FORMULATION AND EVALUATION OF O/W EMULSIONS CONTAINING ARABINOXYLANS FROM RICE BRAN RESIDUES

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for the Degree of Master of Science Program in Pharmaceutical Technology
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การตั้งและประเมินสูตรตำรับอิมัลชันชนิดน้ำมันในน้ำที่มีส่วนผสมของอะราบิโนไซแลนจากกากรำข้าวเจ้า

นางสาว ยุวเรศ เหลืองวิชชเจริญ

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
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ยุวเรศ เหลืองวิชชเจริญ : การตั้งและประเมินสูตรตำรับอิมัลชันชนิดน้ำมันในน้ำที่มีส่วนผสมของอะราบิโนไซแลนจากกากรำข้าวเจ้า (FORMULATION AND EVALUATION OF O/W EMULSIONS CONTAINING ARABINOXYLANS FROM RICE BRAN RESIDUES) อ. ที่ปรึกษาวิทยานิพนธ์หลัก : รศ. ดร. อุบลทิพย์ นิมมานนิตย์, อ. ที่ปรึกษาวิทยานิพนธ์ร่วม : ดร. กฤษณา ศิรเลิศมุกุล, 115 หน้า.

งานวิจัยนี้เป็นการศึกษาการสกัดอะราบิโนไซแลนจากกากรำข้าวเจ้าโดยวิธีการสกัดด้วยน้ำอุ่น วิเคราะห์โครงสร้างทางเคมีของสารสกัดที่ได้ด้วยวิธี FT-IR, NMR และการพัฒนาตำรับอิมัลชันชนิดน้ำมันในน้ำที่มี อะราบิโนไซแลนเป็นสารสำคัญในตำรับ โดยปรับเปลี่ยนอัตราส่วนของสารทำอิมัลชันบริดจ์ 72 ต่อบริดจ์ 721 ในอัตราส่วน 1:4, 2:3, 1:1, 3:2 และ 4:1 ซึ่งตำรับที่พัฒนาขึ้นนี้นำไปใช้เป็นผลิตภัณฑ์เพิ่มความชุ่มชื้นให้กับผิวหนัง โดยทดสอบประสิทธิศักย์ในการให้ความชุ่มชื้น ความยืดหยุ่น และลดริ้วรอยของตำรับในอาสาสมัครเพศหญิง จำนวน 30 คน อายุระหว่าง 30-55 ปี แบบซิงเกิลไบลด์ การสกัดอะราบิโนไซแลน สกัดได้จากกากรำข้าวเจ้าด้วยน้ำอุ่น ได้ปริมาณสารสกัด 0.29 เปอร์เซ็นต์โดยน้ำหนัก ตำรับอิมัลชันน้ำมันในน้ำที่ถูกคัดเลือกประกอบด้วยสารสกัดอะราบิโนไซแลน 1 เปอร์เซ็นต์โดยน้ำหนัก และสารทำอิมัลชันในอัตราส่วนของบริดจ์ 72 ต่อบริดจ์ 721 อัตราส่วน 3:2 ซึ่งเป็นตำรับที่มีความคงตัวทางกายภาพหลังผ่านการทดสอบสภาวะเร่ง ร้อน-เย็น 6 รอบ และผ่านการทดสอบแบบสอบถามความพึงพอใจในคุณสมบัติทางกายภาพและเรื่องความรู้สึกสัมผัส เมื่อนำตำรับอิมัลชันน้ำมันในน้ำที่มีส่วนประกอบของอะราบิโนไซแลนไปทดสอบประสิทธิศักย์ในอาสาสมัครบนใบหน้าบริเวณแก้มและหน้าผากเป็นเวลา 8 สัปดาห์ เพื่อประเมินความชุ่มชื้น และความยืดหยุ่นของผิวหนังโดยใช้เครื่องมือ Skin Diagnostic SD 27 และ DermaLab® ตามลำดับ พบว่าผลิตภัณฑ์นี้เพิ่มความชุ่มชื้นผิว เพิ่มความยืดหยุ่นของผิว (ลดค่ายังส์โมดูลัส) อย่างมีนัยสำคัญ และลดริ้วรอยบนหน้าผากได้ โดยประเมินจากภาพถ่าย ดังนั้น จากการศึกษานี้อาจนำอะราบิโนไซแลนไปใช้ในการเพิ่มความชุ่มชื้นแก่ผิวหนังได้

ภาควิชา วิทยาการเภสัชกรรมและเภสัชอุตสาหกรรม ลายมือชื่อนิสิต.....

สาขาวิชา เทคโนโลยีเภสัชกรรม ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์หลัก.....

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KEYWORDS: ARABINOXYLANS / RICE BRAN / HEMICELLULOSE /EMULSIONS

YUVARED LUANGWITCHAJAROEN : FORMULATION AND EVALUATION OF O/W EMULSIONS CONTAINING ARABINOXYLANS FROM RICE BRAN RESIDUES. THESIS ADVISOR : ASSOC. PROF. UBONTHIP NIMMANNIT, Ph.D., THESIS CO-ADVISOR : KRISANA SIRALERTMUKUL, Ph.D., 115 pp.

In this study arabinoxylans was extracted from rice bran by warm water. The chemical structures were characterized by Fourier Transform-Infrared Resonance (FT-IR) and Nuclear Magnetic Resonance (NMR) Spectroscopy. The o/w emulsions containing arabinoxylans were formulated by varying the ratios of emulsifying agent, Brij 72 to Brij 721 of 1:4, 2:3, 1:1, 3:2 and 4:1. The *in vivo* efficacies were evaluated in 30 healthy female volunteers whose age in range 30-55 years using single blind trials for moisturizer, elasticity and wrinkle determination. The yield of extracted arabinoxylans was 0.29 %w/w. The selected o/w emulsions containing arabinoxylans consisted of 1% w/w of arabinoxylans and the ratio of Brij 72 to Brij 721 was 3:2, which gave the most satisfactory with physical appearances and the skin feeling and spread ability. Moreover, the o/w emulsions containing arabinoxylans was stable and passed 6 heating-cooling cycles. *In vivo* efficacies of o/w emulsion containing arabinoxylans were evaluated for moisture content and elasticity using Skin Diagnostic SD 27 and DermaLab® Elasticity probe instrument, respectively. These efficacy evaluations on the face (cheek and forehead) were investigated for 8 weeks. They show that the product increased the moisturizing property, reduced young's modulus which resulted in increasing skin elasticity with significant difference in 1-8 weeks. The results were also observed by macroscopic photographs that show the reduction of wrinkle line on forehead. It is suggested that arabinoxylans extract may be a candidate for an efficient moisturizing agent.

Department: Pharmaceutics and Industrial Pharmacy Student's Signature: …..…….....……..
Field of Study: Pharmaceutical Technology Advisor's Signature: ...……
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CONTENTS

Page

CHAPTER **Page**

LIST OF TABLES

TABLE **Page**

TABLE **Page**

LIST OF FIGURES

FIGURE **Page**

FIGURE **Page**

LISTS OF ABBREVIATIONS

Brij 72	=	Polyoxyethylene (2) Stearyl Ether
Brij 721	=	Polyethylene (21) Stearyl Ether
cm	=	centimeter
cm^{-1}	=	per centimeter
cm^{2}	=	square centimeter
Co.Ltd	=	company limitted
et al.	=	et alii, and others
E	=	Young's elasticity modulus
FT-IR	=	fourier transform infrared spectroscopy
g	=	gram
GAGs	=	glycosaminoglycans
h	=	hour
LeftC	=	left cheek
LeftF	=	left forehead
M	=	mole per liter
mg	=	milligram
min	=	minute
ml	=	milliliter
mM	=	millimole per liter
MMPs	=	matrix metalloproteinases
MPa.s	=	millipascal.second
MPa	=	mega Pascal
MW	=	molecular weight
NMR	=	nuclear magnetic resonance
No.	=	number
^{o}C	=	Degree Celsius
R	=	Retraction time
RightC	=	right cheek
RightF	=	right forehead
rpm	=	revolution per minute
SD	=	standard deviation

sec	=	second
SELS	=	surface evaluation of the living skin
TEWL	=	transepidermal water loss
UV	=	ultraviolet
VE	=	Visco Elasticity
v/v	=	volume by volume
w/v	=	weight by volume
w/w	=	weight by weight
μg	=	microgram
μm	=	micrometer
μM	=	micromole per liter

CHAPTER I

INTRODUCTION

Rice is extensively planted and consumed as staple diet in many Asian countries especially Thailand. Rice bran is an inexpensive by-product derived from the outer tissue of brown rice in abrasive milling process to produce polished rice. Rice bran and its oil contain large concentrations of several compounds that could apply in nutrition as a low-cost source of protein (Wang et al, 1998), as well as cosmetic and pharmaceutical industry (Ghoneum & Gollapudi, 2003; Ghoneum & Gollapudi, 2006; Gollapudi and Ghoneum, 2008; Noaman et al., 2008). Rice bran is rich in hemicellulose polysaccharide. Hemicellulose, comprise mostly a highly branched heteroxylan type with properties similar to those of exudate gums. Hemicellulose is the main part in rice bran containing arabinoxylans; the complex polysaccharide with a xylan backbone and branches of arabinoses residues.

Arabinoxylans have many functional properties which are interesting for the development of the new cosmetic products. The functional property of arabinoxylans in baked products affects water binding, rheology and starch retrogradation. Water binding is dependent on the arabinose substitution. Chains of arabinoxylans are strengthened by cross-linking ferulic acid dimers, which are ester-linked to the arabinose sugars. Many research papers have reported on the properties of arabinoxylans as antioxidant when substituted with phenolic acids and it can hold water with the capacity of 3.5 to 10 times their weight. Ferulic acid, cinnamic acid and *p*-coumarin are also involved in the linking of hemicellulose (Chaikumpollert et al., 2004).

Recently, many facial products, which are used for increasing moisture and elasticity of skin for reducing wrinkle on the face, have been used and have a variable dosage forms such as gel, cream, patch, and mask. In this study, oil-in-water

emulsions containing arabinoxylans were designed for increasing moisture and elasticity of skin.

The aims of this study were to extract the water extractable arabinoxylans and formulation of o/w emulsions containing arabinoxylans from rice bran residues after rice bran oil extraction. Generally, arabinoxylans hemicellulose can be water-extracted. In this study, crude arabinoxylan hemicellulose was extracted and analyzed using FT-IR, NMR for investigation of its chemical structure. *In vivo* efficacy determination of o/w emulsions containing arabinoxylans was evaluated by using skin diagnosis SD 27 for determination of moisture in the skin, Dermalab® for determination of elasticity and digital camera for determination the wrinkle on the volunteer's face. The standard features were then statistically analyzed and calculated.

Finally, this research provided the development of oil-in-water emulsions containing arabinoxylans. The knowledge from this study will be useful for the consumer who would like to use oil-in-water emulsions containing arabinoxylans for increasing moisture and elasticity of the skin, and also help the researcher who would like to investigate oil-in-water emulsions in pharmaceutical, cosmetics and nutraceutical products.

Objectives

The purposes of this study were as follows:

To extract arabinoxylans from rice bran

To formulate and determine physical appearances of oil-in-water emulsions containing arabinoxylans from rice bran for skin care products

To study the *in vivo* skin moisturizing and elasticity enhancement using formulated oil-in-water emulsions containing arabinoxylans

CHAPTER II

LITERATURE REVIEW

1. Rice

Rice is a seed of a monocot plant *Oryza sativa*. It is an annual plant. The rice plant can grow to 1-1.8 m tall depending on the variety and soil fertility, has slender leaves 50-100 cm long and 2-2.5 cm broad. The seed as a cereal grain is 5-12 mm long and 2-3 mm thick. It is the most important food for a large part of the world's human population. It can survive in tropical areas especially in East, South, Southeast Asia, the Middle East, Latin America, and the West Indies. It is grain with the second highest worldwide production, after maize. The seeds of the rice plant are first milled using a rice huller to remove the outer husks of the grain. In this process, the product is called brown rice. The milling may be continued, removing the 'bran' creating white rice. White rice keeps longer, absences some important nutrients; in a limited diet which does not supplement the rice, brown rice helps to prevent the disease beriberi.

1.1 Rice Bran

A kernel or grain of rice is a seed that contains an embryonic rice plant, stored food and a protective coat. The hull is the outer straw-like protective covering that surrounds the entire grain. It is inedible and must be removed before the grain can be eaten. Under the hull are the germ and the bran. The germ is the plant embryo from which a plant emerges. The bran layers include layers of fibrous tissue with protein, vitamins, minerals and oil. Rice kernel consists of hull, starchy endosperm, bran and germ, contents of which are 20%, 72%, 6%, and 2%, respectively(Juliano et al., 2005). Rice bran is a by-product derived from the outer part of brown rice in milling process. Hemicellulose is the mainly part in rice bran containing arabinoxylans, the complex polysaccharide which has a xylan backbone with branches of arabinoses residues. Arabinoxylans are found in the bran of grasses (Graminiae) such as wheat, oats, barley, rice etc. wheat bran is one of the rich sources of dietary fiber and mainly

consists of 46% non-starch polysaccharides (NSP). The main NSPs present are arabinoxylan, cellulose and (1-3), (1-4)-β-glucan, contents of which are 70%, 24% and 6%, respectively.(Maes and Delcour, 2002).

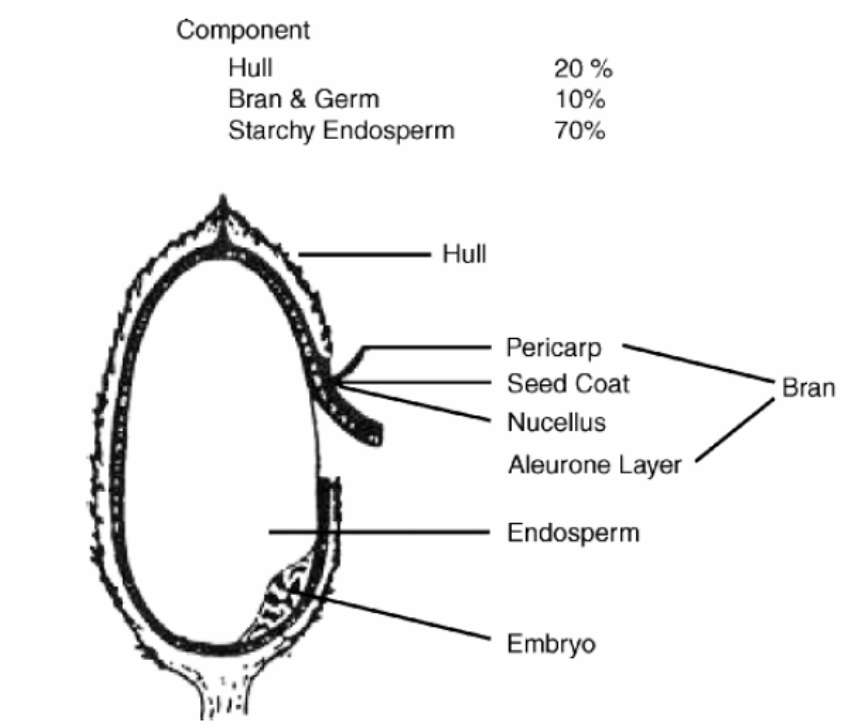


Figure 1 Relative of major rice caryopsis components (Orthoefer, 2005)

1.1.1 Structural unit

Arabinoxylans consist of α-L-arabinofuranose residues attached as branches to β-(1 → 4)-linked D-xylopyranose polymeric backcone as shown in Figure 2. These may be 2- or 3-sustituted or 2- and 3-di-substituted. Arabinose residues may even be substituted by other groups such as acetate, glucoronic acid and ferulic acid.

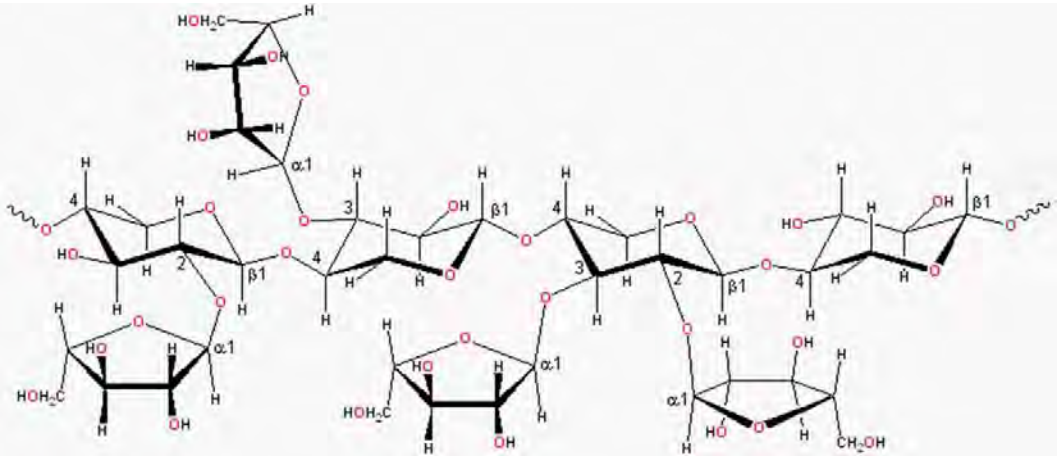


Figure 2 Structure of arabinoxylans(Chaplin, 2009)

1.1.2 Functionality

Wheat and rye arabinoxylan are important functional ingredients in baked products affecting water binding, rheology and starch retrogradation. Water binding is dependent on the arabinose substitution(Chaplin, 2009). Chains of arabinoxylans are strengthened by cross-linking ferulic acid dimers, which are ester-linked to the arabinose sugars. Ferulic acid is also involved in the linking of hemicellulose to lignin(Ralph et al., 1995). Rice endosperm cell walls contain 12 g/kg esterified cinnamic acids comprising ~9 g/kg ferulic, ~2.5 g/kg *p*-coumarin and ~0.5 g/kg diferulic esters.

Mamdouh Ghoneum reported Biobran® MGN-3 or arabinoxylans from rice bran (that has been enzymatically modified with extract from Hyphomycetes mycelia) was tested for anti-HIV activity in primary cultures of peripheral blood mononuclear cells. Biobran® MGN-3 inhibited HIV-1 replication by: 1) inhibition of HIV-a p24 antigen production in a dose dependent manner, 2) inhibition syncytia formation (Acheampong et al., 2005).

1.1.3 Chemical Composition of Cell Walls

Cell walls of crop residues consist mainly of polysaccharides, protein and lignin. The substances, with small amounts of other components, like acetyl group

and phenols, are organized in a complex three-dimensional structure. Other wall components include suberin, cutin, tannins, waxes and minerals.

1.1.4 Polysaccharide

Major polysaccharides in primary cell walls of plants include cellulose, xyloglucan and pectic polysaccharides, while secondary cell walls contain mainly cellulose and xylans.

Hemicelluloses are a wide group of polysaccharides. That basically shares only the property of being soluble in dilute alkali and being able to bind to cellulose by multiple hydrogen bonds and to bind to lignin by covalent bonds. In grasses, the main fraction of hemicellulose is xylans, with a backbone of 4-linked xylose residues and short side chains of arabiose, glucoronic acid and 4-O-methyl-glucoronic acid residues. Most of xylose residues in higher plants are acetylated, mainly on the C-2 hydroxyl groups, but also on C-3. Hemicellulose polysaccharide concentrations in grasses can range anywhere from 150 to 400 g/kg dry matter (DM), whereas in legumes, the concentration is much lower, generally between 80-150 g/kg DM. For both grasses and legumes, xylose usually comprises half or more of total sugars of hemiellulosic fraction. Furthermore, rhamnose only exists in the hemicellulosic polysaccharides of legumes(Qingxiang, 2002).

1.1.5 Proteins

Proteins make up 2 to 10 percent of the primary cell wall of many dicotyledons and some monocotyledons, and may become cross-linked by the formation of isoduthrosine or dityrosine. Cell wall proteins may also be involved in covalent bonding with polysaccharides. Glycoproteins seem to be invariably found in primary cell walls. Apparent covalent protein-lignin linkages have also been observed in wheat internodes.

1.1.6 Lignin

Lignin represents between 5-20 percent of crop residues DM. It is generally recognized that the precursors of these building stones are coniferyl, sinapyl and *p*-coumaryl alcohols, which are transformed into lignin by a complex dehydrogenative polymerization process. Depending upon the number and type of functional groups on the aromatic rings and propane side chains, lignin has variable solubility. Grass lignin is etherified by cinnamic acids, chiefly *p*-coumaric acid through hydroxyls on its monomers. In addition, ether-linked ferulic acids have been observed in lignin from maize stalks, wheat straw, rice straw and bagassse. Lignin in plant cell walls is physically and chemically associated with wall polysaccharides and proteins. The association between lignin and polysaccharides includes glycosidic linkages, ether cross-linkages, ester cross-linkages and cinnamic acid bridges. The strong linkage between lignin and polysaccharides or proteins would definitely prevent cell wall components from enzymatic hydrolysis by ruminal micro-organism, and thus limit the digestion of cell walls.

1.1.7 Others

Other components – including cutin, suberin, tannins, waxes and minerals are also found in the cell walls. Cutin and waxes are attachd to the epidermal walls on plants surface. Suberin is a functional component of cell walls. The polyesters that appear in suberized tissue can be esterified with phenolic monomers, oligomers.

2. Skin

Skin is the largest organ of the human body. The skin barrier prevents foreign material to entering the system. The primary function of the barrier is to prevent water loss. It has other functions such as thermoregulation, protection, metabolism and sensation. It composed of specialized epithelial and connective tissue cells. The skin is composed of three primary layers, which are epidermis, dermis, and subcutaneous tissue(Baumann and Weisberg, 2002).

2.1 Epidermis

Epidermis is the outermost layer. It forms the waterproof, protective wrap over the body's surface. The epidermis helps the skin to regulate body temperature. Epidermis contains no blood vessels, and in the deepest layers is nourished by diffusion from blood capillaries extending to the upper layers of the dermis. It is formed by an ordered arrangement of cells called keratinocytes, whose basic function is to synthesize keratin, a filamentous protein that serves a protective function(Odom R.B, 2000). This keratinized layer of skin is responsible for keeping water in the body, protection from the invasion of foreign substances into the body and making skin a natural barrier to infection. It is very important from a cosmetic standpoint because it is the layer that gives the skin its texture and moisture, and contributes to skin color. The main types of epidermis are keratinocytes, melanocytes, Langerhans cells and Merkels cells.

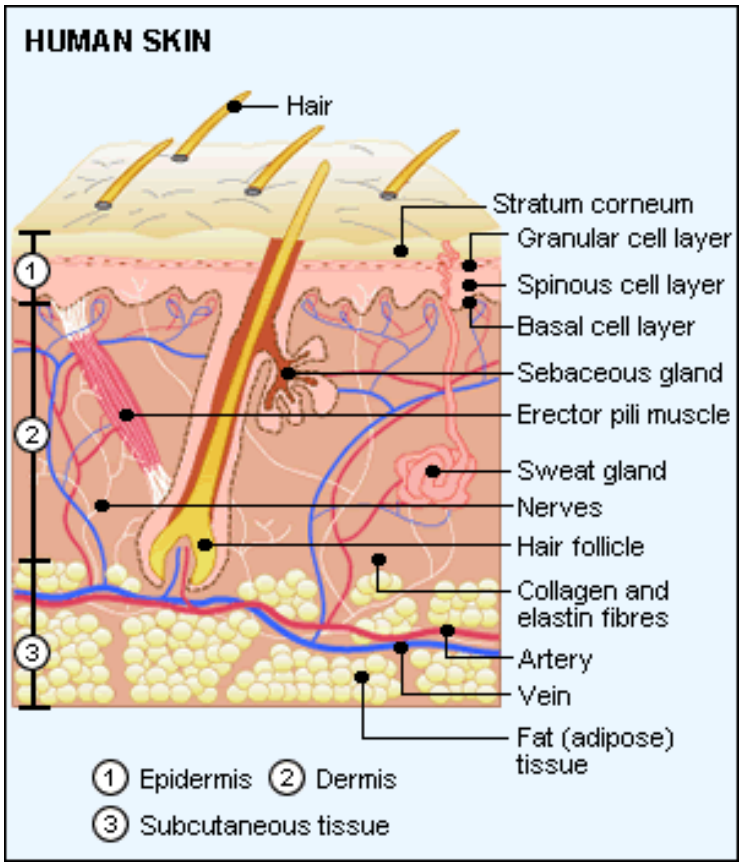


Figure 3 The basic diagram of skin structure

2.1.1 Layers of the epidermis

Epidermis is divided into the following 5 sublayers or strata; Stratum germinativum (stratum basale), Stratum spinosum (spinous layer), Stratum granulosum (ganular layer), Stratum lucidum (clear layer) and Stratum corneum (horny layer). The principal cell of the epidermis is called a keratinocyte, which it gradually migrates to the surface and is sloughed off in a process called desquamation.

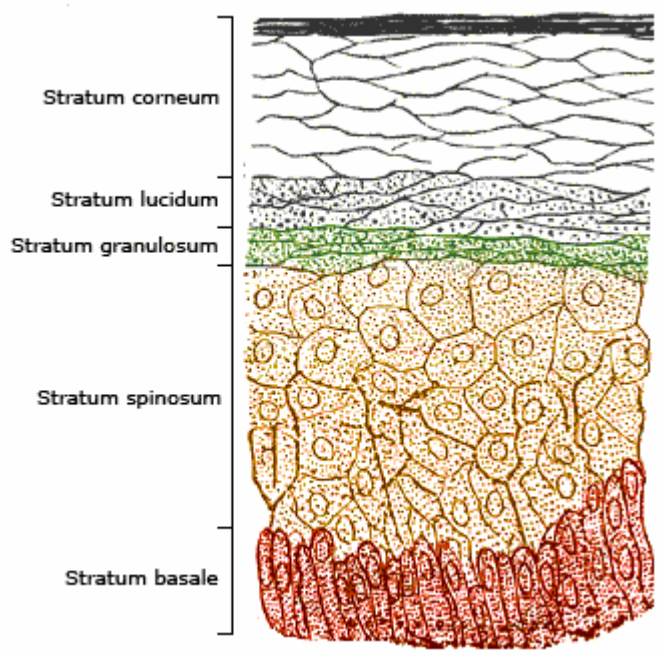


Figure 4 The layers of the epidermis

2.1.1.1 Stratum germinativum (stratum basale)

This is the deepest layer of the epidermis and it is here that new cells are generated for the renewal of the epidermal layers of the skin. A process of cell division referred to a mitotic division which is responsible for the generation of the new epidermal skin cells. After the mitotic division (cell division leading to the formation of a new cell) a newly formed cell will undergo a progressive maturation

called keratinization as it migrates to the surface of the skin. Basal cells are attached to each other by desmosomes that contain cadherins. Basal cell layers attach to the basement membrane (basal lamina) on which they reside via hemidesmosomes that contain integrins. Desmosomes and hemidesmosomes anchor intermediate filaments, which are comprised of keratins in epithelial cells, to the cell surface. Two types of keratins, type I keratins, which are acidic and type II keratins, which are basic, are required to form an intermediate filament. The basal cells are responsible for maintaining the epidermis by continually renewing the cell population(Gartner and Hiatt, 2007).

2.1.1.2 The Stratum spinosum (spinous layer)

The cells that divide in the stratum germinativum soon begin to accumulate many intermediate filaments and desmosomes (structures that join adjacent cells together) on their outer surface. The cells in this layer attain a more flattened shape of the stratum spinosum, which is often called the prickle-cell layer. The cells in this layer arise from the migration from the basal layer and lose their adhesion to the basement membrane and adhere to other keratinocytes.

2.1.1.3 The stratum granulosum (granular layer)

As keratinocyes (these are the basic cell of which the epidermis is composed) progressively mature they accumulate a protein called keratin (this process is called keratinization). In addition, the cells of the stratum granulosum accumulate dense basophilic keratohyalin granules (Granules found in living cells of keratinizing epithelia) these granules contain the protein filaggrin, which serves to bundle the keratin filaments together. The proteins of the cornified cell envelope (involucrin, keratolinin, pancornulins, and loricrin) are cross-linked in this layer by the calcium-requiring enzyme transglutaminase to form the cell envelope. The most superficial layer of the epidermis still has nuclei.

2.1.1.4 The stratum lucidum (clear layer)

This is the second layer of the epidermis and varies in thickness throughout the body depending mainly on frictional forces and is thickest on the palms of the hands and soles of the feet. The stratum lucidum is a thin, clear layer of dead skin cells in the epidermis, and is named for its translucent appearance under a microscope. It contains a clear substance called eleidin, which eventually becomes keratin. The keratinocytes of the stratum lucidum do not feature distinct boundaries and are filled with eleidin, an intermediate form of keratin.

The cells of the stratum lucidum are flattened and contain an oily substance that is the result of exocytosis of lamellar bodies accumulated while the keratinocytes are moving through the stratum spinosum and stratum granulosum. It is this substance that gives the stratum lucidum its waterproof properties, and, thus, it is also called the barrier layer of the skin.

2.1.1.5 The stratum corneum (horny layer)

This layer consists of primarily dead skin cells. As a cell accumulates keratinohyalin granules, it is thought that rupture of lysosomal membranes release lysosomal enzymes (Lysosomal enzymes are those enzymes which are responsible for breaking down complex chemicals within a cell) that eventually cause cell death. The dead and dying cells filled with mature keratin from the stratum corneum. The most superficial layer of the epidermis is the stratum corneum. The keratinocytes that reside in this layer are the most mature and have completed the keratinization process. These keratinocytes have no organelles, and their arrangement resembles a brick wall. The stratum corneum is composed of protein-rich corneocytes embedded in a bilayer lipid matrix arranged in a “brick and mortar” fashion. The “bricks” are composed of keratinocytes, and the “mortar” is composed of the contents extruded from the lamellar granules, including lipids and proteins. The stratum corneum is described as the “dead layer” of cells because these cells do not demonstrate protein synthesis and are unresponsive to cellular signaling(Baumann and Weisberg, 2002). This layer provides 98% of the water retention ability of the epidermis.

2.2 Cell types in epidermis

2.2.1 Keratinocytes

Keratinocytes or squamous cells are the major cell type of the epidermis, making up about 90% of epidermal cells. Keratinocytes found in the stratum spinosum are sometimes refered to as “basal cells” or “basal keratinocytes”. They are pushed up through the layers of the epidermis, undergoing gradual differentiation. While they move to the surface of the skin the keratinocytes are enucleated, flattened and highly keratinized. This layer of dead cells forms an effective barrier to the entry of foreign matter and infectious agents into the body and minimizes moisture loss. The migration process normally takes approximately 28 days(Odom et al., 2000).

2.2.2 Melanocytes

The main function of melanocytes is to produce melanin, which is responsible for the color of the skin. Melanocytes stayed in the basal layer of the epidermis and extend processes that contact many keratinocytes and immediately above the basal layer. They do not form desmosomal connections. Melanocytes produce melanin from tyrosine in specialized organelles called melanosomes, which contain tryosinase, an enzyme critical for melanin production. Keratinocytes are the reservoir for melanin in the skin. Melanocytes increase production of melanin and the numbers of melanosomes. The differences in the number, size and arrangement of melanosomes lead to human skin color. In dark skin, there are more melanosomes, which are larger and distributed throughout the cytoplasm rather than just in the perinuclear area as in light skin.

