Optimization of total carotenoids, phenolic content, and sensory acceptability of java tea-based functional drink enriched with red fruits’ oil emulsion

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Abstract
Red fruits’ oil contains 12,000 ppm carotenoid compounds and among them β-carotene was found as the major carotenoid compound. However, the application of red fruits’ oil as the food ingredient is rarely investigated due to its low solubility in aqueous, phenolic aroma and bitter aftertaste. Therefore, emulsification of red fruits’ oil in water (oil in water, o/w emulsion) using CMC (carboxymethyl cellulose) as the emulsifier and stabilizer agent considered as an alternative to overcome those limitations. In the present work, the optimum concentration of red fruits’ oil emulsion added into Java tea-based functional drink increased the total carotenoid content, gave red color and might enhanced its antioxidant activity. The optimum formula was performed by Response Surface Methodology approach utilizing Design Expert 10.0.7® (DX10) Trial software whereas the emulsion concentration of red fruits’ oil as the variable factor. Red fruits’ oil emulsion which has been prepared from 20% red fruits’ oil and 1.50% CMC as emulsifier and stabilizer was having total carotenoid content of 72.93 ± 1.33 ppm. The optimum concentration of red fruits’ oil emulsion added to Java-tea-based functional drink was 5.96%. The addition of red fruits’ oil emulsion increased the total carotenoid content from 0.37 ± 0.13 ppm to 5.28 ± 0.05 ppm. The results showed that red fruits’ oil emulsion could be used as red colorant agent in the functional drink while increasing the total carotenoid content, however, reduced the flavor sensory acceptance in high amount.

Keywords: red fruit, carotenoid, functional drink from Java tea, red pigment

INTRODUCTION
Red fruit (Pandanus conoideus Lam.) is an endemic plant from Papua that widely used as coloring agent, energy drink, functional food, medicinal plant, etc. [1,2]. According to Sarungallo et al. (2016), the extract of red fruit was edible and could be used for functional food due to its carotenoid and tocopherol content that exhibit antioxidant, anti-tumor, anticancer, anti-diabetic and anti-inflammation activity [3]. Red fruit has a potency due to its high oil content around 11.2-30.7% in dry basis [4]. Red fruits’ oil contains several active compounds such as α-carotene, β-carotene, β-cryptoxanthin, α-tocopherol, oleic acid, linoleic acid, and palmitoleic acid [5,6].

Color is an important visible factor on food and an important characteristic for food quality thus the color change during food storage is critical to be evaluated [7]. The reddish color was generated by red fruit come from the total carotenoid as a pigment. According to Palupi and Martosupono (2009),
red fruits’ oil contains 12,000 ppm carotenoid compounds and among them β-carotene was found as the major carotenoid compound [8]. Carotenoid compounds could act as provitamin A, antioxidant, UV radiation protector, immune regulator and cell proliferation [9]. That biological activities were strengthened by the previous research in which β-carotene from red fruits’ oil could increase the proliferation activity of spleens’ lymphocytes of mice infected by Listeria monocytogenes [10]. Carotenoid as antioxidant agent keeping the cell membrane stability from oxidative damages. Furthermore, carotenoid could protect the DNA and tissues from cancer damage and stimulate enzyme that could remove the carcinogenic compounds [11]. Because of that, red fruits are potential to be used as food functional ingredient.

