DEVELOPMENT OF SUNSCREEN PRODUCT CONTAINING *ARECA CATECHU* EXTRACT

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MASTER OF SCIENCE
IN
COSMETIC SCIENCE

SCHOOL OF COSMETIC SCIENCE
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ABSTRACT

The purpose of this study is to develop sunscreen product containing *Areca catechu* extract. The *A. catechu* extract was prepared by microwave-assisted method and the yield of 16.54% was obtained. It contained phenolic 465 mg GAE/g possessing DPPH radical scavenging and tyrosinase inhibition activities greater than standard trolox and kojic acid, respectively, with IC_{50} of 3.24 µg/mL and 24.32 µg/mL. Physicochemical and biological properties of the sunscreen containing *A. catechu* extract were also evaluated. The product illustrated the SPF value ranging from 8 to 20 when incorporating the extract from 1 to 3% w/w. While the pH was stable, the viscosity and color of the product were conversely changed with decrease in viscosity but rising in delta E when amount of the extract was increased. The product containing higher extract amount exhibited greater phenolic content, antioxidant power and sunscreening effect. High temperature storage decreased phenolic content corresponding to radical scavenging activity and sunscreening capability. With superior cosmetic activity found, the *A. catechu* was promised as a

high potential source for natural sunscreening extraction. However, its color stability enhancement is required to improve the product appearance.

Keywords: *Areca catechu*/Cosmetic activity/Phenolic/Natural sunscreen

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CHAPTER 1

INTRODUCTION

1.1 Background

The concerns of health and qualitative living are now becoming a major influence on various industries. Consumer products consisting entirely, or at least partly, of natural-based ingredients are more attractive to potential buyers than ones consisting entirely of synthetic chemicals on the same shelf because of the perception that natural ingredients are safer, however less effective they are compared to the synthetic ones. Similarly, in cosmetic industry, consumers' concerns on natural products have led to a change in consumer behavior. Consumers nowadays increasingly spend time reading product's labels carefully in order to obtain information about the ingredients - some even check whether the products contain an adequate level of active compounds in order to achieve a certain usage purpose. Since consumers expect more than what basic functions of product can deliver, many cosmetic manufacturers tend to claim the benefits of their products with a plus of natural extracts in order to establish an image of low toxicity, mildness and bio-degradability. Thailand is the origin of varieties of herbs whose extracts have been used in medicines and cosmetics for a long time. Hence, if local wisdoms regarding these herbal properties were merged with the new science of cosmetic technologies to create practical daily-use products, it would offer an alternative for consumers to substitute imported cosmetics with local ones.

Protecting skin against sun exposure is one of the most desirable cosmetic qualification in tropical countries since ultraviolet from sunlight can permanently

cause damages to human's DNA level and several harmful effects on the skin. Therefore, from the first sunscreen commercially appeared in 1928, it has been developed for many decades and not only been promoted as protecting against sunburn, but also preventing other sun-induced adverse effects such as photo-sensitivity, skin aging, and skin cancer. Currently, some of color cosmetics also claimed as protecting against UV from having combination of some ingredients with high refractive index such as Titanium dioxide or Zinc oxide in makeup base and foundation products. Since sunscreen products applied on the skin might lose its protection ability after sun exposure, consumers need to reapply sunscreen frequently during daytime. Ease of application has become consumers' concern since reapplying the product causes uncomfortable feeling to the skin or clogged pores; as a result, the quantity of sunscreen reapplied by consumers might be insufficient because they tend to avoid putting on too much. Water-based sunscreen was then developed to eliminate these undesirable outcomes.

In this study, cold-process sunscreen will be developed using long history Thai herb, *Areca catechu*, as one of the photo-protective agents. *A. catechu* once played a role in Thai economic crops; however, since the government seriously prohibited chewing *A. catechu* as well as did not support people to cultivate *A. catechu* due to its effect on stimulating nervous system such as narcotics, *A. catechu* has then gradually disappeared from Thai cultures. However, the demand for *A. catechu* still exists in other industries, and its export value has grown every year in the past decades with the market price at approximately 37-40 THB per kg for raw nuts and 50-55 THB per kg for dried ones (Ministry of Agriculture [Thailand], n.d.).

Expected sunscreen formulation in this study should practically give a good level of protection as well as commercial sunscreen and might be claimed on other biological properties. The final product will be claimed as a botanically-extracted and easily-applicable sunscreen.

1.2 Objectives

1.2.1 To extract and evaluate biological activity of phenolic compound from *A. catechu*

1.2.2 To develop sunscreen product containing *A. catechu* extract

1.2.3 To evaluate physiochemical properties of developed sunscreen containing *A. catechu* extract

1.3 Scope of the Study

1.3.1 Extract bioactive compound from *A. catechu*. seeds by microwave assisted extraction method

1.3.2 Develop sunscreen containing *A. catechu* extract

1.3.3 Evaluate stability of sunscreen containing *A. catechu* extract

1.3.4 Evaluate sun protection factor value of sunscreen containing *A. catechu* extract

1.3.5 Evaluate phenolic content from developed sunscreen containing *A. catechu*

1.3.6 Evaluate antioxidant capacity from developed sunscreen containing *A. catechu*.

1.4 Benefits

1.4.1 Sunscreen containing *A. catechu* extract can be used as a natural sunscreening agent.

1.4.2 A higher market value is added to *A. catechu.*

CHAPTER 2

REVIEW OF RELATED LITERATURES

2.1 Ultraviolet Radiation Source and Its Harmful

Radiation from the sun is a main source of ultraviolet or UVR which its range of 270-315 nm could be severely harmful to human skin. The longer the wavelength, the more penetration of UV radiation into deeper earth's surface layer and less impacted by atmospheric conditions. When UVR reaches skin's surface, it would not totally absorb into the skin but will reflect some part to the environment. The negative effects of UVR toward human skin are divided into 2 main types: acute cutaneous effects and chronic actinic damage. Acute effect of UVR toward human skin will occur immediately after sun exposure such as erythema, redness, and pigmentation and disappear later, while Chronic effect will result in permanent skin damage such as photocarcinogenesis and photoaging (Gontijol, Pugliesi & Araujo, 2009).

UVA	⟷	long wave: 340-400 nm short wave: 320-340 nm
UVB	⟷	290-320 nm
UVC	⟷	270-290 nm

UVA radiation is the cause of skin damage in stratum corneum, then penetrate more into deeper layer of skin then activate endogenous photosensitizing agents such as porphyrin, riboflavin, quinones to produce free radicals. According to previous study, these free radicals will not directly absorbed by biological targets but drastically alter cell function which leads to stronger photosensitizing action, causes more tanning than erythema (Schalka, Addor, Agelune & Pereira, 2012). In other words, UVA causes photosensitization [both phototoxic and photo-allergic reaction] and work as supported agent to UVB reaction toward human skin (Leelapornpisid,

1989). Melanin oxidation from the effect of UVA can be classified into: Immediate Pigment Darkening (IPD), will occur after seconds of exposure to UVA and visible light then disappear in 2 hours; Persistent Pigment Darkening (PPD), will occurs during 2-24 hours after sun exposure.

UVB contributes 5% of total UVR pass through the earth's surface [in vitro evaluation of sunscreen] UVB is mainly responsible for both acute and chronic skin damage. UVB will interrupt DNA /RNA production and suppress cell mitosis, and probably stimulate carcinogenesis as well. UVB radiation composes of short waves, which have high energy and less penetrate to the ground compared to UVA. However, UVB is an important factor for vitamin D production in human body.

UVC radiation is totally absorb by ozone layer; therefore, this harmful radiation does not exist on the earth's surface.

2.2 Sunscreen Agents

Sunscreen contains molecules or molecular complexes which can absorb, reflect, or scatter ultraviolet radiation. Those complexes are composed of photo-protective agent as active ingredient and vehicle molecules as a carrier in sunscreen formula.

Photo-protective agent or sunscreen agent is mainly divided into chemical type and physical type according to its working mechanism. Since each sunscreen agent has different spectrum protection range, one sunscreen product might combine more than one sunscreen active ingredients to cover broad spectrum protection as shown in Figure 2.1.

Sunscreen agent will act as chromophores to absorb ultraviolet radiation. After UV has been absorbed, the chemical reaction will turn substance into excite state, release energy in form of heat, then return to its steady state; in which the sunscreen agent will recover its ability to absorb UVR. We call this mechanism as photostability. The efficiency of chromophore absorption is directly related to its chemical structure due to its number of conjugated double bonds, the greater amount of the bonds, the more protection ability it does (Gontijol et al., 2009).

In contrast, if there is structure's transformation or degradation occur after the first UV absorption, causes the substance disable to absorb ultraviolet and finally no longer act as protective agent, we call it photo-unstable filter. In addition, the word photo-reactive filter shall be applied to the agent in which their excite state of reaction has release some toxic byproduct or interact with human skin and environment.

Nowadays, there is a hybrid photo-protective agent which enable to reflect and absorb ultraviolet just like the combination of physical and chemical sunscreen. The sample of hybrid sunscreen agent is Bis-Benzotriazolyl Tetramethylbutylphenol or Tinosorb®M (Kasikawatana, 2012).

Table 2.1 Commercial sunscreen agents

Sunscreen Ingredients	Protection		Sunscreen type
	UVA	UVB	
Aminobenzoic acid (PABA)	o	✓✓✓	C
Avobenzone	✓✓✓	✓	C
Cinoxate	✓	✓✓✓	C
Dioxybenzone	✓✓	✓✓✓	C
Escamsule	✓✓✓	✓	C
Homosalate	o	✓✓✓	C
Menthyl anthranilate	✓✓	✓✓✓	C
Octocrylene	✓	✓✓✓	C
Octyl methoxycinnamte	✓	✓✓✓	C
Octylt salicilate	o	✓✓✓	C
Oxybenzone	✓✓	✓✓✓	C
Padimate O	o	✓✓✓	C

Table 2.1 (Continues)

Sunscreen Ingredients	Protection		Sunscreen type
	UVA	UVB	
Phenylbenzimidazole	o	✓✓✓	C
Sulisobenzone	✓✓	✓✓✓	C
Titanium dioxide	✓✓	✓✓✓	P

Note. Protection level: o = minimal, ✓ = limited, ✓✓ = considerable, ✓✓✓ = extensive
Type of sunscreen: c = chemical sunscreen, p = physical sunscreen

Source United States Environmental Protection Agency (2006)

The development of sunscreens has always been based on the concept of protection against the effects of UVR on the skin. As a result, sunscreens used to offer basic protection against UVB and more recently against UVA radiation (Schalka et al., 2012). Some of sunscreen agents are not approved by many countries due to its phototoxic, leads to allergy and carcinogen. The sample of sunscreen which not approved are Aminobenzoic acid or PABA, and Padimate A.

Several most used sunscreen agents are combined together to cover broad spectrum such as Octyl methoxycinnamate requires additional UVB protection filter to achieve SPF>30.

Physical sunscreen agents like Titanium dioxide and Zinc oxide are widely used as well due to its broad spectrum protection and non-reactive property, means less irritate to skin compared to chemical sunscreen agents. The concern on physical sunscreen is its white cast left on skin after application; however, micronized or ultra-fine particles of inorganic sunscreen were developed for better dispersion and absorption properties. Furthermore, dimethicone or silica is added to decrease aggregation of small particles.

2.3 Sun Protection Factors

Sun protection factor is defined as the ratio of Minimal Erythema Dose of protected skin and unprotected skin as followed

$$SPF = \frac{\text{minimum erythemal dose with unprotected skin (MEDu)}}{\text{minimum erythemal dose with protected skin (MEDp)}}$$

Minimum erythemal dose refers to the amount of UV radiation that will produce minimal erythema (sunburn or redness caused by engorgement of capillaries) of an individual's skin within a few hours following exposure (Heckman et al., 2013). Therefore, the ratio of MED before and after treat with sun protection product give a hint to consumers on how protective it does. The higher SPF value, the more protection from the product compared to the lower one at the same amount. Disadvantage of SPF is that it cannot tell if skin starts to get damage from UVA, since UVA affects deeper layer of skin without the sign of redness and burn.

2.4 Natural Sunscreen

Currently, there are many studies claimed that consuming some nutritional supplement such as beta-carotene, lutein, lycopene may help protect the skin against harmful ultraviolet radiation (UV). The studies emphasized on systemic photoprotection which is different from typically way of applying sunscreen. However, the research stressed out that such nutritional supplements cannot be a replacement of sunscreen products due to its sun protection factor level is inadequate under strong sun exposure. Therefore, the studies recommended that both should be used as complementary toward each other which might require several weeks of consumption. Other natural sunscreens for external application are basically known in many kinds of oil such as coconut, sesame, peanut, olive; or high anti-oxidant extracts such as pomegranate, licorice, cinnamon, curcumin, mulberry. The major substance which play a role of sunscreen agent from plants is phenolic compounds. Phenolic compound has at least one aromatic ring with one or more hydroxyl group, therefore their variation in structure affect on the difference in chemical properties. Phenolic compounds from plants is mainly responsible to plant's

color, posses to different biological activities which most important is antioxidative function. Different structures of phenolic compounds create variety of plant's colors as shown in Figure 2.1.

