CHAPTER 1

INTRODUCTION

1.1 Literature Review

1.1.1 Overview of Cosmetic Products

The cosmetic industry is a multibilion-dollar enterprise and is undergoing a rapid evolution where the public in general are very concern about their look and well being, thus are looking for truly effective products. The concern about the appearance of the skin predates modern society. Cosmetics and perfumes have been found at ancient burial sites in Egypt while the use of homemade mixtures to moisturise, rejuvenate dry and ageing skin have been documented in early Greece and Rome (Braddon *et. al.*, 2002, Rodrigues, 1999). Braddon *et. al.* (2002) has also reported that during Elizabeth times, lotions and ointments were prepared with ass's milk, hog lard, honey and beeswax with added embellishments of cherries, rose petals and herbs. Elizabeth I has made a cream from a compound curd to remove wrinkles and a skin lotion from a mixture of egg white, powdered egg shells, alum, borax and white poppy seeds.

By twentieth century, the only moisturisers initially were mass-produced are cold cream (as cleanser and moisturiser) and vanishing cream (as make up base and moisturiser). By 1930s, the beauty industry was in full swing with cosmetic products advertised were to offer smooth and softer skin (Braddon *et. al.*, 2002). Fishman (2000) has reported that cosmeceuticals in skin products became prevalent in the 1980s and 1990s, especially with the use of alpha hydroxy acids (AHAs) as skin exfoliants to treat the

skin ageing problems, which began in 1992. Today the used of herbals, plants and fruits extracts in cosmetic products formulation have increased tremendously. These materials selection are mostly by untiring process of "trial and error" (Dweck, 2002). According to Dweck (2002), plants that contains mucilage, polysaccharides, complex sugars and starch derivatives such as seed oils rich in fatty acids and triglycerides are good for soothing and as emollient agents since they provide an oil layer covering the skin, thus reduce transepidermal water loss and so increase the skin hydration. He has also reported that those plants with anti-inflammatory properties often have high level of flavonoids and those that are used to firm and tone the skin are rich in tannins. The fruits with alpha hydroxy acids property could lighten the skin's colour and reduce skin ageing problems. These benefits have created new concept in cosmetic products formulation, which in turn become trends among the users. Therefore, most cosmetic product's manufacturers today focus on the products functionality to attract consumers to use their products.

1.1.2 Skin Ageing Phenomenon

Cosmetics can't work miracles but they can keep the skin clean, make it moist, soft and supple. Many cosmetic products are designed to protect the skin of people over 30 against dryness and the accompanying wrinkles. Ageing process makes the skin gets thinner, drier and less elastic, all of which cause wrinkles. Skin constantly renews itself but with aged, it can't renew itself as quickly. FDA (2000) has reported the causes to wrinkles, fine lines, sagging skin, freckles, pigmentation, age spots and loss of skin tone are largely due to sun exposure. Sun's ultraviolet (UV) rays destroy collagen fibres in the skin and break down its elastin. According to this report, the average 40-year-old has already lost 30% of the collagen compared with that of a teenager. By mid-20s, the skin's oil production declines and our complexion becomes drier and more sensitive.

Natural ageing process cannot be avoided. But with proper cleansing, moisturising and a well-balanced diet, the skin condition could be improved by reducing its appearance of fine lines and wrinkles. 90% of skin problems are the result due to sun exposure (FDA, 2000). Sun damage is the number one cause of dull, yellowish skin, especially on the cheeks and nose which make our skin complexion rough in texture.

Moisturisers are used by millions of people around the globe every day. As we enter the 21st century, the vital word is innovation. The keys to the new products of tomorrow are ingredients that work better and faster to provide healthy looking, moisturised skin. The ability of moisturisers to improve skin condition and appearance is widely recognized especially since the effects of such products can be perceived by the consumer and measured using a variety of techniques. Moisturisers are usually used to improve dry skin condition and effects of skin photoageing that leads to the formation of lines, wrinkles and loss of elasticity (Marenus, 1998). With the understanding of skin biology, incorporation of modern techniques for products development and use of effective ingredients, modern moisturisers not only rehydrate the skin but could provide distinct benefits to the skin's condition and appearance, leading to more youthful look of the user.

Exfoliation is one of the quickest ways to enliven dull skin tones. The act of exfoliation buffs off dead skin cells that, if left will settle as a thick layer and are impenetrable to moisture, especially on drier areas such as elbows, knees and backs of thighs. Once the dead and tired skin cells are scrubbed away, fresh skin cells will shine through making the skin looking radiant. Buffing away dead skin cells becomes more essential as we grow older. A teenage skin takes around 14 to 20 days to renew itself completely, but as the natural ageing process takes place, the cycle slows down, increasing to about 28

days in our late 20s and 30 days by the time we're 40. Body brushing is one of the easiest ways to help boost the skin tone and improve the lymph and blood circulation. Today, exfoliation of the skin using alpha hydroxy acids (AHAs) product is one of the best way to banish dullness, blotchiness or unevenness that comes with sun damage.

1.1.3 The Skin

Skin is the largest organ in our body, taking up about 10% to 16% of human body weight (Schaefer *et. al.*, 1999, Menon and Norlen, 2002) and is made up of 80% water (The Skin, 2002), Figure 1. The skin is not a uniform surface (Schaefer *et. al.*, 1999,

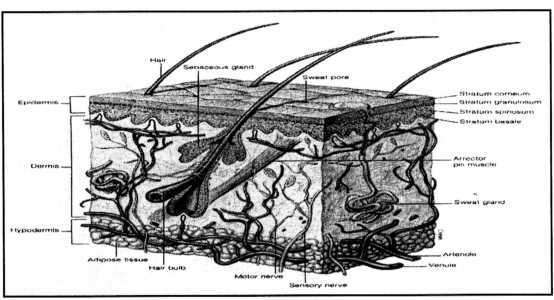

Figure 1.1: Cross section of the skin

Barton and Black, 1998, Kligman *et. al.*, 1985, Fiedler *et. al.*, 1995). Its function is to protect the body systems and internal organs against injury, infection, heat, cold and light exposure, regulate body temperature by controlling moisture loss, store water, fat and vitamins. It must be permeable to certain fluids and gases, must constantly regenerates, must be able to detoxify and manufacture essential compounds for the body.

