Fatty Acid Composition of Sacha Inchi (*Plukenetia volubilis* L.) Oil and Efficacy of Sacha Inchi Lotion

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Abstract

Sacha inchi (*Plukenetia volubilis* L.) or "Inca peanut" is widely cultivated in Northern Thailand provinces such as Phayao, Lampang, Chiang Mai, and Chiang Rai due to the appropriate geographical location and climate. The aims of this study were to determine the fatty acid composition of Sacha inchi oil cultivated in Chiang Rai and evaluate the efficacy of Sacha inchi lotion after topical application to skin. Sacha inchi seed oil was prepared by using soxhlet extraction with *n*-hexane (1:20 w/v). GC-MS analysis was used for analyzing fatty acid composition. Sacha inchi oil was used as an active ingredient in lotion and evaluated by 30 volunteers for its efficacy after application to skin. The main fatty acids in Sacha inchi oil were linoleic acid (45.72%), linolenic acid (42.27%), palmitic acid (6.42%) and stearic acid (4.53%). Sacha inchi lotion containing 2.5% Sacha inchi oil was formulated. The lotion showed no irritation after closed patch test. Sacha inchi lotion increased skin moisture and elasticity significantly ($p<0.001$) after 7 days application and maintained these effects for 28 days. After 28 days application, a significant decrease ($p<0.05$) in melanin value was observed. The Sacha inchi lotion was found to be superior to the placebo control. Sacha inchi oil contains high amounts of linoleic and linolenic acid. The application of Sacha inchi lotion significantly improved skin parameters after 7 days and for 28 days. The result of this study confirm the benefit of Sacha inchi oil for use in cosmetics, which might increase the value of Sacha inchi.

Keywords: *Plukenetia volubilis* L., Sacha inchi, Fatty acid, Lotion, Skin moisturizer

Introduction

Sacha inchi (*Plukenetia volubilis* L.) or "Inca peanut" is a perennial plant of the Euphorbiaceae family. It grows in Amazonian rainforests ranging from the heights of 200 to 1500 m in an environment with water and well-drained acidic soil [1]. The plant is a wild, semi-woody, perennial, oleaginous vine with slightly hairy leaves [2]. It has a star-shaped fruit, which contains dark oval seeds 1.5 - 2.0 cm in diameter (Figure 1). The Amazon natives produce flour and oil from Sacha inchi seeds. They use the products for preparation of different meals and beverages. It has also been used medically in treating rheumatic problems and aching muscles. The oil is also used for cosmetic purposes by women of the native Amazonian tribes [3]. It is traditionally used as everyday skin care oil applied to preserve skin softness and healthiness [1].

According to previous studies, Sacha inchi seed oil has been extracted by various methods such as subcritical fluid extraction with *n*-propane [4], supercritical carbon dioxide extraction [5], hot pressed [6], cold pressed [7] and soxhlet extraction [8]. As reported by those studies, Sacha inchi seed is a good source of oil (35-60%) and proteins (25–27%). The oil contains high levels of essential polyunsaturated fatty acids (PUFAs), namely linolenic acid (C18:3 or omega 3) and linoleic acid (C18:2 or omega 6), representing about 47–51% and 34–37% of total oil content, respectively [2, 7]. Other fatty acid such as oleic acid, palmitic acid, and stearic acid are also present in Sacha inchi seed oil.

PUFAs are not synthesized by the human body. Thus, they must be introduced by food or by cosmetic product application [9]. PUFAs are believed to revitalize the skin and give skin a youthful appearance. A previous report found that PUFAs may restore dry skin conditions as well as have therapeutic effects on skin disorders, such as dry skin, desquamation (scaly skin), wounds failing to heal, loss of humidity, erratic keratinization processes (blocking of follicles), increasing rates of mitosis (disorganized skin layers), tendency towards eczema and itchiness [10].