Source Slater (2012)

Figure 2.2 Fruits and Vegetables Arranged by Colors

Carotenoids found in various plants such as papaya, apricot, carrot, mango, beet root are rich in antioxidants which reduce the negative effects of UVB radiation (Drake, 2011). Similar to other phenolic compounds such as lycopene, catechins, anthocyanins which support skin protection from harmful ultraviolet radiation. Further than the claims of sunscreen function, the skin damage which caused from UV might be added as well; for example, anti-wrinkle or whitening properties. .

2.5 Areca Catechu as Sunscreen Agent

Raw *A. catechu* or Areca nut [Betel nut] for its common name is well known as "Mhark" in Thai (as shown in Figure 2.2, 2.3). As mentioned earlier, *A. catechu* is once Thai economic crops for over 70 years ago. Traditionally, areca nut was thought to be beneficial to oral hygiene, appetite and saliva production but scientists found

that chewing areca nut increases the risk of cancers in mouth and esophagus, and that it can adversely affect the health of children in the womb (Betel nut chewing set in Figure 2.4). Although there are several potentially useful aspects of Areca nut, most experts agree that the risks make the substance unhealthy. In 1941, Health Ministry of Thailand announced the negative effects of consuming Areca nut according to government's intention to lead Kingdom of Thailand step into international culture level.

Source Ploypayom (2011)

Figure 2.2 Inside Areca Nut

Source Ploypayom (2011)

Figure 2.3 A Bunch of Areca Nuts

Source Importfood (n.d.)

Figure 2.4 Betel Nut Chew Set or "Chien Mhark"

The origin of *A. catechu* is still unclear, but it was founded widely in India and South East Asia (Department of Agricultural Extension [Thailand], n.d.). *A. catechu* is used in textile industry as a component in fabric dye to reserve the quality of fabric and extend product life; also, used as a softening active in leather tanning. In term of general consumption, Areca nut is used as a herb to regenerate disorder digestive system and some externally used to relieve skin itching. Areca nut might be preserved in dried form as shown in Figure 2.5.

Source Taj Agro products (n.d.)

Figure 2.5 Dried Areca Nuts

Previous study showed yield capacity in various parts of *A. catechu* extract which obtained by maceration method in selective solvents; petroleum ether, methylene chloride, ethylacetate, methanol, and water. The results in Table 2.1 indicated that the highest %yield extract from *A. catechu* is from 4-month seeds part in methanol at 23.41% and the second-top is 4-month seeds part in water at 23.35%.

Table 2.2 Yield of *A. catechu* extract from various parts calculated from dry weight

Part of *Areca catechu* and extracted solvents	**% Yield of extracts**
Leaves	
Petroleum ether	1.56
Methylene Chloride	1.35
Ethyl acetate	0.65
Methanol	6.69
Water	11.58
Crownshafts	
Petroleum ether	0.21
Methylene Chloride	0.25
Ethyl acetate	0.22
Methanol	0.62
Water	2.15
4-month Seeds	
Petroleum ether	1.97
Methylene Chloride	0.68
Ethyl acetate	0.32
Methanol	23.41
Water	23.35

Table 2.2 (continued)

Part of *Areca catechu* and extracted solvents	% Yield of extracts
8-month Seeds	
Petroleum ether	2.29
Methylene Chloride	1.46
Ethyl acetate	0.59
Methanol	13.77
Water	9.25
4-month fruit shells	
Petroleum ether	0.43

Note. Wetwitayaklung, Phaechamud, Limmatvapirat and Keokitichai (2006)

The same study also pointed out that seed extract in methanol and water had percentage of tannins and total phenols higher than other part extract with high to moderate antioxidant activities according to its IC_{50} values and antioxidant activities of trolox (Wetwitayaklung et al., 2006). The study suggested that %tannin and total phenols were not only factors of anti-oxidant activity, but might contribute of volatile oil or other compounds in *A. catechu* seeds. The results from this study showed that, although the methanol extract of 6 and 8-month seeds had % tannin and total phenols higher than those of 4-month seeds, the anti-oxidant activities of them were lower than 4-month seeds extract.

Other previous studies reported its potential to prevent adverse effects of ultraviolet radiation on skin caused by excessive generation of reactive oxygen species (ROS) which lead to skin cancer and photo-aging. The claims of A. *catechu* which has high amounts of tannic acid and gallic acid are identified as anti-microbial, anti-inflammatory, anti-melanogenesis, anti-elastase, and anti-oxidant. Further claim of active as GABA in stimulating collagen synthesis by buccal fibroblast is found as well. In vitro sun protection factor determination of herbal formulation cream were tested among various kind of herbs at the percentage concentration of 1%, 3%, and 5% accordingly.

The data showed significantly positive result compared to base cream and control, and the formulation contained of A. *catechu* has highest effective value at the concentration of 3% in formula giving SPFs approximately 10.8 (Swarnlata, Sumit, Chanchal & Shailendra, 2012)

CHAPTER 3

MATERIALS AND METHODS

3.1 Chemicals and Instrument

3.1.1 Chemical Materials

Table 3.1 List of chemicals materials

Chemicals	Company
2,2-diphenyl-1-picrylhydrazyl [DPPH reagent]	Sigma Aldrich
3,4 dihydroxyphenylalanine [L-Dopa]	Sigma Aldrich
6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid [Trolox]	Sigma Aldrich
Butylene Glycol	Kyowa Hakko
Consecutive Paraben	Sharon Laboratories
Cyclopentasiloxane	Dow Corning
DI water	
Dipotassium hydrogen phosphate	Ajax Finechem
Dipropylene Glycol	Sigma Aldrich
Disodium EDTA	Dow Corning
Ethanol	Merck
Follin-Ciocalteu reagent	Carlo Erba
Gallic acid	Fluka
Glycerin	Dow Corning
Hydroxyethylcellulose	Dow Chemical
Kojic acid	Sigma Aldrich
Mushroom tyrosinase	Sigma Aldrich
Phenoxyethanol	THOR Specialties

Table 3.1 (continued)

Chemicals	Company
Potassium dihydrogen phosphate	Ajax Finechem
Potassium Hydroxide	SPOLCHEMIE
Sodium Carbonate	Fisher Chem
Sorbeth-30 Tretraisostearate, Sorbitan Sesquiisostearate, PPG-8-Ceteth- 20, Acrylates/Beheneth-25 Methacrylate Copolymer, Dipropylene Glycol, Water	Nikko Chemicals
Tocopheryl Acetate	Eisai Food Chemical
Xanthan gum	CP Kelco

3.1.2 Instrument and Equipment

Table 3.2 List of instrument and equipments

Instrument and equipment	Model	Company
Analytical Balance	XT220A	Precisa
Centrifuge	Micromax	Thermo IEC
Chroma Meter	CR-400	Konica Minolta
Hot Air Oven	Ecocell 222C	MMM
Microwave	FMA25XT	Smeg
pH Meter	Multi-Parameter PCTestr™ 35 Series	Oakton
Refrigerator	SJ-D30M	SHARP
Rotary Evaporater	CCA1110	Eyela
SPF Analyzer	SPF-290S Analyzer system	
UV-Visible Spectrophotometer	10UV	Genesys
Viscometer	DV-II+ Pro	Brookfield
Vortex mixer	Vortex-Genie2	Genie

3.2 Preparation of *A. catechu* extract

Raw *A. catechu* seed was dried, ground, and sieved through 500 μm mesh size. The grinded *A. catechu* nuts was extracted by using 50% ethanol (v/v) at a solvent ratio of 1:10 (w/v), then used microwave assisted extraction at 900 Watt for 30 minutes. The mixture was then filtered and collected. The ethanol was removed by rotary evaporator. *A. catechu* extract was diluted in water or glycerin before adding into the formulations.

3.3 Development of Sunscreen Base Formula

Table 3.3 Base formula

Part	INCI Name	Formulation					
		Base1	**Base2**	**Base3**	**Base4**	**Base5**	**Base6**
A	Glycerin	8.75	5.00	5.00	5.00	5.00	5.00
	Butylene Glycol	5.00	5.00	5.00	5.00	5.00	5.00
	Sorbeth-30 Tretraisostearate, Sorbitan Sesquiisostearate, PPG-8-Ceteth-20, Acrylates/Beheneth-25 Methacrylate Copolymer, Dipropylene Glycol, Water	4.50	4.50	4.50	4.50	4.50	4.50
	Dipropylene Glycol	4.00	1.0	1.0	1.0	1.0	1.0
	Consecutive Paraben	0.50	0.50	0.50	0.50	0.50	0.50
	Phenoxyethanol	0.40	0.40	0.40	0.40	0.40	0.40
	Disodium EDTA	0.03	0.03	0.03	0.03	0.03	0.03
B	DI water	70.22	76.95	76.67	76.37	76.27	76.77
	Xanthan gum	-	-	0.10	0.50	0.50	0.10
	Potassium Hydroxide 10%	0.50	0.50	0.50	0.50	0.50	0.50
	Hydroxyethylcellulose	-	-	0.20	0.10	0.20	0.10
C	Tocopheryl Acetate	0.10	0.10	0.10	0.10	0.10	0.10
D	Cyclopentasiloxane	6.0	6.0	6.0	6.0	6.0	6.0
	Total	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**

Disperse part A and neutralize with part B solution until gel is formed. Then add part C and D accordingly. Each formula was tested and given score from 1-5. The formulation with highest score is selected to develop sunscreen containing *A. catechu* extract.

3.4 Development of Sunscreen Containing Areca Extract

Table 3.4 Sunscreen containing areca extract formulations

Part	INCI Name	Formulation			
		Base	*A.catechu* 1%	*A.catechu* 2%	*A.catechu* 3%
A	Glycerin	5.00	5.00	5.00	5.00
	Butylene Glycol	5.00	5.00	5.00	5.00
	Sorbeth-30 Tetraisostearate, Sorbitan Sesquiisostearate, PPG-8-Ceteth-20, Acrylates/Beheneth-25 Methacrylate Copolymer, Dipropylene Glycol, Water	4.50	4.50	4.50	4.50
	Areca catechu Extract	-	1.00	2.00	3.00
	Dipropylene Glycol	1.00	1.00	1.00	1.00
	Consecutive Paraben	0.50	0.50	0.50	0.50
	Phenoxyethanol	0.40	0.40	0.40	0.40
	Disodium EDTA	0.03	0.03	0.03	0.03
B	DI Water	72.77	71.77	70.77	69.77
	Xanthan gum	0.50	0.50	0.50	0.50
	Potassium Hydroxide 10%	0.50	0.50	0.50	0.50
	Hydroxyethylcellulose	0.20	0.20	0.20	0.20

Table 3.4 (continued)

Part	INCI Name	Formulation			
		Base	*A.catechu* 1%	*A.catechu* 2%	*A.catechu* 3%
C	Tocopheryl Acetate	0.10	0.10	0.10	0.10
D	Cyclopentasiloxane	6.00	6.00	6.00	6.00
Total		**100.00**	**100.00**	**100.00**	**100.00**

3.5 Stability Test

3.5.1 Centrifugation Method

A gram of each formulation was centrifuged by Thermo IEC Micromax at 5,000 rpm for 30 minutes. Any separation was observed.

3.5.2 Heating-Cooling Cycle

The accelerated stability test was conducted by exposing the products to cooling temperature of 4°C for 24 hours, then placing in hot air oven at 45°C for the next 24 hours. After that the products were temperature again for 24 hours to complete one cycle. Stability test was assessed for six heating-cooling cycles and analyzed for any significant changes.

3.5.3 Long Term Stability

The products were kept in 4°C, room temperature and 45°C for 8 weeks. Physiochemical and biological properties were determined every two weeks to observe any significant changes.

3.6 Determination of Physicochemical Properties

3.6.1 pH Measurement

The pH determination was conducted by Oakton Multi-Parameter PCTestr™ 35 Series. The temperature was controlled at 27˚C during the measure.

3.6.2 Viscosity Analysis

The viscosity analysis was conducted by Brookfield DV-II+ Pro Viscometer, using roter no.5 and the speed was set at 30 rpm for 30 seconds each. The temperature was controlled at 27˚C during the measure.

3.6.3 Color Measurement

The color measurement was conducted by Konica Minolta Chroma Meter CR-400, using CIE L*a*b* parameters system.

3.6.4 Determination of Sun Protection Factor (SPFs)

The SPF value was measured following the standard method from Standard Protocol, In-Vitro SPF Testing, Optometric. In-vitro analysis was performed using SPF-290S Sunscreen Protection Factor Analyzer System on Optometric LLC/SPF 290s instrument using the latest version of WinSPF software. A 1 mL syringe was used to dispense 100 or more dabs of sample over a Transpore® tape substrate area (25 cm^2) at the rate of 2 $\mu g/cm^2$, as specified by the U.S. Food and Drug Administration. The results were determined by averaging the value of 6 scans of the sample in different locations on the Transpore® tape substrate. Each scan took a transmittance measurement every 2 nm from 290 to 400 nm. The results were compared to a referenced scan at the same wavelength to compute the sample transmittance. The SPF-290S' WinSPF software computationally converted measurement result to the well-known SPF values, Boots Star ratings.

