

Antimicrobial components from Tamarind (*Tamarindus indica* L.) and
Pomegranate Rinds (*Punica Granatum*, Linn.) in goat's milk lotion

By

Ms. Thipchutha Kosrapunyaapoom

ID. 4735452

A special project snbmitted to the Faculty of Biotechnology,
Assumption University in part fulfillment of the requirements of the
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Level of study: Bachelor of Science

Department: Agro-Industry

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(Dr. Viyada Kunathigan)

Advisor

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Antimicrobial components from Tamarind (*Tamarindus indica* L.) and Pomegranate Rinds (*Punica Granatum*, Linn.) in goat's milk lotion

Abstract

The water soluble components extracted from tamarind pulps and pomegranate rinds were being investigated for their antimicrobial activity against two different types of bacteria; *Staphylococcus aureus* and *Escherichia coli*. There are mainly two methods used through the investigation including an agar disc diffusion method. By doing a primary screening, it was found that the optimum concentrations of water soluble extract were 80% w/v tamarind water extract and 70% w/v pomegranate rinds water extract. Then, these water soluble components were added to goat's milk base body lotion and tested for their efficiency of antimicrobial clear zone.

As a result, the water soluble extract from tamarind pulps exhibited the antimicrobial activity against *S. aureus* and *E. coli*, where the extract from pomegranate rinds exhibited the antimicrobial activity against *S. aureus* only. After the extracts were added into goat's milk lotion, the component was found to give the highest antimicrobial activity at 20 % w/w of 80% w/v tamarind pulps water extract and 10% w/w of 70% w/v pomegranate rinds water extract. Furthermore, the goat's milk body lotion contained the water soluble components has an antimicrobial effect with moisturizing and whitening the skin.

CHAPTER I

INTRODUCTION

Cosmetics are widespread used, especially among women with the worldwide annual expenditures for cosmetics are estimated at U.S. \$18 billion where the popularity of cosmetics has increased rapidly (National Geographic, 2007). With the expanding market in cosmetics, new products are introduced with variety of properties such as moisturizing, anti-aging, anti-brown spot, anti-wrinkle, whitening and much more. These features grab the consumers' attention in trying new products as the prevailing function of fashioned cosmetics in enhancing the appearance or odor of the human body.

One of the ingredients that popularly added to the lotion is milk. Goat's milk is now being popular as an alternative of cow's milk; it is used as a part of food industry, and also in various cosmetics products such as soap, and lotion. With the active ingredients identical to the ones in the natural acid protection layer of human skin, it is easily absorbed by skin (Sirichai, 2006). It results in a mild, non-irritating, soft and smoothing skin, and highly natural moisturizing effect.

Antimicrobial property is one of preference functions that may add into cosmetics such as body lotion. This property can be acquired from modern drugs or chemical substances but they may cause side effects such as rash, nausea and vomit which are not safe for human uses. Natural resources are then being an alternative option as concerning to be harmless to human. With this reason, spices and herbs are becoming much more important as an ingredient in various products including cosmetics. Even though there are variety of spices and herbs available in Thailand, only two of them (Tamarind and Pomegranate) are used to study for their anti-microbial property in which it adds value of agricultural products.

Due to few goats' milk body lotions launched in the market, they only provide the moisturizing or whitening property. None of them is promoting antibacterial property in its lotion. Consequently, the combination of goat's milk, fruits extracted solution and lotion are an alternative choice for those whom want to nourish their skin, remove dead skin cells, and enhance the skin development to look healthy and youthful. Thus, this lotion can ultimately help to reduce the growth of microbial which causes skin infections to human, particularly abscess and boil.

Aim

- To produce the effective antimicrobial goat's milk body lotion that meets the consumer perspective and helps to treat the skin infection causing from bacteria.

Objectives

- To study the antimicrobial property of water-soluble components in fruits.
- To determine which concentration of fruits would provide maximum inhibitory clear zone.
- To differentiate the effectiveness of antimicrobial compound on both gram positive bacteria (*Staphylococcus aureus*) and gram negative bacteria (*Escherichia coli*).
- To produce an antimicrobial goat's milk body lotion.

CHAPTER II

LITERATURE REVIEW

Nowadays, the national awareness of health and nutrition issues renewed consumer interest in a unique natural product. Specifically, the consumption trend in spices and herbs are being popular both domestic and international countries. According to Kasikorn research in 2003, it found that the consumer trend in spices and herbs and those cosmetics contained herbs and spices are dramatically increased by 30 % in the rate of consumption.

Cosmetics are substances used to enhance or protect the appearance or odor of the human body. Cosmetics include skincare creams, lotions, powders, perfumes, lipsticks, baby products, bath oils, bubble baths, bath salts, butters and many other types of products. Their use is widespread, especially among women in Western countries. Skincare product, particularly, lotions are suitable for hairy areas of skin, such as the scalp or chest. Such an advantage, they are thinly spread ability compared to cream and ointment. They are best applied after bathing in order to retain the water in the skin.

Lotions

Lotions are any of various cosmetic preparations, low to medium viscosity medicated or non-medicated liquids, containing insoluble material in the form of a suspension, dispersion or emulsion which intended for external application.

Lotions are easily absorbed and good for mildly dry skin and weeping eczema. Most lotions are oil-in-water emulsions but they can also be water-in-oil emulsions. The main components of a lotion emulsion are consisted of two phases; the aqueous and oil phases. They are composed of various chemical agents where each of them has different properties such as emollient, emulsifier, fragrance, humectant, moisturizer, preservative, stabilizer, thickening agent and UV light absorber.

Lotions can be used in terms of medications such as: antibiotics, antiseptics, antifungal, anti-acne agents and soothing/protective agents. Beside these terms, they can use in the purpose of other accessory such as aiding massage.

The chemical agents are those of:

Emollient/Emulsifier/Stabilizer/Thickening agent (Anonymous^{2,3,6}, 2007)

1. Emollients

They are substances that have the power of softening and soothing the skin which are mainly acquired as a main component in lipstick, lotions and other cosmetic products. Usually, they used to correct dryness and scaling the skin.

2. Emulsifiers

They are known as an emulgent or surfactant. They are a surface-active agent that promotes the formation and stabilization of an emulsion. The emulsion may turn into either oil-in-water (O/W) emulsion or water-in-oil (W/O) emulsion depends upon the volume fraction of both phases and the type of emulsifier.

3. Stabilizer

It is a substance added to another substance or to a system to prevent or retard an unwanted alteration of physical state.

4. Thickening agents/thickeners

They are substances which use to increase the viscosity without substantially modifying the other properties. They provide the body in lotions (viscous solutions or dispersions which impart body), increase the stability as well as improve consistency.

Aloe Vera (*Aloe barbadensis*), in the other name is called Barbados, Cape, Curaiao, Socotrine, and Zanzibar aloe, is derived from the thin-walled mucilaginous cells of the plant. Aloe vera extract is obtained when removing the water from the gel. It contains 95% water, minerals, amino acids, proteins and polysaccharides, therefore making it valuable for moisturizing property. Also, it is a superior humectant by attracting moisture to the skin. Thus, Aloe Vera

prevents immune suppression caused by UVB by repairing damage to epidermal Langerhans cells and it reduces skin irritation. Aloe vera has been used externally to treat various skin conditions such as cuts, burns and eczema. Moreover, it is anti-inflammation, astringent, emollient, anti-fungal, anti-bacteria, and antiviral (Anonymous¹, 2007).

Avocado oil is edible oil which pressed from the fresh pulp of *Persea Americana* fruit. It contains vitamin A, B1, B2, D, E, Pantothenic acid, protein, lecithin, beta-carotene, and unsaturated fatty acids. It is valuable ingredient in cosmetics in terms of regenerative, softening and moisturizing effect. It nourishes the dry and dehydrated skin, eczema, solar keratosis, anti-aging, anti-wrinkle, and improve elasticity. Thus, it is resistant to rancidity and anti-oxidation (Anonymous¹, 2003). Moreover, it helps to reduce spots and heal sun damage and scars. It is said to have a wonderful emollient property (Anonymous¹, 2002).

Cetyl alcohol in IUPAC name is called as 1-Hexadecanol. The other names are Cetanol, Cetyl alcohol, Ethal, Ethol, Hexadecanol, Hexadecyl alcohol, or Palmityl alcohol. The chemical formula is $\text{CH}_3(\text{CH}_2)_{15}\text{OH}$.



Figure 1 The structure of cetyl alcohol

It is a solid organic compound which derived naturally, as in coconut fatty alcohol, or synthetically. At room temperature, cetyl alcohol takes the form of a waxy white solid or flakes. It belongs to the group of fatty alcohols. It is by product of the petroleum industry, or produced from vegetable oils such as palm oil and coconut oil. The production from palm oil would give rise to palmityl alcohol. It is used in a cosmetic industry as a nonionic surfactant, a hair-coating, in shampoos and conditioners, or emollient, emulsifier and thickening agent in

the manufacture of skin creams and lotions or a water based lubricant for fasteners such as nuts and bolts (Field, 2003).

Cucumber (*Cucumis sativus*) **extract** is the juice extracted from the vegetable which has mildly astringent and soothing property. It can help reduce puffiness of the skin. It also has a slight bleaching action which aids in removing dead skin cells, impurities, reduces oiliness, refined pores. Moreover, it has a natural anti-inflammatory actions and skin tightening properties (Anonymous², 2005).

Disodium EDTA or it is called as Ethylene diamine tetra acetic acid, (Ethylendinitilo) Tetra-, Disodium Salt, Disodium Edetate, or Disodium Ethylenediaminetetraacetate. Its chemical formula is $\text{Na}_2\text{C}_{10}\text{H}_{16}\text{N}_2\text{O}_8$.

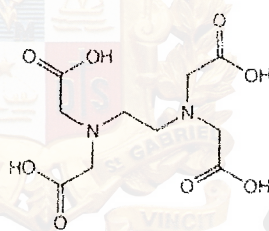


Figure 2 The structure of disodium EDTA

It presents in a white powder which is a substituted diamine and is classed as an alkyl-substituted amino acid. It prevents the ingredients in the formula from binding with trace elements (particularly minerals) that exists in water and other ingredients which would affect in the unwanted product changes such as texture, odor, and consistency of the product. There are no known adverse effects for this chemical compound when used in cosmetic applications. It uses as stabilizer and chelating agent. It helps to build more stable cosmetic product and also used as a viscosity adjuster (Anonymous², 2001).

Glyceryl stearate in other names, it is called Glyceryl monostearate (GMS), Stearin, 1-mono-; (1)-2,3-Dihydroxypropyl stearate, Glycerol stearate, pure Octadecanoic acid, 2,3-dihydroxypropyl ester, or Stearin, 1-mono- (8CI)

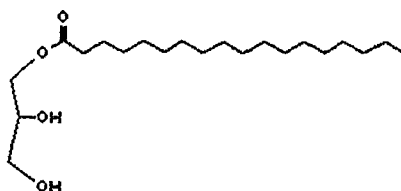


Figure 3 The structure of glyceryl stearate

It is a large group of ingredients that are composed of fats and oils which derived from a compound of glycerin and one molecule of natural stearic acid. At room temperature, the fats are usually white solid and the oils are generally clear liquid. Some tropical oils are liquids in their sites of origin and become solids in cooler or different applications. The color may vary from white to slightly yellowish in the form of hydrophilic wax as pellets. Its production is mainly used for the viscosity adjustment in cosmetic and pharmaceutical O/W emulsions. Typically, it used with another emulsifier such as cetareth-20 or polysorbate-20 in the rate of 1-3%. It acts as an emollient, emulsifier, and thickening agent used in cosmetic preparations such as creams and lotions, providing stability as well as excellent texture. And it is also soften and smooth the skin (Field, 2003).

Jojoba (*Simmondsia Chinensis*) Seed Oil is commonly referred to as the Jojoba plant, this evergreen shrub bears olive-shaped fruits. Jojoba Oil is derived from the bean-like seeds of the desert shurb. It is also called as goat nut. This oil, classified as a light weigh liquid wax, is smooth and non-greasy lubricant in which its chemical closes to our own natural skin oil (sebum). It is the most natural blending oil which is pure, non toxic, and biodegradable. It is not prone to oxidation and does not go rancid. It is shelf-stable when compared with other vegetable oils.

Its physical properties are high viscosity, high dielectric constant, high stability, and low volatility. Because of its balancing, moisturizing and soothing property, it is excellent emollient oil which suits to skin care, hair care and skin nourishment. It is also acted as moisturizer “to improve the skin elasticity and suppleness, containing natural tocopherols to minimize oxidation and lipid peroxidation” (Anonymous, 2000). And, it is easily absorbed by the skin and is especially beneficial in treating inflamed and irritated skin conditions with helps minimize wrinkles and dry skin. Other than using in cosmetics, it uses as antifoam agent, plasticizers, detergents, transformer oil, and leather industry (Undersander *et. al.*, 1990).

Isopropyl myristate or the IUPAC name is Propan-2-yl tetradecanoate. Other names are Tetradecanoic acid, 1-methylethyl ester, estergel, isopropyl tetradecanoate, or Myristic acid isopropyl ester. The chemical formula is $C_{17}H_{34}O_2$.

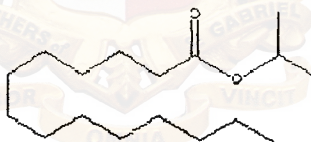


Figure 4 The structure of Isopropyl myristate

It is the ester of isopropyl alcohol and myristic acid. Isopropyl is derived from propane, a gaseous and flammable component of petroleum. Myristic acid, a fatty acid, can be derived from most vegetable and animal fatty acids, but most especially from nutmeg (*Myristica fragrans*), palm seed fats, milk fat and sperm whale (*Physeter catodon*) fat. It is clear, colorless and oily liquid which can be dissolved with organic solvents and insoluble in water. In its pure form, isopropyl myristate is flammable; it re-acts with strong oxidizing agents; it is not a known carcinogen or teratogen (Anonymous⁵, 2005). It is not known to be poisonous to swallow. But it is good spreading capacity for care and decorative preparations. It adds to slip and helps lotions to absorb quickly and easily. It

withstands oxidation and rarely become rancid. It is used as high-grade cosmetic and topical medicinal preparations where good absorption through the skin is desired (Anonymous⁵, 2006).

It can be used as a thickening agent, emollient, emulsifier and moistening agent for cosmetics such as preshaves, aftershaves, shampoos, bath oils, antiperspirants, deodorants, and various creams and lotions.

Olive oil is a vegetable oil which is obtained from the olive (*Olea europaea*), a traditional tree crop of the Mediterranean Basin. It is consisted of high content of monounsaturated fat; oleic acid and polyphenol. Its production is a particularly high quantity of Vitamin E-rich unsaturated Oleic Acid and it is high content in anti-oxidative substances (Anonymous⁶, 2005). Thus, it is a good moisturizing ingredient in which it restores moisture and nourishment to dry, rough skin. It is used as cooking, fuel, cosmetics, pharmaceuticals and soaps. Also, it uses as a cholagogue, laxative, and emollient in the preparation of liniments and foods (Anonymous, 2006).

Stearic acid or IUPAC name is Octadecanoic acid. The chemical formula is $\text{CH}_3(\text{CH}_2)_{16}\text{COOH}$.



Figure 5 The structure of stearic acid

Its chemical is a type of saturated fatty acids that comes from many animal and vegetable fats and oils. It is a waxy solid. The term “stearate” is applied to the salts and esters of stearic acid. It is prepared by treating animal fat with water at a high pressure and temperature, leading to the hydrolysis of triglycerides. It can also be obtained from the hydrogenation of some unsaturated vegetable oils. Common stearic acid is actually a mix of stearic acid and palmitic acid, although purified stearic acid is available separately. Stearic acid undergoes the typical reactions of unsaturated carboxylic acids, notably reduction to stearyl alcohol, and esterification with a range of alcohols. It has

given the outstanding color stability and resistance to rancidity. It uses as an emollient, emulsifier, thickener, fragrance ingredient and cleaning surfactant. Also, it used as a hardening agent in candles, vegetable or paraffin based (Anonymous⁴, 2001).

