

Preparation of *Piper nigrum* Microcapsules by Spray Drying and Optimization with Response Surface Methodology

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Abstract: To overcome the problems of incomplete flavor components from *Piper nigrum* extract and the *Piper nigrum* product easy deterioration in the storage process, microcapsules of the whole *Piper nigrum* were prepared by spray-drying combined with enzymatic hydrolysis. Under the best conditions for the microencapsulation obtained by the response surface methodology, which have been determined as the ratio of core and wall material (1:0.2, w/w), proportion of wall materials (starch sodium octenyl succinate : maltodextrin : xanthan gum) (1:1:0.2, w/w/w), wall material concentration (11%, w/v) and inlet air temperature (180°C), the embedding rate of the prepared *Piper nigrum* microcapsules reached 90.21%. Fourier transform infrared spectroscopy, scanning electron microscopy and particle size distribution studies established that the *Piper nigrum* powder was entrapped within the microcapsules, which had intact morphology and uniform particle size distribution. Besides, gas chromatography-mass spectrometry analysis demonstrated that the prepared *Piper nigrum* microcapsules could preserve the major of the volatile aroma components of *Piper nigrum*, carene, D-limonene, α -phellandrene, and (-)- β -pinene. The obtained results showed that the microcapsules might contribute to the development of preserving original flavor from *Piper nigrum* and have potential applications in the commodity market.

Key words: *Piper nigrum*, microencapsulation, spray drying, response surface methodology, enzymatic hydrolysis

1 Introduction

Piper nigrum, cultivated in warm climate regions worldwide, such as Asia, North America¹⁾, is commonly used spice in the world due to its edibility and commercial value²⁾. The survey suggested that the total *Piper nigrum* production increased by 25% in a decade (from 2006 to 2016). With the development of modern food industry, flavor standardization of natural spices, safety of flavoring agent and eco-friendly techniques of extraction have drawn increasing attention in recent years^{1,2)}. Under the circumstance of advanced technology, the extraction assisted by microwave, ultrasound, pressurized liquid and supercritical fluid has gradually played an essential role in the fields of separation and purification^{3,4)}. However, the property, quantity and composition of substance are the keys to the selection of the extraction methods⁵⁾. It is worth noting that there are significant differences in flavor

compounds between the *Piper nigrum* products and the *Piper nigrum* raw material, and the extracted components are often insufficiency. Meanwhile, the extracts are easily oxidized and deteriorated under conditions of high light, temperature and the like⁶⁾.

The microcapsules consist of one or several different types ingredients as core material, and are coated with another materials. The coating materials is called shell material, wall material or encapsulant. Maltodextrin (MD) is a kind of carbohydrates that can wrap the core material to form a cover, encapsulate flavor and reduce exposure to oxygen. It is cheap, highly water soluble, bland in flavor and low viscosity at a high solid ratio⁷⁾. Gum, such as xanthan gum (XG), is also used for encapsulation due to its good stabilization of emulsion over a wide pH range⁸⁾. Moreover, it is compatible with most starches, carbohydrates, and proteins. Some studies have demonstrated that

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Accepted August 23, 2022 (received for review September 30, 2021)

Journal of Oleo Science ISSN 1345-8957 print / ISSN 1347-3352 online

http://www.jstage.jst.go.jp/browse/jos/ http://mc.manuscriptcentral.com/jjocs



the use of mixtures of MD and gums appeared to offer a good balance between cost and efficiency of microencapsulation using a spray dryer^{9,10}. Starch is a kind of natural macromolecule with hydrophilic properties, which is widely used in the food industry to retain and protect volatile compounds. Starch sodium octenyl succinate (SSOS), as a new kind of modified starch, has amphiphilic molecular structure featured with hydrophobic alkenyl groups, which is widely used in food, pharmaceutical and industrial fields¹¹.

Microencapsulation by spray drying is the most common and economically viable technology to protect liquids, solids or even gases against the surrounding environment into microscopic particles¹². To overcome the problems of incomplete flavor components from *Piper nigrum* extract and the *Piper nigrum* product easy deterioration in the storage process, enzymatic technology^{13,14} and microencapsulation technology¹⁵ can be thought as an advisable alternative to retain the original flavor and stabilize the flavor compounds. Enzymatic technology is used to highly release the active components from *Piper nigrum*, and microencapsulation is performed to stabilize the flavor compounds of the *Piper nigrum* products¹⁶.

Therefore, in order to maintain and stabilize the natural flavor of *Piper nigrum*, microcapsules of the whole *Piper nigrum* were prepared by spray-drying combined with enzymatic hydrolysis, in the meantime, the optimization of the microencapsulation process was performed by response surface methodology (RSM). Besides, the products were evaluated on volatile aroma components, particle size and surface morphology, etc.

2 Material and Methods

2.1 Materials and reagents

Piper nigrum was obtained from Yunnan, China. Starch sodium octenyl succinate (SSOS, 99%) of food-grade was purchased from Guangzhou Huahui Biological Industry Co., Ltd. Xanthan gum (XG, 99%) and maltodextrin (MD, 99%) were food-grade and supplied by Shanghai Yuanpu Biological Technology Co., Ltd. Petroleum ether ($\geq 90\%$), ethanol (99.8%) and methanol (99.8%) were analytical grade and purchased from Sigma Chemical Company. Cellulase (50,000 U/g) and neutral protease (100,000 U/g) were obtained from Henan Yatong Food Raw Material Co., Ltd. Sodium stearyl lactylate (SSL) (99.8%) was food-grade and obtained from Jinan Lanxin Biological Technology Co., Ltd. Mixed standards of *n*-alkanes (C_5 - C_{25}) were purchased from sigma Aldrich (Beijing, China). Distilled water was used for the preparation of all solutions.

