

## Formulation and Characterization of Nanoserum Combination of Carrot (*Daucus carota* L.) Extract and Astaxanthin as Antioxidant

Tiara Bella Pratiwi, Arif Budi Setianto\*, Nuri Ari Efiana, Sri Mulyaningsih

Department of Pharmacy, Faculty of Pharmacy, Universitas Ahmad Dahlan, Jl. Prof. Dr. Soepomo, S.H., Umbulharjo, Yogyakarta, Daerah Istimewa Yogyakarta, 55164, Indonesia

\*Corresponding author: Arif Budi Setianto ([arif.setianto@pharm.uad.ac.id](mailto:arif.setianto@pharm.uad.ac.id))

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### Abstract

Lipophilic antioxidants such as  $\beta$ -carotene and astaxanthin have strong potential to protect the skin against oxidative stress; however, their topical application is limited by low stability and insufficient skin penetration. This study aimed to formulate and evaluate the physical characteristics, stability, and antioxidant activity of a nanoserum combining carrot extract (*Daucus carota* L.) and astaxanthin. Carrot extract was obtained by maceration using 96% ethanol and formulated into a nanoemulsion-based serum containing three extract concentrations: 5% (F1), 10% (F2), and 15% (F3), with 1% astaxanthin. The formulations were evaluated for organoleptic properties, particle size, polydispersity index (PDI), zeta potential, pH, viscosity, and adhesiveness. Stability was observed up to 6 days, while antioxidant activity was determined using ABTS and FRAP assays. All formulations exhibited nanoscale particle sizes, good homogeneity (PDI < 0.5), and a negative zeta potential (< -30 mV), indicating stable dispersions. Formula F2 showed the most favorable characteristics, with a particle size of  $39,21 \pm 0,32$  nm, PDI of  $0,25 \pm 0,02$ , and zeta potential of  $-33,66 \pm 0,27$  mV, along with suitable pH and viscosity for topical application. F2 demonstrated very strong antioxidant activity, with  $IC_{50}$  values of  $9,04 \pm 0,19$  ppm (ABTS) and  $16,78 \pm 0,04$  ppm (FRAP), which remained in the strong category after storage. In conclusion, the carrot extract–astaxanthin nanoserum was successfully developed, with F2 identified as the optimal formulation.

**Keywords:** antioxidant, astaxanthin, carrot, nanoserum, stability

### Introduction

Excessive sun exposure can increase free radical production, triggering oxidative stress in the skin and contributing to premature aging, hyperpigmentation, and cellular damage. Antioxidants play a crucial role in neutralizing free radicals and maintaining healthy skin. As public interest in the use of natural ingredients in cosmetic products increases, studies on natural antioxidant sources continue to expand. Safety is also a key concern, given that some synthetic antioxidants have been reported to potentially cause nephrotoxicity through oxidative stress and toxic accumulation, with long-term exposure associated with an increased risk of chronic kidney disease.<sup>1-3</sup>

Carrots (*Daucus carota* L.) are a source of natural antioxidants rich in  $\beta$ -carotene, vitamin C, flavonoids, and phenolic compounds that contribute to free radical scavenging activity and prevent premature aging. Various studies have shown that carrot extract exhibits significant antioxidant activity, including in lip balm formulations ( $IC_{50} = 10.47$  ppm<sup>4</sup>), lotion formulations ( $IC_{50} = 95.42$  ppm<sup>5</sup>), and face mist formulations ( $IC_{50} =$  very

strong at various concentrations).<sup>6</sup> In addition to carrots, astaxanthin is a natural carotenoid pigment with very high antioxidant activity, reported to be 50–100 times stronger than vitamin E. Astaxanthin is non-prooxidant and effective in protecting the skin from free radicals caused by exposure to ultraviolet radiation.<sup>7</sup> The combination of astaxanthin and carrot extract in this formulation was intended to enhance antioxidant activity through the synergistic effect of both active compounds, as they possess different free radical scavenging mechanisms. This combination was expected to improve the effectiveness and stability of the nanoserum as a topical antioxidant preparation with better protection against free radical-induced skin damage.