The weight of the skin is in the range of 7 to 9 pounds with an average area of 20 square feet. Within one square inch of the skin, there are 650 sweat glands, 65 hair follicles, 19 yards of capillaries, 78 yards of nerves and thousands of sensory nerves (The Skin, 2002). The properties of the skin varies according to the anatomical locations (Schaefer *et. al.*, 1999, Wester and Maibach, 1989) which in turn, varies in their thickness (Marks and Barton, 1983) and composition of the stratum corneum. This is also largely related to the activity of the underlying viable epidermis and to a lesser extent to the density and activity of the sebaceous and sweat glands distribution.

Skin is made up of three layers that is epidermis, dermis and subcutaneous.

(a) Epidermis

Epidermis is the outer layer of the skin that serves as a barrier to the surrounding. It is about 0.04 to1.5 mm (millimetres) thick (Pugliese, 2001, Schaefer *et. al.*, 1999). The epidermis gives the skin its glow, suppleness, youthfulness and texture. The palms and soles consists of five sub-layers while the other parts of the body consists four sub-layers (Pugliese, 2001,Van De Graaf and Fox, 1995). The sub-layers of the epidermis are as follows:

(i) **Stratum Basale/Germinativum** is the deepest sublayer of the epidermis and is 3 to 5 cells thick. It is the active, growing, dividing layer of the epidermis where cell mitosis (reproduction) occurs mostly here. As these cells divide, a series of biochemical reactions or pathways such as protein or keratin synthesis and lipid synthesis are also set in motion. The two types of skin colour- pigments produced here are carotene (orange/yellow) & melanin (brown/black).

Melanin is produced in the melanocytes of the stratum basale. All races have virtually the same number of melanocytes but the amount of melanin produced and the degree of

granular aggregation of the melanin determine whether the individual's skin colour is black, brown, red, tan or white. Melanin is a protective device that guards against the damaging effect of the ultraviolet rays in the sunlight. A gradual exposure to the sunlight promotes the increased production of melanin within the melanocytes, hence tanning of the skin. Carotene is found in the epidermal cells and fatty parts of the dermis. It is abundant in the skin of Asians and together with melanin, it gave a yellow-tan skin colour (Van De Graaf and Fox, 1995). Normal skin colour is caused by the combination of melanin, carotene and haemoglobin. Unlike, melanin and carotene, haemoglobin is not a pigment of the skin but an oxygen-binding pigment found in a red blood cells. Oxygenated blood flowing through the dermis gives the skin its pinkish tones.

(ii) **Stratum Spinosum** is 8 to 10 cells thick and made up of flattened cells with spiny projections. Even though cell reproduction occurs here, but it is less than in the basale layer. The lipids in this layer are not much different from those in the basale layer.

(iii) **Stratum Granulosum** is a grainy layer with 3 to 5 cells thick. At this stage, the keratin proteins are visible as dark aggregates and begin to almost completely fill the cell interior where they are longer than they were in the spiny layer. Lipids substances also change at this stage with free sterols are increased, ceramides, glycolipids and cholesterol sulfate are detected.

(iv) **Stratum Lucidum** is a clear, compact, thin and made up of 3 to 4 cells layers. This layer is thickest on the palms and soles of the feet. Keratin accumulates here.

Stratum Lucidum, Granulosum, Spinosum and Basale are highly cellular which is also called Malpighian layer and do not contains blood vessels or nerves (Pugliese, 2001). This Malphigian layer consists of 80% keratinocytes. Synthesis of keratin precursors and cell envelope, transforming of living cells to cornified cells are done here. The protein keratin in the epidermis layer has a waterproof property. The cornification of the outer layers of the epidermis toughens the mostly dead cells to withstand abrasion and penetration of microorganisms.

(v) **Stratum corneum** (SC) is the outermost layer of the skin which is also called the horny or cornified layer. Zhen *et. al.* (1999), as quoted by Harding and Scott (2002), has reported that SC is comprised of 12 to 16 layers of dried flattened keratinocytes (also called corneocytes), very thin and hardy. The cells in these layers are flat, polyhedral, non-nucleated and hover between life and death. It is about 15-150 μm thick (Pugliese, 2001), approximately 40 μm long and 0.5 μm in diameter (Schaefer *et. al.*, 1999, Harding and Scott, 2002). The SC surface area is also dependant upon age, anatomical locations and conditions that influence epidermal proliferation (Harding and Scott, 2002, Schaefer *et. al.*, 1999, Marks and Barton, 1983). Schaefer *et. al.* (1999) has reported that SC comprises of approximately 15 layers though at sites of increased pressure such as soles of the feet, this number is about 5 to 10 fold increased.

The upper layer of the SC is called *stratum disjunctum* which contains approximately 3 to 5 layers and is constantly undergoing desquamation. The *stratum compactum* (lower three layers) is thicker, more densely packed, more regular and contains structures that more closely reflect the underlying epidermis (Schaefer *et. al.*, 1999, Fartasch, 1997, Hou *et. al.*, 1991). The lower stratum compactum has more water (30% by weight) as compared with stratum disjunctum (15% by weight), though both are

considerably less hydrated than the viable dermis (70% by weight) (Schaefer *et. al.*, 1999, Warner *et. al.*, 1988).

Other important component of SC is lipids, a water-insoluble, oily substances. The SC is able to control its water content through both hygroscopic substances which accounting for 20% of its weight while intercellular lipids control TEWL. SC gives protection to the entrance of microorganisms, UV radiation and toxic substances, besides retaining the water, electrolytes and soluble substances in the skin. Every 2 weeks, this entire layer sloughs off with mild force and new cells move up to replace it.

(b) Dermis

Schaefer *et. al.* (1999) reported that the dermis is 1-2 mm (millimeters) thick and accounts for more than 90% of the skin mass. It is the deeper layer of the skin, underneath the epidermis, consisting of connective tissues, fibrous substances (collagen, reticulin and elastin) which allow the skin to stretch and recoil, and ground substance cells such as fibroblasts, lymphocytes and macrophages. It also contains blood and lymph vessels, hair follicles, sebaceous glands and sweat glands. There are 5 types of nerve endings here which sense pain, heat, cold, touch and pressure.

