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Jiayue Guo – University of Alabama
Peilong Li – Cornell University
Lingyan Kong – University of Alabama
Baojun Xu – Beijing Normal University

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Microencapsulation of Curcumin by Spray Drying and Freeze Drying

Jiayue Guo [a], Peilong Li [b], Lingyan Kong [a, *], Baojun Xu [c,*]

[a] Department of Human Nutrition & Hospitality Management, The University of Alabama, Tuscaloosa, AL 35487, United States

[b] Department of Food Science, Cornell University, Ithaca, NY 14853, United States

[c] Food Science and Technology Program, Beijing Normal University– Hong Kong Baptist University United International College, Zhuhai, Guangdong 519087, China

*Corresponding authors.
Address:
2000 Jintong Road, Tangjiawan, Zhuhai, Guangdong 519087, China. (B. Xu)
482 Russell Hall, 504 University Blvd, Tuscaloosa, AL 35487, USA (L. Kong)
Email:
baojunxu@uic.edu.hk (B. Xu)
lkong@ches.ua.edu (L. Kong)

Abstract

Curcumin is a natural pigment with health benefits and potential uses in food and pharmaceutical industries, but its application is limited by its insolubility and instability. This study was to examine the effect of spray drying and freeze drying methods, as well as 12 combinations of ternary-composite wall materials, on microencapsulation efficiency (MEE) , physicochemical properties (including particle size distribution, morphology, moisture content, and color values), and stability of curcumin against environmental stresses and in a model beverage. Curcumin encapsulated by freeze drying presented higher MEE, whereas spray drying produced microparticles had smaller particle sizes, smoother granule surface, and more regular shapes. Though to different extent, curcumin encapsulated by both methods exhibited improved stability against heat and acidity, and improved stability in a carbonated beverage. This study provides practical information on the selection of wall materials and encapsulation methods for microencapsulating curcumin for various applications.

Keywords: Curcumin; microencapsulation; spray drying; freeze drying; wall materials; microencapsulation efficiency

1. Introduction

Curcumin is a yellow-orange pigment produced by turmeric (Curcuma longa), a member of the ginger family. As a natural phytochemical, curcumin exhibits a wide range of bioactivities, e.g., antioxidative, and anti-inflammatory properties, marking its significance in clinical utilization (Hewlings & Kalman, 2017; Wilken, Veena, Wang, & Srivatsan, 2011). However, free curcumin has little confirmed uses or practical applications in both food processing and medical fields, as it is insoluble in aqueous solutions, susceptible to heat, light, and extreme acidity, meaning that it can be easily degraded under environmental stresses (Liu et al., 2017; Nelson et al., 2017; Wang, Lu, Lv, & Bie, 2009).

Microencapsulation is a widely used technology to improve the stability and bioavailability of bioactive compounds (Gharsallaoui, Roudaut, Chambin, Voilley, & Saurel, 2007). Microcapsules or microparticles are the product of microencapsulation and contains the bioactives in the core surrounded by a coating of wall materials. The wall provides a physical barrier that prevents molecular diffusion and chemical reactions so as to improve the stability of encapsulated compounds (Taguchi, Saito, Uchida, & Tanaka, 2016). Various microencapsulation techniques have been invented, such as spray drying, freeze drying, extrusion, and centrifugal extrusion among others. Among them, spray drying is the most commonly used technique because of its cost-efficiency, flexibility, and continuous operation (Gouin, 2004; Jafari, Assadpoor, He, & Bhandari, 2008; Ré, 2006). Freeze drying is another popular method, especially for the more sensitive and expensive bioactive compounds. Unlike spray drying, freeze drying uses a much lower temperature and operates in the absence of oxygen. Numerous studies employed spray drying and freeze drying techniques to encapsulate curcumin (Cano-Higuita, Malacrida, & Telis, 2015; Sousdaleff et al., 2013; Zuanon, Malacrida, & Telis, 2013),

yet, few research compared various combinations of ternary composite wall materials with both microencapsulation methods.

