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# Preparation of Microemulsion Containing *Sacha Inchi* Seed Oil

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**Abstract**

Microemulsions containing (*Sacha inchi* seed oil and PPG-15 stearyl ether at 1:1 (w/w)) and $S_{mix}$ (polysorbate 20 and sorbitan monolaurate) and deionized water were prepared and analysed. The stable oil in water microemulsion contained $S_{mix}$ less than 50% w/w was SPPG8-11. This formula composed of polysorbate 20 (34.29 ml), sorbitan monolaurate (8.57 ml), *S. inchi* seed oil (2.38 ml), PPG-15 stearyl ether (2.38 ml) and deionized water (52.38 ml). The appearance of SPPG8-11 exhibited yellowish transparent and homogeneous emulsion with particle size of 15.50 ± 0.47 nm, narrow PdI (0.32 ± 0.03) and negative zeta potential charge (-30.03 ± 0.57 mV). The differences on pH value, viscosity, particle size, PdI and zeta potential of SPPG8-11 exhibited less than 10% after 6 heating-cooling cycles. According to the result, a long-term stability has been suggested to be conducted for further usage of microemulsion containing *S. inchi* seed oil in cosmetic applications.

**Keywords:** Microemulsion/ *Sacha inchi* seed oil/ Cosmetics/ Pseudoternary phase diagram

## 1. Introduction

*Sacha inchi* (*S. inchi*) or Inca nut has been known to be enriched with the fatty acids, proteins, tocopherols, phytosterols, phenolic compounds, with antioxidant activities (Chirinos et al., 2013). As *S. inchi* seed oil has excellent profile in term of the unsaturated fatty acids, it renders exceptional benefits for skin, including wound healing, moisturizing effect, and anti-inflammatory properties. For this reason, many personal care products with *S. inchi* seed oil gain popularity amongst consumers. However, *S. inchi* seed oil has been problematic to the formulators due to its stability issue affecting the long-term quality of the finish good.

Microemulsion has been well-known formulation technique to improve the quality of cosmetic ingredients according to its solubilisation, bioavailability, delivery and stability. Most importantly, this form offers easy and spontaneous formation requiring low energy process. The study aims to examine for the appropriate proportion of water, oil, and mixed surfactants to achieve the formation of stable microemulsion containing *S. inchi* seed oil. The examination was executed by constructing pseudo-ternary phase diagram using water titration method, classifying and evaluating the particle size of microemulsion by using the particle size analyser. Furthermore, the study performs stability test by observing its appearance, measuring the viscosity and pH value, and applying accelerated stability test, to ensure the quality of the microemulsion under various factors.

## 2. Research Objectives

2.1 To prepare microemulsion containing *S. inchi* seed oil

2.2 To study the feasibility of the microemulsion formation with appropriate chosen proportion

2.3 To test the physico-chemical stability of microemulsion containing *S. inchi* seed oil

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## 3. Literature Reviews

### 3.1 *S.Inchi*

.*S. inchi*, *Plukenetia volubilis* L., Inca peanut, or mountain peanut, is a perennial plant belonging to the family Euphorbiaceae. The plant is native to the amazon rainforest, Peru, and has been commercially cultivated in Southeast Asia (Hanssen & Schmitz-Hübsch, 2011). Several studies on the nutritional facts of *S. inchi* have revealed that *S. inchi* seed contains interesting number of phytochemicals, which are important health promoting dietary source (Chirinos, et.al., 2013). The chemical components that presents in the *S. inchi* seed are remarkably high particularly on the fatty acid components (Gutiérrez, Rosada & Jiménez, 2011). Additionally, several chemical compounds of phytochemical can be found in *S. inchi* seed, including tocopherol, phenolic compounds and phytosterol.

For centuries, *S. inchi* has been cultivated and used by indigenous people for food and cosmetic. It has also been traditionally used in medicinal purpose for treating aching muscles and rheumatic problems. In a present day, *S. inchi* seed is widely available in the supermarket demonstrating a high economic value. The immense need of *S. inchi* seed product has been introduced to the market due to its nutritional facts that are health-promoting. *S. inchi* seed products are available in many forms such as edible oil, dietary supplement, and personal care products.