Although various cosmetic preparations based on carrot extract have been developed, their effectiveness is still limited due to the relatively large molecular size, which hinders penetration into the skin layers. Therefore, a nanoemulsion-based nanoserum system was developed to improve the penetration and effectiveness of the active ingredients. A nanoserum has a very small particle size, which increases surface area and optimizes absorption.<sup>8</sup> Nanoemulsion technology also allows for better system stability and faster and more effective interaction with biological targets.<sup>9</sup> Several studies have reported that nanoemulsion technology can improve the solubility of lipophilic compounds by reducing particle size and increasing surface area. Nanoemulsion systems can enhance the dispersion and stability of poorly water-soluble compounds by forming nanosized droplets stabilized by surfactants and co-surfactants. Choi and McClements reported that nanoemulsion-based delivery systems improved the water dispersibility and stability of hydrophobic bioactive compounds, including carotenoids and other lipophilic antioxidants.<sup>10</sup> Therefore, this study aimed to develop and evaluate a nanoemulsion-based nanoserum formulation combining carrot extract and astaxanthin as active antioxidant ingredients.

## Method

### Instrument

The instruments used included a magnetic stirrer, oven (Binder), particle size and zeta potential analyzer (PSA) (Malvern), pH meter (Ohaus), UV–Vis spectrophotometer/microplate reader (ELISA reader), water bath (Mettler), viscometer (Rheosys), micropipette, transparent cuvette, vortex mixer, ice bath, and glassware.

### Material

The materials used were pharmaceutical grade, including 96% ethanol p.a. (KimiaARD, Yogyakarta), astaxanthin (Kimia Jaya Abadi, Semarang), niacinamide (Kimia Jaya Abadi, Semarang), vitamin E (Kimia Jaya Abadi, Semarang), jojoba oil (Kimia Jaya Abadi, Semarang), Tween 80 (BY Kimia & Herbal, Yogyakarta), Span 80 (Beli Kimia Jogja, Yogyakarta), sodium dodecyl sulfate (SDS), propylene glycol (BY Kimia & Herbal, Yogyakarta), Viscolam (Beli Kimia Jogja, Yogyakarta), glycerin (Master Sun Chemical, Jakarta), triethanolamine (Master Sun Chemical, Jakarta), DMDM hydantoin (Kimia Jaya Abadi, Semarang), distilled water (Surya Artathama, Yogyakarta), ABTS powder (KimiaARD, Yogyakarta), potassium persulfate ( $K_2S_2O_8$ ) (KimiaARD, Yogyakarta), methanol p.a. (KimiaARD, Yogyakarta), n-hexane p.a. (KimiaARD, Yogyakarta), acetone p.a. (KimiaARD, Yogyakarta), and FRAP reagent consisting of acetate buffer (pH  $\pm$  3.6), TPTZ (KimiaARD, Yogyakarta) in 40 mM HCl, and  $FeCl_3 \cdot 6H_2O$  (KimiaARD, Yogyakarta). Carrot samples used in this study were obtained from Pasar Induk Buah dan Sayur Giwangan (the Giwangan Fruit and Vegetable Wholesale Market) in Yogyakarta. They were processed into simplicia before extraction.

## Procedure

### Carrot Extraction

Extraction was carried out using the maceration method with 96% ethanol for 3 x 24 hours with occasional stirring. The container was covered with aluminum foil. The filtrate was filtered, and the marc was re-macerated with the same solvent. All filtrates were combined and evaporated at 60°C using an evaporator until a thick extract was obtained.

### Extract Evaluation

Organoleptic tests were performed by observing the extract's appearance, color, and odor. Qualitative identification of  $\beta$ -carotene was performed by thin-layer chromatography (TLC) using silica gel 60 F254 as the stationary phase and n-hexane: acetone (6:4, v/v) as the mobile phase. The spots were observed under UV light at 254 nm, and the Rf values were calculated using  $\beta$ -carotene as the reference. The yield was calculated as the percentage of extract weight relative to the initial weight of the simplicia.  $\beta$ -carotene content was determined by preparing a 100 ppm stock solution of the extract in n-hexane, measuring the absorbance at the maximum wavelength, preparing standard solutions (5, 10, 15, 20, and 25 ppm), and calculating the content from the calibration curve.

