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Sunless Tanning Creams from Chlorogenic Acid Extracted of Green Coffee Beans

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The purpose of this study was to develop sunless tanning cosmetic creams from green coffee beans. Chlorogenic acid, which was extracted from green coffee beans, performed the change of the color of keratin in stratum corneum, used as active ingredient in the formulation. Extracted chlorogenic acid from green coffee beans were incorporated into four cosmetic cream bases, and then the tanning effect of the creams were evaluated as well as the stability test of the creams. The results showed that O/W cream base with extracted chlorgenic acid from green coffee beans had the most tanning activity at pH 4.5±0.5 and 40^{o}C. The O/W cream also performed most stable than the other two types of cream. In conclusion, sunless tanning product from green coffee beans might be formulated in O/W type cream.

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Introduction

Many extracts from natural plants can be used in sunless tanning products as stainer or extender are Lawsone found in henna plant, and Juglone (5-hydroxy-1,4-naphthoquinone) derived from walnut[1].The previous study concluded the chlorogenic acid extracted from green coffee beans play role as artificial tanning substances which it can interact with protein on the surface of the skin to produce the brown-colored pigment called melanin[2]. The color tends to wear off after a few days. The chlorogenic acid is recognized as extenders. And it is concluded that sunless tanning product from green coffee beans was potentiated to be continuously studied as a commercialized product.

Therefore, this experiment was planned to determine the concentration of chlorogenic acid, extracted from green coffee beans, as an artificial tanning component or sunless tanning products are also known as self-tanners[3]and develop the formulation in the form of cream.

Materials and Method

Materials

Coffee beans used for the study were obtained from Krabi, a province in the southern part of Thailand. The trade mark of the coffee was Parrot®, which is one of the Krabi province's community agricultural products. The products were harvested and processed in May 2007, and were certified by Thai industrial standard institute, Ministry of industry, and were registered FDA licence as *Coffea robusta* (Rubiaceae family). Three types of coffee beans (green coffee beans, roasted coffee beans and powdered coffee beans) were used in this experiment. All types of coffee beans were evaluated for their moisture content by a moisture analyzer (Mettler®, USA). All cream constituents in this study were cosmetic grade purchased from local chemical representative in Bangkok, Thailand. Other chemicals for analysis were laboratory grade of various trade mark bought from the local chemical representative in Hat Yai, Songkhla, Thailand.

Full thickness flank skins of newborn pigs that had died of natural causes shortly after birth used as the membrane. They were obtained from a local pig farm, Songkhla, Thailand. The average weight of animals for preparing skins was 1.4-1.8 kg. The epidermal flak hairs were carefully removed with an electric hair clipper without damaging it and excised with a scalpel. The subcutaneous fat and underlying tissues was carefully removed from the dermal surface. The skin was rinsed with phosphate buffer ,pH 7.4, blotted with soft household paper, wrapped in aluminum foil and then stored at -20^{o}C for no longer than a month.

Extraction of Chlorogenic Acid from Green Coffee Beans

Chlorogenic acid was extracted employed the procedure of Naidu, *et al*. [4]. Green coffee beans were sterilized at 120^{o}C for 20 min, and flaked into a thickness of between 0.5 and1.0 mm. Coffee flakes were extracted with hexane using Soxhlet apparatus. These flakes were dried at 55±5^{o}C for about 2–3 h, the moisture level was about 10%. The dry flakes were passed through a 30 mesh sieve to get a powder. The 100 g powders were extracted with solvent ratio of 1:10. Solvent mixtures were isopropanol to water in ratio of 60:40 was used for extraction. The 100 ml each of solvent was added 10 times every 30 min contact time for over a period of 5 h. The extracts were drained and pooled. The pooled extracts were distilled on a rotavapor at 50^{o}C under reduced pressure (40 milli bar) and the product stored at 4^{o}C. Coffee conserve was dispersed in water and 1 M ammonium sulfate was added to a final concentration of 20 g/l in order to precipitate proteins by an increase in ionic strength followed by addition of 4% phosphoric acid to render the chlorogenic acid more soluble in ethyl acetate. Extracts were treated with petroleum ether (40–60^{o}C) to remove lipids and pigments. De-pigmented extracts were further treated with chloroform to remove caffeine and wax. The chloroform part was chilled,. The residue was washed with ethyl acetate, distilled and air dried to obtain a light creamy colored powder of chlorogenic acid.

