Preparation and application of freeze-dried astaxanthin nanoemulsion powder with high stability

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Abstract

Background: Astaxanthin has been widely used in food, aquaculture, cosmetics and pharmacy industries for its good capability as antioxidant and coloring agent. However, the currently commercial products of astaxanthin can not meet the needs of diversified applications, due to its drawback of low water solubility and chemical instability. This study aims to prepare astaxanthin nanoemulsion lyophilized powder, further improve the stability of astaxanthin nanoemulsion, and extend the shelf life of the product.

Methods: In this paper, a high-stability freeze-dried astaxanthin nanoemulsion powder was prepared by the low-energy emulsification method and freeze-drying technology. By observing the appearance, particle size change and dissolution time of freeze-dried astaxanthin nanoemulsion, the type and content of the freeze-dried protective agent were determined. And by measuring the retention rate of astaxanthin, the storage stability of freeze-dried astaxanthin nanoemulsion powder was studied.

Results: The astaxanthin nanoemulsion freeze-dried powder has smooth surface, low viscosity, fast reconstitution rate, and has good protective effect on nanostructures, more importantly, it has high stability under the conditions of light and 40℃. The astaxanthin freeze-dried powder was stored for 6 months under the conditions of light, 40℃&60%R.H, and its retention rate was more than 60.10%. Under the same storage conditions, astaxanthin in nanoemulsion was inactivated.

Conclusion: The lyophilized powder prepared by the process is expected to become a new formulation of astaxanthin, which can greatly improve its chemical stability.

Keywords: Astaxanthin; Nanoemulsion; Freezing-drying; Stability.

Introduction.

Astaxanthin, a type of carotenoid, exhibits a stronger antioxidant activity than vitamin E and β-carotene[1,2]. It is widely used in medical treatment, cosmetics, health food and other industries[3]. However, the utilization of astaxanthin is currently limited due to its low bioavailability, poor water-solubility and instability under adverse conditions (such as acidic environment, heat, light, etc.)[4-6].

At present, traditional astaxanthin oil and soft capsule products still occupy the majority of the market, but astaxanthin formulations are becoming more and more abundant, such as micro/nano carriers, which improve the water solubility of astaxanthin[7,8]. At the same time, the micro/nano carrier can overcome the keratin barrier and enhance the permeability of active ingredient. However, it also has certain disadvantages, such as particle aggregation may still occur during long-term storage, which shortens the shelf life of astaxanthin products. And liquid products are prone to leakage and bacterial infection during transportation.

Therefore, this study aims to prepare freeze-dried astaxanthin nanoemulsion powder by freeze-drying technology, further improve the stability of astaxanthin nanoemulsion, and extend the shelf life of the product.

Materials and Methods.

1. Materials

Astaxanthin, Butylated Hydroxytoluene, Diethylhexyl Syringmalonate, Caprylic Caprate Triglyceride, Glycerin, Polyoxyethylene castor oil, Glycine, Sodium dihydrogen phosphate, Disodium phosphate, Protein, Mannitol, Carboxymethyl deacetylated chitosan, Tween-20, Trehalose, Pullulan, Soluble collagen, Collagen, Mannan.

2. Equipments

Freeze Dryer, Analytical Balances, Magnetic stirrer, Malvern particle sizer, UV-Vis Spectrophotometer.

3. Methods

3.1. Preparation of astaxanthin nanoemulsion

Astaxanthin, butylated hydroxytoluene, diethylhexyl syringylmalonate & caprylic acid capric triglyceride, glycerin, polyoxyethylene castor oil were accurately weighed and mixed uniformly as oil phase. Slowly add pure water to the oil phase to form astaxanthin nanoemulsion, which is a red transparent liquid.

3.2. Preparation of astaxanthin nanoemulsion lyophilized powder

The freeze-drying protective agent was sequentially added to the astaxanthin nanoemulsion, stirred evenly, and then placed in a freeze-drying machine for vacuum freeze-drying. After the samples were lyophilized, cap the bottle and observe the appearance.

3.3. Investigation of redispersibility of lyophilized powder

The original volume of deionized water was accurately added to the vial, the time was started after the stopper was added, and the time was stopped by shaking quickly until the solution was clear.

3.4. Particle size, polydispersity index (PDI) analysis

The lyophilized powder samples with good reconstitution properties were selected, and the particle size, PDI of all formulations were measured by Nano ZS90. The required sample volume is 1mL, the measurement temperature is 25℃, and the equilibration time is 60s.