Recent studies indicate a high content of tocopherols (1500-2000 mg/kg) in Sacha inchi seeds that could act as antioxidant agents. Antioxidant agents that display a strong antioxidant activity are considered to promote human health, since they are responsible for critical biological functions [1, 6]. Furthermore, the amino acid profile of Sacha inchi protein fraction showed relatively high levels of

cysteine, tyrosine, threonine, and tryptophan [11]. Moreover, polyphenolic compounds were also detected in Sacha inchi oil derived from cold pressing of the seed; among them, phenyl alcohol, flavonoid, seicoridoid, and lignan classes [7].

Sacha inchi was first grown in Thailand 5-6 years ago in Nong Khai province and Maejo University in Chiang Mai has found that Sacha inchi is able to grow in every part of Thailand, Sacha inchi is now widely cultivated in Thailand, especially in Chiang Rai province. Sacha inchi oil can be added to many formulations such as moisturizing cream and anti-aging lotion. There are many Sacha inchi creams or lotions sold in the market. However, very few studies have been published examining the efficacy of Sacha inchi cream on skin.

Phytochemical screening of Sacha inchi oil derived from soxhlet extraction (with *n*-hexane) of Sacha inchi grown in Chiang Rai revealed the presence of flavonoids and DPPH free-radical scavenging activity [12]. Accordingly, the aims of this study were to evaluate the fatty acid composition of Sacha inchi oil, formulate Sacha inchi oil lotion and evaluate the product efficacy after applying to skin. The results of this study might support further use of Sacha inchi oil in cosmetics and increase the value of this oil.

Figure 1 Visual appearance of Sacha inchi seeds

Methodology

Plant material

Dried Sacha inchi seed (Figure 1) was purchased from Chiangrai Agriculture Development Co., Ltd., Tambon Nanglae, Meuang, Chiang Rai, Thailand. The seed was dried in a hot air oven (Ontherm/06503) at 55ºC until a constant weight was achieved (about 72 h). The moisture content was measured by using a moisture analyzer (Ohaus/MB45).

Chemical and reagents

All the chemicals used in the formulation were cosmetic grade. *n*-Hexane was purchased from Sigma Chemicals Co., USA.

Sacha inchi seed oil extraction

The dried Sacha inchi seed was ground in a blender (Panasonic/ MX-J210GN) and extracted by the soxhlet extraction method of Sawatpakdee and Wuttisin (2017). Soxhlet extractor capacity of 500 ml was used and the extraction time was 5 h using *n*-hexane at a solid to solvent ratio of 1:20 (w/v). The solvent was evaporated at 50°C under reduced pressure by rotary evaporation (Eyela/CCA-1110) and left under a fume hood (48 h) to evaporate the solvent residue. The percentage yield was calculated. Sacha inchi oil was stored at 4°C until used.

Determination of fatty acid composition by Gas chromatography-mass spectrometry (GC-MS)

The fatty acid composition was determined by converting all fatty acids of triglycerides into their corresponding methyl esters followed by GC-MS analysis [13]. Sacha inchi oil (200 µl) was methylated with 0.5% NaOH in methanol (10 ml) and then incubated at 60ºC for 10 min. The methyl esters were extracted with *n*-hexane (8 ml) for 1 min. The *n*-hexane layer was washed with 4 ml distilled water and dried over anhydrous sodium sulfate. Analysis was carried out with gas chromatography (Agilent 6890N) equipped with a capillary column (HP-5MS, 0.25 mm × 30 m × 0.25 µm) and connected to mass selective detector (MSD model MS 5973N). Helium was used as carrier gas. Oven temperatures were set at 140ºC (5 min), 200ºC (15 min) and 250ºC (4.5 min). The injector and detector temperatures were set at 220ºC. Data analysis was carried out using the wiley7n.1 and Pest.1 libraries.

Formulation of lotion containing Sacha inchi oil

Lotion containing Sacha inchi oil was prepared as shown in Table 1. Distilled water was heated up to 75°C and Carbomer 934 was added (2% dispersion). The other

water soluble components were added and the mixture was heated to 80°C. The ingredients in part B were mixed together and heated to 80°C before being slowly mixed into the aqueous phase with continuous stirring until cooling down to 30-40°C. Phenoxyethanol and fragrance were added and mixed until uniform. The control lotion (base) was formulated using the same procedure without Sacha inchi oil.

