การตั้งตำรับครีมรักษาสิวที่มีน้ำคั้นจากส่วนรองรับผลมะม่วงหิมพานต์ในรูปฟรีซดราย

นางสาวอภิมน วุฒิวรวงศ์

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาเภสัชศาสตรมหาบัณฑิต
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FORMULATION OF ACNE CREAM CONTINING FREEZE-DRIED CASHEW APPLE JUICE

Miss APIMON WUTHIWORAWONG

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Thesis Title	Formulation of Acne Cream Containing Freeze-Dried Cashew Apple Juice
By	Miss Apimon Wuthiworawong
Department	Pharmacy
Thesis Advisor	Assistant Professor Panida Vayumhasuwan, Ph.D.
Thesis Co-advisor	Associate Professor Pintip Pongpetch, Ph.D.

Accepted by the Graduate School, Chulalongkorn University in Partial Fulfillment of the Requirements for the Master's Degree.

…….. Dean of Graduate School
(Professor Supawat Chutivongse, M.D.)

THESIS COMMITTEE

…….. Chairman
(Associate Professor Porntip Nimmannitya)

……. Thesis Advisor
(Assistant Professor Panida Vayumhasuwan, Ph.D.)

……. Thesis Co-advisor
(Associate Professor Pintip Pongpetch, Ph.D.)

……. Member
(Assistant Professor Routh Suthisri, Ph.D.)

……. Member
(Nontima Vardhanabhuti, Ph.D.)

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การทดสอบฤทธิ์ยับยั้งเชื้อ *Staphylococcus aureus* (*S. aureus*) จำนวน 29 สายพันธุ์ และ 1 สายพันธุ์มาตรฐาน (ATCC25923) กับน้ำคั้นจากส่วนรองรับผลของมะม่วงหิมพานต์ในรูปฟรีซส์ดราย (FCAJ) ทั้งชนิดผลสีแดงและสีเหลือง ที่มีปริมาณสาร 2, 4, 8 และ 16 มก.ต่อแผ่นทดสอบตามลำดับ โดยใช้วิธี disk diffusion พบว่าแผ่นทดสอบที่มี FCAJ ชนิดผลสีเหลืองในความเข้มข้น 2-4 มิลลิกรัมต่อแผ่นทดสอบ มีขนาดเส้นผ่าศูนย์กลางของพื้นที่ยับยั้งเชื้อใหญ่กว่าชนิดผลสีแดงที่ปริมาณเท่ากัน การทดสอบฤทธิ์ยับยั้งเชื้อ *Propionibacterium acnes* (*P. acnes*) จำนวน 29 สายพันธุ์ ใช้วิธี agar dilution เพื่อหาความเข้มข้นต่ำสุดของ FCAJ ที่สามารถยับยั้งการเจริญของเชื้อ *P. acnes* ได้ร้อยละ 90 พบว่าผลสีแดงมีค่าน้อยกว่าหรือเท่ากับ 5 มก. /มล. และผลสีเหลืองมีค่าน้อยกว่า 2.5 มก./มล. การตั้งตำรับครีมทั้งชนิดน้ำมันในน้ำและน้ำในน้ำมันผสม FCAJ ทั้งชนิดผลสีแดงและสีเหลืองความเข้มข้นร้อยละ 0.25, 0.5, 1, และ 2 (น้ำหนัก/น้ำหนัก)ตามลำดับ การทดสอบฤทธิ์ยับยั้งเชื้อ *S. aureus* 30 สายพันธุ์ (*S. aureus* ที่ไม่ดื้อกับยาเมธิซิลลิน (MSSA) จำนวน 15 สายพันธุ์ และ *S. aureus* ที่ดื้อกับยาเมธิซิลลิน (MRSA) จำนวน 15 สายพันธุ์) และทดสอบกับเชื้อ *P. acnes* จำนวน 29 สายพันธุ์ โดยใช้วิธี cup diffusion เปรียบเทียบกับครีมเปล่า และ benzoyl peroxide (5 เปอร์เซ็นต์) พบว่าครีม FCAJ ชนิดน้ำในน้ำมันทั้งหมด รวมทั้งครีมเปล่าไม่มีฤทธิ์ยับยั้งเชื้อ *S. aureus* และ *P. acnes* เลย ส่วนครีม FCAJ ชนิดน้ำมันในน้ำที่ทุกความเข้มข้น ครีมเปล่า และ benzoyl peroxide (5 เปอร์เซ็นต์) ให้ขนาดเส้นผ่าศูนย์กลางของพื้นที่ยับยั้งเชื้อ MSSA ไม่แตกต่างกัน ในทางตรงกันข้ามครีม FCAJ ชนิดน้ำมันในน้ำเกือบทุกความเข้มข้น มีขนาดเส้นผ่าศูนย์กลางของพื้นที่ยับยั้งเชื้อ MRSA มากกว่าครีมเปล่า และ benzoyl peroxide (5 เปอร์เซ็นต์) และพบว่า benzoyl peroxide (5 เปอร์เซ็นต์) ยับยั้งเชื้อ *P. acnes* ได้ดีกว่าครีม FCAJ ชนิดน้ำมันในน้ำที่ทุกความเข้มข้น และครีมเปล่า โดยมีขนาดเส้นผ่าศูนย์กลางมากกว่าสองเท่า

ภาควิชา..... ลายมือชื่อนิสิต.....

สาขาวิชา..... ลายมือชื่ออาจารย์ที่ปรึกษา.....

ปีการศึกษา 2543 ..... ลายมือชื่ออาจารย์ที่ปรึกษาร่วม.....

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KEY WORD: CASHEW APPLE JUICE / SUSCEPTIBILITY / *Propionibacterium acnes* / *Staphylococcus aureus* / CREAM
APIMON WUTHIWORAWONG : FORMULATION OF ANTIACNE CREAM CONTAINING FREEZE DRIED CASHEW APPLE JUICE. THESIS ADVISOR : ASSIST. PROF. PANIDA VAYUMHASUWAN, Ph.D. THESIS CO-ADVISOR : ASSOC. PROF. PINTIP PONGPECH, Ph.D. 151 pp. ISBN 974-346-651-7

Susceptibility tests of freeze-dried juice of red and yellow cashew apples at 2, 4, 8, and 16 mg/disk, respectively, against twenty-nine strains of *Staphylococcus aureus* (*S. aureus*) and one standard strain *S. aureus* ATCC 25923 were investigated by disk diffusion method. At low amount of freeze-dried cashew apple juice (FCAJ) per disk , the yellow type yielded larger inhibition zone diameters than the red type did at the same concentration. The agar dilution method was applied for testing activities of both red and yellow type FCAJ against 29 strains of *Propionibacterium acnes (P. acnes*). The minimum inhibitory concentration (MIC_{90}) of red type FCAJ was equal to or less than 5 mg/ml and that of yellow type FCAJ was less than 2.5 mg/ml. Oil in water (o/w) and water in oil (w/o) cream bases containing red and yellow type FCAJ at concentrations of 0.25, 0.5, 1, and 2 %w/w, respectively, were formulated. The susceptibility testing against thirty strains of *S. aureus* (fifteen strains of methicillin-sensitive *S. aureus* (MSSA) and fifteen strains of methicillin-resistant *S. aureus* (MRSA)), and twenty-nine strains of *P. acnes* were studied using cup diffusion method. Their cream bases and 5% benzoyl peroxide were used as controls. All w/o creams did not inhibit the growth of *S. aureus* and *P. acnes*. The inhibition zone diameters obtained from o/w creams containing both types of FCAJ at all concentrations, the o/w cream base and 5% benzoyl peroxide against MSSA were not significantly different. On the other hand, almost all concentrations of o/w creams containing both types of FCAJ yielded larger zone diameters against MRSA than the cream base and 5% benzoyl peroxide did. Five percents benzoyl peroxide yielded zone diameters against *P. acnes* that were more than twice as large as the cream base and creams containing both types of FCAJ at all concentrations.

Department…… Student's signature……

Field of study…… Advisor's signature……

Academic year…… Co-advisor's signature……

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LIST OF ABBREVIATIONS

%	=	percent
µg	=	microgram
B. fragilis	=	*Bacteroid fragilis*
cm	=	centimeter
Conc.	=	concentration
CFU	=	colonies forming unit
CV	=	coefficient of variation
FCAJ	=	freeze-dried cashew apple juice
HPLC	=	high performance liquid chromatography
hr	=	hour
IU	=	international unit
k	=	degradation rate constant
kg	=	kilogram
m^3	=	cubic meter
mg	=	milligram
min	=	minute
ml	=	milliliter
mm	=	millimeter
MRSA	=	methicillin-resistant *Staphylococcus aureus*
MSSA	=	methicillin-sensitive *Staphylococcus aureus*
NCCLS	=	National Committee for Clinical Laboratory Standards
nm	=	nanometer
no.	=	number
o/w	=	oil in water
°C	=	degree celsius
P. acnes	=	*Propionibacterium acnes*

CHAPTER I

INTRODUCTION

Acne vulgaris is a chronic inflammatory disease of the pilosebaceous follicles which primarily affects the face and the upper trunk. Usually the onset of acne occurs during adolescence when there is an increase of androgens. It is believed that acne develops only in patients with seborrhea, with the severity of the lesions related to the amount of sebum excretion. Sebum retention in the widely dilated follicular canals filled with lipid, keratin and bacteria plus increased sebum viscosity may cause a physical obstruction and form the comedone. Bacterial infection plays a major role in most acne lesions. The anaerobic Gram positive bacilli bacteria such as *Propionibacteriun acnes* (*P. acnes*) produce lipolytic enzymes, e.g., lipase which act upon the sebum triglycerides to form free fatty acids that are believe to be the major factor in the production of follicle inflammation (Berger, Elias, and Wintroub, 1990).

In addition, an aerobic gram positive cocci bacteria, *Staphylococcus aureus* (*S. aureus*) is a common cause of skin infection such as atopic dermatitis and folliculitis. The principles of acne therapy are comedolysis and prevention of comedo formation, sebosuppression, bacterial therapy and prevention of inflammation.

Early invented antibacterial drugs such as antibiotics, benzoyl peroxide and azelaic acid help reducing inflammation and prevent scarring. The most important topical antibiotics are tetracyclines, erythromycin and clindamycin. Unfortunately, the bacterial resistance emerges as a significant problem. *P. acnes* resistance to the commonly used erythromycin can also be transferred to clindamycin. Azelaic acid has strong antibacterial potency without inducing bacterial resistance similar to benzoyl peroxide. Thus, there is evidence that antibiotics combined therapy with zinc, benzoyl peroxide and azelaic acid increases the bactericidal effect and reduces the risk of resistance (Bojar, Eady, and Jones, 1994). Although benzoyl peroxide induces an irritant dermatitis with erythema and scaling, while only a slight sensation of burning

and a mild erythema can be observed after application of azelaic acid (Gollinick and Schramm, 1998).

A large number of active principles isolated from various plants, for example, anacardic acids isolated from the cashew apple, cashew nut, and cashew shell nut oil; β-caryophyllene identified in green tea flavor; and totarol isolated from the bark of *Podocarpus nagi*, show potent activity against *P. acnes* (Kubo, Muroi, and Kubo, 1994). The application of such natural substances to cosmetic products is interesting. In the southern part of Thailand, cashew apples from cashew nut tree widely grown there are usally neglected. So It is interesting to use the cashew apples as a raw material to make antiacne products.

The colors of fully ripen fruits of the cashew, *Anacardium occidentale L.* (Anacardiaceae), most frequently found are yellow and red. The three anacardic acids in the cashew apple were also previously identified. Himejma and Kubo (1991) and Kubo, Muroi, and Himejima (1993) reported that anacardic acids were found to exhibit potent antibacterial activity against gram-positive bacteria, and weak antifungal activity against mold, but did not exhibit any against gram-negative bacteria. The growth of *P. acnes*, a gram-positive bacteria, was inhibited by the anacardic acids at a MIC of 0.78 μg/ml, and they also showed antibacterial activity against *S. aureus* (MIC 3.13-100 μg/ml). Muroi and Kubo (1996) reported that the inhibitory and bactericidal activity of anacardic acids against methicillin resistant *S. aureus* (MRSA) exhibited a MIC of 6.25 μg/ml.

Furthermore, Muroi, Kubo, and Kubo (1993) reported that aroma components of the fresh cashew apples exhibited antibacterial activity against *P. acnes* with MICs ranging from 6.25 to 800 μg/ml whereas *S. aureus* was the least sensitive gram-positive bacterium with MICs of 400-800 μg/ml. (E)-2-hexenal, one of the ten aroma components studied in fresh cashew apples, exhibited antimicrobial activity against all gram-positve bacteria tested including four Gram-negative bacteria, i.e., *Pseudomonas aeroginosa*, *Enterobacter aerogenes*, *Escherichia coli*, and *Proteus vulgaris* with MICs of 200-400 μg/ml.

The cashew apple juice is an excellent source of vitamin A and vitamin C which can be used to protect fatty tissues from oxidation damage due to ultraviolet. The vitamin A values of the fresh cashew apple juice ranges from 37.5 to 107.5 mg/100 g, and the average vitamin C content in the juice is 261 mg/100 ml which is about six times that of orange juice (Cecchi and Rodriguez-Amaya, 1981).

The above mentioned cashew apple juice consisting of anacardic acids and aroma components may enhance antibacterial activity against *P. acnes* and *S. aureus*. In this study, the antibacterial activity against *P. acnes* and *S. aureus* of freeze-dried cashew apple juice of both red and yellow type was determined.

Objectives

The objectives of this study are as follows:

1. to evaluate the susceptibility of *P. acnes* and *S. aureus* to freeze-dried cashew apple juice.

2. to formulate physically stable creams containing freeze-dried apple juice.

3. to evaluate the susceptibility of *P. acnes* and *S. aureus* to freeze-dried cashew apple juice cream.

CHAPTER II

REVIEW OF LITERATURES

ACNE (Domankos, 1971)

Acne vulgaris is a common disorder involving sebaceous follicles. It is usually first noted during the teenage years. Some degree of acne develops in as many as 80% of adolescents. Acne usually develops at an earlier age in girls than in boys, but the disorder affects boys more frequently and more severely. Acne can persist into mid-adulthood in some persons, and can also present initially in adulthood. It is estimated that 40 to 50% of adult women are affected by a low-grade persistent form of acne (Shalita, Pochi, and Leyden, 1991).

Pathogenesis

A rational approach to the treatment of acne requires a clear understanding of the multifactorial basis of the disorder. The normal pilosebaceous unit is composed of large, multilobulated sebaceous glands, a rudimentary hair and a wide follicular canal lined with stratified squamous epithelium. During the regular turnover process of the skin, desquamated cells from the follicular epithelium are carried up the follicular canal by sebum secreted from the sebaceous glands. If the pilosebaceous unit becomes plugged, the trapped sebum causes bacterial proliferation and inflammation, resulting in the development of acne vulgaris (Figure 1).

Stimulation of the sebaceous follicle by a surge of androgenic hormones appears to be an important factor in the development of acne. Acne usually does not occur until puberty, when hormone changes begin, androgen-sensitive sebaceous follicles enlarge and sebum production increases.

An abnormal desquamation process is required to produce clinical acne vulgaris. This process consists of increased sloughing of the epithelium, which becomes more cohesive and blocks the follicular orifice with the accumulation of dead cells. Within the blocked follicle, the impacted sebum favors the proliferation of *Propionibacterium acnes* (*P. acnes*), an anaerobic diphtheroid organism that normally resides in the pilosebaceous unit. One nutritional requirement of this bacterium is glycerol, obtained through lipolysis of the triglycerides in the

impacted sebum favors the proliferation of *Propionibacterium acnes* (*P. acnes*), an anaerobic diphtheroid organism that normally resides in the pilosebaceous unit. One nutritional requirement of this bacterium is glycerol, obtained through lipolysis of the triglycerides in the sebum, which releases free fatty acids as byproducts. *P. acnes* also releases various chemotactic products, which attract neutrophils to the area. These neutrophils secrete hydrolytic enzymes that cause secondary damage to the follicular wall. The irritating free fatty acids and other bacterial enzymes can then leak into the dermis, creating intense inflammation. In addition, an aerobic gram positive cocci bacteria, *Staphylococcus aureus* (*S. aureus*) is a common cause of skin infections such as dermatitis and folliculitis (Berger et al., 1990).

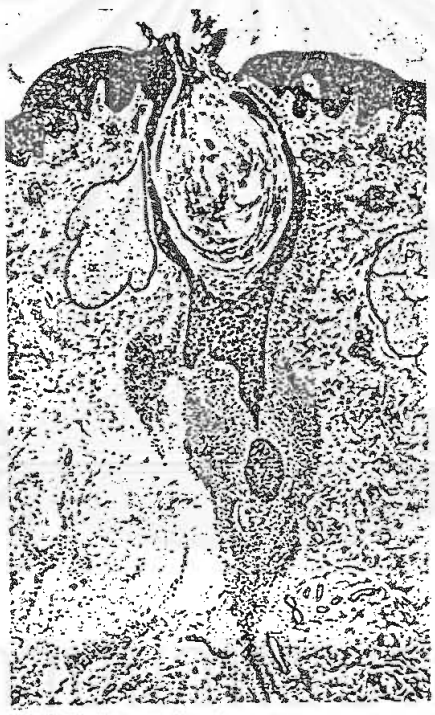


Figure 1 Acne vulgaris; showing comedone and inflammatory infiltration around pilosebaceous apparatus.

From: Domankos, 1971, Andrews' Disease of the skin.

Clinical Manifestations

The hallmark of acne vulgaris is a micro-comedo formed by a sebum-plugged pilosebaceous follicle. The accumulation of sebum results in a visible closed comedo, or

whitehead. Continuing distention of the closed comedo causes protrusion from the follicular orifice, forming an opened comedo, or blackhead. The dark color of a blackhead is due to oxidized lipids, melanin and densely packed keratinocytes. It is not dirt, as commonly assumed. Inflammatory pustules develop when the compacted follicular contents rupture, releasing bacteria and bacterial products, including free fatty acids, into the dermis. When the inflammatory response occurs at a deeper level in the dermis, a papule develops. Unusually intense inflammation can lead to a fluctuant and painful acne cyst, which heals with post-inflammatory pigment changes and scar formation. Comedones can occur anywhere on the body, but are usually found on the forehead and upper cheeks in adolescent patients. Comedones may progress to inflammatory lesions on the lower cheeks, chin, chest, upper back and shoulders, where many pilosebaceous follicles are found (Berger et al., 1990).

Prognostic

While many cases of mild to moderately severe acne resolve over time, most comedones do not usually resolve spontaneously. Larger inflammatory papules and pustules may take several weeks to resolve and the post-inflammatory hyperpigmentation can last for months. Inflammatory cystic lesions can result in acne scars, which may appear as hypotrophic pitted scars or, less commonly, as hypertrophic scars and keloids.

Causes and Factors

Treatment of acne should be discussed with the patient prior to the initiation of treatment. The more the patient knows about acne, the more compliant they will be. Important issues to discuss are the following.

Endocrine

Acne may flare up premenstrually because the sebaceous duct orifice may become more obstructed at this time in the cycle. In females, the possibility of androgenic disorders, such as polycystic ovarian disease and Cushing's syndrome, should be considered; the patient should be asked about menstrual irregularities and evidence of hirsutism should be looked for on physical examination. In young women with acne that does not respond to therapy, hormone testing may reveal an androgen excess, even in the absence of symptoms such as

menstrual abnormality, male-pattern hair loss or hirsutism. An endocrinology consultation may be warranted in complicated cases.

Diet

Although clinical studies have not demonstrated any causal relationship between certain foods and acne, patients should be advised to eat a well-balanced diet and avoid those foods which consistently result in acne flare-ups.

Cleanliness

The development of acne is not related to dirt. Patients should be advised that excessive scrubbing, especially with abrasive cleaning lotions and facial sponges, may actually worsen the condition. Patients who have oily skin should wash their faces using a mild, unscented soap and water.

Environment

Although sunshine can be beneficial in some patients, very humid environments and heavy sweating can worsen acne in other patients. Exposure to pollution and hydrogenated hydrocarbons may aggravate acne.

Mechanical Trauma

Constant pressure, rubbing and humidity from tight or occlusive clothing can aggravate acne. In addition, patients should be warned that repeatedly picking the lesions can result in more inflammation, scarring and pigmentary changes.

Cosmetics

Comedogenic agents such as heavy oils, greases or dyes in cosmetic creams and hair sprays can exacerbate acne. Patients who use cosmetics should be advised to use water-based products instead of occlusive, oil-based products.

Medications

Certain drugs, including corticosteroids, adrenocorticotropic hormone (ACTH), androgens, phenytoin, barbiturates, lithium, isoniazid, cyclosporine, iodides and bromides, are

now known to cause acne. Although some oral contraceptives may provide excellent therapy for acne, those with androgenic and antiestrogenic progesterones may actually promote acne eruptions. Also, gram-negative folliculitis can occur as a complication of chronic broad spectrum antibiotic therapy.

Therapy

Comedonal Acne

Mild noninflammatory acne can be treated with topical antibacterial agents such as benzoyl peroxide or comedolytic agents such as tretinoin (Retin-A®) and salicylic acid. These agents can unplug the blocked follicles with their exfoliative effects. The combination of benzoyl peroxide in the morning and tretinoin at night may be effective when either agent alone has failed. Comedone extraction can accelerate resolution when it is used in addition to topical medications (Arndt, 1989).

Papular Acne

Mild inflammatory lesions can be treated effectively with topical antibiotics. The main action of topical antibiotics is to eliminate *P. acnes* from the sebaceous follicles and thereby suppress free fatty acid production. Some topical antibiotics have anti-inflammatory effects through the inhibition of chemotactic factors. The effectiveness of topical antibiotics in the treatment of acne is limited by their low lipid solubility and consequent difficulty in penetrating sebum-filled follicles. All topical antibiotics are applied twice daily (Leyden, and Shalita, 1986).

Topical Drug Treatment in Acne:

Antibacterial Preparations

Benzoyl peroxide

Azelaic acid

Antibiotic Preparation

Clindamycin

Erythromycin

Meclocycline

Keratolytic Preparations

Tretinoin

Adapalene

Salicylic acid

Sulfur

Benzoyl Peroxide

Benzoyl peroxide is the most commonly used acne medication available without a prescription. It is a potent anti-bacterial oxidizing agent that can decrease the number of *P. acnes* and *S. aureus* on the skin surface of about 2 log cycles and, consequently, the amount of free fatty acids. Its mild comedolytic and exfoliant properties can also unplug obstructed follicles (Fulton, Farzard-Bakshandeh, and Bradley, 1974; Gollnick, 1992). Benzoyl peroxide is the first-line monotherapy for mild acne, and it may be used in combination with other agents in more severe acne (Chalker, Shalita, and Smith, 1983). Benzoyl peroxide is available in over-the-counter preparations of 2.5%, 5%, and 10% gels, creams, lotions or soaps. All concentrations seem to be therapeutically equivalent. The liquid and cream formulations (Benoxyl®) are less irritating and may be useful in patients with dry skin. The gel formulation (Panoxyl®) is more irritating but more effective for patients with oily skin.

Benzoyl peroxide should be applied once or twice daily. Patients should expect mild redness and scaling of the skin during the first week of use. Contact sensitivity is reported in a small percentage of patients.

Azelaic Acid

Azelaic acid is a C9-dicarboxylic acid, which is produced under natural conditions not only by the human flora but also by *Pityrosporrum ovale*. Its effect on acne vulgalis was proven. It demonstrates a comparably good anticomedogenic and antimicrobial effect like other substances such as benzoyl peroxide, tretinoin, erythromycin and tetracycline (Gollnick, and Graupe, 1989). The strong antimicrobial effect of azelaic acid is based on a reduction of *P. acnes* colonization on the skin surface and in the pilosebaceous duct of at least 1

log cycle within 4 weeks (Cunliffe, and Holland, 1989). In addition, azelaic acid reduces the concentration of free fatty acids in skin surface lipids, which may also normalize ductal hypercornification. The advantage of azelaic is due to its lack of prominent side effects. Only a slight sensation of burning or tingling and a mild erythema can be observed after application.

Clindamycin

Its antimicrobial effect is due to a reduction of the follicular microbial colonization. This results in a significant decrease in free fatty acids of the skin surface lipids as a marker of *P. acnes* lipase activity. Clindamycin is available in a 1% concentration prepared as a solution, lotion or gel formulation. Clindamycin is as effective in the treatment of acne as erythromycin (Schachner, Pestana, and Kittles, 1990).

Erythromycin

This agent is available in a 2% solution or gel. Topical erythromycin is considered to be the safest antibiotic for use during pregnancy. It is also available in a 3% gel formulation combined with 5% benzoyl peroxide. This new formulation is probably the most effective topical antibiotic currently used and may be as effective as systemic antibiotics in some patients (Chalker et al., 1983).

Meclocycline

Meclocycline is available in a topical cream (Meclan®). It is less drying but may be less effective than other topical agents.

The most important side effect of topical antibiotics is induction of bacterial resistance and cross-resistance. There has been a dramatic increase in resistance over the past 20 years. For this reason, the treatment with topical antibiotics should be limited to a period of 4-6 weeks (Bojar et al., 1994). There is an evidence that a combined therapy with zinc, benzoyl peroxide and azelaic acid increases the bactericidal effect and thus reduces the risk of resistance.

Tretinoin

This all-trans-retinoic acid is the most effective topical comedolytic agent; it can normalize the desquamation process. Tretinoin decreases the cohesiveness of follicular epithelial cells, thus inhibits the formation of microcomedones, and increases cell turnover resulting in the expulsion of existing comedones. The agent also decreases the thickness of the stratum corneum and potentiates the penetration of other topical antibiotic agents. Tretinoin is available as Retin-A® cream (0.025%, 0.05%, and 0.1%), Retin-A® gel (0.01%, and 0.025%), and Retin-A® liquid (0.05%). Tretinoin therapy should usually be started with the lower strength cream or gel. If no response occurs after a few weeks of treatment, then the higher-concentration liquid formulation can be used. The lubricating cream is favored in patients with dry skin, and the drying gel is best for patients with oily skin (Fulton et al., 1974).

Tretinoin is applied once daily before bedtime to the affected areas. Mild redness and peeling are parts of the therapeutic effect of the medication, but they can decrease compliance. Patients should be aware that improvement may take 6 to 12 weeks, and that flare-ups of acne can occur during the first few weeks of therapy due to surfacing of the lesions onto the skin. It is extremely important that patients avoid excessive sun exposure and use appropriate sunscreens.

Adapalene

Adapalene is a synthetic polyaromatic third-generation retinoid. The results so far have demonstrated that adapalene is as efficient as tretinoin but it shows a potent antiinflammatory effect. Skin irritation caused by adapalene is very much less pronounced than that caused by with tretinoin, for example, transepidermal water loss after occlusive test condition is 10-fold less (Gollinick and Schramm, 1998).

Exfoliants

These agents include salicylic acid, glycolic acid, trichloroacetic acid, elemental sulfur and resorcinol. They are not effective in removing deep comedones and can cause irritation of the skin. Salicylic acid is used in some countries as 1-3% alcoholic solutions. Besides astringent effect, some keratolytic effect on the interfollicular epidermis and the acroinfundibulum can be observed (Gollinick and Schramm, 1998). Sulfur has been known to be

effective in acne for years. A particular form of biological sulfur called MSM (Methylsulfonylmethane) has achieved excellent results in acne cases. It has no side effects.

CASHEW (Morton, and Thomas, 1981)

Family : Anacardiaceae

Genus : Anacardium

Species : occidentale

Ethnic Names : cashew, cashew apple, cajueiro, cashu, acajoiba, acajou, acaju, cajou, jocote, maranon, pomme cajou

Description

A cashew tree is an evergreen tree native of tropical areas. It is very ramiferons with a dense foliage and a globular shape. It is a spontaneous species, however, it can also be cultivated. Its cultivation is quite easy as it does not need much water, nor fertilizer, nor tending and can grow in poor soils such as laterite. In Africa, it is often found in the middle of other plantations. It grows up to 12 meters in height and has a thick and tortuous trunk and branches so winding that they frequently reach the ground.

The cashew tree produces many resources and products. The bark and leaves of the tree are used medicinally. The cashew nut has international appeal and market value, and even the shell around the nut is used medicinally. The cashew fruit is very peculiar as it is really not a fruit at all. The cashew fruit is actually a swollen peduncle that grows behind the real fruit which yields the cashew nut. This large pulpy and juicy part is a pseudo-fruit with a fine sweet flavor and aroma whereas the cashew nut grows externally in its own kidney shaped hard shell at the end of this pseudo-fruit or peduncle. This peduncle, however, is commonly referred to as "cashew fruit" or the “cashew apple" (Figure 2).

compound). It is a valuable raw material for a number of polymer-based industries like marine paints, varnishes, and resins.

Phytochemicals of Cashew Apple

Phytochemicals found in the cashew apple are ascorbic acid, beta-carotene, calcium, hexanal, iron, leucocyanidin, leucopelargonidine, limonene, niacin, phosphorus, protein, riboflavin, anacardic acids, thiamin, and trans-hex-2-enal.

Traditional Pharmacology

Ayurveda, the Indian system of medicine originated 3000 years ago, has accepted this rich nut as a potent remedy as well as a nutritive food. The ayurvedic way of looking at health and healing is very different from the Western medical concepts : it is based on some philosophical aspects (how the body is related to its natural environment, to the cosmos, to time, what is its psychic role, and what are its unconscious levels).

In northern Venezuela, a decoction of the astringent leaves of the cashew trees is a popular treatment for diarrhea. It is currently being acclaimed as an antidiabetic.

In former times, when the cashew tree was common on the Curacao, the leaves were boiled with those of the mango and the decoction was drunk 4 times daily for 9 days after giving birth.

In Colombia, the pulverized bark of the tree is soaked for 24 hours in water, which is then taken as a remedy for diabetes.

In Brazil, a bark decoction is used as an astringent gargle for sore throat and oral inflammation and is drunk as a depurative and tonic.

The juice of cashew apple is much employed as a diuretic and a remedy for vomiting, diarrhea and sore throat.

The shell oil in small doses, diluted, is applied with caution to ulcer, warts and calluses.

The Cuna Indians of Panama drink a sweetened decoction of the bark, boiled with bark of *Spondias*, as a remedy for cold, congestion and asthma.

Applications of Cashew Apple

1. In Food

In addition to valuable nuts, cashew provides vitamin C-rich apples. The vitamin C content of the cashew apple is reported to be 5-7 times greater than that for oranges. The cashew apple is very sour and astringent until fully ripe when it becomes fully edible. It is a very juicy fibrous fruit and can be consumed raw or in the form of jam, marmalade, candy, juice and distilled products. In recent years the cashew apple has increased in value, especially in the countries where it is grown, such as Brazil. In fact, cashew apple juice is now one of the most popular juices in Brazil. As mentioned earlier, the yield of the cashew apple is 4-5 times that of cashew nut.

Although cashew apples have good nutritional value, the problems of distasteful ingredients and poor keeping quality limit their use. Juice extraction remains mostly a small scale; it is locally operated due to the inability of apple storage and transportation for mass production. Approximately 30-40% of the fruit by weight remains after the juice is extracted. The astringent and acrid taste of cashew fruit has been traced to the tannin content and an unidentified oily substance. Two successful methods for removing bad flavors are to steam for 5 minutes at 0.4 kg/m^3 plus subsequent cold water rinsing, and to cook the fruit in a 2% common salt solution for 3-4 min. Cooking in the salt solution results in a slight salty taste. Another method, tannin precipitation with gelatin added to juice has also been successful. There is a considerable flavor difference between cashew apples from different regions as the relative abundance of aroma compounds can vary considerably depending on their cultivations.

Cashew juice is also used to make alcoholic drinks. In Goa, India, people prefer to make cashew brandy. Cashew wine is consumed in a number of countries, such as Guatemala and Mozambique. In West Africa cashew juice is fermented to make an alcoholic drink. However, wine making is not always well controlled and sanitation quality control should be paid attention to. There are also reports that fermented cashew apple juice quite often has an astringent flavor. Brandy, on the other hand, which is made as a distillate of wine under more controlled conditions, retains a cashew apple flavor without astringency. The flavor of cashew apple juice is chemically complex and a wide range of constituents contributes to the overall flavor. All of them must be considered in the design and operation of commercial juice

processing facilities. Such considerations may lead to a stable acceptable product and to increased use of an underutilized resource.

2. In Pharmaceutics

Anacardic acids are active chemicals with a 15 carbon unsaturated side chain found in cashew nuts, cashew apples, and cashew shell nut oil. The side chain with three unsaturated bonds is the most active one against *S. aureus*. However, the activity against *P. acnes* is not affected by the number of unsaturated bonds. Gram positive bacteria which cause tooth decay, acne, and leprosy are also killed by anacardic acids (Kubo et al., 1993).

The cashew apple and its juice also have pharmaceutical properties. Ten of the most abundant flavor compounds of the cashew apple have exhibited potential activity against gram-positive bacteria and weak antifungal activity against molds (Muroi et al., 1993). The antimicrobial activities of the flavor compounds may be applied for use in products such as cosmetics and disinfectants. In addition to their antimicrobial activities, another benefit of these volatile compounds includes the addition of fragrance to the products. However, because the cashew apple is easily spoiled in nature, especially by fungal infection, the use of the antimicrobial activities is limited to local utilization and prevents the exportation of the fresh fruit to other countries.