Chronic sun exposure can stimulate the melanocyte to produce larger melanosomes, thereby making the distribution of melanosomes within keratinocytes resemble the pattern seen in dark-skinned individuals (Odom et al., 2000; Walters and Roberts, 2002).

2.2.3 Langerhans cells

Langerhans cells arise from bone marrow and migrate to the epidermis. These are antigen-presenting cells, mediating T-cell immunity, and play a role in allergic contact dermatitis. Langerhans cells interact with white blood cells called "helper T cells" in immune responses and are easily damaged by UV radiation. They are monocyte derived dendritic cells present in the living layers of the epidermis, especially are found scattered among keratinocytes of the stratum spinosum, or prickle cell layer of the epidermis. They do not contain intermediate filaments (Gartner and Hiatt, 2007; Odom et al., 2000).

2.2.4 Merkel cells

Merkel cells are present in the basal layer of thick skin of hands and soles and located in the deepest layer (stratum basale) of the epidermis of hairless skin, where they are attached to keratinocytes by desmosomes. Merkel cells make contact with the flattened portion of the ending of a sensory neuron (nerve cell), called a tactile (Merkel) disc, and are thought to function in the sensation of touch.

2.3 Dermis

The dermis is a dense, irregular, mesodermally derived, connective tissue, composed of collagen (mostly type I), elastin, and glycosaminoglycans. The dermis has the thick range from 3 – 5 mm consisting of mostly connective tissue (collagen and elastin) and laden with nerves, blood vessels and sweat glands. It is much thicker than the epidermis, comprising 80-90% of the total dermis and epidermis. It contains extensive vasculature, neurons, smooth muscle, and fibroblasts. It is the principal mechanical barrier of skin, remove waste product, control temperature and pressure, mobilize defense forces, and contribute skin color. Its networks of elastic fibers function to support the epidermis and bind the skin to the deeper hypodermis. The upper portion of this layer, which lies beneath the epidermis, is known as the papillary dermis and the lower portion is known as the reticular dermis.

2.3.1 Layers of the dermis

The dermis contains two layers, the papillary layer and the reticular layer.

2.3.1.1 Papillary layer

The papillary dermis contains vascular networks that have two important functions. The first one is that the papillary dermis contains capillary loops being to support the vascular epidermis with vital nutrients and, secondly, to provide a network for thermoregulation. In the papillary dermis, collagen fibers are loosely bundled(Baumann and Weisberg, 2002).

2.3.1.2 Reticular dermis

The reticular layer of the dermis is tightly packed with collagen and elastin for strength and elasticity. This layer also contains glycosaminoglycans to bind water (Baumann, 2002).

2.3.2 Components of the dermis

2.3.2.1 Cellular components

Fibroblasts are the primary cell type in the dermis. They have an important role in producing fiber elements, collagen, elastin, ground substance, other matrix proteins, and enzymes such as collagenase and stromelysin (Aulton, 2002). Immune cells such as mast cells, polymorphonuclear leukocytes (PMNs), lymphocytes, and macrophages are also present in the dermis (Odom et al., 2000).

2.3.2.2 Extracellular matrix (ECM)

The extracellular matrix (ECM) is the material that forms the bulk of the dermis, excluding water and cells (Bernstein and Uitto, 1996). This ECM, produced by keratinocytes and fibroblasts, works not only as a physical support, but

also as an exchange and communication area that allows nutrients, metabolites and growth factors to diffuse between cells. Formation of ECM is essential for processes like growth, wound healing, and fibrosis. The main molecules forming the ECM are collagen, elastin, proteoglycans, fibronectins, and other glycosylated proteins(Thibodeau, 2000).

2.3.2.3 Collagen

Collagen is one of the long fibrous structural proteins whose functions are quite different from those of globular proteins such as enzymes. Strength bundles of collagen called collagen fibers are a major component of the ECM that supports most tissues and gives cells structure from the outside, but collagen is also found inside certain cells. Collagen has great tensile strength, and is the main component of fascia, cartilage, ligaments, tendons, bone and teeth. Along with soft keratin, it is responsible for skin strength and elasticity, and its degradation leads to wrinkles that accompany aging. It strengthens blood vessels and plays a role in tissue development. Collagen fibers are always seen in the final, mature, state of assembly as opposed to elastin, whose immature fibers are seen in the superficial dermis and whose more mature fibers are found in the deeper layer of the dermis. The skin drives its strength from the fibrillar collagens, chiefly collagen type I and III, which from the bulk of the dry weight of the dermis.

2.3.2.4 Elastic fiber

Elastic fibers are composed of at least two distinct proteins, elastin and fibrillin. Elastic fibers are found at the periphery of collagen bundle and endow the skin with recoil properties. These fibers are assembled on bundles of microfibrils composed of fibril. Fibrillin constitutes the fibrillar component of elastic fibers and is analogous to the cloth surrounding a bungee cord, while the stretchy inner component corresponds to elastin. Elastic fibers form a fine network that extends vertically in the dermal papillae and surrounds dermal bloods vessels, while in the reticular dermis the fibers are much thicker and run parallel to the epidermis surrounding the larger

collagen fibers(Baumann and Weisberg, 2002; Bernstein and Uitto, 1996). Elastic fibers are crucial to provide resilience and elasticity, make up only 3-4 percent of the dry weight of the skin and 1 percent of the volume of the dermis.

2.3.2.5 Glycosaminoglycans (GAGs)

Glycosaminoglycans are a family of endogenous mucopolysaccharides that constitute the fundamental substance of the connective tissue. These are electronegative$^{\Theta}$ polymers constituted of repetitive units of disaccharides including an amino-sugar and uronic acid. In international chemical nomenclature, the term “mucopolysaccharides” has been substituted with “glycosaminoglycans” (GAG) as the first was less precise and included products elaborated from epithelial cells and mucous secretion. Moving on to GAG, it can be referred to as a narrower and more defined series of products, the sulphurated and asulphurated acid mucopolysaccharides and more precisely the following: Chondroitin sulphate, Dermatan sulphate, Keratan sulphate, Heparin, Heparan sulphate, Hyaluronic acid. GAGs associated with a protein core are likely termed proteoglycans. Proteoglycans and GAGs maintain dermal hydration, as well as possibly participating in collagen and elastic fiber formation(Baumann and Weisberg, 2002; Bernstein and Uitto, 1996).

2.3.2.6 Matrix metalloproteinases (MMPs)

Matrix metalloproteinases (MMPs) constitute a protein family that participates in the degradation ECM macromolecules. Collectively they are capable of degrading all kinds of ECM proteins, but also can process a number of bioactive molecules. There are more than 200 known metalloproteinases, almost all of them dependent on zinc at the active centre for their catalytic function.

2.4 Subcutaneous layer (hypodermis)

The hypodermis is the deepest section of the skin. The hypodermis refers to the fat tissue below the dermis that insulates the body from cold temperatures and

provides shock absorption. Fat cells of the hypodermis also store nutrients and energy. The hypodermis is the thickest in the buttocks, palms of the hands, and soles of the feet. As we age, the hypodermis begins to atrophy, contributing to the thinning of aging skin. The blood vessels and nerves that it contains are larger than those in the dermis. It may also house the hair follicles when they are in the growing phase(Gray, 2000). One of the functions of this fatty layer may be to act as an insulation to conserve body heat. This layer also contains collagen type I, III, and V. As human age, some of the subcutaneous fat is lost or redistributed into undesired areas. This phenomenon contributes to the aged appearance.

2.5 Skin appendages

Skin appendages are appendages that are associated with the skin and serve a particular function. In humans some of the more common skin appendages are hairs, sebaceous glands, sweat glands, and nails.

2.5.1 Hair follicle

Epidermal buds grow into the dermis. The developing follicle forms at an angle to the skin surface and continues its downward growth. The hair is formed from cells just above the bulb, which also give rise to concentric zones of differentiated epithelial cells destined to form the inner and outer root sheaths. A tiny bundle of muscle fiber that attached to the follicle called the *arrector pili* that is responsible for causing the follicle to become more perpendicular to the surface of the skin, and causing the follicle to protrude slightly above the surrounding skin. This process results in goose bumps or goose flesh. Stem cells are located at the junction of the arrector and the follicle, and are principally responsible for the ongoing hair production during a process known as the Anagen stage. The average growth rate of healthy hair follicles on the scalp is .04 cm per day(Waters and Robert, 2002).

2.5.2 Sebaceous glands

The sebaceous glands are found in greatest abundance on the face and scalp, though they are distributed throughout all skin sites except the palms and soles. Sebaceous glands secrete an oily substance called sebum that is made of fat (lipids) and the debris of dead fat-producing cells. In the glands, sebum is produced within specialized cells and is released as these cells burst; sebaceous glands are thus classified as holocrine glands. Sebum acts to protect and waterproof hair and skin, and keep them from becoming dry, brittle, and cracked. It can also inhibit the growth of microorganisms on skin(Aulton, 2002; Waters and Robert, 2002).

2.5.3 Sweat glands

Sweat glands are normally found in almost every part of the skin, forming tiny coiled tubes embedded in the dermis or subcutaneous fat. The skin contains two different groups of sweat glands, which are apocrine sweat glands and eccrine sweat glands. The glands sweat to cool the surface of the skin and reduce body temperature. This cooling is the primary function of sensible perspiration, and the degree of secretory activity is regulated by neural and hormonal mechanisms. Second, eccrine sweat gland secretion can also provide a significant excretory route for water and electrolytes. Last, eccrine sweat gland secretion provides protection from environmental hazards by diluting harmful chemicals and discouraging growth of microorganisms(Waters and Robert, 2002).

2.5.4 The nails

Anatomically fingernails and toenails are made of a tough protein called keratin and have many different parts. Certainly, nail plate composition, layers of flattened keratinized cells fused into a dense, but somewhat elastic mass, will afford some protection to the highly sensitive terminal phalanx in the keratinization process the cell undergo shape and other changes, similar to those experienced by epidermal cells forming the stratum corneum. The nail plate comprises two major layers (the

dorsal and intermediate layer) with, possibly, a third layer adjacent to the nail bed. There are differences in the chemical composition of the two layers, which further suggests that applied drugs may possess differing partitioning tendencies between the layers (Walters and Roberts, 2002).

3. Emulsion preparation

An emulsion is formed when two immiscible liquids (usually oil and water) are mechanically agitated. One phase is dispersed in the other phased. The most common types of pharmaceutical or cosmetic emulsions include water as one of the phases and oil or a lipid as the other. An oil-in-water (o/w) emulsion consists of oil droplets dispersed in a continuous aqueous phase, and water-in-oil (w/o) emulsion consists of water droplets dispersed in oil (Friberg et al., 1988).

3.1 Oil-in-water emulsions

Emulsions of the o/w type are washable, less oily, and consequently less obvious to the touch than w/o emulsions. This renders o/w types most useful as water-washable drug bases for general cosmetics purposes. An o/w cream is nonocclusive because it dose not deposit a continuous film of water-impervious lipid. When applied o/w emulsions feel light and not greasy. They cause a cooling effect because of the evaporation of the external water phase. However, a correctly formulated cream can deposit lipids and other moisturizers on and in the stratum corneum and so restore the tissues' ability to hydrate; that is, the preparation has emollient properties.

3.2 Water-in-oil emulsions

The advantages of w/o emulsions are close resemblance to the natural protective lipid layer in the stratum corneum and skin protection due to formation of a continuous layer of lipid on the skin after applied. Sustained moisture is formed that reduces evaporation of skin water. Low risk of microbial growth. So the type is usually used for water-resistance sun protection, baby cream, or night cream.

3.3 Components of oil-in-water emulsions

These components are generally used in oil-in-water formulation as shown in Table 1.

Table 1 Components of an oil-in-water emulsions

Material	Concentration (%)	Function
Water	60-95	Diluent
Thickener	0.2-1.0	Improves stability, modifies skin feel, suspending agent
Humectant	2.0-5.0	Improves stability, affects skin feel, solubilizer for preservative
Emulsifier	2.0-5.0	Stabilizes emulsion
Emollient (oil soluble)	5-15	Improve skin feel
Emollient (water soluble)	1-2	Improve skin feel, reduces tackiness
Silicone	2-5	Reduces skin whitening, anti-foaming, improves skin feel
Wax	1-3	Affect skin feel
Alcohol	0-20	Reduces tackiness, provide cooling effect
Color	As needed	Consumer appeal
Fragrance	0.15-0.5	Consumer appeal
Preservative	0.1-1.0	Preservative

3.4 Viscosity

Viscosity is probably one of the most important emulsion properties, since variations are usually quite obvious to the consumer. Viscosity changes often indicate other changes in the product, which may reduce its effectiveness. Viscosity or

consistency plays a considerable part in the patient's assessment of a product, and hence its acceptability.

Viscosity is a measure of a fluid's resistance to change in form due to internal friction. It is a very important parameter in topical emulsions, since the product is designed for a given feel and dispensation from containers of specific types, often with constricted openings.

The unit of viscosity measurement is the poise, which is defined as the tangential force necessary to maintain a velocity of 1 cm^{-1} sec between two planes each 1 cm^2 in area and 1 cm part. The centipoise (cP), which is 0.01 poise, is the common unit for defining viscosity. There are three basic methods for determining viscosity or consistency. The first measures the rate of flow of the product through a capillary or an orifice. The second involves measurement of the rate at which a foreign object will fall or rise through a product. The last measures the torque induced when the product is subject to shear by placing it between a rotating spindle and a stationary cup.

3.5 Emulsion stability

3.5.1 Physical appearances of emulsions

Emulsions can vary in appearance due to viscosity, pour characteristics, gloss, smoothness, opalescence, texture, and opacity; they vary in application and feel due to oiliness, tackiness, wetness, slip, grittiness, spreading qualities, and drying time.

The range of appearances is possible, depending upon the droplets sizes and the difference in refractive indices between the phases. If the droplets are very much larger, the oil phase will become quite distinguishable and apparent. There are three types of emulsion instability: flocculation, creaming, and coalescence.

3.5.1.1 Creaming and sedimentation

Creaming is the upward movement of dispersed droplets relative to the continuous phase, while sedimentation, the reversed process, is the downward

movement of particles. Creaming or sedimentation is depending on the densities of disperse and continuous phase. It means phase separation from the top and the bottom of the preparation after some period of storage and comparing the composition of the two samples by appropriate analysis of water content, oil content, or any suitable constituent but this stability problem can reversed by agitation.

3.5.1.2 Aggregation and coalescence

More serious problem of the stability of emulsions is the processes of aggregation (flocculation) and coalescence.

Aggregation (flocculation) of the dispersed phase may take place before, during, or after creaming. Emulsions involve a joining together of the small water droplets. Flocculation represents a less serious sign of instability, which can be reversed by shaking the system.

Coalescence represents more serious instability. Once coalescence begins, the small droplets grow large enough to settle out (Leopold, 1992; Schramm, 1992). This process is irreversible and lead to separate completely.

3.5.2 Stress Testing

Stress conditions generally employed for evaluation the stability of emulsions. It is important to consider that an accelerated test should speed up only the processes involved in instability under normal storage condition. The most widely used stress in emulsion testing is temperature, changes in temperature can bring into play new reactions. However, it has been demonstrated that many emulsions are stable at 40 or 45 ºC but cannot tolerate temperature in excess of 55 or 60 ºC even for a few hours. A particularly useful means of evaluating shelf life is cycling between two temperatures. Cycling should be conducted between 4 and 45 ºC. An emulsion should be survived at least six or eight heating/cooling cycling between refrigerator temperature and 45 ºC with storage at each temperature (Idson, 1988).

4. Efficacy evaluation of cosmetic products by scientific measurement

Cosmeceutics is a term frequently described various formulations of skin care products such as deodorants, moisturizing cream, sunscreen products and anti-wrinkle creams. Nowadays, cosmeceutic skin care products. These substances have been claimed to exhibit anti-aging and moisturizing properties (Farris, 2005; Suga, 2004). Nowaday, many instruments are used for claming anti-aging and moisturizing properties of cosmetic products.

4.1 Corneometer

An automated instrument is used to measure the amount of moisture in the outer layer of skin or skin hydration and the ability of skin to retain moisture (skin moisture capacity). These measurements are used to characterize skin conditions and determine the effect of products on skin (Sagiv, 2003). The dielectric constant of keratin and lipids are very small compared to that of water. The dielectric constant of a material is a number that reflects its electrical properties. Therefore the dielectric constant of the stratum corneum is determined by its level of hydration. The greater the water content, the larger the dielectric constant (Catherine et al., 2004).

Corneometer is an apparatus that uses this relationship which is shown in Figure 5. It carries a probe acting as a capacitor whose capacitance is proportional to the dielectric constant of the skin and varies according to its state of hydration(Sagiv, 2003). It is possible to distinguish between dehydrated skin, skin with a tendency to dehydration and normal skin can be obtained using this method (James and Anthony, 2002; Sagiv, 2003).

The measurement of the skin moisture is based on the world-wide acknowledged capacitative method, the CORNEOMETER® method. The Skin Diagnostic SD 27 a new type of skin testing instrument has been launched, enabling the beauty therapist, pharmacist or hair dresser to inform his/her customers of their actual skin and hair condition on the basis of scientific measurements. Thus, the right products, independent of any brand, can be recommended for the individual skin and hair type. The Skin Diagnostic SD 27 helps to increase the sales of cosmetic products.

By offering the special service of a skin and hair test, it attracts additional customers and keeps them visiting the salon/shop to monitor their skin and hair condition.

(a) (b)

Figure 5 (a) Skin Diagnostic SD 27, designed to determine the dielectric constant of the stratum corneum; (b) Skin Diagnostic SD 27 in use

The moisture content of the stratum corneum can vary very much depending on its storage capacity. It is a critical parameter for the hydrolipidic film of the skin. This factor plays an important role in our daily life as our skin is dried out due to hazardous effects of the sun, air-conditioned rooms, pollution etc. Dry skin tends to wrinkle. The measurement of the skin moisture is based on the world-wide acknowledged capacitative method, the CORNEOMETER® method. The dry stratum corneum is a dielectric medium The dielectric properties change with the moisture content.

This measurement is based on the completely different dielectric constant of water and other substances. The measuring capacitor shows changes of capacitance according to the moisture content of the samples. A glass lamina separates the metallic tracks (gold) in the probe head from the skin in order to prevent current conduction in the sample. An electric scatter field penetrates the skin during the measurement and the dielectricity is determined. One track builds up a surplus of electrons (minus charge) the other a lack of electrons (plus charge). An electric field between the tracks with alternating attraction is developed.

Type of skin is evaluated by interpretation of the results between 0 to 99. The value < 40 represents very dry skin; 41-60 represents dry skin; > 60 represents normal skin. In practice, however, the technique employing corneometer is used to measure the difference between hydration in stratum corneum before and after the application of a cosmetic or other skin treatment since it is reliable and simple to use (Sagiv, 2003).

Skin Condition	Diodes	Display
		for approx. 20°
very dry	01-05	00-40
normal to dehydrated	06-11	41-60
normal	12-16	61-99

Figure 6 The type of skin condition for moisture content

The results can be influenced by the following factors:

- uneven contact of the probe surface (too much pressure, extreme slant position, uneven support of the surface to be measured, body hair)
- measurements taken during or immediately after physical or psychological stress (e.g. exercise)
- measurements taken in extreme conditions of room humidity and/or room temperatures
- measurements of longer duration (effect of occlusion)
- dirt and damp on the sheet or damage of the sheet covering the probe face.

When testing products, it is recommended not to measure immediately after application as the measurement of water content in the product is more likely instead of moisture content of the skin. The sweat, moisture underlies external and internal influences if air humidity is very high (>80% RH). The volunteers are also recommended not to do making-up or putting on any cream or powders since these products can distort skin moisture. Repeat the measurement not on exactly the same spot but use a neighbored skin area.

4.3 Cutometer

Cutometer is used to measure firmness, gross elasticity and viscoelasticity of the skin under experimental conditions. The principle is based on suction and elongation. The skin area to be measured is drawn into the aperture of the probe due to negative pressure generated by the probe which can be varied between 20 to 500 mbar for two seconds (Fong et al., 1997).

The penetration depth of the skin into the aperture is determined without contact with an optical measuring system. This system consists of a light emitter and a light acceptor. Two opposing glass prisms transmit the light from emitter to acceptor. The light ratio changes proportionally to the penetration depth of the skin (Fong et al., 1997). A built-in database file can calculate the reading R1-R8 automatically as illustrated in Figure 7, each value is described in details below.

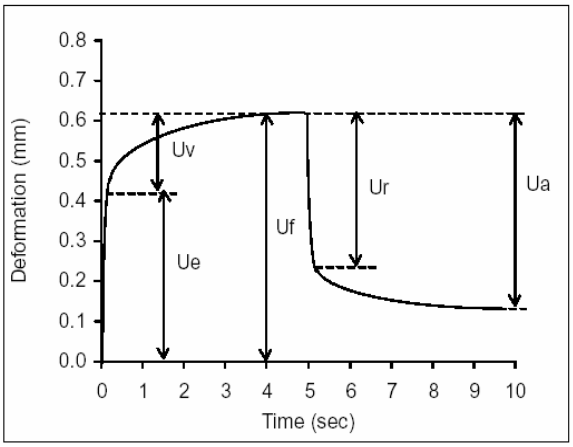


Figure 7 Skin deformation curve obtained with Cutometer

R0: Distensibility value (Ue+Uv or Uf): The first maximum amplitude, the highest point of the first curve, this has an implication for skin firmness. Distensibility value indicates firmness of skin. The smaller value represents the higher skin firmness.

R2: Gross elasticity (Ua/Uf): The relationship between maximum deformation during the first cycle and back formation directly after the first cycle or gross elasticity value. The closer the value is to 1 (100%), indicates the more skin elasticity property.

R3: The highest point of the last maximum amplitude curve compared to the maximum amplitude of the first curve. "Tiring effect" of the skin is shown, visible as the amplitude increases with each new suction.

R4: The last amplitude is evaluated by the last measuring point compared to the amplitude of the first curve. This value indicates the ability of re-deformation decreases with each new suction.

R5: Net elasticity (Ur/Ue): The closer the value to 1, (100%) the more elastic the curve.

R6: Viscoelasticity value (Uv/ Ue): The portion of the viscoelasticity on the elastic part of the curve. This value shows how well skin can return to its original shape. The smaller value representes higher elasticity.

R7: Ur/Uf: The portion of the viscoelasticity on the elastic part of the curve. The smaller represents the higher skin elasticity.

R8: Ua: This value is closer Uf, representing the ability of skin to return to its original state.

Cutometer is a very sensitive instrument. The reading would easily be disturbed by even a slight movement. As this involved muscle contraction and probably a change in skin tension, therefore, resting the volunteers in a proper position for assessment is essential to reduce errors (Fong et al., 1997).

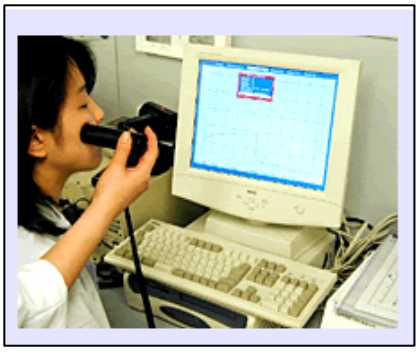
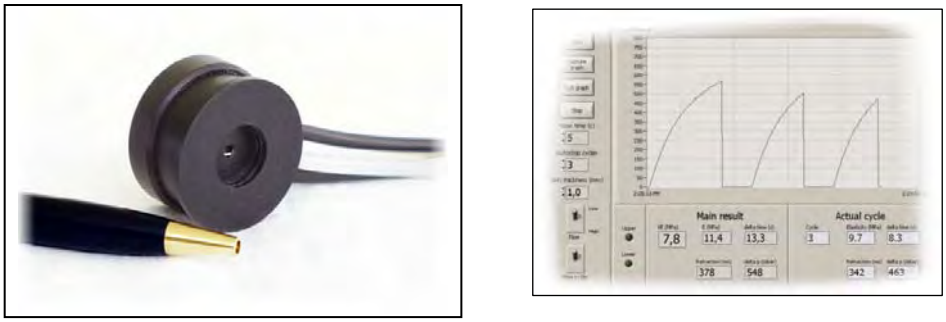


Figure 8 The reading R1-R8 calculated automatically by Cutometer.

Dermalab® Elasticity application module

In order to ensure un-biased readings, the DermaLab® elasticity probe features a light weight probe which, when glued to the skin using a double adhesive sticker,

eliminates movement artifacts from holding the probe. With the probe in place, negative pressure will elevate the skin, and the differential negative pressure needed to lift the skin a predetermined distance is used as input to calculate Young´s modulus. Two additional parameters are presented to describe the skin elasticity: the retraction time (R) and the viscoelasticity (VE), a parameter combining both the elevation and retraction phase in one number. The unit allows for adjusting the airflow according to the measurement site and actual skin condition.



(a) (b)

Figure 9 (a) skin elasticity probe; (b) elasticity screen

The elasticity measurement of the DermaLab USB unit is based on suction applied to the skin surface. The probe provides a vacuum chamber and uses adhesive tape to prevent creeping and folding of the skin under the edge surrounding the measurement chamber.

The suction method features an elevation phase and a retraction phase, the properties of which both contribute to the “feel” of the skin. As an example, young and smooth delicate skin, which is well moisturized, will normally be relatively easy to elevate by applying suction, and it will retract rapidly. Old and loose skin will also be easy to elevate, however, it will not retract rapidly. Therefore, what is usually considered to be skin elasticity (or smoothness, softness, firmness) is of a more complex nature and is best measured by taking both the elevation and retraction phase into account.

The DermaLab offers three descriptive parameters for the skin elasticity: 1) Young’s elasticity modulus (E), 2) the skin retraction time (time to retract from full

extension, R) and 3) a parameter called Visco Elasticity (VE) combining both the elevation and retraction phase. The three parameters are explained below.

1) Young's modulus.

Calculation of Young's elasticity modulus (E) is based on the differential force necessary to elevate the skin surface 1.5 mm between two infrared detection levels inside the probe chamber and calculated using the following equation:

$$\Delta x = \psi \cdot p \cdot \frac{r^4}{E \cdot s^3} \quad \text{where :}$$

Δx = deviation, middle of surface

Ψ = constant

p = surface pressure

E = elasticity modulus

R = radius of the surface

S = thickness of the surface (default skin thickness 1.00 mm, adjustable)

Inserting reasonable assumptions, known probe constants and a default skin thickness of 1.00 mm (may be adjusted) leads to:

$$E = 0.3125 \cdot \frac{\Delta p}{\Delta x}$$

As Δx is 1.5 mm, the elasticity modulus E is entirely depending on the differential force needed to elevate the skin. Young's modulus carries the unit MPa (mega Pascal).

2) Retraction time.

Retraction time (R) is the time in seconds it takes for the skin to retract 1.5 mm from full elevation.

3) Visco Elasticity.

Dividing the elasticity modulus by the retraction time provides a parameter (Visco Elasticity, VE), where both the elevation phase and the retraction phase are taken into account. R is normalized by a retraction time of 260 ms as a typical average of underarm readings (Caucasian skin, age 28 – 51). VE carries the unit MPa (mega Pascal).

$$VE = Young's\ modulus/R_{normalized}\ where\ R_{normalized} = R/260\ ms.$$

Connect the probe to the input connector on the front panel. The use of adhesive rings on the probe surfaces in contact with the skin is recommended in order to obtain the most reliable results. Prior to placing the probe, the skin surface should be clean and dry for the probe to adhere.

As the suction principle applies mechanical stress to the measurement site, attention should be paid to the fact that the measurement cannot be immediately repeated in the exact same position. Allow 30 - 60 minutes between measurements for the skin to recover.

During the measurement, care should be taken to avoid body movement. Do not touch the probe or pull the cables as this will influence the measurement.

CHAPTER III

MATERIALS AND METHODS

Materials

1. Absolute ethanol AR grade (Labscan Asia Co., Ltd., Thailand) Batch No. 07040173
2. Polyoxyethylene (2) Sterayl Ether (Sigma, USA)
3. Polyethylene (21) Stearyl Ether (Sigma, USA)
4. Cetostearyl alcohol (Hong Huat Co., Ltd., Thailand)
5. Propyl paraben (K.H. Co., Ltd., Thailand)
6. Propylene glycol (Srichand United Dispensary Co., Ltd., Thailand) Batch No. 1752124024
7. Rice bran residues obtained after rice bran oil extraction (Thai Edible oil Co.,Ltd.)
8. Stearic acid (S. Tong Chemicals Co., Ltd., Thailand) Batch No. 87173001
9. Disodium hydrogen orthophosphate anhydrous (Fluka Chemi GmbH, Switzerland) Lot. No. 50490
10. Methyl paraben (Acros Organics, USA)
11. Silicone oil (Unison Chemical 1986 Co., Ltd., Thailand)

Apparatus

1. Analytical balance (BA2105, S/N 21203485, Sartorius Basic, Germany)
2. DermaLab® Elasticity probe (Cortex Technology, Denmark)
3. Freeze dryer (model Freeze 6, Labconco, USA)
4. Heating bath (Buchi B-490, Switzerland)
5. Heating magnetic stirrer (VELP Scientifica, Italy)
6. Hot air oven (Model B40, Memmert, Germany)
7. pH-meter (Thermo Electron Cooperation, Model Orion 2 Star, USA)

8. Rotary evaporator (Model R-200, Buchi, Switzerland)
9. Skin Diagnostic (Model SD 27, Courage+Khazaka electronic GmbH, Germany)
10. Ultrasonic bath (Cavitator, Ultrasonic Mettler Electronic, USA)
11. Water bath (Gilson, England)
12. IR (Perkin® El Mer Model Sprectrum One, USA)
13. NMR (Varian® Model Unity Inova, USA)
14. Centrifuge (IEC Centra® MP4R, International Equipment Company, USA)
15. Brookfield DV-II+ programmable viscometer (Model LVDVIIT, USA)

Methods

The experiments were divided into four main parts:

1. Extraction of arabinoxylans from rice bran locally produced in Thailand
2. Determination of chemical structure arabinoxylans from rice bran
3. Preparation of o/w emulsion containing arabinoxylans
4. *In vivo* efficacy evaluation of o/w emulsion containing arabinoxylans from rice bran

1. Extraction of arabinoxylans from rice bran locally produced in Thailand

1.1 Isolation of water-extractable arabinoxylans

1.1.1 Extraction

Extraction method was developed based on water-extractable arabinoxylans. Rice bran from rice locally grown in Thailand was purchased from Thai Edible Oil Co.Ltd, Ayutthaya province, Thailand. Then one hundred gram of the rice bran residues was suspended in 500 ml of water and put in a water bath for 30 min with constant stirring until the temperature was increased to 40 ^{o}C, then continued stirring for 30 minutes. The suspension was centrifuged at 3,000 rpm for 30 min. The precipitated residue was separated out and the supernatant was heated in a water bath for 15 min at 100^{o}C, and then centrifuged at 3000 rpm for 30 min. The soluble components were recovered overnight at 4 °C and precipitated with 70% ethanol. The precipitated component was separated and the precipitate of arabinoxylans was freeze-dried.

1.1.2 Isolation of water-unextractable as non-starch hemicellulose

Water-soluble parts was removed and the remains part was subjected to pulping by delignification process and subsequently with alkaline extraction.