The measurements were performed by using this standard protocol with some modifications. The sunscreen formula was accurately weighed of to 0.22 mg and was injected with syringe 1 ml. Then evenly spread across the entire surface 25 cm^2 of the substrate using Transpore® 3M attached on the square disk using a gloved finger. The SPF values of each formulation were determined by SPF measurement under

transmission between 290 and 400 nm in autoscan program. All of the measurements were performed in triplicate and used for statistical analysis.

3.7 Determination of Cosmetic Biological Activities

3.7.1 Determination of Total Phenolic Content

Extractable phenolics content of *A. catechu* seeds extract was determined according to the Folin-Ciocalteu method (Kumar et al., 2008) with some modifications. The standard phenolic compound used in this study was gallic acid.

Extractable phenolics contents (EPC) of all extracts were expressed as milligram gallic acid equivalents per gram of dried sample (mg GAE/g sample) which calculated from gallic acid standard curve.

3.7.2 Determination of Antioxidant Capacity (DPPH radical scavenging assay)

The decolorizing reaction of 2,2-diphenyl-1-picrylhydrazyl stable radical scavenging activity was employed (Thaipong et al., 2006).

The trolox standard curve is prepared by plotting percentage of inhibition versus trolox concentration. The result was expressed as milligram trolox equivalent antioxidant capacity per gram of dried sample (mg TEAC/ g sample) and the concentration giving 50% inhibitory activity (IC_{50}, µg/mL).

3.7.2 Determination of Tyrosinase Inhibition Activity

The mushroom tyrosinase (EC 1.14.18.1) inhibitory activity of the extract was determined using L-Dopa as the monophenolase substrate (Onar *et al*., 2012).

The kojic acid standard curve is prepared by plotting percentage of inhibition versus kojic acid concentration. The result was expressed as milligram kojic acid equivalent per gram of dried ample (mg KAE/ g sample) and the concentration giving 50% inhibitory activity (IC_{50}, µg/mL).

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Cosmetic Activities of *A. catechu* extract

After extraction and evaporation the solvent out, the *A. catechu* extract was observed as dark brown and viscous liquid. The 16.54 % (w/w) yield was obtained. The extract was further subjected to analyze its cosmetic bioactivity and the result was present in Table 4.1. The *A. catechu* extract contained 465.05 mg GAE/g which was comparable to previously reported by Sangthong, Pintathong and Chaiwut (2013). Noticeably, the antioxidant and tyrosinase inhibitory activities of the extract were substantially high. Its IC_{50} value against DPPH radical (3.24 µg/mL) was lower than that of standard trolox (4.04 µg/mL). The lesser the IC_{50} value, the higher antioxidant power. This cosmetic activity could be used to claim anti wrinkle ability. Similarly, the extract exhibited higher activity against tyrosinase inhibition than the standard kojic acid. It implied that the extract could be also the great skin whitening agent for cosmetic product.

Table 4.1 Phenolic content, antioxidant capacity and tyrosinase inhibitory activity of *A. catechu* extract.

Samples	Phenolic content (mg GAE/g)*	DPPH radical scavenging capacity (IC_{50}, µg/mL)	Tyrosinase inhibitory activity (IC_{50}, µg/mL)
A. catechu extract	465.05 ± 9.44	3.24 ± 0.11	24.32 ± 0.87
Trolox	-	4.04 ± 0.07	-
Kojic acid	-	-	35.65 ± 2.04

Note. *GAE: gallic acid equivalent.

4.2 Development of Sunscreen Base Formula

Table 4.2 Evaluation scores of base formulas

Properties	Score(s)					
	Base1	**Base2**	**Base3**	**Base4**	**Base5**	**Base6**
Appearance	3	3	4	5	5	5
Odor	4	4	3	3	3	3
Color	4	4	5	5	5	5
Spreadability	2	3	3	4	5	3
Absorbability	2	2	3	4	5	3
Centrifuge test	Pass	Pass	Pass	Pass	Pass	Pass
Total	**15**	**16**	**18**	**23**	**25**	**19**

Note. 1 = Poor, 2 = Fair, 3 = Moderate, 4 = Good, 5 = Excellent

After scoring base formulas, Base5 was selected to develop sunscreen base formulation and pass through centrifuge test again to test whether base and *A. catechu* extract are not separated after mix together.

The selected base formula is opaque white with no odor. From the texture appearance, it might be assumed to have high viscosity. However, the absorbability is fast and perform good spread on skin application.

4.3 Stability of Sunscreen Containing *A. catechu* Extract

4.3.1 Stability of pH value

Figure 4.1 showed the pH value of the developed sunscreens stored in temperature of 4˚C for 8 weeks interval in an average of three repeats ± standard deviation. The results base formulation and sunscreen formulations containing *A. catechu* at the concentration of 1%, 2% , and 3% were 6.50±0.08, 5.80±0.03, 5.50±0.16, and 5.20±0.05 accordingly. It can be seen that the pH of the sunscreens were not significantly changed over time. Therefore, pH value of all formulations were stable at 4˚C.

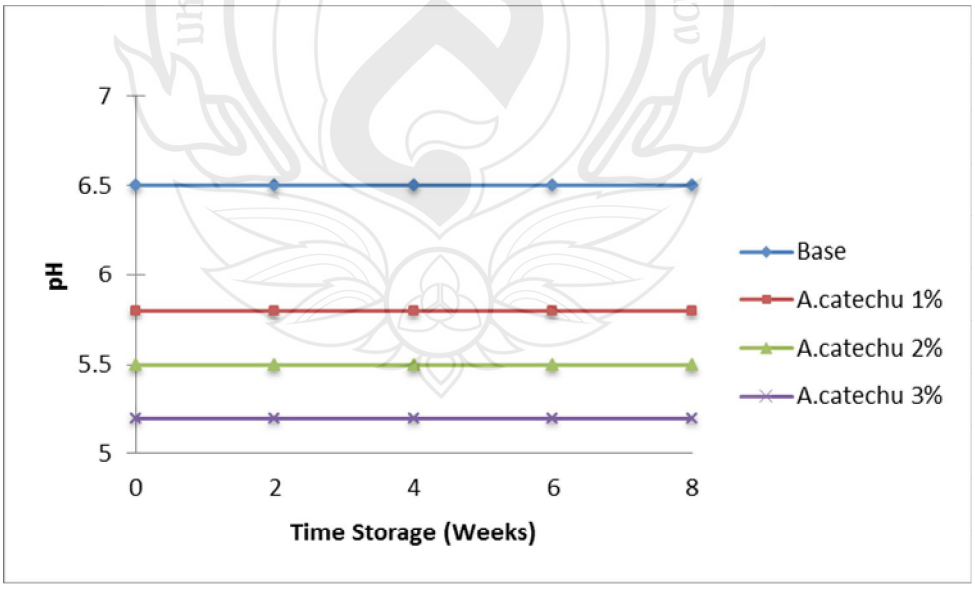


Figure 4.1 pH values of sunscreen stored at temperature of 4˚C for 8 weeks.

Figure 4.2 demonstrated the pH value of the developed sunscreens stored in room temperature for 8 weeks interval in an average of three repeats ± standard deviation. The results of the base formulation and sunscreen containing *A. catechu* at the concentration of 1%, 2%, and 3% were 6.50±0.05, 5.80±0.20, 5.50±0.12, and 5.20±0.06 accordingly. It can be seen that the pH of the sunscreens were not significantly changed over time. Therefore all of the formulations were stable at room temperature.

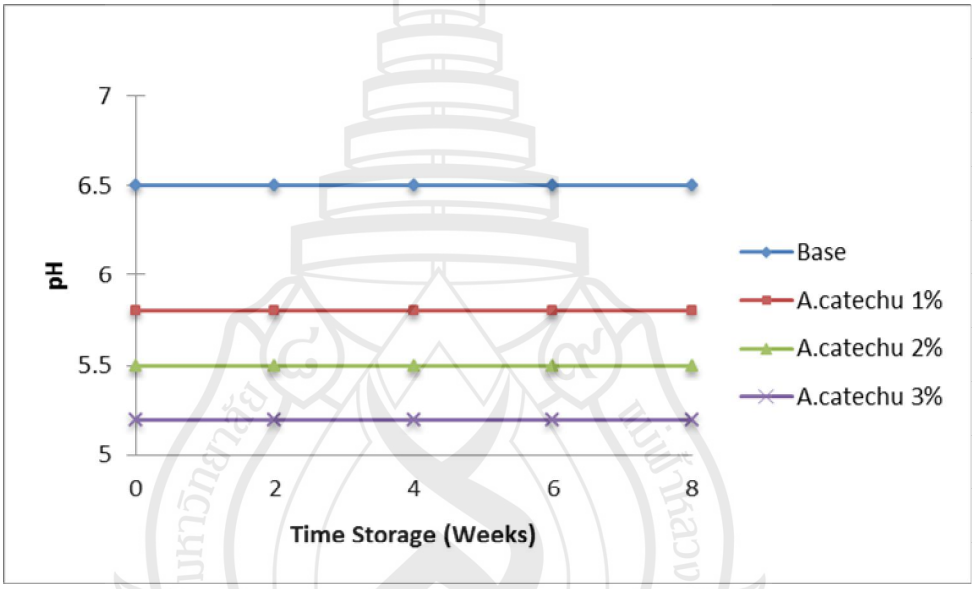


Figure 4.2 pH values of sunscreen stored at room temperature for 8 weeks.

Figure 4.3 illustrated pH values of the developed sunscreens stored in temperature of 45˚C for 8 weeks interval in an average of three repeats ± standard deviation. The results of base formulation and sunscreen containing *A. catechu* at the concentration of 1%, 2% , and 3% were 6.50±0.12, 5.80±0.15, 5.50±0.10, and 5.20±0.08 accordingly. It can be seen that the pH of the sunscreens were not significantly changed over time. Therefore, pH value of all formulations were stable at 45˚C.

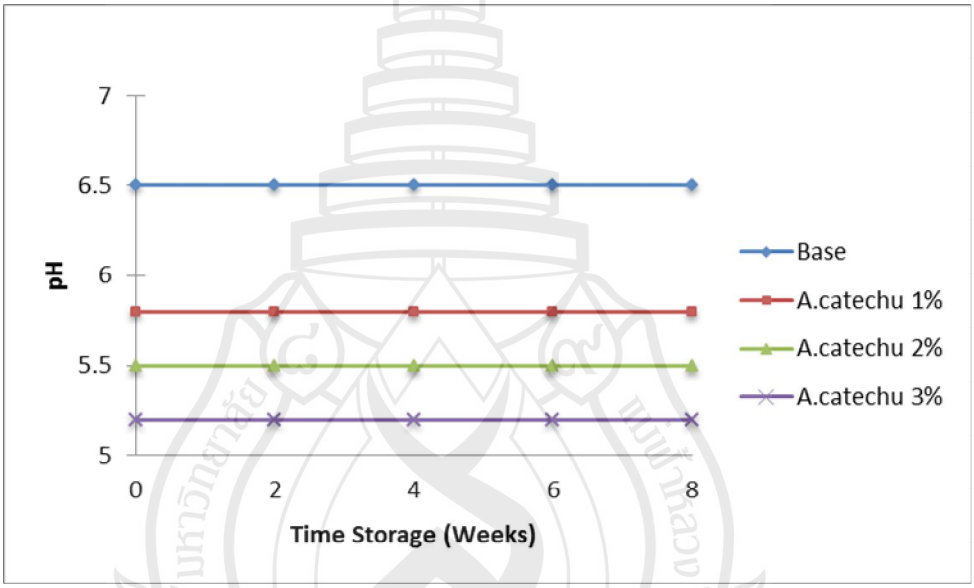


Figure 4.3 pH value of sunscreen stored at temperature of 45˚C for 8 weeks

The graph in figure 4.4 showed pH values of the developed sunscreens stored in accelerated condition for 6 cycles at the initial and end of cycle 6. The results were calculated from a mean of three repeats ± standard deviation of base formulation and sunscreen containing *A. catechu* at the concentration 1%, 2% , and 3% which are 6.50±0.00, 5.80±0.05, 5.50±0.09, and 5.20±0.00 accordingly. It can be seen that the pH of the sunscreens were not significantly changed over time. Therefore, pH value of all formulations were stable in the accelerated condition.

