PHYSICAL STABILITY AND IN VITRO SUN PROTECTION FACTOR (SPF) OF EMULSION CONTAINING NATURAL OIL AND ROASTED BARLEY (*Hordeum vulgare* L.) EXTRACT

PONGSAKORN VITHAYANON

6219603

A thesis submitted to the Theophane Venard School of Biotechnology, Assumption University in part of fulfilment of the requirement for the degree of Master of Science in Food Biotechnology

2019

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Title	:	**Physical Stability and *In Vitro* Sun Protection Factor (SPF) of Emulsion Containing Natural Oil and Roasted Barley (*Hordeum vulgare* L.) Extract**
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Faculty	:	**Theophane Venard School of Biotechnology**
Academic Year	:	**2019**

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ACKNOWLEDGEMENTS

I would like to express my deepest appreciation to all those who provided me the possibility to complete this thesis. A special gratitude I give to my thesis advisor, Asst. Prof. Sireerat Laodheerasiri, whose contribution to stimulating suggestions, encouragement, and helped me to coordinate my thesis. Furthermore, providing raw materials for my thesis.

My grateful thanks are also extended to Dr.-Ing Tatsawan Tipvarakarnkoon who guided me in the part of texture analysis, measurement of viscosity, kinetic energy, and statistical analysis. I would also like to extend my thanks to the technicians of the laboratory of the faculty of biotechnology for their help in offering me the resources in the laboratory. Special thanks to Ms. Kittanan Burapalit who helped me in the part of the heating-cooling cycle stability test. Last but not least, many thanks go to the committees, Asst. Prof. Dr. Phanuphong Chaiwut, Dr.-Ing Tatsawan Tipvarakarnkoon, and Asst. Prof. Dr. Waralee Watcharin for their time attended to my presentation.

Finally, I wish to thank my family for their support and encouragement throughout my thesis.

Pongsakorn Vithayanon

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ABSTRACT

An emulsion containing either chemical absorbers or physical blockers is commonly used to prevent the ultraviolet (UV) radiation from reaching human skin which causing aging, sunburn, and skin cancer. Several natural substances have been recently considered as potential sunscreen resources due to their UV absorption property. This study aimed to evaluate the UV absorbance spectroscopy and sun protective factor (SPF) of roasted barley (*Hordeum vulgare* L.) and natural oils (coconut, avocado, olive, sweet almond, perilla seed oil virgin oils, and *Camellia oleifera* seed oil) to be formulated cosmetic emulsion by compared with the chemical absorber, oxybenzone. The SPF, emulsion stability, and physical characteristics were observed by the heating-cooling cycle. Furthermore, thermal degradation and photooxidation effect on the formulated cosmetic emulsion were examined under accelerated conditions for 28 days storage to estimate the half-life. The results found that all of the natural oils and roasted barley presented the moderately to strongly UV absorption spectrum profile which the virgin perilla seed oil (VPSO) or Nga-Mon provided the most capableness UVB absorber with the highest SPF. The combined between roasted and oxybenzone in the VPSO formulation can promote the board range of UV protection covering both UVA and UVB radiation and it showed the potential to resist a denature of SPF capability by heating-cooling stability test better than oxybenzone alone. Phase separation was not observed after six repeated cycles, the firmness and homogeneity were insignificantly changed. Thus, mixed emulsifiers of Tween 80 and Span 80 can stabilize an emulsion. The SPF reduction was perceived after storage under the varying temperature and light conditions, 45°C / light, and 30°C / light, but not in the 30°C / dark condition. The effect of thermal degradation and photodegradation were followed apparent first-order kinetics, half-life time ($t_{1/2}$) of SPF was 415.89 days when kept at 30°C / dark condition.

KEY WORDS: Roasted barley / natural oil / sun protection factor (SPF) / cosmetic emulsion / emulsion stability

CONTENTS

CONTENTS (continue)

CONTENTS (continue)

LIST OF TABLES

LIST OF TABLES (continue)

LIST OF FIGURES

LIST OF FIGURES (continue)

CHAPTER I
LITERATURE REVIEW

1. Roasted barley

Barley (*Hordeum vulgare* L.), the main grain crop globally, is a cereal plant that has short harvest time and tolerates to the arid climate. It contains several active compounds e.g. β-glucan, tocols, benzoic acid, cinnamic acid, *p*-coumaric acid, ferulic acid, and phenolic compounds. These compounds can give beneficial health e.g. reduce the risk of coronary heart diseases, cancers, and the aging processes (Soto *et al.* 2015).

Figure 1: Roasted barley.

Furthermore, there are the major source of phenolic compounds in barley e.g. benzoic and cinnamic acid derivatives, proanthocyanidins, quinines, flavonols, chalcones, flavones, flavanones, and amino phenolic compounds. Roasting is the processing method used to dry barley seed. Roasted barley is widely consumed because it shows an important role in human nutrition. Phenolic compounds due to the roasting promote the synthesis of Melanoidins by Maillard reactions. Their Maillard reaction products may be considered as the active compounds. It also has antioxidant

activity that can delay some types of cell damage and anti-aging actions (Omwamba *et al.* 2013).

2. Phenolic compounds and flavonoids in barley

Phenolic compounds are plant secondary metabolites which can be found as the main component of the compound in plants. The phenolic compounds e.g. flavonoids, phenolic acids, diterpenes, and tannins have a high anti-oxidative activity which can be reduced the risk of coronary heart diseases, cancers, and the aging processes (Lattanzio, 2013). In the barley, it has many phenol compounds that have antioxidative activity e.g. vanillic acid, *p*-coumaric acid, *p*-hydroxyl-benzoic acid, *p*-hydroxybenzaldehyde, quercetin, and 3,4-dihydroxybenzaldehyde. (Omwamba *et al.* 2013)

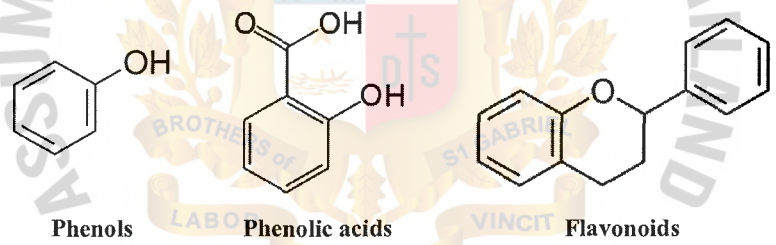

Figure 2: Structure of common phenolic compounds.
(Pondchaleumpong & Rattanapanon, 2013)

Flavonoids are a group of natural products from plant secondary metabolites. It has phenolic structures and can be found in several kinds of fruits, vegetables, grains, bark, roots, stems, flowers, tea, and wine. Flavonoids have several subgroups e.g. chalcones, flavones, flavonols, and isoflavones. It can be applied to the cosmetic product because it has an anti-oxidative activity which helps to prevent the oxidation reaction. Furthermore, they also absorb UVB which protects the harmful of solar radiation from sunlight (Idehen *et al.* 2017).

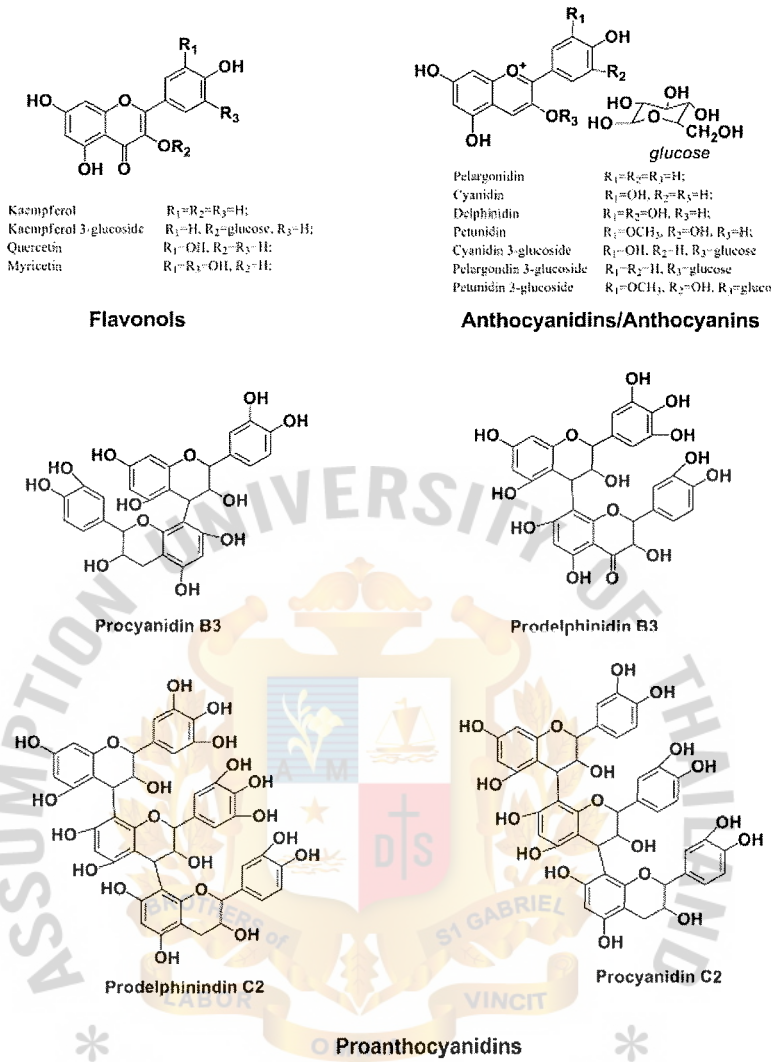

Figure 3: Structures of major flavonoids in barley. (Idehen *et al*. 2017)

3. Antioxidant activity

A compound is antioxidant when it delays or inhibits the oxidation that causes the oxidative damage to a molecule. Oxidation is a reaction that can produce the free radicals which will be scavenged by the antioxidant compound. The lipids, oils and fats, can oxidize into the free radicals when reacting with oxygen gas in the air. The quality of lipids can be decreased and rancid might occur. Other molecules like nucleic acid, proteins are can be oxidized as well.

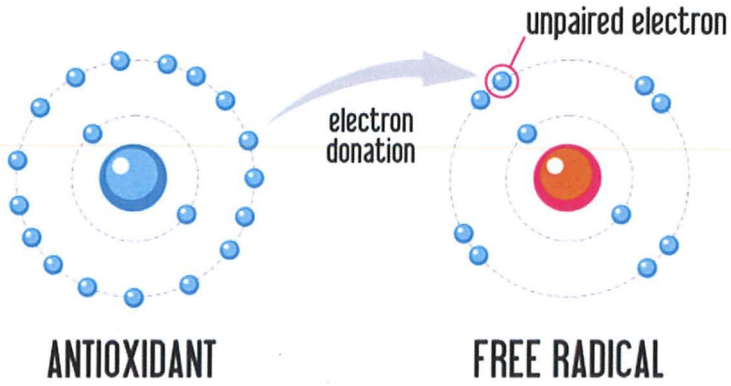

Figure 4: Antioxidant activity. (Nauka, 2015)

Many methods are commonly used to determine the antioxidant activity of the compounds such as DPPH scavenging and ABTS assay which are the rapid method to evaluate the potential of antioxidants (Laguerre *et al.* 2015).

4. Virgin herb oil and herb oil

4.1. Virgin coconut oil (VCO)

Virgin coconut oil (VCO) is the oil that produces from fresh coconut which becomes popular functional food oil. The VCO is extracted by the wet process under controlled temperature which can be retained more vitamins and minerals e.g. pro-vitamin A, vitamin E, and polyphenols. VCO contains a high amount of vitamins content, antioxidants, minerals, medium-chain fatty acids, taste, fragrance. The VCO contains 91.2% of saturated fatty acid (SFA), 6.2% of monounsaturated fatty acid (MUFA), and 1.6% of polyunsaturated fatty acid (PUFA). The predominant fatty acid of VCO is medium-chain saturated fatty acid, lauric acid (C12:0) (Widiyati, 2017). The appearance of VCO is a colorless oil which has good scent and taste. The rancidity of oil will not occur in a short period. Additionally, VCO presence of antioxidant activity. Tocopherols are the natural lipophilic antioxidants that can be found in the vegetable oils. In VCO, there are three types of tocopherols which are β-tocopherol, γ-tocopherol, and δ-tocopherol. Furthermore, VCO is a good

source of phenolic compounds which have high antioxidant properties (Dumancas *et al.* 2016).

4.2. Virgin olive oil (VOO)

Virgin olive oil is produced from fresh olive fruits by the crushing process. There is no heating on the extraction process. The VOO contains high MUFA up to 76.2% of total fat content which provided health benefit. The predominant fatty acid of VOO is oleic acid (C18:1) which is the monounsaturated omega-9 fatty acid (Widiyati, 2017). Furthermore, it can be resistant to oxidation reaction by the molecule of MUFA that has only one double bond (Yubero-Serrano *et al.* 2018). Besides, virgin olive oil (VOO) contains several antioxidant agents e.g. α-tocopherol, polyphenols, and phytosterols which protect the skin from aging process. It might help to reduce the damage and pigmentation from the UV radiation (Raghavamma *et al.* 2016).

4.3. Virgin avocado oil (VAO)

Avocado is a fruit that contains healthy fatty acid and several bioactive compounds that have a positive effect on humans. Avocado oil has been used as a raw material of the cosmetic product. The VAO contains 16.4% SFA, 68.4% of MUFA, and 15.2% of PUFA. The main lipid content in VAO is oleic acid (C18:1) (omega-9), the monounsaturated fatty acid. Besides, the polyunsaturated fatty acids are also found in VAO, the omega-6 to omega-3 ratio is 13:1, which are linoleic acid. (Widiyati, 2017). The natural oil pressed from the pulp of avocado help to retain several healthy components of avocado which provided antioxidant activity e.g. β-sitosterol, α-tocopherol, lutein, and chlorophyll. Also, VAO contains high levels of healthy monounsaturated fats and phytosterols that protect the skin from sunburn and aging process (Raghavamma *et al.* 2016).

4.4. Virgin sweet almond oil (VSAO)

The VSAO is the oil that extracts from almonds by using hydraulic pressure systems and screw presses. VSAO is commonly used in the cosmetic industry because it contains emollient and sclerosant properties that enhance skin hydration, improve skin tone, and reduce the dark spot of scar (Hernandez, 2016). The VSAO contains 11.1% SFA, 70.0% of MUFA, and 18.9% of PUFA (Solomon *et al.* 2014). Furthermore, the main composition of VSAO is long chain monosaturated fatty acid, oleic acid (C18:1), that provided several benefits to the human. Moreover, the long chain of fatty acid in VSAO was shown to prevent the skin damage caused by UV irradiation (Raghavamma *et al.* 2016).

4.5. *Camellia oleifera* seed oil (COSO)

The tea seed oil (*Camellia oleifera* oil) is extracted from *Camellia oleifera* seed which recommended to be used for sensitive and dry skin. The COSO contains 11.8% SFA, 78.9% of MUFA, and 9.3% of PUFA. It contains several unsaturated fatty acids e.g. oleic acid (C18:1) and linoleic (C18:2) acid which reduce the risk of cancer and sunlight protection effect (Choi *et al.* 2020). Tea seed oil has no negative effect on the human skin and can penetrate the different layer of the skin which reduce the wrinkles. Furthermore, the anti-oxidative properties of the oil can be protected the skin from UVB radiation (Ma *et al.* 2010).

4.6. Virgin perilla seed oil (VPSO)

VPSO is commonly used in the cosmetic industry which is the source of polyunsaturated fatty acids, phenolic compounds, and natural antioxidants. The VPSO contains 12.8% SFA, 12.5% of MUFA, and 74.7% of PUFA (Asif, 2020). The seed of *Perilla frutescens* (L.) contains α-linolenic acid (C18:3), linoleic acid (C18:2), and oleic acid (C18:1) which provide antioxidant property and protect the UV radiation (Sirilun *et al.* 2016). Furthermore,

VPSO also contains high contents of unsaponifiable compounds e.g. tocopherols and phytosterols (Chaiyana *et al.* 2018).

Table 1. The profile of virgin herb oil and herb oil.

Oil	Fatty acid component (% w/w)			Predominant fatty acid
	SFA	MUFA	PUFA	
Virgin coconut oil	91.2	6.2	1.6	Lauric acid (C12:0)
Virgin olive oil	13.7	76.2	10.1	Oleic acid (C18:1)
VIrgin avocado oil	16.4	68.4	15.2	Oleic acid (C18:1)
Virgin sweet almond oil	11.1	70.0	18.9	Oleic acid (C18:1)
Camellia oleifera seed oil	11.8	78.9	9.3	Oleic acid (C18:1)
Virgin perilla seed oil	12.8	12.5	74.7	α-Linolenic acid (C18:3)

5. Sun protection factor (SPF)

Sun protection factor (SPF) is commonly used to determine the efficiency of sunscreen which the high value of the SPF might be determined that the sunscreen can protect the skin from solar radiation. The harmful from sunlight radiation can be divided into 3 regions with are UVA (320 to 400 nm), UVB (290 to 320 nm), and UVC (200 to 290 nm). The UVA radiation can be reached into the deeper layer of the skin when compared to the UVB. It can generate the aging of the skin. The UVB radiation cannot be filtered completely by the ozone layer. Thus, the skin can be damaged due to sunburn. The UVC can be filtered by the atmosphere (Zarkogianni *et al.* 2016). The SPF can be determined by *in vivo* or *in vitro* which *in vitro* method is classified into two types. The dilute solution of a sunscreen product is measured of absorption or the transmission of UV by UV-vis spectrophotometer.

Many herb oils have been used to improve skin appearance by hydrating the skin, preventing the loss of water, and reduce the dark spot. Herb oils also show the potential to resist the UV light, they are considered as the radioprotective agents used to reduce the harmful effects of UV radiation (Korać *et al.* 2011).

6. Skin and moisture loss

The skin is an organ that can be affected by the climate. Besides, there are three types of skin which are dry, oily, and combination (Derrick *et al.* 2014). When the skin dry, it may be crack because of the extreme dryness. Cosmetic emulsion, lotion and cream, can help to protect the moisture content of the skin because it helps to absorb the water in the air to the skin. When its coat to the skin, the skin can be retained moisture content. Cosmetics emulsion is the product that makes from two immiscible liquid which are water and oil by using the emulsification method with suitable emulsifiers. The product will become oil in water (O/W) emulsion or water in oil (W/O) emulsion.

7. Hydrophilic-lipophile balance (HLB)

The hydrophilic-lipophile balance (HLB) is the value that shows the balance of the size and strength of the polar and the non-polar groups of the emulsifier (Yamashita & Sakamoto, 2016). It uses to measure the degree of hydrophilic and lipophilic which can be calculated by the values of different regions of the molecule. Lotion was made from two immiscible liquid which were water and oil. The HLB system can be used to determine the optimum range of HLB of emulsifying agents which help to maintain the stability of the lotion. The high HLB value shows the more polar and low value of HLB shows less polar of the compound. The Figure 5 showed HLB values range from 9 to 12 are classified as oil in water (O/W) emulsifying agent and the values range from 3 to 6 are water in oil (W/O) emulsifying agent types (Wilmington, 1980).