### Nanoemulsion Formulation and Nanoserum Production

Preparation began by forming a 30 mL nanoemulsion for each formula. The oil phase (jojoba oil, vitamin E, Tween 80, Span 80, sodium dodecyl sulfate (SDS), and lipophilic active ingredients, including astaxanthin and carrot extract, according to the formula) was homogenized until evenly distributed. In contrast, the aqueous phase (niacinamide, propylene glycol, glycerin, DMDM hydantoin, and distilled water) was homogenized separately. The aqueous phase was added dropwise to the oil phase while stirring with a magnetic stirrer at 300 rpm and 60°C until a clear, homogeneous nanoemulsion formed. The mixture was then evaluated for particle size using a particle-size analyzer (PSA), after which Viscolam was added, and the mixture was stirred continuously until a homogeneous nanoserum was obtained. The composition of the nanoserum formulation combining carrot extract and astaxanthin is presented in Table 1.

**Table 1.** Nanoserum Formulation Composition: Combination of Carrot Extract and Astaxanthin

No	Material	Concentration (%)							
		K1	K2	K3	K4	K5	F1	F2	F3
1.	Carrot extract	-	-	-	-	-	5	10	15
2.	Astaxanthin	-	-	1	-	1	1	1	1
3.	Niacinamide	-	5	-	-	5	5	5	5
4.	Vitamin E	-	-	-	5	5	5	5	5
5.	Jojoba oil	5	5	5	5	5	5	5	5
6.	Span 80	4	4	4	4	4	4	4	4
7.	Tween 80	10	10	10	10	10	10	10	10
8.	Sodium dodecyl sulfate	0,01	0,01	0,01	0,01	0,01	0,01	0,01	0,01
9.	Propylene glycol	10	10	10	10	10	10	10	10

**Table 1.** (Extension)

No	Material	Concentration (%)							
		K1	K2	K3	K4	K5	F1	F2	F3
10.	Viscolam	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.
11.	Glycerin	5	5	5	5	5	5	5	5
12.	DMDM hydantoin	0,5	0,5	0,5	0,5	0,5	0,5	0,5	0,5
13.	Aquadest (ad)	100	100	100	100	100	100	100	100

Information:

K1: Nanoserum base without active ingredients

K2: Nanoserum base containing 5% niacinamide

K3: Nanoserum base containing 1% astaxanthin

K4: Nanoserum base containing 5% vitamin E

K5: Nanoserum base containing a combination of 1% astaxanthin and 5% niacinamide

F1: Combination formula containing 5% carrot extract and 1% astaxanthin

F2: Combination formula containing 10% carrot extract and 1% astaxanthin

F3: Combination formula containing 15% carrot extract and 1% astaxanthin

### Observation of Nanoserum Using a Polarizing Microscope

The nanoserum was applied to a glass slide and covered with a coverslip to form a thin layer. The sample was observed using a polarizing microscope at 100x magnification equipped with a camera, with the polarizer and analyzer set in parallel and crossed positions. Observations focused on particle morphology and optical properties, particularly changes in light intensity and the appearance of interference colors as indicators of birefringence. The results were digitally documented.

### Physical Evaluation of Nanoserum

Organoleptic testing assessed the form, color, and odor of the preparation. The pH was measured using a pH meter and adjusted to fall within the skin pH range. Particle size and size distribution were measured using a particle size analyzer (PSA), and homogeneity was expressed as the polydispersity index (PDI). Zeta potential was measured at 25 °C using a zeta potential analyzer to assess the system's tendency to aggregate. Viscosity was measured using a viscometer (Rheosys) at 5 rpm for approximately 30 seconds until the value stabilized, while adhesiveness was measured using the two-glass slide method (0.25 g of sample pressed with a 1 kg load for 5 minutes, followed by recording the release time under an 80 g load).

### Accelerated Stability Testing

Physical stability was evaluated using the heating–cooling cycle method for three cycles, with one cycle consisting of storage at  $4 \pm 2^\circ\text{C}$  for 24 hours followed by  $40 \pm 2^\circ\text{C}$  for 24 hours. After completion of the cycles, physical parameters (particle size, PDI, zeta potential, pH, viscosity, and adhesiveness) and organoleptic changes were observed and compared with the initial conditions.

