CHAPTER 5

EFFICACY STUDY OF MOISTURISER CONTAINING *Baccaurea motleyana hook f.* FRUIT'S EXTRACT

5.1   Introduction

The desire for an evidence-based approach for cosmetic efficacy has led the cosmetic industry to expand into the world of cosmeceuticals. The consumer increasingly demands that cosmetic claims be backed up with clinical test data. These demands for evidence come in parallel with great progress in the metrics of skin structure and function which can be used to verify the products that work and expose those that do not. Research guidance testing seeks insight into products and prototypes through the analysis of early-in consumer evaluations and the assessments of trained descriptive panels. Some companies rely more heavily on the insights derived from trained panels because their judgements are free of bias and other lean toward tests with naïve consumers because they are the ultimate purchasers of the product. Also, the cost of trained panels can be prohibitive (Braddon *et. al.*, 2002).

5.1.1   Overview of Available Efficacy Testing Methods

Product evaluation study can be performed either by objective or subjective methods. The objective method is either by instrumental or sensory test whilst the subjective can only be obtained from sensory test method which basically represent individual opinions (Ryan, 1996).   According to Ryan (1996) sensory methods measured the product's performance by the senses of sight, hearing, smell, touch and taste. The main

advantage of this method is they measure the performance of product as perceived by the user. In subjective test studies, inferences are drawn from patterns on ideas or opinions offered by consumers whereas in objective evaluation, recommendations are based on statistical inferences from product ratings (Braddon *et. al.*, 2002). According to Braddon *et. al.* (2002), the focus group evaluation is the best known technique for gaining subjective or qualitative in sight into consumer attitudes. Generation of product ideas, benefit, weaknesses in current products as well as how to understand people thinking about branded products, product usage, habits and concepts could be generated. Due to these advantages, subjective research typically precedes objective especially when the project is about new products. Subjective research method can be used to clarify and understand the terms that people actually use to describe moisturisers and their effects on the skin, and also to learn what people think of one or more prototypes.

The sensory test method, either objective or subjective utilise human (usually called panel, assessors or testers) instead of instruments to measure the sensory characteristics of the test products. According to Braddon *et. al.* (2002) and Ryan (1996), the panel of assessors can be classified as follows :

(a)   **Untrained Panel**

Untrained assessors will use their subjective opinions to evaluate products, thus could create a lot of variability in making their judgements since they rely on their experience in dealing with the products. According to Ryan (1996), at least 50 assessors are needed as a panel in research study involving untrained assessors.

(b)   Experienced or Semi-trained Panel

Braddon *et. al.* (2002) has , this group of individuals who either have not participated in a training programme but are experienced in product evaluations or a group that has undergone a very general training programme. The assessors have become experienced through their frequent participation in product evaluations or assessments.

(c)   Trained Panel

Trained assessors would be able to differentiate the different types of product. They could also detect the different levels of ingredient's concentrations used in the products such as the fragrance strength. A properly trained assessors should be able to assessed the products more consistently and a panel of 20 to 30 people is a norm (Ryan, 1996).

(d)   Expert Panel

This is a group of individuals who have undergone a formal and rigorous training (Braddon *et. al.*, 2002, Keane, 1992, Stone, 1992, Munoz and Civille, 1992). The panel evaluates products following common established procedures and they acquired their expertise through their continuous exposure to products evaluations. These assessors have already demonstrated a particular acuity in their work, have developed a good long-term memory and can draw on additional knowledge gained in relevant fields (Ryan, 1996, BSI, 1993). Expert assessors are even more consistent in their judgement and this panel can consist of 1 to 5 people. The most well known sensory experts are wine tasters, tea, coffee tasters, perfumers, dermatologists, dentists and hairdressers.

Moisturisers have dual identities, that is they are experienced as functional and pleasure-giving (hedonic) substances. Some moisturisers are positioned as more functional or therapeutic, thus emphasize the nuances of efficacy and skin healing (Braddon *et. al.*, 2002). To provide direction for product development, moisturiser

terms or attributes must have actionable meanings. The selection of the appropriate test method for the evaluation of moisturisers depends on the development process phase and budget available. However, Braddon *et. al.* (2002) has reported that discriminative-descriptive testing assessments is usually selected for moisturiser product evaluation.

5.1.1 (i)   Discriminative Test

Discriminative test is an objective type of sensory product evaluation test. It is subdivided into two types that is difference test and descriptive test (Ryan, 1996).

5.1.1 (i) (a)   Difference Test

It is used to determine if two products are distinguishable or not. It is quick and inexpensive but provide only limited information since the assessors are required to reply "Yes", "No" or Don't know" (Ryan, 1996). Difference test is useful in quality control to check samples against standards and in R & D for quick screening of prototype formulations. However, it is restricted to comparisons of two products at a time and the most common are the triangle test and duo-trio test. In standard triangle test, two of the samples are the same and one is different. The task is to select the odd product. In a duo-trio test, one product is identified as the reference. The participant is to pick which of the remaining two samples is the same as the reference (Braddon *et. al.*, 2002, Meilgaard *et. al.*, 1991).

5.1.1 (i) (b)   Descriptive Test

This test is sometimes known as profile tests or Quantitative Descriptive Analysis (QDA). It is useful in assessing new product concepts, comparing prototype formulations, documenting product claims and comparing sensory and instrumental methods (Ryan, 1996). According to Braddon *et. al.* (2002), this analysis is the one of the most complex and involved sensory tests used in the evaluation of personal care

products. The technique is used by trained panels to characterise the perceived sensory attributes of a product objectively or subjectively (Braddon et. al., 2002, Stone and Sidel, 1993, Lawless and Heymann, 1998). According to Braddon et. al. (2002), descriptive analysis results provide information not obtained through other methods such as technical and specific information on perceived attributes and personal preferences.

All current skin-feel descriptive evaluations of moisturisers and other personal care products are based on the modified texture profile method (Braddon et. al., 2002, Schwartz, 1975), where the concepts of food texture profile method were adapted for the evaluation of skincare products. Schwartz (1975) classified the main stages of skincare products evaluation as pick-up (the removal of product from the container), rub-out (the application of products on the skin) and after-feel (the evaluation of product's effect on the skin). This work was adapted by all professionals working in skin-feel evaluations for their specific applications (Braddon et. al., 2002). Descriptive skin-feel evaluation was established when the standard practice for descriptive skin-feel analysis of creams and lotions was published by the ASTM committee E18 on sensory evaluation (ASTM-E1490-92, 1999). According to Braddon et. al. (2002), the techniques published in ASTM standard are based on the modified texture profile method for skin care product evaluations.

The main application of descriptive types of data, as suggested by Braddon et. al. (2002) is in the area of

(a)   Product's liking factor identification. Descriptive or attribute data allow the identification of attributes that drive or affect the acceptance of the product.

(b)   Documentation of product's sensory properties. The data provide the product sensory properties and this is crucial in characterising of controls, prototypes   and competitor products.

(c)   Screening of products. This is usually carried out based on specific   sensory attributes such as spreadability, absorbency, smoothness, stickiness, thickness and after-feel sensation.

(d)   Product modifications or maintenance. The data are used to track product characteristics in shelf-life studies and during production (quality control) and to assess differences from and similarities to controls in ingredient or process substitution and cost reduction projects.

(e)   Consumer test design as product guidance or optimisation. Descriptive analysis can be used for product's questionnaire development since it provides evaluated product's attributes information.

(f)   Interpretation of consumer information. The data obtained allow the interpretation of consumer responses.

(g)   Supportive information for claim support. The data support a claim on specific perceived product attributes and performance.

In descriptive test study, the overall and each product attributes could be evaluated separately. In overall descriptive test study for instance, the assessors consider all sensory characteristics in making judgement, therefore, the response reflects overall differences and/or similarities between products. The more focussed attributes descriptive test focuses on a person's ability to detect differences between products on a particular attribute such as the product's greasiness, shininess or fragrance intensity (Braddon et. al., 2002). Braddon et. al. (2002) has reported that moisturiser is appraised in terms of its delivery properties (such as ease of dispensing, viscosity, smoothness,

gloss), pick-up attributes (such as stickiness and stringiness), rub-out properties (such as spreadability, thickness, wetness, whitening effect, absorbency, cooling, warming or tingling sensation,) and after-feel characteristics (such as greasiness, stickiness, moisturise, tautness, burning, tingling and cooling sensation) A detail description of procedures and attributes could be referred to ASTM Standard Practice E 1490-92 (ASTM-E1490-92, 1999). On the other hand, the product's fragrance attribute can be evaluated by itself (that is not in the product) or in the product (Braddon *et. al.*, 2002). When it was evaluated by itself, the fragrance is contained in a glass container whilst when it was in the product, it is applied and the fragrance is sniffed on the skin.