Sunflower seed oil is non-volatile plant oil and a natural source of tocopherols (vitamin E). It is combustible, clear, slightly amber-colored, semidrying oil with a pleasant scent, expressed from the seeds of the common sunflower; soluble in alcohol, ether, and carbon disulfide (Anonymous⁹, 2006). It consists mostly of mixed triglycerides of fatty acids which is a combination of monounsaturated and polyunsaturated fats with low saturated fat levels. The oil provides a rich source of unsaturated linoleic, oleic fatty acids and Vitamin E. It is also soothing, calming and caring to the skin. Moreover, it retains moisture in the skin. It uses as an emollient in cosmetic formulations, cooking oil in restaurants and food manufacturers and medicinal uses (Anonymous⁷, 2005).

Tocopherol known as vitamin E, classifies as a series of organic compounds consisting of a methylated phenols. Its chemical formula is $C_{29}H_{50}O_2$. The various derivatives are also vitamin E. Vitamin E is a fat-soluble antioxidant

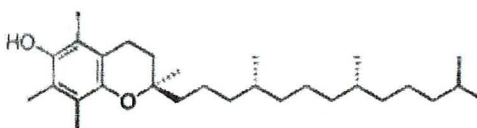


Figure 6 The structure of tocopherol

Alpha-tocopherol is traditionally recognized as the most biological antioxidant in humans. It helps to protect against the damaging effects of free radicals, which may contribute to the development of chronic diseases such as cancer. Vitamin E also may block the formation of nitrosamines, which are carcinogens formed. It also may protect against the development of cancers by

enhancing immune function. In other applications, it uses to reduce scarring, treat heart disease, cancer, cataracts, age-related macular degeneration (AMD), Alzheimer's disease and Parkinson's disease (Anonymous⁷, 2007).

Emulsifier

Ceteareth-20 is a fatty alcohol in the form of white to slightly yellowish flakes. It is the polyoxyethylene ether of cetyl/stearyl alcohol and is classed as an alkoxylated alcohol. It is a universally applicable, non-ionic emulsifier for the manufacture of cosmetic and pharmaceutical O/W emulsions. It is water-soluble and provides exceptionally stable emulsions when used in the combination with another emulsifier such as glyceryl stearate. Typically, it is used in the rate of 1-3%. It uses as a surfactant cleaning agent as well as solubilizing agent. Also, it is widely used as an emulsifying agent (Anonymous¹, 2001).

5. Fragrance

Natural essential oils are health-promoting and life-preserving ingredients. They enhance our wellbeing mentally, spiritually and physically. Thus they stimulate our mind and body, awakening our senses and stimulating the parts of our brains in controlling emotions and regulate hormones. Physically, essential oils promote the circulation and metabolic functions of the skin and its tissues.

5. Humectant

Humectant is also called a hydrator. It is a hygroscopic substance which has the affinity to form hydrogen bonds with molecules of water.

A non-oily ingredient that attracts moisture from atmosphere, retards evaporation and helps to hold water into the skin, thus its function in preventing water loss and drying of the skin. It consists of several hydrophilic groups, mostly hydroxyl groups, and some of amines and carboxyl groups. Humectant is found in many cosmetics where moisturization is desired, for example, lotions,

creams, hair conditioners and so on. Usually, it's concerning on the use of glycerin, propylene glycol, sorbitol, xylitol, maltitol, lactic acid or urea (Anonymous⁴, 2005).

Glycerin or other names are glycerine, glycerol, glycyol alcohol, 1, 2, 3-Propanetriol, 1, 2, 3 -Trihydroxypropane, or propane-1,2,3-triol. The chemical formula is $C_3H_5(OH)_3$.

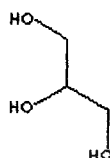


Figure 7 The structure of glycerin

It is colorless, odorless, hygroscopic, and sweet tasting viscous liquid. It presents in all natural lipids (fats), which can be derived from animal or vegetable. It can be manufactured by the hydrolysis of fats and by the fermentation of sugars. It can also be synthetically manufactured. It is a polyhydric alcohol and is classed as a polyol. It has three hydrophilic alcoholic hydroxyl groups (OH) that are responsible for its solubility in water. It shores up the skin's natural protection by filling in the area known as the intercellular matrix and by attracting just the right amount of water to maintain the skin's homeostasis (Anonymous³, 2001). Therefore, it is used to regulate the absorption and release of the water in the skin, creating a moisture balance and helping to soften and smooth the skin. Also, it helps other lipids to perform their work more effectively. It is being replaced by its cheaper counterpart sorbitol. It uses as a fragrance ingredient, skin-friendly humectant, skin-conditioning agent, skin protectant, solvent, emollient, lubricant and viscosity decreasing agent in personal care products (Anonymous³, 2006). It is thus used in toothpaste, mouthwashes, skin care products, shaving cream, hair care products and soaps.

6. Moisturizer

It is complex mixtures of several chemical agents in designing to make the outer skin layers to be softer and smoother. Thus, it uses for imparting or restoring normal levels of hydration to the skin by building such a barrier against the migration of water through epidermis (Marshall, 2007). In other words, it has the mechanism of action which is to prevent the natural oils and water within the skin from escaping to the environment. Moreover, it helps to repair scaly, damaged or dry skin which occurred from external environment aggressions or internal changes, aids in repairing or postponing the aging effects on the skin and nourishes skin moisture content (Anonymous², 2003).

A part of the natural composition in commercial skin moisturizers can be included skin lipids, sterols, oils, humectants, emollients, lubricants, and etc. Usually, it is added into cosmetics such as lotion, cream to counter dryness (Anonymous⁷, 2006).

Ajidew NL-50 in the other names are called Sodium PCA, pyrrolidone carboxylic acid sodium salt, sodium salt of 2-pyrrolidone-5-carboxylic acid, or sodium salt of pyroglutamic acid.

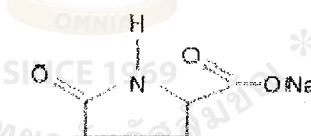


Figure 8 The structure of ajidew NL-50

It is derived from amino acids and is highly water absorbing. At high humidity, it dissolves in its own water hydration to increase softness. It is a naturally occurring component of human skin that is responsible for its moisture-binding capacity in cell. It is highly water -absorbing which can be used as an excellent humectant, emollient and moisturizer. It is considered as a component of the NMF (natural moisturizing factor) and it is one of the best moisture binders available. Typically, it used in the rate of 0.2-4%. However, it should not be used in cosmetic products containing nitrosating agents (Anonymous¹, 2006). It uses as moisturizing products (creams & lotions),

toners, decorative cosmetics/make-up, foam bath, shampoos, conditioner & detangling products, sun protection, after sun product.

Apricot oil is derived from the fruit of *Prunus armeniaca* Kernel oil. It is rich in the essential fatty acids: oleic and linoleic. It contains naturally vitamin A, B1, B2, B6 and vitamin E. It can penetrate the skin without leaving the oil feeling which promotes moisturizing and revitalizing property (Grieve, 2006). Apricot oil is used to resemble almond oil. It is far less expensive and finds considerable employment in cosmetics, for its softening action on the skin. It is commonly used in the manufacture of soaps, cold creams and other preparations of the perfumery trade.

7. Preservative

It is a natural or synthetic chemical substance which is added to prevent spoilage either from microbial growth or undesirable chemical changes. It adds into products such as cosmetics, pharmaceuticals, foods, paints, biological samples, and etc. Even though it is definitely a risk of irritation from using such an ingredient, it is much better than using contaminated products to skin and eyes.

Germaben II is also called as Propylene Glycol, Diazolidinyl Urea, Methylparaben or Propylparaben.

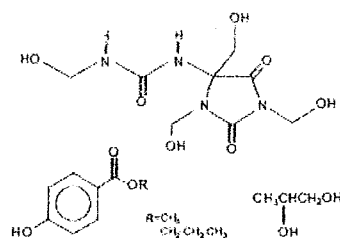


Figure 9 The structure of germaben II

It is clear, and viscous liquid of formaldehyde-releasing preservative. It contains 56% propylene glycol, 30% diazolidinyl urea, 11% methylparaben and

3% propylparaben (Anonymous, 1998). It is a broad spectrum of antimicrobial activity, yeast and mould growth in cosmetics and other personal products. It uses in the rate of 0.5-1% and dissolves completely in wide pH range of 3.0 to 7.5 (Anonymous⁴, 2007). The preferable method is to add it slowly to the product with good stirring just prior to the addition of the fragrance. It can be used without additional co-preservatives and is compatible with essentially all cosmetic ingredients, including surfactants and proteins. It is effective against gram positive and gram negative bacteria and against yeast and mold. It uses as a preservative in lotion, cream, shampoo, hair conditioner and other body care products; especially emulsion products (Anonymous³, 2005).

8. Ultraviolet light absorbers

Ultraviolet irradiation from sunlight is caused a human carcinogen, sunburn inflammation, tanning and systemic immunosuppression.

Ultraviolet light absorbers (UVAs) are substances that used to absorb UV light and reduce the degradation which caused by UV radiation. UVA light is known as “black light” in which it can penetrate through skin in a deep layer. It is thought to be a prime cause of wrinkles since it can damage collagen fibers.

UVB light can cause skin cancer since the radiation excites DNA molecules in skin cells. As the moderate levels of radiation exposed to skin, a brown pigment melanin would be released. It helps to block UV penetration and prevent damage to vulnerable deep skin tissues. Therefore, sunscreen or sun block has been commercially produced as its product contains SPF rating which describes the amount of protection (Anonymous⁸, 2007).

Titanium dioxide or other names are Titanium (IV) oxide, Titania, Rutile, Anatase, or Brookite. Its chemical formula is TiO_2 .

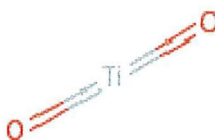


Figure 10 The structure of Titanium dioxide

It is inorganic oxide and naturally occurring mineral oxide of titanium. Its main function is acting as an opacifier, absorbent and white colorant (Anonymous¹¹, 2006). It is inert earth mineral which protects skin from UVA and UVB radiation by reflecting or scattering the radiation away from the skin. Thus, it is considered to have no risk of skin irritation. It uses as thickener, whitening, lubricant, color additive, ultraviolet light absorber and natural physical sunscreen ingredient in cosmetics. It uses in sun-block, and a colorant in toothpastes and cosmetics (Anonymous¹⁰, 2006).

These chemical agents are added as components in making a skincare body lotion. Each compound would vary in its ratio and its function in which it provides different properties to the skin. With the combination of these compounds, it would develop the skincare body lotion with moisturizing and whitening effect.

Somehow, skin infections causing from pathogens such as boils, impetigo, herpes simplex, and so on might be a problem for someone and *Staphylococcus aureus* is one of those microorganisms that might be a cause. *S. aureus* is characterized as gram-positive spherical bacteria (cluster-forming like grapes), nonmotile, nonsporeforming, facultative anaerobe, golden yellow colony on agar with the etymological root of the bacteria's name "*aureus*" which means golden in Latin. It is a pathogen of human which causes a wide range of suppurative infections, as well as food poisoning and toxic shock syndrome.



Figure 11 Gram stain of *S. aureus*

S. aureus is a normal flora of humans found on nasal passages, skin and mucous membranes. *S. aureus* can be spread through contact via an infected wound, skin-to-skin contact with an infected person, and contact of environmental contaminated objects such as towels, clothing, or equipments. Warm, humid environments can also contribute to *staph* infections, so excessive sweating can increase someone's chances of developing an infection. *S. aureus* can grow at a temperature range of 15 to 45 degrees and at NaCl concentrations as high as 15 percent (Todar, 2005).

S. aureus is the most common cause of human diseases, *staph infections*. Around 15-40 % of healthy humans are carriers of *S. aureus*, but they do not cause any active infection or disease. Despite being harmless in most individuals, they are capable to cause various infections of the skin and other organs, ranged from minor skin to life-threatening diseases such as pimples, boils, abscesses, kidney failure, poorly controlled diabetes, severe atopic dermatitis, malnutrition, pneumonia, Immunoglobulin M deficiency, alcoholism, and etc (Stanway, 2002). When the skin is punctured or broken, *S. aureus* would invade to the wound and cause an infection in which they are capable of avoiding the host defense mechanism. Certain strains of *S. aureus* are also a causative agent for Toxic Shock Syndrome and food poisoning.



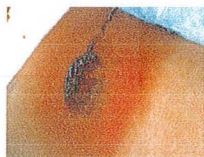



		
Impetigo	Wound infection	Abscess
		
Boil	Scalded skin syndrome	Folliculitis decalvans

Figure 12 Various types of skin infections causing from *Staphylococci*

Even though *S. aureus* is the main causative agent of skin infections, *Escherichia coli* (*E. coli*) is the coliform bacteria which are normally found on human fecal (gut flora). Generally, it is used as the main laboratory microorganism testing. It can cause a skin infection namely gram negative Folliculitis, an acne-like disorder caused by a bacterial infection. Those infections are causing from *E. coli*, *P. aeruginosa*, *S. marcescens*, *Klebsiella* and *Proteus species* (Oakley, 1999).



Figure 13 Gram stain of *Escherichia coli*



Figure 14 Gram negative folliculitis

Since these microbes are caused of skin infections and lotion itself might not be able to treat skin infection, the additional compound should be added to treat with this problem. The natural resources, herbs and spices, are first considered to cause no harm to human. It will also enhance the agricultural product values. Herbs and spices are known to have various properties among variety of species and types. They provide lots of benefit uses in terms of medicine, cosmetics, perfumery, food preservation, religious rituals or as vegetable. The green, leafy part of the plant is often used, and herbal medicine makes use of the roots, flowers, seeds, root bark, inner bark (cambium), berries and sometimes the pericarp or other portions. At least 8,000 species of herbs, medicinal and aromatic plants enter trade globally. Thus, herbs and spices are increased attention to good agricultural and collection practices (Anonymous², 2006).

Pomegranate and Tamarind are classified as fruits. Apart from using as food additives, fruits and additive compounds in some products, their natural compounds may be used to treat skin infections.

Pomegranate rind

Pomegranate rind (Granati Fructi cortex) is the pericarp of the fruit of *Punica Granatum*, Linn., which is a small tree cultivated in the countries bordering the Mediterranean. The fruit is globular, and crowned with a large tubular. Pericarp is hard and granular, brownish-yellow or reddish in colour (Hughes, 2002). The rind of the fruit, separated from the seeds, constitutes the drug. Tannin is the chief constituent of the drug that found in the rind and it is identical with gallotannic acid. The rind also contains a yellow colouring matter, but the alkaloids which characterize the bark of the root and stem have not been detected in the rind. Pomegranate rind is powerfully astringent and it is administered in the form of decoction in diarrhea, dysentery, and as an injection in leucorrhoea (Anonymous, 1995).

According to Chulasiri *et.al.*, 1995, she found that pomegranate rinds showed promising effects upon *B. cereus*, *S. aureus*, *P.aeruginosa*, *C. albicans*, *A. niger* with the appreciable antimicrobial activity after tested by agar disc diffusion method. However, there was no effect upon gram negative bacteria. In addition, the antiseptic mouthwash contained a water soluble component from pomegranate rind (WSC-P) had an antimicrobial activity which exhibited appreciably microbicidal activity upon the tested oral pathogens, namely *S. aureus*, *S. mutans*, and *Lactobacillus spp.* but not *C. albicans*.