The major divisions of the dermis are the papillary layer (most outer part of the dermis and in direct contact with the epidermis) and reticular layer (under the papillary dermis) (Pugliese, 2001). The papillary dermis is thin, containing small and loose elastin and collagen fibers, lymphatic and blood vessels. The reticular dermis has fewer blood vessels, dense collagen bundles and coarse elastin fibers. This is the area that carries most of the physical stress of the skin.

The dermis is the main support structure of the skin. It passively protects the skin against trauma and maintain the body's integrity by virtue of its strong and flexible composite materials (collagen and elastin). Collagen does not stretch. It is the elastin that gives the skin its resiliency and elegant feel. It provides the spring and "snap" to the young face. Elastin is a fibrous protein that makes up to 0.6-2.1% of the dry weight of the skin while for collagen, it is 72%. The nutrition of the skin is provided through the network of the blood vessels and capillaries.

(c) Subcutaneous

This part is 1-2 mm thick (Schaefer *et. al.*, 1999) and contains adipose tissues (fat cells) that acts as "shock absorbers" (Van De Graaf and Fox, 1995).

The smooth, soft and supple skin of a human face is a result of many biochemical and physical factors. Unfortunately, over time these factors are subject to changes both internally and externally such as sun effect, stress, diseases and ageing which alter the structure of the skin, causing it to sag, losing its lustre and suppleness. The epidermis is a very active tissue that is able to replace itself. For adult human skin, the rate of skin proliferation is slower. The SC of the young can be fully replaced in about 14 days while individuals over 50 can require a long as 37 days to replace it (Pugliese, 2001). Changes in the SC send messages to the basal layer to produce cells at different rates. For instance, where there is pressure such as on the sole of the foot, more cells are produced to make a thicker SC.

Anything that disturbs the skin barrier (SC) also increases the transepidermal water loss (TEWL). Pugliese (2001) has reported a study by Reed *et. al.* (1995) which showed that dark skin has more resistant barrier and healed faster than the white or light skin if their

SC is disturbed. A light-skinned African had more water loss than a dark-skinned Caucasian. In his study, Reed *et. al.* (1995) has also reported that race and gender had no influence on the results. Ghadially *et. al.* (1995), as quoted by Pugliese (2001), stated that mature skin has deficient SC compared to young skin, contributing to more permeable condition, heals more slowly and thus, is more susceptible to environment insult.

1.1.3 (i) Importance of Sebaceous and Sweat Glands

These two glands help the skin to retain its moisture. The oil or sebaceous glands are associated with hair follicles since they develop from follicular epithelium of the hair. These glands secrete the oil or sebum onto the shaft of the hair, preventing it from becoming brittle and to the surface of the skin, where it lubricates and waterproofs the SC layer, serves as a barrier against moisture loss. The oily secretions onto the skin surface give the skin an acidic pH in the region of pH 4.0 to 6.8. However, if the sebum secretion pathway is blocked for some reasons, the glands may become infected, resulting in acne. Sex hormones regulate the production and secretion of sebum.

The sweat glands function as a cooling system, releasing the moisture onto the skin surface. They are most numerous on the palms of the hands and soles of the feet, in the axillary and pubic regions and on the forehead. The two types are eccrine which is widely distributed over the body, especially on the forehead, back, palms and soles and function in cooling to thermal and psychological stimuli whereas apocrine glands are found in axillary and pubic regions where they secrete into the hair follicles. This moisture from sweat glands mix with the sebum secreted by the sebaceous glands forming a hydrolipidic film on the skin surface which waterproof and protect the skin

from drying out, keeps it supple and due to its natural acid protection barrier, preventing germs from intruding.

1.1.3 (ii) Skin Hydration

When environmental conditions change, the human body reacts through a series of self-defense mechanisms which replenish the water level, if necessary. These self-regulating mechanisms are extremely sensitive and often do not react quickly enough to perform their tasks if it is no longer young and dynamic. Water is a fundamentally active element in the metabolic functions of cells and tissues and helps to maintain the physical properties of the SC (Morganti, 1999, Warner and Lilly, 1994). Water reaches the epidermis from the dermis of which it constitutes 70%. In the dermis, water is bound reversibly to glucosoaminoglycans (GAGs) and with hyaluronic acid. When GAG is saturated, water becomes free. Both intracellular and extracellular water reaches the stratum corneum and if exceeds the maximum that can be absorbed, will vaporizes as perspiration.

SC must remain hydrated to maintain its integrity and in healthy skin, the tissue contains greater than 10% of water (Harding and Scott, 2002). According to Harding and Scott (2002) and Morganti (1999), SC's flexibility and protective function is tightly linked to its moisture level and is dependent on three factors:

(a) the rate at which water in the dermis reaches the SC

(b) the rate at which water is eliminated by evaporation (TEWL)

(c) the SC's ability to retain water and this is linked with the role of surface lipidic film, natural moisturizing factors (NMFs) and polar lipids (glycolipids, phospholipids and free fatty acids) exists in the intercellular spaces of the SC.

1.1.3 (iii) Cutaneous Lipids

Various kinds of lipids can be found in the SC (Morganti, 1999, Imokawa and Hettori, 1985, Green *et. al.*, 1984, Morganti and Randazzo, 1985):

(a) Lipids from epidermal cells, generally stored in the form of lipidic vacuoles in keratinized cells.

(b) Lipidic lamellas, found in the intercellular spaces of keratinized cells and is recognized as crucial water retention.

(c) Epidermal lipids which make up 5% of surface lipidic film

(d) Sebum lipids produced by sebaceous glands and is 95% of surface lipidic film

(e) Various fatty agents which was applied to the skin, example through cosmetic products.

Lipids can be classified by their electrical charge and by their structure (Pugliese, 2001). By electrical charge identification, two major groups of lipids are polar lipids (with an electrical charge) such as phospholipids, glycolipids and cholesterol and the other one is non-polar lipids (with no electrical charge) such as triglycerides, squalene and waxes. By structural groups classification, there are six major groups; triglycerides, fatty acids, waxes, cholesterol, spingholipids and ceramides. Triglycerides is the most abundant lipids in the body functioning as energy storage compounds which is 12-25% of the lipids in SC. Fatty acids which gives the oily feels, make up to 12-20%, waxes is 6% while cholesterol, spingholipids and ceramides make up to 14-25% of lipids in SC.