5.1.1 (ii) Objective Sensory Test Method

The advantages of this study is the standardized product questionnaire evaluated by all assessors to reach statistically based conclusions. The questionnaire contains primarily closed-ended items using terminology and rating scales selected by the researcher (Braddon *et. al.*, 2002). Braddon *et. al.* (2002) has reported that there are several research designs for the quantitative product assessments such as monadic, sequential monadic, proto-monadic and paired preference (sequential presentation and simultaneous presentation), Table 5.1.

Table 5.1: Research designs for the quantitative assessment

Design Name	Characteristics
Monadic	One product per person. Survey after product use.
Sequential monadic	Two (or more) products used in sequence for equal amounts of time. Balanced order of presentation. Complete survey for all products, including attribute and overall preference.
Proto-monadic	Two products used in sequence for equal amounts of time. Balanced order of presentation. Complete survey for first product. Preference questions in second survey. May also include acceptance questions.

Table 5.1: Research designs for the quantitative assessment, *continued*

Design Name	Characteristics
Paired preference (sequential presentation)	Two products in sequence for equal amounts of time. Balanced order of presentation. No survey after completion of first product, only after both products are used.
Paired preference (simultaneous presentation)	Two products used simultaneously on half the body or face. Product presentation balanced for side of application. Preference questions after both products are used; additional diagnostic items may be included.

Note:
Balanced order of presentation means, for example, half of the assessors use the current control product (assuming A) followed by the new prototype (assuming B) and another half of the assessors will use the products in reverse order that is, the new prototype (assuming B) followed by the current control product (assuming A).

The monadic design is more reflective of normal product use. People rarely use two different brands simultaneously, in alteration or in quick succession for purposes of comparison. Monadic presentation is also aligned with an encounter with a dramatic "break-through" type of product. The sequential design is aligned with a product substitution, upgrade, or brand switching experience. For example, suppose a company wants to substitute a new, improved or less expensive product for a currently marketed product. A sequential monadic design could be used whereby the assessors will be given two products with a balanced order of presentation. Alternatively, a two-cell monadic design could also be employed. All the assessors in the study are screened to be current users of the current marketed product. The monadic assessments for the new formula will now implicitly be carried out with reference to that regular brand. Quantitative testing is either conducted in consumers' homes or at a testing facility. However, home testing most resemblance real life usage.

5.1.1 (iii)   Questionnaire Design

The questionnaire designed should be clear and easy to answer by the assessors. The terms used should be easily understood and the scale rating points must make sense intuitively to the average assessors. Braddon et. al. (2002) suggested that a good questionnaire should comprise the mixture of questions such as comparison to regular brand (scale like much better, equal or much worse), ratings of product's attributes such as softness, smoothness, greasiness etc. (scale like excellent to poor or in points form), directional or intensity type of questions such as "just about right", "too thick", "not at all greasy" to " too much greasy" and open ended form such as preferences of product and comments on overall performance of products.

Another factor in determining the research design is the ability of the test to detect product's differences. When the difference in the product is desired, extending the usage period or employing the half-body procedure is recommended (Braddon et. al., 2002). The half-body technique improves sensitivity because both products are experienced nearly simultaneously. The chances of obtaining reliable data results are enhanced when assessors follow procedures and understand directions.

5.1.1 (iii) (a)   Claim Support Studies

Claim support studies can be divided into two categories that are consumer-based claims and descriptive-based claims (Braddon, et. al., 2002). Advertising claims most often address perceived product attributes and/or differences as perceived by consumers or trained assessors, thus the application of sensory and consumer methods is essential in claim support studies. Many claims support testing is completed using objective sensory descriptive tests because they can provide more objective data regarding the perceived attributes without regard for personal preference (Braddon et. al., 2002).

Consumer-based claims can give a liking/preference or a perceived attribute responses; which can be comparative or non-comparative. Comparative claims can be either parity or superiority claims (ASTM – E1958-98, 1999) such as *"as moisturising as"* (parity claims) or *"more radiant than"* (superiority claims). For non-comparative claims, the results only described the effect of that respective product. For example, *"Our product leaves your skin feeling soft".* *Descriptive-based claims* have the following characteristics when compared to consumer-based claims, that is

(a)   they do not address preference and acceptance or liking

(b)   they address attribute perception by a highly trained panel

(c)   the attributes used are not consumer terms (gentle, moisturising, radiant etc.) but technical and descriptive (oily, greasy, spreadable, sticky etc.)

Any study to support productclaims need to be carefully designed and executed with special attention needs to be paid to sample size (Gacula, 1993), product selection and questionnaire design (Braddon *et. al.*, 2002). As described by Braddon *et. al.* (2002), the consumer-based claim study result will become stronger if it was supported by the descriptive-based study. Descriptive-based claims can also be comparative or non-comparative like those in consumer-based claim study.

5.1.1 (iv)   Product's Adverse Effect

Some products, especially those containing AHA can cause skin irritation or an "adverse reaction" when used by consumers. Typical descriptive terminology includes tingling, stinging, burning, rashing, pulling, tightness and itchiness can also be included in the questionnaire or survey form given to the assessors (Braddon *et. al.*, 2002). However according to Braddon *et. al.* (2002), raising the irritation issue items in a survey may create an exaggerated comments from the assessors. On the other hand, it

could also provide an understanding of the product used if the assessors were told that irritation is part and parcel of the product's positive mode of action. To minimise these concerns, a survey form should be designed with a balanced wording that is free of bias such as *"Did you experience any pleasant or unpleasant sensations after using the products within three weeks?"*, could be used. It is however should be noted that unpleasant sensory stimulation does not necessarily correlate with disliking for a product. Brief stinging may be a sign of product's efficacy that is, it is working to smoothen or exfoliate the skin. As such, the unpleasantness may not negatively influence the assessors. Braddon *et. al.* (2002) has also suggested that studying the product should be in the consumer or assessors normal environment.

5.1.1 (v)   Instrumental Product Evaluation

5.1.1 (v) (a)   Skin Hydration Assessment

Water is the main material of the body substances. In new born, water content is approximately 75% whereas in adults, it is approximately 50%-60% of their weight. Thus, water balance of the body plays an important role. The daily average needs of water for an adult is approximately 2.1 litre. The excretion of water is done mainly by the ureters, by breathing out vapour and through the skin surfaces. The skin's moisture content depends on age (Morganti, 1999, Courage-Khazaka, 1997, Rogiers *et. al.*, 1990). Rogiers *et. al.* (1990) reported that a child's skin hydration is very low, an adult with the age in between of 20-40 years is at its maximum and a senior's skin hydration becomes lower again due to decreasing of water binding capacity of the moisture in stratum corneum layer. A study conducted by Tagami (1988) also stated a similar finding regarding the correlation of skin hydration with age factor, where low hydration values for children was obtained until prepuberal age. Gender, however, is no importance as far as skin hydration is concerned (Rogiers *et. al.*, 1990, Tagami, 1988).

The measured body site also influences skin hydration, especially due to the thickness of the skin, the number and activity of sweat glands (Rogiers *et. al.*, 1990, Blichmann and Serup, 1988). On the forehead and palms of the hands, skin hydration is very high and these places are known to be rich in sweat glands whilst on the arm, it is mostly very dry (Courage-Khazaka, 1997, Rogiers *et. al.*, 1990). Rogiers *et. al.* (1990) have reported skin surface hydration studies on symmetrical sites of the body conducted by Husz and Simon (1964), Clar *et. al.* (1975), Cook *et. al.* (1982) and Thune (1988). They stressed on the importance of measuring skin surface hydration on a well defined body sites area since the results obtained could be vastly different from each other. They reported that the results attained could be different even when it was measured a few centimetres away from the actual areas. In his paper, Morganti (1999) has carried out a study on skin hydration among the Italians (age 26 to 45) in different parts of the region that is in northen, central and southern Italy under conditions of controlled humidity and temperature. He found out that high skin dehydration levels were always related to a constant lack of surface lipids, regardless of age. He also reported that as relative humidity rate increases, skin dehydration decreases (Morganti, 1999 and 1996, Fabrizi and Morganti, 1994).

