FORMULATION AND PRODUCTION OF FACIAL CREAM FROM PAPAYA

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ABSTRACT

The purpose of this research was carried out to formulate and to produce a face cream products from papaya powder. Papaya fruit is typically used for treatment of disease, especially skin. This has led researchers to conduct a study of superfine powder, papaya production by means of freeze drying. After this process, papaya powder will be tested on the presence of the enzyme and its activity in the papaya powder. Papaya powder was also tested for its effectiveness to prevent chemical action of the reaction to produce black melanin. Making face cream is produced by the number of physical tests of stability and viscosity. From the results obtained of this research, the papaya powder can be produced by using freeze drying method with the addition of a few grams of additives that is Maltodextrin. The face cream that has been produced also showed the stability to be saved in the long time and the viscosity obtained was around 90-210 cps. Unfortunately, the melanin synthesis cannot be continued because of some troubleshooting occurred during the cell culture.

ABSTRAK

Tujuan penyelidikan ini dijalankan adalah untuk menggubal dan juga untuk mengeluarkan satu produk krim muka daripada serbuk betik. Buah betik pada kebiasaanya digunakan untuk rawatan penyakit terutamanya kulit. Keadaan ini telah mendorong penyelidik unutk menjalankan satu kajian tentang penghasilan serbuk betik dengan kaedah pembekuan pengeringan. Selepas proses ini, serbuk betik tersebut akan diuji tentang kehadiran enzim dan juga aktiviti enzim tersebut dalam serbuk betik itu. Serbuk betik itu juga diuji dari segi keberkesanannya menghalang tindakan kimia iaitu tindak balas melanin untuk menghasilkan warna hitam. Penghasilan krim muka ini dihasilkan dengan melalui beberapa ujian fizikal iaitu kestabilannya dan juga kelikatannya. Daripada keputusan yang diperolehi dari kajian ini menyatakan bahawa serbuk betik itu boleh dihasilkan dengan menggunakan kaedah pembekuan pengeringan dengan tambahan beberapa gram bahan tambahan iaitu maltodextrin. Krim muka yang telah dihasilkan juga menunjukkan kestabilannya untuk disimpan dalam jangka masa yang panjang dengan memperoleh kelikatan dalam aggaran 90-210 cps. Malangnya, ujian perencatan sintesis melanin itu tidak dapat diteruskan memandangkan sel yang digunakan semasa menternak mengalami beberapa masalah.

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LIST OF SYMBOLS

С	Celsius
Cfu	Colony Forming Unit
Cps	Centipoises
g	Gram
kDa	kilodalton
mL	Mililiter
Ν	Nomality
nm	Nanometer
rpm	Revolution per Minute
µg/ml	Microgram per mililiter

LIST OF ABBREVATIONS

ALES	Ammonium Laureth Sulfate
ALS	Ammonium Lauryl Sulfate
ATCC	American Type Culture Collection
DHI	5, 6- dihyroxyindole
DHICA	Dihydrochinindol-2-carboxyl acid
DOPA	Dihydroxyphenylalanine
DMEM	Dulbecco's Modified Eagles Medium
EDTA	Ethylenediaminetetraacetic acid
FBS	Fatal Bovine Serum
GMP	Good Manufacturing Practice
PBS	Phosphate Buffer Solution
PG	Propylene Glycol
SLES	Sodium Laureth Sulfate
SLS	Sodium Lauryl Sulphate
TCA	Trichloroacetic Acid
TEA	Triethanolamine
UV	Ultraviolet

CHAPTER 1

INTRODUCTION

1.1 Background of Research

Generally there are widely reasons for the change of skin colour. The exposure to the ultraviolet radiation is the main purpose for the changing in colour. UV radiation is the most significance factor influencing human skin pigmentation. UVA rays is the immediate pigment darkening occurs within minutes and persists for several hours followed by persistent pigment darkening, which occurs within several hours and lasts for several days. Basically, the racial or ethnic background are also the factor for the skin colour types such from extremely light or fair to extremely dark. Instead of that, melanocytes density at some area such as back or arms is also virtually identical in all types of skin colour (Howard, 2007). In fair skin, the keratinocytes would cluster poorly their pigment melanosomes above the nuclei. Meanwhile, in the darker skin, the pigmented melanosomes are individually distributed in the keratinocytes. Therefore the absorption of light will be maximum. Melanocytes density may also be vary in different parts of the body such as the difference between skins on palm orsoles compared to the parts of body. Environment also can be ones of the factors to the constitutive of melanocytes density which is by chronic ultraviolet (UV) radiation or by toxic compounds such as hydroquinone. These ultraviolet rays may increase the melanocytes density by 3- or 4-fold. The present of the toxic compound may be serious effect to the skin structure where it can be very selectively and permanently destroy the melanocytes in the skin. Increasing melanocytes density such as freckles or decreasing the melanocytes density such as vitiligo in the skin may be caused by the inherited of pigmented disorder (Howard, 2007).

