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Determination of hydrophilic–lipophilic balance value and emulsion properties of sacha inchi oil



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ABSTRACT

Objective: To determine hydrophilic–lipophilic balance (HLB) value, stability of formulate emulsion and properties of sacha inchi oil.

Methods: The physicochemical characteristics of sacha inchi oil were first investigated. Free radical scavenging property was studied by DPPH assay. HLB value of sacha inchi oil was experimentally determined by preparing the emulsion using emulsifiers at different HLB value. Sacha inchi oil emulsion was prepared using the obtained HLB and its stability was conducted by centrifugation, temperature cycling, and accelerated stability test. The efficiency of the prepared emulsion was clinically investigated by 15 volunteers. The primary skin irritation was performed using closed patch test. Subjective sensory assessment was evaluated by using 5-point hedonic scale method.

Results: Peroxide value of sacha inchi oil was 18.40 meq O₂/kg oil and acid value was 1.86 KOH/g oil. The major fatty acids are omega-3 (44%), omega-6 (35%) and omega-9 (9%). The vitamin E content was 226 mg/100 g oil. Moreover, sacha inchi oil (167 ppm) and its emulsion showed 85% and 89% DPPH inhibition, respectively. The experimental HLB value of sacha inchi oil was 8.5. The sacha inchi oil emulsion exhibited good stability after stability test. The emulsion was classified as non-irritant after tested by primary skin irritation method. The skin hydration value significantly increased from 38.59 to 45.21 ($P < 0.05$) after applying sacha inchi oil emulsion for 1 month and the overall product satisfaction of volunteers after use was with score of 4.2.

Conclusions: This work provides information on HLB value and emulsion properties of sacha inchi oil which is useful for cosmetic and pharmaceutical application.

1. Introduction

Sacha inchi (*Plukenetia volubilis* L.), commonly known as sacha peanut, mountain peanut or inca-peanut, a perennial plant with somewhat hairy leaves, belongs to the Euphorbiaceae family [1]. It is an oilseed and originated from the Amazon Rainforest in Peru [2]. It is now also being cultivated commercially in South East Asia, most notably in Thailand. It has been reported that sacha inchi oil possesses a unique balance of omega-3, 6 and 9 essential fatty acids which were not found in other vegetable oils [3]. The oil contains omega-3 linolenic acid at about 45%–53%, omega-6 linoleic acid 34%–

39% and non-essential omega-9 about 6%–10% of fat content [2,4,5]. In addition, sacha inchi oil has high protein and rich in alpha-tocopherols, beta-sitosterol, stigmaterol and carotenoids [6]. There is also a report on assessing acceptability and side-effects of sacha inchi oil consumption by an oral administration in adult human subjects. The results indicated that sacha inchi oil consumed is safe and has good acceptability [7]. Sacha inchi oil is currently gaining international recognition for its healthy properties and can be used in the food and cosmetic industries. It is registered under the INCI name of *Plukenetia volubilis* seed oil, and is registered to function as emollient (soften and smoothen the skin), humectants, and skin protector [8]. Use of oils in skin care products especially those in emulsion base, the sensory and stability properties of the product are cosmetically concerned [9]. However, there is no published paper regarding sacha inchi oil emulsion. Thus, this study aims to determine hydrophilic–lipophilic balance (HLB) value of sacha inchi oil. Its emulsion was prepared and

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stability was investigated. The clinical studies regarding efficiency and subjective sensory test were studied. Moreover, physiochemical and antioxidative property of the sachai inchi oil was also reported.

2. Materials and methods

2.1. Materials

Sachai inchi oil was purchased from Chiangrai Agriculture Development Co., Ltd (Chiangrai, Thailand). 2,2-diphenyl-1-picrylhydrazyl (DPPH) from Sigma Aldrich. Steareth-2 and steareth-21 from Evonik Industries, Germany. All other ingredients for emulsion formulation were of cosmetics grade.

The peroxide and acid values were determined by Institute of Food Research and Product Development, Kasetsart University, Thailand, using in-house method based on AOAC (2005) 965.33 and AOAC (2009) Cd 3d-63 [10]. Vitamin E content in sachai inchi oil was also determined by Institute of Food Research and Product Development, Thailand, using an in-house method based on BS EN 12823-1:2000 [10].