Tamarind

The scientific or botanical name is *Tamarindus indica* L. under the family of Caesalpiniaceae. Almost all parts of the plant are useful and either edible or used in folk medicine. An oil-like liquid extracted from the fruit pulp is formally used in many pharmacopoeias. In accordance with US patent issued on antibacterial mouthwash in 1998, it prescribed that an effective antibacterial combination is contained a weak carboxylic acid which is mainly tartaric acid, citric acid and benzoic acid. The

carboxylic acid primarily served as an acidulant but contributes to the antibacterial activity of the composition in a synergistic manner. Thus, the chemical constituents in fruit pulps contained both citric acid and tartaric acid. Specifically, the pulp contains tartaric acid in extremely high quantities around 8-14% (Tan, 2006). However, tartaric acid plays an important role chemically by lowering the pH to a level where many undesirable spoilage bacteria cannot live. Moreover, tartaric acid helps to improve skin texture and tone.

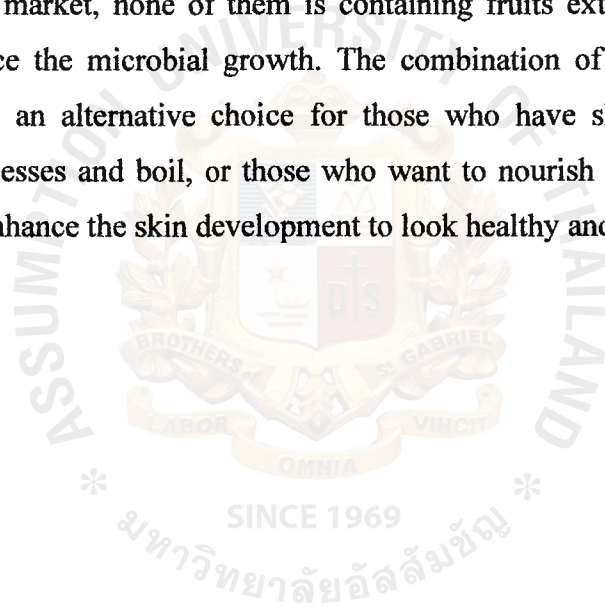
The fruit pulps contain the antibacterial activity and antifungal activity. With 70% ethanol extract, it showed the activity against *S. aureus*, *E. coli*, *B. cereus*, *B. megaterium*, *S. albus*, *Ps. aeruginosa*, and *S. typhimurium*. While 95% ethanol extract, it exhibited activity against *S. aureus*, *E. coli*, *B. subtilis*, *S. typhosa* and *V. cholera*. An undiluted hot water extract showed the activity actively against *E. coli* but equivocal against *S. aureus*. With an ethnomedical use of tamarind, the fruit pulp is able to treat abscesses (Farnsworth and Nuntavan, 1992).

Though there are several extraction methods such as water extraction method, solvent extraction, distillation, maceration, and cold pressing (Anonymous, 2006), the water extraction is the most preferable method used to extract a water soluble compound and it's economically concerned. This method is used to extract the biological compound that is obtained from the extraction, causing no irritation to skin.

The alternative compound may be added to enhance the appearance of human body. Goat's milk (Caprae Lac) is a compound that is rich in nutritious components such as proteins, vitamins, alpha-hydroxy acids and minerals in which it would alleviate allergy to milk products. Researches in Sirichai goat milk's farm have found that goat's milk has the active ingredients identical to the ones in the natural acid protection layer of human skin. Thus, it is naturally homogenized fat molecules and easily absorbed by skin. When fats combined with the natural caprylic acid in its milk, it results in low pH level which closes to the natural pH of human. Moreover, the caprylic acid helps to remove dead skin cells results in a mild, non-irritating, and highly natural moisturizing effect (short protein strands). It would naturally enhance the ability to nourish, and retain its goodness on skin promoting the younger look (anti-aging) and feel of your skin soft and smoothing. It also prevents brown spots (Anonymous⁴. 2006).

By comparison of goat milk, it contains 13% more calcium, 25% more vitamin B-6, 47% more vitamin A, 34% more potassium, 350% more niacin, and 27% more selenium than cow milk. Goat milk is also higher in chloride, copper, and manganese. It is high in protein, triglycerides, Vitamin A, B1, B6, B12, C and E. Minerals such as Zinc help reconstruct collagen fibers encouraging moisture retention and help maintain elasticity. Amino acids and unsaturated fatty acids all help nourish and revitalize dehydrated skin (Jackson-Mitchell, 2004).

Nowadays, goat's milk is used in various products such as butter, cheese, yoghurt, soap bar, body wash and lotion. Even though goat's milk lotions are now available in the market, none of them is containing fruits extract as an ingredient in helping to reduce the microbial growth. The combination of goat's milk, fruits and lotion would be an alternative choice for those who have skin infections problem, particularly abscesses and boil, or those who want to nourish their skin, remove dead skin cells, and enhance the skin development to look healthy and youthful.



CHAPTER III

RESEARCH METHODOLOGY

The materials and methods of this research were used and performed as stated below.

Materials

There are mainly 3 parts of materials including chemical agents, media and equipment and apparatus.

1. Chemical reagents

- | | |
|----------------------|-------------------------|
| 1) Cetereth-20 | 10) Isopropyl myristate |
| 2) Cetyl alcohol | 11) Olive oil |
| 3) Cusson oil* | 12) Price leader oil* |
| 4) Disodium EDTA | 13) Sunflower seed oil |
| 5) Distilled water | 14) Stearic acid |
| 6) Fragrance | 15) Sterile goat's milk |
| 7) Germaben II | 16) Titanium dioxide |
| 8) Glycerin | 17) Tocopheryl acetate |
| 9) Glyceryl stearate | |

Note:

- Cusson oil consists of mineral oil, vitamin E, Aloe Vera extract, and cucumber extract
- Price leader oil consists of mineral oil, jojoba seed oil, avocado oil, and apricot oil.

2. Media

- 1) Nutrient agar (NA)
- 2) Nutrient broth (NB)
- 3) Plate Count Agar (PCA)
- 4) Potato Dextrose Agar (PDA)
- 5) 0.1% Peptone
- 6) Trypticase Soy Agar (TSA) +7.5% NaCl
- 7) Trypticase Soy Broth (TSB) +7.5% NaCl

3. Equipment and apparatus

- 1) Autoclaves (Model HA 300 M2, Hirayama)
- 2) Incubator (Model EB280, Jouan)
- 3) Laminar flow (Model H2, Dwyer Mark 2, “Clean”)
- 4) Munsell Book of Color (2.5R-10 G) volume 1
- 5) Viscometer (Brookfield model RV, Brookfield Engineering Laboratories, Inc.)

Methods

1. Consumers survey

a. Consumers' behavior

The demographic data and consumer behavior were questioned to 115 targeted consumers. The actual trend of body skincare product was demonstrated in order to find out their preferences in buying and using via a descriptive statistic analysis. (Appendix A)

b. Consumers' preference of base lotion

Four samples of lotion were being asked to perform the 9-points scaling hedonic test in identifying the base formula lotion. 100 targeted panelists were involved in the testing in three interval periods of testing; before, during and after applying a lotion as questionnaire provided in Appendix A.

The basic formula of goat's milk lotion is adapted from skin lotion formulation of K.H. Co., Ltd., Aloe Vera body lotion recipe of Somerset Cosmetic Company, LLC. and Goat Milk Lotion ingredients of Sherry Slavish Soap and Bath. (Appendix D) The method of making goat's milk body lotion was stated in figure 15.

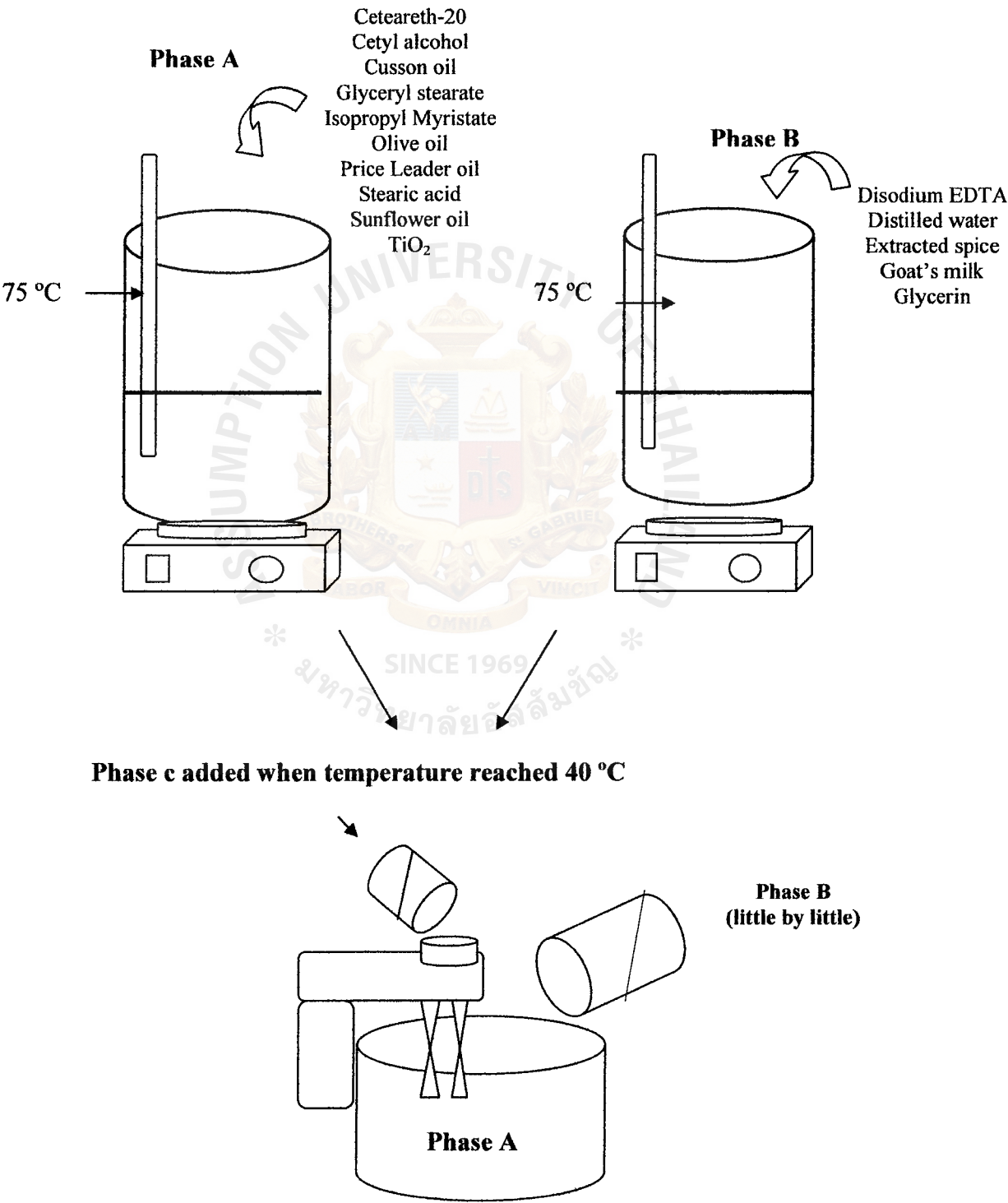


Figure 15 A basic method of making goat's milk body lotion

2. Tamarind and Pomegranate rinds water extraction

Two fruits, tamarind and pomegranate rind, were extracted with water in order to get water soluble compounds for further analysis of antimicrobial activity.

Tamarind was brought from the market to perform the test. The extraction was done by removing the seeds and piths. The remained part, fruit pulps, was then used in the extraction. There were 4 concentrations used to determine the antibacterial property which were 60%, 70%, 80% and 90% w/v of water soluble extraction. In this research, water referred to distilled water.

Pomegranate rind water extraction was done by picking up pomegranate fruits that were a little mature (little red-orange in their color). And then, washed with distilled water and removed the seeds inside. Weigh only the rind at the concentration of 50%, 60%, 70%, and 80% w/v and grinded with blender. Then, added distilled water in the exact amount needed to extract solution.

Since the water soluble compound was expected to have antibacterial property against *S. aureus* and *E. coli*, their solutions were then tested with the primary screening.

3. Primary screening of extracted solution

The microbiological determination was done by testing of an inhibition clear zone using an agar disc diffusion method. Two microorganisms were used to demonstrate in this testing, included *S. aureus* and *E. coli*.

S. aureus was grown in TSA+7.5% NaCl whereas *E. coli*
was grown in NA media



The culture transferred into TSB+7.5% NaCl (*S. aureus*)
and NB (*E. coli*) Then, incubated at 37 °C for 24 hours



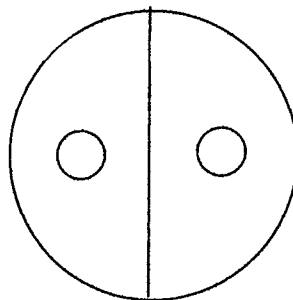
The culture was measured at $\lambda_{650\text{ nm}}$ to have the absorbency 0.5 A.



Swab the culture of *S. aureus* and *E. coli* onto TSA+7.5% NaCl and NA, respectively.



Tamarind water extract and pomegranate rinds water extract solution were soaked with a sterile disc and placed onto the media at different concentration.
(Using blank sterile disc with a diameter of 0.7 cm. as control)



Incubated at 37 °C for 24 hours and then measured the clear zone

Analyzed data using SPSS to find the optimum concentration of extracted solution

Figure 16 Method of primary screening of water extracted compounds

4. Natural antimicrobial Goat's milk Lotions

Fruits were added into goat's milk lotion at various concentrations and tested with an agar disc diffusion method. Before adding tamarind and pomegranate rind water extracted solution into goat's milk body lotion, the optimum concentration of each extracted solution must be done.

Once knowing the optimum concentration, four different amount of extracted solution added into goat's milk body lotion with two conditions of being heat and without heat.

For tamarind in goat's milk lotion, the optimum concentration (80% extraction) was added into lotion at the rate of 5%, 10%, 15% and 20% w/w. Moreover, the optimum concentration of extracted pomegranate rind (70% extraction) was added into goat's milk body lotion at the rate of 2.5%, 5%, 7.5% and 10% w/w. Then, they were further investigated for an antibacterial property on both gram positive and gram negative bacteria.

S. aureus was grown in TSA+7.5% NaCl whereas *E. coli* was grown in NA media



The culture transferred into TSB+7.5% NaCl (*S. aureus*) and NB (*E. coli*).

Then, incubated at 37 °C for 24 hours



The culture was measured at $\lambda_{650 \text{ nm}}$ to have the absorbency 0.5 A.



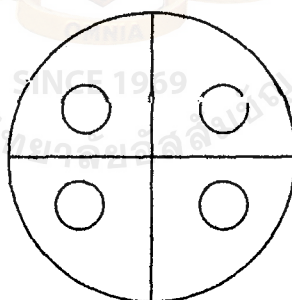
Swab the culture of *S. aureus* and *E. coli* onto TSA+7.5% NaCl and NA, respectively.



There were two conditions; heat and without heat, of extracted solution adding into goat's milk lotion at different concentration.



Tamarind water extract and pomegranate rind goat's milk lotion were dropped by using micropipette 120 μl for 3 spotted samples testing.
(Using goat's milk lotion with no herb added as control)



Incubated at 37 °C for 24 hours and then measured the clear zone



Analyzed data using SPSS to determine the best condition and concentration to be added

Figure 17 Method of testing natural antimicrobial Goat's milk Lotions

5. Physical testing

a) Color

The colors of tamarind and pomegranate rind in goat's milk lotion were measured comparative to the color index in Munsell Book of Color (2.5R-10 G) volume 1.

b) Viscosity

The viscosity of tamarind and pomegranate rind in goat's milk lotion were measured by using a dial Brookfield viscometer model RV.