2.2 Preparation of core material containing piperine

Our previous work has shown that enzymatic hydrolysis can increase the extraction efficiency of piperine from

*Piper nigrum*¹⁷. Therefore, enzymatic hydrolysate containing *Piper nigrum* flavor compounds was as the core material. It was prepared via the following routes. *Piper nigrum* sample was first ground into a fine powder by using a laboratory grinder mill, and then the powder was sieved less than 10 mesh. Then, a mixture of 10 g of *Piper nigrum* farina, 0.008 g of cellulose, 0.01 g of neutral protease, 0.04 g of SSL and 50 mL water was stirred thoroughly at 60°C for about 4 h. The mixture was then quickly heated to 90°C and kept for 5 min in order to inactivate the enzyme, and then quickly cooled to room temperature. After that, the enzymatic hydrolysate was obtained and collected for the next step.

2.3 Preparation of microcapsules by spray-drying

SSOS, MD and XG powders, as wall materials (the ratio of the three materials was 1:1:0.2 (w/w/w)), were dispersed in distilled water (wall material concentration was 11% (w/v)) with vigorous agitation, which was continued until the wall materials completely dissolved.

The enzymatic hydrolysate of *Piper nigrum* was then added into the solution of the wall materials (the ratio of core and wall material was set as 1:0.2 (w/w)) with agitation by means of a high-speed blender (UltraTurrax T-25, IKA Instruments, Germany S25N-18G-ST).

Microencapsulation was performed by spray drying. The emulsion was fed to a mini spray dryer (B290 Büchi, Flawil, Switzerland) at the inlet air temperature of 180°C. The nozzle cleaner was set to level 4 and the aspiration rate was set to 100%. The feed rate of the peristaltic pump was set to 6.67 mL/min, and the speed of the suction pump was set to 100%. The powders, which got from above conditions, were collected and stored at 4°C in a closed container without light^{16,18}.

2.4 Determination of microencapsulation efficiency

The extraction method of piperine from initial *Piper nigrum* powder was that 2 g of *Piper nigrum* powder was dissolved in 20 mL ethanol into a container with ultrasound for about 30 mins¹⁹. While the measurement of piperine in the microcapsule requires that 2 g of the prepared microcapsules was firstly mixed with moderate amount of petroleum ether, and then was shaken gently by hands for 2 mins at room temperature. After that, the mixture was filtered and the retained sample was rinsed three times with 20 mL petroleum ether. The collected sample was ground, and then 2 g of sample were dispersed in 20 mL of ethanol with ultrasound for 30 mins.

The content of piperine was detected at the wavelength of 343 nm using UV-vis spectrophotometer (Shimadzu UV-3600) with Ultraviolet Spectrophotometry²⁰. The external standard method was used for quantitative analysis. The standard curve was $y = 0.2645x + 0.0042$, $R^2 = 0.9996$ ($n = 13$).

The encapsulation efficiency was calculated by the following equation:

$$Y_p = \frac{M_p}{P_p} \times 100\% \quad (1)$$

where Y_p (%) was encapsulation efficiency, M_p (mg/mL) was the content of piperine in produced *Piper nigrum* microcapsules, P_p (mg/mL) was the content of piperine in *Piper nigrum* powder added in the process of the preparation of microcapsules.

2.5 Experimental design

The main factors affecting the microencapsulation efficiency were the ratio of the core and wall material, proportion of wall materials, wall material concentration and inlet air temperature. In order to optimize the microencapsulation process conditions, a single-factor experiment was first performed. Based on the results of the single factor experiment, the ratio of the core and wall material was set of 1:0.2 (w/w).

Then, the analysis experiment using Box-Behnken's response surface method was performed, in which the proportion of wall materials (x_1), wall material concentration (x_2) and inlet air temperature (x_3) were used as the independent variables, and the microencapsulation efficiency (y) was used as the response value. In order to facilitate multiple regression analysis, the actual variables were coded in Table 1.

In Table 2, experiments 1~12 were factorial experiments, and 13~17 were central experiments. The 17 experimental points were divided into factor points and zero points, where the factor point were three-dimensional vertices composed of independent variables with values of x_1 , x_2 , and x_3 , and the zero point was the center point of the region. The zero-point experiment was repeated five times to estimate the experimental error.

In the regression analysis, the relationship among the independent variables (x) and dependent variables (y) was valued by the following second order polynomial equation: $y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \beta_{11} x_1^2 + \beta_{22} x_2^2 + \beta_{33} x_3^2 + \beta_{13} x_1 x_3 + \beta_{12} x_1 x_2 + \beta_{23} x_2 x_3$, where β_0 was constant coefficients of intercept, β_1 , β_2 and β_3 were constant coefficients of linear, β_{11} , β_{22} and β_{33} were constant coefficients of quadratic, β_{13} , β_{12} and β_{23} were constant coefficients of interaction terms.

2.6 Fourier transform infrared spectroscopy (FTIR) analysis

In order to avoid disturbances of other ingredients in the *Piper nigrum* enzymatic hydrolyzate, *Piper nigrum* essential oil extracted by steam distillation was used as the infrared detection sample. Pure wall material, *Piper nigrum* essential oil extracted from *Piper nigrum*, and microcapsules samples were diluted with potassium bromide powder at a mass ratio of 1:100 respectively. Then they were ground and pressed into a transparent wafer

using the specific presser. Finally, the sample wafer was put into the sample cell. The infrared spectra were collected in the range of 400-4000 cm^{-1} at ambient temperature using a Nicolet iS10 FT-IR spectrometer (Thermo Fisher, USA).

