# Emulsion Formulation of Perilla Oil

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## Abstract

This research aimed to determine HLB value and formulate emulsion of Perilla oil. The physiochemical characteristics of Perilla oil were first investigated. Peroxide value of Perilla oil was 4.16 meq $O_2$.$kg^{-1}$ oil and acid value was 3.35 KOH. $g^{-1}$ oil. The major fatty acids are omega-6 (17.44 %), omega-9 (14.04%). The tocopherol content was 90.57 mg.100 $g^{-1}$ oil. Moreover, % DPPH inhibition was determined at 67 ppm and 167 ppm Perilla oil and it showed 65.31 and 85.25 % inhibition, respectively. The HLB value of Perilla oil was 6.0. The emulsion was prepared by using the HLB obtained and it exhibited good stability after being subject to accelerated stability test for 1 month. The product was classified as non-irritation by primary skin irritation method. Subjective sensory assessment was evaluated by using hedonic scale method. The overall of satisfaction of volunteers after use was good.

**Keywords:** Perilla oil/HLB value/Emulsion stability/Sensory test

## Introduction

*Perilla frutescesns* (Lamiaceae family) is a traditional Chinese medicinal plant commonly used for treating a variety of diseases such as depression, inflammation, bacterial and fungal infections, allergy (Lin, Chou, Kuo & Huang, 2010). Perilla provides health-promoting which have been mainly attributed to its content of phenolic acids, flavonoids, and triterpenoids (Hong & Kim, 2010). These components provide the plant with proved antioxidant, anti-inflammatory, antibiotic, and antipyretic properties. Moreover, Perilla seed oil has been shown to be a rich source of unsaturated fatty acids, especially omega-3, omega-6, omega-9 and Vitamin E as a source of natural antioxidant to prolong quality and stability (Eckert et al., 2010). However, there is no published paper regarding Perilla oil emulsion. Thus, this study aims to determine physiochemical characteristics, antioxidant capacity. Also, HLB value, emulsion stability and subjective sensory test of Perilla oil were investigated.

## Objectives

1. To study physiochemical characteristics and antioxidant activities of Perilla oil.
2. To determine HLB value, emulsion stability and sensory test.

## Literature Review

Perilla oil is obtained from the seeds of herbs of the genus Perilla, usually from the species *Perilla frutescens.* The seeds contain 35 to 45 percent oil which is obtained by pressing method. In Asia, Perilla oil is used as edible oil that is valued more for its medicinal benefit than its flavor. The oil is a very rich source of the omega-3 fatty acid alpha-linolenic acid. About 50 to 60% of the oil consists of alpha-linolenic acid (Asif, 2011). The Perilla seed oil is shown in Figure 1.

**Figure 1** The Perilla oil

Emulsions are class of disperse systems consisting of two immiscible liquids. The liquid droplets (the disperse phase) are dispersed in a liquid medium (the continuous phase). Several classes may be distinguished: oil-in-water (O/W), water-in-oil (W/O). To disperse two immiscible liquids, one needs a third component, namely, the emulsifier. The choice of the emulsifier is crucial in the formation of the emulsion and its long-term stability.

HLB (Hydrophilic-Lipophilic Balance) is the ratio of oil-soluble portion to the water-soluble portion of the molecule and firstly developed by Griffin (Altuntas & Yener, 2015). Griffin has directed his activities to select optimal non-ionic emulsifiers ensuring the stability of the emulsion. HLB value of the emulsifier combinations are selected in a way that it is almost equivalent to the substances to be emulsified. If more than one substance will be emulsified at the same time, average weighted HLB value will be calculated based on the HLB value according to their % compositions used in the mixtures of these substances and that the emulsifier is determined according to this HLB value.

## Materials and Methods

### Sample

Perilla oil was purchased from local supermarket (Bangkok, Thailand).

### Physical Characteristics

Peroxide value was determined by using in house method based on AOAC (2005) 965.33. Acid value was determined by using in house method based on The chemical analysis of food (1962) 408-409. Vitamin E was determined by using in

house method based on BS EN 12823-1:2000 (Institute of Food Research and Product Development (IFRPD), Kasetsart University, Thailand).

**Fatty Acid Composition**

Omega-3, omega-6, omega-9 contents were determined by in house method based on Compendium of Methods for Food Analysis, Thailand (2003).