3.5. The effect of the type of lyoprotectant on the lyophilized powder

The type of lyoprotectant directly affects the freeze-drying effect of the sample. In this study, mannitol, trehalose, carboxymethyl deacetylated chitosan, pullulan, hydrolyzed collagen, a mixture of collagen and mannan were selected as the lyoprotectant. The lyophilized powder was prepared as described in “3.2.”. The astaxanthin nanoemulsion without any protective agent was used as a control. After the samples were lyophilized, the appearance was observed and the redispersibility was tested.

3.6. Effect of lyoprotectant concentration on lyophilized powder

This study mainly explored the effects of mannitol and carboxymethyl deacetylated chitosan concentrations on the appearance, particle size and PDI of freeze-dried powder. The prepared freeze-dried powder was re-dispersed in aqueous solution to measure the particle size, and the optimal concentration of the protective agent was screened by the

particle size change of the nano-suspension after re-dispersion and when it was just prepared.

3.7. Chemical stability analysis

The astaxanthin nanoemulsion freeze-dried powder was stored under the conditions of light, 40℃ & 60%R.H for 6 months, and the retention rate of astaxanthin was determined by ultraviolet spectrophotometry regularly, and the astaxanthin nanoemulsion was used as a control.

3.8. Determination of astaxanthin retention

Refer to the method of Hong, L[9] for determination.

4. Statistical analyses

All measurements were carried out in triplicate and were expressed as mean±standard deviation.

Results.

1. The effect of lyophilized protective agent type on lyophilized powder

The addition of lyoprotectant is crucial to the formation of lyophilized powder. According to Table 1, a mixture of 5% mannitol and 0.3% carboxymethyl deacetylated chitosan as lyophilized protectant had a better protective effect. The freeze-dried powder with trehalose, pullulan and hydrolyzed collagen as protective agents had rough surface, and all appeared collapse, shrinkage and loose structure. When 2.5% trehalose and 2.5% pullulan were used as protective agents, cracks appeared on the surface of freeze-dried powder. When 3.25% collagen and 1.75% mannan were used as protective agents, the color of the lyophilized powder was uneven and the surface was rough. In conclusion, the mixture of mannitol and carboxymethyl deacetylated chitosan was selected as the lyoprotectant for subsequent experiments.

Table1 Effect of lyophilized protective agent on lyophilized powder.

Lyophilized protective agent	Sample appearance				Dissolution time(s)
	Color	Surface	Collapse/ shrink	Structural Density	
Free of lyoprotectant	Freeze-dried powder not formed				--
5% Mannitol	Orange	Rough	None	Dense structure	2
5% Trehalose	Uneven color	Rough	Have	Loose structure	--
5% Pullulan	Orange	Bulge	Have	Loose structure	--
2.5% Trehalose, 2.5% Pullulan	Orange	Crack	Have	Loose structure	--
5% Soluble collagen	Orange	Crystalline substance	Have	Loose structure	--
3.25% Collagen, 1.75% Mannitol	Uneven color	Rough	Have	Loose structure	--
5% Mannitol, 0.3% Carboxymethyl deacetylated chitosan	Orange	Smooth	None	Dense structure	5~7

Note: Due to the poor shape of some lyophilized powders, the reconstitution time was not measured.

2. Influence of concentration of lyophilized protectant on lyophilized powder

2.1. Effects of different concentrations of carboxymethyl deacetylated chitosan on freeze-dried powder

The concentration of mannitol was set at 3.6%, and the particle size and PDI of lyophilized powder after redissolution were used as screening indexes to explore the effects of different concentrations of carboxymethyl deacetylated chitosan on lyophilized powder, and the nanoemulsion before lyophilization was used as control. As shown in Fig.1, compared with the particle size of astaxanthin nanoemulsion just prepared, the particle size of samples with lyophilized protectants increased, which was attributed to the increase of viscosity of solution with the increase of carboxymethyl deacetylated chitosan, which may have an impact on the particle size of nanoparticles.

