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Astaxanthin Nanoemulsion Formulation and Evaluation

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Abstract

Astaxanthin has antioxidant activity ten times higher than carotenoids such as -carotene and a hundred times higher than vitamin E. However, its utilization is still limited because its solubility in water is very low which results in low absorption by the skin, resulting in low bioavailability. In this case, to increase the potency of astaxanthin, this research was aimed at the formulation and characterization of astaxanthin nanoemulsions using polysorbate 80 and polyethyleneglycol 400 as a mixture of surfactants with a ratio of 7:1; 8:1 and 9:1 with the method of making a combination of low and high energy emulsification. The data obtained were analyzed using the Kruskal-Wallis test for data on the pH of the preparation and the efficiency of adsorption while the pH test during freeze-thaw stability was analyzed by the Wiloxon test. Based on the test results, it was found that the nanoemulsion preparation with the smix (surfactant mixture) 9:1 formula is the most optimum formula among other formulas, which is to produce preparations with quite good characteristics organoleptically and give a light orange color appearance, clear, distinctive smell with a pH value that meets the SNI standard 16-164399-1996 with pH values ranging from 7.13 to 7.15 and based on the centrifugation test gave stable results and had particle size, polydispersity index and zeta potential values, respectively, 22.9 ± 9.4 nm, 0.435 and -21. ,4 mV and the value of entrapment efficiency ranges from 93.87% to 94.32%. However, the thermodynamic stability is not good enough. This is indicated by the instability of the preparation during the freeze-thaw test with the results of changes in color, transparency and changes in pH.

Keywords: Nanoemulsion, Astaxanthin, Polyethyleneglycol 400, Polysorbate 80, Surfactants

1. Introduction

The potential pharmacological effects of astaxanthin are very large, including anti-cancer, anti-diabetic, anti-inflammatory and antioxidant activities as neuro protective, cardiovascular protective, ocular protective and skin protective [1]. However, the utilization of the potential benefits of astaxanthin in cosmetics and pharmaceuticals is still limited because the solubility of astaxanthin in water is so low that it cannot be absorbed by the skin, resulting in low bioavailability. In addition, astaxanthin is known to be easily degraded

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both during storage and processing. Therefore, it is necessary to develop an efficient and effective delivery strategy.

Several studies have shown a functional increase in astaxanthin formulated in nanodispersions, liposomes and nanoemulsions [2]. Although the nanoemulsion system can improve the function of astaxanthin, its success is highly dependent on the selection and composition of the surfactant used [3, 4]. Tween 80 is a non-ionic hydrophilic surfactant that works by increasing the solubility of one substance with another [5]. Meanwhile, Polyethyleneglycol (PEG) 400 is also a non-ionic hydrophilic surfactant that can support the function of tween 80 in increasing the solubility of astaxanthin. In this study, the formulation and characterization of astaxanthin nanoemulsion preparations will be carried out using variations of tween 80 and polyethylene glycol (PEG) 400 as a mixture of surfactants.

2. Method

In the optimization stage, two steps were carried out, the first by making variations of Smix (a mixture of surfactants) namely tween 80 as a surfactant and polyethyleneglycol 400 (PEG 400) as a co-surfactant with a ratio of 9:1; 8:1 and 7:1, respectively, the ratio of Smix was optimized with sunflower oil as the oil phase. Optimization of Smix and oil phase is done by making five variations of each ratio of Smix, where the ratio of Smix: oil phase is F1 (9:1); F2 (8:2); F3 (7:3); F4 (6:4) and F5 (5:5) with a total Smix and oil phase of 40% (w/w).

Manufacture of astaxanthin nanoemulsion using spontaneous nanoemulsification method, namely by adding an oil phase, surfactant, and co-surfactant into a mixture with the ratio selected to incorporate astaxanthin as much as 5% (w/v). The selection of the amount of astaxanthin to be incorporated is based on research [6] related to the manufacture of lotions from astaxanthin where astaxanthin has the best antioxidant activity at a concentration of 5% with an IC50 of 87.571 ppm so that in this study 5% (w/w) astaxanthin was used. Then stirred using a magnetic stirrer at 200 rpm for 30 minutes then sonicated for 1 hour to form a stable and clear nanoemulsion [7].

