

## Research Article

# Effect of Spray-Drying and Freeze-Drying on the Properties of Soybean Hydrolysates

Huan Wang <sup>1</sup>, Xiaohong Tong,<sup>1</sup> Yue Yuan,<sup>1</sup> Xinhui Peng,<sup>1</sup> Qiaozhi Zhang,<sup>1</sup> Shuang Zhang,<sup>1</sup> Changyuan Xie,<sup>1</sup> Xiaoying Zhang,<sup>1</sup> Shizhang Yan,<sup>1</sup> Jingwen Xu,<sup>1</sup> Lianzhou Jiang <sup>1</sup>, Baokun Qi <sup>1</sup>, and Yang Li <sup>1,2,3,4</sup>

<sup>1</sup>College of Food Science, Northeast Agricultural University, Harbin, China

<sup>2</sup>Department of Food Science, Cornell University, Ithaca, NY, USA

<sup>3</sup>Harbin Institute of Food Industry, Harbin, China

<sup>4</sup>Heilongjiang Institute of Green Food Science, Harbin, China

Correspondence should be addressed to Baokun Qi; [qibaokun22@163.com](mailto:qibaokun22@163.com) and Yang Li; [yangli@neau.edu.cn](mailto:yangli@neau.edu.cn)

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The use of enzyme-assisted aqueous extraction to extract soybean oil will produce soy protein hydrolysates (SPH) that have good antioxidant properties but are bitter and hygroscopic. To microencapsulate these hydrolysates, soy protein isolate/maltodextrin mixtures were used as the carrier. The effects of spray-drying and freeze-drying on the bitterness, hygroscopicity, and antioxidant properties were compared. The properties of different dried samples were compared using solubility, hygroscopicity, moisture content, water activity, flowability, and glass transition temperature ( $T_g$ ). The results showed that the spray-drying was more effective than freeze-drying. Hygroscopicity was reduced to 18.2 g/100 g, and the  $T_g$  value was raised to 80.8°C. The morphology was analyzed using scanning electron microscopy, and the antioxidant properties of the samples were measured using the ABTS<sup>+</sup> radical scavenging activity. The results showed that spray-dried SPH had more carrier masking, which weakened bitterness, reduced moisture absorption, and had no significant negative impact on its oxidation resistance, solubility, and flowability, and spray-drying after carrier encapsulation of SPH improved the recovery rate.

## 1. Introduction

Free radicals are generated within the body during respiration in aerobic organisms. The superabundance of free radicals can lead to a number of different chronic diseases, such as aging, cancer, and cardiovascular diseases [1]. Enzymatic hydrolysis is the main way to produce bioactive protein hydrolysates [2]. Soy protein hydrolysates (SPH) produced using Alcalase have antioxidant properties [3]. The enzyme-assisted aqueous extraction processing (EAEP) is an environmentally friendly and safe alternative method for the simultaneous extraction of oil and protein from oilseeds [4], which gives 4 distinct fractions: an insoluble fraction (residual fraction), a liquid fraction (skim), free oil, and cream. The skim fraction is rich in protein hydrolysates [5]. Hydrolysates are mainly present in the form of low-molecular

weight soybean peptides, some of which have biological activity [6].

Hydrolysis destroys the protein structure, resulting in the exposure of hydrophobic amino acid residues, and this may lead to a strong bitter taste upon contact with taste receptor [7]. In addition, some protein hydrolysates have strong hygroscopicity and/or chemical reactivity [8]. Therefore, it is potentially beneficial to find an effective and practical process to eliminate the defects while retaining biological activity.

Microencapsulation may be an option [9]. Spray-drying (SD) is the most widely used microencapsulation technology because of its low processing costs and the good stability of the final product [10]. Freeze-drying (FD) is a gentle dehydration technology and an ideal process for producing high-value dry products [11]. FD helps retain the stability of

the physicochemical and biological activities of peptides [12].