1.1.2.1 Delignification process

One hundred gram of residue from isolation of water-extractable arabinoxylans were mixed with 1.5 L water and 5.4 ml 96% sulfuric acid, and then 40 gram of sodium chorite was added. The mixture was kept at 70 ºC for 2 h with constant stirring. Holocellulose was recovered by centrifugation (3000 rpm for 30 min), and finally washed with water.

1.1.2.2 Alkaline extraction

Alkaline extraction was performed to remove starch and proteins components. One hundred gram of holocellulose from delignification was extracted with 2.0 L of 0.5 M sodium hydroxide at 40 ºC for 6 h. The supernatant was adjusted to pH 4.8 with sulfuric acid (10%). The acidic supernatant was kept overnight at 4 ºC and precipitate was collected by centrifugation (3000 rpm for 30 min). The clear acidic supernatant was freeze-dried.

The yield of water-extractable arabinoxylans or water-unextractable hemicellulose was calculated from the following equation;

$$\% \text{ yield of arabinoxylans or water-unextractable hemicellulose} = \frac{W_1 \times 100}{W_0}$$

W_1 = weight of arabinoxylans or water-unextractable hemicellulose

W_0 = weight of starting rice bran residues

2. Determination of chemical structure of water-extractable arabinoxylans

2.1 Fourier Transform Infrared Resonance Spectroscopy

The chemical structure was detected by FT-IR spectrophotometer using a KBr disc containing 1 % finely ground samples at the frequency range of 450-4000 cm^{-1}.

2.2 Nuclear Magnetic Resonance Spectroscopy

The ^{1}H-NMR spectra (500.16 MHz) of arabinoxylans in deuterium (D_2O) were recorded at 60 ºC on a NMR spectroscopy (Varian UNITY INOVA, USA). Pulse repetition time was 2.046 s and the pulse angle was 90 degrees.

3. Preparation of o/w emulsions containing arabinoxylans

3.1 Preparation of o/w emulsions containing arabinoxylans

Each formulation of emulsions containing arabinoxylans was prepared by mixing the ingredients of oil phase and water phase as shown in Table 2. The ingredients of oil phase and water phase were separately heated to 70ºC and 75ºC, respectively. The water phase was slowly added to the oil phase while continued stirring and kept stirring at 1000 rpm for 30 min until the emulsion was congealed and homogeneous.

Table 2 Formulations of o/w emulsions containing arabinoxylans

Ingredients		**Formulation (%w/w)**				
		1	**2**	**3**	**4**	**5**
Oil phase	Polyoxyethylene (2) Sterayl Ether (Brij 72)	1	2	2.5	3	4
	Butylated hydroxytoluene	0.01	0.01	0.01	0.01	0.01
	Stearic acid	2	2	2	2	2
	Cetostearyl alcohol	0.5	0.5	0.5	0.5	0.5
Water phase	Arabinoxylans	1	1	1	1	1
	Polyethylene (21) Stearyl Ether (Brij 721)	4	3	2.5	2	1
	Glycerine	1	1	1	1	1
	Paraben concentration	1	1	1	1	1
	Propylene glycol	5	5	5	5	5
	Water qs to	100	100	100	100	100

3.2 Determination of physical properties of o/w emulsions containing arabinoxylans

3.2.1 Determination of sensory evaluation

The physical appearances of emulsions were visually observed such as color, odor, texture and skin feeling. Each formulation was evaluated by 15 female volunteers aged between 20-50 years. The formulation were evaluated for texture, spreadability, tackiness, color and odor by giving scores of 1, 2, 3, 4 and 5 according to the satisfaction from “least satisfied” (1) to “most satisfied” (5).

The graded scores were averaged and the ‘mean score’ was used in the comparison to find any differences of formulations.

3.2.2 Determination of viscosity

Viscosity of each formulation was measured by viscometer. An approximately 250 g of each formulation was measured in triplicate. All formulations used Spindle no. 96. A speed was set at 1 rpm and the spindle was rotated about 60 s or until a constant mean viscosity was obtained. The mean viscosity (mPa.s) was recorded.

3.2.3 Determination of pH

pH meter was used to determine the pH of emulsions.

3.3 Determination of emulsion stability by accelerated temperature test

3.3.1 Temperature cycling (heating-cooling cycle)

The product should pass six cycles of temperature testing at 4ºC and 45ºC. The products were kept at 4ºC for 24 h and subsequently kept at 45ºC for 24 h, which was completed for one cycle. Then, the products were repeated for six cycles (Grimm,

1985). After 6 completed heating-cooling cycle, emulsions were visually observed for physical appearance such as flocculation, creaming, coalescence and phase separation.

4. *In vivo* efficacy evaluation of o/w emulsion containing arabinoxylans

4.1 Protocol of clinical study

4.1.1 Selection of the volunteers

Thirty healthy female volunteers were recruited for this study by following criteria.

4.1.2 Inclusion criteria

1) Healthy females aged between 30-55 years,
2) Showed clearly visible wrinkles on their faces
3) No history of smoking, alcohol drinking or drug use,
4) No history of allergy to cosmetic products or any components
5) Voluntarily enrolled in this study. Subjects will be required to read and sign a consent form summarizing the discussion prior to enrollments and will be assured that they may withdraw from the study any time.

4.1.2 Exclusion criteria

1) Had history of hyper allergic reactions,
2) Had history of eczema and psoriasis within 6 months before this study,
3) Had medical history of steroid, antibiotic, NSAIDs and antihistamine medication or used any drug within 7 days before participated in this study,
4) Had medical history of facial application of any topical agent on the face within 1 month before participating in this study,

5) Had history of major operation within 1 year before participating in this study.

6) Pregnancy or lactating.

4.1.3 Discontinuous criteria

1) Had allergic reactions from using the test product.

2) Concomitantly used any moisturizing cosmetic products during the study.

3) Request to withdraw from the study.

4.2 Study design

Oil in water emulsions contain arabinoxylans from rice bran residues and emulsions base having the same composition but without the active ingredient were used as tested and controlled, respectively. Emulsions were applied on half-side of the face of volunteers. The study design was randomization for treatment to the side of each volunteer's face.

Half gram of each emulsions tested and controlled products were separately applied on each side of the face after skin cleansing in the morning and the night.

In this study, the efficacies of oil-in-water emulsions containing arabinoxylans were compared with controlled emulsion base. The efficacies were focused on skin wrinkle, skin moisture, and skin elasticity. These parameters were determined by instrumental analysis using digital camera, Skin Diagnostic Model SD27, DermaLab® elasticity probe at weeks 0, 1, 2, 4, 6 and 8.

4.3 The data analysis

4.3.1 Determination by instrumental analysis

a) Digital camera image was used in this study. The half of face was photographed for comparison of a reduction in wrinkles.

b) Stratum corneum hydration (SCH) content was investigated using a Corneometer® Skin Diagnostic SD 27. It determines the moisture value of the stratum corneum by measuring its electrical capacitance. The measurements were performed on cheeks three times.

c) The skin elasticity was investigated by Derma Lab® elasticity probe. This instrument measures the vertical deformation of the skin surface when the skin was pulled into the circular aperture (2 mm diameter) of the measuring probe, after application of a constant suction pressure (450 mbar) for 1 second. After the negative pressure was cut off, the skin would be returned into its original shape. The measurements were performed on near sites of left and right forehead.

4.3.2 The statistical analysis

The data of measurements were analyzed by the statistical program for windows software. The mean, standard deviation (SD), minimum and maximum values of the data were determined by descriptive statistics, ANCOVA, paired-sample T-test and split plot design. The significance criterion for the correlation measurements was set at $\alpha = 0.05$.

CHAPTER IV

RESULTS AND DISCUSSION

1. Extraction of arabinoxylans from rice bran locally produced in Thailand

1.1 Extraction

Water-extractable arabinoxylans was extracted from rice bran residues in warm water. The optimum temperature of water bath was used at 40 ºC in this extraction. Arabinoxylans in the supernatant solution was precipitated in aqueous ethanol whereas a large part of the water-unextractable hemicellulose was obtained after alkali-extraction. Ethanol was evaporated out using rotary evaporation. The residues from water-extractable arabinoxylans were delignified with sodium chlorite for lignin removing and represented as holocellulose. Hemicellulose was obtained from holocellulose extracted with sodium hydroxide. After freeze dried, the yellow light powder and aroma of arabinoxylans was received. The yields of water-extractable arabinoxylans and water-unextractable hemicellulose were 0.29 %w/w and 7.6 %w/w, respectively.

2. Determination of chemical structure of arabinoxylans

2.1 Fourier Transform Infrared resonance spectroscopy (FT-IR)

The chemical structure of an arabinoxylans extract was detected by FT-IR spectrophotometer using a KBr Disc containing 1 % finely ground samples at the frequency range of 450-4000 cm^{-1}.

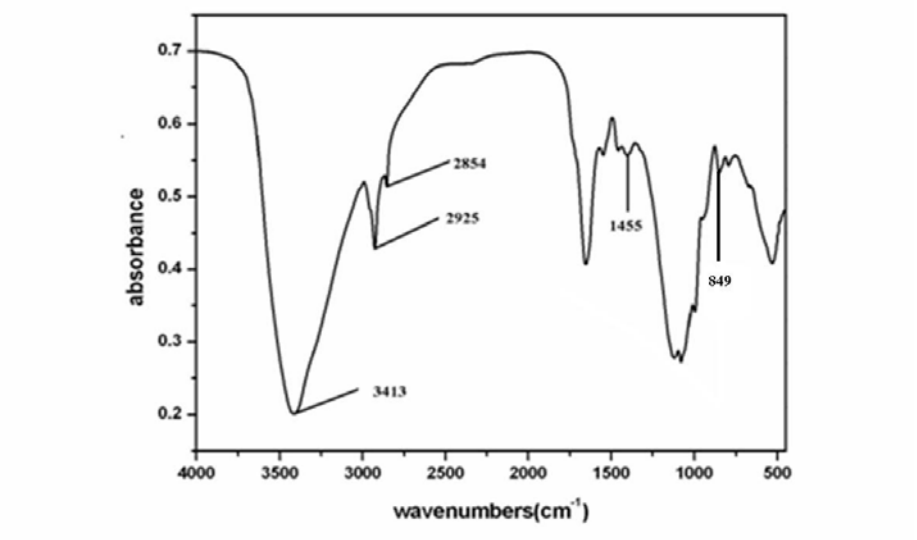


Figure 10 IR-spectrum of water-extractable as arabinoxylans hemicellulose

FT-IR spectra of water-extractable arabinoxylans in the frequency range of 450-4000 cm^{-1} were detected. A narrow band at 849 cm^{-1} was associated to the β–glycosidic linkage between the sugar units. Another main peak appeared at the strong absorption at 3413 cm^{-1} of hydroxyl groups and the CH_2 stretching band was shown at 2854, 2925 cm^{-1} and the CH_2 bending at the 1455 cm^{-1} indicated the presence of alkyl chains as shown in Figure 10.

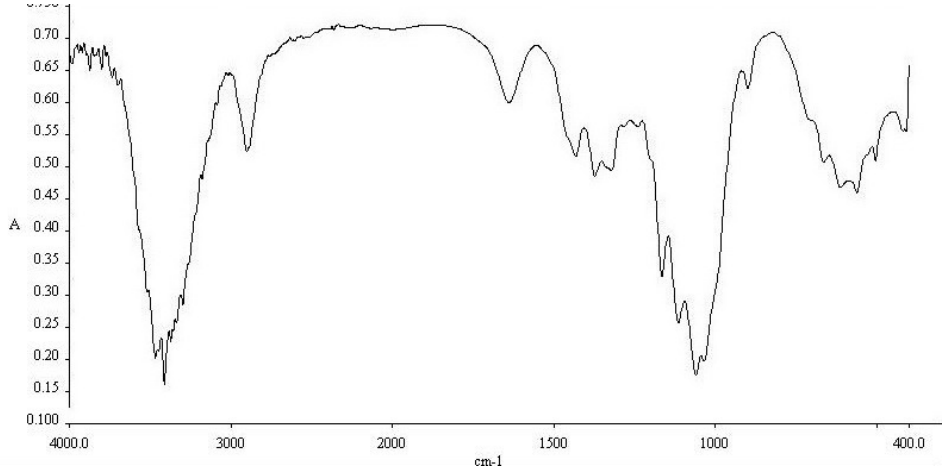


Figure 11 IR-spectrum of water-unextractable hemicellulose

The FT-IR spectra of water-unextractable hemicellulose was shown in Figure 11. The absorption at 1632 cm^{-1} was principally associated with absorbed water, since

the hemicelluloses usually have a strong affinity for water, and in the solid state these macromolecules may have disordered structures which can easily be hydrated (Chaikumpollert et al., 2004). The small bands at 1430, 1316 and 1255 cm^{-1} represented C–H stretch and CH or OH bending in hemicelluloses. The band at 1377 and 1162 cm^{-1} attributed to C–H deformation and C–O–C vibration in hemicelluloses, respectively. Bands between 1112 and 1062 cm^{-1} were typical of xylans. The prominent band at 1056 cm^{-1} was attributed to the C–O, C–C stretching or C–OH bending in hemicelluloses. The sharp band at 896 cm^{-1} corresponding to the C-1 group frequency or ring frequency, was characteristic of β-glycosidic linkages between the sugar units.

2.2 Nuclear Magnetic Resonance Spectroscopy (NMR)

Figure 12 shows proton NMR analysis of this arabinoxylans extract indicated signal at 5.354, and 5.301, 5.267, corresponding to anomeric protons of α-L-arabinofuranoses substituted at O-3 (mono substituted) and at both O-3 and C-2 (disubtituted) of xylose residues, respectively. Signals obtained at 4.518, 4.502 and 4.485 ppm were due to the anomeric protons of β–D-xyloses substituted at C-2 (disubstituted), C-3 (monosubstitued) and unsubstituted residues, respectively. The signals for other protons of arabinose and xylose were observed in the region of 3.225-4.304 ppm and were in close proximity with the signals obtained for oligosaccharides generated from water extractable arabinoxylans from rice bran. The signals assigned here are in close agreement with the signals assigned for purified arabinoxylan isolated from native and malted ragi(Subba Rao and Muralikrishna, 2004).

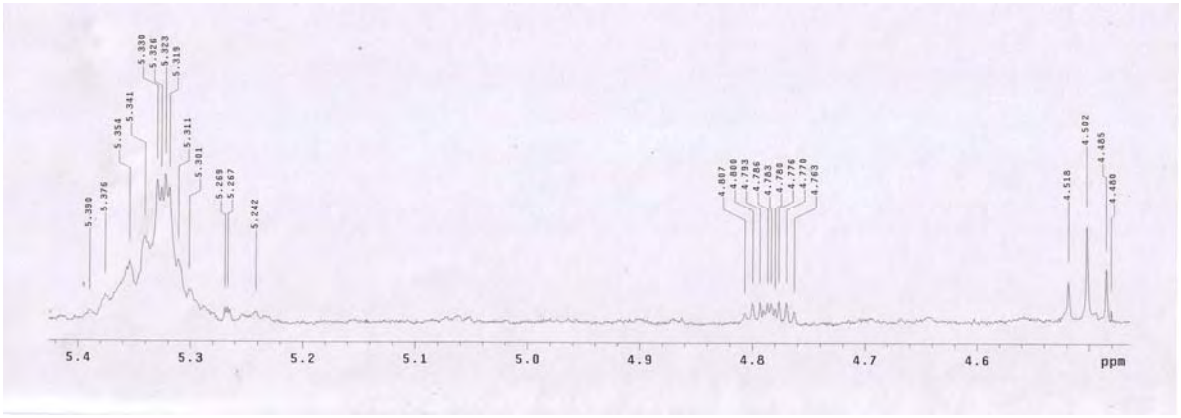


Figure 12 ^{1}H-NMR spectrum of crude water-extractable

The water-extractable arabinoxylans part was formulated and determined physical appearances of oil-in-water emulsions and studied the *in vivo* skin moisturizing and elasticity enhancement for skin care products

3. Preparation of o/w emulsions containing arabinoxylans

3.1 Determination of physical properties

3.2.1 Determination of physical appearance and sensory evaluation

The color, odor, skin feeling, smooth cream mass and spreadability were evaluated by the panelists (n=15). The feelings were ranked at the end of study in 1 to 5 scales of satisfaction: 1 as ‘least’, 2 as ‘slight’, 3 as ‘moderate’, 4 as ‘considerable’ and 5 as ‘most’. The mean scores of satisfactory feelings are shown in Figure 12.

Figure 13 explains the mean score of o/w emulsions containing arabinoxylans from various ratio of Brij 72 to Brij 721. Formulation 4 (ratio of Brij 72 to Brij 721 was 3:2) was evaluated as the most ‘satisfy’ with its skin feeling and speadability. Formulation 3 (ratio of Brij 72 to Brij 721 1:1) was evaluated as the most ‘satisfy’ with its smooth cream mass as same as formulation 4. On the other hand, formulation 5 (ratio of Brij 72 to Brij 721 was 4:1) got the least ‘satisfy’ in all categories.

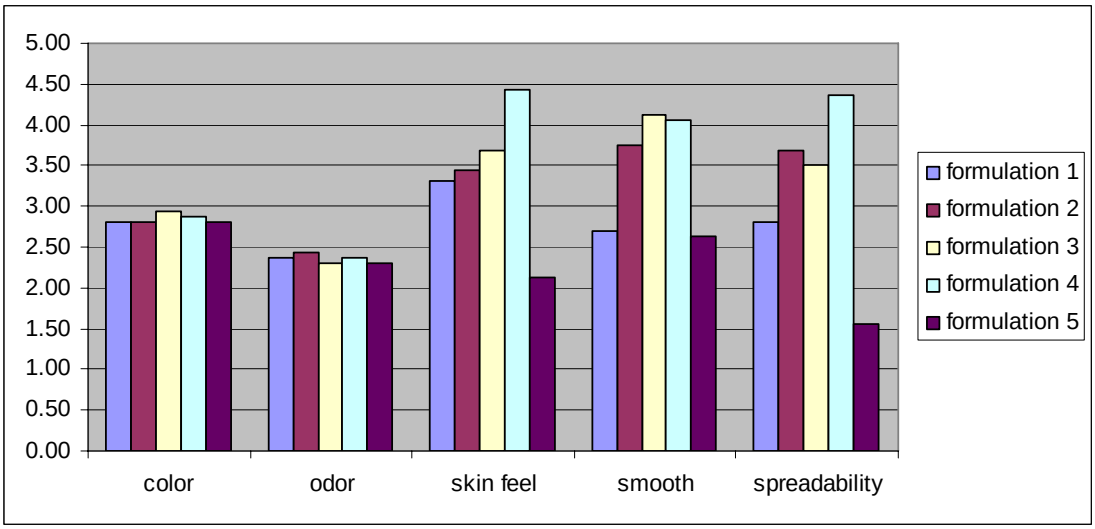


Figure 13 Mean score of satisfactory feel evaluation by the panelists (n=15)

3.2.1 Determination of viscosity

The measured viscosities were ranked from the lowest to highest, as follows: formulation 1 (137.03 ± 1.16 mPa.s), formulation 2 (10368 ± 99.40 mPa.s), formulation 3 (16216.85 ± 303.66 mPa.s), formulation 4 (21827.01 ± 163.76 mPa.s) and formulation 5 (28176.00 ± 172.16 mPa.s). Figure 14 shows that the viscosities were increased with the ratio of Brij 72 to Brij 721, as follows: 1:4, 2:3, 1:1, 3:2 and 4:1.

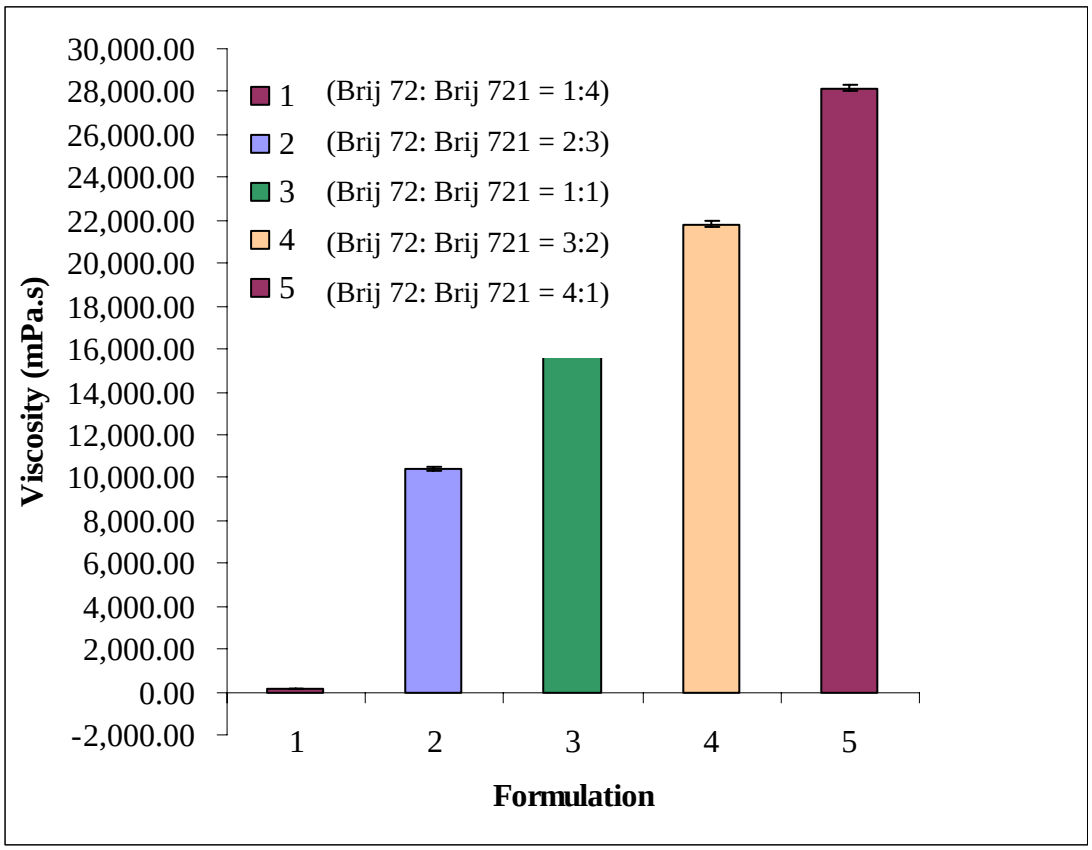


Figure 14 Viscosities of o/w emulsions containing arabinoxylans (each bar represent mean ± SD, n =3)

The differences in viscosities for o/w emulsions containing arabinoxylans from various ratio of Brij 72 to Bij 721 were significant (P<0.05). This indicated that various ratios of Brij 72 to Brij 721 affected viscosity of o/w emulsions containing a constant concentration of arabinoxylans. The ratio 4:1 of Brij 72 to Brij 721 gave the highest viscosity.

3.2.2 Determination of pH

The estimated pH was ranked from the lowest to highest, as follows: formulation 1 (5.52 ± 0.02), formulation 5 (5.55 ± 0.01), formulation 4 (5.57 ± 0.01), formulation 2 (5.60 ± 0.01), and formulation 3 (5.61 ± 0.01) as show in Figure 15.

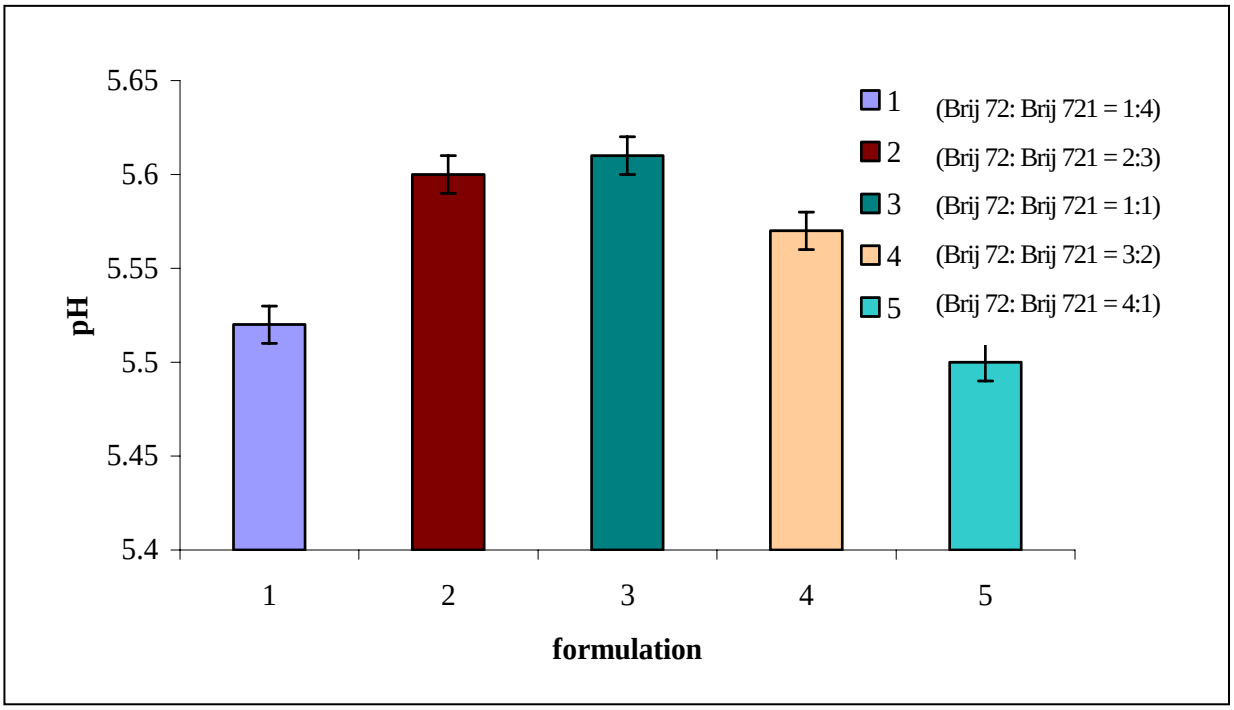


Figure 15 pH of o/w emulsions containing arabinoxylans (Each bar represent mean ± SD, n=3)

The differences in term of pH for o/w emulsions containing arabinoxylans from various ratio of Brij 72 to Brij 721 were not significant (P>0.05). The results show pHs of all ratios of Brij 72 to Brij 721 which were approximately in an acceptable range of skin pH 5.5 ± 0.1.

3.3 Determination the stability of o/w emulsions containing arabinoxylans

3.3.1 The stability of formulations

Formulas were investigated under stress condition at 4 ºC and 45 ºC for 6 cycles. The physical properties were observed by visual evaluation. It appears that all of 5 formulas showed good physical stability in color, creaming/sedimentation, aggregation, flocculation and coalescence.

Table 3 The physical appearances of formulations

Formulation	Cycle	Color	Creaming/ Sedimentation	Aggregation	Flocculation	Coalescence
1	0	white	-	-	-	-
	6	white	-	-	-	-
2	0	white	-	-	-	-
	6	white	-	-	-	-
3	0	white	-	-	-	-
	6	white	-	-	-	-
4	0	white	-	-	-	-
	6	white	-	-	-	-
5	0	white	-	-	-	-
	6	white	-	-	-	-

- Main breakdown processes of emulsion were not observed.

3.3.2 pH of oil-in-water emulsions containing arabinoxylans

The pH values of o/w emulsions containing arabinoxylans were shown in Figure 15. The pHs of all preparations were approximately 5.5 ± 0.1 before temperature cycling. Figure 15 shows that during temperature cycling the pH of all formulations slowly decreased with time to acidic pH. Formulation 3 and 4 show most stability to pH of 5.00 ±0.1. The lowest pH, 4.82 ± 0.01, was found in formulation 1.

Differences in physical stability testing in term of pH of o/w emulsions before and after heating-cooling cycle were significant ($P < 0.05$) for all formulations. The results indicated that heating-cooling cycle affected pH in all ratios of Brij 72 to Brij 721 in o/w emulsions containing arabinoxylans. It was possibly due to hydrolysis of some lipid in the emulsions leading to the formation of free fatty acid which gradually reduced the pH of the system (Klang et al., 1996).

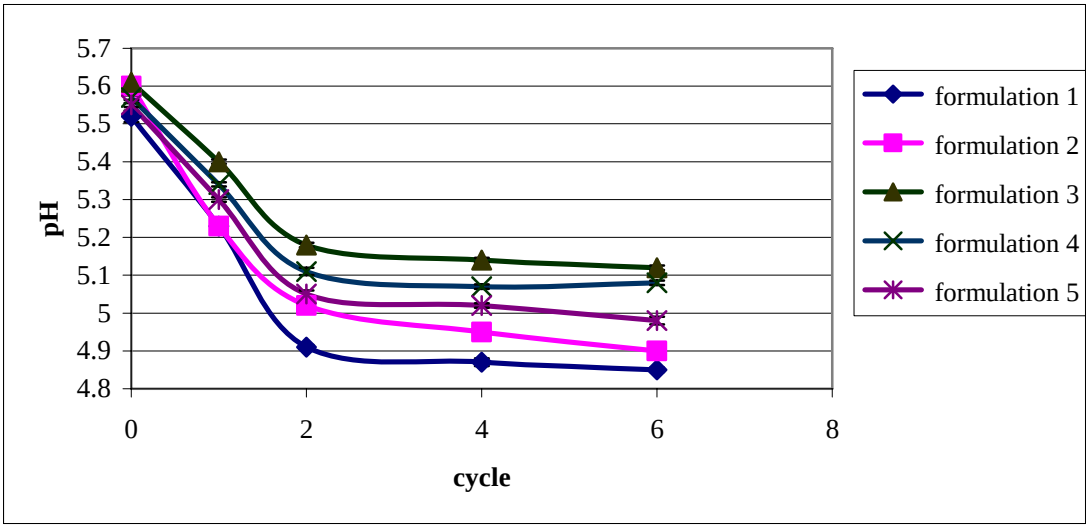


Figure 16 pH of various ratios of Brij 72 to Brij 721 of o/w emulsions containing arabinoxylans during heating-cooling cycle

3.3.3 Viscosity of o/w emulsions containing arabinoxylans

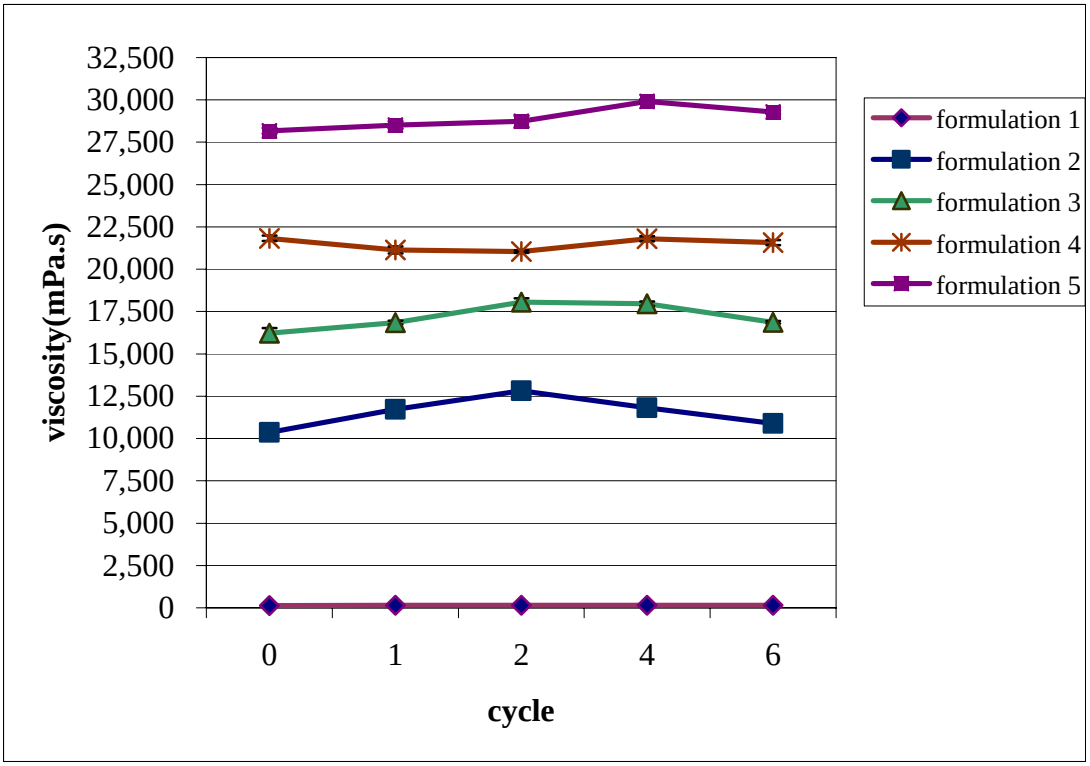


Figure 17 Viscosities of various ratio of Brij 72 to Brij 721 of o/w emulsions containing arabinoxylans during heating-cooling cycle

The apparent viscosity of formulation 1 (ratio of Brij 72 to Brij 721 was 1:4) was 137 ± 1.16 mPa.s at the cycle 0. After that, the apparent viscosity was increased to 141.91 ± 3.46 mPa.s at cycle 2 and slowly changed to 152.40 ± 1.61 mPa.s at the cycle 6.