### 3.2 Microemulsion

Microemulsions are known to have the smallest particle size all of emulsion, which is less than 100 d.nm (Murthy, & Shivakumar, 2010). Microemulsions are a type of emulsion that is thermodynamically stable, kinetically stable, clear, and optically isotropic systems. They are the mixture of a suitable proportion of water, oil, and amphiphile blend. Microemulsions spontaneously form upon simple mixing without any additional input of energy, which ease of manufacturing and scaling up. It can be prepared by water titration method and phase inversion technique. Microemulsions additionally render advantages on improving drug solubilisation, bioavailability, and stability as well as being a vehicle for drug delivery in pharmaceutical and cosmetics applications. Nevertheless, microemulsions can induce dermal irritation as the system requires high amount of surfactant (Alany et. al, 2000).

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### 3.3 Related literatures

Alany, et al., (2000) conducted the study on the effect of co-surfactant to reduce the concentration of surfactant in microemulsion formula. The study used four aliphatic alcohol, including 1-propanol, 1-butanol, 1-hexanol, and 1-octanol, and four 1,2-alkanediol, including 1,2- propanediol (Propylene glycol), 1,2-pentanediol (Pentylene glycol), 1,2-hexanediol, 1,2-octanediol (Caprylyl glycol) as co-surfactants. Polyoxyethylene 20 sorbitan monooleate (Polysorbate 20):sorbitan monolaurate:co-surfactant was at 0.42:0.28:0.30, which was regarded to be an optimal ratio for maximum water solubilisation. Ethyl oleate was used in oil phase. With its ability to solubilize high percentage of both water and oil, 1-butanol and 1,2-hexanediol facilitates the formation of balance microemulsion at low surfactant concentration (Alany et. al, 2000).

Lourith, Kanlayavattanakul, and Ruktanonchai (2016) conducted a study on formulation of microemulsion containing *Moringa oleifera* oil aiming to develop its bioavailability. The test was performed by mixing *Moringa oleifera* oil, Lexol® 865, polysorbate 80 and sorbitan monooleate and using grading system to visually identify the microemulsion. The stability test and antioxidant capacity were conducted afterward. As a result, the prepared microemulsion showed good result on the viscosity (280.00-517.20 mPa•s) as well as the acceptable range of pH (pH 6.75-8.23) (Lourith, Kanlayavattanakul & Ruktanonchai, 2016).

## 4. Research Methodology

### 4.1 Preparation of microemulsion containing *Sacha inchi* seed oil using pseudo-ternary phase diagram

In this experiment, two systems of emulsions were prepared.

4.1.1 The $S_{mix}$ of first system was prepared by using surfactants (tween 20 or TW20 and span 20 or SP20) and co-surfactant (1,2-hexanediol or HXD) (Alany et. al, 2000). The mass ratios of $S_{mix}$ (TW20:SP20:HXD) of this system microemulsion were 42:28:30, 5:65:30 and 10:65:25. The screening process was proceeded to identify the optimal ratio between *S. inchi* seed oil and $S_{mix}$ at 1:9, 3:7, 5:5, 7:3 and 9:1 (w/w) (Lourith, Kanlayavattanakul & Ruktanonchai, 2016).

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One ml of deionized water will be then slowly and progressively added by water titration method (Alany et. al, 2000). The deionized water was added until the total quantity of deionized water reached to 20 ml. The mixtures were blended via magnetic stirrer at 250 rpm until they were completely homogeneous (Preziosi, Perazzo, Caserta, Tomaiuolo, & Guido, 2013).

4.1.2 In the second system, the oil phase contained *S. inchi* seed oil and PPG-15 stearyl ether at 1:1 (w/w) ($O_{mix}$). The mass ratios of $S_{mix}$ (TW20: SP20) of the second system microemulsion were 10:90, 20:80, 30:70, 40:60, 50:50, 60:40, 70:30, 80:20 and 90:10. Accordingly, $O_{mix}$ was mixed into $S_{mix}$ by starting at 1:9, 3:7, 5:5, 7:3 and 9:1 (w/w) ($O_{mix}$: $S_{mix}$) (Lourith, Kanlayavattanakul & Ruktanonchai, 2016). Likewise, the preparation processes of this system were similar to the first system.

