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Impacts of Drying Methods on Physical Properties and Release Kinetics of Complex Coacervated Berberine Microcapsules

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ABSTRACT: Berberine is a multifunctional compound belonging to the Berberidaceae family may be used to promote the quality of industrial food products in the form of dried microcapsules. In this research, the effects of the drying techniques on the coacervated microcapsules as well as the core material's quality and stability were investigated. Berberine microcapsules using *Astragalus rahensis*/gelatin cross-linked by transglutaminase were dried by two different methods The particle size and polydispersity, efficiency, berberine release kinetics were analyzed. Spray-dried (SD) microcapsules were 37.6–49.6 nm in diameter, whereas freeze-drying produces bigger particles with wider size dispersion. The positive interaction between the low-grade flaky tragacanth and gelatin reduces the undesirable stickiness of SD microcapsules and causes higher drying yields by up to 50 %. The porous structure of the freeze-dried (FD) microcapsules may expose sensitive berberine to oxidative reactions. Differential scanning calorimetry thermograms indicated greater thermal stability of SD microcapsules than FD microcapsules. The release profiles of berberine showed a good fit to the modified Korsmeyer-Peppas model, where the SD microcapsules with the greatest burst effect at 5˚ and 80˚C provide the desirable controlled release characteristics. Berberine SD microcapsules may be used as a safe and nutritious powder in heat-treated food formulations.

Keywords: *Astragalus rahensis*, *Berberis vulgaris*, Berberine, Complex Coacervation, Freeze-drying, Spray-drying.

Introduction

Berberine is an isoquinoline-type alkaloid and mostly found in plants belonging to *Berberidaceae* family. Berberine has a long history of extensive usage in traditional Chinese and Indian medicine (Ikuta & Itokawa, 1988). Berberine also offers numerous biological and pharmacological properties including anti-cancer (Wang *et al*., 2020); anti-lipidemic (Zhao *et al*., 2021), anti-viral (Warowicka *et al*., 2020), anti-bacterial (Schmeller & Latz-Brùning, 1997), anti-inflammatory (Liu *et al*., 2013), anti-

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diarrheal (Chen *et al*., 2015), anti-hypertension (Xia & Luo, 2015), and antioxidant activities (Li *et al*., 2014). This compound is obtained in *Berberidaceae* plant species such as *Hydrastis canadensis* L., *Mahonia aquifolium*, *Xanthorhiza simplicissima*, and *Phellodendron amurense* (Yeung *et al*., 2020). *Berberis* are a group of berry fruits, widely distributed in Asian, African and tropical regions and one of the known seedless varieties of the *Berberidaceae* family is *Berberis vulgaris L. var. asperma* (Hatch, 2007; Bhat & Paliyath, 2016). The seedless *Barberry* (*B. vulgaris var. asperma)* is a special fruit belonging to family of Berberidaceae. It is one of the few unique crops grown in Asia, Europe and North Africa. (Hatch, 2007; Alemardan *et al*., 2013). This variety is widely cultivated in Iran, particularly in Southern Khorasan province, located in the eastern part of Iran (Ghosi, 2021). This cultivar has been the subject of intense investigations for the extraction of its multifunctional chemicals for deployment in food and medicinal formulations. Our recent study determined the optimal conditions for berberine extraction in terms of maximum yield, antioxidant activity, and safety by using a co-solvent such as ethanol-water mixture (Keshtkaran *et al*., 2022). One of persistent challenges of using extracted bioactive ingredients such as berberine in food formulations is their susceptibility to degradation and oxidation upon exposure to light, oxygen, heat, and moisture which cause a product with compromised antioxidative activity (Admassu & Kebede, 2019). In order to overcome this challenge, many strategies have been developed to minimize the inconveniences and degradation risks of berberine during oral administrations or processing using microencapsulation technologies (Šeregelj *et al*., 2020).

Microencapsulation involves the manufacture of microcapsules by entrapping the sensitive bioactive compounds within a polymeric matrix or carrier to enhance their stability and bioavailability and to protect them from the damages of harsh environmental or processing conditions such as heat, oxidization, light or moisture and acidity(Yu *et al*. 2017).Microcapsules enable the controlled released of entrapped active substances for an extended period of time (Zuidam & Nedovic, 2010; Ozkan *et al*., 2019). Complex coacervation is a common microencapsulation strategy that is based on separation of colloidal systems into two liquid phases. The associative phase separation between two oppositely charged polymers in an aqueous solution generates a solid complex precipitate (Gouin, 2004; Zuidam & Nedovic, 2010; Ozkan *et al*., 2019; Keshtkaran *et al*., 2022). This is to note that wet microcapsules may not be practically suitable for usage in the food industries, thus, a drying stage is typically required to produce dried microcapsules with a longer shelf life and broader applications (Fernandes *et al*., 2013; Holkem *et al*., 2016). Various drying techniques including freeze-drying, spray drying, and fluidized bed drying have been applied to obtain a dried powder, although freeze- and spray drying have received more attention from academics and occupied the main stream of recent literature (Pang *et al*., 2017; Kanha *et al*., 2021; Qi *et al*., 2021). The choice of drying technique can impart important effects on the microcapsules' characteristics, as well as the core material's quality, stability, and release properties. There are few studies that focused on development of the microcapsulation systems containing berberine (Lam *et al*., 2012; Hu *et al*., 2013); however, the effects of drying

methods on the properties of berberine-entrapped microcapsules that are somewhat unexplored scenarios and need to be investigated with a more comprehensive approach. Given the lack of information on this aspect, this study was devised to engineer a berberine-loaded microcapsule and examine its physical properties and microstructures after drying with two different drying methods (freeze and spray drying). In addition, the solubility, thermal stability, and the release kinetics of the dried microcapsules have been characterized in detail. The findings of this research are envisioned to provide a better comprehension in selecting drying techniques that can be applied toward the manufacture of high quality berberine powder.

Materials and Methods

- *Materials*

The whole *Berberis vulgaris* plant including fruit, leaves, and stems was collected at maturity phase from South Khorasan province (Qayen, Iran) so that all berberine-containing components could be utilized (Rokade *et al*., 2022). Ethanol as an extraction solvent was purchased from Merck (Darmstadt, Germany). The powdered gelatin type B and berberine chloride hydrate (purity of ~90%) were purchased from Sigma-Aldrich Company (St. Louis, Mo., USA). Tragacanth gum (rahensis species) was kindly supplied by the Shahid Beheshti Institute of Nutrition and Food Science (Tehran, Iran). Commercial microbial transglutaminase enzyme (TEGEN 220 DM) with a nominal enzyme activity of 120 U/g was supplied from Benosen Chemical Co., Ltd. (İstanbul, Turkey) and was used as a crosslinking agent. All other chemicals were of analytical reagent grade and were used without further purification.