It is important to choose suitable carriers for microencapsulation. Several common carriers are sodium alginate, carrageenan, maltodextrin (MD), soy protein isolate, and pectin [13, 14]. MD is a good choice as a wall material for microencapsulated using SD because it has favorable biocompatibility, moldability, and physicochemical properties that can effectively prevent aggregation [15]. Encapsulation of whey protein hydrolysates with MD or a mixture of MD/ $\beta$ -cyclodextrin as the wall material can reduce the bitterness of whey protein hydrolysates, reduce hygroscopicity, and enhance stability [8].

Soybean protein isolate (SPI) is produced from defatted soybean meal using alkali solution and acid isolation. It is an inexpensive and renewable raw material, and SPI also has some features useful for encapsulation, such as emulsifying, dissolving, film forming, and water-binding capabilities [16]. Encapsulation of casein hydrolysates using SD with SPI as a wall material can reduce the casein hydrolysate's bitter taste [9]. SPI is usually used as a separate encapsulation material, but protein and carbohydrate mixtures are used as carrier materials to better protect the encapsulated material [17].

The purpose of this study was to evaluate SD and FD using an SPI/MD mixture as a carrier with respect to bitterness, hygroscopicity, stability, antioxidation, and other properties of SPH.

## 2. Materials and Methods

**2.1. Materials.** Full-fat soybean flakes (fat content 20.5%, protein content 40.1%, moisture content 10.0%, and ash content 4.6%) were kindly provided by the Lanshan Group Corp. (Liaocheng, Shandong, China). Alkaline proteases from *Bacillus licheniformis* (Alcalase 2.4 L, EC 3.4.21.62, 2.4 AU/g) were purchased from Novozymes (Beijing, China). MD (DE-15) was purchased from Xiwang Food Co. (Binzhou, Shandong, China). 2,2'-Azinobis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) was purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). SPI was purchased from Shansong Biological Products Co. Ltd. (Linyi, Shandong, China). All other chemicals used were at least of analytical grade and obtained from Tianjin Kemiou Chemical Reagent Co. Ltd. (Tianjin, China).

**2.2. Preparation of SPH.** SPH was prepared using the method of Zhang et al. [6]. In brief, hydrolysis of soybeans was performed using Alcalase 2.4 L (1%, v/w) at pH 8.5 and 55°C for 3 h. After incubation, the reaction was stopped using boiling water for 10 min and centrifuged in a GL-21M refrigerated centrifuge (Xiangyi Instrument Co. Ltd., Changsha, Hunan, China) at 8000 g for 20 min at 4°C. The supernatant is the SPH.

**2.3. Drying.** SPI and MD (1:1, w/w) were used as carriers. The ratio between carrier and full-fat soybean flakes was 1.2:1 and 0.8:1 (w/w). SPI was solubilized in distilled water, and

the pH was adjusted to 8.0 using 1 M NaOH. MD was dissolved in the SPH solution and homogenized with SPI solution using an UltraTurrax T25 mechanical homogenizer (Janke & Kunkel, IKA Labortechnik, Staufen, Germany) at 18,000 rpm for 2 min.

**Spray-drying:** the solution was concentrated using a rotary evaporator (R-300, Buchi Labortechnik AG, Flawil, Switzerland) to a solid content of 20% and dried using a laboratory-scale spray-dryer (B-290, Buchi Labortechnik AG) equipped with a 0.7 mm nozzle atomizer, inlet temperature of 180°C, outlet temperature of 80–90°C, and feed rate of 15 mL/min. The product was unencapsulated SPH (S-1), the encapsulated SPH (S-2) was the 1.2:1 samples, and SPH (S-3) was the 0.8:1 samples.

**Freeze-drying:** an FD5-3 freeze-dryer (Gold Sim International Group, Los Angeles, CA, USA) was used. The equivalent products were F-1, F-2, and F-3, respectively.

**2.4. Spray-Drying Yield (Solids Recovery).** The practical recovery was determined using the method of Daza et al. [18] with slight modifications. It was calculated as the ratio of the final total solids content actually removed from the spray-dryer to the total solids content in the feed mixture, expressed as percentage (%).