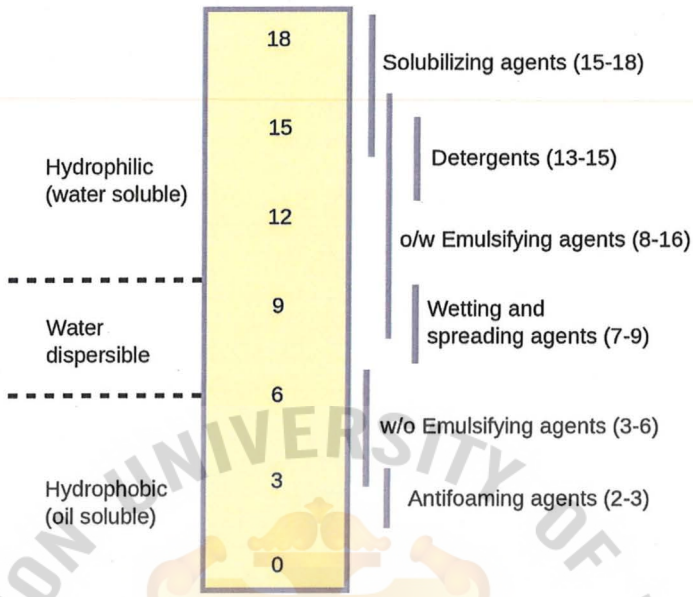

Figure 5: The HLB scale. (As *et al.* 2018)

8. Emulsion

The emulsions are systems which two immiscible liquids can be mixed together. There are several types of emulsions (Figure 6) e.g. oil-in-water (O/W), water-in-oil (W/O), oil-in-water-in-oil (O/W/O), water-in-oil-in-water (W/O/W), etc. The disperse phase is the phase that liquid droplets are dispersed in another liquid medium. To mix two immiscible liquids together, the emulsifier should be mixed into the solution. To maintain stability longer, the emulsifier should be adjusted in the optimum HLB value (Khan *et al.* 2011).

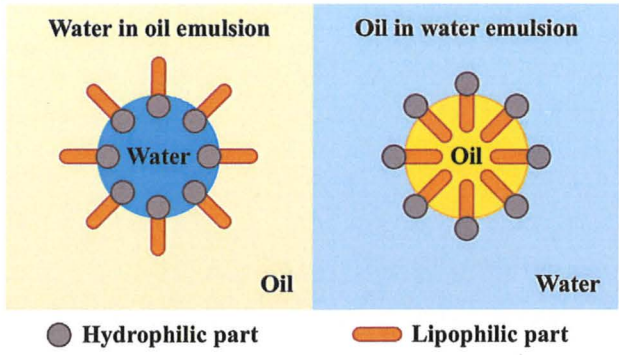

Figure 6: W/O and O/W emulsions. (Khan *et al.* 2011)

9. Occlusion factor

The occlusion factor is the value used to determine the occlusive of the cosmetic emulsion which occlusive performs a physical barrier on the skin to prevent the loss of moisture content from the skin surface. Occlusive ingredients include bee wax, stearic acid, etc. The higher value of the occlusion factor indicates the higher efficiency to maintain moisture in the skin (Wissing *et al.* 2001)

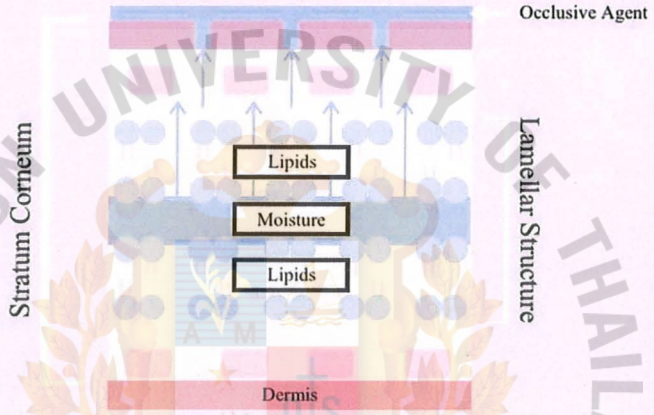

Figure 7: Occlusive agents (Laura, 2019)

10. Stability of emulsion

To maintain the function and quality of the emulsion, the stability should be maintained. Two immiscible liquids will separate without emulsion. Thus, several processes can be breaking the emulsion stability. The creaming and sedimentation process can occur under the thermal motion of droplets with forces. The conditions will make the larger droplets moving to the top or bottom. The flocculation process is the way that droplets change into larger droplets. When the van der Waals attraction is weak, the flocculation will occur. Therefore, it depends on the magnitude of the attractive energy involved. The Ostwald ripening has occurred when the small droplets disappear, and their molecules flowed into groups that the droplet size distribution will move to a greater value over time. The coalescence process has occurred when two or more droplets fusion together into the larger size of droplets. The phase inversion process is the exchanges between the disperse phase and the medium. The emulsion

will change when time pass from O/W to W/O or W/O to O/W emulsions (Tadros, 2013).

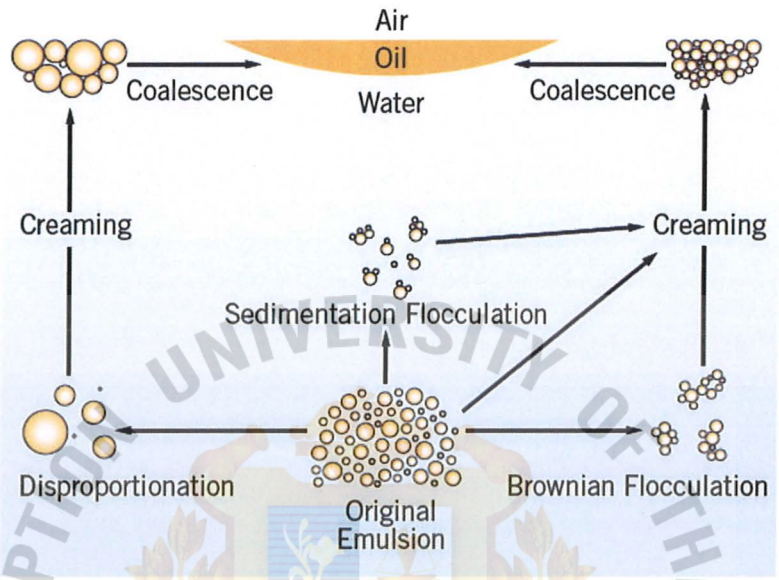

Figure 8: Schematic representation of the various breakdown processes in emulsions. (Lubrizol Life Science, 2020)

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OBJECTIVES

1. To determine the SPF of natural oils, extracted barley and oxybenzone.
2. To determine the effect of extracted barley and oxybenzone on the SPF of emulsion.
3. To formulate and study the physical characteristics of emulsion containing natural oil and roasted barley extract.
4. To study the emulsion stability under heating-cooling cycle stability test.
5. To determine the firmness and cohesiveness of cosmetic emulsion by using the texture analyzer.
6. To determine the occlusion factor of cosmetic emulsion by *in vitro* occlusion test.
7. To study the emulsion stability under light exposure and temperature variation test.

CHAPTER II

EFFECT OF MIXED NATURAL OILS ON BARLEY-VIRGIN OIL BASED O/W COSMETIC EMULSION AND IN VITRO DETERMINATION OF SPF

ABSTRACT

Barley (*Hordeum vulgare* L.) is the main source of several bioactive compounds e.g. phenolic compounds, flavonoids that could absorb the ultraviolet (UV) light. The virgin oils also contain photo-protective compounds that absorb UV radiation. This study was aimed to analyze the effect of barley extraction condition on the total phenolic content, total flavonoid content, and antioxidant activity, furthermore, the sun protection factor (SPF) of extracted barley and virgin oils were determined. The oil in water (o/w) cosmetic emulsion was formulated to study the efficiency of virgin oil types which could aid the physical sunscreen, benzophenone-3, to enhance the SPF. The occlusion factor indicated the prevention of dehydration of water from the skin. Extracted barley at 45°C without microwave preheating revealed the highest total phenolic content (19.62±1.35 mg GAE/g) whereas 45°C extraction of 2 minutes microwave preheating showed the highest total flavonoid content (430.92±1.91 mg QE/g). Radical scavenging DPPH reported the optimum extraction temperature at 45°C which microwave preheating 5 minutes showed the highest the half-maximal inhibitory concentration value (IC_{50}) at 6.23±0.41 mg TE/g. The sun protection factor (SPF) of 10% (w/v) of extracted barley and virgin avocado oil (VAO) were 6.12±0.05 and 8.71±0.37 respectively, whereas virgin coconut oil and virgin olive oil were 2.00±0.08 and 0.71±0.01 respectively. The SPF obtained from cosmetic emulsions E1, E4, and E6 showing higher SPF, above SPF of 5.5, when compared to E2, E3, E5, E7, and E8. The SPF ranged from 4.31 to 5.64. Virgin oils significantly enhanced the benzophenone-3 to exhibit UV absorption. The pH was in the range of alkaline cosmetics which designated to people with psoriasis therapy. All formulae showed occlusion factor varied from 41.81% to 75.40% within 48 hrs.

KEY WORDS: Barley / virgin oil / cosmetic emulsion / sun protection factor / occlusion factor

INTRODUCTION

The harmful effect of solar radiation from sunlight can be damage human skin by the penetration of ultraviolet radiation (UVR). There are 3 different regions of UVR which are UVA (320 nm to 400 nm) that can penetrate into the deeper layer and can induce skin cancer, UVB (290 nm to 320 nm) that can cause sunburn, and UVC (200 nm to 290 nm) that completely filtered by the atmosphere (Zarkogianni *et al.* 2016).

The cosmetics and personal cares market have a trend to use natural compounds as the main ingredient for improving skin appearance, moisturizing to provide long-lasting hydration, reducing wrinkle, etc. Dryness and dehydration of skin occur when skin could not retain sufficient moisture. Regarding the frequent bathing, aging, living in the dry air, or some skin diseases, those can be the cause of the loss of moisture. The cosmetic emulsion is one of the famous products that can be used to maintain moisture in the skin. The one factor that people buy and use this product are the benefits and natural ingredients in the cosmetic emulsion. Therefore, the harmful effect of solar radiation can be prevented by cosmetic emulsion by the mix of roasted barley and oxybenzone and natural oils. The emulsion can be applied to the skin to maintain moisture by the occlusive by the natural oils.

Barley (*Hordeum vulgare* L.) contains a high content of phenolic compounds e.g. flavonoids, phenolic acids, diterpenes, and tannins which have a high anti-oxidative activity that can be reduced the risk of coronary heart diseases, cancers, and the aging processes (Lattanzio, 2013). Furthermore, flavonoids are phytochemical compounds that protect against UVR by UVB absorbing compounds (Idehen *et al.* 2017). During the barley roasting process, the Melanoidins are synthesized by Maillard reactions. The Maillard reaction products (MRPs) may be considered as the active compounds which show antioxidant activity that can delay some actions (Omwamba *et al.* 2013).

The virgin oils are the oils that extract without heating process which the vitamins, minerals, and bioactive compounds will not lose from the oils. Virgin

coconut oil (VCO), virgin olive oil (VOO), and virgin avocado oil (VAO) contain high amounts of vitamins, antioxidants, minerals, medium-chain fatty acids, taste, fragrance. Furthermore, the high amount of anti-oxidative of VCO, VOO, and VAO can prevent skin damage and reduce the appearance of wrinkles. In addition, the sun protection factor (SPF) of herbal oils were reported that contain the efficiency of UVB (290 nm to 320 nm) protection (Kuar *et al.* 2010).

This study aimed to analyze the bioactive compounds presenting in the roasted barley grain and the effect of microwave-heat conditions on the radical scavenging activity. Moreover, in vitro spectrophotometric method was used to determine the SPF of extracted barley solutions, virgin oils, and cosmetics emulsion formulated with extracted barley and virgin oil.

MATERIALS AND METHODS

The roasted barley grains (Edo Baku, Japan), virgin coconut oil (King Island, Thailand), virgin olive oil (Filippo Berio, Italy), virgin avocado oil (Olivado, New Zealand) were obtained by local store.

Barley preparation

The roasted barley was pre-heated by microwave (800 watts) under three heating condition; (1) no heating, (2) 2 min heating, and (3) 5 min heating. The roasted barley (100 grams) was pulverized for 15 sec then the ground roasted barley was poured into the container and kept in a dry place at room temperature.

Extraction of bioactive compounds from barley

The bioactive compounds of ground roasted barley were extracted using a method modified from Omwamba *et al.* 2013. They were extracted using distilled water at the ratio of 1:10 (w/v) at 25°C, 45°C, and 90°C with 200 rpm for 10 min and leave at room temperature 20 min for complete extraction. The extracted solution was lyophilized by freeze drying then kept in dry place.

Total phenolic content

Total phenolic content (TPC) of the barley extracts was determined by Folin-Ciocalteu spectrophotometric method modified from Šimić *et al.* 2017 and Oh, *et al.* 2015. The lyophilized barley was dissolved in distilled water in the concentration of 2 mg/ml. Sample (500 µl) was added into the test tubes followed by 1 ml of 10% (v/v) Folin-Ciocalteu's reagent and 1 ml of 7.5% (w/v) sodium carbonate solution. The tube was allowed to stand for 30 min, then measured the absorbance at 765 nm. TPC was expressed as gallic acid equivalents (GAE) in mg/g material. The calibration curve for gallic acid was $y = 0.0177x$ ($R^2 = 0.9922$) where y is the absorbance and x is the concentration of gallic acid in µg/ml.

Total flavonoid content

Total flavonoid content (TFC) of the barley extracts was determined by aluminum chloride method modified from Oh *et al.* 2015 and Rebaya *et al.* 2014. The 2 mg/ml of lyophilized barley was prepared in distilled water. Sample (500 µl) was added into the test tubes followed by 3 ml of distilled water and 150 µl of 5% (w/v) sodium nitrite. The tubes were allowed to stand for 5 min, then added 150 µl of 10% (w/v) aluminium chloride. The mixture was incubated for 5 min at room temperature, then added 1 ml of 1M sodium hydroxide. The reaction mixture was then incubated for 30 min at room temperature to complete the reaction. The absorbance was measured at 510 nm. TFC was expressed as quercetin equivalents (QE) in mg/g material. The calibration curve for quercetin was $y = 0.001x$ ($R^2 = 0.9978$) where y is the absorbance and x is the concentration of quercetin in µg/ml.

Radical scavenging activity assays

The antioxidant activity was determined by DPPH assay modified from Šimić *et al.* 2017; Oh *et al.* 2015; Rebaya *et al.* 2014. Different dilutions of sample (0.5, 1, and 2 mg/ml) were added by 2 ml of 2,2-diphenyl-1-picrylhydrazyl. Absorbance was measured at 517 nm after 30 min incubated at room temperature without light. Radical scavenging ability was calculated as IC_{50} and expressed as TEAC in mg Trolox/g sample as follows:

$$\text{TEAC (mg TE/g)} = \frac{IC_{50\ Trolox}}{IC_{50\ Sample}}$$

The IC_{50} of Trolox used for calculation of TEAC was 83.78 mg/ml.

SPF Determination

The sun protection factor (SPF) was determined by UV-spectrophotometer using a method modified from Dutra *et al.* 2004. The 1.0 g of all samples were weighed, transferred into a 100 ml volumetric flask, then diluted to volume with ethanol and ultrasonication for 5 min. The samples were filtered through cotton and rejected the ten first ml. A 5.0 ml of sample was transferred into a 50 ml volumetric flask and diluted to volume with ethanol. Then, a 5.0 ml of sample was transferred into a 25 ml volumetric flask and diluted to volume with ethanol. The absorbance values were measured in the range of 290 to 320 nm (5-nm intervals), using ethanol as a blank. Each measurement was determinations three times. The SPF of the samples were calculated using the Mansur equation (Mansur *et al.* 1986) as follows:

$$SPF = CF \times \sum_{320}^{290} EE(\lambda) \times I(\lambda) \times Abs(\lambda)$$

where Correction Factor (CF) = 10, EE = erythemal effect spectrum, I = solar intensity spectrum, and Abs = absorbance of the sample. The values of EE×I are constants, which were determined and are shown in Table 2 (Sayre *et al.* 1979).

Table 2. Normalized product function used in the calculation of SPF

Wavelength (λ nm)	EE×I (normalized)
290	0.0150
295	0.0817
300	0.2874
305	0.3278
310	0.1864
315	0.0839
320	0.0180
Total	1

Emulsification process

Table 3. The formulae of cosmetic emulsion

Ingredient	Cosmetic emulsion (% w/w)							
	E1	E2	E3	E4	E5	E6	E7	E8
Oily phase								
Virgin coconut oil	4	6	6	-	12	-	-	-
Virgin olive oil	4	6	-	6	-	12	-	-
Virgin avocado oil	4	-	6	6	-	-	12	-
Mineral oil	-	-	-	-	-	-	-	12
Benzophenone-3	6	6	6	6	6	6	6	6
Bee wax	5	5	5	5	5	5	5	5
Stearic acid	3	3	3	3	3	3	3	3
Vitamin E-acetate	2	2	2	2	2	2	2	2
Phenoxyethanol (and) chlorphenesin (and) glycerin	1	1	1	1	1	1	1	1
Aqueous phase								
Tween 80	3.06	3.11	3.11	2.95	3.27	2.95	2.95	3.91
Span 80	4.94	4.89	4.89	5.05	4.73	5.05	5.05	4.09
Glycerin	4	4	4	4	4	4	4	4
Triethanolamine	2	2	2	2	2	2	2	2
Aloe vera extract	5	5	5	5	5	5	5	5
Xanthan gum	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Fragrance	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8
Barley extract	5	5	5	5	5	5	5	5
Distilled water	46	46	46	46	46	46	46	46

Notes. Final require HLB; E1=8.39, E2=8.46, E3=8.46, E4=8.25, E5=8.68, E6=8.25, E7=8.25, and E8=9.54

Cosmetic emulsion was done by mixing well of aqueous phase and oily phase shown in Table 3. The oily phase was slowly dropped into the aqueous phase with 1000 rpm using magnetic stirrer (VELP, Italy) until obtain the homogeneous o/w emulsion, then added the preservative. The emulsion was continually agitated for 15 min then kept for further analysis.