2.7 Identification of chemical compounds from *Piper nigrum* powder and microcapsules by solid-phase microextraction (SPME) and gas chromatography (GC)-mass spectrometry (MS)

A certain amount of *Piper nigrum* powder and microcapsules sample (approximately 5.0 g) were placed in a 15 mL glass vial and sealed with a Teflon septum cap, respectively. Each sample was heated at 60°C for 30 mins. After that, the SPME fiber coated with carboxen/polydimethylsiloxane (CAR/PDMS, 65 μm , Bellefonte, PA, USA) was conditioned in the GC-MS injection port for 30 min at 240°C to remove any contaminants, and then was exposed to the vial headspace and inserted into the injection port of the GC system²¹⁾.

The volatile constituents were analyzed with a mass detector model 7000C (Agilent Technologies, Palo Alto, CA, USA) coupled to a 7890A GC, equipped with a HP-5MS capillary column (30 m $\times$ 250 μm $\times$ 0.25 μm) from Agilent. The injector temperature was 240°C with a splitless injector. The oven temperature program was held at 40°C for 5 mins, increased by 5°C/min to 240°C and was maintained for 4 mins. The carrier gas was helium at a constant flow of 1 mL/min. Mass spectra were recorded at 70 eV in the EI mode. The temperature of the quadrupole mass detector, ion source and transfer line were maintained at 150°C, 230°C and 250°C, respectively. The scanning quality range was m/z 40~400. Compound identification was based on mass spectra matching with the standard NIST 2014 MS library and also on the comparison of retention indexes (RI) sourced from the NIST Standard Reference Database and references²²⁾. The calculation of RI was according to references²³⁻²⁵⁾. In addition, the relative contents of individual components were calculated using their GC peak areas²²⁾. All analyses of the volatile compounds were made in triplicate.

2.8 Morphology

The morphology of the microcapsules was observed by a scanning electron microscope (SEM, Sigma 300, Zeiss) under high vacuum conditions at a voltage of 10 kV. The microcapsules were dissolved in the petroleum ether and the mixture was dropped on silicon sheet (10 mm $\times$ 10 mm). The sample was measured until the solution evaporated completely at room temperature.

2.9 Particle size

Particle size of the microcapsules was determined using a particle size analyzer (Nano Brook 90PlusZeta; Brookhav-

en Instruments Ltd., USA). The suspension sample was prepared by mixing the emulsions with distilled water at 25°C.

3 Results and Discussion

3.1 Microencapsulation efficiency and its condition optimization

The experimental results reported in our previous work indicated that surfactant-assisted enzymatic hydrolysis could promote the effective release of piperine in *Piper nigrum*. For the composition of the emulsion, the content of the extracted piperine markedly increased from 0.14% to 4.42% (HPLC), from 0.16% to 4.54% (UV spectrophotometric), respectively¹⁷⁾. With the enhancement of the enzymatic hydrolysis, the content of piperine in the core material (enzymolysis solution) was greatly increased, so that the active ingredients of *Piper nigrum* were distributed in a large amount in the emulsion and the prepared microcapsules contain more piperine, which greatly preserved the original ingredients and the biological activities of *Piper nigrum*. Therefore, enzymatic hydrolysis had a certain effect on the improvement of microencapsulation efficiency.

The polynomial regression model equation, which were calculated for the microencapsulation efficiency, were fitted as follow: $y = 87.24 - 1.29x_1 - 1.32x_2 - 0.33x_3 - 0.74x_1x_2 - 4.42x_1x_3 - 2.89x_2x_3 - 0.23x_1^2 - 1.77x_2^2 - 3.46x_3^2$. The appropriate level range of the operating conditions was obtained through the single factor experiments. The proportion of wall material of 1:1.5:0.2, wall material concentration of 15% and inlet temperature of 170°C were set as zero level (Table 1). The complete design consisted of 17 different combinations and the experimental results were shown in Table 2. Additionally, the results of variance analysis were given by Design-Expert software, which were shown in Table 3. As can be seen from Table 3, the equation regression was significant ($R^2 = 0.9792$; $R^2_{Adj} = 0.9524$). And furthermore, the results of RSM showed that the proportion of wall materials (x_1) and wall material concentration (x_2) had a significant effect on the efficiency of microencapsulation ($p < 0.05$). Among the three wall materials, SOSS was the emulsifier, MD was film foaming, and XG had both functions¹⁶⁾. When the content of MD was high, the

Table 2 Design matrix in the response surface design and obtained experimental results.

Run no.	Coded variables			Microencapsulation efficiency (%)
	A	B	C	
1	-1	-1	0	86.86
2	1	-1	0	85.51
3	-1	1	0	86.44
4	1	1	0	82.12
5	-1	0	-1	80.96
6	1	0	-1	87.46
7	-1	0	1	88.46
8	1	0	1	77.30
9	0	-1	-1	80.79
10	0	1	-1	83.19
11	0	-1	1	86.60
12	0	1	1	77.43
13	0	0	0	86.68
14	0	0	0	88.07
15	0	0	0	86.68
16	0	0	0	88.07
17	0	0	0	86.68

components in the system could not be fully emulsified, the emulsion was not stable enough, and the microcapsule film formed on the surface was not dense enough. However, when the content of MD was low, the active ingredients in the system cannot be embedded, resulting in poor film-forming ability and low microencapsulation efficiency of microcapsules. Therefore, the proportion of functional wall material was one of the key factors for the success of microencapsulation²⁶⁾. Secondly, the wall material concentration was also one of the factors affecting the efficiency of microencapsulation. When the wall material solid content was high, the film formation speed of the microcapsule coating material and the density of the capsule wall were improved²⁷⁾. It would enhance the encapsulation of the core material, reduced the diffusion and migration of the core material to the wall material, and thus improved the embedding rate of the microcapsule. However, when the wall material was too high, the emulsion viscosity

Table 1 Coded levels for independent variables in developing experimental data.