However, red fruits’ oil application as food functional ingredient was limited due to its low solubility in aqua-phase, phenolic aroma and bitter aftertaste [12]. Because of that, emulsification of red fruits’ oil in water (oil in water, o/w emulsion) using CMC as the emulsifier and stabilizer agent could overcome those limited application. The o/w emulsion could facilitate the lipophilic active compound thus the red fruits’ oil could be miscible with water [4,13]. Several studies on red fruits’ oil emulsion have been conducted by Jufri et al. (2009), Sarunagallo et al. (2014), Murtiningrum et al. (2013), and Ferdiansyah et al. (2015) [1,4,5,25]. Emulsion and nano-encapsulate red fruits’ oil using Tween 80 gave the total carotenoid content as 4.21 ± 0.01 and 4.47 ± 0.01 mg/100 g, respectively [25]. Pigment stability on both emulsion and nano-encapsulate was monitored due to no significant absorbance change between blank and after pasteurization, sterilization and light irradiation process. Nanoencapsulation method was stabilized carotenoid pigment thus increased its storage time [25]. However, the nanoencapsulation gave a weak color expression on drinks. It was reported that red fruits’ oil emulsion using CMC (carboxymethyl cellulose) as the emulsifier and stabilizer agent gave the best emulsion stability compared to Tween 20 and Tween 80 [5].

According to Herold (2007), Kordial (2009), Waziiroh (2013), and Eugenie (2015); the antioxidiant activity and color intensity of Java tea based functional drink was decreased during storage time [14,15,16,17]. Therefore, the present research work was carried out to find the optimum concentration of red fruits’ oil in Java tea based functional drink to increase its color impression and antioxidiant capacity thus it would attract the consumers and gave a longer storage time.

EXPERIMENTAL

Materials and equipment

Materials used were Java tea leaves and sappan wood (both from Biofarmaka Study Center LPJM IPB), elephant ginger, lime, kaffir lime, and Javanese turmeric that were obtained from Sinpasa modern market in Serpong. Red fruit oil (long red fruit) from CV. Made Mulya Asih Papua, and carboxyl methyl cellulose (CMC) (PT. Hade Putra Persada). To make the drink, there were some ingredients added, including sucrose (Gulaku), sweeteners (Acet sulfame and Sucralose) from PT. Chemco Prima Mandiri, IMP:GMP (PT. Ajinomoto), xanthan gum (PT. Chemco Prima Mandiri), lemon flavor (PT. KH Roberts Indonesia), and distilled water. For analysis, the materials were Folin-Ciocalteu reagent, gallic acid, sodium carbonate, acetone, sodium hydroxide, and methanol whereas all materials were purchased from Merck.

Equipment used were heat plate stirrer (Barnstead Thermolyne Cimarec), Heidolph RZR-1 Overhead Stirrer, chromometer (Konika Minolta), UV-Vis spectrophotometer (Hitachi U-1800), viscometer (Brookfield Digital Model DV-II), centrifuge (Boeco M-240R), vortex (Barnstead Thermolyne), oven (Memmert), cabinet dryer, dry blender (Philips), analytical balance, pH meter, thermometer, evaporating dishes, beaker glass, cuvette quartz (Hellma), centrifuge tubes, dropping pipette, graduated cylinders, Mohr pipet, volumetric flask, stirring rod, spatula, funnel, sterile bottles, and test tubes.

Characterization of red fruits’ oil emulsion

Preparation of red fruits’ oil emulsion was carried out in a similar manner to the previously described procedure [18] with a minor modification. The used CMC concentrations were 1.00 and 1.50% while the red fruits’ oil emulsion concentrations were 10, 15 and 20%. Briefly, the CMC was dissolved in distilled water by stirring at 1000 rpm for 10 mins. Afterwards, the red fruits’ oil was added, and the mixture was stirred until homogenous. After the emulsion was formed, the particle size, viscosity, emulsion stability, total carotenoid content and total phenolic content were measured. The best sample was chosen for formula optimization for functional drink from cat whiskers.

Formula optimization

The optimum formula was determined using Response Surface Methodology (RSM) with Design Expert 10.0.7% (DX10) Trial software. One Factor Design was used utilizing red fruits’ oil emulsion concentration as the factor. The minimum and maximum values were set at 4 and 8%, respectively, according to our previous report [19]. Seven formula was set determining the total carotenoid content, total phenolic content and optimum sensory acceptance. Analysis of variance (ANOVA) was used to validate the obtained model from RSM analysis with significance of p < 0.05. Furthermore, verification analysis was carried out for the optimum formula.