Table 1 Development of Sacha inchi lotion

Ingredients	%w/w	
	Control lotion	Sacha inchi lotion
Part A		
Distilled water	72.50	72.50
Carbomer 934 (2% dispersion)	14.00	14.00
Glycerin		
Triethanolamine (99%)		
Part B		
Stearic acid	8.25	8.25
Cetyl alcohol		
Glycerylmonostearate		
Isopropylpalmitate		
PEG-40 stearate		
Petrolatum USP		
Mineral oil	5.00	2.50
Sacha inchi oil	-	2.50
Part E		
Phenoxyethanol	0.25	0.25
Fragrance	q.s.	q.s.

Physical properties of Sacha inchi lotion

The pH of Sacha inchi lotion was measured by using a pH meter (Mettler Toledo Seven Easy S20). The viscosity of Sacha inchi lotion was measured by a viscometer (Brookfield RVD-II+P) at 15 rpm under ambient temperature using spindle no.5 (30 sec). The phase separation was tested by centrifugation (Spectrafuge/16M) at 5000 rpm for 30 min.

Patch test

Patch test was performed on inner forearms of 30 volunteers to determine skin irritation. Control lotion and Sacha inchi lotion were put on Finn chambers® and compared with 1% sodium lauryl sulfate (positive control) and distilled water (negative control). After 24 hours occlusion, the patch was removed and observed for any skin redness or irritation. The scores were recorded for the presence of erythema using a score of 5 points, ranging from 0-4; where 0 = absence of erythema, 1 = mild erythema, 2 = redness, 3 = swelling and 4 = severe erythema [14].

Human skin efficacy test

Sacha inchi lotion was evaluated for it efficacy by 30 volunteers, age 21-23 years old who has no history of dermatological diseases or cosmetics allergies. This study was approved by the Ethics in Human Research Committee of Mae Fah Luang University (No. REH-59085).

Efficacy of the Sacha inchi lotion was evaluated using a previously described method [15]. Volunteers were instructed not to use moisturizers, body lotions, and or occlusive cosmetic preparations on the area tested for 12 h prior to the *in vivo* study. All subjects rested in a room maintained at 25±1°C and 40-60% relative humidity for 15 min prior to performing the efficacy test. Skin monitoring was evaluated by using Corneometer® CM 825 (skin moisture), Mexameter® MX 18 (melanin value), and Cutometer® MPA 580 (skin elasticity). Baseline skin data were recorded prior to applying test compounds. The short term skin moisturizing effect was determined after Sacha inchi lotion application for 15, 30 and 45 min compared with control lotion. In addition, volunteers were assigned to apply

control lotion and Sacha inchi lotion for twice a day in 2 areas (1×1 inch/each) at the upper inner arm in randomized single-blind procedure. Skin parameters were recorded before and after using at 7th, 14th, 21st, and 28th day and compared with baseline (initial).

Data analysis

The data were expressed as mean±S.D. of triplicate measurements. The statistical analysis was determined by paired-sample t-test statistics and independent samples test via program IBM SPSS statistics version 21 (Licensed for Mae Fah Luang University).

Results

Sacha inchi seed oil extraction

Sacha inchi oil has an orange-yellow color. The moisture content of Sacha inchi seed before drying at 55ºC was 6.9±0.46% and after drying was 4.3±0.51%, which is similar to a previous reports by Follegatti-Romero *et al.* (2009) [5], Gutiérrez *et al*. (2011) [8] and Chirinos *et al*. (2013) [6]. Thus, the percentage of moisture content was within the range of 0-13% reported to be suitable for storage and processing without microorganism degradation of the triacylglycerol [5]. The percentage yield was 43.55%.

Fatty acid compositions of Sacha inchi oil

Fatty acid composition of Sacha inchi oil was measured by GC-MS. The results in Figure 2 and Table 2 show that the main fatty acids found in Sacha inchi oil were linoleic acid (RT:23.47 min, m/z 279.3, 45.72%), linolenic acid (RT:23.73 min, m/z 277.3, 42.27%), palmitic acid (RT:18.70 min, m/z 255.3, 6.42%), and stearic acid (RT:24.40 min, m/z 283.4, 4.53%).