Kubo et al. (1993) has also indicated that the anacardic acids which are found in the cashew apple juice, show significant (ED_{50} < 20 μg/ml) in vitro cytotoxicity against BT-20 breast carcinoma cells. The active principles from a regularly consumed beverage such as cashew apple juice may be superior as antitumor agents as compared to many non-natural products.

Furthermore, anarcardic acids have exhibited potential activity against gram-positive bacteria such *as S. aureus*, *P. acnes*, *Streptococcus mutans*, and *Brevibacterium ammoniagenes* with MICs of 1.56, 0.39, 0.78, and 0.78 μg/ml , respectively (Kubo, Muroi, and Himejima, 1992). Muroi and Kubo (1996) showed the inhibitory and bactericidal activities of anacardic acids alone and in combination with methicillin against MRSA.

Finally, anacardic acids from cashew apple have been found to exhibit antibacterial acitivity against the gram-negative bacterium *Helicobacter pylori*. They can also inhibit urease, which is now considered to cause acute gastritis (Kubo, Lee, and Kubo, 1999).

3. In Cosmetics

Cashew juice is considered to be a rich source of vitamin C, which is the focus of a great deal of research and is indicated as one of the substances capable of capturing free radicals. In addition, the high amount of mineral salts gives cashew fruit skin remineralizing properties. It also has some conditioning activity due to its proteins and mucilage. Besides making great tasting and highly nutritive snacks and juices, cashew fruit extracts are also used in body care products. Because of its high amount of vitamin C and mineral salts, cashew fruit is used as coadjutant in the treatment of premature aging of the skin and to remineralize the skin. It is also a good scalp conditioner and tonic, which is often used in shampoos, lotions and scalp creams.

Microorganism Related to Acne

S. aureus is a common cause of skin infections such as topic dermatitis and folliculitis. *P. acnes* is an important factor in the pathogenesis of acne. Therefore, the susceptibility of *S. aureus* and *P. acnes* may be used as tests for an antiacne agent.

1. *Staphylococcus aureus*

S. aureus is a gram-positive, nonmotile coccus existing singly, in pairs, in short chains or in irregular clusters. It is frequently found on the skin, nasal and other mucous membranes of human being and in various food products. The disk diffusion test has been primarily the standard method for testing of commonly isolated, rapidly growing *S. aureus* (Berry and Thornsberry, 1985).

2. *Propionibacterium acnes*

The majority of the gram-positive, nonspore-forming anaerobic bacilli isolated from human clinical materials is *P. acnes*. It is a predominant member of the skin flora inhabiting hair follicles and sebaceous glands.

Agar diffusion tests are not recommended because of the complexities and variation introduced by the slow and varied growth rates of anaerobic bacteria. The working group on the standardization of susceptibility tests for anaerobic bacteria, National Committee for Clinical Laboratory Standards [NCCLS] (1982), has published a tentative reference agar dilution procedure which has been shown to give reproducible results. Broth dilution tests, both macro- and microdilution, are convenient procedures in some laboratories. The agar dilution method is convenient for testing a number of strains simultaneously. It is able microbial heterogeneity or contamination. In addition, its reproducibility is slightly better than the broth dilution method (Ericsson and Sherris, 1971).

Susceptibility Testing: Diffusion Tests

Antimicrobics are principal drugs used in the treatment of infectious disease. Once the causative organism of specific disease has been isolated, the physician needs to know, as soon as possible, which antimicrobic will be most effective.

Antimicrobic impregnated paper disks were first used in the late 1940s as penicillin came into widespread use. As the decades rolled by and a multitude of new drugs were discovered, a great deal of experimentation has taken place with the hope for developing a test method that would accommodate the large variety of antimicrobics with a high degree of reliability.

The effectiveness of an antimicrobic in sensitivity testing is based on the size of the zone of inhibition. The zone of inhibition, however, varies with the diffusibility of the agent, the size of the inoculum, the type of medium, and many other factors. Only by taking all these variables into consideration could a reliable method be worked out. The Kirby-Bauer method is such a method and is the accepted procedure in use today. It is sanctioned by the U.S. FDA and the subcommittee on antimicrobial susceptibility testing of the NCCLS. The basic procedure is as follows.

The recommended medium in this test is Mueller-Hinton agar. It should be poured to a uniform thickness of 4 mm in a petri plate. This requires 25 ml of the medium in a 100 mm plate. An inoculation of the medium surface is made with a cotton swab from a broth culture. In clinical

applications, the broth turbidity has to match a defined standard. Care must also be taken to express the excess broth from the swab prior to inoculation.

High potency disks may be placed on the agar with a sterile forceps. Regardless of how they are placed, it is desirable to press down on each disk to ensure close contact of the disk to the medium. After 16 to 18 hr incubation, the plates are examined and the diameters of the zones are measured to the nearest millimeter.

Limitations

Although the Kirby-Bauer test has been accepted as the standard technique for performing disk diffusion susceptibility tests as it gives useful information in most instances, it has a few distinct limitations:

1. Disk diffusion methods are not applicable to slow growing microorganisms such as anaerobes. If prolonged incubation is required to achieve sufficient growth to produce a detectable zone of inhibition, there may be enough deterioration of the diffusing antibiotic to produce imprecise readings.
2. For antibiotics that diffuse slowly in agar, such as polymyxin B, the high polymyxin B disk content of 300 μg/ml counteracts the slow diffusability to some degree; however, results may be unreliable for slow-migration antibiotics, and control must be compared.

Susceptibility Testing: Agar Dilution Test for Anaerobes

The test described below is the tentative agar dilution reference method by NCCLS. Modifications of the incubation time or medium are necessary for unusually slow-growing or nutritionally fastidious strains. Modified procedures should be tested with a recommended control strain.

For each culture to be tested, portions of five or more colonies are inoculated into its appropriate medium. The medium are incubated for 18 to 20 hr, or longer if required, to obtain sufficient growth of the test organism. Just before the test, the turbidity must be adjusted to match that of the barium sulfate standard. An alternative preparation of the inoculum may be

made by suspending the growth from an agar plate (not more than 72 hr. old) into a clear medium such as brucella broth adjusting the turbidity as previously described.

On the day of the test, dilutions of the antimicrobial agents are prepared and incorporated the dilution into Wilkins-Chalgren agar (Wilkins, and Chalgren, 1976). The adjusted inoculum is inoculated onto the surface of the agar of each plate with a 0.001 ml calibrated loop or with a steers replicator. The plates were allowed to dry. The culture is then incubated at 35-37 ^{o}C in anaerobic jars or chamber for 42 to 48 hr. The MIC of each strain is the lowest concentration of drug yielding no growth, one discrete colony, or a fine, barely visible haze.

Before and after the inoculation of each series of antimicrobial agent containing plates, two plates of the Wilkins-Chalgren agar are inoculated without antimicrobial agents. One set of the plates is incubated in the anaerobic jars or chamber to serve as growth controls, while the other set of plates is incubated in the air to determine whether contamination with aerobic or facultatively anaerobic bacteria has occurred.

Susceptibility Testing of Topical Antimicrobial Agents

The cup diffusion test for antibiotics (Cooper and Woodman, 1946) can be used for a new purpose to select the most effective topical antibiotic for treatment of a microbiological contaminant of a burn wound. The clinical value of the cup diffusion procedure for selection of topical agents has been documented in work of Nathan et al. (1977) by serial quantitative swab tests. It may be used to select an effective agent for treatment of any type of surface wound requiring a topical antimicrobial agent.

The cup diffusion method is similar to the disk diffusion method. The only difference is the use of cup containing topical antimicrobial agent instead of the disk antimicrobial.

Emulsions (Idson, 1988)

Emulsions have been defined as heterogeneous systems of one liquid dispersed in another in form of droplets usually exceeding 0.1 μm in diameter. Two liquids are immiscible, chemically unreactive, and form systems charecterized by little to no thermodynamic stability.

Type of Emulsions

Most common types of pharmaceutical or cosmetic emulsions include water as one of the phases and an oil or a liquid as the other. If the oil droplets are dispersed in a continuous aqueous phase the emulsion is termed oil-in-water (o/w); if the oil is the continuous phase, the emulsion is of the water-in-oil type (w/o). It has been observed that o/w emulsion occasionally change into w/o emulsions and vice versa. This change in emulsion type is called inversion.

Emulsions for Topical Use

Skin softness and pliability are directly related to the water content of stratum corneum; the retention of moisture by the skin helps prevent the development of dry chapped skin. Since evaporative moisture loss from the skin surface (transepidermal water loss or TEWL) can be minimized by surface occlusion, a topical application of an occlusive water-impermeable film can facilitate rehydration of dry skin and a hydrophobic oils as efficient moisture occlusion is thwarted by their inelegance. Formulators would prefer to develop a product that rubs into the skin to leave a residue that is undetectable to the eyes and is neither tacky nor greasy. On the other hand, the emulsions whether w/o or o/w can be formulated to provide the requisite occlusivity and reduction in TEWL.

However, even presumably nonvolatile excipients (e.g. glycerin, propylene glycol) evaporate after topical application. Skin permeation by excipients may also occur after application, leading to further compositional change in the applied film on the skin surface. The impact of this evaporative and absorptive loss of adjuvants on formulation composition, skin effect and dry delivery capability of real vehicles increase as the volume of the topical formulation applied is reduced. Increased occlusivity leads to increased hydration of stratum corneum and a correspondingly lower diffusional resistance to permeating solutes. Furthermore, decreased in TEWL results in a slight elevation in skin temperature in the occluded area, and increased solute flux in the affected cutaneous environment

Emulsions may facilitate dry permeation into and through the skin by their occlusiveness or by virtue of their penetration by affecting barrier, i. e., stratum corneum integrity. Surfactants, urea and terpenes can alter stratum corneum and, therefore, enhance the drug penetration. An

increase in thermodynamic activity or solubility of a penetrant can also increase the penetration of drug.

Emulsion of the o/w type are washable, less oily, and consequently less obvious to the touch than w/o emulsion while the w/o type emulsions are used topically as emollient. An increase in concentration gradient of drugs across the stratum corneum can promote percutaneous absorption. An o/w cream is usually nonocclusive because it does not deposit a continuous film of water-impervious lipid. However, a correctly formulated cream can deposit lipids and other moisturizers on and in the stratum corneum and so restores the tissue ability to hydrate.

Components of Creams

In this study, a polar phase was composed of water, glycerin and propylene glycol. Silicone fluids (cyclomethicone, dimethicone, and Arlamol S7®), and cetostearyl alcohol were selected as ingredients in non-polar phase. Water was used as a moisturizer for skin and a water phase for formulating the emulsion.

1. Polar Phase

Glycerin

Glycerin used as a humectant has an ability to rehydrate skin when it is delivered from a cream. It can also help plasticizing the films of certain polymers and are often used as co-solvents during the solubilization of fragrance or oils into products. Glycerin has desirable humectant properties. It exhibits good equilibrium hygroscopicity and is virtually nontoxic except at very high concentrations where a dehydration effect on the skin can be seen. At low temperatures, glycerin tends to supercool rather than crystallize. It was relatively inexpensive, and was readily available. Glycerin is used in creams at levels of 1-5% typically to prevent surface dehydration of the product when it is exposed to air (Gesslein, 1999).

Propylene Glycol

Propylene glycol is a widely used humectant in personal care formulations. It has some antimicrobial activity and enhances the activity of microbial agents. Furthermore, it is a co-solvent for preservatives such as paraben (Wade and Weller, 1994).

2. Non-polar Phase

Cyclomethicone

Volatilte (poly)dimethylcyclosiloxanes (Cyclomethicones) fluid have unique physical properties that benefit for personal care applications. These include volatility, low viscosity, and non-residual transient skin feel. Other attributes of cyclomethicone fluids include: colorless, odorless, nonstaining, good spreading, detackification, water sheeting and transient emolliency properties. The level of use of cyclomethicone in personal care application is typically from 0.1% to 85%; the low end represents use levels which enhance skin feel in creams (e.g., hand and body moisturizers), and the high end use levels are to carry actives to skin and hair (Abrutyn, 1999).

Dimethicone

Linear (poly)dimethylsiloxane or linear PDMS (INCI: Dimethicone) is considered a good skin emollient and lubricant. Dimethicone generally acts as skin-feel modifiers, water barrier protectants, defoamers, desoapers (i.e., eliminators of creamy whitening of a cosmetic formation during the initial rubbing onto skin or hair), and providers of conditioning and emolliency. The tentative final monograph identifies which active can be used at use levels of 1-30% as an active ingredient. The ease of spread and lubricity of a linear PDMS fluid produces the characteristic velvet-like feel typically associated with silicones. As the molecular weight increases for linear PDMS, an increase in residue, oiliness and smoothness can be observed (Abrutyn, 1999).

Arlamol S7®

Arlamol S7® (INCI: Cyclomethicone and PPG-15 stearyl ether) is more convenient for forming cream compared to pure volatile silicone. The solidification point of Arlamol S7® is lower than that of cyclomethicone alone (17°C). This volatile product embodies excellent spreadability for improved skin-feel and gives better water absorption to counteract dry

skin. Furthermore, it exhibits very good compatibility with other cosmetic emollients over a wide pH range and is easy to emulsify. When it is applied, it provides a partial protective barrier that reduces evaporation from the skin's surface while still allowing the skin to breathe (ICI Surfactants, 1995).

Cetostearyl Alcohol

Cetostearyl alcohol is used in cosmetics and oral and topical pharmaceuticals. In pharmaceutical formulations cetostearyl alcohol is used as a stiffening agent and emulsifier in both w/o and o/w emulsions. It acts as an emulsion stabilizer when it is mixed with more hydrophilic primary emulsifier, such as anionic emulsifying agents, to produce o/w emulsion which are stable over a wide pH range (Wade and Weller, 1994).

3. Emulsifiers

The reduction of interfacial tension by an emulsifier is a direct result of their adsorption at the interface. It has long been held that affinity of such surface-active agents (surfactants) for the interfacial region facilitates the formulation of a relatively rigid film of the emulsifier at the interface that acts as a mechanical barrier to droplet adhesion and coalescence. Thus, emulsion stability will increase as the surface viscosity and yield value of the film increase (Block, 1996).

The classification of surfactant types provided in Table 1 is based on the ionic charge of the surfactant. Emulsifier selection is according to emulsion type, oil phase polarity and emulsion viscosity. Furthermore, multifunctional character of surfactants in emulsion systems renders their selection for emulsion systems difficult and empirical at best. The blends of surfactants are often employed as the emulsifier in formulations rather than single surfactants as the resultant emulsions tend to be more stable.

Table 1 Surfactant classification.

Type	Examples
Anionic	Alcohol ether sulfates
	Alkyl sulfates
	Soaps
	Sulfosuccinates
Cationic	Quaternary ammonium compounds
Zwitterionic	Alkyl betaine derivatives
Amphoteric	Fatty amine sulfates
	Difatty alkyl triethanolamine derivatives
Nonionic	Lanolin alcohols
	Polyoxyethylated (POE) alkyl phenols
	POE fatty amide
	POE fatty alcohol ether
	POE fatty amine
	POE fatty ester
	Poloxamers
	POE glycol monoethers
	Polysorbates
	Sorbitan esters

Brij 721® and Brij 72® (ICI Surfactants, 1995)

Brij 721® (INCI: Steareth-21) and Brij 72® (INCI: Steareth-2) are nonionic emulsifiers that in combinations benefit emulsion stability. While they are originally designed for the emulsification of cetostearyl alcohols, combinations of these emulsifiers are well suited for o/w both creams and lotions with attractive surface gloss. Furthermore, these blends of surfactants have been proven in numerous studies to be successful in emulsifying the polar oils (e.g., cyclomethicone, and ester oils) that are favored for their superior emollience, spreading, solvent and carrier properties. The viscosity of the emulsions can be increased when the emulsifier concentration is increased.

Laurylmethicone copolyol

Alkyl dimethicone copolyol such as laurylmethicone copolyol (Dow Corning 5200®) has been used as an emulsifier in preparations of both water in silicone and silicone in water systems. These products provide advantages over traditional hydrocarbon chemicals since they can be used to prepare emulsions without heat. These silicone polymers can be used to prepare products that contain little wax, contain a large concentration of water, and have a light spreadable feel on the skin. The emulsion viscosity is adjusted by varying oil and aqueous phase ratios. Electrolytes are recommended in water phase for emulsion stability (O'Lenick, 1999).

4. Emulsion stabilizers

Emulsion stabilization can be achieved through interference with creaming, droplet flocculation or coalescence. Thus, stability can be conferred by equalizing phase densities, increasing the viscosity of the continuous phase, or by adsorbing stabilizing substances at the oil-water interface. Increasing the viscosity of the continuous phase with lyophilic colloids (e.g., carbomers, polysaccharides, and clays) decreases the rate of creaming or flocculation. The colloids have effectively stabilized emulsions via interfacial adsorption and the subsequent formation of condensed films of high tensile strength that resist droplet coalescence (Block, 1996).

Xanthan gum

Xanthan gum is a lyophilic colloid. It is used as an emulsion stabilizer via interfacial adsorption and the subsequent formation of condensed film of high tensile strength and increasing viscosity of the dispersion medium. Furthermore, it can make lightness and smoothen creams.

5. Preservative

Contamination sources include raw materials, processing equipment and facilities, manufacturing personnel and the consumer. Emulsion formulations are also prone to microbial contamination including those packaged in wide-mouthed containers or in flexible bottles or tubes that draw air back into them. The consequences of microbial contamination have implications for the formulation itself and may be confused with faulty product

development. The inclusion of an antimicrobial agent may be necessary. Obviously, a selection of an appropriate preservative requires considerations of the probable microbial contaminants and the spectrum of activity of the available preservatives (Block, 1996).

Germaben II-E® (Flick, 1991)

The combination of 20% Germall II, 10% methylparaben, 10% propylparaben and 60% propylene glycol is useful for creams and lotions.

Germall II® (diazolidinyl urea) is a superior imidazolidinyl urea because it has a wider spectrum of activity including activity against standard gram-positive and gram-negative bacteria. Not only is Germall II® more active against gram-negative bacteria such as *Pseudomonas*, but also it has increased activity against yeast and mold. Creams and lotions preserved with a Germall II-paraben combination system can retain activity against yeast and mold even when the paraben activity has been diminished by interaction with nonionic surfactants or proteins, or migrated into the oil phase. Incorporation of Germaben II-E® at a level of 1% of the finished formulation is usually required.

5. Antioxidants (Lieberman et al., 1996)

An inclusion of an antioxidant in an emulsion formulation may be necessary to protect not only formulation components but also an active ingredient that are oxygen labile. Oxidation occurring spontaneously under mild conditions generally involves free radical reactions. Oxidative degradation is often preceded by a lag phase corresponding to the gradual increase of free radicals via an initiation reaction. Unless the resultant radicals are stable, radical propagation ensues. If chain branching occurs, reaction kinetics will tend to be more complex. The termination of this oxidative sequence involves the interaction of free radicals with one another to form stable or metastable products.

Kinetic measurements of fat oxidation in o/w emulsions indicate that the rate of oxidation is dependent upon the rate of oxygen diffusion in the system. Trace amounts of metals such as copper, manganese, and iron may also initiate or catalyze the oxidative reactions. Furthermore, some oxidative degradation is pH-dependent as pH affects the degree of ionization of the labile

compound and its corresponding susceptibility to oxidation (Connors, Amidon, and Stella, 1986).

The kinetic behavior in the three phases are described as follows:

Initiation : $RH \longrightarrow R^{\bullet} + H^{\bullet}$

Propagation : $R^{\bullet} + O_2 \longrightarrow ROO^{\bullet}$

$ROO^{\bullet} + RH \longrightarrow ROOH + R^{\bullet}$

Termination : $ROO^{\bullet} + X \longrightarrow$ non-reactive products

$R^{\bullet} + R^{\bullet} \longrightarrow R\text{-}R$

Inhibitions of Oxidation

The extent of drug oxidation degradation in pharmaceutical formulations can be minimized in several ways.

1. Controlling of Temperature

A decrease in temperature usually decreases a reaction rate. Hence, the storage of formulations susceptible to oxidation in a place with low temperature such as in a refrigerator instead of at room temperature would decrease the reaction rate (Stewart and Tucker, 1985).

2. Protection from Light

A decrease in radiation intensity can be achieved by increasing the distance between the radiation source and drug, reducing the time of drug exposure to the radiation, and decreasing the surface area of the drug or drug formulation exposed to the radiation. However, many drugs require more than one protection ways. A lot of techniques used for exclusion of light can be accomplished, for example, wrapping around labels, using various cartoning procedures, using coating containers which some may incorporate ultraviolet absorbing materials, using the pigmented glasses capable of excluding the damaging wavelengths, and using opaque containers that all light is excluded (Connors et al., 1986).

3. Prevention of Oxygen

Many drugs need more processes to protect them from oxygen. Removing oxygen from a formulation is one of the obvious way to prevent oxidation. This can be done by several ways. Oxygen may be expelled from aqueous preparations by boiling water or bubbling nitrogen gas through the solvent to flush the oxygen out of the solution. Another way is to flush the headspace of the containers with nitrogen just prior to filling and sealing or capping. This is done mostly with ampules and bulk powders that are reconstituted at time of dispensing (Connors et al., 1986).

4. Adjustment of pH

The oxidation of many drugs is pH dependent. Most of them degrade more rapidly in neutral to alkaline pH conditions. Increasing of hydrogen ion concentration or decreasing of pH value of drug solutions can decrease the tendency of drug oxidation. A pH range of three to four is generally found to be most useful in retarding the oxidation (Stewart and Tucker, 1985; Connors et al., 1986).

5. Addition of Antioxidants

Antioxidants are often added to emulsion systems by formulators considering the specific kinetic mechanism involved. Some antioxidants function as retardants, and others function as inhibitors. The retardants decrease the oxidation rate while the inhibitors induce or prolong a lag period prior to the onset of oxidation. Combination of retardants and inhibitors may act synergistically, for example, chelating agents and chain terminators used together are more effective than either agent alone. Antioxidants can conveniently be classified as primary antioxidants, reducing agents and chelating agents (Stewart and Tucker, 1985; Connors et al., 1986).

5.1 Primary Antioxidants

Primary antioxidants or free radical inhibitors are the substances that can donate a hydrogen radical or an electron while itself from radicals that are stable and incapable of continuing the propagation chain cycle. This retardation effect can increase the induction

period of oxidation. Examples are alkyl gallates (octyl, propyl, dodecyl), butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and alpha tocopherol.

5.2 Reducing Agents

Reducing agents are compounds that can be oxidized more readily than drugs to be protected. Their mechanisms of oxidation can be either reversible loss of electrons or auto-oxidation. Examples are ascorbic acid, sodium bisulfite, and sodium metabisulfite.

5.3 Chelating Agents

Chelating agents act by binding to metal ions which are known to be one of the initiator of oxidation. Thus, the use of chelating agents in a formulation may markedly improve product stability. Effective chelating agents used pharmaceutically are ethylenediaminetetraacetic acid (EDTA), citric acid, phosphoric acid, tartaric acid and many amino acids. Their metal binding capacities are dependent on their state of ionization. Thus, they lose their chelating capacities at low pH.

CHAPTER III

EXPERIMENTAL

Materials

Acetic acid, AR grade, Lot No. K23679363702 (Merck, Germany).

Acetonitrite, HPLC grade, Batch No. 98080060 (Lab Scan, Ireland).

Arlamol S7® (Uniqema, UK).

Brij 72®, Lot No. 42337 (Uniqema, UK).

Brij 721®, Lot No. 41364 (Uniqema, UK).

Brucella broth (BDH Laboratory, England).

Cashew apples collected during May, 1999 in Chumporn province, Thailand.

Cetostearyl alcohol, Lot No. 376391 (T Chemical, Thailand).

Cholesterol, AR grade, Lot No. 2003532 (Fluka, Switzerland).

Cyclomethicone (Dow Corning Corporation, USA).

Dimethicone (Dow Corning Corporation, USA).

Dow Corning 5200® (Dow Corning Corporation, USA).

Ethanol 95%, AR grade, Lot No. L633508 (BDH Laboratory, England).

Germaben II-E (Wannarat, Thailand).

Glycerol, Lot No. L885 (May and Baker, England).

Methanol, HPLC grade, Lot No. L848602 (BDH Laboratory, England).

Mueller Hinton agar (Merck, Germany).

Panoxyl 5®gel (Stiefel Laboratories, Singapore).

Propyl gallate, Lot No. 40113813 (Merck, Germany).

Sodium metabisulfite (Merck, Germany).

Tryptic soy agar, Lot No. V223258834 (Merck, Germany).

Wilkins Chalgren Broth, Lot Ch-B215548 (Oxoid, England).

Xanthan gum (Keltrol TF®), Lot No. 61908K (Mosanto, Spain).

Equipments

Aluminum replicating device (Supplied by Siriraj Hospital)

Anaerobic chamber, Model 1029 S/N (Forma Scientific, USA)

Analytical balance (Sartorius GMPH, Germany).

Daylight lamps (Oslam Dulux®), 20W (Oslam, Germany).

Digital illuminance meter, Model TES 1332 (TES Electrical Electronic, Taiwan).

High performance liquid chromatography (HPLC) system equipped with:

a turnable absorbance detector, SPD-10A, Shimadzu, Japan,

a constant flow pump, LC10AD, Shimadzu, Japan,

an autoinjector, SIL-10A, Shimadzu, Japan,

a pump oven, CTO-10A, Shimadzu, Japan,

A HPLC cartridge LiChroCart®125-4, LiChrosper®100 RP-18 (5 μm), (Merck, Germany).

Hot air oven, TY ULM700 Schutzort Din 40050-IP20 (Memmert, Germany).

pH meter, Model SA520 (Orion, USA).

STD and BTD Eco tray dryers, FTS systems equipped with:

a freezer, DURA-DRY MP, (Dura-Dry, Japan),

a dryer, DURA-ST OP, (Dura-Dry, Japan).

Turbidity meter, Crystal Spec (Becton Dickinson, USA).

Ultrasonic bath, Bransonic 3210, Branson (SmithKline company, USA).

Viscometer, Brookfield Digital (Brookfield, England).

Method

1. Preparation of Freeze-dried Cashew Apple Juice

Cashew apple juice was prepared by squeezing of fresh cashew apples, and their residue were filtered out using No.1 filter paper. The juice was then frozen at -40 ^{0}C and dried in STD and BTD Eco tray dryers.

The freeze-dried cashew apple juice (FCAJ) was obtained from both yellow and red cashew apples using the same procedure. They had been stored in amber bottles and placed in a desiccator.

2. Antibacterial Susceptibility Test of FCAJ

The bacteria included in this study were 29 clinical isolates of *S. aureus*, 29 clinical isolates of *P. acnes* and two standard strains, *Bacteroides fragilis* (*B. fragilis*) ATCC 25285 and *S. aureus* ATCC 25923.

2.1 Antibacterial Activity Test of FCAJ Against *S. aureus*

The disc agar diffusion method by Bauer (1966) was used. The procedure was as follows.

2.1.1 Preparation of Medium

Thirty-eight grams of Mueller Hinton agar (Difco) was weighed in a one-liter flask. Purified water was added to dissolve the agar and the volume was adjusted. The agar solution was sterilized at 121 ^{0}C under 15 pounds per square inch pressure for 15 min. Twenty milliliters of the sterile Mueller Hinton agar (Difco) was then dispensed into sterile glass petri dishes of 90 mm diameter. The agar was allowed to solidify on a flat level surface. The plates were dried for 20 min at room temperature.

2.1.2 Preparation of Antibacterial Disks

Lyophilized FCAJ were rehydrated with purified water to give end concentrations of 0.08, 0.8 and 8 g/ml, respectively. Twenty microliters of each sample were added to each approximately 6 mm diameter paper disk (Whatman). Thus, the paper disks had sample concentrations of 0.16, 1.60, and 16.00 mg, respectively. The disks were allowed to dry for 30 min at room temperature and were stored under refrigeration until used.

The active sample concentrations per disk were in the range of 1.6 to 16 mg. Another set of sample concentrations per disk (2.0, 4.0, 8.0, and 16.0 mg, respectively) was prepared by the same procedure. Both red and yellow type FCAJ were studied.

2.1.3 Inoculation of Test Plate

In the case of 0.16, 1.6, and 16 mg FCAJ per disk, respectively, 15 *S. aureus* isolates were separately inoculated on Tryptic soy agar (Difco) in petri dishes and incubated at 37 ^{0}C for 20-24 hr. Three to four well-isolated colonies of the same morphological type were inoculated into 4 ml of normal saline to obtain a turbidity standard and was referred to as a Mac Farland 0.5 turbidity standard (about 1x10^{8} CFU/ml). The turbidity was measured by turbidity meter.

A sterile swab was dipped into the culture suspension and the excess suspension was removed by pressing and rotating the swab against the inside of the tube above the fluid level. The plates were inoculated by rubbing the swab throughout the entire surface of the medium. The inoculation was repeated three times in the direction of 60 ^{0} to the previous inoculation. The inoculated plate was then allowed to dry. The antibacterial disks (in 2.1.2) were then placed on the surface of the inoculated plate by sterile forceps. Then, the plates were inverted and placed in an incubator at 37 ^{0}C for 18-20 hr.

In the case of 2.0, 4.0, 8.0, and 16.0 mg FCAJ per disk, respectively, 29 *S. aureus* isolates and *S. aureus* ATCC 25923 were inoculated by the same procedure.

2.1.4 Interpretation and Reading

The plates were examined with transmitted light to visualize complete inhibition zones which were then measured to the nearest whole millimeter by sliding calipers. Faint growth or tiny colonies near the edge of the inhibition zones were ignored if they were present.

2.2 Antibacterial Activity Test of FCAJ against *P. acnes*

The agar dilution method was used for determining of minimum inhibitory concentration of *P. acnes* (Jorgenson, 1993). Briefly, it was described as follows :

2.2.1 Preparation of Medium

Wilkins-Chalgren broth (Difco) (9.9 g) and agar (4.5 g) were weighed and suspended in 270 ml purified distilled water. The medium was boiled until the agar was dissolved completely. Eighteen milliliters of the medium were dispensed into screw-capped test tubes and sterilized in the autoclave at 121 ^{0}C under 15 pounds per square inch pressure for 15 min and allowed to cool in a 50 ^{0}C water bath.

2.2.2 Preparation of Antibacterial Dilution

Four grams of FCAJ were weighed in 10 ml volumetric flasks and sterile distilled water was added to rehydrate the sample and adjust the volume. They were further diluted to make series of two fold dilution of the FCAJ dispersion to obtain final concentrations of 25, 50, 100, 200 and 400 mg/ml, respectively. Both red and yellow type FCAJ were studied.

2.2.3 Preparation of Antibacterial Plates

Two milliliters of each diluted antibacterial suspension were added to 18 ml dissolved and warmed medium. The tubes were then mixed thoroughly but gently. The agar was poured into sterile 90 mm diameter petri dishes and allowed to solidify on a flat level surface. The agar plates would give the final freeze-dried cashew apple juice concentrations of 0.25, 0.5, 1.0, 2.0 and 4.0 mg/ml, respectively.

At least two control plates containing Wilkins-Chalgren agar (Difco) without FCAJ were prepared in each experiment.

2.2.4 Preparation of the Inoculum

Three to five colonies of *P. acnes* from each of the 29 isolated strains were separately incubated in 3 ml Brucella broth (Difco). Each inoculum was then standardized to match a 0.5 turbidity standard of Mac Farland measured by turbidity meter.

The control strain of *B. fragilis* ATCC 25285 was prepared using the same procedure.

2.2.5 Inoculations of Agar Plates

Twenty-nine *P. acnes* cultures and a control strain, *B. fragilis* ATCC 25285, were transferred to appropriate wells in each seed plate containing 30 reservoirs.

An aluminum replicating device (the multipoint inoculator apparatus) was dipped into the wells of inoculum in the seed plates. Approximate 1 μl of the inoculum was inoculated onto the surface of each tested plates. The plates were allowed to dry. Two sets of bacterial inoculation plates were prepared. One set of the inoculation plates was incubated at 37 ^{0}C for 48 hr under an anaerobic condition. The other set was incubated in 37 ^{0}C for 48 hr under an aerobic condition.

2.2.6 Reading of Testing Results

The agar dilution plates were examined for bacterial growth after incubation. First, the control plates without FCAJ in anaerobic chamber were checked for the lowest concentration of *P. acnes* that was capable of growing under experimental condition. A very fine, barely haze or a single colony was disregarded.

3. Effects of Antioxidants on the Red Type FCAJ Dispersions

Twenty grams of the red type FCAJ were weighed in a 100-ml volumetric flask. Distilled water was added to disperse the FCAJ and adjusted the volume. This dispersion was used as a control.

Twenty grams of the red type FCAJ were weighed in a 100-ml volumetric flask. Distilled water was added to disperse the FCAJ. Sodium metabisulfite (0.05 or 1 g) or propyl gallate (0.01 or 0.1 g) was added. The volume was then adjusted using distilled water. In the case of propyl gallate, it was dissolved in ethanol (about 2 ml) prior to adding to the dispersion. Thus, there were a total of five suspensions studied. The FCAJ dispersion (0.5 ml) was pipetted using a micropipette and transferred to 2 ml transparent borosilicated glass vials. The vials were closed with rubber closure and covered with aluminum caps using a hand crimper. The vials were placed in a black plastic box in a hot air oven of which the temperature was controlled at 45 ^{0}C. A 20 W artificial daylight lamp was positioned at the top of each boxes to yield the light luminescence of 1500 $\pm$ 20 lux measured by a Lux meter.