**2.5. Moisture Content and Water Activity ( $A_w$ ).** The moisture content was measured using a moisture analyzer MB35 (Ohaus, Pine Brook, NJ, USA). An Aqualab analyzer (Decagon Devices, Pullman, WA, USA) was used to measure the water activity. The appropriate amount of the sample was evenly spread on the bottom of the plate and read after 1 h [19].

**2.6. Solubility.** The solubility was measured using the method of Daza et al. [18]. The sample (0.5 g) was dispersed in 50 mL of distilled water and stirred at 110 rpm for 30 min, followed by centrifugation at 5000 g for 5 min at 4°C. The supernatant (25 mL) was added to porcelain dishes and dried to constant weight in an oven at 105°C. Solubility was expressed as the ratio of the weight of the supernatant to the weight of sample (%).

**2.7. Hygroscopicity.** Hygroscopicity measurements were done using the method of Correia et al. [20]. Briefly, samples (0.5 g) were placed in an airtight glass container filled with saturated NaCl solution (relative humidity of 75.3%) at 25°C for one week. Hygroscopicity was expressed as the mass of water absorbed per 100 g of sample (g/100 g).

**2.8. Bulk and Tap Density.** A 2 g sample was put into a 10 mL graduated cylinder and the bulk density ( $B_d$ ) was measured and then gently tapped 120 times on a rubber pad to obtain the tap density ( $T_d$ ) [19].

**2.9. Flowability.** The flowability was measured as the Hausner ratio (HR) and Carr's compressibility index (CI) as follows [20]:

$$\text{HR} = \frac{T_d}{B_d},$$

$$\text{CI} = \left[ \frac{(T_d - B_d)}{T_d} \right] * 100. \quad (1)$$

The flowability was classified using HR as follows: (i)  $1.0 < \text{HR} < 1.1$ , free-flowing powders; (ii)  $1.1 < \text{HR} < 1.25$ , medium-flowing powders; (iii)  $1.25 < \text{HR} < 1.4$ , hard-to-flow powders; and (iv)  $\text{HR} > 1.4$ , very-difficult-to-flow powders.

The flowability was classified using CI as follows: (i)  $5 < \text{CI} < 15$ , excellent flowability; (ii)  $15 < \text{CI} < 24$ , good flowability; and (iii)  $\text{CI} > 25$ , poor flowability.

**2.10. Scanning Electron Microscopy (SEM).** The samples were coated with 10 nm gold under vacuum used a fine coat sputter (E-1010, Hitachi Ltd., Tokyo, Japan). The morphology of the samples was observed with an SU-8010 SEM microscope (Hitachi Ltd.) at an accelerating voltage of 5 kV. The observation and measurement were done using the method of Santos et al. [21]. The digital images were captured with a magnification of 5000 and 1000.

**2.11. Glass Transition Temperature ( $T_g$ ).**  $T_g$  was measured using a differential scanning calorimeter (DSC, Auto Q20, TA Instruments, New Castle, DE, USA) equipped with Universal Analysis 2000 software using the method of Liu et al. [22]. The azobenzol and indium were used to calibrate the DSC for temperature and enthalpy. The sample (15–20 mg) was placed in a hermetically sealed aluminum pan, and an empty pan was used as the reference. Each sample was first heated to 60°C at a rate of 10°C/min and then cooled to 0°C at a rate of 10°C/min, and a second heating scan reached 150°C at 10°C/min. The second heating scan was used to measure the  $T_g$ , as the midpoint of the observed heat capacity change.

**2.12. ABTS<sup>+</sup> Radical Scavenging Activity.** The ABTS<sup>+</sup> radical scavenging ability was measured as described by Re et al. [23]. The sample was dissolved in a sodium phosphate buffer solution (0.2 M, pH 7.4) to 2.0 mg/mL. The ABTS<sup>+</sup> solution was prepared by allowing to react a 7 mM ABTS stock solution with 2.45 mM potassium persulfate solution in the dark for 16 h, followed by dilution with sodium phosphate buffer to give an absorbance of  $0.70 \pm 0.02$  at 734 nm using a microplate reader (Tecan Infinite M200; Tecan Inc., Maennedorf, Switzerland). A 50  $\mu\text{L}$  sample solution was added to 4 mL of diluted ABTS<sup>+</sup> solution and allowed to react in the dark for 6 min, and the absorbance was measured at 734 nm. The formula for calculating ABTS<sup>+</sup> free radical scavenging capacity was

