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

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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 & Methods:

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.

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. 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.

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.

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 "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.

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.

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.

8. Determination of astaxanthin retention

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

Results & Discussion:

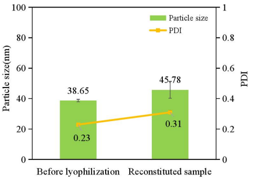
In order to improve the water solubility and chemical stability of astaxanthin, in this study, astaxanthin nanoemulsion freeze-dried powder was prepared by low-energy emulsification and freeze-drying technology.

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. 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. 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.

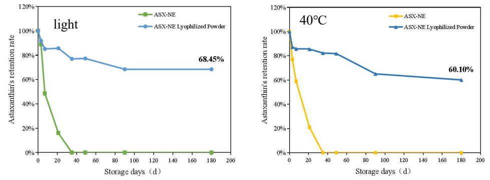
In general, the astaxanthin nanoemulsion freeze-dried powder has smooth surface, low viscosity, fast reconstitution rate, and has good protective effect on nanostructures, more importantly, it has high stability under the conditions of light and 40℃. Therefore, the freeze-dried powder is expected to become a new dosage form of astaxanthin.

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



Particle size and PDI



Stability of astaxanthin nanoemulsion and its freeze-dried powder stored under light, 40℃ conditions for 6 months

Conclusions:

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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