6. Chemical testing

pH was measured by pH meter Model HI 98127 Waterproof pH & temperature meters

7. Product shelf-life testing (Appendix B)

Products were kept for 2 months prior to test for the following groups of microbes

- a) Mesophilic aerobes
- b) Psychrotrophs
- c) Yeast and mold

Period of research

Started from November, 2006- September, 2007

CHAPTER IV

RESULT AND DISCUSSION

In this research, the consumer behavior and consumer acceptance were first studied to find out the consumer trend on body skincare product. And then the antimicrobial components were extracted from fruits, tamarind and pomegranate rind, by a water extraction method. Those components were then determined for their antimicrobial efficacies at different concentrations with two treatments, heat and without heat, to find the optimum concentration of fruits extraction before adding into goat's milk lotion. Finally, the goat's milk lotion with extracted fruits would be produced which would basically contain an antimicrobial property.

Consumers survey

The test has been conducted to find out the actual trend in consumers behavior based on demographic data and consumers buying behavior towards a skincare body product, especially lotion. 115 consumers were asked to answer the questionnaire concerned with their preferences in buying and using body lotion.

The demographic data was gathered from 115 consumers by surveyed on questions related to their gender, age, occupation, monthly income, education, and hobbies. Then, they were asked for circumstantial answers on their behaviors based on type of skin care product, brand, frequency of use, factors to consider in buying, location, property and expectation of consumers.

As a result, it was found that the majority of consumers are female which accounted for 70.4%. They are mostly teenagers (15 and 25 years) and being students. Most of them are graduated with bachelor degree and has monthly income at 5,000-15,000 baht. Their hobbies are mainly focused on watching TV, movie and surfing internet.

Moreover, the rate of consumers used skin care body product was as high as 81.7% in which they were mainly used body lotion. Consumers were highly preferred to use or currently used Nivea brand. Thus, they applied it either once a day or twice a day. Most of them concerned about ingredient as the first factor in buying a body lotion.

and they preferred to buy at supermarket. Additionally, the properties of lotion were expected to have moisturizing as well as whitening. More than half of them have heard about goat's milk body lotion but only 1/3 of them have been used it. To find out the actual consumers perspective and consumers behavior on fruits added into goat's milk lotion, 4 choices of fruits were designed to be chosen. It found that consumers preferred to have tamarind water extract, guava leaves extract, pomegranate rinds water extract and mangosteen peel extract in goat's milk lotion, respectively.

However, only two fruits were chosen to study on antimicrobial effect against *S. aureus* and *E. coli*. Those fruits were tamarind and pomegranate rinds extracted with water. Even though guava leaves extract was preferred by consumers at the highest rate of 2.69 ± 1.05 , it was quite rare to find its tree in urban area. Though, tamarind and pomegranate were easily found in nature and market.

Four formulas of lotion have been used to perform the 9-points scaling hedonic test in identifying the base formula lotion based on consumers' preference. There were 100 panelists involved in testing these goat's milk lotions including students, housewives, and employees. Various attributes were being questioned at 3 interval periods of testing; before, during and after applying a lotion. Those attributes were color, aroma, viscosity, spread ability, absorbency, stickiness, moisture and overall liking.

Table 1 The overall liking score of 4 different base formulae

Formula	Liking score
1	$6.45^a \pm 1.41$
2	$6.98^b \pm 1.10$
3	$7.13^b \pm 1.15$
4	$6.99^b \pm 1.32$

*a, b, c refers to statistical difference

From table1, it illustrated the overall liking score in each formula of lotion. There was a significant effect among formula 1 and other formulas. The highest liking score was determined at $7.13^b \pm 1.15$ on formula 3. Therefore, this formula was used as a based goat's milk body lotion for further studying of antimicrobial goat's milk lotion. Before hand, the primary screening must be done in order to test the inhibitory clear zone activity of fruits extract before adding into goat's milk body lotion.



Primary screening of raw materials

The primary screening of fruits extracted solutions was determined to identify the optimum concentration to be added into goat's milk lotion. Two types of fruits extracted components; tamarind and pomegranate rinds, were used through water extraction method. Even though ethanol extraction may give the better result, the water extraction was more preferable to be used. Because it does not cause any harm to human's skin such as irritation.

Tamarind

Water soluble compounds were gotten from the extraction of four different concentrations of tamarind; 60%, 70%, 80% and 90% w/v extraction. The antimicrobial efficacy of water soluble compounds was tested against the growth of *S. aureus* and *E. coli*.

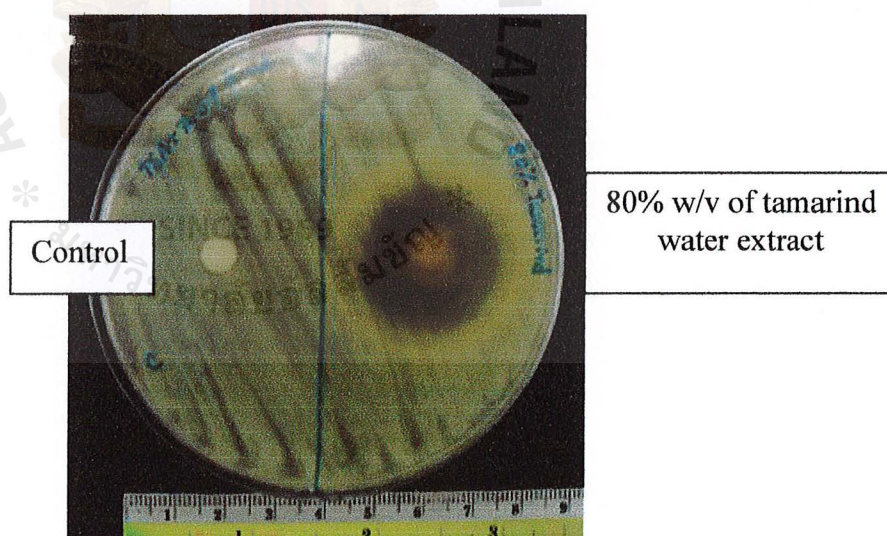


Figure 18 The agar disc diffusion method testing for antimicrobial efficacy at 80% w/v of tamarind water extract.

By performing the antimicrobial test of tamarind water extract, the result was showed in table 2 and 3.

Table 2 The inhibitory zone in each percentage of tamarind water extract on *S. aureus* treated with and without heat condition.

Percentage (w/v) \ Treatment	Clear zone (cm.)	
	Heat	Without heat
60%	1.50 ^b ± 0.55	1.77 ^b ± 0.50
70%	1.64 ^c ± 0.50	2.00 ^c ± 0.46
80%	2.00 ^a ± 0.30	2.33 ^a ± 0.35
90%	2.15 ^a ± 0.24	2.40 ^a ± 0.30

*a, b, c refers to statistical difference

Table 3 The inhibitory zone in each percentage of tamarind water extract on *E. coli* treated with and without heat condition.

Percentage (w/v) \ Treatment	Clear zone (cm.)	
	Heat	Without heat
60%	1.38 ^b ± 0.35	1.57 ^b ± 0.25
70%	1.55 ^c ± 0.29	1.70 ^c ± 0.20
80%	1.64 ^a ± 0.50	1.90 ^a ± 0.30
90%	1.70 ^a ± 0.45	1.97 ^a ± 0.31

*a, b, c refers to statistical difference

Tamarind pulps were used as a raw material in extracting an antimicrobial component since they were found to contain weak carboxylic acids, tartaric acid and citric acid, which contribute to an antimicrobial effect.

As showed in the result tables above, the inhibition clear zone was gradually increased with the concentration of tamarind water extracted solution for both *S. aureus* and *E. coli*. It was clearly seen on the inhibition of *S. aureus* rather than *E. coli*. At 90% w/v tamarind water extracted solution treated without heat, it exhibited the maximum activity of anti-bacterial substance at $2.40^a \pm 0.30$ for *S. aureus* and $1.97^a \pm 0.31$ for *E. coli*. Thus, 90% w/v tamarind water extracted solution treated with heat expressed the maximum activity of anti-bacterial substance at $2.15^a \pm 0.24$ for *S. aureus* and $1.70^a \pm 0.45$ for *E. coli*. However, there was a significant effect between treatment of heat and without heat condition. Without heat condition, it showed the larger inhibitory zone than those of heat condition.

However, the activity has no significantly difference between concentration of 80% and 90% w/v tamarind water extract. Therefore, the concentration at 80% w/v tamarind water extracted solution was used as concerned with an economic issue. However, this result was similar to Farnsworth and Nuntavan research (1992) on which an undiluted hot water extract showed the activity actively against *E. coli* but equivocal against *S. aureus*.

Pomegranate

Water soluble compounds were gotten from the extraction of four different concentrations of pomegranate rinds; 50%, 60%, 70% and 80% w/v water extraction. The antimicrobial efficacy of water soluble compounds was tested against the growth of both *S. aureus* and *E. coli*.

Table 4 The inhibition clear zone in each percentage of pomegranate rind water extract on *S. aureus* treated with and without heat condition.

Treatment Percentage (w/v)	Clear zone (cm.)	
	Heat	Without heat
50%	1.27 ^b ± 0.21	1.08 ^b ± 0.30
60%	1.37 ^b ± 0.21	1.13 ^b ± 0.27
70%	1.67 ^a ± 0.35	1.45 ^a ± 0.30
80%	1.77 ^a ± 0.32	1.59 ^a ± 0.25

*a, b, c refers to statistical difference

The rinds of pomegranate fruits, *Punica Granatum*, Linn., were used as raw materials in extracting antimicrobial component. It was found that rinds of pomegranate fruits contain a chief constituent of drug, tannin, which contributes to an antimicrobial effect.

As a result showed in table 4, it can be seen that 80% w/v pomegranate rinds water extract gave the highest clear zone measurement against *S. aureus*. However, 70% w/v water extraction was used to add into goat's milk body lotion. That was because the antimicrobial activity showed no significant different compared to the compound extracted from 80% w/v pomegranate rinds and also it was economically concerned on raw material usage. However, there was a significant effect between treatment of heat and without heat condition. Heat treatment illustrated the higher inhibitory clear zone than that of without heat treatment.

Nonetheless, the replication of pomegranate rinds water extracted compounds were significantly difference which may caused from different fruits of pomegranate used and the extracted compound may not be steady since the extraction was done by hand after blending. In this study, the antimicrobial activity against *E. coli* was also done but there was no positive result obtained. The result was similar to the research of Chulasiri (1995) that a water soluble component extracted from pomegranate rinds showed the inhibition against *S. aureus*, not any gram negative (*E. coli*). Therefore, it can be concluded that pomegranate rinds water extracted component doesn't contain an antimicrobial compound against *E. coli*.

In conclusion, tamarind at 80% w/v extract and pomegranate rinds at 70% w/v extract were chosen to be used as the preferred concentrations adding into goat's milk body lotion. By comparison of tamarind and pomegranate rinds, it found that tamarind pulps extract showed higher inhibitory activity against *S. aureus* and exhibited the antimicrobial activity against *E. coli*. Unlike pomegranate rinds, it exhibited antimicrobial compound against only *S. aureus*, not *E. coli*.

Natural antimicrobial goat's milk body lotion

The optimum concentration of fruits was chosen after primary screening was done. The optimum concentration of tamarind water extracted was at 80% w/v water extraction where the optimum concentration of pomegranate rinds water extracted was at 70% w/v water extraction.

The based goat's milk lotion that got from the analysis of consumer acceptance was mixed with fruits extracted compounds, tamarind and pomegranate rinds water extracted compounds, and tested for anti-microbial activity against both *S. aureus* and *E. coli*.



Figure 19 Final goat milk lotion

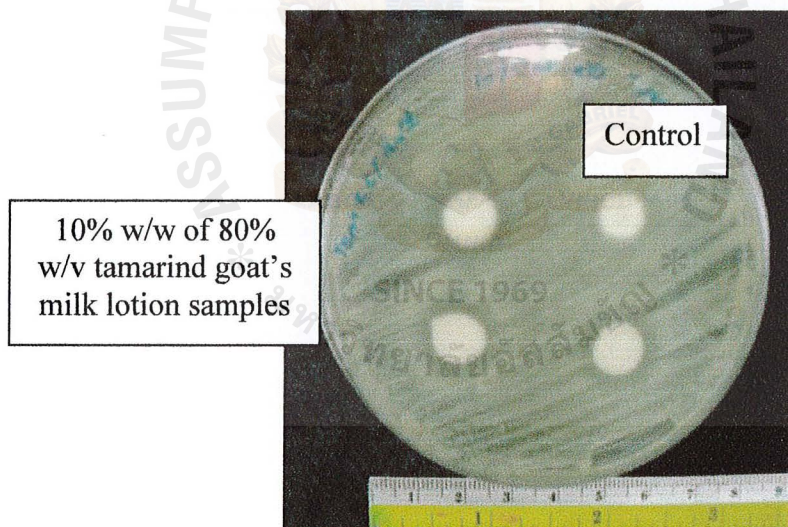


Figure 20 4-spots testing on 10% w/w of 80% w/v tamarind goat's milk lotion

The figures below illustrated the inhibitory zone at different concentrations input (5%, 10%, 15% and 20% w/w) of the original concentration of 80% w/v tamarind water extract (figure 21 and 22). The treatment was performed with heat and without heat condition against both *S. aureus* and *E. coli*.

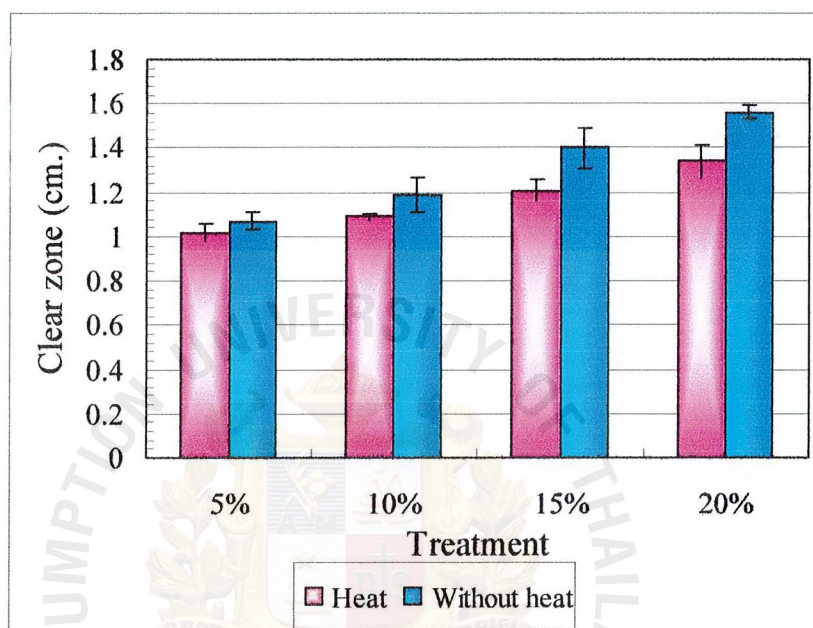


Figure 21 Clear zone compared to heat and without heat treatment of 80% w/v tamarind water extract testing against *S. aureus*

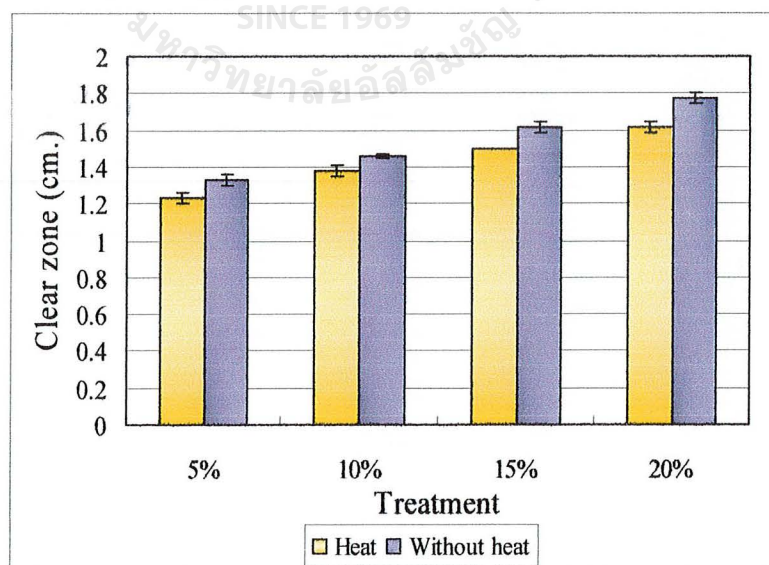


Figure 22 Clear zone compared to heat and without heat treatment of 80% w/v tamarind water extract testing against *E. coli*

Tamarind at 80% w/v water extract was used as original concentration to add into goat's milk body lotion with variation of concentrations at 5%, 10%, 15% and 20% w/w. As a result showed in figure 21 and 22, it exhibited the maximum antimicrobial effect at 20% w/w of 80% w/v tamarind water extraction. This concentration can inhibit both *S. aureus* and *E. coli* but it provided a better result against *E. coli*. And, there was no significant difference between 5% and 10% w/w of 80% w/v tamarind water extraction. But a significant difference was showed between concentration of 15% and 20% w/w of 80% w/v tamarind water extract. Moreover, it exhibited higher inhibitory zone in the condition without heat treatment.