- *Methods*

- *Berberine extraction process*

Before the extraction of berberine, the entire plant was washed, sun-dried outdoors for 7 days, and ground to obtain a uniform powder with particle size of 0.2-0.5 mm using a blender. The ground powder was further dried in a vacuum oven at 75°C for 2 h before storage in a tightly sealed container. In order to extract berberine, ~3 g of the as-prepared powdered sample was mixed with 75% ethanol aqueous solution. The mixture was incubated at 50°C for 30 min while being shaken at 100 rpm in a shaker incubator (Fan Azma Gostar; Tehran, Iran) in accordance to our previous published method (Keshtkaran *et al*., 2022). The extraction suspension was then centrifuged at 9000 rpm for 10 min, and the supernatant was collected and filtered through Whatman® filter paper (Grade 41). A rotary evaporator (HS-2005 VN, Hahn Shin Scientific Co., Korea) was utilized for the complete removal of ethanol from the extract at 55–60 °C and 500 mm Hg. The berberine concentration was determined using a high-performance liquid chromatography system (Knauer, advanced scientific instrument, Berlin, Germany) following our previous report (Keshtkaran *et al*., 2022). A series of standard solutions of berberine with concentrations ranging from 0.7 to 25μg/mL were prepared and the resulting standard curve was used to study the berberine release kinetics from the microcapsules (Wu *et al*., 2015).

- *Preparation of dried coacervated berberine-loaded microcapsules*

Berberine-loaded microcapsules using an optimal extract prepared by the method previously described (Keshtkaran, Mizani, Mousavi, Mohammadifar, & Azizinejad, 2022) with the concentration of 16±0.16

micrograms per milliliter. The wet microcapsules containing berberine were produced by a complex coacervation with wall materials made of three different ratios of *tragacanth*/gelatin (Ps/Pr) of 1:1, 1:2, and 2:1, according to the method and optimal conditions that previously described by Keshtkaran *et al*., (2022). To gain an insight into the effect of drying method on characteristics of microcapsules, two drying processes were utilized: spray-drying and freeze-drying. The microcapsule samples were powdered using a spray-drying method. Briefly, 150 mL of microcapsule suspension was fed into a mini spray-drier (Büchi Mini Spray Dryer B-290, Büchi Labortechnik AG, Flawil, Switzerland) under these conditions: an inlet temperature (T_{inlet}) of 125–130°C, an outlet temperature (T_{outlet}) of 70–75°C. a drying airflow of 50 m^3/h, and pumping rate of 20% (Yu *et al*., 2017). For the Freeze drying, 50 mL of microcapsule suspension was centrifuged at 9,000 rpm for 20 min, before being freeze-dried (ZIRBUS VACO5) for 48 h at -48°C under -0.9 bar pressure (Quispe-Condori *et al*., 2011). All powder samples were separately placed into aluminium bags and stored in dark and cold place until further analysis. Each drying technique was conducted using three ratios of Ps/Pr. Based on the experimental design, spray-dried samples were labeled as (SD 1:1, SD 1:2, and SD 2:1), and those of freeze-dried microcapsules samples were labelled as (FD 1:1, FD 1:2 and FD 2:1).

- ***Evaluation of microstructure and particle size of microcapsules***

The morphological characteristics of the powdered microcapsules through two drying techniques were observed by an environmental scanning electron microscope (ESEM XL30, Philips, Eindhoven, the Netherlands) operating at an accelerating voltage of 20 kV. Using double-sided tapes to fix microcapsules on metal stubs, and then coated with a 20 nm layer of gold using a magnetron sputter (DF 101, Yare Nikane Saleh, Tehran, Iran) for 30 s in a high vacuum evaporator. The SEM images were collected under two magnification levels: 2,000 and 10,000 (Jain, Thakur, Ghoshal *et al*., 2016).

The particle size distribution of the freeze-dried and spray-dried samples were determined using a Malvern Mastersizer 2000 Laser Particle Size Analyzer (Malvern Instruments Ltd., Worcestershire, UK) equipped with a Hydro 2000S automated sample dispersion unit. To measure the size distribution of microcapsules, one milligram of the dried microcapsules was weighed and dispersed in 10 mL deionized water with vigorous stirring for 30 minutes at room temperature. The particle size distribution was expressed as volume-weighted ($D_{4,3}$) and surface-weighted ($D_{3,2}$) mean diameters. All samples were analyzed at least three times.

- ***Product yield and encapsulation efficiency***

The product yield of spray-dried and freeze-dried samples was calculated by dividing the final weight of collected powder sample by the total weight of solid materials as equation1 (Eq.1):

$$\text{Yield \%} = \frac{\text{Weight of Dried Coacervated Microcapsules}}{\text{Total weight of materials used in the preparation of capsules}} \times 100 \quad \text{(Eq.1)}$$

Encapsulation efficiency was determined according to the method described by Rocha *et al*. (2012) with minor modifications.

3 mL of methanol was added to 3 mg of powdered samples. The mixture was shaken with a Heidolph magnetic stirrer at 100 rpm for 5 min and stored in the

darkness for 4 h and it was subsequently centrifuged at 9,000 rpm for 10 min. The supernatant containing unentrapped berberine was used to determine the content of microencapsulated berberine by a double-beam UV-visible spectrophotometer (Cecil CE-7200, UK) at the wavelength of 470 nm. The encapsulation efficiency (EE) was calculated using equation 2 (Eq.2) (Rocha *et al*., 2012):

$$EE\% = \frac{\text{Total initial berberine} - \text{Total free berberine after microcapsulation}}{\text{Total berberine}} \times 100\% \quad \text{(Eq.2)}$$

The standard curve was established by preparing serial dilution of berberine in absolute ethanol (5, 10, 25, 50, 75, and 100 µg/mL). The absorbance values of these standard solutions were determined under similar conditions. At least three measurements of each sample were taken to ensure the repeatability of the results.

