Effect of Emulsifier Variation on The Stability of Carotene Nanoemulsion

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Abstract. Red palm oil has a high content of carotenoids. β-carotene as the highest content of red palm oil acts as antioxidant but with low stability. Nanoemulsion is a system with oil in water emulsion (o/w) with nano size particle scale to increase the solubility and stability of bioactive components contained in red palm oil with 10-1000 nm scale. The purpose of this study was to determine the physical stability and total carotenoids content in nanoemulsion of red palm oil concentrate. The process of making nanoemulsion carotene concentrate was using High Pressure Homogenizer with variation of emulsifier. The emulsifier used was tween 20 and span 80 with ratio as follows 3:7 (F1), 7:3 (F2), 5:5 (F3) and 10:0 (F4). Parameters observed were pH, color, particle size and polydispersity index and stability of nanoemulsion. The results showed that formula F1 had the best physical stability compared to other formulas with particle size of 160.9 nm and total carotene of 1,710 ppm.

1. Introduction
One minor component acting as antioxidant in palm oil is carotene with an amount of around 500-800 ppm [1]. In addition, carotene can also be found in derivative products processed by palm oil, namely red palm oil. Red palm oil is a food source that comes from nature with low availability. Red palm oil is naturally rich in carotene around 500 - 800 ppm, which is 15 times higher than the source of carotene from carrots. Red palm oil, similar to olive oil, is an oil obtained from fruit, other than canola oil and sunflower oil derived from seeds. Red palm oil contains high carotenoids and natural antioxidant in various functional food products.

Nanoemulsion has been widely developed in the fields of food, pharmacy and health. Nanoemulsion is an oil and water emulsion system with particle size ranging from 2 to 500 nm [2,3]. In the form of nanoemulsion, bioactive compounds that are lipophilic become more stable and more easily absorbed in the digestive tract. Nanoemulsion is an alternative form that can increase the solubility and stability of bioactive components in red palm oil. To improve its stability and solubility in water, carotenoids can be dissolved in the oil phase in an oil emulsion in water (o/w) so that it can be easily formulated into pharmaceutical products [4, 5, 6, 7, 8].

In this study carotene nanoemulsions were processed using high pressure homogenizer. The parameters observed were particle size, physical stability and total carotene content. The resulting carotene nanoemulsion from red palm oil is expected to be used as an ingredient for functional foods that can increase absorption of active substances so that it can have more benefits.
2. Method

2.1. Materials
The materials used were red palm oil carotene from the Palm Oil Research Institute in Medan, North Sumatra, Indonesia, tween 20, and span 80. Research was conducted at Indonesian Centre Agricultural Postharvest Research and Development.

The tools used were ultra turrax homogenizers, High Pressure Homogenizer, Zetasizer Nano ZS (Malvern Instruments, Worcestershire, UK), Ch 300 Chromameter, UV-Vis Spectrophotometer, pHmeter, centrifuges, analytical scales and glassware for analysis.

2.2. Nanoemulsion Formulation
Production of carotene concentrate nanoemulsion from red palm oil (oil in water (o / w) was processed using High Pressure Homogenization (HPH) techniques. Carotene concentrates were made in oil emulsions in water (o / w) using tween 20 and span 80 as emulsifiers with various concentrations and aquadest as solvent. Emulsions were made in a base of 100 g, consisted of carotene concentrate from red palm oil, aquadest and a various surfactant concentrations as shown in Table 1 [9,10,11].

Carotene concentrates, tween 20, and span 80 were stirred using ultraturrax (1200 rpm) for 3 minutes as oil phase (M1). Aquadest as the water phase was added into the oil phase (M1) and stirred using ultraturrax for 5 minutes. Afterwards, the emulsion was homogenized using High Pressure Homogenizer (500 bar) for 5 cycles.

| Table 1. Formulation of nanoemulsion |
|-------------------------------------|
| Materials   | Composition (%) | F1 | FII | FIII | FIV |
| Carotene    |                  | 3  | 2   | 2    | 2   |
| Tween 20    |                  | 3  | 7   | 5    | 10  |
| Span 80     |                  | 7  | 3   | 5    | 0   |
| Aquadest    | Ad 100           | Ad 100 | Ad 100 | Ad 100 |

2.3. Analysis

2.3.1. pH measurement
At first the electrodes were calibrated with standard buffer pH 4 and pH 7. Then the electrodes were dipped in samples. pH value appears on the screen and then recorded.

2.3.2. Color Test
Color test to determine "Hue was conducted using Chromameter. The measurement value was displayed with the Hunter color notation system. This tool uses the color system of L, a and b. L shows the brightness with a value of 0 (dark / black) to 100 (bright / white), while a for green (a negative) to red (a positive) and b for blue (b negative) to yellow (b positive). Conversion of L, a and b values to Hue (°) and reddish values (a / b) will showed the chroma colour.

2.3.3. Particle Size and Polydispersity Index
The average droplet diameter and droplet size distribution of carotene concentrated nanoemulsion were determined by a particle size analyzer DeltaTM Nano equipment. The diameter size of the carotene concentrate droplet nanoemulsion was expressed by the average diameter based on the number distribution, and the nanoemulsion droplet size distribution was expressed as a polydispersity index (IP). Polydispersity index values show the distribution of droplet size distributions. The smaller polydispersity index value shows the narrower droplet size distribution, which means the droplet diameter is increasingly homogeneous [3].
2.3.4. Total carotene
120 mg of standard carotene was put into a 100 ml volumetric flask then the volume was filled with hexane up to 100 mL to obtain a concentration of 120 ppm as the liquor. From the mother liquor, 8 different concentrations were diluted with the help of volume pipettes and volumetric flasks, at concentrations of 24, 19.20, 9.60, 3.84, 1.92, 0.96, and 0.48 ppm.