The formation of microemulsions was inspected by visual grading system (Lourith, Kanlayavattanakul & Ruktanonchai, 2016) and the results were recorded and applied to the construction of pseudo-ternary phase diagram. Based on the grading system, the emulsion could be identified into five categories and illustrated on Figure 1 as follows:

1. The microemulsions rapidly form within 1 minute with a clear or bluish appearance.
2. The emulsions rapidly form; however, the emulsions are slightly less clear with bluish-white appearance compared to the first category.
3. The emulsions form within 2 minutes with fine milky appearance.
4. The emulsions appear dull or greyish white with slightly oily appearance. The formation takes more than 2 minutes to emulsify.
5. The emulsions are poorly or minimally emulsified with large oil globules floating on the surface.

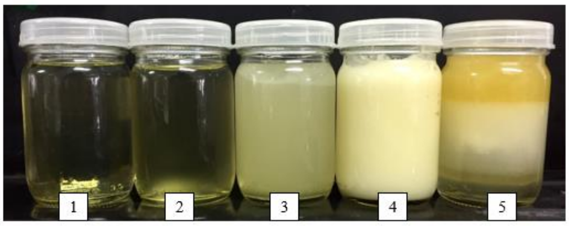


**Figure 1** The comparison of emulsion via grading system

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After the classification of emulsion using grading system, the ratio of microemulsions classified in the first categories was selected and left for 3 days in the ambient condition at 25ºC ± 5ºC allowing the emulsion to complete its transport process. The samples that remained clear and homogeneous with less than 50% (w/w) of $S_{mix}$ were scaled up from 30 ml to 300 ml for further examination on its physico-chemical stability (Lourith, Kanlayavattanakul & Ruktanonchai, 2016).

**4.2 Stability testing on selected microemulsions**

In this study, physico-chemical stability tests of microemulsions were conducted in an accelerated condition.

4.2.1 Heating-cooling cycles

The selected microemulsion was filled in a fully sealed glass jar. These jars were kept under 4ºC for 24 hours and switched to 45 ºC for 24 hours. The processes were replicated for 6 cycles (Shukla et al., 2016). Accordingly, the samples were stable, taken to evaluate the appearance, size, pH and viscosity. These processes were proceeded two times, before and after heating-cooling cycles test (Szumała & Wysocka, 2018).

4.2.2 Particle size analysis

At 25ºC ± 5ºC, the undiluted microemulsion sample was dropped into deionized water at 1:9 (w/w). The sample was proceeded to the particle size analysis process with particle size analyzer.

4.2.3 Viscosity

The viscosity of the microemulsion samples was assessed by using viscometer. The measurement was conducted at 25ºC ± 5ºC with spindle No.2 (Lourith, Kanlayavattanakul & Ruktanonchai, 2016). In each sample, the measurement was triplicated and the average number of the result was calculated and recorded.

4.2.4 pH Value

Under the room temperature (25ºC ± 5ºC), the microemulsions were measured by using pH meter (Lourith, Kanlayavattanakul & Ruktanonchai, 2016). The measurement was triplicated and the average number of the result in each microemulsion sample was calculated and recorded.

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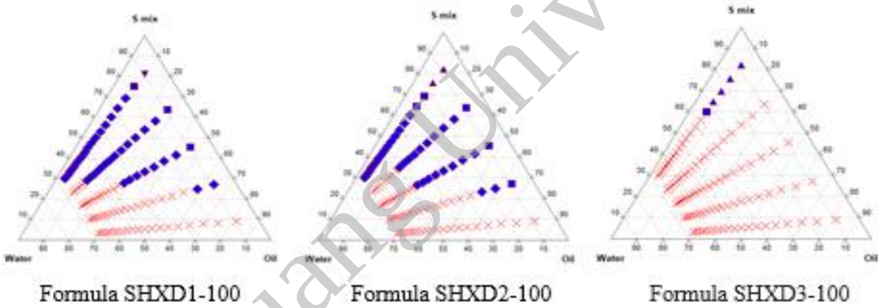
4.3 Conclusions

All collected data were analysed and summarize.