$$\text{ABTS}^+ \text{ radical scavenging activity (\%)} = \left[ 1 - \left( \frac{A_{\text{sample}}}{A_{\text{blank}}} \right) \right] \times 100, \quad (2)$$

where  $A_{\text{sample}}$  is the absorbance of the sample and  $A_{\text{blank}}$  is the absorbance of the blank.

**2.13. Sensory Evaluation.** Sensory evaluation panelists (5 males and 5 females, aged 20 to 35 years) were trained three times a week with quinine standards at room temperature for a period of 1 month. Sample solutions were tested at 1% (w/v). The standard quinine solutions were 0,  $8 \times 10^{-6}$ ,  $1.6 \times 10^{-5}$ ,  $2.4 \times 10^{-5}$ ,  $3.2 \times 10^{-5}$ , and  $4.0 \times 10^{-5}$  g/mL with corresponding scores of 0, 1, 2, 3, 4, and 5 points [24].

**2.14. Statistical Methods.** Results were expressed as the mean  $\pm$  standard deviation (STD) from at least three independent experiments. Statistical analysis was done using one-way analysis of variance (ANOVA) followed by Duncan's multiple range test at a significance level of  $p < 0.05$  using SPSS 22.0 software (SPSS Inc., Chicago, IL, USA).

### 3. Results and Analysis

**3.1. Comparison of Properties of Different Dry Samples.** Protein solubility is a very important functional property, and it has a considerable influence on other functional properties such as emulsification, gelation, and foaming properties [25]. As shown in Table 1, compared to the solubility of nonencapsulated SPH (S-1, F-1), the change with encapsulation with SPH (S-2, S-3, F-2, F-3) is very small. Although the solubility of SPI is not good, MD has excellent solubility, and the solubility of the sample increases with the increase of MD content [26, 27]. Grabowski et al. [28] also found that the solubility of sweet potato powder increased with the amount of maltodextrin increased.

Hygroscopicity is the ability of materials to absorb moisture in the environment. It affects the storage and stability of powdered foods. Samples with less hygroscopicity are easier to handle and pack. As can be seen from Table 1, the hygroscopicity of SPH without carrier encapsulation was 39 and 41 g/100 g after SD and FD, respectively. The hygroscopicity of SPH decreased significantly after encapsulation with carrier giving a lower value. Sarae et al. [9] used SPI as a carrier to reduce the hygroscopicity of casein hydrolysates with SD. Chen et al. [29] reported that the addition of MD decreased the hygroscopicity of a jujube powder. According to Nurhadi et al. [30], the hygroscopicity index of a powder is acceptable if  $< 20$  g/100 g. As shown in Table 1, the hygroscopicity of S-3 meets this criterion.

The moisture content and water activity of powder samples affect the powder stability, storage properties, and other technical properties. After encapsulation, the moisture content of SPH increased. Studies have shown that the higher the protein content, the higher the moisture in the final powder, because the protein has higher water-holding capacity in its amorphous state [20]. SPI as part of the carrier had a slight increase in moisture content. Maltodextrin is less hygroscopic due to its high molecular weight,

TABLE 1: Properties of freeze-dried and spray-dried hydrolysates.