- ***Solubility***

The water solubility of the microcapsules was determined using a gravimetric method. Briefly, 10 mg of powder sample was weighed and added into in a 50 mL beaker containing 10 mL of distilled water and homogenized at 100 rpm for 30 min at room temperature. The stirring was slightly increased to 1,000 and continued for 5 min to ensure homogenous mixing is achieved. The mixture was then stored in the dark environment. The as-prepared solution was then transferred to 50 mL centrifuge tubes and centrifuged at 7,500 rpm for 10 min under normal conditions. An aliquot of 2.5 mL of the supernatant was transferred to a pre-weighed petri dish and oven-dried at 105°C for 2 h to a constant weight. The water solubility was calculated based on the ratio of the mass of solids in the supernatant to the mass of the samples as equation3 (Eq.3):

$$\text{Solubility} = \frac{Wds}{Ws} \times 100\% \quad \text{(Eq.3)}$$

where W_{ds} is the mass of dispersed microcapsules in the supernatant and W_s is the initial mass of the sample (Comunian *et al*., 2016). The measurements were performed in triplicate.

- ***Thermal properties***

The glass transition temperatures (Tg) of berberine-loaded microcapsule powder samples were recorded using an INNUO 500-B differential scanning calorimetry (DSC) instrument (INNUO, Shanghai, China). Briefly, approximately 5 mg of specimens were hermetically sealed into aluminum pans. All samples were scanned from 10 to 300°C at a heating rate of 9.8°C/min with a nitrogen purging flow rate of 50 mL/min. An empty pan was utilized as a reference (Nogueira *et al*., 2017).

- ***Berberine release and kinetic models***

The release characteristics of berberine from spray- and freeze- dried microcapsules were evaluated at two experimental temperatures: 5 °C and 80 °C. 6 mg dried loaded microcapsules were dispersed in 10 mL water and stirred at 100 rpm. At specific time intervals up to 30 days for specimens stored at 5 °C and 100 min for specimens stored at 80 °C, aliquots of 20 µL were taken and filtered through a 0.45 µm pore size filter. The absorbance of each sample was quantified by a UV-Visible spectrophotometer at wavelength of 470 nm (Dong *et al*., 2011). The concentration of berberine in the release medium at each sampling time interval was calculated using a calibration curve. In order to gain an insight on the release mechanisms of berberine from microcapsules, the release data were fitted to three models including zero-order,

Higuchi, and modified Korsmeyer-Peppas models. These models were chosen because they are consistently used to explain the release of bioactive compounds from polymeric microcapsules (Keshtkaran *et al.*, 2022). The zero-order model can be written as equation4:

Zero-Order model: $Qt = K_0 t + C$ (Eq.4)

Where Qt is the amount of the berberine released over time t, C is the amount of berberine in solution prior to release (which is usually zero), and K_0 is the zero-order release rate constant.

The Higuchi model (equation5) can be written as follow:

Higuchi model: $Qt= K_H t^{0.5} + C$ (Eq.5)

Where K_H is the Higuchi rate constant.

To investigate the Fickian and non-Fickian mechanisms, the modified Korsmeyer-Peppas model calculated by equation 6 as follow:

Modified Korsmeyer-Peppas model: $Qt/ Q\infty = K_{K-P} t^{n} + b$ (Eq.6)

Where $Qt/ Q\infty$ is a fraction of released berberine at time t, K_{K-P} is the release kinetic constant, n is the release exponent and b is the intercept, to characterize the burst effect, which represents an abrupt increase in the active compound's initial release. This phenomenon occurs most frequently with microencapsulated flavors and is dependent on the shape and surface porosity of the microcapsule walls (Huang & Brazel, 2001). "n" is an exponent parameter that determines whether the release is controlled by the Fickian mechanism ($n< 0.45$) or both Fickian and swelling phenomena have to be considered ($0.5< n <1$) (Korsmeyer *et al.*, 1983; Ritger & Peppas, 1987; Sheikh *et al.*, 2020). The release rate profile was plotted in Excel. The best-fit model was chosen based on maximum value of the adjusted-R^2.

- ***Statistical analysis***

All the measurements were carried out in triplicate order unless specified otherwise. The analysis of the results was performed through one-way analysis of variance (ANOVA) using a SPSS® ver. 22.0 statistical software (IBM® Co., Armonk, NY, USA). A Duncan test with a probability value of $P < 0.05$ was used to evaluate significant differences between the mean values.

Results and Discussion

- ***Morphological characteristics of the berberine-loaded microcapsules***

The detailed morphology of the berberine-loaded microcapsules after drying with spray and freeze-drying methods were investigated. The scanning electron microscopy (SEM) technique has been also used to examine the effects of different Ps/Pr ratios on the morphological characteristics of the dried microcapsules (Figure 1). The micrographs were presented under two different magnification levels to gain a better understanding of the size and/or sharp images of microcapsules generated by two different drying methods. The spray-drying yielded semi-spherical microcapsules with a few wrinkles and protrusions (Figure 1a, b, and c), which may enhance the release qualities due to the increased surface area, whereas the freeze-drying process resulted in irregular-shaped large microcapsules with a lumpy and visible porous structure (Figure 1d, e, and f). This is because spray-drying often uses a high inlet temperature that causes a rapid evaporation of water within the particle, leading to the formation of a rougher and more concave surface than the

freeze-drying method (Bi *et al*., 2022). Meanwhile, the SEM images suggested that when the protein proportion in the wall material was increased (i.e., Ps/Pr of 1:2), spray-dried microcapsules presented smoother surfaces, smaller sizes, and greater uniformity, as confirmed by previous studies (Alvim & Grosso, 2010; Jain *et al*., 2016). In contrast, freeze drying operates at lower temperatures than spray drying, resulting in microcapsules with higher moisture content, particularly when polymers (proteins) with a higher water-binding capacity (i.e., gelatine and tragacanth) were used as wall materials (Pudziuvelyte *et al*., 2020). Therefore, freeze drying results in the development of sticky microcapsules and agglomerates. Ezhilarasi *et al*. (2013) demonstrated that ice crystal sublimation under vacuum during a typical freeze-drying process may lead to the formation of a porous structure. This porous structure may increase the risk of oxidation (Eratte *et al*., 2014) for the encapsulated core material (i.e., berberine). Therefore, spray drying may be preferred as a method of choice for dehydration of the complex coacervated Berberine microcapsules.

