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ORIGINAL PAPER

Study on the spray-drying encapsulation of lutein in the porous starch and gelatin mixture

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Abstract Spray-drying encapsulation of lutein was conducted to improve its stability and water-solubility. With the porous starch and gelatin as wall material, lutein microcapsules were effectively prepared by spray-drying process. Results showed the optimal technology of lutein encapsulation as follows: the ratio of core to wall material of 1/30, embedding temperature of 60 °C, embedding time 1.5 h, inlet gas temperature of 190 °C, feed flow rate 50 mL/min and drying air flow 60 m^3/h, at which the microcapsules had good encapsulation efficiency (94.4 ± 0.4)% and high yield of product (92.6 ± 1.7)%. Its solubility was increased and it could be directly dissolved in water. The stability of lutein microcapsule in the new carrier mixture against heat, pH, light and oxygen was also greatly improved and their retention rates had been improved about 15–50% than that of free lutein. The results would be helpful to the application of lutein in food industry.

Keywords Lutein · Encapsulation · Spray-drying · Stability

Introduction

As a kind of natural oxygenated carotenoid, lutein can be commonly found in orange, yellow and green fruits and vegetables, such as bananas, kiwi fruit, corn. As a valuable functional ingredient, lutein has strong coloring power and comprehensive pharmacological activity, for example, it can prevent aging, antioxidant and atherosclerosis etc. It is also an important permitted natural colorant used in food, nutritious and pharmaceutical preparations, especially it is applied as a food additive for several years, but its instability and water insolubility limit its wide application in industry [1–6].

As a fat soluble pigment, it is insoluble in aqueous medium. Because of its high degree of unsaturation, it is easily susceptible to light, temperature, pH, reductant and oxidant, so it should be preserved in low temperature and vacuum condition from light [7–10]. Because of these disadvantages, the widespread application of lutein in food processing industry is limited, and it is necessary to find a method to improve its low instability and water insolubility.

Microencapsulation technique can effectively protect food ingredients against deterioration, which has been widely used for the stabilization of labile compounds. It is defined as the technology of packaging solid, liquid and gaseous materials in small capsules that release their content at controlled rates over prolonged periods of time [11–14]. The particles or droplets are surrounded by a coating, or embedded in homogeneous or heterogeneous matrix, to give small capsules with many useful properties. In systems, the stabilization occurs when the wall material act as a physical and permeable barrier and the shelf life of the encapsulated products can be prolonged [15–17].

Some conventional methods, such as spray-cooling, spray-drying, freeze-drying, centrifugal extrusion, molecular inclusion and emulsion freeze-drying, have been used in the microencapsulation techniques. Among these, spray-drying is the most commonly used one due to its low cost, available equipment, continuous production and easiness of

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industrialization. This technique has many other advantages, such as compatible to lots of coating materials, and less effect on its function zone if particles dissolve [17–19].

Microencapsulation efficiency and microcapsules stability are largely dependent on the wall material composition. Wall materials can be selected from a wide variety of natural polymers, such as natural gums. Among them, gelatin, algin, protein, cyclodextrin, maltodextrin and chitosan are often used as wall materials for the lutein microencapsulation, which play a certain embedding role for lutein. However, because of their small hole, low-specific surface area, weak adsorption effect and bad aggregation, the content of lutein in the microcapsules is very low. Furthermore, lutein can easily fall off from the network structure of microcapsules [17–20]. In the meantime, it will disperse and transfer to the surface of the microcapsules, so its water-solubility and stability can be easily influenced by the external environment. The water-solubility and stability of lutein cannot be obviously improved with those natural gums as wall materials, which limits its application in food industry.

In out previous work for the study of curcumin microcapsule, we find the mixture of gelatin and porous starch as the wall material is a good choice due to their good properties of film-formation, water-solubility, edibility, biodegradation and a tendency to form a network upon drying [19, 20]. Porous starch is a kind of denaturated starch, which can be obtained from the raw starch catalyzed by amylases below the gelation temperature. Because of its honeycomb structure, porous starch can improve the absorbability and adhesive property of materials. Results of study indicate that microencapsulation using gelatin mixed with porous starch can obviously improve the color staining effect and enhance the stability of curcumin pigment [19, 20].