Sample	Solubility (%)	Hygroscopicity (g/100 g)	Moisture content (%)	Aw	$T_g$ ( $^{\circ}\text{C}$ )	HR	CI (%)	Recovery rate (%)
S-1	$97 \pm 2^a$	$39 \pm 1^b$	$4.2 \pm 0.0^e$	$0.28 \pm 0.02^a$	$76.1 \pm 0.3^f$	$1.24 \pm 0.01^c$	$19.4 \pm 0.1^e$	$67 \pm 2^c$
S-2	$95 \pm 2^c$	$20 \pm 1^e$	$4.5 \pm 0.1^b$	$0.27 \pm 0.00^a$	$79.3 \pm 0.5^b$	$1.28 \pm 0.02^b$	$21.8 \pm 0.1^c$	$82 \pm 1^b$
S-3	$97 \pm 3^a$	$18 \pm 1^f$	$4.4 \pm 0.01^c$	$0.27 \pm 0.01^a$	$81 \pm 2^a$	$1.30 \pm 0.02^a$	$23.1 \pm 0.1^a$	$84 \pm 2^a$
F-1	$96 \pm 1^b$	$41 \pm 1^a$	$4.3 \pm 0.2^d$	$0.28 \pm 0.00^a$	$76 \pm 1^e$	$1.24 \pm 0.04^c$	$19.1 \pm 0.1^f$	—
F-2	$94 \pm 1^e$	$28 \pm 1^c$	$4.4 \pm 0.1^c$	$0.29 \pm 0.01^a$	$78 \pm 1^d$	$1.27 \pm 0.06^b$	$21.3 \pm 0.2^d$	—
F-3	$95 \pm 2^d$	$27 \pm 0^d$	$4.3 \pm 0.1^a$	$0.28 \pm 0.01^a$	$79 \pm 2^c$	$1.28 \pm 0.04^b$	$22.0 \pm 0.2^b$	—

and therefore, the moisture content of the powder is lower [31]. The similar phenomenon was observed by Grabowski et al. [28]; the addition of maltodextrin reduced the moisture content of amylase hydrolyzed sweet potato powder.

The greater the water activity, the more the free water, which has a negative effect on the shelf life of the product [32]. The Aw values of the individual and encapsulated hydrolysates are similar, and the Aw values of the spray-dried samples range from 0.2 to 0.3, which is typical for industrial spray-drying products [33]. Overall, the Aw values of all samples were within the expected range for powdered products and also within the recommended values to ensure microbial stability (<0.6) [18].

Glass transition temperature ( $T_g$ ) is an indicator of stability during storage [34]. As shown in Table 1, the SD samples had higher  $T_g$  than the FD samples, which leads to better stability at room temperature. The  $T_g$  values increased after SPH was encapsulated, which is due to the addition of MD as a carrier [35]. The  $T_g$  values of S-2 and F-2 were slightly lower than those of S-3 and F-3, respectively, because the more the carrier material, the higher the  $T_g$  value [36]. However, after SPH is encapsulated, the  $T_g$  value rose a little, which was due to the increased water content. Storing at temperatures below the  $T_g$  value can usually lead to longer storage times [37]. The molecular excitation increases when the temperature is above  $T_g$ , contributing to thermodynamic, chemical, and structural transformation, such as stickiness and crystallization [38].

Fluidity is defined as the ability of the sample to flow freely in a constant and regular manner. Table 1 shows that the samples had Hausner ratios (HR) and Carr's compressibility indexes (CI) of 1.24 to 1.30 and 19.1 to 23.1%, respectively. As can be seen from the table, the SPH after FD would flow better than after SD. The size of SPH particles after FD was larger, and the larger the particle size, the smaller the surface area per unit mass. The smaller the chance of interaction between surfaces, the smaller the cohesion and the better the flowability [39]. The best fluidity was F-1. After SPH is encapsulated, the HR and CI values increased. This may be due to the increased moisture content of the sample after encapsulation. Iqbal and Fitzpatrick [40] found that the increase in the moisture content of the powder resulted in an increase in the cohesion between the powder particles, which in turn reduced the flowability. Although the flowability of the SPH and the carrier after SD decreased, the decrease was slight but within the acceptable range.