Independent variable	Unit	Symbol	Coded and actual levels		
			-1	0	+1
proportion of wall materials/ x_1	(w/w/w)	A	1:1:0.2	1:1.5:0.2	1:2:0.2
wall material concentration/ x_2	% (w/v)	B	10	15	20
inlet air temperature/ x_3	°C	C	160	170	180

Table 3 Analysis of variance and statistical parameters of the model.

Source	SS ^a	DF ^b	MS ^c	F-value	p-value	Significance ^d
Model	209.53	9	23.28	36.55	<0.0001	*
x_1	13.34	1	13.34	20.94	0.0026	*
x_2	13.99	1	13.99	21.96	0.0022	*
x_3	0.85	1	0.85	1.34	0.2856	
x_1x_2	2.21	1	2.21	3.46	0.1051	
x_1x_3	77.97	1	77.97	122.39	<0.0001	*
x_2x_3	33.47	1	33.47	52.53	0.0002	*
x_1^2	0.22	1	0.22	0.35	0.5721	
x_2^2	13.24	1	13.24	20.78	0.0026	*
x_3^2	50.42	1	50.42	79.15	<0.0001	*
Residual	4.46	7	0.64			
Lack of fit	2.14	3	0.71	1.23	0.4080	
Pure error	2.32	4	0.58			
Total	213.99	16				

$$R^2 = 0.9792; \quad R^2_{Adj} = 0.9524$$

^a SS= sum of squares; ^b DF =degree of freedom; ^c MS = mean square; ^d significance: * $p < 0.05$ is significant.

would also increase. The obtained emulsion was easy to stick and block around the nozzle, which led to a long time for the flavor substances in *Piper nigrum* to be heated. To some extent, it would cause the loss of active ingredients and affect the evaporation of water and the atomization of liquids. Finally, the embedding rate of the microcapsules was reduced²⁸⁾.

Moreover, the results of RSM also showed that among quadratic and interaction terms of independent variables, x_1x_3 , x_2x_3 , x_2^2 , x_3^2 were significant ($p < 0.05$). It indicated that the experimental factor was not a simple linear relationship to the response value. Thence the experimental parameters were further optimized by the Design-Expert software, and the most favorable conditions were the proportion of wall materials of 1:1:0.2 (SSOS: MD: XG, w/w/w), wall material concentration of 11% (w/v) and inlet air temperature of 180°C. Under the above conditions, the predicted value of microencapsulation efficiency could reach to 90.62%. Finally, the average microencapsulation efficiency of confirmatory experiment carried out 3 times reached 90.21%, which was only 0.41% difference from the theoretical value. It showed that the experimental value was extremely close to the predicted value and there was no significant difference between them. Meanwhile, it revealed that the RSM was feasible to optimize the microencapsulation conditions, and the obtained microencapsulation conditions had certain practical application value.

3.2 FTIR analysis

The microcapsules of *Piper nigrum* were analyzed by FTIR spectroscopy in order to verify the presence of the piperine in the microcapsules. The FTIR spectrum of pure

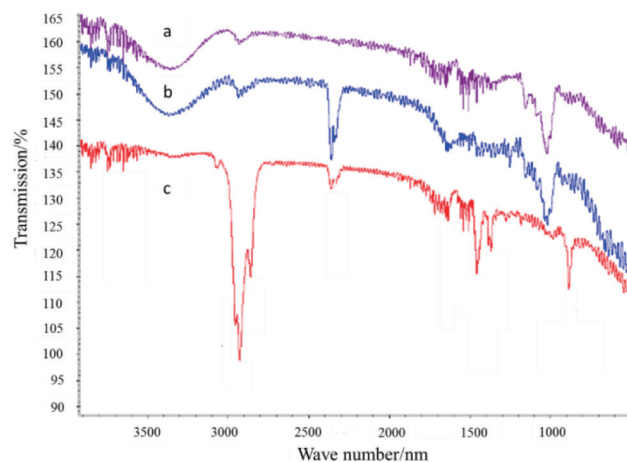


Fig. 1 FTIR of pure wall materials(a), *Piper nigrum* microcapsules(b), and *Piper nigrum* essential oil (c).

wall material, which was shown in **Fig. 1a**, consisted of prominent absorption peaks at 3420 cm^{-1} for the hydroxyl (-OH) groups²⁹⁾, 1448.56 cm^{-1} for -CH₂ bending vibration, 1371.36 cm^{-1} for -CH₃ bending vibration³⁰⁾ and 1060.86 cm^{-1} for C-O stretching vibration²⁹⁾.

On the other hand, the FTIR spectrum of piper nigrum essential oil (**Fig. 1c**), showed that the weak absorption band observed at a wavelength of 3648.71 cm^{-1} was characteristic for hydroxyl (-OH) groups³¹⁾. The peaks presented with strong intensity at approximately 2927.30 cm^{-1} and 2858.41 cm^{-1} belonged to C-H stretching vibration of the methylene groups³²⁾. The signal at 1635.67 cm^{-1} corresponded to the C=C stretching vibration. Furthermore,

the strong absorption band at the 1367.45 cm^{-1} and 1456.76 cm^{-1} was associated to C-H bending vibration⁽³³⁾.

As shown in Fig. 1b, the spectra of the *Piper nigrum* microcapsules also showed peaks characteristic of *Piper nigrum* essential oil, and there was no new chemical bond between the microcapsules and the wall materials spectrums, thus confirming that the *Piper nigrum* essential oil was contained in the wall material and there was no chemical reaction between the core materials and SSOS-MD-XG mixtures. Similar results have been reported by other studies^(32, 34).

