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ที่ใช้น้ำมันรำข้าวเป็นส่วนประกอบ

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DEVELOPMENT AND EVALUATION OF LIPCARE FORMULATIONS CONTAINING RICE BRAN OIL

Miss Jittima Usahanun

A Thesis Submitted Partial Fulfillment of the Requirements
for the Degree of Master of Science in Pharmacy Program in Pharmaceutics

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By	Miss Jittima Usahanun
Field of Study	Pharmaceutics
Thesis Advisor	Vipaporn Panapisal, Ph.D.
Thesis Co-advisor	Associate Professor Porntip Nimmannitya, M.Sc. in Pharm

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Accepted by the Faculty of Pharmaceutical Sciences, Chulalongkorn University in Partial Fulfillment of the Requirements for the Master's Degree

....Dean of the Faculty of Pharmaceutical Sciences

(Associate Professor Pornpen Pramyothin, Ph.D.)

THESIS COMMITTEE

.... Chairman

(Associate Professor Uthai Suvanakoot, Ph.D.)

.... Thesis Advisor

(Vipaporn Panapisal, Ph.D.)

....Thesis Co-advisor

(Associate Professor Porntip Nimmannitya, M.Sc. in Pharm.)

.... Member

(Assistant Professor Panida Vayumhasuwan, Ph.D.)

.... Member

(Pornpen Werawatganone, Ph.D.)

จิตติมา อุสาหะนันท์: การพัฒนาและการประเมินผลสูตรตำรับผลิตภัณฑ์ถนอมริมฝีปากที่ใช้น้ำมันรำข้าวเป็นส่วนประกอบ (DEVELOPMENT AND EVALUATION OF LIPCARE FORMULATIONS CONTAINING RICE BRAN OIL) อ. ที่ปรึกษา : อ.ดร.วิภาพร พนาพิศาล, อ.ที่ปรึกษาร่วม : รศ.พรทิพย์ นิมมานนิตย์ 139 หน้า. ISBN 974-17-4131-6.

งานวิจัยนี้มีจุดมุ่งหมายเพื่อคัดเลือกชนิดและความเข้มข้นของสารต้านออกซิเดชันที่มีประสิทธิภาพดีในการต้านการเกิดออกซิเดชันในน้ำมันรำข้าว พัฒนาสูตรตำรับผลิตภัณฑ์ลิปสติกและทำการประเมินผลการปรับสภาพผิวบริเวณริมฝีปากของลิปสติกน้ำมันรำข้าวทั้งการศึกษานอกร่างกายและในอาสาสมัครสุขภาพดี จากการศึกษาประสิทธิภาพในการต้านการเกิดออกซิเดชันระหว่างเทอเทียรีบิวทิลไฮโดรควิโนน (ทีบีเอชคิว) และบิวทิลเลตเทตไฮดรอกซีอะนิโซล (บีเอชเอ) ภายใต้สภาวะเร่ง 120 องศาเซลเซียสโดยใช้เครื่อง 743 Rancimat® ความเข้มข้นของสารต้านออกซิเดชันอยู่ระหว่างร้อยละ 0.01 ถึง 0.10 โดยน้ำหนัก พบว่าความคงตัวของน้ำมันรำข้าวเพิ่มขึ้นเมื่อความเข้มข้นของสารต้านออกซิเดชันเพิ่มขึ้น ทีบีเอชคิวและบีเอชเอมีประสิทธิภาพเท่าเทียมกันเมื่อใช้ความเข้มข้นต่ำกว่าร้อยละ 0.04 โดยน้ำหนัก แต่เมื่อความเข้มข้นมากกว่า ร้อยละ 0.04 โดยน้ำหนักพบว่าทีบีเอชคิวมีประสิทธิภาพในการต้านการเกิดออกซิเดชันในน้ำมันรำข้าวได้ดีกว่าบีเอชเออย่างมีนัยสำคัญ ดังนั้น ทีบีเอชคิวที่ความเข้มข้น 0.10 เปอร์เซ็นต์โดยน้ำหนักจึงถูกนำมาใช้ในการเพิ่มความคงตัวให้แก่น้ำมันรำข้าวในการเตรียมลิปสติก การตั้งตำรับลิปสติกใช้ส่วนประกอบ 3 ส่วนคือ น้ำมัน ไขมัน และไข ซึ่งสัดส่วนของน้ำมัน:ไขมัน:ไข ที่เหมาะสมหลังจากผ่านการพัฒนาตำรับคือ 76:10:14 โดยน้ำหนัก เมื่อวัดประสิทธิภาพในการป้องกันแสงแดด (เอสพีเอฟ) โดยใช้เครื่องวิเคราะห์ SPF-290s® พบว่าค่าเอสพีเอฟเพิ่มขึ้นเมื่อความเข้มข้นของน้ำมันรำข้าวเพิ่มขึ้น นอกจากนี้น้ำมันรำข้าวยังช่วยทำให้ลิปสติกมีความรู้สึกทางกายที่ดีขึ้นคือ มีความมันวาวเพิ่มขึ้น การทาลื่นขึ้น และความเหนอะหนะลดลง อาสาสมัครหญิงสุขภาพดีจำนวน 45 คน เข้าร่วมการศึกษาการปรับสภาพผิวบริเวณริมฝีปาก โดยวัดความจุน้ำ ปริมาณเมลานิน และปริมาณฮีโมโกลบิน พบว่าลิปสติกที่ไม่มีน้ำมันรำข้าว ลิปสติกน้ำมันรำข้าว และลิปสติกน้ำมันรำข้าวที่เพิ่มปริมาณแกมมาออไรซานอลสามารถเพิ่มความจุน้ำ ลดปริมาณเมลานิน และเพิ่มปริมาณเฮโมโกลบินได้อย่างมีนัยสำคัญเมื่อใช้เป็นระยะเวลานาน 6 สัปดาห์ ลิปสติกน้ำมันรำข้าวและลิปสติกน้ำมันรำข้าวที่เพิ่มปริมาณแกมมาออไรซานอลมีผลต่อสภาพผิวริมฝีปากเหนือกว่าลิปสติกที่ไม่มีน้ำมันรำข้าวอย่างไม่มีนัยสำคัญ อาสาสมัครพอใจลิปสติกน้ำมันรำข้าวที่เพิ่มปริมาณแกมมาออไรซานอลที่สุดเนื่องจากทาง่าย มีความมันวาวดี ใช้แล้วรู้สึกชุ่มชื้น ริมฝีปากแดงขึ้น ลดความคล้ำของริมฝีปาก และไม่เหนอะหนะ

ภาควิชา	เภสัชกรรม	ลายมือชื่อนิสิต........จิตติมา อุสาหะนันท์........
สาขาวิชา	เภสัชกรรม	ลายมือชื่ออาจารย์ที่ปรึกษา........วิภาพร พนาพิศาล
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KEY WORD: RICE BRAN OIL / ANTIOXIDANT / LIP CONDITIONING PROPERTY / LIP CAPACITANCE / LIPSTICK / SENSORY EVALUATION

JITTIMA USAHANUN : DEVELOPMENT AND EVALUATION OF LIPCARE FORMULATIONS CONTAINING RICE BRAN OIL. THESIS ADVISOR : VIPAPORN PANAPISAL, Ph.D., THESIS COADVISOR : ASSOC. PROF. PORNTIP NIMMANNITYA, M.Sc. in Pharm, 139 pp. ISBN 974-17-4131-6.

The purposes of this study were to select the type and concentration of antioxidant with the highest antioxidant activity in rice bran oil (RBO) and to evaluate the lip conditioning properties of the selected RBO lipsticks in healthy volunteers. The antioxidant efficacy study between tertiary butylhydroquinone (TBHQ) and butylated hydroxyanisole (BHA) under accelerated conditions by 743 Rancimat® at 120°C were studied. A series of concentrations for each antioxidant was between 0.01-0.10% by weight. The stability of rice bran oil was increased when using higher concentration of antioxidants. TBHQ and BHA have comparable antioxidant efficacy on rice bran oil at levels less than 0.04%. At levels greater than 0.04%, TBHQ is far more effective than BHA significantly ($P<0.05$). TBHQ at 0.10% by weight was used to stabilize RBO in lipstick preparation. Lipsticks were formulated using oil, fat, and wax. The optimum oil:fat:wax was 76:10:14 by weight. The SPF values of lipsticks measuring by SPF-290s® analyzer were increased when using higher concentrations of rice bran oil. Moreover, rice bran oil lipstick showed high efficacy in improving skin-feel, high gloss, good glide and less tackiness. Forty-five healthy female panelists were participated in the study of lip conditioning properties of lipsticks. Lip capacitance, melanin value and hemoglobin value had been measured weekly for 6 weeks. There is no statistical difference among three treatments in all properties. All treatments could increase lip capacitance and hemoglobin value while decrease melanin value significantly with time goes by. Lipsticks containing RBO and lipsticks containing RBO plus γ-oryzanol induced modest lip conditioning effect compared to control treatment. But lipsticks containing RBO and lipsticks containing RBO plus γ-oryzanol have comparable efficacy in all properties. The sensory evaluation perceived by panelists informed that lipstick containing RBO plus γ-oryzanol was evaluated as the most 'satisfy' with its high slipperiness, high gloss, increase moisturizing effect, increase lip redness, decrease lip darkness, and least tackiness.

Department Pharmacy	Student's signature......Jittima Usahanun......
Field of study Pharmacy	Advisor's signature...Vipaporn Panapisal
Academic year 2005	Co-advisor's signature..Porntip Nimmannitya

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LIST OF ABBREVIATIONS

ANOVA	=	analysis of variance
AOCS	=	American Oil Chemists’ Society
AOM	=	Active Oxygen Method
au	=	arbitrary unit
BHA	=	butylated hydroxyanisole
BHT	=	butylated hydroxytoluene
C.V.	=	coefficient of variation
cm^2	=	square centimeter
Cutina MD®	=	glyceryl stearate
°C	=	degree Celsius
D&C	=	Drugs and Cosmetics
df	=	degree of freedom
et al.	=	et alibi, and others
Eutanol G®	=	octyldodecanol
FDA	=	food and drug administration
FPTRPC	=	Federal provincial territorial radiation protection committee
g	=	gram
h	=	hour
HIDL	=	high intensity discharge lamp
HPLC	=	high performance liquid chromatography
i.e.	=	id est, that is
IPM	=	isopropyl myristate
km	=	kilometer
LSD	=	least significance difference
MED	=	minimal erythemal dose
mg	=	milligram
mL	=	milliliter
mm	=	millimeter
MPF	=	monochromatic protection factor
N	=	Newton

n	=	sample size
nm	=	nanometer
No.	=	number
NOHSC	=	National occupational health and safety commission
OSI	=	Oxidative Stability Index
PM wax®	=	polyethylene wax (and) microcrystalline wax
PUFAs	=	polyunsaturated fatty acids
PV	=	peroxide value
R^2	=	coefficient of determination
RBO	=	rice bran oil
RH	=	relative humidity
rpm	=	round per minute
s	=	seconds
S.D.	=	standard deviation
SCC	=	squamous cell carcinoma
SF1318®	=	MQ Resins/Siloxylicated
Silshine®	=	MQ Resins/Siloxylicated
SPF	=	sun protection factor
TBHQ	=	tertiary butylhydroquinone
UV	=	ultraviolet
v/v	=	volume by volume
W	=	watt
wt	=	weight
w/w	=	weight by weight
α	=	alpha
β	=	beta
γ	=	gamma
δ	=	delta
μL	=	microliter

CHAPTER I

INTRODUCTION

Nowadays, natural products play an important role in medicinal and pharmaceutical area. Customers usually believe that natural products are efficient, natural and safe, less expensive and having less effects than synthetic chemical compounds. Thus, natural products using for beauty and health become more popular and trend to increase other applications.

Rice bran oil (RBO) is the natural oil which is extracted from bran layers of rice and consists of two components. The first one is saponifiable components including saturated and unsaturated fatty acids or essential fatty acids. Saponifiable component provides moiturizing properties to the skin (Schueller and Romanowski, 1999). Another component is unsaponifiable component as beneficial antioxidants. including the most richest source of vitamin E complex (i.e. tocotrienols), vitamin B complex and γ-oryzanol. γ -Oryzanol is a powerful antioxidant found only in rice bran (Xu, Hua, and Godber, 2001). These antioxidants have made RBO to become a strong antioxidant. Several studies have reported that γ -oryzanol have been used as skin nutrition and anti-aging in cosmetics. (Takeshi et al., 1982; Tatsu et al., 1993; Cheruvanky, 2000). Moreover, it has been discovered that RBO is effective as a sunscreening agent in protecting the skin against sunburn upon exposure to ultraviolet radiation in the region from about 295 to 315 nanometers, remains effective for a number of hours, does not discolor or develop odor on exposure to sunlight, nontoxic and non-irritation to the skin (Loo, 1976). The structure of γ -oryzanol is similar to cinnamate structure (Dweck, 1999). Since cinnamate is a strong UVB absorbant and worldwide used (Steinberg, 1996). Therefore, RBO is expected to have the same sunscreening properties as the interesting way to reduce the usage of synthetic sunscreening agents.

Lipstick is one of worldwide used cosmetics which consists of three main ingredients (i.e. oil, fat and wax) either natural or synthetic (Thai Industrial Standard, 1998). Oil has been used in high portion especially in lip-gloss (50-70%) (Schlossman, 2001). Formulation which contains high percentage of oil provides more moisturizing effects (Cadicamo and Cadicamo, 1981). Synthetic oils and vegetable oils are commomly used lipstick preparations. The most favorable vegetable oil is castor oil with two desired properties for lipstick preparation, i.e. the ability to dissolve bromo acid (the universal staining pigment) and having a suitable viscosity to form a stick. Generally it can be used in the range of 25-50%, and sometimes up to 65% (Schlossman, 2001). Besides castor oil, very few vegetable oils have been used even though these vegetable oils can provide nutrition for the skin. One possible reason is that vegetable oils are difficult to preserve (deNaverre, 1975). Therefore, increasing in the stability of vegetable oils will be the way to gain more benefits and give other alternatives for lipstick preparations.

Several antioxidants used by the food industry are effective in extending the shelf life of cosmetics and toiletries. However, synthetic antioxidants meet more of ideal antioxidant than the natural one (Oldfield, 2000). Many studies have investigated the oxidation properties of phenolic antioxidants in various oils. One study reported that tertiary butylhydroquinone (TBHQ) is the best antioxidant for soy bean oil, sunflower oil, canola oil and corn oil when compared with butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and propyl gallate (PG) (Oldfield, 2000). Referring to efficacy comparison study between TBHQ, PG and α-tocopherol in evening primrose oil, TBHQ has the highest antioxidant efficacy (Steab et al.,1998). Similar study about comparing the antioxidant activities of BHA, BHT and PG in RBO has found that BHA is the best antioxidant for RBO (Piyawon chaisena, 2003). Therefore, one of these research objectives is to compare the antioxidant activities between TBHQ and BHA in RBO.

A new automated method, the Oxidative Stability Index (OSI), has chosen for the study of antioxidant activity which uses much less labor and involving fewer variables. Moreover, this method has been shown to produce more reliable and reproducible results than testing by Active Oxygen Method (Arquette, 1997)

The skin in the lip area is different from the other parts of the body. The main reason is that lip does not produce much melanin as a pigment protecting the skin against burning (National Occupational Health and Safety Commission [NOHSC], 1991). Furthermore, lip has thin stratum corneum and does not have sebaceous glands like body skin (Gray, 1959; Hikima et al., 2004). Thus, lips trend to dry easily. In severe case, lip cancer maybe occur (Hikima et al., 2004). Since lip needs to be protected from dryness and sunburn, a moisturizing product with sun protecting effect seems to be an ideal lipcare product.

Many research studies claimed that RBO is the potent sunscreening agent and can be used in vary cosmetic products. However, the actual efficacy of lipcare containing RBO has not been clearly stated. In this present study, the *in vitro* and *in vivo* evaluations are studied to ensure the antioxidant sunscreening and moisturizing effects in the lipcare products. Main objectives are illustrated as following:

The purposes of this study were:

1. To select the type and concentration of antioxidant with the highest antioxidant activity in rice bran oil at the concentration which can be used safely in cosmetics using the oxidative stability index.
2. To formulate the RBO lipstick preparations with adequate lipstick physicochemical properties and to measure *in vitro* SPF values of prepared RBO lipsticks.
3. To evaluate the moisturizing effects of the studied RBO lipsticks in healthy volunteers.

CHAPTER II

LITERATURE REVIEWS

1. Oxidation Protection with Phenolic Antioxidants

Nowadays, the using of natural, botanical personal-care products for emollient properties is particularly desirable. Nevertheless, cosmetic formulators have avoided using natural lipids in most formulations because natural lipids can turn rancid in a short time resulting in short shelf-life product. The challenging of working with natural emollients, especially unsaturated lipids, is overcoming poor oxidative stability.

Fortunately, many antioxidants are effective in extending the shelf life of cosmetics and toiletries. Most antioxidants used in oils are phenolic compounds, and are commonly referred to as phenolic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and tertiary butylhydroquinone (TBHQ).

The phenolic antioxidants inhibit a formation of the fatty free radical and oxidation is terminated at the initiation step (Figure 2.1). The resulting antioxidant free radical is a stable and this resonating structure will not promote future oxidation (Oldfield, 2002).

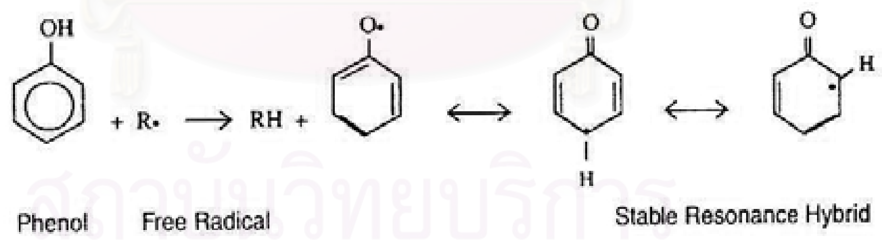


Figure 2.1 Autoxidation of fats and oils

Butylated hydroxyanisole (BHA) is perhaps the most widely used antioxidant in the food industry. The absorption and metabolism of BHA have been studied in rats, rabbits, dogs, monkeys, and humans. The major metabolites of BHA were the glucuronide, ether sulfate, and free phenol (TBHQ) as shown in Figure 2.2 (Madhavi, 1996).

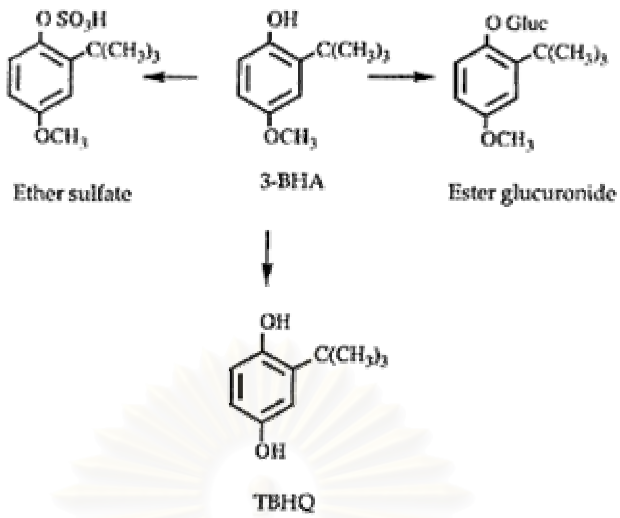


Figure 2.2 Major metabolites of butylated hydroxyanisole.

Since cosmetics are so variable in compositions, it is more practical to evaluate antioxidants in key ingredients known to be susceptible to oxidation rather than in the complete formulation. Most of the following performance data are based on the three test procedures generally accepted by the food industry and specifically by the fat and oil industry. The details of these tests were described as following:

1.1 Active Oxygen Method (AOM)

Following the recommended practice of AOCS Official Method Cd 12-57, AOM measures the time (in hours) required for a sample of fat or oil to attain a predetermined peroxide value under the specific test conditions. The length of this period of time is assumed to be an index of resistance to rancidity. The process begin when air is bubbled through the heated test sample to speed oxidation and shorten testing time. Periodic analyses show when the peroxide value has reached the induction point. AOM is applicable to all normal fats and oils of animal and vegetable but it is not applicable to solid materials (Oldfield, 2002).

1.2 Oxidative Stability Index (OSI)

The oil stablility index (AOCS Official Method Cd 12b-92) is an automated and accelerated method for measuring the stability of fats and oils so that many processors are replacing AOM with the OSI method. This instrument determines the induction period by passing a stream of air through a sample held in a temperature-controlled heating block. The effluent air from the sample is then bubbled through a vessel containing deionized water, where conductivity is continually monitored. The effluent air contains volatile organic acids, swept from the oxidizing oil, that increased the conductivity of the water as oxidation proceeding. Formic acid is the predominant formed organic acid. The length of time before this rapid acceleration of oxidation is the measure of the resistance to oxidation and is commonly referred to as the "induction time." The conductivity of the water is monitored by a computer or strip chart recorder. In Figure 2.3, rancimat curve shows the relationship between the conductivity (μS/cm) and time (h), the Oil Stability Index is defined as the point of maximum change of the rate of oxidation, or mathematically as the maximum of the second derivative of the conductivity with respect to time. The OSI may be run at temperatures of 100, 110, 120, 130 and 140°C. Because by its nature this analysis has this temperature flexibility, all OSI results should specify the OSI time, with the analysis temperature reported immediately (for example, "OSI 11.7 hours at 110°C").The process is shown Figure 2.5 (Oldfield, 2002; Arquette et al., 1997)

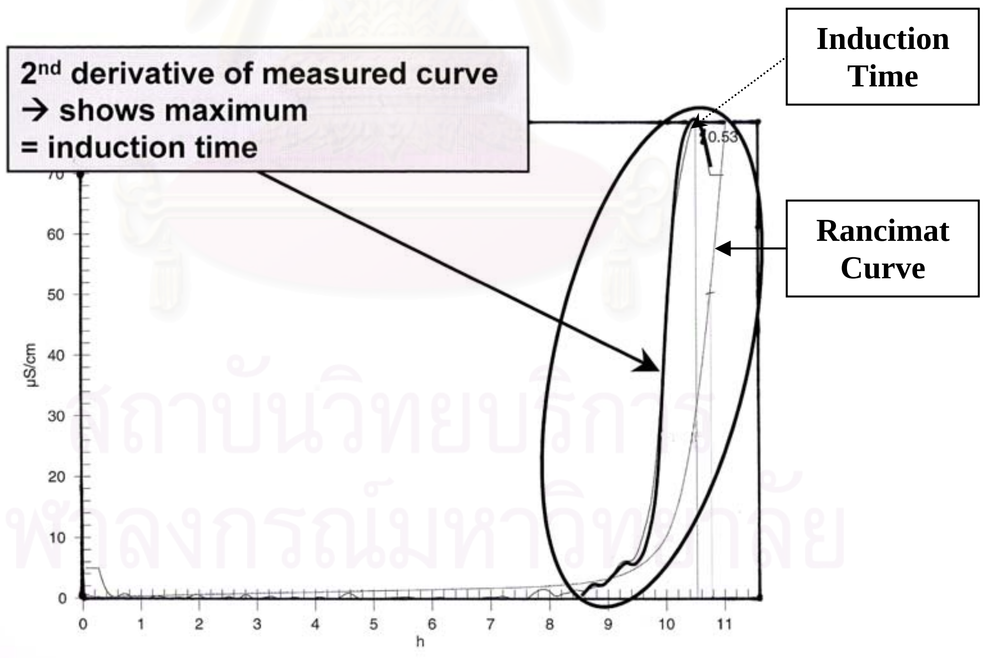


Figure 2.3 Rancimat curve and 2nd derivative of measured curve

Figure 2.4 The OSI instrument named 743Rancimat®.

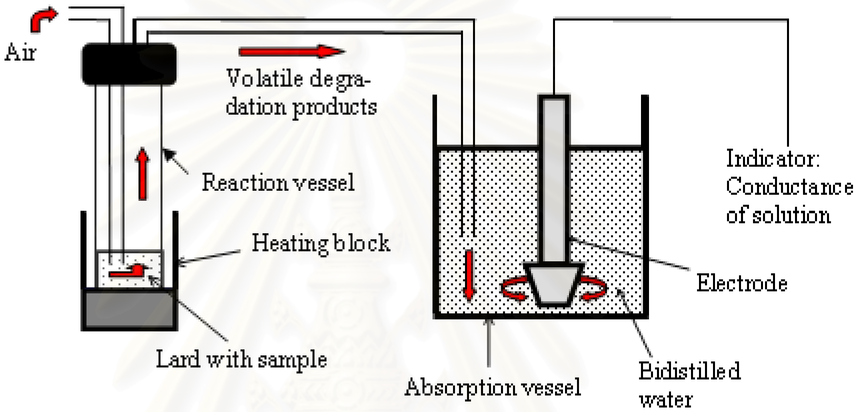


Figure 2.5 The process of OSI

1.3 Oven Storage Test

Oven storage tests, such as the Schaal Oven Stability test, are simply shelf-storage tests conducted at elevated temperatures to speed up the procedure. The oils are stored in an oven at specific temperature. Oxidative stability of the oils is measured by monitoring peroxide value (PV). The duration of monitoring depend on each study. For example, the study of antioxidant activity of rosemary and oregano ethanol extracts in soybean oil was determined in shaal oven test. This test was conducted in a regular laboratory oven adjusted to 63°C for 7 days (Almeida-doria and Regitano-D'Arce, 2000). In addition, the antioxidant property of aframomum danelli spice in oils was conducted in an oven at 63°C and oxidative stability of the oils was measured by monitoring peroxide value (PV) for 28 days (Fasoyiro et al., 2001).

Comparing from these methods, the OSI has been shown to produce more reliable and reproducible results than testing by AOM and oven storage test. It is also much less labour intensive and posing fewer variables (such as manual titration of peroxide value and use of solution requiring standardization).

2. Rice Bran Oil

Rice (*Oryza Sativa L.*) is the principal staple food of about half of the world population. It is grown in more than hundred countries, under a variety of climatic conditions (Kochhar, 2002)

Rice bran is a by-product of rice milling industry. It is the portion of paddy between the hull and the white rice grain (Figure 2.6) (Cheruvanky, 2000). Formerly, rice bran is primarily used as cheap animal feed. Nowadays, many studies confirm that rice bran oil which obtains from stabilized rice bran is considered to be a high-quality health oil, because of its rich phytonutrient content (DerMarderosian and Beutler, eds., 2001; Kochhar, 2002). So that, the oil has gained world wide attention. Rice bran oil not only an animal nutrition but also the high value human nutrition which possess antioxidative and disease-fighting properties.

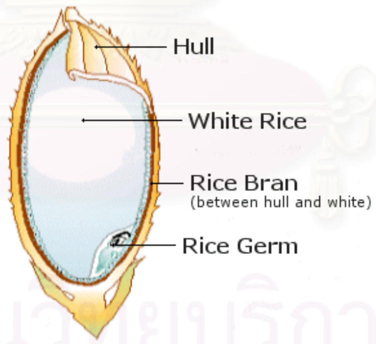


Figure 2.6 Rice bran is the portion of paddy between the hull and the white rice grain

The crude oil is usually dark greenish-brown, depending upon the extraction method (Kochhar, 2002). Moreover, RBO has characteristic odor. Color bodies should be absent, and off-odors are difficult to mask in cosmetic preparations. So that, the demands of the cosmetic formulator are frequently very rigid.

2.1 Rice Bran Oil Components

RBO consists of two components. The first one is saponifiable components including saturated and unsaturated fatty acids or essential fatty acids. Another component is unsaponifiable components as beneficial antioxidants. The lipid composition of crude RBO is presented in Table 2.1.

Table 2.1 Lipid components of crude rice bran oil

Component		% by wt
Saponifiable lipids		**90-96**
Neutral lipids		88-89
Triacylglycerols	83-86%	
Diacylglycerols	3-4%	
Monoacylglycerols	6-7%	
Free fatty acids	2-4%	
Waxes	3-4%	
Glycolipids		6-7
Phospholipids		4-5
Unsaponifiable lipids		**4.2**
Phytosterols	43[a]	
4-methyl sterols	10[a]	
4-dimetyl sterols (triterpene alcohols)**	28[a]	
Hydrocarbons*	18[a]	
Tocopherols and tocotrienols	3[a]	

*Squalene 16-40%, i.e. 0.12-0.3% in oil.

**Mainly oryzanol.

[a]These figures are % of total unsaponifiable lipids.

Source: Sayre and Sounders 1990

2.1.1 Saponifiable Components

The lipid component of RBO consists of neutral lipids, glycolipids and phospholipids. Neutral lipids consist mostly of triacylglycerols, monoacylglycerols and few diacylglycerols, sterols and free fatty acids. The major phospholipids are phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol and phosphatidic acid (Hamavathy and Phabhakar, 1987). Palmitic, oleic and linoleic fatty acids constitue 93-95% of the fatty acid portion of glycerol esters (Kochhar, 2002). Linoleic acid belongs to the polyunsaturated fatty acids (PUFAs) and it is an essential fatty acid because it has to be supplied by the diet (Polo, K. F. 1998). It can be utilized in the skin for its presumed benefit on scaling phenomena with no chemical modification (Rieger, 1994). RBO has linoleic acid about 29-45% of fatty acid portion (Kochhar, 2002). Fatty acid esters are responsible for the excellent slip and lubrication properties of RBO. Thus, RBO is used in skincare for its good moisturizing and emollient properties on dry skin.

2.1.2 Unsaponifiable Components

Rice bran contains 3-5% unsaponifiable lipids (Sayre and Sounders, 1990) which higher than other vegetable oil sources (Roger et al., 1993). Unsaponifiable components of RBO contains many bioactives such as carotenoids, vitamin B, vitamin E, and γ-oryzanol etc. but two unsaponifiable components of RBO have been investigated for possible health benefits. These are vitamin E and γ-oryzanol (Roger et al., 1993; Lloyd et al., 2000)

2.1.2.1 Vitamin E

Vitamin E is a collective name given to a group of naturally occurring tocopherols and tocotrienols found abundantly in plants and plant oils. Both tocopherols and tocotrienols have identical structure with a chromonol head group and phytyl- and farnesyl- side chains respectively (Cheruvanky, 2000) but tocotrienol differ from tocopherols by the presence of three unsaturated bonds in the phytyl side chain (Taylor et al., 1996) as shown in Figure 2.7 and Figure 2.8.

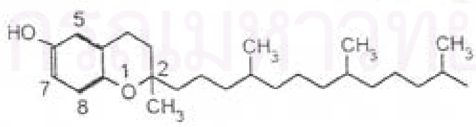


Figure 2.7 Tocopherol

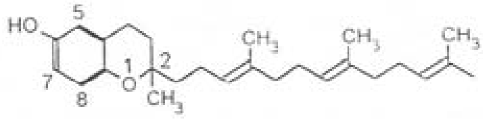


Figure 2.8 Tocotrienol

The four common isomers of tocopherols and tocotrienols that occur in nature are α, β, γ and δ (Rogers, 1993). Tocopherols are powerful antioxidants with a potent vitamin E activity and have higher activity against cardiotoxicity. Furthermore, RBO is relatively rich in tocotrienols which have been investigated for possible health benefits (Cheruvanky, 2000). Like tocopherols, tocotrienols possess antioxidant activity. In addition, other physiological actions attributed to the tocotrienols are decreasing serum cholesterol, decreasing hepatic cholesterol synthesis and having anti-tumor activity (Rogers, 1993).

2.1.2.2 γ-Oryzanol

γ-Oryzanol is an unique antioxidant present in rice bran and its products. Chemically, it is a mixture of ferulic acid esters of triterpene alcohols and phytosterols (Figure 2.9) (Cheruvanky, 2000).

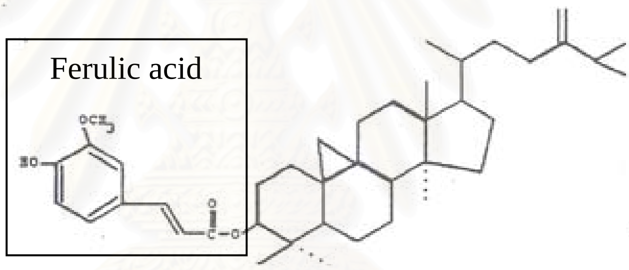


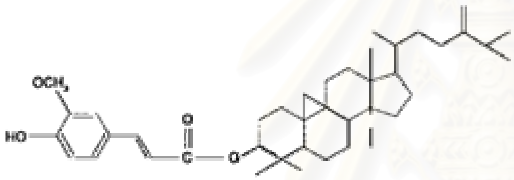
Figure 2.9 Oryzanol

γ-Oryzanol is composed of 4 esters of ferulic acid that is 25 to 50% of campesteryl ferulate, 15 to 25% of β-sitosteryl ferulate, 15 to 30% of cycloartenyl ferulate, and 10 to 40% of 24-methylenecycloartanyl ferulate. (Iijima and Sano, 1986, van Amerongen et al., 2002). γ-Oryzanol has been considered to be the major antioxidant in rice bran oil because the quantity of γ-oryzanol in rice bran oil is up to 10 times higher than vitamin E (Xu et al., 2001). One to two percent of γ-oryzanol can be recovered from RBO (Scavariello and Arellano, 1998; Lai et al., 2005)

γ-Oryzanol has been shown to be very safe (Deckere and Korver, 1996). Side effects have not been reported in animal studies using doses of up to 1,000 mg per day of γ -oryzanol or up to 1,500 mg per day of ferulic acid.