4. Assay of a Selected FCAJ Ingredient

4.1 Chromatographic Conditions

A high pressure liquid chromatographic condition was modified from that of Kubo et al. (1986) for analysis of a selected FCAJ ingredient as follows.

Column : Cartridge LiChroCart® 125-4, LiChrosper® 100 RP-18 (5 μm)

Mobile phase : 10% acetic acid : acetonitrile : methanol = 1 : 2 : 7

Detector wavelength : 254 nm

Flow rate : 1.3 ml/min

Attenuation : 1

Injection volume : 40 μl

Internal standard : 1 mg/ml cholesterol

Retention times : FCAJ ingredient, 10-11 min

Cholesterol, 17-18 min

4.2 Sample Preparation for Assay by HPLC

1 g of freeze-dried cashew apple juice

↓ Add 3 ml purified water

Vortex for 10 second

↓

Centrifuge for 15 min at 5000 rpm

↓

Collect precipitates

↓ Add 2 ml of 1 mg/ml cholesterol in ethanol as internal standard

Sonicate for 15 min

↓

Centrifuge for 15 min at 5000 rpm

↓

Adjust supernatant to 5 ml with 1 mg/ml cholesterol in ethanol

↓

Inject 40 μl of solution into HPLC column

One gram of FCAJ was weighed in a beaker. Three milliliters of purified water was added to disperse the FCAJ and transferred to a 10-ml test tube. The dispersion was vortexed for 10 seconds and then centrifuged for 15 min at 5000 rpm. Its precipitates was collected and 2 ml of 1 mg/ml cholesterol in ethanol was added. The mixture was sonicated for 15 min and centrifuged for 15 minutes at 5000 rpm. The supernatant was collected, adjusted to 5 ml with 1 mg/ml cholesterol in ethanol and ready for HPLC injection.

A stock solution of internal standard was prepared by accurately weighing one hundred milligrams of cholesterol in a volumetric flask. Ethanol was added to dissolve the compound and adjust the final volume.

FCAJ ingredient was prepared as previously described. It was further diluted to obtain final FCAJ concentrations of 12.5, 25, 50, 100 and 200 mg/ml, respectively, for the determination of comparative FCAJ ingredient.

5 Formulation of Creams Containing FCAJ

5.1 Preparation of Cream Bases

Methods of preparation depended upon the types of cream bases. Their details are as follows.

5.1.1 Oil in Water Cream Bases

Compositions of oil in water cream bases are shown in Table 2. The bases were prepared by heating oil phase and water phase separately until their temperature were 70 ^{0}C. The oil phase was slowly poured into water phase with a moderate stir. The mixture were stirred continuously until they were cool to room temperature.

5.1.2 Water in Oil Cream Bases

Compositions of water in oil cream bases are shown in Table 3. They were prepared as follows. The oil phase was stirred intensively by a blender until uniform. The water phase was then slowly added into the oil phase with an intensive stir until uniform. The mixture was then stirred moderately by the blender for 30 min.

5.2 Evaluation of Physical Properties of Cream Bases

The appearances of cream bases were observed. Selection criteria of the cream bases were smoothness, lightness, and spreadability. Viscosity and pH's of the selected formulations were measured before and after 6 heating-cooling cycles (4 ^{0}C for 24 hr and 40 ^{0}C for 24 hr was one cycle) (Grimm and Krummen, 1993).

5.3 Preparation of FCAJ Creams

5.3.1 Preparation of o/w Creams Containing FCAJ

Oil in water creams containing different concentrations of FCAJ (0.25, 0.5, 1 and 2 %w/w, respectively) were prepared. The FCAJ was weighed, dispersed in a small amount of water and added into the cream bases prepared using the same procedure as 5.1.1. An antioxidant, 0.05% w/v sodium metabisulfite, was added in the water phase of the cream base in this study. Both yellow and red cashew apples were studied.

5.3.2 Preparation of w/o Creams Containing FCAJ

Water in oil creams containing different concentrations of FCAJ (0.25, 0.5, 1 and 2 %w/w, respectively) and 0.05% sodium metabisulfite were prepared. The preparation method was the same as those of the cream bases described in 5.1.2 except that the FCAJ was dispersed and 0.05% w/v sodium metasulfite was dissolved in the water phase. Both yellow and red cashew apples were studied.

Table 3 Ingredients of w/o cream bases.

Ingredients (%w/w)	Formulation no.											
	1	2	3	4	5	6	7	8	9	10	11	12
OIL PHASE:												
DC 5200®	2	2	2	2	2	2	2	2	2	2	2	2
Arlamol S7®	15	20	10	12	13	14	14	15	-	-	5	7
Cyclomethicone	-	-	-	-	-	-	-	-	15	15	10	8
Dimethicone	-	-	0.1	0.1	0.1	0.1	1	0.1	0.1	3	0.1	0.1
WATER PHASE:												
Sodium chloride	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Glycerol	4	4	4	4	4	4	4	4	4	4	4	4
Germaben II-E®	1	1	1	1	1	1	1	1	1	1	1	1
Water qs.	100	100	100	100	100	100	100	100	100	100	100	100

6.1 Antibacterial Activity Tests of FCAJ Creams Against *S. aureus*

The cup agar diffusion method was used with the following details.

6.1.1 Preparation of Medium

A medium was prepared by weighing of 38 g Mueller Hinton agar (Difco) in a flask. Purified water was added to dissolve the medium and adjusted to 1000 ml. The medium was sterilized at 121^{0}C under 15 pounds per square inch pressure for 15 min. Twenty milliliters of sterile Mueller Hinton agar (Difco) was dispensed into sterile glass petri dishes of 90 mm diameter. The agar was allowed to solidify on a flat level surface. The plates were dried for 20 min at room temperature.

6.1.2 Inoculation of Test Plate

The 29 isolated strains of *S. aureus* and *S. aureus* ATCC 25923 were separately inoculated on Tryptic soy agar (Difco) in petri dishes and incubated at 37 ^{0}C for 20-24 hr. Three to four well-isolated colonies of the same morphological type and inoculum were inoculated into 4 ml of normal saline to obtain a turbidity standard and was referred to as a Mac Farland 0.5 turbidity standard (about 1x10^{8} CFU/ml) which was measured by the turbidity meter.

The agar medium was inoculated using the same technique as previously described in 2.1.3. Sterile stainless cups with approximately 6.8 mm diameters were filled with different concentrations of FCAJ creams (about 0.35 $\pm$ 0.02 g) and placed on surface of the inoculated plates using sterile forceps. The plates were then incubated at 37 ^{0}C for 20 hr.

6.1.3 Interpretation and Reading

The plates were examined with transmitted light to visualize complete inhibition zones which were measured to the nearest whole millimeter by sliding calipers.

6.2 Antibacterial Activity Tests of FCAJ Creams Against *P. acnes*

The cup agar diffusion method was used with the following details.

6.2.1 Preparation of Medium

A medium was prepared by weighing 33 g of Wilkins-Chalgren broth (Difco) and 15 g agar in a flask. Purified water was added to dissolve the medium and adjusted to 1000 ml. The medium was sterilized at 121^{0}C under 15 pounds per square inch pressure for 15 min. Twenty milliliters of sterile medium was dispensed into 90 mm diameter sterile glass petri dishes. The agar was allowed to solidified on a flat level surface. The plates were dried for 20 min at room temperature.

6.2.3 Inoculation of Test Plates

Three to five colonies of each 29 *P. acnes* isolates were separately incubated in 3 ml of Brucella broth (Difco). The inoculum was then standardized to match a 0.5 turbidity of the standard Mac Farland which was measured by the turbidity meter. The control strain of *B. fragilis* ATCC 25285 was prepared in the same way.

The agar medium was inoculated by the same technique as previously described in 2.1.3 Sterile stainless cups with approximately 6.8 mm diameters were filled with different concentrations of FCAJ creams (about 0.35 ± 0.02 g) that they were then placed on the surface of the inoculated plate by sterile forceps. The plates were incubated in an oven at 37^{0}C for 48 hr under anaerobic condition.

6.2.4 Interpretation and Reading

The plates were examined with transmitted light to visualize complete inhibition zone which was measured to the nearest whole millimeter by sliding calipers. Faint growth or tiny colonies near the edge of the inhibition zones were ignored if they were presented.

7. Validation of the HPLC Method

7.1 Specificity of the method

FCAJ (31.25, 62.50, 125.00, 250.00, and 500.00 mg, respectively) were extracted as previously described in 4.2. The extractions were injected into the HPLC column.

The FCAJ dispersions and the dispersions containing sodium metabisulfite and propyl gallate were forced to decompose by exposing the solutions under 20 W artificial daylight lamp of which the luminance was about 1500 lux at 45 ^{o}C for 6 month. The decomposed dispersion was extracted and injected into the HPLC column.

7.2 Linear Correlation

Five dispersions of samples with FCAJ amount varied from 31.25 to 500.00 mg were extracted and injected. The relationship of peak area ratios of the selected FCAJ ingredient to its internal standard with time was evaluated. The correlation coefficient (r) of the regression line was then determined.

7.3 Precision of the Method

7.3.1 With-run Precision

Three sets of the five extractions of FCAJ (31.25 to 500.00 mg) were injected within one day. The coefficients of variation, %CV, of FCAJ ingredient to its internal standard peak area ratios were calculated.

7.3.2 Between-run Precision

Three sets of the five extractions of FCAJ (31.25 to 500.00 mg) were injected on different days. The coefficients of variation, %CV, of the selected FCAJ ingredient to its internal standard peak area ratios were calculated.

7.4 Accuracy of the Method

Three sets of the five extractions of FCAJ (31.25 to 500.00 mg) were injected. Plots of FCAJ to its internal standard peak area ratios versus concentrations of FCAJ were performed; the slope and intercept were calculated. Then, the percentage of analytical recovery of the selected FCAJ ingredient were calculated by dividing the concentrations fitted from the calibration curve by the known concentrations prepared.

CHAPTER IV

RESULTS AND DISCUSSION

1. **Percent yields of Freeze-dried Cashew Apple Juice**

Percent yields of lyophilized freeze-dried cashew apple juice (FCAJ) obtained from both red and yellow type cashew apples were calculated by (weight of FCAJ lyophilyzed / weight of fresh cashew apple juice) x 100. They were 6.45% and 6.15% for lyophilized FCAJ from red and yellow cashew apples, respectively.

2. **Susceptibility of *S. aureus* to FCAJ**

In order to estimate an appropriate range of FCAJ concentrations in a formulation, the susceptibility test was performed against *S. aureus* by agar diffusion techniques. Table 4 shows the inhibition zone diameters after the application of 3-level amount of FCAJ; 0.16, 1.6 and 16 mg per disc, respectively. Approximately 50% of *S. aureus* strains were resistant to 0.16 mg of both types of FCAJ since no inhibition zone could be observed for about half of *S. aureus* strains tested. It was surprising that almost 50% of *S. aureus* strains were resistant to 16 mg of yellow type FCAJ. Therefore, the susceptibility of *S. aureus* against FCAJ was tested for the amount of FCAJ per disk of 2, 4, 8, and 16 mg, respectively (Table 5 and Figure 3).

S. aureus were more susceptible to red type FCAJ than to yellow type FCAJ. *S. aureus* number 15 and 19 were resistant to both types of FCAJ. For red type FCAJ, the diameters of inhibition zones increased as the amount per disk of FCAJ was increased. However, this trend was not observed in the case of yellow type FCAJ. Neither inhibition zone diameter nor *S. aureus* resistance depended on the amount per disk of yellow type FCAJ. When the same FCAJ amount of red and yellow type were compared, the yellow type FCAJ was more effective at lower amount whereas at greater amount the red type FCAJ was more effective. On the other hand, the organism was more susceptible to lower amount of yellow

Table 4 Inhibition zone diameters of 0.16, 1.6 and 16 mg FCAJ per disk against *S. aureus* .

S. aureus strain no.	Diameters of clear zone (mm)					
	Amount of FCAJ (red) (mg /disk)			Amount of FCAJ (yellow) (mg/disk)		
	0.16	1.6	16	0.16	1.6	16
1	6.5	8	11	6.5	8.5	8
2	8	8	13	8	9	11
3	NZ	8.5	11	NZ	8.5	NZ
4	NZ	8.5	9	7.5	8	NZ
5	9	9	11	8	9	10
6	NZ	7	9	NZ	8.5	9
7	NZ	8.5	13	NZ	8	9
8	NZ	NZ	NZ	NZ	NZ	NZ
9	NZ	8	10	7.5	8	NZ
10	7.5	9	12	8	9	9
11	7.5	8	10	7.5	8	10
12	7.5	10	15	7.5	10	NZ
13	NZ	8.5	15	NZ	10	NZ
14	7	7.5	9	NZ	7.5	NZ
15	NZ	9	14	NZ	10	10

NZ = No inhibition zone

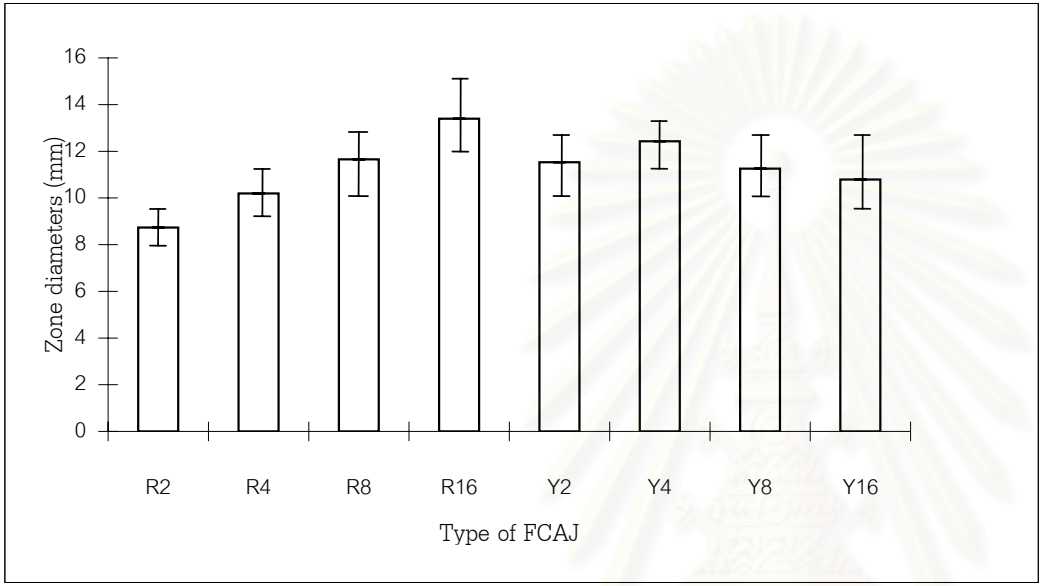
Table 5 Inhibition zone diameters of 2, 4, 8 and 16 mg FCAJ per disk against *S. aureus*.

S. aureus strain no.	Diameters of clear zone (mm)							
	Amount of FCAJ (red) (mg /disk)				Amount of FCAJ (yellow) (mg/disk)			
	2	4	8	16	2	4	8	16
ATCC25923	9	14	14.9	18.6	10	12	13.5	15.5
2	10	11	12	14	13	14	12	NZ
3	10	12	14	16	13.5	14.5	12.5	12
4	10	12	13.5	16	13.5	14	12.5	8
5	7.5	8.5	10.5	12.5	12	11	10.5	9.5
6	8.5	10	11	13	13	13.5	13	10
7	9	11	13	13.5	11.5	12.5	11.5	11.5
8	8	9.5	10	11	10	10.5	10.5	10
9	8	8.5	9	9	10	11	10.5	8.5
10	8	9	9	11	10.5	11	10	NZ
11	8.5	10	11	12	11	12.5	10.5	NZ
12	8	9	10	12	11	12	8.5	NZ
13	9	12	14	17	13.5	11.5	7	9
14	8.5	10	11	10.5	11.5	11.5	10	NZ
15	NZ	NZ	NZ	NZ	NZ	NZ	NZ	NZ
16	8.5	9.5	12	13.5	9	11.5	11	8.5
17	8	9	10	12	10	12	11.5	11
18	7.5	9	10	12	9.5	11	11	12.5
19	NZ	NZ	NZ	NZ	NZ	NZ	NZ	NZ
20	NZ	9	11	13	10.5	13	11.5	12
21	8.5	10.5	13	16	12.5	13.5	12	NZ
22	11	11.5	12	12.5	12	13	NZ	NZ
23	10.5	12.5	14	16	13	15	14.5	14
24	9	10	11	14	11	11.5	9	NZ
25	9	10.5	12	15	12.5	14	15	14

Table 5 Inhibition zone diameters of 2, 4, 8 and 16 mg FCAJ per disk against *S. aureus*.

S. aureus strain no.	Diameters of clear zone (mm)							
	Amount of FCAJ (red) (mg /disk)				Amount of FCAJ (yellow) (mg/disk)			
	2	4	8	16	2	4	8	16
26	8.5	10	12.5	14	12	11.5	11.5	10.5
27	NZ	8.5	10	12	10	12	12	11
28	9	11	14	13	13.5	11.5	11.5	8
29	7.5	9	10	12	10.5	11	11	9.5
30	8	9	12	14	12	10	10	NZ

NZ = No inhibition zone



R2 = red FCAJ 2 mg/disk
R4 = red FCAJ 4 mg/disk
R8 = red FCAJ 8 mg/disk
R16 = red FCAJ 16 mg/disk
Y2 = yellow FCAJ 2 mg/disk
Y4 = yellow FCAJ 4 mg/disk
Y8 = yellow FCAJ 8 mg/disk
Y16 = yellow FCAJ 16 mg/disk

Figure 3 Inhibition zone diameters of FCAJ against *S. aureus*.

type FCAJ than to the greater amount. An analysis of variance and Scheffe tests for Post-Hoc comparisons also yielded the same conclusion (Appendix II).

The theory of agar diffusion can be described as follows. The antimicrobial disks absorb water from the agar medium and thus the drug is dissolved. The antimicrobial agent is then free to diffuse though the adjacent agar medium (Barry and Thornsberry, 1985). The yellow type FCAJ dispersions were more viscous than the red ones, especially, at the greater amount of FCAJ. Since the diffusion coefficient of a molecule is inversely proportional to the medium viscosity, the diffusion coefficients in the FCAJ dispersion of the active ingredients in the yellow type FCAJ dispersions were less. Furthermore, the released of a compound is directly related to its diffusion coefficient. The compound in a more viscous medium were, therefore, release less and slower than that in a less viscous medium. Consequently, the inhibition zone diameters obtained from greater amount of the yellow type FCAJ were smaller or could not be observed in several strains.

3. **Susceptibility of *P. acnes* to FCAJ**

The agar dilution technique was used for the susceptibility testing of *P. acnes* to both red and yellow type FCAJ. The reason of not using the agar diffusion method was that the anaerobic bacterial, *P. acnes*, is a slow growing organism which needs an incubation period of several days. The standard strain of *P. acnes* could not be obtained, *so B. fragilis* ATCC 25285 was chosen as the anaerobic bacterial standard strain for standardizing the anaerobic condition. Results of susceptibility testing of *P. acnes* to red and yellow type FCAJ are illustrated in Table 6. All strains of *P. acnes* were inhibited by yellow type FCAJ at all concentrations tested while only four strains of *P. acnes* and the standard, *B. fragilis*, were resistant to 2.5 mg/ml of red type FCAJ. The MIC_{90} represents the lowest concentration of the antimicrobial agent at which a complete inhibition occurs in 90% of the strains tested . The MIC_{90} of red type FCAJ to *P. acnes* was $\leq$ 5 mg/ml while the MIC_{90} of yellow type FCAJ to *P. acnes* was < 2.5 mg/ml.

Table 6 Inhibitory concentrations of FCAJ against *P. acnes* by agar dilution method.

P. acnes	conc. of red type FCAJ (mg/ml)					conc. of yellow type FCAJ (mg/ml)				
strain no.	2.5	5	10	20	40	2.5	5	10	20	40
1	-	-	-	-	-	-	-	-	-	-
2	-	-	-	-	-	-	-	-	-	-
3	-	-	-	-	-	-	-	-	-	-
4	-	-	-	-	-	-	-	-	-	-
5	+	-	-	-	-	-	-	-	-	-
6	+	-	-	-	-	-	-	-	-	-
7	-	-	-	-	-	-	-	-	-	-
8	-	-	-	-	-	-	-	-	-	-
9	-	-	-	-	-	-	-	-	-	-
10	-	-	-	-	-	-	-	-	-	-
11	-	-	-	-	-	-	-	-	-	-
12	-	-	-	-	-	-	-	-	-	-
13	-	-	-	-	-	-	-	-	-	-
14	-	-	-	-	-	-	-	-	-	-
15	-	-	-	-	-	-	-	-	-	-
16	-	-	-	-	-	-	-	-	-	-
17	-	-	-	-	-	-	-	-	-	-
18	-	-	-	-	-	-	-	-	-	-
19	-	-	-	-	-	-	-	-	-	-
20	-	-	-	-	-	-	-	-	-	-
21	+	-	-	-	-	-	-	-	-	-
22	-	-	-	-	-	-	-	-	-	-
23	-	-	-	-	-	-	-	-	-	-
24	-	-	-	-	-	-	-	-	-	-
25	-	-	-	-	-	-	-	-	-	-
26	-	-	-	-	-	-	-	-	-	-
27	+	-	-	-	-	-	-	-	-	-
28	-	-	-	-	-	-	-	-	-	-
29	-	-	-	-	-	-	-	-	-	-
**B. fragilis*	+	+	+	+	-	+	+	+	+	-

**B. fragilis* ATCC25285 ; + show bacterial growth ; - show bacterial inhibition

4. Effect of Antioxidants on Degradation of a Selected FCAJ ingredient

4.1 Assay of a Selected FCAJ Ingredient

Anacardic acids are thermolabile and decompose on heating to cardanols and carbon dioxide. A large proportion of unsaturated lipids (particularly the 1Z, 4Z dienes and 1Z, 4Z, 7Z triene) in anacardic acids structure (Appendix III) indicated the possibility of autooxidation (Shobha and Ravindranath, 1991). Therefore, the FCAJ creams might be stabilized by an antioxidant. Sodium metabisulfite was chosen as an antioxidant studied because it was effective at the pH of FCAJ dispersions. Propyl gallate was the other antioxidant studied due to its frequency use.

Active ingredients in FCAJ are anacardic acids and aroma components of aromatic flavor in cashew apple (Himejma and Kubo, 1991; Kubo et al., 1993; and Muroi, et al., 1993). Kubo et al. (1986) depicted a chromatogram of anacardic acids (Figure 4) extracted from the freeze-dried fruit juice using hexane. Peaks 4, 5, and 6 are peaks of anacardic acids. In this study, FCAJ ingredients obtained from ethanol extract of FCAJ were analyzed. Peaks A, B, and C in the chromatogram in (Figure 5) was obtained when a HPLC condition employed were similar to that of Kubo et al. (1986). Both YMC pack ODS and LiChroCart columns are C-18 columns. YMC pack column (15 cm x 6 mm i.d.) has a particle size of 5 μm and LiChroCart columns (12.5 cm x 4 mm i.d.) also has a particle size of 5 μm. Since the alcoholic portion was used and the HPLC conditions were similar, it could be assumed that the peaks A, B, and C in Figure 5 were the peaks 4, 5, and 6, respectively, in Figure 4.

Figures 6 and 7 show chromatograms at 280 nm The mobile phase which was methanol : 10% acetic acid : acetonitrite was varied from 8 : 1 : 1 to 7 : 2 : 1. The retention times of the possible anacardic acids were reduced when the volume fraction of acetonitrile was increased. The chromatogram in Figure 8 was obtained when the flow rate was increased. When the wavelength was changed from 280 nm to 254 nm, the chromatogram in Figure 9 was obtained. The absorptivity of a FCAJ ingredient (peak C) at 254 nm was greater than that at 280 nm. Therefore, the wavelength of 254 nm was selected and the FCAJ

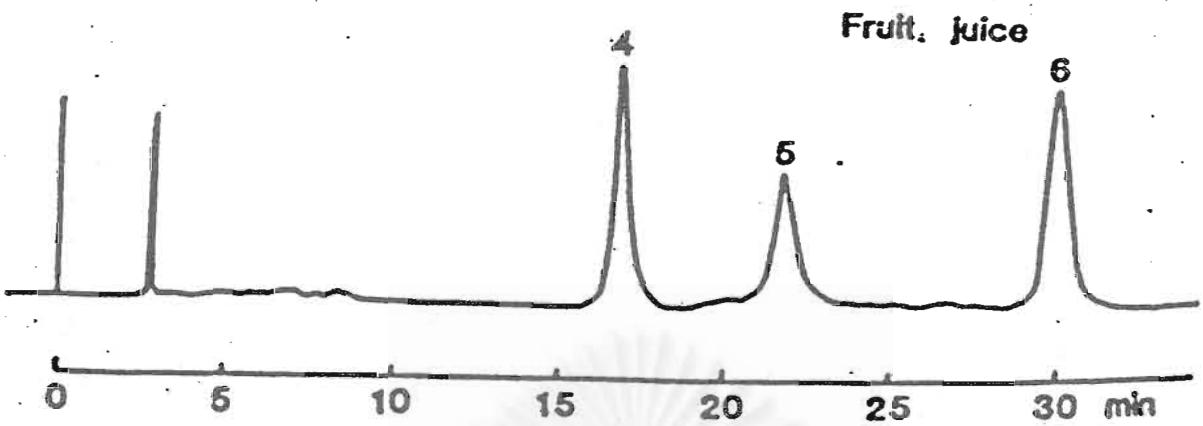


Figure 4 HPLC analyses of the components from the cashew fruit juice: column, YMC pack ODS (15 cm x 6 mm); mobile phase, methanol : 10% acetic acid (9:1); flow rate, 1 ml/min; detection, UV 280 nm.

From: Kubo, 1986, J. Agric. Food. Chem.

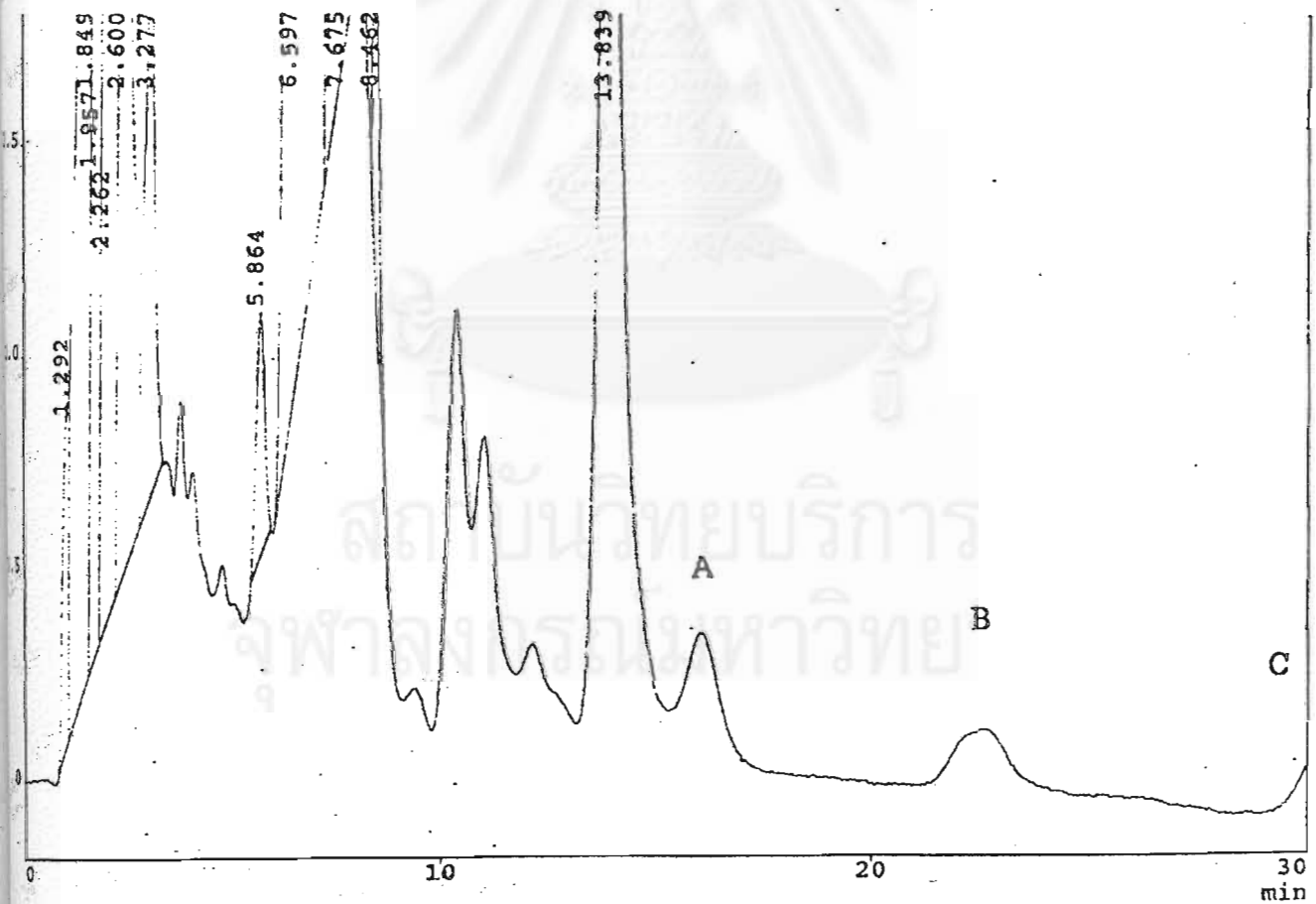


Figure 5 HPLC analyses of the components from the cashew fruit juice: column, LichroCart (125 mm x 4 mm); mobile phase, methanol : 10% acetic acid(9:1); flow rate, 1 ml/min; detection, UV 280 nm.

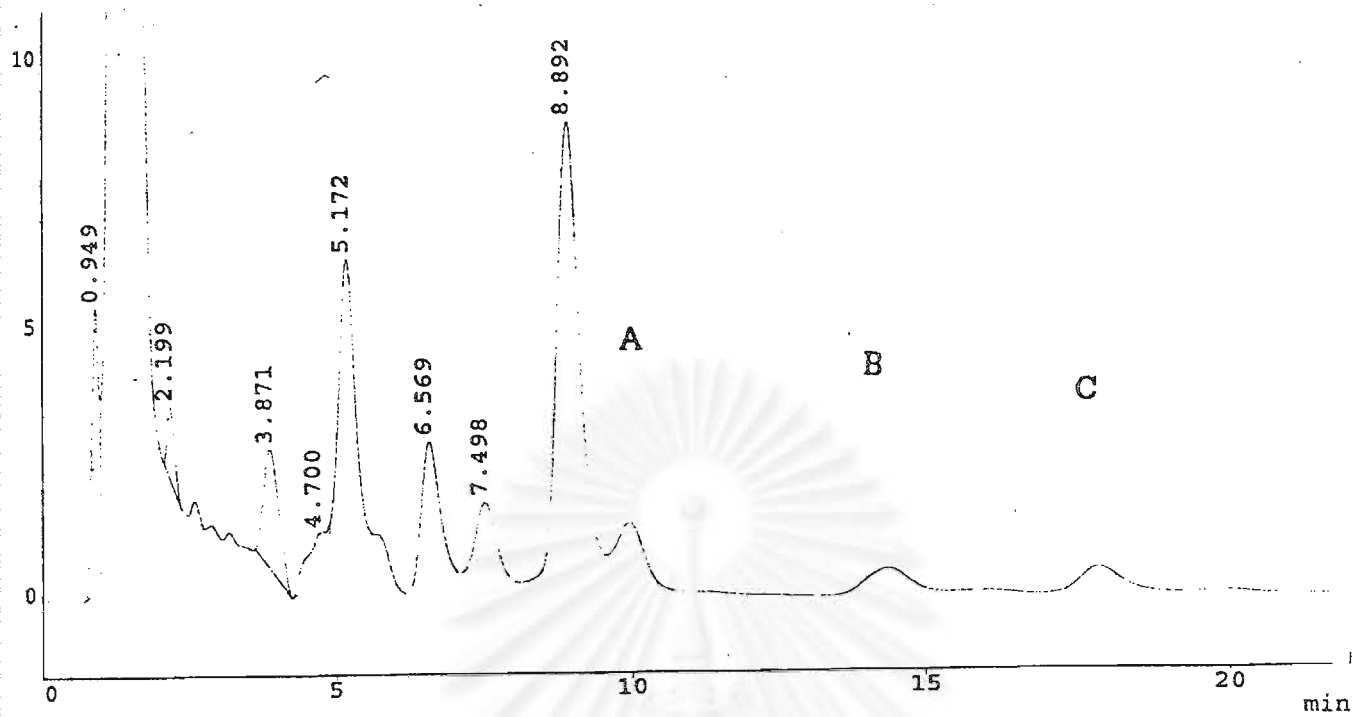


Figure 6 HPLC analyses of the components from the cashew fruit juice: column, LichroCart (125 mm x 4 mm); mobile phase, methanol : 10% acetic acid : acetonitrite (8:1:1); flow rate, 1 ml/min; detection, UV 280 nm.