***In vitro* occlusion test**

The occlusion factor of emulsion was determined using a method modified from Teeranachaideekul *et al.* 2008 and López *et al.* 2015. The beakers (100 ml) were filled with 50 ml of water, covered with Whatman® filter paper grade 42 (surface area = 15.9 cm^2). Samples (200 mg) was spread on the filter surface, using petroleum jelly (Vasaline) as a positive control. The beakers were stored at 32°C and weighed at 4, 6, 24, and 48 hr. The occlusion factor (F) was calculated using the following equation:

$$F = \frac{A - B}{A} \times 100$$

where A refers to the water loss without a sample (reference) and B is the water loss with a sample. An F value of 0 indicates that no occlusive effect compared to the reference. On the other hand, an F value of 100 indicates maximum occlusiveness.

pH measurement

The pH of the cosmetic emulsion was measured in triplicate with a pH meter (HANNA instruments, Thailand). The sample was transferred into the beaker and the pH meter probe was immersed into the container (Suryani *et al.* 2017).

Color measurement

The color value of O/W cosmetic emulsion was measured with Miniscan EZ-4500L spectrophotometer (Hunter Lab Co. Ltd, US). CIE-L*a*b* system, D 65/ 10° standard light source (outdoor daylight), 45/0 angle of illumination/observer was used. The cosmetic emulsion was poured into the petri dish. All the sample were performed in three replications.

Statistical analysis

All experiments were conducted in three replications and statistical analysis was accomplished using ANOVA with Duncan's multiple range tests by the R Statistics (R version 2.15.3) (R Core Team, 2019). Different at $p \leq 0.05$ was considered to be significant level.

RESULTS AND DISCUSSION

Chemical profiles of extracted barley

Roasted barley grained obtained from a local store was treated in microwave 800 watts for 0, 2 and 5 min then barley was subjected to be extracted in distilled water which water-soluble bioactive compounds in the barley grain were obtained (Ferreres *et al.* 2009). The extraction temperatures were varied at 25°C, 45°C, and 90°C to observe the effect of temperature at low, medium, and high temperature on the bioactive compounds of roasted barley. The lyophilization barley was done by using the freeze-drying method to preserve the barley extracts and to prepare the concentration of barley used for the determination of bioactive compounds. Phenolic compounds and flavonoids are the plant secondary metabolites that provide the antioxidant activity. These compounds contain aromatic ring and hydroxyl group which acts as a reducing agent that provided antioxidant activity (Aryal *et al.* 2019). According to the results (Table 4), Barley-IV provided the highest TPC value (19.62± 1.35 mg GAE/g). Flavonoids are a group of natural products from plant secondary metabolites. They have phenolic structures and can be found in several kinds of fruits, vegetables, grains, bark, roots, stems, flowers, tea, and wine (Abidi *et al.* 2015). Barley-V showed the highest TFC value (430.92±1.91 mg QE/g). The preheat had a positive effect on the TFC value because of the release of change of glucosides or bound phenolics into free phenolic derivatives which provided the higher TFC value (Sharma *et al.* 2015), whereas the TPC value was not affected by the preheating. Sulaiman *et al.* reported that the optimum temperature for extracting phenolic compounds and flavonoids were at 60°C to 70°C (Sulaiman *et al.* 2017). At room temperature (25°C), the bioactive compounds might not release out from the lyophilized barley because the extraction temperature was not enough to weaken the linkage. On the other hand, boiling temperature (90°C) was considered as an overheating condition since several bioactive compounds might be degraded. Thus, the barley extracted at 25°C and 90°C provided lower antioxidant activity (IC_{50}) than

barley extracted at 45°C. According to the results (Table 4), Barley-VI showed the significantly highest IC_{50} value at 6.23±0.41 mg TE/ml. The roasted barley grain with 5 min microwave-heating might provide high antioxidant activity because the heating process might enhancement naturally occurring compounds e.g. Maillard reaction products that provided antioxidant activity (Sharma *et al.* 2015). Furthermore, the TPC and TFC value from table 4 also showed that the higher TPC and TFC provided more antioxidant activity

Table 4. Chemical profiles of extracted barley using different extraction temperature

Sample	Temperature (°C)	Microwave heat (min)	Chemical profile of extracted barley		
			TPC (mg GAE/g)	TFC (mg QE/g)	TEAC (mg TE/g)
I	25	0	11.52 ± 0.17 ^{e}	387.08 ± 0.80 ^{f}	4.69 ± 0.42 ^{b}
II		2	12.54 ± 0.24 ^{d}	402.67 ± 4.00 ^{d}	3.96 ± 0.45 ^{c}
III		5	13.00 ± 0.17 ^{cd}	378.00 ± 1.05 ^{g}	4.39 ± 0.24 ^{bc}
IV	45	0	19.62 ± 1.35 ^{a}	403.33 ± 3.50 ^{d}	5.64 ± 0.18 ^{a}
V		2	14.75 ± 1.17 ^{b}	430.92 ± 1.91 ^{a}	4.50 ± 0.13 ^{bc}
VI		5	14.76 ± 0.42 ^{b}	394.58 ± 3.54 ^{e}	6.23 ± 0.41 ^{a}
VII	90	0	13.49 ± 0.14 ^{c}	406.67 ± 1.97 ^{c}	4.01 ± 0.09 ^{c}
VIII		2	12.92 ± 0.29 ^{cd}	417.17 ± 1.69 ^{b}	3.89 ± 0.09 ^{c}
IX		5	11.72 ± 0.54 ^{e}	387.42 ± 2.50 ^{f}	3.92 ± 0.14 ^{c}

Note. means with the same letter in the same column are not significantly different ($p>0.05$)

SPF of barley extract and virgin oils

The scanning wavelength of the 10% (w/v) extracted barley and all virgin oils are shown in Figure 9 that they can absorb the UVA (320 to 400 nm) and UVB (290 to 320 nm) which it might be able to used as a natural sunscreen agent in cosmetic emulsion.

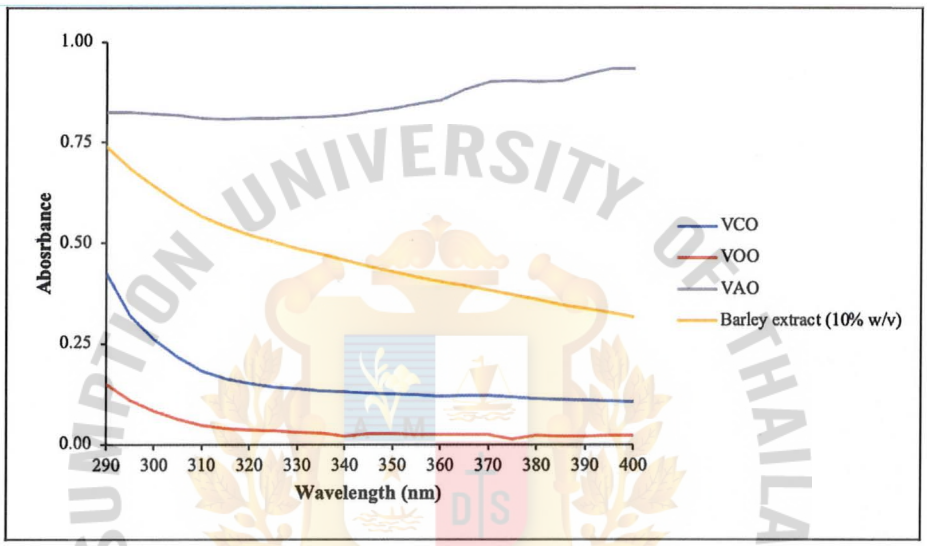

Figure 9: Scanning wavelength of virgin oils and extracted barley.

The SPF of extracted barley and all virgin oils was determined. The VCO contain 92% (w/w) of saturated fatty acid (SFA), 6% (w/w) of monounsaturated fatty acid (MUFA), and 2% (w/w) of polyunsaturated fatty acid (PUFA). The VOO contain 14% (w/w) of SFA, 83% (w/w) of MUFA, and 3% (w/w) of PUFA. The VAO contain 12% (w/w) of SFA, 71% (w/w) of MUFA, and 13% (w/w) of PUFA (Widiyati, 2017). The SFA has no double bond between the carbon atoms of the fatty acid chain which shows lower efficiency of UV absorption. In contrast to unsaturated fat, one or more double bonds are found in the fatty acid chain. The double covalent bond is weaker than a single bond, so oils containing unsaturated fatty acids as the main part are able to absorb UV light. PUFA contains more than one double bond that provides the photoprotective effect on the oil. The higher PUFA content, the higher UV absorption value. (Anil *et al.* 2013). According to Table 5, VAO revealed a significantly high in

SPF value which related to Figure 9 when compared with the other sample by the highest amount of PUFA. In contrast, the VCO contains high SFA but low unsaturated fat. It should show lower UV absorption. Anil *et al.* 2013 reported that the VCO presence of a small number of nonbonding electrons which provide some higher absorptions in UV radiation (Anil *et al.* 2013).

Table 5. SPF of 10% (w/v) barley and virgin oils

Sample	SPF
Barley extract	6.12 ± 0.05^{b}
Virgin coconut oil	2.00 ± 0.08^{c}
Virgin olive oil	0.71 ± 0.01^{d}
Virgin avocado oil	8.71 ± 0.37^{a}

Note. means with the same letter in the same column are not significantly different ($p>0.05$)

SPF of barley-virgin oil based cosmetic emulsion

To develop the o/w cosmetic emulsion, barley and virgin oils were used to study their photo-protective efficiency by formulate 8 different formulae of emulsion (Table 3). The barley extract (10% w/v) was used to enhance the SPF value in the cosmetic emulsion. In addition, the organic sunscreens are involved with the aromatic ring which in roasted barley containing the flavonoids that help to absorb UVB radiation (Idehen *et al.* 2017). The fixed ratio of barley extract (10% w/v) and synthetic UV absorber, benzophenone-3, was used to formulate cosmetic emulsions, only the amount of virgin oil was varied in order to examine the effect of natural oil on the SPF value. The results in Table 6 showed that the SPF values found for E1-E7, containing virgin oil, were in between 4.97 to 5.64 whereas E8, containing mineral oil, was 4.31. The formulae contained virgin oil as a photo-protective ingredient exhibited the higher SPF significantly than mineral oil. According to this result, it was suggested that virgin oils could enhance and aids benzophenone-3 to promote the higher SPF approximately up to 23.58% compared with mineral oil. Furthermore, o/w emulsion formulae containing VOO (E1, E2, E4 and E6) showed SPF value above 5.55. Even

though the SPF determination of only VOO indicated SPF value of 0.71 which was the lowest SPF while compared with other, the higher SPF of these o/w cosmetic emulsions might be resulted from the VOO had reaction with benzophenone-3 or other ingredients as emulsifier or extraction solvent that caused it showed the higher SPF than other formulae. The chemical reaction among the VOO with other ingredients might be taken in the formulated emulsion which could be resulting in the increase of SPF. Thus, this should be further examined. The pH of cosmetic emulsions were in the range of alkaline (pH 7.94-8.68) which could be designated for people with psoriasis therapy that the pH of the skin shifts to the acidic side (Dutta *et al.* 2018). The color measurement reported that color of the cosmetic emulsions contained VAO was dark yellow which was the resulting of color of VAO itself.

Table 6. SPF, pH, and color of cosmetic emulsion

Cosmetic emulsion	SPF	pH	Color of cosmetic emulsion		
			L*	a*	b*
E1	5.58 ± 0.05^{ab}	7.94 ± 0.01^{g}	73.50 ± 0.12^{b}	-0.24 ± 0.03^{d}	18.36 ± 0.24^{c}
E2	5.55 ± 0.02^{b}	8.39 ± 0.01^{f}	74.66 ± 0.12^{a}	0.09 ± 0.05^{ab}	13.78 ± 0.09^{e}
E3	5.30 ± 0.06^{c}	8.43 ± 0.02^{e}	73.58 ± 0.51^{b}	-0.69 ± 0.16^{e}	19.72 ± 0.09^{b}
E4	5.57 ± 0.02^{ab}	8.45 ± 0.01^{d}	74.39 ± 0.19^{a}	-0.17 ± 0.02^{cd}	19.75 ± 0.29^{b}
E5	4.97 ± 0.02^{e}	8.68 ± 0.01^{a}	70.57 ± 0.20^{d}	-0.30 ± 0.10^{d}	11.02 ± 0.02^{g}
E6	5.64 ± 0.07^{a}	8.61 ± 0.02^{b}	68.88 ± 0.08^{f}	0.25 ± 0.14^{a}	16.99 ± 0.19^{d}
E7	5.18 ± 0.04^{d}	8.67 ± 0.01^{a}	69.58 ± 0.23^{e}	-1.00 ± 0.12^{f}	24.65 ± 0.32^{a}
E8	4.31 ± 0.01^{f}	8.58 ± 0.02^{c}	71.81 ± 0.16^{c}	-0.03 ± 0.09^{bc}	12.37 ± 0.08^{f}

Note. means with the same letter in the same column are not significantly different ($p>0.05$)

In vitro occlusion test

The occlusion factor (F) indicates the occlusive of the emulsion which used to maintain the moisture in the skin. Petroleum jelly, Vaseline brand, (R) was use as a positive reference by the high potential of occlusive. VCO, VOO, and VAO can function as occlusive agent because of their saturated and unsaturated fatty acid contents. Thus, this occlusive property provides the forming of the barrier that blocks water from evaporation. Furthermore, oils also have an emollient property that can

replace natural skin oils (Vaughn *et al.* 2017). According to the result (Figure 10), Vaseline showed the highest occlusion factor which the water loss from the system only 0.7% at 48 hr. Furthermore, the cosmetic emulsion contained mineral oil (E8) had significantly high F value (2.36% water loss at 48 hr.) when compared with other formulae which mineral oil and petrolatum are two of the most effective occlusive ingredients. The petroleum jelly and mineral oil are the byproduct of the refining crude oil which is a hydrocarbon. It cannot absorb into the skin which forms a thin film that can be prevented the evaporation of water from human skin (Petry *et al.* 2017). The water loss from the cosmetic emulsion formulae E1-E7 range from 2.86% to 3.20% which slightly different from the reference and E8 at 48 hr.

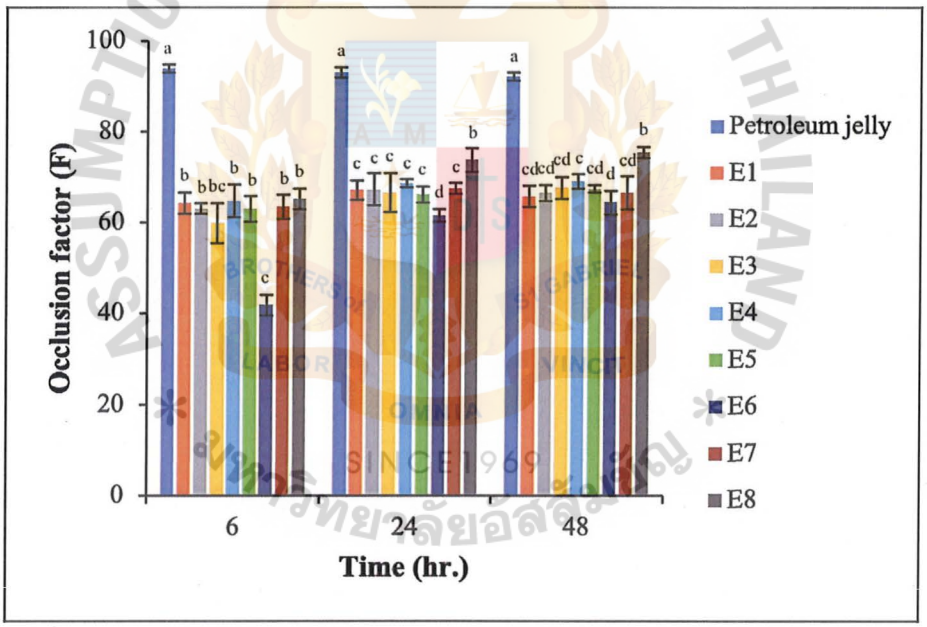

Figure 10: Occlusion factor of cosmetic emulsion.

CONCLUSIONS

In this study, it can be concluded that the extraction temperature had affected the bioactive compounds of barley extract which extracted at 45°C provided the highest TPC, TFC and antioxidant activity. Therefore, 5 minutes of microwave preheating showed the highest antioxidant activity (IC_{50} value). The *in vitro* UV spectrophotometric method revealed that the barley had photo-protective compounds which absorb the UVB radiation. Besides, all virgin oils also provided SPF value, VAO represented the most effective of sunscreen protection. Barley and virgin oil could be recommended as a natural UV absorption to be effective in preventing skin cancer, sunburn, and wrinkle reduction. The barley-virgin oil based cosmetic emulsion exhibited the occlusion factor that prevents dehydration.

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CHAPTER III

POTENTIAL OF HERB OILS AND ROASTED BARLEY AS THE PHYTO-ULTRAVIOLET PROTECTIVE

ABSTRACT

Ultraviolet radiation (UVR) has been reported to cause human skin irritation includes skin aging, pigment darkening, sunburn, and skin cancer. Sunscreen containing UVA (320-400 nm) and UVB (290-320 nm) blockers have been widely used to protect the harmful UVR. Many herbal extracts provided phyto-ultraviolet protective agents have recently used as an ingredient of sunscreen formulation. This study aimed to evaluate the potential of roasted barley (*Hordeum vulgare* L.) and five herb oils (virgin coconut, virgin avocado, virgin olive, virgin sweet almond, virgin perilla seed, and *Camellia oleifera* seed oils) to use for natural chemical absorber in the sunscreen. Roasted barley contains several bioactive compounds such as phenolic compounds and flavonoids that can absorb the UVR and provide antioxidant activity. Furthermore, herb oils contain natural sunscreens that resist UVR and emollient properties that moisturize and smooth skin. The results found that roasted barley and herb oils exhibited the absorbance spectrum in the three distinct bands of UVA, UVB, and UVC in the different potential. The 20% (w/v) roasted barley extract showed a significantly shifted spectrum indicating the more absorb of UVR than 10% (w/v) concentration. Moreover, it increased the sunblock property which showed a high in SPF value of 72.55% Furthermore, UV absorbance spectroscopy of four herb oils indicated the potential of natural substances in oils can provide the resist of UVA and UVB radiations except for *Camellia oleifera* seed oil (COSO) which absorbed UVC (200-290 nm). UV absorbance spectrum of Virgin perilla seed oil (VPSO) showed a maximum absorbance value and also showed the significantly highest SPF value at 9.52±0.12. Therefore, the roasted barely and VPSO contain the effectiveness natural phyto-UV protective substances to be used as the chemical absorbers ingredients to resist the UVA and UVB rays in sunscreen cosmetic products to protect the skin.