## 5. Results and Discussion

### 5.1 Result on preparation of microemulsion containing *Sacha inchi* seed oil using pseudoternary phase diagram

For the first system, a total numbers of tested formulas were 300 formulas. According to Figure 2, pseudoternary phase diagram prepared by using *S. inchi* seed oil and $S_{mix}$ at 1:9, 3:7, 5:5, 7:3 and 9:1 (w/w), respectively. The emulsions were classified by grading system, resulted in 6 formulas of grade 1, 1 formula of grade 2, 9 formulas of grade 3, 75 formulas of grade 4 and 209 formulas of grade 5.




**Note.** ( ▲ = Grade 1, ▼ = Grade 2, ■ = Grade 3, ◆ = Grade 4, × = Grade 5)

**Figure 2** Pseudoternary phase diagram of microemulsion containing $O_{mix}$ using TW20 : SP20 in a different mass ratio as a $S_{mix}$.

For the second system, this experiment produced 900 formulas. Figure 3 represented the pseudoternary phase diagram of microemulsion. The mixtures of $O_{mix}$: $S_{mix}$ were prepared at 1:9, 3:7, 5:5, 7:3, and 9:1 (w/w). Classifying by grading system, there were 27 formulas of grade 1, 7 formulas of grade 2, 28 formulas of grade 3, 482 formulas of grade 4 and 356 formulas of grade 5.

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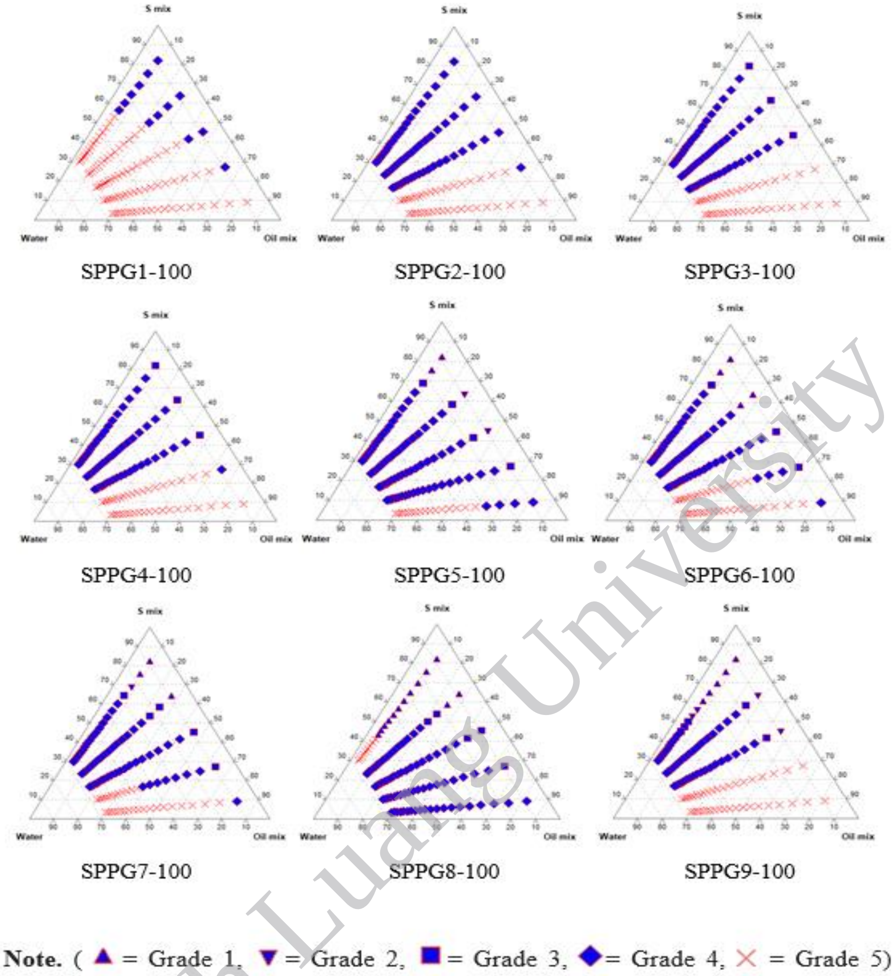