The apparent viscosity of formulation 2 (ratio of Brij 72 to Brij 721 was 2:3) was 10368.76 ± 99.40 mPa.s at the cycle 0. The apparent viscosity was increased to 12819.68 ± 131.77 mPa.s at cycle 2. After that, the apparent viscosity was decreased to 10897.67 ± 57.27 mPa.s until complete temperature cycling.

The apparent viscosity of formulation 3 (ratio of Brij 72 to Brij 721 was 1:1) was 16216.85 ± 303.66 mPa.s at the cycle 0. The apparent viscosity was increased to 18060.11 ± 219.86 mPa.s at cycle 4. After that, the apparent viscosity was decreased to 16871.40 ± 74.98 mPa.s until complete temperature cycling.

The apparent viscosity of formulation 4 (ratio of Brij 72 to Brij 721 was 3:2) was 21827.00 ± 163.76 mPa.s at the cycle 0. After that, the apparent viscosity was decreased to 21049.28 ± 75.04 mPa.s at cycle 2 and slowly changed to 21570.40 ± 112.48 mPa.s at the cycle 6.

The apparent viscosity of formulation 5 (ratio of Brij 72 to Brij 721 was 1:4) was 28176.00 ± 172.16 mPa.s at the cycle 0. The apparent viscosity was increased to 29910.87 ± 131.52 mPa.s at cycle 4. After that, the apparent viscosity was decreased to 29281.25 ± 112.47 mPa.s until complete temperature cycling.

Differences in physical stability testing in term of viscosity of oil-in-water emulsions before and after heating-cooling cycles were not significant ($P>0.05$) for all formulations. The results indicated that heating-cooling cycle did not affect the viscosity in all various ratio of Brij 72 to Brij 721 of o/w emulsions containing arabinoxylans. The selected formula for *in vivo* testing was formulation 4.

4. *In vivo* efficacy evaluation of o/w emulsion containing arabinoxylans

In this investigation, 30 healthy females volunteers aged between 30-55 years were participated in the study.

4.1 Moisturizing effect of o/w emulsions containing arabinoxylans

Skin hydration was evaluated by Skin Diagnostic SD 27. Tested areas of thirty volunteers' face were studies, a half left of the face was treated with o/w emulsions containing arabinoxylans from rice bran and a half right of face was treated with emulsions base. The results of skin hydration was measured as moisture content at tested area on left cheek (LeftC), right cheek (RightC), left forehead (LeftF) and right (RightF) forehead. The results were shown in Table 4.

Table 4 The average skin hydration after using o/w emulsions containing arabinoxylans at week 0, 1, 2, 4, 6 and 8 (n=30)

Moisture Content				
Group	**Week**	**Mean(%)±SD**	**Group**	**Mean(%)±SD**
LeftC (o/w emulsion containing arabinoxylans)	0	16.8780±16.70	RightC (emulsion base)	15.4583±20.22
	1	31.1500±17.21		29.8667±20.36
	2	31.1250±20.75		31.4750±22.10
	4	35.2000±10.96		27.5000±14.09
	6	35.3833±15.77		32.0667±14.87
	8	30.0833±17.95		29.3667±17.97
LeftF (o/w emulsion containing arabinoxylans)	0	27.8167±20.98	RightF (emulsion base)	31.0083±21.48
	1	34.1917±20.15		35.6250±21.38
	2	39.9167±25.29		39.8583±23.99
	4	36.7083±17.79		35.9250±17.90
	6	38.6583±25.02		38.5500±19.25
	8	42.2833±22.98		40.6000±20.91

Table 5 Paired samples test of moisture content by SPSS program

Paired Samples Test		Paired Differences		Sig. (2-tailed)
		Mean(%)	Std. Deviation	
Pair 1	leftC_week0 - leftC_week1	-14.2720*	16.6098	0.000
Pair 2	leftC_week0 - leftC_week2	-14.2470*	29.9750	0.014
Pair 3	leftC_week0 - leftC_week4	-18.3220*	16.8309	0.000
Pair 4	leftC_week0 - leftC_week6	-18.5053*	24.9306	0.000
Pair 5	leftC_week0 - leftC_week8	-13.2053*	23.8418	0.005
Pair 6	rightC_week0 - rightC_week1	-14.4083*	19.7977	0.000
Pair 7	rightC_week0 - rightC_week2	-16.0167*	31.3367	0.009
Pair 8	rightC_week0 - rightC_week4	-12.0417*	17.1869	0.001
Pair 9	rightC_week0 - rightC_week6	-16.6083*	25.3504	0.001
Pair 10	rightC_week0 - rightC_week8	-13.9083*	24.7705	0.005
Pair 11	leftF_week0 - leftF_week1	-6.3750*	16.3499	0.041
Pair 12	leftF_week0 - leftF_week2	-12.1000*	23.3776	0.008
Pair 13	leftF_week0 - leftF_week4	-8.8917*	15.2207	0.003
Pair 14	leftF_week0 - leftF_week6	-10.8417*	22.6143	0.014
Pair 15	leftF_week0 - leftF_week8	-14.4667*	20.9601	0.001
Pair 16	rightF_week0 - rightF_week1	-4.6167	17.3741	0.156
Pair 17	rightF_week0 - rightF_week2	-8.8500*	23.3190	0.047
Pair 18	rightF_week0 - rightF_week4	-4.9167	14.1404	0.067
Pair 19	rightF_week0 - rightF_week6	-7.5417	20.5982	0.054
Pair 20	rightF_week0 - rightF_week8	-9.5917*	25.4334	0.048

* = Significant difference ($P<0.05$)

Table 6 Paired samples test of moisture content of each formulation at same week by SPSS program

Paired Samples Test of moisture content	Week	Paired Differences			Sig. (2-tailed)
		Mean(%)	Std. Deviation	Std. Error Mean	
LeftC – RightC	0	1.41967	9.39200	1.71474	0.414
	1	1.28333	11.00778	2.00974	0.528
	2	-0.35000	16.14517	2.94769	0.906
	4	7.70000*	10.25208	1.87177	0.000
	6	3.31667	10.14080	1.85145	0.084
	8	0.71667	14.02641	2.56086	0.782
LeftF - RightF	0	-3.19167	8.85824	1.61729	0.058
	1	-1.43333	13.65270	2.49263	0.570
	2	0.05833	14.86298	2.71360	0.983
	4	0.78333	12.43738	2.27075	0.733
	6	0.10833	14.06538	2.56797	0.967
	8	1.68333	13.13375	2.39788	0.488

* = Significant difference (P<0.05)

The average skin hydration after using o/w emulsions containing arabinoxylans at week 0, 1, 2, 4, 6 and 8 (n=30) were shown in Table 4. The paired samples tests for moisture content of each formulation from week 0 to week 8 were shown in Table 5. The results show that group of o/w emulsions containing arabinoxylans were significant difference from week 1 to week 8 on LeftC and LeftF. The moisture content of treatment on LeftC, RightC and LeftF was increased every week with significant difference from week 0. Moisture content of each group after using o/w emulsions containing arabinoxylans was long-lasting for 8 weeks.

The paired samples tests for moisture content of each formulation from week 0 to week 8 were shown in Table 6. The results show that group of o/w emulsions containing 1%w/w arabinoxylans was not significant difference from week 0 to week 8 on cheek and forehead, exception week 4 of cheek.

The estimated marginal means of moisture content of each group were shown in Table 7. The pairwise comparisons of each group were shown in Table 8. When the

results were compared between o/w emulsions containing arabinoxylans and emulsions base, the increasing of moisture on the RightC was less than the leftC as same as LeftF and RightF. The results were also observed by the macroscopic photograph as shown in Figures 18-31.

Table 7 The estimated marginal means of moisture content of each group

Group	Mean(%)±SD	Group	Mean(%)±SD
LeftC (o/w emulsion containing arabinoxylans)	32.4926±1.42	RightC (emulsion base)	30.1507±1.42
LeftF (o/w emulsion containing arabinoxylans)	39.2494±1.48	RightF (emulsion base)	37.2140±1.48

a. Covariates appearing in the model are evaluated at the following values: baseline_week(0) .

Table 8 Pairwise comparisons of moisture content of each group by SPSS program

Group (I)	Group (J)	Mean Difference (I-J) (%)	Std. Error	Sig.(a)
LeftC (o/w emulsion containing arabinoxylans)	RightC (emulsion base)	2.3419	2.0048	0.244
LeftF (o/w emulsion containing arabinoxylans)	RightF (emulsion base)	2.0354	2.0913	0.331

* = Significant difference ($P< 0.05$)

Table 9 The estimated marginal means of moisture content of each week using split plot design

Week	Moisture Content on Cheek	Moisture Content on Forehead
	Mean(%)±SD	Mean(%)±SD
0	16.168±2.28	29.413±2.78
1	30.508±2.28	34.908±2.78
2	31.300±2.28	39.888±2.78
4	31.350±2.28	36.317±2.78
6	33.725±2.28	38.604±2.78
8	29.725±2.28	41.442±2.78

Table 10 Pairwise Comparisons of moisture content of each week on cheek using split plot design

(I) Week	(J) Week	Mean Difference (I-J) (%)	Std. Error	Sig.(a)
0	1	-14.340*	3.227	0.000
	2	-15.132*	3.227	0.000
	4	-15.182*	3.227	0.000
	6	-17.557*	3.227	0.000
	8	-13.557*	3.227	0.000
1	0	14.340*	3.227	0.000
	2	-0.792	3.227	0.806
	4	-0.842	3.227	0.794
	6	-3.217	3.227	0.320
	8	0.783	3.227	0.808
2	0	15.132*	3.227	0.000
	1	0.792	3.227	0.806
	4	-0.050	3.227	0.988
	6	-2.425	3.227	0.453
	8	1.575	3.227	0.626
4	0	15.182*	3.227	0.000
	1	0.842	3.227	0.794
	2	0.050	3.227	0.988
	6	-2.375	3.227	0.462
	8	1.625	3.227	0.615
6	0	17.557*	3.227	0.000
	1	3.217	3.227	0.320
	2	2.425	3.227	0.453
	4	2.375	3.227	0.462
	8	4.000	3.227	0.216
8	0	13.557*	3.227	0.000
	1	-0.783	3.227	0.808
	2	-1.575	3.227	0.626
	4	-1.625	3.227	0.615
	6	-4.000	3.227	0.216

a. Adjustment for multiple comparisons: Least Significant Difference (equivalent to no adjustments).

* = Significant difference (P<0.05)

Table 11 Pairwise Comparisons of moisture content of each week on forehead using split plot design

(I) Week	(J) Week	Mean Difference (I-J) (%)	Std. Error	Sig.(a)
0	1	-5.496	3.937	0.164
	2	-10.475*	3.937	0.008
	4	-6.904	3.937	0.080
	6	-9.192*	3.937	0.020
	8	-12.029*	3.937	0.002
1	0	5.496	3.937	0.164
	2	-4.979	3.937	0.207
	4	-1.408	3.937	0.721
	6	-3.696	3.937	0.348
	8	-6.533	3.937	0.098
2	0	10.475*	3.937	0.008
	1	4.979	3.937	0.207
	4	3.571	3.937	0.365
	6	1.283	3.937	0.745
	8	-1.554	3.937	0.693
4	0	6.904	3.937	0.080
	1	1.408	3.937	0.721
	2	-3.571	3.937	0.365
	6	-2.288	3.937	0.562
	8	-5.125	3.937	0.194
6	0	9.192*	3.937	0.020
	1	3.696	3.937	0.348
	2	-1.283	3.937	0.745
	4	2.288	3.937	0.562
	8	-2.838	3.937	0.472
8	0	12.029*	3.937	0.002
	1	6.533	3.937	0.098
	2	1.554	3.937	0.693
	4	5.125	3.937	0.194
	6	2.838	3.937	0.472

a. Adjustment for multiple comparisons: Least Significant Difference (equivalent to no adjustments).

* = Significant difference (P<0.05)

The estimated marginal means of moisture content on forehead of each week by split plot design were shown in Table 9. The pairwise comparisons of moisture content of each week on cheek by split plot design were shown in Table 10. When the moisture content on cheek of each week were compared they show significant difference between week 0 and week 1 to week 8 (P<0.05). The pairwise comparisons of moisture content of each week on forehead using split plot design were shown in Table 11. When the moisture content on forehead of each week were compared they show significant difference between week 0 and week 2, week 6 and week 8 (P<0.05).

Table 12 Analysis of variance for moisture content on cheek as a function of treatment and week

Source		**Type III Sum of Squares**	**df**	**Mean Square**	**F**	**Sig.**
Treatment	Hypothesis	496.062	1	496.062	0.631	0.430
	Error	45,608.922	58	786.361		
Week	Hypothesis	12,020.716	5	2,404.143	11.043	0.000*
	Error	63,132.808	290	217.699		
treatment * week	Hypothesis	622.770	5	124.554	0.572	0.721
	Error	63,132.808	290	217.699		
Subject(treatment)	Hypothesis	45,608.922	58	786.361	3.612	0.000*
	Error	63,132.808	290	217.699		
week * subject(treatment)	Hypothesis	63,132.808	290	217.699	.	.
	Error	0.000	0	.(c)		

a. MS(subject(treatment))

b. MS(week * subject(treatment))

c. MS(Error)

* = Significant difference (P<0.05)

The analysis of variance for moisture content on cheek as a function of treatment and week were shown in Table 12. The results show that there was not significant interaction between treatment and week of moisture content on cheek

(*P*=0.721). There was a significant main effect of week and subject on moisture content, *P*<0.05. However, the main effect of treatment was not significant difference (*P*=0.430). The results show that the moisture content on cheek of o/w emulsions containing 1%w/w arabinoxylans was not significant difference from emulsions base after using the products for 8 weeks.

Table 13 Analysis of variance for moisture content on forehead as a function of treatment and week

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Treatment	Hypothesis	9.917	1	9.917	0.005	0.942
	Error	109,256.606	58	1,883.735		
Week	Hypothesis	5,562.670	5	1,112.534	6.143	0.000*
	Error	52,523.434	290	181.115		
treatment * week	Hypothesis	225.636	5	45.127	0.249	0.940
	Error	52,523.434	290	181.115		
Subject(treatment)	Hypothesis	109,256.606	58	1,883.735	10.401	0.000*
	Error	52,523.434	290	181.115		
week * subject(treatment)	Hypothesis	52,523.434	290	181.115	.	.
	Error	0.000	0	.(c)		

a. MS(subject(treatment))

b. MS(week * subject(treatment))

c. MS(Error)

* = Significant difference (*P*<0.05)

The analysis of variance for moisture content on forehead as a function of treatment and week were shown in Table 13. The results show that there was not significant interaction between treatment and week on moisture content of forehead (*P*=0.940). There was a significant main effect of week and subject on moisture content, *P*<0.05. However, the main effect of treatment was not significant difference (*P*=0.942). The results show that the moisture content on forehead of o/w emulsions

containing 1%w/w arabinoxylans was not significant difference from emulsions base after using the products for 8 weeks.

4.2 The elasticity measurement using DermaLab® Elasticity probe

The elasticity (Young's modulus value) was measure by DermaLab® Elasticity probe, the differences of Young's modulus between week 0 and 8 were calculated. The macroscopic photographs were observed to evaluate an improvement of the area of test product. Three measurements were performed in each testing and the results were shown in Table 14. The paired samples tested of Young's modulus by SPSS program were shown in Table 15.

Table 14 The average young's modulus after using o/w emulsions containing arabinoxylans at week 0, 1, 2, 4, 6 and 8 (n=30)

Young's modulus				
Group	**Week**	**Mean(Mpa)±SD**	**Group**	**Mean(Mpa)±SD**
LeftC (o/w emulsion containing arabinoxylans)	0	7.3380±1.77	RightC (emulsion base)	7.5137±2.06
	1	6.1533±1.87		6.4180±1.56
	2	6.5540±1.81		7.3267±4.58
	4	7.9427±2.08		7.5403±2.14
	6	6.9207±1.95		7.0743±2.01
	8	6.4083±1.90		6.7723±2.16
LeftF (o/w emulsion containing arabinoxylans)	0	10.2973±2.05	RightF (emulsion base)	10.0470±2.33
	1	9.8810±2.11		10.3593±1.92
	2	10.0593±1.78		10.0570±1.70
	4	10.4480±2.17		10.4617±2.50
	6	10.7363±2.43		10.6250±2.62
	8	10.8867±2.02		10.7073±2.19

Table 15 Paired samples test of young's modulus by SPSS program

Paired Samples Test (Young's modulus)		Paired Differences		Sig. (2-tailed)
		Mean(Mpa)	Std. Deviation	
Pair 1	leftC_week0 - leftC_week1	1.1847*	1.7414	0.001
Pair 2	leftC_week0 - leftC_week2	0.7840*	1.7352	0.019
Pair 3	leftC_week0 - leftC_week4	-0.6047*	1.5992	0.047
Pair 4	leftC_week0 - leftC_week6	0.4173	1.6353	0.173
Pair 5	leftC_week0 - leftC_week8	0.9297*	1.7111	0.006
Pair 6	rightC_week0 - rightC_week1	1.0957*	1.8307	0.003
Pair 7	rightC_week0 - rightC_week2	0.1870	4.8023	0.833
Pair 8	rightC_week0 - rightC_week4	-0.0267	1.6998	0.932
Pair 9	rightC_week0 - rightC_week6	0.4393	1.5179	0.124
Pair 10	rightC_week0 - rightC_week8	0.7413*	1.7671	0.029
Pair 11	leftF_week0 - leftF_week1	0.4163	1.8894	0.237
Pair 12	leftF_week0 - leftF_week2	0.2380	1.5499	0.407
Pair 13	leftF_week0 - leftF_week4	-0.1507	1.6697	0.625
Pair 14	leftF_week0 - leftF_week6	-0.4390	1.6302	0.151
Pair 15	leftF_week0 - leftF_week8	-0.5893	1.8407	0.090
Pair 16	rightF_week0 - rightF_week1	-0.3123	1.7818	0.345
Pair 17	rightF_week0 - rightF_week2	-0.0100	1.9797	0.978
Pair 18	rightF_week0 - rightF_week4	-0.4147	1.7423	0.203
Pair 19	rightF_week0 - rightF_week6	-0.5780	2.0303	0.130
Pair 20	rightF_week0 - rightF_week8	-0.6603	1.9489	0.074

* = Significant difference ($P< 0.05$)

Table 16 Paired samples test of Young's modulus of each formulation at same week by SPSS program

Paired Samples Test of Young's modulus	Week	Paired Differences			Sig. (2-tailed)
		Mean(MPa)	Std. Deviation	Std. Error Mean	
LeftC – RightC	0	-0.17567	1.57707	0.28793	0.547
	1	-0.26467	1.77561	0.32418	0.421
	2	-0.77267	4.68676	0.85568	0.374
	4	0.40233	1.18021	0.21548	0.072
	6	-0.15367	1.65628	0.30239	0.615
	8	-0.36400	1.05315	0.19228	0.068
LeftF - RightF	0	0.25033	1.90490	0.34779	0.477
	1	-0.47833	1.81196	0.33082	0.159
	2	0.00233	1.52975	0.27929	0.993
	4	-0.01367	2.06274	0.37660	0.971
	6	0.11133	1.82026	0.33233	0.740
	8	0.17933	1.48110	0.27041	0.512

* = Significant difference (P<0.05)

The Young's modulus values of the skin were reduced resulted from higher elasticity of the skin. Thus, the elasticity of the skin was increased due to the elevating its skin tension and firming on the cheek which show the significant differences between week 0 to week 1-8 but in RightC the differences were shown only at week 1 and 8. In LeftF and RightF the results were not significant differences (P>0.05).

The estimated marginal means of Young's modulus of each group were shown in Table 17. The pairwise comparison of each group was shown in Table 18. The results show that group of o/w emulsions containing arabinoxylans was not significant difference from emulsions base when used on cheek and forehead. The macroscopic photographs were observed in Figures 18-31 which show reducing in wrinkle on forehead.

The average young's modulus after using o/w emulsions containing arabinoxylans at week 0, 1, 2, 4, 6 and 8 (n=30) were shown in Table 14. The results

show that group of o/w emulsion containing 1%w/w arabinoxylans was not significant difference from week 0 to week 8 on cheek and forehead. The paired samples tests for Young's modulus of each formulation at same week from week 0 to week 8 were shown in Table 16.

Table 17 The estimated marginal means of Young's modulus of each group

Group	**Mean(Mpa)±SD**	**Group**	**Mean(Mpa)±SD**
LeftC (o/w emulsion containing arabinoxylans)	6.847±0.17	RightC (emulsion base)	6.975±0.17
LeftF (o/w emulsion containing arabinoxylans)	10.322±0.13	RightF (emulsion base)	10.523±0.13

a. Covariates appearing in the model are evaluated at the following values: baseline_week(0) .

Table 18 Pairwise comparison of Young's modulus of each group by SPSS program

Group (I)	**Group (J)**	**Mean Difference (I-J) (MPa)**	**Std. Error**	**Sig.(a)**
LeftC (o/w emulsion containing arabinoxylans)	RightC (emulsion base)	-0.1279	0.2388	0.593
LeftF (o/w emulsion containing arabinoxylans)	RightF (emulsion base)	-0.2011	0.1895	0.290

* = Significant difference ($P< 0.05$)

Table 19 The estimated marginal means of Young's modulus of each week using split plot design

Week	**Young's Modulus on Cheek**	**Young's Modulus on Forehead**
	Mean(Mpa)±SD	**Mean(Mpa)±SD**
0	7.423±0.30	10.169±0.29
1	6.284±0.30	10.117±0.29
2	6.938±0.30	10.055±0.29
4	7.739±0.30	10.452±0.29
6	6.995±0.30	10.678±0.29
8	6.588±0.30	10.794±0.29

Table 20 Pairwise Comparisons of Young's modulus of each week on cheek using split plot design

(I) Week	(J) Week	Mean Difference (I-J) (MPa)	Std. Error	Sig.(a)
0	1	1.140*	0.417	0.007
	2	0.486	0.417	0.245
	4	-0.316	0.417	0.449
	6	0.428	0.417	0.305
	8	0.835*	0.417	0.046
1	0	-1.140*	0.417	0.007
	2	-0.654	0.417	0.118
	4	-1.456*	0.417	0.001
	6	-0.711	0.417	0.089
	8	-0.304	0.417	0.466
2	0	-0.486	0.417	0.245
	1	0.654	0.417	0.118
	4	-0.802	0.417	0.055
	6	-0.057	0.417	0.891
	8	0.350	0.417	0.402
4	0	0.316	0.417	0.449
	1	1.456*	0.417	0.001
	2	0.802	0.417	0.055
	6	0.744	0.417	0.075
	8	1.151	0.417	0.006
6	0	-0.428	0.417	0.305
	1	0.711	0.417	0.089
	2	0.057	0.417	0.891
	4	-0.744	0.417	0.075
	8	0.407	0.417	0.330
8	0	-0.835*	0.417	0.046
	1	0.304	0.417	0.466
	2	-0.350	0.417	0.402
	4	-1.151*	0.417	0.006
	6	-0.407	0.417	0.330

a. Adjustment for multiple comparisons: Least Significant Difference (equivalent to no adjustments).

* = Significant difference ($P<0.05$)

The estimated marginal means of Young's modulus on cheek of each week using split plot design were shown in Table 19. The pairwise comparisons of Young's modulus of each week on cheek using split plot design were shown in Table 20. When the Young's modulus on cheek of each week was compared they were significant difference between week 0 and week 1 and week 8. Furthermore Young's modulus of each week on cheek was significant difference between week 1 and week 4.

Table 21 Pairwise Comparisons of Young's modulus of each week on forehead using split plot design

(I) Week	(J) Week	Mean Difference (I-J) (MPa)	Std. Error	Sig.(a)
0	1	0.052	0.396	0.896
	2	0.114	0.396	0.773
	4	-0.283	0.396	0.475
	6	-0.509	0.396	0.200
	8	-0.625	0.396	0.115
1	0	-0.052	0.396	0.896
	2	0.062	0.396	0.875
	4	-0.335	0.396	0.398
	6	-0.561	0.396	0.158
	8	-0.677	0.396	0.088
2	0	-0.114	0.396	0.773
	1	-0.062	0.396	0.875
	4	-0.397	0.396	0.316
	6	-0.623	0.396	0.117
	8	-0.739	0.396	0.063
4	0	0.283	0.396	0.475
	1	0.335	0.396	0.398
	2	0.397	0.396	0.316
	6	-0.226	0.396	0.569
	8	-0.342	0.396	0.388
6	0	0.509	0.396	0.200
	1	0.561	0.396	0.158

(I) Week	(J) Week	Mean Difference (I-J) (MPa)	Std. Error	Sig.(a)
6	2	0.623	0.396	0.117
	4	0.226	0.396	0.569
	8	-0.116	0.396	0.769
8	0	0.625	0.396	0.115
	1	0.677	0.396	0.088
	2	0.739	0.396	0.063
	4	0.342	0.396	0.388
	6	0.116	0.396	0.769

a. Adjustment for multiple comparisons: Least Significant Difference (equivalent to no adjustments).

* = Significant difference ($P<0.05$)

The pairwise comparisons of Young's modulus of each week on forehead using split plot design were shown in Table 21. When the Young's modulus on forehead of each week was compared they were not significant difference between week 0 and week 1 to week 8 ($P>0.05$).

Table 22 Analysis of variance for Young's modulus on cheek as a function of treatment and week

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Treatment	Hypothesis	4.408	1	4.408	0.284	0.596
	Error	901.221	58	15.538		
Week	Hypothesis	84.757	5	16.951	5.381	0.000*
	Error	913.555	290	3.150		
treatment * week	Hypothesis	10.816	5	2.163	0.687	0.634
	Error	913.555	290	3.150		
Subject(treatment)	Hypothesis	901.221	58	15.538	4.932	0.000*
	Error	913.555	290	3.150		
week * subject(treatment)	Hypothesis	913.555	290	3.150	.	.
	Error	0.000	0	.(c)		

a. MS(subject(treatment))

b. MS(week * subject(treatment))

c. MS(Error)

* = Significant difference (P<0.05)

The analysis of variance for Young's modulus on cheek as a function of treatment and week were shown in Table 22. The results show that there was not significant interaction between treatment and week on Young's modulus (P=0.634). There was a significant main effect of week and subject on Young's modulus, P<0.05. However, the main effect of treatment was not significant difference (P=0.596). The results show that the Young's modulus on cheek of o/w emulsions containing 1%w/w arabinoxylans was not significant difference from emulsions base after using the products for 8 weeks.