Another fruit goat's milk lotion is consisted of pomegranate rinds. The graphs below illustrated the inhibitory clear zone at different concentrations input (2.5%, 5%, 7.5% and 10% w/w) of the original concentration of 70% w/v pomegranate rinds water extract. The treatment was performed with heat and without heat condition against both *S. aureus* and *E. coli*.

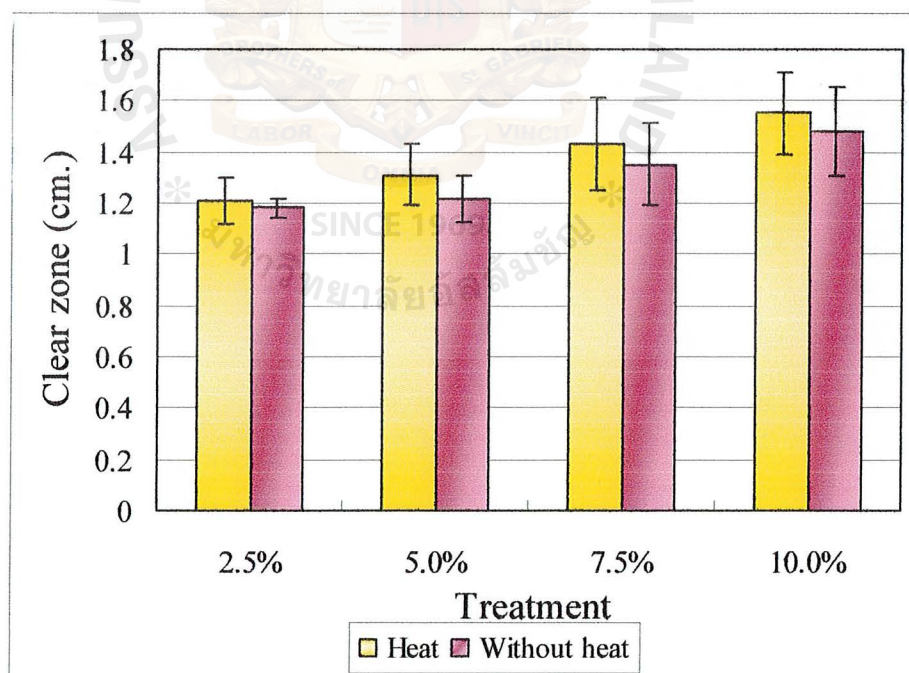


Figure 23 Clear zone compared to heat and without heat treatment of 70% w/v pomegranate rinds water extract testing against *S. aureus*

The original concentration of 70% w/v pomegranate rinds water extract was added into goat's milk body lotion at different concentration of 2.5%, 5%, 7.5% and 10% w/w. The experimental test was done against both *S. aureus* and *E. coli*. As a result, it found that pomegranate rinds goat's milk body lotion expressed the antimicrobial activity against *S. aureus*, not *E. coli* (data not showed). According to a primary screening, it dictated that pomegranate rinds were able to inhibit only *S. aureus*. And the heat treatment showed the better resolution in inhibiting the growth of *S. aureus* (figure 23). Furthermore, it is found the maximum antimicrobial activity against *S. aureus* at the concentration of 10% w/w of 70% w/v pomegranate rinds water extraction. With each concentration of 70% w/v pomegranate rinds water extraction input into goat's milk body lotion, there was no significant difference.

After testing for antimicrobial test against both *S. aureus* and *E. coli*, the physical and chemical tests including viscosity, color, and pH of fruits goat's milk lotion were examined.

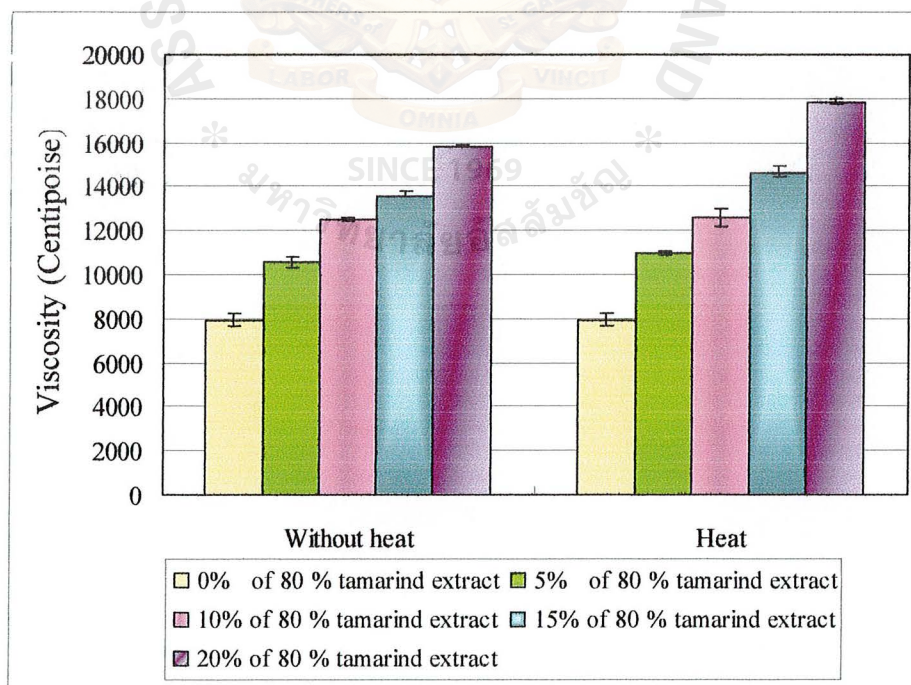


Figure 24 Viscosity of 80% w/v tamarind goat's milk body lotion

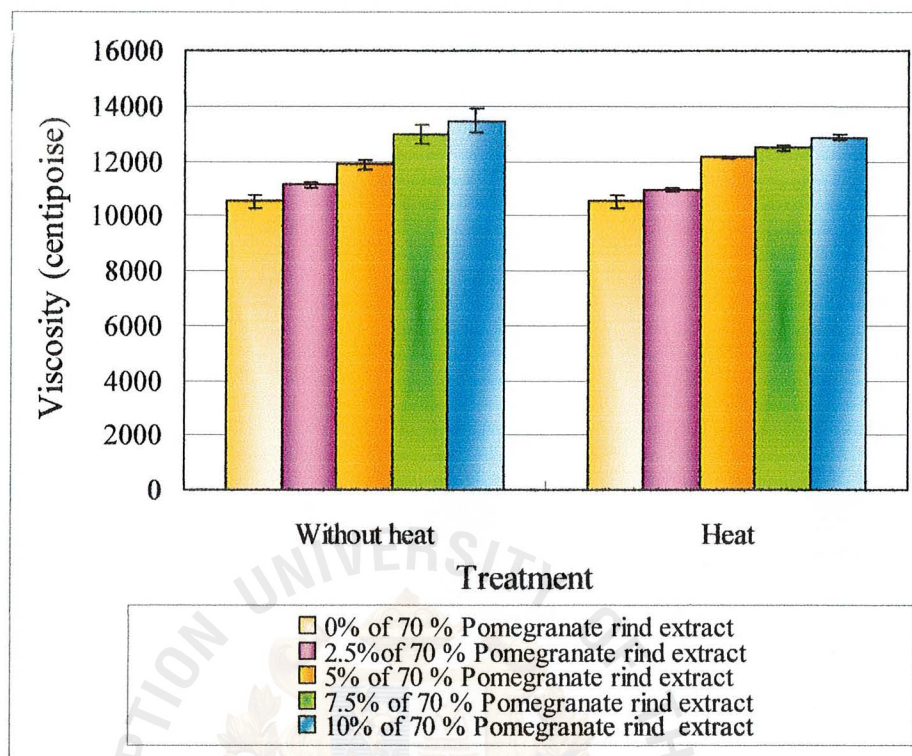


Figure 25 Viscosity of 70% w/v pomegranate rinds goat's milk body lotion

The viscosity of pomegranate rinds and tamarind goat's milk body lotion were measured using the viscometer (figure 25). For tamarind goat's milk body lotion, it can be seen that the viscosity was gradually increased as the higher concentration of fruits added while its viscosity was slightly increased for pomegranate rinds goat's milk body lotion. In tamarind goat's milk body lotion, it found higher viscosity in heat treatment than that of without heat treatment due to removing of volatile compound. Thus, it may affect the consumers' acceptance. On the other hand, the pomegranate rinds goat's milk body lotion was found to have higher viscosity in the condition without heat treatment than that of heat treatment. That may cause from an error of dial viscometer.

Then, the color measurement was evaluated according to the standard Munsell Book of color (2.5R-10 G) volume 1. The finished fruits goat's milk body lotion showed in the figure 26 and 27.

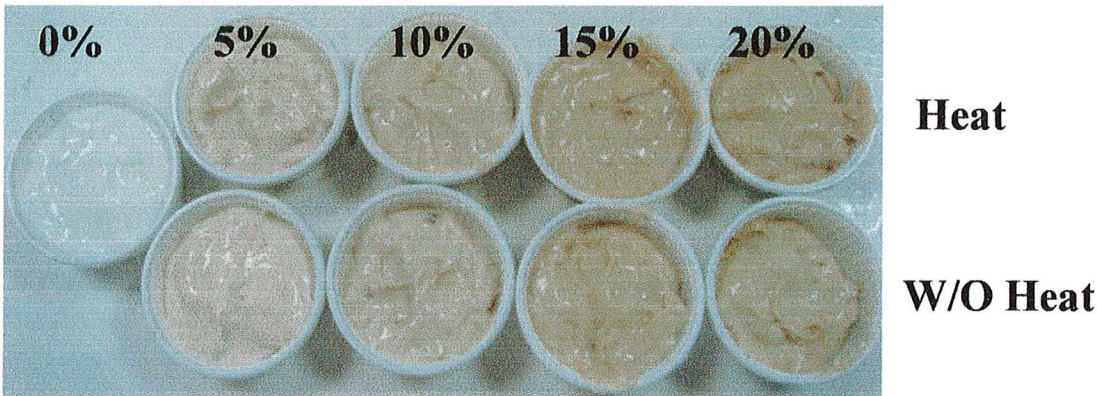


Figure 26 The color of tamarind goat's milk lotion with and without heat treatment of tamarind water extracted.

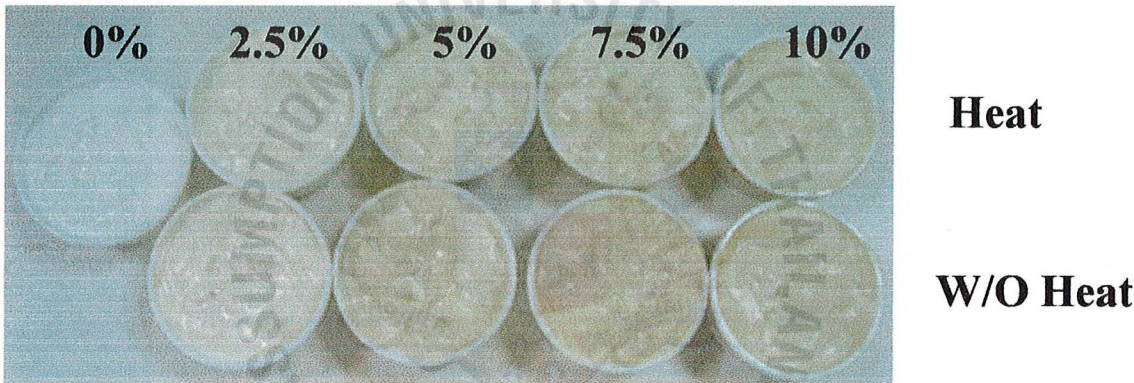


Figure 27 The color of pomegranate rinds goat's milk lotion with and without heat treatment of pomegranate rinds water extracted.

The Munsell Book of color was used as a reference of color indication. Color of tamarind goat's milk lotion and pomegranate rinds goat's milk lotion were measured as shown in figure 26 and 27. It can be seen that the color of both tamarind and pomegranate rinds goat's milk body lotion were slightly increased as higher concentration of extracted components input. Tamarind goat's milk lotion with the heat treatment showed the darker brown tone resulting from removing of volatile compounds. On the one hand, the pomegranate rinds goat's milk lotion promised the darker yellow tone to that of without heat treatment. However, high concentration of extracted components added into goat's milk lotion may affect the consumers' acceptance.

The chemical test, pH measurement, was analyzed with pH meter for both tamarind and pomegranate rinds goat's milk body lotion. The pH values were plotted as showed in the figures 28 and 29.

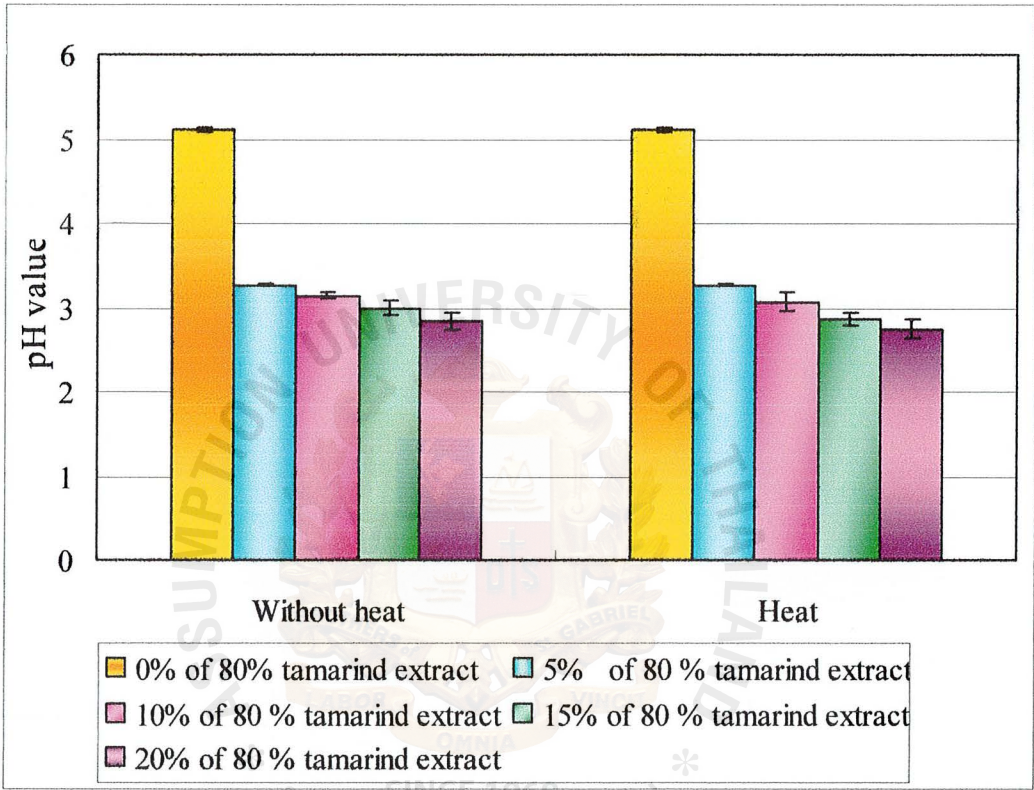


Figure 28 pH measurement of tamarind goat's milk body lotion.