Morganti (1999) has reported that lipids change dramatically in quality and quantity as soon epidermal cells leave the basal layer to turn gradually into desquamating cells. In basale cells, phospholipids, cholesterol, free fatty acids, triglycerides and glycolipids prevail. On the other hand, the horny cell lipids are made up half of ceramides and half

of a mixture of cholesterol and free fatty acids. The lipids in the basal cell differ from those in the SC since mostly they are phospholipids and neutral lipids. In the basale layer, there are more polar lipids and less free fatty acids and almost no sphingolipids.

The polar lipids (cholesterol esters and glycolipids) are fundamental components of the water barrier and help to maintain the physical integrity of the lower part of the SC. Besides the formation of hydrolipidic film on the skin surface to retain the water of the SC, the highly structured intercellular lipids also provide a very effective barrier to the passage of water through the SC, holding the water in SC and preventing the highly water-soluble natural moisturising factor components from leaching out of the surface layers of the skin.

1.1.3 (iv) Skin Natural Moisturising Factors (NMF)

Skin NMF play a major role in skin hydration. As reported by Morganti (1999), qualitative and quantitative changes in the composition of both surface lipidic film and NMF components were shown in many skin diseases. NMF components have the ability to bind water and thereby maintain skin tissue hydration. It consists of a mixture of amino acids, organic acids, urea and inorganic ions where they present at high concentrations within the cell and may represent 10% dry weight of the SC (Harding and Scott, 2002). The major constituents of NMF apart from amino acids are sodium lactate, urea and pyrrolidone carboxylic acid (PCA). Sodium lactate and PCA salt are intensely hygroscopic, thus absorbing atmospheric water and dissolve in their own water of hydration thereby acting as very efficient humectants. This property allows the outermost layers of the SC to feel plasticized, keeping it resilient by preventing cracking and flaking that might occur due to mechanical stresses. The pH value of a 2% NMF solution is 4.7. This substance also shows a mild surface-active property, hence reduce

the skin's surface tension and control the normal water repellent power of the keratin. (Morganti, 1999).

Under normal conditions, the skin produces enough NMF to preserve the right moisturise level in the SC whereas hardly any NMF was produced under pathological conditions such as psoriasis. A loss of NMF was shown to completely prevent the SC from binding water and noticeably reduced the uptake of moisture by keratins. Under normal conditions, water-soluble substances represent 30% of the SC but upon exposure to the sun's rays, they decrease to 23% and may drop to 7% in psoriasis.

1.1.4 Alpha Hydroxy Acids (AHAs)

Throughout the ages, women have turned to natural acids to improve the integrity of their skin. Cleopatra's flawless skin was attributed to her famous sour milk baths and the ladies of the French court of Louis XIV applied aged wine to their faces achieving a luxuriously soft and blemish-free complexion (Rodrigues, 1999). Fruit acids commonly known as AHAs have become popular in the cosmetic industry. AHAs appear to be the miracle in the cosmetic ingredients of the 90s. AHAs are weak acids derived from natural substances and naturally present in fruit, milk and many plant extracts. Examples of AHAs are citric acid which can be derived from all citrus fruits such as lemon and orange, malic acid from apples, lactic acid from sour milk, glycolic acid from sugar cane and tartaric acid from fermented grapes. The most commonly used AHAs in cosmetic products are glycolic acid, lactic acid and citric acids (Scalia *et. al.*, 1998, Wickett, 1996). Glycolic acid has small molecular weight and size, hence better capacity to penetrate the skin. Lactic acid has larger molecular weight but is capable of being converted *in vivo* to pyruvic acid (an alpha keto acid) which is presumed to be a more effective exfoliating agent (Sepp, 1998).

1.1.4 (i) Definition of AHAs

AHAs are also known as chemical exfoliators. Chemically, alpha-hydroxy acid (AHA) is a low molecular weight organic acid. An organic acid is defined as a molecule that possesses a carboxylic acid (-COOH) group. An AHA has a hydroxyl group (-OH) present on the carbon atom immediately adjacent to the acid group. This position is defined as "alpha" because it is the first carbon next to the acid group. Beta-hydroxy acids are thus molecules where the hydroxy group is on the second carbon next to the acid group. Both malic acid and citric acid are alpha and beta hydoxy acids (FDA, 1999). Example of AHAs are glycolic acid ($CH_2OH.COOH$), lactic acid ($CH_3CHOH.COOH$), malic acid ($CH_2CHOH.2(COOH)$), tartaric acid ($2(CHOH.COOH)$) and citric acid ($((CH_2)_2COH.3(COOH)$).

1.1.4 (ii) Benefits of AHAs To The Skin

1.1.4 (ii) (a) Moisturising Agent

A well-established effect of AHAs is in increasing the elasticity of the SC *in vitro* (Middleton, 1974, Hill *et. al.*, 1995). AHAs are hydrophilic. Thus, can diffuse freely throughout the intercelullar watery phase of the skin epidermis. Compounds that increase elasticity are thought to do so either by increasing the SC water content through hydration effect or by direct interaction with SC proteins. There is also substantial experimental evidence that both the acid and its alpha hydroxy functional groups are required for this plasticizing effect to occur (Wickett, 1996, Takahashi *et. al.*, 1985). A few other studies were also conducted which showed an increased in ceramides level of SC (Rawlings *et. al.*, 1994) and polysaccharide hyaluronic acid in both epidermis and dermis (Berstein and Uitto, 1995) after AHAs treatment. Wickett (1996) reported that Van Scott and Yu (1983, 1980) have filed a series of patents claiming lactic, glycolic, other AHAs and alpha keto acid (AKA), pyruvic acid for the

treatment of both common and severe dry skin. Various products at 5% AHA, including both lactic and glycolic acid were tested on 10 subject panels and they were reported to alleviate these symptoms.

According to Wickett (1996), Alderson *et. al.*(1984) has patented AHAs from 6 to10 carbons as skin conditioning agent while Grove (1994) and DiNardo *et. al.* (1995a, 1995b) have shown the increase of skin moisture content after application of products containing AHAs on the skin. In their study, Grove (1994) and DiNardo *et. al.* (1995a) use the electrical conductance measurement to detect skin moisture content. According to Wickett (1996), DiNardo *et. al.* (1995a) has reported that 8% glycolic acid was as effective as 12% ammonium lactate for treatment of dry skin.