**Figure 3** Pseudoternary phase diagram of microemulsion containing $O_{mix}$ using TW20 : SP20 in a different mass ratio as a $S_{mix}$.

**5.2 Result on preliminary screening of microemulsion containing *S. inchi* seed oil**

After the grading process, the preliminary screening of the microemulsion was initiated by selected the grade 1 emulsion that contained $S_{mix}$ less than 50% (w/w). The selected samples were kept at 25ºC ± 5ºC for three days to let them complete their emulsification process. Accordingly, the emulsions that remained grade 1 emulsion (Table 1) were collected and proceeded to physico-chemical stability test.

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**Table 1** Grade 1 microemulsion formulas containing *S. inchi* seed oil.

| Formula | TW20 (ml) | SP20 (ml) | HXD (ml) | PPG-15 stearyl ether (ml) | *S. inchi* (ml) | Water (ml) | Appearance |
|---|---|---|---|---|---|---|---|
| SHXD2-1 | 8.18 | 40.91 | 32.73 | - | 9.09 | 9.09 | Turbid yellowish liquid |
| SHXD2-2 | 7.50 | 37.50 | 30.00 | - | 8.33 | 16.67 | Turbid yellowish liquid |
| SHXD3-4 | 9.64 | 38.57 | 16.07 | - | 7.14 | 28.58 | Turbid yellowish liquid |
| SPPG8-8 | 40.00 | 10.00 | - | 2.78 | 2.78 | 44.44 | Phase separation |
| SPPG8-9 | 37.89 | 9.47 | - | 2.63 | 2.63 | 47.38 | Phase separation |
| SPPG8-10 | 36.00 | 9.00 | - | 2.50 | 2.50 | 50.00 | Clear yellowish liquid |
| SPPG8-11 | 34.29 | 8.57 | - | 2.38 | 2.38 | 52.38 | Clear yellowish liquid |

Referring to the Table 1, seven formulas were left to be equilibrated in a condition at 25ºC ± 5ºC for three days. As a result, SHXD2-1, SHXD2-2. SHXD3-4, SPPG8-8 and SPPG8-9 exhibited turbidity and phase separation. These emulsions were excluded from the experiment due to the stability issue (Lourith, Kanlayavattanakul & Ruktanonchai, 2016). On the other hand, the change in the characteristic of the microemulsion did not exhibit in SPPG8-10 and SPPG8-11. The selected microemulsions were formula SPPG8-10 and SPPG8-11 because the emulsions remained clear after 3 days.

### 5.3 Result on the stability testing on selected microemulsions

Selected microemulsions containing *S. inchi* seed oil including SPPG8-10 and SPPG8-11 were proceeded to undergo stability test. The result of the evaluation on microemulsions' stability was shown on Figure 4 and Table 2.

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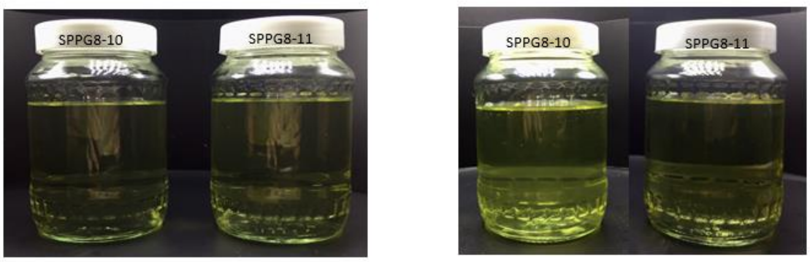


(A) (B)