3. Results

3.1 Optimization Results

Astaxanthin nanoemulsion was made based on the optimization results of 27 nanoemulsion formulas with various concentrations of surfactant, cosurfactant and oil. The selected formula is F1 from each ratio of smix (9:1; 8:1 and 7:1). Furthermore, each formula is made in triples to get accurate and valid results. The temperature in the manufacture of astaxanthin nanoemulsions is very important, where the temperature for the manufacture of astaxanthin should not exceed the temperature of 50^{o}C because it will cause the astaxanthin to undergo structural isomerization and completely degrade within 32 hours [8]. Therefore, the process of making astaxanthin nanoemulsions using a low energy combination method using a magnetic stirrer is carried out at room temperatures ranging from 25-30^{o}C. A high energy sonicator is used with a final temperature not exceeding 50^{o}C. Sonification using a probe sonicator aims to reduce particle size by utilizing ultrasonic waves that can convert electrical energy into physical vibrations that can reduce particle size to a nanometer size range of 20-200 nm.1.

Table 1. Smix formulation

Nama Bahan	Smix 9:1 (% b/v)	Smix 8:1 (% b/v)	Smix 7:1 (% b/v)
Polisorbat 80	32,4	32	31,5
PEG 400	3,6	4	4,5
Sunflower oil	4	4	4
Aquadest	Ad 100	Ad 100	Ad 100

Furthermore, astaxanthin is incorporated into the base using the same manufacturing method.

Characterization

3.2 Organoleptic Test

Organoleptic test was carried out visually by observing the color, shape and smell of the preparation.

3.3 pH test

The pH test was carried out in triples to get accurate and valid results. The pH test for the astaxanthin nanoemulsion preparation has met the required range of topical preparations as can be seen in table 2.

Table 2. pH test for astaxanthin nanoemulsion

Formulation	Batch	average pH ± SD
9:1	1	7,13 ± 0,020
	2	7,15 ± 0,026
	3	7,13 ± 0,011
8:1	1	7,14 ± 0,023
	2	7,13 ± 0,015
	3	7,11 ± 0,015
7:1	1	7,13 ± 0,035
	2	7,13 ± 0,015
	3	7,12 ± 0,012

3.4 Particle Size Test and Polydispersion Index

Particle size test was carried out to determine the particle size of astaxanthin nanoemulsion preparations. According to [9] which states that a preparation is said to be a nanoemulsion if it has a size of 20-200 nm. The results of particle size testing carried out at the Print-G Laboratory of Universitas Padjadjaran using the Horiba S Z-100 Particle Size Analyzer measured at a temperature of 25oC can be seen in Table 3.

Table 3. Particle Size Test and Polydispersion Index

Formulation	Particle Size	Polydispersion Index
9:1	22,9 ± 9,4 nm	0,435
8:1	28,8 ± 13,8 nm	0,475
7:1	28,2 ± 15,2 nm	0,541

3.5 Zeta Potential Test

The zeta potential test was carried out to determine the overall charge of a particle that could describe the stability of a nanoemulsion. Zeta potential can describe the stability of a system containing dispersed particles because this potential regulates the degree of repulsion between dispersed particles of the same charge and close to each other [10]. The results of the potential zeta test were carried out at the Print-G Laboratory of the University of Padjadjaran using the Horiba S Z-100 Particle Size Analyzer which was measured at a temperature of 25^{o}C.

Zeta potential measurements were carried out only on samples of astaxanthin smix 9:1 nanoemulsion which were selected based on the results of the most optimum particle size and polydispersity index test results. The results of the zeta potential measurement on the astaxanthin smix 9:1 nanoemulsion sample showed a zeta potential value of -21.4 mV.

Note:
Qt: The number of drugs used

The results of the adsorption efficiency test on the astaxanthin nanoemulsion can be seen in table 4.

3.6 Centrifugation Test

Based on the test results that all formulas did not experience changes in either phase separation, precipitation, creaming or cracking after the test which indicated a picture of the stability of the preparation against gravity.

3.7 Adsorption Efficiency Test

The adsorption efficiency test was carried out to measure the amount of drug adsorbed in the nanoemulsion system as a carrier. The measurement of the absorption efficiency of the active substance in the nanoemulsion system is carried out indirectly by measuring the amount of astaxanthin that is not adsorbed in the nanoemulsion where the amount can be calculated from the results of the absorbance measurement which can be calculated by the equation for the percentage of adsorption efficiency:

$$EE\ (\%) = \frac{(Qt-Qs)}{Qt} \times 100\%$$

Qs : The amount of drug that is not adsorbed in the nanoemulsion
[11]