### ABTS Antioxidant Activity Assay

The ABTS assay was performed by generating  $\text{ABTS}^{\bullet+}$  radicals via the reaction of 7 mM ABTS with 2.45 mM  $\text{K}_2\text{S}_2\text{O}_8$ . The mixture was incubated for 12 hours in the dark until stable radicals were formed. The extract was prepared as a 1,000 ppm stock solution in methanol and then diluted into a series of concentrations. Nanoserum samples were prepared at concentrations of 7.8125, 15.625, 31.25, 62.5, and 125  $\mu\text{g}/\text{mL}$ .

A total of 20  $\mu\text{L}$  of sample was mixed with 200  $\mu\text{L}$  of ABTS<sup>•+</sup> working solution in a microplate and incubated for 30 minutes at room temperature in the dark. The absorbance was measured using a microplate reader at 736 nm. The percentage of radical inhibition was determined by comparing the absorbance of the sample with that of the control, and the IC<sub>50</sub> value was determined from the curve representing the relationship between sample concentration and percentage inhibition.

### FRAP Antioxidant Activity Assay

The FRAP assay was performed using freshly prepared reagents consisting of acetate buffer (pH  $\pm$  3.6), 10 mM TPTZ solution in 40 mM HCl, and 20 mM FeCl<sub>3</sub>·6H<sub>2</sub>O solution. The three solutions were mixed in a specific ratio to obtain the FRAP reagent. Samples at various concentrations were reacted with the FRAP reagent and incubated for 10 minutes at 37°C. The reduction of Fe<sup>3+</sup> to Fe<sup>2+</sup> produced a blue complex, with color intensity proportional to the antioxidant activity of the sample. The absorbance was measured at 593 nm. Antioxidant activity was expressed as reducing ability, and the IC<sub>50</sub> value was determined from the curve relating sample concentration to reduction activity.

### Data Analysis

Data were tested for normality (Kolmogorov–Smirnov test) and homogeneity. The selection of the best formula was analyzed using one-way ANOVA if the assumptions were met. Changes before and after stability testing were analyzed using a paired-sample t-test. If the data did not meet parametric assumptions, appropriate nonparametric tests were applied. All analyses were performed at a 95% confidence level using SPSS version 30.

## Result

### Carrot Extraction

The 96% ethanol extract of carrots obtained was thick and homogeneous. The extract was orange to brownish-orange, indicating the presence of carotenoid pigments. Organoleptically, the extract had a characteristic carrot odor without any rancid or off-odors. An extract yield of 47.81% was obtained from 412 g of simplicia, yielding 197 g of thick extract, as shown in Table 2.

**Table 2.** Percentage (%) Yield of Carrot Extract

Sample Weight (g)	Solvent	Extract Weight (g)	Yield (%)
412	Ethanol 96%	197	47,81

### Extract Characterization

### Phytochemical Screening Test

Phytochemical screening of the carrot extract revealed the presence of secondary metabolites. The complete test results are presented in Table 3.

**Table 3.** Phytochemical Screening Results of Carrot Extract (*Daucus carota* L.)

Compound Groups	Reagent	Results	Information
Flavonoid	Mg–HCl	(+)	Color change observed
Alkaloid	Mayer/Dragendorff	(+)	A brown precipitate formed

**Table 3.** (Extension)

Compound Groups	Reagent	Results	Information
Tannin	FeCl <sub>3</sub>	(-)	No precipitate formed
Saponin	Test the foam	(+)	Stable foam formed

### Thin-Layer Chromatography (TLC) Test of $\beta$ -Carotene

Observations showed spots in the carrot extract with Rf values close to that of the  $\beta$ -carotene standard, indicating the presence of  $\beta$ -carotene in the extract. The spots were clearly visible under UV light at 254 nm. The results are presented in Figure 1, and the Rf values from the TLC analysis of the carrot extract and standard are presented in Table 4.



a b

**Figure 1.** TLC results of  $\beta$ -carotene

Information:

- (a) Carrot extract sample
- (b) Standard

**Table 4.** TLC Results of  $\beta$ -Carotene in Carrot Extract

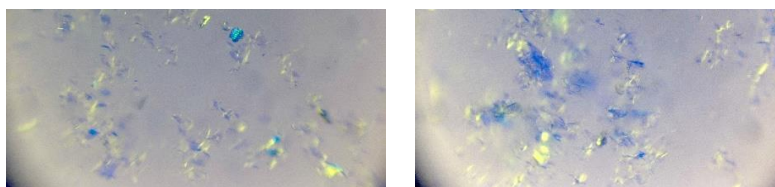
Sample	Spot distance (cm)	Solvent front distance (cm)	Rf	Color
Standard	6,2	8	0,77	Bright yellow-orange
Carrot extract	6,1	8	0,76	Yellow-orange

### Determination of $\beta$ -Carotene Content

The  $\beta$ -carotene determination showed that the extract contained 15.97% (w/w)  $\beta$ -carotene. This value reflects a relatively high content of the main carotenoid. The high  $\beta$ -carotene content indicates that the extraction method using 96% ethanol effectively extracted carotenoids while preserving the stability of the active compounds.

### Results of Nanoserum Observations Using a Polarizing Microscope

The nanoserum was observed under a polarizing microscope to determine the morphology and optical properties of the particles formed. The results of the nanoserum observation using a polarizing microscope are presented in Figure 2.











**Figure 2.** Nanoserum observation using a polarizing microscope at 100x magnification

### Physical Evaluation of Nanoserum

Organoleptically, all nanoserum preparations, including the controls and formulas F1–F3, appeared homogeneous without phase separation upon initial observation. The main difference was observed in color intensity: formulas containing carrot extract exhibited greater intensity than the controls as extract concentration increased. Visual documentation of the controls and formulas F1–F3 is presented in Table 5.

**Table 5.** Visual Documentation and Physical Evaluation of Nanoserum Preparations

				
<b>a</b>	<b>b</b>	<b>c</b>	<b>d</b>	<b>e</b>
				
	<b>f</b>	<b>g</b>	<b>h</b>	

Information:

- (a) Control K1 (nanoserum base)
- (b) Control K2 (nanoserum with niacinamide)
- (c) Control K3 (nanoserum with vitamin E)
- (d) Control K4 (nanoserum with astaxanthin)
- (e) Control K5 (nanoserum without extract)
- (f) Formula F1 (nanoserum with 5% carrot extract)
- (g) Formula F2 (nanoserum with 10% carrot extract)
- (h) Formula F3 (nanoserum with 15% carrot extract)

A summary of all physical evaluation parameters for the controls and formulas is presented in Tables 6 and 7.

**Table 6.** Summary of Physical Evaluation of Nanoserum (Particle Size, PDI, Zeta Potential) (Mean  $\pm$  SD)

Sample	Particle Size (nm)	PDI	Zeta potential (mV)
F1	46,03 $\pm$ 0,27	0,24 $\pm$ 0,01	-30,44 $\pm$ 0,518
F2	39,21 $\pm$ 0,32	0,25 $\pm$ 0,02	-33,66 $\pm$ 0,273
F3	48,68 $\pm$ 0,83	0,28 $\pm$ 0,01	-30,69 $\pm$ 1,003
Base Control	54,31 $\pm$ 0,58	0,37 $\pm$ 0,01	-34,46 $\pm$ 0,304
Niacinamide Control	54,99 $\pm$ 0,78	0,38 $\pm$ 0,003	-32,73 $\pm$ 1,074
Astaxanthin Control	47,57 $\pm$ 2,15	0,32 $\pm$ 0,009	-32,18 $\pm$ 0,194
Vitamin E Control	52,74 $\pm$ 0,71	0,35 $\pm$ 0,01	-25,79 $\pm$ 0,373
Control without Extract	53,63 $\pm$ 1,25	0,35 $\pm$ 0,04	-32,66 $\pm$ 0,964

Based on Table 6, all nanoserum formulations were within the nanoscale range, with formula F2 having the smallest particle size, a PDI value  $<$  0.5, and a zeta potential below  $-30$  mV, indicating good system stability. Based on these characteristics, viscosity, pH, and adhesiveness were subsequently evaluated, as presented in Table 7.