- ***Particle size distribution of microcapsules***

Particle size is an important parameter when it comes to the applications of microcapsules in different food formulations. The particle size and polydispersity index of microcapsules may be realized as the most influential aspects of the release patterns of microencapsulated compounds (Li *et al*.,

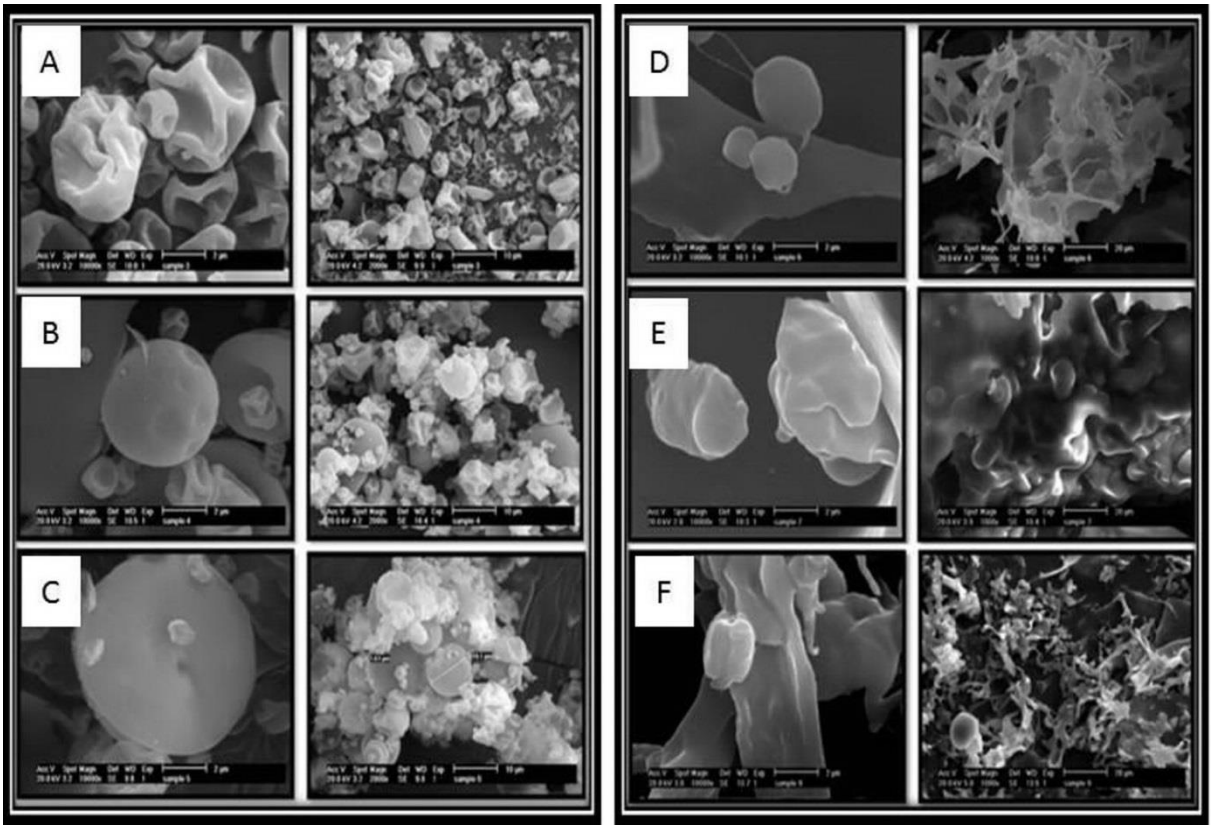

Fig. 1. Scanning electron micrograph of complex coacervated berberine microcapsules with different ratios of *Astragalus rahensis* to gelatin A) SD1:1, B) SD1:2, and C) SD2:1 and D) FD 1:1, E) FD1:2, and F) FD 2:1. The images magnifications are 2000X (left) and 10000X (right)

2016). The particle size characteristics (D [3,2], D [4, 3], and span) of the berberine-loaded microcapsules dried by two different drying methods are given in Table 1. The particle size data varied in the range of 37.56–183.55 µm while the span values spread from 1.61 to 2.61. The average size of microcapsules obtained by spray-drying and freeze-drying was 40.97 µm and 177.46 µm, respectively. It is important to note that the spray-dried particle sizes lie in the range that is commonly reported for the complex coacervated microcapsules (Comunian *et al*., 2016). The average sizes of the freeze-dried microcapsules were in the range of 158–184 µm with the distribution slightly shifted to larger particle sizes (100−1000 µm). Indeed, the particle sizes of the spray-dried microcapsule were smaller than those of freeze-dried microcapsules. Spray drying produces smaller microcapsules because of atomizing small droplets in the drying chamber and potential volatilization/dehydration upon exposure to hot air (Kaushik *et al*., 2016; Bi *et al*., 2022). We found a larger particle size for microcapsule powder dried by freeze-drying. This is because freeze drying is often operated at lower temperatures compared with spray drying. A low operational temperature results in the production of microcapsules with higher moisture content. As a result, freeze drying may result in the development of large and sticky microcapsules as was previously comprehended from SEM analysis. However, the wall material seems to have a slight change in the particle size distribution. As shown in Table 1, when using Ps/Pr of 1:2 as the wall material, the spray-dried microcapsules had the lowest particle size of 37.56 µm.

- ***Microencapsulation yield and efficiency***

As given in Table 1, the yields of berberine microencapsulation were in the range of 24–50% for the spray-drying and (34–42%) freeze-drying processes. The spray-dried berberine microcapsule with a Ps/Pr of 1:2 had the highest yield (50%) amongst others. This may be ascribed to the ice crystallizing during freezing that leads to liberation/release of some of the core material and lower yields (Kanha *et al*., 2020). The literature has often reported contradictory outcomes on the yields of spray-drying and freeze-drying methods. According to Kaushik *et al*., 2016, freeze drying could have a higher yield, up to 90%, and spray drying was found to have a minimum yield of ~50, which may contribute to adhesion of the particles to the walls of the drying chamber, causing higher loss of core material in the dried product. A recent work by Carra *et al*. (2022) showed that the size of the spray drying chamber and the efficiency of the cyclone could be considered the two most effective processing factors to reduce the likelihood of deposition of dried particles and loss of yield. Based on results from Table 1, the differences between the yields obtained from the two drying techniques in our research were somewhat lower than in previous research (Kaushik *et al*., 2016). The main reason for this observation could be related to using tragacanth gum as one of the wall components, which may create a sticky texture in the dried products for both of the drying methods. In terms of Ps/Pr ratios in the wall material, spray-dried microcapsules with more protein components (Ps/Pr=1:2) had a significantly higher yield (Table 1). It can be assumed that a mixture of tragacanth gum and other biopolymers such as protein may prevent or reduce the stickiness of the spray-dried microcapsules, resulting in higher yields (Saffari *et al*., 2013). The higher yields observed in the present study

could be partially related to the high efficiency of the cyclone that trapped a large number of smaller particles and/or larger specific surface areas of particles, which enabled more contacts between the drying air and the particle surface. It may be emphasized that applying the spray drying method further to complex coacervation may have fewer negative effects on microcapsule quality as compared to using this drying method for microcapsulation (Rojas-Moreno *et al*., 2018; Glomm *et al*., 2021).