Table 23 Analysis of variance for Young's modulus on forehead as a function of treatment and week

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Treatment	Hypothesis	0.006	1	0.006	0.000	0.986
	Error	1,208.405	58	20.835		
Week	Hypothesis	29.085	5	5.817	3.944	0.002*
	Error	427.765	290	1.475		
treatment * week	Hypothesis	5.050	5	1.010	0.685	0.635
	Error	427.765	290	1.475		
Subject(treatment)	Hypothesis	1,208.405	58	20.835	14.125	0.000*
	Error	427.765	290	1.475		
week * subject(treatment)	Hypothesis	427.765	290	1.475	.	.
	Error	0.000	0	.(c)		

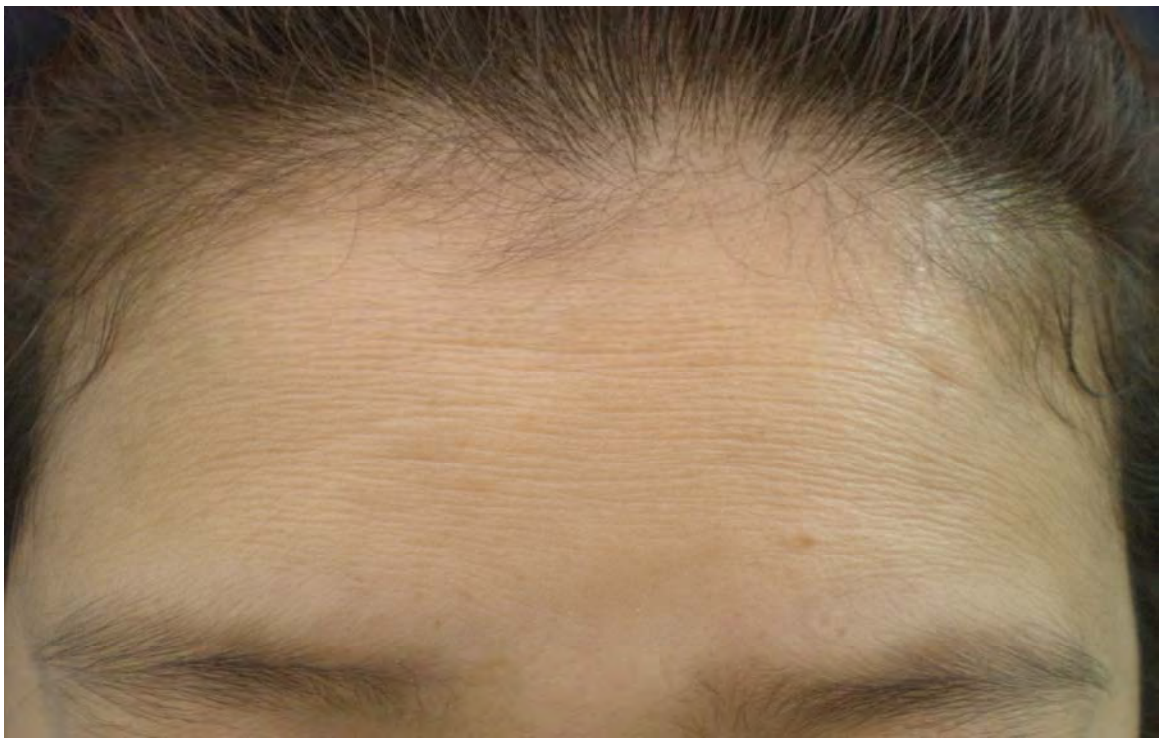
a. MS(subject(treatment))

b. MS(week * subject(treatment))

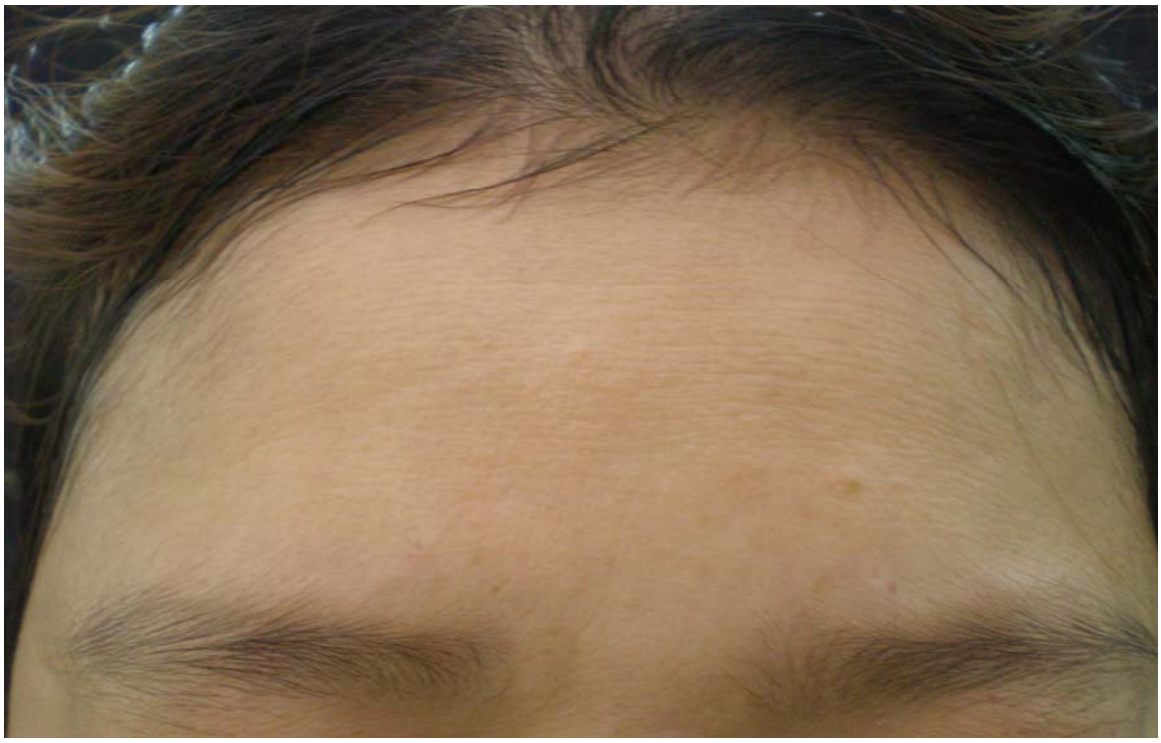
c. MS(Error)

* = Significant difference (P<0.05)

The analysis of variance for Young's modulus on forehead as a function of treatment and week were shown in Table 23. The results show that there was not significant interaction between treatment and week on Young's modulus of forehead (P=0.635). There was a significant main effect of week and subject on Young's modulus, P<0.05. However, the main effect of treatment was not significant difference (P=0.986). The results show that the Young's modulus on forehead of o/w emulsions containing 1%w/w arabinoxylans was not significant difference from emulsions base after using the products for 8 weeks.

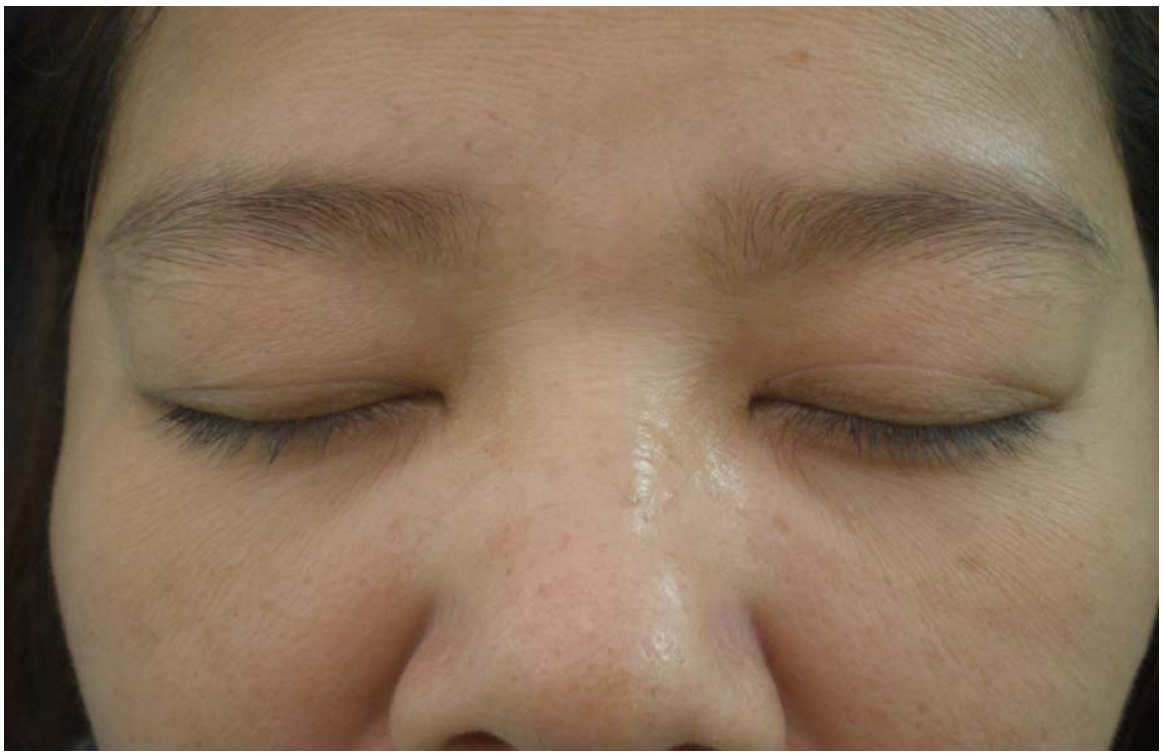


(a)

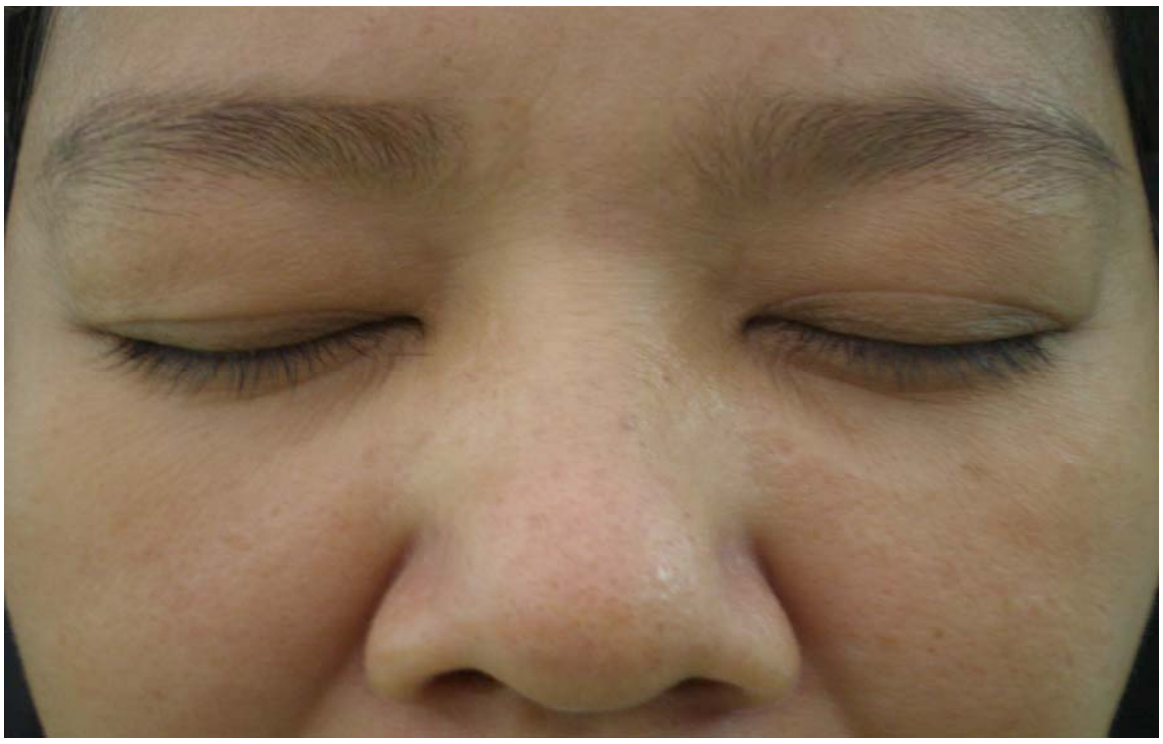


(b)

Figure 18 The macroscopic photograph on forehead of volunteer No.4: (a) before application (week 0) and (b) after application (week 8) of controlled and tested products

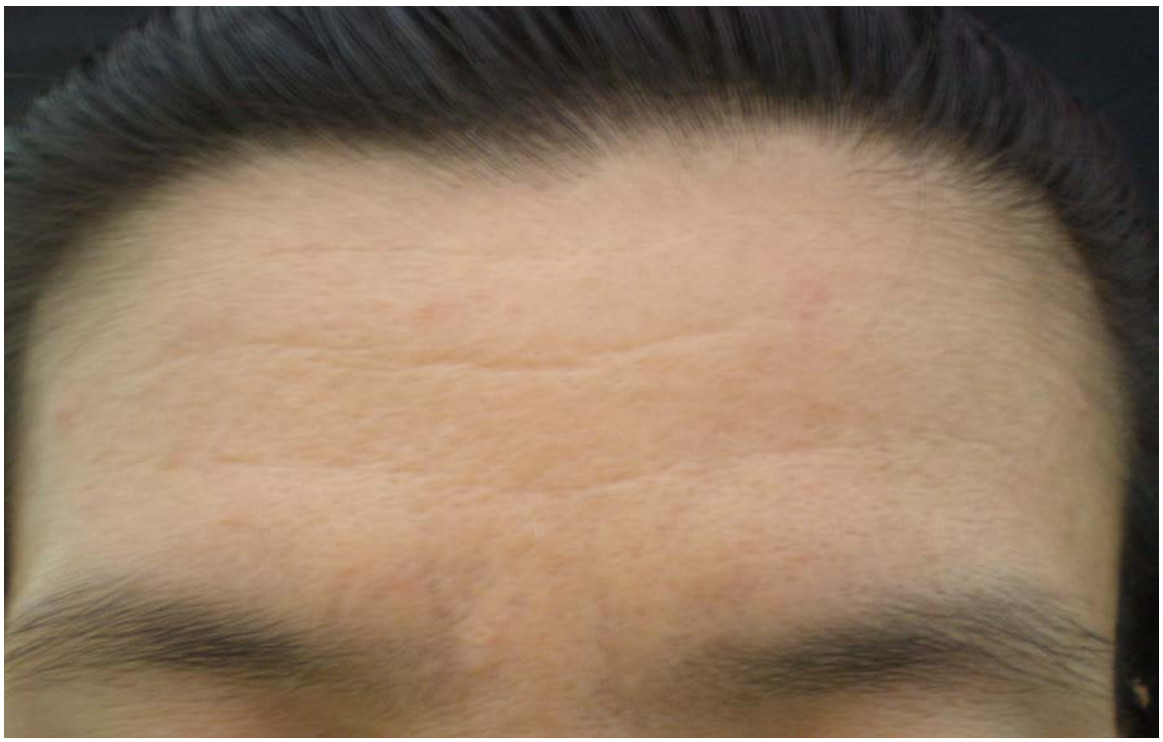


(a)

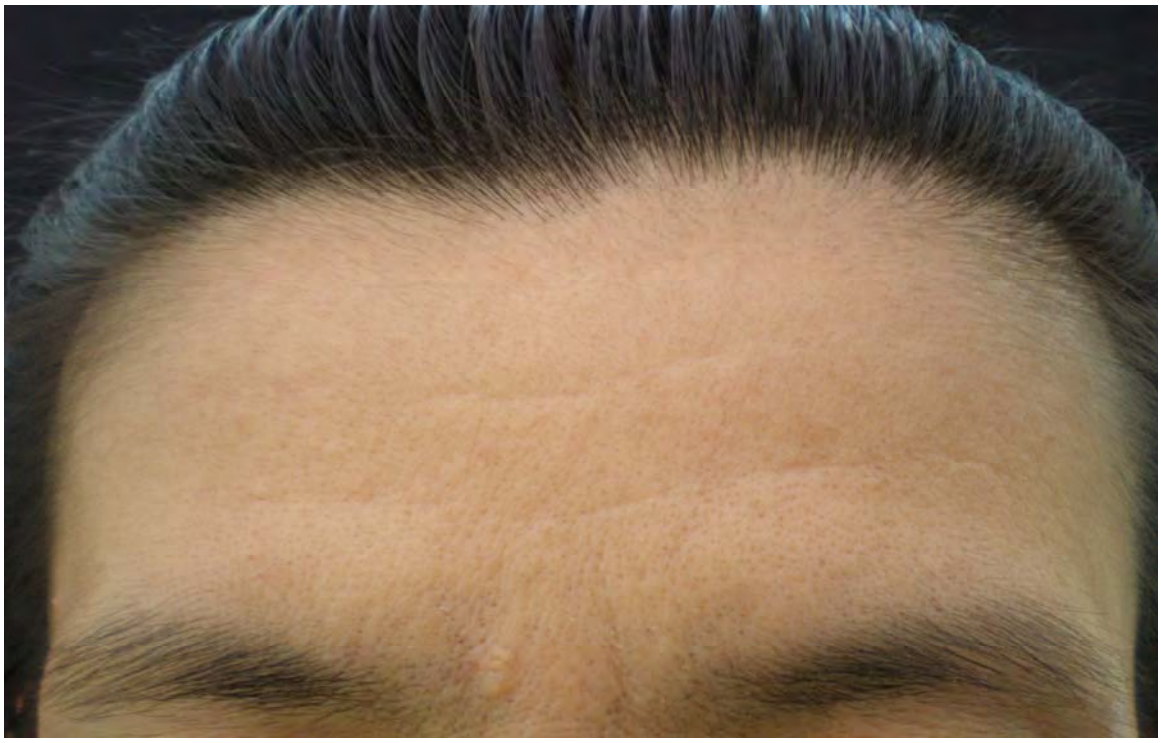


(b)

Figure 19 The macroscopic photograph on cheek of volunteer No.4: (a) before application (week 0) and (b) after application (week 8) of controlled and tested products

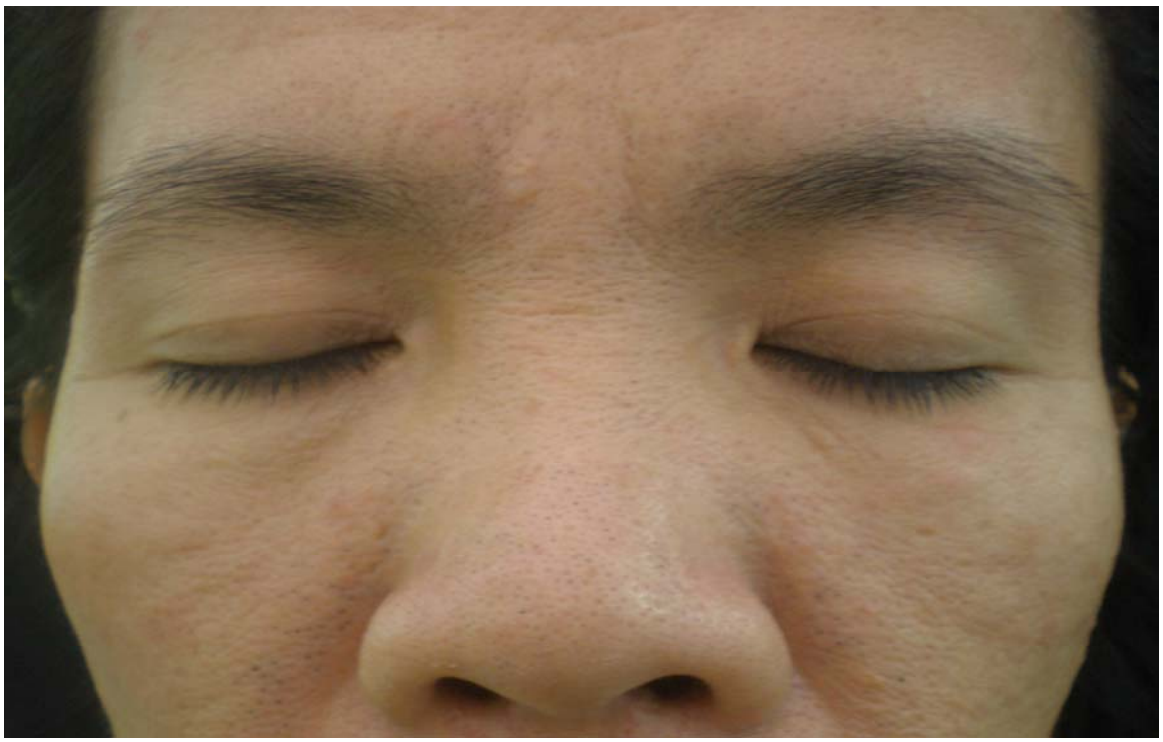


(a)

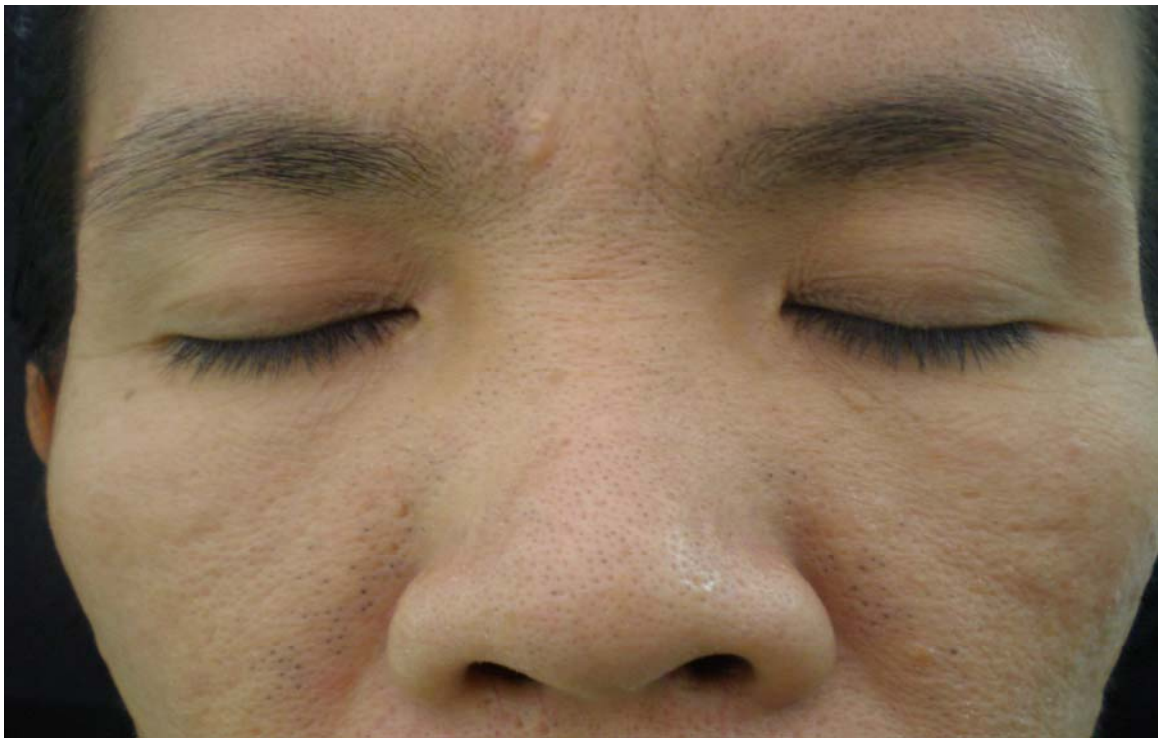


(b)

Figure 20 The macroscopic photograph on forehead of volunteer No.7: (a) before application (week 0) and (b) after application (week 8) of controlled and tested products

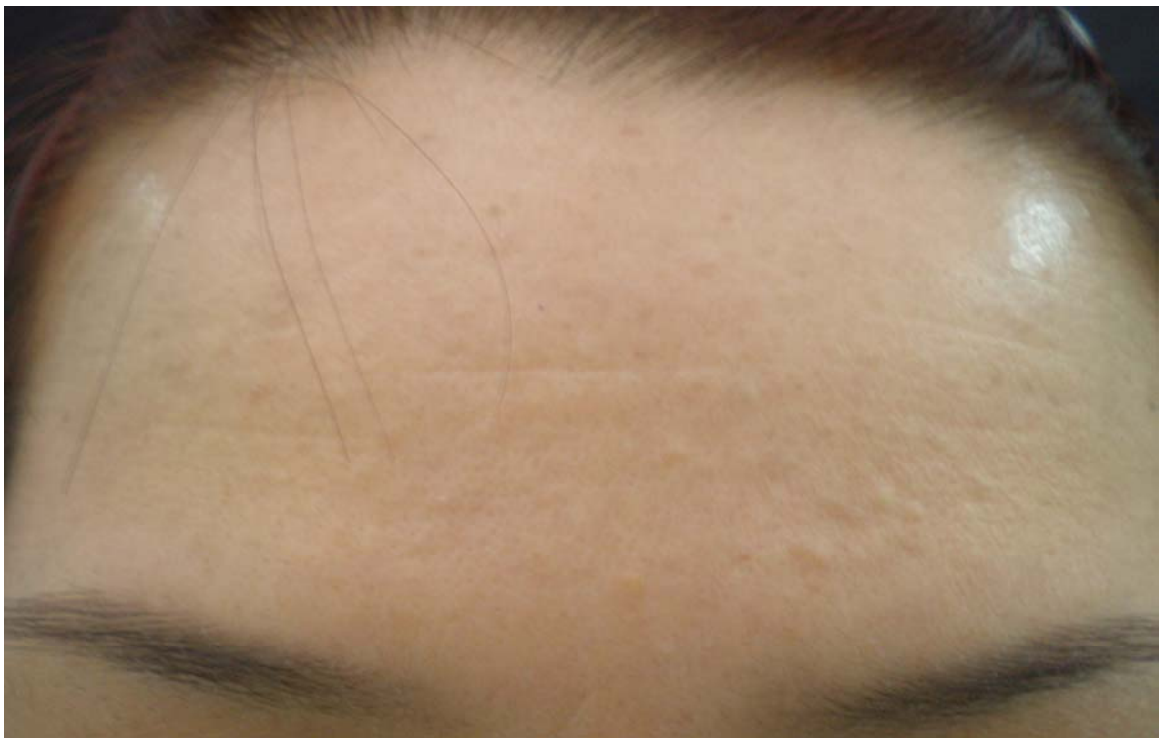


(a)



(b)

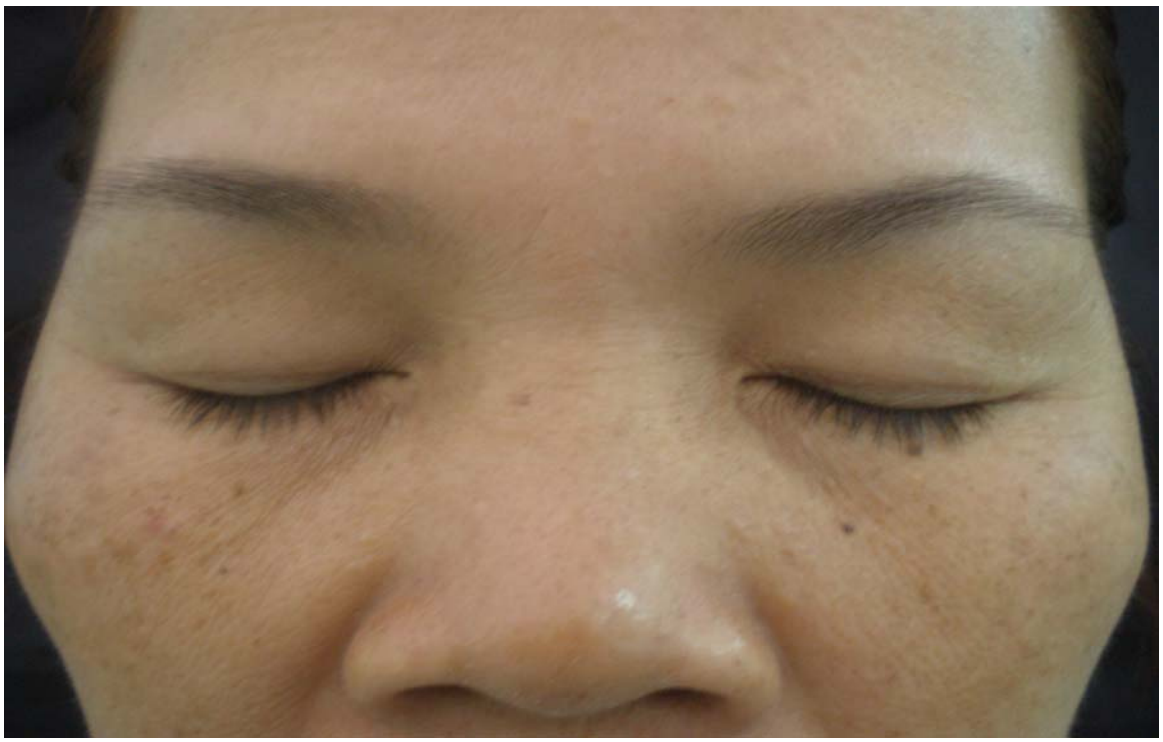
Figure 21 The macroscopic photograph on cheek of volunteer No.7: (a) before application (week 0) and (b) after application (week 8) of controlled and tested products



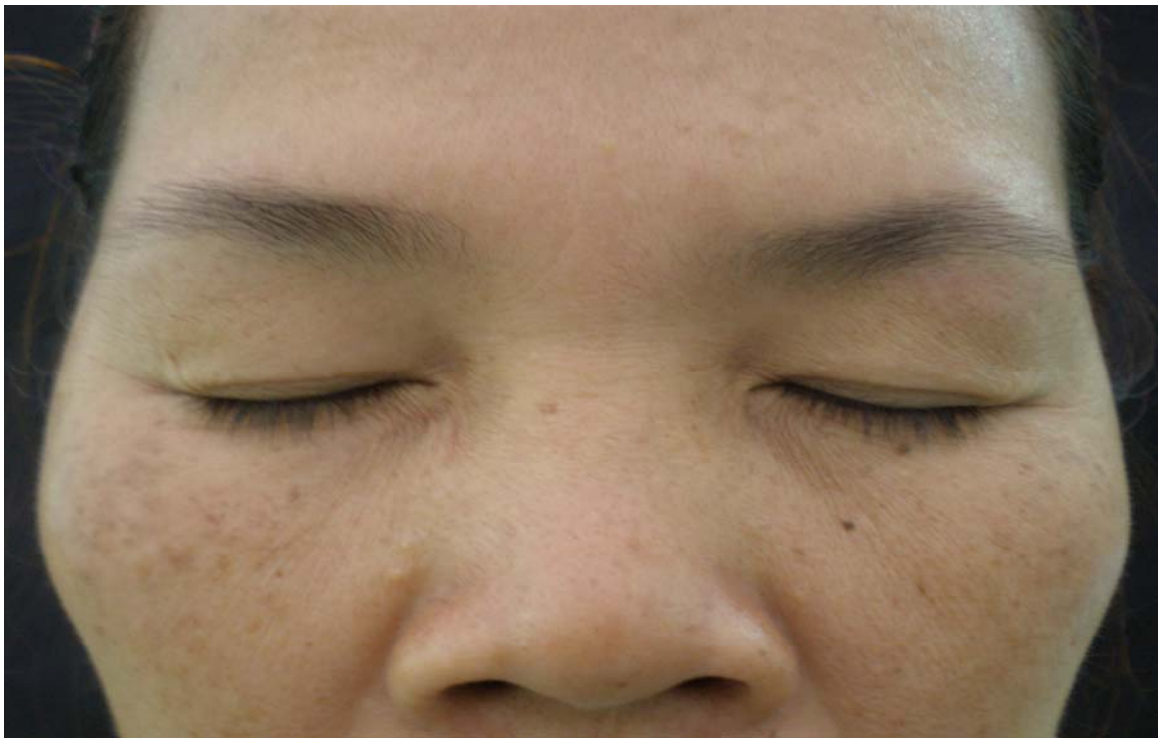
(a)

(b)

Figure 22 The macroscopic photograph on forehead of volunteer No.10: (a) before application (week 0) and (b) after application (week 8) of controlled and tested products

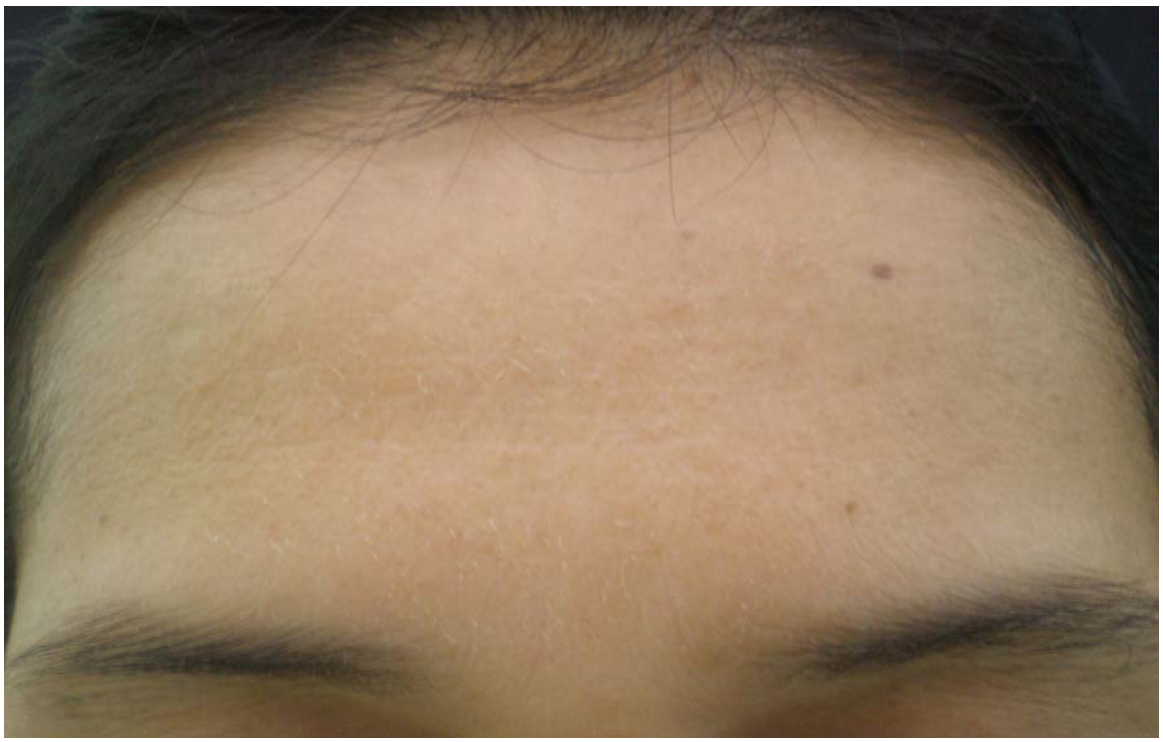


(a)