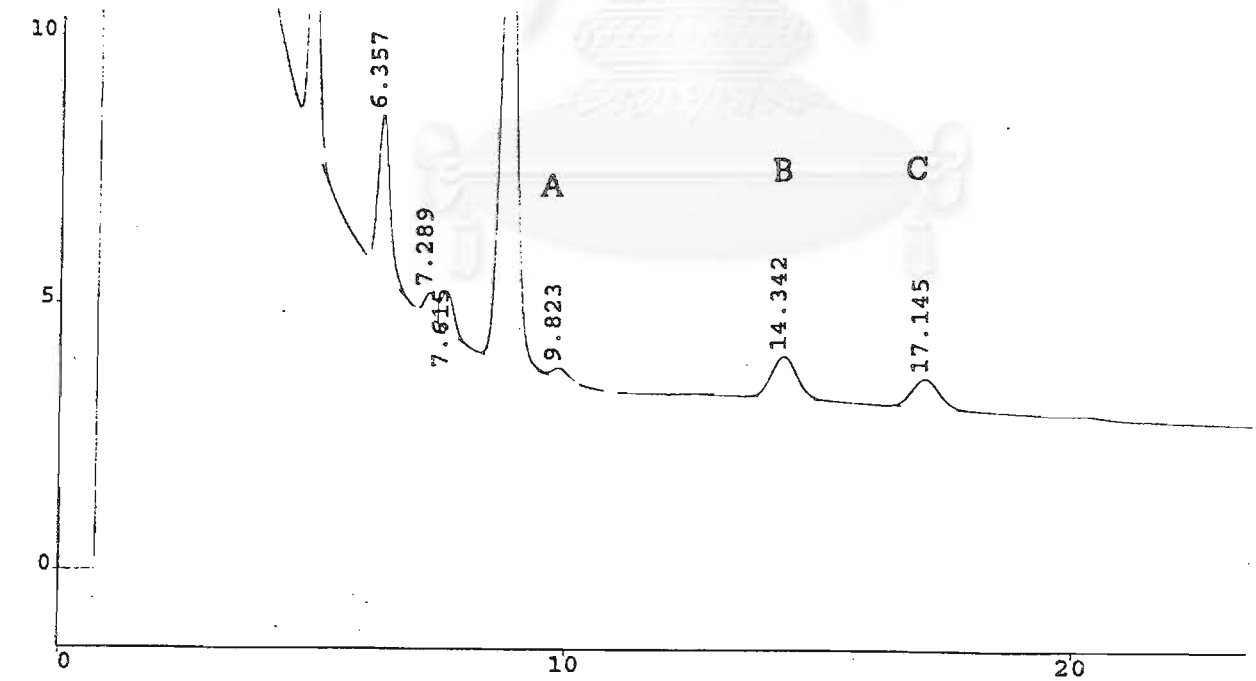


Figure 7 HPLC analyses of the components from the cashew fruit juice: column, LichroCart (125 mm x 4 mm); mobile phase, methanol : 10% acetic acid : acetonitrite (7:1:2); flow rate, 1 ml/min; detection, UV 280 nm.

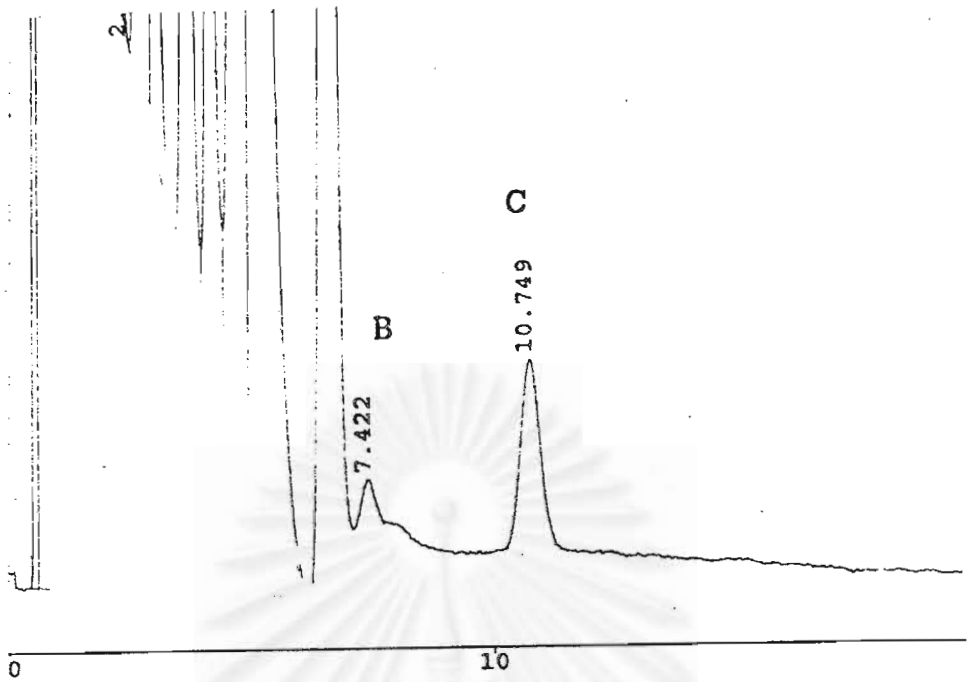


Figure 8 HPLC analyses of the components from the cashew fruit juice: column, LichroCart (125 mm x 4 mm); mobile phase, methanol : 10% acetic acid : acetonitrite (7:1:2); flow rate, 1.3 ml/min; detection, UV 280 nm.

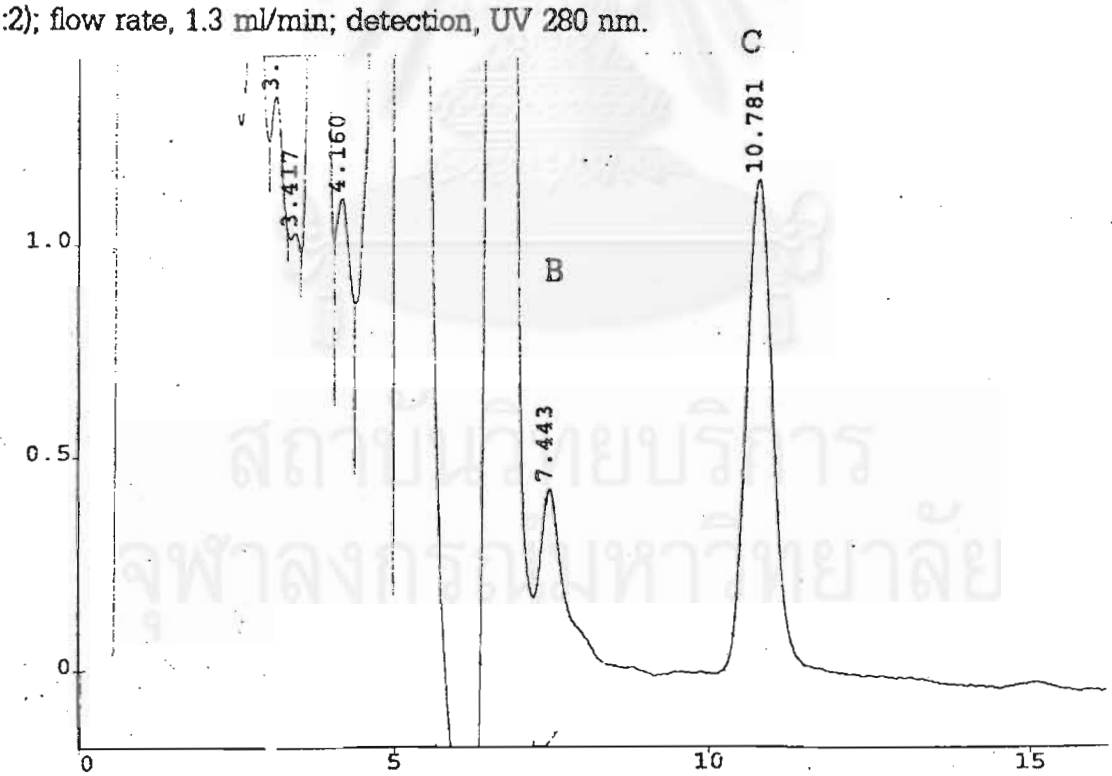


Figure 9 HPLC analyses of the components from the cashew fruit juice: column, LichroCart (125 mm x 4 mm); mobile phase, methanol : 10% acetic acid : acetonitrite (7:1:2); flow rate, 1.3 ml/min; detection, UV 254 nm.

ingredient at peak C was chosen for further analyses. The chromatogram in Figure 10 was obtained when cholesterol was used as an internal standard; the retention time of cholesterol was about 18 min (Peak D).

Histograms in Figure 11 display the FCAJ ingredient to cholesterol peak area ratios which were 0.3636 ± 0.0061 and 1.8113 ± 0.0288 for 25 %(w/w) dispersions of red type FCAJ and yellow type FCAJ, respectively. This finding was consistent with their antibacterial activities described previously, of which the yellow type FCAJ was more active against *S. aureus* when there was no viscosity effect. Furthermore, The MIC_{90} of yellow type FCAJ against *P. acnes* was less than MIC_{90} of the red type.

4.2 Degradation Kinetics of the Selected FCAJ Ingredient

The fundamental kinetic principles are mostly conveniently described by solution kinetics which are best elucidated. Orders and rates of reactions are two important parameters indicating the kinetics of a chemical reaction. The order of reaction determines the shape of the concentration-time profile of drugs or drug products, whereas the rate constant is determined by its slope (Connors et al., 1986).

When a plot of concentration of drug remaining in a degradation process versus time is a straight line, the reaction kinetic is described as zero order. The reaction kinetics is first-order when a plot of natural log of concentration versus time gives a straight line. While the second-order is the result of the straight line of a plot of 1/(concentration) versus time. In this study, however, a pure chemical of anacardic acids could not be obtained. Therefore, a calibration curve could not be drawn. Since a peak area ratio (PAR) of a drug to its internal standard from HPLC analysis was directly related to the drug concentration, the PAR could also be applied to use for the degradative kinetic determination. The linear regression analysis was used to determine the kinetic order of reaction and their details are shown in Appendix IV. Their correlation coefficients (r) are presented in Table 7. Since the reaction kinetics (zero-order, first-order, and second-order) could not be distinguished from the statistical standpoint, the zero-order was proposed because the shelf-life calculated would be the shortest and thus it would be safe for a patient. As the zero-order kinetics was assumed, the rate constants (k)

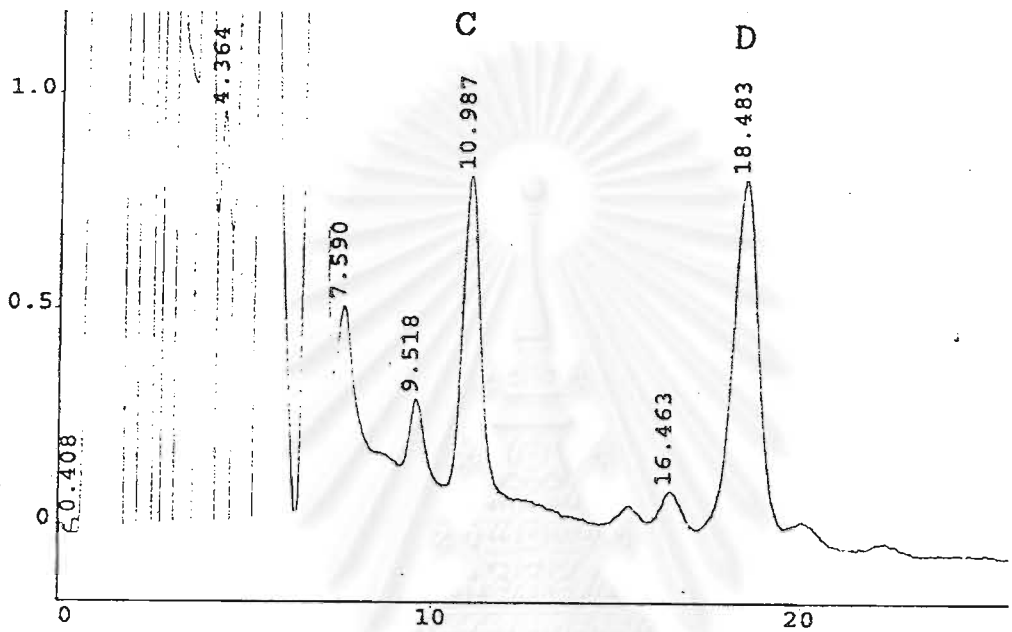


Figure 10 HPLC analyses of the components from the cashew fruit juice and cholesterol: column, LichroCart (125 mm x 4 mm); mobile phase, methanol : 10% acetic acid : acetonitrite (7:1:2); flow rate, 1.3 ml/min; detection, UV 254 nm.

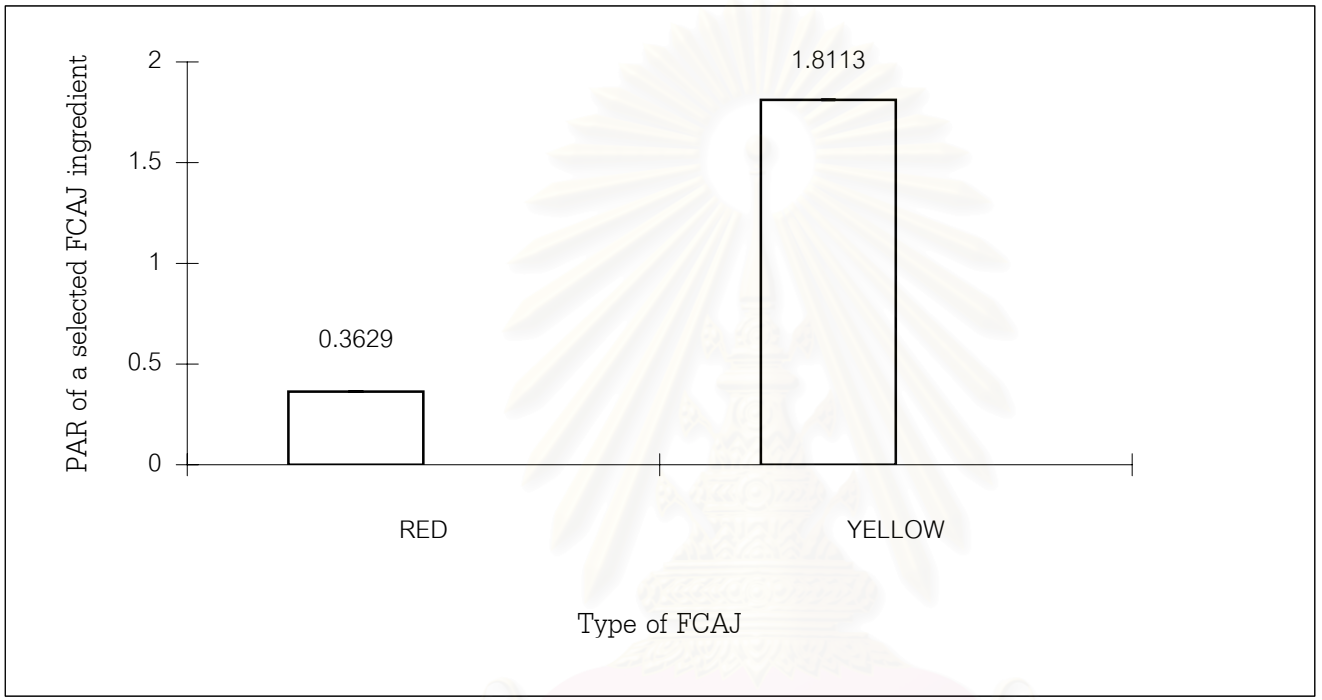


Figure 11 Peak area ratios of the selected ingredient from red and yellow type FCAJ.

Table 7 Correlation coefficients (r) of regression lines of zero, first and second order kinetics.

Antioxidants added	Correlation coefficient (r)		
	Zero order	First order	Second order
None	0.969	0.972	0.971
0.05% Sodium metabisulfite	0.946	0.945	0.942
1.00% Sodium metabisulfite	0.961	0.961	0.958
0.01% Propyl gallate	0.933	0.936	0.938
0.10% Propyl gallate	0.919	0.927	0.933

Table 8 Zero order degradation rate constants of the selected FCAJ ingredient in the presence of antioxidants.

Antioxidant added	Rate constant (x 103 hr-1)	Estimated shelf-life (days)
None	8.75	47.62
0.05% Sodium metabisulfite	5.72	75.84
1.00% Sodium metabisulfite	5.86	71.1
0.01% Propyl gallate	6.15	67.75
0.10% Propyl gallate	6.28	66.35

would be obtained from the PAR versus time plots (Figure 12) and they are presented in Table 8. Furthermore, the autocorrelations and the distribution of all models could indicate good fitness of the model. The autocorrelations of all models displayed in Appendix IV show no outliers. The TSPlots of all models also showed equal variances and normal distributions of the data. So, all models displayed good fitness.

4.3 Effect of Antioxidants

4.3.1 Effect of Sodium Metabisulfite

Sodium metabisulfite is an antioxidant that has been widely used. It has been used in formulation pH range of 3.5-5. The pH of FCAJ dispersion was 4.55. Two concentration levels of sodium metabisulfite (0.05 and 1.00% w/v) were studied.

When 0.05 and 1.00% w/v of sodium metabisulfite were added to FCAJ dispersions, the degradation rate constants were decreased significantly as indicated by ANCOVA in Appendix IV. However, two concentrations of sodium yielded comparable rate constants from the statistical standpoint. The estimated shelf-life of FCAJ dispersions containing sodium metabisulfite were longer than that of FCAJ dispersion.

4.3.2 Effect of Propyl Gallate

Propyl gallate was a free radical inhibitor studied because they have been widely used. Two concentration levels of propyl gallate (0.01 and 0.10% w/v) were studied.

When 0.01 and 0.10% w/v propyl gallate were added to the FCAJ dispersion, the degradation rate constants were reduced. However, the ANCOVA analysis shown in Appendix IV informed that the decreases in rate constants were not significant. The estimated shelf-life of FCAJ dispersions containing propyl gallate were longer than the FCAJ dispersion alone but they were shorter than the ones containing sodium metabisulfite. Therefore, 0.05% sodium metabisulfite sholud be included in the formulation.

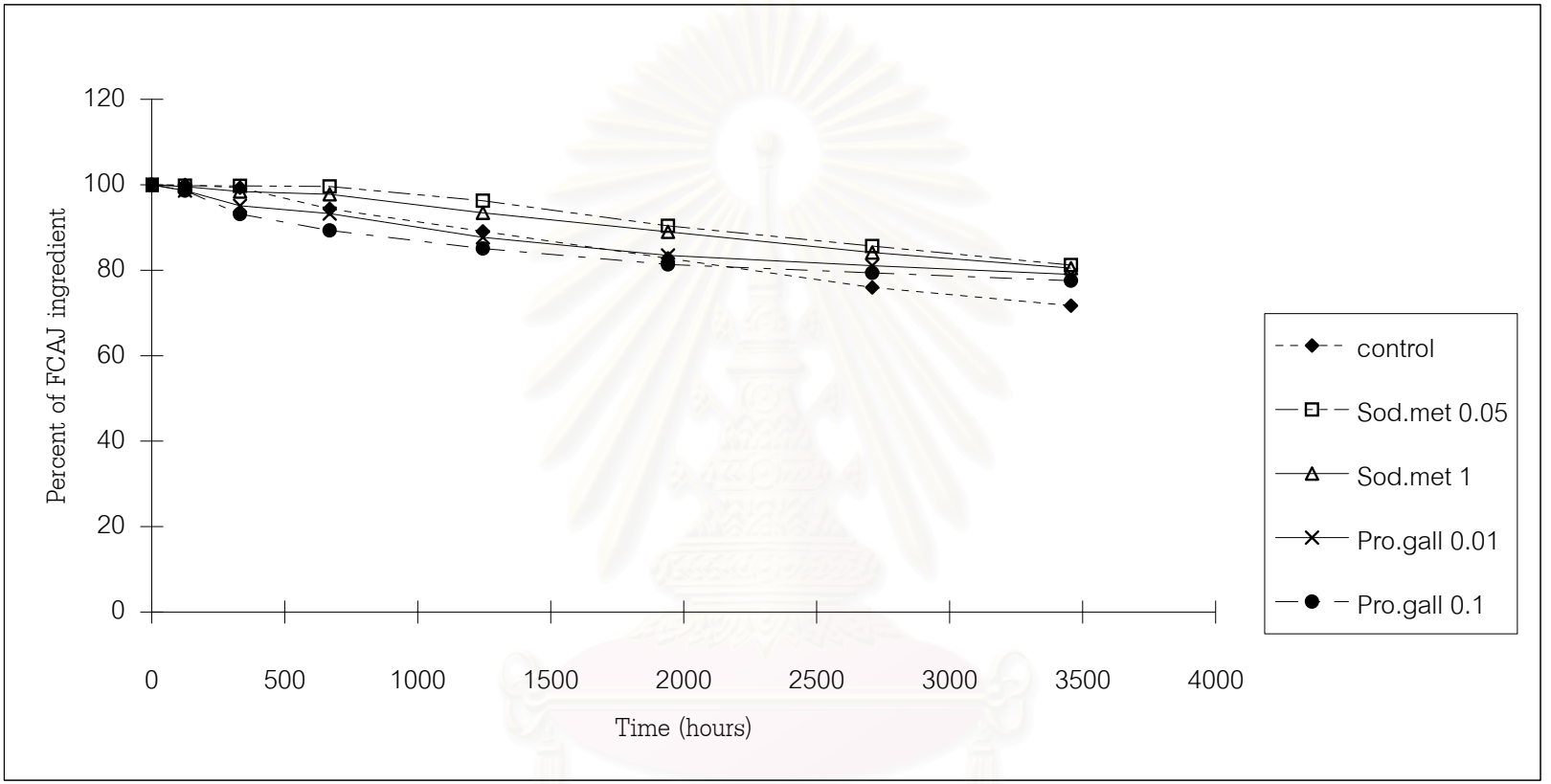


Figure 12 Effect of antioxidants on degradation of a selected FCAJ ingredient.

5. Formulation of FCAJ Cream

5.1 Selection of O/W Cream Base

Brij 72® and Brij 721® were chosen as emulsifiers in this o/w cream base because it could emulsify Arlamol S7®. Arlamol S7® was chosen as a volatile silicone because it provided excellent spreadability and improved skin-feel. Keltrol® yielded smooth and light cream. Glycerol was used as a humectant and Germaben II-E® was used as a preservative which were useful for the cream. Criteria for selection of an oil in water cream base were based on the data in Tables 9 and 10. The glossy cream bases with appropriate thickness were chosen. The chosen base should neither be oily nor leave white residue after application. The viscosity and pH of a preferred cream base should not change after six heating-cooling cycles.

Brij 72® and Brij 721® at ratios of 2:1 (formulation no. 1), 2:2 (formulation no. 2), and 3:1 (formulation no. 3) could not form stable creams. Whereas, Brij 72® and Brij 721® at ratios exceeding 3:2 (formulation no. 4-18) could form stable creams. Increasing Brij 72® fraction would make the creams glossier. Arlamol S7® is a combination of cyclomethicone and polyoxypropylene-15 stearyl ether that acts as volatile silicone and emollient-solvent, respectively. If more than 10% of Arlamol S7® was added into the formulations (formulation no. 4-9), the skin would feel oily and tacky after application. Cetostearyl alcohol was used as a thickening agent and stabilizer in the formulations. When 2.5 and 3 g of cetostearyl alcohol were added into the formulations containing Brij 72® and Brij 721® at a ratios of less than 4:2 (formulation no. 4, 7, 8, and 10), white residue occurred after application.

Six heating-cooling cycles did not seem to affect pH and viscosities of the cream bases (Table 10). The pH values of o/w cream bases was slightly increased, while their viscosities was slightly decreased after six heating-cooling cycles.

Formulation number 15 was chosen as the best o/w cream base due to its good texture, good skin-feel and little change after six heating-cooling cycles.

Table 9 Appearances of o/w cream bases after two-day storage.

Appearances	Formulation no.											
	1	2	3	4	5	6	7	8	9	10	11	12
Texture of cream:												
Glossy	separation of oil from cream			++	++	++	++	++	++	+	++	++
Fluidity				+	++	++	+	+	+	+	+	+
Skin-feel after application:												
Tackiness				++	+	-	++	++	+	++	+	-
Oiliness	separation of oil from cream			++	++	++	+	+	+	-	-	-
White residue				+	-	-	+	+	-	+	-	-

Appearances	Formulation no.					
	13	14	15	16	17	18
Texture of cream:						
Glossy	++	+++	+++	+++	+++	++
Fluidity	++	+	-	++	+	+++
Skin-feel after application:						
Tackiness	-	-	-	-	-	-
Oiliness	-	-	-	-	-	-
White residue	-	-	-	-	-	-

- lowest ; +++ highest

Table 10 pH and viscosities of o/w cream bases after six heating-cooling cycles.

Formulation no.	before 6 heating-cooling cycles		after 6 heating-cooling cycles	
	pH	viscosity (cps)	pH	viscosity (cps)
7	4.37	162391.60	4.69	139192.80
8	4.42	158459.60	4.77	152168.40
9	4.63	151185.40	4.91	137816.60
10	4.41	168093.00	4.76	157673.20
11	4.69	164161.00	5.03	150202.40
13	5.38	157673.20	5.64	138209.80
14	4.32	158066.40	4.66	152561.60
15	4.34	157476.60	4.67	151775.20
16	4.54	158263.00	4.91	150399.00
17	4.92	158066.40	4.89	139192.80

5.2 Selection of W/O Cream Base

Dow corning 5200® (laurylmethicone polyol) was chosen as an emulsifier studied in the cream base because could provide products that contained a small amount of wax and the products obtained offered a light spreadable feel on the skin. Selection of the best w/o cream base was based on the data in Tables 11 and 12. The selection criteria were similar to those of the o/w cream bases. Table 11 informs the appearances of all w/o cream bases after two-day storage. Dow corning 5200® that was used as a silicone emulsifier was able to form cream. However, the creams formed were influenced by water content. Increasing water content would yield greater viscosity, which was in agreement with Forster (1997). When the water content was greater than 79.8%, the creams could not be formed (formulation number 3 and 4) or it would be hardened (formulation no. 5).

Cyclomethicone alone could not form creams (formulation no. 9 and 10) as the separation of the oil and water phases occurred. Arlamol S7® alone was able to form cream (formulation no. 1, 2, 6, 7, and 8); however, the creams obtained were poorly spreaded on the skin. Depended upon the ratios of Arlamol S7® and cyclomethicone, their combination could make a well spreaded cream. The formulations with more than 10% Arlamol S7® (formulation no. 14-17) yielded poorly spreaded creams, while less than 10% Arlamol S7® (formulation no. 11-12) would yielded too fluid creams. The Arlamol S7® and cyclomethicone ratio of 10:5 (formulation no. 13 and 18) was appropriate for making well spreaded and beautiful texture creams. Dimethicone was used as an emollient and for thickness increment. Cream with 1% dimethicone (formulations no. 18) was tacky. Therefore, 0.1% of dimethicone was used.

Table 12 shows pH and viscosities of the chosen w/o cream bases before and after six heating-cooling cycles. Both pH and viscosities decreased after the six heating-cooling cycles. Especially, the reduction in viscosities were very significant. Forster (1997) also showed that

Table 12 pH and viscosities of w/o cream bases after six heating-cooling cycles.

Formulation no.	before 6 heating-cooling cycles		after 6 heating-cooling cycles	
	pH	viscosity (cps)	pH	viscosity (cps)
1	4.57	160425.60	3.08	75985.90
6	4.9	188342.80	4.22	80999.20
7	5.07	167896.40	3.59	79033.20
8	4.46	166323.60	3.28	68810.00
11	6.76	129362.80	separation of oil from cream	
12	5.69	128576.40	4.82	78541.70
13	5.47	151185.40	4.64	77067.20
14	5.71	152954.80	4.17	79131.50
15	5.47	124644.40	4.32	78738.30
17	5.43	156100.40	4.47	76280.80
18	5.04	150202.40	5.02	82375.40

the viscosities of silicone emulsions containing water were decreased when they were kept at higher temperature. Consequently, formulation no. 13 was chosen for further study.

5.3 Formulation of FCAJ Creams

Both red and yellow types of FCAJ at concentrations of 0.25, 0.5, 1, and 2%, respectively, in the selected o/w and w/o cream bases were studied (Tables 13 and 14). The inclusion of FCAJ did not significantly affect the pH and viscosities of the o/w creams. The pH and viscosities of o/w FCAJ creams slightly increased after six heating-cooling cycles.

FCAJ reduced the pH of w/o creams, but did not have much effect on their viscosities. The heating-cooling cycles did not affect pH of w/o FCAJ creams significantly, but decreased its viscosities greatly.

6. Susceptibility of *S. aureus* and *P. acnes* to FCAJ Creams

The susceptibility tests of *S. aureus* and *P. acnes* to o/w and w/o creams containing FCAJ as compared with their cream bases and Panoxyl 5® gel were evaluated using agar diffusion methods.

6.1 Susceptibility Tests of *S. aureus* and *P. acnes* to FCAJ o/w Cream

6.1.1 Susceptibility Test of *S. aureus*

The inhibition zone sizes from varied concentration of FCAJ in o/w creams as compared with the cream base and Panoxyl 5® gel are shown in table 15 and Figure 13. Only *S. aureus* strain no. 6, 15, and 19 were resistant to o/w cream base and FCAJ creams but not resistant to Panoxyl 5® gel. The inhibition zone diameters from both red and yellow type FCAJ creams and Panoxyl 5® gel were slightly larger than those from o/w cream base. A possible explanation was that the preservative in all formulations showed activity against *S. aureus*,

Table 13 pH and viscosities of o/w FCAJ creams after six heating-cooling cycles.

FCAJ cream	before 6 heating-cooling cycles		after 6 heating-cooling cycles	
(%w/w)	pH	viscosity (cps)	pH	viscosity (cps)
Yellow type				
0.25	4.31	146073.80	4.86	159835.80
0.5	4.01	148826.20	4.55	156690.20
1	4.49	167896.40	4.65	164554.20
2	4.53	173794.40	4.89	168486.20
Red type				
0.25	4.06	153151.40	4.65	164357.60
0.5	4.24	159639.20	4.79	165537.20
1	4.12	148236.40	4.28	165733.80
2	4.34	155117.40	4.53	162981.40

Table 14 pH and viscosities of w/o FCAJ creams after six heating-cooling cycles.

'CAJ cream (%w/w	before 6 heating-cooling cycles		after 6 heating-cooling cycles	
	pH	viscosity (cps)	pH	viscosity (cps)
Yellow type				
0.25	4.42	152365.00	4.54	79229.80
0.5	4.68	148826.20	4.24	74118.20
1	4.25	156493.60	4.39	82866.90
2	4.3	152561.60	4.38	81982.20
Red type				
0.25	3.91	168289.60	4.24	80999.20
0.5	4.38	172614.80	4.64	76575.70
1	3.92	176940.00	4.62	88470.00
2	4.65	161998.40	4.45	80016.20

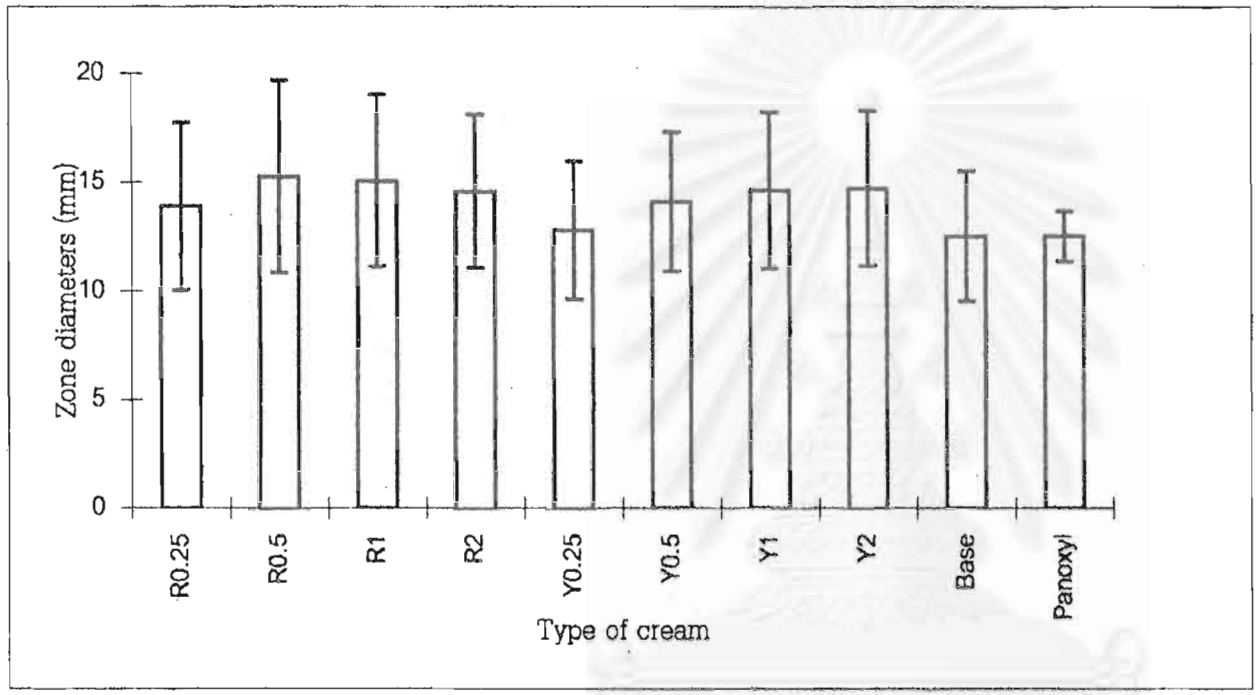
Table 15 Inhibition zone diameters against S. aureus of o/w creams containing red and yellow type FCAJ compared with the cream base and Panoxyl 5 gel.

