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아스타잔틴을 포함하는 나노에멀젼의 안정성 향상을 위한 신규 Glyceryl Ester 이용 캡슐화

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Novel Encapsulation with New Glyceryl Ester Vesicle Enhances Stability of Nanoemulsion Containing Astaxanthin

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요 약: 아스타잔틴을 포함하는 수중유형 형태의 나노에멀젼을 고압균질기를 이용하여 제조하였다. 이에 유화조건, 유화제 종류, 유화제 농도 그리고 아스타잔틴 농도에 따라 최적화를 되었다. 나노에멀젼의 안정성은 제타포텐셜, FF-SEM, 입도분석기, 색차계를 이용하여 측정하였다. 제조된 아스타잔틴 나노에멀젼의 입도는 160 ~ 190 nm로 균일하였으며 레시친에 의한 나노에멀젼 보다 glyceryl citrate/lactate/linoleate/oleate에 의한 나노에멀젼이 더욱 안정하고 균일한 입도분포를 가졌다. 아스타잔틴의 봉입도는 HPLC, FF-SEM을 이용하여 확인 하였으며, 제형 구성 후 보관조건에서의 안정도 및 제타 포텐셜 값도 −41의 우수한 결과를 나타내었다.

Abstract: Oil in water nanoemulsion of astaxanthin was prepared by high pressure homogenization. The emulsifying conditions including emulsifier type, concentration and astaxanthin concentration were optimized. Stability of nanoemulsion was measured using zetasizer, freeze-fracture scanning electron microscope (FF-SEM), particle analyzer and colorimeter. The mean diameter of the dispersed particles containing astaxanthin ranged from 160 to 190 nm. Size distribution was unimodal and extended from 40 to 200 nm. The nanoemulsion prepared by glyceryl citrate/lactate/linoleate/oleate had smaller particle size and narrow size distribution. Stable incorporation of astaxanthin in nanoemulsion was performed and checked using high performance liquid chromatography (HPLC), freeze-fracture scanning electron microscope (FF-SEM). Physical stability of nanoemulsion was not significantly changed during storage at both light and thermal condition for a month with zeta potential value of -41 mV meaning stable colloid.

Keywords: *nanoemulsion, astaxanthin, high pressure homogenation, zeta potential*

1. Introduction

In recent years, researchers have further shown that carotenoids posses certain health benefiting functions which might be helpful in the prevention in health disorders[1]. Especially in cosmetic industry, butylated hydroxyl toluene (BHT), acerora and tocopherol are the conventionally used antioxidant for improving formulation stability and durability. Astaxanthin (3, 3'-dihydroxy-β-β'-carotene-4-4'-dione) is a ketocarotenoid, used as a preferred pigment in aqua-culture feeds. Due to high antioxidant activity[2,3], it can be used as a potential prophylactic agent against skin cancer and a possible chemopreventive agent. Despite the availability of syn-

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Table 1. Prescription of Formulation for Astaxanthin Nanoemulsion

Phase	Ingredients	% by weight
Lipid	Ethanol	10
	Ceramide	0.2
	Cholesterol	0.3
	Co-antioxidant	1.0
	Oil	2.0
Aqueous	Emulsifier (glyceryl citrate/lactate/linoleate/oleate, hydrogenated lecithin)	3.0
	Glycerin	2.5
	Tween60	0.3
	Water	To 100
	Astaxanthin from *Hamatococcus Pluvialis*	q.s

thetic astaxanthin, astaxanthin from natural sources still received more interest due to its greater antioxidant activity and stability[4].

However, like most carotenoids, astaxanthin is a highly unsaturated molecule and thus it is highly sensitive to high temperature, light, and oxidative conditions which may promote the isomerization of astaxanthin into cis form which possesses less activity than their corresponding trans configuration[5]. In additions, genericity of use in the food and cosmetic field are not much enough because of following reasons. Low solubility, color change and Photostability are of that[6]. One of the approaches that can be used to improve the solubility and bioavailability of carotenoids such as β-carotene is to incorporate them in the fine particles of oil-in water (O/W) emulsions. But systemic study with active incorporation into a nanoemulsion using carotenoids especially, astaxanthin, has not been proposed. Besides, physicochemical influence of emulsifier concentration, homogenation condition, temperature as well as variety of co-antioxidants on astaxanthin incorporated nanoemulsion was not examined.