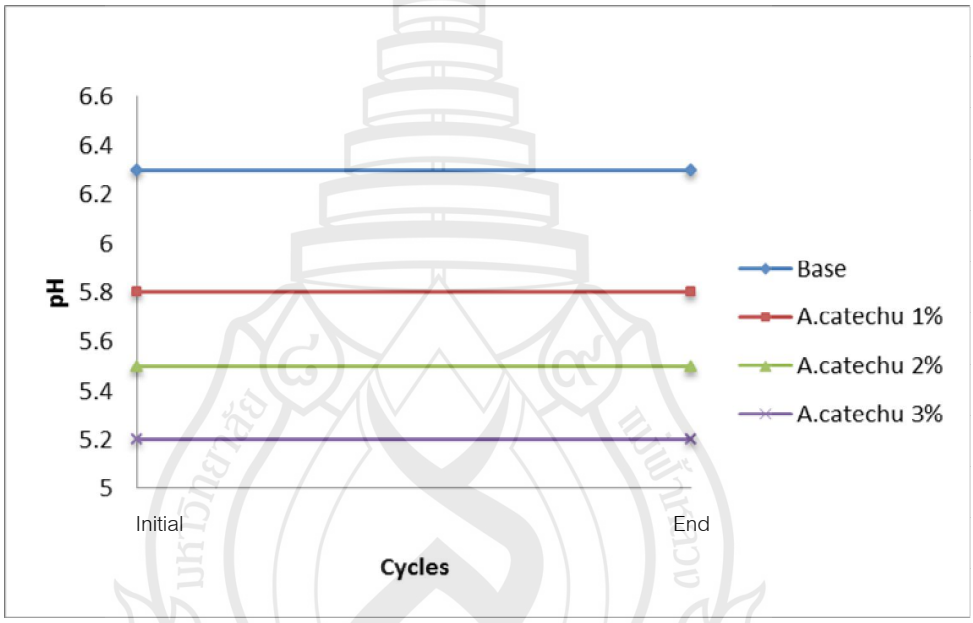


Figure 4.4 pH value of sunscreen stored in accelerated condition at the initial and the end of cycle 6.

4.3.2 Stability of product viscosity

Figure 4.5 showed viscosity values of sunscreen stored in 4˚C for 8 weeks in a form of an average of three repeats ± standard deviation. The result of base formulation and sunscreen containing *A. catechu* at the concentration of 1%, 2% , and 3% were 9325±463, 8293±548, 7979±336, and 7683±41 accordingly. It can be seen that the viscosities of the sunscreens were not significantly changed over time. Therefore, all of the formulations were stable at 4˚C.

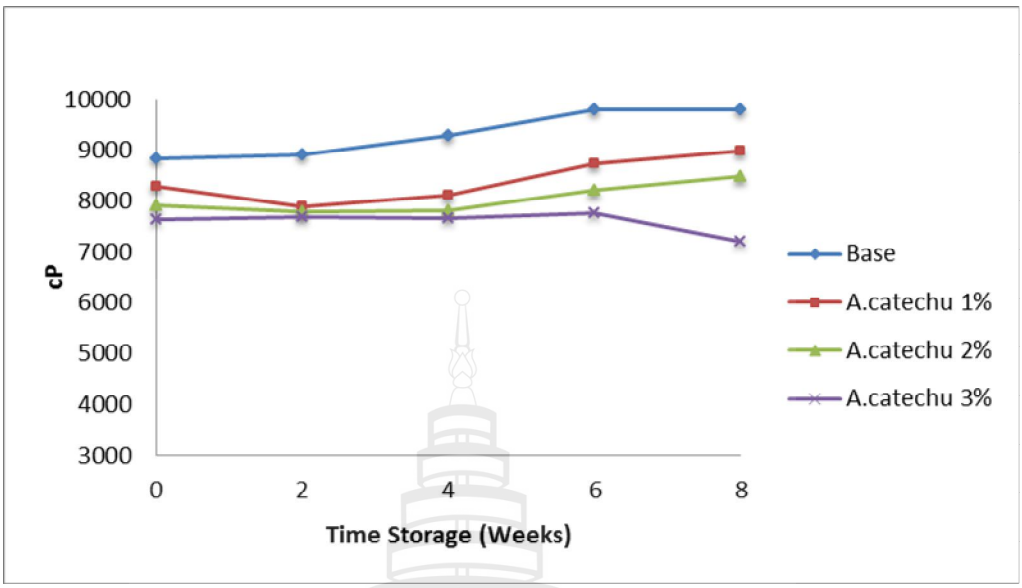


Figure 4.5 Viscosity of sunscreen stored at temperature of 4˚C for 8 weeks

Figure 4.6 demonstrated viscosity values of sunscreen stored in room temperature for 8 weeks in a form of a mean of three repeats ± standard deviation. The results of base formulation and sunscreen containing *A. catechu* at the concentration of 1%, 2%, and 3% were 8851±36, 7756±18, 7639±21, and 7340±254 accordingly. It can be seen that the viscosities of the sunscreens were not significantly changed over time. Therefore, all of the formulations were stable at room temperature.

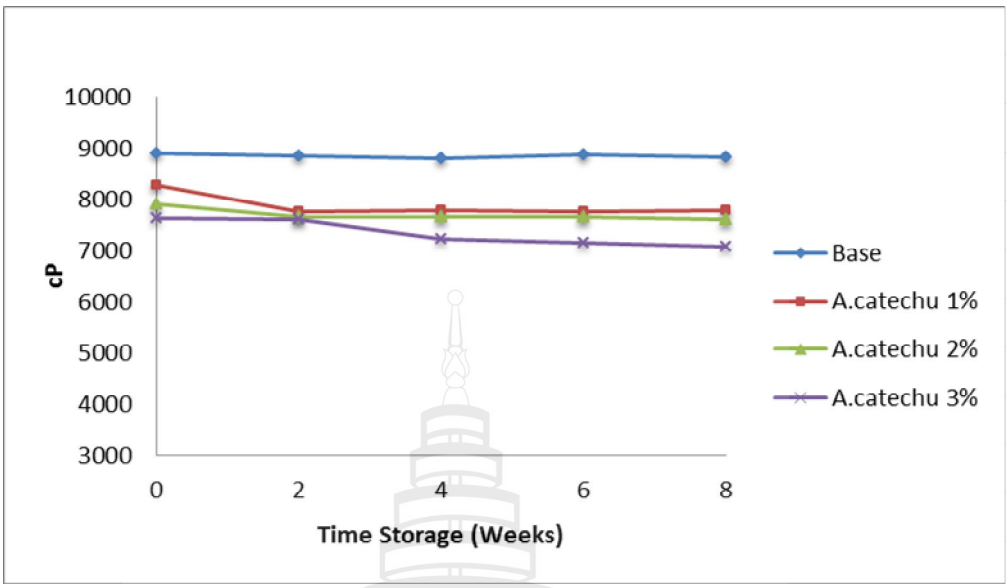


Figure 4.6 Viscosity of sunscreen stored in room temperature for 8 weeks.

The graph in figure 4.7 showed viscosities of sunscreen stored in 45˚C in a form of an average of three repeats ± standard deviation. The results of base formulation and sunscreen containing *A. catechu* at the concentration of 1%, 2% , and 3% were 8591±273, 7619±59, 7320±346, and 5427±1598 accordingly. It can be seen that the viscosities of base formulation and sunscreens containing *A. catechu* at 1% and 2% were not significantly changed over time. However, the formulation containing 3% of *A. catechu* showed a significant decrease in viscosity after 2 weeks time. Therefore, only formulations of base and sunscreen containing *A. catechu* 1%, and 2% were stable at 45˚C.

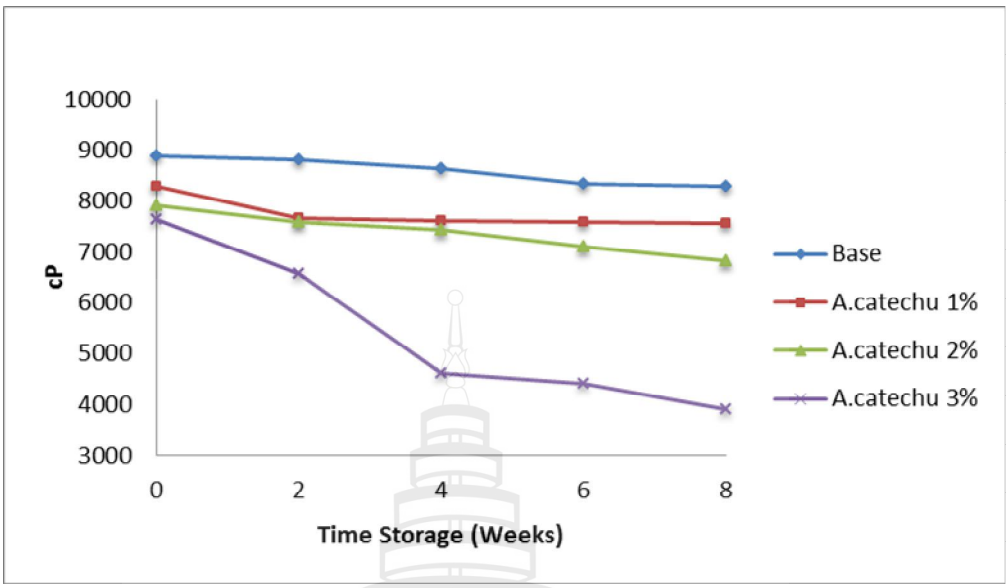


Figure 4.7 Viscosity of sunscreen stored at temperature of 45˚C for 8 weeks

The graph in figure 4.8 demonstrated viscosity values of sunscreen at the beginning and the end of accelerated condition. The results in a form of an average of three repeats ± standard deviation were 8854±84, 7630±122, 7579±82, and 7694±105 which belong to base formulation, sunscreen contained *A. catechu* at the concentration at 1%, 2%, and 3% accordingly. It can be seen that the viscosities of the sunscreens were not significantly changed over time. Therefore, all of the formulations were stable in accelerated condition.

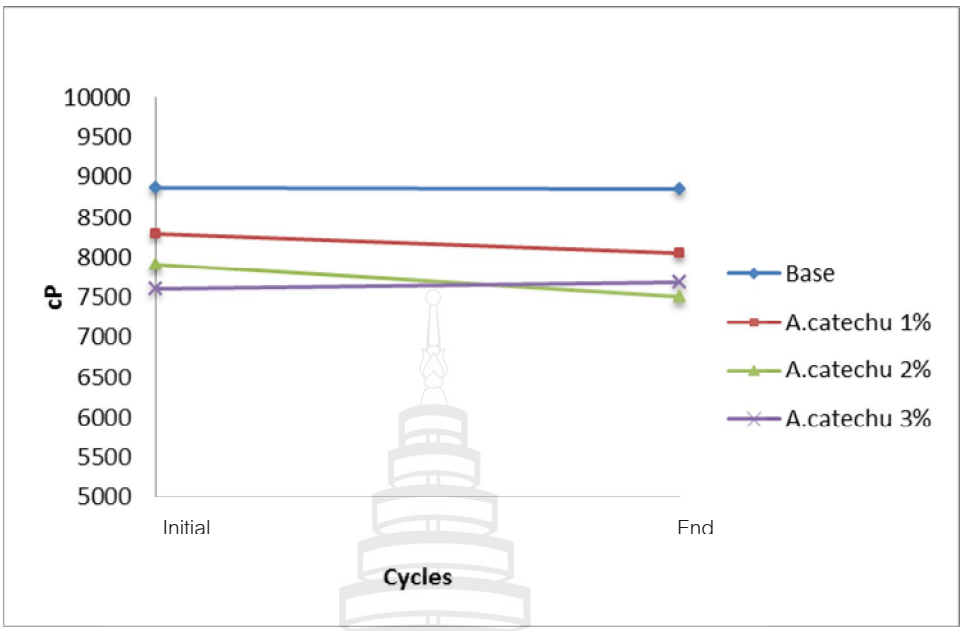


Figure 4.8 Viscosity of sunscreen stored in accelerated condition at initial and end of the of cycle 6.

4.3.3 Stability of product color

The color of base formulation did not change over storage time at the temperature of 4°c, while sunscreen formulations contained *A. catechu* at the concentration of 2% and 3% were slightly get darker over storage time. However, color difference of sunscreen containing *A. catechu* 1% was drastically increased in the first two weeks interval and noticeably became red-brown shade. The maximum color difference (ΔE) of base formulation and sunscreen containing *A. catechu* at 1%, 2%, and 3% were 0.90, 8.20, 4.16 ,and 4.06 accordingly in Figure 4.9

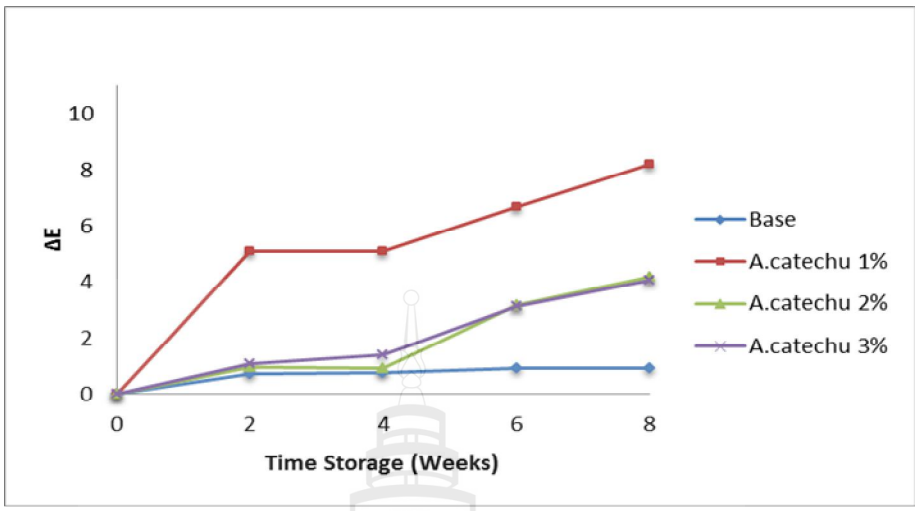


Figure 4.9 Color difference of sunscreen stored at 4˚C for 8 weeks.