2.3.5. Stability of Nanoemulsion
Cycling test was conducted to determine the stability of nanoemulsion with 6 cycles at low temperatures (4 ± 2°C) for 24 hours, then transferred at high temperatures (40 ± 2°C) for 24 hours (1 cycle). The experiment was repeated for 6 cycles and the changes were observed and compared with nanoemulsion before cycling test.

3. Results and Discussion

3.1. Color
L value show brightness with values (0 dark / black) to 100 (bright / white), while a for green (a negative) to red (a positive) and b for blue (b negative) until yellow (b positive). Convert the value L, a and b to the value of Hue (°Hue) and obtain the chromaticity color range. By using the Hunter color system and °Hue values to find out the area of chromaticity. In this experiment the °Hue value was between 95.9-107.6 (Table 2). So these four formulas showed that the chromatic color range was in the range 90-126 which indicated the intensity of yellow (Y) color in this nanoemulsion. This yellow color was from carotene concentrate from red palm oil [3].

| Formula | °Hue Value | Particle Size | Carotene Total | pH   |
|---------|------------|---------------|----------------|------|
| 1       | 107.6      | 160.95 ± 0.070| 1.710 ± 0.309 | 6.61 |
| 2       | 95.9       | 50.54 ± 0.0176| 4.817 ± 0.705 | 6.01 |
| 3       | 107        | 138.05±0.212  | 6.106 ± 0.253 | 6.35 |
| 4       | 103        | 139.30 ± 1.626| 3.046 ± 0.556 | 5.33 |

3.2 Particle Size
The droplet size of nanoemulsion was expressed by the average diameter (Z-avarage) based on the number distribution. The nanoemulsion droplet size distribution was expressed as amount of droplets at each peak of the distribution curve. The value of PDL showed the diversity of samples. Low pdl values (<0.3) indicated monodispersed samples [3]. The pdl value > 0.4 indicated that the nanoemulsion had wider particle size distribution which showed the lower particle uniformity [12,13,14].

3.3. Total carotene
Carotene concentrate nanoemulsion from red palm oil before stability test ranged between 1.710 ppm-6.106 ppm. Nanoemulsion after stability testing increased with any various value (Table 3). The carotene content tended to decreased by increasing temperature. The decreased of total carotene levels at high temperatures was caused by the nature of carotenoids which are unstable to heat and can trigger carotene degradation causing a decrease in total carotene levels at high temperatures. Carotene levels (β-carotene) are very susceptible to degradation by exposure to air, light and heat [9].

Table 2. Hue value, particle size, total carotene and pH
Table 3. Carotene total from various temperature

| Formula | Temperature | Carotene total |
|---------|-------------|----------------|
| 1       | Early       | 1.710 ± 0.309  |
|         | 4°C         | 1.274 ± 0.131  |
|         | 29°C        | 1.673 ± 0.316  |
|         | 50°C        | 1.399 ± 0.118  |
| 2       | Early       | 4.817 ± 0.705  |
|         | 4°C         | 2.975 ± 0.420  |
|         | 29°C        | 2.994 ± 0.533  |
|         | 50°C        | 1.845 ± 0.034  |
| 3       | Early       | 6.106 ± 0.253  |
|         | 4°C         | 3.602 ± 0.036  |
|         | 29°C        | 3.746 ± 0.160  |
|         | 50°C        | 2.218 ± 0.035  |
| 4       | Early       | 2.006 ± 0.292  |
|         | 4°C         | 1.176 ± 0.021  |

3.4. Particle stability testing at various temperature storage

Particle size measurements at 50°C showed that the particle size increased. It can be said that the preparation of nanoemulsion was stable at low temperatures but not stable at high temperatures. Nanoparticle at high temperature of 50°C can cause an increase in particle size [15].

From Table 4, it can be seen that the particle was not stable, there was a constant change in size. Particle size generally increased due to agglomeration. Only particles in formula F3 were generally more stable.

Table 4. Nanoemulsion particle size from another temperature

| Weeks | Formula | Particle Size (29°C) | Particle Size (50°C) |
|-------|---------|----------------------|----------------------|
| 0     | 1       | 160.9                | 160.9                |
| 1     | 2       | 50.54                | 50.54                |
|       | 3       | 138.05               | 138.05               |
|       | 4       | 13.93                | 13.93                |
|       | 1       | 159.0                | 154.5                |
| 1     | 2       | 55.15                | 101.9                |
|       | 3       | 140.2                | 120.2                |
|       | 4       | 180.5                | 87.49                |
|       | 1       | 159.0                | 192.3                |
| 2     | 2       | 53.62                | 127.2                |
|       | 3       | 135.4                | 111.8                |
|       | 4       | 190.4                | 520.9                |

4. Conclusion

Particle size of concentrate carotene nanoemulsion particle size were 160.9 nm, 50.53 nm, 137.9 nm and 12.78 nm for formula 1 to 4. The pH value ranged from 5.33 to 6.61. From the color intensity showed that the carotene nanoemulsion formula had a yellow color. Total carotene levels of formula 1 to 4 were 1.710 ppm, 4.817 ppm, 6.106 ppm and 3.046 ppm respectively. From storage showed that concentrate carotene changed in color and odor, damaged by heating, and decreased in total carotene. The best formula was formula F3 that most stable than the other formulas.
5. References

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