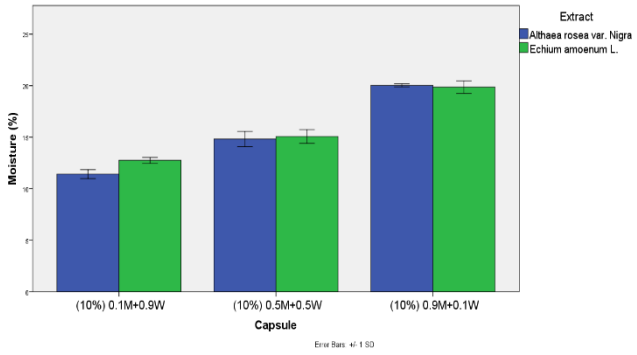

Fig. 1. The effect of wall type on the moisture content of the powders.

3.2. The effect of wall material on the solubility of powders

According to the results obtained, the higher the amount of whey protein in the samples, the lower the solubility of the samples. This problem is probably due to the fact that whey protein is a heavier compound than maltodextrin, and maltodextrin, which is made of sugar, dissolves easily in water, but proteins have lower solubility due to their more complex structure. In the samples, the solubility of BE extract was also higher than that of BHE. BHE extract has more complex compounds with higher molecular weight, i.e. heavier phenolic compounds and lower solubility compared to BE. Therefore, BE extract, which does not have many solids, makes the capsule more soluble when its solids enter the capsule. In all samples, the solubility increased with the increase of maltodextrin. (25) investigated the effect of spray drying conditions and the use of coating compounds on the physical properties of powdered black currant extract, their results showed that 2% maltodextrin and 6% gum Arabic had the highest solubility (about 87%).

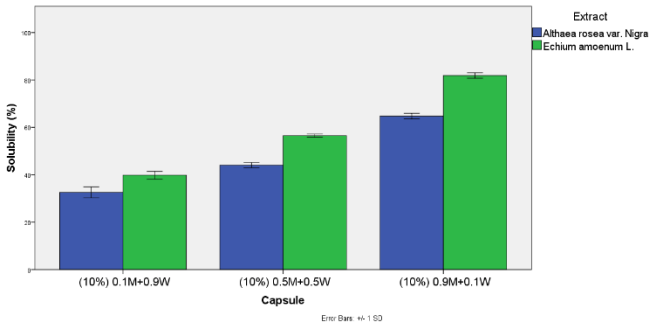

Fig. 2. The effect of wall type on the solubility content of the powders.

3.3. The effect of wall material on the density of powders

Bulk density is one of the most important parameters measured in powders. This is important in terms of transportation, storage and packaging in the industry, the density of mass is one of the characteristics of food that depends on the size, shape, surface characteristics and powder particles. Thus, smooth and uniform powders have a higher bulk density. Comparison of the mean bulk density of the samples showed that with increasing maltodextrin and decreasing whey protein, the bulk density decreased. Due to the weight and complexity of the protein structure, the protein density increased compared to the carbohydrates and with this decrease, the density also decreased. Comparison of bulk density of encapsulated extract of BE and BHE showed no significant difference. And the type of extract had no effect on the powder density. (18) showed that the higher the molecular weight of the powder, the higher the volumetric density. Heavier materials move more easily between the spaces of the particles, so they are more voluminous in less space.

3.4. The effect of wall material on anthocyanins

As shown in Fig. 4, with an increase in maltodextrin and a decrease in its whey protein, anthocyanin levels decreased. The amount of anthocyanin encapsulation increased when the capsule had a higher whey protein content (90 protein to 10 maltodextrin). Because whey protein has a stronger protein and a more solid capsule, the whey protein was able to capture more of the anthocyanin. By reducing the whey protein and increasing the maltodextrin, the capsule wall became thinner

and probably some of the anthocyanins were removed and destroyed. In 2010, Renata et al. showed a negative effect on the amount of anthocyanins. The researchers state in their results that the rate of further degradation of anthocyanins at higher temperature may be due to the presence of sugar and protein in the product structure, which ultimately leads to the Maillard reaction during the production process or during storage. (26) argued that micronization of black carrot by spray drying technique at 160 ° C and the use of maltodextrin coating with grade 30 had the highest anthocyanin level. Maltodextrin and gum Arabic are highly soluble substances. Therefore, the mixture is spray dried. As a result, the resulting powder consists of hollow particles whose crust is a matrix containing both carriers and trapped juices, which can be considered as microcapsules juice.