The studies described above showed that AHAs can perform as moisturisers for dry skin. Controlled clinical studies have shown significant improvements in skin condition such as in hands (Middleton, 1974) and legs (DiNardo *et. al.*, 1995b) after treatment with moisturisers containing lactate salts or lactic acid. According to Wickett (1996), the first study on the use of lactic acid and sodium lactate as skin conditioning agents in both in vitro and human clinical studies was reported by Middleton (1974). In his study, Middleton (1974) argued that both sodium lactate and lactic acid improve the hand skin condition significantly better than the control products at 95% confidence by analysis of variance. However, he found out that the difference between sodium lactate and lactic acid was not statistically significant.

The influence of hydroxy acids on the rheological properties of SC in vitro was also studied by Takahashi *et. al.* (1985). In his study, Takahashi *et. al.* (1985) found out that sodium lactate had more effect on water absorption than lactic acid but lactic acid

increased extensibility more than sodium lactate. The authors attributed this difference to interaction of lactic acid with SC proteins.

1.1.4(ii) (b) Exfoliating Agent

Products with AHAs are originally developed to reduce the appearance of fine lines and wrinkles but today are also used for the treatment of acne, to lighten skin discolourations and even for stretch marks. AHAs at concentrations lower than 10% are regularly formulated into everyday use cream (FDA, 2000). At higher concentrations, AHAs function as peeling agents which act more rapidly and at a deeper level (FDA, 2000, Wickett, 1996), increasing cell turnover rate and decreasing the thickness of the skin's outer layer. This effect however, depends on the product's pH level, the AHA concentration and AHA vehicle cream, as well as how the product is used (for example, frequency of use and where on the skin it is applied) (FDA, 1999).

Dermatologists often use a procedure called chemical peel to treat facial skin conditions. Peeling is used to diminish signs of photoageing (Wickett, 1996, Van Scott and Yu, 1989a and 1989b, Rubin, 1994, Roenigk, 1995, Goodman and Richards, 1994, Griffin and Van Scott, 1991). According to Wickett (1996), Murad *et. al.* (1995) and Moy *et. al.* (1993), peeling the skin with 70% glycolic acid is reported to be less likely to cause skin scarring or hyperpigmentation compared to peeling with trichloroacetic acid (TCA) or phenol.

In another studies, Murad *et. al.* (1995), Rubin (1994) and Moy *et. al.* (1993) have all discussed the use of 50% and 70% of glycolic acid as chemical peeling agents. The treatment using these concentrations of glycolic acid is carried out within 3 to 5 minutes, followed by washing of the face with either water or a sodium bicarbonate solution. Treatments may be repeated every few weeks for three or four treatments

(Moy *et. al.*, 1993). According to Murad *et. al.* (1995), stinging or burning sensations are always reported by the patients during acid peels treatment. He has also carried out comparisons between the effects caused by TCA and glycolic acid peels. From his study, TCA effects were reported to be primarily in the epidermis whereas the effects of glycolic acid were reported to be more pronounced in the papillary dermis (Murad *et. al.*, 1995). 70% glycolic acid has a pH of 0.5. Elson (1993), as quoted by Wickett (1996), treated his patients with 70% glycolic acid partially neutralised to pH 2.75 with ammonium hydroxide, and noticed a decrease in their fine lines and wrinkles, resulting with smoother skin. Removal of the outermost layer of the skin stimulates the cells in lower layers to grow and divide, causing the skin to thicken and thus, diminishing visible signs of ageing. The more the skin is exfoliated, the more cell divisions will occur in the lower skin layers.

The perceived efficacy of AHA products against the sign of photoageing, especially fine lines and wrinkles has largely driven the remarkable consumer acceptance of these products (Wickett, 1996, Sargisson, 1994, Hwang, 1994). Both Scholz *et. al.* (1994) and Smith (1994a and 1993) have reported about faster SC turnover after AHA treatment. Wickett (1996) reported that Smith (1994a and 1993) judged the efficacy of AHAs tested (lactic, glycolic, pyruvic, citric and acetic acids) primarily by their effect on SC turnover using dansyl chloride method. In his study, Smith (1994a and 1993) has presented evidence that this effect is diminished with continued AHA treatment as the skin accommodates to the AHA. He has also evaluated skin irritation by a combination of subjective stinging and changes in redness using Minolta Chromameter. According to Wickett (1996), there is also evidence from several sources that AHAs can reduce the appearance of fine lines, wrinkles and improve skin appearance, both through

instrumental and clinical studies (Smith 1994a, 1993, Morganti *et. al.*, 1994, Nole, 1995).

As ageing progress, less collagen is produced and long term UV radiation exposure also degrades collagen in the dermis layer of the skin. Sepp (1998) said that premature aged skin brought about by long term UV radiation exposure (actinic ageing) is partly caused by thickening of SC resulting from increased corneocyte cohesion. Although most of the evidence on how AHAs work seems pointing to exfoliation and the resulting turnover of new cells in the outer epidermal layer of the skin, there is increasing evidence that AHAs may be working at a much deeper level of the skin. One study showed that topical treatment twice a day for 3 months with 5% glycolic acid cream affected surface and epidermal changes while the same treatment but with 12% glycolic acid cream, reached deeper and influenced both the epidermis and the deeper dermis layer, resulted in increased epidermal and dermal firmness and thickness. This thickening is accompanied by increased synthesis of glycosaminoglycans (GAGs) and collagen (Sepp, 1998, Ditre *et. al.*, 1995).

The effects of AHAs on epidermal and dermal histology support the argument that AHAs alter the skin structure (Wickett, 1996, Rubin *et. al.*, 1995, Rubin, 1994, Elson, 1993). According to Johnson (2002) and Wickett (1996), the effects caused by AHAs mostly reported consistently are decreased in SC thickness (DiNardo *et. al.*, 1995a, 1995b, Leyden, 1995) at pH lower concentrations due to reduction in corneocytes cohesion (Sepp, 1998, Van Scott and Yu, 1989a), forming a thinner stratum corneum which is more compact and flexible, increased in epidermis thickness (DiNardo *et. al.*, 1995a, 1995b, Ditre *et. al.*, 1995, 1994) and increased in glycoaminoglycans (GAGs) in the dermis (DiNardo *et. al.*, 1995a, 1995b, Ditre *et. al.*, 1995, 1994). Increased in collagen deposition in the dermis has also been reported (Van Scot and Yu, 1989a and

1975, DiNardo *et. al.*, 1995a). All of the studies described above indicate hyper-proliferative effects of the skin cells which finally affected the structure of the skin. At higher concentrations, AHAs operate at a deeper level and cause detachment of keratinocytes (Wickett, 1996).