Furthermore, the effect of astaxanthin nano emulsion during storage which is very important parameter of emulsions has not been studied thoroughly.

Therefore, we report the stability evaluation of astaxanthin nanoemulsion prepared by high pressure homogenization under various conditions using new vesicle, glyceryl citrate/lactate/linoleate/oleate and it is thought be very useful for cosmetic industry.

2. Materials & Experiments

2.1. Extraction of astaxanthin

Astaxanthin were collected from *Hamatococcus pulvialis* (Honghao Chemical Co., Ltd, China). The astaxanthin was specially extracted with a continuous-flow super critical-carbon dioxide extraction apparatus (Lab SFE, Supercritical labs, Korea).

2.2. Preparation of astaxanthin nanoemulsion

Oil-in-water (O/W) nanoemulsion prepared using liquid paraffin (seojin chemical, Korea), Ceramide (Doosan biotech, Korea), cholesterol as dispersed phase and glyceryl citrate/lactate/linoleate/oleate (Imwitor 375, Sasol, Germany), ester of mono- and diglycerides of unsaturated edible fatty acids with citric acid and lactic acid, hydrogenated lecithin (Lipoid 100 - 3, Lipoid GMBH, Germany), polysorbate 60 (Croda Korea, Korea), ethanol (Korea alcohol, Korea), propylene glycol (SKC Chem, Korea), deionized water as continuous phase. Prescription of formulation for astaxanthin nanoemulsion was mentioned on table 1. The continuous and dispersed phases were each heated to 70 ~ 75 ℃ and then mixed to form pre-mix. The pre-mix was then homogenized using ho-

Table 2. Condition of Elution

Time (min)	% solvent A	% solvent B	% solvent C	Flow (mL/min)
0.0	81	15	4	1.0
15.0	66	30	4	1.0
23.0	16	80	4	1.0
27.0	16	80	4	1.0
35.0	81	15	4	1.0

Calculation of Astaxanthin contents as follow.
Contents of Astaxanthin (mg/g) = [(S × a × b × 1,000)/[sample weight (mg) × 1,000]
S : Solution concentration (mg/mL)
a : Total amount of test solution (mL)
b : Dilution factor

momixer (T.K Homo mixer, Osaka, Japan) for 5 min to pre-emulsify, and then, high-pressure homogenization (Microfluidics, Panda, Italy) was performed at 1,000 bar.

2.3. Analysis of astaxanthin concentration

Standard astaxanthin and astaxanthin in the extract were analyzed by a high-performance liquid chromatography (HPLC, Agilent 1,200, and Japan) series, equipped with a diode array detector. The standard and extract solutions were diluted with dichloromethane/methanol (1 : 3, v/v) and injected through the auto sample and separated with a reversed-phase C18-YMCA Carotenoid column (5 μm, 4.6 · 250 mm) at 25 ℃. Gradient elution was performed. The detection wavelength was kept at 474 nm. The gradient elution condition was shown in table 2.

2.4. Identification and analytical study of particle size and distribution

The average particle size and size distribution of nanoemulsion were determined by dynamic light scattering using Mastersizer (Mastersizer 2,000 Hydro, Malvern Instrument, and Worcestershire, UK). The particle size of the emulsions was described by mean diameter and size distribution was described by volume per diameter and size distribution graph .

2.5. Evaluation of emulsion stability

The emulsion stability of the nano emulsion was evaluated using particle size analyzer, zeta potential (Zetasizer 3000HS, Malvern, UK), change of C, E value were carried out by colorimeter (Croma Meter CR200, Minolta, Japan), the nanoemulsions were sampled immediately after the preparation and divided into three groups which store at 5 ℃, Cycle (-5 ℃ ~ 45 ℃), for four weeks, Light irradiated by suntester (Suntest CPS$^+$, Atlas, USA)

Stability of nanoemulsion was evaluated according to the potential difference by zetasizer as well as change of size and color for certain period of time.

The zeta potential was calculated from the following equation:

$$\zeta = \mu U_e / \varepsilon_d \quad (1)$$

(ζ = zeta potential, μ = viscosity of solution, U_e = electronflowrate, ε_d = permittivity).

