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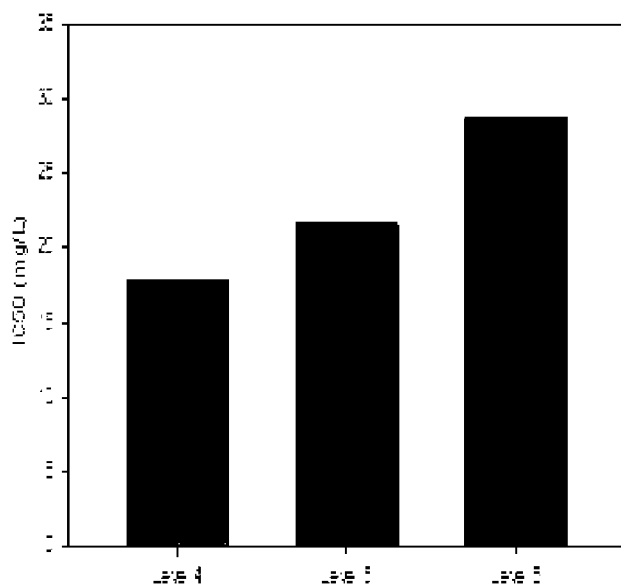
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(54) Title: THE INHIBITORY MELANOGENESIS BY COFFEE OIL EXTRACTED BY SUPERCRITICAL CO₂



(57) Abstract: The coffee oil extracted by supercritical carbon dioxide is useful for skin whitening by inhibition of melanogenesis. A effective dosage of coffee oil wherein said dosage of 10 mg/L of treating oil, the total melanin content was decreased over 25%. The coffee oil inhibits the early period of melanogenesis is which was proved by the down-regulation of 8 identified proteins including -MSH, MC1R, ACTHR, MITF, PKC-, SCF, TYR and TRP-2.

Description

Title of Invention: THE INHIBITORY MELANOGENESIS BY COFFEE OIL EXTRACTED BY SUPERCRITICAL CO₂

Technical Field

- [1] The present invention relates to the inhibitory melanogenesis of coffee oil extracted by supercritical carbon dioxide. This coffee oil is useful for skin whitening by inhibition the early period of melanogenesis which was proved by the down-regulation of 8 identified proteins including -MSH, MC1R, ACTHR, MITF, PKC-, SCF, TYR and TRP-2.

Background Art

- [2] Robusta (*Coffea Robusta*) is a species of coffee which has its origins in central and western sub-Saharan Africa and was introduced into Southeast Asia by French colonists in the late 19th century. According to the statistics of the International Coffee Organization, approximately one-third of the coffee produced in the world is Robusta, and Vietnam has been the world's single largest exporter of Robusta in recent years.
- [3] Benefits of coffee on human health are natural because of bioactive compounds. Chlorogenic acids are a family of esters formed between trans-cinnamic acids and quinic acid. The commonest individual chlorogenic acid is formed between caffeic acid and quinic acid. It has been shown that both chlorogenic acid and caffeic acid are strong antioxidants in vitro. Coffee beans are one of the richest dietary sources of chlorogenic acid and for many consumers this will be their major dietary source. The roasting of coffee beans dramatically increases their total antioxidant activity. A roasting time of 10 minutes (medium-dark roast) was found to produce coffee with optimal oxygen scavenging and chain breaking activities in vitro. The total antioxidant activities of different plant phenol- containing beverages have been compared and it has been shown that coffee has significantly more total antioxidant activity than either cocoa, green tea, black tea or herbal tea. It can be concluded that coffee possesses greater in-vitro antioxidant activity than other beverages, due in part to intrinsic compounds such as chlorogenic acid, in part to compounds formed during roasting such as melanoidins and in part to as yet unidentified compounds.
- [4] The melanogenesis is a process of production of special pigment in our body called melanin. Human epidermis contains special cells - melanocytes - which are responsible for melanogenesis. And the amount of melanin in human epidermis defines the skin color. The synthesis and distribution of melanin in the epidermis involves several steps: transcription of melanogenic proteins, melanosome biogenesis, sorting of melanogenic proteins into the melanosomes, transport of melanosomes to the tips of

melanocytes dendrites and finally transfer into keratinocytes. Melanin formation and transformation in human skin is an important mechanism for the protection of skin from the UV light. Side effects like hypopigmentation or hyperpigmentation can occur due to numerical mutations. Abnormal melanism such as liver spots, freckles, nevus freckles, and dark spots are also a serious problem in dermatology and the beauty care area. Bernard et al. have made efforts to identify the mechanism of melanin formation and transformation in human skin that relate to the control of melanogenesis and its side effects. Up to now, the regulation of melanogenesis has not been fully understood. However various factors, involved in melanogenic synthesis have been gradually identified.