Microencapsulation efficiency is another key quality that was investigated in this study. Table 1 indicates the microencapsulation efficiencies of freeze and spray-dried microcapsules varied in the ranges of 70–75% and 65–68%, respectively. The nature of the wall material has obviously a substantial effect on the retention of the core material. Electrostatic interaction between negative-charged carboxyl groups of tragacanth and positive-charged amine groups of gelatin in aqueous media has been recently studied in detail (Molaahmadi Bahraseman *et al*., 2022) and the results revealed the potential of this protein/polysaccharide system to form a continuous strong film layer around the core material. However, microencapsulation efficiency increased ($P > 0.05$) when the ratio of Ps/Pr of spray-dried microcapsules decreased. Similar findings were reported by Carpentier *et al*. (2022) who found a significant increase in encapsulation efficiency when the Ps/Pr ratio was changed from 1:1 to 1:2. The main explanation for the improvement in percentage of efficiency after adding protein to the carbohydrate wall system was attributed to the lowering of the solvent diffusability through the matrix due to the creation of a strong continuous phase.

- ***Solubility***

The solubility of microcapsules is a critical factor for the quality of the powder as it shows its ability to absorb water. Complex coacervation usually increases microcapsule insolubility, creating better conditions for controlled release patterns in food formulations (Baracat *et al*., 2012; Dong *et al*., 2011). As a result, microcapsules with low solubility may be formed during coacervation, despite the fact that each of the polymeric components (i.e., gelatine and tragacanth) has a high-water solubility (Molaahmadi Bahraseman *et al*., 2022). This study revealed that the freeze-dried microcapsules possessed the maximum water solubility (40–53%), while those of spray-dried samples were relatively insoluble, with solubility values ranging from 4 to 16% (Table 1). The key parameters affecting the solubility appear to be the drying techniques and conditions, particle sizes, and textural porosity of the dried microcapsules. As it was understood from SEM analysis, freeze drying produced larger particles with a porous structure, which is apparently responsible for more solubility of the powder. On the other hand, the high temperature at the beginning of the spray drying process could accelerate the formation of a hardened case of wall material, rendering it less soluble by preventing the transfer of water molecules (Chegini & Ghobadian, 2007), and more appropriate for controlled release processes. It should be noted that the higher inlet temperatures result in producing larger, hygroscopic particles with a porous structure, while using lower temperatures may produce shrunken and smaller particles (Pui & Saleena, 2022). This point may explain why the spray-dried microcapsules produced in the current study at low inter temperatures (130) exhibited lower solubility in comparison to those produced in previous

research at high temperatures (180) (Ho *et al*., 2022).

- ***Thermal characteristics***

Figure 2 shows the thermograms obtained from differential scanning calorimetry (DSC) analysis to examine the thermal properties of dried microcapsules including the glass transition temperature (T_g). T_g was determined as the midpoint of the heat capacity change (Fredlake *et al*., 2004). Indeed, the T_g is a key parameter that if properly tuned, can avoid physical and structural changes of the encapsulating material during processing and storage (Zhang *et al*., 2021). The T_g values of microencapsulated berberine powders are summarized in Table 1. As shown in Table 1, there was a significant difference ($P < 0.05$) between the T_g values for the freeze-dried and spray-dried microcapsules, with the higher T_gs of the spray-dried samples (80-85 ˚C) indicating better thermal stability than the freeze-dried microcapsules (Durrieu & Gandini, 2006; Cruz *et al*., 2017; Lu *et al*., 2022). It is well known that the Tg values of carbohydrates in a carbohydrate-protein system will shift to higher temperatures (Górska *et al*., 2017). The T_g value of the native species of tragacanth (*Astragalus rahensis)* used in the current research is 49–49.5˚C (Delta Cp= 0.055 J/g˚K), which is significantly lower than the Tg levels previously reported for other species such as *Astragalus compactus* (Saffari *et al*., 2012; Martín-Alfonso *et al*., 2019). It seems that the lower Tg of *Astragalus rahensis* is attributable to the higher concentration of saccharides with lower molecular weights (Furlán *et al*., 2011) such as arabinose (Mw=150.1) as compared to *Astragalus compactus, which* is rich in fucose with a high Mw (Mw=363.9) (Taghavizadeh Yazdi *et al*., 2021; PubChem, 2022; Chemical aid, 2022). Meanwhile, the T_g value of gelatin is ~25-30 °C (Bohidar *et al*., 1993; Singh *et al*., 2007) It is well known that the Tg values of carbohydrates in a carbohydrate-protein system will shift to higher temperatures (Górska, 2017). The Tg value of the native species of tragacanth (Astragalus rahensis) used in the current research is 49–49.5˚C (Delta Cp= 0.055 J/g˚K), which is significantly lower than the Tg levels previously reported for other species such as Astragalus compactus (Saffari *et al*., 2012; Martín-Alfonso *et al*., 2019). It seems that the lower Tg of *Astragalus rahensis* is attributable to the higher concentration of saccharides with lower molecular weights (Furlán *et al*., 2011) such as arabinose (Mw=150.1) as compared to Astragalus compactus, which is rich in fucose with a high Mw (Mw=363.9) (Taghavizadeh Yazdi *et al*., 2021; PubChem, 2022; Chemical aid, 2022). Meanwhile, the Tg value of gelatin is ~25-30 °C (Bohidar *et al*., 1993; Singh *et al*., 2007) and this means that, the Tg of the microcapsules may be expected to be higher than the Tg of each component, and the results of Table 1 confirm this point for both of the drying methods. In addition, the level of the residual moisture in the dried microcapsules may significantly affects the Tg values (Frascareli *et al*., 2011; Dianawati *et al*., 2013; Górska *et al*., 2017). The high inlet temperature of the spray drying process may have caused it to lower the moisture content more effectively than freeze-drying, and the hygroscopic texture of the freeze-dried samples, which was already discussed in the morphological study (section 3.1), may confirm this result. It may be concluded that all Berberine microcapsule powders may be in their glassy state ($T<Tg$) at ambient temperature, with limited thermal motion in the polymeric structure of the wall material (Kirk *et al*., 2004) resulting