The objective of this study is to develop a spray-drying method and establish an optimal technology for the preparation of lutein microcapsules in the new carrier mixture using gelatin mixed with porous starch as wall materials for the first time. Furthermore, the physicochemical properties of the powders in the new carrier mixture were evaluated to provide some potential useful parameters for encapsulation of lutein in food industry.

Materials and methods

Wall and core materials

Wall materials used here included edible gelatin (purity 96%, G200, Shanghai Chemical Reagent Corporation, China) and porous starch (purity 98%, Chongqing Taiwei ecoagriculture Ltd., China).

Core materials used here were lutein samples (purity 80%, Hangzhou Lvtian Biotechnology Ltd., China).

Emulsification process

Gelatin and porous starch (mass ratio, 1/8) were mixed and dissolved in 60 °C hot distilled water with stirring to form an aqueous solution. Lutein sample (mass ratio of core to wall material, M_c/M_w, 1/10, 1/20 and 1/30), preheated to dissolve in ethanol (solution concentration was 10%) and emulsified with food grade soybean phospholipid (SPY type, purity 60%, Changsha Yama Biotechnology Co., Ltd., China), was dripped into the gelatin and starch solution to form a coarse emulsion at the following conditions (shown in Table 1): embedding temperature, T_{et}, 50, 60 and 70 °C; embedding time, t_{et}, 1, 1.5 and 2 h. All the experiments were repeated three times, the data were calculated as means.

Spray-drying process

The spray-drying of emulsion was carried out on a Model YC-105 Spray Dryer (Pilotech Instrument & Equipment Co., Ltd., Shanghai, China) equipped with a spray-drying chamber with dimensions of 150 cm height and 80 cm diameter, a cyclone separator, plus a hot air blower and a exhaust blower. For the Model YC-105 Spray Dryer, outlet gas temperature is not a controllable parameter. The emulsion was then fed into the spray dryer at the following conditions (shown in Table 2): feed flow rate of microencapsulating composition, W_{ff}, 50, 60 and 70 mL/min; inlet

Table 1 Orthogonal design for the optimization of lutein microencapsulation

Number	T_{et} (°C)	M_c/M_w	T_{et} (h)	EE (%)
1	(−1) 50	(−1) 1:10	(0) 1.5	62.0 ± 1.2
2	(−1) 50	(0) 1:20	(−1) 1	76.3 ± 2.7
3	(−1) 50	(1) 1:30	(1) 2	85.2 ± 0.5
4	(0) 60	(−1) 1:10	(−1) 1	90.2 ± 1.1
5	(0) 60	(0) 1:20	(1) 2	92.7 ± 0.7
6	(0) 60	(1) 1:30	(0) 1.5	94.4 ± 0.4
7	(1) 70	(−1) 1:10	(1) 2	70.1 ± 2.3
8	(1) 70	(0) 1:20	(0) 1.5	79.5 ± 1.4
9	(1) 70	(1) 1:30	(−1) 1	81.2 ± 3.2
K_{-1} (%)	223.5 ± 1.8	222.3 ± 0.7	247.7 ± 3.2	
K_0 (%)	277.3 ± 0.9	248.3 ± 2.5	235.9 ± 2.6	
K_1 (%)	230.8 ± 2.1	260.8 ± 1.7	248.0 ± 1.5	
R (%)	53.8 ± 1.6	38.5 ± 1.8	12.1 ± 2.4	

K_{-1} the total EE of each factor in its first level, K_0 the total EE of each factor in its second level, K_1 the total EE of each factor in its third level, R the range EE of each factor in its each level

Table 2 Orthogonal design for the optimization of spray-drying operation conditions