Physical properties of Sacha inchi lotion

The pH of Sacha inchi lotion was 5.5. The viscosity was 11,506±59 cP. Sacha inchi lotion and control lotion both had a white color as shown in Figure 2. After 1 month storage there was no change in color or pH and no phase separation occurred.

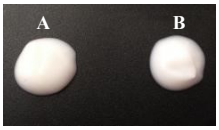

Figure 2 Sacha inchi lotion (A) and control lotion (B)

Patch test

All 30 volunteers reported no irritation and no severe erythema after the closed patch test and during the 1 month use for both control lotion and Sacha inchi lotion. This indicated that Sacha inchi lotion was safe for long-term use.

Human skin efficacy and satisfaction test

A short term skin moisturizing effect was determined after application of Sacha inchi lotion for 15, 30 and 45 min compared with control lotion. The results in Table 3 show that Sacha inchi lotion increased skin moisture after application and prolonged this moisturizing effect for 45 min ($p<0.05$, compared with initial) while base lotion only increased skin moisture after 15 min application ($p<0.05$, compared with initial).

The skin improvement after continuous use was measured as skin moisture, skin elasticity and melanin value. The results in Table 4 show that Sacha inchi lotion significantly increased skin moisture and elasticity after 7 days application ($p<0.001$) and maintained these effects during 28 days use. Moreover, melanin value also significant decreased ($p<0.05$) after 28 days application.

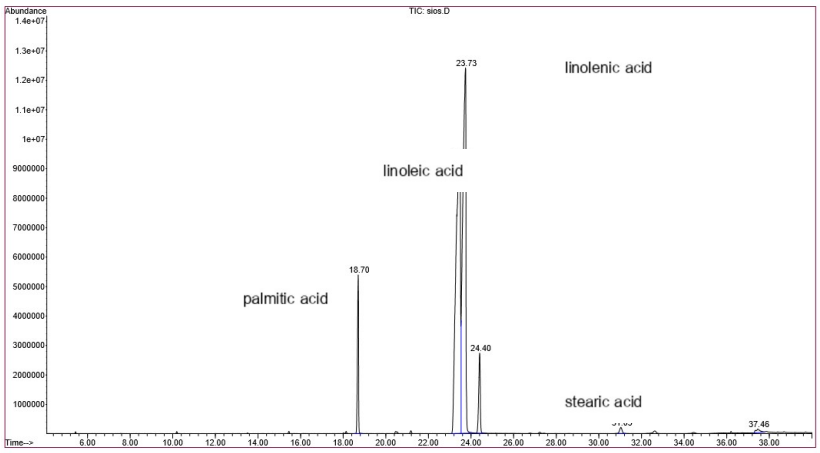

Figure 2: Chromatogram showing fatty acid composition of Sacha inchi oil analyzed by GC-MS

Table 2 Fatty acid composition of Sacha inchi oil.

Linolenic	Linoleic	Palmitic	Stearic	Oleic	Reference
42.27	45.72	6.42	4.53	-	Present study
45.20	36.80	4.50	3.20	9.60	Hamaker *et al.*, 1992
50.41	34.08	4.24	2.50	8.41	Follegatti-Romero *et al.*, 2009
50.80	33.40	4.40	2.40	9.10	Gutiérrez *et al.*, 2011
46.80	36.20	4.30	3.00	9.00	Fanali *et al.*, 2011
12.80-16.00	12.40-14.10	1.60-2.00	1.10-1.30	3.50-4.70	Chirinos *et al.*, 2013
44.00	40.00	4.00	3.00	9.00	Liu *et al.*, 2014
48.32	34.10	4.70	3.30	8.90	Cisneros *et al.*, 2014
48.00	35.00	5.00	2.00	9.00	Gonzalez-Aspajo *et al.*, 2015
-	40.45	41.29	7.63	-	Sawatpakdee and Wuttisin, 2017

Table 3 Short term skin moisturizing effect after Sacha inchi lotion application.