**Table 7.** Summary of Physical Evaluation of Nanoserum (Viscosity, pH, Adhesiveness) (Mean  $\pm$  SD)

Sample	Viscosity (cPs)	pH	Adhesiveness (seconds)
F1	65,35 $\pm$ 0,57	5,38 $\pm$ 0,04	45 $\pm$ 1
F2	69,66 $\pm$ 1,00	5,34 $\pm$ 0,07	51,66 $\pm$ 0,57
F3	76,34 $\pm$ 1,35	5,24 $\pm$ 0,04	58 $\pm$ 1
Base Control	18,25 $\pm$ 0,30	6,34 $\pm$ 0,02	23 $\pm$ 1
Niacinamide Control	29,40 $\pm$ 0,48	6,40 $\pm$ 0,05	34,66 $\pm$ 0,57
Astaxanthin Control	19,27 $\pm$ 0,22	5,36 $\pm$ 0,01	40,33 $\pm$ 1,52
Vitamin E Control	39,43 $\pm$ 0,75	6,42 $\pm$ 0,02	60,66 $\pm$ 0,57
Control without Extract	49,68 $\pm$ 0,62	5,54 $\pm$ 0,26	60,33 $\pm$ 0,57

Based on Table 7, formula F2 showed optimal viscosity, a pH within the skin pH range, and higher adhesiveness compared to the controls, while F1 and F3 remained within the acceptable range. F2 was selected as the optimal formulation, and antioxidant activity testing was continued using several control formulations.

### Antioxidant Activity (ABTS and FRAP)

A summary of the ABTS and FRAP antioxidant activity assay results for the extract, controls, and formulations is presented in Table 8.

**Table 8.** ABTS and FRAP Antioxidant Assay Results (IC<sub>50</sub>, ppm; Mean  $\pm$  SD)

Sample	ABTS	FRAP
Carrot Extract	90,21 $\pm$ 0,13	80,45 $\pm$ 0,15
Quercetin	7,86 $\pm$ 0,02	16,84 $\pm$ 0,08
K1. Base	69,62 $\pm$ 0,31	53,48 $\pm$ 0,73

**Table 8.** (Extension)

Sample	ABTS	FRAP
K2. Niacinamide	34,64 ± 0,18	46,34 ± 0,29
K3. Vitamin E	28,42 ± 0,71	38,89 ± 1,31
K4. Astaxanthin	25,53 ± 0,72	28,97 ± 0,109
K5. Without Extract	20,55 ± 0,14	26,66 ± 0,49
F2 (10%) Day 0	9,04 ± 0,19	16,78 ± 0,04
F2 (10%) Day 6	10,55 ± 0,09	19,86 ± 0,23

Based on Table 8, quercetin and formula F2 showed the highest antioxidant activity, as indicated by the lowest IC<sub>50</sub> values compared to the other samples.

### Heating–Cooling Stability Test

A summary of the stability test results for the physical parameters, organoleptic properties, and antioxidant activity of formula F2 on Day 0 and Day 6 is presented in Table 9.

**Table 9.** Nanoserum F2 Stability Test Results (Day 0 and Day 6)

Parameter	Day 0 (Mean ± SD)	Day 6 (Mean ± SD)
Particle size (nm)	43,51 ± 1,06	55,13 ± 0,09
PDI	0,16 ± 0,01	0,21 ± 0,006
Zeta potential (mV)	–33,97 ± 0,86	–30,31 ± 0,58
Viscosity (cPs)	79,45 ± 0,35	78,42 ± 0,26
pH	5,19 ± 0,01	5,31 ± 0,09
Adhesiveness (seconds)	52,66 ± 1,52	54,00 ± 1,00
Color	Bright red–orange, homogeneous	Red–orange slightly darker/duller
Smell	Mild carrot odor, not pungent	Mild carrot odor, no rancid odor
Texture	Medium liquid–gel, stable, no phase separation	Medium liquid–gel, slightly thicker to thinner, remained stable
ABTS (IC <sub>50</sub> , ppm)	9,04 ± 0,19	10,55 ± 0,09
FRAP (IC <sub>50</sub> , ppm)	16,78 ± 0,04	19,86 ± 0,23

Formula F2 selected for the heating–cooling stability test showed good physical stability until Day 6, with particle size remaining within the nanoscale range, a PDI value < 0.5, and a zeta potential below –30 mV, as presented in Table 9. The parameters of viscosity, pH, adhesiveness, and organoleptic properties did not show significant changes, and antioxidant activity remained within the acceptable range.