Number	T_{gi} (°C)	W_{ff} (ml/min)	W_{af} (m^3/h)	Y (%)
1	(−1) 170	(−1) 50	(−1) 40	79.2 ± 2.5
2	(−1) 170	(0) 60	(0) 50	76.0 ± 2.1
3	(−1) 170	(1) 70	(1) 60	78.1 ± 1.5
4	(0) 180	(−1) 50	(0) 50	84.4 ± 3.3
5	(0) 180	(0) 60	(1) 60	82.9 ± 0.9
6	(0) 180	(1) 70	(−1) 40	79.8 ± 4.0
7	(1) 190	(−1) 50	(1) 60	92.6 ± 1.7
8	(1) 190	(0) 60	(−1) 40	86.3 ± 2.2
9	(1) 190	(1) 70	(0) 50	88.0 ± 1.8
K_{-1} (%)	233.3 ± 3.7	256.2 ± 3.1	245.3 ± 4.3	
K_0 (%)	247.1 ± 4.5	245.2 ± 2.8	248.4 ± 2.1	
K_1 (%)	266.9 ± 3.3	245.9 ± 2.3	253.6 ± 3.7	
R (%)	33.6 ± 3.8	11.0 ± 2.6	8.3 ± 3.2	

K_{-1} the total Y of each factor in its first level, K_0 the total Y of each factor in its second level, K_1 the total Y of each factor in its third level, R the range Y of each factor in its each level

gas temperature, T_{gi}, 170, 180 and 190 °C; drying air flow, W_{af}, 40, 50 and 60 m^3/h.

Samples of the particles were collected during the experiments.

The quantification of lutein microcapsule

One hundred mg lutein microcapsule was ultrasound dissolved in 5 mL purified water and was made up to 100 mL constant volume with absolute ethyl alcohol. After it was diluted 50 times, its absorbance was determined by a Model 752 UV spectrophotometry (Shanghai Jinghua Instrument Ltd., China) at $\lambda_{max} = 445$ nm with ethanol as the blank control [1]. The content of lutein was calculated according the Eq. 1:

$$C = \frac{A_{445} \times 5,000}{2,550 \times M} \times 100\% \quad (1)$$

where M refers to the mass of sample, while A_{445} refers to the absorbance value at $\lambda_{max} = 445$ nm. The value 2,550 is the extinction coefficient of lutein in alcohol, the value 5,000 is the total dilution.

The encapsulation efficiency (EE)

The encapsulation efficiency is an important indicator for microencapsulated particles. The EE was calculated as the following Eq. 2:

$$\mathrm{EE}(\%) = \frac{C}{M_t} \times 100\% \quad (2)$$

where M_t refers to total added lutein mass and C refers to the content of lutein in the microcapsule.

The yield of product obtained by spray-drying (Y)

The yield of product is the ratio between the final mass of microcapsules solids after spray-drying (M_a) and the initial mass of raw microcapsules solids (M_b), as Eq. 3:

$$Y = \frac{M_a}{M_b} \times 100\% \quad (3)$$

The solubility evaluation of lutein microcapsule

The encapsulated lutein solubility in water was determined as follows: 10 mg lutein microcapsule was added in 50 mL water at room temperature, and the mixture was gently stirred with the magnetic stirrer, the time for complete dissolution of lutein microcapsule was recorded visually [1, 19].

The experiment was carried out with free lutein in the liquid form as a blank control.

The stability evaluation of lutein microcapsule

To evaluate the stability of lutein, the retention rate (R) of the lutein microcapsule was determined under different condition such as pH, temperature, time, light and oxygen. The active ingredient is within the shell material, and the net structure of encapsulation controlled the release of lutein. If the lutein microcapsule is dealt with treatments, a little lutein will release from the encapsulation, and the content of lutein will change. So the retention rates of the lutein microcapsule are calculated according to the Eq. 4:

$$R(\%) = \frac{C_a}{C_b} \times 100\% \quad (4)$$

where C_b refers to the content of lutein in the sample before treatments, while C_a refers to the content of lutein in the sample after treatments.