MC1R is standing for melanocortin 1 receptor where it is the ones of the pigment determinant of the skin. The quantity and quality of melanins production is regulate by the G protein-coupled receptor. The function of MC1R is controlled by the agonists' α -melanocyte stimulating hormone (α MSH) and adrenocorti-cotropic hormone (ACTH) and also by an antagonist, Agouti signalling protein (ASP) (Howard, 2007). Melanogenic cascade and eumelanin synthesis are stimulate by the activation of MC1R by an agonist. Meanwhile the ASP may reverse the effect and elicit the production of of pheomenlanin.

Skin pigmentation can be determine through a several way such as the migration of melanoblasts to that tissue during development, their survival and differentiation to melanocytes, the density of melanocytes, the expression or function of enzymatic and structural constituents of melanosomes, the synthesis of different types of melanin (euor pheomelanin), the transport of melanosomes to dendrites, the transfer of melanosomes to keratinocytes and finally the distribution of melanin in suprabasal layers of the skin.

The regulation of human skin pigmentation has been a longstanding goal for cosmetic and pharmaceutical applications (Howard, 2007). There are several implications according to this skin pigmentation regarding to the social standing, cosmetic appearance and also photoprotection of the skin against cancer and photoaging. A number of approaches to stimulate pigmentation have been tried, including activation of MC1R by agonists and bioactive derivatives, topical application of factors that bypass the MC1R, factors to stimulate tyrosinase function, and factors to increase melanosomes transfer (Howard, 2007). Therefore this chapter will discuss the problem statement, research objective, research question/ hypothesis, scope of study, expected result and also rationale and significance of the study.

1.2 Problem Statement

Most people obtain darker skin through to ultraviolet light (Clemente et al., 2010).Ultraviolet radiation is one of the types light radiation. Usually ultraviolet rays will penetrate into the skin when it reaches the outer surface of the skin. The exposure to the sunlight radiation may give effect to the skin colour (Stanojevic, 2004).

The skin colour is a function of size, number and the distribution of melanin cells. Melanin cell of darkly pigmented skin have thicker, longer and branched dendrites. Tyrosinase is a rate-limiting enzyme of this biosynthetic pathway. Activities of tyrosinase give effect on the skin colour. Melanin cells are produced from the black pigment due to the increasing of tyrosinase activity. Therefore, this is the main reason why this research is conducted that are to inhibit the tyrosinase activity in order to reduce the synthesis of melanin through the extraction of papaya using freeze drying process(Mediderm Laboratory, n.d).

1.3 Research Objective

There are three objectives to conduct this research:

- a) To extract the papaya using freeze drying method
- b) To investigate the effect of papaya on inhibitory mechanisms of melanin synthesis
- c) To test the physical characteristic of developed milk lotion with the papaya extract

1.4 Research Question/Hypothesis

The research questions of this research are:

- a) The application of papaya extract is significant as a main agent in reducing the dark pigment
- b) The higher concentration of extract papaya can inhibit the melanin synthesis

c) The physical characteristic of developed milk lotion is due stable

1.5 Scope of Research

To fulfil the objectives, the following scope of research was carried out:

- a. The concentration of the maltodextrin used during freeze drying process
- b. The concentration of papaya extract
- c. The test of the milk lotion physical characteristic

1.6 Rationale and Significance of Research

The objectives of this research are to extract papaya using freeze drying, to investigate the effect of papaya extract on inhibitory mechanisms of melanin synthesis and to test the physical characteristic of developed milk lotion. The removal of dark pigment from skin is very necessary due to maintain whitening agent in face skin. Besides that, it is also to avoid any skin disease. Therefore this research would identify that freeze drying is the potential way to preserve whitening agent in the skin. In the other hand, papaya extract can be use to induce the tyrosinase activity so that the raw material used in the whitening cosmetic may show a high stability and superior whitening effect.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

Plants and plant ingredients are common and of major importance in the fields of pharmacy, food and cosmetic (Schurch.G, 2007). Since the ancient time, plants including it parts such as leaves, fruits, seeds, flowers, stems, barks, and roots are very well known among the cosmetic and pharmaceutical industries. In cosmetic industries, the application of plants and plant extracts are widely used and various of purpose such as moisturizing, whitening, tanning, colour cosmetic, sunscreens, radical-scavenging, anti-oxidant, immunostimulant, washing, preservatives, ands thickeners (Blum et al., 2007). The spectrum of used of plants or parts of plants is broad and ranges for different types of plants (Blum et al., 2007).