**3.2. Spray-Drying Yield (Solids Recovery).** When the product recovery rate is greater than 50%, the effect of SD is better, which is closely related to the production cost and efficiency [41], so the SD recovery rate is important. Figure 1 shows that after encapsulation, the recovery rate of the sample increased, and recovery increased as the carrier content increased. The SPI in the carrier preferentially migrates to the droplet-air interface and forms a high protein content film on the surface of the particles. Hot air in the dryer causes the film to be converted to a glassy skin with a high  $T_g$  value. The glassy skin reduces the interaction between the walls and the particles in the SD chamber, which in turn improves the recovery rate [15]. As mentioned above, the addition of MD increased the  $T_g$  value, which also increased the SD recovery [42]. Wang et al. [35] used SD of soy sauce with whey protein and MD to significantly increase product recovery.

**3.3. SEM.** S-1, S-2, and S-3 in Figure 1 had spherical and smooth surfaces. It is also possible to observe irregular, concave, and wrinkled particles on the surface. The formation of concavity and wrinkles is due to the rapid evaporation of moisture during the SD process and the formation of protective films on the surface of the material droplets [43].

Favaro-Trindade et al. [44] observed that encapsulating casein hydrolysates with a mixture of MD and SPI also produced a similar morphology with SD. As indicated by the white arrow in Figure 1(d), some particles have pores, especially particles without depressions. The diffusion of the solvent occurs at a slower rate than the heat transfer to the interior of the droplets, and the pressure inside the droplet increases causing expansion of the droplet. As the shell of the surface film becomes thinner, allowing faster diffusion, the poorer permeable of the shell can cause the formation of pores, and even rupture, in the surface layer [45].

Figures 1(a), 1(c), and 1(e) show the SEM micrographs of F-1, F-2, and F-3. The FD showed a broken layered structure, indicating that the FD process did not contribute to microsphere formation.

**3.4. Analysis of ABTS<sup>+</sup> Radical Scavenging Activity.** ABTS<sup>+</sup> is a water-soluble free radical cation that can easily react with antioxidants in aqueous systems, resulting in a decreased absorbance at 734 nm [46]. As can be seen in Figure 2, the free radical scavenging activities of S-1 and F-1 are similar, which are 33.54% and 32.88%, respectively, indicating that spray-drying and freeze-drying have similar