S. aureus strain no.	Diameters of clear zone (mm)									
	%(w/w) of red type FCAJ in cream				%(w/w) of yellow type FCAJ in cream				Cream Base	Panoxyl 5 gel
	0.25	0.5	1	2	0.25	0.5	1	2		
ATCC 25923	12.1	13.4	9.4	10.2	12.9	14.1	15.9	13.9	12.6	15.3
2	16.4	20.1	19	19.5	13.9	15.3	14.8	15.5	14	11.7
3	10.9	11	9.5	10.7	8.8	9.1	11	11.6	8.7	12
4	9.2	9.2	11.1	10.8	9.3	10.6	11.4	11	9.2	12.4
5	9.1	10.7	12.2	11.7	8.8	10.7	10.3	11.2	9.2	11.6
6	NZ	NZ	NZ	NZ	NZ	NZ	NZ	NZ	NZ	12.5
7	10	11	9.4	11.7	9.1	10	9.5	9.5	8.9	12
8	9.2	10	11.4	9.2	10	11.1	12.7	12.7	10	13
9	10.2	10.5	10.8	12	10.3	10.5	10.7	10.8	10.7	12.3
10	10	10.2	12.7	11	9.8	12.4	12.3	10.9	9.6	12.8
11	10.2	10.7	10.2	10	9.3	9.8	10.6	10.8	9.6	12.5
12	15.9	19.7	15.9	15.4	11	13	10.9	12.4	11.2	13.4
13	9.6	10.5	9.8	10.6	10.7	13.3	12.7	12	9.8	12
14	10.8	11.5	11.3	11.6	10.8	11.3	10	10.1	10.3	13.8
15	NZ	NZ	NZ	NZ	NZ	NZ	NZ	NZ	NZ	12.5

Table 15 Inhibition zone diameters against S. aureus of o/w creams containing red and yellow type FCAJ compared with the cream base and Panoxyl 5 gel.

S. aureus strain no.	Diameters of clear zone (mm)									
	%(w/w) of red type FCAJ in cream				%(w/w) of yellow type FCAJ in cream				Cream Base	Panoxyl 5 gel
	0.25	0.5	1	2	0.25	0.5	1	2		
16	14.2	16.9	18.1	16	14.6	16.5	17.8	17.9	14.4	14
17	15.8	16.8	18.7	19.3	15.8	19.4	18.2	17.8	15.8	12.1
18	17.7	19	17.7	16	16.5	17	18.7	17.5	15	11.5
19	NZ	NZ	NZ	NZ	NZ	NZ	NZ	NZ	NZ	11.8
20	13.6	16.2	17.1	14	11.3	12	12.4	13.1	11.4	12
21	13.7	18.8	18.7	16.1	12.1	14.3	16.5	17.2	12.1	12.5
22	17.1	19.4	18.6	17.4	17	17.9	20.4	21	17.4	17.2
23	15.3	16	18	14.6	16.8	17.1	17.6	19.5	14.6	13.6
24	18.9	21	20	19.6	19	19.4	19.3	19	19	13
25	14.7	14.4	16.4	15.9	11.8	14	13.8	15.3	11.3	11.9
26	21.1	21.5	19.3	17.8	14.4	16	17.7	17.6	14	11.2
27	22.4	23.6	22.8	21.8	14.2	16	16.5	18.1	14.2	11.3
28	16.5	19	18.5	18.5	16.8	15.3	17.9	18.9	16.4	12.3
29	13.7	14.4	16.1	16	13.6	17.5	18.1	14.3	13	12.3
30	17.2	17.5	15.25	17.3	17.5	18.4	18.2	18.8	16.9	11.4

NZ = No inhibition zone



R0.25 = 0.25% (w/w) red FCAJ cream

R0.5 = 0.50% (w/w) red FCAJ cream

R1 = 1.00% (w/w) red FCAJ cream

R2 = 2.00% (w/w) red FCAJ cream

Y0.25 = 0.25% (w/w) yellow FCAJ cream

Y0.5 = 0.5% (w/w) yellow FCAJ cream

Y1 = 1.00% (w/w) yellow FCAJ cream

Y2 = 2.00% (w/w) yellow FCAJ cream

Base = o/w cream base

Panoxyl = 5% Panoxyl gel

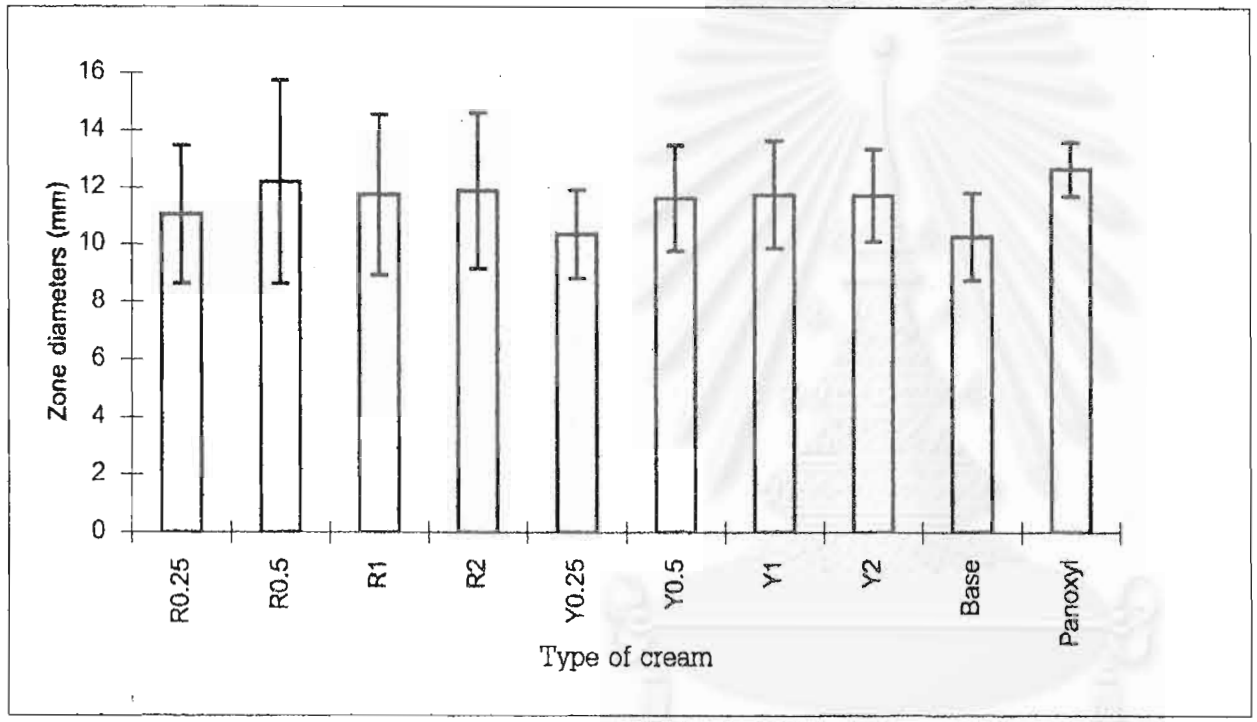
Figure 13 Zone diameters of FCAJ creams compared with the cream base and Panoxyl 5® gel against *S. aureus*.

Germaben II-E®, which was the preservative used in the o/w cream base and FCAJ creams, was composed of 0.1% propylparaben, 0.1% methylparaben and 0.2% diazolidinyl urea in propylene glycol. They are effective against *S. aureus*, gram-positive and gram-negative bacteria, and mold (Flick, 1991).

S. aureus strain no. 2-15 used were methicillin-sensitive *S. aureus* (MSSA) while *S. aureus* strain no. 16-30 used were methicillin-resistant *S. aureus* (MRSA). Thus, zones of inhibition were separately evaluated. The diameters of inhibition zones were not significantly different among all of the samples against MSSA (Figure 14). The zone diameters of Panoxyl 5® gel was the smallest, but it was not significantly different when it was compared with 0.25% yellow type FCAJ creams and cream bases (Figure 15). Nevertheless, the inhibition zone of cream base was significantly smaller than 0.5% and 1% red type FCAJ creams by using an analysis of variance and Student-Newman-Keuls tests for Post-Hoc comparisons (Appendix II). This result exhibited that MRSA were more susceptible to FCAJ than to Panoxyl 5® gel and the preserved cream. Muroi and Kubo (1996) reported that the growth of MRSA was inhibited by 6.25 μg/ml anacardic acids at any stage of growth.

6.1.2 Susceptibility Tests of *P. acnes*

Table 16 depicts the inhibition zones of varied concentrations of FCAJ creams compared with the cream base and Panoxyl 5® gel. *P. acnes* strain no.27 and *B. fragilis* ATCC 25285 were resistant to FCAJ creams and the cream base but not resistant to Panoxyl 5® gel. Generally, the inhibition zones of Panoxyl 5® gel were more than twice larger than the others (Figure 16). The zone diameters of the cream base was smaller than 0.5, 1 and 2% yellow type FCAJ creams. The diameters of inhibition zones from red and yellow type FCAJ creams were slightly different. Nevertheless, FCAJ creams from both red and yellow types were more effective against *P. acnes* than the cream base. Germaben II-E® used in the o/w cream base was also effective against *P. acnes*, gram positive anaerobic bacteria.



R0.25 = 0.25% (w/w) red FCAJ cream

R0.5 = 0.50% (w/w) red FCAJ cream

R1 = 1.00% (w/w) red FCAJ cream

R2 = 2.00% (w/w) red FCAJ cream

Y0.25 = 0.25% (w/w) yellow FCAJ cream

Y0.5 = 0.5% (w/w) yellow FCAJ cream

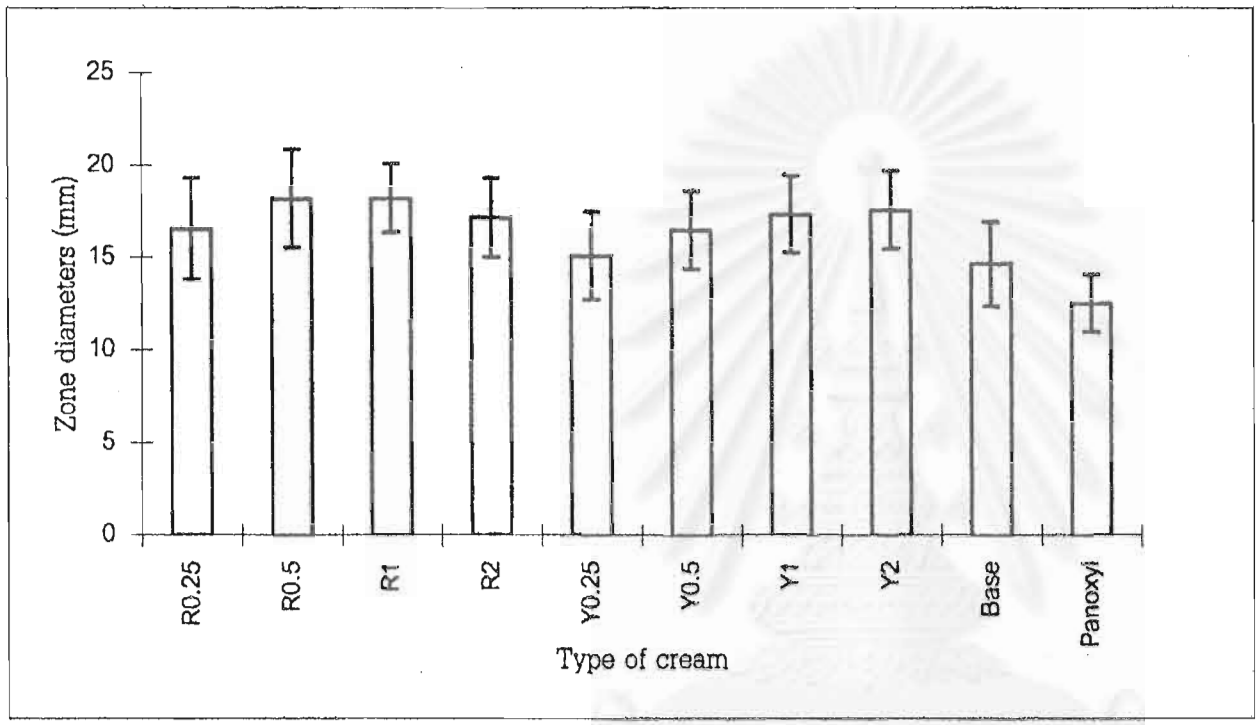
Y1 = 1.00% (w/w) yellow FCAJ cream

Y2 = 2.00% (w/w) yellow FCAJ cream

Base = o/w cream base

Panoxyl = 5% Panoxyl gel

Figure 14 Zone diameters of FCAJ creams compared with the cream base and Panoxyl 5® gel against MSSA.



R0.25 = 0.25% (w/w) red FCAJ cream

R0.5 = 0.50% (w/w) red FCAJ cream

R1 = 1.00% (w/w) red FCAJ cream

R2 = 2.00% (w/w) red FCAJ cream

Y0.25 = 0.25% (w/w) yellow FCAJ cream

Y0.5 = 0.5% (w/w) yellow FCAJ cream

Y1 = 1.00% (w/w) yellow FCAJ cream

Y2 = 2.00% (w/w) yellow FCAJ cream

Base = o/w cream base

Panoxyl = 5% Panoxyl gel

Figure 15 Zone diameters of FCAJ creams compared with the cream base and Panoxyl 5® gel against MRSA.

Table 16 Inhibition zone diameters against *P. acnes* of o/w creams containing red and yellow type FCAJ compared with the cream base and Panoxyl 5® gel.

P. acnes strain no.	Diameters of clear zone (mm)									
	%(w/w) of red type FCAJ in cream				%(w/w) of yellow type FCAJ in cream				Cream base	Panoxyl 5 gel
	0.25	0.5	1	2	0.25	0.5	1	2		
1	10.6	11.7	11.3	14.4	11.3	12	10.7	11.2	9.8	31.5
2	10.2	10.7	10.8	10.4	12.3	13.4	15	17.7	11.5	31.8
3	10.9	12	12.4	13	12.3	13.1	13.8	13.6	9.7	32
4	22.4	26.5	28.3	29.2	23.4	27.3	29.4	30.7	18.7	45.2
5	9.1	9.2	9.65	9.65	9.5	11	11.1	10.5	8.6	26.8
6	9.4	10.5	10.8	11.2	10.5	11.3	11.7	10	9.2	26.3
7	10.3	11.3	12.3	10.9	10.5	12.2	16.6	16.6	8.6	29.7
8	12.1	14.1	15.3	13.7	12.1	15.5	16.1	14.7	10.9	37.4
9	9.6	9.9	10	10.2	10.5	11.8	11.9	12.4	9.85	37.3
10	19.7	20.1	20.85	21.8	16.5	17.85	19.7	20.2	15	41.5
11	10.6	12.1	10.8	10.8	9	9.5	9.4	10	8.8	28.8
12	8.9	9.7	10.5	10.6	8.4	9.1	9.8	9.7	8.1	27
13	10.9	11.7	12	11.8	11.7	12.5	13.3	14	9.2	30.75
14	9.5	11.6	11.8	12	11.5	11.8	11	10.7	9.2	31.7
15	10.6	11	11.4	10.4	9.2	9.9	10.8	12	9.1	28.6

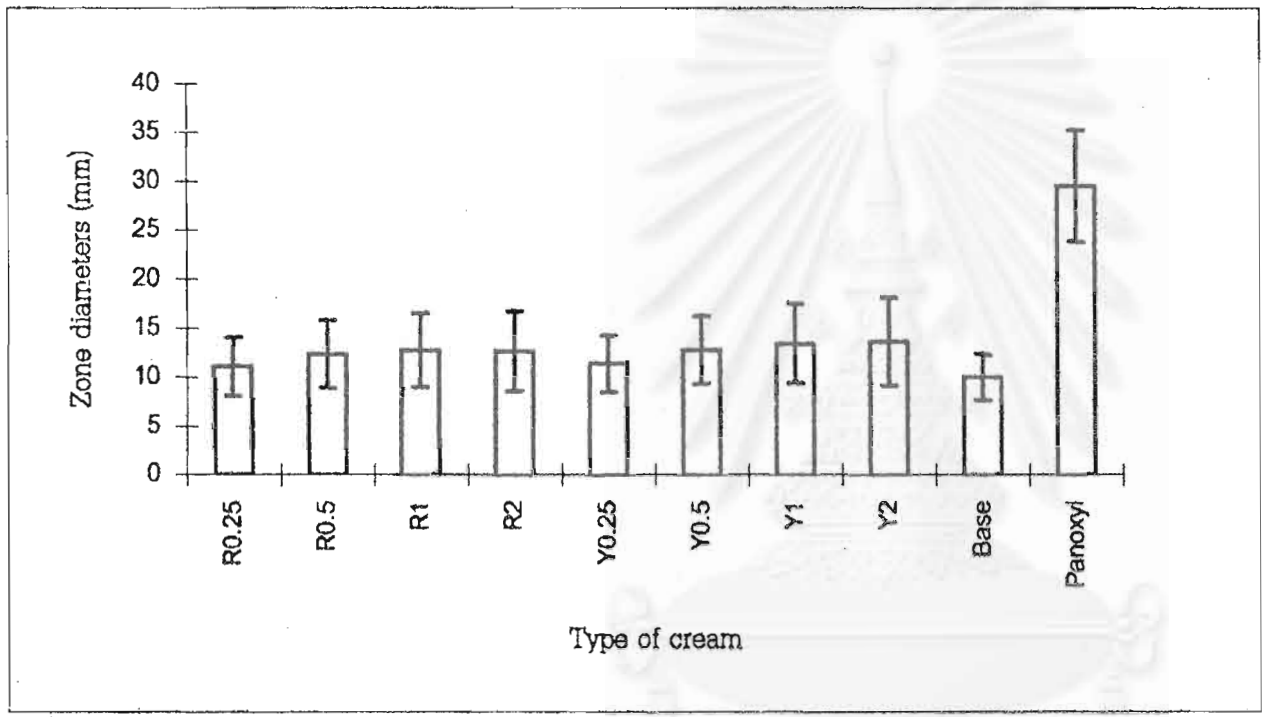
Table 16 Inhibition zone diameters against *P. acnes* of o/w creams containing red and yellow type FCAJ compared with the cream base and Panoxyl 5® gel.

P. acnes strain no.	Diameters of clear zone (mm)									
	%(w/w) of red type FCAJ in cream				%(w/w) of yellow type FCAJ in cream				Cream base	Panoxyl 5 gel
	0.25	0.5	1	2	0.25	0.5	1	2		
1	10.6	11.7	11.3	14.4	11.3	12	10.7	11.2	9.8	31.5
2	10.2	10.7	10.8	10.4	12.3	13.4	15	17.7	11.5	31.8
3	10.9	12	12.4	13	12.3	13.1	13.8	13.6	9.7	32
4	22.4	26.5	28.3	29.2	23.4	27.3	29.4	30.7	18.7	45.2
5	9.1	9.2	9.65	9.65	9.5	11	11.1	10.5	8.6	26.8
6	9.4	10.5	10.8	11.2	10.5	11.3	11.7	10	9.2	26.3
7	10.3	11.3	12.3	10.9	10.5	12.2	16.6	16.6	8.6	29.7
8	12.1	14.1	15.3	13.7	12.1	15.5	16.1	14.7	10.9	37.4
9	9.6	9.9	10	10.2	10.5	11.8	11.9	12.4	9.85	37.3
10	19.7	20.1	20.85	21.8	16.5	17.85	19.7	20.2	15	41.5
11	10.6	12.1	10.8	10.8	9	9.5	9.4	10	8.8	28.8
12	8.9	9.7	10.5	10.6	8.4	9.1	9.8	9.7	8.1	27
13	10.9	11.7	12	11.8	11.7	12.5	13.3	14	9.2	30.75
14	9.5	11.6	11.8	12	11.5	11.8	11	10.7	9.2	31.7
15	10.6	11	11.4	10.4	9.2	9.9	10.8	12	9.1	28.6

Table 16 Inhibition zone diameters against *P. acnes* of o/w creams containing red and yellow type FCAJ compared with the cream base and Panoxyl 5 gel (Continued).

P. acnes strain no.	Diameters of clear zone (mm)									
	%(w/w) of red type FCAJ in cream				%(w/w) of yellow type FCAJ in cream				Cream base	Panoxyl 5 gel
	0.25	0.5	1	2	0.25	0.5	1	2		
16	10	11	11.7	10.6	11.7	13.5	14.2	13.3	10.8	28.2
17	11	12.5	13	11.7	10.8	13.8	15.3	13.2	10	30.2
18	10.9	12	12	12.1	11.7	13	13.8	14	11.1	32.2
19	11.9	12.9	13.5	15	11	11.5	13.3	16.6	10	27.3
20	11.5	12.8	13.1	12.9	12.7	14.7	15	15.3	11	30.4
21	9.5	13	13.5	13	13.5	16.4	17.5	18.2	12.9	23
22	11.4	12.3	12.9	11.4	9.7	10.8	10.2	9.8	7.5	27.9
23	9.4	10.9	12.3	10.3	10.3	11.4	11.2	11.8	9	21.7
24	10.2	11.5	12.4	11	10.9	12.1	13	12.5	8.4	20.5
25	10	13.1	14.1	14.4	11.6	12.4	13.3	11.8	9.7	30.5
26	8.8	9.2	9.2	10	10	11.2	11.2	10.2	9.2	34.9
27	NZ	NZ	NZ	NZ	NZ	NZ	NZ	NZ	NZ	20.9
28	10.9	11.9	10	9.6	9	11	10	8.65	7.5	22.4
29	11	11.6	11.6	12.7	9.5	10.1	9	NZ	8.3	28.6
**B. fragilis*	NZ	NZ	NZ	NZ	NZ	NZ	NZ	NZ	NZ	23

**B. fragilis* ATCC25285 NZ = No inhibition zone



R0.25 = 0.25% (w/w) red FCAJ cream

R0.5 = 0.50% (w/w) red FCAJ cream

R1 = 1.00% (w/w) red FCAJ cream

R2 = 2.00% (w/w) red FCAJ cream

Y0.25 = 0.25% (w/w) yellow FCAJ cream

Y0.5 = 0.5% (w/w) yellow FCAJ cream

Y1 = 1.00% (w/w) yellow FCAJ cream

Y2 = 2.00% (w/w) yellow FCAJ cream

Base = o/w cream base

Panoxyl = 5% Panoxyl gel

Figure 16 Zone diameters of FCAJ creams compared with the cream base and Panoxyl 5® gel against *P. acnes*.

6.2 Susceptibility Tests of *S. aureus* and *P. acnes* to FCAJ w/o Creams

The results of susceptibility tests of *S. aureus* and *P. acnes* to w/o creams containing FCAJ as compared with the cream base and Panoxyl 5® gel showed no inhibition zones from all samples observed. A possible explanation was that FCAJ and Germaben II-E® used as a preservative were in the water phase and wrapped with the silicone. Thus, they could not dissolved in water from the agar medium and did not have a chance to act.

7. HPLC Method Validation

7.1 Specificity of the Method

Chromatograms of varied amount of the selected FCAJ ingredient and cholesterol are shown in Figure 17. FCAJ ingredient and cholesterol were eluted at 10-11 min and 17-18 min, respectively.

A stability indicating assay is an important methodology to ensure that the capability of the method used in the stability studies is high enough to separate the parent drug from its decomposition products. However, the degradation product(s) could not be seen in this study although the decomposition of the selected ingredient in FCAJ dispersion was about 60-70% as seen in Figure 18. There was no interference from the peak(s) of degradation product(s) during the time period studied.

Furthermore, sodium metabisulfite and propyl gallate added to the FCAJ dispersions did not interfere with the peak of the selected FCAJ ingredient (Figure 19).

7.2 Linear Correlation

Table 17 shows PAR and amount data of FCAJ. The plot of FCAJ amount versus the peak area ratio of the selected FCAJ ingredient to its internal standard (Figure 20) showed

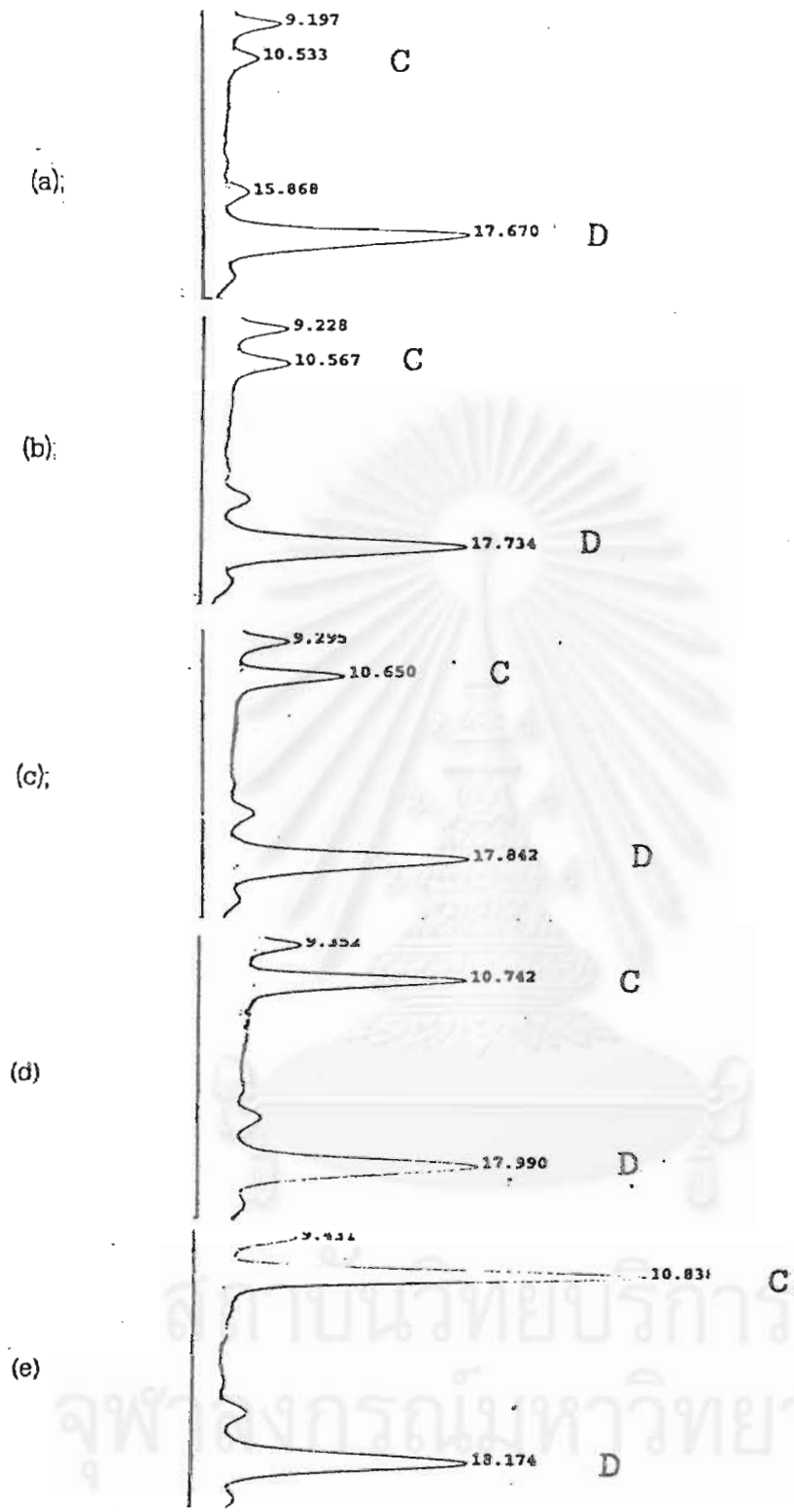


Figure 17 HPLC chromatograms of a selected FCAJ ingredient: 31.25 mg (a); 62.50 mg (b); 125.00 mg (c); 250.00 mg (d); and 500.00 mg (e).

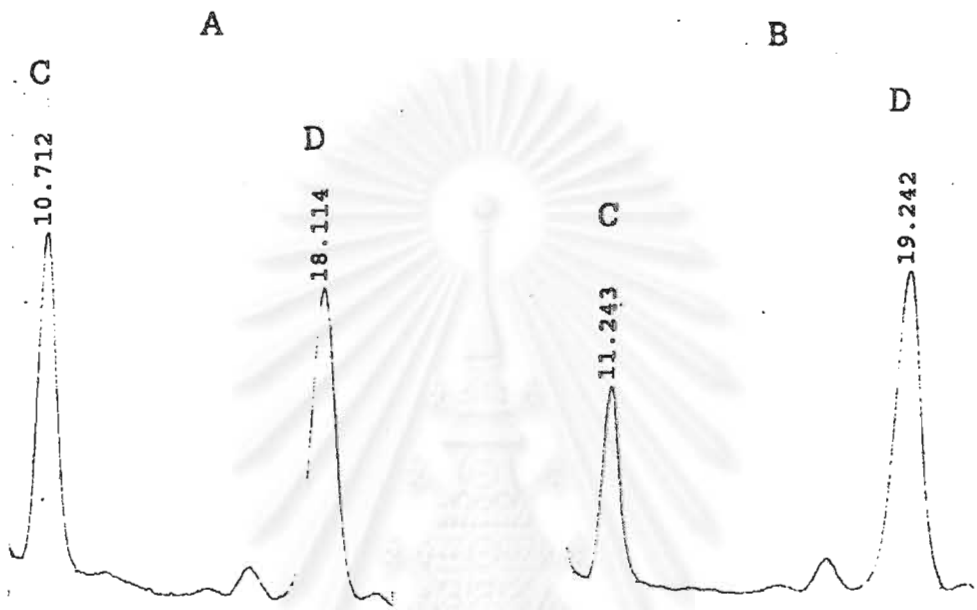


Figure 18 HPLC chromatograms of a selected FCAJ ingredient analysed at 0 (A) and 4 (B) month, respectively.

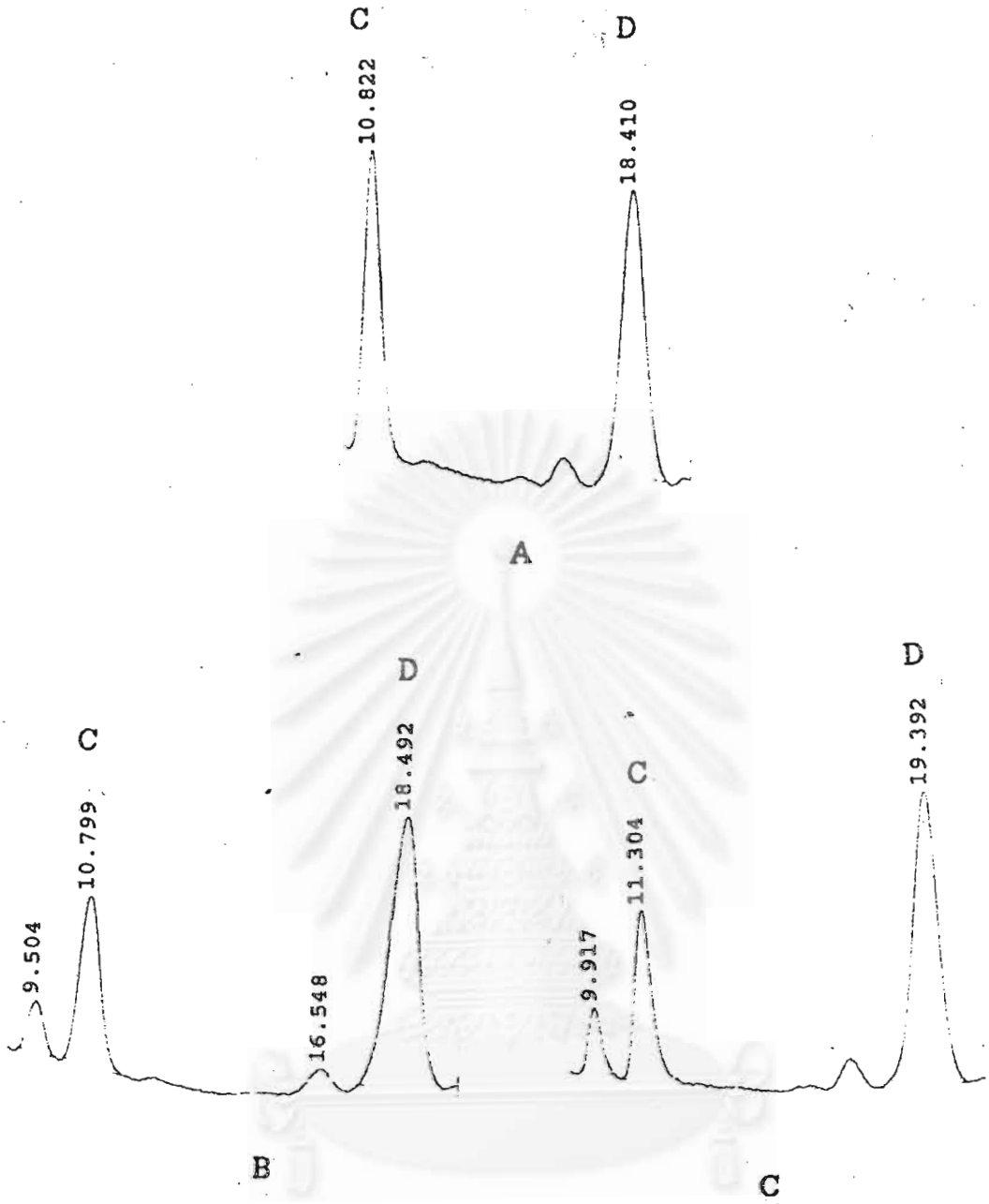


Figure 19 HPLC chromatograms of a selected FCAJ ingredient: no antioxidant at 0 month (A); with sodium metabisulfite at 6 month (B); and with propyl gallate at 6 month (C).