KEY WORDS: Roasted barley / herb oils / phyto-UV protective / sun protection factor

INTRODUCTION

The harmful effect of solar radiation can be induced the skin cancer and damage to human skin by ultraviolet (UV) region of the electromagnetic spectrum. UVR can classify into three regions which are UVA (320 to 400 nm), UVB (290 to 320 nm), and UVC (200 to 290 nm) (Dutra *et al.* 2004). UVA radiation can penetrate into a deeper layer of human skin which generates aging skin and skin cancer. UVB radiation is not completely filtered out by the ozone layer which can cause sunburn on human skin. In contrast, UVC radiation is completely filtered out by the ozone layer. Many different sunscreen products such as cream, lotion, gel or stick are launched in the cosmetics and pharmaceutical market, people use these sunscreen products to protect the harmful of UVR. Sun protection factor (SPF) is the value that use to measure the efficiency of the sunscreen product which the higher SPF value, the more efficiency of photo-protective effect. Sunscreen products nowadays they contain both physical and chemical sunscreen agents, the physical blockers reflect and scatter light thus the UVR cannot penetrate into the skin whereas the chemical blockers absorb the UVR before they reach the skin. Physical blocker ingredients in the sunscreen products are mostly used titanium oxide and zinc oxide which are inorganic compounds providing the broad-spectrum UVA and UVB protection. Most chemical blocker ingredients provide the narrow-spectrum UVB protection such as avobenzone (Korać *et al.* 2011; Lathe *et al.* 2013). Highly use of sunscreen products containing synthetic sunscreen blockers might cause the skin irritation.

Recently researches have shown the photoprotective agents derived from plants or herbs can provide the UVA and UVB protection, these natural extracts may be less harmful than synthetic blockers. Recent trends of sunscreen product not only have the beneficial effects in reducing the skin problems, but they have to contain the herbal ingredients to improve the efficiency of sun protection (Ngoc *et al.* 2019).

Roasted barley (*Hordeum vulgare* L.) is a plant that has short harvest time and tolerates to the arid climate. It contains of several bioactive compounds e.g. β-glucan,

tocols, benzoic acid, cinnamic acid, *p*-coumaric acid, ferulic acid, phenolic compounds, and flavonoids. Flavonoids are a group of natural products from plant secondary metabolites that have phenolics structure. The previous research found that a high concentration of flavonoids can be used to prevent UV-induced oxygen free radical generation (Ebrahimzadeh *et al.* 2014).

Virgin herb oil is the oil that extracts without heating process which maintains several bioactive compounds, vitamins, and mineral which provide benefit to human skin. Various oils have shown the characteristic of UV blocker that can absorb the sun light before it damages the skin. Researchers have found that coconut, olive, cotton seed and sesame oils can resist approximately 20-30% of UV light (Korać *et al.* 2011). The fatty acid contents in the virgin herb oil can affect to the SPF value. Polyunsaturated fatty acid in the oil contains more than one double bond which provides a photo-protective effect.

Therefore, five virgin herb oils, coconut virgin oil, olive virgin oil, avocado virgin oil, sweet almond virgin oil and perilla seed virgin oil (VCO, VOO, VAO, VSAO, and VPSO), and one herb oil, *Camellia oleifera* seed oil (COSO) were selected to evaluate the efficiency of herbal oil on the UV protection In addition, the roasted barley was also extracted and investigated the UV protection. The UVA and UVB absorbance profiles were examined. Moreover, the SPF value was determined by UV/VIS spectrophotometry method.

MATERIALS AND METHODS

MATERIALS

The roasted barley grain (Edo Baku, Japan) was obtained from the local store in Bangkok, Thailand. The grain was ground and stored for further extraction. Six herb oils, five virgin oils and one oil, were obtained from the local store in Bangkok, Thailand. Virgin coconut oil – VCO (King Island, Thailand), *Camellia oleifera* seed oil – COSO (Pat Pat, Thailand), and virgin perilla seed oil – VPSO (Lemon Farm, Thailand) were the products of Thailand. Virgin olive oil – VOO (Filippo Berio, Italy), virgin avocado oil – VAO (Olivado, New Zealand), and virgin sweet almond oil – VSAO (Olivado, New Zealand) were the products from Italy and New Zealand, respectively. Herb oils were collected and used to analyze in the further step.

METHODS

Extraction of bioactive compounds from barley

The roasted barley (100 grams) was pulverized then the ground roasted barley was poured into the container and kept in a dry place at room temperature. The bioactive compounds of ground roasted barley were extracted using distilled water at the ratio of 1:10 (w/v) and 1:5 (w/v) at 45°C with 200 rpm for 10 min and leave at room temperature 20 min for complete extraction. The extracted solution was filtered and then determined the UV protection characteristic.

Evaluation of UV absorbance spectrum and SPF determination

The 1.0 g of all samples (herb oils and barley extract) were weighed, transferred into a 100 ml volumetric flask, then diluted to volume with ethanol and ultrasonication for 5 min. The samples were filtered through cotton. The determination of UV absorbance spectrum of herb oils and barley extract was measured between 200-400 nm by UV/VIS spectrophotometer (Napagoda *et al.* 2016). The sun protection factor (SPF) was determined using a method modified from Dutra *et al.* 2004. The

absorbance values were measured in the range of 290 to 320 nm (5-nm intervals), using ethanol as a blank. Each measurement was determined three times. The SPF of the samples were calculated using the Mansur equation as follows:

$$SPF = CF \times \sum_{320}^{290} EE(\lambda) \times I(\lambda) \times Abs(\lambda)$$

where Correction Factor (CF) = 10, EE = erythemal effect spectrum, I = solar intensity spectrum, and Abs = absorbance of the sample. The values of EE×I are constants, which were determined and are shown in Table 7 (Sayre *et al.* 1979).

Table 7. Normalized product function used in the calculation of SPF

Wavelength (λ nm)	EE×I (normalized)
290	0.0150
295	0.0817
300	0.2874
305	0.3278
310	0.1864
315	0.0839
320	0.0180
Total	1

Statistical analysis

All experiments were conducted in three replications and statistical analysis was accomplished using ANOVA with Duncan's multiple range tests by the R Statistics (R version 2.15.3) (R Core Team, 2019). Different at $p \leq 0.05$ was considered to be a significant level.

RESULTS AND DISCUSSION

1. UV absorbance spectrum of roasted barley and herb oils

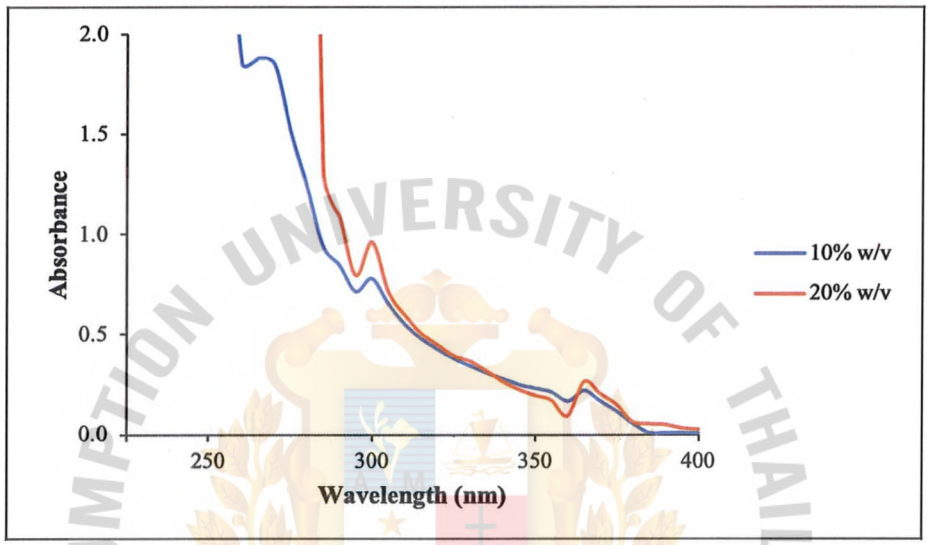

Figure 11: UV absorbance spectrum of roasted barley extracts.

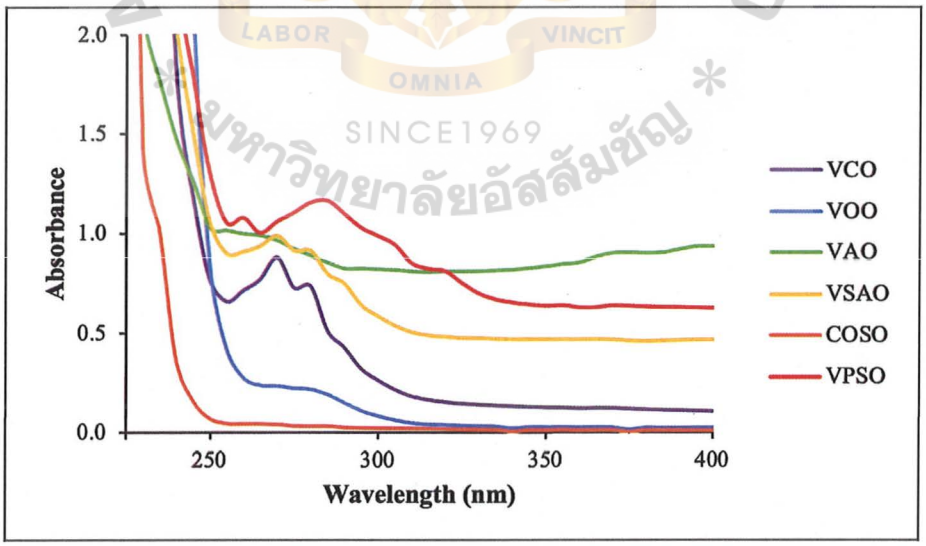

Figure 12: UV absorbance spectrum of herb oils.

UV absorbance spectroscopy of roasted barley (Figure 11) and herb oils (Figure 12) was observed at the wavelength 200 nm to 400 nm which in the range of UVA (320 to 400 nm), UVB (290 to 320 nm) and UVC (200 to 290 nm). The results from figures 11 and 12 were shown that roasted barley and herb oils contain phyto-UV protective agents that absorb the UVR in all three regions. However, the most effective UV absorption was in a range of 200-290 nm of UVC radiation since the spectrum showed the highest peak. Besides, the phenolics and flavonoids in barley provided high antioxidant activity which prevents the UV-induced oxygen free radical generation and lipid peroxidation (Ebrahimzadeh *et al.* 2014). Thus, the higher concentration of roasted barley solution, the more able to absorb the UVC light. In contrast, UVA and UVB spectrum of herb oils except VOO and COSO were higher than roasted barley. Herb oils are rich in fatty acids that offer the chemical UV blocker. Thus, the narrow-UVB spectrum can be absorbed. VPSO's UV spectrum showed the most effectiveness in the wavelength of 290-320 nm. VPSO contains one of the highest proportions of omega-3 polyunsaturated fatty acid which reported to decrease UVB-induced sunburn (Rhodes *et al.* 1994). Furthermore, VAO showed a higher of spectrum in the UVA region than other herb oils. The cold pressing extraction of VAO increased a concentration of and squalene (Satriana, *et al.* 2018) which protect UVA rays (Dessì *et al.* 2002). Therefore, UV absorbance spectroscopy could be used to examine the photoprotective characteristic of roasted barley and herb oils, all of these samples exhibited the active compounds that can absorb the UV light thus they could be used as the ingredient to formulate the chemical sunscreen products.

2. SPF of roasted barley and herb oils

The determination of SPF values for roasted barley, virgin herb oils, and herb oil was measured using the UV/VIS spectrophotometric method and the Mansur equation was used for calculation. Roasted barley contains several bioactive compounds especially flavonoids. Flavonoids are plain secondary phenolics that provide antioxidant activity. Furthermore, it has the potential to absorb the UV radiation from sunlight which provides the SPF values (Saewan *et al.* 2013). The

results in Table 8 showed that the calculated SPF of 20% (w/v) barley extract showed significantly different which increased 72.55% of the SPF value when compared with 10% (w/v) of barley extract. Thus, the higher amount of roasted barley can provide more bioactive compounds which increased the SPF value of the extracted barley.

Table 8. Calculated SPF values of roasted barley extracts

Barley extract (% w/v)	Sun protection factor (SPF)*
10	6.12 ± 0.05
20	10.56 ± 0.05

Note. [*] means significant different ($p \leq 0.05$)

Virgin oil is the oil that extracts without heat or control temperature. This process helps to maintain the bioactive compounds, vitamins, and minerals in the oil. Therefore, the fatty acids in the oil also provided the photo-protective property. The fatty acids can classify into two types which are saturated fatty (SFA) acids and unsaturated fatty acids (UFA). The carbon atoms in SFA are connected with single strong σ-bonds which are not able to absorb the energy in shorter wavelengths. Thus, the oil that contains SFA as the main part might not able to absorb the UV radiation when compared with the oil that contains UFA as the main part. Furthermore, the UFA can be classified into two types which are monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA). VOO, VAO, VSAO, COSO contain oleic acid as a major fatty acid which is the major MUFA. It contains one double bond in the fatty acid chain. On the other hand, linoleic acid is the main PUFA which has more than one double bond. Thus, the oil that contains PUFA as the main part can absorb more UV radiation (Anil Kumar *et al.* 2013). The results from Table 9 showed that VPSO provided a significantly high SPF value due to the highest contents of PUFA (74.7% w/w) (Asif, 2020). Furthermore, VAO and VSAO also provide the SPF value better than VCO, VOO, and COSO because of the higher PUFA content. In contrast, VCO contains high SFA which should not be able to absorb the UV radiation. But, VCO presence of a small number of nonbonding electrons which provide some higher absorptions in UV radiation (Anil Kumar *et al.* 2013). COSO showed the lowest SPF

value by the effect of the extraction process which does not control the temperature. Thus, the bioactive compounds that can provide SPF might loss and the saturated fatty acid can be increased (Abbas *et al.* 2017).

Table 9. Calculated SPF values of virgin herb oils

Natural oil	Sun protection factor (SPF)
Virgin coconut oil	2.00 ± 0.08^{d}
Virgin olive oil	2.06 ± 0.02^{d}
Virgin avocado oil	8.71 ± 0.37^{b}
Virgin sweet almond oil	5.08 ± 0.02^{c}
Virgin perilla seed oil	9.52 ± 0.12^{a}
Camellia oleifera seed oil	0.22 ± 0.04^{e}

Note. means with the same letter in the same column are not significantly different ($p>0.05$)

Perilla seed or Nga-Mon is widely cultivated especially in the northern part of Thailand, it contains bioactive compounds with functioning as antioxidant, anti-allergic, anti-inflammatory, and neuroprotective substances. Oil isolated from perilla seed is rich in n-3 polyunsaturated fatty acid includes omega-3 (α-linolenic acid), omega-6 (linoleic acid), and omega-9 (oleic acid) which have demonstrated a wide range of health-related benefits (Sirlun *et al.* 2016). The results study reported that the highest contents of n-3 polyunsaturated fatty acids could absorb the UVR to protect skin from the harmful UVA and UVB.

CONCLUSIONS

In conclusion, the roasted barley extract and virgin herb oils have the potential to absorb the UVR. The UV absorbance spectrum of roasted barley and herb oils showed that they exhibited the chemical blocker property which absorbs the UV light especially the UVA and UVB which can damage the skin and cause skin irritation. Therefore, the herbal oils showed the spectrum in a board-UVA and UVB which could be used as ingredients in sunscreen products. Furthermore, the concentration of the roasted barley can affect the SPF value which the higher concentration of barley, the higher SPF value. The 20% (w/v) Barley extract can increased the SPF value to 72.55% compared with 10% (w/v) barley extract. Moreover, the PUFA content in the virgin herb oils also has an efficiency of photoprotective which VPSO contained the highest PUFA and showed the highest SPF value at 9.52. Therefore, extracted barley at 20% (w/v) and VPSO can be used as a natural phyto-UV protective substance to supplement the synthetic UV absorbers in the cosmetic emulsion on the future experiment.

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CHAPTER IV
THE EFFECT OF HEATING-COOLING CYCLE STABILITY ON THE SPF OF VIRGIN PERILLA SEED OIL COSMETIC EMULSION AND ITS PHYSICAL CHARACTERISTICS

ABSTRACT

The Heating-cooling cycle stability test is the method to accelerate the storage time of the product in which emulsion stability is an important indicator of the quality of the product. High polyunsaturated fatty acids content of virgin perilla seed oil (VPSO) has been exhibited a strongly ultraviolet (UV) absorption spectrum thus it can provide sun protection factor (SPF) which protects human skin from ultraviolet radiations (UVR). Roasted barley (*Hordeum vulgare* L.) and oxybenzone were used as additional UV absorber agent which roasted barley contains several bioactive compounds such as phenolic compounds and flavonoids that can absorb the UVB and oxybenzone can absorb the UVA. This study aimed to evaluate the potential of cosmetic emulsion formulated from VPSO contributing with combined and either roasted barley or oxybenzone to be used as sunscreen product. The SPF and physical characteristics including pH, color, occlusion factor, and apparent viscosity were determined. Furthermore, the effect of heating-cooling cycle stability on the physical stability of VPSO cosmetic emulsion (pH, color, texture analysis, and emulsion stability) was also determined. The results found that VPSO cosmetic emulsion formulated with a combined oxybenzone and roasted barely (+oxybenzone, +roasted barley) showed an increase of SPF value due to their board range capability of the different regions of UV absorbing, the SPF value was 14.53±0.16. The firmness and homogeneity of VPSO cosmetic emulsion were increased by oxybenzone and Tween 80 regarding the apparent viscosity values. The degradation of SPF, pH, and color of cosmetic emulsion occurred under heating-cooling (H/C) cycle stability test exclude emulsion stability. The SPF of different VPSO cosmetic emulsions were decreased by 17.01% to 66.39% at the end of 6 H/C cycles. The formulations containing roasted barley, (−oxybenzone, +roasted barley) and (+oxybenzone, +roasted barley), showed

the potential to resist the damage better than oxybenzone alone. Although cosmetic emulsion stability was maintained constant, the extreme condition in the H/C cycles can change the physical characteristic of VPSO cosmetic emulsion. Therefore, VPSO could be recommended to use as a natural UV absorber and moisturizing ingredient by providing the SPF value and occlusive. Formulation with the combined oxybenzone and roasted barley can promote the effectiveness of sunscreen products and increase stability.

KEY WORDS: Heating-cooling cycle stability / emulsion stability / virgin perilla seed oil / roasted barley / sun protection factor

INTRODUCTION

Emulsion stability of the cosmetic emulsion is an important indicator during the storage which indicates the quality of the products. Emulsifiers play an important role in the formation of stable cosmetic emulsion, combined nonionic emulsifiers are the most common ingredients used in cosmetic emulsion. Several factors could destabilize the emulsion e.g. increasing of temperature, the ratio between oil and water, type of emulsifier that can promote flocculation, creaming, sedimentation and change the physical appearance of emulsion (Tadros, 2013).