All samples of the solutions were prepared with lutein microcapsule dissolved only in water (1% w/v). Experiments were carried out with free lutein in the liquid form as blank controls.

Experiments were repeated three times, and the data were calculated as means.

Temperature stability

Ten mL solutions were heated for 10 min at a certain temperature, T_h, 0, 40, 50, 60, 70, 80, 90 and 100 °C, respectively. The absorbance values were determined at $\lambda_{max} = 445$ nm, and the retention rates of lutein then could be obtained.

Ten mL solutions were kept at 100 °C for a certain heating time, t_{ht}, 10, 20, 30, 40, 50 and 60 min, respectively. Absorbance values were also determined and their retention rates of lutein then could be obtained.

pH stability

Ten mL solutions at room temperature for 1 h were tested at pH 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 and 11, respectively. The different pH could be obtained with 1 mol/L chlorohydric acid or 1 mol/L sodium hydroxide solution added in the solutions. Absorbance values were determined at 445 nm and their retention rates of lutein then could be obtained.

Light stability

One hundred mL solutions at pH 7 were exposed in daylight at room temperature for certain days (illumination time), t_i, 0, 5, 10, 15, 20, 25 and 30 day. Absorbance values were determined and their retention rates of lutein then could be obtained.

Oxygen stability

One hundred mL solutions at pH 7 were exposed in high oxygen (oxygen content was 70%) at room temperature for certain times, 0, 2, 4, 6, 8 and 10 h. Absorbance values were determined and their retention rates of lutein then could be obtained.

Statistical analysis

All the experiments were repeated three times each with three replicates (three fingers for one replicate). The data were expressed as means ± standard deviations of triplicate determinations ($n = 3\times3$). One-way analysis of variance (ANOVA) and Duncan's multiple range test (DMRT) were carried out to assess the significance of the differences between means using Statistical Analysis System software. The significance of level was set at $p < 0.05$.

Results and discussion

The technology of lutein encapsulation

For establishing the technology of lutein encapsulation, the optimization of encapsulation pretreatment and spray-drying process was necessary.

The best of EE was (94.4 ± 0.4)% when the encapsulation pretreatment condition was as following: T_{et}, 60 °C; M_c/M_w, 1/30; t_{et}, 1.5 h (shown in Table 1). Along with the increase in the embedding temperature from 50 to 60 °C, the encapsulation efficiency increased. While the embedding temperature was from 60 to 70 °C, the encapsulation efficiency decreased. At the 0.05 level, $p < 0.05$, the EE were significantly different. The embedding temperature was the major factor which determined the density of lutein microcapsules.

Mass ratio of core to wall material was also the relative important factor. Microencapsulation using gelatin mixed with porous starch can enhance the encapsulation efficiency of curcumin [19, 20]. In this study, porous starch had the same function. Because of its honeycomb structure, porous starch could improve the absorbability and adhesive property of material; furthermore, it had good emulsibility, which improved the emulsification of soybean phospholipid. They could emulsify core and wall material and made all material well dispersed, so the encapsulation efficiency of lutein might be improved [14, 19–21].

For embedding time, if it was short, lutein could not be completely encapsulated; if it was long, it would cause unnecessary economic loss, although it might be beneficial for the complete encapsulation, so the embedding time (t_{et}, 1.5 h) was the optimal time.

The best of Y was (92.6 ± 1.7)% when the spray-drying operation condition was $T_{gi} = 190$ °C, $W_{ff} = 50$ mL/min and $W_{af} = 60$ m^3/h (shown in Table 2). At the 0.05 level, $p < 0.05$, the Y were significantly different. It was obvious that the best important factor was inlet gas temperature. With the increasing of inlet gas temperature, the volatile material on the surface of encapsulation lutein (such as water and ethanol) could be volatilized, and its content decreased; furthermore, the content of solid matter (such as lutein, edible gelatin and porous starch) fed into the Spray Dryer was increased, which contributed to the yield of product [11, 22]. However, if the inlet gas temperature was very high, lutein would lost its activity, so 190 °C was the optimal temperature.