The temperature regulation mechanism of the body also influences skin hydration. The human body is usually warmer than the environment. The body balances this difference by evaporating water. The hydration of skin surface is one of the most important parameters to evaluate the barrier function of the skin. The more balanced the hydrolipidic film, the higher is skin hydrated. The types of product used is another factor influences skin hydration. The w/o creams had more effect on skin hydration than the o/w emulsions, considering that w/o emulsions are more occlusive than o/w

emulsions, thus better preventing excessive water loss from the skin (Rogiers *et. al.*, 1990, Choudhury *et. al.*, 1985, Barnett, 1972).

Among the factors that affecting skin water content as reported by Bashir and Maibach (2000) and Courage-Khazaka (1997) are

(a)   water diffusion from viable epidermis

This water is usually retained by stratum corneum barrier. The thicker this layer, the more difficult the water to vapour out from the skin.

(b)   air humidity factor and room temperature

Direct light can warm up the skin and may cause higher transpiration, but at the same time decreasing sebum secretion, thus changing hydrolipidic film enormously. The equilibrium between skin's horny layer water content and ambient air is very important due to the movement of water from the skin to the atmosphere (vice versa) which depends on atmosphere external humidity. The higher the room temperature and the air humidity, the higher will be the moisture level of the skin. The climate also plays an important role in effecting the moisture content of the skin. At higher temperature, transpiration is higher but due to the increased in air humidity, less water can evaporate from the skin producing a more hydrated stratum corneum condition.

(c)   fixed water in the horny layer

Water in this form is bound to the natural moisturising factors (NMF) in the stratum corneum such as urea, pyrrolidone carboxylic acid, amino acids and lactic acids.

(d)   moisturising agents

These may hydrate the skin by forming an occlusive layer of oils and fats on the skin surface and/or by fixing water in the stratum corneum with humectants. w/o emulsions provide more moisture to the skin than o/w emulsions.

Methods to Determine Skin Hydration

The measurement is based on the principle that water is a major contributor to the electrical properties of the skin. Thus, the flow of electric current through the skin is correlated with the water level in the stratum corneum (Morganti, 1999). Dry stratum corneum is typically a weak medium of electrical conduction whose electrical properties change greatly upon hydration. Morganti (1999) has reported that measuring water level in the upper layers of the skin is proven to be sensitive mostly to changes in moisture level occurring in extra dry and dehydrated skin such as psoriasis. He also stated that a number of in vitro and in vivo tests widely showed that impedance decreases with an increase in the skin moisture level. Thus, both bound and free water in the stratum corneum can be detected by alternatively using capacitance and conductance (impedance) electrical methods, which are extremely useful especially when investigating hyperkeratosis (Morganti, 1999, Hashimoto *et. al.*, 1993, Leveque and De Rigal, 1983, Serban *et. al.*, 1981).

Among the devices commonly used to measure skin hydration based on this electrical properties are Corneometer® CM 825 (Courage-Khazaka, Cologne), Nova Dermal Phase Meter®, Skin Surface Hygrometer - Skicon-200 and Infrared Spectroscopy. In this study, Corneometer® CM 825 (Courage-Khazaka, Cologne) is used. This instrument measures capacitance of the skin in arbitrary units. The measuring probe consists of an interdigital grid of gold-covered electrodes, covered by a low-dielectric vitrified material which is 20 μ thick. Thus, there is no direct galvanic contact between the electrodes and the skin. The probe head consists of a spring which ensures the application of a constant pressure (1.6 N/m^2) when the probe is placed on the skin. The electrical field present in the upper epidermis is a function of the dielectric material covering the electrodes and the capacitance of the skin in contact with the electrode.

The total capacitance is changed by changes in dielectric constant of the skin surface. Data output is expressed in arbitrary capacitance units (ACU) which read from approximately 30 – 40 in dry skin to 120 in very hydrated skin. This instrument measures entire stratum corneum and upper viable epidermis (depth 20 – 40 millimeters).

Rogiers *et. al.* (1990) carried out a study to measure hydration state of the epidermis using both Skicon-100 and Corneometer CM 820®. He found out that both instruments are suitable in measuring skin hydration but Corneometer CM 420® gave a better accuracy in the data acquired. The data obtained showed the changes in skin hydration down to a depth of about 0.1 mm, accounting for most body site regions. Relative humidity and RT are two factors influencing the assessment of skin hydration (Courage-Khazaka, 1997, Rogiers *et. al.*, 1990, Tagami, 1988, Mosely *et. al.*, 1985) although capacitance seems to be less affected by changes in relative humidity than conductance (Rogiers, 1990, Mosely *et. al.*, 1985).

5.1.1 (v) (b)   Skin Colour Assessment

Clarys *et. al.* (2000) reported that skin colour is predominantly determined by pigments such as hemoglobin, melanin, bilirubin and carotene. These skin colour determining components can be altered significantly by UV light and several substances such as drugs and irritants. The constituent elements that determine skin colour can be classified as chromophores or scatterers (Bashir and Maibach, 2000, Kollias, 1995), Table 5.2. Chromophores absorb light whereas scatterers are structures which have a different index of reflection from the medium in which they are embedded. According to Bashir and Maibach (2000) and Kollias (1995), the concentration and distribution of these two

components varies throughout the skin, leading to different optical properties at different sites.

Table 5.2: Chromophores and scatterers in the skin (Kollias, 1995)

Skin Layer	Chromophore	Scatterer
Stratum Corneum	Melanin	Melanin
Viable Epidermis	Melanin	Melanin
		Cellular Structures
Dermis	Melanin	Collagen
	Hemoglobin	Melanin in Macrophages
	Billirubin	Erythrocytes

Methods to Determine Skin Colour

Skin's colour can be measured by several instruments such as scanning reflectance spectrophotometers (Clarys et. al., 2000, Bjerring, 1995, Anderson and Bjerring, 1995, Wilhelm and Maibach, 1995), tristimulus colorimetirc instruments (Clarys et. al., 2000, Westerhof et. al., 1986, Seitz and Whitmore, 1988, Queille, 1991, Chan and Li, 1993) and narrow-band simple reflectance meters (Clarys et. al., 2000, Takiwaki et. al., 1994, Farr and Diffey, 1984, Diffey et. al., 1984, Diffey and Farr, 1991). Scanning reflectance spectrophotometers, however are very expensive, cumbersome and not portable enough for routine clinical work. and are mainly used for fundamental laboratory research (Clarys et. al., 2000, Bjerring, 1995, Anderson and Bjerring, 1995, Wilhelm and Maibach, 1995, Anderson, 1995). Different hand-held tristimulus reflectance meters are very handy, portable and inexpensive. Clarys et. al. (2000) has mentioned the study conducted by Westerhof (1995) in discussing the CIE colorimetry method to measure skin colour. The most popular types are Minolta Chromameter CR200 and CR300 which are considered more or less as a standard instrument for colour measurements in dermato-cosmetic field (Clarys, 2000, Westerhof, 1995).

Another simpler portable skin reflectance instruments developed are based on the difference in absorption between melanin and haemoglobin at well chosen wavelengths. Clarys *et. al.* (2000) has reported several studies conducted using this instrument to measure the skin colour. Among them are the study made by Takiwaki and Serup (1995) in measuring erythema and melanin indices of skin colour. Takiwaki *et. al.* (1994) in comparing skin colour measurements using narrow band reflectance spectrophotometer and tristimulus colorimetric, Farr and Diffey (1984) measure cutaneous erythema induced by ultraviolet radiation, Diffey *et. al.* (1984) in quantifying erythema effect induced from ultraviolet radiation; and Diffey and Farr (1991) in studying the quantitative aspects of ultraviolet erythema. The commercially available narrow band reflectance spectrophotometers are Erythema Meter (Diastron, Andover, UK, DermaSpectrometer (Cortex Technology, Hadsund, Denmark) and Mexameter (Courage-Khazaka Electronic, Koln, Germany) compute only erythema and melanin indices which are mainly used in dermatological research (Clarys *et. al.*, 2000, Takiwaki *et. al.*, 1994, Farr and Diffey, 1984, Diffey *et. al.*, 1984, Diffey and Farr, 1991).

Skin-surface colorimeters use heterogenous wavelengths that lie within visual spectrum to simulate daylight. Tristimulus colorimeters emit wavelengths of 700 nanometers (nm), 564.1 nm and 435.8 nm, which correspond to red, green and blue light (Andreassi and Flori, 1995). For skin colour measurements, the L*a*b* system (Cielab) is commonly used (Bashir and Maibach, 2000, Clarys *et. al.*, 2000). In this study, the colour of the skin is measured using Minolta Chromameter CR300 and Mexameter MX18.