Table 4. Adsorption Efficiency Test

Formula	**Batch**	**% EE 1**	**% EE 2**	**% EE 3**	**Rata-rata ± SD**
9:1	I	94,55 %	94,55 %	93,87 %	94,32% ± 0,003925982
	II	93,87 %	93,87 %	93,87 %	93,87% ± 0

	III	94,55 %	93,87 %	93,87 %	94,10% ± 0,003925982
8:1	I	92,49 %	92,49 %	93,18 %	92,72% ± 0,003983717
	II	93,18 %	92,49 %	93,18 %	92,95% ± 0,003983717
	III	93,18 %	93,18 %	92,49 %	92,95% ± 0,003983717
7:1	I	91,80 %	91,80 %	92,49 %	92,03% ± 0,003983717
	II	93,18 %	92,49 %	92,49 %	92,72% ± 0,003983717
	III	91,80 %	92,49 %	92,49 %	92,26% ± 0,003983717

3.8 Freeze-thaw Stability Test

Freeze thaw test was conducted to determine the effect of temperature on the physical stability of nanoemulsion preparations. This test was carried out for six cycles, where one cycle consisted of storage at a temperature of 4˚C ± 2˚C for 24 hours and at a temperature of 40˚C ± 2˚C for 24 hours. The results of the freeze-thaw stability test can be seen in Table 5.

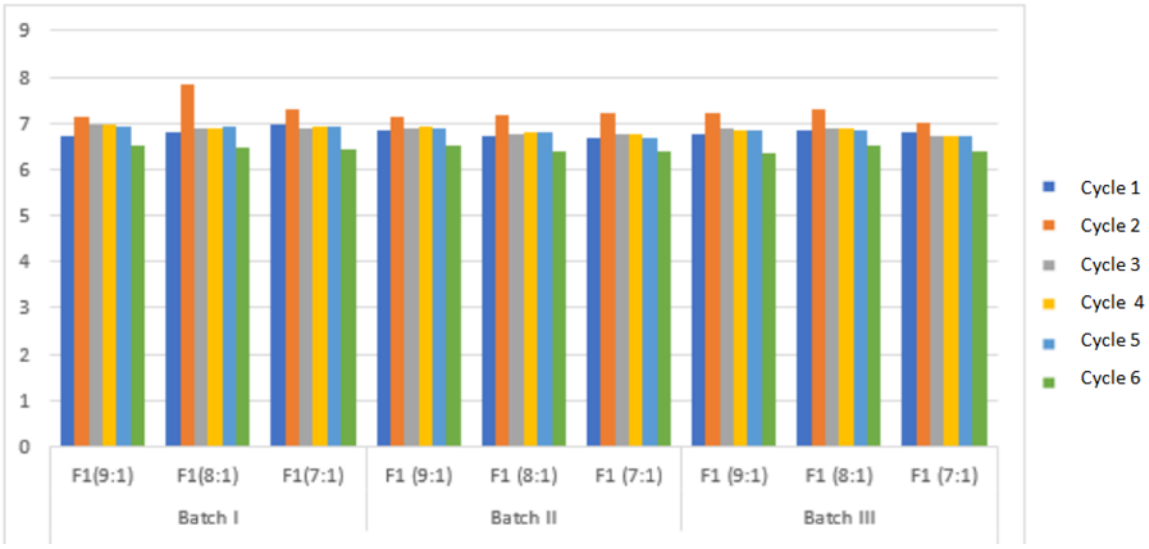
Table 5. Freeze-thaw stability test on organoleptic results

Batch	**Formula**	**Parameter**	**Cycle**					
			1	**2**	**3**	**4**	**5**	**6**
I	F1 (9:1)	Discoloration	-	-	-	+	+	+
		Phase separation	-	-	-	-	-	-
		Transparency	-	-	-	-	-	-
	F1 (8:1)	Discoloration	-	-	-	+	+	+
		Phase separation	-	-	-	-	-	-
		Transparency	-	-	-	+	+	+
	F1 (7:1)	Discoloration	-	-	-	+	+	+
		Phase separation	-	-	-	-	-	-
		Transparency	-	-	-	+	+	+
II	F1 (9:1)	Discoloration	-	-	-	+	+	+
		Phase separation	-	-	-	-	-	-
		Transparency	-	-	-	+	+	+
	F1 (8:1)	Discoloration	-	-	-	+	+	+
		Phase separation	-	-	-	-	-	-
		Transparency	-	-	-	+	+	+
	F1 (7:1)	Discoloration	-	-	-	+	+	+
		Phase separation	-	-	-	-	-	-
		Transparency	-	-	-	+	+	+
III	F1 (9:1)	Discoloration	-	-	-	+	+	+
		Phase separation	-	-	-	-	-	-
		Transparency	-	-	-	+	+	+
	F1 (8:1)	Discoloration	-	-	-	+	+	+