The harmful from solar radiation can cause several skin diseases e.g. sunburn and skin cancer which the ultraviolet radiation (UVR) can be classified into three regions; UVA (320-400 nm), UVB (290-320 nm), and UVC (200-290 nm). UVA radiation can penetrate in a deeper layer of human skin which generates aging skin and skin cancer. UVB radiation is not completely filtered out by the ozone layer which can cause sunburn on human skin. In contrast, UVC radiation is completely filtered out by the ozone layer (Dutra *et al.* 2004). Sunscreen products in the market are commonly contained the physical sunscreen, chemical sunscreen, or mixed physical-chemical sunscreen ingredients to produce the most effective preventing the skin from UVR. An oxybenzone, derivative of benzophenone, is an organic compound that can be found in several flowering plants (Djalil *et al.* 2018). It commonly used in many sunscreen products due to the UVA absorber potential that can increase the sun protection factor (SPF) value of the product. Sunscreen product containing the natural UV blocker is an alternative way to prevent human skin without the using of synthetic chemical sunscreen ingredients which able to cause skin allergy.

Virgin perilla seed oil (VPSO) or Nga-Mon virgin oil is a good natural source of antioxidants. It contains a high content of polyunsaturated fatty acids (PUFA, 74.7%) as a predominant fatty acid that provides the anti-oxidative property and photoprotective (Torri *et al.* 2019). Various kinds of herb oils including VPSO have been reported that oils could absorb the UVR to protect the skin from the harmful of

radiation. Moreover, roasted barley grain (*Hordeum vulgare* L.) consists of phenolic compounds and flavonoids which exhibit antioxidant activity and photoprotective activity (Ebrahimzadeh *et al.* 2014).

Therefore, this study aimed to evaluate the SPF of oil in water cosmetic emulsion contained different oxybenzone, roasted barley extract, and VPSO. Furthermore, a heating-cooling cycle stability test was used to accelerate the storage condition of emulsion in order to examine the changing of SPF and physical characteristics including pH, color, texture, and viscosity.

MATERIALS AND METHODS

MATERIALS

The roasted barley grain (Edo Baku, Japan) was ground and stored for further extraction. Virgin perilla seed oil – VPSO or Nga-Mon (Lemon Farm, Thailand) produced from the northern part of Thailand was used to formulate sunscreen cosmetic emulsion. Both materials were obtained from the local store in Bangkok, Thailand. Oxybenzone, benzophenone derivative, was used as the chemical sunscreen ingredient to compare and evaluate the efficiency of VPSO. Tween 80 and Span 80, nonionic emulsifiers, were chosen to perform an O/W cosmetic emulsion.

METHODS

Extraction of bioactive compounds from barley

The roasted barley (100 grams) was pulverized, then the ground roasted barley was poured into the container and kept in a dry place at room temperature. The bioactive compounds of ground roasted barley were extracted using distilled water at the ratio of and 1:5 (w/v) at 45°C with 200 rpm for 10 min and leave at room temperature 20 min for complete extraction. The extracted solution was filtered and then determined the UV protection characteristic.

Evaluation of UV absorbance spectrum and SPF determination

The 1.0 g of oxybenzone (0.01%, 0.05%, and 0.10% w/v) and cosmetic emulsion were weighed, transferred into a 100 ml volumetric flask, then diluted to volume with ethanol and ultrasonication for 5 min. The samples were filtered through cotton. The determination of the UV absorbance spectrum of oxybenzone was measured between 200-400 nm by UV/VIS spectrophotometer (Napagoda *et al.* 2016). The sun protection factor (SPF) of oxybenzone and cosmetic emulsion were determined using *in vitro* UV spectrophotometry method, a method modified from Dutra *et al.* 2004. The absorbance values were measured from 290 to 320 nm at 5 nm

interval, using ethanol as a blank. Each measurement was determined three times. The SPF of the samples were calculated using the Mansur equation as follows:

$$SPF = CF \times \sum_{320}^{290} EE(\lambda) \times I(\lambda) \times Abs(\lambda)$$

where Correction Factor (CF) = 10, EE = erythemal effect spectrum, I = solar intensity spectrum, and Abs = absorbance of the sample. The values of EE×I are constants, which were determined and are shown in Table 10 (Sayre *et al.* 1979).

Table 10. Normalized product function used in the calculation of SPF

Wavelength (λ nm)	EE×I (normalized)
290	0.0150
295	0.0817
300	0.2874
305	0.3278
310	0.1864
315	0.0839
320	0.0180
Total	1

Emulsification process

Oil in water (O/W) cosmetic emulsion contained different of VPSO, mineral oil, and oxybenzone were prepared (Table 11 and 12). The require HLB value of each formulae was calculated by the following equation (Wilmington, 1980):

$$\text{Require HLB} = \frac{\text{Amount of material} \times \text{RHLB of material}}{\text{Total oil}}$$

The ratio of emulsifiers was calculated by the following equation (Wilmington, 1980):

$$\%\,(A) = \frac{X - HLB_B}{HLB_A - HLB_B} \times 100 \quad \text{and} \quad \%\,(B) = 100 - \%\,(A)$$

where **X** refer to required HLB, **%(A)** refer to ratio of Tween 80, **%(B)** refer to ratio of Span 80, $\mathbf{HLB_A}$ refer to HLB of Tween 80, $\mathbf{HLB_B}$ refer to HLB of Span 80.

Table 11. Formulation of cosmetic emulsion

Ingredient	Cosmetic emulsion (% w/w)			
	E1	**E2**	**E3**	**E4**
Oily phase				
Mineral oil	12	12	12	12
Oxybenzone	-	-	0.1	0.1
Bee wax	5	5	5	5
Stearic acid	3	3	3	3
Vitamin E acetate	2	2	2	2
Phenoxyethanol (and) chlorphenesin (and) glycerin	1	1	1	1
Aqueous phase				
Tween 80	4.4	4.4	4.3	4.3
Span 80	3.6	3.6	3.7	3.7
Glycerin	4	4	4	4
Triethanolamine	2	2	2	2
Roasted barley extract	-	10	-	10
Xanthan gum	0.2	0.2	0.2	0.2
Distilled water	q.s. ad 100	q.s. ad 100	q.s. ad 100	q.s. ad 100

Notes. Final require HLB; E1 and E2 = 10.23, E3 and E4 = 10.21, RHLB of mineral oil = 10, RHLB of bee wax = 12, RHLB of stearic acid = 15, HLB of Tween 80 = 15.0, HLB of Span 80 = 4.3.

Table 12. Formulation of VPSO cosmetic emulsion

Ingredient	VPSO Cosmetic emulsion (% w/w)			
	CE1	CE2	CE3	CE4
Oily phase				
Virgin perilla seed oil	12	12	12	12
Oxybenzone	-	-	0.1	0.1
Bee wax	5	5	5	5
Stearic acid	3	3	3	3
Vitamin E acetate	2	2	2	2
Phenoxyethanol (and) chlorphenesin (and) glycerin	1	1	1	1
Aqueous phase				
Tween 80	3.6	3.6	3.5	3.5
Span 80	4.4	4.4	4.5	4.5
Glycerin	4	4	4	4
Triethanolamine	2	2	2	2
Roasted barley extract	-	10	-	10
Xanthan gum	0.2	0.2	0.2	0.2
Distilled water	q.s. ad 100	q.s. ad 100	q.s. ad 100	q.s. ad 100

Notes. Final require HLB; E1 and E2 = 9.14, E3 and E4 = 9.13, RHLB of VPSO = 8, RHLB of bee wax = 12, RHLB of stearic acid = 15, HLB of Tween 80 = 15.0, HLB of Span 80 = 4.3.

The emulsification of O/W cosmetic emulsion was done by mixing well of aqueous phase ingredients (each formula) in Tables 11 and 12 in a beaker, then heating in a double boiler at 80°C and vigorously shaking. All of the oily phase ingredients (each formula) shown in Tables 11 and 12 were mixed in another beaker, then heated in a double boiler at 80°C and vigorously shaken. The oil phase was slowly dropped wise into the aqueous phase with 1000 rpm using a magnetic stirrer for 15 min. The preservative was added, then kept in a glass bottle for further analysis.

pH measurements

The pH of VPSO cosmetic emulsions (CE1;CE4) were measured in triplicate with a pH meter (HANNA instruments, Thailand). The sample was transferred into the beaker and the pH meter probe was immersed into the container (Suryani *et al.* 2017).

Color measurements

The color value of VPSO cosmetic emulsions (CE1;CE4) were measured with Miniscan EZ-4500L spectrophotometer (Hunter Lab Co. Ltd, US). CIE-L*a*b* system, D 65/10° standard light source (outdoor daylight), 45/0 angle of illumination/ observer. The emulsion was poured into the petri dish. The measurement was performed in three replications.

Emulsion stability index

The VPSO cosmetic emulsion stability index was performed immediately after the emulsion (CE1;CE4) were prepared to determine the initial stability. Direct visual observations were used to determine the total heights of the opaque cream, turbid layer and the transparent layer of the cosmetic emulsion inside the test tube and to determine the stability index calculated by the equation:

$$\% \text{ Stability index} = \frac{H_{opaque\ cream}}{H_{total}} \times 100$$

***In vitro* occlusion test**

The occlusion factor of VPSO cosmetic emulsions (CE1;CE4) were determined using a method modified from Teeranachaideekul *et al.* 2008 and López *et al.* 2015. The beakers (100 ml) were filled with 50 ml of water, covered with Whatman® filter paper grade 42 (diameter=50.27 cm^3). The 200 mg of sample was spread on the filter surface, using petroleum jelly (Vaseline) as a positive control. The beakers were stored at 32°C and weighed at 6, 24, and 48 hr. The occlusion factor (F) was calculated using the following equation:

$$F = \left(\frac{A - B}{A}\right) \times 100$$

where A refers to the water loss without a sample (reference) and B is the water loss with a sample. An F value of 0 indicates that no occlusive effect compared to the reference. Therefore, an F value of 100 indicates maximum occlusive.

Texture analysis

The texture analysis of VPSO cosmetic emulsion (CE1;CE4) was measured by the backward extrusion method using texture analyser model TA-XT2 (Stable Micro System, London, UK). The cosmetic emulsion was prepared in 50 mm internal diameter glass. The measurements obtained using a 40 mm diameter of the compression disc. The following experimental protocol was employed to collect the data:

Test Mode: Compression

Pre-Test Speed: 1.50 mm s^{-1}

Test Speed: 1.00 mm s^{-1}

Post-Test: 2.00 mm s^{-1}

Target Mode: Strain

Strain: 65.0%

Trigger Type: Auto (Force)

Trigger Force: 5.0 g

Break Mode: off

Stop Plot at: Start Position (70 mm)

Tare Mode: Auto

Advanced Options: on

Return distance: 70 mm

Return speed: 10 mm s^{-1}

Contact force: 20 g

The result was shown as values of firmness (g) and cohesiveness (g). The firmness (F1) was determined by the mean of the maximum value of force in the plot of force vs. time at compression. The cohesiveness (F2) was determined by the mean of the maximum value of the force of the negative peak at back extrusion.

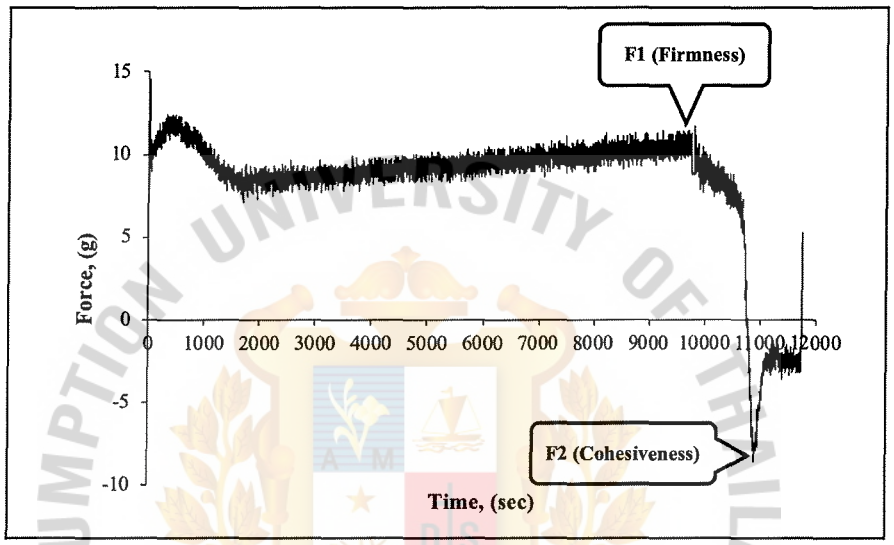


Figure 13: The graph of texture analysis.

Measurement of apparent viscosity

The values of apparent viscosity (η_a) of VPSO cosmetic emulsions (CE1;CE4) were determined by using Brookfield DV2T rotational viscometer (Brookfield, US) using the spindle (LV) no. 63 for 1 minute at 25°C. The viscosity values at 10 and 50 rpm were selected.

Physical stability test using heating-cooling cycle (H/C cycle)

The physical stability of VPSO cosmetic emulsions (CE1;CE4) was studied by H/C cycle modified by Suryani *et al.* 2017. The method of cycling tests was performed one cycle when the preparation emulsions (CE1;CE4) were stored at 4°C for 24 hours and then moved and placed in an incubator at a temperature of 45±2°C for 24 hours. This experiment was repeated for 6 cycles. The SPF, pH, color and emulsion stability index of VPSO cosmetic emulsion were observed for each cycle. The different of the

color between H/C cycle was calculated using the following equation (Mokrzycki, *et al.* 2011):

$$\Delta E^* = \sqrt{(L_2^* - L_1^*)^2 + (a_2^* - a_1^*)^2 + (b_2^* - b_1^*)^2}$$

- $0 < \Delta E^* < 1$ = observer does not notice the difference
- $1 < \Delta E^* < 2$ = only experienced observer can notice the difference
- $2 < \Delta E^* < 3.5$ = unexperienced observer also notices the difference
- $3.5 < \Delta E^* < 5$ = clear difference in color is noticed
- $5 < \Delta E^*$ = observer notices two different colors

Statistical analysis

All experiments were conducted in three replications and statistical analysis was accomplished using ANOVA with Duncan's multiple range tests by the R Statistics (R version 2.15.3) (R Core Team, 2019). Different at $p \leq 0.05$ was considered to be a significant level.

RESULTS AND DISCUSSIONS

1. UV absorbance spectrum of oxybenzone

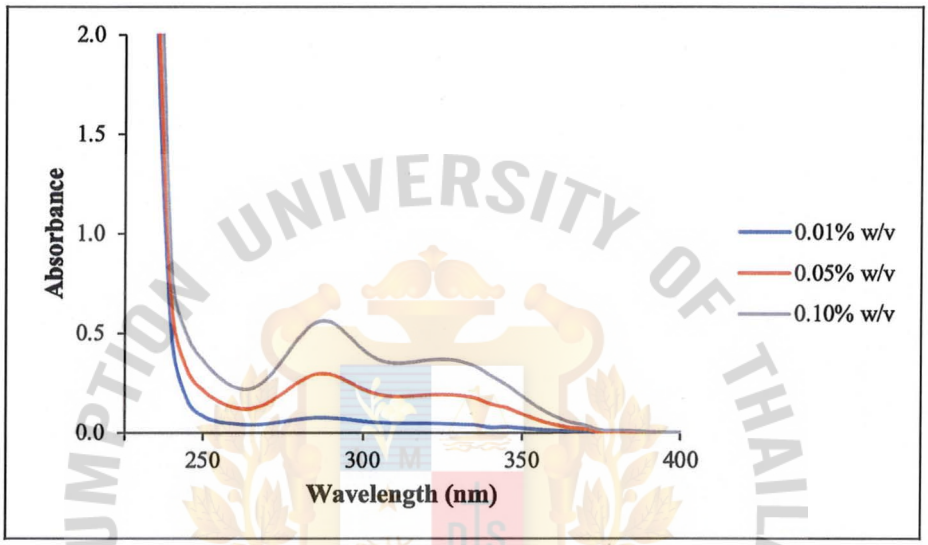


Figure 14: UV absorbance spectrum of oxybenzone.

Absorbance spectroscopy of oxybenzone was measured in the ultraviolet spectral region from 200 to 400 nm (Figure 14), the absorption maximum was above 2.0 at 210 nm of short wave ultraviolet (UVC). Furthermore, the absorption spectrum of oxybenzone in the medium and long-wave ultraviolet (UVA and UVB) was shown at 290-375 nm. It was revealed that the absorption peak in the UVB region was higher than in the UVA region, the absorption spectrum of oxybenzone with the absorption of 0.555 at 290-295 nm of wavelength and the concentration in 0.10% w/v was shown. Furthermore, it had the potential to absorb at a wavelength of 320-335 nm which in the range of UVA region with the absorption of 0.369 of concentration in 0.10% w/v. The higher concentration of oxybenzone can increase the absorption intensity in both UVB and UVA regions. Even though oxybenzone, benzophenone's derivative, absorbs mostly in UVB (peak absorption=288 nm) but it is considered as UVA (peak absorption=325 nm) absorber as well according to its spectrum profile (Wang *et al.*

2011). Oxybenzone is commonly used in chemical sunscreen products as a UV blocker against rays (Gabros *et al.* 2020). Food and drug administration (FDA) has allowed the use of oxybenzone as an ingredient in sunscreen products. Therefore, oxybenzone can be used in the cosmetic emulsion as a UVA absorber. Besides, oxybenzone contains hydroxyl and methoxyl groups that can donate the electron. Thus, it causes a shift toward the longer wavelengths (Widiyati, 2017).

2. SPF determination of oxybenzone

The determination of the SPF value of oxybenzone was measured using UV/ VIS spectrophotometric method and the Mansur equation was used for calculation. The result in Table 13 showed that SPF of 0.10% (w/v) oxybenzone provided the significantly highest SPF value which increased 121.11% of the SPF value when compared with 0.05% (w/v) of oxybenzone respectively. Thus, the higher concentration of oxybenzone can provide more efficiency of photoprotective which increased the SPF value of the oxybenzone.

Table 13. SPF of oxybenzone

Oxybenzone (% w/v)	Sun protection factor (SPF)
0.01	0.33 ± 0.04^{c}
0.05	1.80 ± 0.01^{b}
0.10	3.98 ± 0.01^{a}

Note. means with the same letter in the same column are not significantly different ($p>0.05$)

3. Effect of roasted barley and oxybenzone on the SPF of cosmetic emulsion

Figure 15: Cosmetic emulsion containing roasted barley and oxybenzone; E1 (−oxybenzone, −roasted barley), E2 (−oxybenzone, +roasted barley), E3 (+oxybenzone, −roasted barley), and E4 (+oxybenzone, +roasted barley).