(a)   Chromameter CR 300

With this instrument, the skin surface is illuminated by a pulsed xenon arc lamp. The light reflected perpendicular to the surface is collected and analysed at 450, 560 and 600 nm using the L*a*b* colour system (Cielab). The L* parameter expressed colour brightness, varying between 100 for a white surface and 0 for a black surface, a* parameter represents changes along red-green axis with changes from +60 for red surface to –60 for green surface and b* parameter shows changes from +60 for yellow surface to –60 for blue surface. This instrument is calibrated using a white calibration plate. Bashir and Maibach (2000) has also reported the work carried out by Yamamoto (1995), a Japanese plastic surgeon who used Chromameter CR-300 (Osaka, Japan) to study the skin colour for grafting purpose. By comparing E* value in his study, Yamamoto concluded that the colour of the cheek is most similar to the dorsum of the foot and the flexor forearm.

(b)   Mexameter MX18

Mexameter MX18 measures the content of melanin and erythema of the skin. These two components are mainly responsible for the skin colour. When the skin is exposed to UV radiation, it will protect itself by producing more pigments or melanin. On the other hand, the overstrain of the protection mechanisms of the body will lead to erythema. An erythema is a critical reaction to radiation, the border at which a burn develop. An erythema looks red because the arterial vessels extend under the damaged part of the skin to allow transportation of nutritive and moisturising substances for the healing. There are three different phases of erythema (Courage-Khazaka, 1997):

(a)   **Initial phase**: This is the time between sun exposure and the first reddening of the skin. In this phase, colour differences are not yet visible with human eye. On cell basis however, substances are already released leading to extension of the vessels.

(b)   **Early phase**: Painful burns as well as reddening of the skin and the feeling of stressed skin are typical in this phase. It lasts approximately 8 hours, then the skin will go back to normal condition.

(c)   **Late phase**: In this phase, the cells are destroyed through radiation decay, leading to typical scaling of the skin.

Besides skin burns, there are also other influences such as mechanical pressure, friction, heat, irritation, chemical or toxic substances that might lead to erythema. The measurement using this instrument is based on the absorption and reflection principle. The special probe of Mexameter MX18 emits light of three defined wavelengths, 568 nm (green), 660 nm (red) and 880 (infrared). A photodectector measures the light reflected by the skin. This instrument measures the absorbed and reflected light at wavelengths in green and red for haemoglobin and the wavelengths in red and near-infrared for melanin. A melanin index is computed from the intensity of the absorbed and reflected light at respectively 660 nm and 880 nm. Erythema index is computed from the intensity of the absorbed and reflected light at 568 nm and 660 nm respectively.

In general, when evaluating the colour of the skin, the researcher may consider the overall colour or focus on the parameter of interest. For example, a* value may be of prime interest in the study of skin exfoliation and irritation since it is parallel with erythema scores (Wilhelm *et. al.,* 1989, Bashir and Maibach, 2000) whereas the L* value is important in the study of skin exfoliation and the result is parallel to melanin scores. In their comparison study using three different skin colour instruments that are Chromameter CR200 (Minolta), Mexameter MX16 (Courage-Khazaka) and DermaSpectrometer (Cortex Technology), Clarys *et. al.* (2000) reported about a

significant correlation was seen between a* and erythema value, and L* with melanin value. In the study, as a* value increases, erythema indexes measured also increased. Similar result was observed with L* value which shows a reduction in melanin value when L* value measured increases. The similar correlations from the study conducted by Clarys *et. al.* between a* and L* colour factor (Chromameter) and erythema and melanin indices (DermaSpectrometer) was also observed by Takiwaki *et. al.* (1994).

5.1.1 (v) (c)   Skin pH Assessment

pH value of the skin results from water-soluble substances contained in stratum corneum and the secretion of perspiration and sebum as well as the secretion of carbonic acid. Its value varies depending on the tested skin area and different external factors. On the cheek, pH value is higher than on the forehead whereas the armpit and soles of the feet have the highest pH value. The normal skin pH of men is slightly lower than for a women which is in the range of pH 4.3-5.5 whilst for a women it is from pH 4.5-5.5. Less than this, the skin pH would be considered acidic whereas higher than pH 5.5, it is considered as alkaline. In general, the pH value for a women is taken as 5.5 and for men, it is slightly lower which is approximately 5 (Courage-Khazaka, 1997). This means the skin pH is in the acidic range and this condition gives the acid protection layer of the skin. This condition influences the bactericide and fungicidal effect of the skin which is very important for health. The pH value is an age factor dependent and increases with age.

Methods to Determine Skin pH

The measurement of skin pH is not very much influenced by environmental conditions (temperature and air humidity) or the physical condition (fat or thin) of the test person. Extreme conditions such as high transpiration or the application of cosmetic products, however, can change the pH of the skin. Constant maltreatment of the skin with

cosmetic and pharmaceutical products or chemicals may lead to desiccation of the skin which is indicated by damage and premature ageing. The skin usually needs up to 5 hours to restore its original pH-value after cleansing or application of cosmetic products (Courage-Khazaka, 1997). Thus, it is possible to see the undesired effects of products or environmental influences. On the other hand, a sensitive skin may regain its natural condition over a longer period of time. The skin pH can be measured using the skin-pH-meter PH900 (Courage & Khazaka, 1997).

5.1.2 Objective of the Study

To study the efficacy of moisturiser product's containing 15wt/wt% *Baccaurea motleyana hook f.* fruit's extract using

(a)   instrumental product evaluation

(b)   objective descriptive (sensory) product evaluation

5.2 Materials and Method

5.2.1 Instrumental Product Evaluation

The purpose of the study is to find out the effects after using moisturiser containing 15wt/wt% of *Baccaurea motleyana hook f.* fruit's extract for 4 weeks.

(i)   Product

Two types of test products were used in this study that is moisturiser containing 15wt/wt% *Baccaurea motleyana hook f.* fruit's extract, MR, and the placebo (moisturiser formulated without *Baccaurea motleyana hook f.* fruit's extract), MP will be known as moisturiser and placebo respectively in this study. A complimentary skincare product that is facial cleanser lotion is also given to the assessors.

(ii)   Method

This study is carried out using human assessors with three range of age groups. The assessors are briefed on the benefit and adverse effects of using the products. The consent from the assessors taking part in this test was also acquired (Appendix L). Only an experienced user of skincare products were selected to be involve in this study for an obvious reason that they are familiar in using the product and also could compare the efficacy of the product tested with their currently used moisturiser purchased from the market. The assessors were required to stop using their present skincare products during the period of study.

Each assessors was given two types of product that is a facial cleanser and a test product which is either a moisturiser or a placebo. The test product samples were coded and the assessors were given a choice to choose the product randomly. A similar facial cleanser product samples were given to all assessors taking part in this study for facial cleansing and standardisation purpose. The assessors were shown on how to apply the product on the face. They were required to cleanse their face each time with facial cleanser sample given prior to product application. The moisturiser product is applied on the face three times per day that is once in the morning (7.30-7.45 am), once in the afternoon (1.45-2.00 pm) and once at night (9.30-10.00 pm) during a study period of 4 weeks.

(iii)   Assessment of Product's Efficacy

The product's efficacy could be easily detected with changes in skin colour such as decreasing of melanin value and increasing of L* value, a* value and erythema value (Clarys *et. al.,* 2000, Bashir and Maibach, 2000, Wilhelm *et. al.,* 1989). The effect on skin pH and skin hydration after product's application were also studied. In this study, a

single measurement taken is calculated from a mean value of three readings performed. The instruments used in this study are listed in Table 5.3.

Table 5.3: Instrument for assessing the efficacy of products

No.	Instruments	Function	Manufacturers
1.	Chromameter CR300	Measure skin colour (L*, a* value)	Minolta, Osaka, Japan.
2.	Mexameter MX18	Measure melanin and erythema of the skin	Courage-Khazaka, Koln, Germany
3.	Skin pH-Corneo-Sebumeter	Measure skin pH and skin hydration	Courage-Khazaka, Koln, Germany

The face or body site of the assessors were divided into 2 zones that is cheek and T-zone (comprise of forehead and chin). For each assessors, 8 spots were measured that is 2 spots each on the right and left site of the cheek, 1 spot each on the right and left forehead and 1 spot each on the right and left chin. 3 age groups range were measured that is 21 to 30 years, 31 to 40 years and 41 to 50 years. The body sites of assessors were measured before and after 4 weeks of products application. The assessors were asked to cleanse their face using facial cleanser 5 hours (Rogiers *et. al.*, 1990) before any measurement were taken, to allow the skin to restore its natural pH, moisture and sebum level (Courage-Khazaka, 1997). They were also let to rest for at least 30 minutes prior taking measurement to get their blood circulation back to normal condition (Courage-Khazaka, 1997). The information recorded were age, gender, races and body sites measured. The skin hydration measurement was performed at 20°C and 43%-45% of relative humidity.