**Evaluation of Radical Scavenging Activity**

The radical scavenging activity of Perilla oil and emulsion was assayed by using DPPH method. The DPPH solution was prepared at 0.1 mM. The standard solution was prepared by dissolving 1 mg of vitamin C then added absolute alcohol 1 ml. The standard solution 300 µl was mixed with DPPH 1000 µl. Control solution was prepared by adding 300 µl of absolute alcohol then mixed with DPPH 1000 µl in test tube. The oil sample was prepared at 67 ppm and 167 ppm in absolute alcohol. The Perilla emulsion (5% w/w of seed oil in formulation) 1 g were extracted by absolute alcohol 3 ml stirred. Then, the mixture was centrifuged at 5000 rpm for 5 minutes. The clear solution of samples (300 µl) was mixed with DPPH 1000 µl in test tube. Each mixture was kept in the dark for 30 minutes and the absorbance was measured at 517 nm against a blank using UV-VIS Spectrophotometer. The ability of Perilla oil and emulsion to scavenge DPPH radical was calculated as % inhibition by the following equation:

$$\% \text{ inhibition} = \left[ \frac{\text{A control} - \text{A standard or A sample}}{\text{A control}} \right] \times 100$$

A control = Absorbance of DPPH

A standard = Absorbance of Vitamin C

A sample = Absorbance of sample

**HLB Value Screening**

The HLB value of Perilla oil was determined according to the reported method (Ferreira et al., 2010), with modifications. The Perilla emulsion was prepared at the different amount of steareth-2 (HLB 5) and steareth-21 (HLB15) in total 5% w/w of emulsifiers. HLB range was obtained from 5.0 – 15.0. The emulsion of seed

oil was homogenized at 6,500 rpm for 5 minutes then phase separation was observed at 24 hours. The less creaming index with best texture formulation was chosen to do second run. The HLB screening emulsion of second run formulation after 48 hours that provided the less creaming index with best texture will be conducted to do emulsion.

$$\% \text{ Creaming Index (CI)} = (CC/CT)*100$$

CC = Total height of cream layer

CT = Total height of emulsion

**Preparation of Emulsion**

The Perilla emulsion was prepared by using the ratio of stearerth-2 and stearerth-21 that provided the less creaming index with best texture formulation of the second run. Auxiliary emulsifiers and thickening agents were added to provide better stability of emulsion.

**Centrifugation Test**

The emulsion sample (1 g) was centrifuged at 5,000 rpm for 30 minutes and phase separation was observed. Those formulations that did not show any phase separation will be taken for heating-cooling cycle test.

**Heating-Cooling Cycle Test**

The 6 cycles test was performed by alternating conditions between 4 $^0$C (±2 $^0$C) for 24 hours and 45 $^0$C (±2 $^0$C) for 24 hours of each cycle. The pH and viscosity of emulsion were measured and phase separation, color, and odor were visually observed at cycle 0 and cycle 6.

**Accelerated Stability Test**

Perilla emulsion was kept in ambient temperature, 4 $^0$C (±2 $^0$C) and climate chamber at 45 $^0$C (±2 $^0$C). The properties included color, odor, pH, viscosity, phase separation were checked and recorded every week for 4 weeks.

### Primary Skin Irritation Test

Patch test was performed on 15 volunteers both male and female aged between 18–50 years old. Enrolled volunteers were done closed patch test by using Finn chamber with 0.2 g per each of emulsion base, Perilla emulsion, 0.1% sodium lauryl sulfate as positive control and deionized water as negative control. The patch test material was removed at the end of the 24 hours period and it was checked if any reaction such as erythema and edema occurred after 30 minutes.

### Subjective Sensory Assessment

Subjective sensory assessment was evaluated by using hedonic scale method scoring: 1 slightly; 2 few; 3 medium; 4 good; 5 excellent. The evaluation form was done by volunteers after being finished the testing period on W4.

## Results and Discussion

### Physiochemical Characteristics

The Perilla oil showed peroxide value of 4.16 meq $O_2.kg^{-1}$ oil and acid value 3.35 $KOH.g^{-1}$ oil. It can be seen that peroxide value of Perilla oil is lower than the limit (15 meq $O_2$•kg-1 oil) established by The Codex Alimentarius Commission for cold–pressed and non– refined oil (FAO, 1999). Acid value is within the limit (4.0 mg KOH•g-1 oil) established by The Codex Alimentarius Commission for cold–pressed and non– refined oil (FAO, 1999). Peroxide value is a measure of the concentration of peroxides and hydroperoxides formed in the initial stages of lipid oxidation, one of the most widely used tests for oxidative rancidity. The acid number is a measure of the amount of carboxylic acid groups in a chemical compound, such as a fatty acid, or in a mixture of compounds. According to the results, it is expected that the Perilla oil will exhibit good stability overtime.