In Figure 4.10, the color of base formulation did not change over storage time at room temperature, while sunscreen formulations contained *A. catechu* at the concentration of 1%, 2%, and 3% developed to darker shade every two weeks interval. The maximum color difference (ΔE) of base formulation and sunscreen containing *A. catechu* at 1%, 2%, and 3% were 0.46, 9.21, 4.88 ,and 3.87 respectively.

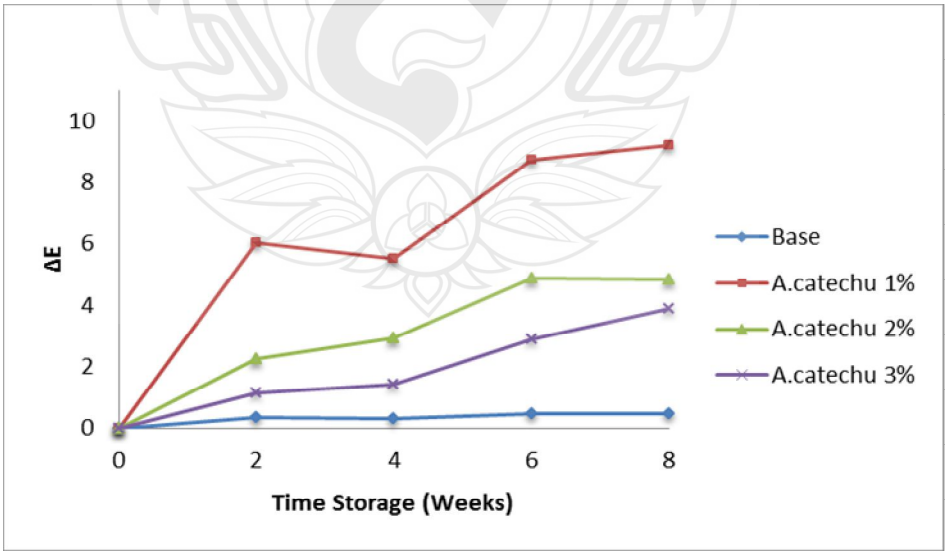


Figure 4.10 Color difference of sunscreen stored in room temperature for 8 weeks

Color difference(ΔE) range of base formulation and sunscreen containing *A.catechu* at the concentration of 1%, 2% , and 3% had slightly changed over the first 4 weeks, then sharply increased at week 6 and 8. The maximum color difference of base formulation and sunscreen containing A. *catechu* were 1.90, 8.68, 5.99, and 4.06 accordingly.

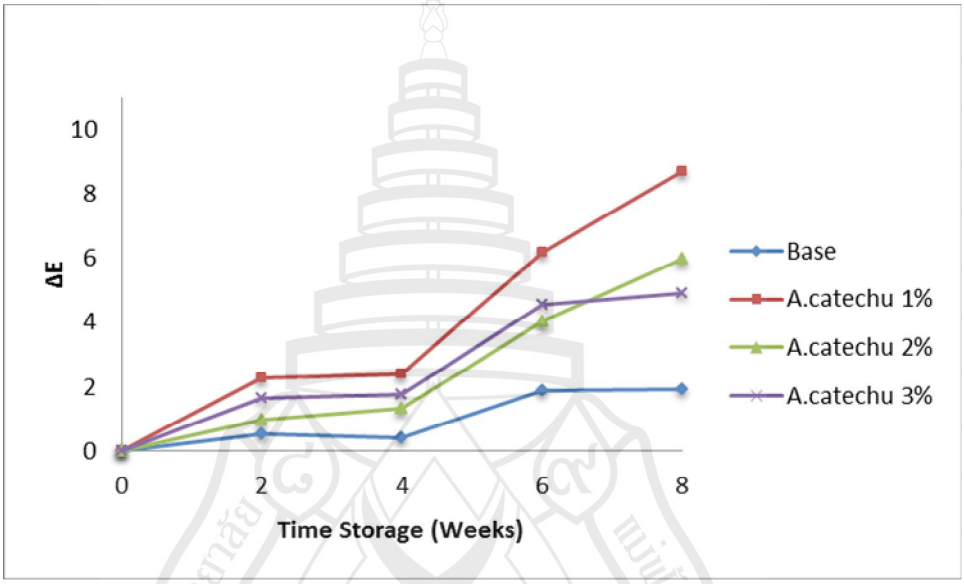


Figure 4.11 Color difference of sunscreen stored at 45˚C for 8 weeks

The color of base formulation and sunscreen containing A. catechu extract at the concentration of 1% almost not changed over storage time at the initial and end of total heating - cooling cycles, while other sunscreen formulations which contained *A. catechu* at the concentration of 2% and 3% were slightly get darker. The color difference (ΔE) of base formulation and sunscreen containing *A. catechu* at 1%, 2%, and 3% were 0.36, 0.60, 3.56 ,and 2.21 respectively.

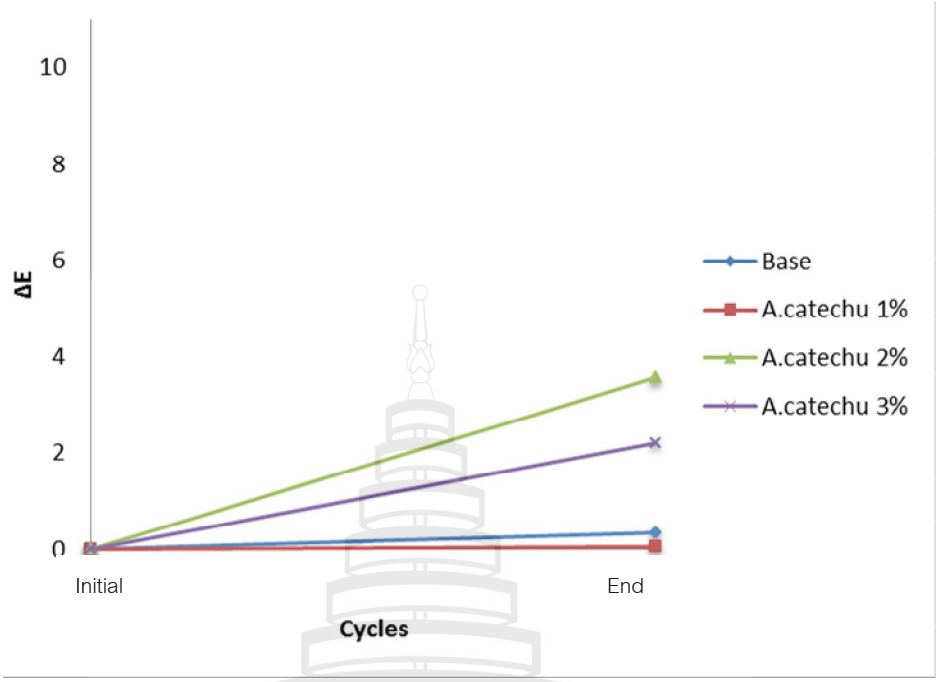


Figure 4.12 Color difference of sunscreen stored in accelerated condition at initial and end of cycle 6.

. **4.3.4 Stability of Sun Protection Factor (SPF) in sunscreen formulas**

According to Figure 4.13 and 4.14, it can be seen that SPF value of base formulation almost not changed from the beginning, which are 2.03 ± 0.11 for the one stored at room temperature and 1.74 ± 0.46 for the one stored in 45˚C. Other sunscreen formulations containing *A. catechu* were also remain SPF value along storage time at room temperature; which are, 7.62 ± 0.23 for sunscreen containing *A. catechu* 1%, 13.34 ± 0.25 for sunscreen containing *A. catechu* 2%, and 13.34 ± 0.25 for sunscreen containing *A. catechu* 3%. Two commercial also added to test SPF value in comparison, naming Product I and Product II which has claimed SPF value at 30 and 50 accordingly. The average of two commercial sunscreens are 12.06 ± 0.20 and 13.29 ± 0.22 at room temperature. However, after stored all products at 45˚C, some of formulations still performing well and remain its SPF value or has only slightly drop such as sunscreen containing *A. catechu* 1% which has an averaged SPF of 11.04 ± 2.13. As same as sunscreen containing *A. catechu* 2% which SPF was

dropped more than the previous formulation, but still giving moderate SPF level at 16.69 ± 3.31. Although some of the formulation such as Product II, has an averaged SPF 10.05 ± 2.90 at 45˚C storage, the final week result did not come out well as it has SPF only 6.87. SPF value of sunscreen containing *A. catechu* 3% also dropped over storage time; however, the final week SPF result was 11.90 with 16.69 ± 3.31 averagely.

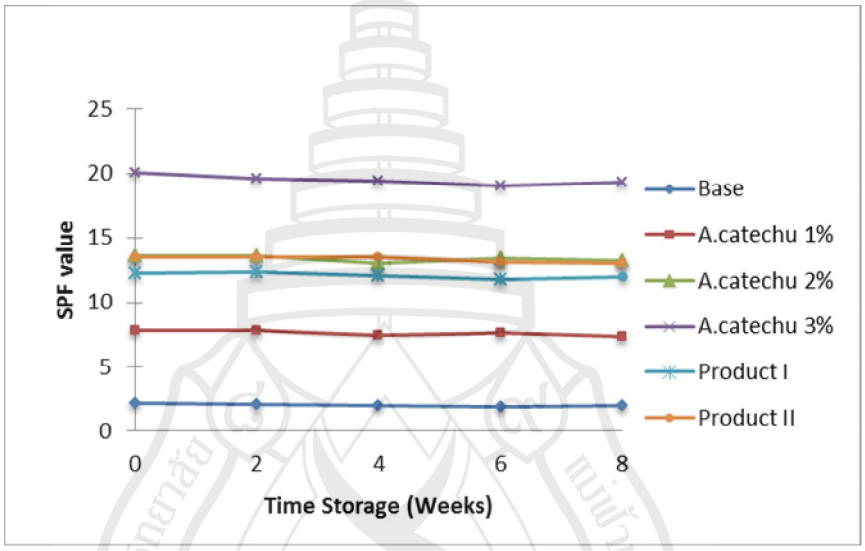


Figure 4.13 SPF evaluation of sunscreen stored at room temperature for 8 weeks

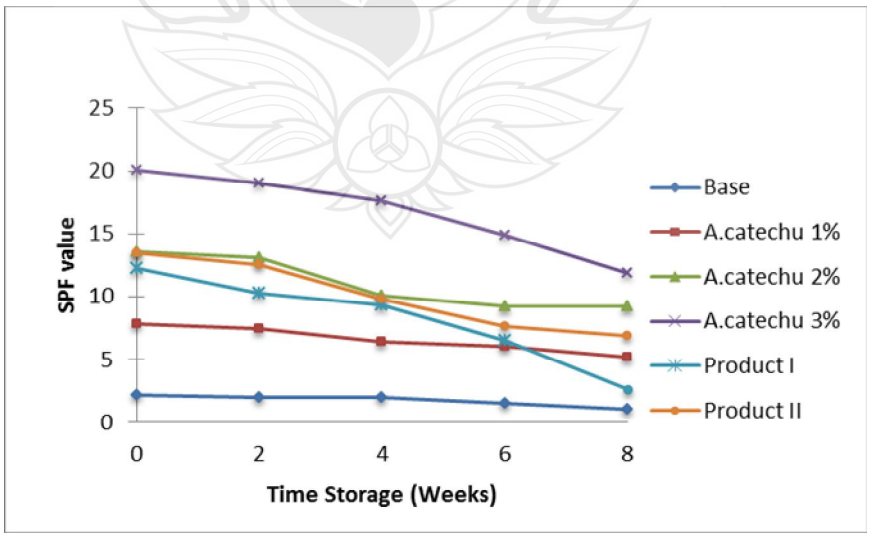


Figure 4.14 SPF evaluation of sunscreen stored at 45˚C for 8 weeks

4.3.5 Stability of Total Phenolic Content in sunscreen formulas

The determination of total phenolic content was done by Folin-Ciocalteu method. The results showed that the phenolic content increased according to the increase amount of A. catechu in the formulations. From figure 4.15, it can be seen that the phenolic content of each formulation was steady during 8 weeks time in room temperature. The total phenolic content in the formulations storing at 45°C over 8 weeks was presented in Figure 4.16. Even though the highest phenolic content was found in the 3% .catechu formulation at 25.25mg GAE/g , there was a significantly decrease in the amount of phenolic in the 3% A. *catechu* formulation over the time. This might be due to the sensitivity of phenolic compound to Therefore the recommend dosage of A. *catechu* that would provide a constant amount of phenolic compound was at 2%.