The objective of this study was to examine the effect of both spray drying and freeze drying methods, as well as 12 different combinations of ternary composite wall materials, on microencapsulation efficiency and stability of curcumin against environmental stresses. Additionally, this study also examined the application of microencapsulated curcumin in a carbonated beverage, which would provide reference information for the food industry.

2. Materials and methods

2.1. Materials:

Curcumin (90-95% purity) was provided by Yafuxingyuan Food Industry Co., Ltd. (Zhuhai, China). Modified corn starch (MS, water soluble corn starch) and maltodextrin (MD) with dextrose equivalent of 20 were obtained from Zhiyou Technology Co., Ltd. (Changchun, China). Inulin (IN), gelatin (GE), tamarind gum (TG) were obtained from Beneo Orafti SA (Belgium), Amin Biological Muslim Gelatin Co., Ltd. (Lanzhou, China), and BLX Food Materials Co., Ltd. (Zhengzhou, China), respectively. β-Cyclodextrin (CD) and pectin (PE) were obtained from Huayue Chemical Products Co., Ltd. (Zhengzhou, China). All the chemical reagents used were of analytical grade.

2.2. Formulation and preparation of emulsion

To prepare the emulsions for spray drying and freeze drying, 12 combinations of wall materials were formulated according to **Table 1**. Wall materials (30 g) were dissolved in distilled water and mixed at 1,008 g until complete dissolution. After setting still overnight, 1 g of curcumin powder in 40 mL of absolute ethanol was poured into the wall materials solution and mixed using a high-speed homogenizer (T25 digital Ultra Turrax, IKA, Germany) at 28,672 g for

10 min in cold water bath to reach complete emulsification. A small amount of distilled water was used to rinse the beaker and the curcumin residue to the emulsion, and the emulsion was made up to 100 mL.

2.3. Microencapsulation by spray drying and freeze drying

Spray drying was carried out using a lab-scale spray drier (Laiheng L-217, Beijing, China), equipped with a spray-drying chamber, a high-speed atomizer, a cyclone separator, a hot air blower, an air compressor with a maximum exhaust pressure of 0.75 MPa, and a spray nozzle of 0.7 mm. The homogenized emulsion was fed into the chamber with inlet temperature of 190 °C and feed rate of 600 mL/h, atomized by the hot air (blow rate of 28 m^3/s), and the spray-dried microparticles were collected in a glass collector.

For freeze drying, core-wall emulsions were frozen at -80 °C for more than 12 h, and then placed in a Labconco FreeZone 1 freeze drier (Kansas City, MO, USA) at -45 °C, lower than 10 Pa for 2 -3 days. The freeze dried samples were then ground into fine powder with a mortar and pestle.

2.4. Determination of microencapsulation efficiency (MEE)

The amount of curcumin was quantified by spectrophotometric measurement at 425 nm using a spectrophotometer (Jingje, Shanghai, China). The calibration curve (R^2=0.9998) was established using a series of curcumin solutions in ethanol ranging from 1 to 5mg/L. The microencapsulation efficiency (MEE) of curcumin microparticles was calculated according to Eq. 1.

$$MEE = (\text{Total curcumin} - \text{superficial curcumin}) / \text{Total curcumin} \times 100\% \quad \text{(Eq. 1)}$$

Where superficial curcumin is the amount of curcumin that was partitioned into the ethanol phase when the microparticles were mixed into absolute ethanol, and total curcumin is the amount of curcumin extracted into ethanol after dissolving the microparticles in water.

2.5. Morphological analyses

The particle size distribution of curcumin microparticles was analyzed by a laser diffraction particle size analyzer (LA-920, Horiba Ltd., Tokyo, Japan) in the wet mode, with measuring range from 0.02 to 2000 μm. Powder samples were suspended in isopropyl alcohol, sonicated with a microtip probe, and particle size distribution was determined after successive readings became constant. For the evaluation of surface morphology, powder samples were deposited on carbon double-sided adhesive tape mounted on stubs, which were observed using a scanning electron microscope (SEM, JSM-6390A, JEOL, Tokyo, Japan) with an accelerating voltage of 15 keV.