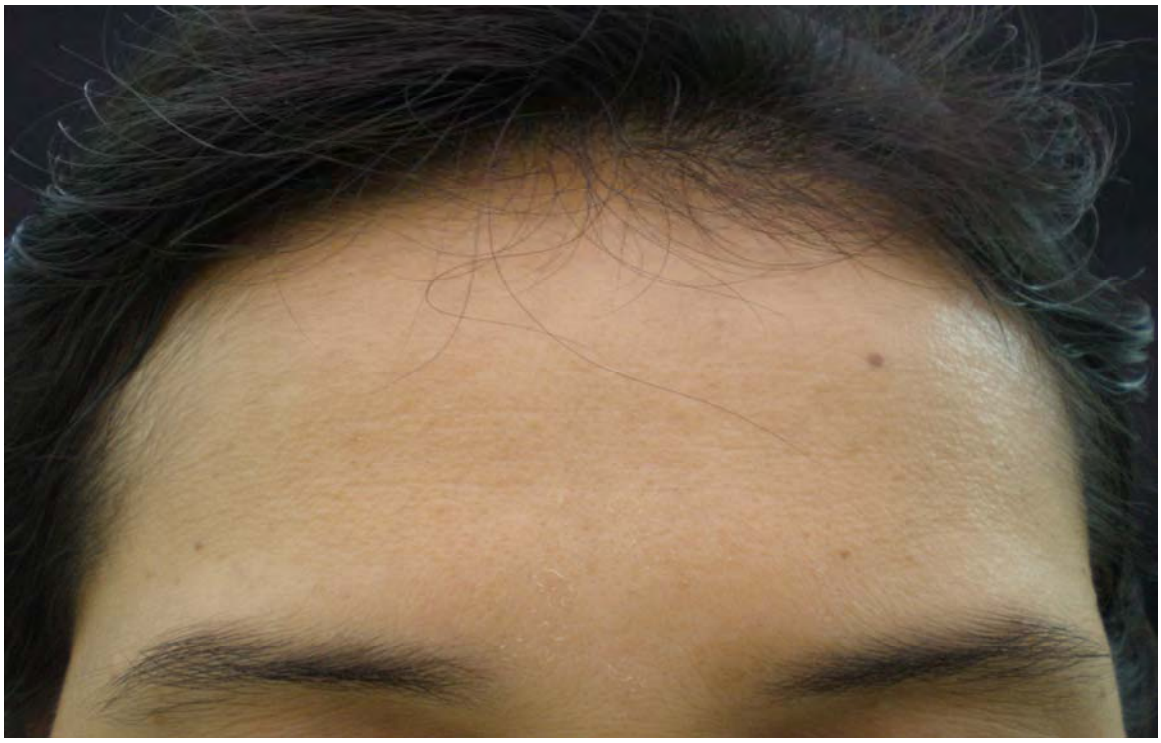


(b)

Figure 23 The macroscopic photograph on cheek of volunteer No.10: (a) before application (week 0) and (b) after application (week 8) of controlled and tested products

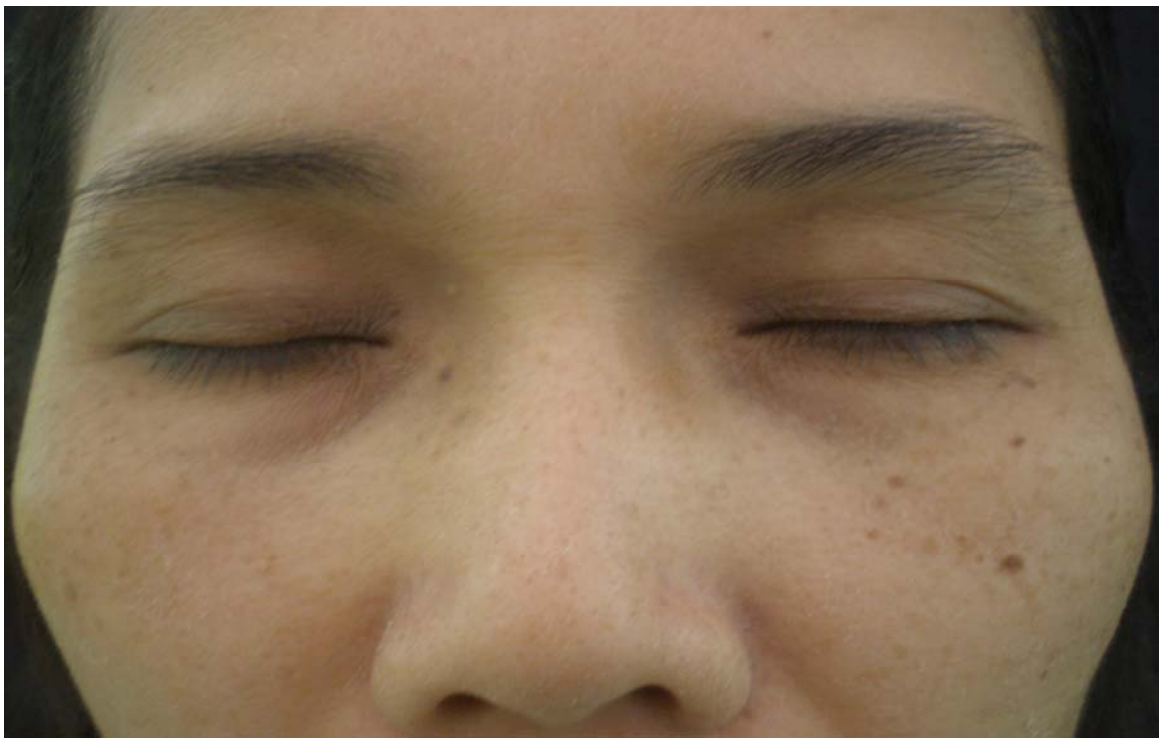


(a)

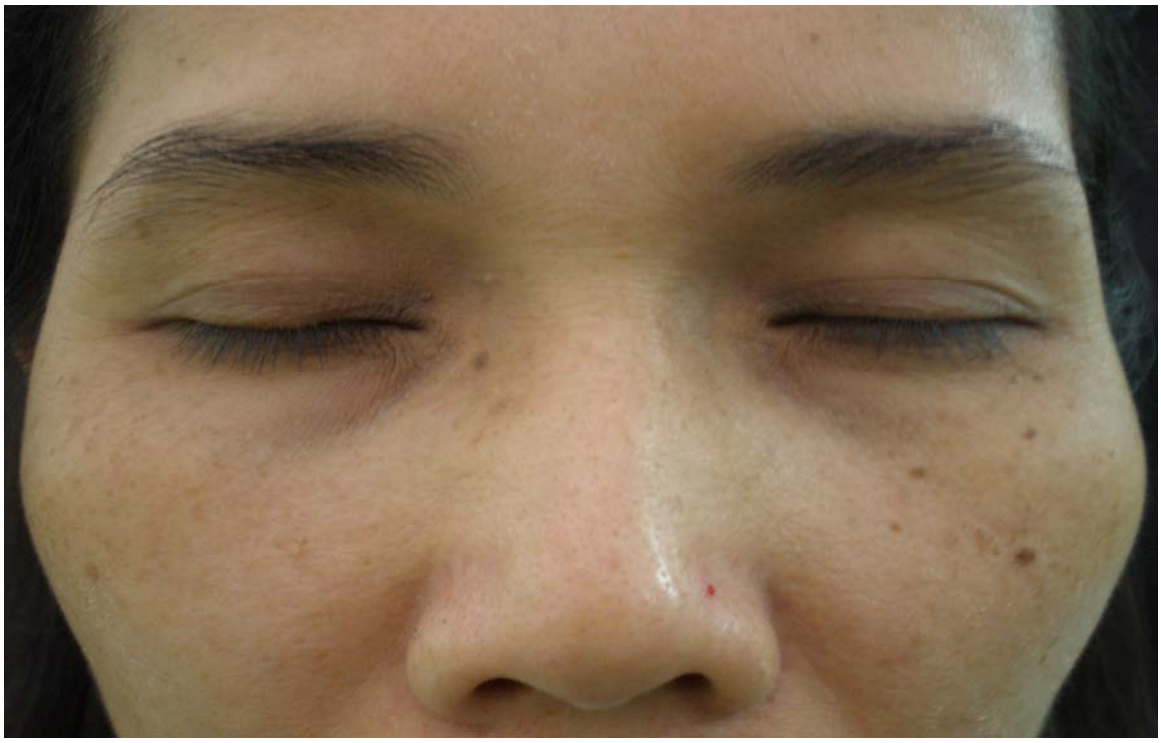


(b)

Figure 24 The macroscopic photograph on forehead of volunteer No.12: (a) before application (week 0) and (b) after application (week 8) of controlled and tested products

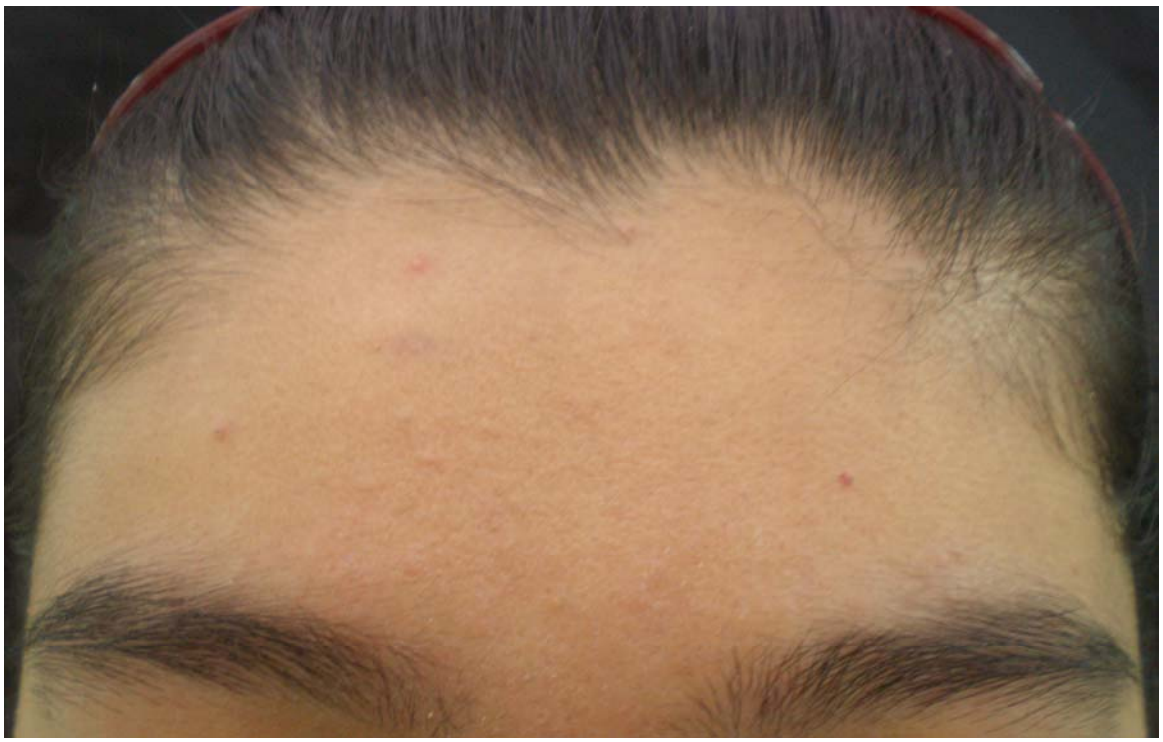


(a)

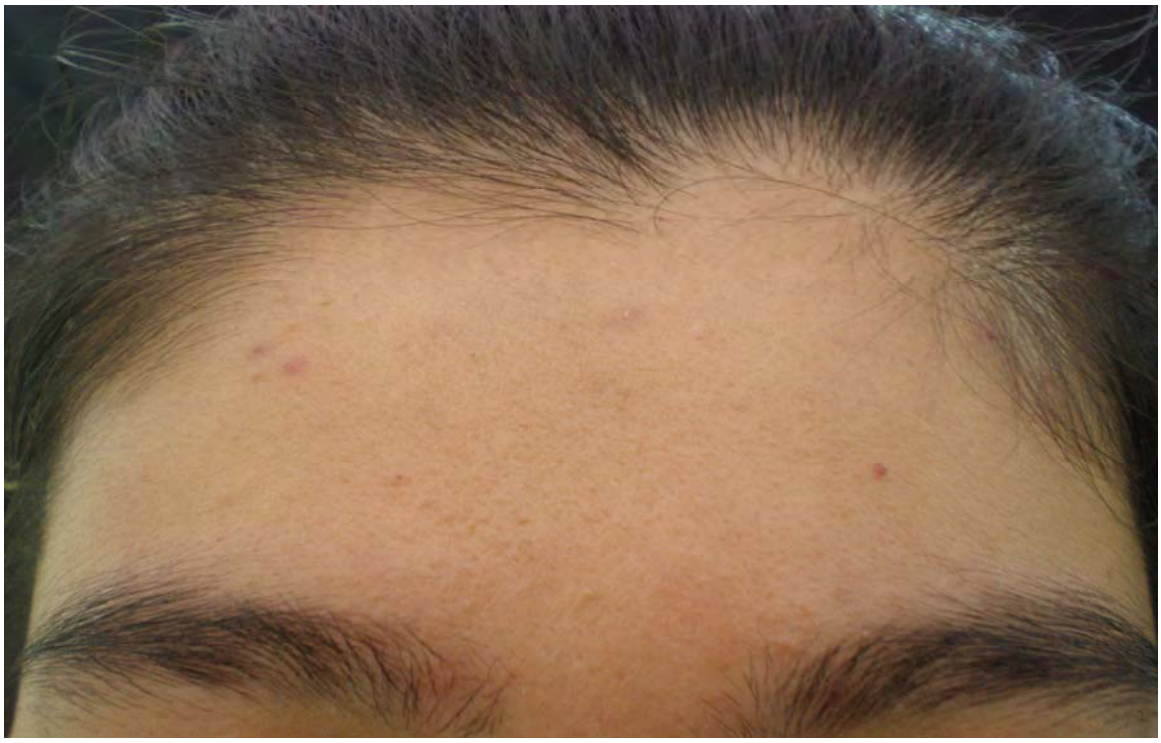


(b)

Figure 25 The macroscopic photograph on cheek of volunteer No.12: (a) before application (week 0) and (b) after application (week 8) of controlled and tested products

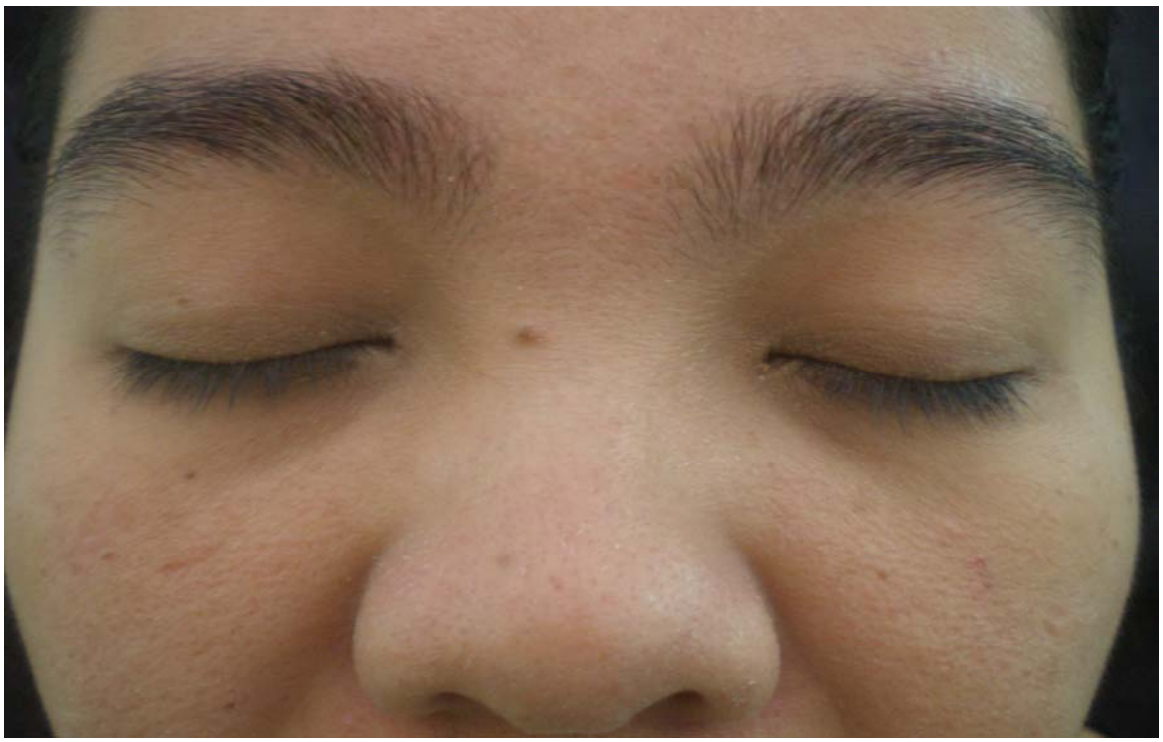


(a)

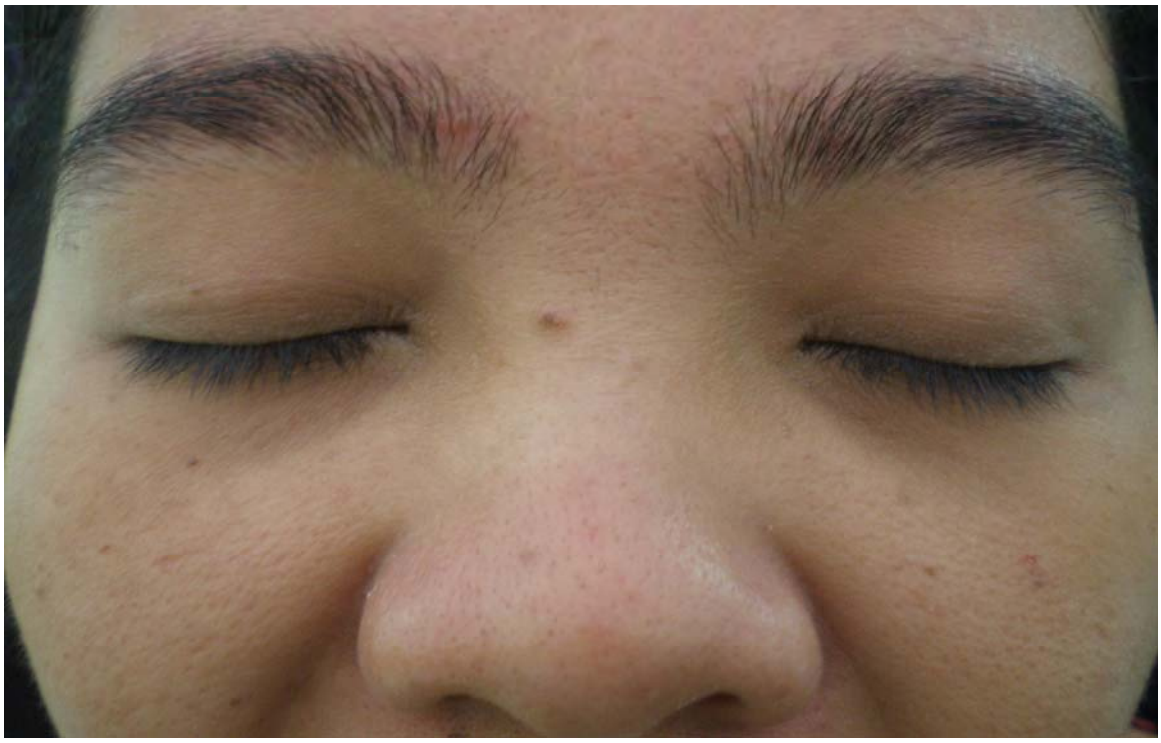


(b)

Figure 26 The macroscopic photograph on forehead of volunteer No.16: (a) before application (week 0) and (b) after application (week 8) of controlled and tested products

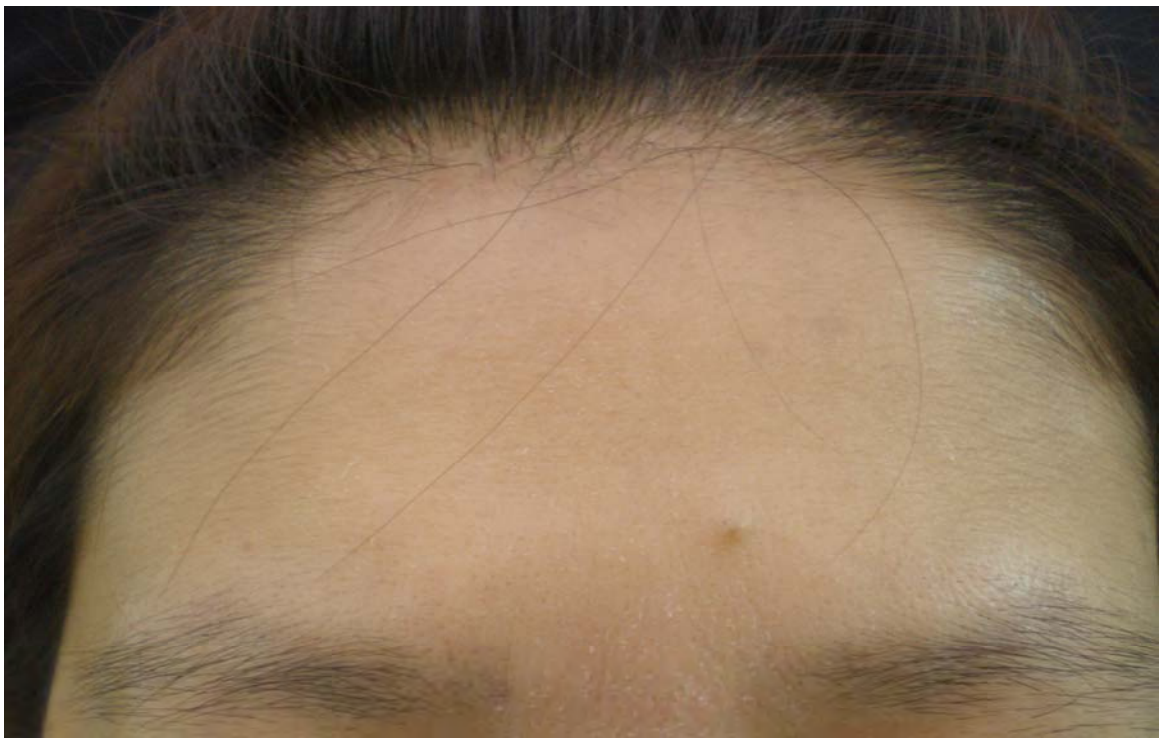


(a)

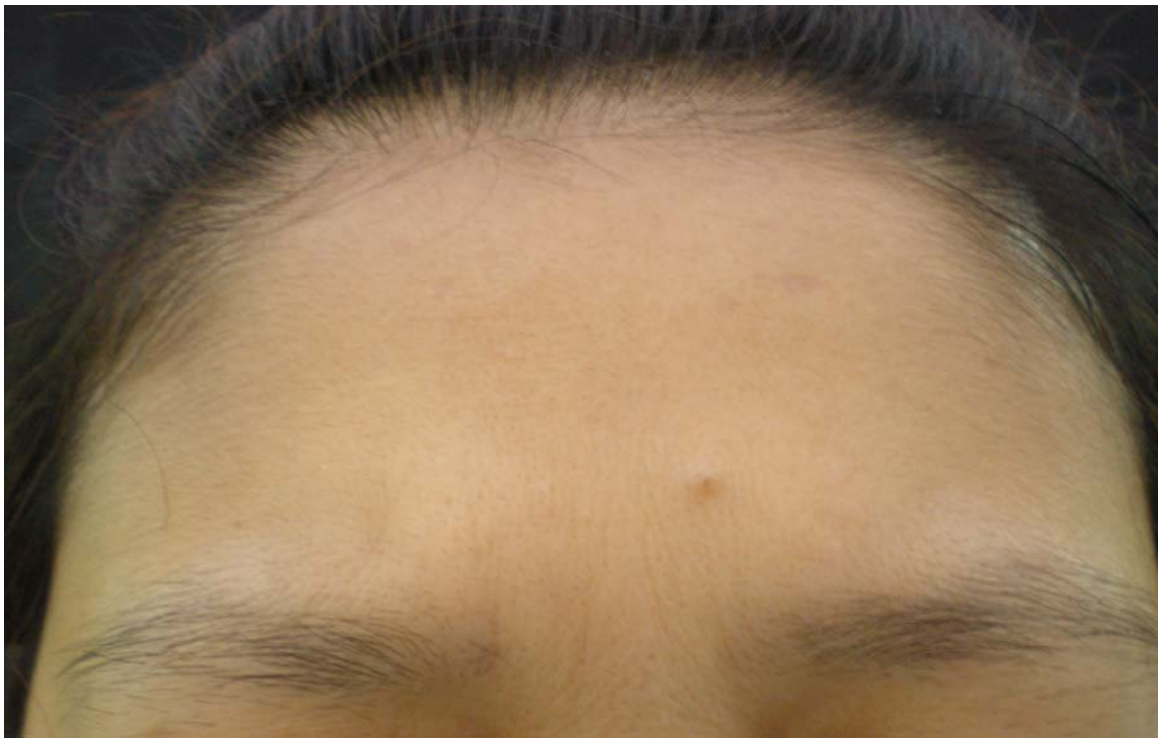


(b)

Figure 27 The macroscopic photograph on cheek of volunteer No.16: (a) before application (week 0) and (b) after application (week 8) of controlled and tested products

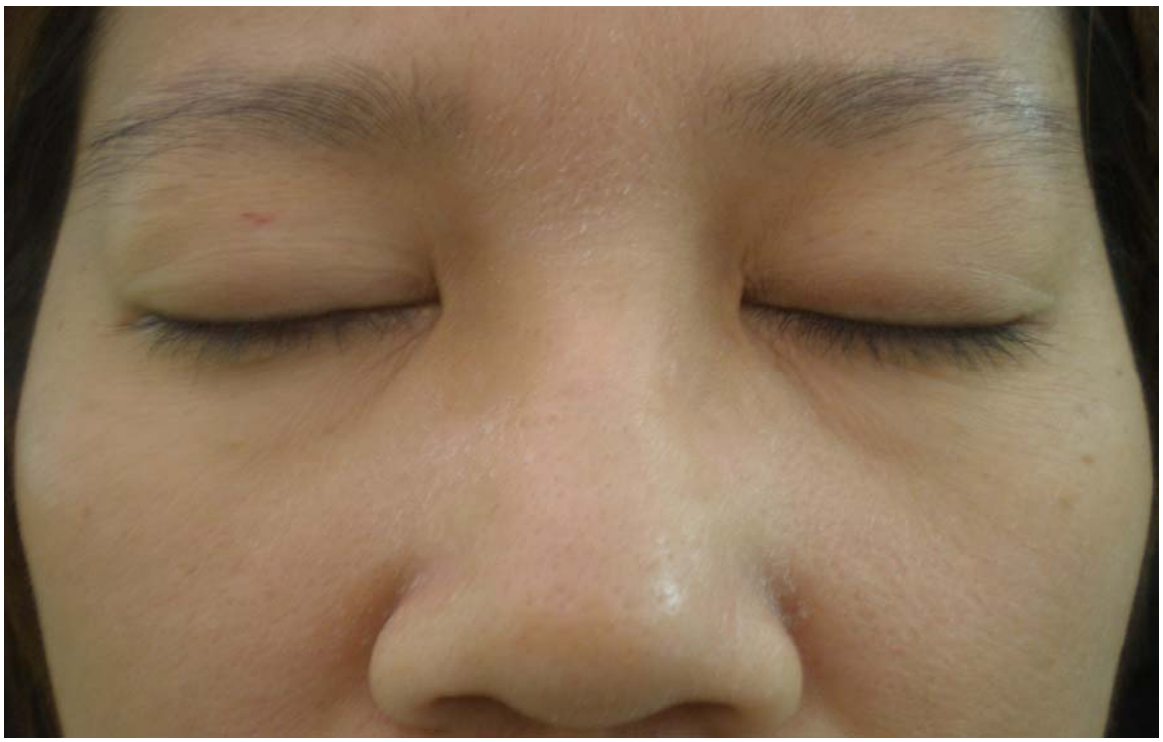


(a)

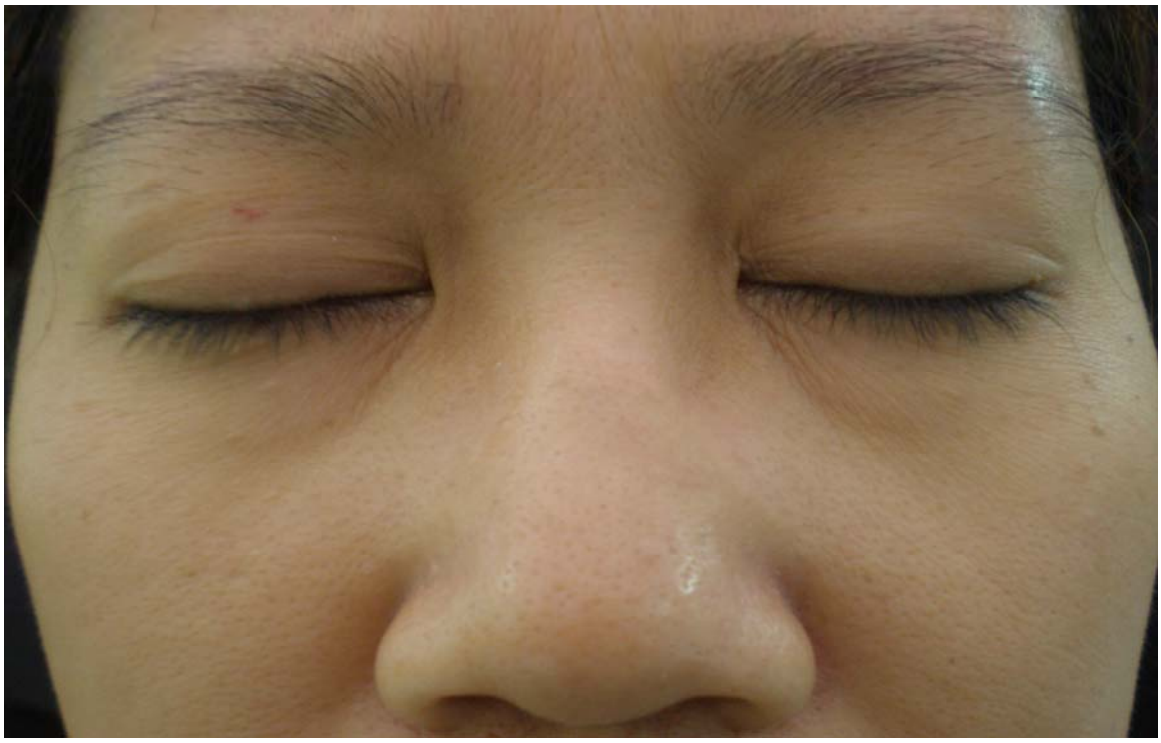


(b)