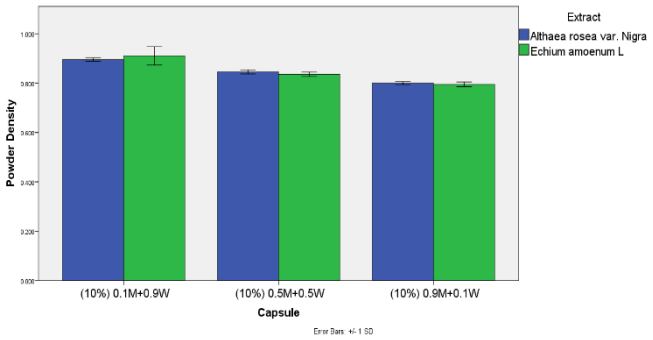

Fig. 3. The effect of the type of wall material on the density of the powders.

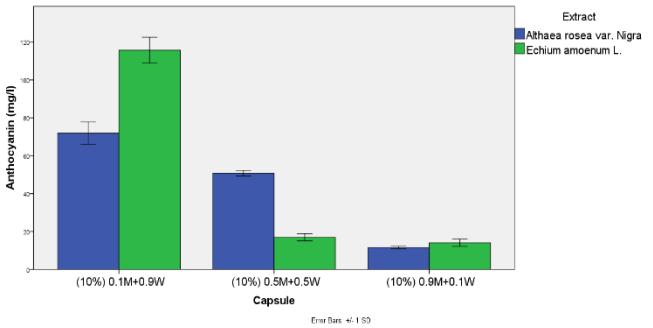

Fig. 4. The effect of the type of wall material on the Anthocyanin of the powders.

3.5. The effect of wall material on the encapsulation efficiency

Encapsulation efficiency is a measure of how much of the core material was successfully encapsulated and can be used to evaluate the efficiency of the encapsulation process. The results of investigating of encapsulation efficiency show that with decreasing protein content, the strength of the capsule was reduced. And as a result, the capsule's efficiency declined. In the sample with a protein ratio of 90 to 10 maltodextrin, the highest encapsulation efficiency (above 80%) was observed. (27) concluded that increasing concentration of maltodextrin as wall material from 5 to 15% gave rise to a significant increase in encapsulation efficiency of pomegranate peel anthocyanin. More recently, the encapsulation efficiency of catechin was shown to increase significantly by increasing the concentration of wall materials from 5 to 10% (w/w) (28).

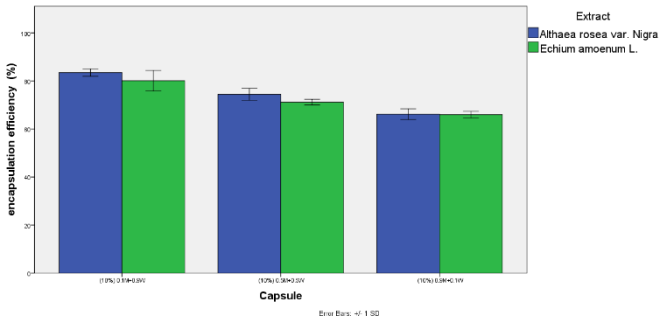

Fig. 5. The effect of the type of wall material on the encapsulation efficiency.

4. Conclusion

This study assessed the effectiveness of freeze-drying methodology in the microencapsulation of BE and BHE extract, employing different concentrations and ratios of maltodextrin and whey protein. According to the results obtained from the microcoating efficiency, it was observed that as the concentration of whey protein increased, the efficiency also increased. The density test results also showed that the density decreased as the whey protein concentration increased. This means that by increasing the whey protein, large and bulky capsules were obtained. Therefore, the optimal treatment was the capsule with a concentration of 90% whey protein and 10% maltodextrin.

References

1. Monjotin N, Amiot MJ, Fleurentin J, Morel JM, Raynal S. Clinical evidence of the benefits of phytonutrients in human healthcare. *Nutrients*. 2022;14(9):1712.
2. Nwabor OF, Singh S, Marlina D, Voravuthikunchai SP. Chemical characterization, release, and bioactivity of Eucalyptus camaldulensis polyphenols from freeze-dried sodium alginate and sodium carboxymethyl cellulose matrix. *Food Quality and Safety*. 2020;4(4):203-12.
3. Gruskiene R, Bockuviene A, Sereikaite J. Microencapsulation of bioactive ingredients for their delivery into fermented milk products: A review. *Molecules*. 2021;26(15):4601.
4. Tomé AC, da Silva FA. Alginate based encapsulation as a tool for the protection of bioactive compounds from aromatic herbs. *Food Hydrocolloids for Health*. 2022;2:100051.
5. Souza AL, Hidalgo-Chávez DW, Pontes SM, Gomes FS, Cabral LM, Tonon RV. Microencapsulation by spray drying of a lycopene-rich tomato concentrate: Characterization and stability. *LWT*. 2018;91:286-92.
6. Mehran M, Masoum S, Memarzadeh M. Improvement of thermal stability and antioxidant activity of anthocyanins of Echium amoenum petal using maltodextrin/modified starch combination as wall material. *International Journal of Biological Macromolecules*. 2020;148:768-76.
7. Zannou O, Pashazadeh H, Ghellam M, Ibrahim SA, Koca I. Extraction of anthocyanins from Borage (*Echium amoenum*) flowers using choline chloride and a glycerol-based, deep eutectic solvent: Optimization, antioxidant activity, and in vitro bioavailability. *Molecules*. 2021;27(1):134.
8. Karimi E, Oskoueian E, Karimi A, Noura R, Ebrahimi M. Borago officinalis L. flower: A comprehensive study on bioactive compounds and