2.6. Moisture content analysis

The moisture content of microparticle samples was determined using a direct drying method based on the AOAC method with slight modifications (AOAC, 2005). In detail, dry and clean aluminum pans were weighed (m_0) and loaded with 0.5 g of microparticle powder. Samples were then dried for 4 h at 70 °C in vacuum oven (Memmert Co., Shanghai, China) for complete dehydration. Aluminum pans with samples after drying were then weighed again (m_1) until the dried weight stayed the same. Moisture content of microparticles were calculated as $[1 - (m_1 - m_0 / 0.5)] \times 100\%$.

2.7. Color analysis

Color values of curcumin microparticle samples were measured using a HunterLab D25 colorimeter (Hunter Lab Associates Inc, Reston, VA, USA). The colorimeter was calibrated with

a CR-A43 calibration white plate. Values including *L** (lightness), *a** (redness and greenness), and *b** (yellowness and blueness) were recorded. In detail, *L** stands for the lightness ranging from 0 (black) to 100 (white); *a** indicates redness with "+" and greenness with "-"; while *b**indicates yellowness with "+" and blueness with "-".

2.8. Stability tests

The heat stability of curcumin was measured as the retention (%) of curcumin after the microparticle suspensions (0.2%, w/v) were heated at 90 °C for 20 min. The acid stability of curcumin was determined by its retention (%) after the microparticle suspensions (0.2%, w/v) were adjusted to pH of 1 by adding hydrochloric acid. Stability of free curcumin was measured using its ethanol solution. The amount of curcumin was determined by the same spectrophotometric method as described in 2.4.

2.9. Stability of microparticles in carbonated beverage

The microparticles were mixed into a transparent carbonated beverage, Sprite (Coca-Cola, Atlanta, GA, USA), to reach a 0.05% (w/v) dispersion. The beverage was then set still at room temperature for 48 h for the quantitative evaluation of stability (% retention), and visual evaluation of clarity and sediment formation.

2.10. Statistical analysis

All the experiments were conducted in triplicates, and the results were expressed as mean value ± standard deviation and analyzed by one-way analysis of variance (ANOVA) with Duncan's Multiple Range Test to determine the significant differences among the means ($p < 0.05$), using the SPSS 17.0 software.

3. Results and discussion

3.1. Microencapsulation efficiency (MEE) of curcumin

The MEE of curcumin in spray dried and freeze dried microparticles are summarized in **Table 2**. In general, freeze dried microparticles showed higher MEE than the spray dried samples, except that freeze dried formula B, J, and L resulted in lower MEE. The MEE of most freeze dried microparticles was above 70 % except for the two formula using inulin (IN) and β-cyclodextrin (CD) (J and L). Formula I, which used IN, maltodextrin (MD), and tamarind gum (TG) as wall materials, resulted in the highest MEE among all freeze dried and spray dried microparticles, i.e., 89.44% and 82.50%, respectively. The higher retention of active compounds by freeze drying than by spray drying was also reported by other researchers (Ballesteros, Ramirez, Orrego, Teixeira, & Mussatto, 2017; Murali, Kar, Mohapatra, & Kalia, 2015; Ramírez, Giraldo, & Orrego, 2015). The reason can be attributed to the milder processing conditions of freeze drying (low temperature and absence of oxygen) compared with spray drying (high temperature and presence of oxygen) (Cano-Higuita et al., 2015; Chen, Zhong, Wen, McGillivray, & Quek, 2013). An elevated temperature (190 °C in spray drying) and air flow providing oxygen accelerated the degradation of curcumin (Aniesrani Delfiya, Thangavel, Natarajan, Kasthuri, & Kailappan, 2015). Therefore, for sensitive materials like curcumin, freeze drying could be a better choice for its microencapsulation. As for different formulas, the lowest MEE of curcumin (54%) in freeze dried samples was resulted from formula L (IN, CD, and TG). TG is a group of structural heteropolysaccharides from tamarind plants, which is composed of a 1,4-linked β-D glucan main chain and α-D xylan side chains (Keonakhone et al., 2017). Although being widely used in industrial applications, such as glue, thickener, and paper adhesive manufacturing, TG has many drawbacks such as low solubility in cold water and