In general, AHAs can speed up the normal process of skin cell regeneration and sloughing. The exfoliating action of AHAs occurs as a result of their ability to break the bonds between dead skin cells that form at the surface of the skin. This result in increased flexibility of the skin as well as decreased formation of large dry skin flakes at the surface of the skin. Van Scott and Yu (1989a and 1984) have frequently speculated that AHAs reduce corneocyte cohesion by interference with ionic bonding. AHAs also speed up SC turnover (Smith, 1994a, 1994b and 1993). This effect was shown by the removal of squames from the surface due to reduced corneocyte cohesion (Van Scot and Yu, 1989a).

Bartolone *et. al.* (1995a and 1995b) and Wolf *et. al.* (1995) have investigated the effects of glycolic acid and lactic acid treatment on cultured human epidermal keratinocytes and dermal fibroblasts. Bartolone *et. al.* (1995a) found that glycolic and lactic acid were mitogenic, stimulating keratinocyte proliferation and causing a dose-dependant increase in the synthesis of collagen by dermal fibroblasts. In his patent, Bartolone *et. al.* (1995a) claimed L-lactic acid stimulates keratinocyte proliferation more than D-lactic acid. Wolf *et. al.* (1995) has also reported in the increased of keratinocyte proliferation and stimulation of GAG production *in vitro* after AHAs treatment.

Since AHAs may thin the SC, it is possible that they affect the barrier function of the skin. The concentration and pH of AHAs will be important in determining to what

extend the skin will be affected. Peeling of the SC and part of the epidermis with 50% and 70% concentrations is certain to compromise the skin barrier integrity. The increase in TEWL value indicates that the treatment with AHA led to significant degradation in skin barrier function (Wickett, 1996, Frosch and Kurte, 1995). The large decreases in Minimal Erythemal Dose (MED) after treatment with 4% glycolic acid as reported by Sauder (1995), may also be related to thinning of the SC, thus weakening its barrier function to UV radiation.

1.1.4 (ii) (c) Treating Dermatological Conditions

According to Wickett (1996), Van Scott and Yu (1975) has reported significant improvement in lamellar ichthyosis on AHA and AKA treatments. Even though the treatments were performed on only seven subjects, but some of the AHAs led to complete clearing of the ichthyotic condition. In contrast, Wickett (1996) has reported that AHAs is ineffective for treating either psoriasis (Green and Cole, 1994) and seborrheic keratosis (Klaus *et. al.*, 1990).

AHAs are also claimed effective in acne treatments. Van Scott and Yu have claimed activity for AHAs and AKAs against acne both in publications (1994, 1989a and 1989b) and patents (1975, 1977). Another study conducted by Rubin (1994) and Lauber *et. al.* (1995) also claim that AHAs are effective against acne, but present no statistical analysis of their data. Rubin (1994) has described the use of 70% glycolic acids peels solutions as addition to acne therapy whereas Lauber *et. al.* (1995) use 35% glycolic acid by weekly application to treat patients with comedonal acne for approximately three months. Murad *et. al.* (1992) also claimed that chronic AHA treatment improves acne. In his study, 8% of glycolic acid solution was given to 500 patients for up to 8

months and many patients also got and addition of light chemical peels after the treatment period.

1.1.4 (iii) Factors Influencing Efficacy of AHAs

Most often AHAs used in cosmetics are glycolic acid and lactic acid, although there are others and many are used in combination (Johnson, 2002, DiSalvo, 2000, FDA, 1999, Wickett, 1996). As reported by Wickett (1996), the efficacy of AHAs used as an exfoliating agent is independent upon the specific types of alpha hydroxy acid (AHA) used. It depends upon the concentration of the AHA and the pH of the medium in which it is used.

(a) pH of product

AHAs are hydrophilic, therefore are freely diffuse throughout the intercellular watery phase of the skin. In this respect however, DiSalvo (2000) has expressed whether AHAs have more direct actions on epidermal metabolism or not is still unknown. He has quoted what Smith (1994a, 1993) has pointed out that:

> *Chronic treatment with low-pH-based formula is likely to induce changes in pH in the living layers of the skin. Numerous skin enzymes, lipases, phosphatases, etc., have maximal activity at pH 5 or less and it is possible that acid treatment may alter various enzyme relationship, resulting in a direct increase in proliferation.*

Sepp (1998) has reported that glycolic acid and lactic acid applied at equal concentrations and at the same pH have just about the same cell renewal rates. All organic acids tested showed a decrease in cell renewal with increasing pH. The optimum pH for cell renewal stimulation was at about 3.0. At pH greater than 6.0, very

little stimulation was observed for any of the acids. Sepp (1998) and DiSalvo (2000) have also stated that AHAs at pH 6.0 or greater generally behave more like moisturisers than exfoliating agents. The influence of the product's pH on both SC turnover and skin's irritation is also obvious from Smith's work. All the AHAs tested led to the increased in SC turnover and were irritating at pH 3 whereas this effect is not much produced at pH 7. From his study, Smith (1994a, 1993) has also used tricholoacetic acid (TCA) which gave the most irritating and greatest SC turnover compared to the other AHAs. This finding might indicates that the greater the skin is irritated, the higher is the rate of SC turnover.

DiSalvo (2000) has suggested for a product to have a pH in the range of about 4.0 which is considered a compromised between its skin cell renewal benefit with the product's irritation effect. Since the efficacy of the AHA in the product formulation is dependent on the product's pH, then any product sold to the consumer should be labelled with not only the identity of the AHA, but also with its concentration and the pH of the product. According to Johnson (2002), the most widely available commercial AHA products have a concentration of 8% glycolic acid and a pH of 3.8. Wickett (1996) reported that Middleton (1974) has found both lactic acid and lactate increased the extensibility and elasticity of SC and this effect is greater at lower pH value.