Poor absorption appears to be the reason for the lack of side effects associated with higher doses (Hirose et al., 1999).

Many reports has indicated that γ-oryzanol is effective in absorbing ultraviolet light (Loo, 1976; Cheruvanky, 2000; DerMarderosian and Beutler, eds., 2001) and so in protecting the skin against sunburn upon exposure to ultraviolet radiation in the region from about 295 to 315 nanometers. γ-Oryzanol remains effective for a number of hours, does not discolor or develop odor on exposure to sunlight, is both nontoxic and non-irritating to the skin (Loo, 1976). The structure of γ-oryzanol (4-hydroxy-3-methoxycinnamic acid) is similar to cinnamate structure, which is the popular sunscreening agent in cosmetic, as referred to ethyl cinnamate (Figure 2.10) (Dweck, 1999). Therefore, it may be the reason why γ-oryzanol is strong photoprotective agent.



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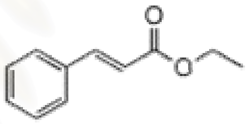


II

Figure 2.10 Comparison of chemical structure between oryzanol (I) and ethyl cinnamate (II)

2.2 The Advantages of Rice Bran Oil

2.2.1 Overall Uses of Rice Bran Oil

Many studies have been claimed that RBO has various pharmacological effects and therefore is used for medical uses. Tocotrienol, γ-oryzanol, and linoleic acid in RBO have been shown lowering plasma cholesterol levels (Xu et al., 2001; Deckere and Korver, 1996). It is also reported to decrease early atherosclerosis (Cheruvanky, 2000), inhibit platelet aggregation (DerMarderosian and Beutler, eds., 2001) and decrease hepatic cholesterol biosynthesis (Kochhar, 2002). Moreover, Hirose et al. (1999) has reported that phytic acid inhibits hepatic and mammary carcinogenesis.

RBO has been used as antioxidant in high linolenic edible oils (e.g. soy and rapeseed oils) by the addition of about 2-5% (w/w) that is processed to retain unsaponifiable matter; physically refined RBO may also be used (Taylor et al., 1996). The stability of RBO is probably due to the combined protective effects of oryzanol, phytosterols, squalene, tocopherols and tocotrienols. These make the oil a premium choice for frying high quality products with delicate flavours (Kochhar, 2002).

2.2.2 Cosmetic Uses of Rice Bran Oil

The components in RBO, oryzanol and ferulic acid, have actions in preventing skin aging and smoothening wrinkles without any adverse effects on living bodies when used as components for general pharmaceutical and cosmetic preparations such as ointments, creams and lotions (Tatsu et al., 1993). Functions of RBO and oryzanol in skin formulations are skin conditioning agent, emollient, moisturizer and SPF booster (Wenniger and McEwen, 1992).

DL-alpha tocopherol (vitamin E) and ferulic acid in RBO prevented facial hyperpigmentation by suppressing melanogenesis induced by UV light. In addition, the γ-oryzanol has a powerful UV absorbency and is used in skin lotions and suntan creams (Cheruvanky, 2000).

3. Lipstick

Lipstick is a solid made from the mixture of waxes, fats, oils, and/or pigments (Thai Industrial Standard, 1998). The ideal lipstick must be apply easily and give even color. Color provided to the lips should be the same as that of the lipstick itself without any change in color. The application must not be greasy. It should have a pleasant taste and not come off easily onto the cups or drinking glasses. Furthermore, the lipstick must be only slightly affected by temperature difference between 10°C and 40°C. Free from sweating, bloom, changes in hardness, rancidity even on aging, the ideal lipstick should also be firm but not brittle in texture (Finkenaur, 1986; deNaverre, 1975). Formulators have directed their efforts to fulfilling all these criteria, but because of effects of color, gloss and other characteristics, no product has met all criteria.

The lipstick materials differ from other cosmetics because lipstick materials should be nontoxic and edible. The wax gives lipstick its shape and ease of application. The traditional waxes such as beeswax, candelilla wax, carnauba wax are important ingredients. Many formulations contain these waxes because they give the molded stick resilience and hardness (deNaverre, 1975). But they don’t permit good transparent application due to crystallization and polymorphism phenomena (Zanotti et al., 1998).

Castor oil is one of the better solvents for bromo acid (the universal staining pigment). It can be bought in a highly refined grade having practically no odor or taste. Some lipstick on the market contain as much as 65% castor oil. Generally 25-50% is the amount used (deNaverre, 1975). Other than castor oil, few vegetable oils are used now. The main drawback with vegetable oils is that they are difficult to preserve. The synthetic oils such as isostearyl alcohol, octyldodecanol are preferred as lipstick materials because their low viscosity and freedom from rancidication (Finkenaur, 2000). In recent years, ingredients such as moisturizers, vitamin E, aloe vera, collagen, amino acids, and sunscreen have been added to lipstick. The extra components keep lips soft, moist, and protected from the elements.

Lipstick gets its color from a variety of added pigments. These pigments are subject to ingestion. Among them are bromo acid, D&C Red No. 21, and related dyes. Other common lipstick dyes are D&C Red No. 27 and insoluble dyes known as lakes, such as D&C Red No. 34, Calcium lake, and D&C Orange No. 17. Pink shades are made by mixing titanium dioxide with various shades of red (Berdick, 1986). Furthermore, lipstick perfume is important. It is necessary to review the composition of perfumes used in lipstick first, then discuss lipstick allergy (deNaverre, 1975). Because fragrances and dyes used in lipsticks can cause an allergic reaction which subsequently can cause discolouration of the lips.

3.1 Examinations of the Finished Lipstick

Because each material potentially can cause multiple variations in the final product, a development laboratory must adapt methods and processes in order to obtain predetermined values which will serve as parameters in the development of the ideal formula, as well as in future quality control of the manufactured product.

3.1.1 Break Strength Test

Women apply lipstick with varying degrees of force. There is a research reported that the average force for each application using between 50 and 120 grams and the maximum lipstick used by the average user was 73 mg per day and the average use was 22 mg per day in a group of 222 women (Finkenaur, 2000). The method for determining breaking point or rupture point has been developed. In the past, breaking strength test used the instrument look like Figure 2.11. Lipstick A is put in the holder B and inserted into stationary block C and through movable block E. Block E rides on guide rods D. Weights are put at F, with a 30-second interval between each addition of weight. When stick ruptures, E moves on guide rods D to reach microswitch M, which light up bulp. Weights plus weight of E is the pressure required for rupture (deNaverre, 1975; Gouvea, M. C. 1978)

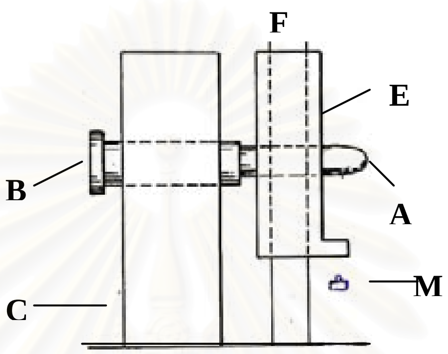


Figure 2.11 The design of break strength test in the past

Nowadays, the testing of the break strength of lipstick is performed more accurately with force gauges attached to a motorized platform with controls for the speed of lift (Finkenaur, 2000). Figure 2.12 is an example of break strength application named TA.XT2I Texture analyzer.

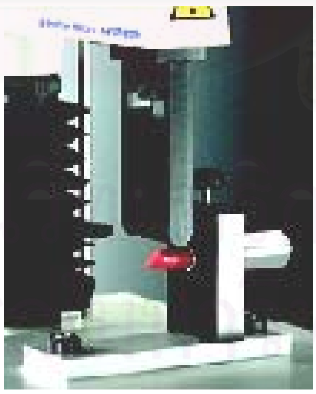


Figure 2.12 TA.XT2I Texture analyzer

3.1.2 Hardness Measurement

Lipstick Hardness is an important determination which specifies the use of a needle penetration test (Figure 2.13). Besides determining lipstick hardness, this test may also indicate the presence of unwanted trapped air bubbles, or a "grainy" texture as a result of either incomplete colorant dispersion or the working and chilling processes during manufacture (Finkenaur, 2000).

Figure 2.13 Hardness measurement

3.1.3 Color Control

In color control it is important to remember that each time a sample or batch is re-heated, the color may change slightly. In visual color control, the "master" and the new batch are preferably applied to white paper or the lips using one-half of lip for standard and other half for new batch, allowing the applications to run into each other. The difference in shade is noted and corrected accordingly (deNaverre, 1975).

3.1.4 Melting Point Test

There are many methods of determining the melting point of a lipstick. From the USP method XVIII, this is the capillary tube method which fixes the melting temperature at the point where the lipstick mass rises in the tube during heating. In making the test, the lipstick is allowed to come to ambient temperature, the capillary is then forced through the stick to about a 10 mm height. The melting point is determined as mentioned above. Nowadays, the automatic melting point equipment is also available such as Mettler FP62 (Figure 2.14). The most used measurement with this apparatus is the drop point or the point where the mass changes sufficiently from its gel point to a liquid allowing the lipstick to pass an electric eye and registering the temperature when this occurs. This method is reproducible and does not required a technician present during the testing. An acceptable lipstick has a melting point between 55 and 65°C (Finkenaur, 2000).

Figure 2.14 Mettle FP62 Melting Point Apparatus

From Thai Industrial Standard 234-2541, dropping point test has been used to determine the characteristic of lipstick. This test looks like melting point apparatus. Lipstick is melted in crucible. Then, thermometer is dipped into liquid lipstick and allows to cool at room temperature. After that, thermometer is put into test tube and heat in water bath. Lipstick is melted until reaching the drop point. The drop point is the point where the mass changes sufficiently from its gel point to a liquid.

3.1.5 Thermal Stability

Thermal stability is the property that ensures that a lipstick is usable at any temperature that may be encountered in the market in which it is sold. It is tested by placing lipsticks horizontally in constant temperature chambers at 25, 35, 45 and 55°C. The lipstick should not droop or distort after 24 hours at 55°C. The lipstick should minimally remain stable and not distort at 45°C and should be usable at 35°C after two months. The samples at 25°C will act as the control for all other temperatures. However, these samples must be checked for six months to ensure that no changes have occurred in the texture hardness (Finkenaur, 2000). But Thai Industrial Standard 234-2541 has different criteria that is the lipstick should not droop or distort exceed 5 mm after 24 hours at 45°C.

3.1.6 Sweating Test

Sweating is a seasonal phenomena (under ambient conditions) and is usually observed after a large temperature fluctuation or increase in temperature taking place over a short period of time. It is the excretion of oil on the surface of a lipstick. In all cases, the oil excretion was found to be pure castor oil, lacking any trace elements of other components (Dweck, 1981).

Sweating test is determined by keeping the lipstick preferably in its own container, in large (500 cc) dry closed transparent jars, placed in an oven at temperature of 43-52°C for at least 24 hours; then bringing to room temperature while still in closed jar. Sweating shows up as droplets of oil left on the surface of the stick or as a powdered like deposit when cool. It is due to poor manufacturing molding methods or incorrect formulation or both (deNavarre, 1975).

3.2 Useful Materials (Finkenaur, 1986)

These materials are commonly used in the development of lipstick base, excluding colorants, preservatives and perfumes.

3.2.1 Castor oil: It helps in the dispersion of colorants including solubilizing bromo-acid colorants and it has acceptable color, odor and taste. Formulations containing more than 50% have limited stability and leave a heavy, greasy feel on the lip.

3.2.2 Eutanol G® (octyldodecanol): It is a clear, slightly yellow, odourless oil of low polarity. Moreover, it is stable to hydrolysis.

3.2.3 Vegetable oils: They are often used today to provide claims support due to their natural vitamin or mineral contents, or their skin benefit claims. With the exception of castor oil, they are not used as primary component of modern formulations because they are difficult to preserve and have characteristic tastes that can be difficult to mask.

3.2.4 Isopropyl myristate (IPM): It is fatty acid esters and widely used in classically formulated lipsticks. It has poor bromo-acid solvent properties. However, the primary use in modern formulas is the reduction of quantity of castor oil used in the lipstick.

3.2.5 Beeswax: It is mostly used for the productions of cosmetics and pharmaceutical emulsions, creams, and ointments and also for lipsticks. It shrinks much when chilled, aiding in the de-molding of the lipstick

3.2.6 Carnauba wax: It is typically used in conjuction with amorphous waxes such as ozokerite and microcrystalline waxes to raise the melting point of the formula and to enhance high temperature stability.

3.2.7 Candelilla wax: It provides high shine, rigidity, and hardness without graininess associated with carnauba wax. However, the melting point of candelilla is less than carnauba and must be used at significantly high levels.

3.2.8 Ozokerite wax: It is a solid white to off white wax and typically used to raise the melting point of the lipstick in conjunction with other waxes such as carnauba.

3.2.9 Ceresin wax: It is a mixture of ozokerite or microcrystalline wax with paraffin. It shrinks much like beeswax when chilled, aiding in the de-molding of the lipstick.

3.2.10 Higher alcohols: Cetyl alcohol ($C_{16}H_{33}OH$) and stearyl alcohol ($C_{18}H_{37}OH$) are higher alcohols. They are primarily used to provide creamy textures at lower concentrations. In addition, their lower melting points may cause a softening of the stick.

4. The Lips

The lips are two fleshy folds surrounding the oral orifice. The size and curvature of the exposed red-lip surfaces are subjected to considerable individual, male-female and world variation. The junction between the external, hair-bearing skin and the red, hairless surface in the upper lip almost invariably takes the form of a double-curved Cupid's bow (Gray, 1959).

4.1 Anatomy and Physiological Characteristics of the Lips

The lips are continuous with the mucosa at the transitional or vermillion border, a reddish zone which depending upon the degree of melanization, covered by thin keratinized epithelium. The colour of this region is due to the proximity of blood vessels to the epithelial surface (Gray, 1959). The skin in the lip area is different from the other parts of the body. The main reason is that lip does not produce much melanin as a pigment protecting the skin against burning (National Occupational Health and Safety Commission [NOHSC], 1991). Furthermore, lip has thin stratum corneum and does not have oil produce glands like body skin, (Gray, 1959; Hikima et al., 2004). This layer is function as the barrier to evaporation without it lips retain less fluid. As shown in Figure 2.15, lips do not have the same complement of oil and sweat glands like regular skin. Sweat glands add moisture to skin, but for lips only source of moisture is saliva inside the mouth. Moreover, lip cells have a faster rate of turnover which is approximately twice than face (Arai et al., 1990). Thus, harsh winter wind, cold, sun, and dryness both indoors and outdoors make lips a vulnerable target for chapping.

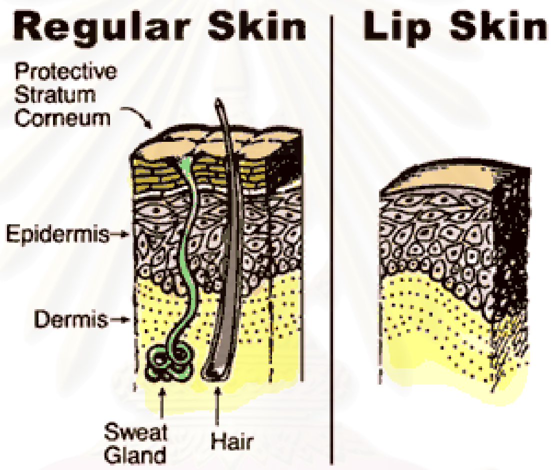


Figure 2.15 Lip skin compared with regular skin (http://www.blistex.com)

4.2 Lips Problems

4.2.1 Sun Damage

The lips in their natural state are not adequately shielded from the sun because the outer layer of the lip is so thin. In addition, lips have almost no melanin, the natural pigment in skin that helps screen out the sun's harmful rays. As a result, lips rarely tan, but they can easily burn. And because lips are located on the face, they are rarely covered, and thus constantly exposed to sun damage. Furthermore, the lower lip is especially exposed to solar ultraviolet radiation (Federal Provincial Territorial Radiation Protection Committee (FPTRPC), 1998). When unprotected lips are exposed to too much sun, the collagen changes causing lips to wrinkle and fine lines to form around the mouth. Squamous cell carcinoma (SCC) is one of skin cancers. The dangerous of this skin cancer is in the medium level and it can occur on the lips particularly the lower lip (NOHSC, 1991). In fact, the thickness assessment of photoprotective lipsticks has been shown that photoprotective lipsticks are applied in a much thinner layer than recommended by international standards (2 mg/cm^2). Furthermore, the frequency of application is too low for adequate protection (Maier, 2003). So that, photoprotective lipsticks with high and ultrahigh SPF should be recommended, especially for individuals with high risk for the development of the lip malignancies.

4.2.2 Lip Chapping

Lip chapping is abnormal desquamation along with scaling. Because the lip has thin stratum corneum and does not have oil produce glands like body skin, lip chapping can occur easily. Whenever lips are dry and chapped, they automatically tend to look dark. And the hydration was also lower in areas of lip chapping. Cathepsin D-like and chymo-trypsin-like proteinase, which are also present in skin as desquamation –regulating proteinases, were detected in lip corneocytes, though only cathepsin D-activity was found to decrease in severely chapped lips. The application of nutritional essence increased cathepsin D-activity and improved chapping severity (Hikima et al., 2001).

4.2.3 Cosmetic Dermatitis

Cosmetic dermatitis is an unpredictable itchy, red reaction to a cosmetic applied to the face or lips. In generally, it is caused by skin-irritating chemicals present in a cosmetic, but may also represent a true allergy or sensitivity to one or more of the components of the cosmetic. Such an allergy may lead to more severe itching, burning, and swelling of the skin and lips (Meynadier et al., 1994).

Cheilitis is one of cosmetic allergy persistent inflammation of one or both lips. It is usual allergic picture to lipstick. The sensitizers present in the lipsticks are preservatives and perfumes, although not colorants. The base constituents such as oils, waxes and cocao butter can also trigger allergic cheilitis. Allergic cheilitis due to lipstick is different from that induced by toothpaste, which extends largely on each side of the mouth. Scaling, cracking, and swelling may occur on the vermilion border, which is the area where lip mucosa meets the normal skin of the face (Meynadier et al., 1994).

5. Solar Radiation

5.1 Solar Radiation

The radiation striking the earth is approximately 50% visible (wavelength 400-800 nm), 40% infrared (wavelength 1300-1700 nm), and 10% UV (10-400 nm). The UV spectrum is divided for convenience into UV-A, from 320 to 400 nm; UV-B, from 290 to 320 nm; UV-C, from 100 to 290 nm (Lowe and Friedlander, 1997). The high energetic electromagnetic radiations, emitted by the sun, lethal to all living organism, are absorbed by the atmosphere of the Earth. Only one part of ultraviolet (UV), visible light, and infrared radiation reach the surface of the Earth. The ozone layer contains about 10 ppm of ozone and is about 2 mm thick. It is located in the stratrosphere at a height of between 20 and 30 km. It filters out the UV-C and reduced UV-B- radiation. The UV radiation, which remains about 5% of the solar spectrum, has the shortest wavelength and the highest energy. It can produce photochemical reactions causing both immediate and delayed damages to skin (Polo, 1998).

The remaining UV that reaches the ground is about 10% UV-B and 90% UV-A at midday. The UV-B intensity declines from the noontime apex, but UV-A intensity remains relatively constant throughout the day (Lowe and Friedlander, 1997).

In the case of UV-A energy, erythema of the skin produced as a result of exposure to this radiation attains its maximum intensity at about 72 hours after exposure, while in the case of UV-B radiation, the erythemal reaction reaches its maximum intensity within 6-24 hours after exposure (Wilkinson and Moore, eds., 1982). The main harmful effects of sources in the different ultraviolet radiation bands are summarized in Table 2.2.

Table 2.2 Sources and effects of ultraviolet radiation (FPTRPC, 1998)

UV-C	UV-B	UV-A
Wavelength: 100-280 nm Higher energy per photon.	**Wavelength:** 280-315 nm Intermediate energy per photon.	**Wavelength:** 315-400 nm Lower energy per photon
Sources: • Sun (UV-C is absorbed by molecular oxygen, ozone and water vapour in the upper atmosphere) • Germicidal lamps • Arc welding equipment • High intensity discharge lamps (HIDL)	**Sources:** • Sun (5% of UVR at ground level, only wavelengths > 297 nm) • Germicidal lamps • Arc welding equipment • HIDL • Therapeutics lamps • Medical and industrial lasers	**Sources:** • Sun (95% of UVR at ground level) • Black light lamps • Germicidal lamps • Arc welding equipment • HIDL • Therapeutics lamps • Tanning devices (sunbeds)
Penetration: • Photons between 100 to 200 nm are absorbed in air. • Absorbed by keratin in the epidermis, does not penetrate to the dermis.	**Penetration:** • Partially absorbed by ozone in the upper atmosphere • Penetrates to the dermis	**Penetration:** • Not absorbed by ozone • Penetrates deeper into the skin than any other form of UVR.
Effects: • DNA damage on unprotected cells: epithelium, cornea and bacteria.	**Effects:** • Responsible for vitamin D_3 production and delayed tanning. • Most effective in causing acute and chronic harmful effects. • Sunburn, immunosuppression, cellular damage, skin cancer, solar urticaria, photo aging and, photokerato-conjunctivitis, cataract, and pterygium.	**Effects:** • Causes immediate tanning. • Can potentiate some carcinogenic effects of UV-B. • Thermal burns • Sunburn, immuno-suppression, cellular damage, photoallergy, phototoxicity, photo-aging, photokerato-conjunctivitis, cataract and pterygium, solar retinitis.

5.2 Protective Mechanism of the Skin

The two factors which are mainly responsible for the skin’s natural protection against sunburn are the thickness of the stratum corneum and the pigmentation of the skin. The solar radiation increases the mitotic rate of epidermal cells, causing a thickening of the stratum corneum in the course of 4-7 days, and making it thereby more impervious to the passage of erythemogenic radiation. Moreover, some degree of protection against sunburn is conferred by an increased melanin content of the epidermis. Granules of melanin which are formed in the basal cell layer of the skin following the action of UV-B radiation migrating upwards towards the stratum corneum and the skin surface, where they are believed to be oxidized by radiation of the UV-A range. These granules are eventually shed during exfoliation, causing the skin to loss its immunity to sunburn (Wilkinson and Moore, eds., 1982).

5.3 The Effects of Solar Radiation on Skin

5.3.1 Effects of UV-B Radiation

Besides causing lesions to DNA of epidermal cells and solar erythema, UV-B radiation leads to a reduction of sebaceous glands activity (drying out of the skin) (Polo, 1998). Furthermore, the long term or chronic effects, caused by too frequent or too long exposure to ultraviolet radiation are dose-dependent and cumulative. With a weakening or disappearance of autoprotective mechanisms, the long term effects are skin aging, with lesions of dermal collagen and elastin fibers, and skin cancer.

5.3.2 Effects of UV-A Radiation

During a lifetime the skin receives large doses of the UV-A. It is the cause of solar elastosis (formation of wrinkles by photoaging). The long photoaging also leads to a decrease in the synthesis and cross-linking of collagen fibers. UV-A does damage to blood-vessels in the skin. The minimal erythemal dose (MED) for UV-A is about 100 to 1000 fold greater than UV-B (Polo, 1998).

6. Sunscreens

The incidence of sunlight-induced skin aging and skin cancers has been increasing in many part of the world. In particular, the incidence of melanoma skin cancer has shown a well-documented increase in several continents over the last several years (NOHSC, 1991).

Most modern sunscreens have highly efficient absorption or reflecting capabilities throughout the UV-B, partly the UV-A, and in some instances, infrared wavelengths. Over the last several years, more efficient sunscreening ingredients have been developed to improve protection of the population (Lowe and Friedlander, 1997).

6.1 Frequency of Use (Steinberg, 1996)

The sunscreen agents in cosmetics are divided in two functions. First, it is used as sunscreening agent in sunscreen products. Second, it is used to prevent UV degradation of the formulation. DEA-methoxycinnamate and Benzophenone-4, 8 are mainly used for the secondary function. The frequency of sunscreen use in 1996, as reported in the U.S. Food and Drug Administration (FDA) voluntary reporting database are shown in Table 2.3.

Table 2.3 Frequency of sunscreen use in 1996, as reported in the FDA voluntary reporting database

Sunscreen	Frequency
Octyl methoxycinnamate	688
Benzophenone-4	462
Benzophenone-3	408
Octyl dimethyl PABA	327
Octyl salicylate	95
DEA-methoxycinnamate	56
PABA	42
Butyl methoxydibenzoylmethane	30
Homosalate	21
Phenylbenzimidazole sulfonic acid	19
Menthyl anthranilate	13
Benzophenone-8	10
Octylcrylene	10
TEA-salicylate	8

From Table 2.3, Octyl methoxycinnamate are the most popular sunscreen protecting the UV-B portion of the electromagnetic spectrum.

6.2 Toxicity of Sunscreens

Many studies have shown about toxicity of synthetic sunscreens. For example, it would be prudent not to apply oxybenzone to large surface areas of skin for extended and repeated periods of time because oxybenzone has low acute toxicity in animal studies yet little is know about its chronic toxicity and disposition after topical application in people (Hayden et al., 1997).

Dermatologists became aware that PABA was a fairly common sensitizer and that it tended to cross-sensitize with compounds of similar chemical structure both in contact with the skin and given as systemic drugs. Furthermore, continued exposure to chemicals of this type could lead to autoimmune responses especially systemic lupus erythematosus and dermatomyositis (Mackie and Mackie, 1997). Besides, Padimate-O or octyldimethyl PABA is harmless in the dark but mutagenic in sunlight because it attack DNA directly (Knowland et al., 1997). Titanium dioxide has been an unsafe physical sunscreen because it reflects and scatters UV-B and UV-A in sunlight (Danford et al., 1995).

Sunscreens are widely used to avoid sunburn, photoageing, and skin cancer, and to minimise various photosensitivities and phototoxicities. However, the usage of synthetic sunscreens in cosmetic has been limited because of their toxicity.

7. Evaluations of Lips Using Instruments

7.1 Skin Hydration Measurement (Gall and Chappuis, 1994)

The Corneometer CM 825® (Courage-Khazaka, Germany) was used to measure the skin hydration in this study. The measurement was based on the physical principle of a common capacitor; two metal plates electrically insulated by a medium that acts as a dielectric. An excess electron is built up on one plate (negative charge) and an electron deficiency (positive charge) on the other plate. This condition will remain unchanged even when the voltage source is removed. The dielectric constants of keratin and epidermal lipids are very small compared with that of water. Therefore the dielectric constant of the stratum corneum is principally determined by its level of hydration: the greater the water content, the larger the dielectric constant. The corneometer is an apparatus with a probe that is placed in contact with the skin. The probe acts as a capacitor, in which the dielectric material is the skin on which it is applied. The capacitance thus measured is proportional to the dielectric constant of the skin, and varies according to its state of hydration. The device measures capacitance in arbitrary units, which in theory are proportional to stratum corneum water content. In practice, owing to the absence of a physical significance of this unit of measurement, this technique is confined to the measurement of variation in stratum corneum hydration between initial and final states (before and after). The advantages of this device are its simplicity of operation and reproducibility. Its main disadvantage is the artefactual increase in the dielectric constant of the skin due to the presence of electrolytes.

7.2 Lip Pigmentation Measurement (Thibodeau and D'Ambrosio, 1997)

Pigmentation is one of the most evident factors in distinguishing the main geographical races. The amount of melanin in the keratinocytes determines the pigmentation of the skin and hair (Farinelli et al., 1994).

In this study, a narrow-band reflectance spectrophotometer (MEXAMETER® MX 18) was used to measure melanin and hemoglobin pigmentation in the lips. The instrument consists of 2 diodes that emit light peak bands centered at 568 nm for hemoglobin and 660 nm for melanin. The instrument is portable, non-invasive, has a rapid processing time and is fitted with a 5 mm diameter probe which allows for the measurement of relatively small surface areas. Reflectance data are automatically transformed into numerical indices for both erythema and melanin. The indices, which increase in value with increasing levels of erythema (hemoglobin) and melanin pigmentation, are defined as:

Erythema index = $100 \times \{\log_{10}(/r_{568\ nm}) - \log_{10}(1/r_{655\ nm})\}$

Melanin index = $100 \times \log_{10}(1/r_{655\ nm})$

r = intensity of reflected light at the specified wavelength

8. An Instrument for *In Vitro* Determinations of Sun Protection Factor (Anderson et al., 1997)

Sun protection factor (SPF) is the definitive measure of the effectiveness of any sunscreen product. *In vivo* SPF testing using human subjects is time-consuming and expensive. Although this should always be carried out on the finalized products before they are marketed, it is not feasible to measure every trial formulations during development of a new sunscreen product.

The method involves a spectroradiometric measurement, in which a tape substrate is used. The tape, Transpore (3M Industries), was chosen because it is UV-transparent and has a textured surface that distributes topically applied radiation transmitted through the substrate, with and without sunscreen applied is determined automatically by recording photocurrent in 5-nm steps from 290 to 400 nm.

The instrument used (Figure 2.16) comprises a continuous UV-VIS source, color compensating filters, diffusion plates, a grating monochromator, and a photomultiplier detector. UVA and UVB radiation is provided by a 75-W xenon arc lamp. Both the operation of the scannings monochromator and the collection of UV light transmission readings are controlled by a computer. The computer also perform all the mathematical calculations necessary to determine the SPF and the UVA/UVB ratio.

Product is applied at 2 μL/cm^2 by applying a series of small dots over the surface of the tape and then spreading these evenly using a gloved finger.

Measurements of monochromatic protection factor (MPF) are made at each 5-nm increment between 290-400 nm. MPF is defined as the ratio of the detector signal intensity without sunscreen applied to the Transpore tape, to that with sunscreen applied to the tape:

$$\text{MPF}(\lambda) = \frac{\text{mean photocurrent at wavelength } (\lambda) \text{ without sunscreen applied}}{\text{mean photocurrent at wavelength } (\lambda) \text{ with sunscreen applied}}$$

The SPF is calculated using the following formula:

$$SPF = \sum_{290}^{400} E(\lambda)\varepsilon(\lambda) / \sum_{290}^{400} \frac{E(\lambda)\varepsilon(\lambda)}{MPF(\lambda)}$$

Where *E(λ)* is the spectral irradiance of terrestrial sunlight under defined condition (e.g. midday midsummer sunlight for southern Europe (latitude 40 N; solar zenith angle 20°; ozone layer thickness 0.305 cm); and ε(λ) is the relative effectiveness of UV radiation, at wavelength λ nanometers, in producing delayed erythema in human skin (the erythema action spectrum), and *MPF(λ)* is the monochromatic protection factor at wavelength λ nanometers.

The UVA/UVB ratio is also determined using this method. This is defined as the ratio between the mean absorbance in the UVA region (320-400 nm) and the mean absorbance in the UVB region (290-320 nm). This is one way of assessing the extent of UVA protection provided by a product, and is the basis of a rating system in the United Kingdom to indicate the level of UVA protection provided by a product.

However, the so-called Diffey method suffers from several shortcomings. The Transpore tape is of variable quality, with pore size varying from batch to batch and even between different parts of the same roll. This can lead to wide variations in results, because the pore size affects the spreading of the sample. Also, some cosmetic ingredients seem to react with either the tape itself or the adhesive on it, leading to inflated SPF values. The tape cannot mimic any chemical or biochemical effect that skin may exert on the efficacy of the product. Nevertheless, this in vitro method is a valuable screening tool for the formulator. Development samples can be measured rapidly (a single measurement takes less than 10 min), allowing the formulator to judge which formula modifications increase SPF.