biodegradability. Such drawbacks limited its efficiency in forming microparticles with high MEE as one of the wall materials. Similar results have been reported by Keonakhone et al. (2017), indicating that TG was not completely dissolved in water and presented a higher viscosity that caused droplet aggregation, thus resulting a lower EE value of microparticles produced. In addition, the only difference between formula L and I was the auxiliary material used: CD vs. MD, but the MEE of formula I (IN, MD, and TG) was much higher than that of formula L ($p < 0.05$). Similar results were also found among microparticles embedded with IN as structural material: high-MEE formulas G and H vs. low-MEE formulas J and K in freeze drying, and H and I vs. K and L in spray drying. Therefore, based on the above results, it can be inferred that IN worked better with MD than CD for trapping curcumin into the microparticles, with higher MEEs obtained. Another study investigating encapsulating carbohydrates also indicated that the mixture of octenylsuccinylated starch, inulin, and MD was the best combination of encapsulating wall material, which produced jussara pulp microparticles of desired properties (Lacerda et al., 2016).

3.2. Particle size and morphology of curcumin microparticles

The particle size distribution patterns of curcumin microparticles are presented in **Figure 1**. Spray drying tended to create smaller particles than freeze drying; most spray dried microparticles distributed around 10 μm, while all the freeze dried particles distributed around 100 μm. Nearly half of spray dried samples presented unimodal size distribution, while almost all freeze dried microparticles exhibited bimodal distribution pattern. Spray drying of formulas A and C exhibited the finest and most uniform microparticles, with 99% of particles having diameter below 100 μm. The difference in particle size distribution, especially affected by the two methods, was because of the materials and processes involved. Spray drying applies

powerful atomization, whereas in freeze drying, the final particle size only depended on the grinding procedure instead of the drying process (Cano-Higuita et al., 2015).

The morphological structures of microparticles examined by SEM are presented in **Figure 2**. In addition to their difference in particle size, which is in agreement with the particle size distribution patterns of **Figure 1,** microparticles created by the two drying methods resulted in very different morphological characteristics. Spray dried microparticles were more spherical and regular in shape, and their surface appeared relatively smoother than the freeze dried ones, which exhibited more cracks or fractures. However, some spray dried microparticles tended to fuse together (B, F, I, and K), which may be due to the viscosity, hygroscopicity, and concentration of the wall materials, as well as their film forming capacity.

3.3. Physical characteristics of curcumin microparticles

Physical properties of curcumin microparticles, including moisture content, color, and pigment value, are shown in **Table S1** in Supplementary Materials. The moisture content of curcumin microparticles ranged from 1.9% to 10.1 % (spray dried) and from 1.4% to 8.6 % (freeze dried), while most treatments resulted in moisture content below or around 5 %. This range agreed with Cano-Higuita et al. (2015), in which the moisture content of curcumin microparticles was all below 5% using different wall material compositions by both freeze drying and spray drying. Furthermore, the two drying methods created significant difference in moisture content. Specifically, freeze dried microparticles exhibited lower moisture content in most cases excepting formula G, indicating more dehydration through freeze drying. Group J (IN + CD + GE) exhibited a very high moisture content by both drying methods (8% - 10%). By spray drying, microparticles embedded with formulas A, B, and F also presented high moisture

contents (> 4%), which served as one reasonable explanation why the particles stuck or merged together as observed from the SEM images.