Disclosure of Invention

Technical Problem

- [5] Melanogenesis is under complex regulatory controlled by multiple agents. The most important positive regulator of melanogenesis is the MC1 receptor with its ligands melanocortin peptides. MC1R activates the cyclic AMP (cAMP) response-element binding protein (CREB). Increased expression of MITF and its activation by phosphorylation (P) stimulate the transcription of tyrosinase (TYR), tyrosinase-related protein 1 (TRP1), and dopachrome tautomerase (TRP2), which produce melanin. Melanin synthesis takes place within specialized intracellular organelles named melanosomes. Melanin-containing melanosomes then move from the perinuclear region to the dendrite tips and are transferred to keratinocytes.
- [6] A need exists for inhibitory melanogenesis agent with specific above action mechanisms. In addition, low cytotoxicity is required for the agent above.

Solution to Problem

- [7] An invention was carried out to evaluate the skin whitening efficacy of Vietnamese Robusta oil. Vietnamese Robusta green beans were roasted at six different levels and extracted oil by supercritical carbon dioxide. After measuring the radical scavenging activity and tyrosinase inhibition activity of coffee oils, the good roasting condition was chosen for in vitro test on B16F1 mouse melanoma cells. First, MTT assay was conducted to identify the toxicity of the coffee oil. After that, melanin content was measured to evaluate the inhibition of Vietnamese Robusta oil on melanin production. Finally, proteome analysis using 2-D was conducted to observe the changes of proteins that are involved in melanogenesis.

Advantageous Effects of Invention

- [8] Coffee oil was found to inhibit well melanogenesis of mouse melanoma B16F1 cells. Coffee at roasting level 4 (230, 15min) has stronger melanogenesis inhibition activity with SC50 value for DPPH scavenging is 400 mg/L and IC50 value for Tyrosinase

Inhibitor is 17.73 mg/L. Melanin content of melanoma cells treated with 10 mg/L VN Robusta oil decrease about 25%. The coffee oil inhibits the early period of melanogenesis which was proved by the down-regulation of 8 identified proteins including -MSH, MC1R, ACTHR, MITF, PKC-, SCF, TYR and TRP-2.

Brief Description of Drawings

- [9] For the more complete understanding of present invention, and for further details and advantages thereof, reference is now made to the following detailed description taken in conjunction with the accompanying drawings, in which,
- [10] Fig. 1. is a bar chart showing the IC₅₀ (mg/L) of Tyrosinase Inhibition Activity of the coffee oils. Oils extracted from 3 roasting levels 4 (230, 15min), 5 (250, 15min) and 6 (250, 20min) were tested for tyrosinase inhibition activity. The IC₅₀ of the coffee oils were calculated. At every roasting level, the tyrosinase inhibition activity of coffee oil is in concentration dependent manner. Following the roasting levels from 4 to 6, the tyrosinase inhibition properties of the coffee oil decreased regularly. Based on the IC₅₀ values, the coffee oil at roasting level 4 was chosen as the efficient condition.
- [11] Fig. 2. is a graph showing the Melanin content (%) in Melanoma B16F1 treated by the coffee oil. The melanin contents formed inside and released outside of the cells treated by the coffee oil at 0.1; 0.5; 1; 5 and 10 mg/L were measured. The higher concentration of oil treated, the lower intracellular and extracellular melanin contents were. At 10 mg/L of treating oil, the total melanin content was decreased over 25%. This proved that the coffee oil had the ability to inhibit melanin production in melanoma cells.
- [12] Fig. 3. is a 2D-PAGE images of proteins extracted from melanoma B16F1 treated by 10 mg/L of the coffee oil at roasting level 4. The left image is control, and the right image is the 2D-PAGE image of melanoma treated with the coffee oil. The changes of melanogenesis related proteins were observed by comparing the control 2-D gel and sample 2-D gel which was treated by 10 mg/L of the coffee oil at roasting level 4. On control gel and sample gel, there were 625 and 666 detected spots respectively. Among them, 398 spots were matched in which 186 spots were up-regulated and 212 spots were down-regulated. Based on isoelectric point (pI) and molecular weight (MW), 8 proteins were identified. Almost the identified proteins participate in the early period of melanogenesis. Noticeably, in the transcription of tyrosinase gene there were 4 identified proteins down-regulated. Among them, melanocortin 1 receptor (MC1R), the most important positive regulator of melanogenesis, decreased more than 60% after treating with VN Robusta oil. When activated by one of the variants of MSH, typically -MSH, MC1R initiates a complex signaling cascade that leads to the production of the brown or black pigment eumelanin. These lead to a decrease of the formation of Ty-