(b) Concentration of AHAs in the product

There is no optimum concentration for the use of AHA since this depends upon how it is being used. For example, use of AHAs as peeling agent can go up to 30% to 70% concentration and for use in cosmetic products, the concentration recommended is up to 10% (FDA, 2000, DiSalvo, 2000). For trained cosmetologists, Cosmetic Ingredient Review (CIR) panel concluded that formulations of glycolic acid and lactic acid at 30%

or less and at pH 3.0 or greater, intended for only "brief" use at one time followed by thorough rinsing and daily use of sun protection products were safe. The CTFA study also recommended that retail products containing 10% or less AHA to maintain a pH at least 3.0. In both cases, they recommended that the person treated with AHAs should use sun protection of at least SPF 15 (FDA, 2000, Sepp, 1998).

1.1.4 (iv) AHAs Safety Concerns

Sustained by the quest for a youthful appearance, the use of AHAs in skincare products has grown dramatically throughout the last decade. Despite the adverse effects issues regarding the products containing AHAs was brought up, cosmetic products with AHAs ingredients on the product's labels seems to attract customers and has boost cosmetic sales which outweighs their potential risks of use. The Cosmetic & Ingredient Review (CIR), an independent panel formed by the CTFA (Cosmetic, Toiletry and Fragrance Association) began reviewing the safety of AHAs in 1994. They acknowledged that in most respects, there were no concerns about the safety of AHAs (Johnson, 2002, CIR, 1998). According to Johnson (2002), there was much data from which to conclude that AHAs are not mutagenic or carcinogenic, are not toxic and skin sensitizers.

Johnson (2002) and Wickett (1996) have stated the areas that CIR identified for particular consideration were irritation potential of AHAs, the exfoliating effect of AHAs leading to potentially enhance penetration of other ingredients and/or increase the sensitivity of skin to solar UV rays. The CIR Expert Panel also concluded that there were studies indicating no need for concern about use of AHA enhancing the penetration of other chemicals. They noted that AHAs themselves do penetrate skin readily but because of low systemic toxicity, this was not a concern (Johnson, 2002).

(a) Single-Treatment of AHAs Effects

According to Wickett (1996), the most consistent reported side effect of topical AHA application is stinging, tingling, burning and irritation sensation experienced shortly after product application. However, there were data indicating acceptable limits for concentration or pH of AHAs for leave-on skin products (Johnson, 2002, FDA, 2000). Wickett (1996) has reported a study carried out by Murad *et. al.* (1995) and Moy *et. al.* (1993) about a single treatment on the face with lactic or glycolic acid at 50% to 70% concentration. According to them, the subjects experienced severe stinging within 3 to 5 minutes. These AHAs concentrations peel the epidermis and may cause scarring (Murad *et. al.*, 1995) or hypopigmentation (Boschert, 1994) in some cases.

(b) Short, Cumulative and Long Term Effects

Concentrations in the range of 2% to 20% have been reported to cause transient stinging sensations on the face (Lauber *et. al.*, 1995, Jackson, 1994, Smith, 1994a and 1993, Green and Cole, 1994), legs (Rogers, 1989, Wehr *et. al.*, 1986), heels (Siskin *et. al.*, 1993) and forearms. Other reports of adverse reactions on human use studies mentioned that subjects experienced stinging sensations (Wickett, 1996).

As reported by Wickett (1996), cumulative irritation tests reported shows AHAs are potential to produce significant levels of skin irritation, probably due to daily product application. In contrast, when the test is carried out with alternate day of product's application, the result obtained showed little or no irritation, even from 8% glycolic acid at pH 3.8. There is however, little information about their long-term safety aspect and this needs to be further explored.

(c) Skin Sensitivity Due To UV Radiation

AHAs do not contain UV absorbing chromophores and thus would not be expected to cause photoallergy or phototoxicity directly (Wickett, 1996, Kornhauser *et. al.*, 1982, Harber and Baer, 1972). However, AHAs may potentiate the ability of other compounds to cause photoallergy or phototoxicity by increasing their penetration into the skin (Wickett, 1996) and increasing the skin's sensitivity to UV radiation (Johnson, 2002). According to Johnson (2002), the CIR Expert Panel pointed out that there are evidence showing an increase in sun sensitivity after use of AHA products. Few studies were carried out using sunburn cell (SBC) production as the endpoint indicating for UV rays penetration into the skin. After 12 weeks of AHA treatment, Johnson (2002) reported that there was a small but significant increase in SBC formation after UV rays challenge. In this study, it was found that an addition of a sunscreen with an SPF 2 to an AHA product containing 10% glycolic acid at pH 3.5 would be sufficient to eliminate the AHA-induced increased in UV rays sensitivity. A recent publication by Johnson *et. al.* (2000) also supported this study's finding whereas the commercial AHA products containing 8% and 4% glycolic acid at pH 3.8 with SPF 4 sunscreen agent do not increase sun sensitivity over 6 months study period with twice application on the forearms.

The study sponsored by FDA's Office of Women's Health involved Caucasian volunteers with varying degrees of susceptibility to sunburn. The study has confirmed that applying an AHA to the skin can make people more susceptible to the damaging effects of the sun, including sunburn. The study has shown that topical application of glycolic acid in a cream base causes sensitivity to UV radiation. However, the study also indicate that, at least in the case of glycolic acid, the AHA most commonly used in cosmetics, this effect tends to be quickly reversible and does not last long after a person

stops using the product. One week after discontinuing using of the cream, researchers found no significant differences in UV sensitivity among the various skin sites (FDA, 2000).

In 1997 based on industry-sponsored studies, the CIR Expert Panel concluded that products containing glycolic and lactic acids and their related chemical compounds are safe for use in products intended for consumer use under the following conditions (Johnson, 2002, DiSalvo, 2000, FDA, 2000 and 1999, Wickett, 1996):

(a) The AHA concentration is 10% or less

(b) The final product has a pH of 3.5 or greater

(c) The final product formulated in such a way that it protects the skin from increased sun sensitivity or its package directions tell consumers to use sunscreen product.