Table 1. Different physical characteristics of the complex coacervated berberine microcapsules dried by spray-drying and freeze-drying methods

Samples[1]	Yield (%)	Efficiency (%)	Solubility (%)	T_g (°C)	D[4,3] (µm)	D[3,2](µm)	Span
SD 1:1	24.80±0.32^{d}	68.00±1.21^{c}	4.00±0.03^{f}	80.95±4.63^{a}	46.68±2.13^{c}	26.28±3.46^{c}	1.89±0.34^{b}
SD 1:2	50.00±0.42^{a}	67.00±1.35^{c}	8.00±0.03^{e}	80.38±4.56 ^{a}	37.56±2.48^{d}	18.01±4.12^{d}	1.76±0.31^{b}
SD 2:1	36.00±0.33^{c}	65.00±1.19^{d}	16.00±0.02^{d}	83.87±4.61^{a}	38.68±3.12^{d}	19.41±2.72^{d}	2.61±0.41^{a}
FD 1:1	34.80±0.45^{c}	71.14±1.31^{b}	47.00±0.04^{b}	50.49±4.23^{b}	169.30±4.11^{b}	90.74±3.23^{b}	1.61±0.36^{b}
FD 1:2	42.44±0.28^{b}	75.34±1.23^{a}	40.00±0.02^{c}	49.38±4.44^{b}	179.54±4.15^{a}	96.39±3.81^{a}	1.89±0.21^{b}
FD 2:1	40.35±0.35^{b}	70.21±1.24^{b}	53.33±0.02^{a}	56.59±4.32 ^{b}	183.55±4.45^{a}	98.48±3.67^{a}	1.69±0.43^{b}

[1] Berberine microcapsules were produced with different ratios of tragacanth/gelatin (Ps/Pr) (1:1, 1:2 and 2:1). SD and FD are the abbreviations for spray-drying and freeze-drying, respectively

Values with different superscript letters in the same column indicate significant difference ($P \leq 0.05$) when analyzed by Duncan's multiple range test.

Note: Results are expressed as mean value± standard deviation

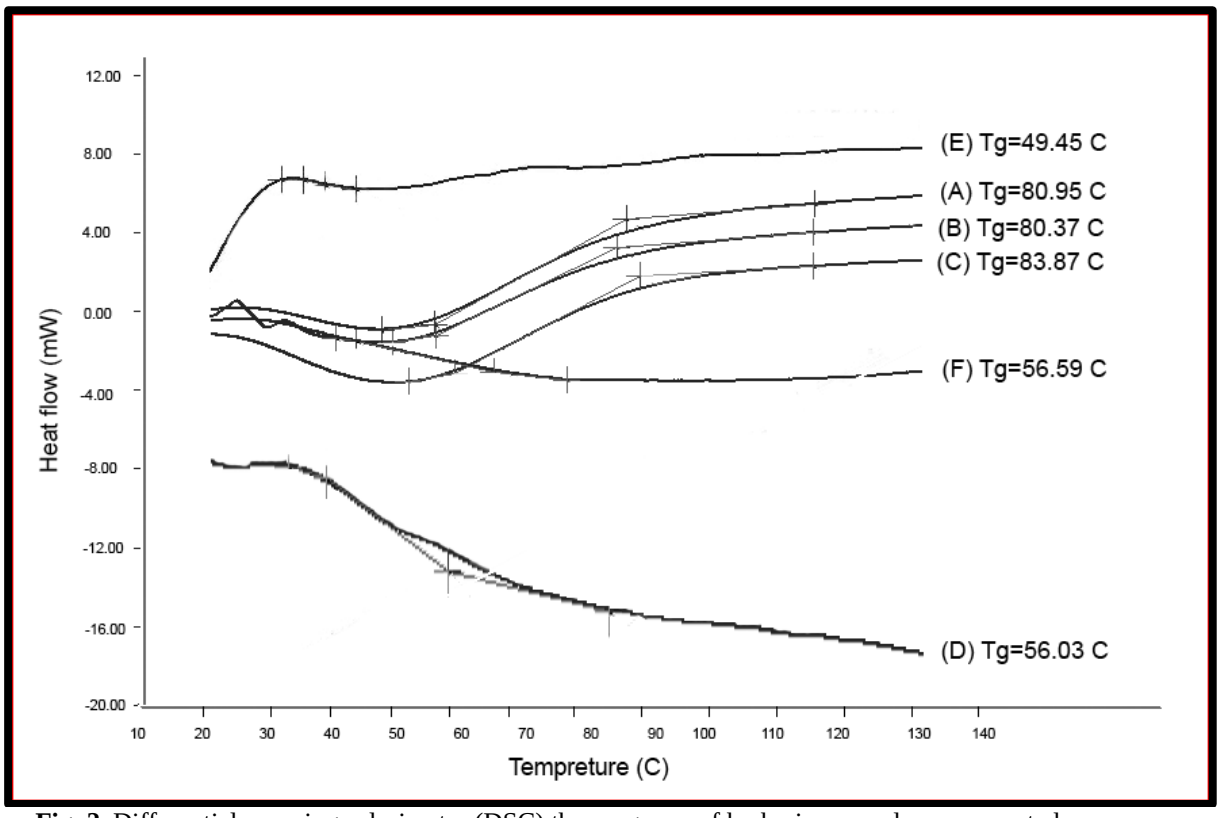

Fig. 2. Differential scanning calorimetry (DSC) thermograms of berberine complex coacervated microcapsules with different ratios of tragacanth: gelatin: A) SD1:1, B) SD1:2, and C) SD 2:1 D) FD 1:1, E) FD1:2, and F) FD 2:1

in low oxygen permeability and protecting the sensitive core material (Berberine) from oxidation (Jin *et al*., 2018; Kanha *et al*., 2021).