Instead of known the plants benefits, there are also a certain limitations can occur when to use the plants or plant material such as availability can be restricted through seasons, limited stock, protection of the plant, problems in cultivation and bad harvest. Besides that the inconstant of quality through seasonal changes, different cultivation methods, geographical differences, delivery sources, clone types, pollution and also physical state (Schurch et al., 2007). These facts complicate the use of certain plants in a cosmetic application (Blum et al., 2007).

In this chapter the things that will be discuss is the chemical profile of unripe papaya, freeze drying and melanogenesis.

2.2 **Properties of Unripe Papaya**

Papaya is a fruit of the papaya tree or also known as Carica papaya, native of Central America. The fruit ripens from 4 to 6 months depend on the climate where it is grown (Salunkhe and Kadam, 1995). The other name of papaya is Papaw or Paw Paw where it is belongs to the group of Caricaceae (Oleyede, 2005). The plant can be monoecious, dioecious or hermaphroditic (Purseglove, 1968, Janick, 19880. Papaw fruit is a berry, developing from syncarpous superior ovary with parietal placentation (Kochhar, 2986, Rice et al., 1987). Mostly, plantation of papaya is used for its fruits, and it is very familiar among people because they used them as their breakfast and also as an ingredient in jellies, preserves, or cooked in various ways. The juice makes a popular beverage, young leaves, shoots and fruits cooked as vegetable (Oleyede, 2005). Plant extracts have pronounced bactericidal activity against Staphylococcus aureus, Bacillus cereus, Esherichia coli, Pseudomonas aeruginosa and Shigella flax neri (Oleyede, 2005).Proximate analysis, mineral composition and phytochemical analysis were carried out on dried sample of unripe papaya to determine the unripe papaya properties (Oleyede, 2005).

2.2.1 Phytochemical Analysis

Phytochemical analysis also known as phytochemical screening procedures which had been carried out adapted from the previous work on plant analysis (Odebiyi et al., 1979). The purpose of this screening procedure is to detect the biologically active non-nutritive compounds that contribute to the flavour, colour and other characteristics of plant parts such as alkaloids, tannins, cardiac glycisides, saponin and others (Oleyede, 2005).

2.3 Natural Ingredients for Facial Cream

Natural is available from nature without chemical modification or intermediate synthesis. Cosmetic preparations become more advantageous when using antioxidant as an active ingredient. There is growing interest in the natural antioxidants found in plants. Many antioxidatively acting compounds are isolated from natural herbs and extracts and used as potential antioxidants in cosmetics, (Akhtar, 2011). The most successful recent and natural skin whitening agents are vitamin C, kojic acid, litoric extract, burnet root extract, scutellaria extract, and mulberry, (Thongchai, Liawruangrath, & Liawruangrath, 2007). The following table are describing a few of antioxidants from plant extraction as natural ingredient in cosmetic.

Ingredients	Formulation	Testing	References
Glycrrhiza	W/O creams,	Stability tests-	(Naveed Akhtar,
glabra	paraffin	physical analysis,	2011)
	oil,beeswax,coconut	types of emulsion, pH	
	oil and surfactant	determination,	
	(ABIL-EM 90)	electrical conductivity	
		and centrifugation	
		tests.	
		Patch test	
		Panel test	
		Dermatological test	
		Statistical analysis	
Cucumber	Paraffin oil, Cetyl-	Pharmaceutical	(Akhtar, 2011)
	Dimethicone	stability test-	
	copolyol with HLB	centrifugation, pH	
	5, lemon oil	determination,	
		electrical conductivity	
		,temperature, colour,	
		creaming and	
		liquefaction	
		Burchard tests	
Fenugreek	Paraffin oil, abil-	Stability test-	(Waqas, 2010)