		Phase separation	-	-	-	-	-	-
		Transparency	-	-	-	+	+	+
	F1 (7:1)	Discoloration	-	-	-	+	+	+
		Phase separation	-	-	-	-	-	-
		Transparency	-	-	-	+	+	+

Note :
(-) : No change
(+) : There is a change

In addition, a freeze thaw stability test was also carried out on pH
Figure 1. Freeze-thaw stability on pH results



4. Discussion Characterization

4.1 Organoleptic test

The resulting astaxanthin nanoemulsion is a clear and transparent single-phase liquid with a light orange color and a special odor. This shows that the nanoemulsion formulation process is running well.

4.2 Ph measurement

The results of statistical analysis showed that in the normality test, the data distribution was not normal in the smix 9:1 formula for the second batch and smix 7:1 for the third batch with a significance value of 0.000 < 0.05, in addition to the homogeneity test, a significance value of 0.001 < 0.05 so that the data is also said to be inhomogeneous. Therefore, the statistical test used is the Kruskal-Wallis test, where the results of the Asymp test are obtained. Sign 0.010 < 0.05 so it can be concluded that there is a significant difference in pH in each formula. This indicates that there is a significantly different effect between increasing the concentration of smix on the pH value of the preparation.

4.3 Particle Size Test and Polydispersion Index

Based on the results of particle size measurements, the astaxanthin nanoemulsion formula with a ratio of 9:1 smix; 8:1 and 7:1 are in the range of particle sizes required for nanoemulsion preparations, where astaxanthin nanoemulsion with 9:1 smix has the smallest particle size, this is due to the composition in smix where the amount of polysorbate 80 surfactant used is more than

the two. other formulas so as to further lower the surface tension, stabilize the new surface during the homogenization process and produce smaller particles [9]. Particle size measurement is a very important factor to determine the stability of a nanoemulsion preparation. The stability of nanoemulsions depends on the size of the particles in the dispersed phase. The smaller the particle size, the smaller the rate of incorporation so that the nanoemulsion is not easily creamed. In addition, the small particle size results in good optical clarity and can be stored longer, is not easily damaged, does not change easily and is easily absorbed by the body [12].

The polydispersity index test was carried out to describe the level of uniformity of particle size in a nanoemulsion, the value of the polydispersity index is important to know because it is related to the uniformity of the size of the nanoemulsion, a small polydispersity index value indicates better size uniformity, this test was carried out at the Print-G Laboratory of the University Padjadjaran using the Particle Size Analyzer Horiba S Z-100 measured at a temperature of 25^{o}C, the results of the polydispersity index test measurements can be seen in Table 3. Based on the measurement results of the three astaxanthin nanoemulsion formulas with smix 9:1; 8:1 and 7:1 have polydispersity indexes of 0.435, respectively; 0.475 and 0.541. These results according to [13] fall into the range of 0.3-0.7 where the polydispersity index value of a nanoemulsion from 0.3-0.7 is polydispersity, which indicates that the particle size is uniform but has different shapes and distributions. the particles are wide.

4.4 Zeta Potential Test

The results of the zeta potential measurement on the astaxanthin smix 9:1 nanoemulsion sample showed a zeta potential value of -21.4 mV. The results of the measurement of the zeta value which is negative (-) indicates that the majority of the surface charge of the droplet is anionic, resulting in a decrease in the surface charge of the droplet to be negative [14]. The zeta potential value is -21,4 mV which is greater than -30mV so it is possible that the preparation has a repulsive force that is not good enough to achieve colloid physical stability as stated by [15] that a good zeta potential value to produce a stable preparation is less than -30 mV or more than +30 mV because it can avoid particle aggregation with repulsive forces exceeding the attractive forces of the dispersed particles so that they can stabilize themselves, on the contrary if the potential zeta value is outside What is required is the possibility of the preparation to produce aggregation and flocculation of very large particles because there is a van der Waals force that produces physical instability in the preparation. The low value of zeta potential can be caused by the surfactant and the amount of surfactant used, in this study non-ionic surfactants were used which have a constant hydrophobic group and tend to reduce the potential zeta value [16].