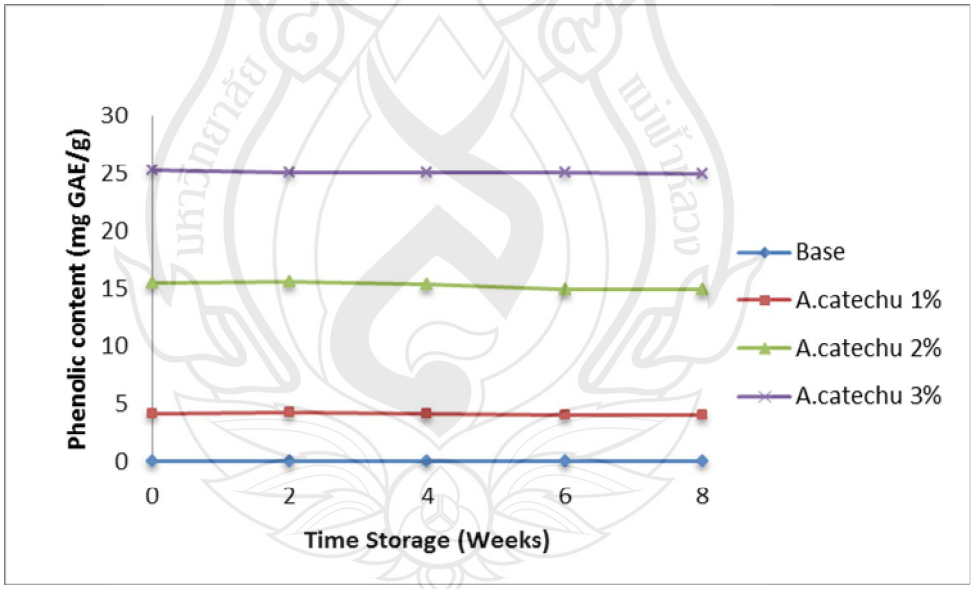


Figure 4.15 Total phenolic content of sunscreen stored at room temperature for 8 weeks

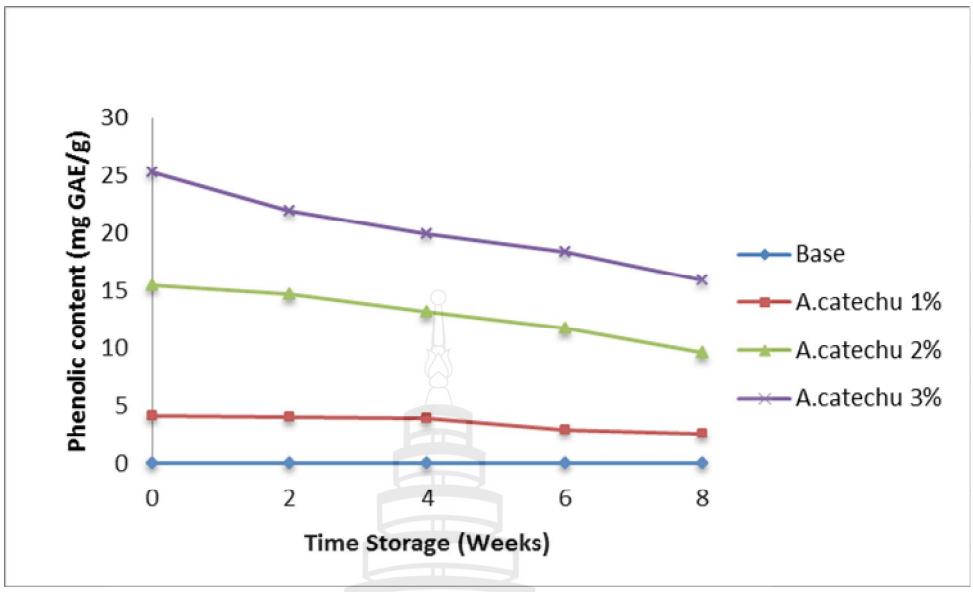


Figure 4.16 Total phenolic content of sunscreen stored at 45˚C for 8 weeks

4.3.5 Stability of Antioxidant Capacity in sunscreen formulas

Figure 4.16 illustrated the ability of the formulated products to scavenge DPPH radical over 8 weeks time at room temperature. It can be seen the base formulation has no antioxidant property. However the antioxidant capacity of the products increased according to the increased percentage of A catechu in the formulation, 3% of a catechu provided the highest antioxidant powder at approximately 13mg TEAC throughout the storage time.

The antioxidant capacity was also evaluated at 45℃ for 8 weeks. Figure 4.17 presented an increase in antioxidant ability as the amount of A. catechu increased in the formulations. The line graph also showed a decreased in the antioxidant powder in the formulation containing 3% A. catechu after 4 weeks. The 2% A. catechu formulation also showed a slight decrease in antioxidant capability in week 8, but the decrease was not statistically significant. Therefore, it can be concluded that the maximum amount of A. catechu in the formulation that was stable throughout 8 weeks was at 2%

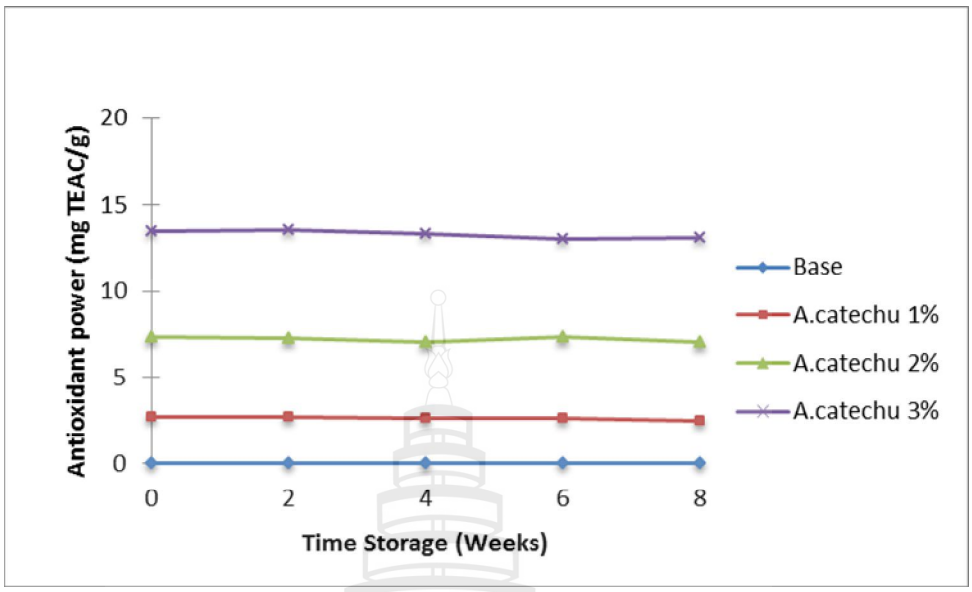


Figure 4.17 Determination of antioxidant capacity (DPPH radical scavenging assay) at room temperature

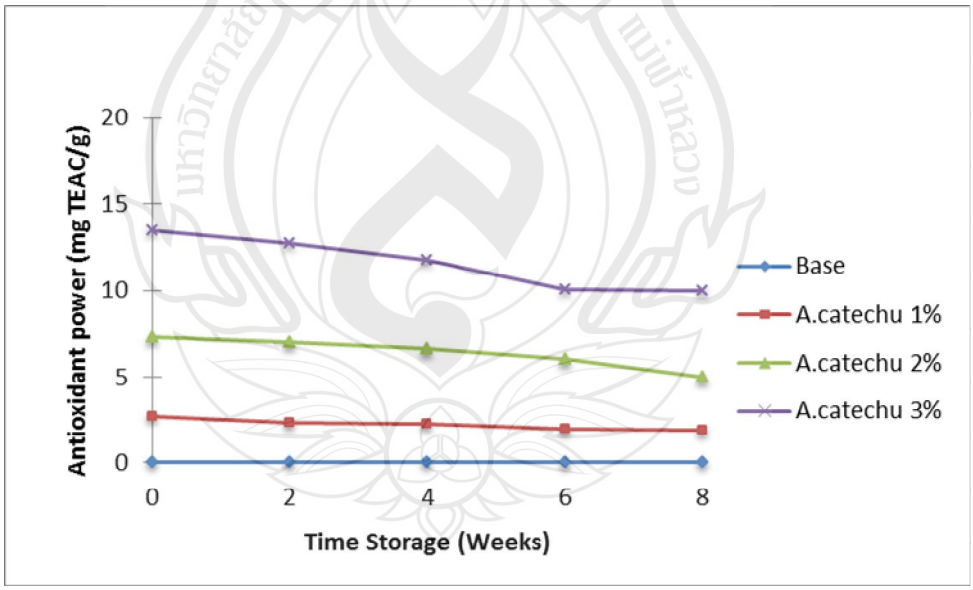


Figure 4.18 Determination of antioxidant capacity (DPPH radical scavenging assay) at 45˚C

CHAPTER 5

CONCLUSION AND RECOMMENDATION

5.1 Conclusion

Extraction of *A. catechu* seeds by microwave assisted method provided a yield of 16.54% (w/w) containing phenolic 465.05 mg GAE/ g with activity against DPPH radical and tyrosinase enzyme of 3.24 µg/mL and 24.32 µg/mL, respectively. These values were respectively superior to the standard trolox and kojic acid.

Increased amount of *A. catechu* extract exhibited low stability of the sunscreen formulas with darker color and viscosity dropped. High temperature storage also caused to the same observation for viscosity and color changing.

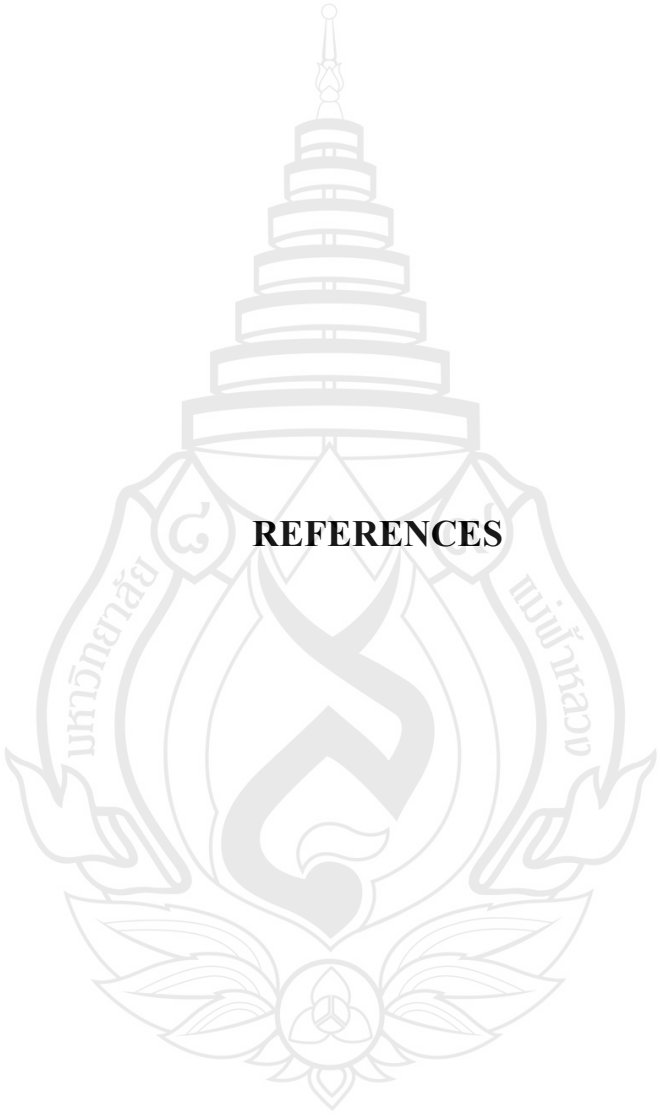
Although some physical properties were vulnerable along different temperature, the evaluation result of sun protection factor was mostly stable during storage time at room temperature. The formulation given the highest SPF value was the sunscreen containing 3% *A. catechu* extract. This extract amount also provided the highest phenolic content which remained stable until the final storage week at room temperature. The product also showed the antioxidant capacity and SPF value corresponded to the amount of phenolic content. However, the increase in temperature results in the decrease of phenolic content in sunscreen formulas.

Therefore, it can be illustrated that the *A. catechu* extract would be substantial natural sunscreening agent with possessing other potential cosmetic features including, antiaging and whitening capacities. However, improvement for color and viscosity changing would be needed in further study.

5.2 Recommendation

A. catechu can be claimed as multi functional active ingredient due to its various kind of bioactive compounds. Further study might focus on anti-oxidant in *A. catechu* extract, since the results of this study suggested that there should be other substance rather than phenolic compounds performing as anti-oxidative function.

Any further study which develop product containing *A. catechu* extract should concern on color sensitivity as well, since *A. catechu* color can be changed rapidly after oxidation. Otherwise, package of *A. catechu* product should be properly selected.



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APPENDICES

APPENDIX A

Color measurement
(CIE L*a*b* parameters system)

Table A1 Color measurement of sunscreen which stored at temperature of 4˚c for 8 weeks.