- ***Berberine release kinetics***

The in vitro release of berberine from both groups of the dried microcapsules in water was investigated at two distinct temperatures (5 °C and 80 °C) and time intervals (Figure 3 a, b). At both temperatures, the general release pattern was similar, consisting of an initial rapid release phase (burst effect) followed by a slow and sustained one. As it may be expected, the release rates were

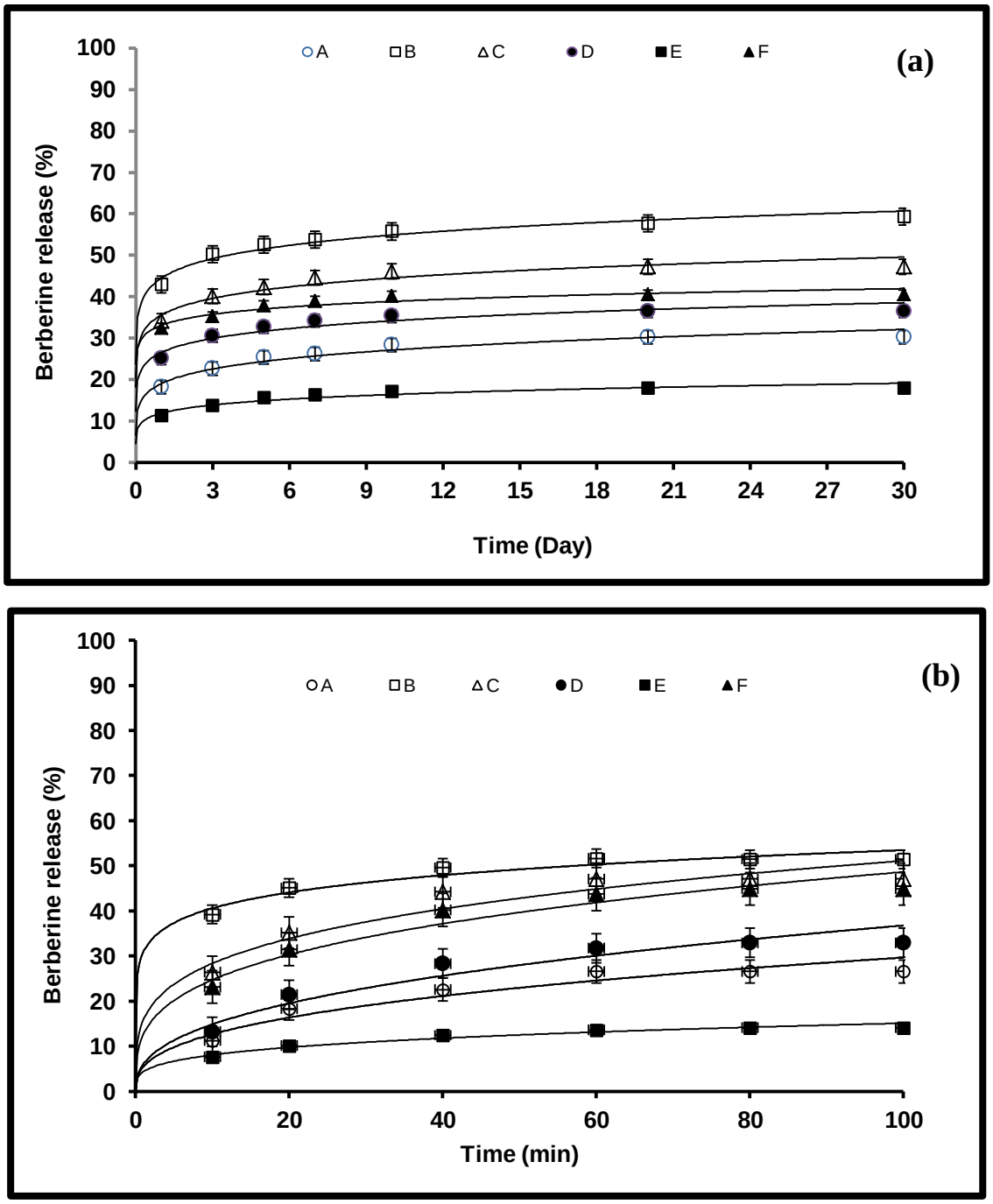

Fig. 3. Berberine release rate from dried microcapsules with different ratios of *Astragalus rahensis*: gelatin: A) SD1:1, B) SD1:2, and C) SD2:1 D) FD 1:1, E) FD 1:2, and F) FD 2:1 at 5 °C (a) & 80 °C (b)

considerably higher at 80˚ C (pasteurization temperature) than at 5˚C (refrigerator temperature). Therefore, about 50% release from spray-dried microcapsules took about five days at 5˚C and only one hour at 80˚C. According to Cui *et al*. 2021, the higher temperatures of the release medium increase the Brownian motion and kinetic energy of the core materials, leading to a larger diffusion rate. Therefore, it may be concluded that berberine microcapsules were more stable in low-temperature conditions and released relatively slowly over an extended length