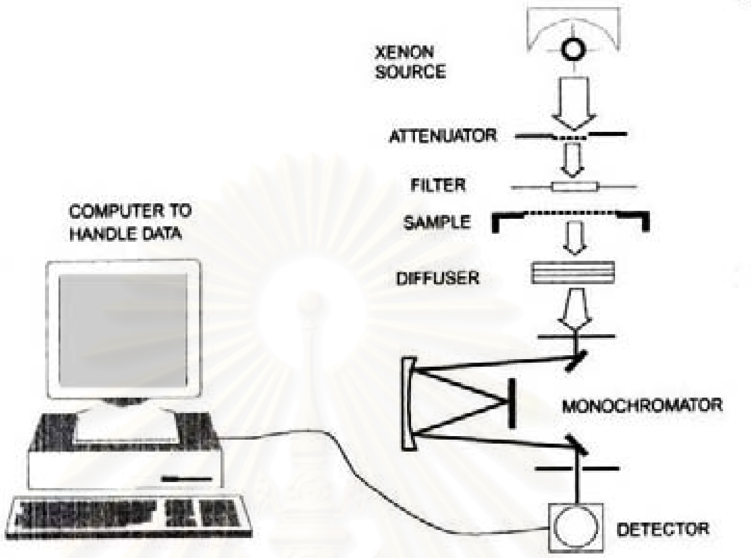


Figure 2.16 Optometrics

CHAPTER III

MATERIALS AND METHODS

Materials

1) γ-Oryzanol (Tsuno Rice Fine Chemicals Co., Ltd., Japan) Lot No. F03170
2) 3- tert-butyl-4-hydroxyanisole (BHA), (Fluka, Switzerland) Lot No. 428515/1
3) Acetonitrile HPLC grade (Labscan Asia Co., Ltd., Ireland) Batch No. 04060049
4) Anhydrous Lanolin (Srichand United Dispensary Co., Ltd., Thailand) Lot No. S30207
5) Beeswax (Supplied by Hong Huat Co., Ltd., Thailand) Lot No. P12064
6) Candelilla wax (Supplied by Hong Huat Co., Ltd., Thailand) Lot No. 2323470
7) Carnauba wax (Supplied by Hong Huat Co., Ltd., Thailand) Batch No. 4F1009
8) Castor oil (Thai Castor oil Industries Co., Ltd., Thailand) Lot No. 402301
9) Ceresin wax (S. Tong Chemicals Co., Ltd., Thailand) Lot No. P083127
10) Cetyl alcohol (S. Tong Chemicals Co., Ltd., Thailand) Lot No. FPG2631-2328
11) Cutina MD® (Cognis Thai Co., Ltd., Thailand) Lot No. 2669526
12) Eutanol G® (Henkel Co., Ltd., Germany) Lot No. 490952
13) Fuller's earth (Supplied by Chaiyo Agro Industry Co., Ltd.) Lot No. F142
14) Isopropanol HPLC grade (Labscan Asia Co., Ltd., Ireland) Batch No. 03090150
15) Isopropyl myristate (S. Tong Chemicals Co., Ltd., Thailand) Lot No. 495137
16) Methanol HPLC grade (Labscan Asia Co., Ltd., Ireland) Batch No. 04120015
17) Ozakerite wax (Supplied by Hong Huat Co., Ltd., Thailand) Batch No. 103029
18) Petrolatum® (Nam Siang Trading Co., Ltd., Thailand) Batch No. 295937
19) PM wax® (Supplied by Adinop Co., Ltd., Thailand) Lot No. 111104
20) Propyl paraben (Srichand United Dispensary Co., Ltd., Thailand) Lot No. L12011
21) Rice bran oil (Donated from Chaiyo Agro Industry Co., Ltd., Thailand) Lot No. 201203
22) SF 1318® (Supplied by Chemico Inter Corporation Co., Ltd., Thailand) Lot No. RF067
23) Silshine 151® (Supplied by Chemico Inter Corporation Co., Ltd., Thailand) Lot No. 041422

24) Spermaceti wax (Supplied by Hong Huat Co., Ltd., Thailand) Batch No. CD40550001
25) Stearyl alcohol (S. Tong Chemicals Co., Ltd., Thailand) Lot No. CJBF09
26) tert-Butylhydroquinone (TBHQ), (Fluka, Switzerland) Lot No. 112941

Apparatus

1) 743Rancimat® (Metrohm Co. Ltd., Switzerland)
2) Analytical balance (Model 1615, Sartorius, Germany)
3) CAT mixer (R18, CAT M. Zipper GmbH, Germany)
4) Centrifuge (Labofuge 610, Heraeus-Christ GMBH, Germany)
5) Corneometer® (CM 825, Courage + Khazaka electronic GmbH, Germany)
6) High Performance Liquid Chromatography (LC-10 AD, Shimadzu, Japan)
   - Autosampler (SIL-10A, Shimadzu, Japan)
   - Communications Bus Module (CBM-10A, Shimadzu, Japan)
   - UV detector (SPD-10A, Shimadzu, Japan)
   - Pumps (LC-10 A, Shimadzu, Japan)
7) Mexameter® (MX 18, Courage + Khazaka electronic GmbH, Germany)
8) Refrigerated incubator (FOC 225I, VELP Scientifica, Italy)
9) SPF 290s analyzer (Optometrics Ltd., USA)
10) Tensiometer (Model H1K-S, Tinius Olsen, USA)
11) Vortex mixer (Vortex Genies-2, Scientific Industries, Inc., USA)

Methods

1. Antioxidant Selection for Rice Bran Oil

1.1 Comparing the Antioxidant Efficacy between Butylated Hydroxyanisole (BHA) and Tertiary Butylhydroquinone (TBHQ) when Accelerated by 743 Rancimat®

Rice bran oil with and without antioxidant were studied of the antioxidant efficacy using 743 Rancimat® at accelerated temperature 120°C and exposed to a stream of air. Rice bran oil oxidation were compare between two different antioxidants; butylated hydroxyanisole and tertiary butylhydroquinone, to determine the difference in antioxidant activity. First, each antioxidant was added separately into

30 g of RBO. A series of concentrations for each antioxidant was 0.01%, 0.02%, 0.03%, 0.04%, 0.05%, 0.06%, 0.08% and 0.10% by weight. Each sample was determined the amount of γ-oryzanol using HPLC before accelerated by 743 Rancimat® as a stating point.

Then, 3.0 g of each sample were accelerated by 743 Rancimat®. This apparatus has worked following the standard of oil stability index (OSI). In the Rancimat Method, the samples were exposed to a stream of air at 120°C. The volatile oxidation products were transferred to the measuring vessel by the air steam and absorbed there in the measuring solution (distilled water). When the conductivity of this measuring solution was recorded continuously and an oxidation curve was obtained where point of inflection was known as “the induction time”. The long induction time means the long oxidative stability. The induction times were compared by one-way ANOVA followed by least significant difference (LSD) at a significant level (α) of 0.05.

After that, the remaining amount of γ-oryzanol was measured by HPLC. The remaining amount of γ-oryzanol and the induction time were used as the antioxidant selection criteria. Antioxidant that has the highest antioxidant activities (i.e. the highest remaining amount of γ-oryzanol and the longest induction time) at the low concentration was chosen.

1.2 Analysis of γ-Oryzanol by HPLC Method (Piyawon Chaisena, 2003)

HPLC method was used to quantify γ-oryzanol because of its specificity and high sensitivity.

1.2.1 Rice Bran Oil Sample Preparation

Samples of RBO were dilute with the mixture of acetonitrile, methanol, isopropanol and water (45:45:5:5), to achieve 1% v/v and were vigorously vortexed for 5 min. Slightly emulsions were formed and could be broken by centrifugation at 3000 rpm for 30 min. Aliquots were taken for HPLC analysis. These conditions allowed for the extraction of the tocol and oryzanol components from the oil into the solvent (acetonitrile : methanol : isopropanol : water) and the remaining oil droplet at the lower phase containing high concentrations of the more nonpolar triglycerides, which could be easily discarded. One hundred microliters of the supernatant was diluted with mobile phase to 10 mL.

1.2.2 Chromatographic Conditions

The HPLC conditions for the analysis of γ-oryzanol were shown as following:

Column	: μ-Bondapak (C18, 10 μm)
Mobile phase	: 70:30 methanol : isopropanol
Injection volume	: 50 μL
Flow rate	: 1.0 mL/min
Detector	: UV detector at 325 nm
Temperature	: ambient
Run time	: 10 min

The mobile phase was thoroughly mixed, filtered through 0.45 μm membrane filter and then degassed by sonication for 30 min prior to use.

2. Lipstick Base Formulation

Morshauser and Kalish's formulation was used as a reference lipstick base and modified accordingly to obtain the desired lipstick base. (deNaverre, 1975) The formulation is illustrated in Table 3.1.

Table 3.1 Morshauser and Kalish's lipstick formulation

Ingredients	% by weight
castor oil	65
isopropyl myristate	5
anhydrous lanolin	10
beeswax	7
candelilla wax	7
carnauba wax	3
ozokerite	3

The desired lipstick base was developed where many considerations were taken to account such as the dropping point, mold release property and other physical characteristics which will be described in details in section 3. The selected lipstick base was then incorporated with RBO. Stepwise development of lipstick base scheme is presented in Figure 3.1.

Selected waxs and amount of total waxs

⇩

Selected fat

⇩

Adjusted amount of wax and fat

⇩

Select oils and amount of oils

⇩

Selected the best formulation

Figure 3.1 Lipstick development scheme

Lipsticks were prepared as followings. First, waxes and fats were melted beginning with the highest melting point wax or fat to the lowest melting point one while being agitated. Second, 60ºC heated oil portion was added to melted waxes and brought to 80ºC to obtain a homogeneous mixture and deaerated. After that, the melted lipstick base was cooled to 70ºC and poured into the prepared molding cavities which were kept warm at 45ºC. The lipstick base was allowed to cool at room temperature for 35 minutes until it solidified at the top of the cavities and excess was scraped off. Lipstick bullets were then stripped off from the molds. The prepared lipstick base were evaluated and selected according to the selection criteria which are described as followings:

1. Good physical appearances and skin-feel.
2. Good physical properties referred to dropping point test, sweating test, drooping test and break strength test.

The criteria for choosing lipstick was shown in Table 3.2.

Table 3.2 The criteria for choosing lipstick

Physical Test	Criteria
Sweating test	no sweating
Drooping test	not droop more than 5 mm. at 45°C
Dropping test	dropping point higher than 60°C
Break Strength test	breaking point higher than 2 N

2.1 Wax Selection and Amount of Total Waxes

2.1.1 Wax Selection

Wax is considered one of a critical lipstick components. Each wax must work in conjuction with others to achieve the desired melting point and thermal stability of the finished product (deNaverre, 1975). Mixtures of different waxes were used in this part because a single wax may not exhibit the good quality of finished product (Zanotti, 1998). From Morshauser and Kalish's formulation (Table 3.1), 20% by weight of total amount of original waxes were replaced by several waxes and the details were presented in Table 3.3.

2.1.2 Determination of Suitable Amount of Total Waxes

Since certain oils provide more emollient and moisturizing effects, the amount of waxes used in 2.1.1 (20% by weight) would be reduced to allow more oil to be used. Using a system with high efficiency waxes allows a system to be more moisturizing and more emollient but still held on good physical appearances and good physical properties (Cadicamo and Cadicamo, 1981). The amounts of total waxes were studied at 10%, 12%, 14%, 16% and 18% by weight which were shown in Table 3.4. Lipstick bases were prepared. The formulation with the least amount of total waxes was chosen while both physical appearances and good physical properties still maintained.

Table 3.3 Compositions of lipstick with several waxes

Ingredient	Formula No.																							
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
Oil																								
castor oil	65	65	65	65	65	65	65	65	65	65	65	65	65	65	65	65	65	65	65	65	65	65	65	65
IPM	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
Fat																								
anhydrous lanolin	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
Wax																								
beeswax	7	7	7	7	7	7	4	-	7	7	7	7	7	5.5	7	7	7	7	7	7	7	6	6	6
carnauba wax	3	3	3	3	3	3	3	3	3	4	3	4	4	7	3	3	3	3	3	3	3	3	3	3
candelilla wax	7	7	7	7	7	7	7	7	8	5	8	5	5	-	7	7	7	7	7	7	6	6	6	6
ozokerite wax	3	-	-	-	-	-	3	3	-	-	-	-	-	7.5	-	1.5	-	-	-	-	-	2	3	2
ceresin wax	-	3	-	-			3	7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
cetyl alcohol	-	-	3	-	-	-	-	-	2	4	-	-	-	-	1.5	1.5	-	2	1	-	1	1.5	-	-
stearyl alcohol	-	-	-	3	-	-	-	-	-	-	2	4	-	-	-	-	-	-	-	-	-	-	-	-
microcrystalline	-	-	-	-	3	-	-	-	-	-	-	-	-	-	1.5	-	-	-	-	-	-	-	-	-
spermaceti wax	-	-	-	-	-	3	-	-	-	-	-	-	4	-	-	-	-	-	-	1	1	-	-	1
PM wax®	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3	1	2	2	2	1.5	2	2

Table 3.4 Compositions of lipstick with different amount of waxes

Ingredient	Formula No.									
	25	26	27	28	29	30	31	32	33	34
Oil										
castor oil	75	73	71	69	67	75	73	71	69	67
IPM	5	5	5	5	5	5	5	5	5	5
Fat										
anhydrous lanolin	10	10	10	10	10	10	10	10	10	10
Wax										
beeswax	3.5	4.2	4.9	5.6	6.3	3.0	3.6	4.2	4.8	5.4
carnauba wax	1.5	1.8	2.1	2.4	2.7	1.5	1.8	2.1	2.4	2.7
candelilla wax	3.5	4.2	4.9	5.6	6.3	3.0	3.6	4.2	4.8	5.4
ozokerite wax	-	-	-	-	-	1.5	1.8	2.1	2.4	2.7
ceresin wax	-	-	-	-	-	-	-	-	-	-
cetyl alcohol	-	-	-	-	-	-	-	-	-	-
stearyl alcohol	-	-	-	-	-	-	-	-	-	-
microcrystalline	-	-	-	-	-	-	-	-	-	-
spermaceti wax	0.5	0.6	0.7	0.8	0.9	-	-	-	-	-
PM wax®	1.0	1.2	1.4	1.6	1.8	1.0	1.2	1.4	1.6	1.8
% of total wax	10	12	14	16	18	10	12	14	16	18

2.2 Fat Selection

Several fats were chosen for this study such as anhydrous lanolin, petrolatum and Cutina MD®. Ten percent by weight of anhydrous lanolin was replaced by several fats. Single or combinations of fats was studied as shown in Table 3.5. Lipstick bases were prepared. The physical appearances and physical tests were evaluated. The lipstick base formulation with good physical appearances and passed physical tests was chosen for the next section.

Table 3.5 Compositions of lipsticks with several fats

Ingredient	Formula No.				
	35	36	37	38	39
Oil					
castor oil	69	69	69	69	69
IPM	5	5	5	5	5
Fat					
anhydrous lanolin	-	-	5	5	-
petrolatum	10	-	5	-	5
cutina MD®	-	10	-	5	5
Wax					
beeswax	4.8	4.8	4.8	4.8	4.8
carnauba wax	2.4	2.4	2.4	2.4	2.4
candelilla wax	4.8	4.8	4.8	4.8	4.8
ozokerite wax	2.4	2.4	2.4	2.4	2.4
ceresin wax	-	-	-	-	-
cetyl alcohol	-	-	-	-	-
stearyl alcohol	-	-	-	-	-
microcrystalline	-	-	-	-	-
spermaceti wax	-	-	-	-	-
PM wax®	1.6	1.6	1.6	1.6	1.6

2.3 Determination of Suitable Amount of Total Waxes and Fats

According to Thai Industrial Standard, lipstick should has dropping point above 60ºC which can be predetermined by solid content i.e., waxes and fats (Thai Industrial Standard, 1998). The amount of each waxes and fats were varied as shown in Table 3.6 and Table 3.7 respectively. Lipstick bases were prepared. The physical appearances and physical tests were evaluated. The lipstick base formulation with good physical appearances and passed physical tests was chosen for the next section.

Table 3.6 Compositions of lipsticks with varied amount of each wax

Ingredient	Formula No.					
	40	41	42	43	44	45
Oil						
castor oil	69	69	69	69	69	69
IPM	5	5	5	5	5	5
Fat						
anhydrous lanolin	-	-	-	-	-	-
petrolatum	5	5	5	5	5	5
cutina MD®	5	5	5	5	5	5
Wax						
beeswax	4.8	4.8	4.8	4.8	4.8	3.2
carnauba wax	1.6	2.4	1.6	1.6	1.6	2.4
candelilla wax	4.8	4.0	4.0	3.2	3.6	4.0
ozokerite wax	2.4	3.2	3.2	4.0	4.0	4.8
ceresin wax	-	-	-	-	-	-
cetyl alcohol	-	-	-	-	-	-
stearyl alcohol	-	-	-	-	-	-
microcrystalline	-	-	-	-	-	-
spermaceti wax	-	-	-	-	-	-
PM wax®	2.4	1.6	2.4	2.4	2.0	1.6

Table 3.7 Compositions of lipsticks with varied amount of each fat

Ingredient	Formula No.					
	46	47	48	49	50	51
Oil						
castor oil	69	69	69	69	69	69
IPM	5	5	5	5	5	5
Fat						
anhydrous lanolin	-	-	-	-	-	-
petrolatum	6	7	8	6	7	8
cutina MD®	4	3	2	4	3	2
Wax						
beeswax	4.8	4.8	4.8	4.8	4.8	4.8
carnauba wax	1.6	1.6	1.6	2.4	2.4	2.4
candelilla wax	3.6	3.6	3.6	4.0	4.0	4.0
ozokerite wax	4.0	4.0	4.0	3.2	3.2	3.2
ceresin wax	-	-	-	-	-	-
cetyl alcohol	-	-	-	-	-	-
stearyl alcohol	-	-	-	-	-	-
microcrystalline	-	-	-	-	-	-
spermaceti wax	-	-	-	-	-	-
PM wax®	2.0	2.0	2.0	1.6	1.6	1.6

2.4 Oil Selection

There are two types of oils were used i.e. vegetable oil and synthetic oils. Selected formulation from 2.3 was contained castor oil (vegetable oil) and IPM (synthetic oil). Several synthetic oils were used in lipstick formulations such as Eutanol G®, SF 1318® and Silshine151® with different properties. Where Eutanol G® provides skin moisturizing and less tackiness. SF 1318® exhibits good spreadibility, and Silshine151® is non volatile oil with high gloss. Therefore these three synthetic oils were used to substitute castor oil at suitable portion (details as follows).

2.4.1 Eutanol G® Substitution

Vary Eutanol G® concentrations (5.0%, 10.0% and 15.0% by weight) were used in lipstick base formulations as shown in Table 3.8.

Table 3.8 Compositions of lipstick for the selection of amount of Eutanol G®

Ingredient	Formula No.		
	52	53	54
Oil			
castor oil	64	59	54
IPM	5	5	5
eutanol G®	5	10	15
Fat			
petrolatum	8	8	8
cutina MD®	2	2	2
Wax			
beeswax	4.8	4.8	4.8
carnauba wax	2.4	2.4	2.4
candelilla wax	4.0	4.0	4.0
ozokerite wax	3.2	3.2	3.2
ceresin wax	-	-	-
cetyl alcohol	-	-	-
stearyl alcohol	-	-	-
microcrystalline	-	-	-
spermaceti wax	-	-	-
PM wax®	1.6	1.6	1.6

2.4.2 Silshine 151® and SF1318® Substitution

Silshine 151® and SF1318® were replaced Eutanol G® in selected formulation from 2.4.1 from 5.0% to 10.0% by weight as shown in Table 3.9.

Table 3.9 Compositions of lipstick for the selection amount of Silshine 151® and SF1318®

Ingredient	Formula No.		
	55	56	57
Oil			
castor oil	54	54	54
IPM	5	5	5
silshine 151®	5	-	5
SF 1318®	-	5	5
eutanol G®	10	10	5
Fat			
petrolatum	8	8	8
cutina MD®	2	2	2
Wax			
beeswax	4.8	4.8	4.8
carnauba wax	2.4	2.4	2.4
candelilla wax	4.0	4.0	4.0
ozokerite wax	3.2	3.2	3.2
ceresin wax	-	-	-
cetyl alcohol	-	-	-
stearyl alcohol	-	-	-
microcrystalline	-	-	-
spermaceti wax	-	-	-
PM wax®	1.6	1.6	1.6

Lipstick base were prepared. The physical appearances and physical tests were evaluated. The lipstick base formulation with good physical appearances and passed physical tests was chosen for RBO incorporation.

3. Physical Testings of Lipsticks

3.1 Break Strength Test

The strength of lipstick was measured using Tensiometer with standard cutting blade as shown in Figure 3.2. The method was as following: first, lipstick was inserted horizontally into stationary block. Then, lipstick was cut by the blade which moved downward in constant rate of 0.5N/15s until lipstick rupture. The measurements of all formulations were performed in triplicate.

Figure 3.2 Break strength test

3.2 Drooping Test

The lipstick was placed in holder and stored in an oven at constant temperature of 45ºC. After 24 hours, drooping or distortion should not be occurred.

3.3 Dropping Test

Lipstick was melted in casserole. Then, thermometer was dipped into melted lipstick and allowed to cool at room temperature. After that, thermometer was positioned above 75 mL of water inside 250 mL Erlenmeyer flask as shown in Figure

3.3. The whole setting was warmed using water bath untill reached drop point. The drop point is the point where the mass changes sufficiently from its gel point to a liquid.

Figure 3.3 Dropping test

3.4 Sweating Test

Sweating test was best determined by keeping the lipstick preferably in its own container, in a large (500 mL) dry closed transparent beaker as shown in Figure 3.4. The beaker was then stored in an oven at 45ºC for 24 hours after that was taken to room temperature while still in the closed beaker. Sweating was presented as a powder deposit on the lipstick when it cooled. The formulations which showed sweating were rejected.

Figure 3.4 Sweating test

4. Skin-feeling Evaluation

The prepared lipsticks were evaluated for their gloss, slipperiness, odor, stickiness and taste. Recorded scores were 1, 2, 3, 4 and 5 according to the degree of satisfaction from “least satisfied” (1) to “most satisfied” (5).

5. Determination of Suitable Amount of RBO with Regarding to Sunscreen Efficacy

5.1 The Preparation of Lipstick Containing RBO

Crude RBO was decolorized and deoderized by followings: Crude RBO 300 g was mixed with selected antioxidant from section 1 study by CAT R18 Mixer for 5 minutes. Fuller’s earth was selected to used as adsorbent with the ratio of 10 : 1.5 RBO:Fuller’s earth (Piyawon Chaisena, 2003). The mixture was stirred for 15 minutes and left at room temperature for one day. Finally, the mixture was filtered through Wattman No. 1 membrane filter to remove Fuller’s earth.

Various lipstick formulations containing RBO (Table 3.10) were prepared same way as lipstick base preparation. The physical appearance, physical tests, and sunscreen efficacy regarding to SPF measurement (details in section 5.2) were used as the selection criteria.

The criteria for selecting the lipstick formulation containing RBO were as followings:

1. The formulation with the highest SPF value
2. The formulation with good in physical properties
3. The formulation with aesthetic appeals such as high spread ability, good smell and and not greasy.

Table 3.10 Compositions of lipstick for the selection amount of RBO

Ingredient	Formula No.		
	58	59	60
Oil			
castor oil	45	36	27
RBO	9	18	27
IPM	5	5	5
silshine 151®	5	5	5
SF 1318®	5	5	5
eutanol G®	5	5	5
Fat			
petrolatum	8	8	8
cutina MD®	2	2	2
Wax			
beeswax	4.8	4.8	4.8
carnauba wax	2.4	2.4	2.4
candelilla wax	4.0	4.0	4.0
ozokerite wax	3.2	3.2	3.2
ceresin wax	-	-	-
cetyl alcohol	-	-	-
stearyl alcohol	-	-	-
Microcrystalline	-	-	-
spermaceti wax	-	-	-
PM wax®	1.6	1.6	1.6

5.2 Determination of SPF of Lipsticks Containing RBO by SPF-290s Analyzer

The SPFs of prepared sunscreen lipsticks were evaluated by SPF-290s analyzer (Figure 3.5). A reference (or baseline) was set by measuring the transmissions of UVB and UVA wavelengths (290-400 nm) through the transpore® tape which was placed in the incident beam. The reference preparation data would be used to normalize for wavelength which was depending on variable in the source, substrate, monochromator, and detector. Tested lipstick was weighted and loaded onto the transpore® tape in rows of small "dabs" or "spots" and was rubbed gently. An area of approximately 9.0X7.0 cm^2 should be completely covered with the tested sample or resulting in a 2 mg/cm^2 sample layer with even thickness. Transmissions of UVA and UVB wavelengths were done in similar ways as a reference run. When all runs were completed, the means of MPF values and their standard deviations were calculated for each wavelength. The SPF was then calculated from the MPF (Diffey and Robson, 1989).

Figure 3.5 SPF-290s analyzer

6. The Addition of γ-Oryzanol in Lipstick Containing RBO

The SPF values of lipsticks containing RBO were not significant higher than lipstick base; therefore, γ-oryzanol was added into lipstick containing RBO for higher sunscreen efficacy.

6.1 The Solubility of γ-Oryzanol in RBO and in the Oil Combinations Used in Lipstick Formulations

The solubility of γ-oryzanol in edible oils and fats is found to be very low. Thus, the γ-oryzanol solubility testing in edible oils was done. Excess γ-oryzanol was added into RBO or the combination of oils in three separated sets. Each sample was mixed on a vortex mixer for 10 minutes. Then, all sample were rotated by suspension mixer at 25°C for 72 hours or 84 hours. Slight emulsions may formed and could be broken by centrifugation at 3000 rpm for 30 minutes. Aliquots were analyzed for γ-oryzanol using HPLC analysis as described in section 1.2.

6.2 Determination of the Suitable Amount of γ-Oryzanol Added into Lipstick Containing RBO

Various γ-oryzanol concentrations (0.25%, 0.50%, 0.75%, 1.00%, 1.25%, and 1.50% by weight) were added into the selected lipstick containing RBO from the section 5.1. Lipsticks were prepared the same ways as before and SPF values were measured using SPF 290 analyzer. The formulation which exhibited the highest SPF value was selected.

7. Clinical Study of Lipsticks Containing RBO and RBO Plus γ-Oryzanol

The tests are carried out under medical supervision with the approval of the regional medical ethics committee.

7.1 Selection of Panelists

Forty-eight healthy female panelists were recruited according to the following criteria.

Inclusion criteria

1. Females at the age of 18-60.
2. Potential for full participation in the study.
3. Permission to write her informed consent before participating in the study.

Exclusion criteria

1. Allergic and/or hypersensitivity to cosmetics.
2. History of skin disease such as eczema and psoriasis on their faces.
3. Not on any medicines both orally and topically on her lips for at least one week before and during the study.

A questionnaire was used to inquire the information presented in Appendix. The panelists were allowed to quit the study at any time. Once the allergic or hypersensitivity occurred, the subjects must quit the study and were monitored by a physician for any undesirable effects that might have occurred during the study.

7.2 Study Design

The recruited panelists were grouped randomly into 3 groups: control (without RBO), RBO and RBO plus γ-oryzanol. The panelists were asked not to use any lip coloring and other lip care products during the study. Uses of other cosmetics were permitted. At week 0, the measurements of lip hydration, melanin level, and hemoglobin level were taken and referred to as “baseline”. The panelists were asked to apply approximately 0.4 g per week of the test lipstick at home, four times a day in the morning, afternoon, evening after meals and at night for 6 weeks. They were instructed to carry the lipstick in their inside pocket and drink eight glasses of water per day. The measurement of lip hydration, melanin level, and hemoglobin level were taken in five times once a week for 6 weeks. The lip area where the measurements of lip hydration, melanin level, and hemoglobin level had been taken was at the middle of lower lip. The study took place in the evaluation room where temperature and humidity were controlled.

7.3 *In Vitro* Moisturization Measurements of Lipsticks

7.3.1 Determination of Lip Hydration

The probe of the Corneometer® CM 825 was placed at the middle of lower lip. The lip capacitance was read in five times at 5 adjacent spots and the average value was calculated and further used in statistical analysis.

7.3.2 Determination of Melanin and Hemoglobin Level

The probe of the Mexameter MX® 18 was placed at the middle of lower lip. The melanin and hemoglobin level were read in five times at 5 adjacent spots and the average values were calculated and further used in statistical analysis.

Repeated measures on each experimental unit provide information on the time trend of the response variable under different treatment conditions. Time trend can reveal how quickly the panelists respond to lipsticks or how long the lipsticks effects are manifest on the panelists of the study. Differences in trends among the lipsticks also can be evaluated by the split plot analysis.

7.4 Sensory Evaluation by Panelists

The panelists evaluated the ease of applying, odor, stickiness, taste and gloss on the lip after applying the test lipsticks. They also evaluated some changes in their lip. These included lip moisturizing, softness, color of lip and overall perception. The attributed were graded and recorded as 1, 2, 3, 4, and 5 according to the degree of satisfaction. Their details were as follows:

"easy to apply", +5	→	+1, "hard to apply"
" least tacky", +5	→	+1, "most tacky"
"least gloss", +5	→	+1, "most gloss"
"most skin moisturizing", +5	→	+1, "least skin moisturizing"
"most skin softness", +5	→	+1, "least skin softness"
"most satisfy with overall liking", +5	→	+1, "least skin moisturizing"

The graded scores were averaged and the 'mean score' was used in the comparison to find any differences between the control, RBO and RBO plus more γ-oryzanol lipsticks in provision of such attributes. Then, the significant differences between each pair of the lipsticks in provision of an attribute were determined using the nonparametric Wilcoxon's Mann Whitney test.

CHAPTER IV

RESULTS AND DISCUSSION

1. Antioxidant Selection for Rice Bran Oil

Although rice bran oil (RBO) contains highly unsaturated fatty acids and high content linolenic acid leading to oxidative deterioration, it has good oxidative stability due to the significant levels of natural antioxidants, such as oryzanol, phytosterols, squalene, tocotrienols and tocopherols (Rogers et al., 1993). Unfortunately, almost 83-95% of the original antioxidants contents were lost during refining (Kochhar, 2002). In order to prevent or retard oxidative deterioration, additional antioxidants have been widely used. They may act as free radical quenchers, reducing compounds, singlet oxygen scavengers and as pro-oxidant metals suppressor (Oldfield, 2002).

1.1 Comparing the Antioxidant Efficacy between Butylated Hydroxyanisole (BHA) and Tertiary Butylhydroquinone (TBHQ) when Accelerated by 743 Rancimat®

In this study, the comparison of antioxidant activity between BHA and TBHQ was performed under accelerated conditions using 743 Rancimat®. The induction times are shown in Table 4.1. RBO without antioxidant was used as control. The results showed that the induction times increased with the antioxidant concentrations. It was found that RBO with 0.10% TBHQ had the longest induction time where the long induction time means the long oxidative stability. From the induction time study, 0.10% TBHQ showed the longest oxidative stability. There were significant differences between the induction times of RBO with and without antioxidant. From the least significant difference (LSD), it was found that TBHQ and BHA have comparable antioxidant efficacy on rice bran oil at levels less than 0.04%. At levels greater than 0.04%, TBHQ is far more effective than BHA significantly. The induction times of RBO with 0.01% BHA and RBO with 0.01% TBHQ were not significantly different from the control ($p>0.05$). But the induction times of RBO with 0.02%-0.10% BHA and RBO with 0.02%-0.10% TBHQ were significantly different from the control ($p<0.05$). So that the concentration of antioxidant used in RBO should be more than 0.02% to be effective. Moreover, a typical antioxidant level in food products is 0.02% (Oldfield, 2002). But the expected shelf life of a food product is usually shorter than for a cosmetic product; and food products are typically stored in the refrigerator. Since oxygen exposure is the major cause of oxidation, the type of packaging and expected shelf life of the product should be taken into account when considering the use and level of an antioxidant. If the product has a large surface area that is exposed to oxygen on a regular basis and it must last a very long time, then the need of an effective antioxidant is greater and the level of antioxidant may need to be higher (Oldfield, 2002).

Table 4.1 The oxidative stability with TBHQ and BHA at concentrations up to 0.10% in rice bran oil

Formulation	Induction time (h) at 120°C			
	Sample 1	Sample 2	Sample 3	Mean±S.D.
RBO	7.25	7.33	7.34	7.31±0.05
RBO+BHA0.01%	7.33	7.62	7.64	7.53±0.17
RBO+BHA0.02%	7.84	7.96	8.12	7.97±0.14*
RBO+BHA0.03%	8.43	7.47	8.28	8.06±0.52*
RBO+BHA0.04%	8.36	7.97	8.21	8.18±0.20*
RBO+BHA0.05%	8.61	8.26	8.28	8.38±0.20*
RBO+BHA0.06%	8.12	8.32	8.18	8.21±0.10*
RBO+BHA0.08%	8.16	8.05	8.24	8.15±0.10*
RBO+BHA0.10%	8.10	8.26	8.30	8.22±0.11*
RBO+TBHQ0.01%	7.68	7.50	7.70	7.63±0.11
RBO+TBHQ0.02%	7.92	7.52	7.74	7.73±0.20*
RBO+TBHQ0.03%	8.37	7.55	8.02	7.98±0.41*
RBO+TBHQ0.04%	8.30	8.68	8.18	8.39±0.26*
RBO+TBHQ0.05%	9.05	8.40	9.02	8.82±0.37*
RBO+TBHQ0.06%	9.18	9.17	9.20	9.18±0.02*
RBO+TBHQ0.08%	9.25	9.30	9.36	9.30±0.06*
RBO+TBHQ0.10%	9.59	9.74	9.84	9.72±0.13*

* Significant difference ($p<0.05$)

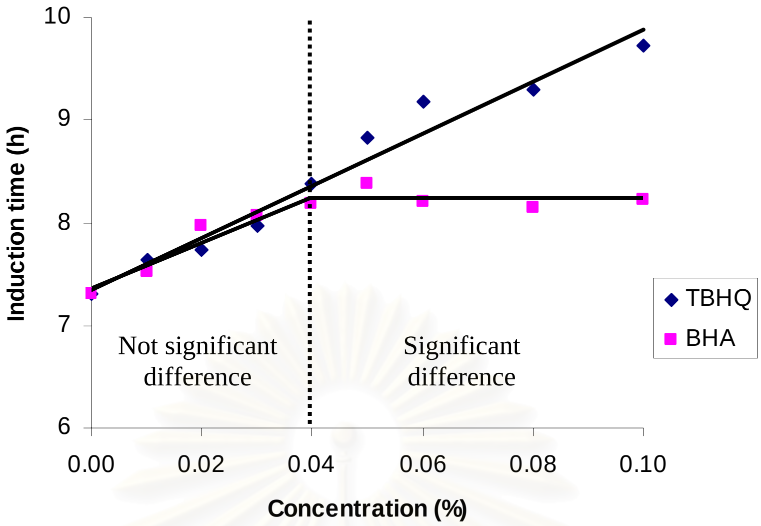


Figure 4.1 The induction times of RBO with antioxidants at 120°C

Figure 4.1 shows that the induction times increase with the TBHQ concentration and the same as for BHA. However, higher BHA concentration (above 0.04%) did not show any changing in the induction time.