Sunless Tanning Cream Formulation and Preparation

Cosmetic cream bases were developed and prepared at least 4 formulations as shown in Table.1. Then chlorogenic acid extracted from green coffee bean was incorporated into the cream bases with the concentration of 1, 5, and 10%w/w.

Thus, 12 preparations with 3 concentrations of active compound were provided.

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Table 1.Cream bases formulated for preparation of sunless tanning cream with chlorogenic acid extracted from green coffee bean

Components	Quantity (%w/w)				Categories
	R_X1	R_X 2	R_X 3	R_X 4	
Palm oil	25	25	35	35	Moisturizer, antioxidant
Tween 20	2.86	-	2.58	-	Emulsifying agent
Span 20	7.14	-	7.42	-	
Tween 60	-	6.10	-	5.87	
Span 60	-	3.90	-	4.13	
Cetyl alcohol	5	5	5	5	Thickening agent
Paraben concentrate	1	1	1	1	Preservative
Purified water q.s.to	100	100	100	100	Vehicle

The cosmetic cream bases were studied for some physical properties, such as, textures, colors, odors, and viscosity. The stability test was also done by 6-freeze-thraw cycles to determine the best formulation of sunless tanning cream with chlorogenic acid extracted from green coffee beans.

Tanning Effect of the Sunless Tanning Cream with Green Coffee Beans Extract

All twelve preparations were done percutaneous absorption using the modified Franz diffusion cell; the 4.5x4.5 cm prepares pig skin. The skin was first rubbed by buffer solution with various pH of 2, 4, 6, 8, and 10. The 1.0 gram of each formulation was loaded in a donor compartment, spread on the surface of the skin. The cells was filled with phosphate buffer at pH 7.4 and 37^{o} C. Samples in the receptor compartment would be withdrawn at 0.5, 1, and 2 hours, collected in the vial for melanoidins content analysis (interactive product from Maillard reaction between chlorogenic acid and epidermal protein). The samples on the skin was removed by draining with buffered phosphoric acid solution pH 7 and collected for chlorogenic acid content analysis [5].
Melanoidins were separated by dialysis using 40-45 kDa cutoff membranes (Sartorious®, USA). The measurements were taken by HPLC method and calibration with standard curve. The color of the skin was also observed at 0.5, 1, and 2 hours[6].

Statistical Analysis

All data were subjected to an analysis of t-test and average mean, and separated by the least significant difference (LSD) test at the 5% level using the statistical SPSS 17.0. When duplicates differed more than 10%, sample extraction and measurement were repeated.

Results

The results shown in Table 2 found that R_X2 is most dependable and stable of the 4 formulated cream bases, hence, it was chosen as the cream base for preparation of sunless tanning cream. (Scale most value = 5, unvalued = 1). The chlorogenic acid could be penetrated into the skin, and can be calculated by the following equation, when the chlorogenic acid concentration on the skin and the concentration of chlorogenic acid in the receptor were analyzed (shown in figure 1).