rosinase - the most important enzyme forming melanin from tyrosine - about 20%. Furthermore, PKC- and SCF were also observed that down-regulated, as a result the activation of tyrosinase was also decreased. Consequently, the enzyme tyrosinase was not only decreased the amount but also declined the activity. In addition, the amount of tyrosinase related protein-2 (TRP-2) was decreased nearly 40%, which in particular inhibits the melanin production in melanoma cells.

Industrial Applicability

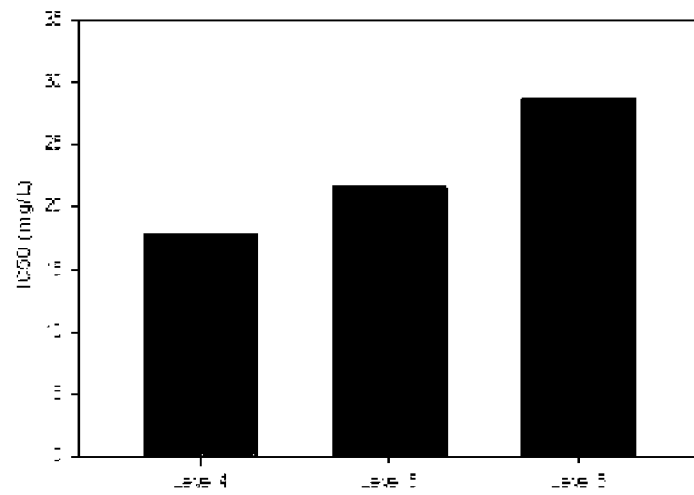
[13] The coffee oil prepared by present invention is applicable to skin whitening agent for cosmetic industry. The coffee oil is also applicable to food industry especially in the area of beauty foods.

[14]

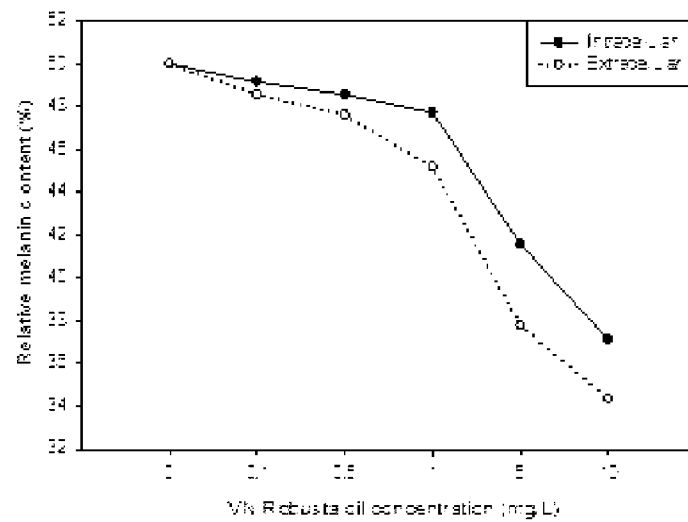
Claims

- [Claim 1] A method of promoting the inhibitory melanogenesis of skin comprising delivering a effective dosage of coffee oil wherein said dosage inhibits the synthesis of proteins related to melanin synthesis in human skin including -MSH, MC1R, ACTHR, MITF, PKC-, SCF, TYR and TRP-2.
- [Claim 2] The method of claim 1 wherein the coffee oil preparation comprises extraction from coffee roasted more than 10 minutes at the temperature of 200 and higher.
- [Claim 3] The method of claim 1 wherein the coffee oil preparation comprises extraction with supercritical carbon dioxide.
- [Claim 4] The method of claim 3 wherein the supercritical carbon dioxide comprises carbon dioxide at boundary conditions of temperature between 40 to 90 and of pressure 100 bar and higher.