### Fatty Acid Compositions

The major fatty acids of Perilla oil are omega-6 (17.44 %) and omega-9 (14.04%). However, the oil contains no omega-3, which is different from the reported paper (Asif, 2011). The area crops, harvesting time, processing, storage conditions are

affected the amount of essential fatty acids and other substances and these may explain the finding in this work. Vitamin E content in Perilla oil was 90.57 mg.100g $^{-1}$ oil. It acts as natural antioxidant and retards the rancidity of seed oil (Choe & Min, 2006).

**Evaluation of Radical Scavenging Activity**

In this work, radical scavenging activity of Perilla oil was investigated by DPPH assay. This method is based on a single electron transfer mechanism and measures the ability of the antioxidants in oil to reduce a stable DPPH radical (Prescha et al., 2014). The standard solution of Vitamin C (1 ppm) showed 94.80 % inhibition. The Perilla oil 67 ppm and 167 ppm showed 65.31 and 85.25 % inhibition, respectively. The Perilla emulsion prepared at 5% w/w oil showed 89.61 % inhibition. However, Perilla oil still has the lower % inhibition than that of Vitamin C.

**HLB Value**

In order to determine the HLB value of Perilla oil, the emulsion was prepared by using steareth-2 (HLB5) and steareth-21 (HLB 15) as emulsifiers. The total concentration of steareth-2 and steareth-21 was fixed at 5.0 % w/w and the amount ratio was varied to obtain the HLB in the range of 5 to 15. The seed oil in formulation was fixed 5.0 % w/w. Then, the % CI values were determined from the ratio between the total height of cream layer (CC) and the total height of emulsion layer (CT). CC and CT were measured directly in storage glass bottle after being stored for 24 hours. The emulsion showed the lowest % CI is that with HLB value 6.0 (FP2, Table 1) which will be taken to do the second run.

**Table 1** HLB value and % creaming index of Perilla oil at 24 hours

| **Formula** | **% w/w Steareth-2** | **% w/w Steareth-21** | **HLB** | **CC** | **CT** | **%CI** |
|---|---|---|---|---|---|---|
| FP1 | 5.00 | 0.00 | 5.0 | 0.2 | 4.5 | 4.4 |
| FP2 | 4.50 | 0.50 | 6.0 | 0.1 | 4.5 | 2.2 |
| FP3 | 4.00 | 1.00 | 7.0 | 0.5 | 4.5 | 11.1 |

| | | | | | | |
|---|---|---|---|---|---|---|
| FP4 | 3.50 | 1.50 | 8.0 | 0.3 | 4.5 | 6.7 |
| FP5 | 3.00 | 2.00 | 9.0 | 0.3 | 4.5 | 6.7 |
| FP6 | 2.50 | 2.50 | 10.0 | 0.5 | 4.5 | 11.1 |
| FP7 | 2.00 | 3.00 | 11.0 | 0.4 | 4.5 | 8.9 |
| FP8 | 1.50 | 3.50 | 12.0 | 0.4 | 4.5 | 8.9 |
| FP9 | 1.00 | 4.00 | 13.0 | 0.4 | 4.5 | 8.9 |
| FP10 | 0.50 | 4.50 | 14.0 | 0.6 | 4.5 | 13.3 |
| FP11 | 0.00 | 5.00 | 15.0 | 0.6 | 4.5 | 13.3 |

**Note.** HLB = Hydrophilic Lipophilic Balance

The second run experiments were then repeated with emulsifiers in different amount for 3 formulations at HLB 5.5, 6.0 and 6.5. It was found that the prepared emulsion with HLB 6.0 (FP13), steareth-2 4.50 % w/w and steareth-21 0.50 % w/w exhibited lowest % CI and good texture. Thus, the HLB of Perilla oil was approximately 6.0. HLB value and % creaming index are shown in Table 2. Emulsions of Perilla oil with different HLB value are shown in Figure 2.

**Table 2** HLB and % Creaming index of Perilla oil calculated at 24 and 48 hours

| **Formulation** | **% w/w Steareth-2** | **% w/w Steareth-21** | **HLB** | **24 hours** | | | **48 hours** | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | **CC** | **CT** | **%CI** | **CC** | **CT** | **%CI** |
| FP12 | 4.75 | 0.25 | 5.5 | 0.2 | 4.5 | 4.4 | 0.2 | 4.5 | 4.4 |
| FP13 | 4.50 | 0.50 | 6.0 | 0.1 | 4.5 | 2.2 | 0.1 | 4.5 | 2.2 |
| FP14 | 4.25 | 0.75 | 6.5 | 0.4 | 4.5 | 8.9 | 1.0 | 4.5 | 22.2 |