Preparation of Java tea based functional drink enriched with red fruit oil emulsion

The functional drink from Java tea leaves was prepared from simplisia extract (Java tea leaves, ginger elephant, hardwood, and Javanese turmeric), lime extract, kaffir lime extract, sucrose, 1% acesulfame stock solution, 1% sucralose stock solution, 1% xanthan gum stock solution, IMP:IGP taste testers, salt, lemon flavor, and water. All the ingredients were mixed according to the previously reported formula. Then red fruits’ oil emulsion was added and homogenized using rotor-stator homogenizer. The drink was pasteurized at 80 °C for 15 mins, then carried out of shock cooling in cold water and stored at refrigerator for further analysis.

Total carotenoid content analysis

Determination of total carotenoid content was done in a similar manner to the previously described procedure [20] with a minor modification. As much as 100 mg sample was extracted with 10 mL of 80% acetone. The extract was centrifuged at 3000 rpm for 15 mins. The supernatant was separated, and the pellet was re-extracted with 5 mL of 80% acetone until colorless. All supernatant was collected, and the absorbance was measured at 480, 645 and 663 nm using a
spectrophotometer UV-Vis. Total carotenoid content was calculated by the following equation:

\[
\text{Carotenoid content (mg/g)} = \frac{A_{660} - 0.14 A_{480} - 0.638 A_{453}}{a \times 1000 w V}
\]

where:
- \(a\) = light path on the sample (1 cm)
- \(V\) = extract volume (mL)
- \(w\) = sample mass (g)

**Total phenolic analysis**

Total phenolic content was determined using a Folin-Ciocalteu method [21]. Gallic acid (GAE) was used as the control. As much as 1 mL of sample was mixed with 2 mL of diluted (10x) Folin-Ciocalteu reagent and then neutralized with 7.5 b/v sodium carbonate solution. The mixture was incubated for 30 mins at room temperature and the absorbance was measured at 725 nm using a UV-Vis spectrophotometer. The total phenolic content was expressed in mg GAE/g unit.

**Sensory evaluation**

Sensory evaluation was carried out through a hedonic test to express the panel’s acceptance toward the tested product. Hedonic test included 70 panels for evaluating the product with 7-point scale (1 = very dislike, 2 = dislike, 3 = little dislike, 4 = neutral, 5 = little like, 6 = like, 7 = very like). As much as 15-20 mL sample was served in a plastic glass with 3 numbers code. Before sensory test, panels have to drink the water to neutralize their tongue. Then the samples were tasted for taste, aroma, color and overall acceptance and then neutralized again with water.

**RESULTS AND DISCUSSION**

**Characterization of red fruits’ oil emulsion**

The characteristic red fruits’ oil emulsion with various concentration of red fruits extract and CMC was shown in Table 1. The particle size was decreased, as well as the emulsion stability and viscosity were increased, by decreasing the CMC concentration. Meanwhile, the emulsion stability was decreased, as well as the particle size and viscosity were increased, by increasing the concentration of red fruits’ oil emulsion. The formula was chosen from the emulsion stability, total carotenoid content and total phenolic content. As shown in Table 1, the most stable emulsion was A2B3 formula as high as 97 ± 1 %. The higher concentration of CMC gave thicker gel formation in the continuous phase in which the oil droplets will be trapped in its network thus stabilized the emulsion [22]. Based on those parameters, A2B3 formula was chosen because of it has (91%), high total carotenoid and phenolic contents as 72.93 ± 1.3 ppm and 2.040 ± 0.026 mg GAE/g, respectively.

**Determination on Optimum Emulsion Concentration**

In determining the best red fruit emulsion formulation to optimize Java tea-based functional drink was analyzed by Response Surface Methodology (RSM) utilizing DX 7 Trial software. The critical parameters were total carotenoid, total phenolic content, and emulsion stability. The results were listed in Table 2.