Time (min)	Control lotion		Sacha inchi lotion	
	Value	ΔChange (Min, Max)	Value	ΔChange (Min, Max)
Initial	68.40±11.22	-	55.48±7.52	-
15	81.80±6.05*	13.41±7.59 (3.99,22.83)	73.37±10.24*	17.89±11.68 (3.38,32.40)
30	78.66±5.41	10.27±12.22 (-4.90,25.44)	77.59±8.32*	22.11±7.34 (12.99,31.23)
45	80.35±8.25	11.95±8.24 (1.72,22.19)	79.11±11.13*	23.63±10.72 (10.33,36.94)

ΔChange = After-Initial. Values are expressed in Mean±SD (n=5). * Significantly difference ($p<0.05$) when compared with initial.

Table 4 Skin efficacy evaluation after Sacha inchi lotion application.

Time (day)	Control lotion		Sacha inchi lotion	
	Value	ΔChange (Min, Max)	Value	ΔChange (Min, Max)
Skin moisture				
Initial	51.43±14.38	-	46.42±13.53	-
7th	56.87±16.88	5.44±8.34 (2.33,8.56)	49.68±13.79**	3.26±1.83 (2.58,3.95)
14th	59.05±14.82	7.62±12.14 (3.09,12.16)	61.48±11.93**	15.06±10.46 (11.16,18.97)
21st	65.16±14.31**	13.73±14.09 (8.47,18.99)	67.11±13.57**	20.68±12.10 (16.16,25.20)
28th	65.04±12.72**	13.60±13.22 (8.67,18.55)	77.08±10.37**	30.66±11.92 (26.21,35.11)
Elasticity				
Initial	1.00±0.13	-	0.91±0.08	-
7th	1.00±0.10	-0.01±0.08 (-0.04,0.02)	1.08±0.16**	0.17±0.19 (0.10,0.24)
14th	0.95±0.09	-0.06±0.08 (-0.09,-0.03)	1.05±0.17**	0.14±0.21 (0.06,0.22)
21st	0.96±0.08	-0.04±0.07 (-0.07,-0.01)	1.12±0.13**	0.21±0.18 (0.15,0.28)
28th	1.02±0.08	0.01±0.10 (-0.02,0.05)	1.40±0.19**	0.50±0.23 (0.41,0.58)
Melanin value				
Initial	175.73±31.13	-	181.42±34.81	-
7th	192.48±38.26	16.75±20.79 (8.99,24.51)	186.41±34.45	4.99±15.65 (-0.85,10.83)
14th	183.01±31.45	7.28±19.58 (-0.02,14.60)	172.34±31.10	-9.08±19.87 (-16.50,-1.66)
21st	176.50±31.25	0.77±12.41 (-3.86,5.40)	185.23±30.60	3.82±23.39 (-4.92,12.55)
28th	167.94±31.67*	-7.79±14.09 (-13.05,-2.52)	165.20±30.37**	-16.21±21.69 (-24.31,-8.11)

ΔChange = After-Initial. Values are expressed in Mean±SD (n=30). ** Significantly difference ($p<0.001$) when compared with initial. * Significantly difference ($p<0.05$) when compared with initial.

Discussion and Conclusion

In Thailand, Sacha inchi is cultivated in many areas for seeds to consume because it is rich in omega-3 or linolenic acid, which is reported to be beneficial to health [17, 18]. In this study, Sacha inchi seed oil was prepared by using soxhlet extraction with *n*-hexane. Fatty acid composition was determined and we found the presence of linoleic (45.72%), linolenic (42.27%), palmitic (6.42%), and stearic acids (4.53%). The fatty acid composition of Sacha inchi oil in this study was quite different to previous studies, as shown in Table 2. The present study shows that Sacha inchi oil cultivated in Chiang Rai contains high levels of linoleic acid and linolenic acid, which makes it a rich source of omega-6 and omega-3 fatty acids. The differences that we saw in the fatty acid content might be due to using different cultivars, geographical effects, changes in climate and growing conditions, the harvesting time of the seeds [19] and the processing (e.g. roasting prior to extraction) as well as differences in the method of oil extraction [6] and the quantification methods used in the analysis.