of time. In order to comprehend the process of berberine release from tragacanth/gelatin microcapsules, the release profile was fitted with three known kinetic models: zero-order, Higuchi, and modified Korsmeyer-Peppas. The constants and coefficient of determination (R^2) for each model are listed in Tables 2 and 3. The zero-order and Higuchi models were deemed unsatisfactory ($R^2 < 0.8$) for modeling the release of berberine from microcapsules. Nonetheless, the modified Korsmeyer-Peppas model best explained the release data ($R^2 > 0.90$). The Korsmeyer-Peppas model has been found to accurately describe the release data of essential oils (de Oliveira *et al*., 2014), curcumin (Xiao *et al*., 2015) from nanoparticles, and polyphenols (Pulicharla *et al*., 2016). The n parameter, determined from the Korsmeyer-Peppas equation, represents the diffusional exponent as an indicator of the release mechanism. All studied samples had n values smaller than 0.45, indicating the Fickian diffusion release mechanism from swellable spherical particles (Ritger & Peppas, 1987). The spray-dried and freeze-dried microcapsules with a Ps/Pr ratio of 1:2 had the highest (Kk-p =44.5, b = 43) and lowest (Kk-p =11.93, b= 11.34) release rates at 5 °C, respectively (Figure 3a, Table 2). The variations in release rate constants may be attributable to the following factors: a) the size and specific area of the microcapsules b) The thermal conditions used in drying procedures c) The Ps/Pr ratio of the wall material and hydrophilicity of each component d) Temperature of the release medium influencing the hydrating potential of the polymeric wall material. According to Table 1, the spray-dried microcapsules with PS/Pr 1:2 were the smallest sized samples compared to the freeze-dried samples; thus, the largest specific area might provide a greater potential for the diffusion through the wall material of this sample (Jovanovi´c *et al*., 2021). The high inlet temperature of the spray drying process is another parameter which is previously reported to alter the release rate due to formation a hard shell on the microcapsule surfaces and reducing the diffusability of the wall material (Wardhani *et al*., 2020) It has been distinguished that the effect of this parameter was not dominant in the current study since the inlet temperature (130 ˚C) applied in the spray drying process was not as high as in the earlier research (180˚-200˚C). Microcapsules, on the other hand, do not case-harden due to the low temperature of freeze-drying, but the larger-sized, lumpy particles may be expected to slow down the release rate. Comparing the release data for sample (SD2:1) with that of sample (FD2:1) indicated that there is no big difference between these two samples produced by two different drying procedures at both temperatures (Tables 2, 3). This unexpected result may be attributable to the higher solubility values observed for the freeze-dried samples, which were described previously (Table 1). On the other hand, the hydration of the polymeric wall material may expedite the diffusion of the core material into the surrounding liquid by creating a porous structure that is more susceptible to release. Therefore, the freeze-dried samples with a higher Ps/Pr ratio (FD-2:1) exhibited a greater release rate compared to (FD 1:2), because of the greater proportion of tragacanth with more hydrophilicity than gelatine in the wall material (Zajic *et al*., 1976; De Oliviera *et al*., 2014). Meanwhile, applying a release medium at a high temperature (80˚C) may alter the water sorption ability of the wall material. According to Alexandre *et al*. 2019, some degradation may happen in the

Table 2. The release kinetic parameters of spray-dried and freeze-dried berberine microcapsules stored at 5˚C

Model	Kinetic parameter	Samples[1]					
		SD1:1	**SD1:2**	**SD2:1**	**FD1:1**	**FD1:2**	**FD2:1**
Zero-order	K_0	0.34	0.43	0.34	0.29	0.18	0.22
$Q_0 = K_0 t + C$	C	22.21	48.59	39.39	29.84	13.79	35.57
	R^2	0.67	0.67	0.57	0.55	0.59	0.55
Higuchi	K_H	0.03	0.03	0.03	0.02	0.02	0.02
$Q_H = K_H t^{0.5} + C$	C	4.71	6.97	6.27	5.45	3.71	5.45
	R^2	0.64	0.0.65	0.55	0.53	0.57	0.54
Modified	K_{K-P}	19.07	44.51	35.61	26.56	11.93	33.12
Korsmeyer-Peppas	*n*	0.15	0.11	0.11	0.11	0.14	0.11
$Q_t/Q_0 = K_{K-P} t^n + b$	b	18.23	43.00	34.12	25.12	11.34	32.41
	R^2	0.95	0.95	0.91	0.91	0.92	0.90

[1] Berberine microcapsules were produced with different ratios of tragacanth/gelatin (Ps/Pr) (1:1, 1:2 and 2:1). SD and FD are the abbreviations for spray-drying and freeze-drying, respectively.

Table3. The release kinetic parameters of spray-dried and freeze-dried berberine microcapsules stored at 80˚C

Model	Kinetic parameter	Samples[1]					
		SD1:1	**SD1:2**	**SD2:1**	**FD1:1**	**FD1:2**	**FD2:1**
Zero-order	K_0	0.16	0.12	0.21	0.21	0.06	0.02
$Q_0 = K_0 t + C$	C	13.93	41.83	30.37	16.34	8.51	26.35
	R^2	0.76	0.71	0.72	0.79	0.81	0.78
Higuchi	K_H	0.02	0.01	0.02	0.02	0.01	002
$Q_H = K_H t^{0.5} + C$	C	3.72	6.47	5.14	4.04	2.92	5.14
	R^2	0.73	0.71	0.76	0.75	0.78	0.76
Modified	K_{K-P}	5.39	3.08	15.73	5.99	4.32	12.55
Korsmeyer-Peppas	*n*	0.37	0.12	0.26	0.39	0.27	0.29
$Q_t/Q_0 = K_{K-P} t^n + b$	b	11.2	39.2	26.41	13.22	7.64	23.12
	R^2	0.91	0.92	0.91	0.93	0.96	0.94

[1] Berberine microcapsules were produced with different ratios of tragacanth/gelatin (Ps/Pr) (1:1, 1:2 and 2:1). SD and FD are the abbreviations for spray-drying and freeze-drying, respectively.

polymeric matrix of the cross-linked polysaccharide/gelatine coacervates at temperatures around 100˚C due to losing free and bound water. Consequently, it may be supposed that these structural changes may slow down the release rate at high temperatures (i.e., 80˚C). The burst effect and the b factor may be taken into consideration when recommending a drying process for berberine microcapsules from a controlled release standpoint. According to Huang and Brazel (2001), an appropriate burst effect (a high b factor) is preferable for certain applications, such as encapsulated food ingredients. Therefore, the spray drying technique with the greatest burst effect at both temperatures may be chosen to produce the microcapsule samples with desirable controlled release characteristics (Ps/Pr = 1:2, and Ps/Pr = 2:1) (Tables 2 and 3).

Conclusion

In this research the Berberine coacervates prepared by using different ratios of Astragalus rahensis/gelatin were dried by spray drying and freeze drying and the effects of two drying techniques on the characteristics of microcapsule powders were investigated. The rahensis species, a low-grade flaky tragacanth with economic value, was utilized for the first time in the formation of Berberine

microcapsules and exhibited desirable functional properties in combination with bovine gelatin. Due to the lumpy and porous structure of the freeze-dried microcapsules, the sensitive core material (Berberine) may be exposed to oxidative reactions. The Tg values as a thermal stability index, for spray-dried were greater than those for freeze dried products. After 60 minutes at 80°C and one month at 5°C, the maximum release rate of spray-dried microcapsules with 1:2 Ps/Pr ratios was 51% and 58%, respectively. of 51 % and 58 %, respectively. The hydrophilicity of tragacanth from one side and structural changes of gelatin polymeric at high temperatures from the other side, have governed the structural diffusability of the wall polymeric matrix and release pattern.

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