**Effect of Optimum Emulsion Concentration**

ANOVA results were listed in Table 3. The variable response such as total carotenoid content, total phenolic content, taste and overall acceptability follow cubic model while aroma and color acceptance follow quadratic model. All model was obtained with p-value < 0.05 showing that red fruits’ oil emulsion concentration significantly influenced all response on the functional drink from cat whiskers. While the p-value for all fit models were higher than 0.05 except for color and taste acceptability demonstrating that the model was almost fit enough to predict the parameters.

As shown in Table 3, the addition of a certain amount of red fruit emulsion increased color acceptance of the drink. However, when it was gradually added, the acceptance became lower. This might be due to red fruit oil incorporated in the emulsion possessed high carotenoid pigments that impart intense red color thus the addition of higher amount decreased consumer acceptance. In addition, the red color of red fruit is closely related to its carotenoids compound that have at least seven conjugated bonds in which the higher the number of the double bonds results in the hue of carotenoids become more red [3, 23]. Furthermore, by increasing the red fruits’ oil emulsion concentration, the aroma sensory was decreased which might due to the presence of several volatile compounds, such as 1,3-dimethyl-benzene, N-glicyl-L-alanine, trichloromethane, dan ethane [24]. In the Java tea-based functional drink, as much as 80-100% was dominated by monoterpenene(-)-limonene (d-limonene) and 5% of oxygenated terpene [16].

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| Table 1. Physicochemical properties of red fruits’ oil emulsion |
|---------------------------------------------------------------|
| **Formula** | **[CMC]** (% | **[Red fruits’ oil]** (%) | **Physical properties** | **Chemical properties** |
|--------------|---------------|----------------------------|------------------------|------------------------|
|              |               |                            | Physical properties    |                        |
|              |               |                            |                         | Total carotenoid (ppm) | Total phenolic (mg GAE/g) |
| A1B1         | 1.00          | 10                         | 31.43 ± 1.05           | 1130 ± 20              | 87 ± 1                  | 61.3662 ± 1.5531         | 1.0933 ± 0.0971 |
| A1B2         | 1.00          | 15                         | 46.64 ± 0.91           | 1366 ± 19              | 83 ± 1                  | 72.0918 ± 1.4285         | 1.6117 ± 0.0196 |
| A1B3         | 1.00          | 20                         | 56.64 ± 1.37           | 1823 ± 9.0             | 79 ± 1                  | 80.0035 ± 1.5908         | 2.0203 ± 0.0318 |
| A2B1         | 1.50          | 10                         | 20.96 ± 1.47           | 3038 ± 34              | 97 ± 1                  | 60.9464 ± 1.9379         | 1.0777 ± 0.0333 |
| A2B2         | 1.50          | 15                         | 33.04 ± 0.98           | 3426 ± 39              | 95 ± 1                  | 71.0236 ± 1.5204         | 1.6141 ± 0.0209 |
| A2B3         | 1.50          | 20                         | 42.81 ± 0.48           | 3709 ± 57              | 91 ± 1                  | 79.9255 ± 1.3348         | 2.0420 ± 0.0261 |

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### Table 2. Results of RSM toward formula from DX10 program

| Run | Emulsion concentration (%) | Total carotenoid (ppm) | Total phenolic (mg GAE/g) | Aroma | Color | Taste | Overall |
|-----|-----------------------------|------------------------|---------------------------|-------|-------|-------|---------|
| 1   | 4                           | 4.0176                 | 0.3399                    | 5.23  | 4.80  | 5.37  | 5.31    |
| 2   | 4                           | 4.0675                 | 0.3405                    | 5.27  | 4.81  | 5.41  | 5.40    |
| 3   | 7                           | 5.8926                 | 0.4223                    | 4.83  | 4.52  | 4.91  | 4.76    |
| 4   | 8                           | 6.8181                 | 0.4578                    | 4.37  | 3.24  | 3.53  | 3.57    |
| 5   | 5                           | 4.8183                 | 0.3735                    | 5.20  | 5.01  | 5.30  | 5.21    |
| 6   | 6                           | 5.2842                 | 0.3998                    | 4.89  | 4.54  | 5.01  | 5.11    |
| 7   | 8                           | 6.8518                 | 0.4572                    | 4.40  | 3.27  | 3.56  | 3.64    |