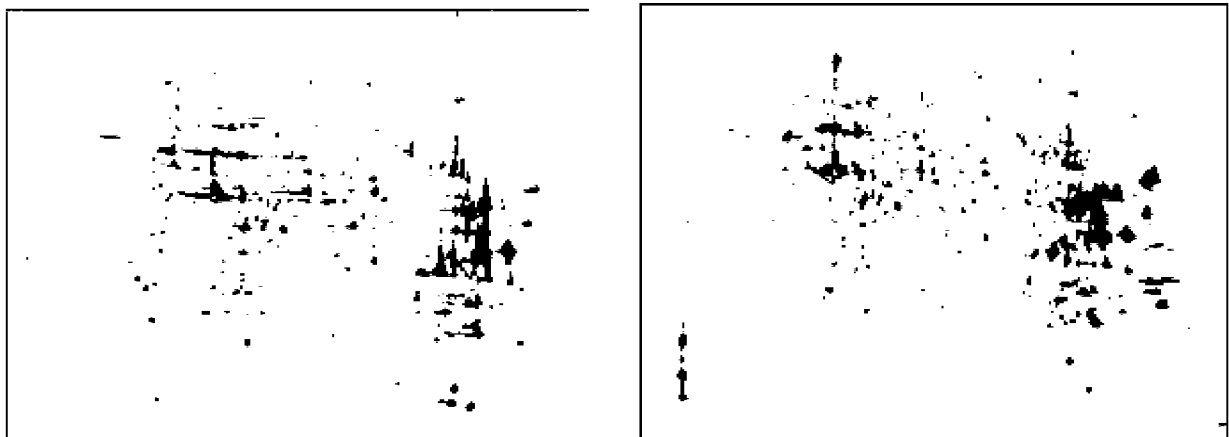
[Fig. 1]



[Fig. 2]



[Fig. 3]



INTERNATIONAL SEARCH REPORT

International application No.
PCT/KR2012/008909**A. CLASSIFICATION OF SUBJECT MATTER****A61K 36/74(2006.01)i, A61K 8/97(2006.01)i, A61P 17/00(2006.01)i, A61Q 19/02(2006.01)i**

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

A61K 36/74; A61K 8/02; A61K 36/185; A61Q 19/00; C11B 13/00; A61K 8/97; A61P 17/00; A61Q 19/02

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Korean utility models and applications for utility models

Japanese utility models and applications for utility models

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

eKOMPASS(KIPO internal) & Keywords: supercritical carbon dioxide, inhibition of melanogenesis, coffee oil, melanin

C. DOCUMENTS CONSIDERED TO BE RELEVANT

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X	WO 2012-013764 A2 (NESTEC S.A. et al.) 02 February 2012 See abstract, claims 1-4, 7, 13-14, column 8, lines 29-35.	1-2
A	US 2006-0018986 A1 (BRETON, L.) 26 January 2006 See abstract, claims 1-4, paragraphs [0004]-[0005].	1-4
A	PARK, K. S., "The Study of Cosmeceutical Activities from Coffee Beans and Application of MLV Liposomes on Advanced Formulation", Thesis of Graduate School of Daegu Haanny University, 2010. See pages 47-58.	1-4
A	KR 10-2011-0077722 A (NATURAL LIVE PLANKTON CO., LTD.) 07 July 2011 See abstract, claims 1-11.	1-4
A	KR 10-2007-0015569 A (VDF FUTURECEUTICALS, INC.) 05 February 2007 See abstract, claims 1, 14.	1-4



Further documents are listed in the continuation of Box C.



See patent family annex.

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Date of the actual completion of the international search

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.

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