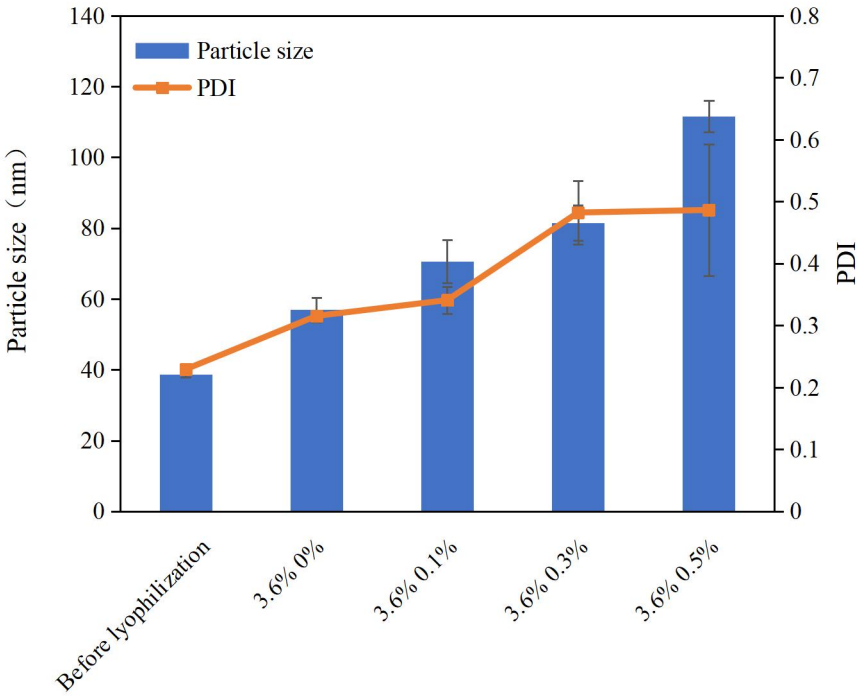

Fig.1 Effects of different concentrations of carboxymethyl deacetylated chitosan on freeze-dried powder.

Note: 3.6% represents the content of mannitol, 0.3% indicates the content of carboxymethyl deacetylated chitosan.

2.2. The effect of different concentrations of mannitol on freeze-dried powder

The concentration of carboxymethyl deacetylated chitosan was set at 0.3%, and the particle size and PDI of lyophilized powder after redissolution were used as screening indexes to explore the effects of different concentrations of mannitol on lyophilized powder, and samples before lyophilization were used as control. As can be seen from Fig.2, compared with the size of astaxanthin nanoemulsion just prepared, the size of samples added with lyophilized protectant increased. The size of samples added with 5% mannitol and 0.3% carboxymethyl deacetylated chitosan had the smallest change, with a particle size of 45.78±5.53 nm and PDI of 0.31±0.01.

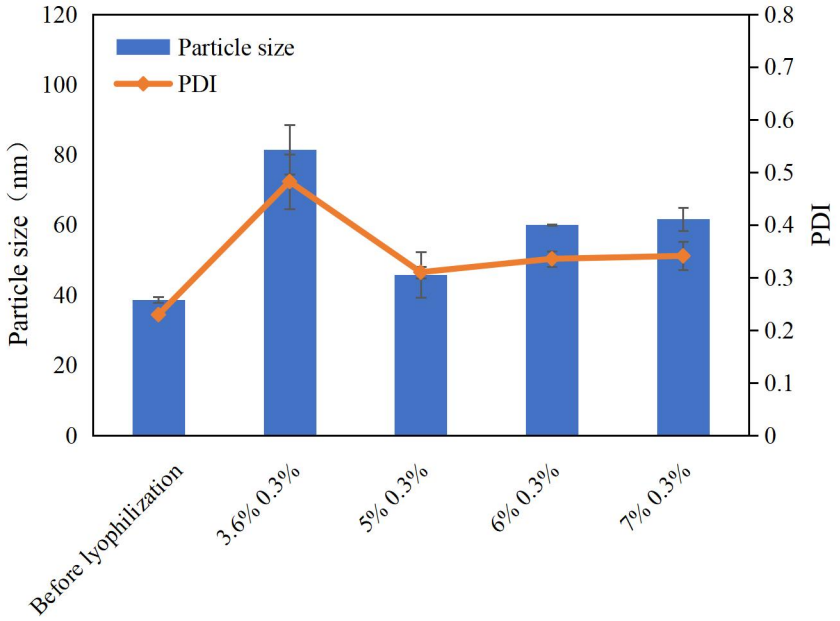

Fig.2 Effect of mannitol on freeze-dried powder.

Note: 3.6%,5%,6% and 7% represent different amounts of mannitol, 0.3% represents the content of carboxymethyl deacetylated chitosan.