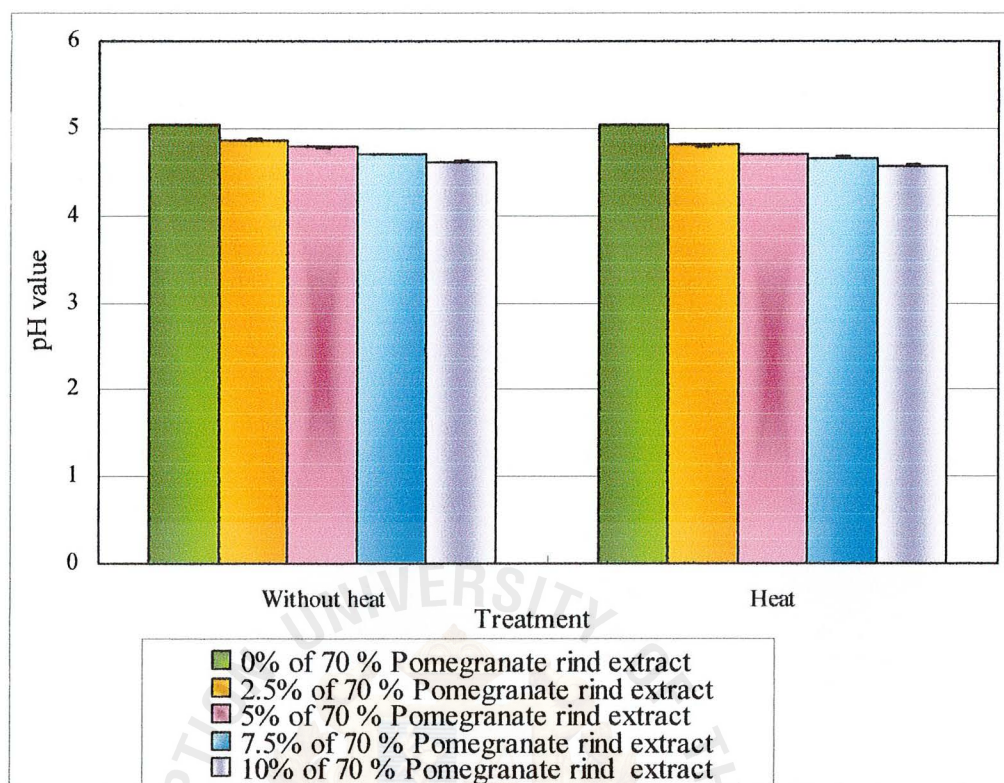


Figure 29 pH measurement of pomegranate rinds goat's milk body lotion.

The pH value of goat's milk was originally set to be around 5.0 compared to the standard of pH of human skin. The components of tamarind and pomegranate rinds water extracted were added into goat's milk body lotion. As consequences, the pH of tamarind goat's milk body lotion was dramatically reduced as compared to the original lotion since tamarind water extracted solution contained weak carboxylic acid, tartaric acid and citric acid. These acids would lower pH where undesirable spoilage bacteria cannot live. Moreover, there was no significant difference between heat and without heat treatment. On the contrary, the pH of pomegranate rinds goat's milk body lotion was slightly decreased as compared to the original lotion. That was because pomegranate rinds contained tannin constituent, which would lower pH. And, there was no significant difference between heat and without heat treatment.

After done physical and chemical tests, products were kept for 2 months (at room temperature) in determining the shelf-life. Mesophilic aerobes, psychrotrophs and yeast and mold were experimental test for their growth. Hence, there was no growth observed. Therefore, the products after kept for 2 months still saved and be able to sell on the market for at least 2 months.

The final tamarind and pomegranate rinds goat's milk body lotion was produced to contain antibacterial property, which was the additional function to cosmetic products. Moreover, these lotions would easily absorb to the skin, nourish the skin, remove dead skin cells, be highly natural moisturizing effect, soften and smoothing the skin layers.



CHAPTER V

CONCLUSION AND RECOMMENDATION

By studying the antimicrobial components contained in Tamarind and Pomegranate rinds water extract in goat's milk lotion, it was found that the water soluble component from tamarind pulps can inhibit *S. aureus* and *E. coli* growth where water soluble component from pomegranate rinds can inhibit *S. aureus* growth but not *E. coli*. By comparison of the activity between tamarind pulps and pomegranate rinds, it was found that the water soluble tamarind water extract showed higher inhibitory activity against *S. aureus*.

At 80% w/v tamarind water extract and 70% w/v pomegranate rinds water extract, they were found to be the optimum concentration in adding to goat's milk lotion. After adding these extracted fruits components into goat's milk lotion, the most antimicrobial inhibitory zone was found with the use of 20% w/w of 80% w/v tamarind water extract and 10% w/w of 70% w/v pomegranate rinds water extract added.

The combination of goat's milk, tamarind or pomegranate rinds and goat's milk lotion are best applied to those whom have skin infections problem and those whom want to nourish, moisturize and whiten their appearance of skin to look healthy and youthful.

The further investigation should be done with other extraction methods such as solvent extraction or distillation in which they can control the composition of extracted substance to be steadily produced and the identification of other components containing in tamarind and pomegranate rinds may be study. In addition, other microorganisms such as *Propionibacterium acne*, and *Plasmodium ovale*, the major causative microorganisms of skin infection, may be tested for its antimicrobial activity. The product shelf-life should also continue studying and determine any method to keep it for longer lifetime.

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Assumption University

Faculty of Biotechnology

I am undergraduate student studying in faculty of Biotechnology, Assumption University, and currently enrolling in AI 4290 Special Project. As a partial fulfillment of the requirement of the degree of Bachelor Degree of Science in Biotechnology, I am requested to conduct field surveys on consumption behavior of consumers.

As such, to be able to fulfill the requirements, I would like to kindly request few minutes of your valuable time to answer this questionnaire. Your responses will be treated confidential and will be strictly used for this course's purpose. Your kind cooperation and assistance would be highly appreciated.

Questionnaire No. _____

Part I: Demographic

Gender: ☐ Male ☐ Female

Age: ☐ Less than 15 ☐ 16-25 ☐ 26-35 ☐ 36-45 ☐ More than 46

Occupation: ☐ Student ☐ Private company's employee

☐ Government's employee ☐ Business owner

☐ Housewife ☐ Others

Monthly income: ☐ Less than 5,000 ☐ 5,000-15,000 ☐ 15,001-25,000
(Baht)

☐ 25,001-35,000 ☐ 35,001-45,000 ☐ More than 45,000

Education: ☐ Less than High school ☐ High school ☐ Certificate

☐ Bachelor Degree ☐ Master Degree ☐ Doctor Degree

Hobbies: ☐ Watching TV, movie ☐ Reading books ☐ Sports

☐ Fitness ☐ Listen to radio ☐ Surfing internet

☐ Cooking ☐ Others

Part II: Consumer behavior

1. Do you use any skincare body product?
☐ Yes ☐ No (Proceed to question 8)
2. Which type of skin care body product do you often use?
☐ Body lotion ☐ Body cream ☐ Body gel
☐ Body scrub ☐ others
3. Which brand(s) of body lotion do you currently using/ prefer to use?
☐ Nivea ☐ Johnson& Johnson ☐ Citra
☐ Jergens ☐ Vaseline ☐ Others.....
4. How often do you normally apply body lotion?
☐ Once/day ☐ Twice/day ☐ Once/week ☐ Twice/week
☐ Others.....
5. Please rank the factor that you would consider in buying body lotion
(1-main factor → 4- least factor) Note: no rank repetition allowed
..... Price Promotion Brand Ingredients
6. Where do you buy body lotion?
☐ Supermarket (eg. Tops, Market place, Gourmet market)
☐ Hypermarket (eg. Tesco, Carrefour, Big C)
☐ Specialty store (eg. Marks & Spencer, Body Shop, Oriental Princess)
☐ Convenient store (eg. 7-eleven, Family mart, 108 shops, V-shop)
☐ Direct sale (eg. Amway, Mistine)
☐ Others
7. Which property do you expect to have in body lotion?
☐ Moisture ☐ Whitening ☐ Others.....
8. Have you ever heard about goat's milk lotion?
☐ Yes ☐ No
9. Have you ever used goat's milk body lotion?
☐ Yes ☐ No

10. Which spices do you prefer?

- ☐ Guava leaves extract ☐ Mangosteen extract ☐ Tamarinds extract
- ☐ Pomegranate extract (ทับทิม) ☐ others

11. Please rank the desirable order of spice that would put into goat's milk lotion
(1-most desire → 4- least desire) Note: no rank repetition allowed

- | | |
|----------------------------|---------------------------|
| Guava leaves extract | Mangosteen extract |
| Tamarinds extract | Pomegranate extract |

Thank you very much for your kind cooperation



Hedonic Scaling Test

Product: Goat's milk Lotion

Name: _____

Date: _____

- Instruction:
- 1) Please clean your hands and arms before applying.
 - 2) Please observe the appearance of samples before applying.
 - 3) In the order presented from left to right, take 1 spoon of each sample and apply to your left arm. Spread it until the sample is totally dispersed. Then determine attributes of sample during and after using.
 - 4) Then, evaluate hedonic scale in each attribute of sample by using the following numbers:

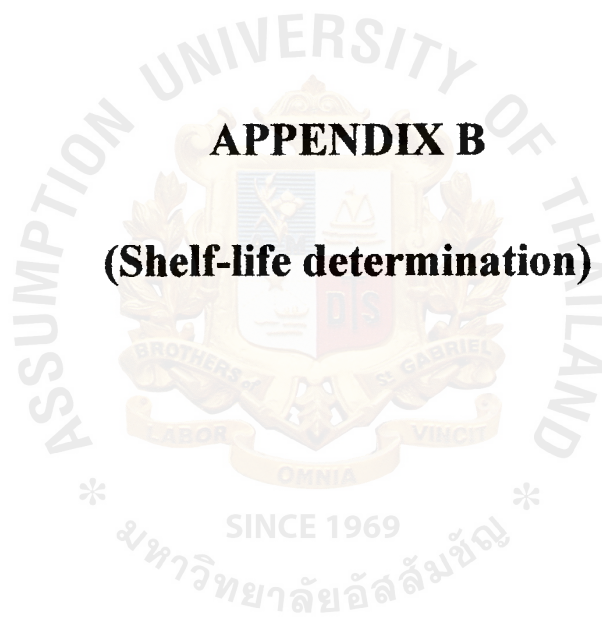
1 = Dislike extremely
 2 = Dislike very much
 3 = Dislike moderately
 4 = Dislike slightly
 5 = Neither like nor dislike

6 = Like slightly
 7 = Like moderately
 8 = Like very much
 9 = Like extremely

Sample			
<u>Before</u>			
Color			
Aroma			
Viscosity			
<u>During</u>			
Spread ability			
Absorbency			
<u>After</u>			
Stickiness			
Moisture			
Overall			

Comments _____

Thank you for your cooperation



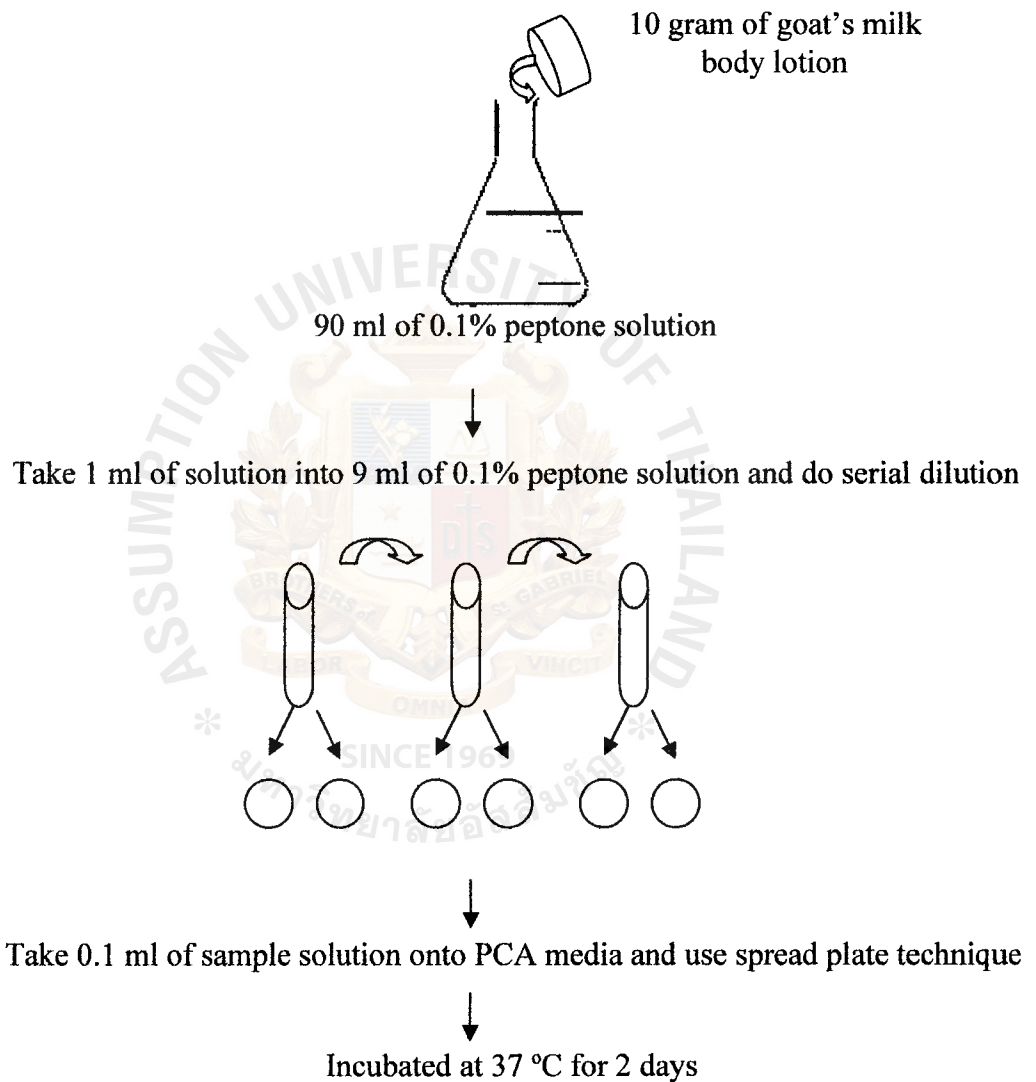
APPENDIX B

(Shelf-life determination)

The final products were kept for 2 months prior to test for shelf-life.