Color values reflected the lightness and saturation degree of curcumin microparticle products (**Table S1** in Supplementary Material). All curcumin microparticles presented high lightness with L^* ranging from 79.58 to 93.62. Most of the a^* values were slightly above 0, indicating a small tendency to redness, whereas the high b^* values above 0 indicated significant yellow tendency due to their curcumin content. Spray dried samples embedded with MS and CD (E and F) even possessed b^* values greater than 90. The b^* value could be an important indicator for the quality of curcumin microparticles. The spray dried and freeze dried microparticles exhibited b^* values ranging from 75.12 (H) to 91.48 (E), and from 58.29 (I) to 81.77 (G), respectively. Therefore, the overall yellowness of spray dried microparticles were more than that of freeze dried samples, although freeze dried microparticles showed generally higher MEE (**Table 2**). It was possible that higher surficial curcumin contributed more to the yellowness than curcumin trapped in the core and coated with white wall materials. In particular, the microparticles made with formula I by freeze drying presented the lowest b^* value (58.29), yet, gave the highest MEE (89.44%, **Table 2**).

3.4. Stability of curcumin microparticles against environmental stresses

The stability of microencapsulated curcumin against high temperature and acidic conditions, expressed as percent retention, is presented in **Table 2**. The retention rates of free curcumin under the same environmental stresses were 60.8% and 79.9%, respectively. Both were significantly lower than any of the microencapsulated curcumin. The results were consistent with the current literature. A recent review on curcumin encapsulation reported that the chemical stability of curcumin is enhanced by encapsulating it in microemulsions (Jiang, Liao, &

Charcosset, 2020). Yet, there was no simple trend that spray dried microparticles demonstrated higher stability than freeze dried samples, or vice versa. The highest thermal stability was achieved using formula D by spray drying and formula A by freeze drying. The highest acid stability was seen in spray dried formula D & F, and in freeze dried formula F. The wall materials used and morphological characteristics should all affect the heat and acid resistance of the microparticles. In several wall material combinations, modified corn starch as the structural material tended to provide better protection against environmental stresses than inulin. For instance, formula I with the highest MEE did not show high thermal and acid stability. As most food products present pH values higher than 2, it was ensured that curcumin microparticle could be applied to highly acidic foods with acceptable resistance to acid even when the pH value was 1.

3.5. Application of curcumin microparticles in carbonated beverage

Most carbonated beverages, e.g., Sprite (pH=3.3) used in this study, are slightly acidic with pH in the range of 3 to 4, due to dissolved carbon dioxide gas and added organic acids. All the curcumin microparticles were soluble or dispersible in the beverage samples. The retention rates of curcumin after 2 days in Sprite beverage are shown in **Table 2**. Different extents of sediment formation occurred among samples with different wall materials (**Table S2** in Supplementary Material) and the retention of curcumin ranged between 55% and 97%. Among them, sample groups E, F, G, H, K performed well in carbonated beverage for the clarity and absence of sedimentation with no matter which drying method was applied. Formulas A, G, H, and J produced by spray drying, as well as groups G and J by freeze drying exhibited high retention of curcumin after the 2-day settling, which were above 90%. It seemed that inulin performed better than modified corn starch as a structural material for the application in carbonated beverage. In

addition to its stabilizing effect in beverage, inulin could also serve as a supplement of dietary fiber, and could be welcomed by customers pursuing healthy eating, which marked its potential application in the beverage industry for its use in microencapsulation.

4. Conclusion

Curcumin microparticles formulated with 12 combinations of wall materials were produced by spray drying and freeze drying. Microencapsulation efficiency of freeze drying was generally higher than that of spray drying. Spray drying presented finer, and more regular and spherical particles. The average moisture content of microparticles was around or below 5% in most samples and all of the samples exhibited similar lightness. Spray dried microparticles were more yellow than freeze dried ones, possibly owing to more superficial curcumin and less encapsulated curcumin. Microencapsulated curcumin showed enhanced stability against high temperature and acidic conditions than free curcumin. While formulas with modified starch as structural material presented higher thermal and acid stability than those with inulin. Inulin-based formulas performed better in carbonated beverage, making it a good choice as coating material for its encapsulating performance and health beneficial effects. This study provides invaluable reference on the selection of wall materials and processes for future studies on the microencapsulation of curcumin and other bioactive compounds.

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