### Table 3. ANOVA model analysis from the response data of the tested functional drink

| Response           | Model     | p-value model | p-value lack of fit | Adj R-Squared | Graph |
|--------------------|-----------|---------------|---------------------|----------------|-------|
| Total carotenoid   | Cubic     | <0.0001       | 0.1311              | 0.9983         |       |
| Total phenolic     | Cubic     | <0.0001       | 0.0636              | 0.9996         |       |
| Aroma acceptance   | Quadratic | 0.0006        | 0.0569              | 0.9621         |       |
| Color acceptance   | Quadratic | 0.0021        | 0.0033              | 0.9311         |       |
| Taste acceptance   | Cubic     | 0.0023        | 0.0124              | 0.9754         |       |
Formula optimization
To obtain the optimum formula of red fruits’ oil emulsion, each factor and response were listed according to its significance as shown in Table 4. Significance and goal of all response were set as maximal to obtain the optimum parameters in the Java tea-based functional drink. The optimum formula was listed in Table 5 with predicted desirability value is 0.663 which is high enough for consumer preferable.

Verification
In the verification step, the predicted response was compared with the actual optimum formula in Table 5. It was found that both actual and experimental data was in agreement, which is remarkable. The verification result shows that all responses were in range of either CI or PI demonstrating that the predicted model was valid enough to be applied.

Profile of Found Formula and Its Comparison to the Control
The comparison between final product and control was listed in Table 6. The statistical analysis shows that the total carotenoid and phenolic content in that functional drink were significantly different (p < 0.05) with the un-added functional drink. By addition of red fruits’ oil emulsion, the color of Java tea-based functional drink was changed to become reddish color while increasing the total carotenoid content that might yield higher antioxidant activity. To sum up, the result of final product enriched with red fruit emulsion is lower than java tea-based functional drink without addition of red fruit oil emulsion, although still in the same level of acceptance. The highest difference was on aroma acceptance showing that the consumer did not prefer the final product. While for taste acceptance, the greasy sensation was found by the consumers might reduce the taste acceptability. The color acceptance was slightly higher than the control in which the color of both drinks was shown in Figure 1. Even though so, the more critical parameters were total carotenoid and total phenolic content, which were much higher in the enriched drink than in the original functional drink.

| Table 4. Parameters’ criteria for formula optimization |
|-----------------------------------------------|
| Factor dan Response | Goal      | Lower limit | Upper limit | Importance |
| Emulsion concentration (%) | is in range | 4 | 8 | 5 |
| Total carotenoid (ppm) | Maximize | 4.0176 | 6.8518 | 5 |
| Total phenolic (mg GAE/g) | Maximize | 0.3399 | 0.4578 | 5 |
| Aroma acceptance | Maximize | 4.37 | 5.27 | 5 |
| Color acceptance | Maximize | 4 | 5.01 | 5 |
| Taste acceptance | Maximize | 4 | 5.41 | 5 |
| Overall acceptance | | 4 | 5.40 | 5 |

| Table 5. Verification of the optimum final product |
|-----------------------------------------------|
| Factors and response | Actual | Prediction | SE Mean | 95 % CI | SE Pred | 95 % PI |
| Total Carotenoid (ppm) | 5.28 | 5.262 | 0.0343 | 5.2288 | 5.4475 | 0.060 | 3.15 | 5.53 |
| Phenolic Content (mg GAE/g) | 0.40 | 0.395 | 0.0007 | 0.3965 | 0.4008 | 0.001 | 0.39 | 0.40 |
| Aroma | 5.07 | 5.019 | 0.0513 | 4.8472 | 5.1319 | 0.090 | 4.74 | 5.24 |
| Color | 5.03 | 4.834 | 0.1347 | 4.4111 | 5.1593 | 0.240 | 4.13 | 5.44 |
| Taste | 5.10 | 5.182 | 0.0890 | 4.8864 | 5.4562 | 0.160 | 4.67 | 5.67 |
| Overall | 5.21 | 5.151 | 0.0345 | 5.0227 | 5.2425 | 0.081 | 4.94 | 5.33 |