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Table 2. Physical properties of formulated cream bases

Physical properties	R_X1	R_X 2	R_X 3	R_X 4
Textures-consistency	+5	+5	+5	+5
Greasability	+3	+3	+4	+5
Spreadability	+3	+4	+3	+2
Color	Slightly yellow	Slightly yellow	Slightly yellow	Slightly yellow
Odor	+5	+5	+5	+5
viscosity	+4	+4	+3	+4
pH	5.00±0.09	4.66±0.25	4.61±0.18	4.50±0.11

***Chlorogenic acid in the skin = chlorogenic acid in the receptor site – chlorogenic acid on the* skin**

So

Total chlorogenic acid = chlorogenic acid in the receptor site + chlorogenic acid on the skin + chlorogenic acid in the skin

Then percentage concentration of chlorogenic acid in receptor could be calculated by the following equation.

$$\textbf{\textit{Chlorogenic acid concentration in percent}} = \frac{\textbf{\textit{Concentration of cholorogenic acid in the receptor}}}{\textbf{\textit{Total cholorogenic acid cocentration}}}$$

Total percentage concentration of chlorogenic acid were 1%, 5%, and 10% There was no significant difference between 3 concentration of chlorogenic acid because the chlorogenic acid was react with protein keratin in the skin according to the Maillard reaction, produced the melanoidin (browning color substance). The results of tanning effects to the skin were comparatively concluded in Figure 2. It was concluded that more chlorogenic acid, the more melanoidin occurred. The most effective and rapidly appeared the brown color was 10% by weight of chlorogenic acid. After the chlorogenic acid concentration on the skin was analyzed, the concentration of chlorogenic acid in the skin could be calculted by the following equation.

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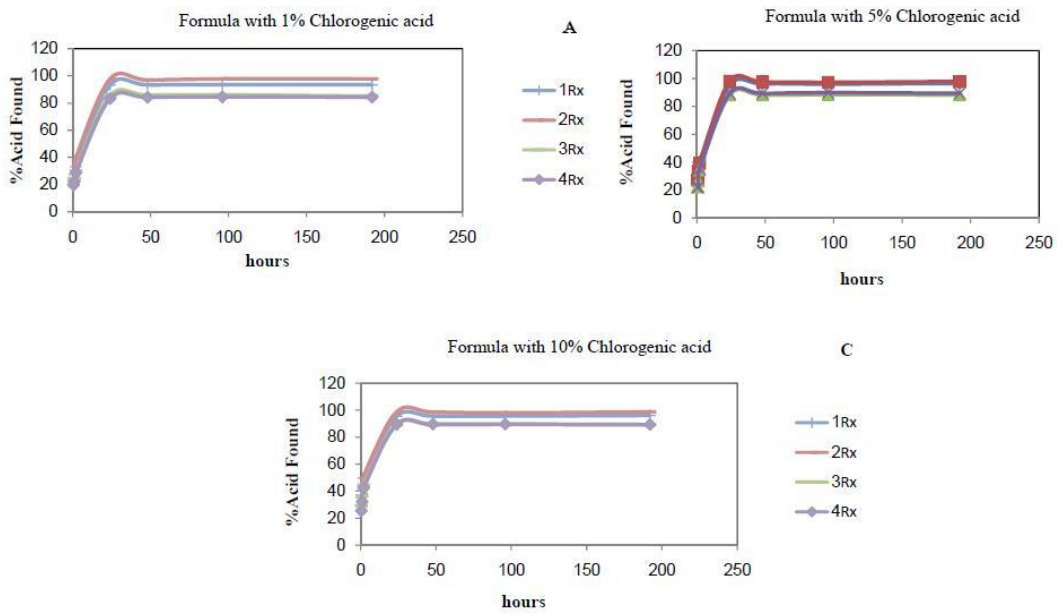

Fig.1. Chlorogenic acid concentration found in the receptor site of Franz diffusion cell analyzed from 4 formulas with various concentration (% by weight) of chlorogenic acid. (a = 1%, b = 5% and c = 10%) at various times of reaction and penetration.

Although chlorogenic acid was found in the receptors. But there were not melanoidin in the receptors, because there was no any protein in the receptor. On the other hand, melanoidins were found in the skin (shown in figure 2) and it was found that the more chlorogenic acid concentration, the more melanoidin concentra- tion was found.