The cosmetic emulsions were formulated into four formulae (E1-E4) by varying the use of roasted barley and oxybenzone to compare UV photoprotection efficiency. Formula E1 (−oxybenzone, −roasted barley) was performed and used as negative control which containing only mineral oil that is not considered as a photoprotective agent whereas formulae E2 (−oxybenzone, +roasted barley), E3 (+ oxybenzone, − roasted barley) and E4 (+oxybenzone, + roasted barley) were performed and used to examine the effect of those ingredients on the SPF of cosmetic emulsion (Figure 15). According to Table 14, negative control cosmetic emulsion (E1) showed the minimum SPF of 0.31±0.02 which could be considered that mineral oil and other ingredients like emulsifiers, triethanolamine, stearic acid, etc. had no or contain a low capability of photoprotective against UV rays. Besides, the presenting of an individual of either oxybenzone or roasted barley and mixed of oxybenzone and roasted barley showed a significantly different SPF of 7.02±0.06, 5.25±0.24 and 12.26±0.22 of formulae E2, E3 and E4 respectively. The increasing of SPF in cosmetic emulsion E2 occurred due to barley grain is the natural source of flavonoids

that exhibit the potential of UVB absorber which provides SPF value to the cosmetic emulsion (Saewan *et al.* 2013). In addition, oxybenzone in cosmetic emulsion E3 also showed the SPF value due to its UV protection ability. Furthermore, the enhanced SPF of combined oxybenzone and roasted barley in cosmetic emulsion E4 was significantly increased from cosmetic emulsion E2 and E3 about 74.64% and 133.52% respectively. Therefore, a cosmetic emulsion containing combined barley and oxybenzone can increase the SPF value by the potential to absorb UVB.

Table 14. SPF of cosmetic emulsion containing roasted barley and oxybenzone

Cosmetic emulsion	Sun protection factor (SPF)
E1	0.31 ± 0.02^{d}
E2	7.02 ± 0.06^{b}
E3	5.25 ± 0.24^{c}
E4	12.26 ± 0.22^{a}

Note. means with the same letter in the same column are not significantly different ($p>0.05$)

4. SPF determination and physical characteristics of VPSO cosmetic emulsion

Figure 16: Formulated VPSO cosmetic emulsion;
CE1 (−oxybenzone, −roasted barley), CE2 (−oxybenzone, +roasted barley),
CE3 (+oxybenzone, −roasted barley), and CE4 (+oxybenzone, +roasted barley).

4.1. SPF determination of VPSO cosmetic emulsion

Virgin perilla seed oil was used as the main ingredient in the oily phase of the cosmetic emulsion to study the enhancing of SPF value from natural herb oil. Four different formulae were prepared as shown in Figure 16, including formula CE1 (−oxybenzone, − roasted barley), formal CE2 (−oxybenzone, + roasted barley), formula CE3 (+oxybenzone, −roasted barley) and formula CE4 (+oxybenzone, + roasted barley). According to Table 15, SPF of cosmetic emulsion CE1, CE2, CE3, and CE4 were significantly different with the SPF value of 3.60±0.10, 10.17±0.03, 9.63±0.09, and 14.53±0.16 respectively. The cosmetic emulsion CE4 showed the highest significant SPF which is the resulting from combined oxybenzone and roasted barley. The substitution of mineral oil with VPSO in the formation of cosmetic emulsion showed a moderately increasing of SPF. Thus, it could be revealed that VPSO contained natural photoprotective to absorb UVR while compared between E1-E4 formulae (Table 14) and CE1-CE4 formulae (Table 15). VPSO cosmetic emulsion formula CE4 showed the significantly highest of SPF value compared with other

formulae which increased by 18.52% from E4. Moreover, the SPF of VPSO cosmetic emulsion formula CE3 also increased from cosmetic emulsion formula E3 about 83.43%. According to the UV absorbance spectrum of VPSO in the previous study, it showed that VPSO can absorb radiation in the UVB region more than the UVA region which the cosmetic emulsion formula CE3 contained only oxybenzone that provided the ability as UVA absorber. Thus, it can increase the SPF value when combining the UV blocker agents which can absorb different regions of UVR. Furthermore, the SPF value of VPSO cosmetic emulsion contained only roasted barely (CE2) also increased by 44.87% from E2. Roasted barely contains flavonoids which provided the ability to absorb UVB the same as VPSO. Therefore, it can slightly increase the value of SPF by mixing of the UV blocker agents that can absorb the same region of UVR. Hence, there has been an enhancing of photoprotective activity shown in cosmetic emulsion formulated by the use of VPSO, VPSO had obviously exhibited the capability against the board range of UVA and UVB. VPSO is rich in omega-3 fatty acids (53.6% to 64%), especially α-linolenic acid or ALA (18:3, n-3, 52.58% to 61.98%) which is a precursor of long chains polyunsaturated fatty acids such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (Zhou *et al.* 2014). Polyunsaturated fatty acid (PUFA) contains two or more C−C double bonds that can absorb in the wavelength 200 to 700 nm according to their electron transitions to the exit state (Anil *et al.* 2013). Thus, a cosmetic emulsion containing VPSO showed more SPF because this natural oil contains up to 64% of PUFA. This result aided to develop herbal oil cosmetics emulsion agains UVB for replacing the synthetic chemical absorber.

Table 15. SPF of VPSO cosmetic emulsion

VPSO cosmetic emulsion	Sun protection factor (SPF)
CE1	3.60 ± 0.10^{d}
CE2	10.17 ± 0.03^{b}
CE3	9.63 ± 0.09^{c}
CE4	14.53 ± 0.16^{a}

Note. means with the same letter in the same column are not significantly different ($p>0.05$)

4.2. pH determination of VPSO cosmetic emulsion

The pH of VPSO cosmetic emulsions were measured. Table 16 showed that the pH of all 4 formulae were ranged in alkaline (pH 8.61 to 8.73) which could be designated for people with psoriasis therapy that the pH of the skin shifts to the acidic side (Dutta *et al.* 2018). The pH of cosmetic emulsion contained roasted barley (CE2 and CE4) were showed the significantly highest compared with other formulae without roasted barley but increased only 0.1 from CE1 and CE3. The pH of cosmetic emulsion might increase from other ingredients e.g. triethanolamine and emulsifiers. Besides, triethanolamine was used as a thickening agent for the cosmetics emulsion which also increased the pH value.

Table 16. pH of VPSO cosmetic emulsion

VPSO cosmetic emulsion	pH
CE1	8.65 ± 0.02^{bc}
CE2	8.70 ± 0.02^{ab}
CE3	8.61 ± 0.04^{c}
CE4	8.73 ± 0.02^{a}

Note. means with the same letter in the same column are not significantly different ($p>0.05$)

4.3. Color measurement of VPSO cosmetic emulsion

The color of VPSO cosmetic emulsions were measured using CIE-L* a* b* system which L* indicated lightness of the sample, a* indicated red (+) or green (−), and b* indicated yellow (+) or blue (−). According to Table 17, the results showed that cosmetic emulsion CE1 and CE3 indicated more lightness than the formulae CE2 and CE4. The b* value of all 4 formulae were positive value which indicated yellow. Therefore, the cosmetic emulsion formulae CE 2 and CE4 provided more b* value which came from the color of roasted barley. Thus, roasted barley can affect the lightness and yellowish of the product.

Table 17. Color measurement of VPSO cosmetic emulsion

VPSO cosmetic emulsion	Color of VPSO cosmetic emulsion		
	L*	a*	b*
CE1	81.85 ± 0.10 ^{a}	-1.15 ± 0.02 ^{d}	9.52 ± 0.27 ^{d}
CE2	70.21 ± 0.06 ^{c}	2.41 ± 0.01 ^{b}	16.22 ± 0.04 ^{b}
CE3	81.53 ± 0.09 ^{b}	-0.93 ± 0.02 ^{c}	10.62 ± 0.03 ^{c}
CE4	69.54 ± 0.05 ^{d}	3.02 ± 0.01 ^{a}	16.99 ± 0.04 ^{a}

Note. means with the same letter in the same column are not significantly different (p>0.05), L* = CIE lightness coordinate, a* = CIE red (+) / green (−) colour attribute, b* = yellow (+) / blue (−) colour attribute

4.4. Texture analysis of VPSO cosmetic emulsion

The texture analysis of the VPSO cosmetic emulsion was measured by using the backward extrusion method. The graph of texture analysis was used to calculate the firmness and cohesiveness of VPSO cosmetic emulsions and compared with commercial brands cosmetic emulsion. The firmness was used to determine the force required to fully compress the product between thumb and forefinger (Savary *et al.* 2019). Besides, the texture of the emulsion can break very easily with a low value of cohesiveness. According to the results (Table 18), the firmness and cohesiveness of VPSO cosmetic emulsions were significantly different from the commercial brand's cosmetic emulsion. The formulae CE3 and CE4 which contained oxybenzone provided more firmness value among CE 1 and CE2. On the other hand, the cohesiveness of all VPSO cosmetic emulsions were not significantly different.

Table 18. Texture analysis of VPSO cosmetic emulsion

VPSO cosmetic emulsion	Firmness	Cohesiveness
Reference	102.95 ± 0.52 ^{a}	73.60 ± 2.60 ^{a}
CE1	19.90 ± 1.93 ^{d}	17.63 ± 3.40 ^{b}
CE2	23.01 ± 1.99 ^{c}	12.98 ± 0.39 ^{b}
CE3	26.51 ± 0.50 ^{b}	15.37 ± 0.33 ^{b}
CE4	26.20 ± 0.48 ^{b}	15.87 ± 0.84 ^{b}

Note. means with the same letter in the same column are not significantly different (p>0.05)

4.5. Occlusion factor of VPSO cosmetic emulsion

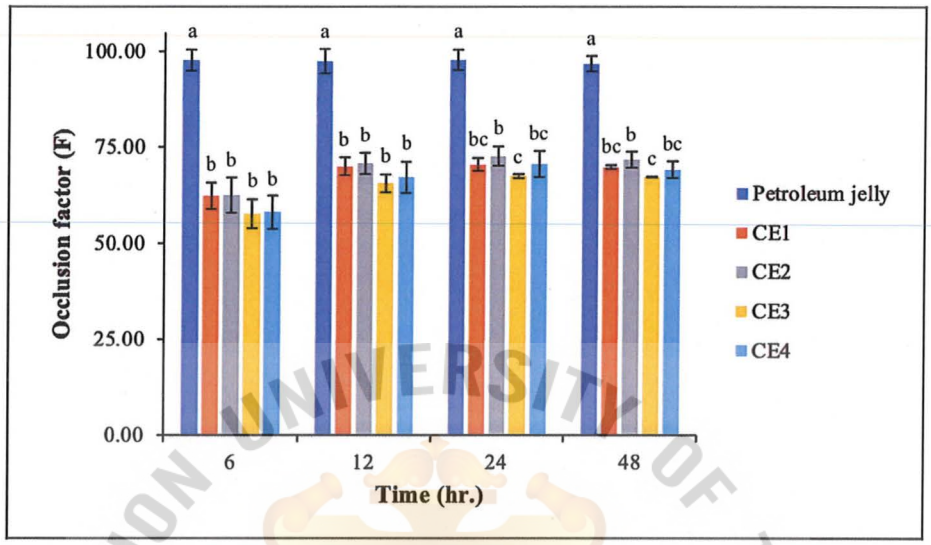


Figure 17: Occlusion factor of VPSO cosmetic emulsion.

The occlusion factor (F) indicates the occlusive of the cosmetic emulsion which used to prevent moisture loss from the skin layer. Petroleum jelly, Vaseline brand (R), was used as positive control by the high potential of occlusive. The petroleum jelly is the byproduct of the refining crude oil which is hydrocarbon. It cannot absorb into the skin which form a thin film that can be prevented the evaporation of water from human skin (Petry *et al.* 2017). Fatty acid contents, saturated and unsaturated fatty acid, in the oil can provide occlusive. Thus, VPSO can be forming of the barrier to block moisture evaporated out from the container. The results from Figure 17 showed that positive control, Vaseline, had the highest occlusion factor (96.80%) which the water loss from the system only 0.28% at 48 hr. Furthermore, the occlusion factor of cosmetic emulsion CE1, CE2, and CE4 were not significantly different at 48 hrs., the occlusion factor varied from 67.27% to 71.82%. On the other hand, the cosmetic emulsion CE3 was significantly different from CE2 at 24 and 48 hrs. The water loss from the cosmetic emulsion formulae CE1 to CE4 were ranged from 2.47% to 2.88%. Therefore, the use of VPSO as the oil to provide the

occlusiveness in the cosmetic emulsion was successfully prevent the loss of water from the skin.

Table 19. Occlusion factor of VPSO cosmetic emulsion

Sample	Occlusion factor (%)			
	6 hrs.	12 hrs.	24 hrs.	48 hrs.
Vaseline	97.70 ± 2.76 ^{a}	97.48 ± 3.14 ^{a}	97.75 ± 2.65 ^{a}	96.80 ± 2.04 ^{a}
CE1	62.31 ± 3.44 ^{b}	70.07 ± 2.31 ^{b}	70.57 ± 1.67 ^{bc}	69.87 ± 0.56 ^{bc}
CE2	62.55 ± 4.58 ^{b}	70.77 ± 2.73 ^{b}	72.68 ± 2.54 ^{b}	71.82 ± 2.09 ^{b}
CE3	57.68 ± 3.78 ^{b}	65.60 ± 2.31 ^{b}	67.45 ± 0.59 ^{c}	67.27 ± 0.05 ^{c}
CE4	58.12 ± 4.30 ^{b}	67.16 ± 4.05 ^{b}	70.60 ± 3.41 ^{bc}	69.24 ± 2.22 ^{bc}

Note. means with the same letter in the same column are not significantly different (p>0.05)

4.6. Viscosity of VPSO cosmetic emulsion

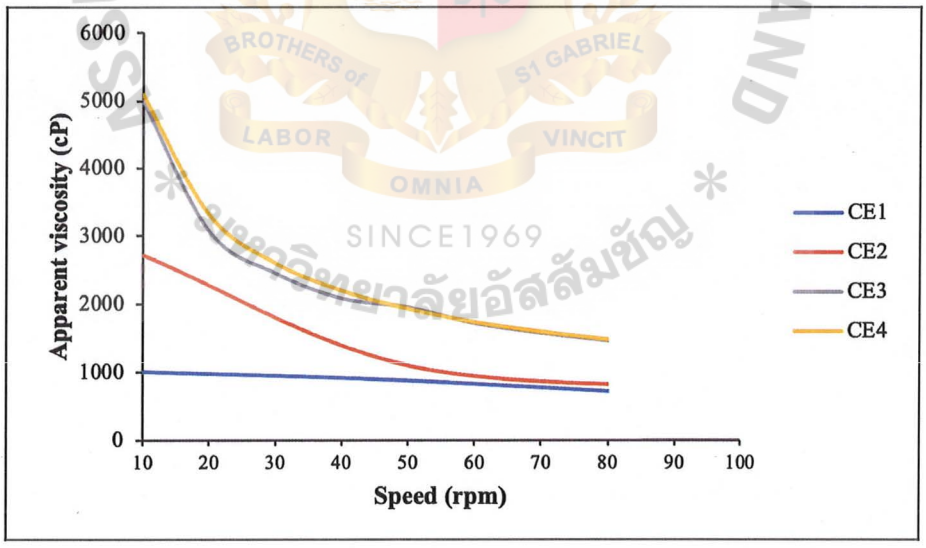


Figure 18: Apparent viscosity of VPSO cosmetic emulsion.

The viscosity of the emulsion is expected to be affected by many factors such as volume fraction of dispersed phase or continuous phase, the viscosity of dispersed phase or continuous phase, characteristic and concentration of emulsifying agents, average droplet size and temperature (Dan *et al.* 2006). Therefore, the apparent

viscosity (η_a) of VPSO cosmetic emulsions were measured by the DV2T rotational viscometer (Brookfield, US) using the spindle (LV) no. 63 for 1 minute with the speed of 10 rpm to 80 rpm at room temperature, Figure 18 showed that different concentrations of ingredients used to perform the four cosmetic emulsions affected the apparent viscosity. According to the results, cosmetic emulsions containing the chemical absorber oxybenzone in the CE3 and CE4 had the close values of apparent viscosity at all speeds and their apparent viscosity were significantly higher than CE1 and CE2. Moreover, the CE1 cosmetic emulsion which had no oxybenzone showed the lowest apparent viscosity that had remained constant at all speed rotations. Furthermore, apparent viscosity measured at speed rotations of 10 rpm and 50 rpm were reported in Figures 19 and 20, it was found that the apparent viscosity of cosmetic emulsions CE3 (4992 cP at 10 rpm and 1939 cP at 50 rpm) and CE4 (5088 cP at 10 rpm and 1922 cP at 50 rpm) were increased approximately 87.61% and 76.75%, respectively, while compared with CE1 (1004 at 10 rpm and 876 rpm at 50 rpm). Considering in the preparation of 4 cosmetic emulsions, the ratio of emulsifying agents were different which CE1 and CE2 contained a higher amount of Tween 80 than CE3 and CE4, the viscosity apparent values showed that CE1 and CE2 were less viscous than CE3 and CE4. Therefore, the applying of oxybenzone leads to increase in the viscosity of these VPSO cosmetic emulsions. Previous research found that increasing viscosity of formulation can be used to retard the human skin penetration of the sunscreen oxybenzone which high dose can cause skin irritation (Cross, *et al.* 2001). However, the viscous formulation may sometimes enhance the efficacy of penetration of other active ingredients through epidermal skin.

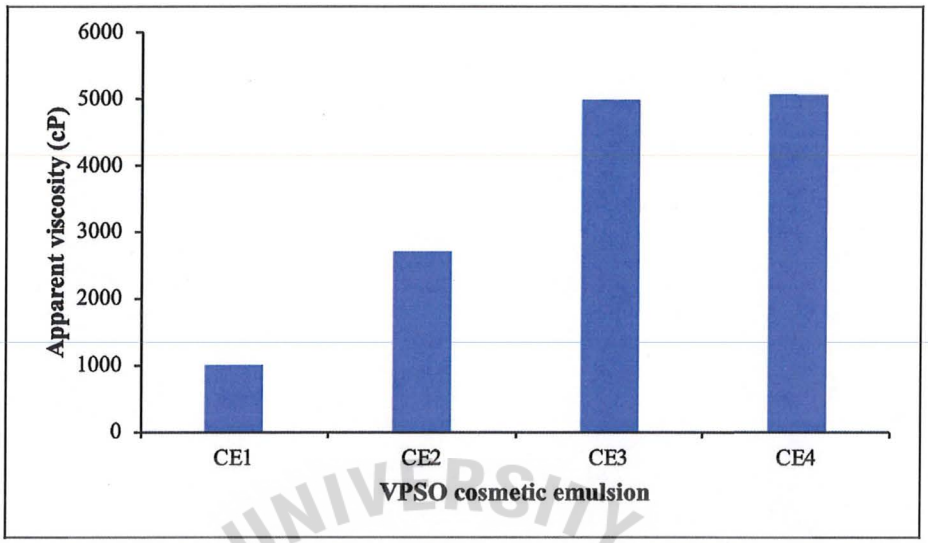


Figure 19: Apparent viscosity of VPSO cosmetic emulsion at speed rotation of 10 rpm.