3. Appearance and redispersibility of lyophilized powder

The appearance of the astaxanthin nanoemulsion freeze-dried powder is shown in Fig.3A. The freeze-dried sample has no shrinkage or collapse, and is an orange powder with smooth surface and certain strength. Add the same volume of deionized water as before lyophilization to the lyophilized sample, shake it manually, it can be completely dispersed within 5~7s and become a clear and transparent red liquid (Fig.3B), with good redispersibility, and the particle size after reconstitution was 45.78±5.53 nm, and the PDI was 0.31±0.01 (Fig.4).

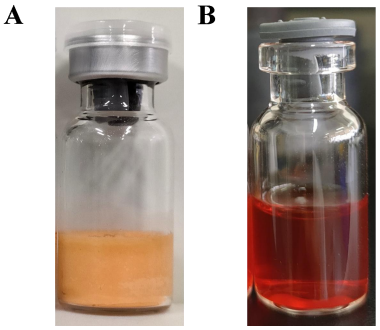

Fig.3 Astaxanthin nanoemulsion freeze-dried powder (A), redissolved astaxanthin nanoemulsion freeze-dried powder (B).

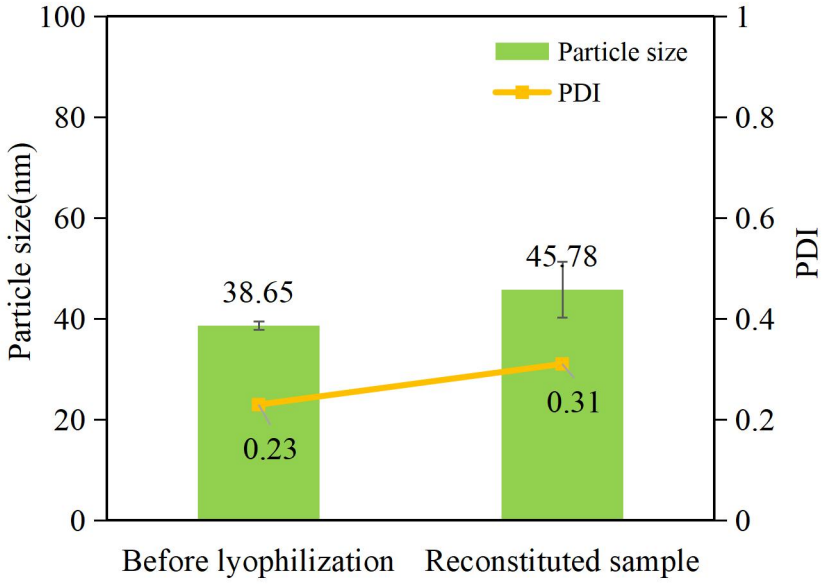

Fig.4 Changes of particle size and PDI of the prepared astaxanthin nanoemulsion before freeze-drying and after reconstitution.

4. Chemical stability analysis

In order to determine the chemical stability of astaxanthin nanoemulsion freeze-dried powder, it was stored in natural light, 40℃ & 60% R.H for 6 months, and the astaxanthin retention rate was measured regularly. The results were shown in Fig.5 and Fig.6. The astaxanthin nanoemulsion freeze-dried powder prepared in this paper still has good stability under the conditions of light, 40℃ & 60% R.H. The astaxanthin nanoemulsion was stored under the condition of light or 40℃, 60%R.H for one month, and the astaxanthin retention rate was zero; while the astaxanthin nanoemulsion freeze-dried powder was stored under the light condition for 6 months, and the retention rate was 68.45%; stored at 40℃, 60% R.H for 6 months, the retention rate was 60.10%, and the retention rate of astaxanthin in freeze-dried powder was significantly improved.