**Figure 2** Emulsions of Perilla oil with different HLB value

**Formulation of Perilla Emulsion**

Perilla emulsion was prepared by using 4.50 % w/w steareth-2, 0.50 % w/w steareth-21, 5.0 % w/w Perilla oil and co-emulsifiers (such as stearic acid 5.0 % w/w, cetyl alcohol 5.0 % w/w, glyceryl monostearate 2.0 % w/w), thickening agent (ammonium acryloyldimethyl taurate/ VP copolymer 0.5 % w/w) were added to provide better stability in the formulation. In addition, dimethicone 2.0 % w/w, butylated hydroxytoluene 0.05 % w/w, glycerine 3.0 % w/w, triethanolamine 0.50 % w/w and preservative 1.0 % w/w were also added. The emulsion obtained has creamy pale yellow color with pH 4.62 and characteristic seed oil odor.

**Centrifugation Test**

Gravitational stability test of Perilla emulsion was evaluated by centrifugation at 5,000 rpm for 30 minutes and there was no phase separation observed.

**Heating-Cooling Cycle Test**

After the centrifugation test was done. The emulsions were stored at 4 $^{0}$C (±2 $^{0}$C) and then in climate chamber at 40 $^{0}$C (±2 $^{0}$C) for 6 cycles. The pH and viscosity were recorded at cycle 0 and cycle 6. The results showed that pH and

viscosity of Perilla emulsion decreased from 4.63 to 4.34 and from 37,226 cP to 21,066 cP. However, emulsion showed no phase separation.

**Accelerated Stability Test**

The Perilla emulsion was kept in ambient temperature, 4 $^0$C (±2 $^0$C) condition and 45 $^0$C (±2 $^0$C) condition for 1 month. The products stored at ambient temperature and 4 $^0$C (±2 $^0$C) condition showed slight change after the first week in term of color, odor, pH and viscosity. However, emulsion stored at heating condition showed obvious change. The color of emulsion changed to more yellow at W4. The pH decreased from 4.62 to 4.48 and viscosity reduced from 37,226 cP to 22,895 cP. However, there was no phase separation and emulsion showed good stability.

**Primary Skin Irritation Test**

The skin irritation test of emulsion was tested by using closed patch test finn chamber for 24 hours. The patch test was removed at the end of the test period. There were no irritation on the skin of volunteers. The products are classified as non-irritation by primary skin irritation method.

**Subjective Sensory Assessment**

Subjective sensory assessment was evaluated by using hedonic scale method scoring: 1 slightly; 2 few; 3 medium; 4 good; 5 excellent. The 15 volunteers were evaluated satisfaction after being finished the test at W4. The average result score showed that color was the highest score 4.2 and odor was the lowest score 3.8. The satisfaction average score was 4.2. The overall of satisfaction of volunteers after use was good, Table 3. Thus, if the Perilla oil will be used in the emulsion product, the fragrance or essential oil should be added in order to obtain a cosmetically acceptable product.

**Table 3** Subjective sensory assessment

| **Parameter** | **Score** | **±SD** |
|---|---|---|
| Texture of product | 3.8 | 0.81 |
| Color of product | 4.3 | 0.80 |
| Odor of product | 3.3 | 1.40 |
| Spreading on skin | 3.7 | 0.88 |
| Penetration on skin | 3.9 | 0.83 |
| Moisturization after use | 4.0 | 0.93 |
| Elasticity after use | 4.1 | 0.70 |
| Smoothness after use | 4.1 | 0.64 |
| Satisfaction after use | 4.3 | 0.80 |

## Conclusion

In this work, The physiochemical characteristics of Perilla oil were first investigated. Peroxide value of Perilla oil was 4.16 meq $O_2.kg^{-1}$ oil and acid value was 3.35 $KOH.g^{-1}$ oil. The major fatty acids are omega-6 (17.44 %), omega-9 (14.04%). The tocopherol content in the oil was 90.57 mg.100 $g^{-1}$ oil. Moreover, % DPPH inhibition was determined and it was found that the Perilla oil at 67 ppm and167 ppm showed 65.31 and 85.25 % inhibition, which is considered lower when compared with Vitamin C. The HLB value of Perilla oil was determined and it has HLB of 6.0. It exhibited good stability after being subject to accelerated stability test for 1 month. The emulsion is classified as non-irritation by primary skin irritation method. Subjective sensory assessment was evaluated by using hedonic scale method. The average result score showed that color was the highest score 4.2 and odor was the lowest score 3.8. The satisfaction average score was 4.2.

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