1.1.5 *Baccaurea motleyana hook. f (Rambai)* Fruit

Many cosmetic manufacturers add extracts to enhance the performance of their cosmetic products. These extracts are mostly derived from plants, herbs and fruits. Among the fruits extracts used in cosmetic products are bearberry (*Arctostaphylos uva-ursi*), bilberry (*Vaccinium myrtillus*), cucumber (*Cucumis sativus*), papaya (*Carica papaya L.*) and grapefruit (*Citrus grandis*). However, not all of the fruit extracts have AHA properties. Apple Secrets extract, (Gattefosse, 2000) contains malic acid and this extract is proposed to be used as an exfoliating agent. Other examples of commercially AHAs extract promoted as skin exfoliating agent are Lemon Secrets extract which contain citric acid (Gattefosse, 2000), Sugar Cane Amidroxy contains glycolic acid (Alban Muller International, 1996) and Actiplex® 1177, Fruit Acid Complex which contains glycolic, citric acid and ascorbic acid (active organics, 1996).

Rambai, Figure 2, is a fruit that belongs to a family known as Euphorbiaceae. The scientific name for *Rambai* is *Baccaurea motleyana hook. f.*, called *Rambai* in Malaysia, *rambi* in Philippines and *maifai-farang, ramai, lam-khae* (Pattani) and *raa-maa tee-ku* (Narathiwat) in Thailand (Subhadrabandhu, 2001, Morton, 1987). *Rambai* is commonly cultivated in the lowlands and valued for its fruits, which are eaten raw, made into jam or wine. According to Subhadrabandhu (2001), the tree is about 15 to 25 meter tall, has a thick trunk, broad, dense, rounded crown and silky-hairy branchlets. The leaves are evergreen, 152 mm to 330 mm long, 76 mm to 152 mm wide, dark green, glossy with conspicuously indented veins on the upper surface; greenish-brown and hairy below. The small fragrant male and female flowers are petalless. The fruits appear in showy strands dangling from older branches and trunk are oval, about 25.4 mm to 44.45 mm inches long and 25.4 mm thick, with thin, salmon-coloured or brownish yellow, velvety skin becoming wrinkled after ripening. The full ripe fruit is translucent, white, sweet-to-sourish taste contains brown, flat seed(s) about 12.7 mm

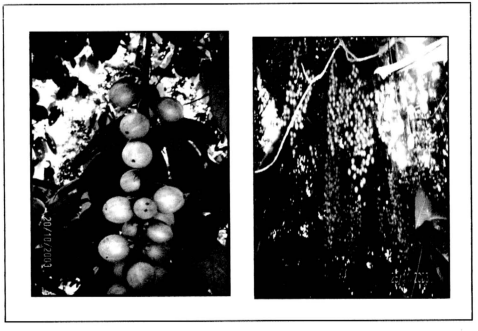

Figure 1.2: *Baccaurea motleyana hook f.* fruits

long. Within each fruits, there are a variable number of seeds enclosed in a translucent white pulp.

In Thailand, this fruit is regarded as under utilized tropical fruits which has potential to be developed for home-garden use (Subhadrabandhu, 2001). Presently this fruit is taken as food, either eaten raw, made into jam or the skin of the fruit is cooked with coconut milk made into *rendang*. According to Morton (1987), the bark of *Rambai* is used as dyes and also employed to relieve eye inflammation. In Indonesia, this fruit is prepared as pickled and served with curries (Subhadrabandhu, 2001). In Malaysian market, a skincare product made from *Rambai* trunk is commercially available in a powder form which is used as *lulur* by women to prepare themselves before married or after giving birth. This *Rambai* powder is used to scrub away all the dead skin cells from the body thus making the skin soft, smooth and supple.

Subhadrabandhu (2001) has also reported the study carried out by Anon that *Rambai*'s fruit has a low vitamin content that is per 100 gm edible portion, it contains 55 mg Vitamin C, 0.03 mg vitamin B_1 (thiamine), 0.09 mg vitamin B_2 (riboflavin), 2 mg calcium and 20 mg phosphorus. Mohamed *et. al.* (1994) has studied the antimicrobial property activity of *Rambai* fruit's skin together with other few tropical fruit wastes. In his study, he has dried the *Rambai*'s skin at room temperature and grounded the samples where these samples were then extracted by using a general extraction procedure with petroleum ether, chloroform and ethanol. The *Rambai*'s skin extract was then evaluated for the antimicrobial activity against gram positive and gram negative bacteria, yeast and fungi. Mohamed *et. al.* (1994) has reported that *Rambai* skin extract showed strong inhibiting activity against all bacteria tested but poor against

yeast or fungi. He concluded that *Rambai* skin's extract has potential use against bacteria.

1.2 Objective of the Study

Looking at the world scenario on skincare products presently and demand by consumers, it can be concluded that among other things, people are looking for natural products with anti-ageing and whitening properties. Both of these effects can be achieved by using fruit's (AHAs) extract. Malaysia has many fruits to be considered but *Rambai* fruit has been chosen in this study due to the following criteria:

(a) no literature on this fruit was found in the area of cosmetic

(b) it is not a popular fruit, thus it is cheap

(c) easy to grow, bear lots fruits and abundant

(d) contains lots of juice and easy to extract.

(e) create better income for farmers through higher added value products developed

Even though *Rambai* is a seasonal type of fruit, it can be produced by batches, preserved and stored for use in cosmetic products preparation.

This research is conducted with the following objectives:

- to develop a stable moisturiser product containing *Baccaurea motleyana hook f.* fruit's extract.
- to study the efficacy of the product's developed and compare its performance to the commercial moisturiser product.

1.3 Thesis Outline

Chapter 2 describes the extraction procedure of *Baccaurea motleyana hook f.* fruits, its extract's physical properties, the types of AHAs in the extract and its quantity. The extract's stability study at room temperature (RT), 4°C and freeze-thaw condition were also discussed.

Chapter 3 discuss about the development of moisturiser product base and those containing *Baccaurea motleyana hook f.* fruit's extract. The moisturiser product's stability study at 50°C and freeze-thaw condition are also described. An attempt was made to increase the concentration of the extract used in the product's developed without jeopardizing the stability of the product.

Chapter 4 explains about the rheological properties of the moisturiser product's developed. The product's yield stress value, hysteresis value and their correlation with the product's stability are also studied.

Chapter 5 covers about the moisturiser product's containing 15wt/wt% *Baccaurea motleyana hook f.* fruit's extract's efficacy study. The efficacy studies were conducted using objective method by instrumental and objective descriptive (sensory) product evaluation. Also presented in this chapter is the result and discussion of the product's efficacy comparative study between the product's developed and the commercial product.

Chapter 6 gave overall conclusions of the studies carried out and recommendations for future work.