3.3 Analysis of volatile aroma components

By using GC-MS analysis, twenty-one and fourteen odor-active volatile compounds were detected in *Piper nigrum* powder and *Piper nigrum* microcapsules, respectively (Fig. 2). The odor-active volatile compounds identified from *Piper nigrum* powder and *Piper nigrum* microcapsules were presented in Table 4. As it can be seen, the quantities of alkenes in *Piper nigrum* powder and microcapsules were both high. More specifically, the most important volatile compounds for aroma in these two samples were carene, D-limonene, α -phellandrene, and (-)- β -pinene. Furthermore, the highest content of the two samples was D-limonene. Indeed, similar results were obtained in previous studies focusing on *Piper nigrum*^(21, 30).

The recovery percentages of most volatile compounds were relatively low (no more than 15%) while some main volatile compounds, such as D-limonene and carene, were higher recovery percentages in microcapsules. Meanwhile some volatile components, such as β -caryophyllene and (+/-)- δ -elemene, were not detected in the microcapsules. Previous studies reported that the volatile retention quantity of carbohydrate had no relation to the relative content of aroma compounds, but with the volatile molecular weight increasing, it would increase correspondingly. In addition, it was established that some volatile compounds were lost during microencapsulation^(35, 36).

However, most of main components from *Piper nigrum* powder had a little change before and after microencapsulation, which proved that most of the volatile components could be maintained by microencapsulation and the natural flavor of *Piper nigrum* could be retained to the greatest extent.

3.4 Morphology

Figure 3 revealed that the microcapsules had a broad size distribution with an irregular shape (Fig. 3A). In general, microcapsules that produced by spray drying generally had a spherical or irregular shape and various sizes^(29, 30, 37). Those microcapsules produced with SSOS, MD and XG powders as wall materials were characterized by larger deformations and irregular surfaces (folds) (Figs. 3B-3D). These irregular surfaces were mainly represented by many

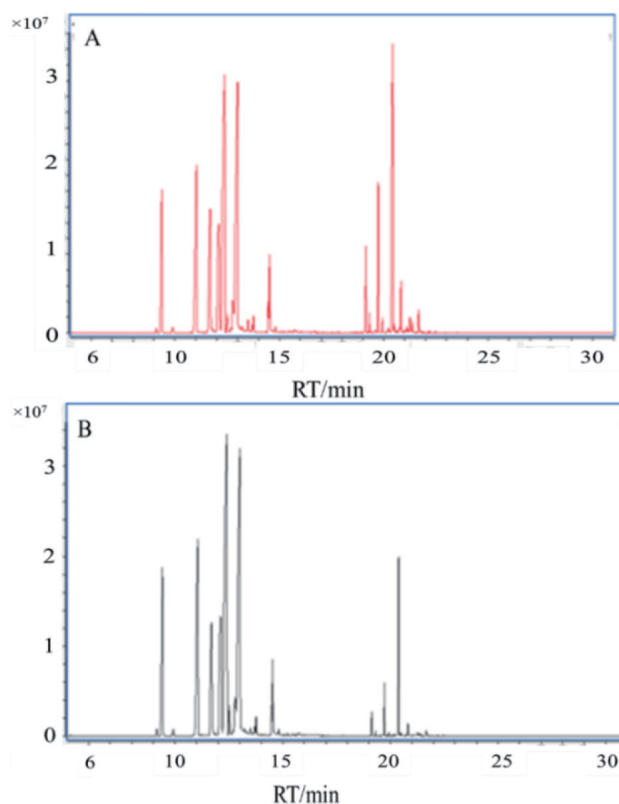


Fig. 2 Chromatograms for odour active volatile of *Piper nigrum* powder (A) and *Piper nigrum* microcapsules (B).

shrinkages and dents on the surface of the microcapsules, which were consistent with some previous reports^(38, 39). The shape irregularity of the microcapsules was closely related to the components of core material including flavor compounds, essential oil and other cellulose and lignin, etc. Besides, the presence of depressions on the surface of the microparticles has been associated to the shrinkage at the early stages of the drying and subsequent cooling^(40, 41).

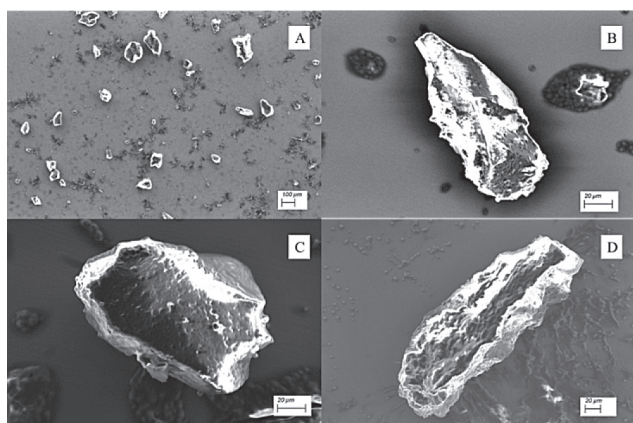
3.5 Particle size determination

The particle size distribution curve of *Piper nigrum* microcapsules emulsions was presented in Fig. 4. As it can be seen in Fig. 4, the particle size distribution of emulsions was uniform. More specifically, the average particle size distributions of the emulsions were $0.15\text{ }\mu\text{m}$, which were slightly lower than those previous studies on different microcapsules^(28, 38). The enormous differences between studies mainly arose from the emulsification methods⁽²⁸⁾ and the properties of wall and core materials. In addition, recent studies have shown that the average particle size distributions of the microcapsules carried out by the ultrasonic emulsification method were generally smaller⁽⁴²⁾, which may be attributed to cavitation and mechanical function of ultrasound^(43, 44).