5.2.1 (i)   Statistical Analysis

The instrumental data obtained are statistically analysed using a widely used significance test known as the t test since the total number of assessors and body sites measured is less than 30 (Ryan, 1996). This is to know whether the data acquired

(moisturiser and placebo) is really difference or purely a chance occurrence. Since the similar body sites area from the same age group assessors were measured and compared, but using a different type of products (moisturiser and placebo), a paired t-test is used to analyse the data acquired. To carry out this test, the null hypothesis need to be formulated (Ryan, 1996) as follows:

Null hypothesis, H_0:

The true mean of the difference (between moisturiser and placebo) is equal to zero,

$\mu_{difference} = 0$

Alternative hypothesis, H_1:

The true mean of the difference (between moisturiser and placebo) is not equal to zero,

$\mu_{difference} < \text{ or } > 0$

The test value (t value) is calculated using the following formula:

$$\text{Test value, } t = \frac{| x_{difference} - \mu_{difference} | (n)^{0.5}}{s}$$

whereby

$x_{difference}$ = the mean of the difference from two sets of data (moisturiser and placebo)

$\mu_{difference}$ = true mean of the difference between two sets of data (moisturiser and placebo)

s = standard deviations of the differences between two sets of data (moisturiser and placebo)

n = sample size of the data

The t value obtained from this calculation (t_{cal}) is then compared to the t value obtained from the t distribution table (t_{table}). If t_{cal} value obtained is larger than t_{table}, we can reject the null hypothesis and conclude that there is a significant difference between the two samples (Ryan, 1996). One-way analysis of variance (ANOVA) is then

calculated to know significant differences among the three different age groups that use moisturiser product. Duncan analysis is also carried out to see which of the three age groups means are significantly different from each other. The paired t test, one-way ANOVA and Duncan analysis was analysed using SPSS for Windows (version 11.5). All probability values were two tailed and those with probability value (p) < 0.05 were considered significant.

5.2.2 Objective Descriptive (Sensory) Product Evaluation

The purpose of this study is to find out the acceptability of moisturiser product containing 15wt/wt% *Baccaurea motleyana hook f.* fruit's extract in terms of the product's attributes (physical properties) and its efficacy on the skin. The comparable study between the moisturiser product's efficacy compared to the currently used commercial product by the assessors is also explored.

(i)   Product

Moisturiser product containing 15wt/wt% *Baccaurea motleyana hook f.* fruit's extract referred to as MR and a complimentary skincare product that is facial cleanser lotion is given to the assessors.

(ii)   Method

The assessors were required to evaluate the moisturiser product given in terms of the product's attributes and its efficacy. The questionnaires were designed in three parts. Questionnaire Part 1 is designed for the assessors to test the product at the back of their palms. The purpose is to let the assessors feel the product, evaluate its physical properties and have confidence in it prior to testing the product on their faces. If they like it, they were to proceed to questionnaires Part 2 and Part 3; which is to use the

product on the face for about 4 weeks. Finally, the assessors were also requested to evaluate and compare the moisturiser product given with the currently used commercial product. Using the commercial product as the benchmark with the rating scale of 5, the assessors were asked to evaluate the given product's physical properties, after rubbing effect and effectiveness. This test is carried out to know the acceptability of the developed product compared to the commercial ones.

The procedure and frequency of using the product on the face is similar to the method which has been explained earlier under instrumental product evaluation section. The assessors were also explained in details and shown on how to apply the moisturiser product at the back of the palm and on the face. The questionnaire forms were explained in details so that all the assessors are clear and can evaluate the product with the similar understanding and perspectives.

(iii)   Definition of Product's Attributes

The product's attributes are classified into 4 sections and defined as follows (Wiechers and Wortel, 2000, IFSCC, 1998):

(a)   Product Physical Characteristics – Product Evaluation Form - Part 1 and 2

- Scent of product   - smell or odour of the product in its container.

- Strength of product's scent – the intensity of the product's odour in its container.

- Viscosity of product – thickness of the product is evaluated by pouring out the product from its container.

- Colour of product - the product's colour in its container.

- Texture of product – Feel, smoothness, appearance and consistency (uniformity) of the product.

- Easiness of product's application – Easy to spread or apply the product onto the skin.

(b)   **After Rubbing Effect of Product – Product Evaluation Form -**
    Part 1 and 2

- Endurance of product's scent – The lastingness of product's scent after applying the product onto the skin within 2 hours of product's application.

- Absorption into the skin –The rate of moisturiser product being absorbed into the skin.

- Moisturising of the skin – Degree to which the product moisturise the skin.

- Tackiness of the skin – Degree to which the product stick to the skin.

- Oiliness of the skin – Degree to which the product feels oily or slippery on the skin.

(c)   **Effectiveness After 4 Weeks of Product's Application – Product Evaluation Form - Part 2**

- Decrease pigmentation/freckles – Degree to which the product manage to reduce or lessen of pigmentation or freckles on the skin.

- Moisturising skin effect – Degree to which the product moisturise the skin.

- Exfoliating of the skin– Degree to which the product manage to cause skin exfoliation effect.

- Lightening skin colour effect – Degree to which the product causes the skin to become fairer.

- Softening of the skin – Degree to which the product is able to soften the skin.

- Reducing skin pore size – Degree to which the skin pore size is reduced.

- Inactivate acne growth – Degree to which the product is able to stop the growth of acne.

- Reducing acne scar – Degree to which the product manage to reduce acne mark.

(d)   **Overall Product Liking Evaluation – Product Evaluation Form – Part 1 and 2**

This means the evaluation of the product after taking consideration all its attributes and performance effect on the skin.

(e)   **Product's Adverse Effect – Product Evaluation Form - Part 3**

- Tingling – Condition where the skin feels tingly or prickly.

- Stinging – Condition where the skin feels stinging sensation.

- Warming – Condition where the skin feels warm.

- Tightness – Condition where the skin feels stiff and taut.

- Itchiness – Condition where the skin feels itchy.

5.2.3   Rating Scale System

The product attributes are evaluated using a rating scale from 1 to 5 (Ryan, 1996, Harding, 1996, IFSCC, 1998) and the rated points are defined in Table 5.3 below.

Table 5.4: Moisturiser product's attributes rating scale

Product Attributes	Product's Rating Scale				
	1	2	3	4	5
Physical characteristics					
1. Scent of product	Very bad	Bad	Acceptable	Good	Very good
2. Strength of product's scent	Very weak (Very bad)	Weak (Bad)	Moderate (Acceptable)	Strong	Very strong
Product' rubbing effect					
3. Viscosity of product	Very thin	Thin	Acceptable	Viscous	Very viscous
4. Colour of product	Very bad	Bad	Acceptable	Good	Very good
5.Texture of product	Very bad	Bad	Acceptable	Good	Very good
6. Easiness of product's application	Very bad	Bad	Acceptable	Good	Very good
7. Endurance of product's scent	Very short	Short	Moderate	Long	Very long
8. Absorption into the skin	Very slow	Slow	Moderate	Quick	Very quick
9. Moisturising effect	Very little	Little	Moderate	Much	Very much
10. Tackiness of the skin	Very much	Much	Moderate	Slight	Very slight
11. Oiliness of the skin	Very much	Much	Moderate	Slight	Very slight
Effectiveness of Product					
Moisturising effect	Not effective	Slight effective	Moderate	Effective	Very effective
Decrease pigmentation/freckles	Not effective	Slight effective	Moderate	Effective	Very effective
Lighten skin colour	Not effective	Slight effective	Moderate	Effective	Very effective
Soften of the skin	Not effective	Slight effective	Moderate	Effective	Very effective

Table 5.4: Moisturiser product's attributes rating scale, *continued*

Product Attributes	Product's Rating Scale				
	1	2	3	4	5
Reduce skin pore size	Not effective	Slight effective	Moderate	Effective	Very effective
Inactivate acne growth	Not effective	Slight effective	Moderate	Effective	Very effective
Exfoliating effect	Not effective	Slight effective	Moderate	Effective	Very effective
Reduce acne scars	Not effective	Slight effective	Moderate	Effective	Very effective
Overall product evaluation	Very bad	Bad	Acceptable	Good	Very good

In general, the higher the product is rated, the better is the product. The data obtained is analysed and discussed in a percentage form.