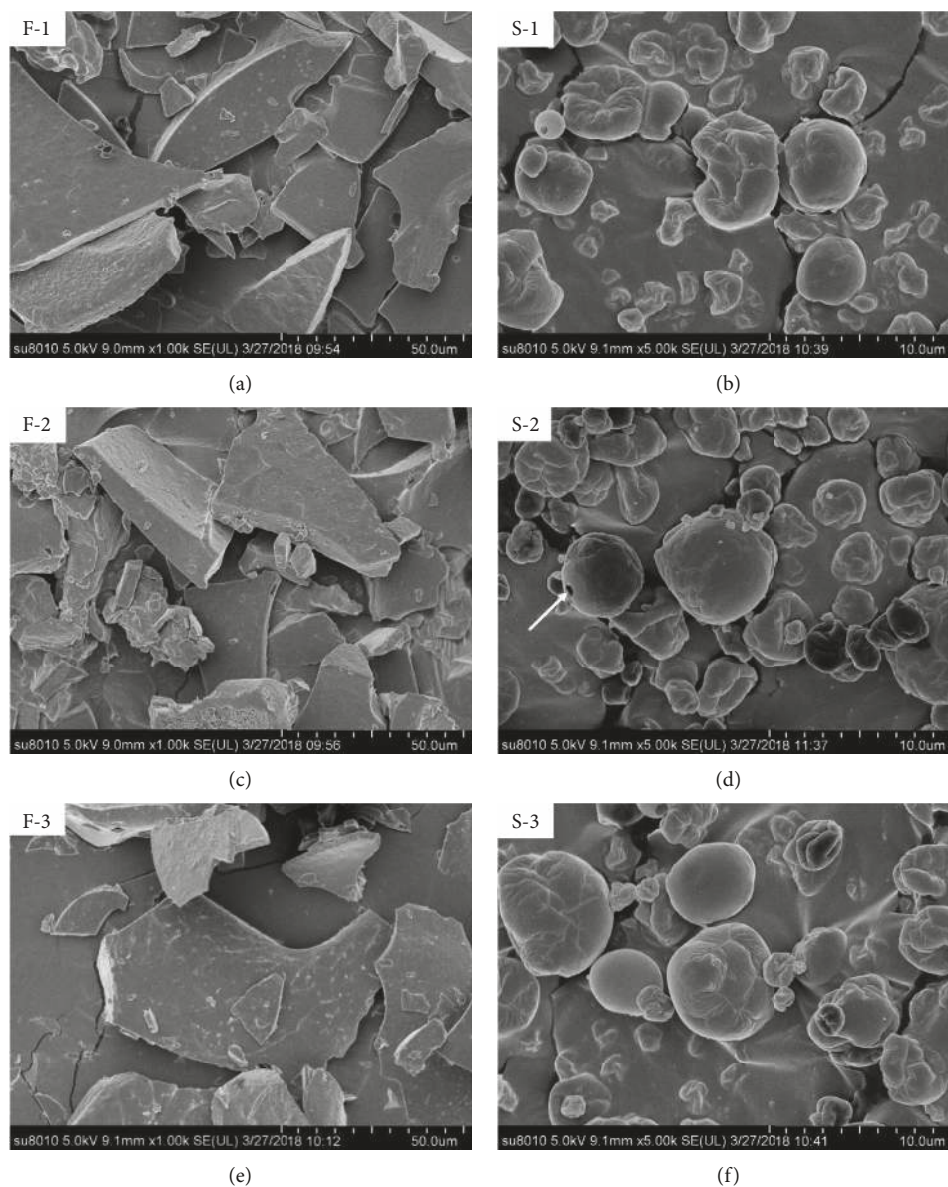


FIGURE 1: Morphological characteristics (SEM micrographs) of spray-dried and freeze-dried samples. (b) S-1, (d) S-2, and (f) S-3 are magnified 5000-fold. (a) F-1, (c) F-2, and (e) F-3 are magnified 1000-fold.

effects on the antioxidant properties of SPH. The free-radical scavenging activities of S-2, S-3, F-2, and F-3 were 28.35%, 27.39%, 27.36%, and 26.21%, respectively, indicating that the antioxidation of SPH decreased after encapsulation, which may be because the carriers (SPI and MD) were weak in oxidation resistance. But, from the figure, it can be seen that encapsulated SPH is less in the reduction of antioxidation, indicating that the soy protein isolate/maltodextrin mixture as a carrier has no obvious negative effect on the antioxidant properties of SPH.

**3.5. Sensory Evaluation.** The bitterness intensity was evaluated sensorially. As shown in Figure 3, the bitter tastes of S-1 and F-1 were similar and far higher than those of the other 4 groups. The high bitterness values indicated that the

unencapsulated SPH has a significantly stronger bitterness than the encapsulated SPH. SD had a greater protective effect than FD. This is consistent with the observation that SD reduced the bitterness of casein hydrolysates as reported by Favaro-Trindade et al. [44]. Experiments by Ma et al. [12] showed that SD using whey proteins or whey protein/sodium alginate mixtures as a carrier reduced the bitterness and hygroscopicity without impairing the immunoregulatory activity of the whey protein hydrolysates better than FD. The bitterness values of S-2 and S-3 were 0.7 and 0.5, respectively, and the bitterness values were different but not significant. The differences in the bitterness values of S-2 and S-3 may be due to the difference in the ratio of SPH and carrier.

Although FD can maintain the original chemical composition and physical properties of the product, FD takes a

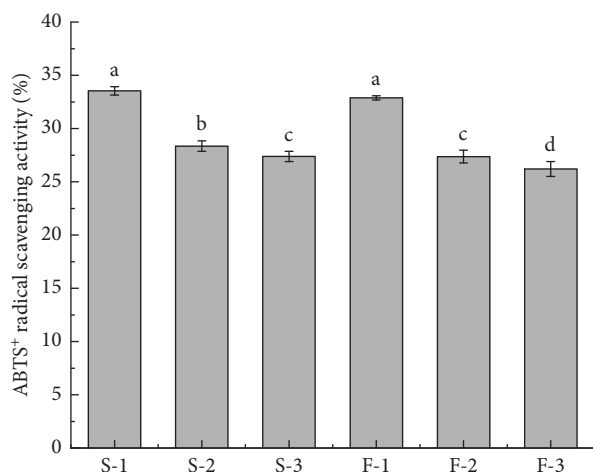


FIGURE 2: ABTS<sup>+</sup> radical scavenging activity of spray-dried and freeze-dried samples. Data are expressed as the mean  $\pm$  standard deviation,  $n=3$ . Means with different letters indicate significant differences ( $p < 0.05$ ).