Table 4 Comparison of all compounds in *Piper nigrum* powder and *Piper nigrum* microcapsules (n = 3 for each analysis).

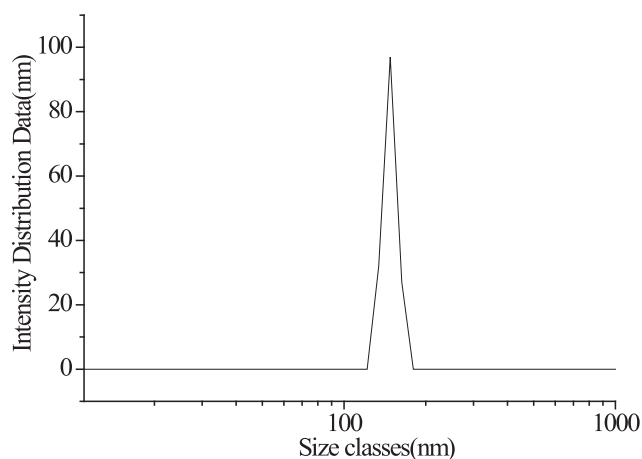
No.	Name	Piper nigrum powder				Piper nigrum microcapsules			
		RI ^C	RI ^L	Relative contents (%)	Base of ID ^a	RI ^C	RI ^L	Relative contents (%)	Base of ID ^a
1	1R-(+)- α -Pinene	930	935	6.16	MS,RI	932	935	8.07	MS,RI
2	(-)-Camphene	944	949	0.21	MS,RI	945	949	0.31	MS,RI
3	(-)- β -Pinene	976	978	16.46	MS,RI	977	978	20.13	MS,RI
4	α -Phellandrene	1007	1007	8.18	MS,RI	1007	1007	13.29	MS,RI
5	Carene	1017	1011	19.36	MS,RI	1017	1011	21.50	MS,RI
6	D-Limonene	1039	1033	23.41	MS,RI	1039	1033	23.95	MS,RI
7	(Z)- β -Ocimene	1040	1040	0.51	MS,RI			–	–
8	γ -Terpinene	1066	1064	0.47	MS,RI	1066	1064	0.63	MS,RI
9	Terpinolene	1091	1089	2.89	MS,RI	1091	1089	3.26	MS,RI
10	Linalool	1104	1104	0.33	MS,RI	1104	1104	1.36	MS,RI
11	2,6-dimethyl-octa-2,4,6-triene	1149	1147	0.46	MS,RI	1149	1147	0.48	MS,RI
12	α -Terpineol	1181	1192	0.05	MS	1180	1192	0.10	MS
13	(+/-)- δ -Elemene	1351	1340	1.77	MS	–	–	–	–
14	(-)- α -Cubebene	1364	1354	0.38	MS,RI	1363	1354	0.50	MS
15	α -Copaene	1393	1387	3.32	MS,RI	1392	1387	3.06	MS,RI
16	β -Elemene	1399	1394	0.41	MS,RI	1340	1394	0.10	MS,RI
17	(-)- α -Gurjunene	1407	1408	0.18	MS,RI	–	–	–	–
18	β -Caryophyllene	1445	1451	12.40	MS,RI	–	–	–	–
19	10,10-dimethyl-2,6-dimethylenebicyclo[7.2.0]undecane	1439	1440	0.21	MS,RI	–	–	–	–
20	(+)- β -Selinene	1517	1496	0.45	MS	–	–	–	–
21	(+)- δ -Cadinene	1542	1528	0.49	MS	–	–	–	–

– Non-detected; Relative percentage content (%) = peak area alone/total peak area $\times$ 100; RI^C: calculated retention index, RI^L: reported retention index, ^a MS: compounds were identified by MS spectra; RI: compounds were identified by comparison to references.

**Fig. 3** SEM of the *Piper nigrum* microcapsules.

4 Conclusions

In conclusion, the produced *Piper nigrum* enzymatic hydrolyzate, the wall material used and the spray drying conditions were effective in keeping the natural chemical compounds in the *Piper nigrum* microcapsules. The best conditions for the microencapsulation have been determined as the ratio of core and wall material (1:0.2, w/w), proportion of wall materials (1:1:0.2, SSOS : MD : XG, w/w/

**Fig. 4** Particle size of *Piper nigrum* microcapsules emulsions.

w), wall material concentration (11 %, w/v) and inlet air temperature (180°C). Under the optimal conditions determined by RSM, the microencapsulation efficiency of *Piper nigrum* could achieve 90.21%. In the microscopic observation, the shape of the microcapsules was irregular and the average particle size of the emulsion was 0.15 μ m.

Based on the findings, this study could provide a useful guideline for the microencapsulation of *Piper nigrum*.

Acknowledgements

This work was supported by the National Key Research and Development Program of China (2017YFC1600401-3), Beijing Municipal Science and Technology Project (Z171100001317015) and National Natural Science Foundation of China (31871749).