2.2. HLB value screening and preparation of emulsion

The HLB value of sachai inchi oil was determined according to the reported method [11] with modifications. A series of sachai inchi oil emulsion was prepared at the different amount of emulsifiers: steareth-2 (HLB 5) and steareth-21 (HLB 15) in total 5% w/w emulsifiers. HLB range was obtained from 5 to 15. The emulsion was homogenized at 6500 rpm for 5 min at $(75 \pm 2)^\circ\text{C}$, then it was cooled to room temperature and phase separation was observed at 24 h. Creaming index (CI) was determined from the total height of serum layer over the total height of emulsion layer [12,13]. The less % CI with best texture formulation was chosen to do the second run. The HLB screening emulsion of second run formulation after 48 h that provided the less CI with best texture will be used to prepare sachai inchi oil emulsion for stability and efficiency study.

Sachai inchi oil emulsion was prepared by using 3.25% w/w steareth-2, 1.75% w/w steareth-21, 5.0% w/w sachai inchi oil. Co-emulsifiers (such as stearic acid 5.0% w/w, cetyl alcohol 5.0% w/w, glyceryl monostearate 2.0% w/w), and thickening agent (ammonium acryloyldimethyltaurate/VP copolymer 0.5% w/w) were added in the formulation to provide better stability. In addition, dimethicone 2.0% w/w, butylated hydroxytoluene 0.05% w/w, glycerine 3.0% w/w, triethanolamine 0.50% w/w and preservatives 1.0% w/w were also added.

2.3. Stability of sachai inchi oil emulsion

Gravitational stability test of sachai inchi oil emulsion was evaluated by centrifugation at 5000 rpm for 30 min and there was no phase separation observed. After the centrifugation test was done, the emulsion was subject to heating-cooling cycle test and accelerated stability test. Emulsion (100 g) was filled in glass jars to permit easy observation and physical measurements at intervals. The emulsion was stored at ambient temperature, $(4 \pm 2)^\circ\text{C}$ for 24 h and then in climate chamber at $(45 \pm 2)^\circ\text{C}$, 24 h, for 6 cycles. The pH and viscosity of emulsion were measured and phase separation, color, and odor were visually observed at cycle 0 and cycle 6 in heating-cooling cycle test.

And the properties included color, odor, pH, viscosity, phase separation were checked and recorded every week for 4 weeks in accelerated stability test.

2.4. DPPH radical scavenging activity

The radical scavenging activity of sachai inchi oil and its emulsion was assayed by using DPPH method [14]. This method is based on a single electron transfer mechanism and measures the ability of the antioxidants of oil to reduce a stable DPPH radical [15]. The DPPH solution was prepared at 0.1 mM. The standard solution was prepared by dissolving 1 mg of vitamin C in 1 mL absolute alcohol. 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 sachai inchi oil emulsion (5% w/w of seed oil in formulation) 1 g was extracted by dissolving in 3 mL absolute alcohol and the mixture was then centrifuged at 5000 rpm for 5 min. 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 min and the absorbance was measured at 517 nm against a blank using UV-VIS spectrophotometer. The ability of sachai inchi oil and its emulsion to scavenge DPPH radical was calculated as % inhibition by the following equation:

$$\% \text{Inhibition} = \left[\frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \right] \times 100$$

where A control is the absorbance of the control reaction (containing all reagents except the test compound), and A sample is absorbance of test sample.

2.5. Clinical study

2.5.1. Primary skin irritation test

Patch test was performed on 15 volunteers both male and female aged between 18 and 50 years old. Closed patch test was done on the enrolled volunteers by using Finn chamber with approximately 0.2 g of sachai inchi oil emulsion, 0.1% sodium lauryl sulfate as positive control and deionized water as negative control. The patch was removed at the end of the 24 h period and it was checked if any reaction such as erythema and edema occurred after 30 min and 24 h after patch removal.