Zeta potential is a scientific term for electro kinetic potential in colloidal systems. Zeta potential is electric potential in the interfacial double layer at the location of the slipping plane versus a point in the bulk fluid away from the interface. The significance of zeta potential is that its value can be related to the stability of colloidal dispersions. When the potential is low, attraction exceeds repulsion and the dispersion will break and flocculate. So, colloids with high zeta potential (approximately over ± 40) are electrically stabilized whereas colloids with low zeta potentials tend to coagulate or flocculate.

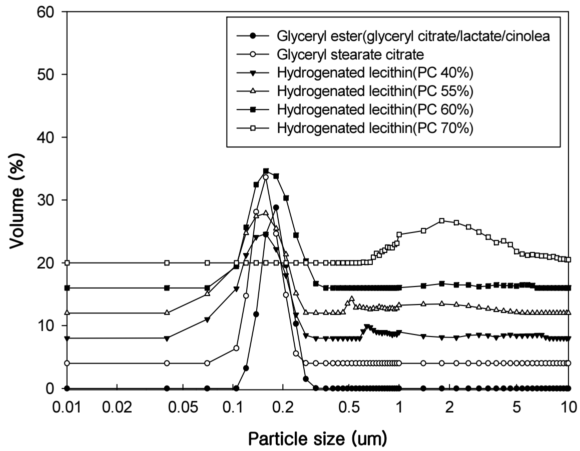

Figure 1. Particle size distribution on vesicle.

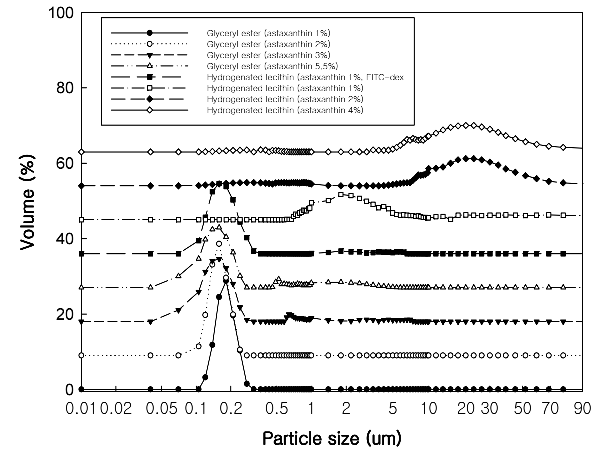

Figure 2. Particle size distribution on astaxanthin concentration.

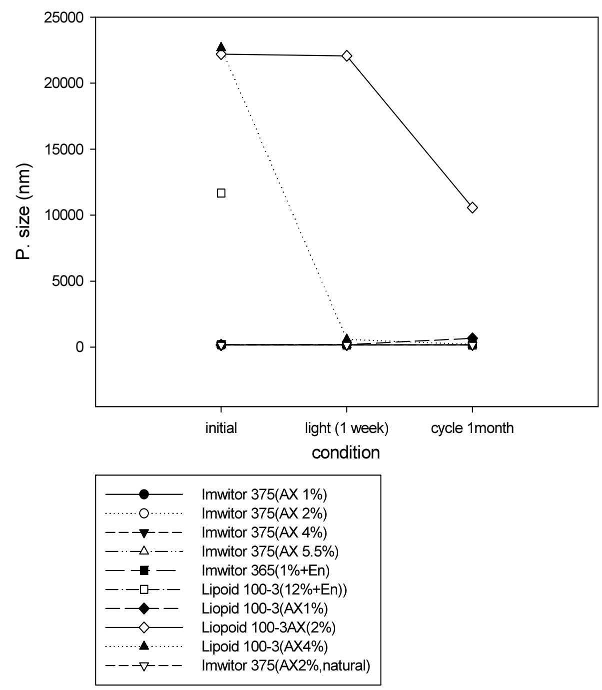

Figure 3. Particle size distribution on astaxanthin concentration under storage condition.

2.6. Encapsulation morphology by electron microscopy

The measurement of astaxanthin incorporation status was carried out by freeze-fracture scanning electron microscope (FF-SEM, JEM1400, and Jeol, Japan) and transmission electron microscope (TEM, H-7600, and Hitachi, Japan). A small amount of sample was immersed into liquid ethane which was immediately frozen in liquid nitrogen, and morphology checked using FF-SEM with propane gas.

3. Results & Discussion

3.1. Effect of the emulsifier type and homogenization pass times on the particle size parameter of nanoemulsion

Unimodal nanoemulsion sized about 170 nm was observed when used glyceryl citrate/lactate/linoleate/oleate as an emulsifier. but broad and larger size distribution of nanoemulsion was observed in the use of lecithin which may has much contents of phosphatidyl cholin.