### Discussion

Carrot extract obtained by maceration in 96% ethanol exhibited organoleptic characteristics as a thick, homogeneous mass with a distinctive orange-to-brownish-orange color, indicating the presence of carotenoid pigments, especially β-carotene as the dominant component. This distinctive color serves as a visual indicator of carotenoids

that play an important role in antioxidant activity. The absence of any detectable rancid or off-odors indicates that the extraction process maintained the stability of the active compounds without significant oxidative degradation.<sup>11,12</sup> The presence of  $\beta$ -carotene in the extract was also confirmed by thin-layer chromatography (TLC), in which the extract's Rf value was close to that of the  $\beta$ -carotene standard, indicating that the main compound extracted was a lipophilic carotenoid contributing to antioxidant activity.

The formulation results showed that all nanoserum preparations successfully formed a nanoemulsion system with particle sizes in the nanometer range and a relatively homogeneous size distribution. Small particle size is an important characteristic of nanosystems because it is associated with increased surface area, dispersion stability, and the potential for enhanced penetration of active ingredients into the skin layers.<sup>8,9</sup> Formula F2, containing 10% carrot extract, showed the smallest particle size with a polydispersity index (PDI) value  $< 0,5$ , indicating a uniform particle size distribution and a stable dispersion system. The particle size obtained in this study was  $39,21 \pm 0,32$  nm, which remained within the nanosize range and demonstrated good nanoemulsion characteristics. A droplet size of 15,00 nm at a carrot extract concentration of 20% has also been reported in an SNEDDS-based formulation. The difference in particle size may be attributed to variations in the delivery system, surfactant composition, oil phase, and the type and concentration of active compounds used. Nevertheless, the present formulation still exhibited good stability and homogeneity of the nanoemulsion system. The zeta potential values of all formulations were below  $-30$  mV, indicating sufficient electrostatic repulsion between particles to prevent aggregation, thereby supporting the physical stability of the nanoemulsion during storage.<sup>13,14</sup>

The viscosity of the nanoserum increased with increasing carrot extract concentration, which was associated with the greater amount of dispersed solids in the system. Statistical analysis using SPSS showed that F1 and F2 were not significantly different ( $p > 0.05$ ), even as the carrot extract concentration increased from 5% to 10%, indicating that the nanoemulsion system maintained good flow properties and dispersion stability within this concentration range. However, F3 containing 15% carrot extract exhibited a more pronounced increase in viscosity and differed significantly from the other formulations ( $p < 0.05$ ), likely due to enhanced intermolecular interactions and greater flow resistance within the system. Adequate viscosity helps maintain physical stability and increase the preparation's contact time with the skin.<sup>15</sup> The pH values of all formulations were within the physiological skin pH range of approximately 5.2–5.4; therefore, the formulations were considered safe and unlikely to irritate.<sup>16</sup>

Observation of the nanoserum using a polarizing microscope at 100 $\times$  magnification showed variations in light intensity and the appearance of interference colors in several areas of the preparation under both parallel and crossed polarizer–analyzer settings. This phenomenon indicates differences in local refractive indices related to the size, distribution, and orientation of particles in the nanoserum system. The appearance of these interference colors can be associated with weak birefringence properties commonly found in colloidal systems containing anisotropic particles or microaggregates, as reported in studies using polarizing microscopy to characterize nanosystems and dispersed materials.<sup>17,18</sup> Furthermore, irregular light distribution in the microscopic image indicates that, although the preparation is macroscopically homogeneous, variations in particle size or orientation remain at the microscopic level. This finding is consistent with the principle that polarizing microscopy is sensitive to differences in optical properties and particle anisotropy; therefore, areas containing larger particles or aggregates appear more contrasted than areas with finely dispersed particles.<sup>19</sup>