2.5.2. Skin efficacy test

Efficacy test was performed with the enrolled 15 volunteers by applying 2 mg/cm^2 of emulsion on an inner forearm twice a day for 4 weeks. During the testing period, the volunteers were not allowed to use any skin care products on the forearm where the product would be used. Then, skin hydration was measured by Corneometer CM825 (Courage and Khazaka, Germany), skin scaliness measured by Skin Visioscan VC98 (Courage and Khazaka, Germany), at W0 and W4.

2.5.3. Subjective sensory assessment

Subjective sensory assessment was evaluated by using 5-score hedonic scale method where 1 is dislike extremely, 2 is dislike, 3 is neither like nor dislike, 4 is like and 5 is like extremely. The evaluation form was done by volunteers after being finished the testing period on W4.

2.6. Statistical analysis

The comparison of all parameters was accomplished by analysis of SPSS version 21 (SPSS Inc, Chicago, USA). Significant differences between means were determined by pair *t* test. Finally, *P* values less than 0.05 were considered statistically significant.

2.7. Ethical approval

The clinical study was ethically approved by the Mae Fah Luang University Research Ethics Committee on Human Research (REH-59099).

3. Results

3.1. Physiochemical characteristics of sachai inchi oil

The oil was transparent with light yellow in color. Sachai inchi oil showed peroxide value of 18.40 meq O₂/kg oil. It had an acid value of 1.86 KOH/g oil. Additionally, fatty acid compositions in sachai inchi oil was also determined [10]. The major fatty acids were omega-3 (44.11%), omega-6 (35.53%) and omega-9 (8.96%). Tocopherol content in sachai inchi oil was 225.96 mg/100 g oil.

3.2. HLB value screening

The emulsion showed the lowest % CI was that with HLB value 9.0 (FS5, Table 1) which was taken to do the second run. The second run experiments were then repeated with emulsifiers in different amount for 3 formulas at HLB 8.5, 9.0 and 9.5 (Table 2). It was found that the prepared emulsion with HLB 8.5 (FS12) exhibited lowest % CI with good texture. Thus, it was concluded that the HLB of sachai inchi oil was approximately 8.5.

Table 1

HLB value and % CI of sachai inchi oil emulsion at 24 h.

Formula code	HLB	% (w/w)		% CI
		Stearth-2	Stearth-21	
FS1	5.0	5.00	0.00	11.1
FS2	6.0	4.50	0.50	11.1
FS3	7.0	4.00	1.00	13.3
FS4	8.0	3.50	1.50	11.1
FS5	9.0	3.00	2.00	2.2
FS6	10.0	2.50	2.50	6.7
FS7	11.0	2.00	3.00	22.2
FS8	12.0	1.50	3.50	20.0
FS9	13.0	1.00	4.00	22.2
FS10	14.0	0.50	4.50	22.2
FS11	15.0	0.00	5.00	11.1

Table 2

HLB and CI of sachai inchi oil emulsion at 24 h and 48 h.

Formulation	HLB	% (w/w)		% CI	
		Stearth-1	Stearth-20	24 h	48 h
FS12	8.5	3.25	1.75	2.2	2.2
FS13	9.0	3.00	2.00	2.2	22.2
FS14	9.5	2.75	2.25	6.7	44.4

3.3. Formulation of sachai inchi oil emulsion

The emulsion obtained showed white color with creamy texture and characteristic oil odor. The emulsion exhibited pH of 4.79 and viscosity of 30407 cP.

3.4. Stability of sachai inchi oil emulsion

However, emulsion showed no phase separation, which indicated that, the formula possesses relatively stable property. And it was found that the products stored at ambient temperature and (4 ± 2) °C showed only slight change after in term of color, odor, pH and viscosity. However, emulsion stored at (45 ± 2) °C showed obvious change. The color of emulsion changed from white to light yellow at W4. The pH decreased from 4.79 to 4.48 and viscosity reduced from 30 407 cP to 18 450 cP (Table 3). However, there was no phase separation.

3.5. Evaluation of radical scavenging activity

The standard solution of vitamin C (1 ppm) showed 94.80% inhibition. The sachai inchi oil at 67 ppm and 167 ppm showed 65.31% and 85.25% inhibition, respectively. Additionally, the sachai inchi oil emulsion prepared with 5% w/w oil showed 89.61% DPPH inhibition.