Base formulation				
Week	**L***	**a***	**b***	**ΔE**
0	64.12±0.06	-1.44±0.03	-3.63±0.02	0
2	64.23±0.60	-1.41±0.02	-4.33±0.04	0.71
4	64.39±0.42	-1.43±0.01	-4.32±0.02	0.74
6	64.57±0.77	-1.22±0.02	-4.38±0.05	0.90
8	64.02±0.10	-1.18±0.02	-4.48±0.06	0.90
Sunscreen contained *A. catechu* 1%				
Week	**L***	**a***	**b***	**ΔE**
0	58.46±0.33	9.39±0.06	16.56±0.05	0
2	54.47±0.29	8.02±0.03	13.72±0.04	5.08
4	54.42±0.94	8.03±0.09	13.76±0.18	5.10
6	52.01±0.73	8.38±0.04	15.16±0.30	6.68
8	50.86±0.74	8.06±0.07	13.79±0.16	8.20
Sunscreen contained *A. catechu* 2%				
Week	**L***	**a***	**b***	**ΔE**
0	60.47±0.20	12.11±0.02	16.81±0.10	0
2	60.12±0.61	11.47±0.05	16.16±0.13	0.95
4	60.40±0.08	11.47±0.04	16.15±0.05	0.93
6	58.59±0.60	12.33±0.05	19.34±0.13	3.15
8	58.11±0.92	12.61±0.07	20.20±0.26	4.16

Table A1 (continued)

Sunscreen contained *A. catechu* 3%				
Week	**L***	**a***	**b***	**ΔE**
0	58.45±0.28	13.49±0.03	16.83±0.07	0
2	59.43±0.26	13.12±0.02	17.01±0.05	1.07
4	59.79±0.31	13.07±0.03	16.84±0.13	1.40
6	57.81±0.67	13.79±0.07	19.86±0.37	3.11
8	57.89±0.50	14.08±0.04	20.81±0.16	4.06

Table A2 Color measurement of sunscreen which stored at room temperature for 8 weeks

Base formulation				
Week	**L***	**a***	**b***	**ΔE**
0	68.77±0.08	-1.51±0.01	-3.36±0.01	0
2	69.08±0.69	-1.53±0.03	-3.45±0.03	0.33
4	69.07±0.17	-1.56±0.02	-3.38±0.04	0.31
6	68.37±0.60	-1.27±0.02	-3.37±0.03	0.46
8	69.06±0.14	-1.21±0.01	-3.37±0.01	0.46
Sunscreen contained *A. catechu* 1%				
Week	**L***	**a***	**b***	**ΔE**
0	59.7±0.14	9.63±0.07	16.47±0.14	0
2	53.68±0.42	9.69±0.04	16.17±0.15	6.03
4	54.2±0.70	9.61±0.06	16.09±0.16	5.51
6	51.75±0.19	11.72±0.07	19.41±0.19	8.74
8	50.67±0.75	11.31±0.01	17.05±0.05	9.21

Table A2 (continued)

Sunscreen contained *A. catechu* 2%				
Week	**L***	**a***	**b***	**ΔE**
0	61.69±0.17	11.89±0.03	16.77±0.04	0
2	59.48±0.30	12.05±0.02	16.26±0.06	2.27
4	58.81±0.37	12.10±0.05	16.43±0.11	2.91
6	56.97±0.07	12.95±0.02	17.42±0.06	4.88
8	57.42±0.51	13.82±0.03	17.87±0.12	4.82
Sunscreen contained *A. catechu* 3%				
Week	**L***	**a***	**b***	**ΔE**
0	59.19±0.13	13.60±0.04	17.02±0.18	0
2	58.34±0.45	13.47±0.04	17.76±0.14	1.14
4	58.07±0.61	13.53±0.10	17.85±0.21	1.40
6	57.06±6.50	14.50±0.04	18.76±0.05	2.90
8	56.09±0.30	15.20±0.05	18.70±0.14	3.87

Table A3 Color measurement of sunscreen which stored at temperature of 45˚c for 8 weeks.

Base formulation				
Week	**L***	**a***	**b***	**ΔE**
0	67.61±0.27	-1.53±0.01	-3.26±0.04	0
2	67.19±0.44	-1.53±0.01	-3.53±0.00	0.51
4	67.88±0.43	-1.53±0.02	-3.54±0.01	0.39
6	69.48±0.31	-1.42±0.01	-3.25±0.02	1.87
8	69.39±033	-1.33±0.02	-2.63±0.02	1.90

Table A3 (continued)

Sunscreen contained *A. catechu* 1%				
Week	**L***	**a***	**b***	**ΔE**
0	56.32±0.23	12.63±0.04	15.68±0.07	0
2	55.15±0.10	13.44±0.03	17.47±0.02	2.28
4	54.89±0.35	13.40±0.10	17.43±0.21	2.39
6	52.01±0.22	15.00±0.06	19.41±0.13	6.17
8	49.86±0.42	16.16±0.09	20.28±0.21	8.68
Sunscreen contained *A. catechu* 2%				
Week	**L***	**a***	**b***	**ΔE**
0	54.23±0.11	14.62±0.05	13.90±0.23	0
2	55.04±0.34	14.65±0.05	14.39±0.05	0.95
4	55.46±0.33	14.65±0.02	14.41±0.02	1.33
6	52.53±0.41	16.23±0.12	17.17±0.26	4.03
8	51.58±0.55	16.92±0.12	18.75±0.21	5.99
Sunscreen contained *A. catechu* 3%				
Week	**L***	**a***	**b***	**ΔE**
0	50.56±0.35	15.70±0.08	13.55±0.39	0
2	51.99±0.54	15.77±0.06	14.33±0.24	1.07
4	52.11±0.40	15.78±0.03	14.34±0.12	1.40
6	51.13±0.39	16.94±0.06	17.87±0.18	3.11
8	48.23±0.68	17.77±0.24	17.35±0.57	4.06

Table A4 Color measurement of sunscreen which stored at accelerated state for 6 cycles.

Base formulation				
	L*	**a***	**b***	**ΔE**
Initial	68.76±0.25	-1.60±0.03	-3.67±0.03	0
End of cycle 6	68.60±0.55	-1.65±0.03	-4.00±0.01	0.36
Sunscreen contained *A. catechu* 1%				
	L*	**a***	**b***	**ΔE**
Initial	54.52±0.18	11.79±0.09	16.59±0.24	0
End of cycle 6	54.36±0.30	11.87±0.03	17.17±0.09	0.60
Sunscreen contained *A. catechu* 2%				
	L*	**a***	**b***	**ΔE**
Initial	56.74±0.11	13.03±0.03	12.83±0.02	0
End of cycle 6	56.16±0.66	13.20±0.10	16.34±0.19	3.56
Sunscreen contained *A. catechu* 3%				
	L*	**a***	**b***	**ΔE**
Initial	52.71±0.08	14.56±0.03	14.30±0.02	0
End of cycle 6	52.86±0.21	14.24±0.03	14.66±0.06	2.21

APPENDIX B

PROCEEDING

DEVELOPMENT OF SUNSCREEN PRODUCT CONTAINING *ARECA CATECHU* EXTRACT

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ABSTRACT

The purpose of this study is to develop sunscreen product containing *Areca catechu* extract. The *A. catechu* extract was prepared by microwave assisted method and the yield of 16.54% was obtained. It contained phenolics 465 mg GAE/g possessing DPPH radical scavenging and tyrosinase inhibition activities greater than the standard trolox and kojic acid, respectively, with IC_{50} of 3.24 µg/mL and 24.32 µg/mL. Physicochemical and biological properties of the sunscreen containing *A. catechu* extract were also evaluated. The product illustrated the SPF value ranging from 8 to 20 when incorporating the extract from 1 to 3% w/w. While the pH was stable, the viscosity and color of the product were conversely changed with decrease in viscosity but rising in delta E when amount of the extract was increased. The product containing higher extract amount exhibited greater phenolic content, antioxidant power and sunscreening effect. High temperature storage decreased phenolic content corresponding to radical scavenging activity and sunscreening capability. With superior cosmetic acitivity found, the *A. catechu* was promised as a high potential source for natural sunscreening extraction. However, its color stability enhancement is required to improve the product appearance.

Keywords: *Areca catechu*/ Cosmetic activity/ Phenolic/ Natural sunscreen

INTRODUCTION

The concerns of health and qualitative living are now becoming a major influence on various industries. Consumer products consisting entirely, or at least partly, of natural-based ingredients are

more attractive to potential buyers than ones consisting entirely of synthetic chemicals on the same shelf because of the perception that natural ingredients are safer, however less effective they are compared to the synthetic ones. Similarly, in cosmetic industry, consumers' concerns on natural products have led to a change in consumer behavior. Consumers nowadays increasingly spend time reading product's labels carefully in order to obtain information about the ingredients - some even check whether the products contain an adequate level of active compounds in order to achieve a certain usage purpose. Since consumers expect more than what basic functions of product can deliver, many cosmetic manufacturers tend to claim the benefits of their products with a plus of natural extracts in order to establish an image of low toxicity, mildness and bio-degradability. Thailand is the origin of varieties of herbs whose extracts have been used in medicines and cosmetics for a long time. Hence, if local wisdoms regarding these herbal properties were merged with the new science of cosmetic technologies to create practical daily-use products, it would offer an alternative for consumers to substitute imported cosmetics with local ones.

Protecting skin against sun exposure is one of the most desirable cosmetic qualification in tropical countries since ultraviolet from sunlight can permanently cause damages to human's DNA level and several harmful effects on the skin (Gontijol, *et al.,* 2004) Therefore, from the first sunscreen commercially appeared in 1928, it has been developed for many decades and not only been promoted as protecting against sunburn, but also preventing other sun-induced adverse effects such as photo-sensitivity, skin aging, and skin cancer. Currently, some of color cosmetics also claimed as protecting against UV from having combination of some ingredients with high refractive index such as Titanium dioxide or Zinc oxide in makeup base and foundation products (Schalka, *et al*., 2012). Since sunscreen products applied on the skin might lose its protection ability after sun exposure, consumers need to reapply sunscreen frequently during daytime. Ease of application has become consumers' concern since reapplying the product causes uncomfortable feeling to the skin or clogged pores; as a result, the quantity of sunscreen reapplied by consumers might be insufficient because they tend to avoid putting on too much. Water-based sunscreen was then developed to eliminate these undesirable outcomes.

In this study, cold-process sunscreen will be developed using long history Thai herb, *Areca catechu*, as one of the photo-protective agents (Sangthong, *et al*., 2012). *A. catechu* once played a role in Thai economic crops; however, since the government seriously prohibited chewing *A. catechu* as well as did not support people to cultivate *A. catechu* due to its effect on stimulating nervous system such as narcotics, *A. catechu* has then gradually disappeared from Thai cultures. However, the demand for *A. catechu* still exists in other industries, and its export value has grown every year in the past decades with the market price at approximately 37-40 THB per kg for raw nuts and 50-55 THB per kg for dried ones (Ministry of Agriculture [Thailand], 2013).

Expected sunscreen formulation in this study should practically give a good level of protection as well as commercial sunscreen and might be claimed on other biological properties. The final product will be claimed as a botanically-extracted and easily-applicable sunscreen.

MATERIALS AND METHODS

Plant extract is provided by microwave assisted method using 50% ethanol (v/v) at a solvent ratio of 1:10 (w/v). Cold-process sunscreen base is developed and varied the percentage of extract at 1%, 2%, and 3%. Physiochemical and biological stability of product are evaluated among different storage temperature conditions. The determination of sun protection factor was done by SPF 290-S analyzer system. The changes in color are evaluated by Konica Minolta Chroma meter CR-400 and interpreted the results in CIE L*a*b* parameters system. Total phenolic content was determined according to Folin-ciocalteu method, using gallic acid as a standard. DPPH radical scavenging assay was done to examine antioxidant capacity using trolox for standard.

RESULTS AND DISCUSSION

The results of biological activities determination in *A. catechu* extract are shown in table below. The value of IC_{50} indicates stronger antioxidant capacity of *A. catechu* extract than standard trolox, as same as tyrosinase inhibition activity in which *A. catechu* extract had better performance compared to standard kojic acid.

Samples	Phenolic content (mg GAE/g)*	DPPH radical scavenging capacity (IC_{50}, µg/mL)	Tyrosinase inhibitory activity (IC_{50}, µg/mL)
A. catechu extract	465.05 ± 9.44	3.24 ± 0.11	24.32 ± 0.87
Trolox	-	4.04 ± 0.07	-
Kojic acid	-	-	35.65 ± 2.04

The pH stability of sunscreen containing *A. catechu* at different storage temperature in which remain unchanged along storage time are aligned in Figure 1.. The average pH value of sunscreen base formulation and those contained *A. catechu* extract 1%, 2%, and 3% were 6.5, 5.7, 5.5, and 5.2 accordingly.

Viscosity stability of sunscreen products are shown in figure 2-5. Base formulation and sunscreen containing *A. catechu* extract at 1% and 2% almost remained their stable viscosity along storage time at room temperature. However, the higher temperature (45˚c) caused sharply drop in viscosity of sunscreen containing *A. catechu* extract 3% and the lower temperature (4˚c) caused viscosity of all formulation to slightly increase. Nonetheless, all viscosity at initial and end of accelerated condition storage remain stable for every formulation.