| Table 6. Comparison between final optimized product and control (p<0.05) |
|-----------------------------------------------|
| Parameters | Control | Final product |
| Total titrated acid (%) | 0.30±0.00 | 0.30±0.01 |
| Viscosity (cP) | 23±3 | 35±0 |
| Hue, color | 101.5±0.77, yellow | 43.44±1.59, red |
| Total carotenoid (ppm) | 0.3691±0.1347 | 5.2791±0.0465 |
| Total phenolic (mgGAE/g) | 0.2716±0.0120 | 0.4008±0.0056 |
| Aroma acceptance | 4.89±1.20 | 5.03±1.35 |
| Color acceptance | 4.89±1.20 | 5.10±1.16 |
| Taste acceptance | 5.61±0.89 | 5.70±0.73 |
| Overall acceptance | 5.70±0.73 | 5.21±0.98 |

*Best values are shown in bold
CONCLUSIONS

The red fruits’ oil emulsion which obtained from 1.50% CMC and 20% red fruits’ oil having total carotenoid content of 72.93±1.33 ppm. Based on the RSM optimization, it was found that the optimum concentration of red fruits’ oil emulsion can be added into the Java tea-based functional drink was 5.9%. Carotenoid compounds acted as the red coloring agent in that drink which also as antioxidant. Additionally, the total carotenoid content was increased from 0.37±0.13 to 5.28±0.05 ppm. However, the consumers’ acceptance of the Java tea-based functional drink enriched with red fruit emulsion was slightly lower than its un-original particularly its flavor sensory attributes. Thus, further sensory optimization, and also the emulsion stability should be studied further.

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Figure 1. Functional drink based on cat whiskers: control (left) and after the addition of red fruits’ oil emulsion (right)
Abstrak
Minyak buah merah mengandung total karotenoid sebesar 12000 ppm, dan β-karoten merupakan karotenoid paling dominan pada buah merah. Pemanfaatan minyak buah merah sebagai ingredient pangan masih terbatas karena masalah kelarutan dan cita rasanya yang asing, aroma fenolik, dan aftertaste pahit. Pembuatan minyak buah merah menjadi emulsi minyak dalam air (o/w) dengan penambahan CMC sebagai pengemulsi dan penstabil diharapkan dapat mengatasi kendala-kendala tersebut. Penelitian ini bertujuan untuk mendapatkan emulsi minyak buah merah yang ditambahkan pada minuman fungsional berbasis kumis kucing meningkatkan total kandungan karotenoid sebagai pigmen yang memberikan warna merah dan peningkatan aktivitas antioksidan. Penentuan formula optimum menggunakan Response Surface Methodology (RSM) dengan program Design Expert 10.0.7® (DX7) Trial dengan konsentrasi emulsi minyak buah merah sebagai faktor variabel. Emulsi minyak buah merah diperoleh dari 1.50 % CMC dan 20 % minyak buah merah dengan nilai total karotenoid 72.93 ± 1.33 ppm. Konsentrasi optimum emulsi minyak buah merah yang ditambahkan ke minuman fungsional berbasis kumis kucing didapatkan sebesar 5.86 %. Penambahan emulsi minyak buah merah dapat meningkatkan total karotenoid pada minuman fungsional berbasis kumis kucing dari 0.37 ± 0.13 ppm menjadi 5.28 ± 0.05 ppm. Emulsi minyak buah merah berpotensi sebagai pewarna alami pada minuman fungsional minuman kumis kucing, namun masih perlu perbaikan dari segi sensori aroma dan rasa.

Kata kunci: buah merah, karotenoid, minuman fungsional kumis kucing, pigmen merah