1.2 Analysis of γ-Oryzanol by HPLC Method

Although the 743 Rancimat® was much less labour intensive and posing fewer variables, it had some weaknesses such as sample preparation errors and cleaning problems. High performance liquid chromatography (HPLC) was used to confirm the results of the oxidative stability. The chromatogram of γ-oryzanol in RBO is shown in Figure 4.2 and the results of γ-oryzanol remainings before and after tested by 743 Rancimat® are shown in Table 4.2.

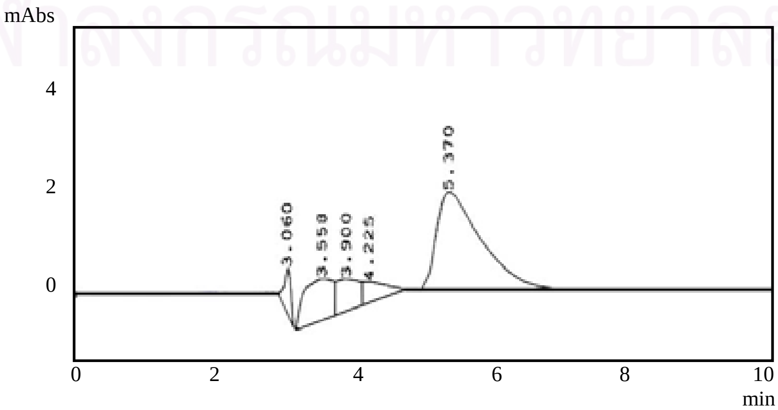


Figure 4.2 HPLC chromatogram of γ-oryzanol in rice bran oil

Table 4.2 The results of γ-oryzanol remainings before and after tested by 743 Rancimat®

Type	Inversely concentration (ng/ml)		Induction time(h)	Heating time(h)
	Before[a]	After[a]		
RBO	858.33 ± 51.49	158.15 ± 2.22	7.25	8.43
RBO+BHA0.01%	859.88 ± 60.26	172.47 ± 3.74	7.33	8.43
RBO+BHA0.02%	858.70 ± 11.35	357.02 ± 12.86	7.84	8.43
RBO+BHA0.03%	877.11 ± 26.93	462.46 ± 17.51	8.43	8.43
RBO+BHA0.04%	855.19 ± 4.58	426.33 ± 10.17	8.36	9.05
RBO+BHA0.05%	859.92 ± 16.58	485.11 ± 5.84	8.61	9.05
RBO+BHA0.06%	851.37 ± 68.43	311.53 ± 6.52	8.32	8.32
RBO+BHA0.08%	859.48 ± 25.46	410.97 ± 9.37	8.05	8.32
RBO+BHA0.10%	875.75 ± 10.36	517.26 ± 1.74	8.26	8.32
RBO+TBHQ0.01%	868.08 ± 6.73	194.54 ± 8.52	7.68	8.68
RBO+TBHQ0.02%	854.93 ± 13.42	384.89 ± 6.42	7.92	8.68
RBO+TBHQ0.03%	878.27 ± 36.82	473.36 ± 2.83	8.37	8.68
RBO+TBHQ0.04%	861.21 ± 32.44	551.23 ± 15.06	8.30	9.05
RBO+TBHQ0.05%	865.40 ± 12.71	637.43 ± 13.24	9.05	9.05
RBO+TBHQ0.06%	874.72 ± 44.23	393.86 ± 10.29	9.20	9.84
RBO+TBHQ0.08%	877.30 ± 7.50	462.06 ± 12.20	9.36	9.84
RBO+TBHQ0.10%	855.75 ± 14.04	620.63 ± 3.75	9.84	9.84

[a] Mean ± S.D., n = 3

The comparison of γ-oryzanol remainings between RBO plus BHA and RBO plus TBHQ after tested 743 Rancimat® has shown in Figure 4.3. The γ-oryzanol remainings were not related to each individual antioxidant concentration because each sample was heated at different time duration due to the machine operation.

The 743 Rancimat® is equipped with two heating blocks each with 4 measuring positions as shown in Figure 4.4. Each block can be individually heated. When the reaction reached the longest induction point among four sample cells, each block was shut down automatically. The time that begin oxidized sample until block was shut down is called 'heating time'. The heating time is shown in the last column of Table 4.2.

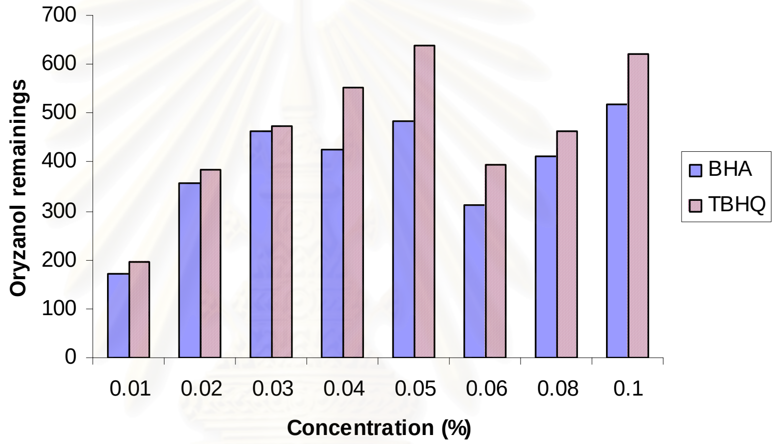


Figure 4.3 The comparison of γ-oryzanol remainings between RBO plus BHA and RBO plus TBHQ after tested by 743 Rancimat®

Even though a sample reached the induction time, it still remained heating where there was one that had not reached the induction time. Sample with long heating time means it has been oxidized by hot air for a long time. Thus the γ-oryzanol remaining of this sample would be reduced more than other sample which has short heating time. But in case of TBHQ, it found that there were a lot of γ-oryzanol remainings though they had long heating time. For example, 0.10% TBHQ was heated 9.84 hours but the γ-oryzanol remainings of 0.10% TBHQ was higher than the γ-oryzanol remainings of 0.10% BHA which was heated 8.32 hours as shown in Figure 4.3.

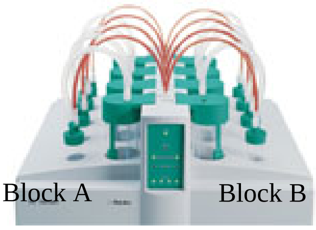


Figure 4.4 Heating blocks of 743 Rancimat®

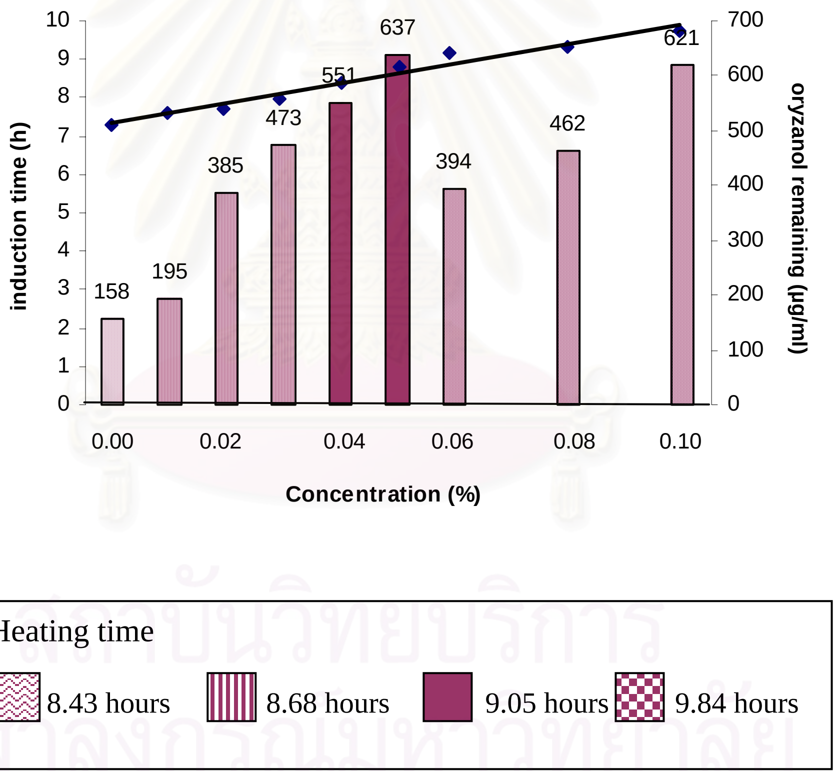


Figure 4.5 Induction times and γ-oryzanol remainings of RBO plus TBHQ

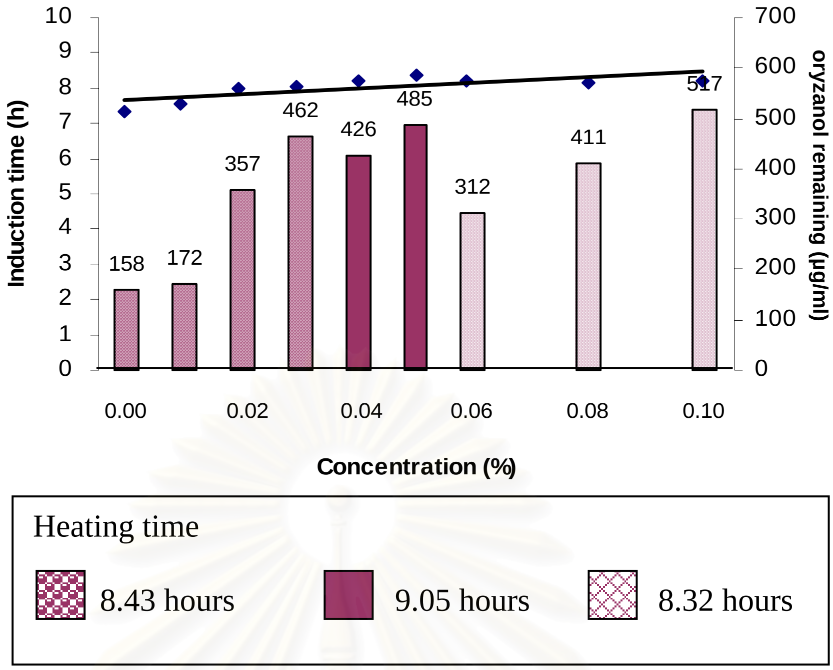


Figure 4.6 Induction times and γ-oryzanol remainings of RBO plus BHA

Figure 4.5 and Figure 4.6 show the relation of induction time and γ-oryzanol remainings of TBHQ and BHA respectively. The induction time is shown in line graph and the γ-oryzanol remaining is shown in bar chart. The same filled pattern of bar shows the same heating time.

The results showed that TBHQ was the premier antioxidant for vegetable oil including RBO than BHA which correlates very well with other studies (Steab et al.,1998; Oldfield, 2000).

In agreement with Steab et al (1998), referring to the efficacy comparison study between TBHQ, PG and α-tocopherol in evening primrose oil, TBHQ had the highest antioxidant efficacy. In addition, TBHQ was found to be the most effective antioxidant for soybean oil in the oven test when compared with rosemary extract and BHA+BHT (Almeida-doria and Regitano-D'Arce, 2000).

Furthermore, odor and taste should be considered before selecting the antioxidant for lipstick preparation. Among these antioxidants, TBHQ has smell better than BHA (Oldfield, 2002). When consider the antioxidant efficacy and physical properties (odor and taste), 0.10%TBHQ was selected for further study.

2. Lipstick Base Formulation

With the ability of so many new materials in recent years, the comparative simple formulation of classic lipsticks has become more complex and requires more study and experimentation. The new materials were added in the formulas as replacements for the traditional ingredients.

2.1 Wax Selection and Amount of Total Waxes

Wax is an important component in the formulation of stick systems. The most efficient wax system simply means wax or waxes which can produce the hardest structure with the least amount used. In order to choose the most efficient wax system, several attempts to reduce the total percentage of waxes were made without sacrificing structure. This reduction of waxes, allows more oils to be used where certain oils produce emolliency and moisturizing. On the other hand, using a system with high efficiency waxes allows a system to be more moisturizing and more emollient (Cadicamo and Cadicamo, 1981).

2.1.1 Wax Selection

The skin-feel, molding and physical property results are presented in Table 4.3. Formula 1 through 24 showed the effects of various waxes on a lipstick system. Lipsticks containing spermaceti wax 3% and 4% by weight (Formula 6 and Formula 13, respectively) gave good skin feel but they are too soft and have very low dropping point. The possible reason was that spermaceti wax has lowest melting point (53.5°C) when compare with other waxes. Lipsticks containing ceresin wax (Formula 7 and 8) were quite difficult to mold. Lipsticks containing stearyl alcohol and cetyl alcohol (Formula 9 through 12) showed dull looking and low dropping point. Lipstick containing cetyl alcohol at 4% (Formula 10) had the lowest breaking point and not passed the criteria as shown in Figure 4.7. This finding is consistent with Schlossman (2002) that stearyl alcohol and cetyl alcohol are higher alcohols which used to provide creamy texture at lower concentration. They can dull the finished lipstick and the associated film on application. In addition, their lower melting points may soften the stick.

Formula 17 through 24 showed the effects of PM wax® on a lipstick system. The use of oxidized polyethylene wax and oxidized microcrystalline wax greatly improves the wax lattice stability, which is reflected in the reduction of sweating and also in the better and longer lasting gloss obtained (Dweck, 1981) PM wax® contains both oxidized polyethylene wax and oxidized microcrystalline wax with melting point of 80°C. It showed high gloss and produced the hardest structure with the least amount used. With all these superb properties, PM wax® was selected to improve the lipstick characteristics.

Lipstick containing 3% by weight PM wax® (Formula 17) met good stability requirements and showed high gloss while lipstick containing 1% by weight PM wax® (Formula 18) had better skin-feel compared with Formula 17 but its dropping point did not meet the standard requirement. Formula 19 through 24 met good stability requirements and good skin-feels but Formula 20 and 23 showed better skin-feel than other formulas. From this study, Formula 20 and 23 were selected for further study.

Table 4.3 The properties of lipsticks containing various waxes

Testing		**Formula No.**					
		1	**2**	**3**	**4**	**5**	**6**
Skin-feel							
Gloss[a]		5	3	4	3	2	4
Slipperiness[a]		3	3	3	3	2	4
Odor[a]		4	2	3	3	3	4
Softness[a]		3	3	3	3	3	3
Tackiness[a]		3	3	3	3	2	3
Taste[a]		3	3	3	3	3	3
Smearing[b]		x	x	x	x	x	x
Molding[c]		3	3	3	3	3	3
Physical Testing							
Dropping test (°C)	1	68.0	68.0	69.0	66.0	70.0	53.5
	2	70.0	69.0	69.5	65.0	67.0	54.5
	3	70.5	68.0	69.0	66.5	68.0	52.5
	$\overline{X}$	69.5	68.3	69.2	65.8	68.3	53.5*
Drooping test (cm.)	X_1[d]	1.6	1.5	1.7	1.5	1.5	1.7
	X_2[e]	1.6	1.5	1.5	1.5	1.4	1.7
	X_1-X_2[f]	0	0	0.2	0	0.1	0
Breaking test (N)	1	3.23	6.34	4.10	3.54	5.02	2.80
(at 30°C)	2	3.10	5.66	3.76	4.00	5.04	4.11
	3	3.42	4.89	3.22	3.40	4.58	3.90
	$\overline{X}$	3.25	5.63	3.70	3.65	4.88	3.60
Sweating test[g]		x	x	x	x	x	x

Table 4.3 The properties of lipsticks containing various waxes (continued)

Testing		Formula No. 7	8	9	10	11	12
Skin-feel							
Gloss[a]		2	4	3	4	2	1
Slipperiness[a]		3	2	3	4	4	4
Odor[a]		3	3	3	3	2	3
Softness[a]		3	2	3	3	3	3
Tackiness[a]		3	4	3	3	3	3
Taste[a]		3	3	3	3	3	3
Smearing[b]		x	x	x	x	x	x
Molding[c]		3	1	3	1	3	3
Physical Testing							
Dropping test (°C)	1	68.0	64.0	70.0	70.0	68.0	67.0
	2	65.0	65.0	69.0	70.0	69.0	68.0
	3	66.5	65.0	68.0	70.0	67.0	68.0
	$\overline{X}$	66.5	64.7	69.0	70.0	68.0	67.7
Drooping test (cm.)	X_1[d]	2.2	1.7	1.8	1.7	2.0	2.1
	X_2[e]	2.0	1.5	1.5	1.5	1.5	1.4
	X_1-X_2[f]	0.2	0.2	0.3	0.2	0.5	0.7*
Breaking test (N) (at 30°C)	1	6.84	5.25	4.76	1.34	5.11	4.79
	2	5.96	5.87	4.62	1.43	5.63	4.66
	3	6.12	6.14	4.54	1.28	5.24	4.70
	$\overline{X}$	6.31	5.75	4.64	1.35	5.33	4.72
Sweating test[g]		x	x	x	x	x	x

Table 4.3 The properties of lipsticks containing various waxes (continued)

Testing		Formula No. 13	14	15	16	17	18
Skin-feel							
Gloss[a]		5	4	4	4	5	4
Slipperiness[a]		3	3	4	4	3	4
Odor[a]		4	3	4	4	4	4
Softness[a]		4	3	3	4	3	5
Tackiness[a]		3	4	4	4	3	4
Taste[a]		3	3	3	3	3	3
Smearing[b]		x	x	x	x	x	x
Molding[c]		5	1	3	3	4	4
Physical Testing							
Dropping test (°C)	1	51.0	70.5	69.0	71.0	71.0	57.0
	2	52.0	71.0	69.5	70.0	71.0	56.0
	3	53.0	70.0	69.0	70.5	70.0	56.0
	$\overline{X}$	52.0*	70.5	69.2	70.5	70.7	56.3*
Drooping test (cm.)	X_1[d]	1.4	1.8	1.6	1.6	1.7	1.6
	X_2[e]	0	1.6	1.5	1.4	1.5	1.3
	X_1-X_2[f]	1.4*	0.2	0.1	0.2	0.2	0.3
Breaking test (N) (at 30°C)	1	4.68	5.47	4.12	4.32	3.44	2.96
	2	4.87	4.84	4.04	4.32	4.00	2.63
	3	4.79	4.65	4.14	4.68	3.48	2.82
	$\overline{X}$	4.78	4.99	4.10	4.44	3.64	2.80
Sweating test[g]		x	x	x	x	x	x

Table 4.3 The properties of lipsticks containing various waxes (continued)

Testing		Formula No. 19	20	21	22	23	24
Skin-feel							
Gloss[a]		4	4	3	4	4	4
Slipperiness[a]		4	4	3	4	4	4
Odor[a]		4	4	4	4	4	4
Softness[a]		3	4	4	3	5	3
Tackiness[a]		4	4	4	4	4	4
Taste[a]		3	3	3	3	3	3
Smearing[b]		x	x	x	x	x	x
Molding[c]		4	4	4	4	4	4
Physical Testing							
Dropping test (°C)	1	64.0	66.0	67.5	67.0	71.5	64.0
	2	65.0	69.0	70.5	66.0	69.0	66.0
	3	64	65.0	65.5	66.0	70.5	68.0
	$\overline{X}$	64.3	66.7	67.8	66.3	70.3	66.0
Drooping test (cm.)	X_1[d]	1.7	2.0	1.7	1.7	1.7	1.7
	X_2[e]	1.5	1.7	1.5	1.5	1.6	1.6
	X_1-X_2[f]	0.2	0.3	0.2	0.2	0.1	0.1
Breaking test (N) (at 30°C)	1	2.81	3.97	3.46	3.03	4.08	3.39
	2	2.94	4.04	2.84	3.48	4.46	3.40
	3	2.92	3.85	3.22	5.27	3.78	3.56
	$\overline{X}$	2.89	3.95	3.17	3.93	4.10	3.45
Sweating test[g]		x	x	x	x	x	x

[a] very good, 5 ⟶ bad, 1

[b] no smear (x)

[c] mold well, 5 ⟶ difficult, 1

[d] height before drooping test (X_1)

[e] height after drooping test (X_2)

[f] The difference between height before and after drooping test (X_1-X_2)

[g] no sweat (x)

* not passed the criterion

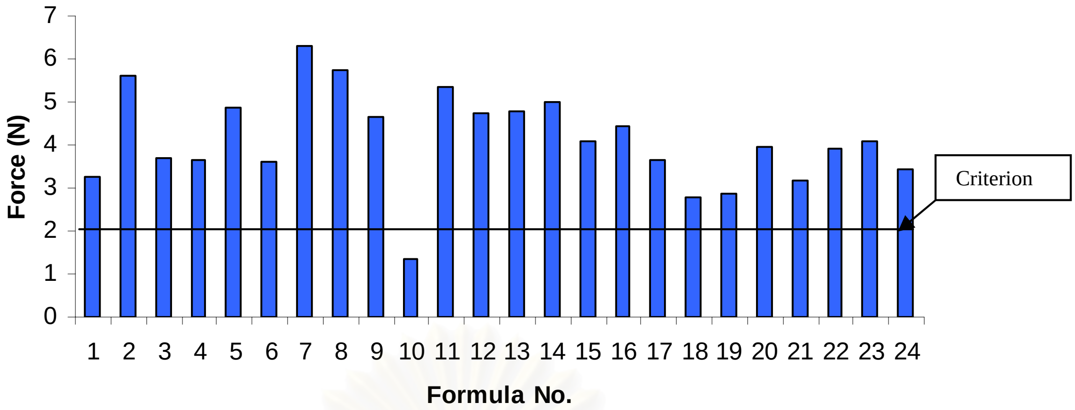


Figure 4.7 Break strength test results of Formula 1 through 24 at 30°C

2.1.2 Determination of Suitable Amount of Total Waxes

In this section, total amount of waxes would be reduced to allow more oils to be used. Formula 25-29 were developed from Formula 20 and Formula 30-34 were developed from Formula 23. Varied percentages of waxes were studied i.e., 10%, 12%, 14%, 16% and 18% by weight. The skin-feel, molding and physical property results are presented in Table 4.4. Sticks could be made in all lipsticks containing spermaceti wax (Formula 25-29). But lipsticks containing spermaceti wax at 10% (Formula 25) and 12% by weight (Formula 26) were too soft. Lipsticks containing ozokerite wax (Formula 30-34) could be formed when the amount of the total waxes not less than 12% by weight for example lipsticks containing 10% by weight ozokerite wax (Formula 30) could not form the stick. Figure 4.8 illustrates the break strength test results of Formula 25 through 34. Lipsticks containing 10%, 12% by weight spermaceti wax and 10%, 12% by weight ozokerite wax (Formula 25, 26, 30 and 31, respectively) did not pass the criteria of break strength test. Lipsticks containing 16%, 18% by weight ozokerite wax (Formulas 33 and 34) were much stronger than lipsticks containing 16%, 18% by weight spermaceti wax (Formulas 28 and 29).

Table 4.4 The properties of lipsticks containing various amounts of total waxes

Testing		Formula No.									
		25	26	27	28	29	30	31	32	33	34
% of total wax		10%	12%	14%	16%	18%	10%	12%	14%	16%	18%
Skin-feel											
Gloss[a]		5	5	4	4	3	-	4	4	4	4
Slipperiness[a]		4	4	4	3	3	-	4	4	4	3
Odor[a]		3	3	3	3	3	-	3	3	3	3
Softness[a]		4	4	3	3	3	-	4	4	4	3
Tackiness[a]		3	3	3	3	3	-	3	3	3	3
Taste[a]		3	3	3	3	3	-	3	3	3	3
Smearing[b]		x	x	x	x	x	-	x	x	x	x
Molding[c]		4	4	5	5	5	-	4	5	5	5
Physical Testing											
Dropping test (°C)	1	60.0	66.5	66.5	69.5	67.5	62.0	68.5	67.5	71.5	70.0
	2	62.5	66.5	67.0	69.0	66.5	62.0	69.0	66.5	67.0	70.0
	3	62.0	67.0	66.5	68.5	66.5	61.5	68.0	68.5	68.0	68.0
	$\overline{X}$	61.5	66.7	66.7	69.0	66.8	61.8	68.5	67.5	68.8	69.3
Drooping test (cm.)	X_1[d]	1.7	1.3	1.5	1.8	2.0	-	2.0	1.4	1.6	1.2
	X_2[e]	0	0	1.5	1.7	1.8	-	1.5	1.0	1.5	1.1
	X_1-X_2[f]	1.7*	1.3*	0	0.1	0.2	-	0.5	0.4	0.1	0.1
Breaking test (N) at 30°C	1	1.87	1.67	2.47	2.22	2.87	-	1.86	2.42	3.34	3.78
	2	1.57	1.59	2.14	2.65	2.32	-	1.78	2.52	3.17	4.11
	3	1.64	1.78	2.21	2.73	2.60	-	2.08	2.15	3.25	4.76
	$\overline{X}$	1.69*	1.68*	2.27	2.53	2.60	-	1.91*	2.37	3.25	4.22
Sweating test[g]		x	x	x	x	x	x	x	x	x	x

[a] very good, 5 ⟶ bad, 1

[b] no smear (x)

[c] mold well, 5 ⟶ difficult, 1

[d] height before drooping test (X_1)

[e] height after drooping test (X_2)

[f] The difference between height before and after drooping test (X_1-X_2)

[g] no sweat (x), - can not form stick, * not passed the criterion

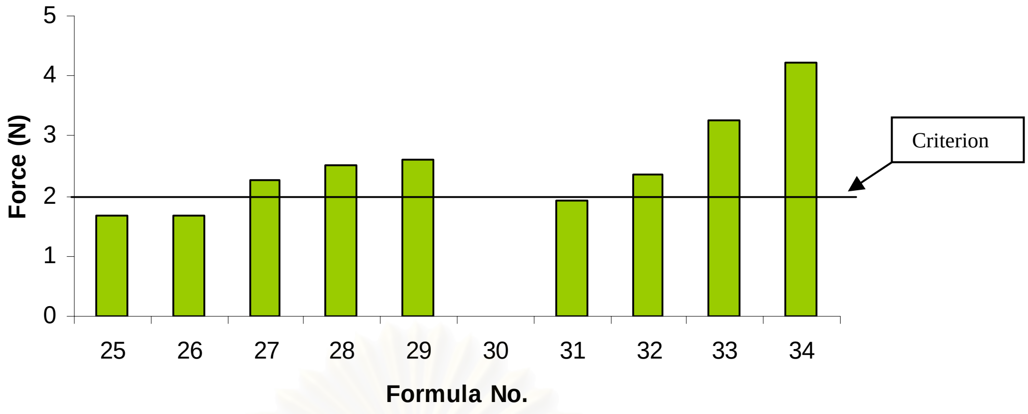


Figure 4.8 Break strength test results of Formula 25 through 34 at 30°C

Formulas 32 and 33 had better characteristic than other formula such as good molding property, high glossy, and good slippery. But formula 32 was selected for further study because the lowest amount of total waxes was needed to form the stick.

2.2 Fat Selection

Anhydrous lanolin is useful in preventing sweating and good emollient (deNaverre, 1975). But there are many reports related with its allergic and odor problems (Wakelin, 2001; Banham, 2002). To further develop the selected formulation from 2.1.2, ten percent (10%) by weight of anhydrous lanolin was replaced by petrolatum and Cutina MD® (glyceryl sterate). The skin-feel, molding and physical property results are presented in Table 4.5 The physical properties of all formulations were similar. Only lipsticks containing 10% petrolatum by weight (Formula 35) showed the weakest stick where the average breaking point was 1.98 N. However, Formula 35 had good slippery and less sticky but did not molded very well. Lipsticks containing 10% Cutina MD® by weight (Formula 36) were less glossy and too much drag. Lipsticks containing anhydrous lanolin / petrolatum at the ratio of 1:1 (Formula 37) and lipsticks containing anhydrous lanolin / Cutina MD® at the ratio of 1:1 (Formula 38) gave similarly good skin-feel. Lipsticks containing petrolatum / Cutina MD® at the ratio of 1:1 (Formula 39) gave better skin-feel than the other formula. Morover, it also showed good slippery, acceptable smell, high moisturizing, less sticky and mold well. So that Formula 39 was selected for further study.

Table 4.5 The properties of lipsticks containing various fats

Testing		**Formula No.**				
		35	**36**	**37**	**38**	**39**
Skin-feel						
Gloss[a]		3	2	4	4	3
Slipperiness[a]		4	2	3	3	4
Odor[a]		3	4	3	3	4
Softness[a]		4	2	4	4	4
Tackiness[a]		4	4	3	3	4
Taste[a]		3	3	3	3	3
Smearing[b]		x	x	x	x	x
Molding[c]		1	4	4	5	5
Physical Testing						
Dropping test (°C)	1	68.0	69.5	64.5	67.0	67.5
	2	68.0	70.5	65.0	68.0	66.0
	3	67.0	69.5	65.0	69.0	67.5
	$\overline{X}$	67.7	69.8	64.8	68.0	67.0
Drooping test (cm.)	X_1[d]	1.9	1.9	1.9	1.5	1.8
	X_2[e]	1.8	1.9	1.9	1.3	1.6
	X_1-X_2[f]	0.1	0	0	0.2	0.2
Breaking test (N)	1	2.14	3.60	2.72	2.71	2.92
(at 30°C)	2	2.12	3.32	2.98	2.66	3.13
	3	1.68	3.06	2.42	2.64	2.78
	$\overline{X}$	1.98*	3.33	2.71	2.67	2.94
Sweating test[g]		x	x	x	x	x

[a] very good, 5 ⟶ bad, 1

[b] no smear (x)

[c] mold well, 5 ⟶ difficult, 1

[d] height before drooping test (X_1)

[e] height after drooping test (X_2)

[f] The difference between height before and after drooping test (X_1-X_2)

[g] no sweat (x)

* not passed the criterion

2.3 Determination of Suitable Amount of Total Waxes and Fats

After waxes and fats were selected from previous sections, the amount of waxes and fats were varied to obtained the optimal amounts of both. Lipsticks were prepared using formula selected from 2.2. Formulas 40 through 45 were varied amount of each wax. All waxes in these formulas composed of bee wax, carnauba wax, candelilla wax, ozokerite wax, and PM wax®. The skin-feel, molding and physical properties of lipsticks with varied amount of each waxes are presented in Table 4.6. Similar findings were reported by others; carnauba wax in conjunction with ozokerite wax makes for better success which produces the molded stick toughness. Ozokerite without carnauba wax produces a soft, easily crushed mass (deNaverre, 1975). Since Formula 40 had the lowest amounts of ozokerite wax and carnauba wax, it gave the lowest breaking point compared with other formulas. There was no significant difference between other formulas in the physical properties. However, Formula 41 and Formula 44 showed better skin-feel than other formulas with good slippery, high glossy and mold well. Therefore, Formulas 41 and 44 were selected for next step.

The amount of fats were varied to obtain the optimal amount of each. Formula 46-48 were developed from Formula 44 and Formula 49-51 were developed from Formula 41. Formula 46-48 were using petrolatum/Cutina MD® at the ratio of 6:4, 7:3 and 8:2 respectively. For Formula 49-51, petrolatum/Cutina MD® were used in the same way. The skin-feel, molding and physical properties of lipsticks with varied amounts of each fat are presented in Table 4.7. When the amount of petrolatum increased, the breaking point trended to reduce as illustrated in Figure 4.10. Same skin-feel was showed in all formulas. Only Formula 51 showed better slip than other formulas.