5.3   Result and Discussions

5.3.1   Instrumental Product Evaluation

The total number of assessors taking part in this test are 12. Six of them used moisturiser containing 15wt/wt% of *Baccaurea motleyana hook f.* fruit's extract (later known as product) and the other six used placebo. Four different spots were measured at each cheek and t-zone body sites for each assessor. Therefore the total number of sample size, N, measured in this study is 24. The statistical results analysed consider the changes before and after using the product and placebo. Comparison of the results obtained with three different age groups, two different body sites (cheek and t-zone area) and six variables (L*, a*, melanin, erythema, pH and skin hydration values) are also discussed. The details of the data measured before and after using the product and placebo can be referred to Appendix P.

All the t values calculated using the SPSS for Windows (version 11.5) (known as t_{cal})are 2 tailed and calculated at 0.05 significant level. The t values taken from t

distribution table (IFSCC Monograph, Number 1) known as t_{table} are 2 tailed and read at 0.05 significant level. Detail of the t-paired test, ANOVA and Duncan analysis results could be referred to Appendix Q, R and S respectively.

(i)   Skin Colour Evaluation - L* Value

L* value shows fairness or darkness of the skin. The higher the L* value, the fairer is the colour of the skin. The result is presented in the Figure 5.1. There is an obvious L* mean value result difference between the product and placebo for all the three age

Note: Product-C: Product(Cheek)   Product-T: Product(T-Zone)
         Placebo-C: Placebo(Cheek)   Placebo-T: Placebo(T-Zone)
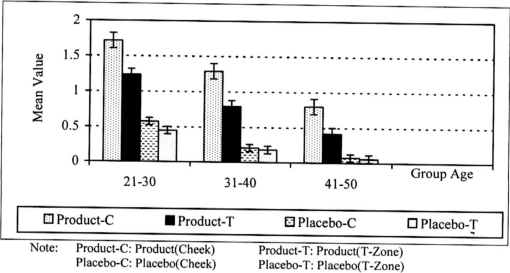
Figure 5.1: Skin colour evaluation (L* value) with different age groups

groups at different body sites (cheek and t-zone). The product's L* mean value measured on the cheek is 1.713, 1.292 and 0.8 for 21-30, 31-40 and 41-50 age group respectively whereas the result measured at t-zone area is 1.238, 0.796 and 0.417 for 21-30, 31-40 and 41-50 age group respectively. The placebo's L* mean value measured on the cheek is 0.575, 0.204 and 0.075 for 21-30, 31-40 and 41-50 age group respectively whilst the result measured at t-zone area is 0.45, 0.179 and 0.058 for 21-30, 31-40 and 41- 50 age group respectively. The L* mean value of the product is much higher than placebo since the product contains *Baccaurea motleyana hook. f* fruit's extract. The

frequent use of the product accelerates and increases the rate of natural skin exfoliation process, causing the skin to become fairer in a shorter time and this is shown with greater increase in L* mean value of the product. Even though an increase in L* value after using placebo is observed, but the increase is much lower when compared to the product, possibly due to the natural skin exfoliation process that occurred during the 4 weeks period of study.

The product's L* mean value measured on the cheek is slightly higher compared to t-zone body site for all the different age groups, might be due to the following reason. Since t-zone body sites contain higher number of sebaceous and sweat glands, thus higher secretion of oily and water soluble components from these two glands were expected, forming a thicker layer of hydrolipidic film on the skin surface which give better protection of its stratum corneum (SC) layer when compared to cheek body site. Physical action by cleansing the face or through AHAs exfoliating action (in the product), however, will reduce the thickness of the hydrolipidic film and fasten the SC exfoliation process. Since the hydrolipidic film formed is thinner on the cheek, hence this part would be faster exfoliated and the resultant showed a higher L* value attained on the cheek compared to the t-zone area. The L* mean value also decrease with age due to slower skin exfoliation process.

(ii)   Skin Colour Evaluation - a* Value

The skin colour a* value contributes the redness colour of the skin and the result is presented in Figure 5.2. There is also an obvious a* mean value result differences between the product and placebo for the three age groups at different body sites (cheek and t-zone). The product's a* mean value measured on the cheek is 2.212, 1.658 and 1.238 for 21-30, 31-40 and 41-50 age group respectively whilst the result measured at t-zone area is 1.742, 1.254 and 0.696 for 21-30, 31-40 and 41-50 age group
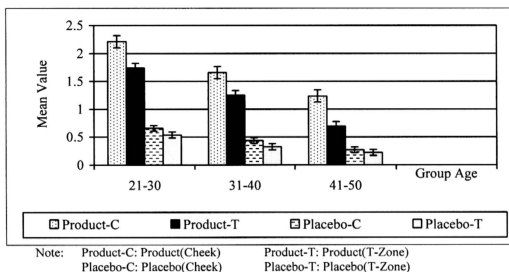
Note: Product-C: Product(Cheek)   Product-T: Product(T-Zone)
        Placebo-C: Placebo(Cheek)   Placebo-T: Placebo(T-Zone)

Figure 5.2: Skin colour evaluation (a* value) with different age groups

respectively. There is also an increase in a* value after using placebo but the increase is much lower when compared to the product. The placebo's a* mean value measured on the cheek is 0.658, 0.433 and 0.271 for 21-30, 31-40 and 41-50 age group respectively whilst the result measured on the t-zone area is 0.538, 0.325 and 0.225 for 21-30, 31-40 and 41- 50 age group respectively. The small increase of a* value for placebo might be due to natural skin exfoliation process during 4 weeks period of study, causing the skin to become more red than before. On the other hand, the higher increase of the product's a* mean value might be due to the presence of *Baccaurea motleyana hook. f* fruit's extract in the product, increasing the rate of skin exfoliation process, causing the skin to appear more red in shorter time. The product's a* mean value results measured on the cheek is slightly higher when compared to t-zone area for each age groups, probably because the cheek is more sensitive than t-zone area such that upon skin exfoliation process, the skin redness appear more obviously. The a* mean value however, decrease as ageing progress, due to slower skin exfoliation process.

(iii)   Melanin Evaluation

The greater melanin mean value result differences for the product compared to placebo for each age groups at cheek and t-zone body sites is illustrated in Figure 5.3. The product's melanin mean value measured on the cheek is -53.58, -43.71 and -33.5
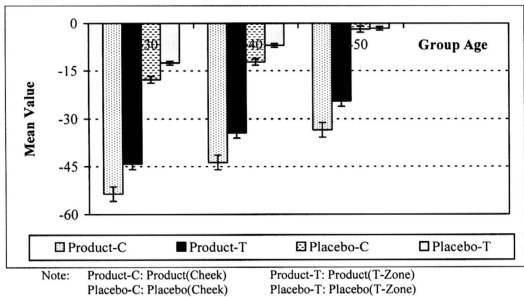

Note: Product-C: Product(Cheek)   Product-T: Product(T-Zone)
         Placebo-C: Placebo(Cheek)   Placebo-T: Placebo(T-Zone)

Figure 5.3: Melanin evaluation with different age groups

for 21-30, 31-40 and 41-50 age group respectively whilst the result measured at t-zone area is -44.25, -34.46 and -24.54 for 21-30, 31-40 and 41-50 age group respectively. The placebo's melanin mean value measured on the cheek is -17.79, -12.17 and -1.88 for 21-30, 31-40 and 41-50 age group respectively whilst the result measured at t-zone area is -12.54, -7.04 and -1.58 for 21-30, 31-40 and 41-50 age group respectively.

The negative melanin values means a reduction in the melanin value after using the product and placebo within 4 weeks period of study. The product contains *Baccaurea motleyana hook. f* fruit's extract, therefore the rate of skin exfoliation process is expected to be faster when using the product compared to the natural skin exfoliation process. This phenomenon caused the skin to become fairer in shorter time and the

result is shown with higher reduction in melanin mean value of the product. A reduction in melanin value after using placebo is observed, possibly due to natural skin exfoliation process during the 4 weeks period of study. The melanin mean value also decreased with age due to slower skin exfoliation process.