References

- 1) Baenas, N.; Belovic, M.; Ilic, N.; Moreno, D.A.; Garcia-Viguera, C. Industrial use of pepper (*Capsicum annum* L.) derived products: Technological benefits and biological advantages. *Food chem.* **274**, 872-885 (2019).
- 2) Andrade, K.S.; Trivellin, G.; Ferreira, S.R.S. Piperine-rich extracts obtained by high pressure methods. *J. Supercrit Fluid.* **128**, 370-377 (2017).
- 3) Mazzutti, S.; Riehl, C.A.S.; Ibañez, E.; Ferreira, S.R.S. Green-based methods to obtain bioactive extracts from *Plantago major*, and *Plantago lanceolata*. *J. Supercrit Fluid.* **119**, 211-220 (2017).
- 4) Viganó, J.; Zobot, G.L.; Martínez, J. Supercritical fluid and pressurized liquid extractions of phytonutrients from passion fruit by-products: Economic evaluation of sequential multi-stage and single-stage processes. *J. Supercrit Fluid.* **122**, 88-89 (2017).
- 5) Wang, Y.; Jiang, Z.T.; Li, R. Composition comparison of essential oils extracted by hydrodistillation and microwave-assisted hydrodistillation from black pepper (*Piper nigrum* L.) grown in China. *J. Essent. Oil Bear. Plants* **12**, 374-380 (2009).
- 6) Lee, Y.K.; Ahn, S.I.; Kwak, H.S. Optimizing microencapsulation of peanut sprout extract by response surface methodology. *Food Hydrocoll.* **30**, 307-314 (2013).
- 7) Pourashouri, P.; Shabanpour, B.; Razavi, S.H.; Jafari, S.M.; Shabani, A.; Aubourg, S.P. Oxidative stability of spray-dried microencapsulated fish oils with different wall materials. *J. Aquat. Food Prod. Technol.* **23**, 567-578 (2014).
- 8) Fu, D.W.; Deng, S.M.; McClements, D.J.; Zhou, L.; Zou, L.Q. et al. Encapsulation of β -carotene in wheat gluten nanoparticle-xanthan gum-stabilized Pickering emulsions: Enhancement of carotenoid stability and bioaccessibility. *Food Hydrocoll.* **89**, 80-89 (2019).
- 9) Apintanapong, M.; Noomhorm, A. The use of spray drying to microencapsulate 2-acetyl-1-pyrroline, a major flavour component of aromatic rice. *J. Food Sci. Tech.* **38**, 95-102 (2003).
- 10) Bhandari, B.R.; Dumoulin, E.D.; Richard, H.M.J.; Noleau, I.; Lebert, A. Flavor encapsulation by spray drying: Application to citral and linalyl acetate. *J. Food. Sci.* **57**, 217-221 (1992).
- 11) Fang, J.M.; Fowler, P.A.; Tomkinson, J.; Hill, C.A.S. An investigation of the use of recovered vegetable oil for the preparation of starch thermoplastics. *Carbohydr Polym.* **50**, 429-434 (2002).
- 12) Soliman, E.A.; Moghazy, A.Y.E.; Mohy, E.D.M.S.; Masoud, M.A. Microencapsulation of essential oils within alginate: Formulation and *in vitro* evaluation of anti-fungal activity. *J. Encapsulation Adsorpt. Sci.* **3**, 48-55 (2013).
- 13) Dutta, S.; Bhattacharjee, P. Nanoliposomal encapsulates of piperine-rich black pepper extract obtained by enzyme-assisted supercritical carbon dioxide extraction. *J. Food Eng.* **201**, 49-56 (2017).
- 14) Tomazelli Junior, O.; Kuhn, F.; Padilha, P.J.M.; Vicente, L.R.M.; Costa, S.W. et al. Microencapsulation of essential thyme oil by spray drying and its antimicrobial evaluation against *Vibrio alginolyticus* and *Vibrio parahaemolyticus*. *Braz. J. Biol.* **78**, 311-317 (2018).
- 15) Paini, M.; Aliakbarian, B.; Casazza, A.A.; Lagazzo, A.; Botter, R.; Perego, P. Microencapsulation of phenolic compounds from olive pomace using spray drying: A study of operative parameters. *LWT-Food Sci. Technol.* **62**, 177-186 (2015).
- 16) Madene, A.; Jacquot, M.; Scher, J.; Desobry, S. Flavour encapsulation and controlled release - A review. *Int. J. Food Sci. Technol.* **4**, 1-21 (2006).
- 17) Yu, Y.; Hu, S.Q.; Fu, D.J.; Zhang, X.X.; Liu, H.Q. et al. Surfactant-assisted enzymatic extraction of piperine from *Piper nigrum* L. *Int. J. Food Prop.* **23**, 52-62 (2020).
- 18) Shaikh, J.; Bhosale, R.; Singhal, R. Microencapsulation of black pepper oleoresin. *Food Chem.* **94**, 105-110 (2006).
- 19) Rathod, S.S.; Rathod, V.K. Extraction of piperine from *Piper longum* using ultrasound. *Ind. Crops. Prod.* **58**, 259-264 (2014).
- 20) Gorgani, L.; Mohammadi, M.; Najafpour, G.D.; Nikzad, M. Sequential microwave -ultrasound-assisted extraction for isolation of piperine from black pepper (*Piper nigrum* L.). *Food Bioprocess Technol.* **10**, 2199-2207 (2017).
- 21) Matsushita, T.; Zhao, J.J.; Igura, N.; Shimoda, M. Authentication of commercial spices based on the similarities between gas chromatographic fingerprints. *J. Sci. Food Agric.* **98**, 2989-3000 (2018).
- 22) Chandran, J.; Amma, K.P.P.; Menon, N.; Purushothaman, J.; Nisha, P. Effect of enzyme assisted extraction on quality and yield of volatile oil from black pepper and cardamom. *Food Sci. Biotechnol.* **21**, 1611-1617