Table 4.6 The properties of lipsticks with varied amount of each wax

Testing		Formula No. 40	41	42	43	44	45
Skin-feel							
Gloss[a]		3	4	3	3	4	3
Slipperiness[a]		3	4	3	3	4	3
Odor[a]		4	4	4	4	4	4
Softness[a]		4	4	4	4	4	4
Tackiness[a]		3	3	3	3	3	3
Smearing[b]		x	x	x	x	x	x
Taste[a]		3	3	3	3	3	3
Molding[c]		4	4	4	5	5	4
Physical Testing							
Dropping test (°C)	1	69.0	67.0	66.0	66.0	68.5	68.0
	2	67.0	66.0	68.0	69.0	68.0	68.0
	3	68.0	68.0	68.5	68.0	68.0	68.5
	$\overline{X}$	68.0	67.0	67.5	67.7	68.2	67.7
Drooping test (cm.)	X_1[d]	1.5	1.7	1.4	1.6	1.8	1.8
	X_2[e]	1.4	1.5	1.4	1.5	1.5	1.7
	X_1-X_2[f]	0.1	0.2	0	0.1	0.3	0.1
Breaking test (N)	1	2.27	3.30	3.79	3.77	2.70	3.26
(at 30°C)	2	3.46	4.33	4.58	4.32	2.86	3.34
	3	1.99	3.79	4.63	3.79	2.58	3.09
	$\overline{X}$	2.58	3.81	4.33	3.96	2.71	3.23
Sweating test[g]		x	x	x	x	x	x

[a] very good, 5 ⟶ bad, 1

[b] no smear (x)

[c] mold well, 5 ⟶ difficult, 1

[d] height before drooping test (X_1)

[e] height after drooping test (X_2)

[f] The difference between height before and after drooping test (X_1-X_2)

[g] no sweat (x)

Table 4.7 The properties of lipsticks with varied amount of each fat

Testing		Formula No.					
		46	47	48	49	50	51
Skin-feel							
Gloss[a]		3	3	3	3	3	3
Slipperiness[a]		3	3	3	3	3	4
Odor[a]		4	4	4	4	4	4
Softness[a]		4	4	4	4	4	4
Tackiness[a]		3	3	3	3	3	4
Smearing[b]		x	x	x	x	x	x
Taste[a]		3	3	3	3	3	3
Molding[c]		4	4	4	4	4	4
Physical Testing							
Dropping test (°C)	1	67.0	68.0	64.5	65.5	68.0	67.0
	2	66.0	66.0	65.0	66.0	66.5	67.0
	3	66.0	66.0	65.0	64.0	67.5	65.5
	$\overline{X}$	66.3	66.7	64.8	65.2	67.3	66.5
Drooping test (cm.)	X_1[d]	2.0	1.9	1.8	1.8	1.9	1.8
	X_2[e]	1.8	1.9	1.7	1.8	1.9	1.8
	X_1-X_2[f]	0.2	0	0.1	0	0	0
Breaking test (N)	1	2.94	2.94	2.37	2.77	3.06	2.50
(at 30°C)	2	3.20	2.56	2.79	3.08	2.57	2.59
	3	3.27	2.80	2.19	2.61	2.74	2.63
	$\overline{X}$	3.14	2.76	2.45	2.82	2.79	2.57
Sweating test[g]		x	x	x	x	x	x

[a] very good, 5 ⟶ bad, 1

[b] no smear (x)

[c] mold well, 5 ⟶ difficult, 1

[d] height before drooping test (X_1)

[e] height after drooping test (X_2)

[f] The difference between height before and after drooping test (X_1-X_2)

[g] no sweat (x)

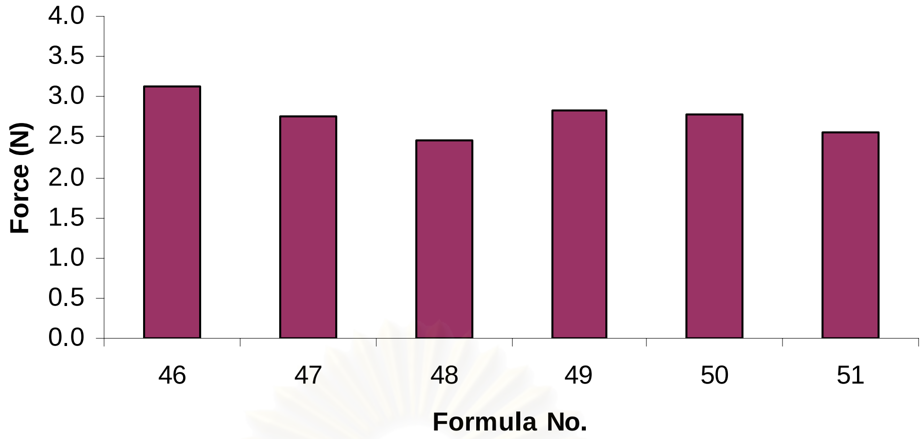


Figure 4.9 Break strength test results of Formula 46 through 51 at 30°C

2.4 Oil Selection

2.4.1 Eutanol G® Substitutions

Eutanol G® or octyldodecanol, is a clear, slightly yellow, odourless oil of low polarity. Due to its chemical structure, Eutanol G® is stable to hydrolysis and can therefore be used without any problems both in the alkaline and the acid ranges. Eutanol G® was partially used instead of castor oil for tackiness reduction with different concentrations: 5.0% , 10.0% and 15.0% by weight (Formula 52, 53 and 54 respectively). The skin-feel, molding and physical properties of lipsticks with varied amount of Eutanol G® were presented in Table 4.8. When the amount of Eutanol G® increased, the breaking point was reduced as illustrated in Figure 4.11. Formula 54 showed higher glossy and less tackiness than other formulas. So that formula 54 was selected for next study.

Table 4.8 The properties of lipsticks containing various amounts of Eutanol G®

Testing		Formula No. 52	Formula No. 53	Formula No. 54
Skin-feel				
Gloss[a]		3	3	3
Slipperiness[a]		3	4	5
Odor[a]		4	4	4
Softness[a]		4	4	4
Tackiness[a]		3	4	4
Smearing[b]		x	x	x
Taste[a]		3	3	3
Molding[c]		4	4	4
Physical Testing				
Dropping test (°C)	1	66.5	65.5	66.0
	2	66.5	65.5	65.0
	3	67.5	67.0	64.5
	$\overline{X}$	66.8	66.0	65.2
Drooping test (cm.)	X_1[d]	1.8	1.7	1.5
	X_2[e]	1.6	1.5	1.4
	X_1-X_2[f]	0.2	0.2	0.1
Breaking test (N)	1	3.14	2.82	2.68
(at 30°C)	2	2.96	2.48	2.49
	3	2.70	2.60	2.77
	$\overline{X}$	2.94	2.63	2.65
Sweating test[g]		x	x	x

[a] very good, 5 ⟶ bad, 1

[b] no smear (x)

[c] mold well, 5 ⟶ difficult, 1

[d] height before drooping test (X_1)

[e] height after drooping test (X_2)

[f] The difference between height before and after drooping test (X_1-X_2)

[g] no sweat (x)

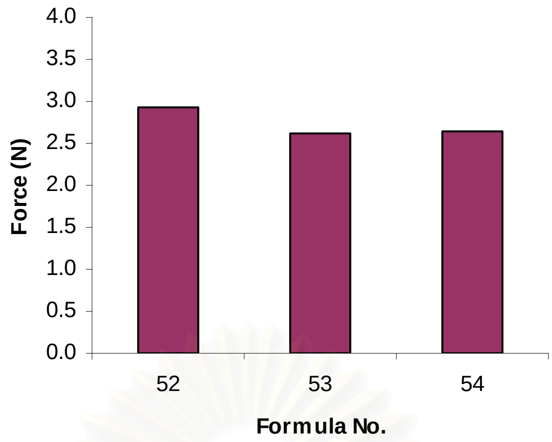


Figure 4.10 Break strength test results of Formula 52 through 54 at 30°C

2.4.2 Silshine 151® and SF1318® Substitutions

SilShine 151® is silicone resin. It is an excellent multi-functional, film-forming material that helps deliver visually perceptible high gloss to lips, boosts SPF performance and may increase the compatibility of organic sunscreens in a formulation. And SF 1318® is light yellow, copolymer of a silicone resin and organic ester of isostearic acid used for emollient/film former in protective skin, sunscreen, facial make-up and color cosmetic products. It exhibits good lubricity and spreadability. Silshine 151® and SF1318® were replaced Eutanol G in Formula 54 from 5.0% to 10.0% by weight. The skin-feel, molding and physical properties of lipsticks with varied amount of oils are presented in Table 4.9. Lipsticks containing SilShine 151® 5.0% by weight (Formula 55) were tackier than other formulas. Lipsticks containing SF 1318® 5.0% by weight (Formula 56) were slipper and less tackiness than Formula 55. Lipsticks containing both SilShine 151® 5.0% by weight and SF 1318® 5.0% by weight (Formula 57) showed higher skin-feel than other formulas. In addition, it was high gloss, good slip, less odor, less tackiness and no smearing. So that formula 57 was selected as lipstick base formulation and later be incorporated with RBO.

Table 4.9 The properties of lipsticks containing various amount of Silshine 151® and SF1318®

Testing		Formula No.		
		55	56	57
Skin-feel				
Gloss[a]		5	5	5
Slipperiness[a]		4	5	5
Odor[a]		4	4	5
Softness[a]		5	4	5
Tackiness[a]		4	5	5
Smearing[b]		x	x	x
Taste[a]		4	4	5
Molding[c]		4	4	5
Physical Testing				
Dropping test (°C)	1	65.5	67.0	65.0
	2	65.5	66.5	65.0
	3	64.0	67.0	65.0
	$\overline{X}$	65.0	66.8	65.0
Drooping test (cm.)	X_1[d]	1.9	1.9	1.8
	X_2[e]	1.8	1.8	1.7
	X_1-X_2[f]	0.1	0.1	0.1
Breaking test (N)	1	2.09	2.04	2.59
(at 30°C)	2	2.31	2.48	2.23
	3	2.19	2.22	2.34
	$\overline{X}$	2.20	2.24	2.39
Sweating test[g]		x	x	x

[a] very good, 5 ⟶ bad, 1

[b] no smear (x)

[c] mold well, 5 ⟶ difficult, 1

[d] height before drooping test (X_1)

[e] height after drooping test (X_2)

[f] The difference between height before and after drooping test (X_1-X_2)

[g] no sweat (x)

3. Determination of Suitable Amount of RBO with Regarding to Sunscreen Efficacy

RBO is found in several supplements and cosmetics products due to its natural vitamins or mineral contents, or its claimed skin benefits. Also undocumented is the belief that animal or plant-derived lipids may be safer than substances of comparable texture derived from petroleum products (Rieger, 1994). Moreover, RBO helps protect the skin against freckles and aging. In the literature, γ-oryzanol is said to have good absorption of ultraviolet and will help to protect against sunburn (DerMarderosian and Beutler, 2001).

Unfortunately, the crude rice bran oil is usually dark greenish-brown, depending upon the extraction method, bran condition, and composition. The colour pigments include carotenoids, chlorophyll and Millard browning products (Kochhar, 2002). Arquette et al. (1997) determined color values using a Lovibond Tintometer and found that RBO showed higher lovibond yellow than other natural cosmetic oils. Hence, RBO has limited use in colored products containing titanium dioxide and other pigments. Furthermore, RBO gives bad odor which is difficult to mask in cosmetic preparations. So that the incorporated amount of RBO was determined to produce a lipstick with high sun protection and acceptable physical appearance.

3.1 The Preparation of Lipstick Containing RBO

Various percentages of RBO were used (i.e., 9, 18, and 27%w/w). The skin-feel, molding and physical properties of lipsticks with varied amount of RBO are presented in Table 4.10. RBO showed high efficacy in improving skin-feel. Lipsticks gave high gloss, good glide and less tackiness in all formulations. Lipstick containing RBO 9.0% by weight (Formula 58) showed good physical appearance as well as lipsticks containing RBO 18.0% by weight (Formula 59). Lipsticks containing RBO 27.0% by weight (Formula 60) gave bad odor and unacceptable taste.

All formulas also passed the physical testings. When the amount of RBO increased, the breaking point was reduced as illustrated in Figure 4.11.

Table 4.10 The properties of lipsticks containing various amount of RBO

Testing		Formula No.		
		58	**59**	**60**
Skin-feel				
Gloss[a]		5	5	5
Slipperiness[a]		5	5	5
Odor[a]		3	3	2
Softness[a]		5	5	4
Tackiness[a]		5	5	5
Smearing[b]		x	x	x
Taste[a]		4	4	3
Molding[c]		5	5	5
Physical Testing				
Dropping test (°C)	1	64.0	64.5	63.5
	2	65.0	65.0	64.0
	3	64.5	64.0	63.0
	$\bar{X}$	64.5	64.5	63.5
Drooping test (cm.)	X_1[d]	1.8	1.8	1.7
	X_2[e]	1.7	1.7	1.7
	X_1-X_2[f]	0.1	0.1	0
Breaking test (N)	1	2.52	2.16	1.89
(at 30°C)	2	2.34	2.30	2.02
	3	2.40	2.43	1.76
	$\bar{X}$	2.42	2.30	1.89
Sweating test[g]		x	x	x

[a] very good, 4 ⟶ bad, 1

[b] no smear (x)

[c] mold well, 4 ⟶ difficult, 1

[d] height before drooping test (X_1)

[e] height after drooping test (X_2)

[f] The difference between height before and after drooping test (X_1-X_2)

[g] no sweat, x

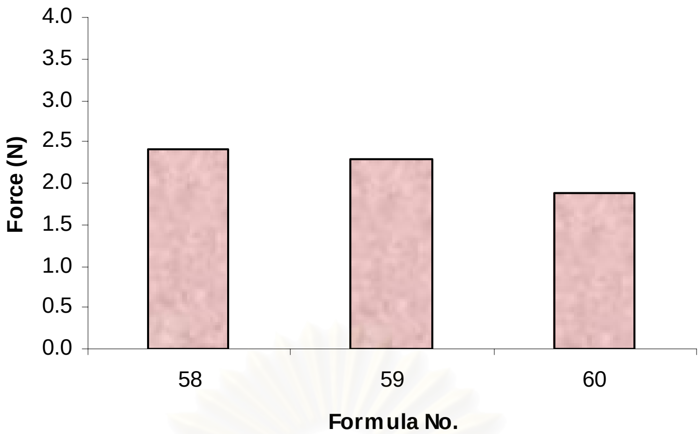


Figure 4.11 Break strength test results of Formula 58 through 60 at 30°C

3.2 Determination of SPF of Lipsticks Containing RBO by SPF-290s Analyzer

SPF-290s analyzer was used in SPF determinations for lipsticks containing RBO. Lipstick which showed high efficacy in sun protection and good physical appearance was selected for further study. The SPF values and UVA values of lipsticks containing varied percentages of RBO are shown in Table 4.11.

Table 4.11 SPF values and UVA values of lipsticks containing varied % by weight of RBO

Lipstick	SPF value[a]	UVA value[a]
Lipstick base	1.283 ± 0.072	1.100 ± 0.000
Lipstick with RBO 9% [b]	1.442 ± 0.079	1.158 ± 0.051
Lipstick with RBO 18%[b]	1.550 ± 0.067	1.167 ± 0.049
Lipstick with RBO 27%[b]	1.608 ± 0.138	1.233 ± 0.049

[a] mean ± S.D., n = 12

[b] % by weight

SPF valus and UVA values increased by increasing RBO or γ-oryzanol. In conclusion, lipstick with RBO 27% by weight (Formula 60) showed high efficacy in sun protection but it was too soft stick. The SPF values and UVA values of lipsticks with RBO 9% by weight (Formula 58) were less than lipsticks with RBO 18% by weight (Formula 59). Although, Formula 59 had higher % of RBO than Formula 58 but it showed acceptable odor and taste like Formula 58. So that Formula 59 was selected for further study.

4. The Addition of γ-Oryzanol in Lipstick Containing RBO

Normally, γ-oryzanol can be recovered from RBO at a level of 1 to 2 % (Scavariello and Arellano, 1998; Lai et al., 2005). On the other hand, γ-oryzanol was significantly reduced during the refining process of RBO. In order to maintain sunscreen efficacy, an additional γ-oryzanol was added to lipstick formulation.

4.1 The Solubility of γ-Oryzanol in RBO and in the Oil Combinations Used in Lipstick Formulations

The solubility of γ-oryzanol in edible oils or fats is very low. One report shows that the solubility of γ-oryzanol in oil is 1 g/100 g (oil) (Iijima and Sano, 1986). Thus, the solubility test of γ-oryzanol in the oil combinations used in lipstick formulation oil was determined before it was added into lipsticks. The results of this section confirmed that γ added -oryzanol would not excess the solubility of γ-oryzanol in. The results of solubility test show in Table 4.12.

Table 4.12 The γ-oryzanol remaining in solubility test

Replicate	Concentration of γ-oryzanol remaining (g/100g)			
	Oil combinations [a]		Rice Bran Oil	
	72 hr.	96 hr.	72 hr.	96 hr.
1	4.268	4.242	3.265	3.052
2	4.240	4.291	3.040	3.098
3	4.258	4.254	3.094	3.155
Mean ± S.D.	4.256 ± 0.014	4.262 ± 0.025	3.133 ± 0.118	3.102 ± 0.052

[a] The oil combination used in lipstick formulation contain castor oil, RBO, IPM, Silshine 151®, SF 1318®, and Eutanol G® at the ratio of 36:18:5:5:5:5.

The solubility of γ-oryzanol in 72 hr. and 96 hr. did not different; it confirms that was the solubility of γ-oryzanol in combination oil and RBO under studied conditions. From the presented results (Table 4.12), γ-oryzanol was more soluble in oil combinations than RBO. So that γ-oryzanol was dissolved in oil combinations before preparing the lipstick.

4.2 Determination of the Suitable Amount of γ-Oryzanol Added into Lipstick Containing RBO

Lipsticks containing RBO 18% by weight were added with γ-oryzanol at varying concentrations (0.25%, 0.50%, 0.75%, 1.00%, 1.25%, and 1.50% by weight). The SPF values of these lipsticks are illustrated in Table 4.13.

Table 4.13 The SPF values and UVA values of lipsticks in addition of γ-oryzanol

Lipstick		SPF value[a]	UVA value[a]
RBO (% by wt)	γ-oryzanol (% by wt)		
18%	-	1.550 ± 0.067	1.167 ± 0.049
18%	0.25%	1.975 ± 0.296	1.408 ± 0.144
18%	0.50%	2.158 ± 0.520	1.458 ± 0.144
18%	0.75%	2.450 ± 0.342	1.567 ± 0.144
18%	1.00%	2.642 ± 0.526	1.667 ± 0.277
18%	1.25%	3.525 ± 0.781	2.017 ± 0.319
18%	1.50%	3.725 ± 0.693	2.233 ± 0.337

[a] mean ± S.D., n = 12

The SPF values of lipsticks with RBO + γ-oryzanol at all concentrations were significant different from lipsticks containing only RBO ($p<0.05$). The average SPF value of lipsticks with RBO + 1.50% by weight γ-oryzanol was not significantly different from the one with RBO + 1.25% by weight γ-oryzanol ($p=0.343$). In the contrary, the average UVA value of lipsticks with RBO + 1.50% by weight γ-oryzanol was significantly different from the one RBO + 1.25% by weight γ-oryzanol. So that 1.50% by weight γ-oryzanol was selected to add into lipstick formulation.

From section 4.1, the solubility of γ-oryzanol in the oil combinations was found to be about 4.26% by weight. Formula 59 contains 74% by weight total oil after adding 1.50% by weight γ-oryzanol which is equivalent to 2.03% by weight of oil combinations. Thus, it may confirm that 1.50% by weight γ-oryzanol dissolved in the lipstick.

5. Clinical Study of Lipsticks Containing RBO and RBO Plus γ-Oryzanol

A questionnaire (Appendix E) was used to obtain information about prevalence of skin disorder, skin hypersensitivity, lip care product used and overall habit. Forty-eight panelists participated initially in this study. However, one panelist developed mild desquamation after using lipstick containing RBO. Some panelists were excluded because they broke the rule. Finally there were 45 panelists participating in the study or 15 panelists per group. Epstein and Simion (2001) proposed that usually ten or more panelists were enough to statistically differentiate the moisturizing effect. Panelists in group 1 obtained lipstick base, panelists in group 2 obtained lipstick with RBO, and panelists in group 3 obtained lipstick with RBO plus γ-oryzanol, respectively. All 45 panelists were healthy women aged from 18 to 59. The panelists could be grouped into 4 aged ranges: 18-25 (n = 20, 44.4%), 26-35 (n = 11, 24.4%), 36-45 (n = 7, 15.6%), and 46-59 (n = 7, 15.6%) while the age distribution between groups was not the same due to the randomization as seen in Figure 4.12.

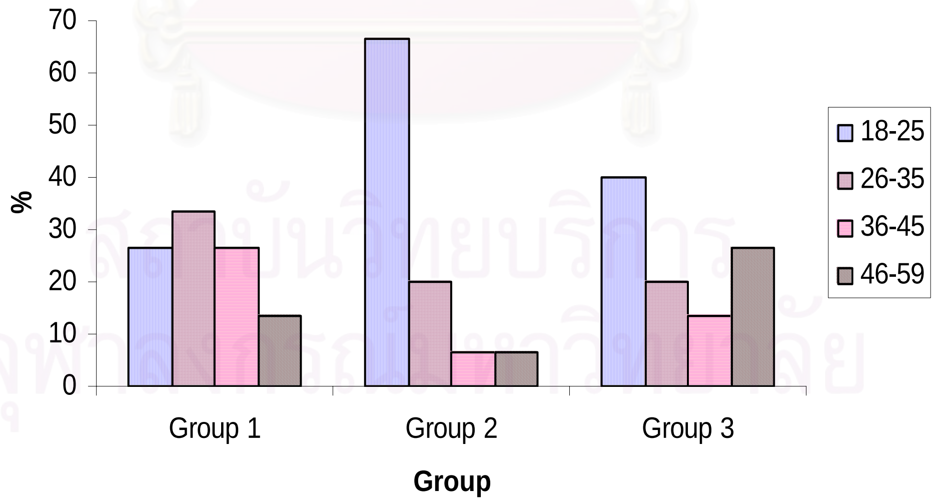


Figure 4.12 The age report of each group

The lip hydration, melanin level, and hemoglobin level were investigated after apply four times a day in the morning, afternoon, evening after meals and at night for 6 weeks using the Corneometer® CM 825 and Mexameter MX® 18, respectively. Each measurement was taken five times. The measurements of lip hydration, melanin level, and hemoglobin level was taken at the middle of lower lip.

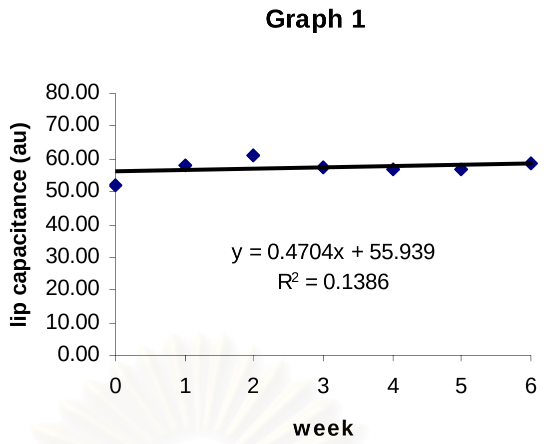
The lip conditioning properties of 3 lipstick groups were compared using the repeated measures (split-plot) designs. The repeated measures analysis provides information on the time trend of the response variable under different treatment conditions (Bolton, 1997). Time trends can reveal how quickly the units respond to treatment or how long the treatment effects are manifest on the units of the study. Differences in trends among the treatments also can be evaluated. The split plot analysis of variance mean squares can be used to test hypothesis about the treatment means and their interactions with time.

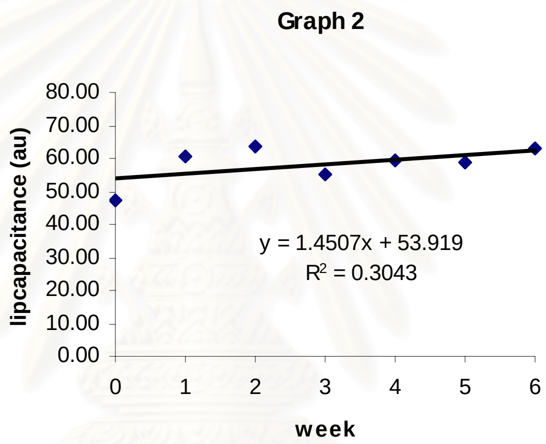
5.1 Determination of Lip Hydration

Table 4.14 and Figure 4.13 show the average lip capacitance of fifteen panelists for each lipstick.

Table 4.14 Average lip capacitances of 15 panelists (Mean±S.D.)

Week	Lip capacitance (au)		
	Group 1	Group 2	Group 3
0	52.20±12.53	47.04±10.24	50.23±10.07
1	57.91±9.31	60.62±9.44	57.92±12.15
2	61.24±10.77	63.53±11.28	60.58±10.52
3	57.68±7.47	55.41±10.63	60.40±10.49
4	56.79±9.72	59.34±9.30	58.27±9.43
5	56.85±9.01	58.71±9.86	62.48±8.37
6	58.78±6.71	63.25±11.82	60.82±11.69





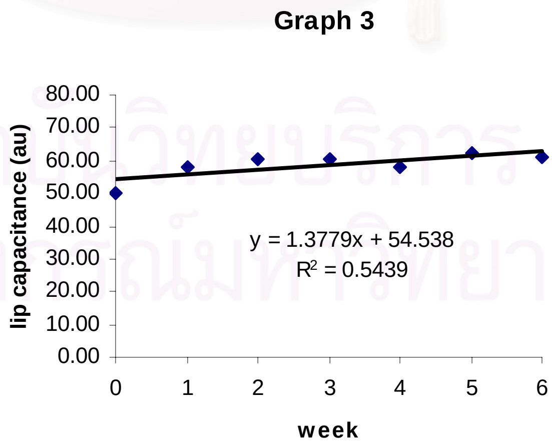


Figure 4.13 Average lip capacitances of 15 panelists

Table 4.15 Split-plot analysis of variance for repeated measures from the hydration study in a completely randomized design

Source of variation	*Degrees of Freedom*	*Sum of Squares*	*Mean Square*	F_0	F_{table}
Total	314	36809.83			
Formulation	2	243.84	121.92	0.3943	3.23
Error(1) sub(trt)	42	12987.86	309.23		
Time	6	3875.14	645.86	8.7017*	2.10
trt*time	12	999.16	83.26	1.1218	1.83
Error(2)	252	18703.83	74.22		

* Significant difference compared with the baseline values

The application of all group of lipstick resulted in increases lip capacitance. The slopes of group 1, 2, and 3 from Figure 4.14 are 0.47, 1.45, and 1.38, respectively. These results showed that lipstick containing RBO (group 2) and lipstick containing RBO plus oryzanol (group 3) had higher efficacy in increased lip capacitance than lipstick base (group 1) almost three times of slope after using for 6 weeks. The lip capacitance value was used as the response variable for split-plot analysis of variance. It found that the post-treatment differences among lip capacitance of these three treatments did not show a significant difference ($F_{table} > F_0$) as shown in Table 4.15. But all treatments could increase lip capacitance significantly with time goes by.

5.2 Determination of Melanin Level

Table 4.16 and Figure 4.14 show the average melanin value of fifteen panelists for each lipstick.

Table 4.16 Average melanin values of 15 panelists (Mean±S.D.)

Week	Melanin value		
	Group 1	Group 2	Group 3
0	102.65±58.30	137.67±84.32	133.25±85.01
1	90.21±63.31	122.05±70.35	115.57±74.78
2	80.24±43.14	117.40±69.52	113.75±75.74
3	82.84±45.40	106.51±59.17	104.96±74.95
4	78.11±52.82	106.95±65.72	107.63±82.44
5	74.76±44.65	110.72±59.99	111.93±77.40
6	73.85±43.62	101.53±63.04	102.68±73.07

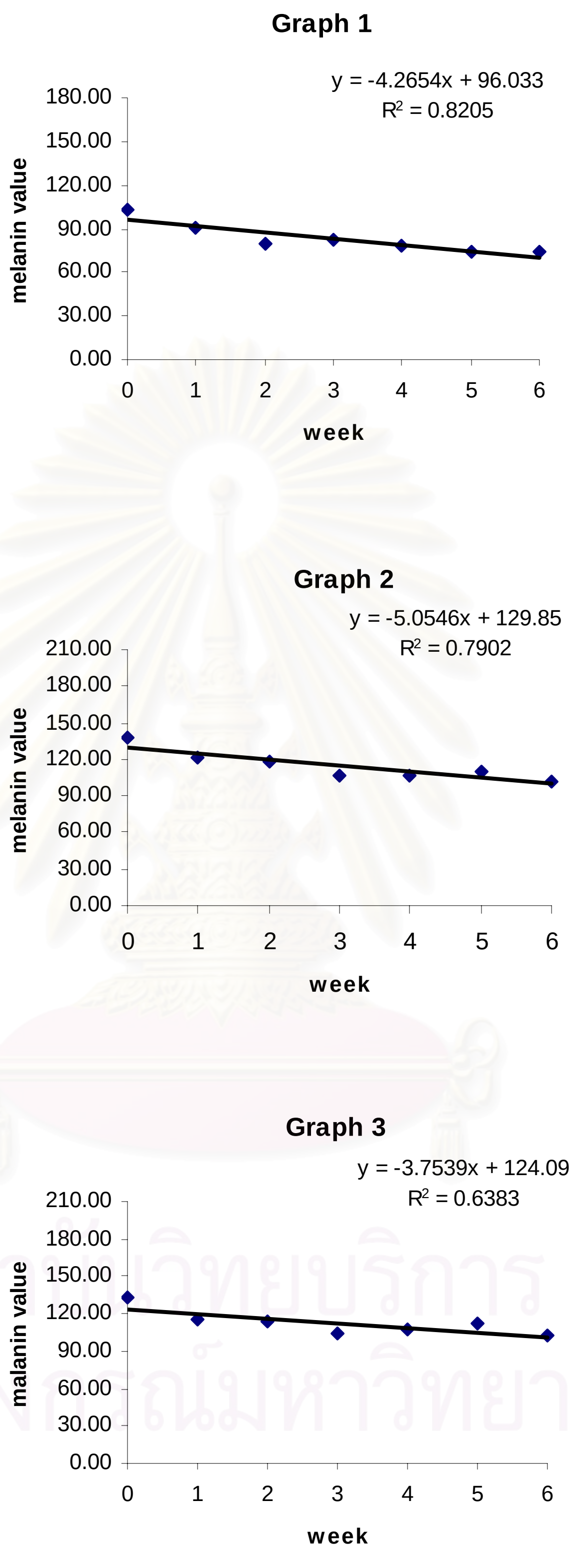


Figure 4.14 Average melanin values of 15 panelists

Table 4.17 Split-plot analysis of variance for repeated measures from the Melanin value study in a completely randomized design

Source of variation	*Degrees of Freedom*	*Sum of Squares*	*Mean Square*	F_0	F_{table}
Total	314	1387251.27			
Formulation	2	64675.30	32337.65	1.1584	3.23
Error(1) sub(trt)	42	1172475.02	27916.07		
Time	6	28599.56	4766.59	10.0092*	2.10
trt*time	12	1493.17	124.43	0.2613	1.83
Error(2)	252	120008.22	476.22		

* Significant difference compared with the baseline values

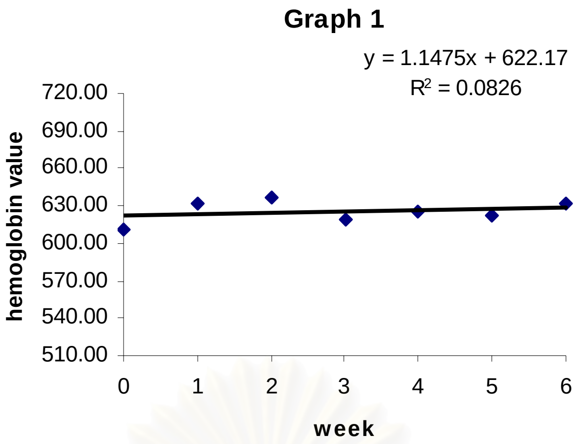
The application of all group of lipstick resulted in decreases melanin value. The slopes of group 1, 2, and 3 from Figure 4.15 are -4.26, -5.05, and -3.75, respectively. This result showed that lipstick containing RBO (group 2) had highest efficacy in decreased melanin value than other groups after using for 6 weeks. The melanin value was used as the response variable for split-plot analysis of variance. It found that the post-treatment differences among melanin value of these three treatment did not show a significant difference ($F_{table} > F_0$) as shown in Table 4.17. But all treatments could decrease melanin value significantly with time goes by.

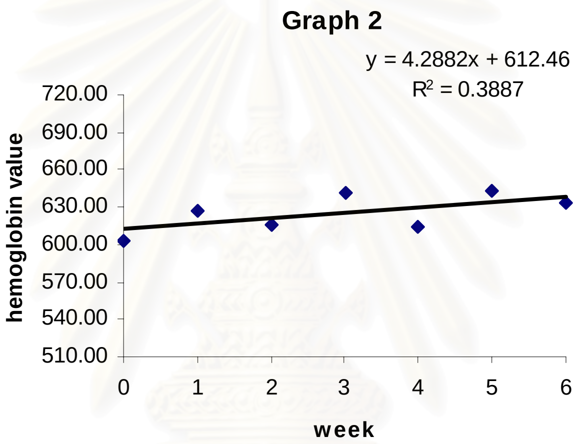
5.3 Determination of Hemoglobin Level

Table 4.18 and Figure 4.15 show the average hemoglobin value of fifteen panelists for each lipstick.

Table 4.18 Average hemoglobin values of 15 panelists (Mean±S.D.)