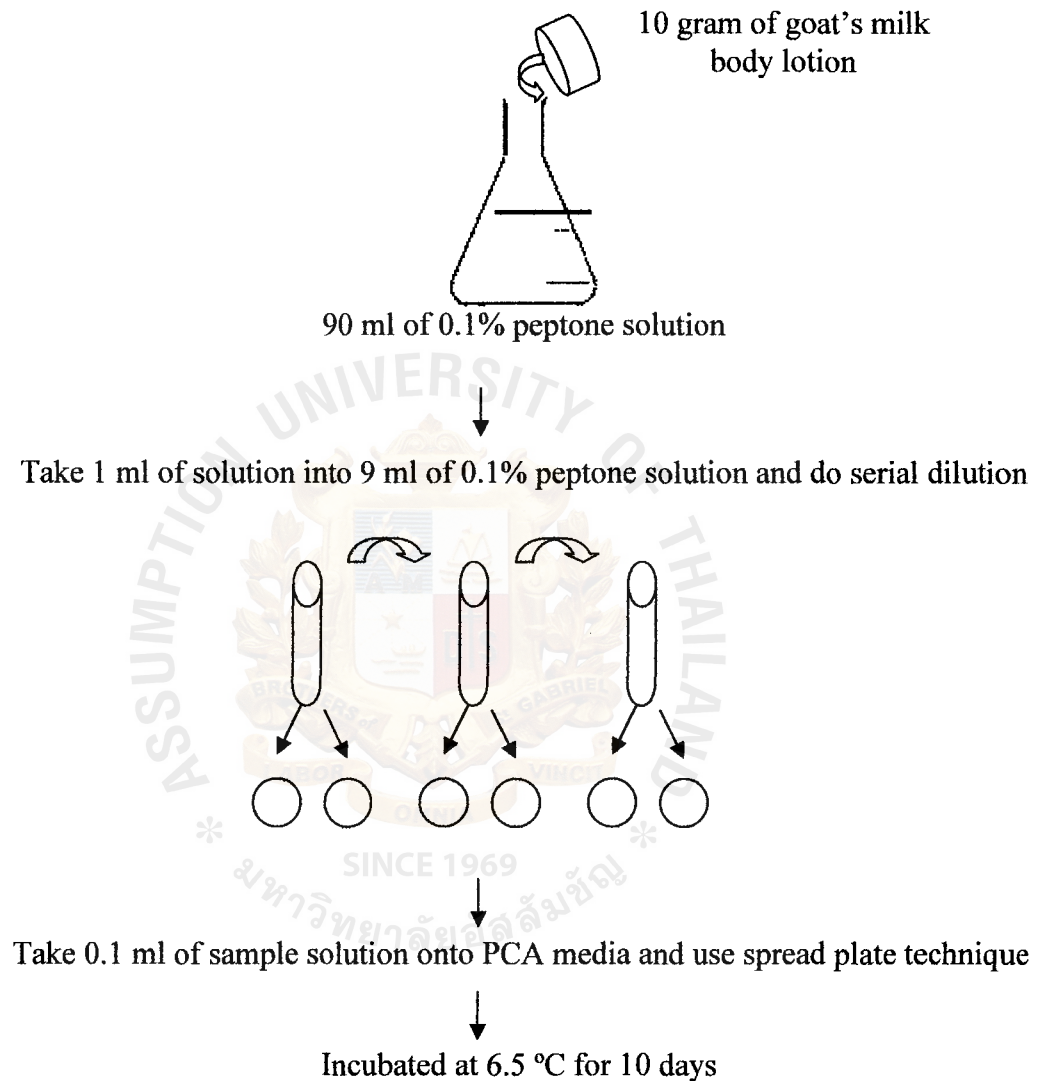
a) Mesophilic aerobes

10 gram of goat's milk body lotion blended with 90 ml of 0.1% peptone solution



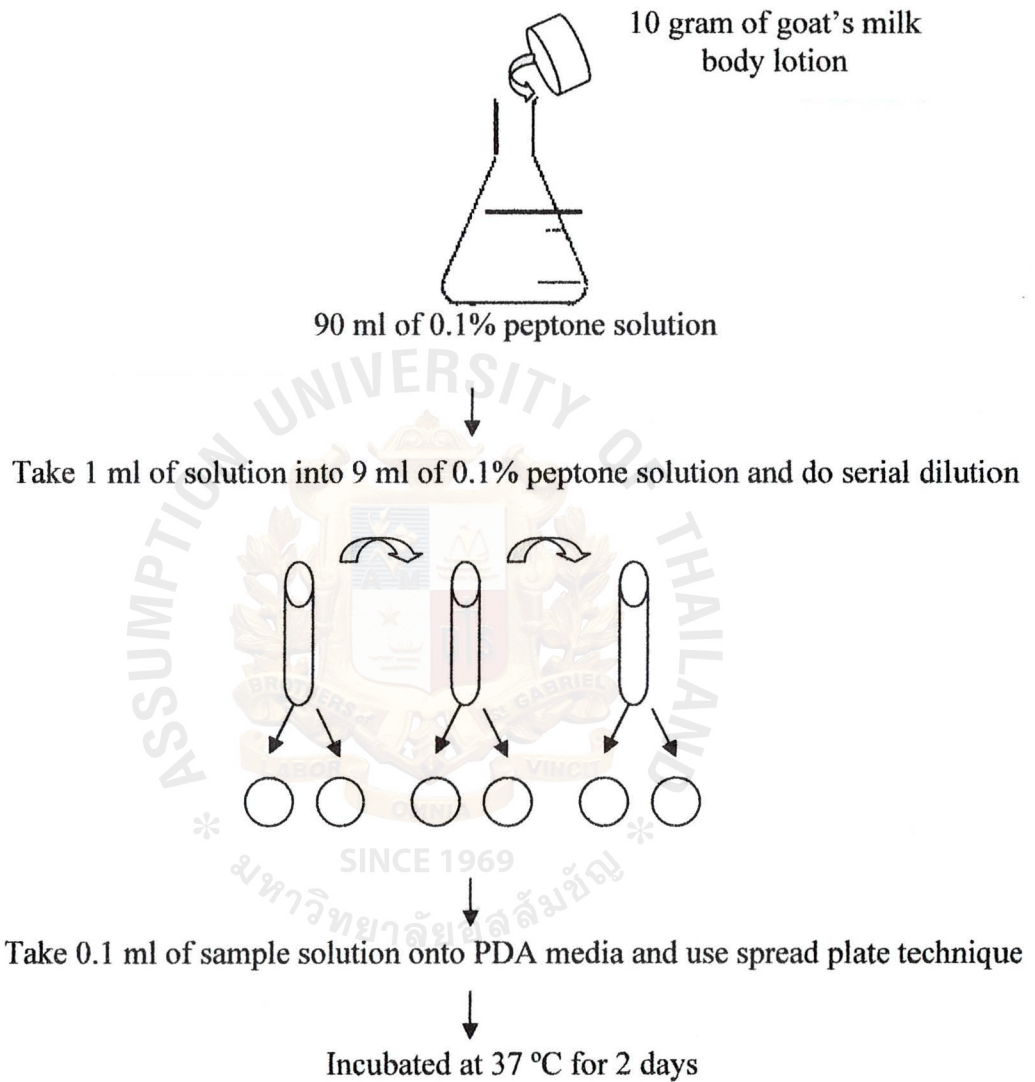
b) Psychrotrophs

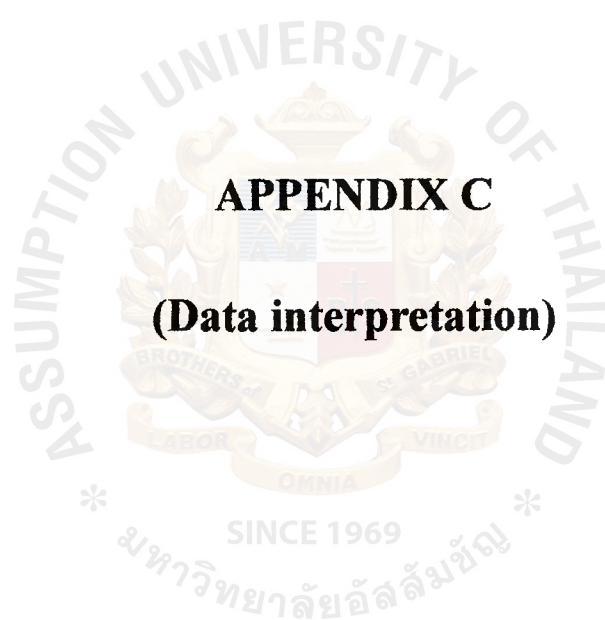
10 gram of goat's milk body lotion blended with 90 ml of 0.1% peptone solution



c) Yeast and mold

10 gram of goat's milk body lotion blended with 90 ml of 0.1% peptone solution





A. Consumers’ survey

Consumers’ acceptance is done by screening of consumer’s behavior and consumers’ preference. Among a hundred and fifteen consumers, they are being asked to answer the questionnaire on consumer’s behavior. However, only a hundred consumers are being asked to perform the hedonic nine-points scaling test on consumers’ preference of goat’s milk lotion.

a) Consumers’ behavior

Table A- 1 Descriptive statistics in frequencies of gender in consumer behavior

Gender				
		Frequency	Percent	Cumulative Percent
Valid	Male	34	29.6	29.6
	Female	81	70.4	100.0
	Total	115	100.0	

Table A- 2 Descriptive statistics in frequencies of age in consumer behavior

Age				
		Frequency	Percent	Cumulative Percent
Valid	Less than 15 years	1	.9	.9
	15-25 years	104	90.4	91.3
	26-35 years	4	3.5	94.8
	36-45 years	4	3.5	98.3
	More than 46 years	2	1.7	100.0
	Total	115	100.0	

Table A- 3 Descriptive statistics in frequencies of occupation in consumer behavior

Occupation		Frequency	Percent	Cumulative Percent
Valid	Student	98	85.2	85.2
	Private company's employee	8	7.0	92.2
	Government's employee	4	3.5	95.7
	Business owner	2	1.7	97.4
	Housewife	1	.9	98.3
	Others	2	1.7	100.0
	Total	115	100.0	

Table A- 4 Descriptive statistics in frequencies of income in consumer behavior

Income		Frequency	Percent	Cumulative Percent
Valid	Less than 5,000 baht	31	27.0	27.0
	5,000-15,000 baht	67	58.2	85.2
	15,001-25,000 baht	6	5.2	90.4
	25,001-35,000 baht	7	6.1	96.5
	35,001-45,000 baht	3	2.6	99.1
	More than 45,000 baht	1	.9	100.0
	Total	115	100.0	

Table A- 5 Descriptive statistics in frequencies of education in consumer behavior

Education		Frequency	Percent	Cumulative Percent
Valid	Less than High School	1	.9	.9
	High School	18	15.6	16.5
	Certificate	1	.9	17.4
	Bachelor Degree	92	80.0	97.4
	Master Degree	3	2.6	100.0
	Total	115	100.0	

Table A- 6 Descriptive statistics in frequencies of hobbies in consumer behavior

Hobbies		Frequency	Percent	Cumulative Percent
Valid	Watching TV & movie	72	26.1	26.1
	Reading books	43	15.6	41.7
	Sports	27	9.8	51.4
	Fitness	14	5.1	56.5
	Listen to radio	42	15.2	71.7
	Surfing Internet	50	18.1	89.9
	Cooking	12	4.3	94.2
	Others	16	5.8	100.0
	Total	276	100.0	

**Table A- 7 Descriptive statistics in frequencies of
“Do you use any skincare body product?” in consumer behavior**

Skincare

		Frequency	Percent	Cumulative Percent
Valid	Yes	94	81.7	81.7
	No	21	18.3	100.0
	Total	115	100.0	

**Table A- 8 Descriptive statistics in frequencies of
“Which type of skin care body product do you often use?” in consumer behavior**

Type

		Frequency	Percent	Cumulative Percent
Valid	Body lotion	78	71.6	71.6
	Body cream	17	15.6	87.2
	Body gel	3	2.7	89.9
	Body scrub	10	9.2	99.1
	Others	1	.9	100.0
	Total	109	100.0	

**Table A- 9 Descriptive statistics in frequencies of
“Which brand(s) of body lotion do you currently using/ prefer to use?” in
consumer behavior**

Brand

		Frequency	Percent	Cumulative Percent
Valid	Nivea	30	27.0	27.0
	Johnson & Johnson	16	14.4	41.4
	Citra	8	7.2	48.6
	Jergens	11	9.9	58.5
	Vaseline	22	19.9	78.4
	Others	24	21.6	100.0
	Total	111	100.0	

**Table A- 10 Descriptive statistics in frequencies of
“How often do you normally apply body lotion?” in consumer behavior**

Often

		Frequency	Percent	Cumulative Percent
Valid	Once/day	40	43.0	43.0
	Twice/day	38	40.9	83.9
	Once/week	3	3.2	87.1
	Twice/week	7	7.5	94.6
	Others	5	5.4	100.0
	Total	93	100.0	

**Table A- 11 Descriptive statistics in descriptives of
“Please rank the factor that you would consider in buying body lotion
(4main factor --> 1- least factor)” in consumer behavior**

Note: no rank repetition allowed

Descriptive Statistics

	N	Minimum	Maximum	Mean	Std. Deviation
Price	93	1.0	4.0	2.419	.9816
Promotion	93	1.0	4.0	1.828	.9163
Brandname	93	1.0	4.0	2.957	.9881
Ingredient	93	1.0	4.0	2.796	1.2297

**Table A- 12 Descriptive statistics in frequencies of
“Where do you buy body lotion?” in consumer behavior**

Where

		Frequency	Percent	Cumulative Percent
Valid	Supermarket	40	36.4	36.4
	Hypermarket	25	22.7	59.1
	Specialty Store	23	20.9	80.0
	Convenient store	11	10.0	90.0
	Direct sale	5	4.5	94.5
	Others	6	5.5	100.0
	Total	110	100.0	

**Table A- 13 Descriptive statistics in frequencies of
“Which property do you expect to have in body lotion?” in consumer behavior**

Property		Frequency	Percent	Cumulative Percent
Valid	Moisture	62	64.6	64.6
	Whitening	30	31.2	95.8
	Others	4	4.2	100.0
	Total	96	100.0	

**Table A- 14 Descriptive statistics in frequencies of
“Have you ever heard about goat’s milk lotion?” in consumer behavior**

Heard		Frequency	Percent	Cumulative Percent
Valid	Yes	62	53.9	53.9
	No	53	46.1	100.0
	Total	115	100.0	

**Table A- 15 Descriptive statistics in frequencies of
“Have you ever used goat’s milk body lotion?” in consumer behavior**

Used		Frequency	Percent	Cumulative Percent
Valid	Yes	21	18.3	18.3
	No	94	81.7	100.0
	Total	115	100.0	

**Table A- 16 Descriptive statistics in frequencies of
“Which spices do you prefer?” in consumer behavior**

Prefer

		Frequency	Percent	Cumulative Percent
Valid	Guava leaves extract	25	21.7	21.7
	Mangosteen extract	22	19.1	40.9
	Tamarinds extract	30	26.1	67.0
	Pomegranate extract	24	20.9	87.8
	Others	14	12.2	100.0
	Total	115	100.0	

**Table A- 17 Descriptive statistics in descriptives of
“Please rank the desirable order of spice that would put into goat’s milk lotion
(4-most desire --> 1- least desire)” in consumer behavior**

Note: no rank repetition allowed

Descriptive Statistics

	N	Minimum	Maximum	Mean	Std. Deviation
Guava leaves	115	1.0	4.0	2.687	1.0460
Mangosteen	115	1.0	4.0	2.287	1.0904
Tamarinds	115	1.0	4.0	2.670	1.1217
Pomegranate	115	1.0	4.0	2.357	1.1712

b) Consumers’ preference of base lotion

Four formulas of goat’s milk lotion were used to demonstrate the based lotion for further experiment used in adding the spices. The based formula is found by using hedonic nine - points scale testing among a hundred targeted consumers.

Table A- 18 Compare means in One-Way Anova of overall liking in goat's milk lotion

Overall				
Sample		N	Subset for alpha = .05	
			1	2
Duncan ^a	314	100	6.45	
	524	100		6.98
	941	100		6.99
	236	100		7.13
	Sig.		1.000	.428

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 100.000.