Table 2.1: Antioxidants from plant extraction as natural ingredient in cosmetic

seeds	EM 90, Lemon Oil,	temperature, colour,	
	W/O emulsion	liquefaction, phase	
		separation	
		Centrifuge test	
		Electrical conductivity	
		рН	
		Dermatological test-	
		melanin,erythema,skin	
		moisture content, skin	
		sebum content, trans	
		epidermal water loss	
Twigs of	Arbutin, kojic acid,	In vivo in 20 human	(Omboon luanratana,
Morus alba	trans-resveratrol,	subjects	2005)
	mushroom		
	tyrosinase		
Phlai rhizome	Male and female	Adult albino rabbits of	(Reungpatthanaphong,
and mulberry	rabbit, weighting 2-	New Zealand white	2010)
leaves	3 kg	hybrid-acute dermal	
		irritation test	
Yuzu seed		Inhibitory effects on	(Oryza Oil & Fat
		melanin formation	Chemical Co., LTD,
			2004)

2.3.1 Synthetic Cosmetic to Avoid

According to the Dr, Samuel S. Epstein (Safe Shopper's Bible pages 182 &183), there are more than 38,000 cosmetic injuries requiring medical attention were reported in the United States. In 1989, the United States National Institute of Occupational Safety and Health recognized 884 poisonous substances from a list of 2,983 chemicals used in the fragrance industry that are capable of causing cancer, birth defects, central nervous system disorders, allergic respiratory reactions, skin and eye irritations. Mostly,

chemicals that used in cosmetic do not show an obvious sign of toxicity but it is showing in the slowly way to the users. The following table is showing the synthetic ingredient that should be avoided in cosmetic, (Hampton, 2000).

Table 2.2: Synthetic ingredient that should be avoided in cosmetic

Synthetic Ingredient	Description	
Methyl, propyl, butyl, ethyl paraben	This parabens is used as inhibitors of microbial	
	growth and to extend shelf life of the product.	
	This synthetic cosmetic are widely used even it	
	is well know that they are toxicity. This	
	paraben have cause many allergic reaction and	
	skin rashes.	
Sodium lauryl sulphate(SLS) &	Commonly these synthetic ingredients were	
Sodium Laureth Sulfate (SLES),	used in shampoos for its detergents and	
Ammonium Laureth Sulfate (ALES)	foaming-build abilities. It causes eye	
& Ammonium Lauryl Sulfate (ALS)	irritations, skin rashes, hair loss, scalp scurf	
	similar to dandruff and allergic reaction. It is	
	frequently disguised in pseudo-natural	
	cosmetic with the parenthetic explanation	
	'comes from coconut'	
Synthetic colour	Also known as FD&C Colour pigments is a	
	synthetic colour that make from coal tar,	
	containing heavy metals salts that deposit	
	toxins onto the skin, causing skin sensitivity	
	and irritation. Absorption of certain colours	
	can cause depletion of oxygen in the body and	
	death. Animal studies have shown almost all of	
	them to be carcinogenic. No alternative	
	necessary.	
Fragrances	This synthetic ingredient is containing animal	

Source: (Hampto	on, 2000)
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	urine or feces. Fragrance on a label can
	indicate the presence of up to four thousand
	separate ingredients, many toxic or
	carcinogenic. Symptoms reported to the FDA
	include headaches, dizziness, allergic rashes,
	skin discoloration, violent coughing and
	vomiting and skin irritation. As a clinical
	observation proves that fragrance can affect
	the central nervous system, causing
	depression, hyperactivity, irritability, inability
	to cope, and other behavioural changes.
	Alternatives such as:
	Aromatherapeutic, organic essential oils.
Formaldehyde	Also known as DMDM hydantein diazolidinyl
	urea sodium hydroxymethyl glycinate, N-
	(Hydroxy methyl) glycine, monosodium salt
	and quaternium-15, is a general ingredient
	used in a number of beauty products. Some of
	them are used as a preservative for an
	alternative to paraben. Formaldehyde was
	categorized as a human carcinogen in the
	middle of 2004 and is known to cause irritated
	threats, eyes and nerves as well as the cause of
	cancer and asthma with recurrent usage.
Petrolatum	This ingredient is made off from crude oil and
	its look like petroleum jelly or ointment. It is
	frequently used in the cosmetic industry
	because basically it is really cheap. Some facts
	about this oily substance that might not know
	are that it actually provoke acne in skin and
	can cause premature aging. Its immunity to
	contamination is low and it can be invaded by
	harmful substances such as 1,4-dioxane, a