The technology of lutein encapsulation was then established. When the optimal condition was as follows: the ratio of core to wall material of 1/30, embedding temperature of 60 °C, embedding time 1.5 h, inlet gas temperature of 190 °C, feed flow rate 50 mL/min and drying air flow 60 m^3/h, the lutein microcapsules had good encapsulation efficiency (94.4 ± 0.4)% and high yield of product (92.6 ± 1.7)%.

The solubility of lutein microcapsule

In the water-solubility experiment, free lutein could not be dissolved with water as solvent at room temperature while encapsulated lutein could be dissolved immediately after 120 s. There was no deposit in the solution, whose color and luster of solution were vivid and transparent.

Porous starch improved the absorbability and adhesive property of material. It made all material well dispersed, so the solubility of encapsulation lutein might be improved. It showed when encapsulated lutein turned into powders after spray-drying process, the solubility of encapsulated lutein was improved.

Shell and net structures were formed when the wall materials (edible gelatin and porous starch) were dealt with spray-drying and then the core material (lutein) was entrapped in net structures [11, 14, 19–22]. At the optimal of encapsulation condition, lutein could be well emulsified and dispersed, and the diameter of net structures could also be well controlled, so uniform particles were formed. When particles after atomizing passed through gaseous medium, spherical particles were produced and oil phase was embedded in aqueous phase at this moment. The moisture in aqueous phase evaporated through the heating zone of spray dryer and then its envelope turned into the hydrophilic wall film [19–22], so the solubility of encapsulated lutein increased.

Temperature stability

Effects of heating temperature for 10 min (as the example) on the stability of free lutein, lutein microcapsule before and after spray-drying were shown in Fig. 1.

For three forms of lutein, when heating temperature was below 70 °C, the effect of heating temperature on the lutein stability was not obvious. Along with increasing temperature, the lutein retention rate of free lutein fell off rapidly while those of lutein microcapsule before and after spray-drying declined tardily. Compared with the retention rate values variation of lutein at the temperature from 0 to 100 °C, the reducing ratio of retention rate value for free lutein was 6.2%, while these for lutein microcapsule before and after spray-drying were 1.2 and 0.8%, respectively. At the 0.05 level, $p < 0.05$, the retention rate of lutein for lutein microcapsule after spray-drying were significantly different from others.

Effects of heating time on the stability of free lutein, lutein microcapsule before and after spray-drying at 100 °C were shown in Fig. 2. For lutein at this temperature, along with the increase in heating time, the lutein retention rate of free lutein decreased rapidly while those of lutein microcapsule before and after spray-drying changed less. Compared with their retention rate values variation of lutein from 0 to 60 min, the reducing ratio of retention rate for free lutein was 54.4%, while those of lutein microcapsule before and after spray-drying were 14.3 and 7.5% respectively. At the 0.05 level, $p < 0.05$, the retention rate of lutein for lutein microcapsule after spray-drying were significantly different from others, so it was tested that lutein microcapsule after spray-drying had best temperature stability.

When lutein was embedded in net structures using gelatin mixed with porous starch as wall material, its lutein retention rate had been greatly improved about 46.9% than that of free lutein, and lutein microcapsule especially after spray-drying had a better heat resistance stability.