Week	Hemoglobin value		
	Group 1	Group 2	Group 3
0	611.72±71.97	602.99±70.66	608.09±54.52
1	631.63±67.06	627.04±46.96	636.93±65.11
2	636.71±65.68	615.32±52.33	615.28±67.70
3	618.85±71.84	641.68±50.94	641.29±66.33
4	625.53±63.42	614.95±70.60	620.84±66.63
5	622.88±66.78	642.35±58.98	625.11±65.96
6	631.99±67.79	632.93±62.25	627.08±63.26





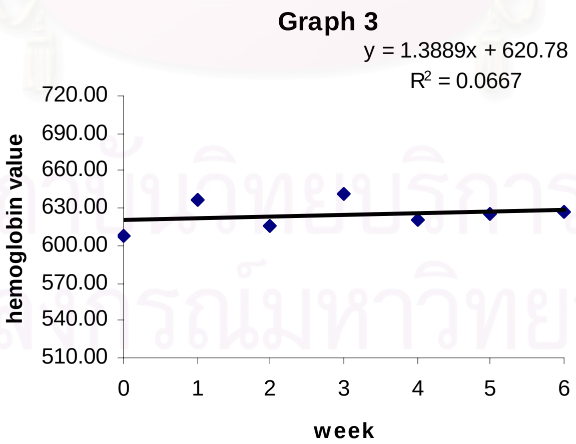


Figure 4.15 Average hemoglobin values of 15 panelists

Table 4.19 Split-plot analysis of variance for repeated measures from the Hemoglobin value study in a completely randomized design

Source of variation	*Degrees of Freedom*	*Sum of Squares*	*Mean Square*	F_0	F_{table}
Total	314	1288781.32			
Formulation	2	6198.86	3099.43	0.1355	3.23
Error(1) sub(trt)	42	960598.45	22871.39		
Time	6	22695.39	3782.57	3.3475*	2.1
trt*time	12	14539.30	1211.61	1.0723	1.83
Error(2)	252	284749.32	1129.96		

* Significant difference compared with the baseline values

The observed trends over time for the three treatments in the hemoglobin value study were shown in Figure 4.15. The profile plots exhibited increases in the observed hemoglobin value. The slopes of group 1, 2, and 3 from Figure 4.16 are 1.15, 4.29, and 1.39, respectively. This result showed that lipstick containing RBO (group 2) had highest efficacy in increased hemoglobin value than other groups after using for 6 weeks. The hemoglobin value was used as the response variable for split-plot analysis of variance. It found that the post-treatment difference among hemoglobin value of these three treatment did not show a significant difference ($F_{table} > F_0$) as shown in Table 4.19. But all treatments could increase hemoglobin value significantly with time goes by.

Although the assumption of this study was lipstick containing RBO plus oryzanol (group 3) might showed the highest efficacy in increase moisturizing effect, increase lip redness, and decrease lip darkness because it contains highest amount of γ-oryzanol which is strong moisturizing and sunscreening agent. But in this study, it was surprising that lipstick containing RBO (group 2) had comparable efficacy as lipstick containing RBO plus oryzanol.

Environmental temperature and relative humidity are very important factors when the lip moisture is measured (Wild, 1993); therefore, all measurements of skin parameters were done under constant ambient conditions. The average room temperature and relative humidity during the measurements in this study were controlled at 25 ± 5 °C and 60 ± 5 % RH, respectively. Another factor influencing skin moisture is age. Most of the panelist participated in group 2 (use lipstick containing RBO) have ages within the range of 18-25 years old but in the range of 45-59 years old in group 3 by chance. Normally an adult (between the ages of 20-40 years old) reaches the maximum skin moisture while a senior's skin moisture contents becomes lower due to the decreasing of storing capacity of the stratum corneum (Rogiers et al., 1990). This may be the reason why panelists in group 2 showed the highest efficacy in hydration property.

Dry skin is likely to be aggravated by a low humidity or an abnormal cornification of epidermal cells causing an imbalance of the lipid composition as well as an impaired maturation and barrier function of the stratum corneum. In this study, all groups of lipstick could increase the lip hydration. The possible mechanism could be that cathepsin D-like and chymo-trypsin-like proteinase, which are also present in skin as desquamation-regulating proteinases, were detected in lip corneocytes, though only cathepsin D-activity was found to decrease in severely chapped lips. The reduced cathepsin D-activity may be one of the mechanisms that is further decreased by low hydration. And the enhancement of cathepsin D-activity by lip moisture may be effective to improve lip chapping (Hikima et al., 2004).

The colour of lip is due to the proximity of the blood vessels to the epithelial surface (Gray, 1959). The γ-oryzanol is a triterpene alcoholic ester of ferulic acid extracted and purified from rice bran, and has an activity of increasing the skin temperature due to the percutaneous absorption, increasing the amount of local blood flow and the amount of sebum secretion (Muneaki et al., 1982). From this study, lipstick containing RBO and lipstick containing RBO plus oryzanol showed higher efficacy in increased hemoglobin level when compare with lipstick base.

Because the outer layer of the lip is so thin (Gray, 1959), the lips in their natural state are not adequately shielded from the sun. In fact, lips have almost no melanin (NOHSC, 1991), the natural pigment in skin that helps screening out the sun's harmful rays. As a result, lips rarely tan but they can easily burn. Lipstick containing sunscreening agent may reduced this effect. Moreover, lipstick containing natural sunscreening agent like γ-oryzanol may be the safer way for lip protection.

Moreover, there is a research support the use of lipstick with high SPF. Maier H. (2003) reported that photoprotective lipsticks are applied in a much thinner layer than recommended by international standards for other sunscreen products (2 mg/cm^2). Furthermore, the frequency of application is too low for adequate protection. Therefore, the sun protection factor (SPF) should be assessed for an area density that reflects the actual usage patterns. As long as the test protocol is not adapted to the reduced area density, photoprotective lipsticks with high and ultrahigh SPF should be recommended, especially for individuals with increased risk for the development of lip malignancies.

5.4 Sensory Evaluation

Parallel sensory evaluation was performed. Each panelist would evaluate only a single product, but in depth. The benefit of this study is each panelist would rate many elements within a single product, providing a detailed measurement of that panelist's reaction to the elements in that category. Thus the data would be "solid" on an individual-by-individual basis (Moskowitz, 1996).

The feel of slipperiness, gloss, taste, odor, lip moisturizing, lip redness, lip darkness, lip tackiness, and overall liking were evaluated by the panelists. The feel was ranked at the end of the study into 5 scales of satisfaction: 1 as 'least', 2 as 'slight', 3 as 'moderate', 4 as 'considerable', and 5 as 'most'. The mean scores of the satisfactory feel evaluated are shown in Table 4.20 and Figure 4.16. Table 4.21 shows the details of the feel evaluated and are presented as percentages. The Wilcoxon's Mann Whitney test was used to identify which pair of the lipsticks that were significantly different and the results are also shown in Table 4.20.

Group 3 (lipstick containing RBO plus oryzanol) was evaluated as the most 'satisfy' with its high slipperiness, high gloss, increase lip moisturizing, increase lip redness, decrease lip darkness, and least tackiness. Moreover, it got the highest score in the overall preference. On the contrary, Group 1 (control) got the least scores in almost all categories.

For the remaining attributes, the panelists felt that Group 1, Group 2 (lipstick containing RBO) and Group 3 had similar results in taste and odor. Group 2 got the medium score in almost all categories.

There were significant differences between three groups in these attributes. The liking of panelists in Group 3 significant different from Group 2 only in slipperiness ($p= 0.005$). And the liking of panelists in Group 3 was significant different from Group 1 in gloss, lip moisturizing, redness, darkness and overall liking ($p<0.05$).

Table 4.20 Scores obtained from sensory evaluation by the panelists (satisfaction: 1 = least, 2 = slight, 3 = moderate, 4 = considerable, 5 = most)

Attributes	Lipsticks	Mean scores	Sig. diff. (p<0.05)
Slipperiness	Group 1	4.00	
	Group 2	3.73	Group 2 VS Group 3
	Group 3	4.40	
Gloss	Group 1	3.60	Group 1 VS Group 3
	Group 2	3.80	
	Group 3	4.13	
Taste	Group 1	3.87	
	Group 2	4.13	
	Group 3	3.93	
Odor	Group 1	3.87	
	Group 2	3.67	
	Group 3	3.87	
Lip moisturizing	Group 1	4.00	Group 1 VS Group 3
	Group 2	4.33	
	Group 3	4.60	
Redness	Group 1	3.47	Group 1 VS Group 3
	Group 2	3.47	Group 2 VS Group 3
	Group 3	4.00	
Darkness	Group 1	3.00	Group 1 VS Group 3
	Group 2	3.33	
	Group 3	3.87	
Tackiness	Group 1	3.13	
	Group 2	3.67	
	Group 3	3.80	
Overall liking	Group 1	3.87	Group 1 VS Group 3
	Group 2	4.07	
	Group 3	4.40	

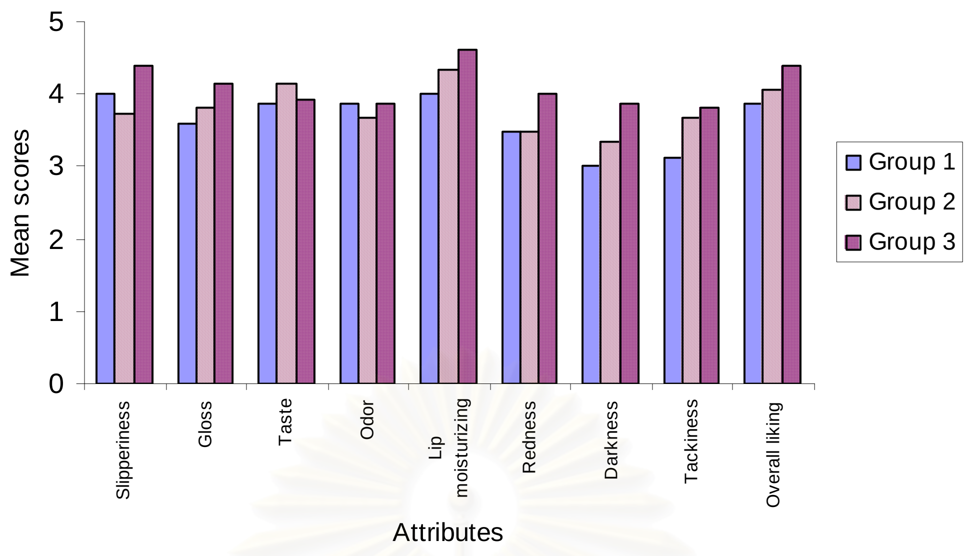


Figure 4.16 Mean scores of satisfactory feel evaluated by the panelists.

Table 4.21 Percentages of satisfactory feel evaluated by the panelists

Lipstick	Satisfactory level	Percentage of satisfaction			
		Slipperiness	Gloss	Taste	Odor
gr. 1	Most (5)	26.7	13.3	13.3	20.0
	Considerable (4)	46.7	33.3	60.0	46.7
	Moderate (3)	26.7	53.3	26.7	33.3
	Slight (2)	0.0	0.0	0.0	0.0
	Least (1)	0.0	0.0	0.0	0.0
gr. 2	Most (5)	6.7	6.7	33.3	20.0
	Considerable (4)	60.0	66.7	53.3	33.3
	Moderate (3)	33.3	26.7	6.7	40.0
	Slight (2)	0.0	0.0	6.7	6.7
	Least (1)	0.0	0.0	0.0	0.0
gr. 3	Most (5)	40.0	26.7	26.7	26.7
	Considerable (4)	60.0	60.0	40.0	46.7
	Moderate (3)	0.0	13.3	33.3	13.3
	Slight (2)	0.0	0.0	0.0	13.3
	Least (1)	0.0	0.0	0.0	0.0

Table 4.21 Percentage of satisfactory feel evaluated by the panelists (continued)

Lipstick	Satisfactory level	Percentage of satisfaction				
		Lip moisturizing	Lip Redness	Lip Darkness	Lip Tackiness	Overall liking
gr. 1	Most (5)	26.7	13.3	6.7	13.3	13.3
	Considerable (4)	46.7	20.0	13.3	13.3	60.0
	Moderate (3)	26.7	66.7	60.0	53.3	26.7
	Slight (2)	0.0	0.0	13.3	13.3	0.0
	Least (1)	0.0	0.0	6.7	6.7	0.0
gr. 2	Most (5)	53.3	13.3	6.7	20.0	26.7
	Considerable (4)	26.7	20.0	33.3	33.3	53.3
	Moderate (3)	20.0	66.7	53.3	40.0	20.0
	Slight (2)	0.0	0.0	6.7	6.7	0.0
	Least (1)	0.0	0.0	0.0	0.0	0.0
gr. 3	Most (5)	60.0	26.7	33.3	33.3	46.7
	Considerable (4)	40.0	46.7	20.0	26.7	46.7
	Moderate (3)	0.0	26.7	46.7	33.3	6.7
	Slight (2)	0.0	0.0	0.0	6.7	0.0
	Least (1)	0.0	0.0	0.0	0.0	0.0

The increase in lip moisturizing perceived by the panelists could be related with the lip capacitance measured by the Corneometer® CM 420. But the reduction in melanin value and the increase in hemoglobin value which measured by the Mexameter® did not agree with the results perceived by the panelists. The panelists had the most satisfied skin-feel such as increasing in redness and reducing in darkness for the lipstick containing RBO plus oryzanol (group 3) significantly while melanin values and hemoglobin values which measured by the Mexameter® did not significantly different among three groups.

The long-term study duration 6 weeks in this case maybe a barrier of this study. This study was conducted in the middle of May which was close to the beginning of a semester. The majority of the panelists are university's personnel. It was found that the lip conditioning properties of almost overall panelists became worse than 2 weeks ago when the school opening had come. One possible reason is that the panelists might have the stress from a hard working causing the lips became dry and looked dark. By controlling this influencing factor or other factors which may affect the lips could result in more precising data. But sometimes with some limitations could make it more difficult.

More sophisticated method which uses to study the activities of desquamation-regulating proteinases in lip chapping is the application of scanning electron microscopy and the results can be evaluated as quickly as 2 weeks of use (Hikima et al., 2004). Therefore, this technique can be an alternative way to improve the sensitivity of the study relating to the moisturizing efficacy of the lipstick.

RBO is an emollient that softens the skin and has an occlusive action which reduces water lost from the skin. γ-Oryzanol is an effective sunscreening agent in protecting the skin against sunburn. So that RBO is very useful in photoprotective lipsticks. And this might be proposed that γ-oryzanol should be used with other synthetic sunscreening agents so that this could reduce the amount of synthetic sunscreening agents then side effects from those sunscreening agents may be reduced. Furthermore, RBO is extracted from rice bran which is cultivated widely in Thailand. The use of RBO in cosmetic products can add value to rice bran which is a by-product in the rice industry.

CHAPTER V

CONCLUSION

1. Addition of an antioxidant in rice bran oil, Tertiary Butylhydroquinone (TBHQ) is more effective antioxidant than Butylated Hydroxyanisole (BHA) under accelerated conditions by 743 Rancimat® at 120°C.

2. The stability of rice bran oil was increased when using higher concentration of antioxidants. TBHQ and BHA have comparable antioxidant efficacy on rice bran oil at levels less than 0.04%. At levels greater than 0.04%, TBHQ is far more effective than BHA.

3. Antioxidant efficacy of the TBHQ and BHA concentrations at least 0.02% by weight were significantly different from control one without antioxidant.

4. In the development of lipsticks, the suitable lipsticks were 1) no sweating, 2) not droop more than 5 mm at 45°C, 3) dropping point higher than 60°C and 4) breaking point higher than 2 N. Three lipstick components (oil, fat, wax) were varied and the optimum ratio of lipstick components was 76:10:14 by weight of oil:fat:wax.

5. The SPF values of lipsticks were increased when using higher concentrations of rice bran oil. Moreover, rice bran oil lipstick showed high efficacy in improving skin-feels. Lipsticks gave high gloss, good glide and less tackiness in all formulations. However, using rice bran oil in lipsticks was limited because of bad odor and unacceptable taste. Thus, 18% by weight rice bran oil was selected to use in the lipsticks.

6. γ-Oryzanol was added into lipstick containing RBO for higher sunscreen efficacy. The SPF values of lipsticks were higher as increasing in γ-oryzanol and it comes to the conclusion that the greater γ-oryzanol, the higher the sun protection according to SPF value.

7. The lip conditioning properties studies of 3 lipstick groups were lip capacitance, melanin value and hemoglobin value. There is no statistical difference among three treatments in all properties. All treatments could increase lip capacitance and hemoglobin value while decrease melanin value significantly with time goes by.

8. The sensory evaluation perceived by panelists informed that Group 3 (lipstick containing RBO plus oryzanol) was evaluated as the most 'satisfy' with its high slipperiness, high gloss, increase lip moisturizing, increase lip redness, decrease lip darkness, and least tackiness. Moreover, it got the highest score in the overall liking. On the contrary, Group 1 (control) got the least scores in almost all categories. There were significant differences between three groups in these attributes. Only slipperiness in Group 3 is significantly different from Group 2 (lipstick containing RBO) ($p = 0.005$) according to the liking from the panelists. But gloss, lip moisturizing, redness, darkness and overall liking in Group 3 are all significantly different from Group 1 ($p<0.05$). In further study, the sensory evaluation may be use crossover design then every panelist can evaluate all formulations.

9. The clinical study of lipsticks containing RBO and RBO plus γ-oryzanol has high variation because there are many factors influencing the results during the study. For example, the stress from a hard working may lead to the extreme dehydration of the skin resulting in dry and chapped lip. High variation can be reduced by a very well planned and controlled study or by using more sensitive method like scanning electron microscopy study.

10. RBO shows many possible efficacies such as moisturizing efficacy and sunscreen efficacy. Moreover, RBO is extracted from rice bran which is cultivated widely in Thailand. The use of RBO in cosmetic products can add value to rice bran which is a by-product in the rice industry.

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APPENDICES

APPENDIX A

Validation of HPLC Method

APPENDIX A

Validation of the HPLC Method

Validated analytical parameters were precision, accuracy, and linearity. The validation of an analytical method was the process by which performance characteristics of the method were established to meet the USP 27, 2004 requirements for the intended analytical applications.

1. Linearity

The linearity of an analytical method is its ability to elicit test results that are directly, or by a well-defined mathematical transformation, proportional to the concentrations of analyte in samples within a given range. The preparation of linearity test was done as following.

Ten mg of γ-oryzanol was accurately weighted and transferred into a 100 ml volumetric flask. The substance was dissolved and the solution was adjusted to volume with isopropanol to produce the stock solution. This stock solution had a final concentration of 0.1 mg/ml.

Standard solutions of γ-oryzanol were prepared by pipetting 0.01, 0.03, 0.05, 0.07, 0.10 and 0.13 ml of the γ-oryzanol stock solution into 10 ml volumetric flasks, respectively. The solutions were adjusted to volume with mobile phase so that the concentrations of the standard γ-oryzanol solutions were 100, 300, 500, 700, 1000 and 1300 ng/ml, respectively. Three sets of standard solutions were prepared for each HPLC run. The calibration curve was obtained by plotting the peak area of γ-oryzanol versus the corresponding concentration of γ-oryzanol by means of linear regression. Standard curve was obtained from the average of three determinations. The standard curve of γ-oryzanol is shown in Table 1A and Figure 3A.

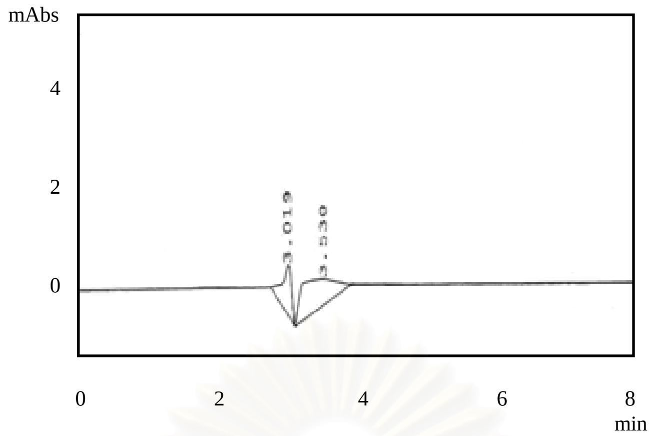


Figure 1A HPLC chromatogram of blank (Methanol : Isopropanol 70:30)

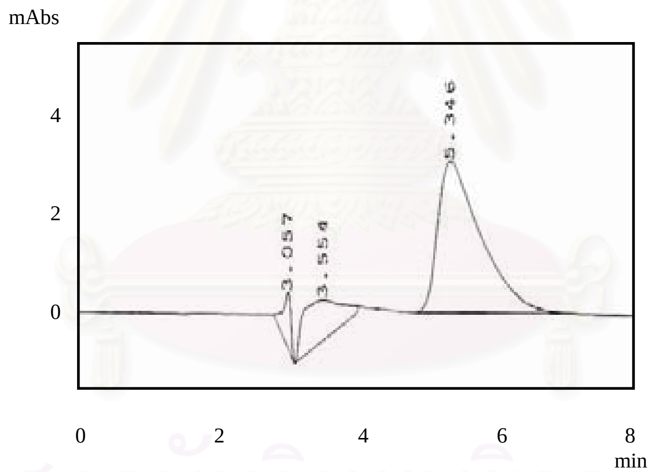


Figure 2A HPLC chromatogram of 1200 ng/ml γ-oryzanol

Table 1A Data for standard curve of γ-oryzanol by HPLC method

concentration (ng/ml)	Peak area	Inversely estimated concentration (ng/ml)	%Recovery
100	7216.67	101.90	101.90
300	25346.33	297.01	99.00
500	44101.33	498.84	99.77
700	62805.33	700.13	100.02
1000	91155.00	1005.23	100.52
1300	118258.00	1296.90	99.76
		Mean	100.16
		S.D.	0.43
		%C.V.	0.43

1. $r^2 = 0.9999$, Y = 92.92X – 2251.70
2. Inversely estimated concentration = (peak area + 2251.70)/92.92
3. %Recovery = (Inversely estimated concentration / Known concentration) x 100
4. % C.V = (S.D./ Mean) x 100

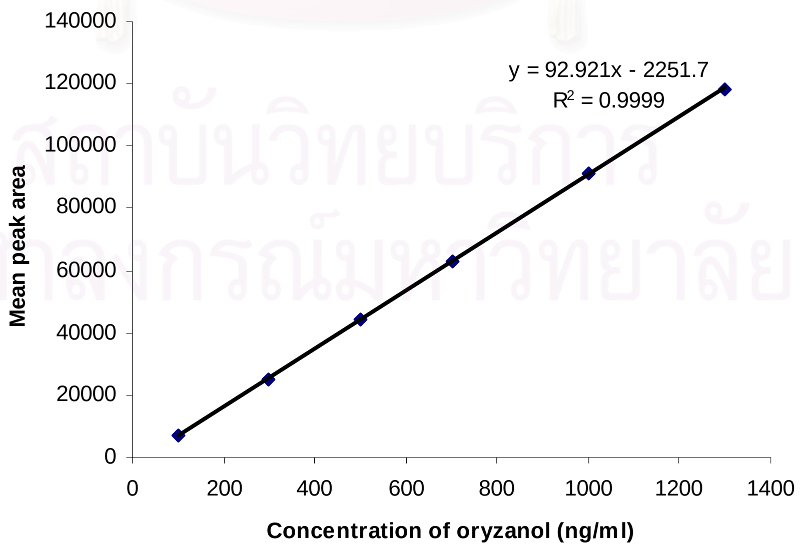


Figure 3A Standard curve of γ-oryzanol by HPLC method

2. Accuracy

Three sets of quality control samples (low, medium and high) of γ-oryzanol solution were prepared for analysis of γ-oryzanol in terms of percent recovery. Percent recovery of each concentration was calculated from the ratio of inversely estimated concentration to known concentration of γ-oryzanol multiplied by 100. The accuracy was determined by using five determinations per concentration.

Acceptance criteria:

For accuracy, the percent recovery should be within ±2 percent of each nominal concentration whereas the percent coefficient of variations for both the within run and between run should be less than 2 percent (USP 27, 2004).

The accuracy of an analytical method is the closeness of test results obtained by that method to the true value. Accuracy may often be expressed by as percent recovery by the assay of know, added amount of analysis. The percentages of analytical recovery of each γ-oryzanol concentration are shown in Table 2A All the percentages analytical recovery of all γ-oryzanol concentrations with mean of 99.97% and a %C.V. of 0.51 indicated that this method could be used for analysis of γ-oryzanol in all concentrations studied with high accuracy.

Table 2A The percentages of analytical recovery of γ-oryzanol by HPLC method

concentration	Inversely estimated concentration (ng/ml)					Mean	%recovery
(ng/ml)	1	2	3	4	5		
200	200.07	201.62	198.78	200.33	203.04	200.77	100.39
800	797.6	800.86	790.57	800.48	786.91	795.28	99.41
1200	1200.35	1190.04	1207.13	1199.86	1210.22	1201.52	100.13

Mean % recovery = 99.97, S.D. = 0.51, C.V. = 0.51%

Each data was determined using five determinations per concentration

3. Precision

The precision of an analytical method is the degree of agreement among individual test results when the procedure is applied repeatedly to multiple sampling of a homogeneous sample. The precision of an analytical method is usually expressed as the relative standard deviation (coefficient of variation).

3.1 Within run precision

Within run precision was ascertained by analyzing three sets of quality control samples (low, medium and high) in the same day. The percent coefficient of variation (%C.V.) of the estimated concentration of γ-oryzanol of each concentration was then discovered. The precision was determined by using five determinations per concentration.

3.2 Between run precision

Between run precision was determined by comparing the estimated concentration of γ-oryzanol of three sets of quality control samples (low, medium and high) for five different days. The percent coefficient of variation (% C.V.) of the estimated concentration of γ-oryzanol of each concentration was determined.

Table 3A and 4A illustrated the data of within run precision and between run precision, respectively. All coefficients of variation values were small, as 0.65-0.81 % and 0.88-1.50 %, respectively. The coefficient of variation of an analytical method should generally be less than 2% (USP 27, 2004). Therefore, the HPLC method were used for quantitative analysis of γ-oryzanol in the studied range.

Table 3A Data of within run precision by HPLC method

concentration (ng/ml)	Inversely estimated concentration (ng/ml)					Mean±SD	%C.V.
	1	2	3	4	5		
200	200.07	201.62	198.78	200.33	203.04	200.77±1.62	0.81
800	797.60	800.86	790.57	800.48	786.91	795.28±6.24	0.78
1200	1200.35	1190.04	1207.13	1199.86	1210.22	1201.52±7.80	0.65

Each data was determined using five determinations per concentration

Table 4A Data of between run precision by HPLC method

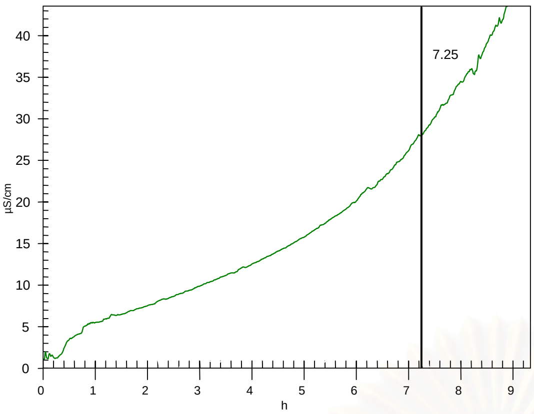
concentration (ng/ml)	Inversely estimated concentration (ng/ml)					Mean±SD	%C.V.
	1	2	3	4	5		
200	200.07	192.60	204.46	202.66	196.32	199.22±4.80	1.50
800	797.60	804.60	776.91	793.55	789.66	792.46±10.30	1.30
1200	1200.35	1192.90	1195.43	1204.88	1177.42	1194.20±10.45	0.88

Each data was determined using five determinations per concentration

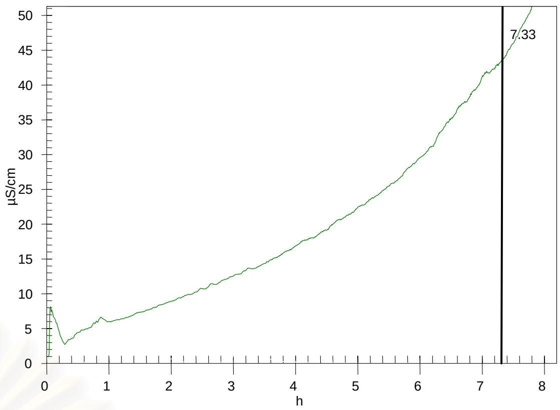
In conclusion, the analysis of γ-oryzanol by developed HPLC method showed good linearity, accuracy and precision. Thus this method was used for the determination of the content of γ-oryzanol in the study.

APPENDIX B

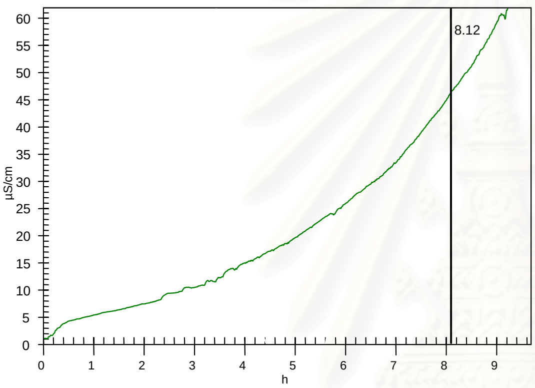
Rancimat Curve of RBO Containing Various Antioxidants



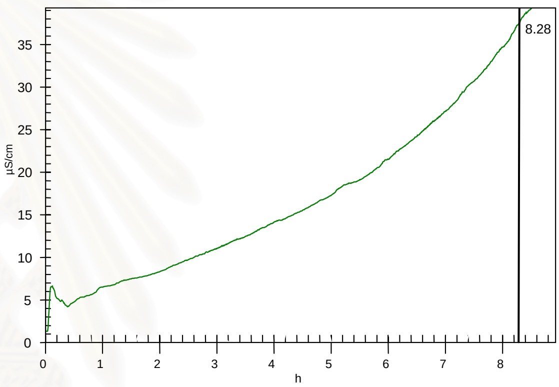
RBO



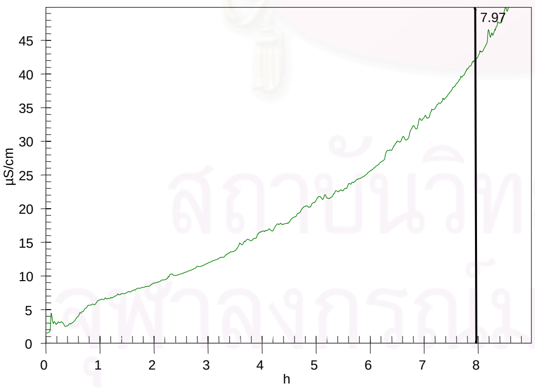
RBO+BHA 0.01%



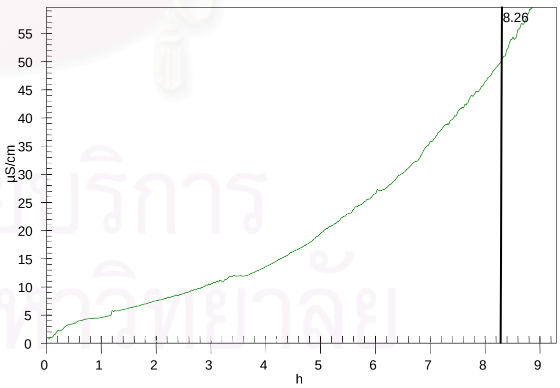
RBO+BHA 0.02%



RBO+BHA 0.03%

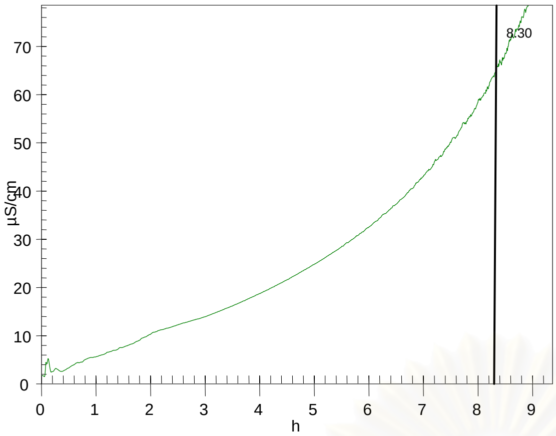


RBO+BHA 0.04%

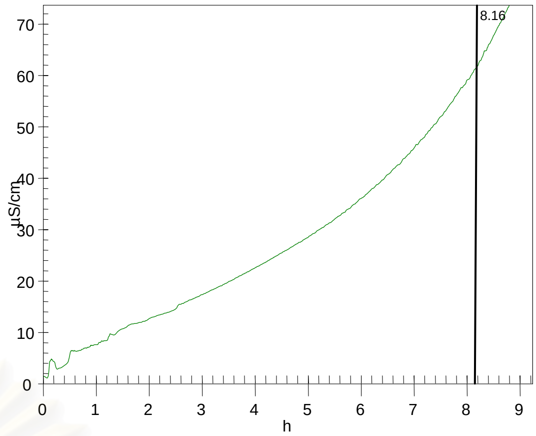


RBO+BHA 0.05%

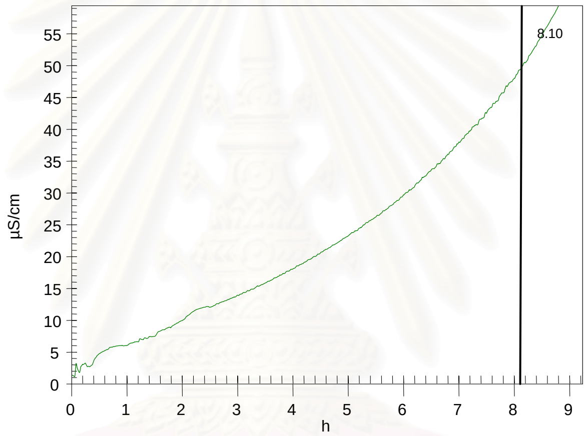
Figure 1B Rancimat curve of RBO containing 0.01%-0.10% BHA at 120°C