4.5 Centrifugation Test

The centrifugation test was carried out to determine the stability of the preparation against the force of gravity and as an illustration of the stability of the preparation against shocks during distribution with the test parameters being the occurrence of phase separation, precipitation, creaming or cracking [17,

18]. Based on the test results that all formulas did not experience changes in either phase separation, precipitation, creaming or cracking after the test which indicated a picture of the stability of the preparation against gravity. This shows that all formulas are stable and there is no phase separation

4.6 Adsorption Efficiency Test

Based on Table 4, it was found that the average adsorption efficiency of the astaxanthin nanoemulsion was more than 90% in each formula made. This high adsorption efficiency can be attributed to the high solubility of astaxanthin in sunflower oil as the oil phase, which according to [6], sunflower oil can dissolve astaxanthin well up to a concentration of 100 mg/L. The results showed that nanoemulsion with smix 9:1 had greater adsorption efficiency than nanoemulsion with smix 8:1 and 7:1 so that it could also be related to the amount of surfactant used which was associated with increasing surfactant concentration causing lower surface tension between droplets so that prevent incorporation which can thus increase the solubility of astaxanthin in nanoemulsions[19] .

The results of statistical analysis showed that in the normality test, the data distribution was not normal in the entire formula with a significance value of 0.000 < 0.05 so that the data distribution was concluded to be abnormal, in addition to the homogeneity test, the significance value was 0.106 > 0.05 so that the data was said to be homogeneous, Therefore, the statistical test used is the Kruskal-Wallis test because the data are not normally distributed even though it is homogeneous, where based on the results of the Kruskal-Wallis test, the Asymp test results are obtained. Sign 0.005 < 0.05 so it can be concluded that there is a difference in the value of the adsorption efficiency in each formula. This indicates that there is a significantly different effect between increasing the concentration of smix on the adsorption efficiency.

4.7 Freeze-thaw Stability Test

Results in table 5 state that all formulas have changed either in color change or transparency, the stability of the resulting astaxanthin nanoemulsion can be predicted beforehand by looking at the potential zeta value, where the 9:1 formula has a particle size and the best polydispersity index but has a zeta potential value that is not good enough, causing the preparation to have a repulsion force that is not good enough to achieve colloid physical stability, as evidenced by the test results that the preparation is unstable after the third cycle is characterized by a change in color in the preparation caused by the presence of an oxidation reaction during the test, as well as the other formulations. In addition to the color change, phase separation and transparency, other parameters that can be seen are the pH of each formula, and it can be concluded that there is a change in the pH of each formula from the 1st cycle to the 6th cycle. Changes in pH during the test were caused by hydrolysis after the preparation was stored at 40^{o}C, but when viewed based on the requirements for the pH range during the test, it was still acceptable according to SNI 16-4399-1996 which stated that the ideal pH for topical preparations was between pH 4.5- 8.0.

The results of statistical analysis from figure 1 showed that in the normality test, the data distribution was not normal in the 3rd batch smix 9:1 formula, smix 8:1 batch 1, smix 7:1 batch 1, smix 7:1 batch 3

before the freeze-thaw test test and there was an abnormal distribution of data in the 2nd batch of smix 9:1 formula and smix 7:1 batch 3 on the data after the freeze-thaw test with a significance value of 0.000 <0.05 so that the data distribution was concluded abnormal. The statistical test used was the Wiloxon test to compare the effect of the freeze-thaw test on the initial pH (cycle 0) and final pH of the preparation (cycle 6) in each formula. The Wiloxon test was used because the data distribution was not normal, the Wiloxon test results showed the asymp value. Sig of 0.000 <0.05 so it can be concluded that there is a significant difference in the pH value of each formula between before and after the freeze-thaw test

5. Conclusions

Nanoemulsion preparation with the smix 9:1 formula is the most optimum formula, which is to produce preparations with quite good characteristics organoleptically and give a light orange color appearance, clear, distinctive smell with a pH value that met the SNI standard 16-164399-1996 with pH values ranging from 7.13 to 7.15 and based on the centrifugation test gave stable results and had particle size, polydispersity index and zeta potential values, respectively, 22.9 ± 9.4 nm, 0.435 and -21,4 mV and the value of entrapment efficiency ranges from 93.87% to 94.32%. However, the thermodynamic stability is not good enough. This is indicated by the instability of the preparation during the freeze-thaw test with the results of changes in color, transparency and changes in pH.

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