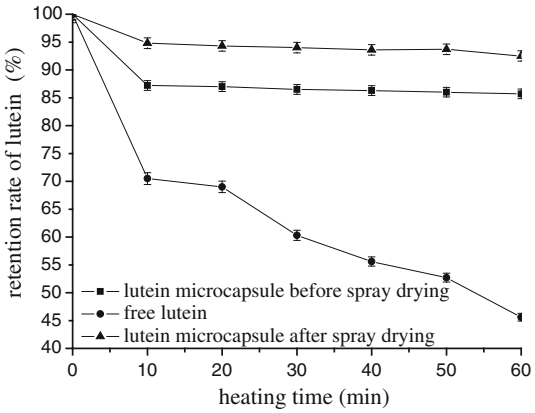

Fig. 2 Effect of heating time on lutein stability at temperature 100 °C

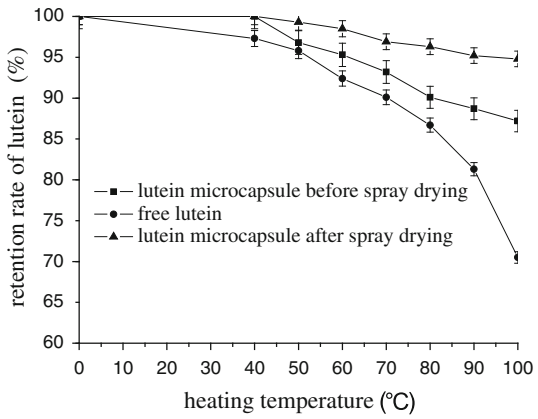

Fig. 1 Effect of heating temperature on lutein stability

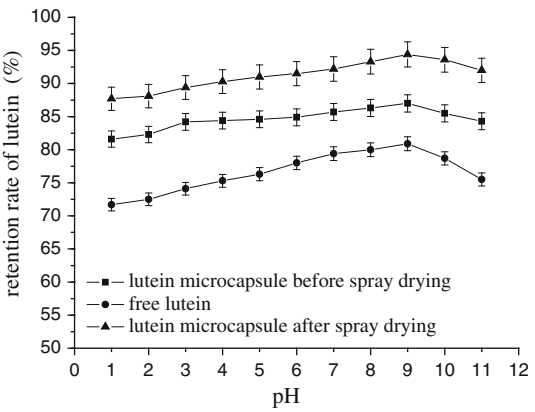

Fig. 3 Effect of pH on acid and alkali fastness stability of lutein

pH stability

Effects of pH on the stability of free and lutein microcapsule before and after spray-drying were shown in Fig. 3.

Along with increasing of pH, the retention rates of lutein in the three samples increased initially and then decreased. From pH 1 to pH 9, their retention rates increased, while from pH 9 to pH 11, their rates decreased. Their retention rates of lutein in the three samples were relatively better from pH 5 to pH 9, and their stability was relatively lower under high acid and alkali conditions.

The retention rates of the lutein microcapsule before and after spray-drying were higher than that of free lutein. From pH 1 to pH 11, the retention rates of the lutein microcapsule before and after spray-drying were about 85 and 90%, while that of free lutein was about 75%. At the 0.05 level, $p < 0.05$, the retention rate of lutein for lutein microcapsule after spray-drying was significantly different from others, and its lutein retention rate had been improved about 15% than that of free lutein, so it showed that lutein microcapsule after spray-drying had best pH stability Microencapsulation of lutein especially after spray-drying was well embedded in net structures [11, 14, 19–22], so it had better acid and alkali fastness stability, and spray-drying further improved its compactness and the acid and alkali fastness stability of lutein.

Light stability

Effects of illumination on the stability of free lutein, lutein microcapsule before and after spray-drying were shown in Fig. 4.

When their solutions were exposed to light, orange color was stable. From 1 to 5 days, the lutein retention rate value of free lutein changed less. Along with increase in the illumination time from 5 to 30 days, the retention rate values of free lutein decreased rapidly. The whole reducing ratio from 1 to 30 days was about 42.7%, while those of lutein microcapsule before and after spray-drying were minor, which were 17.7 and 10.5%. At the 0.05 level, $p < 0.05$, the retention rate of lutein for lutein microcapsule after spray-drying was significantly different.

Microencapsulation of lutein also had better stability to illumination for a long times, especially lutein microcapsule after spray-drying had best light resistance stability, and the lutein retention rate had been improved about 32.2% than that of free lutein.

Oxygen stability

Effects of oxygen on the stability of free lutein, lutein microcapsule before and after spray-drying were shown in Fig. 5.