(v)   Erythema Evaluation

There is an increase in erythema mean values after using the product and placebo for each age groups at cheek and t-zone body sites, Figure 5.4. A slight increase in erythema value after using placebo is probably due to natural skin exfoliation process

Note: Product-C: Product(Cheek)   Product-T: Product(T-Zone)
          Placebo-C: Placebo(Cheek)   Placebo-T: Placebo(T-Zone)
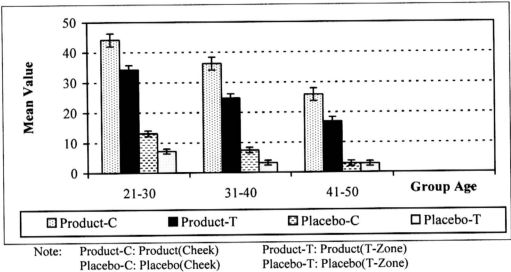
Figure 5.4: Erythema evaluation with different age groups

within 4 weeks period of study, causing the skin to become slightly red than before. The result acquired on the cheek is 13.08, 7.46 and 2.88 for 21-30, 31-40 and 41-50 age group respectively whilst the result measured at t-zone area is 7.17, 3.17 and 2.79 for 21-30, 31-40 and 41- 50 age group respectively.

In contrast, the erythema mean value measured after using the product is much higher with the measured value on the cheek is 44.13, 36.25 and 25.83 for 21-30, 31-40 and

41-50 age group respectively whereas the result measured at t-zone area is 34.29, 24.75 and 16.96 for 21-30, 31-40 and 41-50 age group respectively. The presence of AHA in *Baccaurea motleyana hook. f* fruit's extract expedite and increase the rate of skin exfoliation process, causing the skin to appear more red resulting from the expansion of blood vessels under the skin to allow transportation of nutritive and moisturising substances to heal the skin.

The product's erythema mean value results measured on the cheek and t-zone area for each age groups are also different where the result is higher on the cheek compared to t-zone area, probably because the cheek is more sensitive and contains more blood vessels compared to t-zone area usually contains lots of sebaceous and sweat glands, such that upon skin exfoliation process, these blood vessels appear more obvious on the cheek. The erythema mean value however, decrease as ageing progress due to slower skin exfoliation process.

(vi)   pH Evaluation

The pH of the skin measured before applying the product or placebo is in the range of 4.4 to 4.9 (21-30 years), 4.9 to 5.2 (31-40 years) and 5.1 to 5.5 (41-50 years), showed that the skin's pH value increased with age. The pH at t-zone body site is lower (more acidic) than cheek due to higher presence of sebaceous and sweat glands. These glands secrete oily and water soluble substances on the skin's surface. The greater the number of these glands, the higher is the amount of these substances were found on the skin surface, forming a thicker hydrolipidic film on the skin and reducing its pH. However in this study, only the difference before and after using the product and placebo is considered. The result is presented in Figure 5.5.
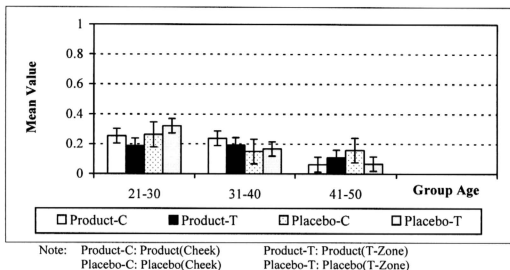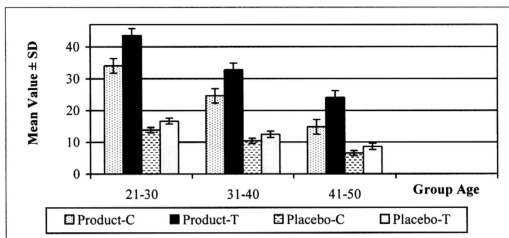
Note:　Product-C: Product(Cheek)　　　Product-T: Product(T-Zone)
　　　　Placebo-C: Placebo(Cheek)　　　Placebo-T: Placebo(T-Zone)

Figure 5.5　pH evaluation with different age groups

There is no clear pH trend observed after using the product and placebo among all the age groups at cheek and t-zone body sites. The changes in the skin's pH after using the product and placebo is also small which is in the range of 0.1-0.3.

(vii)　Skin Hydration Evaluation

Skin hydration analysis indicates the water content of the skin. In this study, the skin hydration mean values results obtained, Figure 5.6, are much higher after applying the product than placebo for each age groups, measured on both cheek and t-zone body sites, with the measured value on the cheek is 34.04, 24.58 and 14.79 for 21-30, 31-40 and 41-50 age group respectively whilst the result measured at t-zone area is even higher, 43.54, 32.75 and 24 for 21-30, 31-40 and 41-50 age group respectively. T-zone body site generally contains higher number of sebaceous and sweat glands. These sebaceous and sweat glands produce sebum and water soluble compounds, secrete them onto the skin surface, forming a layer of hydrolipidic film that covers the skin surface and reduce the evaporation of water from the skin. Therefore, the skin hydration values measured at this area gave a higher result when compared to the cheek.

Figure 5.6: Skin hydration evaluation with different age groups

The greater skin hydration mean values attained after using the product might be due to the following reason. As already explained in Section 1.1.2, SC consists of two layers, namely *stratum disjunctum* (upper layer of SC) and *stratum compactum* (lower layer of SC) whereby the *stratum compactum* is more hydrate than *stratum disjunctum* (Schaefer *et. al.,* 1999, Warner, 1988). By applying the product, AHAs help to fasten and increase the rate of skin exfoliation process and this is achieved by firstly eliminating the *stratum disjunctum*, thus revealing *stratum compactum*. After 4 weeks of product's application, it is possible that it is the *stratum compactum* that is being measured which gave higher skin hydration reading.

There is also an increase in skin hydration mean value after using placebo but very much less and might be due to the natural skin exfoliation process. The result acquired for the cheek is 13.83, 10.42 and 6.46 for 21-30, 31-40 and 41-50 age group respectively whereas the result measured at t-zone area is higher 16.67, 12.46 and 8.63 for 21-30, 31-40 and 41- 50 age group respectively. The increase of the mean value

after using the placebo and the product is however, lesser with age, due to slower rate of skin exfoliation process and also less production of sebum.

5.3.2   Statistical Analysis

Statistical analysis performed for all six variables measured (L*, a*, melanin, erythema, pH and skin hydration) showed that paired t-test analysis carried out between the product and placebo, and the cheek and t-zone body sites for all age groups are significant when analysed at 0.05 significant level, except the pH value for 21-30 years and 31-40 years age groups, which is insignificant. ANOVA and Duncan analysis carried out also showed a significant difference among the three different group age for all the variables analysed at 0.05 significant level.

## 5.3.3   Objective Descriptive (Sensory) Product Evaluation

25 people taking part in this study are all Malays and females. The age is in the range of 21 to 46 years. All of the assessors are current users of skincare products. They are grouped as experience or semi-trained assessors since all of them have become experienced through their frequent participations in cosmetic product evaluations (Braddon *et. al.*, 2002). By taking into consideration on the application of the product at the back of the palm and on the face, for both product's efficacy studies (self-evaluation and commercial compatibility study), the results are discussed in terms of the product's physical properties, after rubbing effect and overall product liking. The adverse effect of using the product is also discussed.

Generally the product is rated between 3 (acceptable/moderate) and 4 (good) on the product's physical properties and its effectiveness aspects whereas for the product's after rubbing effect, the assessors have mostly rated 4 (good) and 5 (very good). The

product's evaluation forms and the details of the results can be referred in Appendix L, M, N and O.

(a)   **Product's Physical Properties**

The product's physical properties, either applied at the back of the palm or on the face showed similar result, indicates a consistency on the product's assessment carried out by all assessors. In general, the assessors have rated most of the product's physical properties between 3 and 4, except its viscosity which is rated between 4 and 5, Figure 5.7. The product's viscosity is considered viscous (rating scale 4) by 52% of the assessors while another each of the 24% rated it as very viscous (rating scale 5) and acceptable (rating scale 3), which means that the product thickness is good enough and accepted by all the assessors. 68% and 32% of the assessors rated the product's colour as 3 (acceptable) and 4 (good) respectively. The texture of the product is also appraised between 3 (acceptable) and 4 (good) by 72% and 28% of the assessors respectively.
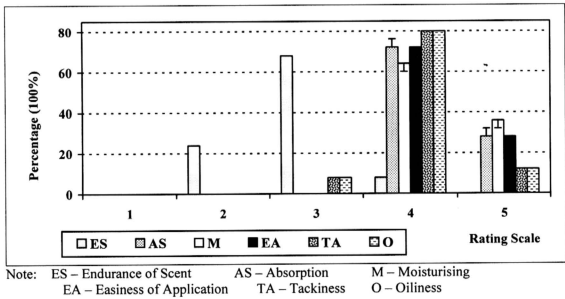

Note: S – Scent   SS - Scent Strength   V – Viscosity   C- Colour
        TE – Texture
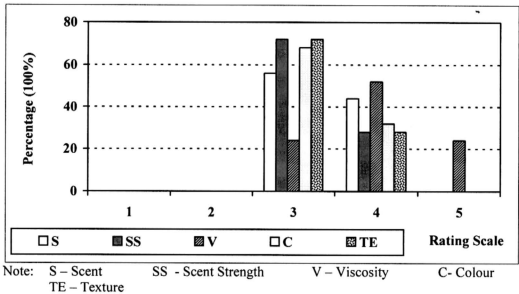
Figure 5.7: Physical properties product evaluation
(Back of the palm and on the face)

Both of the product's fragrance and its strength are also rated between 3 and 4. 56% and 44% of the assessors graded the product's scent as acceptable and good respectively while the product's strength is evaluated as moderate/acceptable by 72% and strong by 28% of the assessors. It is well known that fragrance and its strength is a very subjective matter. There are people who like product with strong fragrance. In contrast, there are also people who do not like fragrance at all. This result implies that all assessors can accept the fragrance used in the product and they are quite happy with its strength in the product.