Table 17 Data for correlation curve of PAR and amount of FCAJ.

Amount of FCAJ (mg)	Peak area ratios		%CV
	Mean	SD	
31.25	0.0870	0.0039	4.4682
62.50	0.1711	0.0016	0.9129
125.00	0.3580	0.0045	1.2696
250.00	0.6902	0.0120	1.7326
500.00	1.3602	0.0061	0.4460

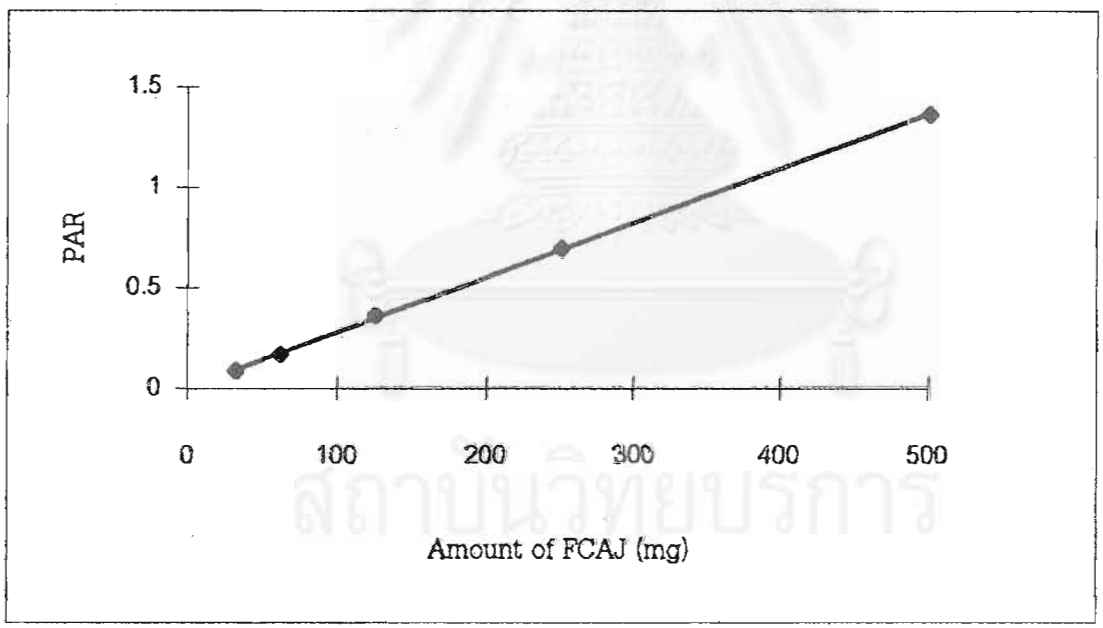


Figure 20 The correlation curve of PAR and amount of FCAJ.

$$PAR = 0.0027 \text{ Amount} + 0.0077$$

$$R^2 = 0.9998$$

Table 18 Within-run precision data.

Amount of FCAJ (mg)	Peak area ratios		%CV
	Mean	SD	
31.25	0.0870	0.0039	4.4682
62.50	0.1711	0.0016	0.9129
125.00	0.3580	0.0045	1.2696
250.00	0.6902	0.0120	1.7326
500.00	1.3602	0.0061	0.4460

Table 19 Between-run precision data.

Amount of FCAJ (mg)	Peak area ratios		%CV
	Mean	SD	
31.25	0.0894	0.0032	3.5427
62.50	0.1793	0.0067	3.7507
125.00	0.3567	0.0023	0.6395
250.00	0.6944	0.0240	3.5112
500.00	1.3677	0.0068	0.4977

linear correlation in the amount range studied, 31.25-500.00 mg. The correlation coefficient (r^2) of this line was 0.9998.

7.3 Precision of the Method

The data of within-run and between-run precision are shown in Tables 18 and 19, respectively. The coefficient of variation values of each amount of FCAJ indicated that this method gave the relatively unchanged results for analyzing the peak area ratio of the selected FCAJ ingredient and its internal standard any time.

7.4 Accuracy of the method

The percentages of analytical recovery of each drug amount are shown in Table 20. All of the mean values were comparable indicating that the percentage of analytical recovery did not depend on drug amount. The mean value of % analytical recovery was 102.71% with % CV of 2.68 indicated the high accuracy of this method. Therefore, it could be used for analysis of the selected FCAJ ingredient.

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Table 20 Percentages of analytical recovery of the seleced FCAJ ingredient.

Known amount of FCAJ (mg)	Amount calculated from calibration curve (mg)	% Analytical recovery
31.25	31.99	102.37
	30.92	98.94
	33.77	108.06
62.50	63.73	101.97
	62.70	100.31
	63.66	101.85
125.00	132.84	106.27
	134.1	107.28
	130.77	104.64
250.00	254.55	101.82
	260.47	104.19
	251.81	100.72
500.00	504.58	100.91
	501.21	100.24
	505.47	101.09
		Mean = 102.71
		SD = 2.75
		%CV = 2.68

CHAPTER V

CONCLUSION

The results of susceptibility tests of FCAJ dispersions and creams containing FCAJ against *S. aureus* and *P. acnes* can be summarized as follows:

1. Both red and yellow type FCAJ could inhibit the growth of *S. aureus* as evaluated by agar diffusion technique. At low concentrations, inhibition zone diameters obtained from the yellow type FCAJ were larger than those obtained from red type, while at high concentrations, the zone diameters obtained from red type FCAJ were larger than those obtained from yellow one.

2. The agar dilution technique was used in the susceptibility testing of red and yellow type FCAJ against *P. acnes*. The MIC_{90} of the red type FCAJ was equal to or less than 5 mg/ml, while that of the yellow type FCAJ was less than 2.5 mg/ml.

3. The selected o/w cream base was composed of 4% Brij 72®, 2% Brij 721®, 10% Arlamol S7®, 3% cetostearyl alcohol, 0.1% Ketrol®, 4% glycerol, 1% Germaben II-E® and 0.05% sodium metabisulfite. And the selected w/o cream base was composed of 2% DC 5200®, 10% Arlamol S7®, 5% cyclomethicone, 0.1% dimethicone, 0.1% Ketrol®, 4% glycerol, 1% Germaben II-E® and 0.05% sodium metabisulfite.

4. The w/o cream base and creams containing FCAJ could not inhibit the growth of *S. aureus* and *P. acnes* when there were evaluated by the cup agar diffusion method.

5. The susceptibility of MSSA to red and yellow type FCAJ o/w creams, o/w cream base, and Panoxyl 5® gel were not significantly different. However, the inhibition zone diameters of the red and yellow type FCAJ o/w creams against MRSA were larger than the cream base and Panoxyl 5® gel.

6. The inhibition zone diameters obtained from Panoxyl 5® gel against *P. acnes* were larger than those obtained from the cream base and the red and yellow type FCAJ o/w creams.

7. The susceptibility study showed that 1% yellow type FCAJ o/w cream should be chosen for antiacne purpose.

This study concentrated on the antimicrobial activities against *S. aureus* and *P. acnes*. However, there are other areas that FCAJ can be applied, and more work needs to be done, e.g., the extraction of FCAJ for pure anacardic acids, the stability study, other activities of FCAJ. Further research will be, therefore, helpful.

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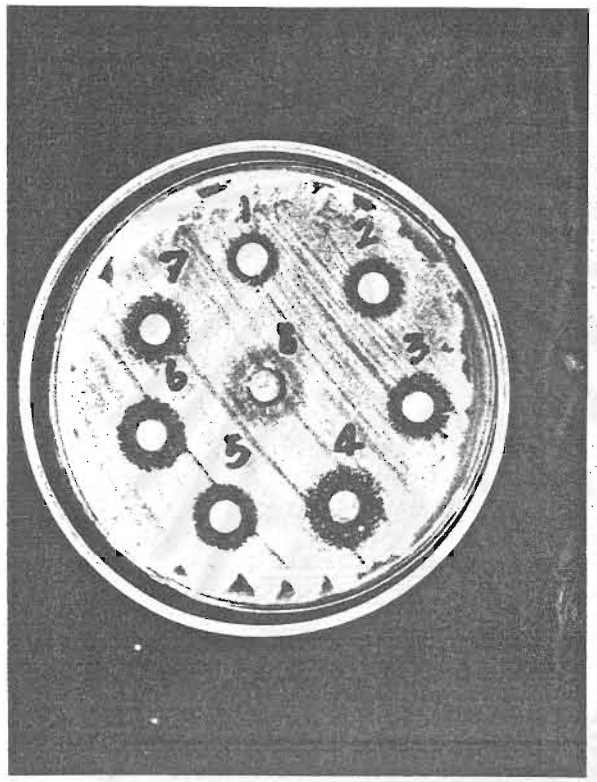
APPENDIX I

Susceptibility Tests

SUSCEPTIBILITY TEST OF FCAJ TO *S. AUREUS* BY DISK DIFFUSION METHOD

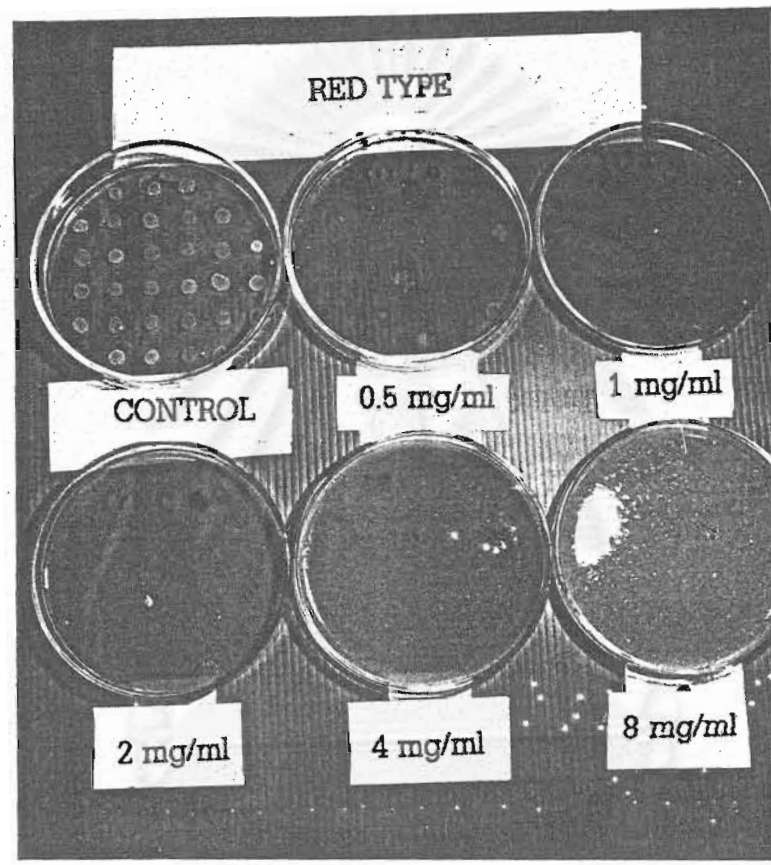


1 = red FCAJ 2 mg/disk; 2 = red FCAJ 4 mg/disk; 3 = red FCAJ 8 mg/disk;

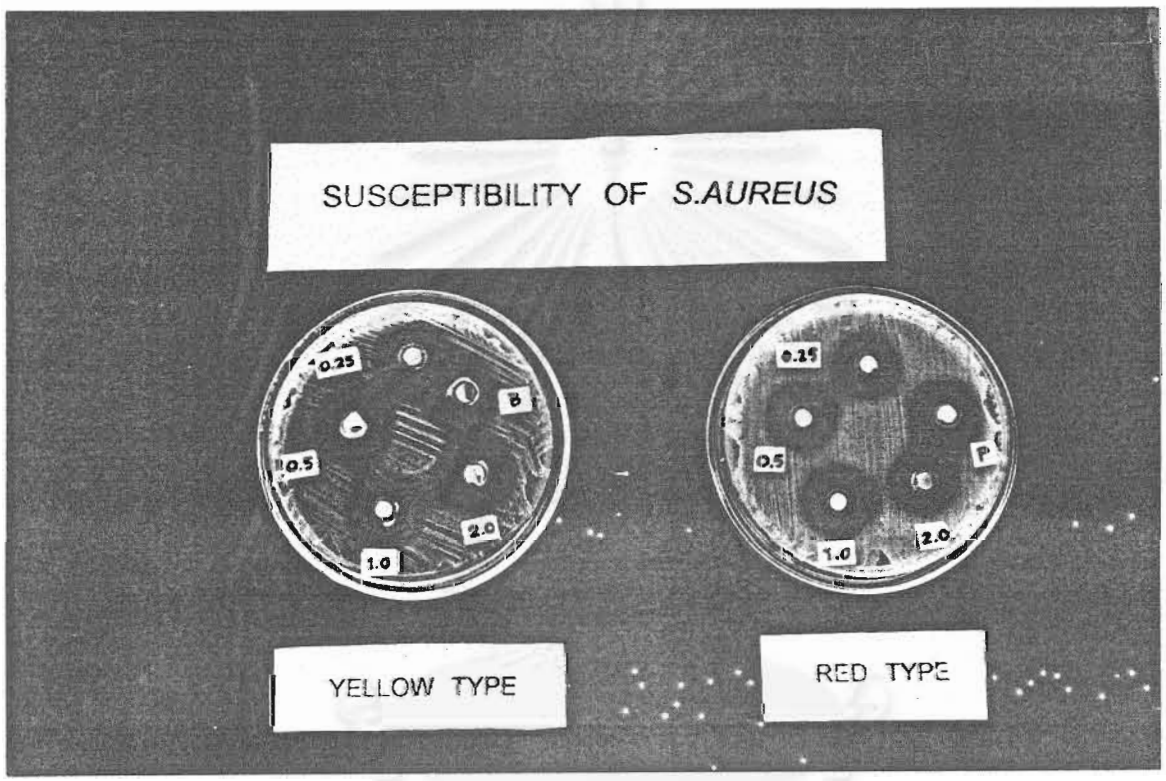
4 = red FCAJ 8 mg/disk; 5 = yellow FCAJ 2 mg/disk; 6 = yellow FCAJ 4 mg/disk;

7 = yellow FCAJ 8 mg/disk; 8 = yellow FCAJ 16 mg/disk.

SUSCEPTIBILITY TEST OF FCAJ TO *P. ACNES* BY AGAR DILUTION METHOD

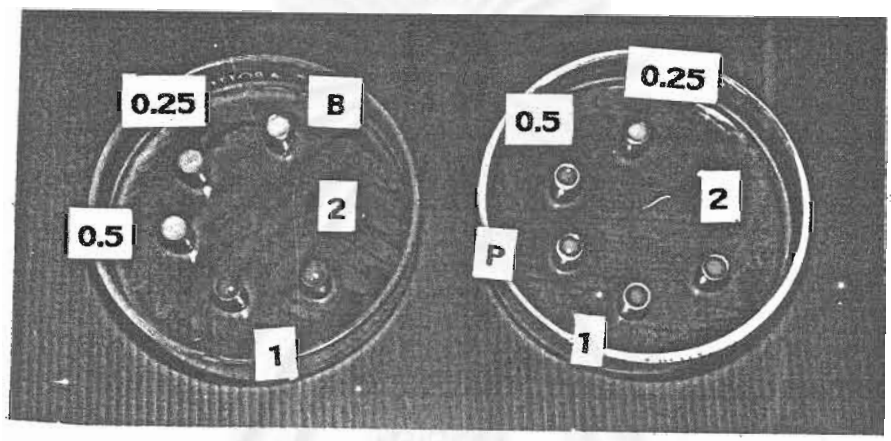


SUSCEPTIBILITY TEST OF FCAJ CREAMS COMPARED TO CREAM BASE AND PANOXYL 5® TO *S. AUREUS* BY USING CUP DIFFUSION METHOD



0.25 = 0.25% FCAJ cream, 0.5 = 0.5% FCAJ cream, 1 = 1% FCAJ cream, 2 = 2% FCAJ cream, B = cream base, P = Panoxyl 5®

SUSCEPTIBILITY TEST OF FCAJ CREAMS COMPARED TO CREAM BASE AND PANOXYL 5® TO *P. ACNES* BY USING CUP DIFFUSION METHOD



RED TYPE YELLOW TYPE

0.25 = 0.25% FCAJ cream, 0.5 = 0.5% FCAJ cream, 1 = 1% FCAJ cream, 2 = 2% FCAJ cream, *B* = *cream base*, *P* = *Panoxyl* 5®

APPENDIX II

Statistics of Susceptibility Tests

Normal Distribution Test of Zone Diameters of FCAJ Dispersions Against *S. aureus*

One-Sample Kolmogorov-Smirnov Test

		LOGS.AU
N		196
Normal Parameters[a,b]	Mean	1.0422
	Std. Deviation	8.466E-02
Most Extreme Differences	Absolute	.088
	Positive	.060
	Negative	-.088
Kolmogorov-Smirnov Z		1.235
Asymp. Sig. (2-tailed)		.094

a. Test distribution is Normal.

b. Calculated from data.

One-way ANOVA of zone diameters of FCAJ dispersions against *S. aureus*

Levene's Test of Equality of Error Variances[a]

	F	df1	df2	Sig.
LOGS.AU	.951	6	189	.460

Tests the null hypothesis that the error variance of the dependent variable is equal across groups.

a. Design: Intercept+TYPE

Tests of Between-Subjects Effects

Dependent Variable: LOGS.AU

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Noncent. Parameter	Observed Power[a]
Corrected Model	.655[b]	6	.109	27.772	.000	166.630	1.000
Intercept	212.873	1	212.873	54163.033	.000	54163.033	1.000
TYPE	.655	6	.109	27.772	.000	166.630	1.000
Error	.743	189	3.930E-03				
Total	214.271	196					
Corrected Total	1.398	195					

a. Computed using alpha = .05

b. R Squared = .469 (Adjusted R Squared = .452)

Post-hoc of zone diameters of FCAJ dispersions against *S. aureus*

LOGS.AU

Scheffe[a,b]

type	N	Subset 1	2	3	4
2r	28	.9273			
4r	28		1.0047		
8y	28		1.0369	1.0369	
2y	28		1.0577	1.0577	
8r	28		1.0623	1.0623	1.0623
4y	28			1.0845	1.0845
16r	28				1.1217
Sig.		1.000	.071	.237	.056

Means for groups in homogeneous subsets are displayed.
Based on Type III Sum of Squares
The error term is Mean Square(Error) = 3.930E-03.

a. Uses Harmonic Mean Sample Size = 28.000.

b. Alpha = .05.

Normal distribution test of zone diameters of FCAJ creams against MSSA

One-Sample Kolmogorov-Smirnov Test

		zone
N		150
Normal Parameters[a,b]	Mean	10.9753
	Std. Deviation	2.6101
Most Extreme Differences	Absolute	.096
	Positive	.096
	Negative	-.090
Kolmogorov-Smirnov Z		1.179
Asymp. Sig. (2-tailed)		.124

a. Test distribution is Normal.

b. Calculated from data.

One-way ANOVA of zone diameters of FCAJ creams against MSSA

Levene's Test of Equality of Error Variances[a]

	F	df1	df2	Sig.
zone	1.143	9	140	.336

Tests the null hypothesis that the error variance of the dependent variable is equal across groups.

a. Design: Intercept+TYPE

Tests of Between-Subjects Effects

Dependent Variable: zone

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Noncent. Parameter	Observed Power[a]
Corrected Model	88.484[b]	9	9.832	1.485	.159	13.369	.688
Intercept	18068.691	1	18068.691	2730.073	.000	2730.073	1.000
TYPE	88.484	9	9.832	1.485	.159	13.369	.688
Error	926.575	140	6.618				
Total	19083.750	150					
Corrected Total	1015.059	149					

a. Computed using alpha = .05

b. R Squared = .087 (Adjusted R Squared = .028)

Post-hoc of zone diameters of FCAJ creams against MSSA

zone

Student-Newman-Keuls[a,b]

type	N	Subset 1
base	15	9.8267
0.25y	15	9.8867
0.25r	15	10.4800
0.5y	15	10.9867
2y	15	11.0667
1r	15	11.0867
1y	15	11.0933
2r	15	11.2000
0.5r	15	11.4733
panoxyl	15	12.6533
Sig.		.078

Means for groups in homogeneous subsets are displayed.
Based on Type III Sum of Squares
The error term is Mean Square(Error) = 6.618.

a. Uses Harmonic Mean Sample Size = 15.000.

b. Alpha = .05.

Normal Distribution Test of Zone Diameters of FCAJ Creams Against MRSA

One-Sample Kolmogorov-Smirnov Test

		zone
N		150
Normal Parameters[a,b]	Mean	15.7870
	Std. Deviation	3.5035
Most Extreme Differences	Absolute	.108
	Positive	.072
	Negative	-.108
Kolmogorov-Smirnov Z		1.325
Asymp. Sig. (2-tailed)		.060

a. Test distribution is Normal.

b. Calculated from data.

One-way ANOVA of Zone Diameters of FCAJ Creams Against MRSA

Levene's Test of Equality of Error Variances[a]

	F	df1	df2	Sig.
zone	.613	9	140	.785

Tests the null hypothesis that the error variance of the dependent variable is equal across groups.

a. Design: Intercept+TYPE

Tests of Between-Subjects Effects

Dependent Variable: zone

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Noncent. Parameter	Observed Power[a]
Corrected Model	339.577[b]	9	37.731	3.547	.001	31.922	.987
Intercept	37384.405	1	37384.405	3514.280	.000	3514.280	1.000
TYPE	339.577	9	37.731	3.547	.001	31.922	.987
Error	1489.300	140	10.638				
Total	39213.283	150					
Corrected Total	1828.877	149					

a. Computed using alpha = .05

b. R Squared = .186 (Adjusted R Squared = .133)

Post-hoc of Zone Diameters of FCAJ Creams Against MRSA

zone

Student-Newman-Keuls[a,b]

type	N	Subset 1	Subset 2
panoxyl	15	12.5400	
base	15	14.1533	14.1533
0.25y	15	14.5467	14.5467
0.5y .	15		15.8400
0.25r	15		15.9133
2r	15		16.4733
1y	15		16.6600
2y	15		16.8533
0.5r	15		17.4200
1r	15		17.4700
Sig.		.211	.120

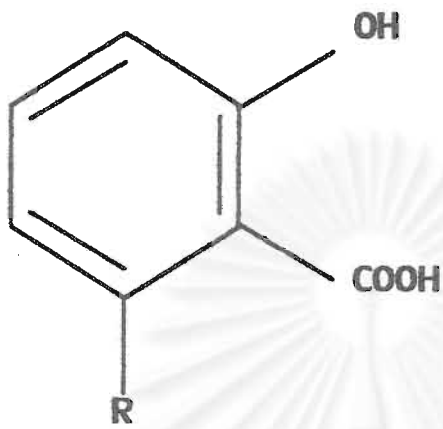
Means for groups in homogeneous subsets are displayed.
Based on Type III Sum of Squares
The error term is Mean Square(Error) = 10.638.

a. Uses Harmonic Mean Sample Size = 15.000.

b. Alpha = .05.

APPENDIX III

Calibration Curve and Stability Data of FCAJ



1: R = 8Z,11Z,14-pentadecatrienyl

2: R = 8Z,11Z-pentadecadienyl

3: R = 8Z-pentadecenyl

4: R = pentadecenyl

Structure of Anacardic Acids

Calibration curve data A

Amount of FCAJ (mg)	Peak area ratios (PAR)		
	Set no.1	Set no.2	Set no.3
31.25	0.09428	0.0889	0.0892
62.50	0.1728	0.1759	0.16813
125.00	0.3715	0.3640	0.3721
250.00	0.6496	0.6742	0.6653
500.00	1.3524	1.3852	1.3412

PAR = 0.0097 + 1.3454 Amount ; r = 0.9991

Calibration curve data B

Amount of FCAJ (mg)	Peak area ratios (PAR)		
	Set no.1	Set no.2	Set no.3
31.25	0.0952	0.0963	0.0957
62.50	0.1652	0.1759	0.1611
125.00	0.3675	0.3608	0.3752
250.00	0.7945	0.7907	0.7932
500.00	1.6549	1.6769	1.6145

PAR = - 0.0344 + 1.6747 Amount ; r = 0.9993

Calibration curve data C

Amount of FCAJ (mg)	Peak area ratios (PAR)		
	Set no.1	Set no.2	Set no.3
31.25	0.0864	0.0835	0.0912
62.50	0.1721	0.1693	0.1719
125.00	0.3587	0.3621	0.3531
250.00	0.6873	0.7033	0.6799
500.00	1.3624	1.3533	1.3648

PAR = 0.0077 + 1.3563 Amount ; r = 0.9998

Calibration curve data D

Amount of FCAJ (mg)	Peak area ratios (PAR)		
	Set no.1	Set no.2	Set no.3
31.25	0.0952	0.1001	0.0948
62.50	0.1891	0.1916	0.1869
125.00	0.3528	0.3438	0.3363
250.00	0.6545	0.6662	0.6842
500.00	1.2695	1.2326	1.2525

PAR = 0.0345 + 1.2272 Amount ; r = 0.9992

Calibration curve data E

Amount of FCAJ (mg)	Peak area ratios (PAR)		
	Set no.1	Set no.2	Set no.3
31.25	0.0890	0.0883	0.0864
62.50	0.1854	0.1774	0.187
125.00	0.3542	0.3629	0.3515
250.00	0.7215	0.7139	0.72
500.00	1.3754	1.4004	1.3648

PAR = 0.0115 + 1.3773 Amount ; r = 0.9995

Calibration curve data F

Amount of FCAJ (mg)	Peak area ratios (PAR)		
	Set no.1	Set no.2	Set no.3
31.25	0.0824	0.0908	0.0897
62.50	0.1798	0.1869	0.1826
125.00	0.3634	0.3434	0.3564
250.00	0.6478	0.6669	0.6452
500.00	1.2712	1.2344	1.2578

PAR = 0.0289 + 1.2328 Amount ; r = 0.9992

Calibration curve data G

Amount of FCAJ (mg)	Peak area ratios (PAR)		
	Set no.1	Set no.2	Set no.3
31.25	0.0927	0.0934	0.0946
62.50	0.1805	0.1834	0.1843
125.00	0.3487	0.3571	0.3617
250.00	0.6743	0.681	0.6914
500.00	1.3654	1.3842	1.3521

PAR = 0.0117 + 1.3538 Amount ; r = 0.9999

Calibration curve data H

Amount of FCAJ (mg)	Peak area ratios (PAR)		
	Set no.1	Set no.2	Set no.3
31.25	0.0821	0.0735	0.07657
62.50	0.1602	0.1632	0.1531
125.00	0.3312	0.3478	0.3298
250.00	0.6312	0.6433	0.6296
500.00	1.2241	1.2411	1.1937

PAR = 0.0151 + 1.2135 Amount ; r = 0.9991

Stability Data of the Selected FCAJ Ingredient

Time (hr)	Cal.*	Percent of the selected FCAJ ingredient				
		n1	n2	n3	Average	SD
0	A	102.01	97.20	100.79	100.00	2.50
125	B	99.21	102.47	98.23	99.97	2.22
332	C	96.12	100.76	101.04	99.31	2.76
669	D	93.00	99.14	91.01	94.38	4.24
1245	E	85.37	93.13	88.79	89.10	3.89
1941	F	85.22	83.16	80.02	82.80	2.62
2709	G	77.74	76.06	74.18	75.99	1.78
3456	H	67.85	72.77	74.47	71.70	3.44

Stability Data of the Selected FCAJ Ingredient

Containing 0.05% Sodium Metabisulfite

Time (hr)	Cal.*	Percent of the selected FCAJ ingredient				
		n1	n2	n3	Average	SD
0	A	98.06	102.02	99.92	100.00	1.98
125	B	97.52	102.13	99.72	99.79	2.31
332	C	96.95	100.38	101.89	99.74	2.53
669	D	99.51	97.47	101.86	99.61	2.20
1245	E	96.82	98.30	93.68	96.27	2.36
1941	F	92.85	90.20	88.06	90.37	2.40
2709	G	87.05	86.73	83.13	85.64	2.18
3456	H	84.96	77.93	80.69	81.19	3.54

Stability Data of the Selected FCAJ Ingredient

Containing 1% Sodium Metabisulfite

Time (hr)	Cal.*	Percent of the selected FCAJ ingredient				
		n1	n2	n3	Average	SD
0	A	102.21	99.91	97.87	100.00	2.17
125	B	102.66	98.08	97.96	99.57	2.68
332	C	98.97	95.86	100.42	98.42	2.33
669	D	98.35	98.59	96.41	97.78	1.20
1245	E	96.24	93.26	90.80	93.43	2.72
1941	F	87.09	91.56	88.36	89.00	2.30
2709	G	85.95	83.87	82.59	84.14	1.70
3456	H	81.36	76.80	83.36	80.51	3.36

Stability Data of the Selected FCAJ Ingredient

Containing 0.01% Propyl gallate

Time (hr)	Cal.*	Percent of the selected FCAJ ingredient				
		n1	n2	n3	Average	SD
0	A	102.06	97.76	100.19	100.00	2.16
125	B	100.90	97.85	97.11	98.62	2.01
332	C	91.79	97.17	96.26	95.07	2.88
669	D	94.71	91.17	93.93	93.27	1.86
1245	E	84.50	90.83	87.81	87.71	3.17
1941	F	84.74	85.70	80.09	83.51	3.00
2709	G	83.97	80.25	78.91	81.04	2.62
3456	H	75.02	80.36	81.70	79.03	3.53

Stability Data of the Selected FCAJ Ingredient

Containing 0.1% Propyl gallate

Time (hr)	Cal.*	Percent of the selected FCAJ ingredient				
		n1	n2	n3	Average	SD
0	A	100.33	101.27	98.39	100.00	1.47
125	B	96.25	99.14	100.49	98.63	2.17
332	C	94.62	93.17	91.62	93.14	1.50
669	D	89.50	88.53	89.84	89.29	0.68
1245	E	84.17	82.93	88.09	85.06	2.69
1941	F	84.76	84.26	78.60	82.54	3.42
2709	G	82.38	76.71	79.06	79.38	2.85
3456	H	80.03	76.80	75.83	77.55	2.20

APPENDIX IV

Statistics for Comparison of Degradation Rate Constants

Zero Order Kinetics of FCAJ Alone

Variables Entered/Removed[b]

Model	Variables Entered	Variables Removed	Method
1	time[a]	.	Enter

a. All requested variables entered.

b. Dependent Variable: CONTROL

Model Summary[b]

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate	Durbin-Watson
1	.969[a]	.940	.937	2.7659	2.185

a. Predictors: (Constant), time

b. Dependent Variable: CONTROL

ANOVA[b]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	2629.501	1	2629.501	343.710	.000[a]
	Residual	168.308	22	7.650		
	Total	2797.809	23			

a. Predictors: (Constant), time

b. Dependent Variable: CONTROL

Coefficients[a]

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	100.612	.837		120.197	.000
	time	-8.75E-03	.000	-.969	-18.539	.000

a. Dependent Variable: CONTROL

Residuals Statistics[a]

	Minimum	Maximum	Mean	Std. Deviation	N
Predicted Value	70.3778	100.6119	89.1549	10.6923	24
Residual	-4.3538	4.3792	1.658E-14	2.7051	24
Std. Predicted Value	-1.756	1.072	.000	1.000	24
Std. Residual	-1.574	1.583	.000	.978	24

a. Dependent Variable: CONTROL

Autocorrelations of Zero Order of FCAJ Alone

Partial Autocorrelations: ZRE_1 Standardized Residual

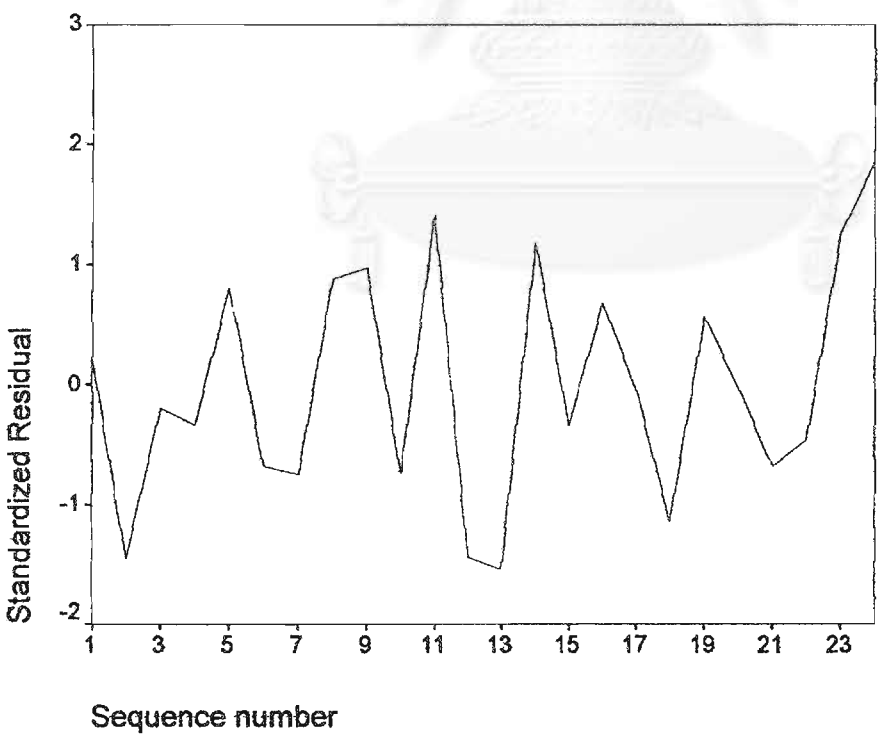
Lag	Pr-Aut-Corr.	Stand. Err.	-1 -.75 -.5 -.25 0 .25 .5 .75 1
1	-.122	.204	**I
2	-.247	.204	*****I
3	.017	.204	*
4	-.247	.204	*****I
5	.094	.204	I**
6	-.071	.204	*I
7	-.147	.204	***I
8	.056	.204	I*
9	-.019	.204	*
10	.122	.204	I**
11	-.124	.204	**I
12	-.048	.204	*I
13	-.118	.204	**I
14	-.077	.204	**I
15	.175	.204	I****
16	.083	.204	I**

Plot Symbols: Autocorrelations * Two Standard Error Limits .