	suspected toxin to humans.
Triclosan	A synthetic 'antibacterial' ingredient with a
	chemical structure similar to agent orange. The
	EPA registers it as a pesticide, giving it high
	scores as a risk to both human health and the
	environment. It is classified as a chlorophenol,
	a class of chmicals suspected of causing cancer
	in humans. Its manufacturing process may
	produce dioxin, hormone-disrupting chemical
	with toxic effects measured in the parts per
	trillion. Hormone disruptors pose enormous
	long-term chronic health risks by interfering
	with the way hormones perform, such as
	changing genetic material, decreasing fertility
	and sexual function and fostering birth defects.
	It can temporarily deactivate sensory nerve
	endings, so contact with it often causes little or
	no pain. Internally, it can lead to cold sweats,
	circulatory collapse and convulsions. Stored in
	body fat, suppression of immune function,
	brain haemorrhages, and heart problems. Tufts
	University School of Medicine says that
	triclosan is capable of forcing the emergence
	of 'super bugs' that it cannot kill. Its
	widespread use in popular antibacterial
	cleansers, toothpastes and household products.
Stearalkonium Chloride	A chemical used in hair conditioners and
	creams. Causes allergic reactions.
	Stearalkonium chloride was developed by the
	fabric industry as a fabric softener, and is a lot
	cheaper and easier to use in hair conditioning
	formulas than proteins or herbals, which do
	help hair health. Toxic.

Triethanolamine	Most used in cosmetics to adjust pH, and used
	with many fatty acids to convert acid to salt
	(stearate), which then becomes the base fpr a
	cleanser. TEA causes allergic reactions
	including eye problems, dryness of hair and
	skin, and could be toxic if absorbed into the
	body over a long period of time.
Propylene glycol (PG) and Butylene	body over a long period of time. Ideally this is vegetable glycerine mixed with
Propylene glycol (PG) and Butylene Glycol	body over a long period of time. Ideally this is vegetable glycerine mixed with grain alcohol, both of which are natural.
Propylene glycol (PG) and Butylene Glycol	body over a long period of time. Ideally this is vegetable glycerine mixed with grain alcohol, both of which are natural. Usually it is synthetic petrochemical mix used
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Propylene glycol (PG) and Butylene Glycol	body over a long period of time. Ideally this is vegetable glycerine mixed with grain alcohol, both of which are natural. Usually it is synthetic petrochemical mix used as humectants. Has been known to cause allergic and toxic reactions.

2.4 Advantages of Using Facial Cream

The most reason why people purchase a skin facial cream is to restore a more youthful complexion, as a means to enhancing personal beauty. Moreover, it helps ailments of skin. Ranging from age spots, to other skin discoloration due to hyper pigmentation. According to Author Ken Turner that has been studying and doing research for over 20 years on natural herbal remedies and nutrition, including natural skin whitening creams. He owned an herbal health and vitamin store and has helped thousands of people solve their health problems in a very safe and natural way, (Turner, 2010).

A facial cream also helps in whitening the colour of skin and also adds moisture and protective layer on the skin. Instead of that it is also can covered the dark spots, blemishes and discolorations to make them unnoticeable. The facial creams acts as removal of the flaws from skin, maintaining the smoothness and moistness of skin (Hussey, 2009).

2.5 Development of Skin Care Product

Starting material quality is the most important part to ensure the cosmetic product quality are good and followed GMP regulation. According to GMP guidelines for cosmetic products include that all starting material should correspond to the agreed specification and consistently be of good quality. The requirement is equally to chemical and physical product parameters and microbial purity. The cosmetic starting material and material mixtures need protection against microbial contamination during transportation, storage and product application. The contamination of starting material will introduce to the load or overload of production and the product preservation capacity. Therefore, an essential condition for the manufacture of cosmetics is the use of starting materials containing the lowest possible level of microbes, where it is possible fewer than 10 colony forming units (cfu) per gram. The ingredients and packaging of cosmetic products must be ensured that all the information must be clearly identifiable and bear such as product name, batch number, and number of items, gross weight and tare. All the aspect such as the quality of the quality of cosmetics ingredients, product and storage stability, adequate preservation and the compatibility of cosmetic ingredients and packaging are all checked during the development stage and appropriate specification of the cosmetic ingredients are defined. According to the GMP guidelines indicate that production should be carried out by qualified personnel in suitable premises and equipment. Measurement and instruments should be calibrated and service regularly. A comprehensive system of records should be established to provide documentation of consistent good quality of production, storage and testing. Cosmetic ingredients must be produced in a clean and rigorously hygienic environment to exclude any form of contamination. A crucial requirement for the production of cosmetic ingredients with low microorganism content is the use starting materials must with low micro-organism content. The GMP requirements also have stated that the quarantined and released materials must be clearly separated and labelled (Scholtyssek, 2004).