Figure 28 The macroscopic photograph on forehead of volunteer No.18: (a) before application (week 0) and (b) after application (week 8) of controlled and tested products

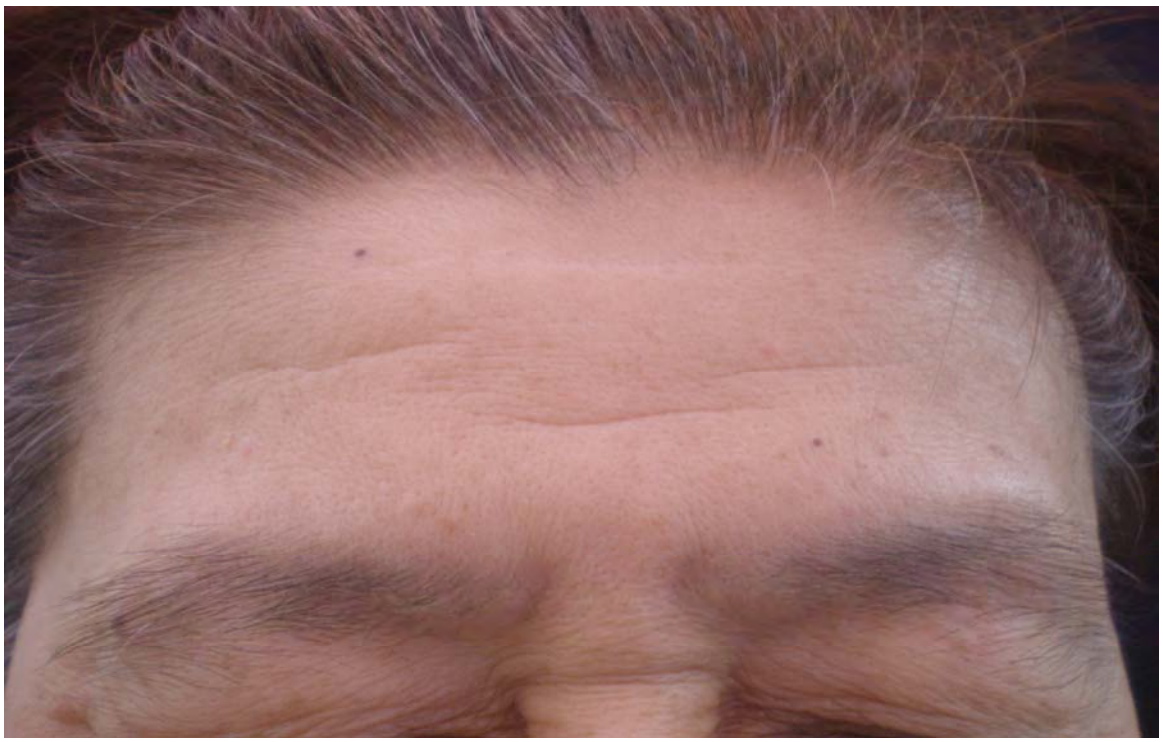


(a)

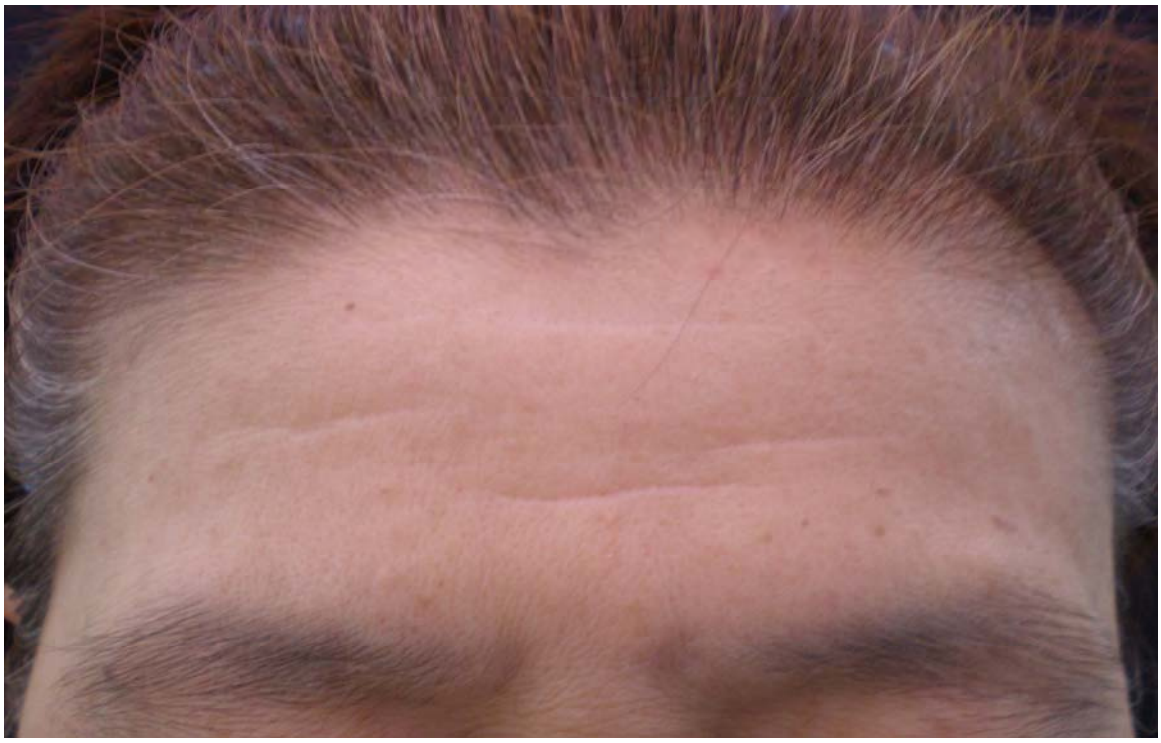


(b)

Figure 29 The macroscopic photograph on cheek of volunteer No.18: (a) before application (week 0) and (b) after application (week 8) of controlled and tested products

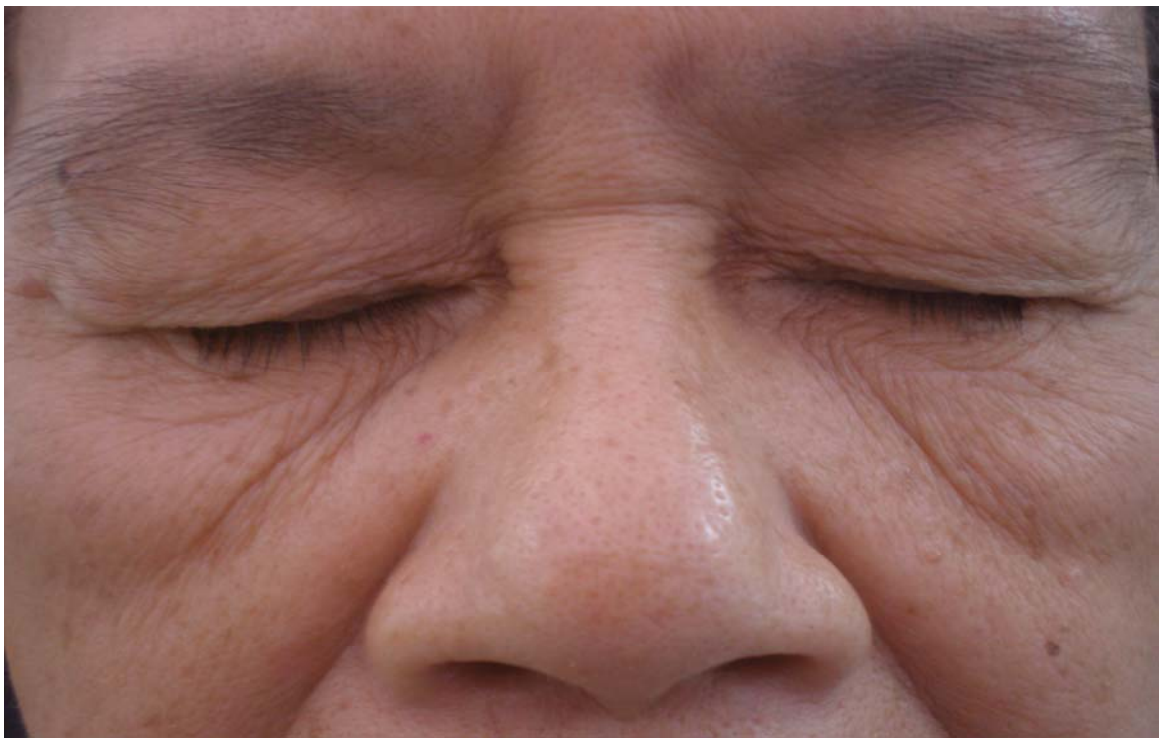


(a)

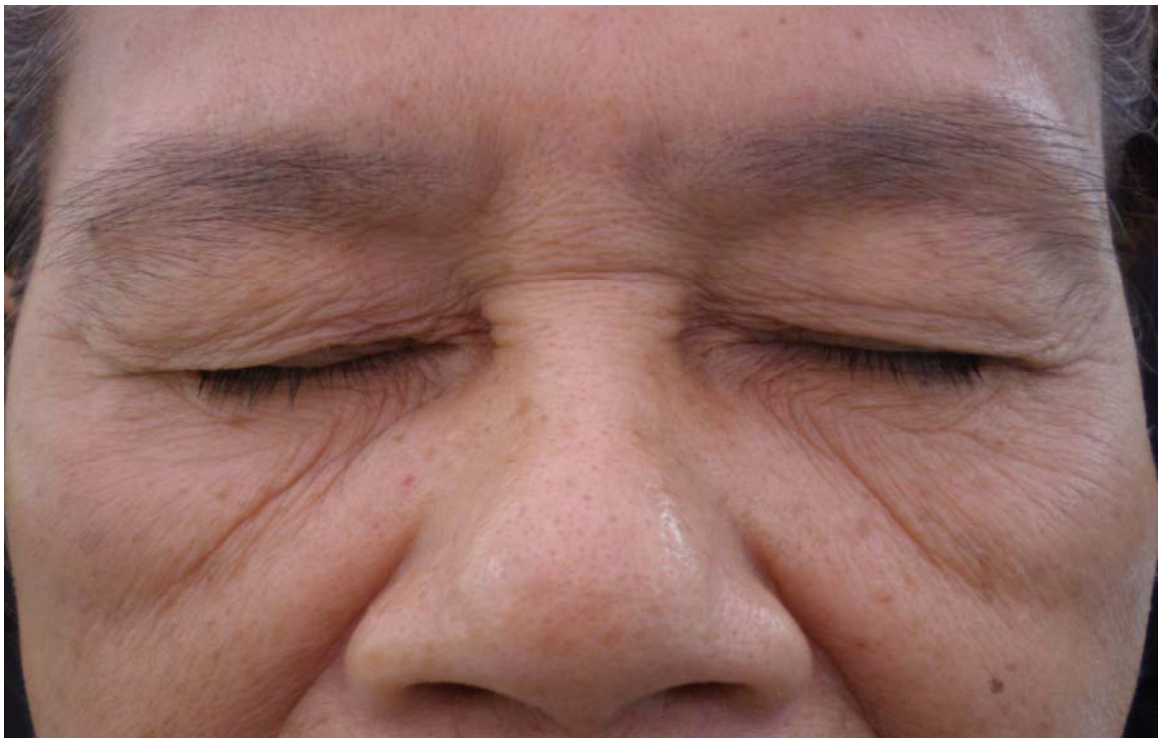


(b)

Figure 30 The macroscopic photograph on forehead of volunteer No.25: (a) before application (week 0) and (b) after application (week 8) of controlled and tested products



(a)



(b)

Figure 31 The macroscopic photograph on cheek of volunteer No.25: (a) before application (week 0) and (b) after application (week 8) of controlled and tested products

CHAPTER V

CONCLUSIONS

Rice bran is a staple diet in Asia. Rice bran is a by-product derived from the outer part of brown rice in milling process. Rice bran is rich of hemicelluloses polysaccharide. One of the main part is arabinoxylans, which have functional properties that were interested in development of cosmetic product. The studies were focused on extraction of arabinoxylans, formulation of oil-in-water emulsions containing arabinoxylans and *in vivo* skin moisturizing and elasticity efficacy determination of oil-in-water emulsions containing arabinoxylans. The results were concluded as following:

Water-extractable arabinoxylans were extracted from rice bran in warm water. The suitable temperature of water bath was 40^{o}C. Arabinoxylans in the supernatant solution was precipitated in aqueous ethanol. After arabinoxylans was separated and freeze dried. It gave yellow light powder and aroma. The yield of water-extractable arabinoxylans was 0.29 %w/w.

Structure of arabinoxylans extract was detected by FT-IR and NMR. FT-IR spectrum of crude arabinoxylan hemicellulose was detected in the frequency range of 450-4000 cm^{-1}. It showed a narrow band at 849 cm^{-1} which was associated to the β–glycosidic linkage between the sugar units. Another main peak appeared at the strong absorption at 3413 cm^{-1} of hydroxyl groups and the CH_2 stretching band was shown at 2854, 2925 cm^{-1} and the CH_2 bending at the 1455 cm^{-1} indicating the presence of alkyl chains.

^{1}H-NMR analysis of this arabinoxylans indicated signal at 5.354, and 5.301, 5.267, corresponding to anomeric protons of α-L-arabinofuranoses substituted at O-3 (mono substituted) and at both O-3 and C-2 (disubtituted) of xylose residues, respectively. Signals obtained at 4.518, 4.502 and 4.485 ppm were due to the anomeric protons of β–D-xyloses substituted at C-2 (disubstituted), C-3 (monosubstitued) and unsubstituted residues, respectively. The signals for other protons of arabinose and xylose were observed in the region of 3.225-4.304 ppm and

were in close proximity with the signals obtained for oligosaccharides generated from water extractable arabinoxylans from rice bran.

Formulation of oil-in-water emulsions containing arabinoxylans were developed by varying the weigh ratio of Brij 72 to Brij 721 1:4, 2:3, 1:1, 3:2, and 4:1. The physical properties, viscosities and pH of these formulations were evaluated. The various ratios of Brij 72 to Brij 721 affected the viscosity of o/w emulsion containing arabinoxylans. The suitable viscosity that gave the most satisfying skin feeling and spreadability were 21827.01 mPa.s in formulation 4.

The pH for o/w emulsions containing arabinoxylans from various ratios of Brij 72 to Brij 721 were not significantly different (P>0.05). The results show pHs of all various ratio of Brij 72 to Brij 721 were approximately in an acceptable range of skin pH 5-5.5.

Differences in physical stability testing in term of viscosity of oil-in-water emulsions before and after heating-cooling cycles were not significant (P>0.05) for all formulations. The results indicated that heating-cooling cycle did not affect viscosity in all various ratios of Brij 72 to Brij 721 of o/w emulsions containing arabinoxylans.

The selected formulation of o/w emulsions containing 1%w/w arabinoxylans consisted of the ratio of oil phase to water phase of 1:9 and the ratio of Brij 72 to Brij 721 of 3:2. It gave most satisfactory with its skin feeling and spreadability.

The *in vivo* skin hydration and elasticity efficacy determination of oil-in-water emulsions containing arabinoxylans were evaluated in the 30 healthy female volunteers. They were recommended to applied oil-in-water emulsions containing arabinoxylans 2 times a day after skin cleansing in the morning and night for 8 weeks. The moisture content and elasticity (Young' modulus value) were measured by Skin Diagnostic SD 27 and Elasticity probe DermaLab® instruments, respectively.

The group of o/w emulsions containing arabinoxylans was significant difference from week 1 to week 8 on LeftC and LeftF. The moisture content of treatment on LeftC, RightC and LeftF was increased significant difference from week 0 to week 1 to week 8. When the results were compared between o/w emulsions containing arabinoxylans and emulsions base, on the RightC the increasing of moisture was less than the leftC as same as LeftF and RightF.

The pairwise comparisons of moisture content of each week on cheek using split plot design, the results show that the moisture content on cheek of each week was significant difference between week 0 and week 1 to week 8. The pairwise comparisons of moisture content of each week on forehead using split plot design the results show that the moisture content on forehead of each week were difference they show significant difference between week 0 and week 2, week 6 and week 8.

The elasticity of the skin was increased due to the elevating its skin tension and firming on cheek which show the significant differences between week 0 to week 1-8 but in RightC the differences were shown only at week 1 and 8. When the Young's modulus on cheek in each week was compared they were significant difference between week 0 and week 1 and week 8. Furthermore Young's modulus in each week on cheek was significant difference between week 1 and week 4.

The moisture values were significantly increased. The Young's modulus values were significantly decreased that resulted in the improvement of elasticity of the skin. When the pairwise comparisons of moisture content and Young's modulus in each week on cheek and forehead using split plot design the results show that they were significant difference. There was a significant main effect of week and subject on moisture content and Young's modulus on cheek and forehead. The results show that the moisture content on cheek of o/w emulsions containing 1%w/w arabinoxylans was not significant difference from emulsions base after using the products for 8 weeks.

Recommendations for further study

- The increasing amount of arabinoxylans should be more effective for moisturizing and elasticity in skin care product.
- The extraction process of arabinoxylans should be modified for increasing amount of arabinoxylans.
- The adding of enhancer in formulations should be enhanced penetration for increasing moisturizer and reducing wrinkle lines.

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APPENDICES

APPENDIX A

Arabinoxylans Extract Powder

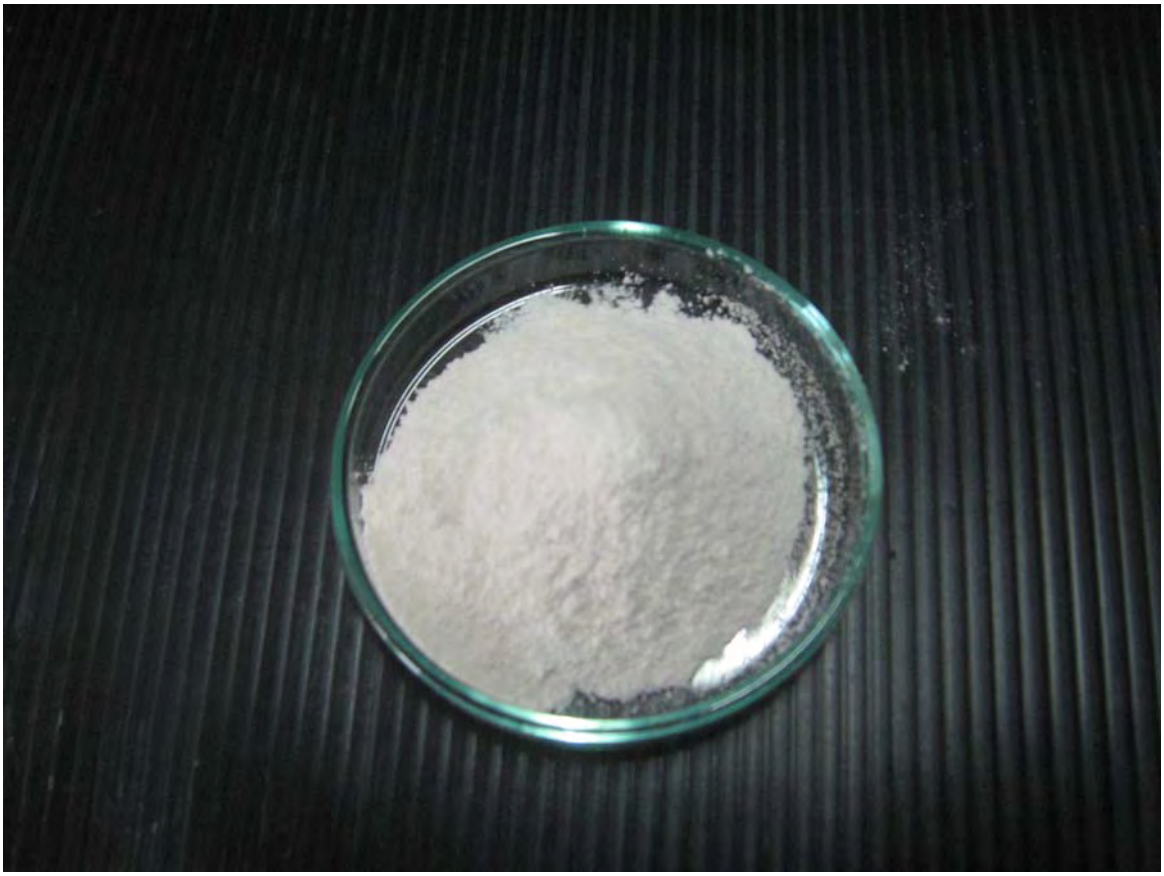

Figure 32 Arabinoxylans extract powder from rice bran

APPENDIX B

Statistical Analysis

Table 24 The Average of Moisture content of left and right cheeks

No.	The Average of Moisture content (%)											
	LeftC (o/w emulsion with arabinoxylans)						RightC (emulsion base)					
	wk0	wk1	wk2	wk4	wk6	wk8	wk0	wk1	wk2	wk4	wk6	wk8
1	14.75	13.50	44.25	37.50	37.25	15.75	4.25	23.00	41.25	18.75	35.00	21.00
2	15.25	5.75	32.50	34.25	25.25	18.00	4.00	10.00	30.25	8.25	15.25	8.50
3	0.00	18.00	29.25	30.25	34.50	23.25	0.00	14.50	12.00	11.75	28.00	16.25
4	6.25	49.00	82.75	51.75	98.50	78.50	8.25	81.00	97.50	21.50	87.00	70.00
5	51.50	51.75	18.50	47.50	41.25	29.00	51.00	55.25	17.00	43.00	41.00	26.00
6	0.00	36.75	37.25	21.25	24.75	10.75	0.00	32.50	2.25	26.25	23.50	30.25
7	6.75	49.25	57.50	46.50	56.50	54.00	14.00	50.50	26.25	39.25	43.75	30.75
8	23.75	13.50	35.50	27.25	43.75	33.50	5.50	7.25	24.75	20.00	40.75	8.50
9	1.00	32.25	46.50	37.50	42.75	63.75	1.25	10.25	41.00	27.25	12.75	25.75
10	36.50	48.50	23.00	28.75	48.50	55.75	14.75	35.75	38.75	32.00	44.25	58.25
11	0.00	6.00	42.25	24.50	42.50	12.25	0.00	4.00	28.00	0.25	40.25	10.75
12	1.00	31.50	31.50	36.50	28.00	25.25	3.25	21.25	15.00	13.50	21.00	11.00
13	8.00	25.75	60.25	48.50	49.00	52.50	6.00	21.25	52.50	48.75	22.25	58.00
14	32.75	28.75	41.50	40.50	43.50	31.50	35.50	10.50	21.00	23.25	45.00	34.25
15	0.00	23.75	1.75	25.25	28.25	26.00	0.00	8.50	0.00	13.25	18.50	8.75
16	14.75	37.00	0.25	24.25	33.75	42.00	0.75	31.00	2.50	27.00	27.50	31.00
17	0.00	17.25	57.25	24.50	25.75	15.00	0.00	26.00	69.00	29.00	17.75	25.25
18	1.25	40.00	18.50	47.00	39.00	28.50	0.00	32.50	12.75	36.00	37.25	18.50
19	4.50	5.00	32.75	37.00	25.75	34.25	0.00	2.50	40.50	30.25	29.75	66.00
20	1.00	20.00	8.00	29.00	34.25	20.50	1.00	36.00	29.25	21.25	37.00	25.25
21	11.25	33.00	70.25	43.75	44.00	42.00	6.25	41.00	70.75	48.75	47.75	56.75
22	24.00	55.25	21.75	28.75	30.50	12.50	8.50	46.50	22.50	25.50	45.50	19.50
23	25.50	38.50	10.75	20.75	32.75	17.75	33.00	40.75	31.25	23.25	34.25	24.00
24	30.75	42.00	42.00	56.50	16.25	31.75	38.75	55.50	55.75	53.50	13.75	37.00
25	6.50	13.00	4.50	24.75	15.75	5.50	11.75	4.00	16.50	9.75	23.00	9.75
26	43.00	20.50	14.25	41.75	15.25	35.25	45.00	27.25	25.75	44.00	39.50	33.50
27	26.25	29.00	24.25	22.25	25.00	7.75	27.75	20.00	35.50	15.50	22.50	18.50
28	28.34	33.75	5.25	29.75	28.50	5.25	35.00	34.50	18.50	37.50	26.25	10.25
29	34.00	33.25	15.75	28.75	25.75	32.50	23.75	35.75	54.00	20.75	19.50	39.00
30	57.75	83.00	24.00	59.50	25.00	42.25	84.50	77.25	12.25	56.00	22.50	48.75

Table 25 The Average of Moisture content of left and right foreheads

No.	The Average of Moisture content (%)											
	LeftF (o/w emulsion with arabinoxylans)						RightF (emulsion base)					
	wk0	wk1	wk2	wk4	wk6	wk8	wk0	wk1	wk2	wk4	wk6	wk8
1	10.50	32.75	45.00	47.25	42.00	29.50	15.00	24.75	18.25	30.75	20.25	11.00
2	24.50	15.25	20.00	20.50	26.25	24.75	17.00	7.50	9.00	13.75	23.50	15.00
3	11.00	21.25	20.00	29.75	26.75	35.75	11.00	14.00	5.00	2.25	31.25	29.50
4	24.75	40.00	99.00	53.75	97.00	54.25	21.25	59.50	99.00	31.75	97.75	47.25
5	50.75	61.50	45.00	40.50	33.00	45.50	59.50	55.25	49.75	43.25	28.75	26.50
6	5.00	42.25	35.50	38.00	28.25	37.00	7.75	32.25	28.50	24.75	26.75	34.00
7	29.75	63.00	68.50	54.75	53.50	56.00	29.25	49.25	59.50	35.00	43.25	33.25
8	30.25	34.00	38.50	37.25	53.75	43.50	22.50	7.00	32.00	28.50	38.25	47.50
9	18.25	35.50	33.00	40.50	51.75	53.75	35.75	32.25	16.50	30.00	28.75	28.75
10	21.50	33.25	48.75	35.75	22.00	44.25	25.75	61.25	37.75	21.50	24.00	28.00
11	0.00	14.00	25.25	17.50	49.25	23.75	6.00	2.25	9.50	7.75	35.00	52.00
12	24.75	32.75	42.75	31.25	29.25	12.00	27.75	29.25	42.50	41.25	37.75	30.00
13	13.75	52.50	61.25	49.25	43.00	95.00	20.00	42.75	59.00	58.00	39.50	93.75
14	53.75	24.25	72.00	48.75	68.50	47.25	54.50	31.25	45.50	43.75	60.50	43.75
15	4.25	15.25	9.75	24.50	31.50	36.50	12.00	22.00	5.75	28.25	26.75	42.25
16	58.75	42.25	36.50	42.50	62.75	88.00	43.25	43.75	44.50	40.25	51.75	81.75
17	3.75	6.75	44.00	13.00	6.50	19.50	2.00	15.25	51.50	14.25	6.00	19.50
18	11.75	11.50	10.75	13.25	8.75	4.50	16.00	26.00	18.75	27.75	35.00	24.75
19	29.25	8.00	22.25	27.25	21.50	55.00	31.50	10.00	43.75	40.25	42.25	72.00
20	23.25	29.25	32.75	40.25	33.00	56.75	22.00	43.00	44.25	39.00	27.00	48.50
21	39.00	59.75	86.00	50.75	91.25	58.50	44.50	57.50	81.75	55.50	69.25	71.25
22	21.50	28.75	37.00	30.50	27.00	29.00	15.00	53.25	47.25	46.75	44.00	36.25
23	49.00	35.25	31.25	34.50	32.00	35.75	62.75	34.75	41.50	41.00	46.00	35.75
24	47.25	49.00	55.50	60.50	46.50	62.50	52.00	64.75	73.50	61.50	34.75	57.25
25	44.00	38.50	33.50	34.25	32.00	36.75	33.00	33.00	46.25	34.50	42.00	30.00
26	35.00	19.75	6.50	35.50	8.00	24.75	65.00	51.00	38.50	42.50	27.25	26.25
27	25.75	48.00	17.00	16.75	14.50	4.50	38.50	30.50	46.00	27.50	38.00	12.00
28	5.50	7.50	15.50	25.25	17.25	21.00	16.00	15.25	13.00	33.00	17.25	22.50
29	19.00	25.00	5.75	8.75	6.50	39.25	24.75	21.25	9.00	35.75	27.75	44.25
30	99.00	99.00	99.00	99.00	96.50	94.00	99.00	99.00	78.75	97.75	86.25	73.50

Table 26 The Average of Young's modulus of left and right cheeks

No.	The Average of Young's Modulus (MPa)											
	LeftC (o/w emulsion with arabinoxylans)						RightC (emulsion base)					
	wk0	wk1	wk2	wk4	wk6	wk8	wk0	wk1	wk2	wk4	wk6	wk8
1	7.18	8.18	7.28	10.52	9.52	7.84	8.06	9.81	9.33	10.77	7.59	8.25
2	7.84	8.82	7.20	8.23	8.30	6.54	7.52	7.03	7.47	7.16	8.17	6.50
3	9.71	5.84	6.90	8.12	8.27	5.79	8.46	8.24	5.78	8.75	7.51	7.26
4	10.99	8.74	6.03	9.36	8.69	9.33	11.66	7.11	9.35	7.64	8.65	9.31
5	8.08	6.90	6.33	8.19	6.70	6.67	7.54	5.42	7.30	7.89	8.24	6.27
6	5.67	5.38	8.09	8.68	6.08	7.00	11.41	6.31	6.97	8.47	8.63	8.18
7	4.25	2.77	2.32	4.81	3.12	4.73	4.60	4.89	2.36	4.07	2.08	3.73
8	7.40	7.53	8.11	8.33	7.86	6.61	7.57	5.69	8.87	8.39	6.55	7.83
9	10.84	8.32	7.32	9.99	10.02	9.47	10.97	6.80	9.12	9.93	9.47	8.23
10	6.83	3.97	5.14	7.63	8.76	5.02	6.03	4.63	29.50	4.51	8.00	3.87
11	6.31	7.97	5.87	7.37	6.77	9.39	7.66	7.02	6.66	8.33	8.45	7.48
12	6.90	4.09	6.17	6.45	9.70	4.01	9.36	5.41	5.43	6.70	7.45	5.45
13	5.68	5.40	4.67	3.92	5.22	4.23	5.33	7.42	3.38	5.25	7.59	3.45
14	6.73	4.61	7.19	8.10	5.04	6.24	7.75	6.08	5.56	7.92	8.08	7.58
15	4.46	4.05	4.98	6.82	4.01	5.35	6.67	5.66	6.55	7.80	6.07	7.71
16	8.44	6.64	6.49	8.46	4.96	5.73	7.75	5.41	6.50	7.44	6.10	6.71
17	5.27	6.54	5.94	6.39	6.55	3.77	4.89	4.87	7.19	7.07	6.86	5.15
18	6.52	5.97	5.40	6.03	4.54	4.49	6.29	5.61	4.75	4.51	5.29	4.54
19	7.91	8.69	5.35	9.61	8.26	10.14	7.08	6.46	6.97	10.77	9.93	11.80
20	7.22	4.80	5.88	7.99	6.92	7.14	5.89	4.34	4.25	5.88	5.35	6.57
21	8.14	6.37	9.95	7.84	6.95	4.42	5.44	7.00	7.89	5.82	3.06	5.28
22	3.69	3.26	2.47	2.54	2.81	2.68	3.06	2.14	2.06	2.25	1.59	1.37
23	7.99	6.80	8.63	11.91	9.69	7.06	10.37	6.91	6.79	9.64	9.61	7.19
24	9.41	6.77	8.57	8.41	6.77	5.99	9.32	8.60	8.82	9.55	8.33	6.87
25	9.14	8.50	8.73	11.81	8.22	7.86	8.31	8.51	6.26	10.28	7.66	8.08
26	7.26	8.12	9.72	9.75	8.40	8.67	7.24	7.32	7.66	10.07	7.38	9.75
27	6.47	6.50	8.22	5.47	5.69	4.90	5.36	6.68	6.66	4.89	5.75	4.84
28	6.40	4.71	5.00	8.62	7.84	6.57	6.60	5.05	5.78	7.35	7.09	7.50
29	8.85	6.08	6.52	7.38	6.77	6.09	7.39	7.89	8.04	8.44	7.86	6.61
30	8.56	2.28	6.15	9.55	5.19	8.52	9.83	8.23	6.55	8.67	7.84	9.81