Total cases: 24 Computable first lags: 23

TSPLOT of Zero Order of FCAJ Alone

MODEL: MOD_7.



First Order Kinetics of FCAJ Alone

Variables Entered/Removed[b]

Model	Variables Entered	Variables Removed	Method
1	time[a]	.	Enter

a. All requested variables entered.

b. Dependent Variable: ln_control

Model Summary[b]

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate	Durbin-Watson
1	.972[a]	.944	.942	3.09E-02	2.121

a. Predictors: (Constant), time

b. Dependent Variable: ln_control

ANOVA[b]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	.357	1	.357	374.135	.000[a]
	Residual	2.097E-02	22	9.533E-04		
	Total	.378	23			

a. Predictors: (Constant), time

b. Dependent Variable: ln_control

Coefficients[a]

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	4.616	.009		494.037	.000
	time	-1.02E-04	.000	-.972	-19.343	.000

a. Dependent Variable: ln_control

Residuals Statistics[a]

	Minimum	Maximum	Mean	Std. Deviation	N
Predicted Value	4.264012	4.616122	4.482693	.124524	24
Residual	-4.7E-02	4.86E-02	-5.9E-16	3.02E-02	24
Std. Predicted Value	-1.756	1.072	.000	1.000	24
Std. Residual	-1.514	1.573	.000	.978	24

a. Dependent Variable: ln_control

Autocorrelations of First Order of FCAJ Alone

Partial Autocorrelations: ZRE_2 Standardized Residual

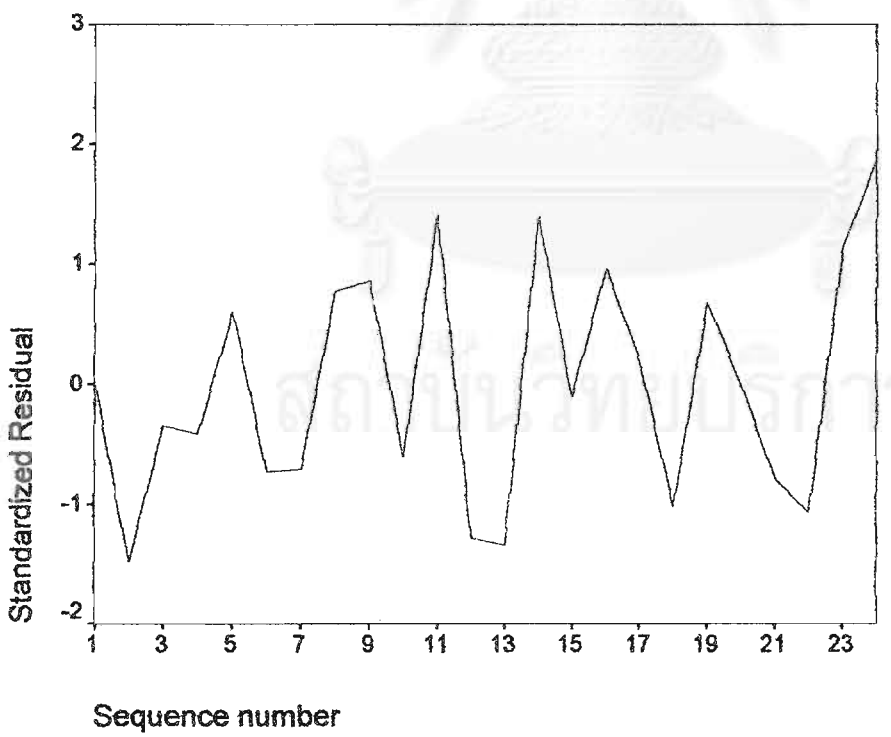
Lag	Pr-Aut-Corr.	Stand. Err.	-1 -.75 -.5 -.25 0 .25 .5 .75 1
1	-.090	.204	**I
2	-.277	.204	******I
3	-.004	.204	*
4	-.247	.204	*****I
5	.107	.204	I**
6	-.114	.204	**I
7	-.106	.204	**I
8	.042	.204	I*
9	.072	.204	I*
10	.172	.204	I***
11	-.066	.204	*I
12	-.003	.204	*
13	-.136	.204	***I
14	-.098	.204	**I
15	.101	.204	I**
16	.101	.204	I**

Plot Symbols: Autocorrelations * Two Standard Error Limits .

Total cases: 24 Computable first lags: 23

TSPLOT of First Order of FCAJ Alone

MODEL: MOD_9.



Second Order Kinetics of FCAJ Alone

Variables Entered/Removed[b]

Model	Variables Entered	Variables Removed	Method
1	time[a]	.	Enter

a. All requested variables entered.

b. Dependent Variable: inv_control

Model Summary[b]

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate	Durbin-Watson
1	.971[a]	.944	.941	3.66E-04	1.944

a. Predictors: (Constant), time

b. Dependent Variable: inv_control

ANOVA[b]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	4.923E-05	1	4.923E-05	367.549	.000[a]
	Residual	2.946E-06	22	1.339E-07		
	Total	5.217E-05	23			

a. Predictors: (Constant), time

b. Dependent Variable: inv_control

Coefficients[a]

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	9.826E-03	.000		88.725	.000
	time	1.197E-06	.000	.971	19.172	.000

a. Dependent Variable: inv_control

Residuals Statistics[a]

	Minimum	Maximum	Mean	Std. Deviation	N
Predicted Value	9.83E-03	1.40E-02	1.14E-02	1.46E-03	24
Residual	-5.8E-04	7.75E-04	7.23E-20	3.58E-04	24
Std. Predicted Value	-1.072	1.756	.000	1.000	24
Std. Residual	-1.583	2.119	.000	.978	24

a. Dependent Variable: inv_control

Autocorrelations of Second Order of FCAJ Alone

Partial Autocorrelations: ZRE_3 Standardized Residual

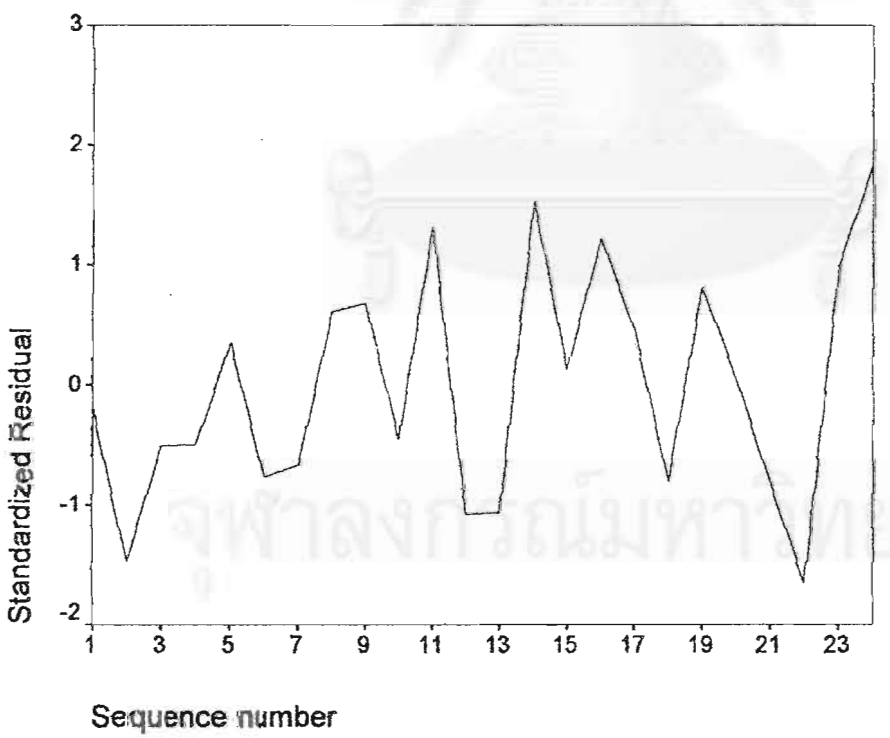
Lag	Pr-Aut-Corr.	Stand. Err.	-1 -.75 -.5 -.25 0 .25 .5 .75 1
1	-.001	.204	*
2	-.252	.204	*****I
3	.041	.204	I*
4	-.169	.204	***I
5	.151	.204	I***
6	-.135	.204	***I
7	-.037	.204	*I
8	.015	.204	*
9	.129	.204	I***
10	.121	.204	I**
11	-.093	.204	**I
12	-.047	.204	*I
13	-.186	.204	****I
14	-.128	.204	***I
15	.043	.204	I*
16	.099	.204	I**

Plot Symbols: Autocorrelations * Two Standard Error Limits .

Total cases: 24 Computable first lags: 23

TSPLOT of Second Order of FCAJ Alone

MODEL: MOD_11.



Zero Order Kinetics of a FCAJ Dispersion Containing 0.05% Sodium Metabisulfite

Variables Entered/Removed[b]

Model	Variables Entered	Variables Removed	Method
1	Time[a]	.	Enter

a. All requested variables entered.

b. Dependent Variable: sod.met0.05

Model Summary[b]

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate	Durbin-Watson
1	.946[a]	.896	.891	2.4412	1.961

a. Predictors: (Constant), Time

b. Dependent Variable: sod.met0.05

ANOVA[b]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	1125.690	1	1125.690	188.892	.000[a]
	Residual	131.108	22	5.959		
	Total	1256.798	23			

a. Predictors: (Constant), Time

b. Dependent Variable: sod.met0.05

Coefficients[a]

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	101.572	.739		137.486	.000
	Time	-5.72E-03	.000	-.946	-13.744	.000

a. Dependent Variable: sod.met0.05

Residuals Statistics[a]

	Minimum	Maximum	Mean	Std. Deviation	N
Predicted Value	81.7902	101.5722	94.0760	6.9959	24
Residual	-3.8641	4.1128	-2.37E-15	2.3875	24
Std. Predicted Value	-1.756	1.072	.000	1.000	24
Std. Residual	-1.583	1.685	.000	.978	24

a. Dependent Variable: sod.met0.05

Autocorrelations of Zero Order of a FCAJ Dispersion Containing 0.05% Sodium Metabisulfite

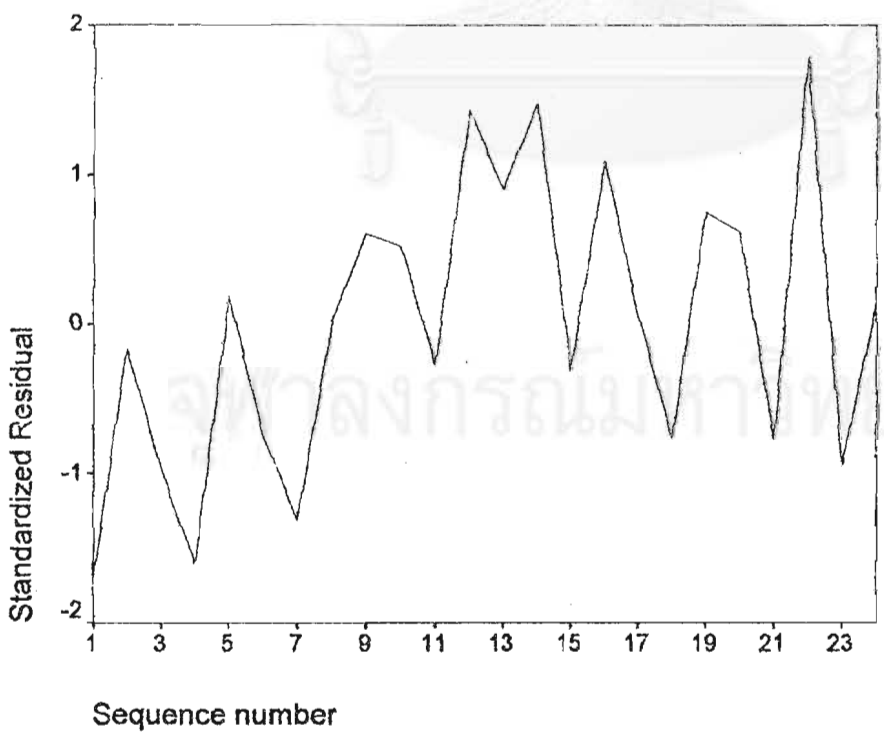
Partial Autocorrelations: ZRE_1 Standardized Residual

Lag	Pr-Aut- Corr.	Stand. Err.	-1 -.75 -.5 -.25 0 .25 .5 .75 1
1	.018	.204	* .
2	.266	.204	I***** .
3	.396	.204	I********
4	.031	.204	I* .
5	-.263	.204	*****I .
6	-.182	.204	****I .
7	-.136	.204	***I .
8	.079	.204	I** .
9	-.187	.204	****I .
10	.007	.204	* .
11	-.041	.204	*I .
12	.072	.204	I* .
13	-.175	.204	****I .
14	.141	.204	I*** .
15	-.042	.204	*I .
16	-.125	.204	**I .

Plot Symbols: Autocorrelations * Two Standard Error Limits .

Total cases: 24 Computable first lags: 23

TSPLOT of Zero Order of a FCAJ Dispersion Containing 0.05% Sodium Metabisulfite



First Order Kinetics of a FCAJ Dispersion Containing 0.05% Sodium Metabisulfite

Variables Entered/Removed[b]

Model	Variables Entered	Variables Removed	Method
1	Time[a]	.	Enter

a. All requested variables entered.

b. Dependent Variable: ln_sod.m0.05

Model Summary[b]

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate	Durbin-Watson
1	.945[a]	.893	.888	2.72E-02	1.902

a. Predictors: (Constant), Time

b. Dependent Variable: ln_sod.m0.05

ANOVA[b]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	.135	1	.135	183.264	.000[a]
	Residual	1.627E-02	22	7.394E-04		
	Total	.152	23			

a. Predictors: (Constant), Time

b. Dependent Variable: ln_sod.m0.05

Coefficients[a]

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	4.623	.008		561.830	.000
	Time	-6.28E-05	.000	-.945	-13.537	.000

a. Dependent Variable: ln_sod.m0.05

Residuals Statistics[a]

	Minimum	Maximum	Mean	Std. Deviation	N
Predicted Value	4.406220	4.623254	4.541011	7.68E-02	24
Residual	-5.0E-02	4.29E-02	5.92E-16	2.66E-02	24
Std. Predicted Value	-1.756	1.072	.000	1.000	24
Std. Residual	-1.856	1.579	.000	.978	24

a. Dependent Variable: ln_sod.m0.05

Autocorrelations of First Order of a FCAJ Dispersion Containing 0.05% Sodium Metabisulfite

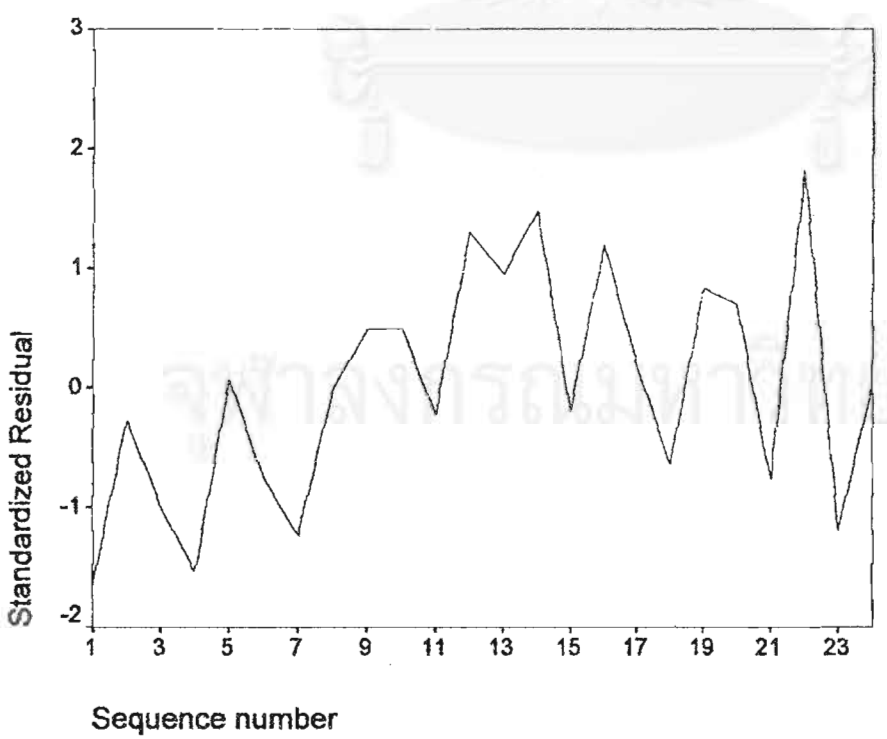
Partial Autocorrelations: ZRE_2 Standardized Residual

Lag	Pr-Aut-Corr.	Stand. Err.	-1 -.75 -.5 -.25 0 .25 .5 .75 1
1	.049	.204	I*
2	.307	.204	I******
3	.402	.204	I********
4	.002	.204	*
5	-.262	.204	*****I
6	-.141	.204	***I
7	-.148	.204	***I
8	.028	.204	I*
9	-.226	.204	*****I
10	-.014	.204	*
11	-.034	.204	*I
12	.088	.204	I**
13	-.134	.204	***I
14	.145	.204	I***
15	-.034	.204	*I
16	-.134	.204	***I

Plot Symbols: Autocorrelations * Two Standard Error Limits .

Total cases: 24 Computable first lags: 23

TSPLOT of First Order of a FCAJ Dispersion Containing 0.05% Sodium Metabisulfite



Second Order Kinetics of a FCAJ Dispersion Containing 0.05% Sodium Metabisulfite

Variables Entered/Removed[b]

Model	Variables Entered	Variables Removed	Method
1	Time[a]	.	Enter

a. All requested variables entered.

b. Dependent Variable: inv_sod.m0.05

Model Summary[b]

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate	Durbin-Watson
1	.942[a]	.887	.882	3.08E-04	1.840

a. Predictors: (Constant), Time

b. Dependent Variable: inv_sod.m0.05

ANOVA[b]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	1.643E-05	1	1.643E-05	173.142	.000[a]
	Residual	2.088E-06	22	9.492E-08		
	Total	1.852E-05	23			

a. Predictors: (Constant), Time

b. Dependent Variable: inv_sod.m0.05

Coefficients[a]

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	9.791E-03	.000		105.015	.000
	Time	6.916E-07	.000	.942	13.158	.000

a. Dependent Variable: inv_sod.m0.05

Residuals Statistics[a]

	Minimum	Maximum	Mean	Std. Deviation	N
Predicted Value	9.79E-03	1.22E-02	1.07E-02	8.45E-04	24
Residual	-4.8E-04	6.51E-04	2.17E-19	3.01E-04	24
Std. Predicted Value	-1.072	1.756	.000	1.000	24
Std. Residual	-1.555	2.113	.000	.978	24

a. Dependent Variable: inv_sod.m0.05

Autocorrelations of Second Order of a FCAJ Dispersion Containing 0.05% Sodium Metabisulfite

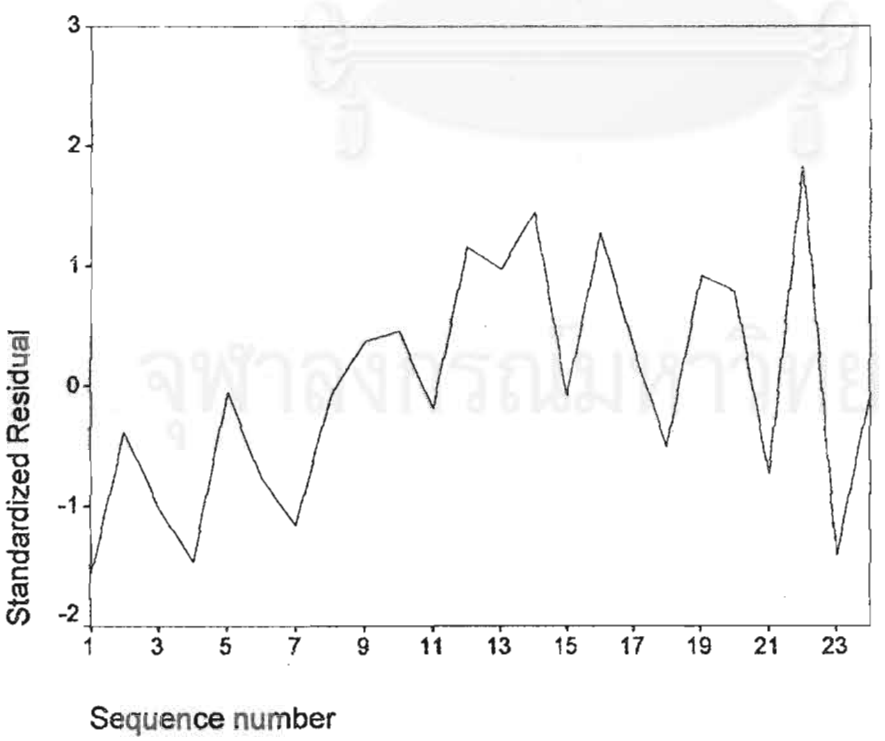
Partial Autocorrelations: ZRE_3 Standardized Residual

Lag	Pr-Aut-Corr.	Stand. Err.	-1 -.75 -.5 -.25 0 .25 .5 .75 1
1	.083	.204	. I** .
2	.344	.204	. I******* .
3	.399	.204	. I********
4	-.033	.204	. *I .
5	-.256	.204	. *****I .
6	-.095	.204	. **I .
7	-.157	.204	. ***I .
8	-.027	.204	. *I .
9	-.253	.204	. *****I .
10	-.027	.204	. *I .
11	-.032	.204	. *I .
12	.091	.204	. I** .
13	-.094	.204	. **I .
14	.150	.204	. I*** .
15	-.020	.204	. * .
16	-.131	.204	. ***I .

Plot Symbols: Autocorrelations * Two Standard Error Limits .

Total cases: 24 Computable first lags: 23

TSPLOT of Second Order of a FCAJ Dispersion Containing 0.05% Sodium Metabisulfite



Zero Order Kinetics of a FCAJ Dispersion Containing 1% Sodium Metabisulfite

Variables Entered/Removed[b]

Model	Variables Entered	Variables Removed	Method
1	time[a]	.	Enter

a. All requested variables entered.

b. Dependent Variable: sod.met1

Model Summary[b]

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate	Durbin-Watson
1	.961[a]	.923	.920	2.107257	2.465

a. Predictors: (Constant), time

b. Dependent Variable: sod.met1

ANOVA[b]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	1178.773	1	1178.773	265.458	.000[a]
	Residual	97.692	22	4.441		
	Total	1276.465	23			

a. Predictors: (Constant), time

b. Dependent Variable: sod.met1

Coefficients[a]

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	100.526	.638		157.633	.000
	time	-5.86E-03	.000	-.961	-16.293	.000

a. Dependent Variable: sod.met1

Residuals Statistics[a]

	Minimum	Maximum	Mean	Std. Deviation	N
Predicted Value	80.283211	100.5263	92.855325	7.158981	24
Residual	-3.484191	3.076591	-2.9E-14	2.060938	24
Std. Predicted Value	-1.756	1.072	.000	1.000	24
Std. Residual	-1.653	1.460	.000	.978	24

a. Dependent Variable: sod.met1

Autocorrelations of Zero Order of a FCAJ Dispersion Containing 1% Sodium Metabisulfite

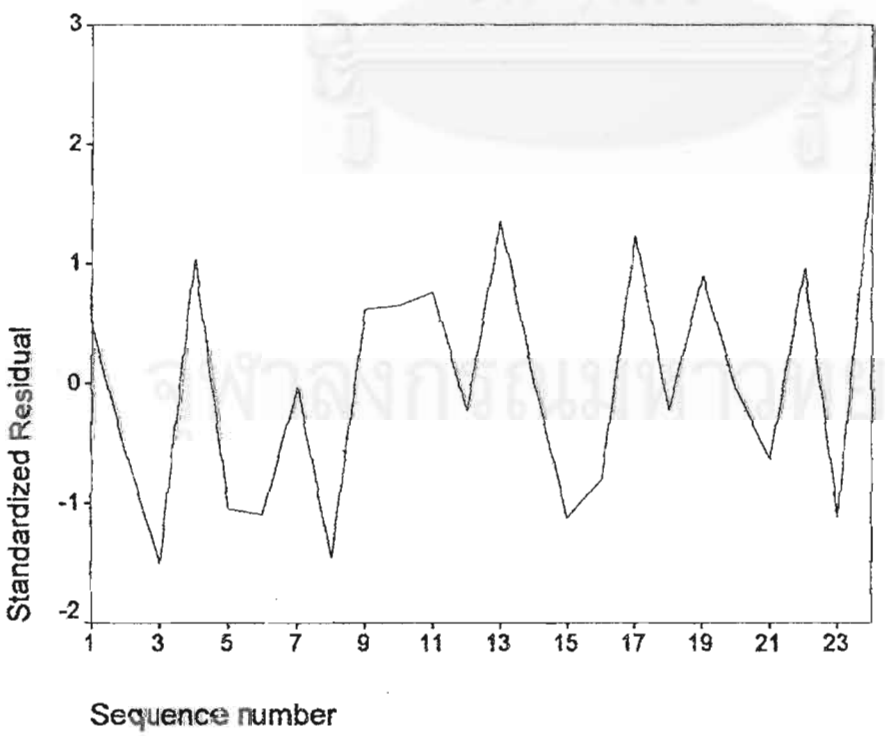
Partial Autocorrelations: ZRE_1 Standardized Residual

Lag	Pr-Aut-Corr.	Stand. Err.	-1 -.75 -.5 -.25 0 .25 .5 .75 1
1	-.259	.204	*****I
2	-.002	.204	*
3	.098	.204	I**
4	-.131	.204	***I
5	-.059	.204	*I
6	-.044	.204	*I
7	.063	.204	I*
8	-.061	.204	*I
9	.001	.204	*
10	-.046	.204	*I
11	-.037	.204	*I
12	-.068	.204	*I
13	.229	.204	I*****
14	-.100	.204	**I
15	.099	.204	I**
16	-.190	.204	****I

Plot Symbols: Autocorrelations * Two Standard Error Limits .

Total cases: 24 Computable first lags: 23

TSPLOT of Zero Order of a FCAJ Dispersion Containing 1% Sodium Metabisulfite



First Order Kinetics of a FCAJ Dispersion Containing 1% Sodium Metabisulfite

Variables Entered/Removed[b]

Model	Variables Entered	Variables Removed	Method
1	time[a]	.	Enter

a. All requested variables entered.

b. Dependent Variable: ln_sod.m1

Model Summary[b]

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate	Durbin-Watson
1	.961[a]	.923	.919	2.35E-02	2.448

a. Predictors: (Constant), time

b. Dependent Variable: ln_sod.m1

ANOVA[b]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	.144	1	.144	262.174	.000[a]
	Residual	1.211E-02	22	5.502E-04		
	Total	.156	23			

a. Predictors: (Constant), time

b. Dependent Variable: ln_sod.m1

Coefficients[a]

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	4.613	.007		649.777	.000
	time	-6.48E-05	.000	-.961	-16.192	.000

a. Dependent Variable: ln_sod.m1

Residuals Statistics[a]

	Minimum	Maximum	Mean	Std. Deviation	N
Predicted Value	4.388764	4.612705	4.527845	7.92E-02	24
Residual	-4.8E-02	3.48E-02	-1.5E-16	2.29E-02	24
Std. Predicted Value	-1.756	1.072	.000	1.000	24
Std. Residual	-2.028	1.483	.000	.978	24

a. Dependent Variable: ln_sod.m1

Autocorrelations of First Order of a FCAJ Dispersion Containing 1% Sodium Metabisulfite

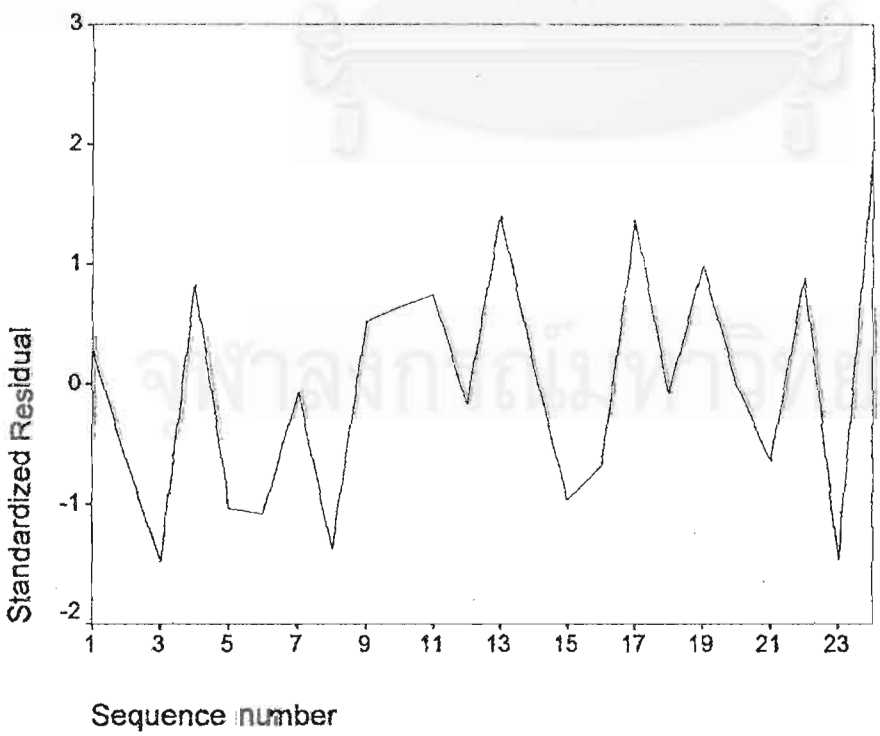
Partial Autocorrelations: ZRE_2 Standardized Residual

Lag	Pr-Aut-Corr.	Stand. Err.	-1 -.75 -.5 -.25 0 .25 .5 .75 1
1	-.246	.204	*****I
2	.043	.204	I*
3	.117	.204	I**
4	-.103	.204	**I
5	-.021	.204	*
6	-.021	.204	*
7	.096	.204	I**
8	-.037	.204	*I
9	-.026	.204	*I
10	-.108	.204	**I
11	-.057	.204	*I
12	-.087	.204	**I
13	.179	.204	I****
14	-.120	.204	**I
15	.094	.204	I**
16	-.181	.204	****I

Plot Symbols: Autocorrelations * Two Standard Error Limits .