3.2. Effect of the astaxanthin extract concentration on the particle size parameter in the astaxanthin nano emulsion

Astaxanthin incorporated nanoemulsion was investigated according to the amount of astaxanthin concentration. Particle distribution on different vesicles between new vesicle, glyceryl citrate/lactate/linoleate/oleate and normal hydrogenated lecithin was studied. Tendency for unimodal particle size distribution was observed in the use of new vesicle, glyceryl citrate/lactate/linoleate/oleate compared to that of hydrogenated lecithin. Size distribution rate dependant on concentration from 1 to 5.5%

(a) (b) (c) (d) (e)

Figure 4. Comparison of the clarity of astaxanthin emulsions made with glyceryl ester, with increasing astaxanthin concentration. (a) 1%, (b) 2%, (c) 4%, (d) 5.5%, (e) Astaxanthin 1% + FITC Dextran.

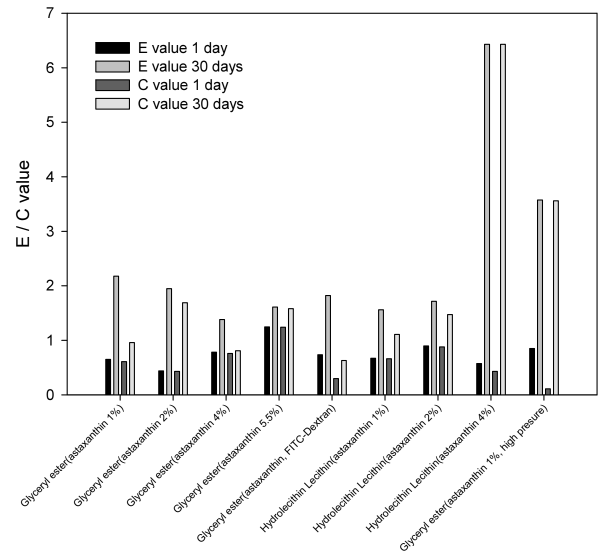

Figure 5. color change of astaxanthin nanoemulsion on vesicle concentration under storage condition.

and change of size distribution after storage under light and thermal condition were observed in figure 2 and 3. Also unimodal particle size can also make diluted solution transparent even though incorporated maximum 5.5% in Figure 4.

3.3. Observation of color change

Colour change valued as E and C were also observed in Figure 5, The degree of which depended on the concentration of astaxanthin. Figure 5 shows the colour change of astaxanthin nanoemulsion under the light, and heat. Also it shows the result after the storage at cycle condition from 5 to 45 ℃. The nanoemulsions with glyceryl ester tended to show good colour stability with concentration of astaxanthin in the range of 1 ~ 4%. However, the nanoemulsion with hydrogenated lecithin shows serious colour change with concentration of astaxanthin at 2% or higher.

3.4. Detection of astaxanthin in the nanoemulsion by HPLC

The results from Table 3 show that the astaxanthin content of the nanoemulsions remained at > 90% after these physical stresses indicating the robustness of the nanoemulsion's ability to retain the incorporated astaxanthin under typical potentially destabilizing physical stresses.

3.5. Observation of zeta potential

One of the methods frequently used in the cosmetic stability is measurement of potential difference called ze-

Table 3. Analytical Data of Astaxanthin

	Solution concentration (ug/mL)	Sampleweight (mg)	Total amount of test solution	Contents of astaxanthin (mg/g)	%
Nanoemulsion (initial)	1,710	884.1	50	0.1934	100
Nanoemulsion (Sun light irradiated, 1 week)	1.637	975.4	50	0.1678	86.7
Nanoemulsion (Cycle test, 1 month)	1.510	799.1	50	0.1890	97.8

Table 4. Zeta Potential Measurement of The Nanoemulsions on Vesicle under Storage Condition

	Zeta potential (mV)		
	Initial	(1 month)	Light irradiation (12 hrs)
Glyceryl/Lactate/Linoleate/Oleate (astaxanthin 4%)	-52.6	-78.9	-48.1
Glyceryl/Lactate/Linoleate/Oleate (Astaxanthin 5.5%)	-47.2	-52.5	-48.7
Hydrogenated lecithin (Astaxanthin 4%)	-56.8	-47.7	-36.1