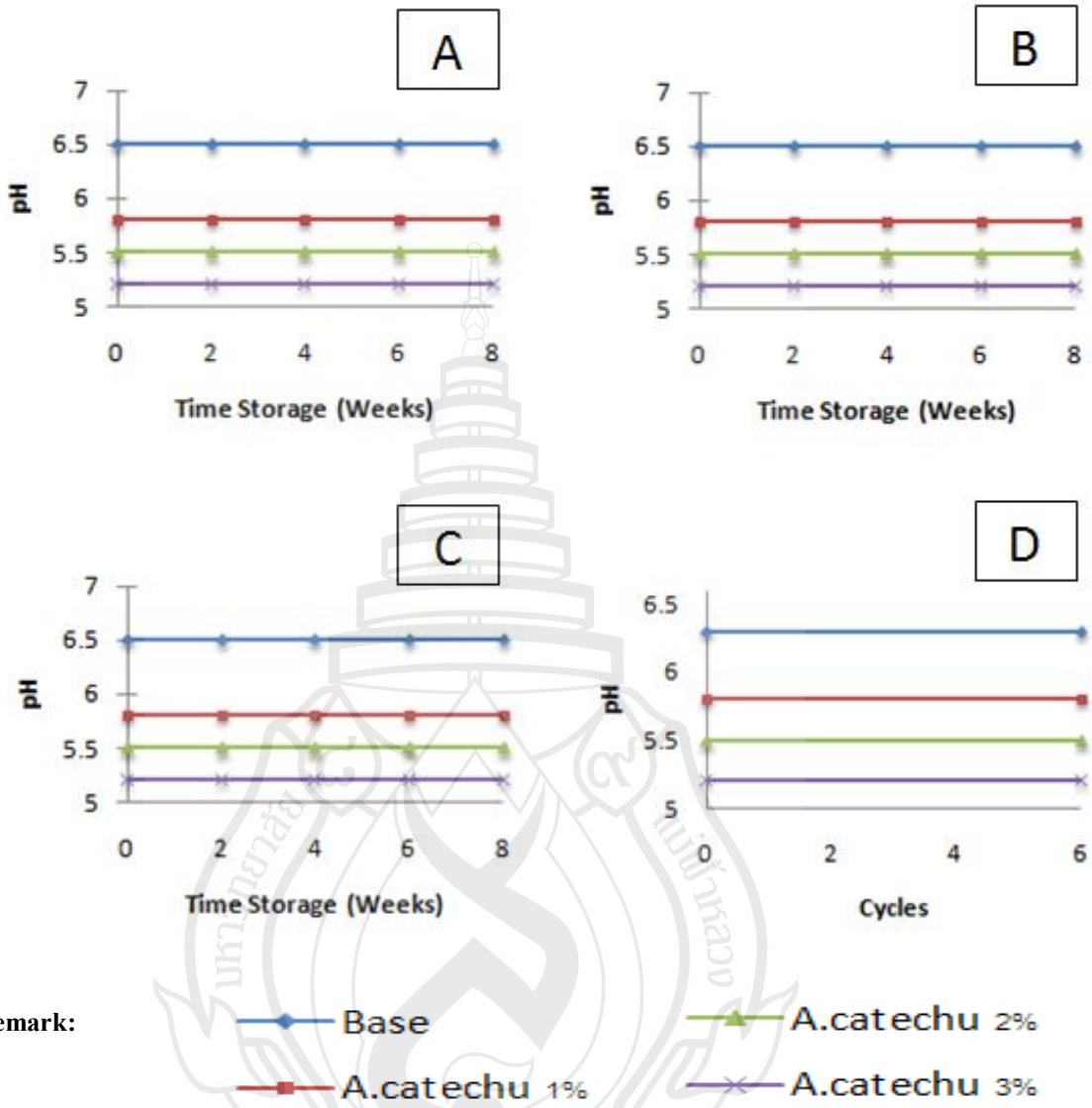

Figure B1 pH stability of sunscreen products stored at 4°C (A), room temperature (B) and 45˚C (C) and treated with heating-cooling 4 cycles (D).

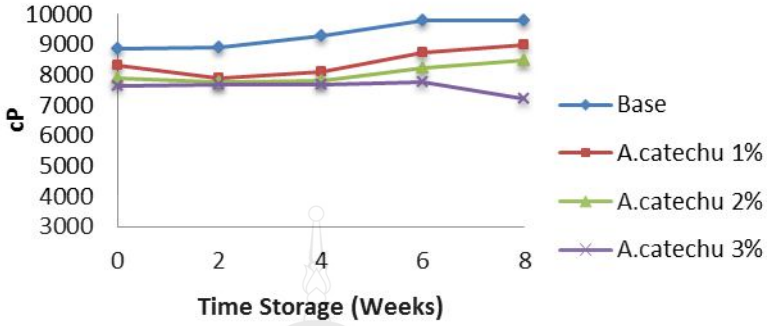

Figure B2 Viscosity stability of sunscreen stored at temperature of 4˚C for 8 weeks

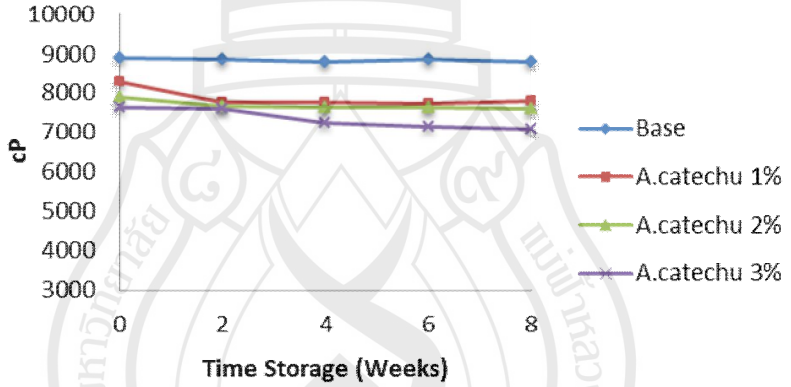

Figure B3 Viscosity stability of sunscreen stored in room temperature for 8 weeks.

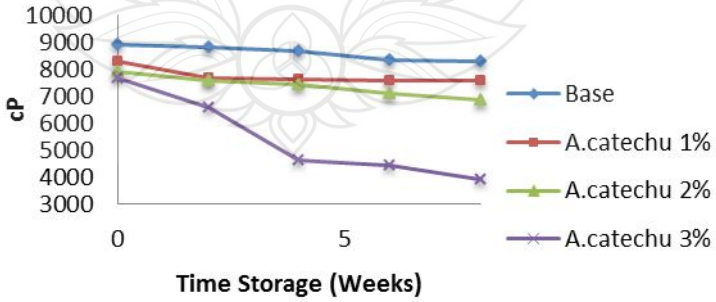

Figure B4 Viscosity stability of sunscreen stored at temperature of 45˚C for 8 weeks.

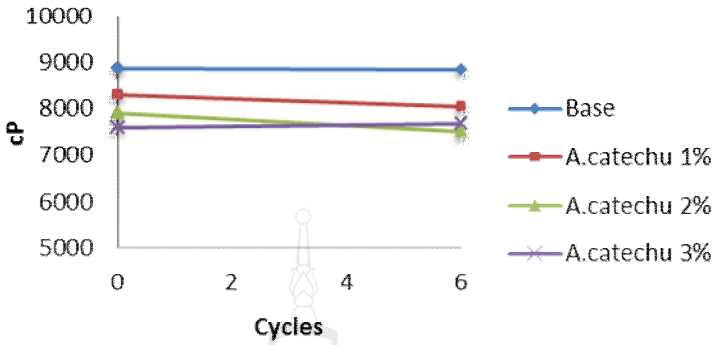

Figure B5 Viscosity of sunscreen stored in accelerated condition at initial and end of cycle 6.

Color stability of the products are shown in figure 6-9.. The color of base formulation did not change over storage time in every temperature condition. Color difference (ΔE) of sunscreen containing *A. catechu* extract 1% indicates the higher value than other formulations after second weeks storage. The color of sunscreen containing *A. catechu* 1% is observed that it was obviously changed into red-brown shade while those of 2% and 3% extract slightly became more dark-brown.

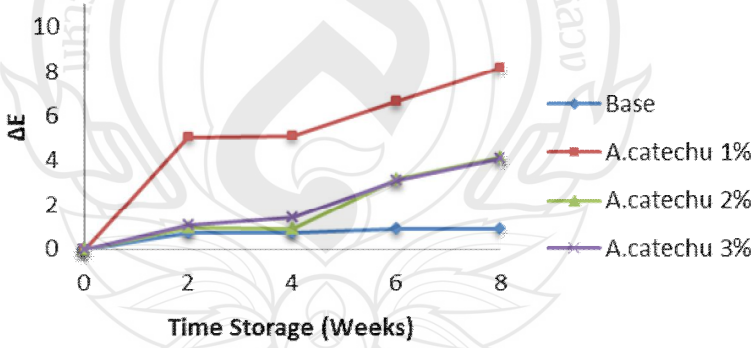

Figure B6 Color stability of sunscreen stored at 4°C for 8 weeks.

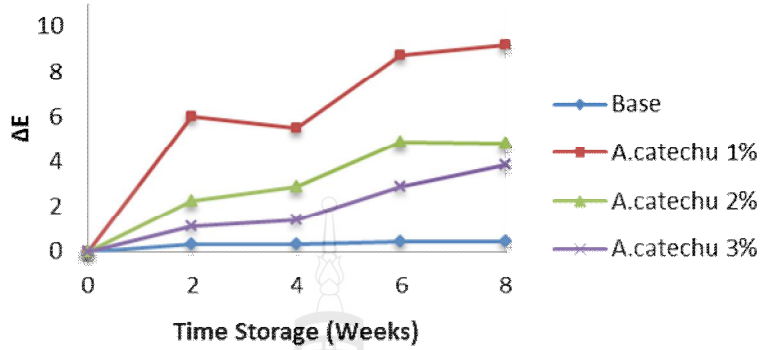

Figure B7 Color stability of sunscreen stored in room temperature for 8 weeks.

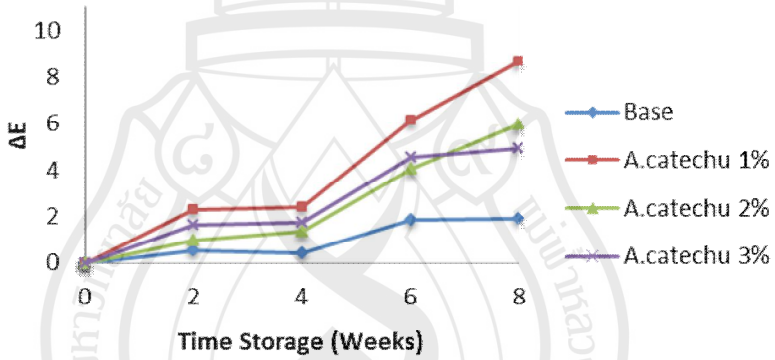

Figure B8 Color stability of sunscreen stored at 45˚C for 8 weeks.

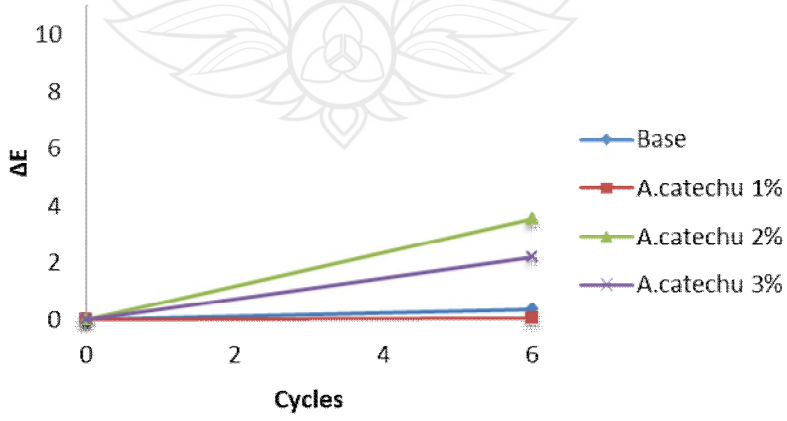

Figure B9 Color difference of sunscreen stored in accelerated condition at initial and end of cycle 6.

CONCLUSION

Extraction of *A. catechu* seeds by microwave assisted method provided a yield of 16.54% (w/w) containing phenolics 465.05 mg GAE/ g with activity against DPPH radical and tyrosinase enzyme of 3.24 µg/mL and 24.32 µg/mL, respectively. These values were respectively superior to the standard trolox and kojic acid. Increased amount of *A. catechu* extract exhibited low stability of the sunscreen formulas with darker color and viscosity dropped. High temperature storage also caused to the same observation for viscosity and color changing. Although some physical properties were vulnerable along different temperature, the evaluation result of sun protection factor was mostly stable during storage time at room temperature. The formulation given the highest SPF value was the sunscreen containing 3% *A. catechu* extract. This extract amount also provided the highest phenolic content which remained stable until the final storage week at room temperature. The product also showed the antioxidant capacity and SPF value corresponded to the amount of phenolic content. However, the increase in temperature results in the decrease of phenolic content in sunscreen formulas. Therefore, it can be illustrated that the *A. catechu* extract would be substantial natural sunscreening agent with possessing other potential cosmetic features including, antiaging and whitening capacities. However, improvement for color and viscosity changing would be required in further study.

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