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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 physiochemical 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 O_2/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, stigmasterol 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 sacha inchi oil was also reported.

2. Materials and methods

2.1. Materials

Sacha inchi oil was purchased from Chiangrai Agricultur 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 sacha 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 sacha inchi oil was determined according to the reported method [11] with modifications. A series of sacha 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 ± 2)°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 sacha inchi oil emulsion for stability and efficiency study.

Sacha inchi oil emulsion was prepared by using 3.25% w/w steareth-2, 1.75% w/w steareth-21, 5.0% w/w sacha 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 sacha inchi oil emulsion

Gravitational stability test of sacha 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 ± 2) °C for 24 h and then in climate chamber at (45 ± 2) °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 sacha 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 sacha 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 sacha inchi oil and its emulsion to scavenge DPPH radical was calculated as % inhibition by the following equation:

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

where A control is s 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 sacha 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.