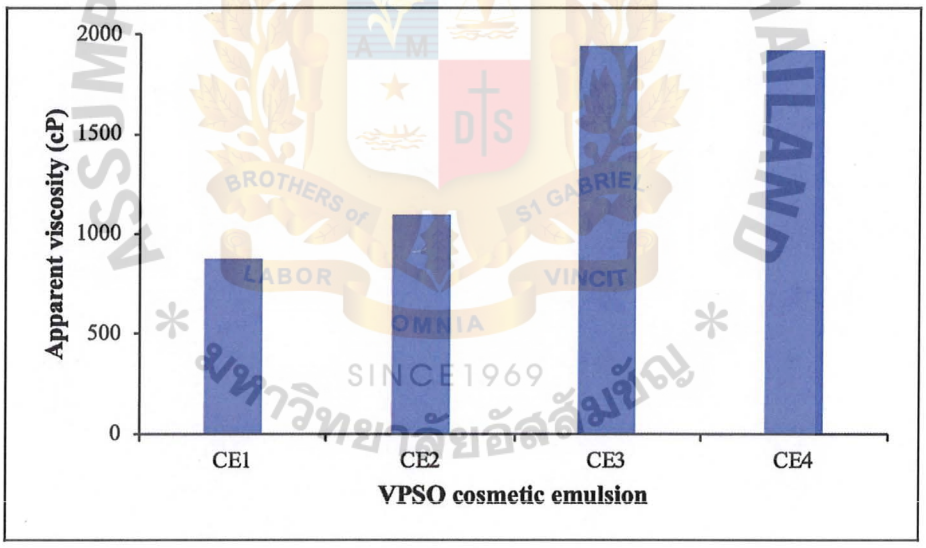


Figure 20: Apparent viscosity of VPSO cosmetic emulsion at speed rotation of 50 rpm.

5. Physical stability test using heating-cooling cycle

The effect of the storage condition of VPSO cosmetic emulsions or thermal stress test was examined by using a heating-cooling cycle test to accelerate the storage time. The heating-cooling cycle was 24 hrs. at 4°C and 24 hrs. at 45±2°C, the cycle was repeated 6 times. The SPF, pH, color, and emulsion stability of VPSO cosmetic emulsions were measured from each cycle.

5.1. SPF determination of VPSO cosmetic emulsion

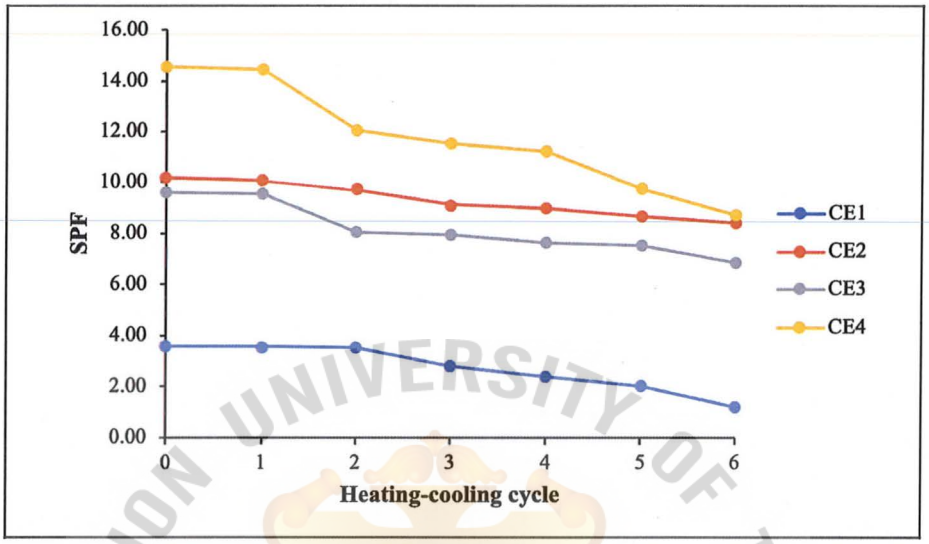


Figure 21: Heating-cooling cycle test of VPSO cosmetic emulsion on SPF

The SPF of VPSO cosmetics emulsion in Figure 21 showed that the SPF of all 4 different cosmetic emulsions (CE1;CE4) were gradually decreased from cycle 0 to cycle 6 which offered decreased by 17.01% to 66.39%. The result revealed that SPF slightly decreased non-significantly after 48 hrs. storage of cycle 1. However, after 96 hrs. storage onward (cycle 2 to cycle 6) SPF moderately decreased significantly (Table 20). Based on the percentage of decreasing of SPF, cosmetic emulsion CE2 showed the most stable of photoprotective activity after storage in 288 hrs. (six H/C cycles) because its SPF decreased by 17.01%. Other formulae, CE1, CE3 and CE4, their effectiveness to protect the skin from UV radiation were less stable because their SPF decreased by 66.39%, 29.07%, and 39.85% respectively. According to the results, cosmetic emulsions containing oxybenzone alone and oxybenzone combined with roasted barley in CE3 and CE4 respectively revealed the more unstable of SPF after examined by the thermal stress test, whereas, the applying of roasted barley alone in CE2 exhibited the more stable of UV protection. Thus, roasted barley can resist the damage of photoprotective activity from the H/C cycles better than oxybenzone. Consequently, oxybenzone was revealed to be sufficiency stable UV-filter after 48 hrs.

of cool and high-temperature exposure but it could be degraded by the repeating of a heating-cooling cycle that leads to the loss of the sun protection ability (Ceresole *et al.* 2013).

Table 20. Effect of H/C cycle on the SPF of VPSO cosmetic emulsion

H/C cycle	Sun protection factor (SPF)			
	CE1	CE2	CE3	CE4
0	3.60 ± 0.10^{Da}	10.17 ± 0.03^{Ba}	9.63 ± 0.09^{Ca}	14.53 ± 0.16^{Aa}
1	3.55 ± 0.09^{Da}	10.07 ± 0.16^{Ba}	9.57 ± 0.07^{Ca}	14.47 ± 0.15^{Aa}
2	3.52 ± 0.01^{Da}	9.72 ± 0.04^{Bb}	8.05 ± 0.02^{Cb}	12.05 ± 0.07^{Ab}
3	2.81 ± 0.02^{Db}	9.12 ± 0.03^{Bc}	7.94 ± 0.12^{Cb}	11.54 ± 0.16^{Ac}
4	2.37 ± 0.02^{Dc}	8.98 ± 0.08^{Bd}	7.62 ± 0.08^{Cc}	11.23 ± 0.04^{Ad}
5	2.01 ± 0.11^{Dd}	8.66 ± 0.05^{Be}	7.52 ± 0.05^{Cc}	9.77 ± 0.05^{Ae}
6	1.21 ± 0.04^{De}	8.44 ± 0.01^{Bf}	6.83 ± 0.07^{Cd}	8.74 ± 0.11^{Af}

Notes. A:D determine means in the same roll are not significantly different ($p>0.05$), a:f determine means in the same column are not significantly different ($p>0.05$)

5.2. pH of VPSO cosmetic emulsion

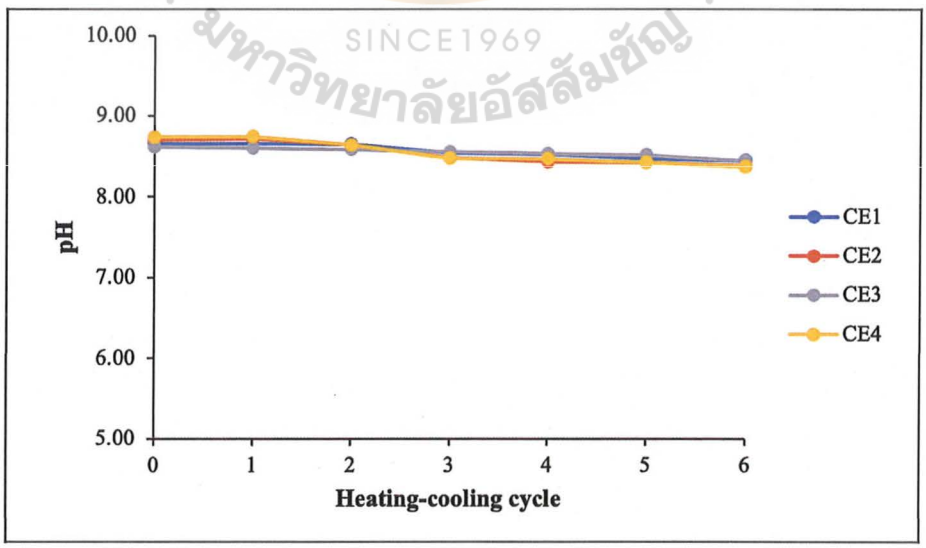


Figure 22: Heating-cooling cycle test of VPSO cosmetic emulsion on pH

Determining the pH value is used for assessing the stability of the emulsion. The pH of VPSO cosmetic emulsion was measured at the end of each H/C cycle for 6 cycles. No change of pH was observed in the 48 hrs. of the first H/C cycle. However, a slight decreased in pH were observed after 48 hrs. onward during the second to the sixth of the H/C cycle. It could be due to the destabilization of emulsion caused by the hydrolysis process at cold and heat temperature, the rancidity of VPSO caused by the changing of temperature. Previous research reported that the increase of acidity (drop in pH) can be occurred by the increase of the rancidity of the product (Oyelese *et al.* 2013). However, the overall pH changed were not have any influence on the stability of this VPSO cosmetic emulsion because the pH at the end of the sixth cycle was in the alkaline range which were above 8.37. In addition, α-tocopherol (vitamin E acetate) was the recommended ingredient that provided antioxidant property in the oily phase of the cosmetic emulsion. The pH of human skin usually varies from 4.5 to 6.0 so it is essential to formulate the lotion within this pH range. However, VPSO cosmetic emulsion had a pH in an alkaline range of approximately to 8.37-8.73, this formulation has an efficiency to use with some skin diseases such as psoriasis that causes red and scaly patches of skin (Dutta *et al.* 2018).

Table 21. Effect of H/C cycle on the pH of VPSO cosmetic emulsion

H/C cycle	pH			
	CE1	CE2	CE3	CE4
0	8.65 ± 0.02^{BCa}	8.70 ± 0.02^{ABa}	8.61 ± 0.04^{Ca}	8.73 ± 0.02^{Aa}
1	8.65 ± 0.03^{Ba}	8.71 ± 0.01^{Aa}	8.60 ± 0.03^{Ca}	8.74 ± 0.04^{Aa}
2	8.65 ± 0.02^{Aa}	8.63 ± 0.04^{Ab}	8.58 ± 0.03^{Bab}	8.63 ± 0.02^{Ab}
3	8.54 ± 0.02^{Ab}	8.47 ± 0.04^{Bc}	8.55 ± 0.01^{Abc}	8.47 ± 0.03^{Bc}
4	8.52 ± 0.03^{Ab}	8.43 ± 0.04^{Bd}	8.53 ± 0.02^{Ac}	8.46 ± 0.03^{Bcd}
5	8.47 ± 0.02^{Bc}	8.42 ± 0.02^{Cd}	8.52 ± 0.03^{Ac}	8.42 ± 0.03^{Cd}
6	8.45 ± 0.01^{Ac}	8.38 ± 0.01^{Bd}	8.44 ± 0.01^{Ad}	8.37 ± 0.01^{Be}

Notes. A:C determine means in the same roll are not significantly different ($p>0.05$), a:e determine means in the same column are not significantly different ($p>0.05$)

5.3. Color measurement of VPSO cosmetic emulsion

The L*, a*, and b* values of the cosmetic emulsions were measured at the end of each cycle to observe the change in color of the samples. The results from Table 22 showed that the lightness values of emulsions were significantly decreased at the end of H/C cycles. The formulae that contained roasted barley (CE2 and CE4) showed darker color by the color of barley extract. Furthermore, a* and b* also increased at the end of H/C cycles which the temperature of storage condition affected the color of VPSO cosmetic emulsion. The difference in the color of the cosmetic emulsion can be examined by ΔE* value. At the end of cycle 1, all formulae (CE1;CE4) cannot notice the different in color by the ΔE* values were between 0 and 1. Only experienced observers can notice the difference in the color of CE1 CE2, and CE3 at the end of cycle 2 except CE4 which unexperienced observers also notice the difference in color. At cycles 3, 4, and 5, ΔE* value of formulae CE1, CE2, and CE3 were higher than 2 which inexperienced observers can notice the difference in color. The cosmetic emulsion CE4 showed a clear difference in color at the end of cycles 4, 5, and 6 due to ΔE* value higher than 3.5. Furthermore, ΔE* values of CE1 and CE2 also higher than 3.5 at the end of cycle 6.

Table 22. Effect of H/C cycle on the color of VPSO cosmetic emulsion (L*)

H/C cycle	L* value			
	CE1	CE2	CE3	CE4
0	81.85 ± 0.10^{Aa}	70.21 ± 0.06^{Ca}	81.53 ± 0.09^{Ba}	69.54 ± 0.05^{Da}
1	81.29 ± 0.08^{Ab}	69.43 ± 0.03^{Bb}	81.11 ± 0.14^{Aa}	68.99 ± 0.14^{Cb}
2	80.22 ± 0.17^{Ac}	68.70 ± 0.07^{Cc}	79.78 ± 0.05^{Bb}	67.15 ± 0.12^{Dc}
3	79.94 ± 0.23^{Ac}	67.23 ± 0.13^{Cd}	79.26 ± 0.05^{Bc}	66.35 ± 0.29^{Dd}
4	79.09 ± 0.30^{Ad}	67.22 ± 0.36^{Bd}	78.82 ± 0.45^{Acd}	66.12 ± 0.10^{Cd}
5	79.05 ± 0.22^{Ad}	67.00 ± 0.07^{Cd}	78.66 ± 0.10^{Bd}	66.08 ± 0.29^{Dd}
6	78.52 ± 0.19^{Ae}	66.41 ± 0.47^{Be}	78.58 ± 0.54^{Ad}	65.45 ± 0.49^{Ce}

Notes. A:D determine means in the same roll are not significantly different ($p>0.05$), a:e determine means in the same column are not significantly different ($p>0.05$), L* = CIE lightness coordinate

Table 23. Effect of H/C cycle on the color of VPSO cosmetic emulsion (a*)

H/C cycle	a* value			
	CE1	CE2	CE3	CE4
0	-1.15 ± 0.02 ^{Da}	2.41 ± 0.01 ^{Bc}	-0.93 ± 0.02 ^{Ca}	3.02 ± 0.01 ^{Ac}
1	-1.12 ± 0.01 ^{Da}	2.47 ± 0.01 ^{Bc}	-0.95 ± 0.00 ^{Ca}	3.04 ± 0.10 ^{Ac}
2	-1.41 ± 0.02 ^{Db}	2.80 ± 0.03 ^{Bb}	-1.15 ± 0.04 ^{Cb}	3.24 ± 0.02 ^{Ab}
3	-1.51 ± 0.03 ^{Dc}	2.99 ± 0.04 ^{Ba}	-1.22 ± 0.03 ^{Cc}	3.26 ± 0.01 ^{Ab}
4	-1.55 ± 0.02 ^{Dcd}	3.02 ± 0.03 ^{Ba}	-1.28 ± 0.03 ^{Cd}	3.26 ± 0.00 ^{Ab}
5	-1.54 ± 0.02 ^{Dd}	3.06 ± 0.06 ^{Ba}	-1.24 ± 0.01 ^{Cc}	3.30 ± 0.04 ^{Aa}
6	-1.55 ± 0.02 ^{Dd}	3.05 ± 0.07 ^{Ba}	-1.33 ± 0.01 ^{Ce}	3.38 ± 0.05 ^{Aa}

Notes. A:D determine means in the same roll are not significantly different ($p>0.05$), a:d determine means in the same column are not significantly different ($p>0.05$), a* = CIE red (+) / green (−) colour attribute

Table 24. Effect of H/C cycle on the color of VPSO cosmetic emulsion (b*)

H/C cycle	b* value			
	CE1	CE2	CE3	CE4
0	9.52 ± 0.27 ^{Dd}	16.22 ± 0.04 ^{Bc}	10.62 ± 0.03 ^{Cc}	16.99 ± 0.04 ^{Ad}
1	9.68 ± 0.05 ^{Dcd}	16.24 ± 0.03 ^{Bc}	10.69 ± 0.03 ^{Cc}	17.04 ± 0.10 ^{Ac}
2	9.99 ± 0.13 ^{Dbc}	15.22 ± 0.03 ^{Bd}	10.68 ± 0.01 ^{Cc}	17.60 ± 0.03 ^{Ac}
3	10.13 ± 0.33 ^{Db}	16.01 ± 0.17 ^{Bc}	10.73 ± 0.02 ^{Cc}	17.90 ± 0.08 ^{Ab}
4	10.54 ± 0.29 ^{Da}	16.77 ± 0.08 ^{Bb}	10.96 ± 0.08 ^{Cc}	17.92 ± 0.05 ^{Ab}
5	10.68 ± 0.13 ^{Da}	17.08 ± 0.21 ^{Ba}	11.35 ± 0.33 ^{Cb}	18.20 ± 0.17 ^{Aa}
6	10.76 ± 0.06 ^{Da}	17.22 ± 0.24 ^{Ba}	11.81 ± 0.48 ^{Ca}	18.35 ± 0.18 ^{Aa}

Notes. A:D determine means in the same roll are not significantly different ($p>0.05$), a:d determine means in the same column are not significantly different ($p>0.05$), b* = yellow (+) / blue (−) colour attribute

Table 25. Different in color of VPSO cosmetic emulsion

H/C cycle	ΔE*			
	CE1	CE2	CE3	CE4
1	0.61 ± 0.07	0.79 ± 0.05	0.42 ± 0.18	0.56 ± 0.12
2	1.72 ± 0.16	1.85 ± 0.01	1.76 ± 006	2.50 ± 0.17
3	2.05 ± 0.15	3.04 ± 0.08	2.29 ± 0.04	3.33 ± 0.34
4	2.97 ± 0.31	3.10 ± 0.35	2.75 ± 0.40	3.56 ± 0.15
5	3.07 ± 0.25	3.39 ± 0.04	2.98 ± 0.21	3.69 ± 0.26
6	3.59 ± 0.17	3.99 ± 0.43	3.22 ± 0.62	4.33 ± 0.45

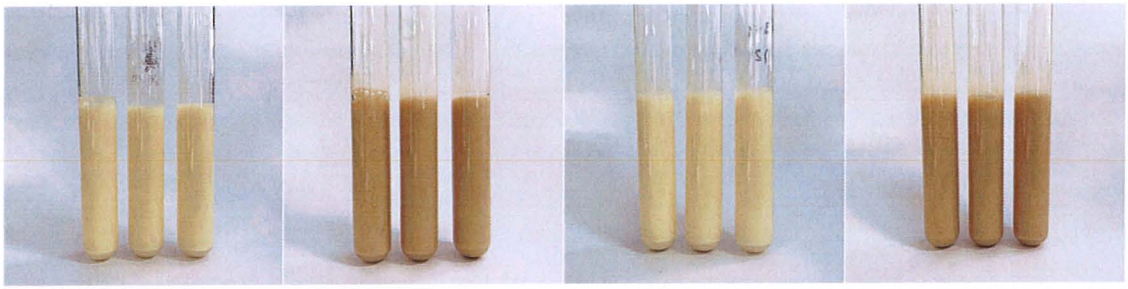
5.4. Emulsion stability of VPSO cosmetic emulsion

The emulsion stability of the VPSO cosmetic emulsion was monitored at the end of each H/C cycle to observe the effect of storage condition on the emulsion stability. According to the results showed in Table 26, emulsion stability of all cosmetic emulsions (CE1;CE4) were stable. The mixed of emulsifiers of Span 80 and Tween 80 can increase emulsion stability which provided the specific number of required HLB values of the oil contained in the cosmetic emulsion. Thus, mixed emulsifiers were recommended to increase emulsion stability.