At present, Sacha inchi oil is added to many cosmetic formulations such as moisturizing creams, anti-aging lotions, and body oils. These products are distributed widely in local markets around Thailand without scientific testing of their efficacy. In the present study, the Sacha inchi lotion containing 2.5% Sacha inchi oil was formulated and tested by applying to volunteers' skin. Topically applied Sacha inchi lotion was found to increase skin moisture and elasticity. Sacha inchi lotion increased skin moisture significantly ($p<0.05$) after application and prolong the moisturizing effect for 45 min while base lotion that contained mineral oil increased skin moisture significantly ($p<0.05$) only after 15min application. This indicated that Sacha inchi lotion could prolong the moisturizing effect for longer than base lotion. The reason for this might be due to the occlusive effect of Sacha inchi seed oil blocking

transepidermal water loss (TEWL) from the skin surface, thereby increasing hydration of the stratum corneum. Additionally, the humectant activity of Sacha inchi lotion is likely to contribute to the moisturizing effect because it helps to retain moisture from the environment and restore water to the skin. Sacha inchi oil contains high levels of linoleic, linolenic and palmitic acids. These fatty acids are the most frequently used fatty acids in cosmetic products. They are emollients and have occlusive properties to reduce TEWL through the skin, mainly by means of making a protective layer on the epidermis. They also soften the stratum corneum and reduce inflammation of the skin. [20]. Linoleic acid is key part of ceramide I which is the most important barrier substance in the horny layer of the epidermidis. Linolenic acid improves skin moistening, activates regeneration of damaged lipid barrier of the epidermis, heals inflammation and stabilizes skin metabolism [20-21]. It also stimulates the synthesis of barrier lipids of the skin and proteins precursors of natural moistening factors [20]. Palmitic acid is an important component of both the skin barrier and the acid layer of the skin [21]. It has the most potent skin permeation enhancing effect, which is used mainly as the vehicle for other active ingredients [22-23].

When skin is routinely exposed to stressful factors from the environment, such as UV radiation, smoke, and pollutants, an elevated number of free radicals will be produced, which accelerates skin aging [24]. Free radical activation results in the production of collagenases which degrade collagen by kinase pathways [25]. Based on this theory, an effective approach to delay skin aging is to externally supply antioxidants through skincare products to either suppress production or neutralize excess free radicals [26]. Sacha inchi oil contains antioxidant compounds such as tocopherols, phenolics and flavonoids [6, 12, 17]. Thus, application of sacha inchi lotion is an effective approach to delay the skin aging process.

Additionally, a decrease of melanin in the skin was observed after 28 days of Sacha inchi lotion application. This might be due to the UV absorption property of Sacha inchi oil. Previous studies have found that Sacha inchi oil has UV-absorption properties between 260-320 nm (UVB) and might be used as sunscreen application [8, 12].

Decreases in skin moisture and elasticity are considered to promote wrinkle formation. Exposure to UV radiations also results in skin damage [25]. Thus, Sacha inchi lotion might be effective as anti-aging product. Further investigations are needed for determining the other active chemical components of Sacha inchi. The efficacy of pure Sacha inchi oil on skin also needs to be investigated. In addition, studies about the suitable conditions for Sacha inchi cultivation in Thailand are recommended to determine how to control the quality of the raw material.

In conclusion, Sacha inchi oil contains important quantities of essential fatty acids especially linoleic and linolenic acid. Sacha inchi lotion had significant effects on skin, enhancing skin moisture, increasing skin elasticity and decreasing melanin content. Consequently, Sacha inchi oil is suitable to use as moisturizer and emollient for anti-aging, whitening and as a sun screen product. Finally, this present study indicates that Sacha inchi oil is an active ingredient for cosmetics which can be safely used on the skin.

Acknowledgements

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