**Figure 4** The appearance of selected microemulsions before (A) and after (B) heating-cooling cycles

**Table 2** Physico-chemical stability of selected microemulsion before and after heating-cooling cycles

| Parameters | SPPG8-10 | | SPPG8-11 | |
|---|---|---|---|---|
| | **Before** | **After** | **Before** | **After** |
| Appearance | Clear yellowish liquid | Clear yellowish liquid | Clear yellowish liquid | Clear yellowish liquid |
| pH Value | 6.31 ± 0.01 | 6.02 ± 0.01 | 6.07 ± 0.00 | 6.41 ± 0.01 |
| Viscosity* | 466.50 ± 0.50 | 450.17 ± 0.29 | 440.00 ± 0.00 | 438.00 ± 0.00 |
| Particle Size (d.nm) | 14.05 ± 0.90 | 19.46 ± 1.86 | 15.50 ± 0.47 | 15.74 ± 0.92 |
| PdI | 0.26 ± 0.02 | 0.46 ± 0.03 | 0.32 ± 0.03 | 0.29 ± 0.00 |
| Zeta Potential (mV) | -43.20 ± 0.40 | -24.1 ± 1.21 | -30.03 ± 0.57 | -28.07 ± 1.50 |

**Note.** *The viscosity was measured with #2, 80 rpm, %torque ≥ 80, and at 25ºC ± 5ºC.

According to the Table 2 and Figure 4, both formulas exhibited light yellowish clear and homogeneous microemulsions (grade 1 emulsion). The particle size measurements of SPPG8-10 and SPPG8-11 were identified within the range of 10 to 100 nm. The zeta potential measurement of both formulas owned negative charge, although the mixed surfactants and oils of both formulas were non-ionic. The negative charge was given by deionized water that used in the formulas.

After the heating-cooling cycles, SPPG8-10 became slightly turbid. Nevertheless, the PdI value of SPPG8-10 considerably increased, indicating the issue on the possibility of the emulsion droplet to aggregate and lead to the state of emulsion destabilization (Sharma, 2018). As a result, SPPG8-10 was not considered to be a stable microemulsion that it was eventually excluded in this experiment, whereas the PdI value of SPPG8-11 remained ≤ 0.4, which was acceptable (Sharma, 2018). In a general emulsion system, zeta potential requires more than plus or minus 30 mV implicating that the system is stable (Sharma, Shukla, Misra, & Mishra, 2014). The difference between the before and after heating-cooling cycles of SPPG8-11 presented pH value of 5.66 ± 0.10%, viscosity of 0.45 ± 0%, particle size of 5.68 ± 5.23%, PdI of 9.68 ± 8.80% and zeta potential of 6.54 ± 4.74% respectively. Henceforth, the stability of SPPG8-11 was generally acceptable according to zeta potential. SPPG8-11 exhibited a good stability after the heating-cooling cycle. In

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addition to this study, long-term stability of SPPG8-11 should be conducted aiming to investigate the product shelf-life in different conditions.

**6. Conclusions and Suggestions**

A stable microemulsion containing *S. inchi* seed oil can be prepared by using low energy method at the optimum ratio of TW20 : SP20 at 80:20 (w/w) and the $O_{mix}$ containing *S. inchi* seed oil:PPG-15 stearyl ether at 1:1 (w/w), which was SPPG8-11. The appearance of SPPG8-11 was light yellowish clear liquid with particle size (15.50 ± 0.47 d.nm), narow PdI (0.32 ± 0.03) and negative zeta potential charge (-30.03 ± 0.57 mV). The differences on pH value, viscosity, particle size, PdI and zeta potential of SPPG8-11 exhibited less than 10% after heating-cooling 6 cycles.

Nevertheless, long term stability test of the microemulsion are encouraged to be conducted. Additionally, the extended study on $O_{mix}$ mass ratio is encouraged. The mass ratio in a future study may conducted at 6:4, 7:3, 8:2, or 9:1 (w/w). Also, the efficacy test of microemulsion containing *S. inchi* seed oil is suggested to be proceeded for further application in cosmetic products.

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