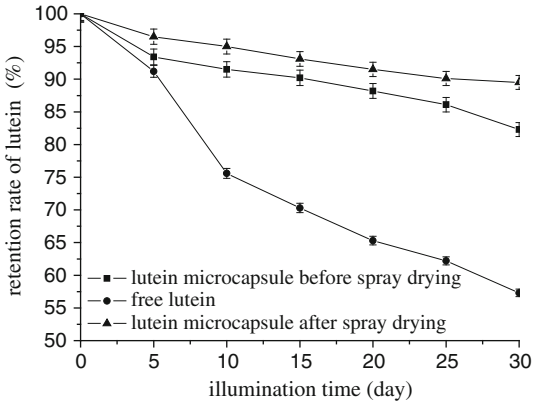

Fig. 4 Effect of illumination on light stability of lutein

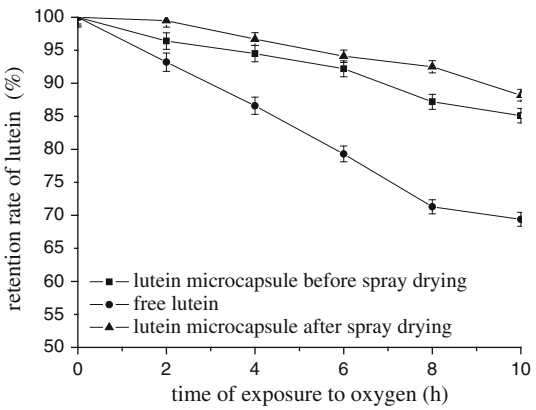

Fig. 5 Effect of oxygen on antioxidant stability of lutein

When the three forms of lutein were exposed in oxygen for a short time, their retention rates of lutein were relatively stable. While after 2 h, the rates cut down obviously. Up to 10 h, the rate of free lutein decreased to 69.4%, while the rates of lutein microcapsule before and after spray-drying were 85.1 and 88.2%, respectively. At the 0.05 level, $p < 0.05$, the retention rate of lutein for lutein microcapsule after spray-drying were significantly different from the others. So it showed microencapsulation could well reduce the effect of oxygen on lutein. When lutein microcapsule was after spray-drying, its oxygen stability was further improved and its lutein retention rate had been improved about 18.8% than that of free lutein.

Conclusion

Lutein microcapsules in the new carrier mixture were well produced using a wall system consisting of gelatin and porous starch for the first time. Compared with the wall material of single natural gum, such as gelatin, algin, protein, cyclodextrin, maltodextrin and chitosan, the mixture of gelatin and porous starch could further improve the

efficiency and stability of lutein microcapsules. Porous starch improved the absorbability and adhesive property of material, and made all material well dispersed, which was due to its good properties of film-formation, honeycomb structure, water-solubility, high absorbability and a tendency to form a network upon drying. The dense mesh structure of lutein microcapsules was then formed when the wall material and the lutein were dealt with spray-drying. Results of the study indicated, when lutein embedded in net structures using the mixture of gelatin and porous starch as wall material, its solubility increased and it could be directly dissolved in water. The stability of lutein microcapsule in the new carrier mixture against heat, pH, light and oxygen was also greatly improved and their retention rates had been improved about 15–50% than that of free lutein.

The technology of lutein encapsulation by spray-drying method using gelatin mixed with porous starch as wall materials was successfully established for the first time. EE and Y were significantly affected by embedding temperature, the ratio of core to wall materials and inlet gas temperature. When the optimal condition was as follows: the ratio of core to wall material of 1/30, embedding temperature of 60 °C, embedding time 1.5 h, inlet gas temperature of 190 °C, feed flow rate 50 mL/min and drying air flow 60 m^3/h, the lutein microcapsules had good encapsulation efficiency (94.4 ± 0.4)% and high yield of product (92.6 ± 1.7)%. This study would be helpful to the application of lutein in food industry.

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