Table 27 The Average of Young's modulus of left and right foreheads

No.	The Average of Young's Modulus (MPa)											
	LeftF (o/w emulsion with arabinoxylans)						RightF (emulsion base)					
	wk0	wk1	wk2	wk4	wk6	wk8	wk0	wk1	wk2	wk4	wk6	wk8
1	9.85	10.24	9.23	9.81	8.65	6.41	8.57	8.98	9.29	7.45	6.97	7.27
2	13.31	11.50	11.00	12.88	12.67	12.13	14.20	14.70	12.26	14.80	13.57	11.24
3	5.42	7.84	7.34	9.13	8.32	9.52	6.60	9.84	9.85	8.74	9.21	10.60
4	9.18	11.31	10.60	9.62	12.01	10.95	9.19	10.80	11.75	10.18	12.05	11.66
5	8.78	7.28	8.72	8.18	10.70	10.38	12.40	10.71	11.62	12.65	13.31	12.81
6	10.98	9.33	8.74	10.56	8.60	9.37	10.39	10.45	11.08	12.44	9.62	11.99
7	9.12	7.34	8.01	6.28	9.20	8.66	9.39	7.88	7.42	6.18	9.28	7.07
8	9.03	10.86	10.74	10.35	9.94	12.07	9.26	12.03	11.54	11.95	12.67	12.72
9	10.84	12.15	12.86	11.65	11.08	11.92	10.78	12.49	13.29	11.32	12.67	12.65
10	11.72	11.32	11.46	10.66	10.23	10.06	9.05	11.57	11.25	12.50	12.12	8.05
11	11.58	11.99	9.41	12.35	13.65	10.52	9.28	12.08	8.15	13.02	12.20	12.29
12	7.02	6.63	7.88	7.52	4.09	9.49	10.59	10.95	9.58	9.38	4.30	7.92
13	6.81	10.92	10.30	8.64	10.09	11.51	7.31	9.90	9.02	9.25	5.78	10.88
14	11.26	9.77	10.77	10.63	11.58	12.11	8.17	8.55	7.96	6.65	9.25	8.52
15	7.23	6.47	6.53	8.64	7.26	7.24	2.99	4.96	6.26	4.59	5.21	5.40
16	9.05	9.11	9.30	9.84	9.49	10.14	10.34	7.84	8.33	8.20	9.59	8.98
17	9.07	8.67	9.76	11.78	10.30	10.01	7.20	9.71	9.98	7.65	9.38	9.91
18	9.09	10.22	8.34	8.56	10.32	9.02	10.59	9.32	10.00	9.39	11.71	9.85
19	10.63	10.79	11.82	11.53	9.99	12.70	10.25	12.61	10.70	11.11	7.92	12.02
20	8.64	3.38	6.23	4.57	5.38	6.35	7.39	7.72	6.99	7.71	9.07	7.89
21	11.71	10.96	11.04	11.81	12.72	12.16	11.68	8.72	8.79	12.26	9.84	9.28
22	12.07	11.35	11.08	9.30	12.24	11.15	10.91	9.91	10.30	9.59	12.62	12.12
23	12.79	10.87	12.31	12.55	13.11	13.57	11.79	10.24	9.32	12.74	13.10	12.33
24	11.28	10.80	11.31	10.79	11.14	11.85	11.00	10.48	11.10	10.36	12.34	11.11
25	11.42	10.87	10.63	13.23	13.18	12.89	13.49	12.67	9.03	13.60	13.22	13.50
26	11.69	13.35	12.08	13.93	13.51	13.66	11.38	11.55	10.93	11.63	11.15	12.45
27	13.15	12.38	12.46	11.12	13.16	11.76	11.67	11.98	11.81	13.65	12.79	12.44
28	13.56	9.53	9.63	13.04	13.30	13.29	9.84	10.26	11.49	12.48	11.37	12.80
29	10.81	9.98	9.73	11.15	12.25	11.80	12.12	9.97	10.77	11.15	12.04	11.84
30	11.83	9.22	12.47	13.34	13.93	13.91	13.59	11.91	11.85	11.23	14.40	13.63

Table 28 Paired Samples Statistics of Moisture Content

Paired samples test		Mean(%)	N	Std. Deviation	Std. Error Mean
Pair 1	Left C_week0	16.8780	30	16.70138	3.04924
	LeftC_week1	31.1500	30	17.20523	3.14123
Pair 2	LeftC_week0	16.8780	30	16.70138	3.04924
	leftC_week2	31.1250	30	20.74629	3.78774
Pair 3	LeftC_week0	16.8780	30	16.70138	3.04924
	LeftC_week4	35.2000	30	10.95846	2.00073
Pair 4	LeftC_week0	16.8780	30	16.70138	3.04924
	LeftC_week6	35.3833	30	15.76562	2.87840
Pair 5	LeftC_week0	16.8780	30	16.70138	3.04924
	LeftC_week8	30.0833	30	17.94956	3.27713
Pair 6	RightC_week0	15.4583	30	20.21640	3.69099
	RightC_week1	29.8667	30	20.35046	3.71547
Pair 7	RightC_week0	15.4583	30	20.21640	3.69099
	RightC_week2	31.4750	30	22.09466	4.03391
Pair 8	RightC_week0	15.4583	30	20.21640	3.69099
	RightC_week4	27.5000	30	14.09206	2.57285
Pair 9	RightC_week0	15.4583	30	20.21640	3.69099
	RightC_week6	32.0667	30	14.86809	2.71453
Pair 10	RightC_week0	15.4583	30	20.21640	3.69099
	RightC_week8	29.3667	30	17.96821	3.28053
Pair 11	LeftF_week0	27.8167	30	20.98336	3.83102
	LeftF_week1	34.1917	30	20.14545	3.67804
Pair 12	LeftF_week0	27.8167	30	20.98336	3.83102
	LeftF_week2	39.9167	30	25.28998	4.61730
Pair 13	LeftF_week0	27.8167	30	20.98336	3.83102
	LeftF_week4	36.7083	30	17.78561	3.24719
Pair 14	LeftF_week0	27.8167	30	20.98336	3.83102
	LeftF_week6	38.6583	30	25.02305	4.56856
Pair 15	LeftF_week0	27.8167	30	20.98336	3.83102
	LeftF_week8	42.2833	30	22.98320	4.19614
Pair 16	RightF_week0	31.0083	30	21.48300	3.92224
	RightF_week1	35.6250	30	21.38106	3.90363
Pair 17	RightF_week0	31.0083	30	21.48300	3.92224
	RightF_week2	39.8583	30	23.99306	4.38051
Pair 18	RightF_week0	31.0083	30	21.48300	3.92224
	RightF_week4	35.9250	30	17.89639	3.26742
Pair 19	RightF_week0	31.0083	30	21.48300	3.92224
	RightF_week6	38.5500	30	19.24506	3.51365
Pair 20	RightF_week0	31.0083	30	21.48300	3.92224
	RightF_week8	40.6000	30	20.90935	3.81751

Table 29 Paired Samples Test of Moisture Content

		Paired Differences					t	df	Sig. (2-tailed)
		Mean(%)	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	LeftC_wk0 - left_wk1	-14.2720	16.6098	3.0325	-20.4742	-8.0698	-4.7063	29	0.0001
Pair 2	LeftC_wk0 - left_wk2	-14.2470	29.9750	5.4727	-25.4398	-3.0542	-2.6033	29	0.0144
Pair 3	LeftC_wk0 - left_wk4	-18.3220	16.8309	3.0729	-24.6067	-12.0373	-5.9625	29	0.0000
Pair 4	LeftC_wk0 - left_wk6	-18.5053	24.9306	4.5517	-27.8146	-9.1961	-4.0656	29	0.0003
Pair 5	LeftC_wk0 - left_wk8	-13.2053	23.8418	4.3529	-22.1080	-4.3027	-3.0337	29	0.0051
Pair 6	RightC_wk0 - right_wk1	-14.4083	19.7977	3.6146	-21.8009	-7.0157	-3.9862	29	0.0004
Pair 7	RightC_wk0 - right_wk2	-16.0167	31.3367	5.7213	-27.7180	-4.3153	-2.7995	29	0.0090
Pair 8	RightC_wk0 - right_wk4	-12.0417	17.1869	3.1379	-18.4593	-5.6240	-3.8375	29	0.0006
Pair 9	RightC_wk0 - right_wk6	-16.6083	25.3504	4.6283	-26.0743	-7.1423	-3.5884	29	0.0012
Pair 10	RightC_wk0 - right_wk8	-13.9083	24.7705	4.5225	-23.1578	-4.6589	-3.0754	29	0.0046
Pair 11	LeftF_wk0 - leftF_wk1	-6.3750	16.3499	2.9851	-12.4801	-0.2699	-2.1356	29	0.0413
Pair 12	LeftF_wk0 - leftF_wk2	-12.1000	23.3776	4.2681	-20.8293	-3.3707	-2.8350	29	0.0083
Pair 13	LeftF_wk0 - leftF_wk4	-8.8917	15.2207	2.7789	-14.5752	-3.2081	-3.1997	29	0.0033
Pair 14	LeftF_wk0 - leftF_wk6	-10.8417	22.6143	4.1288	-19.2860	-2.3973	-2.6259	29	0.0137
Pair 15	LeftF_wk0 - leftF_wk8	-14.4667	20.9601	3.8268	-22.2933	-6.6400	-3.7804	29	0.0007
Pair 16	RightF_wk0 - rightF_wk1	-4.6167	17.3741	3.1721	-11.1043	1.8709	-1.4554	29	0.1563
Pair 17	RightF_wk0 - rightF_wk2	-8.8500	23.3190	4.2574	-17.5574	-0.1426	-2.0787	29	0.0466
Pair 18	RightF_wk0 - rightF_wk4	-4.9167	14.1404	2.5817	-10.1968	0.3634	-1.9045	29	0.0668
Pair 19	RightF_wk0 - rightF_wk6	-7.5417	20.5982	3.7607	-15.2332	0.1498	-2.0054	29	0.0543
Pair 20	RightF_wk0 - rightF_wk8	-9.5917	25.4334	4.6435	-19.0887	-0.0947	-2.0656	29	0.0479

Table 30 Paired Samples Statistics of Young's Modulus

		Mean (MPa)	N	Std. Deviation	Std. Error Mean
Pair 1	Left C_week0	7.3380	30	1.77260	.32363
	LeftC_week1	6.1533	30	1.86978	.34137
Pair 2	LeftC_week0	7.3380	30	1.77260	.32363
	leftC_week2	6.5540	30	1.80081	.32878
Pair 3	LeftC_week0	7.3380	30	1.77260	.32363
	LeftC_week4	7.9427	30	2.08127	.37999
Pair 4	LeftC_week0	7.3380	30	1.77260	.32363
	LeftC_week6	6.9207	30	1.95306	.35658
Pair 5	LeftC_week0	7.3380	30	1.77260	.32363
	LeftC_week8	6.4083	30	1.89553	.34607
Pair 6	RightC_week0	7.5137	30	2.06411	.37685
	RightC_week1	6.4180	30	1.55999	.28481
Pair 7	RightC_week0	7.5137	30	2.06411	.37685
	RightC_week2	7.3267	30	4.58114	.83640
Pair 8	RightC_week0	7.5137	30	2.06411	.37685
	RightC_week4	7.5403	30	2.13774	.39030
Pair 9	RightC_week0	7.5137	30	2.06411	.37685
	RightC_week6	7.0743	30	2.00819	.36664
Pair 10	RightC_week0	7.5137	30	2.06411	.37685
	RightC_week8	6.7723	30	2.16188	.39470
Pair 11	LeftF_week0	10.2973	30	2.04740	.37380
	LeftF_week1	9.8810	30	2.10616	.38453
Pair 12	LeftF_week0	10.2973	30	2.04740	.37380
	LeftF_week2	10.0593	30	1.77869	.32474
Pair 13	LeftF_week0	10.2973	30	2.04740	.37380
	LeftF_week4	10.4480	30	2.17004	.39619
Pair 14	LeftF_week0	10.2973	30	2.04740	.37380
	LeftF_week6	10.7363	30	2.42916	.44350
Pair 15	LeftF_week0	10.2973	30	2.04740	.37380
	LeftF_week8	10.8867	30	2.01811	.36845
Pair 16	RightF_week0	10.0470	30	2.33477	.42627
	RightF_week1	10.3593	30	1.92065	.35066
Pair 17	RightF_week0	10.0470	30	2.33477	.42627
	RightF_week2	10.0570	30	1.69580	.30961
Pair 18	RightF_week0	10.0470	30	2.33477	.42627
	RightF_week4	10.4617	30	2.49923	.45630
Pair 19	RightF_week0	10.0470	30	2.33477	.42627
	RightF_week6	10.6250	30	2.62328	.47894
Pair 20	RightF_week0	10.0470	30	2.33477	.42627
	RightF_week8	10.7073	30	2.19201	.40020

Table 31 Paired Samples Test of Young's Modulus

		Paired Differences					t	df	Sig. (2-tailed)
		Mean (MPa)	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	LeftC_wk0 - left_wk1	1.1847	1.7414	0.3179	0.5344	1.8349	3.7261	29	0.0008
Pair 2	LeftC_wk0 - left_wk2	0.7840	1.7352	0.3168	0.1361	1.4319	2.4748	29	0.0194
Pair 3	LeftC_wk0 - left_wk4	-0.6047	1.5992	0.2920	-1.2018	-0.0075	-2.0710	29	0.0474
Pair 4	LeftC_wk0 - left_wk6	0.4173	1.6353	0.2986	-0.1933	1.0280	1.3978	29	0.1728
Pair 5	LeftC_wk0 - left_wk8	0.9297	1.7111	0.3124	0.2907	1.5686	2.9758	29	0.0058
Pair 6	RightC_wk0 - right_wk1	1.0957	1.8307	0.3342	0.4121	1.7793	3.2780	29	0.0027
Pair 7	RightC_wk0 - right_wk2	0.1870	4.8023	0.8768	-1.6062	1.9802	0.2133	29	0.8326
Pair 8	RightC_wk0 - right_wk4	-0.0267	1.6998	0.3103	-0.6614	0.6080	-0.0859	29	0.9321
Pair 9	RightC_wk0 - right_wk6	0.4393	1.5179	0.2771	-0.1275	1.0061	1.5853	29	0.1238
Pair 10	RightC_wk0 - right_wk8	0.7413	1.7671	0.3226	0.0815	1.4012	2.2979	29	0.0290
Pair 11	LeftF_wk0 - leftF_wk1	0.4163	1.8894	0.3449	-0.2892	1.1218	1.2069	29	0.2372
Pair 12	LeftF_wk0 - leftF_wk2	0.2380	1.5499	0.2830	-0.3408	0.8168	0.8411	29	0.4072
Pair 13	LeftF_wk0 - leftF_wk4	-0.1507	1.6697	0.3048	-0.7741	0.4728	-0.4942	29	0.6249
Pair 14	LeftF_wk0 - leftF_wk6	-0.4390	1.6302	0.2976	-1.0477	0.1697	-1.4750	29	0.1510
Pair 15	LeftF_wk0 - leftF_wk8	-0.5893	1.8407	0.3361	-1.2767	0.0980	-1.7537	29	0.0901
Pair 16	RightF_wk0 - rightF_wk1	-0.3123	1.7818	0.3253	-0.9777	0.3530	-0.9601	29	0.3449
Pair 17	RightF_wk0 - rightF_wk2	-0.0100	1.9797	0.3614	-0.7492	0.7292	-0.0277	29	0.9781
Pair 18	RightF_wk0 - rightF_wk4	-0.4147	1.7423	0.3181	-1.0652	0.2359	-1.3036	29	0.2026
Pair 19	RightF_wk0 - rightF_wk6	-0.5780	2.0303	0.3707	-1.3361	0.1801	-1.5593	29	0.1298
Pair 20	RightF_wk0 - rightF_wk8	-0.6603	1.9489	0.3558	-1.3881	0.0674	-1.8558	29	0.0737

Table 32 Estimates moisture content of left and right cheeks

Estimates moisture content				
Dependent Variable: variate_week(n)				
Group	**Mean (%)**	**Std. Error**	**95% Confidence Interval**	
			Lower Bound	**Upper Bound**
LeftC (o/w emulsion containing arabinoxylans)	32.4926	1.4171	29.7035	35.2818
RightC (emulsion base)	30.1507	1.4171	27.3616	32.9398
a. Covariates appearing in the model are evaluated at the following values: baseline_week0 = 16.1682.				

Table 33 Pairwise comparisons moisture content of left and right cheeks

Pairwise Comparisons moisture content						
Dependent Variable: variate_week(n)						
(I) treatment	**(J) treatment**	**Mean Difference (I-J) (%)**	**Std. Error**	**Sig.(a)**	**95% Confidence Interval for Difference(a)**	
					Lower Bound	**Upper Bound**
LeftC (o/w emulsion containing arabinoxylans)	Right C (emulsion base)	2.3419	2.0048	0.2437	-1.6040	6.2879
RightC (emulsion base)	Left C (o/w emulsion containing arabinoxylans)	-2.3419	2.0048	0.2437	-6.2879	1.6040
Based on estimated marginal means						
a. Adjustment for multiple comparisons: Least Significant Difference (equivalent to no adjustments).						

Table 34 Estimates moisture content of left and right foreheads

Estimates moisture content				
Dependent Variable: variate_week (n)				
Group	**Mean (%)**	**Std. Error**	**95% Confidence Interval**	
			Lower Bound	**Upper Bound**
Left F (o/w emulsion cotaining arabinoxylans)	39.2494	1.4767	36.3430	42.1557
Right F (emulsion base)	37.2140	1.4767	34.3076	40.1203
a. Covariates appearing in the model are evaluated at the following values: baseline_week0 = 29.4125.				

Table 35 Pairwise comparisons moisture content of left and right foreheads

Pairwise comparisons moisture content						
Dependent Variable: variate_week (n)						
(I) treatment	**(J) treatment**	**Mean Difference (I-J) (%)**	**Std. Error**	**Sig.(a)**	**95% Confidence Interval for Difference(a)**	
					Lower Bound	**Upper Bound**
LeftF (o/w emulsion containing arabinoxylans)	RightF (emulsion base)	2.0354	2.0913	0.3312	-2.0808	6.1516
RightF (emulsion base)	LeftF (o/w emulsion with arabinoxylans)	-2.0354	2.0913	0.3312	-6.1516	2.0808
Based on estimated marginal means						
a. Adjustment for multiple comparisons: Least Significant Difference (equivalent to no adjustments).						

Table 36 Estimates Young's modulus of left and right cheeks

Estimates Young's modulus				
Dependent Variable: variate_week (n)				
Group	**Mean (MPa)**	**Std. Error**	**95% Confidence Interval**	
			Lower Bound	**Upper Bound**
LeftC (o/w emulsion containing arabinoxylans)	6.8471	0.1688	6.5149	7.1793
RightC (emulsion base)	6.9750	0.1688	6.6428	7.3072
a. Covariates appearing in the model are evaluated at the following values: baseline_week0 = 7.4258				

Table 37 Pairwise Comparisons Young's modulus of left and right cheeks

Pairwise Comparisons Young's modulus						
Dependent Variable: variate_week (n)						
(I) treatment	**(J) treatment**	**Mean Difference (I-J) (MPa)**	**Std. Error**	**Sig.(a)**	**95% Confidence Interval for Difference(a)**	
					Lower Bound	**Upper Bound**
LeftC (o/w emulsion containing arabinoxylans)	RightC (emulsion base)	-0.1279	0.2388	0.5927	-0.5979	0.3422
RightC (emulsion base)	LeftC (o/w emulsion containing arabinoxylans)	0.1279	0.2388	0.5927	-0.3422	0.5979
Based on estimated marginal means						
b.Adjustment for multiple comparisons: Least Significant Difference (equivalent to no adjustments).						

Table 38 Estimates Young's modulus of left and right foreheads

Estimates Young's modulus				
Dependent Variable: variate_week (n)				
Group	**Mean (MPa)**	**Std. Error**	**95% Confidence Interval**	
			Lower Bound	**Upper Bound**
LeftF (o/w emulsion containing arabinoxylans)	10.3216	0.1339	10.0581	10.5852
RightF (emulsion base)	10.5227	0.1339	10.2592	10.7863
a. Covariates appearing in the model are evaluated at the following values: baseline_week0 = 10.1722				

Table 39 Pairwise Comparisons Young's modulus of left and right foreheads

Pairwise Comparisons Young's modulus						
Dependent Variable: variate_week (n)						
(I) treatment	**(J) treatment**	**Mean Difference (I-J) (MPa)**	**Std. Error**	**Sig.(a)**	**95% Confidence Interval for Difference(a)**	
					Lower Bound	**Upper Bound**
LeftF (o/w emulsion containing arabinoxylans)	RightF (emulsion base)	-0.2011	0.1895	0.2895	-0.5741	0.1719
RightF (emulsion base)	LeftF (o/w emulsion containing arabinoxylans)	0.2011	0.1895	0.2895	-0.1719	0.5741
Based on estimated marginal means						
a. Adjustment for multiple comparisons: Least Significant Difference (equivalent to no adjustments).						

Table 40 Paired samples test of moisture content of each formulation at same week

Paired samples test		Paired Differences					t	df	Sig. (2-tailed)
		Mean (%)	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	left_week0 - right_week0	1.4197	9.3920	1.7147	-2.0874	4.9267	0.828	29	0.414
Pair 2	left_week1 - right_week1	1.2833	11.0078	2.0097	-2.8270	5.3937	0.639	29	0.528
Pair 3	left_week2 - right_week2	-0.3500	16.1452	2.9477	-6.3787	5.6787	-0.119	29	0.906
Pair 4	left_week4 - right_week4	7.7000	10.2521	1.8718	3.8718	11.5282	4.114	29	0.000
Pair 5	left_week6 - right_week6	3.3167	10.1408	1.8514	-0.4700	7.1033	1.791	29	0.084
Pair 6	left_week8 - right_week8	0.7167	14.0264	2.5609	-4.5209	5.9542	0.280	29	0.782
Pair 7	leftF_week0 - rightF_week0	-3.1917	8.8582	1.6173	-6.4994	0.1161	-1.973	29	0.058
Pair 8	leftF_week1 - rightF_week1	-1.4333	13.6527	2.4926	-6.5313	3.6647	-0.575	29	0.570
Pair 9	leftF_week2 - rightF_week2	0.0583	14.8630	2.7136	-5.4916	5.6083	0.021	29	0.983
Pair 10	leftF_week4 - rightF_week4	0.7833	12.4374	2.2707	-3.8609	5.4275	0.345	29	0.733
Pair 11	leftF_week6 - rightF_week6	0.1083	14.0654	2.5680	-5.1438	5.3604	0.042	29	0.967
Pair 12	leftF_week8 - rightF_week8	1.6833	13.1338	2.3979	-3.2209	6.5876	0.702	29	0.488

Table 41 Paired samples test of Young's modulus of each formulation at same week

Paired samples test		Paired Differences					t	df	Sig. (2-tailed)
		Mean (MPa)	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	left_week0 - right_week0	-0.1757	1.5771	0.2879	-0.7646	0.4132	-0.610	29	0.547
Pair 2	left_week1 - right_week1	-0.2647	1.7756	0.3242	-0.9277	0.3984	-0.816	29	0.421
Pair 3	left_week2 - right_week2	-0.7727	4.6868	0.8557	-2.5227	0.9774	-0.903	29	0.374
Pair 4	left_week4 - right_week4	0.4023	1.1802	0.2155	-0.0384	0.8430	1.867	29	0.072
Pair 5	left_week6 - right_week6	-0.1537	1.6563	0.3024	-0.7721	0.4648	-0.508	29	0.615
Pair 6	left_week8 - right_week8	-0.3640	1.0532	0.1923	-0.7573	0.0293	-1.893	29	0.068
Pair 7	leftF_week0 - rightF_week0	0.2503	1.9049	0.3478	-0.4610	0.9616	0.720	29	0.477
Pair 8	leftF_week1 - rightF_week1	-0.4783	1.8120	0.3308	-1.1549	0.1983	-1.446	29	0.159
Pair 9	leftF_week2 - rightF_week2	0.0023	1.5298	0.2793	-0.5689	0.5736	0.008	29	0.993
Pair 10	leftF_week4 - rightF_week4	-0.0137	2.0627	0.3766	-0.7839	0.7566	-0.036	29	0.971
Pair 11	leftF_week6 - rightF_week6	0.1113	1.8203	0.3323	-0.5684	0.7910	0.335	29	0.740
Pair 12	leftF_week8 - rightF_week8	0.1793	1.4811	0.2704	-0.3737	0.7324	0.663	29	0.512

APPENDIX C

Questionnaires

แบบสอบถามประเมินความพึงพอใจของผลิตภัณฑ์

ชื่อ.....นามสกุล.....อายุ.....

กรุณาทำเครื่องหมาย / ในช่องที่เห็นว่าเหมาะสมที่สุด

ผลิตภัณฑ์หมายเลข 1

ความพึงพอใจ	พอใจมากที่สุด	พอใจมาก	พอใจปานกลาง	พอใจน้อย	พอใจน้อยที่สุด
1. สี					
2. กลิ่น					
3. ความนุ่มนวล					
4. ความเนียนของเนื้อครีม					
5. การกระจายตัวและการดูดซึมบนผิว					

ข้อเสนอแนะ_____

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ผลิตภัณฑ์หมายเลข 2

ความพึงพอใจ	พอใจมากที่สุด	พอใจมาก	พอใจปานกลาง	พอใจน้อย	พอใจน้อยที่สุด
1. สี					
2. กลิ่น					
3. ความนุ่มนวล					
4. ความเนียนของเนื้อครีม					
5. การกระจายตัวและการดูดซึมบนผิว					

ข้อเสนอแนะ_____

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ผลิตภัณฑ์หมายเลข 3

ความพึงพอใจ	พอใจมากที่สุด	พอใจมาก	พอใจปานกลาง	พอใจน้อย	พอใจน้อยที่สุด
1. สี					
2. กลิ่น					
3. ความนุ่มนวล					
4. ความเนียนของเนื้อครีม					
5. การกระจายตัวและการดูดซึมบนผิว					

ข้อเสนอแนะ_____

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ผลิตภัณฑ์หมายเลข 4

ความพึงพอใจ	พอใจมากที่สุด	พอใจมาก	พอใจปานกลาง	พอใจน้อย	พอใจน้อยที่สุด
1. สี					
2. กลิ่น					
3. ความนุ่มนวล					
4. ความเนียนของเนื้อครีม					
5. การกระจายตัวและการดูดซึมบนผิว					

ข้อเสนอแนะ_____

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ผลิตภัณฑ์หมายเลข 5

ความพึงพอใจ	พอใจมากที่สุด	พอใจมาก	พอใจปานกลาง	พอใจน้อย	พอใจน้อยที่สุด
1. สี					
2. กลิ่น					
3. ความนุ่มนวล					
4. ความเนียนของเนื้อครีม					
5. การกระจายตัวและการดูดซึมบนผิว					

ข้อเสนอแนะ_____

_____

แบบบันทึกข้อมูลอาสาสมัคร

ชื่อ-นามสกุล _____

เพศ _____

อายุ _____

โรคประจำตัว _____

แพ้ยา _____

ค่าความชุ่มชื้น

ซ้าย							ขวา						
No.	**wk0**	**wk1**	**wk2**	**wk4**	**wk6**	**wk8**	**No.**	**wk0**	**wk1**	**wk2**	**wk4**	**wk6**	**wk8**
1							1						
2							2						
3							3						
เฉลี่ย							เฉลี่ย						

ความความยืดหยุ่น

ซ้าย							ขวา						
No.	**wk0**	**wk1**	**wk2**	**wk4**	**wk6**	**wk8**	**No.**	**Wk0**	**wk1**	**wk2**	**wk4**	**wk6**	**wk8**
1							1						
2							2						
3							3						
เฉลี่ย							เฉลี่ย						

ค่าการวัดสีผิว

ซ้าย							ขวา						
No.	**wk0**	**wk1**	**wk2**	**wk4**	**wk6**	**wk8**	**No.**	**Wk0**	**wk1**	**wk2**	**wk4**	**wk6**	**wk8**
1							1						
2							2						
3							3						
เฉลี่ย							เฉลี่ย						

APPENDIX D

Study Protocol Approval

Protocol Review No. 09-33-จธม.

Study Protocol Approval

The Ethics Committee of The Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok, Thailand has approved the following study to be carried out according to the protocol dated and/ or amended as follows:

Study Title: Formulation and evaluation of o/w emulstions containing arabinoxylan from rice bran residues

Study Code: -

Centre: CHULALONGKORN UNIVERSITY

Principal Investigator : Miss Yuvared Luangwitchajaroen

Protocol Date : **December 18, 2009**

A list of the Ethics Committee members and positions present at the Ethics Committee meeting on the date of approval of this study has been attached.

This Study Protocol Approval Form will be forwarded to the Principal Investigator.

Chairman of Ethics Committee: .....

(Rungpetch Sakulbumrungsil, Ph.D.)

Secretary of Ethics Committee: Suyanee Pongthananikorn .....

(Suyanee Pongthananikorn, Ph.D.)

Date of Approval: June 23, 2009

VITA

Ms. Yuvared Luangwitchajaroen was born on December 2, 1983 in Nakornsawan, Thailand. She received her Bachelor's Degree of Pharmaceutical Sciences from the Faculty Pharmaceutical Sciences, Huachiew Chalermprakiet University in 2005. After graduation, she entered the Master's Degree program in Pharmaceutical Technology at Chulalongkorn University.