RBO+BHA 0.06%

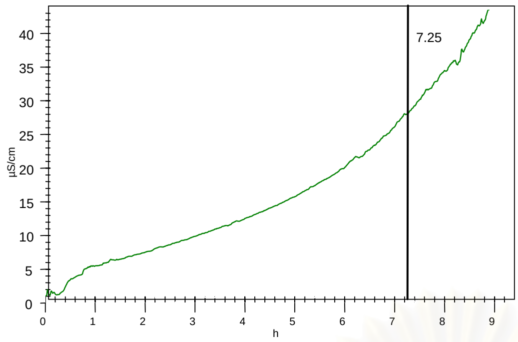


RBO+BHA 0.08%

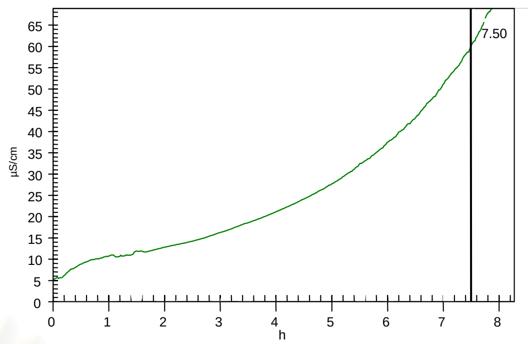


RBO+BHA 0.10%

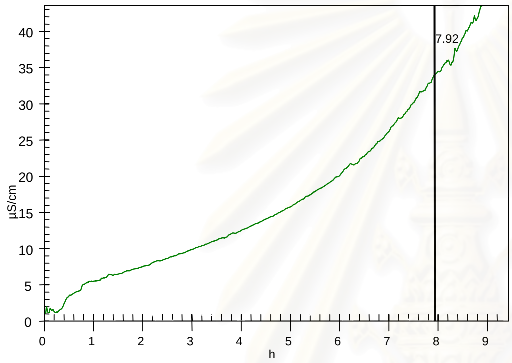
Figure 1B Rancimat curve of RBO containing 0.01%-0.10% BHA at 120°C (continued)



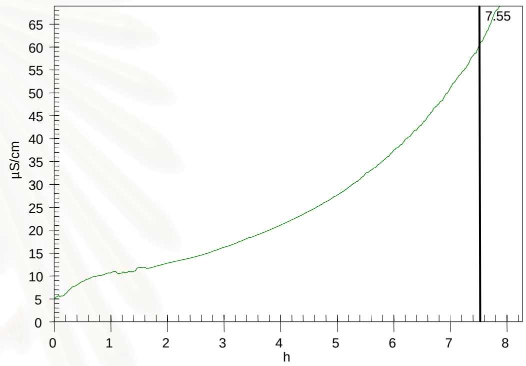
RBO



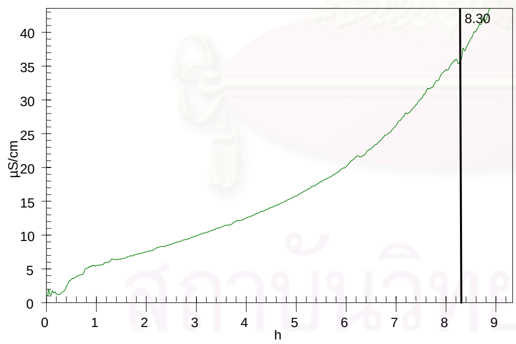
RBO+TBHQ 0.01%



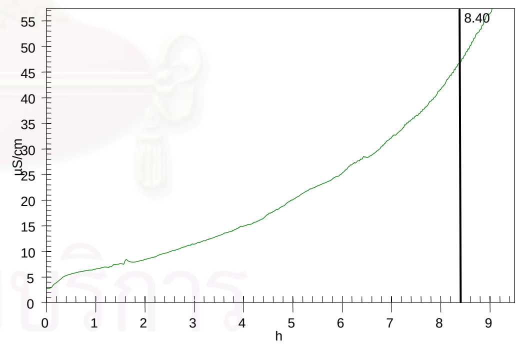
RBO+TBHQ 0.02%



RBO+TBHQ 0.03%

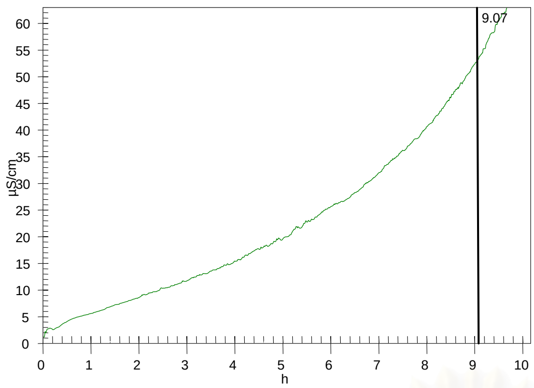


RBO+TBHQ 0.04%

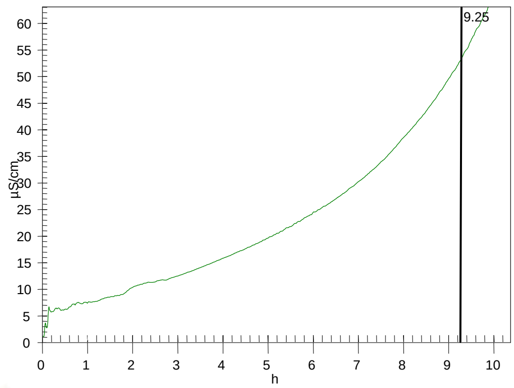


RBO+TBHQ 0.05%

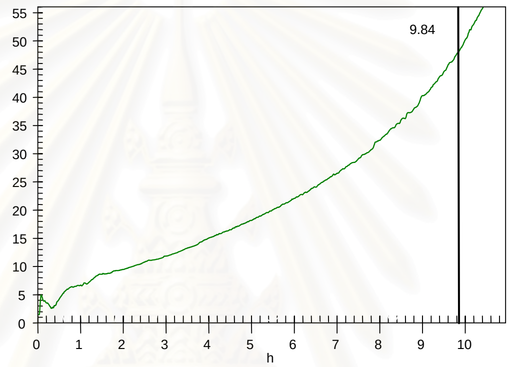
Figure 2B Rancimat curve of RBO containing 0.01%-0.10% TBHQ at 120°C



RBO+TBHQ 0.06%



RBO+TBHQ 0.08%



RBO+TBHQ 0.10%

Figure 2B Rancimat curve of RBO containing 0.01%-0.10% TBHQ at 120°C (continued)

APPENDIX C

SPF and UVA Value of Lipstick Containing RBO

Table 1C SPF value and UVA value of lipsticks containing vary % by weight of RBO

Lipstick	SPF value												SPF	S.D
	1	2	3	4	5	6	7	8	9	10	11	12		
Lipstick base	1.2	1.3	1.3	1.2	1.2	1.2	1.3	1.3	1.3	1.3	1.4	1.4	1.283	0.072
Lipstick with RBO 9%	1.4	1.3	1.4	1.5	1.4	1.5	1.4	1.4	1.4	1.5	1.6	1.5	1.442	0.079
Lipstick with RBO 18%	1.6	1.6	1.6	1.6	1.4	1.5	1.5	1.5	1.6	1.6	1.5	1.6	1.550	0.067
Lipstick with RBO 27%	1.7	1.5	1.4	1.6	1.5	1.4	1.6	1.8	1.7	1.8	1.6	1.7	1.608	0.138

Table 2C The SPF value and UVA value of lipsticks in addition of γ-oryzanol

Lipstick	SPF value												SPF	S.D
	1	2	3	4	5	6	7	8	9	10	11	12		
Lipstick with RBO 18%	1.6	1.6	1.6	1.6	1.4	1.5	1.5	1.5	1.6	1.6	1.5	1.6	1.550	0.067
Lipstick with RBO 18% + oryzanol 0.25%	2.5	1.9	2.2	2.1	2.1	2	2.2	1.9	1.5	1.5	1.7	2.1	1.975	0.296
Lipstick with RBO 18% + oryzanol 0.50%	1.9	3	1.7	1.8	1.9	1.4	2.2	2.8	2.9	2.3	2.3	1.7	2.158	0.520
Lipstick with RBO 18% + oryzanol 0.75%	2	2.1	3.2	2.3	2.2	2.8	2.3	2.3	2.8	2.6	2.4	2.4	2.450	0.342
Lipstick with RBO 18% + oryzanol 1.0%	2	2.6	2.6	3.3	3.3	2.2	2.3	2.2	2.1	3.1	3.5	2.5	2.642	0.526
Lipstick with RBO 18% + oryzanol 1.25%	3	3.1	2.6	3.6	4.8	3.3	3	4.2	2.9	5.1	3.2	3.5	3.525	0.781
Lipstick with RBO 18% + oryzanol 1.50%	2.7	3.4	5.1	3.7	3.1	4.2	3.2	4.3	3.2	4.3	3.3	4.2	3.725	0.693

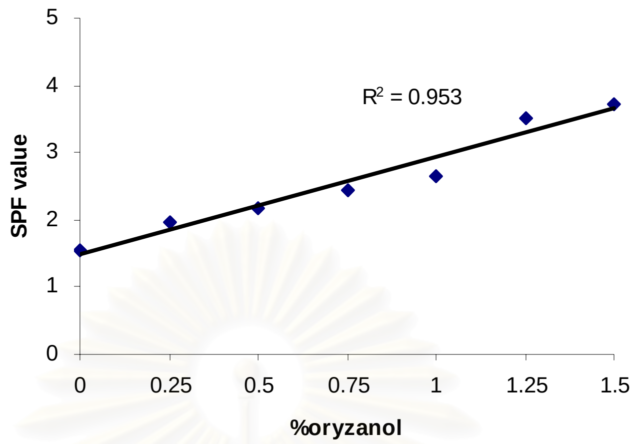


Figure 1C SPF value of lipsticks in addition of γ-oryzanol

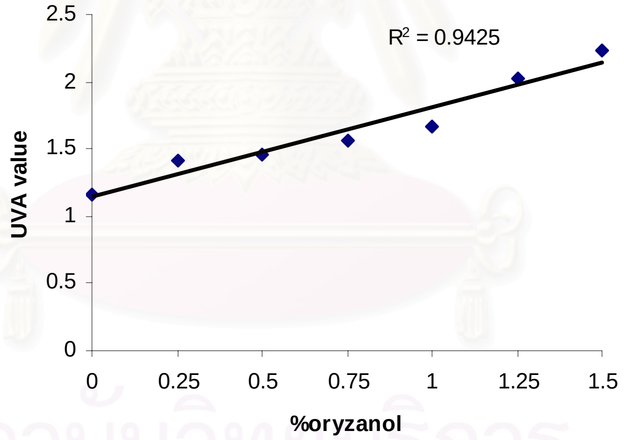


Figure 2C UVA value of lipsticks in addition of γ-oryzanol

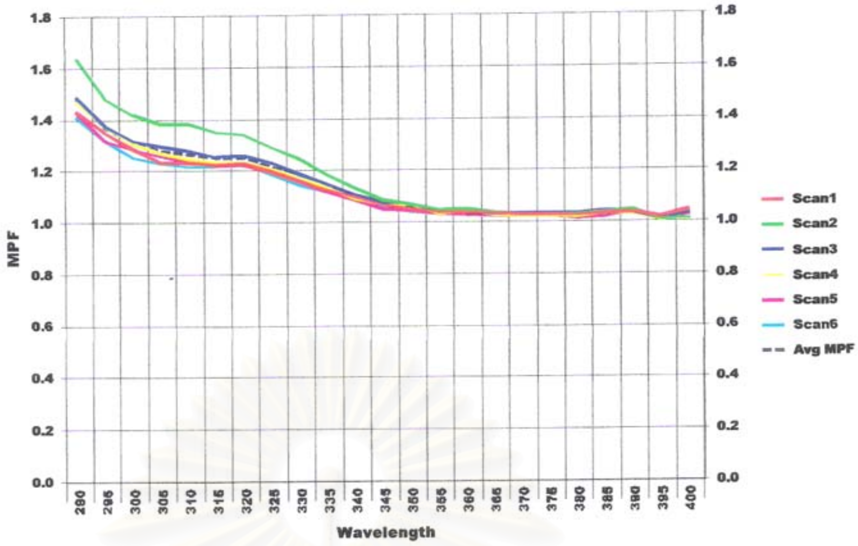


Figure 3C SPF-290 Graph Report of Lipstick Base

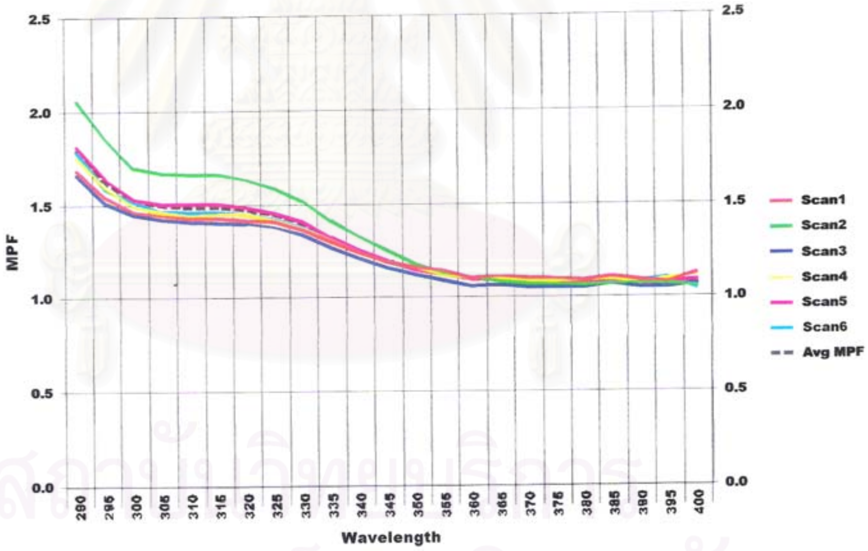


Figure 4C SPF-290 Graph Report of Lipstick with RBO 9%

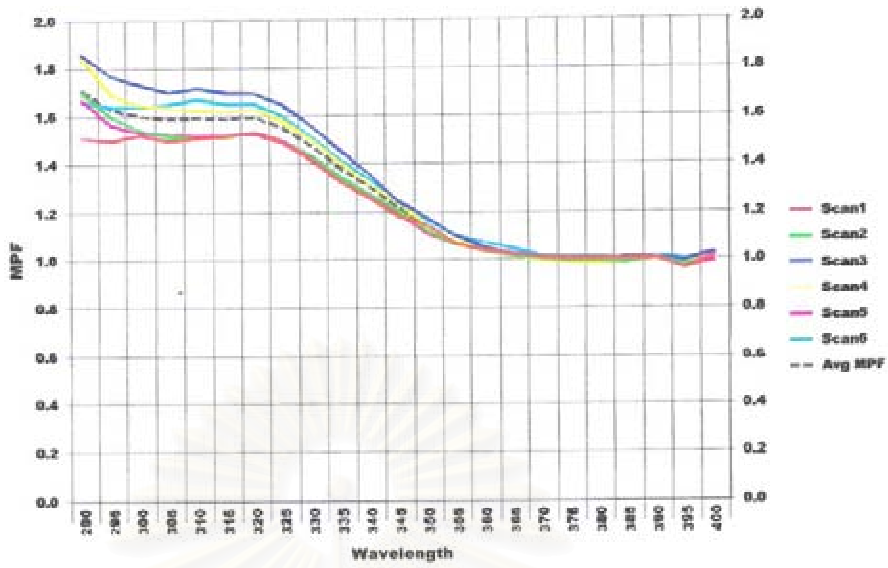


Figure 5C SPF-290 Graph Report of Lipstick with RBO 18%

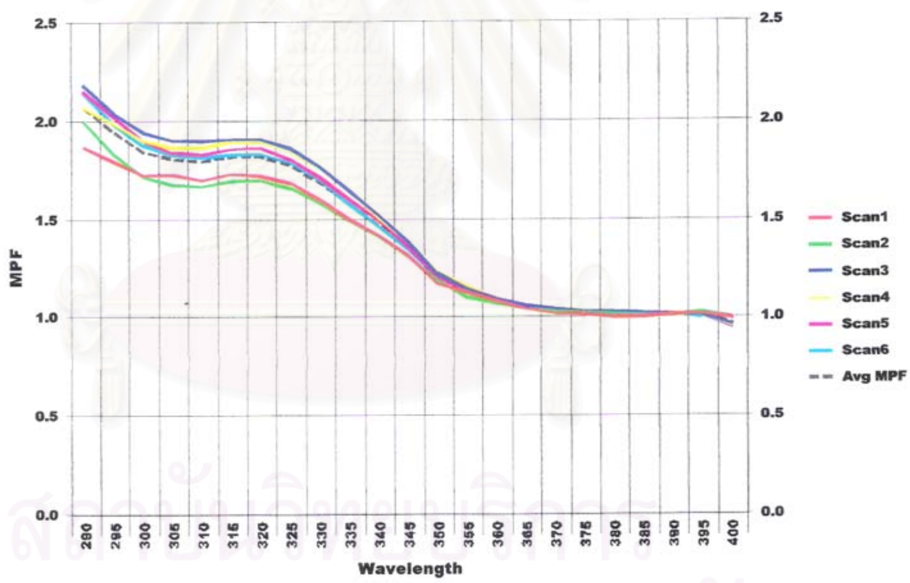


Figure 6C SPF-290 Graph Report of Lipstick with RBO 27%

APPENDIX D

Split-Plot Model and Data of Lip Conditioning Properties

APPENDIX D

1. Split-Plot Model

The split-plot analysis of variance mean squares can be used to test hypotheses about the treatment means and their interactions with time. The panelists in the study are equivalent to whole plots for the three treatments and repeated measures on the panelists are equivalent to subplot treatments. The linear statistical model for the split-plot experiment is

$$y_{ijk} = \mu + \alpha_i + d_{ik} + \beta_j + (\alpha\beta)_{ij} + e_{ijk}$$

μ is the general mean

α_i is the effect of the *i*th treatment

d_{ik} is the random experimental error for panelists within treatments with variance σ_d^2

β_j is the effect of the *j*th time

$(\alpha\beta)_{ij}$ is the interaction between treatment and time

e_{ijk} is the normally distributed random experimental error on the repeated measures with variance σ_e^2

2. Data of Lip Conditioning Properties

2.1 Lipstick Base

Sub. No.	Wk	Lip capacitance 1	2	3	4	5	Mean	Melanin Value 1	2	3	4	5	Mean	Hemoglobin Value 1	2	3	4	5	Mean
1	0	82.10	81.70	81.70	82.50	84.10	**82.42**	76	80	95	104	59	**83**	678	653	631	605	614	**636**
	1	54.40	54.90	56.60	55.60	59.10	**56.12**	99	80	47	87	108	**84**	644	653	640	650	643	**646**
	2	39.70	42.20	44.20	51.20	43.60	**44.18**	129	64	96	123	124	**107**	576	567	589	601	583	**583**
	3	56.30	52.60	59.20	63.60	64.10	**59.16**	87	91	83	89	78	**86**	627	673	614	601	636	**630**
	4	62.40	65.60	57.60	58.80	70.20	**62.92**	109	45	115	93	98	**92**	608	607	605	610	580	**602**
	5	53.20	53.00	55.30	57.10	55.00	**54.72**	36	38	48	46	45	**43**	694	647	625	659	612	**647**
	6	58.20	57.10	54.60	57.90	59.40	**57.44**	78	80	66	55	70	**70**	648	687	674	684	698	**678**
2	0	54.90	56.50	54.80	48.50	49.70	**52.88**	98	150	158	92	124	**124**	520	587	556	518	528	**542**
	1	68.50	67.30	72.40	73.70	66.40	**69.66**	151	171	163	153	189	**165**	527	539	537	505	546	**531**
	2	70.80	64.30	63.00	70.00	68.10	**67.24**	101	108	90	104	106	**102**	574	610	584	590	583	**588**
	3	50.70	56.60	52.50	60.40	51.90	**54.42**	138	108	146	131	117	**128**	605	552	605	525	542	**566**
	4	56.40	57.90	62.00	59.30	63.60	**59.84**	73	109	91	155	86	**103**	680	604	586	521	576	**593**
	5	63.60	63.80	63.20	60.00	58.30	**61.78**	85	128	92	86	88	**96**	581	568	558	600	587	**579**
	6	55.00	65.40	63.40	61.80	62.60	**61.64**	89	58	67	59	70	**69**	620	653	639	639	643	**639**
3	0	52.60	53.40	52.50	54.60	54.00	**53.42**	95	126	135	120	83	**112**	708	739	680	720	723	**714**
	1	56.70	53.30	56.70	61.40	61.50	**57.92**	128	130	123	141	127	**130**	716	737	704	736	742	**727**
	2	49.70	50.10	48.60	54.30	49.40	**50.42**	132	130	125	135	140	**132**	660	656	692	662	655	**665**
	3	55.50	58.10	64.70	63.10	60.80	**60.44**	134	127	127	97	111	**119**	615	640	646	653	637	**638**
	4	37.50	38.20	42.40	43.00	42.50	**40.72**	158	164	166	157	152	**159**	633	661	680	642	661	**655**
	5	45.30	41.70	45.00	44.70	42.80	**43.90**	130	141	120	135	125	**130**	641	632	620	637	618	**630**
	6	58.30	55.60	50.00	61.20	57.10	**56.44**	132	136	145	116	121	**130**	593	628	655	626	641	**629**
4	0	55.40	59.10	54.10	60.40	58.80	**57.56**	36	49	41	32	50	**42**	543	580	597	585	606	**582**
	1	50.60	55.00	56.90	55.10	56.30	**54.78**	20	20	25	32	25	**24**	638	607	614	578	579	**603**
	2	68.60	72.60	69.10	73.30	70.60	**70.84**	22	38	22	46	41	**34**	585	576	632	572	590	**591**
	3	50.00	53.40	54.70	49.30	48.90	**51.26**	39	21	23	43	25	**30**	584	593	575	560	595	**581**
	4	53.00	62.60	58.50	65.80	61.30	**60.24**	27	21	25	22	24	**24**	613	578	549	584	611	**587**
	5	68.00	66.80	66.80	63.00	65.40	**66.00**	36	32	38	48	31	**37**	681	554	672	635	576	**624**
	6	53.90	53.20	56.10	53.60	55.10	**54.38**	46	38	48	49	47	**46**	520	576	562	549	549	**551**
5	0	19.00	25.60	26.00	27.90	25.00	**24.70**	107	82	100	92	82	**93**	665	655	684	647	664	**663**
	1	35.30	41.00	44.10	45.80	46.20	**42.48**	30	30	54	30	39	**37**	663	643	629	643	596	**635**
	2	40.50	39.90	45.60	42.90	47.30	**43.24**	37	46	16	50	35	**37**	701	694	715	692	694	**699**
	3	52.90	57.10	58.50	55.80	55.20	**55.90**	42	38	46	51	23	**40**	639	675	630	668	625	**647**
	4	34.70	35.00	35.80	38.50	37.30	**36.26**	12	43	44	24	34	**31**	597	678	671	664	667	**655**
	5	34.60	34.90	35.00	37.00	38.20	**35.94**	40	46	46	50	43	**45**	712	697	708	694	701	**702**
	6	48.50	44.80	44.60	47.10	52.90	**47.58**	37	45	27	51	53	**43**	682	718	717	733	718	**714**
6	0	57.90	63.60	63.50	63.20	64.50	**62.54**	109	84	88	84	85	**90**	480	354	477	374	439	**425**
	1	38.30	38.20	37.90	38.40	39.10	**38.38**	38	43	56	49	38	**45**	496	510	552	532	568	**532**
	2	59.70	66.00	67.30	63.30	62.40	**63.74**	92	64	68	75	52	**70**	470	515	492	470	541	**498**
	3	40.80	41.80	43.50	42.80	42.30	**42.24**	86	80	88	70	69	**79**	465	435	440	434	433	**441**
	4	43.00	42.80	44.00	44.60	43.70	**43.62**	57	46	58	42	41	**49**	427	480	491	507	504	**482**
	5	53.50	59.60	57.50	61.70	59.80	**58.42**	42	50	45	54	56	**49**	502	459	437	356	492	**449**
	6	47.80	46.60	49.10	47.20	47.50	**47.64**	50	64	57	51	59	**56**	436	483	495	425	521	**472**
7	0	42.60	47.50	50.60	49.40	51.00	**48.22**	234	245	246	213	206	**229**	519	534	542	566	554	**543**
	1	55.20	55.50	58.30	55.80	55.50	**56.06**	223	252	191	230	219	**223**	570	559	554	576	581	**568**
	2	69.90	69.00	71.30	70.20	73.30	**70.74**	158	147	144	153	149	**150**	622	581	662	582	596	**609**
	3	63.20	76.70	65.50	75.90	68.40	**69.94**	167	165	194	171	186	**177**	546	610	558	623	570	**581**
	4	57.20	59.10	63.00	59.10	66.00	**60.88**	195	183	174	191	176	**184**	512	573	569	543	593	**558**
	5	63.30	63.70	61.80	62.60	62.30	**62.74**	175	150	170	165	177	**167**	515	588	557	572	526	**552**
	6	64.90	59.30	62.30	63.70	61.90	**62.42**	175	161	133	189	182	**168**	508	605	525	604	488	**546**

Sub. No.	Wk	Lip capacitance					Mean	Melanin Value					Mean	Hemoglobin Value					Mean
		1	2	3	4	5		1	2	3	4	5		1	2	3	4	5	
8	0	33.60	37.60	40.60	39.40	42.90	**38.82**	155	114	98	112	119	**120**	569	647	589	603	579	**597**
	1	60.20	59.60	57.60	59.50	58.70	**59.12**	86	67	54	86	78	**74**	635	668	707	634	641	**657**
	2	48.90	44.10	43.40	46.00	47.70	**46.02**	134	162	92	132	119	**128**	643	680	618	680	664	**657**
	3	61.20	60.40	61.70	61.90	63.10	**61.66**	81	115	58	135	154	**109**	718	679	636	580	604	**643**
	4	50.30	46.90	48.90	49.50	50.30	**49.18**	105	44	76	43	45	**63**	694	698	680	694	701	**693**
	5	42.80	46.00	42.50	44.70	44.90	**44.18**	98	94	96	63	89	**88**	656	653	645	625	658	**647**
	6	46.60	44.70	50.90	49.70	46.20	**47.62**	101	95	103	47	97	**89**	656	672	643	662	647	**656**
9	0	49.80	54.20	52.00	61.70	60.40	**55.62**	32	44	45	48	74	**49**	691	726	702	691	640	**690**
	1	65.80	67.70	67.90	71.10	68.00	**68.10**	29	28	18	31	50	**31**	680	680	735	680	695	**694**
	2	71.00	69.90	63.80	72.20	71.00	**69.58**	44	50	34	39	37	**41**	717	673	691	695	699	**695**
	3	45.00	48.00	55.00	47.80	53.20	**49.80**	40	39	33	30	41	**37**	677	687	694	695	736	**698**
	4	59.80	66.00	63.30	69.40	67.40	**65.18**	80	76	29	61	52	**60**	652	630	677	626	687	**654**
	5	65.60	67.30	69.40	68.40	70.30	**68.20**	45	50	57	51	59	**52**	727	714	727	721	725	**723**
	6	59.40	58.10	66.10	62.40	63.90	**61.98**	36	23	40	47	26	**34**	685	706	685	687	694	**691**
10	0	56.40	58.10	58.70	62.20	63.20	**59.72**	31	22	54	29	34	**34**	655	681	630	685	653	**661**
	1	66.30	66.70	69.80	68.70	68.00	**67.90**	15	30	29	16	26	**23**	681	701	731	691	698	**700**
	2	48.20	54.20	55.10	57.10	57.50	**54.42**	29	12	15	17	24	**19**	678	758	721	741	704	**720**
	3	70.20	69.90	67.90	70.60	70.70	**69.86**	19	22	32	18	18	**22**	721	721	731	776	718	**733**
	4	63.90	64.80	67.00	71.40	68.50	**67.12**	25	34	23	30	33	**29**	727	674	698	712	711	**704**
	5	57.70	58.40	59.10	59.10	57.00	**58.26**	31	28	42	40	22	**33**	649	665	655	629	675	**655**
	6	67.00	63.60	71.20	69.40	66.40	**67.52**	21	21	18	21	13	**19**	682	666	749	682	746	**705**
11	0	35.60	39.30	43.20	45.80	43.30	**41.44**	94	97	61	63	94	**82**	626	641	627	636	626	**631**
	1	53.90	55.40	67.20	65.10	66.90	**61.70**	104	90	116	118	91	**104**	607	707	700	685	710	**682**
	2	55.80	58.10	61.80	62.90	65.80	**60.88**	63	56	46	69	53	**57**	614	682	710	685	692	**677**
	3	52.30	57.70	55.50	60.50	59.00	**57.00**	67	26	48	51	78	**54**	675	703	614	623	630	**649**
	4	50.60	54.10	54.00	57.00	55.00	**54.14**	36	54	44	42	31	**41**	728	713	716	700	691	**710**
	5	62.10	66.60	65.60	62.50	63.70	**64.10**	78	65	68	48	76	**67**	572	652	543	663	593	**605**
	6	60.70	62.00	63.50	61.40	64.20	**62.36**	82	47	73	45	95	**68**	694	759	701	716	658	**706**
12	0	48.50	49.40	55.90	59.50	54.20	**53.50**	57	47	59	30	45	**48**	658	698	701	647	635	**668**
	1	40.70	43.60	49.40	49.70	51.20	**46.92**	125	125	108	143	101	**120**	613	657	634	609	606	**624**
	2	65.90	72.40	70.70	72.90	73.20	**71.02**	107	129	108	114	94	**110**	653	636	651	639	674	**651**
	3	76.00	67.90	62.70	57.50	58.10	**64.44**	121	57	87	65	89	**84**	628	632	668	632	619	**636**
	4	64.70	59.90	62.10	65.00	67.00	**63.74**	117	132	84	98	103	**107**	584	568	561	568	535	**563**
	5	62.10	57.50	57.20	58.20	63.20	**59.64**	84	74	87	73	79	**79**	558	580	584	605	563	**578**
	6	58.60	56.20	63.80	61.90	64.00	**60.90**	90	101	78	100	98	**93**	582	641	609	614	607	**611**
13	0	45.60	46.10	50.10	49.60	54.20	**49.12**	253	245	195	241	198	**226**	603	640	651	622	636	**630**
	1	64.50	65.00	67.20	67.10	67.90	**66.34**	201	175	197	202	168	**189**	229	626	651	622	650	**556**
	2	73.80	74.20	73.30	75.90	76.40	**74.72**	122	121	104	136	108	**118**	652	662	669	623	637	**649**
	3	47.00	47.80	55.20	50.90	52.20	**50.62**	145	129	151	146	155	**145**	630	641	633	572	540	**603**
	4	59.90	59.90	65.10	63.70	67.20	**63.16**	171	145	141	138	147	**148**	644	620	651	653	665	**647**
	5	52.80	57.40	53.90	53.30	54.90	**54.46**	158	138	158	170	151	**155**	654	622	658	607	608	**630**
	6	60.70	68.10	68.00	66.50	66.30	**65.92**	125	166	155	104	170	**144**	623	629	643	654	636	**637**
14	0	36.50	39.50	44.90	45.60	44.70	**42.24**	116	117	81	116	81	**102**	558	565	539	664	592	**584**
	1	47.10	49.30	51.50	52.50	50.90	**50.26**	58	49	55	61	42	**53**	560	620	599	590	590	**592**
	2	43.20	43.90	48.30	49.20	49.10	**46.74**	80	67	53	66	71	**67**	561	569	579	536	489	**547**
	3	32.70	30.70	30.60	32.30	34.00	**32.06**	99	67	86	80	69	**80**	580	512	529	535	536	**538**
	4	62.00	59.40	62.30	62.80	63.50	**62.00**	46	52	60	36	53	**49**	612	612	588	605	592	**602**
	5	61.30	66.00	60.70	61.60	62.40	**62.40**	59	48	64	44	55	**54**	625	651	651	607	648	**636**
	6	60.20	61.40	60.10	63.30	65.40	**62.08**	26	47	33	54	23	**37**	608	624	582	617	607	**608**

Sub. No.	Wk	Lip capacitance 1	2	3	4	5	Mean	Melanin Value 1	2	3	4	5	Mean	Hemoglobin Value 1	2	3	4	5	Mean
15	0	56.90	59.60	57.20	58.30	59.30	**58.26**	84	137	83	146	88	**108**	561	649	641	575	622	**610**
	1	64.70	69.80	62.70	70.40	69.20	**67.36**	67	61	33	20	72	**51**	804	712	723	713	691	**729**
	2	61.80	63.40	69.00	66.70	70.10	**66.20**	29	28	42	26	24	**30**	722	695	760	715	722	**723**
	3	56.40	52.50	48.70	55.80	56.70	**54.02**	69	40	50	60	52	**54**	698	704	692	675	711	**696**
	4	68.70	69.70	68.50	72.10	71.00	**70.00**	28	33	35	28	38	**32**	672	668	698	697	648	**677**
	5	45.30	46.30	48.60	46.90	47.80	**46.98**	31	25	20	26	26	**26**	691	704	626	735	679	**687**
	6	55.20	51.80	59.00	63.60	54.70	**56.86**	36	33	48	44	53	**43**	665	659	582	672	613	**638**