Nanoserum analysis using ImageJ software yielded quantitative data on particle area, intensity, orientation angle, and length. Particle length was used to represent

particle size in polarizing microscope observations, with an average of  $53,049 \pm 14,426$  pixels and a range of 28,636–67,469 pixels, indicating size variation but a relatively uniform distribution. The average particle area of  $54,000 \pm 14,399$  pixels<sup>2</sup> indicates that the particles were dispersed in small sizes without the formation of large aggregates. Random particle orientation, indicated by an average angle value close to zero, is characteristic of a stable nanoemulsion system and supports the particle size measurements obtained using a particle size analyzer (PSA), which indicated system homogeneity.

The antioxidant activity of the nanoserum, evaluated using the ABTS and FRAP assays, showed that formula F2 exhibited very strong antioxidant activity, with IC<sub>50</sub> values much lower than those of the carrot extract alone or the controls. The decrease in IC<sub>50</sub> values after formulation into a nanoserum indicates that the nanoformulation enhanced the effectiveness of the antioxidant compounds. This enhancement is attributed to the increased particle surface area and more uniform distribution of the active ingredients, resulting in more effective interactions between antioxidants and free radicals.<sup>20,21</sup> The combination of  $\beta$ -carotene from carrot extract and astaxanthin is presumed to produce a synergistic effect in free radical scavenging.  $\beta$ -Carotene is known to have limited stability to heat and oxidation. In contrast, astaxanthin is a carotenoid with very high antioxidant capacity and acts through multiple mechanisms, including electron transfer, hydrogen atom transfer, and protection against mitochondrial oxidative stress.<sup>11,22,23</sup>

After the heating–cooling stability test, the antioxidant activity of formula F2 showed a slight increase in IC<sub>50</sub> values but remained within the strong to very strong category. These changes indicate partial degradation of the active compounds during storage; however, the nanoserum system maintained overall biological activity. These findings confirm that the nanoemulsion system effectively protects lipophilic antioxidant compounds and maintains their stability and activity during storage.<sup>24,25</sup>

Overall, the results of the study indicate that the nanoserum formulation combining carrot extract and astaxanthin significantly improved physical characteristics, stability, and antioxidant activity compared to the conventional extract. The particle size results showed that F2, containing 10% carrot extract, produced the smallest particle size compared to F1 and F3, despite the increasing concentration of the active substance. This finding indicates that particle size was influenced not only by extract concentration but also by the balance among the oil phase, surfactant system, and dispersed active compounds within the nanoemulsion. At the 10% concentration, the formulation was presumed to be in optimal condition, allowing for more effective reduction of interfacial tension and the formation of smaller, more homogeneous droplets. In contrast, increasing the extract concentration to 15% in F3 likely increased system viscosity and intermolecular interactions, leading to droplet aggregation and larger particle size. These findings are consistent with those of Iskandar et al., who reported that nanoemulsion particle size is strongly influenced by the surfactant/co-surfactant ratio and the stability of the dispersion system.<sup>26</sup> Therefore, Formula F2 (10%) was identified as the optimal formulation because it exhibited small particle size, good system homogeneity, adequate physical stability, and high antioxidant activity, indicating its potential for further development as an effective and safe natural-based antioxidant cosmetic preparation for topical application.<sup>26</sup>

## Conclusion

The nanoserum formulation combining carrot extract and astaxanthin was successfully developed, exhibiting nanometer-scale physical characteristics and demonstrating good system homogeneity, as indicated by particle size and polydispersity index (PDI) values. Formula F2 (10%) was identified as the optimal formulation because it exhibited the smallest particle size, a low PDI value, a negative

zeta potential that supports dispersion stability, a pH suitable for topical preparations, and adequate viscosity and adhesiveness. The antioxidant activity of formula F2 was classified as very strong in both the ABTS and FRAP assays, with low IC<sub>50</sub> values. After storage up to Day 6, antioxidant activity was maintained despite an increase in IC<sub>50</sub> values. The stability test results showed that F2 remained physically and organoleptically stable, without phase separation, indicating that this formulation has potential for further development as an antioxidant nanoserum.

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