Total cases: 24 Computable first lags: 23

TSPLOT of First Order of a FCAJ Dispersion Containing 1% Sodium Metabisulfite



Second Order Kinetics of a FCAJ Dispersion Containing 1% Sodium Metabisulfite

Variables Entered/Removed[b]

Model	Variables Entered	Variables Removed	Method
1	time[a]	.	Enter

a. All requested variables entered.

b. Dependent Variable: inv_sod.m1

Model Summary[b]

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate	Durbin-Watson
1	.958[a]	.919	.915	2.68E-04	2.399

a. Predictors: (Constant), time

b. Dependent Variable: inv_sod.m1

ANOVA[b]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	1.779E-05	1	1.779E-05	248.604	.000[a]
	Residual	1.574E-06	22	7.157E-08		
	Total	1.937E-05	23			

a. Predictors: (Constant), time

b. Dependent Variable: inv_sod.m1

Coefficients[a]

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	9.897E-03	.000		122.249	.000
	time	7.196E-07	.000	.958	15.767	.000

a. Dependent Variable: inv_sod.m1

Residuals Statistics[a]

	Minimum	Maximum	Mean	Std. Deviation	N
Predicted Value	9.90E-03	1.24E-02	1.08E-02	8.80E-04	24
Residual	-4.0E-04	6.37E-04	-5.1E-19	2.62E-04	24
Std. Predicted Value	-1.072	1.756	.000	1.000	24
Std. Residual	-1.504	2.380	.000	.978	24

a. Dependent Variable: inv_sod.m1

Autocorrelations of Second Order of a FCAJ Dispersion Containing 1% Sodium Metabisulfite

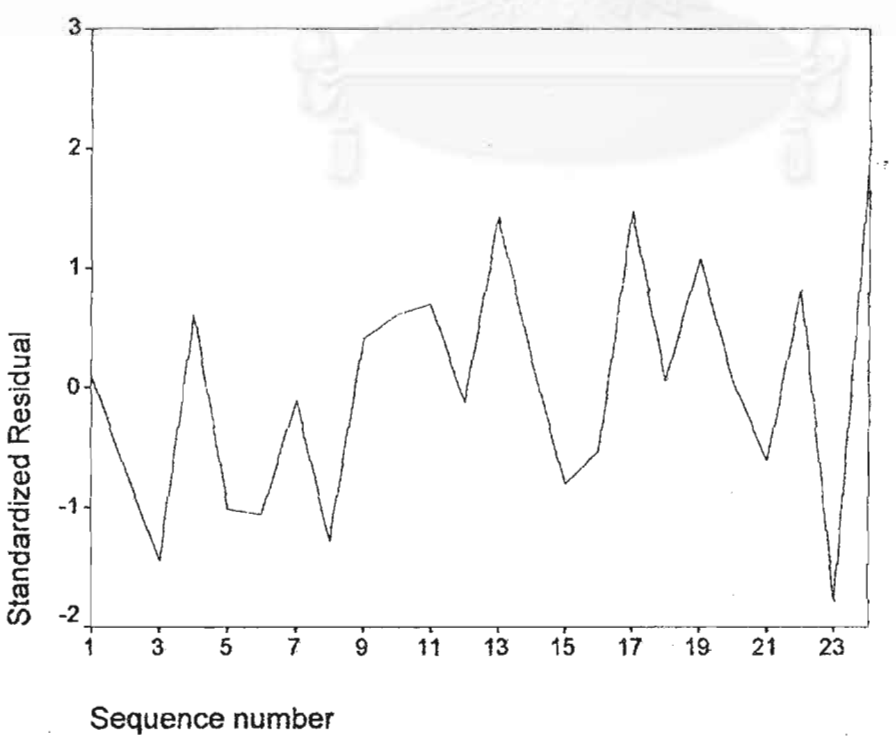
Partial Autocorrelations: ZRE_3 Standardized Residual

Lag	Pr-Aut-Corr.	Stand. Err.	-1 -.75 -.5 -.25 0 .25 .5 .75 1
1	-.216	.204	****I
2	.103	.204	I**
3	.144	.204	I***
4	-.077	.204	**I
5	.011	.204	*
6	-.011	.204	*
7	.111	.204	I**
8	-.030	.204	*I
9	-.068	.204	*I
10	-.173	.204	***I
11	-.074	.204	*I
12	-.096	.204	**I
13	.134	.204	I***
14	-.126	.204	***I
15	.098	.204	I**
16	-.158	.204	***I

Plot Symbols: Autocorrelations * Two Standard Error Limits .

Total cases: 24 Computable first lags: 23

TSPLOT of Second Order of a FCAJ Dispersion Containing 1% Sodium Metabisulfite



Zero Order Kinetics of a FCAJ Dispersion Containing 0.01% Propyl Gallate

Variables Entered/Removed[b]

Model	Variables Entered	Variables Removed	Method
1	time[a]	.	Enter

a. All requested variables entered.

b. Dependent Variable: pro.g0.01

Model Summary[b]

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate	Durbin-Watson
1	.933[a]	.870	.864	2.9731	1.755

a. Predictors: (Constant), time

b. Dependent Variable: pro.g0.01

ANOVA[b]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	1298.599	1	1298.599	146.908	.000[a]
	Residual	194.470	22	8.840		
	Total	1493.069	23			

a. Predictors: (Constant), time

b. Dependent Variable: pro.g0.01

Coefficients[a]

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	97.833	.900		108.732	.000
	time	-6.15E-03	.001	-.933	-12.121	.000

a. Dependent Variable: pro.g0.01

Residuals Statistics[a]

	Minimum	Maximum	Mean	Std. Deviation	N
Predicted Value	76.5858	97.8329	89.7815	7.5140	24
Residual	-5.8135	5.1135	8.882E-15	2.9078	24
Std. Predicted Value	-1.756	1.072	.000	1.000	24
Std. Residual	-1.955	1.720	.000	.978	24

a. Dependent Variable: pro.g0.01

Autocorrelations of Zero Order of a FCAJ Dispersion Containing 0.01% Propyl Gallate

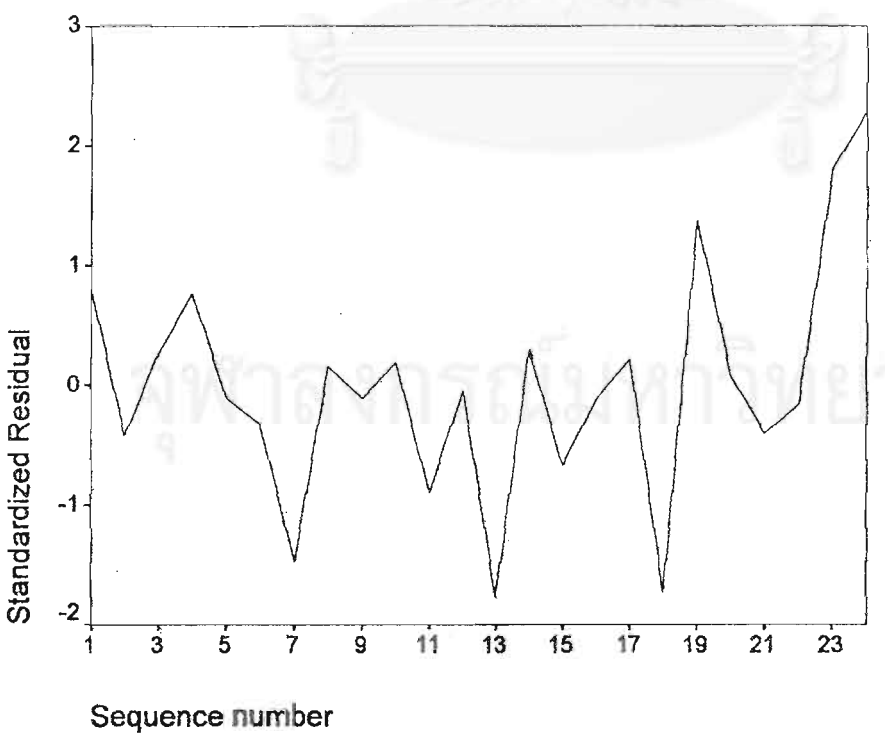
Partial Autocorrelations: ZRE_1 Standardized Residual

Lag	Pr-Aut-Corr.	Stand. Err.	-1 -.75 -.5 -.25 0 .25 .5 .75 1
1	.021	.204	. * .
2	.059	.204	. I* .
3	-.027	.204	. *I .
4	.131	.204	. I*** .
5	.172	.204	. I*** .
6	-.218	.204	. ****I .
7	.061	.204	. I* .
8	-.070	.204	. *I .
9	-.108	.204	. **I .
10	-.137	.204	. ***I .
11	.019	.204	. * .
12	-.293	.204	. ******I .
13	.010	.204	. * .
14	.005	.204	. * .
15	.065	.204	. I* .
16	-.039	.204	. *I .

Plot Symbols: Autocorrelations * Two Standard Error Limits .

Total cases: 24 Computable first lags: 23

TSPLOT of Zero Order of a FCAJ Dispersion Containing 0.01% Propyl Gallate



First Order Kinetics of a FCAJ Dispersion Containing 0.01% Propyl Gallate

Variables Entered/Removed[b]

Model	Variables Entered	Variables Removed	Method
1	time[a]	.	Enter

a. All requested variables entered.

b. Dependent Variable: ln_prog0.01

Model Summary[b]

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate	Durbin-Watson
1	.936[a]	.876	.870	3.27E-02	1.884

a. Predictors: (Constant), time

b. Dependent Variable: ln_prog0.01

ANOVA[b]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	.166	1	.166	155.387	.000[a]
	Residual	2.348E-02	22	1.067E-03		
	Total	.189	23			

a. Predictors: (Constant), time

b. Dependent Variable: ln_prog0.01

Coefficients[a]

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	4.584	.010		463.667	.000
	time	-6.95E-05	.000	-.936	-12.465	.000

a. Dependent Variable: ln_prog0.01

Residuals Statistics[a]

	Minimum	Maximum	Mean	Std. Deviation	N
Predicted Value	4.344333	4.584456	4.493464	8.49E-02	24
Residual	-6.6E-02	5.87E-02	8.51E-16	3.20E-02	24
Std. Predicted Value	-1.756	1.072	.000	1.000	24
Std. Residual	-2.035	1.797	.000	.978	24

a. Dependent Variable: ln_prog0.01

Autocorrelations of First Order of a FCAJ Dispersion Containing 0.01% Propyl Gallate

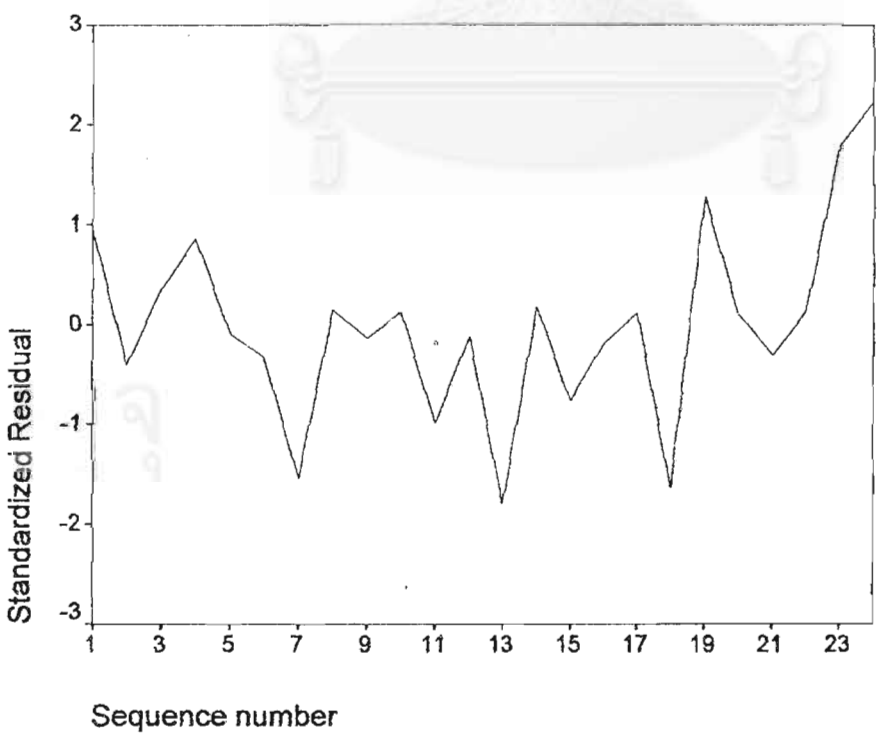
Partial Autocorrelations: ZRE_2 Standardized Residual

Lag	Pr-Aut-Corr.	Stand. Err.	-1 -.75 -.5 -.25 0 .25 .5 .75 1
1	.083	.204	I**
2	.118	.204	I**
3	-.003	.204	*
4	.120	.204	I**
5	.148	.204	I***
6	-.238	.204	*****I
7	.031	.204	I*
8	-.070	.204	*I
9	-.136	.204	***I
10	-.148	.204	***I
11	.035	.204	I*
12	-.292	.204	******I
13	.022	.204	*
14	.036	.204	I*
15	.031	.204	I*
16	-.059	.204	*I

Plot Symbols: Autocorrelations * Two Standard Error Limits .

Total cases: 24 Computable first lags: 23

TSPLOT of First Order of a FCAJ Dispersion Containing 0.01% Propyl Gallate



Second Order Kinetics of a FCAJ Dispersion Containing 0.01% Propyl Gallate

Variables Entered/Removed[b]

Model	Variables Entered	Variables Removed	Method
1	time[a]	.	Enter

a. All requested variables entered.

b. Dependent Variable: INV_PG01

Model Summary[b]

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate	Durbin-Watson
1	.938[a]	.879	.874	3.65E-04	1.999

a. Predictors: (Constant), time

b. Dependent Variable: INV_PG01

ANOVA[b]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	2.137E-05	1	2.137E-05	160.191	.000[a]
	Residual	2.935E-06	22	1.334E-07		
	Total	2.430E-05	23			

a. Predictors: (Constant), time

b. Dependent Variable: INV_PG01

Coefficients[a]

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	1.019E-02	.000		92.220	.000
	time	7.887E-07	.000	.938	12.657	.000

a. Dependent Variable: INV_PG01

Residuals Statistics[a]

	Minimum	Maximum	Mean	Std. Deviation	N
Predicted Value	1.02E-02	1.29E-02	1.12E-02	9.64E-04	24
Residual	-6.8E-04	7.62E-04	-2.9E-19	3.57E-04	24
Std. Predicted Value	-1.072	1.756	.000	1.000	24
Std. Residual	-1.859	2.087	.000	.978	24

a. Dependent Variable: INV_PG01

Autocorrelations of Second Order of a FCAJ Dispersion Containing 0.01% Propyl Gallate

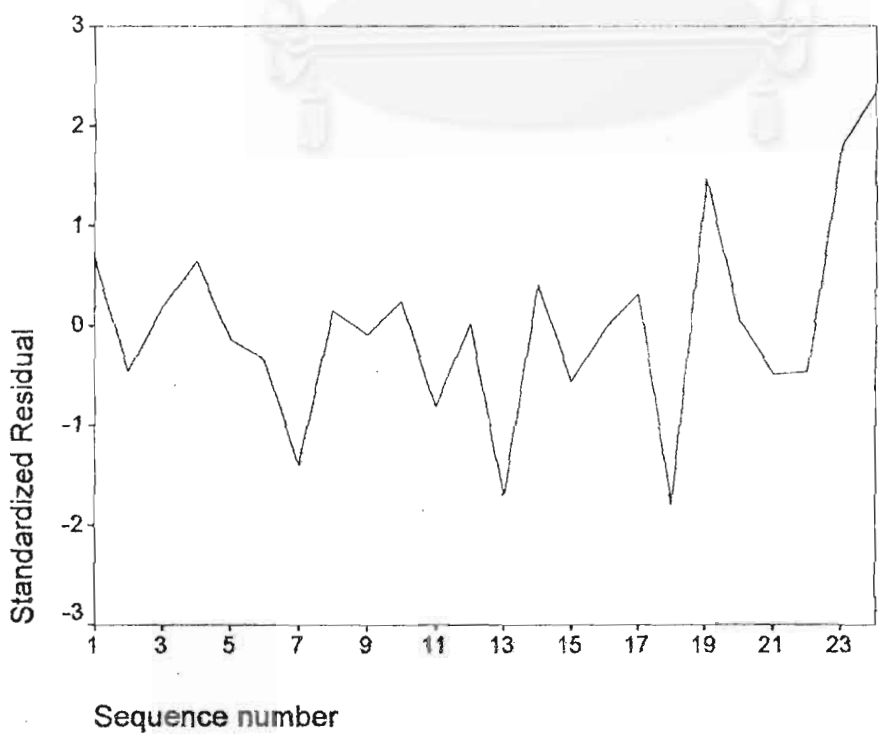
Partial Autocorrelations: ZRE_3 Standardized Residual

Lag	Pr-Aut-Corr.	Stand. Err.	-1 -.75 -.5 -.25 0 .25 .5 .75 1
1	-.033	.204	*I
2	-.007	.204	*
3	-.063	.204	*I
4	.136	.204	I***
5	.188	.204	I****
6	-.195	.204	****I
7	.097	.204	I**
8	-.066	.204	*I
9	-.071	.204	*I
10	-.114	.204	**I
11	.006	.204	*
12	-.288	.204	******I
13	-.003	.204	*
14	-.034	.204	*I
15	.092	.204	I**
16	-.021	.204	*

Plot Symbols: Autocorrelations * Two Standard Error Limits .

Total cases: 24 Computable first lags: 23

TSPLOT of Second Order of a FCAJ Dispersion Containing 0.01% Propyl Gallate



Zero Order Kinetics of a FCAJ Dispersion Containing 0.1% Propyl Gallate

Variables Entered/Removed[b]

Model	Variables Entered	Variables Removed	Method
1	time[a]	.	Enter

a. All requested variables entered.

b. Dependent Variable: prog0.1

Model Summary[b]

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate	Durbin-Watson
1	.919[a]	.845	.838	3.3602	1.066

a. Predictors: (Constant), time

b. Dependent Variable: prog0.1

ANOVA[b]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	1354.031	1	1354.031	119.922	.000[a]
	Residual	248.400	22	11.291		
	Total	1602.430	23			

a. Predictors: (Constant), time

b. Dependent Variable: prog0.1

Coefficients[a]

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	96.420	1.017		94.818	.000
	time	-6.28E-03	.001	-.919	-10.951	.000

a. Dependent Variable: prog0.1

Residuals Statistics[a]

	Minimum	Maximum	Mean	Std. Deviation	N
Predicted Value	74.7247	96.4205	88.1990	7.6727	24
Residual	-5.6745	5.3010	-4.74E-15	3.2863	24
Std. Predicted Value	-1.756	1.072	.000	1.000	24
Std. Residual	-1.689	1.578	.000	.978	24

a. Dependent Variable: prog0.1

Autocorrelations of Zero Order of a FCAJ Dispersion Containing 0.1% Propyl Gallate

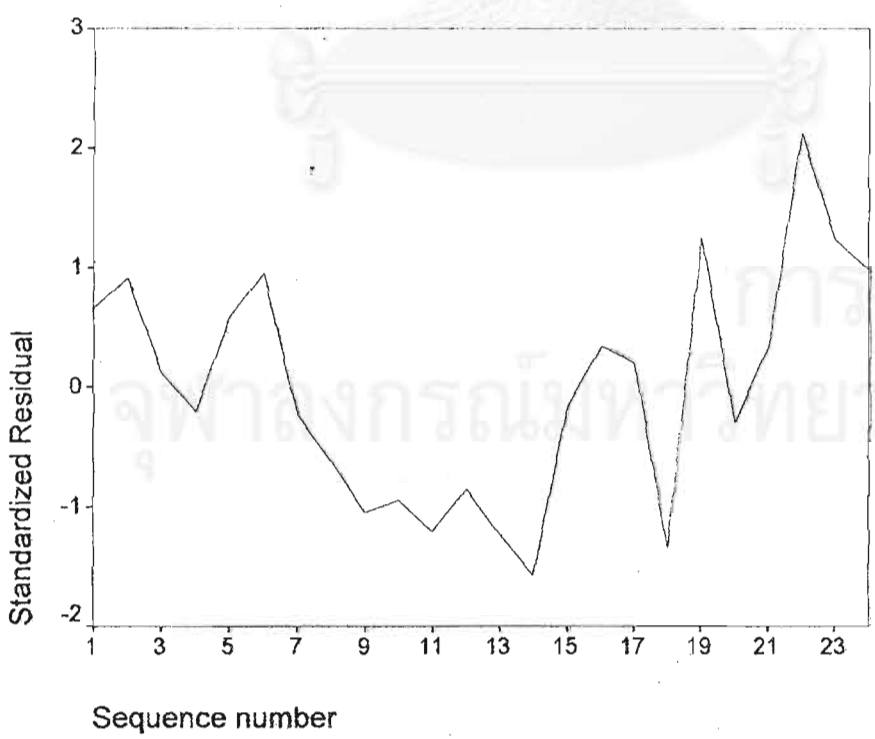
Partial Autocorrelations: ZRE_1 Standardized Residual

Lag	Pr-Aut-Corr.	Stand. Err.	-1 -.75 -.5 -.25 0 .25 .5 .75 1
1	.469	.204	. I******* .
2	.122	.204	. I** .
3	.129	.204	. I*** .
4	-.055	.204	. *I .
5	-.085	.204	. **I .
6	-.136	.204	. ***I .
7	-.044	.204	. *I .
8	-.338	.204	.*******I .
9	-.051	.204	. *I .
10	-.070	.204	. *I .
11	-.021	.204	. * .
12	-.208	.204	. ****I .
13	.051	.204	. I* .
14	.022	.204	. * .
15	.027	.204	. I* .
16	-.014	.204	. * .

Plot Symbols: Autocorrelations * Two Standard Error Limits .

Total cases: 24 Computable first lags: 23

TSPLOT of Zero Order of a FCAJ Dispersion Containing 0.1% Propyl Gallate



First Order Kinetics of a FCAJ Dispersion Containing 0.1% Propyl Gallate

Variables Entered/Removed[b]

Model	Variables Entered	Variables Removed	Method
1	time[a]	.	Enter

a. All requested variables entered.

b. Dependent Variable: ln_prog0.1

Model Summary[b]

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate	Durbin-Watson
1	.927[a]	.860	.853	3.63E-02	1.280

a. Predictors: (Constant), time

b. Dependent Variable: ln_prog0.1

ANOVA[b]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	.177	1	.177	134.593	.000[a]
	Residual	2.894E-02	22	1.316E-03		
	Total	.206	23			

a. Predictors: (Constant), time

b. Dependent Variable: ln_prog0.1

Coefficients[a]

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	4.569	.011		416.284	.000
	time	-7.18E-05	.000	-.927	-11.601	.000

a. Dependent Variable: ln_prog0.1

Residuals Statistics[a]

	Minimum	Maximum	Mean	Std. Deviation	N
Predicted Value	4.321223	4.569319	4.475305	8.77E-02	24
Residual	-6.6E-02	6.11E-02	-2.6E-16	3.55E-02	24
Std. Predicted Value	-1.756	1.072	.000	1.000	24
Std. Residual	-1.808	1.685	.000	.978	24

a. Dependent Variable: ln_prog0.1

Autocorrelations of First Order of a FCAJ Dispersion Containing 0.1% Propyl Gallate

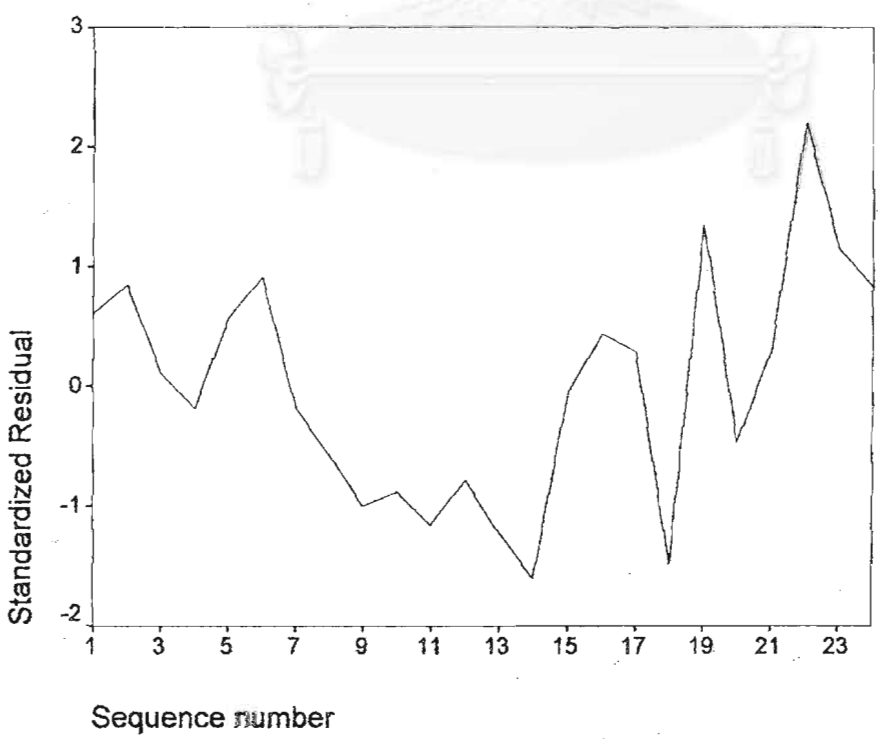
Partial Autocorrelations: ZRE_2 Standardized Residual

Lag	Pr-Aut-Corr.	Stand. Err.	-1 -.75 -.5 -.25 0 .25 .5 .75 1
1	.383	.204	I********
2	.134	.204	I***
3	.142	.204	I***
4	-.049	.204	*I
5	-.069	.204	*I
6	-.098	.204	**I
7	-.039	.204	*I
8	-.337	.204	********I
9	-.087	.204	**I
10	-.067	.204	*I
11	-.021	.204	*
12	-.204	.204	****I
13	.002	.204	*
14	.042	.204	I*
15	.043	.204	I*
16	-.016	.204	*

Plot Symbols: Autocorrelations * Two Standard Error Limits .

Total cases: 24 Computable first lags: 23

TSPLOT of First Order of a FCAJ Dispersion Containing 0.1% Propyl Gallate



Second Order Kinetics of a FCAJ Dispersion Containing 0.1% Propyl Gallate

Variables Entered/Removed[b]

Model	Variables Entered	Variables Removed	Method
1	time[a]	.	Enter

a. All requested variables entered.

b. Dependent Variable: inv_prog0.1

Model Summary[b]

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate	Durbin-Watson
1	.933[a]	.871	.865	3.96E-04	1.505

a. Predictors: (Constant), time

b. Dependent Variable: inv_prog0.1

ANOVA[b]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	2.337E-05	1	2.337E-05	148.794	.000[a]
	Residual	3.456E-06	22	1.571E-07		
	Total	2.683E-05	23			

a. Predictors: (Constant), time

b. Dependent Variable: inv_prog0.1

Coefficients[a]

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	1.036E-02	.000		86.334	.000
	time	8.248E-07	.000	.933	12.198	.000

a. Dependent Variable: inv_prog0.1

Residuals Statistics[a]

	Minimum	Maximum	Mean	Std. Deviation	N
Predicted Value	1.04E-02	1.32E-02	1.14E-02	1.01E-03	24
Residual	-7.1E-04	7.66E-04	-2.5E-18	3.88E-04	24
Std. Predicted Value	-1.072	1.756	.000	1.000	24
Std. Residual	-1.791	1.932	.000	.978	24

a. Dependent Variable: inv_prog0.1

Autocorrelations of Second Order of a FCAJ Dispersion Containing 0.1% Propyl Gallate

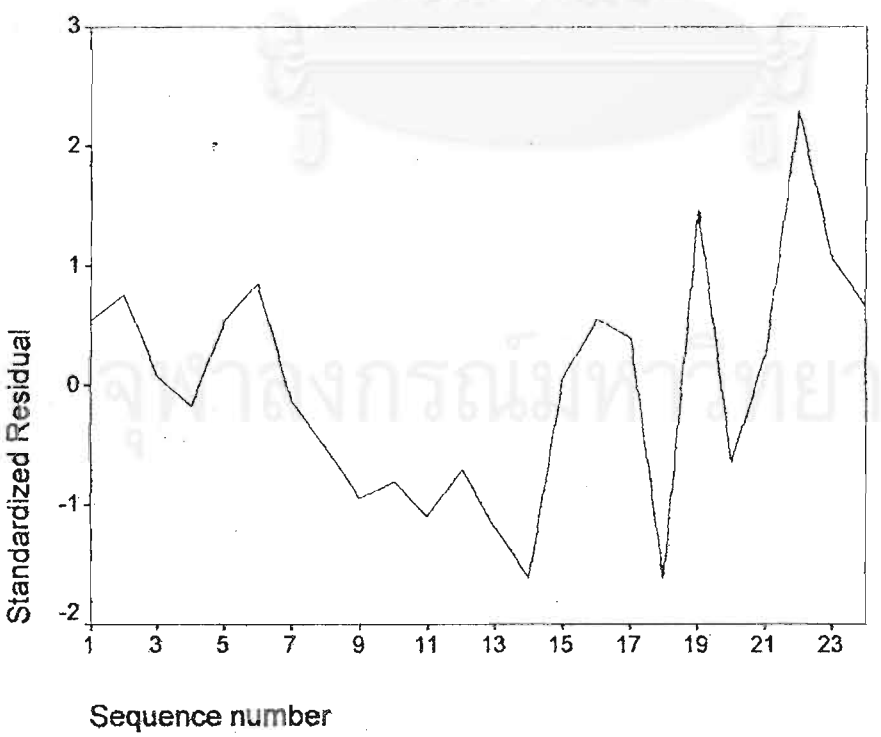
Partial Autocorrelations: ZRE_3 Standardized Residual

Lag	Pr-Aut-Corr.	Stand. Err.	-1 -.75 -.5 -.25 0 .25 .5 .75 1
1	.283	.204	I******
2	.130	.204	I***
3	.155	.204	I***
4	-.041	.204	*I
5	-.054	.204	*I
6	-.055	.204	*I
7	-.024	.204	*
8	-.332	.204	*******I
9	-.123	.204	**I
10	-.068	.204	*I
11	-.019	.204	*
12	-.199	.204	****I
13	-.048	.204	*I
14	.050	.204	I*
15	.063	.204	I*
16	-.011	.204	*

Plot Symbols: Autocorrelations * Two Standard Error Limits .

Total cases: 24 Computable first lags: 23

TSPLOT of Second Order of a FCAJ Dispersion Containing 0.1% Propyl Gallate



ANCOVA of Degradation of the Selected FCAJ Ingredient

Levene's Test of Equality of Error Variances[a]

	F	df1	df2	Sig.
PAR	1.204	4	115	.313

Tests the null hypothesis that the error variance of the dependent variable is equal across groups.

a. Design: Intercept+ANTIOX+TIME+ANTIOX * TIME

Tests of Between-Subjects Effects

Dependent Variable: PAR

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Noncent. Parameter	Observed Power[a]
Corrected Model	7702.567[b]	9	855.841	87.551	.000	787.958	1.000
Intercept	278437.3	1	278437.3	28483.625	.000	28483.625	1.000
ANTIOX	71.392	4	17.848	1.826	.129	7.303	.540
TIME	7028.787	1	7028.787	719.032	.000	719.032	1.000
ANTIOX * TIME	213.134	4	53.284	5.451	.000	21.803	.971
Error	1075.288	110	9.775				
Total	1005335	120					
Corrected Total	8777.855	119					

a. Computed using alpha = .05

b. R Squared = .877 (Adjusted R Squared = .867)

Estimated Marginal Means

Antioxidants

Estimates

Dependent Variable: PAR

Antioxidants	Mean	Std. Error
control	89.154909	.638
SM0.05	94.075984	.638
SM1	92.855325	.638
PG0.01	89.781475	.638
PG0.1	89.781475	.638

Control = do not add antioxidants ; SM0.05 = 0.05%(w/w) sodium metabisulfite ;

SM1 = 1%(w/w) sodium metabisulfite ; PG0.01 = 0.01%(w/w) propyl gallate ;

PG0.1 = 0.1%(w/w) propyl gallate.

Pairwise Comparisons

Dependent Variable: PAR

(I) Antioxidants	(J) Antioxidants	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
control	SM0.05	-4.921075*	.903	.000	-7.507	-2.335
	SM1	-3.700416*	.903	.000	-6.286	-1.115
	PG0.01	-.626566	.903	.489	-3.212	1.959
	PG0.1	-.626566	.903	.489	-3.212	1.959
SM0.05	SM1	1.220659	.903	.179	-1.365	3.806
	PG0.01	4.294508*	.903	.000	1.709	6.880
	PG0.1	4.294508*	.903	.000	1.709	6.880
	control	4.921075*	.903	.000	2.335	7.507
SM1	SM0.05	-1.220659	.903	.179	-3.806	1.365
	PG0.01	3.073849*	.903	.001	.488	5.659
	PG0.1	3.073849*	.903	.001	.488	5.659
	control	3.700416*	.903	.000	1.115	6.286
PG0.01	SM0.05	-4.294508*	.903	.000	-6.880	-1.709
	SM1	-3.073849*	.903	.001	-5.659	-.488
	PG0.1	1.04E-13	.903	1.000	-2.586	2.586
	control	.626566	.903	.489	-1.959	3.212
PG0.1	SM0.05	-4.294508*	.903	.000	-6.880	-1.709
	SM1	-3.073849*	.903	.001	-5.659	-.488
	PG0.01	-1.04E-13	.903	1.000	-2.586	2.586
	control	.626566	.903	.489	-1.959	3.212

Based on estimated marginal means

*. The mean difference is significant at the .05 level.

Univariate Tests

Dependent Variable: PAR

Source	Sum of Squares	df	Mean Square	F	Sig.	Noncent. Parameter	Observed Power[a]
Contrast	460.646	4	115.162	11.781	.000	47.123	1.000
Error	1075.288	110	9.775				

Each F tests the simple effects of Antioxidants within each level combination of the other effects shown. These tests are based on the linearly independent pairwise comparisons among the estimated marginal means.

a. Computed using alpha = .05

Vita

Miss Apimon Wuthiworawong was born on December 10, 1968 in Bangkok, Thailand. She received her Bachelor of science in Pharmacy Degree from the faculty of Pharmacy, Mahidol University, Bangkok, Thailand in 1994. After graduation, she had worked at Surin Hospital for two years. Next, she had worked at Children Hospital for two years before she entered the Master's Degree program in Pharmacy at Chulalongkorn University.