3.6. Clinical study

The skin hydration value was 38.59 ± 8.88 at W0 and it was found to be 45.21 ± 7.55 and the skin scaliness value was 0.093 ± 0.060 on W0 and decreased to 0.059 ± 0.050 on W4.

The average score showed that volunteers feel that the sachai inchi oil emulsion gives high skin moisture feel, high skin smoothness feel, and high skin high elasticity feel. The overall

Table 3

Stability evaluation of sachai inchi emulsion for 1 month.

Condition	Week	pH	Viscosity (cP) ^a
Ambient temperature	0	4.79	30 407
	4	4.65	30 390
4 °C	0	4.79	30 407
	4	4.61	30 185
45 °C	0	4.79	30 407
	4	4.48	18 450

^a Spindle #7 100 rpm.

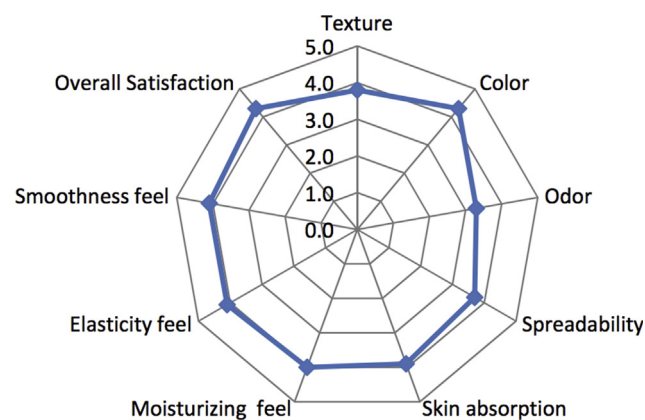


Figure 1. Subjective sensory evaluation of sachai inchi oil emulsion.

product satisfaction was also quite high with average score of 4.2. However, the result of the product's odor was low at 3 (Figure 1).

3.7. Primary skin irritation test

The patch test was removed at the end of the 24 h test period. The results showed that there was no irritation, redness or itching on the skin of volunteers. Then, the mean irritation index (M.I.I) was calculated from the sum of irritation grade per total number of subject which resulted in mean irritation index M.I.I. value of 0. Thus, the sacha inchi oil emulsion was classified as non-irritating.

4. Discussion

Sacha inchi (*Plukenetia volubilis* L.) oil is traditionally used in Peru as an everyday skin care oil to help preserving skin softness and healthiness [16]. The oil is now also being cultivated commercially in South East Asia, most notably in Thailand. Sacha inchi oil possesses a unique balance of omega-3, 6 and 9 and is currently gaining international recognition for its health properties and can be used in the food and cosmetic industries. However, there is no published paper regarding sacha inchi oil emulsion as the use of natural oils in skin care emulsion base is cosmetically sensorial and stability concerned. This study reported the physiochemical, HLB value and emulsion characteristics of sacha inchi oil. The oil possessed peroxide value of 18.40 meq O₂/kg oil which is higher than the limit of 15 meq O₂/kg oil and its acid value is 1.86 mg KOH/g oil, within the limit of 4.0 mg KOH/g oil standard, which was established by the FAO in Codex Standard for Edible Fats and Oils not Covered by Individual Standards (CODEX STAN 19-1981) [17]. Peroxide value is a measurement 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 value represents the amount of free fatty acids in the compound. The acid value and peroxide value are parameters that indicate the quality or conservation state of oils and fats, and are not used as identification parameters. The lower the value, the lower the extent of oxidation and hydrolysis is. According to the result which is slightly high in peroxide value, it may indicate that the sacha inchi oil probably had undergone processing and it is expected that the oil may exhibit high rancidity overtime. The major fatty acids are omega-3 (44.11%), omega-6 (35.53%) and omega-9 (8.96%). Tocopherol content in sacha inchi oil is 225.96 mg/100 g oil. The obtained results are slightly different but in the relative same range with the other studies [3,5,6,18]. The area crops, harvesting time, processing, and storage conditions affected the amount of essential fatty acids and other substances and these may explain the finding in this work.