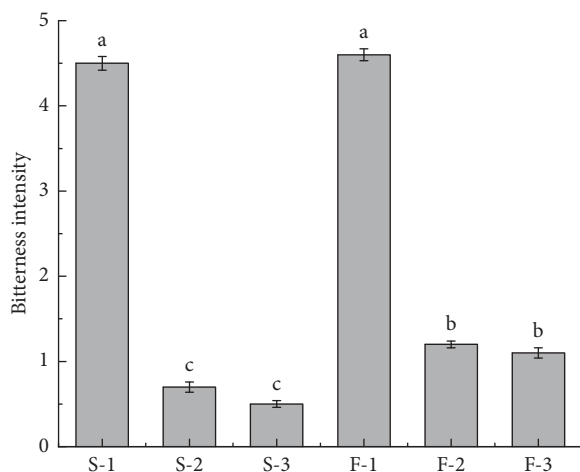


FIGURE 3: Bitterness values of spray-dried and freeze-dried samples. Data are expressed as the mean  $\pm$  standard deviation,  $n=3$ . Means with different letters indicate significant differences ( $p < 0.05$ ).

longer time, and the need to maintain a vacuum at low temperature consumes more energy, thus limiting the large-scale production. Although the temperature is high during SD, the time is short (a few sec) and the raw material is a liquid. Most of the heat will be removed through water evaporation, so the temperature of the material during drying will not be too high. The quality of the sample after drying does not change much, and the equipment is simple to operate with a large output at low cost.

#### 4. Conclusion

The main conclusions are as follows: (1) the bitterness of the enzymatic hydrolysates encapsulated with SPI/MD was significantly reduced, and the bitterness value of the samples after SD was minimal. (2) After soybean protein hydrolysates are encapsulated, hygroscopicity was reduced.

(3) Stability was increased. (4) SD was better than FD. And, (5) the solubility, flowability, and oxidation resistance decreased slightly after encapsulation, but the decrease was within the acceptable range for powder products. This work provides a theoretical basis for the preparation of SPH products with lower bitterness, better functionality, and antioxidant activity.

#### Data Availability

All of the data used to support the findings of this study are included within the article. The data used to support the findings of this study have not been made available because we are applying for a patent with the data.

#### Conflicts of Interest

The authors declare no conflicts of interest.

#### Authors' Contributions

Huan Wang and Xiaohong Tong contributed equally to this work.

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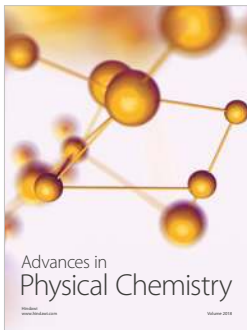
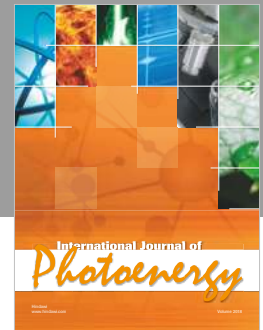
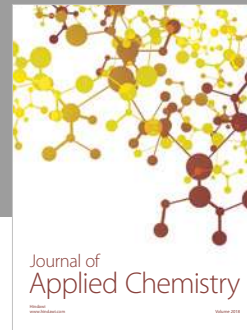
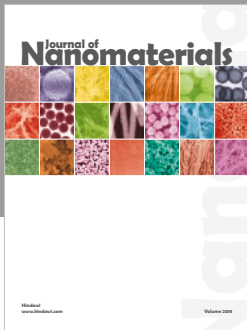
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