B. Primary screening

The water extracted components of fruits, tamarind and pomegranate rinds, are used to perform the primary screening on antimicrobial test against *S. aureus* and *E. coli*. Antimicrobial testing against *S. aureus* is done by using TSA+7.5% NaCl media whereas antimicrobial testing against *E. coli* is done by using NA media.

a) Tamarind water extracted

1. Analyzed result on TSA+7.5% NaCl media (testing against *S. aureus*)

Table B- 1 General linear model in Univariate of clear zone (cm.) in different percentages of tamarind water extracted for without heat treatment by Duncan method.

Clear zone (cm.)					
	Percentage	N	Subset		
		1	2	3	1
Duncan(a,b)	60% w/v tamarind water extract	3	1.7667		
	70% w/v tamarind water extract	3		2.0000	
	80%w/v tamarind water extract	3			2.3333
	90% w/v tamarind water extract	3			2.4000
	Sig.		1.000	1.000	.439

Means for groups in homogeneous subsets are displayed.

Based on Type III Sum of Squares

The error term is Mean Square(Error) = .010.

a Uses Harmonic Mean Sample Size = 3.000.

b Alpha = .05.

Table B- 2 General linear model in Univariate of clear zone (cm.) in different percentages of tamarind water extracted for heat treatment by Duncan method.

Clear zone (cm.)

	Percentage	N	Subset		
		1	2	3	1
Duncan(a,b)	60% w/v tamarind water extract	3	1.5000		
	70% w/v tamarind water extract	3		1.6445	
	80%w/v tamarind water extract	3			2.0000
	90% w/v tamarind water extract	3			2.1500
	Sig.		1.000	1.000	.350

Means for groups in homogeneous subsets are displayed.

Based on Type III Sum of Squares

The error term is Mean Square(Error) = .005.

a Uses Harmonic Mean Sample Size = 3.000.

b Alpha = .05.

2. Analyzed result on NA media (testing against *E. coli*)

Table B- 3 General linear model in Univariate of clear zone (cm.) in different percentage of tamarind water extracted for without heat treatment by Duncan method.

Clear zone (cm.)

Percentage	N	Subset		
	1	2	3	1
Duncan(a,b) 60% w/v tamarind water extract	3	1.5667		
70% w/v tamarind water extract	3		1.7000	
80%w/v tamarind water extract	3			1.9000
90% w/v tamarind water extract	3			1.9667
Sig.		1.000	1.000	.223

Means for groups in homogeneous subsets are displayed.

Based on Type III Sum of Squares

The error term is Mean Square(Error) = .004.

a Uses Harmonic Mean Sample Size = 3.000.

b Alpha = .05.

Table B- 4 General linear model in Univariate of clear zone (cm.) in different percentage of tamarind water extracted for heat treatment by Duncan method.

Clear zone (cm.)

Percentage	N	Subset		
	1	2	3	1
60% w/v tamarind water extract	3	1.3778		
70% w/v tamarind water extract	3		1.5445	
Duncan(a,b) 80% w/v tamarind water extract	3			1.6445
90% w/v tamarind water extract	3			1.7000
Sig.		1.000	1.000	.235

Means for groups in homogeneous subsets are displayed.

Based on Type III Sum of Squares

The error term is Mean Square(Error) = .010.

a Uses Harmonic Mean Sample Size = 3.000.

b Alpha = .05.

b) Pomegranate rinds water extracted

3. Analyzed result on TSA+7.5% NaCl media (testing against *S. aureus*)

Table B- 5 General linear model in Univariate of clear zone (cm.) in different percentage of pomegranate rind water extracted for without heat treatment by Duncan method.

Clear zone (cm.)			
Percentage		N	Subset
		1	2
Duncan(a,b)	50% w/v pomegranate rinds water extract	3	1.0778
	60% w/v pomegranate rinds water extract	3	1.1333
	70% w/v pomegranate rinds water extract	3	1.4445
	80% w/v pomegranate rinds water extract	3	1.5889
	Sig.		.295

Means for groups in homogeneous subsets are displayed.
Based on Type III Sum of Squares
The error term is Mean Square(Error) = .013.
a Uses Harmonic Mean Sample Size = 3.000.
b Alpha = .05.

Table B- 6 General linear model in Univariate of clear zone (cm.) in different percentage of pomegranate rind water extracted for heat treatment by Duncan method.

Clear zone (cm.)			
Percentage		N	Subset
		1	2
Duncan(a,b)	50% w/v pomegranate rinds water extract	3	1.2667
	60% w/v pomegranate rinds water extract	3	1.3667
	70% w/v pomegranate rinds water extract	3	1.6667
	80% w/v pomegranate rinds water extract	3	1.7667
	Sig.		.315

Means for groups in homogeneous subsets are displayed.
Based on Type III Sum of Squares
The error term is Mean Square(Error) = .020.
a Uses Harmonic Mean Sample Size = 3.000.
b Alpha = .05.

C. Fruits in goat’s milk body lotion

The optimum concentrations of tamarind and pomegranate rinds water extraction were chosen. For tamarind, it was chosen at eighty percent w/v extraction where pomegranate rinds were chosen at seventy percent w/v extraction. There were two conditions, heat and without heat, involved in the antimicrobial test after adding components extracted into goat’s milk lotion.

Tamarind water extracted in Goat’s milk Lotion (80% w/v of tamarind water extraction used)

- Analyzed for antibacterial against *S.aureus*

Table C- 1 General linear model in Univariate of clear zone (cm.) in different percentage of tamarind water extracted for without heat treatment by Duncan method.

Clear zone (cm)					
	Percentage	N	Subset		
		1	2	3	1
Duncan(a,b)	5% w/w of 80% w/v tamarind water extract	4	1.0667		
	10% w/w of 80% w/v tamarind water extract	4	1.1889		
	15% w/w of 80% w/v tamarind water extract	4		1.4000	
	20% w/w of 80% w/v tamarind water extract	4			1.5556
	Sig.		.320	1.000	1.000

Means for groups in homogeneous subsets are displayed.
Based on Type III Sum of Squares
The error term is Mean Square(Error) = .011.
a Uses Harmonic Mean Sample Size = 6.000.
b Alpha = .05.

Table C- 2 General linear model in Univariate of clear zone (cm.) in different percentage of tamarind water extracted for heat treatment by Duncan method.

Clear zone (cm)					
	Percentage	N	Subset		
		1	2	3	1
Duncan(a,b)	5% w/w of 80% w/v tamarind water extract	4	1.0222		
	10% w/w of 80% w/v tamarind water extract	4	1.0889		
	15% w/w of 80% w/v tamarind water extract	4		1.2111	
	20% w/w of 80% w/v tamarind water extract	4			1.3445
	Sig.		.295	1.000	1.000

Means for groups in homogeneous subsets are displayed.

Based on Type III Sum of Squares

The error term is Mean Square(Error) = .015.

a Uses Harmonic Mean Sample Size = 4.000.

b Alpha = .05.

- Analyzed for antibacterial against *E. coli*

Table C- 3 General linear model in Univariate of clear zone (cm.) in different percentage of tamarind water extracted for without heat treatment by Duncan method.

Clear zone (cm)						
	Percentage	N	Subset			
		1	2	3	4	1
Duncan(a,b)	5% w/w of 80% w/v tamarind water extract	4	1.3333			
	10% w/w of 80% w/v tamarind water extract	4		1.4556		
	15% w/w of 80% w/v tamarind water extract	4			1.6223	
	20% w/w of 80% w/v tamarind water extract	4				1.7667
	Sig.		1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

Based on Type III Sum of Squares

The error term is Mean Square(Error) = .010.

a Uses Harmonic Mean Sample Size = 4.000.

b Alpha = .05.

Table C- 4 General linear model in Univariate of clear zone (cm.) in different percentage of tamarind water extracted for heat treatment by Duncan method.

Clear zone (cm)						
	Percentage	N	Subset			
		1	2	3	4	1
Duncan(a,b)	5% w/w of 80% w/v tamarind water extract	4	1.2333			
	10% w/w of 80% w/v tamarind water extract	4		1.3778		
	15% w/w of 80% w/v tamarind water extract	4			1.5000	
	20% w/w of 80% w/v tamarind water extract	4				1.6223
	Sig.		1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

Based on Type III Sum of Squares

The error term is Mean Square(Error) = .020.

a Uses Harmonic Mean Sample Size = 4.000.

b Alpha = .05.

Pomegranate rinds water extracted in Goat's milk Lotion (70% w/v of pomegranate rinds water extraction used)

- Analyzed for antibacterial against *S.aureus*

Table C- 5 General linear model in Univariate of clear zone (cm.) in different percentage of pomegranate rinds water extracted for without heat treatment by Duncan method.

Clear zone (cm)			
	Percentage	N	Subset
		1	1
Duncan(a,b)	2.5% w/w of 70% w/v tamarind water extract	4	1.1778
	5% w/w of 70% w/v tamarind water extract	4	1.2222
	7.5% w/w of 70% w/v tamarind water extract	4	1.3445
	10% w/w of 70% w/v tamarind water extract	4	1.4778
	Sig.		.138

Means for groups in homogeneous subsets are displayed.
Based on Type III Sum of Squares
The error term is Mean Square(Error) = .015.
a Uses Harmonic Mean Sample Size = 4.000.
b Alpha = .05.

Table C- 6 General linear model in Univariate of clear zone (cm.) in different percentage of pomegranate rinds water extracted for heat treatment by Duncan method.

Clear zone (cm)			
	Percentage	N	Subset
		1	1
Duncan(a,b)	2.5% w/w of 70% w/v tamarind water extract	4	1.2111
	5% w/w of 70% w/v tamarind water extract	4	1.3111
	7.5% w/w of 70% w/v tamarind water extract	4	1.4333
	10% w/w of 70% w/v tamarind water extract	4	1.5445
	Sig.		.134

Means for groups in homogeneous subsets are displayed.
Based on Type III Sum of Squares
The error term is Mean Square(Error) = .011.
a Uses Harmonic Mean Sample Size = 4.000.
b Alpha = .05.

D. Viscosity measurement of final goat’s milk body lotion

Table D- 1 Raw data of dial reading of viscosity and pH value of tamarind goat’s milk body lotion.

Condition	Percentage	Viscosity (Torque)	pH
Control	No tamarind water extract	39.57	5.12 ± 0.03
Heat	5% w/w of 80 % w/v tamarind water extract	54.83	3.27 ± 0.01
	10% w/w of 80 % w/v tamarind water extract	62.97	3.14 ± 0.04
	15% w/w of 80 % w/v tamarind water extract	73.07	3.00 ± 0.09
	20% w/w of 80 % w/v tamarind water extract	89.30	2.84 ± 0.10
Without heat	5% w/w of 80 % w/v tamarind water extract	52.87	3.27 ± 0.01
	10% w/w of 80 % w/v tamarind water extract	62.50	3.07 ± 0.12
	15% w/w of 80 % w/v tamarind water extract	67.90	2.86 ± 0.08
	20% w/w of 80 % w/v tamarind water extract	79.10	2.75 ± 0.12

Viscosity (mPa.S or centipoise) = Dial reading (Torque) × Factor (200)

For example, viscosity = 39.57 × 200 = 7914 centipoise

Table D- 2 Raw data of dial reading of viscosity and pH value of pomegranate rinds goat's milk body lotion.

Condition	Percentage	Viscosity (Torque)	pH
Control	No pomegranate rind water extract	52.67	5.05 ± 0.00
Heat	2.5% w/w of 70 % w/v pomegranate rinds water extract	54.80	4.81 ± 0.01
	5.0% w/w of 70 % w/v pomegranate rinds water extract	60.70	4.7 ± 0.01
	7.5% w/w of 70 % w/v pomegranate rinds water extract	62.40	4.66 ± 0.01
	10.0% w/w of 70 % w/v pomegranate rinds water extract	64.30	4.57 ± 0.01
Without heat	2.5% w/w of 70 % w/v pomegranate rinds water extract	55.67	4.87 ± 0.01
	5.0% w/w of 70 % w/v pomegranate rinds water extract	59.33	4.78 ± 0.01
	7.5% w/w of 70 % w/v pomegranate rinds water extract	64.83	4.7 ± 0.01
	10.0% w/w of 70 % w/v pomegranate rinds water extract	67.33	4.61 ± 0.01

E. The color measurement with the standard Munsell Book of color

Table E- 1 Raw data of color measurement reading as Hue, Value/ Chroma of tamarind goat's milk body lotion for heat and without heat treatment.

Treatment	Condition	Hue, Value/Chroma
Control	No tamarind water extracted	White
Without heat	5% tamarind water extracted	10 YR 9/2
	10% tamarind water extracted	2.5 Y 9/2
	15% tamarind water extracted	2.5 Y 8/4
	20% tamarind water extracted	10 YR 6/4
With heat	5% tamarind water extracted	10 YR 9/2
	10% tamarind water extracted	2.5 YR 8/4
	15% tamarind water extracted	2.5 Y 8/4
	20% tamarind water extracted	10YR 7/4

Table E- 2 Raw data of color measurement reading as Hue, Value/ Chroma of pomegranate rinds goat's milk body lotion for heat and without heat treatment.

Treatment	Condition	Hue, Value/Chroma
Control	No pomegranate rind water extracted	White
Without heat	2.5% pomegranate rind water extracted	7.5 Y 9/2
	5% pomegranate rind water extracted	7.5 Y 8.5/2
	7.5% pomegranate rind water extracted	5 Y 8.5/4
	10% pomegranate rind water extracted	5 Y 8.5/4
With heat	2.5% pomegranate rind water extracted	7.5 Y 9/2
	5% pomegranate rind water extracted	7.5 Y 8.5/2
	7.5% pomegranate rind water extracted	7.5 Y 8.5/4
	10% pomegranate rind water extracted	7.5 Y 8/4



Base goat's milk lotion

Phase A

Cetareth-20	1.5 %
Cetyl alcohol	1.0 %
Cusson oil	2.5 %
Glyceryl stearate	4.0 %
Isopropyl Myristate	1.5 %
Olive oil	1.0 %
Price Leader oil	2.0 %
Stearic acid	1.0 %
Sunflower oil	2.0 %
TiO ₂	0.3 %

Phase B

Disodium EDTA	0.1 %
Distilled water	Q.S.
Extracted herb	vary concentration
Goat's milk	1.0 %
Glycerin	0.7 %

Phase C

Ajidew	2.0 %
Fragrance	0.2 %
Germaben II	0.5 %

Process of making the goat's milk lotion (water in oil emulsion)

Phase A and phase B were separately heated in beakers until the temperature reached 75 °C and then phase B was added into phase A little by little. Stirred thoroughly until it became a homogenous lotion and temperature reduced to 40 °C (around 15 minutes). Then, added phase C and gentle stir (5 minutes). Let it cooled down at room temperature before packaging.

Four basic formulas of goat's milk body lotion produced and asked for consumers' preference with hedonic 9 points scaling test among 100 targeted consumers.

Table 1- 1 The basic formulas of goat's milk body lotion.

Ingredients	Formula 1	Formula 2	Formula 3	Formula 4
Percentage (%)				
Phase A				
Sunflower oil	2.0	2.0	2.0	2.0
Olive oil	1.0	1.0	1.0	1.0
Cusson oil	2.0	2.0	2.0	2.0
Leader price oil	2.5	2.0	2.5	2.5
Glyceryl stearate	6.0	2.0	4.0	3.0
Isopropyl Myristate	3.0	1.0	1.5	1.5
Cetyl Alcohol	2.0	1.0	1.0	1.0
Cetereth-20	3.0	1.0	1.5	1.5
Stearic acid	1.0	0.5	1.0	1.0
Titanium dioxide	0.5	0.3	0.3	0.3
Phase B				
Distilled water	72.5	82.7	78.7	79.7
Goat's milk	1.0	1.0	1.0	1.0
Glycerin	0.7	0.7	0.7	0.7
EDTA 2 Na	0.1	0.1	0.1	0.1
Additional phase				
Germaben II	0.5	0.5	0.5	0.5
Fragrance	0.2	0.2	0.2	0.2
Ajidew	2.0	2.0	2.0	2.0