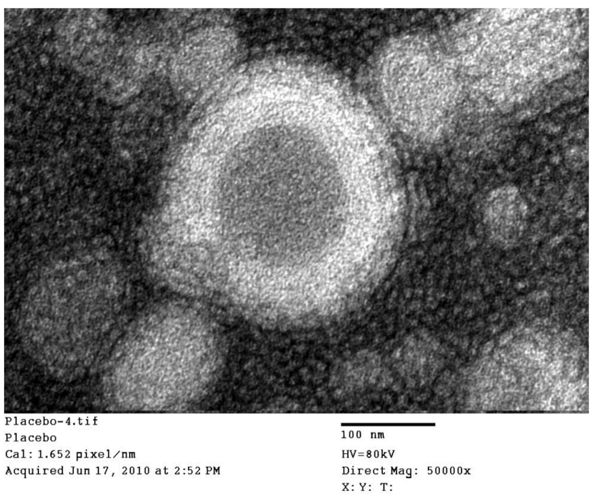

Figure 6. FF-SEM of astaxanthin contains nanoemulsion in glyceryl citrate/lactate/linoleate/oleate.

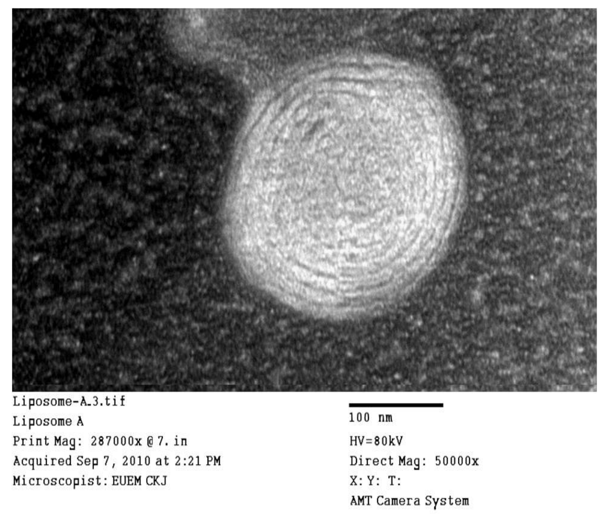

Figure 7. FF-SEM of astaxanthin contains nanoemulsion in hydrogenated lecithin.

ta potential. Zeta potential was measured for stability check of nanoemulsion. Stable astaxanthin incorporation was predicted by figure valued over -10 mV of zeta potential. Zeta potential (mV) value was measured initial -47.2 mV to −52.5 mV after 1months, even measured -48.7 mV after light irradiation for 12 hours. But as it is shown in Table 4, lecithin derived nanoemulsion was found increase of particle size which means unstable.

3.6. Nano emulsion morphology by FF–SEM

The FF-SEM observation of the astaxanthin nanoemulsions was made immediately after the emulsion was prepared. Resultant micrographs are presented in the Figure 5 and 6. Particles appear spherical or multi-layer shape which is able to check well incorporation of astaxanthin as you see in the Figure 6 but different result was observed on the nanoemulsion made by hydrogenated lecithin shown in Figure 7.

4. Conclusion

In this study, new vesicle, glyceryl citrate/lactate/linoleate/oleate, was used as emulsifier instead of using conventional hydrogenated lecithin originated from bean or egg. New vesicle also helps emulsion stabilization by forming surface layer strong resulting in better stabilization of astaxanthin incorporated nanoemulsion, transparency and emulsion stability against storage condition. Nanoemulsion prepared in this study was sized about 160 ~190 nm and -40 mV for zeta potential. Especially, combination of ethyl alcohol makes the emulsion easy penetration when spreading on the skin by increasing flexibility and intended for convenient use with low viscosity emulsion. Due to the superior property against oxidation, astaxanthin easily causes color change, strange odor by light and heat. In terms of using cosmetic industry, using lots of astaxanthin causes sticky texture too. By integrated investigation of astaxanthin in this study it is said that it carries weight with resolving above problem of astaxanthin.

In this study, we enhanced nanoemulsion with astaxanthin showed excellent light and thermal stability on the new vesicle. We anticipate that this study provides cosmetic industry the way how astaxanthin can be used as an active cosmeceutical ingredient in skin care production having the properties of anti-wrinkle, anti-aging and h2umectancy.

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