its health-promoting properties. *Journal of Food Measurement and Characterization*. 2018;12:826-38.
9. Abolhassani M. Antiviral activity of borage (*Echium amoenum*). *Archives of Medical Science*. 2010;6(3):366-9.
10. Patocka J, Navratilova Z. Bioactivity of *Echium amoenum*: A mini review. *Pharmacology*. 2019;20(2):14915-7.
11. Nazir S, Ahmad MK, Ali F, Ganie SA. Phytochemical analysis and antibacterial potential of *Onosma hispidium* and *Alcea rosea*. *Biomedicine*. 2022;42(1):47-52.
12. Nowicka P, Wojdyło A. Anti-hyperglycemic and anticholinergic effects of natural antioxidant contents in edible flowers. *Antioxidants*. 2019;8(8):308.
13. Fahamiya N, Shiffa M, Aslam M. A comprehensive review on *Althaea rosea* Linn. *Journal of Pharmaceutical Research*. 2016;6(11).
14. Yourdkhani E, Jafarpour A. The effect of aqueous and ethanolic extract of Hollyhock black (*Alcea rosea*) on physicochemical and antioxidant properties of ketchup sauce. *Food & Health*. 2021;4(3)19-23.
15. Hu Y, Li Y, Zhang W, Kou G, Zhou Z. Physical stability and antioxidant activity of citrus flavonoids in arabic gum-stabilized microcapsules: Modulation of whey protein concentrate. *Food Hydrocolloids*. 2018;77:588-97.
16. Rabiei K, Bekhradnia S, Nabavi S, Nabavi S, Ebrahimzadeh M. Antioxidant activity of polyphenol and ultrasonic extracts from fruits of Crataegus pentagyna subsp. elburensis. *Natural Product Research*. 2012;26(24):2353-7.
17. Bae E, Lee S. Microencapsulation of avocado oil by spray drying using whey protein and maltodextrin. *Journal of Microencapsulation*. 2008;25(8):549-60.
18. Goula AM, Adamopoulos KG. Spray drying of tomato pulp in dehumidified air: I. The effect on product recovery. *Journal of Food Engineering*. 2005;66(1):25-34.
19. Garcia-Amoedo LH, Almeida-Muradian LBD. Comparação de metodologias para a determinação de umidade em geléia real. *Química Nova*. 2002;25:676-9.
20. Zhu J, Li X, Liu L, Li Y, Qi B, Jiang L. Preparation of spray-dried soybean oil body microcapsules using maltodextrin: Effects of dextrose equivalence. *LWT*. 2022;154:112874.
21. Association of Official Analytical Chemists, Association of Official Agricultural Chemists (US). Official methods of analysis of the Association of Official Analytical Chemists. Association of Official Analytical Chemists.; 1920.
22. Afzaal M, Khan AU, Saeed F, Ahmed A, Ahmad MH, Maan AA, et al. Functional exploration of free and encapsulated probiotic bacteria in yogurt and simulated gastrointestinal conditions. *Food Science & Nutrition*. 2019;7(12):3931-40.
23. Grabowski J, Truong VD, Daubert C. Spray-drying of amylase hydrolyzed sweetpotato puree and physicochemical properties of powder. *Journal of Food Science*. 2006;71(5):E209-E17.
24. Adhikari B, Howes T, Bhandari B, Troung V. Effect of addition of maltodextrin on drying kinetics and stickiness of sugar and acid-rich foods during convective drying: experiments and modelling. *Journal of Food Engineering*. 2004;62(1):53-68.
25. Fazaeli M, Emam-Djomeh Z, Kalbasi-Ashtari A, Omid M. Effect of process conditions and carrier concentration for improving drying yield and other quality attributes of spray dried black mulberry (*Morus nigra*) juice. *International Journal of Food Engineering*. 2012;8(1):1-20.
26. Ersus S, Yurdagel U. Microencapsulation of anthocyanin pigments of black carrot (*Daucus carota* L.) by spray drier. *Journal of food Engineering*. 2007;80(3):805-12.
27. Sharayei P, Azarpazhooh E, Ramaswamy HS. Effect of microencapsulation on antioxidant and antifungal properties of aqueous extract of pomegranate peel. *Journal of Food Science and Technology*. 2020;57(2):723-33.
28. Ruengdech A, Siripatrawan U. Improving encapsulating efficiency, stability, and antioxidant activity of catechin nanoemulsion using foam mat freeze-drying: The effect of wall material types and concentrations. *LWT*. 2022;162:113478.