The HLB of sacha inchi oil was experimentally determined. HLB is the ratio of the water-soluble portion to oil-soluble portion of the molecule and was firstly developed by Griffin [19]. In order to use sacha inchi oil in emulsion, it is crucial to select optimal non-ionic emulsifiers as this will ensure the emulsion's stability. HLB value of the emulsifier combinations are selected in a way that it is almost equivalent to the substances to be emulsified. The experimental HLB value of sacha inchi oil was approximately 8.5 as its emulsion showed the least

phase separation after 2 days. The sacha inchi oil cosmetic emulsion was then prepared using the obtained HLB. The stability was conducted by centrifugation, temperature cycling, and accelerated stability test. The emulsion showed good stability after centrifugation. Centrifugation is an accelerated method to predict the physical stability compared to the lengthy storage studies and causes the droplets to collide and coalesce at a rapid rate that will result in destabilization. The suitable conditions for emulsion formulation, *i.e.*, proper emulsifier type and concentration, homogenization speed and other auxiliaries' stabilizers might have prevented the breakage of the formulations during testing. Resistance to phase separation in preliminary test gives good idea about developing stable formulation [20]. The emulsion also showed no separation after 6 cycles of temperature cycling test which is designed to monitor the physical properties under rapid change environment. However, the pH changed from 4.79 to 4.40 and viscosity decreased from 30407 cP to 16080 cP. The results indicate that there might be the physiochemical interaction occurs as the pH and viscosity values obviously decreased. The formulation with ingredient that can help stabilize the system at wide range of temperatures may need to be considered. The sacha inchi oil emulsion was then tested for accelerated stability program for 1 month. The product showed good stability at ambient temperature and (4 ± 2) °C. However, emulsion stored at (45 ± 2) °C showed obvious change in color from white to light yellow. The pH and viscosity results showed same tendency as obtained in temperature cycling test. Considering the pH of the product which decreased from 4.79 to 4.48 in elevated temperatures, it may suggest that in order for a formulation to possibly gain admission for topical application, the emulsion that contains sacha inchi oil should have pH of about 4.5–7.0 which falls in to the pH values of 4–7 of human skin [21]. The sacha inchi oil formulated in this work had a pH value of 4.79, which is close to the skin's pH. The high temperature may cause the hydrolysis of sacha inchi oil, but it did not affect the overall quality of emulsion because the pH values remained around skin pH, which is cosmetically acceptable.

Sacha inchi oil (67 and 167 ppm) and its emulsion showed 65%, 85% and 89% DPPH inhibition, respectively. There has been report regarding antioxidant properties of sacha inchi oil using the oxygen radical absorbance capacity assay. The total antioxidant capacity was within the ranges of 6.5–9.8 μmol Trolox equivalent per gram seed [6]. No studies in the literature have reported the % DPPH inhibition of sacha inchi oil. Vitamin E which presents in sacha inchi oil is a natural antioxidant and may contribute to the exhibited radical scavenging activity [6,22]. It is also may help retarding the rancidity of seed oil thus stabilizing the emulsion [23]. The results obtained are useful for cosmetic application as the sacha inchi oil may function as active oil for anti-aging products.

The sacha inchi oil emulsion is considerate non-irritant tested by primary skin irritation method. It significantly helps increasing skin hydration from dry skin to sufficiently moisturized skin and also decrease skin scaliness after being applied tested in 15 volunteers for 1 month. The results indicate that the sacha inchi oil emulsion increased the skin moisture and help decreased the dryness of the skin. The sacha inchi oil may contribute to the results obtained by acting as skin emollient. Moreover, the volunteers showed fairly high product satisfaction

especially on the skin moisturizing, skin smoothness and skin elasticity feel. The results supported its function in cosmetics as it is registered as emollient (soften and smoothen the skin), humectants, and skin protector [8]. However, the oil itself possessed somewhat strong characteristic odor, if it will be using in the emulsion product, the fragrance or essential oil should be added in order to obtain a cosmetically acceptable product.

In conclusion, this work has reported for the first time the HLB value and emulsion properties of sacha inchi oil in term of stability and clinical effectiveness. The information is useful for utilization of sacha inchi oil in cosmetic industry.

Conflict of interest statement

The authors declare that they have no conflict of interest.

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