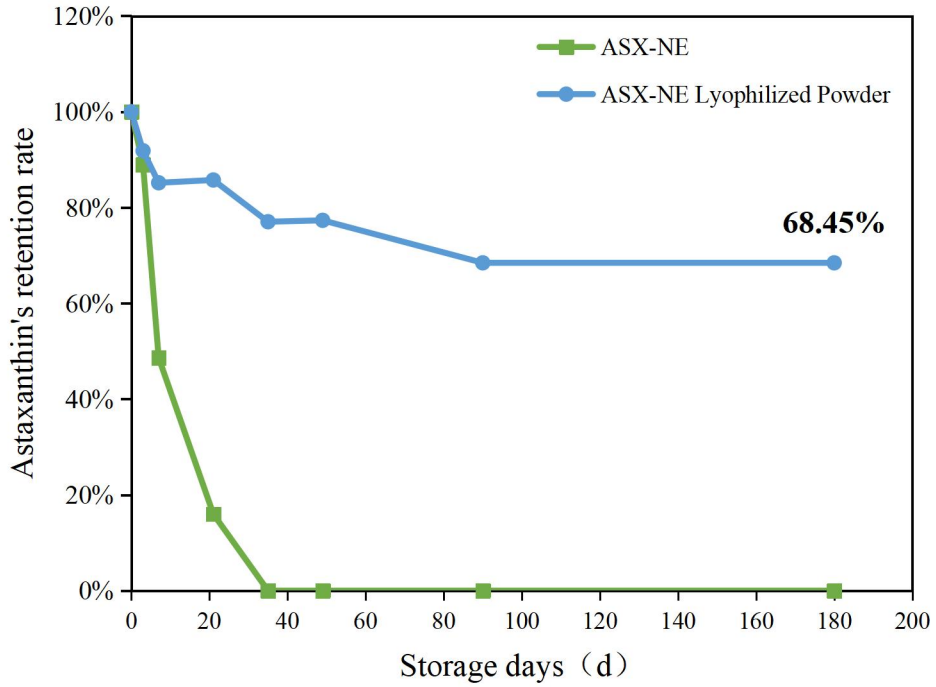

Fig.5 Stability of astaxanthin nanoemulsion and its freeze-dried powder stored under light conditions for 6 months

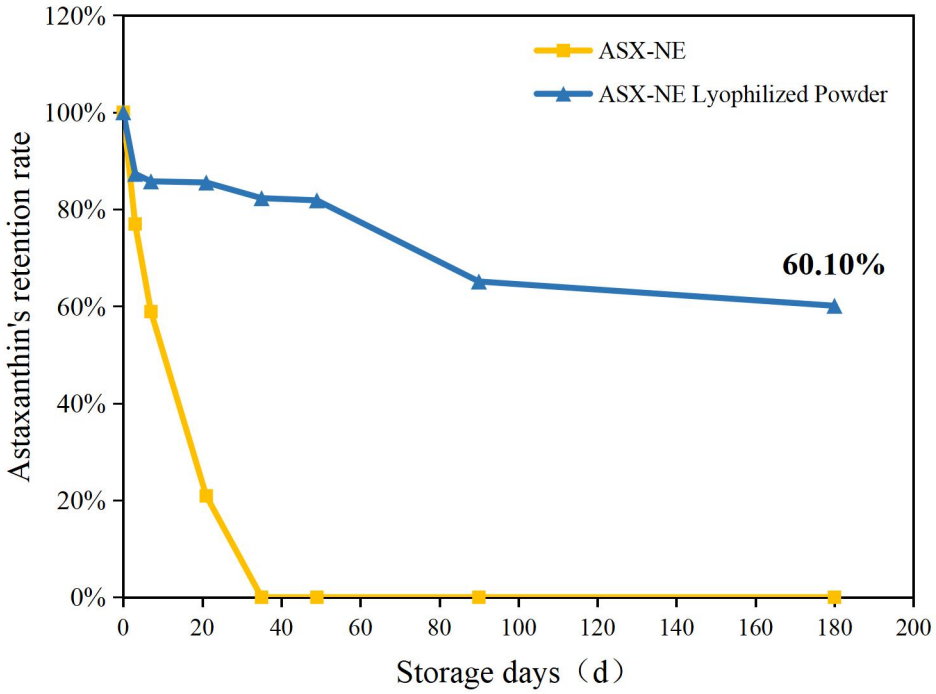

Fig.6 Stability of astaxanthin nanoemulsion and its freeze-dried powder stored at 40℃, 60%R.H for 6 months

Discussion.

In order to improve the water solubility and chemical stability of astaxanthin, in this study, astaxanthin nanoemulsion freeze-dried powder was prepared by low-energy emulsification and freeze-drying technology. During the freeze-drying process of nanoemulsion, adding a freeze-drying protective agent can effectively improve its stability. Different types of protective agents have different protective effects on nanoemulsions. Saccharide protective agents can prevent the nanoemulsion from being damaged by ice crystals during the freeze-drying process; alcohol-based protective agents can obtain lyophilized formulations with full appearance, and the combination of different types of freeze-drying protective agents can achieve a more ideal freeze-drying effect.