- (2012).
- 23) Prodhon, M.A.I.; Sleman, A.A.; Kim, S.; McClain, C.; Zhang, X. Generalization of reference system for calculating the second dimension retention index in GC $\times$ GC-MS. *J. Anal. Test.* **2**, 263-273 (2018).
 - 24) Wang, Z.; Wei, J.W.; Wang, Y.; Zhu, T.T.; Huang, M.Q. *et al.* A new method to predict the content changes of aroma compounds during the aging process of Niulanshan Baijiu using the GM(1,1) gray model. *Flavour Frag. J.* **37**, 5-19 (2022).
 - 25) Zheng, Y.; Sun, B.G.; Zhao, M.M.; Zheng, F.P.; Huang, M.Q. *et al.* Characterization of the key odorants in Chinese zhima aroma-type Baijiu by gas chromatography-olfactometry, quantitative measurements, aroma recombination, and omission studies. *J. Agric. Food Chem.* **64**, 5367-5374 (2016).
 - 26) Reineccius, G.A. The spray drying of food flavors. *Dry Technol.* **22**, 1289-1324 (2007).
 - 27) Balasubramani, P.; Viswanathan, R.; Vairamani, M. Response surface optimisation of process variables for microencapsulation of garlic (*Allium sativum* L.) oleoresin by spray drying. *Biosyst. Eng.* **114**, 205-213 (2013).
 - 28) Tontul, I.; Topuz, A. Influence of emulsion composition and ultrasonication time on flaxseed oil powder properties. *Powder Technol.* **264**, 54-60 (2014).
 - 29) Sanchez-Reinoso, Z.; Osorio, C.; Herrera, A. Effect of microencapsulation by spray drying on cocoa aroma compounds and physicochemical characterisation of microencapsulates. *Powder Technol.* **318**, 110-119 (2017).
 - 30) Fernandes, R.V.B.; Borges, S.V.; Silva, E.K.; Silva, Y.F.; Barboza, H.J. *et al.* Study of ultrasound-assisted emulsions on microencapsulation of ginger essential oil by spray drying. *Ind. Crop Prod.* **94**, 413-423 (2016).
 - 31) Santiago-Adame, R.; Medina-Torres, L.; Gallegos-Infante, J.A.; Calderas, F.; González-Laredo, R.F. *et al.* Spray drying-microencapsulation of cinnamon infusions (*Cinnamomum zeylanicum*) with maltodextrin. *LWT-Food Sci. Tech.* **64**, 571-577 (2015).
 - 32) Banerjee, S.; Chattopadhyay, P.; Ghosh, A.; Goyary, D.; Karmakar, S.; Veer, V. Influence of process variables on essential oil microcapsule properties by carbohydrate polymer-protein blends. *Carbohydr. Polym.* **93**, 691-697 (2013).
 - 33) Che Man, Y.B.; Syahariza, Z.A.; Mirghani, M.E.S.; Jinap, S.; Bakar, J. Analysis of potential lard adulteration in chocolate and chocolate products using Fourier transform infrared spectroscopy. *Food Chem.* **90**, 815-819 (2005).
 - 34) Huang, G.Q.; Sun, Y.T.; Xiao, J.X.; Yang, J. Complex coacervation of soybean protein isolate and chitosan. *Food Chem.* **135**, 534-539 (2012).
 - 35) Jeleñ, H.H.; Gracka, A. Analysis of black pepper volatiles by solid phase microextraction-gas chromatography: A comparison of terpenes profiles with hydrodistillation. *J. Chromatogr. A* **1418**, 200-209 (2015).
 - 36) Rulkens, W.H.; Thijssen, H.A.C. The retention of organic volatiles in spray-drying aqueous carbohydrate solutions. *Int. J. Food Sci. Tech.* **7**, 95-105 (1972).
 - 37) Chew, S.C.; Tan, C.P.; Nyam, K.L. Microencapsulation of refined kenaf (*Hibiscus cannabinus* L.) seed oil by spray drying using beta-cyclodextrin/gum arabic/sodium caseinate. *J. Food Eng.* **237**, 78-85 (2018).
 - 38) Carneiro, H.C.F.; Tonon, R.V.; Grosso, C.R.F.; Hubinger, M.D. Encapsulation efficiency and oxidative stability of flaxseed oil microencapsulated by spray drying using different combinations of wall materials. *J. Food Eng.* **115**, 443-451 (2013).
 - 39) Daza, L.D.; Fujita, A.; Fávoro-Trindade, C.S.; Rodrigues-Ract, J.N.; Granato, D.; Genovese, M.I. Effect of spray drying conditions on the physical properties of Cagaita (*Eugenia dysenterica* DC.) fruit extracts. *Food Bioprod. Process.* **97**, 20-29 (2016).
 - 40) Hijo, A.A.C.T.; Da Costa, J.M.G.; Silva, E.K.; Azevedo, V.M.; Malta, M.R.; Borges, S.V. Physical and thermal properties of oregano (*Origanum vulgare* L.) essential oil microparticles. *J. Food Process Eng.* **38**, 1-10 (2015).
 - 41) Da Costa, J.M.G.; Silva, E.K.; Hijo, A.A.C.T.; Azevedo, V.M.; Malta, M.R. *et al.* Microencapsulation of Swiss cheese bioaroma by spray-drying: Process optimization and characterization of particles. *Powder Technol.* **274**, 296-304 (2015).
 - 42) Jafari, S.M.; He, Y.H.; Bhandari, B. Role of powder particle size on the encapsulation efficiency of oils during spray drying. *Dry Technol.* **25**, 1081-1089 (2007).
 - 43) Shen, L.L.; Chen, J.P.; Bai, Y.J.; Ma, Z.C.; Huang, J.; Feng, W. Physical properties and stabilization of microcapsules containing thyme oil by complex coacervation. *J. Food Sci.* **81**, 2258-2262 (2016).
 - 44) Spada, J.C.; Marczak, L.D.F.; Tessaro, I.C.; Cardozo, N.S.M. Effect of ultrasound treatment on particle size, rheology, microstructure, colour and water holding capacity of a soy-based dessert. *Acta Alimentaria* **45**, 242-249 (2016).

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