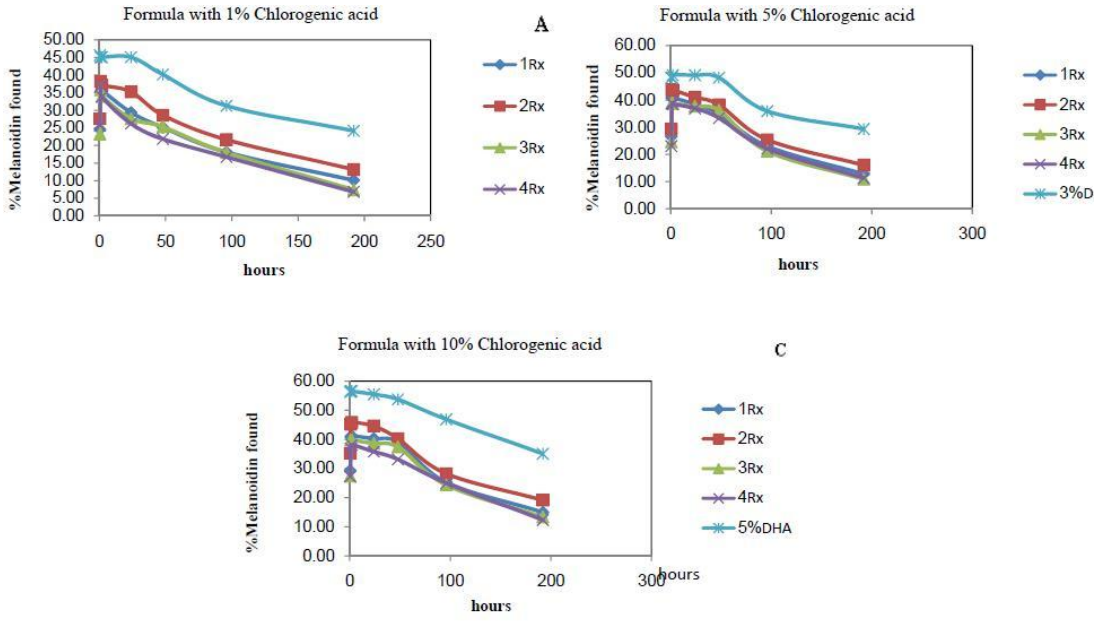

Fig.2. Melanoidin concentration found in the skin analyzed from 5 formulas with various concentration (% by weight) of chlorogenic acid and Dihydrocyacetine (DHA) (a = 1%chlorogenic acid and 1%DHA, b = 5% chlorogenic acid and 3%DHA and c = 10% chlorogenic acid and 10%DHA) at various times of reaction and penetration.

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Moreover, the concentration of melanoidins were determined when apply to the skin rubbed with various pH buffer solution, and the result was shown in figure 3. It can be concluded that melanoidin was most stable at pH 4, because the chlorogenic acid in skin was also stable at pH 4[7].