Table1 shows that the lyophilized powder has a good appearance when mannitol is used as protective agent, and the combination of mannitol and carboxymethyl deacetylated chitosan is the best: smooth surface, no collapse, no shrinkage, and compact structure. Probably because saccharide protective agents can form a glass state during dehydration. In this state, the viscosity of the system is high and the molecular diffusion coefficient is low. Therefore, the glassy saccharide are around the biomolecules, which hinders the movement of the biomolecules and inhibits the stretching and aggregation of the molecules, thereby maintaining the stability of their structures[10].

The effect of lyophilization changes when the amount of lyophilization protectant is changed. According to Fig.2, the particle size of the nanoemulsion before lyophilization was 38.65±0.79 nm, and the PDI was 0.229±0.004. The concentration of carboxymethyl deacetylated chitosan remained unchanged, when the concentration of mannitol was 3.6%, the particle size of the reconstituted lyophilized powder dispersion increased to 81.45±7.02 nm, and the PDI increased to 0.482±0.052. Due to the low content of mannitol, the structure of the nanoemulsion was not protected. With the increase of mannitol content, the change in particle size of freeze-dried astaxanthin nanoemulsion decreases, indicating that mannitol has a better protective effect, and plays an excellent supporting and protective role in the freeze-dried solution, effectively avoiding the agglomeration between particles [11].

When the content of mannitol increased to 6% and 7%, the particle size of the lyophilized powder redispersed liquid increased slightly, indicating that the concentration of the protective agent should not be too high. This may be because excessive mannitol

forms crystals[12], and the structure of nanoparticles is destroyed[13], which promotes the aggregation and fusion of nanoparticles, thereby increasing the particle size. Therefore, in this paper, 5% mannitol and 0.3% carboxymethyl deacetylated chitosan were selected as the freeze-drying protective agent to prepare astaxanthin nanoemulsion freeze-dried powder.

The stability of astaxanthin is affected by environmental factors, such as light, temperature, oxygen, etc. The degradation rates of astaxanthin under UV and sunlight irradiation were 13 times and 7.9 times higher than those under dark conditions, respectively [14]. The experimental results in Fig.5, Fig.6 show that under the conditions of light, high temperature and high humidity, the retention rate of astaxanthin nanoemulsion decreased significantly within 7 days, and dropped to zero after one month. The retention rate of astaxanthin in astaxanthin nanoemulsion freeze-dried powder was still as high as 77.03% (light) and 82.28% (40 ℃, 60% R.H).

On the one hand, freeze-drying technology improves the stability of the active ingredient by reducing the oxygen concentration of the product, and the freeze-dried powder avoids the exposure of astaxanthin to adverse environmental factors, which is beneficial for extending shelf life. Studies have shown that the stability of astaxanthin can be improved by selecting packaging materials with good shading and low oxygen permeability[14,15], or by introducing nitrogen into the packaging bottle to protect astaxanthin[16].

On the other hand, it may be due to the compact structure of the freeze-dried powder after adding the mannitol and carboxymethyl deacetylated chitosan, which can reduce the leakage of astaxanthin in the preservation process. Moreover, the amount of sunlight shining into the interior of the freeze-dried powder is reduced, and the degradation rate of astaxanthin is more effectively reduced.

According to Fig.6, the freeze-dried powder still showed good stability under the storage condition of 40℃ and 60% R.H, and the retention rate of astaxanthin after 6 months was 60.10%. The reason is that mannitol has no hygroscopicity and is used as an additive to make the product less susceptible to moisture, thus showing good protection at 60.10% humidity.

Conclusion.

In this paper, astaxanthin nanoemulsion freeze-dried powder was prepared by low energy emulsification and freeze-drying technology. The effect of protective agent type and concentration on freeze-dried powder was investigated by taking the appearance of freeze-dried powder, particle size and PDI after redissolution as indexes. The optimal ratio was as follows: 5% mannitol, 0.3% carboxymethyl deacetylated chitosan. The lyophilized powder prepared in this study had good redispersibility in water, the particle size after reconstitution was 45.78±5.53, and the PDI was 0.31±0.01. The retention rate of astaxanthin for 6 months was determined by ultraviolet spectrophotometry, and the astaxanthin nanoemulsion was used as a control. The results show that the freeze-dried powder can significantly improve the retention rate of astaxanthin under the conditions of light and 40℃&60% R.H. Meanwhile, it is convenient to transport and store, and no preservatives are added, which greatly reduces skin irritation, and is expected to become a new dosage form of astaxanthin.

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Conflict of Interest Statement.

NONE.

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