(b)   Product's After Rubbing Effect

Most of the product's after rubbing effect attributes results either applied at the back of the palm or on the face showed quite similar rating points, Figure 5.8. Six product's

Note: ES – Endurance of Scent   AS – Absorption   M – Moisturising
         EA – Easiness of Application   TA – Tackiness   O – Oiliness

Figure 5.8: After rubbing effect product evaluation
(Back of the palm or on the face)

attributes were evaluated in this category with 5 of them are appraised between rating scale of 4 and 5. Only the endurance of product's scent attribute is evaluated low, which

is 2 (short), 3 (moderate) and 4 (long) by 24%, 68% and 8% of the assessors respectively. 72% of the assessors valued the easiness of the product's application on the skin 4 (good) and 28% of them valued it 5 (very good). The rate of product absorption into the skin is appraised as quick (rating scale 4) by 72% - 76% of the assessors while another 28% - 24% evaluated it as very quick (rating scale 5). 60% to 64% of the assessors evaluated the product's moisturising effect as 4 (much) and another 40% to 36% rated it as 5 (very much). The tackiness and oiliness product's attributes effect are rated 4 (slight) and 5 (very slight) by 80% and 12% of the assessors respectively. Only 8% of the assessors rated it as moderate.

These product's attributes effects are interrelated to each other. The easiness of product's application make the product easy to be spread on the skin while the evenly distributed product characteristic will make it easier to be absorbed into the skin. The fast absorption rate of the product into the skin prevent the skin from feeling oily or tacky. On the other hand, although the product is fast absorbing, all the assessors still find the product moisturising.

(c)   Effectiveness After 4 Weeks of Product's Application

8 product's attributes effectiveness after 4 weeks of continuous product's application were evaluated and the results were presented in Figure 5.9. Both product's self-evaluation and commercial compatibility studies conducted showed similar result on product's effectiveness effects for each of the product's attributes, clearly demonstrate the consistency of the assessors in appraising the product. Obviously most of the results obtained fall between rating scale of 3 to 4. Most of the assessors taking part in this study have considered the product effective in inactivating the growth of acne, softening and moisturising of the skin. About 60% of the assessors have rated 4 (effective) and

40% have graded 5 (very effective) on the product's moisturising effectiveness after 4
weeks of product's application. 76% of them have regarded the product as effective
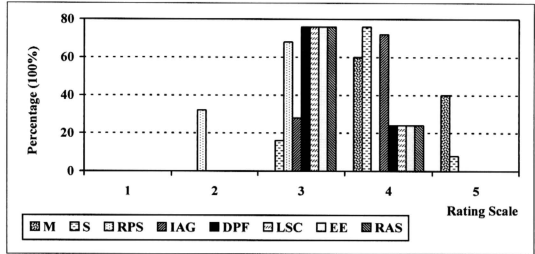
Note: M – Moisturising   S – Soften skin   RPS – Reduce skin pore size
          IAG – Inactivate acne   DPF – Decrease pigmentation/freckles
          LSC – Lightening skin colour   EE – Exfoliating Effect   RAS – Reduce acne scar

**Figure 5.9: Product effectiveness evaluation (After 4
Weeks of product's application)**

(rating scale 4), 8% considered very effective (rating scale 5) whereas another 16%
valued moderate (rating scale 3) in softening of the skin. 72% of the assessors rated the
product as 4 (effective) in inactivated the acne growth while another 28% appraised it as
3 (moderate). About 76% of the assessors have evaluated the product's effectiveness in
relation to its ability to reduce the skin's pigmentation or freckles, increase skin's
exfoliation effect, lightening skin colour and reducing acne scars as 3 (moderate). They
have also found the product is moderately effective (rating scale 3) in reducing the
skin's pore size.

(d)   Overall Product Liking Evaluation

By considering all the product's attributes such as its physical properties, after rubbing
effects and effectiveness after   applying the product in 4 weeks   time, 44% of the
assessors have evaluated this product 4 (good) while another 56% has rated 3

(acceptable), Figure 5.10. The result showed that the product developed is fairly accepted by all the assessors taking part in this study.
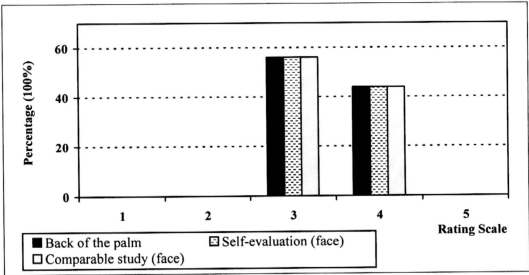

Figure 5.10: Overall product liking evaluation (Back of the palm and face)

5.3.4 Product Evaluation (Part 3) – Adverse Effect After Product's Application

Out of 25 assessors taking part in this study, only 5 of them experienced adverse effect after using the moisturiser. This might be due to the fact that these assessors might have a more sensitive skin compared to the other 20. Only one of the assessors has an immediate unpleasant effect whilst the other experienced it after one week (one person), two weeks (one person) and three weeks (two persons) using the product. One assessor felt strong stinging sensation, two experienced slight warming sensation, one felt mild tightness on the cheek and the other one experienced moderate itchiness on the forehead. Two assessors start to feel the effect immediately, another two felt it after 5 – 10 minutes of product application and one of them felt the sensation within 30 minutes. However these adverse effects sensations are not lasting and were felt within 30 minutes after product's application. Even though this product's adverse effect did

influence 4 out of 5 assessors' opinion on the product tested, but all of them can take on the effects and could accept the product.

5.3.5 Conclusion

(i) Instrumental Product Evaluation

The performance of the products containing 15wt/wt% of rambai extract was evaluated before use and after application on the assessors faces 3x a day for 4 weeks. The products demonstrated better exfoliation than placebo with an increased L* and reduction in the melanin value. However, even the placebo exhibited some exfoliating effect due to natural renewal by the skin.

The different age groups experienced different hydration effects – the younger the group, the better the hydration. Theoretically, with aging, less sebum is secreted with a thinner layer of hydrolipidic film formed on the skin, increasing the moisture loss.

The different facial areas also experienced different hydration effects. The t-zone was better hydrated than the cheeks, possibly because the t-zone skin had more sebaceous and sweat glands to form a thicker hydrolipidic layer, reducing skin moisture evaporation.

The t-test showed that the skin colour and hydration on the cheek and t-zone were improved over the effects by the placebo at $p = 0.05\%$. There were no significant differences with the placebo for skin pH for the age groups 21 – 30 and 31 – 40. ANOVA and Duncan's test confirmed the significant differences between the age groups for all the variables analysed at 5% level.

(ii) Objective Descriptive (Sensory) Product Evaluation

Generally a group of 25 Malay females of 21 to 46 years old rated the product's physical properties and its effectiveness 3 (acceptable/moderate) and 4 (good) whereas the product's after rubbing effect is rated good by 60% to 80%, except the product's scent lastingness , rated 3 (moderate) by 68%. The skin exfoliation effect is evaluated 3 by 76% of the assessors which means the product is able to cause skin whitening but at a moderate rate.

56% of the assessors found the product as acceptable after taking consideration all the product's attributes such as its physical properties, after rubbing effects and effectiveness after application in 4 weeks and another 44% rated it good, which implies that the product developed is fairly accepted by all the assessors taking part in this study. In both product's self-evaluation and commercial compatibility studies, the overall product evaluation is rated between 3 and 4 which shows that this product is potentially marketable. However, 20% of them experienced itchiness, stinging feel and tightness after application for only 30 minutes.