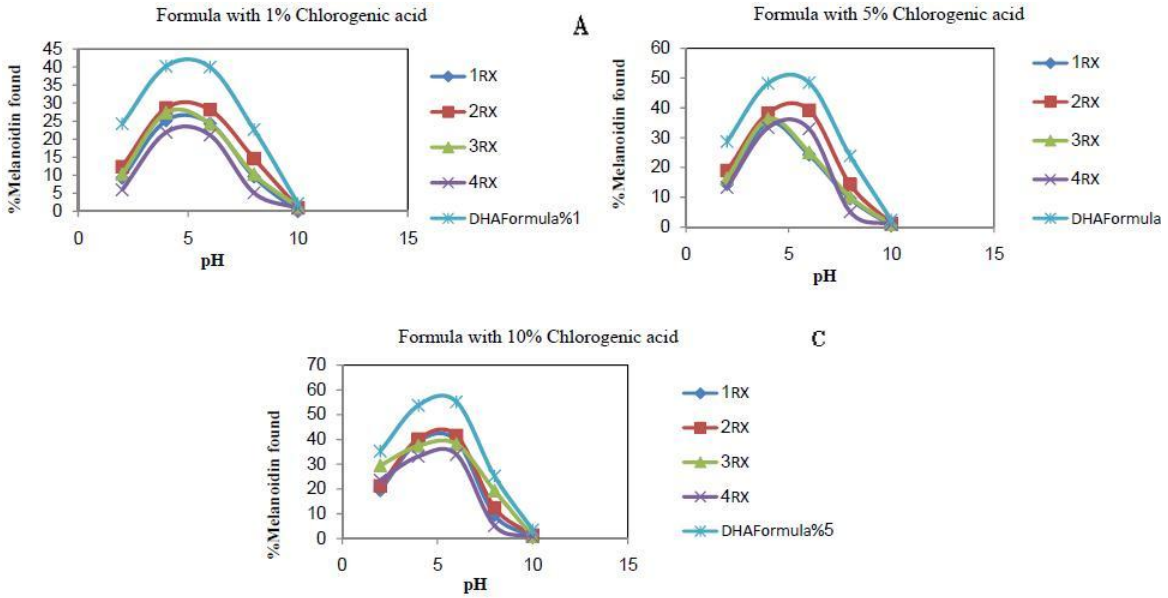

Fig.3. Melanoidin concentration found in the skin firstly rubbed with various pH analyzed from 5 formulas with various concentration (% by weight) of chlorogenic acid. (a = 1%, b = 5% and c = 10%) at 2 days application and reaction time.

Conclusion

Sunless tanning products are also known as self-tanners [3]. Their use has become more popular as people become more aware of the dangers of long term sun exposure. The main ingredient in sunless tanning can be divided in: stainers (based on dihydroxyacetone, DHA), bronzers (which basically are dyes), tan accelerators (based on tyrosine and psolarens), and solaria (method used in sunbeds and sunlamps) [1]. Tanning accelerators are ineffective and also may be dangerous. Long-term exposure to artificial sunbeds increases risk of developing skin cancer. Two other sunless tanning products, bronzers and extenders, are considered cosmetics for external use and are not harmful. Bronzers, made from color additives approved by FDA for cosmetic use, stain the skin when applied and can be washed off with soap and water. Extenders, when applied to the skin, interact with protein on the surface of the skin to produce color. The color tends to wear off after a few days. The only color additive approved for extenders is dihydroxyacetone. Now-a-day the cosmetic manufacturing industry has become interested in using natural extracts as ingredients. They are popular believed to be effective, safe and friendly to human skin. So do the sunless tanning products, natural products are interesting to be applied into cosmetic. Many extracts from natural plants can be used in sunless tanning products as stainer or extender are Lawsone found in henna plant, and Juglone (5-hydroxy-1,4-naphthoquinone) derived from walnut. Another plant's extract that assumed to be sunless tanning ingredient in this study is the extract from green coffee beans.

Coffee is among the most widely consumed pharmacologically active beverages in the world [8]. The major components in coffee are caffeine and other polyphenol: chlorogenic acid, an ester formed between *trans* cinnamic acids and quinic acid.Chlorogenic acid and other acids in coffee bean are still not concerned in the cosmetic means. It is an antioxidant [9, 10]. The supposition of its tanning activity is due to maillard reaction or browning reaction. Chlorogenic acid may be considered a

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reacting with free amino groups available as amino acid, peptides, and proteins supplied by the keratin to form chromophores referred to as melanoidins, a golden brown hue, and no skin damage. The site of action of sunless tanning chlorogenic acid is the stratum corneum. In this study, extracted chlorogenic acid from green coffee beans could be incorporated into cosmetic cream base, and it gave the tanning effect with stability test. The O/W cream base with extracted chlorgenic acid from green coffee beans had the most tanning activity with pH 4.5±0.5 and 40^{o}C. The O/W cream also performed most stable than the other two types of cream. In conclusion, sunless tanning product from green coffee beans might be formulated in O/W type cream and apply with the pH more than 7.

Conflict of interest

Author certifies that no actual or potential conflict of interest in relation to this article exists.

Acknowledgement

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