2.2 Lipstick containing RBO

Sub. No.	Wk	Lip capacitance					Mean	Melanin Value					Mean	Hemoglobin Value					Mean
		1	2	3	4	5		1	2	3	4	5		1	2	3	4	5	
1	0	53.70	45.00	53.90	47.30	43.90	**48.76**	125	104	115	142	140	**125**	668	646	656	643	671	**657**
	1	66.10	65.40	58.50	68.50	66.60	**65.02**	120	169	188	170	168	**163**	664	675	711	691	623	**673**
	2	84.60	74.10	72.70	67.40	69.30	**73.62**	187	185	171	190	220	**191**	612	667	614	655	589	**627**
	3	63.70	72.10	73.70	73.80	72.50	**71.16**	134	145	157	163	130	**146**	674	616	659	674	639	**652**
	4	50.50	58.10	49.90	53.90	58.90	**54.26**	194	117	186	199	197	**179**	591	708	617	590	559	**613**
	5	76.20	72.40	72.40	71.60	73.70	**73.26**	100	128	111	140	120	**120**	673	639	699	644	740	**679**
	6	80.10	80.70	82.70	83.40	87.60	**82.90**	115	131	112	116	121	**119**	695	700	717	712	667	**698**
2	0	30.50	34.10	39.00	32.30	44.10	**36.00**	22	54	21	30	37	**33**	701	633	713	688	694	**686**
	1	48.50	53.20	52.50	49.60	52.40	**51.24**	65	52	41	37	24	**44**	691	685	730	727	705	**708**
	2	49.70	51.80	51.10	56.40	56.90	**53.18**	41	41	19	52	42	**39**	714	738	741	729	732	**731**
	3	35.50	38.10	36.60	42.60	40.40	**38.64**	34	27	30	26	28	**29**	655	701	704	697	723	**696**
	4	35.60	38.70	39.30	42.00	42.50	**39.62**	25	37	46	20	30	**32**	710	732	704	710	710	**713**
	5	49.30	49.70	49.20	52.10	51.90	**50.44**	25	24	27	25	24	**25**	733	730	724	735	722	**729**
	6	48.10	45.80	45.10	46.50	46.20	**46.34**	29	23	23	21	16	**22**	688	713	704	711	726	**708**
3	0	28.60	27.80	38.40	36.20	35.10	**33.22**	154	210	178	199	155	**179**	563	554	584	564	563	**566**
	1	52.40	50.60	56.90	47.80	53.30	**52.20**	189	109	171	150	192	**162**	568	617	577	626	567	**591**
	2	54.00	58.10	56.10	58.50	61.20	**57.58**	91	82	106	83	84	**89**	644	617	621	607	605	**619**
	3	49.40	48.30	55.80	48.60	53.00	**51.02**	87	69	83	65	90	**79**	644	628	646	651	666	**647**
	4	66.50	68.20	62.20	70.40	67.40	**66.94**	80	54	54	64	46	**60**	607	654	646	640	614	**632**
	5	54.80	52.80	52.40	52.10	51.50	**52.72**	130	96	156	103	103	**118**	765	688	643	688	706	**698**
	6	54.50	57.90	57.20	58.60	56.80	**57.00**	41	58	48	43	54	**49**	727	763	803	758	729	**756**
4	0	46.20	55.40	51.20	59.60	61.70	**54.82**	92	121	56	78	55	**80**	617	655	624	627	669	**638**
	1	65.60	67.30	68.70	71.60	68.40	**68.32**	62	56	121	77	85	**80**	600	642	621	619	602	**617**
	2	65.00	71.60	68.00	75.30	73.70	**70.72**	111	80	68	71	76	**81**	598	629	636	636	612	**622**
	3	53.70	54.30	58.40	53.00	58.30	**55.54**	80	98	86	62	60	**77**	645	698	668	688	663	**672**
	4	58.30	56.30	60.20	65.60	59.90	**60.06**	63	29	38	58	55	**49**	661	697	646	642	629	**655**
	5	38.20	40.80	41.10	38.60	39.80	**39.70**	82	82	74	96	83	**83**	662	623	632	633	591	**628**
	6	72.30	72.00	72.50	73.50	75.40	**73.14**	36	51	49	52	39	**45**	695	704	688	688	684	**692**
5	0	36.30	52.70	43.20	57.50	50.00	**47.94**	36	36	73	54	51	**50**	541	503	516	490	452	**500**
	1	49.00	58.40	60.10	58.10	62.50	**57.62**	38	51	50	46	45	**46**	612	600	537	631	609	**598**
	2	73.40	69.80	76.60	75.80	83.50	**75.82**	42	46	41	28	38	**39**	598	620	593	615	566	**598**
	3	54.70	57.70	57.80	61.90	63.20	**59.06**	71	56	50	58	49	**57**	664	688	676	720	666	**683**
	4	58.20	63.30	69.30	60.90	60.90	**62.52**	48	73	76	77	65	**68**	514	481	506	489	481	**494**
	5	63.10	67.70	71.60	67.90	69.50	**67.96**	77	81	85	85	86	**83**	597	626	614	606	609	**610**
	6	88.30	85.60	85.70	81.70	83.60	**84.98**	52	51	83	76	61	**65**	568	663	568	584	600	**597**
6	0	35.60	45.20	41.40	47.80	43.30	**42.66**	65	79	64	90	93	**78**	666	694	664	677	687	**678**
	1	71.90	72.50	71.70	73.20	70.10	**71.88**	52	54	58	53	62	**56**	637	640	619	620	624	**628**
	2	81.70	80.30	79.50	79.80	79.10	**80.08**	78	76	96	94	69	**83**	695	666	680	673	699	**683**
	3	60.40	61.80	58.50	61.30	55.90	**59.58**	74	69	78	85	62	**74**	678	684	623	636	673	**659**
	4	77.40	77.70	77.60	70.70	73.50	**75.38**	90	65	61	87	71	**75**	615	645	641	627	614	**628**
	5	71.40	70.70	71.90	70.00	70.40	**70.88**	64	54	61	72	59	**62**	662	681	650	634	652	**656**
	6	66.20	68.80	67.20	66.50	68.10	**67.36**	40	45	32	39	35	**38**	715	713	676	719	691	**703**
7	0	46.50	48.80	56.10	54.30	55.50	**52.24**	40	59	27	58	24	**42**	611	640	636	620	646	**631**
	1	59.20	60.10	61.70	58.70	61.50	**60.24**	41	21	42	33	43	**36**	660	697	650	676	664	**669**
	2	55.30	65.60	59.60	68.30	57.40	**61.24**	34	45	54	45	46	**45**	636	657	620	621	631	**633**
	3	50.30	56.50	50.90	54.60	53.90	**53.24**	75	59	59	62	50	**61**	651	712	667	685	725	**688**
	4	52.10	41.50	45.20	47.70	46.30	**46.56**	56	75	54	72	58	**63**	612	644	627	647	629	**632**
	5	58.30	62.20	59.00	61.40	60.40	**60.26**	102	92	90	74	71	**86**	665	691	658	688	650	**697**
	6	57.20	58.30	56.00	61.90	59.60	**58.60**	45	48	51	39	48	**46**	598	597	606	632	642	**615**

Sub. No.	Wk	Lip capacitance					Mean	Melanin Value					Mean	Hemoglobin Value					Mean
		1	2	3	4	5		1	2	3	4	5		1	2	3	4	5	
8	0	64.20	66.20	65.50	64.70	66.70	**65.46**	17	13	16	11	17	**15**	691	681	694	704	663	**687**
	1	51.50	48.00	45.60	46.90	49.40	**48.28**	24	22	29	32	35	**28**	698	717	650	745	657	**693**
	2	61.90	63.70	57.60	60.80	62.90	**61.38**	13	29	20	14	13	**18**	627	711	652	669	623	**656**
	3	42.80	43.20	42.20	51.10	50.10	**45.88**	23	14	12	19	24	**18**	639	698	636	688	620	**656**
	4	66.50	72.00	73.30	74.00	74.40	**72.04**	15	8	18	7	17	**13**	721	713	679	669	701	**697**
	5	50.60	54.40	53.50	53.10	56.60	**53.64**	27	15	15	29	14	**20**	713	745	745	711	713	**725**
	6	45.60	40.80	47.00	43.30	44.90	**44.32**	33	36	42	21	31	**33**	604	584	563	606	532	**578**
9	0	39.90	37.00	43.40	40.50	41.60	**40.48**	102	109	110	130	98	**110**	613	600	594	581	596	**597**
	1	76.60	73.70	69.90	74.40	74.00	**73.72**	88	118	89	131	110	**107**	668	671	669	618	642	**654**
	2	70.00	63.10	67.00	64.60	69.20	**66.78**	94	80	96	74	86	**86**	607	627	600	628	619	**616**
	3	58.30	56.70	63.20	61.00	62.90	**60.42**	89	61	88	46	73	**71**	573	640	619	612	626	**614**
	4	58.00	59.50	65.00	61.40	59.60	**60.70**	102	69	61	74	66	**74**	556	595	577	574	580	**576**
	5	67.00	73.50	68.10	72.00	67.40	**69.60**	82	106	80	57	91	**83**	603	623	601	660	581	**614**
	6	70.70	71.10	64.80	69.50	65.00	**68.22**	120	110	92	82	85	**98**	540	594	592	582	615	**585**
10	0	57.80	60.90	63.90	61.60	60.60	**60.96**	197	257	204	229	141	**206**	589	568	636	566	611	**594**
	1	65.30	63.00	62.00	61.10	59.60	**62.20**	130	146	130	162	118	**137**	573	605	594	617	642	**606**
	2	66.80	67.00	65.90	65.60	66.80	**66.42**	190	189	186	201	182	**190**	570	607	621	589	596	**597**
	3	77.50	77.00	75.10	74.90	75.20	**75.94**	133	133	138	176	176	**151**	702	699	687	633	667	**678**
	4	65.00	62.50	62.90	64.40	63.10	**63.58**	154	141	126	155	152	**146**	601	582	556	577	510	**565**
	5	63.50	62.80	67.70	63.90	67.40	**65.06**	201	152	192	151	193	**178**	614	522	642	637	635	**610**
	6	72.10	70.40	73.60	72.60	75.60	**72.86**	143	212	162	182	200	**180**	586	550	569	576	541	**564**
11	0	30.00	37.10	42.50	41.70	37.50	**37.76**	238	224	242	234	238	**235**	429	427	442	450	448	**439**
	1	56.30	56.30	60.10	57.10	60.70	**58.10**	193	208	219	219	197	**207**	557	548	544	546	559	**551**
	2	40.70	39.60	39.00	42.90	42.70	**40.98**	220	227	227	213	238	**225**	493	531	526	494	500	**509**
	3	38.60	39.60	40.70	37.90	36.70	**38.70**	230	230	224	231	227	**228**	484	500	500	505	492	**496**
	4	51.50	50.30	56.00	54.50	60.10	**54.48**	215	205	238	230	212	**220**	503	523	482	509	519	**507**
	5	56.50	62.60	59.70	59.40	58.10	**59.26**	246	245	246	231	203	**234**	487	516	491	495	481	**494**
	6	65.40	62.50	65.20	60.10	68.70	**64.38**	182	201	194	196	220	**199**	490	502	480	496	494	**492**
12	0	45.10	52.70	49.70	59.00	54.70	**52.24**	200	219	234	208	253	**223**	588	676	680	663	683	**658**
	1	54.20	57.10	61.30	58.30	64.10	**59.00**	202	209	212	162	215	**200**	619	612	605	581	598	**603**
	2	41.10	47.10	42.20	43.60	46.60	**44.12**	209	212	209	203	216	**210**	603	592	576	555	580	**581**
	3	46.30	43.00	46.30	47.00	48.60	**46.24**	192	134	137	156	172	**158**	667	705	685	654	629	**668**
	4	62.40	62.80	63.90	62.60	64.80	**63.30**	117	151	143	130	132	**135**	788	761	743	705	753	**750**
	5	44.30	41.50	46.70	47.60	44.50	**44.92**	184	162	166	174	174	**172**	598	659	615	588	651	**622**
	6	54.80	57.70	58.90	58.30	55.50	**57.04**	167	152	143	181	166	**162**	639	662	640	684	648	**655**
13	0	29.10	30.30	30.80	30.80	35.30	**31.26**	241	223	204	233	194	**219**	550	565	596	573	601	**577**
	1	59.70	61.50	57.70	61.70	63.50	**60.82**	151	149	161	155	154	**154**	646	626	643	618	611	**629**
	2	56.60	59.40	58.20	60.00	59.10	**58.66**	128	134	151	167	146	**145**	659	680	605	577	611	**626**
	3	54.80	52.90	57.60	54.30	59.20	**55.76**	171	150	144	176	162	**161**	585	607	650	598	613	**611**
	4	57.30	54.20	55.50	56.00	58.10	**56.22**	198	195	180	171	204	**190**	572	555	641	608	610	**597**
	5	53.30	51.20	49.60	53.60	52.80	**52.10**	179	161	187	182	201	**182**	632	619	619	598	585	**611**
	6	51.90	56.70	56.20	53.30	54.70	**54.56**	176	155	175	169	151	**165**	584	652	605	623	632	**619**
14	0	53.20	59.10	58.20	52.40	62.70	**57.12**	265	237	233	215	249	**240**	545	569	547	551	540	**550**
	1	81.50	80.00	77.30	71.30	73.80	**76.78**	126	166	156	185	190	**165**	721	625	627	614	603	**638**
	2	75.90	63.10	70.40	73.40	64.70	**69.50**	144	120	178	114	154	**142**	576	552	545	578	555	**561**
	3	66.20	56.20	75.50	61.40	67.10	**65.28**	112	167	129	151	151	**142**	595	570	650	583	629	**605**
	4	49.30	47.80	52.60	52.10	55.20	**51.40**	94	121	117	100	108	**108**	629	602	563	609	557	**592**
	5	68.20	65.00	61.80	57.60	66.20	**63.76**	93	92	87	84	82	**88**	594	646	625	659	639	**633**
	6	58.30	53.60	60.30	68.40	55.10	**59.14**	102	111	131	118	147	**122**	658	649	637	630	618	**638**

Sub. No.	Wk	Lip capacitance					Mean	Melanin Value					Mean	Hemoglobin Value					Mean
		1	2	3	4	5		1	2	3	4	5		1	2	3	4	5	
15	0	40.60	45.40	46.40	43.90	47.40	**44.74**	235	270	208	232	208	**231**	574	585	592	601	586	**588**
	1	42.80	41.10	45.10	44.30	46.40	**43.94**	225	253	232	256	260	**245**	539	543	558	547	555	**548**
	2	73.80	74.70	73.70	72.20	70.30	**72.94**	173	200	184	149	190	**179**	560	583	568	556	582	**570**
	3	50.90	52.70	58.00	57.90	54.20	**54.74**	141	171	132	140	142	**145**	594	601	614	599	591	**600**
	4	63.00	62.20	64.10	64.70	61.20	**63.04**	226	223	179	164	183	**195**	561	576	576	574	572	**572**
	5	56.70	56.10	57.40	56.50	58.50	**57.04**	156	113	127	122	120	**128**	636	657	633	625	599	**630**
	6	57.40	56.90	56.70	58.90	60.00	**57.98**	172	158	187	185	202	**181**	600	571	597	601	600	**594**

2.3 Lipstick containing RBO+oryzanol

Sub. No.	Wk	Lip capacitance					Mean	Melanin Value					Mean	Hemoglobin Value					Mean
		1	2	3	4	5		1	2	3	4	5		1	2	3	4	5	
1	0	68.30	61.40	62.20	61.20	58.50	**62.32**	254	245	237	241	291	**254**	578	583	602	574	585	**584**
	1	69.00	78.00	78.60	71.90	75.00	**74.50**	253	237	249	229	245	**243**	559	498	563	514	572	**541**
	2	57.30	56.80	60.80	62.90	64.10	**60.38**	244	245	222	214	249	**235**	585	542	577	573	553	**566**
	3	79.90	87.20	80.50	84.90	85.50	**83.60**	211	220	211	207	189	**208**	556	542	587	561	574	**564**
	4	70.70	69.70	71.80	73.50	73.50	**71.84**	257	257	267	258	266	**261**	541	539	535	504	527	**529**
	5	69.50	70.20	67.50	67.00	69.40	**68.72**	279	258	245	245	232	**252**	566	578	555	544	553	**559**
	6	72.00	71.40	68.80	73.20	67.70	**70.62**	249	197	170	221	224	**212**	612	587	549	582	478	**562**
2	0	47.20	42.20	50.00	41.20	41.30	**44.38**	142	132	148	151	148	**144**	532	586	542	565	561	**557**
	1	35.60	36.00	43.20	44.40	49.30	**41.70**	104	133	110	125	115	**117**	647	630	620	616	612	**625**
	2	53.30	58.30	69.80	63.50	65.70	**62.12**	162	176	147	160	188	**167**	607	599	588	593	524	**582**
	3	68.20	55.70	53.30	50.10	60.90	**57.64**	157	179	131	175	145	**157**	635	589	591	594	555	**593**
	4	44.30	50.00	48.60	54.70	53.40	**50.20**	150	176	140	144	159	**154**	553	554	564	602	549	**564**
	5	64.40	64.20	70.10	65.30	68.40	**66.48**	188	179	172	182	154	**175**	533	544	557	542	611	**557**
	6	56.00	56.30	59.80	60.60	55.60	**57.66**	155	156	169	144	170	**159**	623	618	617	632	563	**611**
3	0	47.00	46.20	53.20	45.20	48.30	**47.98**	85	80	90	85	87	**85**	713	659	680	703	676	**686**
	1	59.60	70.00	68.90	67.30	64.40	**66.04**	98	55	88	47	85	**75**	687	751	722	729	721	**722**
	2	43.20	49.20	47.00	49.70	50.30	**47.88**	134	124	127	153	144	**136**	683	716	659	711	719	**698**
	3	44.10	46.70	55.20	46.20	61.50	**50.74**	98	116	87	111	104	**103**	702	717	680	728	741	**714**
	4	47.90	52.90	57.10	55.40	59.90	**54.64**	79	128	113	132	109	**112**	735	744	769	624	756	**726**
	5	69.40	71.00	68.20	71.40	70.00	**70.00**	104	97	84	115	90	**98**	747	741	754	659	647	**710**
	6	80.80	79.80	85.40	90.70	86.80	**84.70**	98	104	66	79	73	**84**	706	722	741	727	720	**723**
4	0	33.00	42.60	37.10	45.20	38.50	**39.28**	98	75	78	91	56	**80**	698	698	721	687	699	**701**
	1	35.80	38.00	39.20	37.90	40.50	**38.28**	65	49	38	54	21	**45**	721	723	701	732	719	**719**
	2	39.70	39.00	41.50	41.80	45.70	**41.54**	44	69	41	46	65	**53**	607	658	667	656	671	**652**
	3	43.70	48.90	48.20	49.20	50.90	**48.18**	68	61	58	54	46	**57**	705	716	705	691	678	**699**
	4	41.10	46.10	44.50	48.90	49.40	**46.00**	71	56	50	58	49	**57**	664	688	676	720	666	**683**
	5	47.50	46.40	47.80	48.70	51.90	**48.46**	61	52	58	58	46	**55**	708	681	701	701	717	**702**
	6	53.30	52.40	56.30	52.30	55.20	**53.90**	47	30	45	54	51	**45**	681	717	685	714	684	**696**
5	0	30.70	33.50	30.50	36.60	31.70	**32.60**	163	186	175	176	161	**172**	706	672	666	683	677	**681**
	1	52.10	53.50	53.20	51.70	57.10	**53.52**	141	159	113	133	129	**135**	728	706	694	725	677	**706**
	2	73.10	75.00	78.30	78.40	75.60	**76.08**	119	170	139	149	146	**145**	699	720	691	628	675	**683**
	3	43.10	39.70	45.90	46.10	42.00	**43.36**	145	122	135	122	155	**136**	758	743	747	757	743	**750**
	4	55.90	48.20	53.50	58.60	52.30	**53.70**	112	139	125	133	130	**128**	723	671	707	687	698	**697**
	5	65.70	65.20	67.20	66.00	68.50	**66.52**	51	100	85	83	88	**81**	653	677	701	687	699	**683**
	6	59.20	60.70	59.30	60.00	64.70	**60.78**	163	140	129	191	142	**153**	629	651	663	655	630	**646**
6	0	65.30	63.10	67.50	67.40	67.30	**66.12**	359	340	304	330	271	**321**	609	620	625	588	593	**607**
	1	76.00	75.90	75.70	73.80	72.70	**74.82**	242	271	312	279	241	**269**	691	673	575	661	665	**653**
	2	70.30	72.90	72.90	73.70	71.10	**72.18**	298	271	279	257	264	**274**	569	573	595	561	592	**578**
	3	71.60	74.20	73.80	74.90	74.00	**73.70**	279	260	229	312	276	**271**	574	601	496	593	531	**559**
	4	65.20	72.70	71.40	73.80	71.20	**70.86**	311	277	290	280	299	**291**	564	535	615	576	586	**575**
	5	62.90	67.90	64.30	68.70	64.60	**65.68**	320	234	299	265	281	**280**	550	553	511	542	589	**549**
	6	61.60	63.60	62.30	61.80	60.80	**62.02**	276	271	271	271	256	**269**	562	615	555	629	561	**584**
7	0	56.20	59.10	56.40	63.10	62.50	**59.46**	20	13	24	30	21	**22**	569	588	658	627	641	**617**
	1	47.30	45.50	41.10	47.50	44.50	**45.18**	32	23	18	13	18	**21**	662	641	607	606	598	**623**
	2	50.00	57.20	55.60	58.00	60.50	**56.26**	7	36	18	19	26	**21**	596	639	609	652	649	**629**
	3	63.10	67.10	63.80	65.10	67.40	**65.30**	33	30	7	29	13	**22**	601	637	602	691	653	**637**
	4	70.90	68.00	66.00	72.40	69.90	**69.44**	13	13	18	10	6	**12**	607	623	610	637	656	**627**
	5	46.00	45.00	47.70	49.40	48.20	**47.26**	24	20	34	27	37	**28**	705	708	732	682	688	**703**
	6	54.80	57.30	58.60	57.10	57.50	**57.06**	15	16	32	35	22	**24**	591	653	594	653	617	**622**

Sub. No.	Wk	Lip capacitance					Mean	Melanin Value					Mean	Hemoglobin Value					Mean
		1	2	3	4	5		1	2	3	4	5		1	2	3	4	5	
8	0	42.90	44.90	46.70	42.50	44.30	**44.26**	182	178	188	202	188	**188**	602	569	582	624	632	**602**
	1	62.10	66.00	69.80	68.30	70.20	**67.28**	140	121	178	146	118	**141**	694	641	681	746	666	**686**
	2	62.80	72.50	61.60	70.50	68.60	**67.20**	103	107	94	92	106	**100**	606	631	624	624	636	**624**
	3	69.00	71.80	71.30	72.50	69.50	**70.82**	65	84	60	70	54	**67**	675	643	653	641	633	**649**
	4	48.40	44.90	46.50	56.70	50.10	**49.32**	93	114	102	101	79	**98**	596	623	590	606	531	**589**
	5	57.70	58.40	59.10	59.10	57.00	**58.26**	97	97	103	103	107	**101**	583	575	581	606	565	**582**
	6	62.40	64.00	61.10	63.20	63.10	**62.76**	67	71	93	62	96	**78**	642	622	541	609	528	**588**
9	wk0	50.70	57.60	54.50	56.20	60.00	**55.80**	86	78	80	81	90	**83**	475	492	510	476	511	**493**
	wk1	72.50	68.50	74.50	71.80	81.80	**73.82**	94	85	77	86	87	**86**	503	532	516	528	513	**518**
	wk2	73.70	74.30	70.50	76.50	78.20	**74.64**	70	66	41	66	53	**59**	489	491	484	478	479	**484**
	wk3	49.00	54.00	53.80	58.00	57.60	**54.48**	68	45	45	49	43	**50**	492	546	531	490	530	**518**
	wk4	49.70	48.70	53.50	49.50	55.50	**51.38**	54	70	79	72	74	**70**	519	523	543	522	524	**526**
	wk5	65.00	67.60	64.40	65.80	66.90	**65.94**	64	65	61	65	55	**62**	500	524	497	518	503	**508**
	wk6	73.10	76.00	76.30	80.00	79.10	**76.90**	58	43	38	33	37	**42**	561	535	551	508	547	**540**
10	0	36.90	38.00	38.00	39.60	43.90	**39.28**	57	62	38	40	59	**51**	614	583	552	601	568	**584**
	1	48.90	50.00	51.80	45.60	50.00	**49.26**	51	46	39	49	42	**45**	678	627	663	685	688	**668**
	2	44.20	45.40	56.20	47.60	48.90	**48.46**	57	70	58	67	63	**63**	601	594	598	565	573	**586**
	3	59.50	58.70	58.40	62.00	62.50	**60.22**	41	37	29	45	32	**37**	672	645	652	604	658	**646**
	4	71.90	76.00	74.10	70.70	76.60	**73.86**	29	21	42	23	25	**28**	638	593	615	612	618	**615**
	5	50.60	50.60	51.50	52.50	52.10	**51.46**	45	46	65	56	51	**53**	629	607	609	630	615	**618**
	6	46.30	46.70	48.70	52.20	49.60	**48.70**	76	76	54	45	61	**62**	478	598	547	607	565	**559**
11	0	43.20	45.60	47.50	45.00	46.00	**45.46**	45	54	42	56	45	**48**	581	623	600	578	637	**604**
	1	46.60	43.90	48.10	50.00	52.00	**48.12**	41	33	26	32	31	**33**	591	595	552	607	589	**587**
	2	52.80	60.40	61.20	61.10	64.20	**59.94**	32	21	39	40	21	**31**	643	632	656	639	642	**642**
	3	65.60	64.70	71.40	66.00	73.90	**68.32**	20	27	17	13	33	**22**	594	624	647	588	688	**628**
	4	59.10	55.60	59.50	62.10	61.80	**59.62**	12	20	21	18	10	**16**	634	643	688	678	652	**659**
	5	61.20	58.10	58.00	58.90	61.60	**59.56**	18	16	33	24	33	**25**	645	641	678	647	655	**653**
	6	71.10	69.10	68.90	71.60	70.00	**70.14**	20	24	21	22	30	**23**	741	694	766	791	684	**735**
12	0	64.50	59.00	54.60	65.20	65.70	**61.80**	60	56	66	54	61	**59**	630	629	545	584	657	**609**
	1	52.00	51.50	62.60	58.40	58.30	**56.56**	80	86	62	55	59	**68**	680	646	691	644	653	**663**
	2	63.30	69.40	65.10	66.00	73.70	**67.50**	39	41	39	27	52	**40**	708	609	701	605	632	**651**
	3	58.50	58.10	59.70	61.90	60.90	**59.82**	57	45	76	36	50	**53**	705	731	754	720	760	**734**
	4	56.40	51.90	51.30	58.40	55.50	**54.70**	30	28	32	23	33	**29**	694	728	705	743	711	**716**
	5	72.50	79.30	73.70	73.20	73.70	**74.48**	57	55	68	65	54	**60**	751	736	649	652	687	**695**
	6	63.60	66.00	66.50	68.90	69.90	**66.98**	42	29	27	29	55	**36**	692	795	687	718	604	**699**
13	0	55.10	57.50	59.00	59.70	60.10	**58.28**	214	218	228	207	224	**218**	587	621	601	619	643	**614**
	1	65.70	67.20	66.00	63.60	65.90	**65.68**	167	179	178	197	206	**185**	613	655	660	662	677	**653**
	2	55.20	59.90	65.60	60.70	63.40	**60.96**	169	165	162	169	160	**165**	653	695	670	620	691	**666**
	3	53.60	58.40	58.60	63.70	61.20	**59.10**	182	208	201	205	193	**198**	662	691	679	676	672	**676**
	4	65.80	68.10	67.20	61.90	62.00	**65.00**	135	160	138	145	127	**141**	564	603	547	574	622	**582**
	5	72.20	72.50	75.30	69.60	72.70	**72.46**	169	176	185	175	181	**177**	606	653	650	627	626	**632**
	6	52.40	49.40	51.30	51.90	54.30	**51.86**	148	156	152	140	145	**148**	572	679	608	652	615	**625**
14	0	39.50	47.00	45.50	46.30	38.00	**43.26**	140	120	135	119	129	**129**	532	552	549	561	543	**547**
	1	57.80	56.70	63.30	65.10	63.30	**61.24**	156	161	156	186	152	**162**	477	555	540	577	542	**538**
	2	61.60	70.20	63.90	68.40	65.10	**65.84**	149	152	153	141	134	**146**	460	464	509	525	478	**487**
	3	52.60	53.20	53.80	57.60	62.70	**55.98**	112	96	111	113	101	**107**	589	605	619	622	623	**612**
	4	51.80	54.10	53.40	56.10	55.30	**54.14**	84	116	127	121	124	**114**	543	590	532	572	558	**559**
	5	60.10	62.30	65.90	63.80	61.30	**62.68**	125	134	117	116	118	**122**	551	584	626	525	611	**579**
	6	43.10	43.70	43.60	44.10	48.30	**44.56**	125	123	126	122	119	**123**	493	582	587	585	569	**563**

Sub. No.	Wk	Lip capacitance					Mean	Melanin Value					Mean	Hemoglobin Value					Mean
		1	2	3	4	5		1	2	3	4	5		1	2	3	4	5	
15	0	48.40	50.90	56.10	55.30	55.00	**53.14**	108	141	171	150	155	**145**	659	627	639	636	619	**636**
	1	52.00	51.20	53.50	54.70	52.50	**52.78**	87	143	92	151	69	**108**	632	655	677	629	664	**651**
	2	45.50	45.80	47.60	49.90	49.70	**47.70**	76	53	83	75	74	**72**	691	698	738	680	698	**701**
	3	55.10	52.80	55.90	56.40	53.50	**54.74**	86	72	97	108	71	**87**	651	661	604	641	652	**642**
	4	49.70	48.60	48.20	49.80	50.60	**49.38**	85	116	104	116	94	**103**	684	666	659	658	657	**665**
	5	58.00	58.70	57.50	60.20	61.50	**59.18**	93	114	115	119	108	**110**	641	643	641	651	649	**645**
	6	42.70	42.40	42.70	46.50	43.90	**43.64**	100	85	74	65	80	**81**	627	671	658	661	645	**652**

APPENDIX E

Questionnaires

แบบประเมินก่อนการใช้ผลิตภัณฑ์

ชื่อ นาง/น.ส..............................นามสกุล...ชื่อเล่น................

อายุ...............................ปี เบอร์โทรศัพท์ที่ติดต่อได้สะดวก.......................................

กรุณากรอกข้อมูลดังต่อไปนี้

1. การแพ้เครื่องสำอาง ☐ ง่าย ☐ ปานกลาง ☐ ไม่แพ้

ผลิตภัณฑ์ที่แพ้คือ...

.2. ผลิตภัณฑ์ที่ใช้บำรุงริมฝีปาก ☐ ไม่ใช้ ☐ ใช้ ระบุ..

3. ผลิตภัณฑ์ที่ใช้แต่งสีริมฝีปาก ☐ ไม่ใช้ ☐ ใช้ ระบุ...

4. ขณะนี้กำลังรับประทานยา ☐ ไม่ใช้ ☐ ใช่ ระบุ..

5. ปกติดื่มน้ำวันละ

☐ น้อยกว่า 4 แก้ว ☐ 4-6 แก้ว ☐ 6-8 แก้ว ☐ มากกว่า 8 แก้ว

วันที่เริ่มทำการทดลอง ☐ อ.17/05/48 ☐ พ.18/05/48 ☐ พฤ.19/05/48

แบบสอบถามประเมินความพึงพอใจต่อผลิตภัณฑ์เมื่อสิ้นสุดการทดลอง

ได้รับผลิตภัณฑ์หมายเลข..........

ชื่อ นาง/น.ส..................... นามสกุล... อายุ....................ปี

สิ้นสุดการทดลองวันที่...

กรุณาทำเครื่องหมาย √ ในช่องที่เห็นว่าเหมาะสมที่สุด

ความพึงพอใจ	พอใจมากที่สุด	พอใจมาก	พอใจปานกลาง	พอใจน้อย	พอใจน้อยที่สุด
ประเมินความพึงพอใจลิปสติก 1. ความนุ่มลื่นในการทาลิปสติก					
2. ความมันวาวของแท่งลิปสติก					
3. รสชาติ					
4. กลิ่น					
ประเมินความพึงพอใจหลังการใช้ลิปสติก 5. ความชุ่มชื้นของริมฝีปาก					
6. ริมฝีปากแดงขึ้นหลังใช้					
7. ริมฝีปากดำลดลงหลังใช้					
8. ความเหนอหนะ					
9. ความพึงพอใจต่อผลิตภัณฑ์					

ข้อเสนอแนะอื่นๆ____

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VITA

Miss Jittima Usahanun was born on September 5, 1978 in Uttaradit, Thailand. She received her Bachelor degree of Science in Pharmacy from Faculty of Pharmacy, Chiangmai University, Chiangmai, Thailand in 2000.

From 2001 up to now, she has worked at Lampang Cancer Center. In 2003, She granted from Lampang Cancer Center to entered the Master's Degree program in Pharmacy at Chulalongkorn University.