Table 26. Effect of H/C cycle on the emulsion stability of VPSO cosmetic emulsion

H/C cycle	Emulsion stability index (%) [NS]			
	CE1	CE2	CE3	CE4
1	–	–	–	–
2	–	–	–	–
3	–	–	–	–
4	–	–	–	–
5	–	–	–	–
6	–	–	–	–

Notes. NS refer to no significant different ($p>0.05$), emulsion stability (+) indicated separation and (–) indicated no separation.

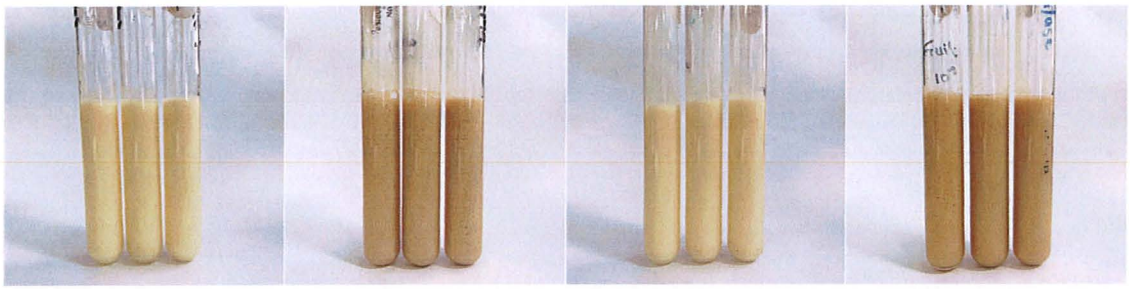


CE1 (cycle 1) **CE2 (cycle 1)** **CE3 (cycle 1)** **CE4 (cycle 1)**

CE1 (cycle 2) **CE2 (cycle 2)** **CE3 (cycle 2)** **CE4 (cycle 2)**

CE1 (cycle 3) **CE2 (cycle 3)** **CE3 (cycle 3)** **CE4 (cycle 3)**

Figure 23.1: Emulsion stability of VPSO cosmetic emulsion



CE1 (cycle 4) CE2 (cycle 4) CE3 (cycle 4) CE4 (cycle 4)

CE1 (cycle 5) CE2 (cycle 5) CE3 (cycle 5) CE4 (cycle 5)

CE1 (cycle 6) CE2 (cycle 6) CE3 (cycle 6) CE4 (cycle 6)

Figure 23.2: Emulsion stability of VPSO cosmetic emulsion

CONCLUSIONS

Virgin perilla seed oil or VPSO contained the activity against the UV radiation by absorbing the UVR in the UVA and UVB regions which resulting from rich of PUFA in oil. The formulation of cosmetic emulsion from organic sunscreen and natural plant extract enhanced the sun protection capability as found in the combined oxybenzone and roasted barley extract. Moreover, oxybenzone increased the firmness and homogeneity of cosmetic emulsion compared with other formulae regarding the apparent viscosity values. Furthermore, the pH values of VPSO cosmetic emulsion were ranged in alkaline pH between 8.61 to 8.73 that has an efficiency to treat some skin diseases that pH of skin shift in acidic. VPSO was a very effective occlusive moisturizer because it provided the forming of protective film to prevent the skin from losing moisture. The heating-cooling cycle stability test did not affect the emulsion stability, there was no separation between the disperse phase and continuous phase after 288 hrs., or 6 cycles of incubation. However, other parameters, SPF, pH, and color, were mildly affected by the heating-cooling cycle by decreasing the values. Although cosmetic emulsion stability was maintained constant, the variation of temperature in the heating-cooling cycle can change the physical characteristic of VPSO cosmetic emulsion.

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CHAPTER V

EFFICIENCY OF STABILITY OF VIRGIN PERILLA SEED OIL COSMETIC EMULSION UNDER LIGHT EXPOSURE AND TEMPERATURE VARIATION TEST

ABSTRACT

Thermal degradation and photooxidation can affect the SPF and physical characteristics of cosmetic emulsion which decrease the quality of the product. The accelerated stability test was performed to reduce the storage time compared with real-time storage. Virgin perilla seed oil (VPSO) and roasted barley (*Hordeum vulgare* L.) are natural sources that provided a potential of ultraviolet absorber by the high content of polyunsaturated fatty acids of oil and flavonoid in barley. This study aimed to evaluate emulsion stability includes SPF, pH, color, texture analysis, and emulsion stability under accelerated conditions. The cosmetic emulsion was stored in different conditions which were 30°C / dark, 30°C / light, and 45°C / light for 28 days. The SPF and physical characteristics of the cosmetic emulsion were measured at 0, 7, 14, 21, and 28 days of storage. The results showed the effect of thermal degradation and photooxidation on the SPF value which decreased SPF about 28.8% and 38.4% compared with the freshly prepared emulsion. The SPF can be maintained more than a year by the half-life time of SPF was equal to 415.89 days when kept at 30°C / dark condition. Furthermore, pH, color, and emulsion stability were effect by the high temperature of storage which the decrease in pH indicates the rancidity of the oil. The difference in the color of cosmetic emulsion storage at 45°C / light can be detected at the end of storage time. Moreover, the phase separation of emulsion storage at 45°C / light occurred at 21 days of storage. Therefore, cosmetic emulsion should be kept at room temperature and packed in the container that can protect light exposure in order to prevent thermal degradation and photooxidation of SPF and other physical characteristics of cosmetic emulsion.

KEY WORDS: Accelerated stability test / virgin perilla seed oil / roasted barley / thermal degradation / photooxidation

INTRODUCTION

The effect of sunlight can be damaged the human skin directly by the ultraviolet radiation (UVR). Three regions of UVR and be classified by the different of the wavelength which are UVA (320 nm to 400 nm), UVB (290 nm to 320 nm), and UVC (200 nm to 290 nm). The ozone layer can completely filter the UVC but UVA and UVB. Both UVA and UVB can be damaged human skin which can cause skin cancer and sunburn respectively (Dutra *et al.* 2004). The cosmetic emulsion provides sun protection factor (SPF) that can be applied to protect the skin from UVR. SPF is the value that use to evaluate the efficiency of the sunscreen product which the higher of SPF value, the more potential to protect skin from UVR.

Virgin perilla seed oil (VPSO) and roasted barley (*Hordeum vulgare* L.) are the good natural sources that provide the potential to absorb the UVR. VPSO contains a high content of polyunsaturated fatty acids (PUFA) about 74.7% (Asif, 2020) which presents the anti-oxidative property and photoprotective activity. Furthermore, roasted barley is the main source of phenolic compounds and flavonoids which show the radical scavenging activity and UV protective activity (Ebrahimzadeh *et al.* 2014).

The accelerated stability test is the method that use to evaluate the thermal degradation and photooxidation on the SPF and physical characteristics of cosmetic emulsion. Therefore, emulsion stability is the most important parameter to observe the quality of the product which accelerated stability tests can be used to decrease the storage time compare with real-time storage.

This study aimed to examine the changing of SPF and physical characteristics including pH, color, texture, and emulsion stability of VPSO- roasted barley cosmetic emulsion by thermal degradation and photooxidation under the varying of temperature and light conditions in order to investigate the stability.

MATERIALS AND METHODS

MATERIALS

The roasted barley grain (Edo Baku, Japan) was ground and stored for further extraction. Virgin perilla seed oil – VPSO or Nga-Mon (Lemon Farm, Thailand) produced from the northern part of Thailand was used to formulate sunscreen cosmetic emulsion. Both materials were obtained from the local store in Bangkok, Thailand. Tween 80 and Span 80, nonionic emulsifiers, were chosen to perform an O/W cosmetic emulsion.

METHODS

Extraction of bioactive compounds from barley

The roasted barley (100 grams) was pulverized, then the ground roasted barley was poured into the container and kept in a dry place at room temperature. The bioactive compounds of ground roasted barley were extracted using distilled water at the ratio of and 1:5 (w/v) at 45°C with 200 rpm for 10 min and leave at room temperature 20 min for complete extraction. The extracted solution was filtered and then determined the UV protection characteristic.

Emulsification process

Oil in water (O/W) cosmetic emulsion contained VPSO and roasted barley were prepared (Table 27). The require HLB value of each formulae was calculated by the following equation (Wilmington, 1980):

$$\text{Require HLB} = \frac{\text{Amount of material} \times \text{RHLB of material}}{\text{Total oil}}$$

The ratio of emulsifiers was calculated by the following equation (Wilmington, 1980):

$$\%\,(A) = \frac{X - HLB_B}{HLB_A - HLB_B} \times 100 \quad \text{and} \quad \%\,(B) = 100 - \%\,(A)$$

where **X** refer to required HLB, **%(A)** refer to ratio of Tween 80, **%(B)** refer to ratio of Span 80, $\mathbf{HLB_A}$ refer to HLB of Tween 80, $\mathbf{HLB_B}$ refer to HLB of Span 80.

Table 27. Formulation of cosmetic emulsion

Ingredient	Percent weight by weight (% w/w)
Oily phase	
Virgin perilla seed oil	12
Bee wax	5
Stearic acid	3
Vitamin E acetate	2
Aqueous phase	
Tween 80	3.6
Span 80	4.4
Glycerin	4
Triethanolamine	2
Roasted barley extract	10
Xanthan gum	0.2
Preservative	1
Distilled water	q.s. ad 100

Notes. RHLB of VPSO = 8, RHLB of bee wax = 12, RHLB of stearic acid = 15, HLB of Tween 80 = 15.0, HLB of Span 80 = 4.3.

The emulsification of O/W cosmetic emulsion (Table 27) was done by mixing well of aqueous phase ingredients in a beaker, then heating in a double boiler at 80°C and vigorously shaking. All of the oily phase ingredients (each formula) shown in

Table 27 were mixed in another beaker, then heated in a double boiler at 80°C and vigorously shaken. The oil phase was slowly dropped wise into the aqueous phase with 1000 rpm using a magnetic stirrer for 15 min then kept in a glass bottle for further analysis.

Accelerate stability test

The formulated cosmetic emulsion was kept in the incubator for 1 month-long protocol of accelerated stability testing performed following Shah *et al.* 2013. The tests were performed on VPSO cosmetic emulsions kept at these followings; (1) 30°C without light, (2) 30°C with light (LED lamp 4W;300 Lumens), and (3) 45°C with light (LED lamp 4W;300 Lumens) The SPF, pH, color, texture analysis and emulsion stability of formulated cosmetic emulsion were analyzed at 7 days interval during 28 days of storage. These experiments were performed in triplicate.

***In vitro* SPF determination**

The sun protection factor (SPF) of cosmetic emulsion was determined using *in vitro* UV spectrophotometry method, a method modified from Dutra *et al.* 2004. The 1.0 g of cosmetic emulsion was weighed, transferred into a 100 ml volumetric flask, then diluted to volume with ethanol and ultrasonication for 5 min. The samples were filtered through cotton. The absorbance values were measured from 290 to 320 nm at 5 nm interval, using ethanol as a blank. Each measurement was determined three times. The SPF of the samples were calculated using the Mansur equation as follows:

$$SPF = CF \times \sum_{320}^{290} EE(\lambda) \times I(\lambda) \times Abs(\lambda)$$

where Correction Factor (CF) = 10, EE = erythemal effect spectrum, I = solar intensity spectrum, and Abs = absorbance of the sample. The values of EE×I are constants, which were determined and are shown in Table 28 (Sayre *et al.* 1979).

SPF of VPSO cosmetic emulsion was analyzed at 0, 7, 14, 21, and 28 days of storage. The percentage of initial SPF of VPSO cosmetic emulsion of each storage condition was investigated.

Table 28. Normalized product function used in the calculation of SPF

Wavelength (λ nm)	EE×I (normalized)
290	0.0150
295	0.0817
300	0.2874
305	0.3278
310	0.1864
315	0.0839
320	0.0180
Total	1

pH measurements

The pH of VPSO cosmetic emulsion was measured with a pH meter (HANNA instruments, Thailand). The pH tests were performed 0, 7, 14, 21, and 28 days of storage (Suryani *et al.* 2017).

Color measurements

The color value of VPSO cosmetic emulsion was measured with Miniscan EZ-4500L spectrophotometer (Hunter Lab Co. Ltd, US). CIE-L*a*b* system, D 65/ 10° standard light source (outdoor daylight), 45/0 angle of illumination/observer. The emulsion was poured into the petri dish. The color measurement was performed for VPSO cosmetic emulsion after preparation and after storage at 7, 14, 21, and 28 days. The different of the color between H/C cycle was calculated using the following equation (Mokrzycki, *et al.* 2011):

$$\Delta E^* = \sqrt{(L_2^* - L_1^*)^2 + (a_2^* - a_1^*)^2 + (b_2^* - b_1^*)^2}$$

- $0 < \Delta E^* < 1$ = observer does not notice the difference
- $1 < \Delta E^* < 2$ = only experienced observer can notice the difference
- $2 < \Delta E^* < 3.5$ = unexperienced observer also notices the difference
- $3.5 < \Delta E^* < 5$ = clear difference in color is noticed
- $5 < \Delta E^*$ = observer notices two different colors

Texture analysis

The texture analysis of VPSO cosmetic emulsion was measured at 0, 7, 14, 21, and 28 days of storage under three different conditions by the backward extrusion method using texture analyser model TA-XT2 (Stable Micro System, London, UK). The cosmetic emulsion was prepared in 50 mm internal diameter glass. The measurements obtained using a 40 mm diameter of the compression disc. The following experimental protocol was employed to collect the data:

Test Mode: Compression

Pre-Test Speed: 1.50 mm s^{-1}

Test Speed: 1.00 mm s^{-1}

Post-Test: 2.00 mm s^{-1}

Target Mode: Strain

Strain: 65.0%

Trigger Type: Auto (Force)

Trigger Force: 5.0 g

Break Mode: off

Stop Plot at: Start Position (70 mm)

Tare Mode: Auto

Advanced Options: on

Return distance: 70 mm

Return speed: 10 mm s^{-1}

Contact force: 20 g

The result was shown as values of firmness (g) and cohesiveness (g). The firmness (F1) was determined by the mean of the maximum value of force in the plot of force vs. time at compression. The cohesiveness (F2) was determined by the mean of the maximum value of the force of the negative peak at back extrusion.

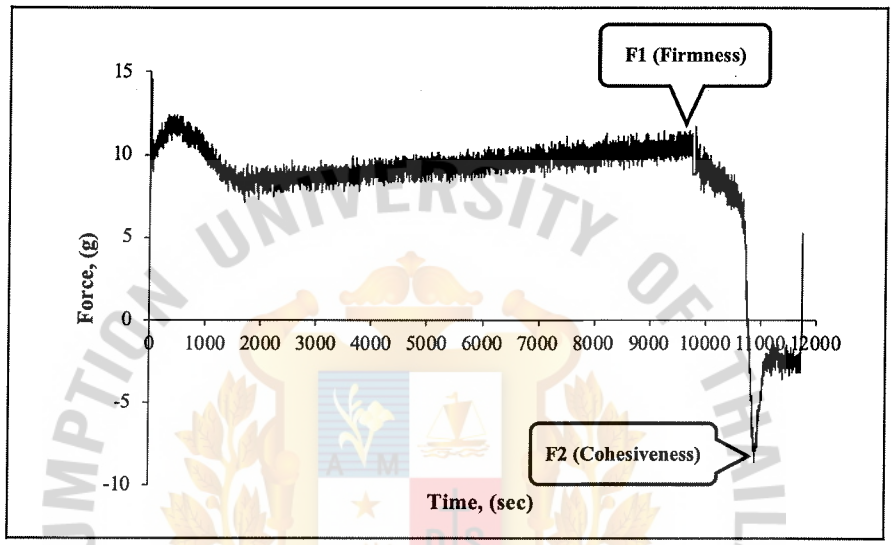

Figure 24: The graph of texture analysis.

Emulsion stability index

The VPSO cosmetic emulsion stability index was performed immediately after preparation to determine the initial stability. The stability index was repeated after 7, 14, 21, and 28 days of storage. Direct visual observations were used to determine the total heights of the opaque cream, turbid layer and the transparent layer of the cosmetic emulsion inside the test tube and to determine the stability index calculated by the equation:

$$\% \text{ Stability index} = \frac{H_{opaque\ cream}}{H_{total}} \times 100$$

Statistical analysis

All experiments were conducted in three replications and statistical analysis was accomplished using ANOVA with Duncan's multiple range tests by the R Statistics (R version 2.15.3) (R Core Team, 2019). Different at $p \leq 0.05$ was considered to be a significant level.

RESULTS AND DISCUSSION

1. Evaluation of sun protection factor of cosmetic emulsion

Oil in water (O/W) cosmetic emulsion was formulated using virgin perilla seed oil and roasted barley as the main ingredients. The cosmetic emulsion was placed in different storage conditions which were 30°C without light, 30°C with light, and 45°C with light for 28 days in the incubator. The SPF of the cosmetic emulsion was evaluated during the accelerated stability test at 7 days interval during the storage time to observe the effect of temperature and light on the cosmetic emulsion.

The freshly prepared of VPSO cosmetic emulsion provided the SPF value of 8.75±0.08 which could be classified into low protection level regarding the SPF classification of Lionetti, *et al.* 2017. VPSO contains a high percentage of polyunsaturated fatty acids (PUFA) that contain α-linolenic acid (ALA) as a major part which ranged from 52.58% to 61.98% (Zhou, *et al.* 2014). PUFA contains two or more C−C double bonds which provided the potential to absorb UVR (Anil, *et al.* 2013). Roasted contained phenolic compounds and flavonoids that provided high anti-oxidative properties that prelude the UV-induced oxygen free radical generation and lipid peroxidation (Ebrahimzadeh *et al.* 2014). Even though the preparation of VPSO cosmetic emulsion was classified into a range of low protection level because of its SPF value but this SPF determination result suggested that VPSO and roasted barley can be used as the natural anti-UV agents. Moreover, using these natural ingredients combine with other organic sunscreen ingredients like oxybenzone, titanium oxide and etc. can promote the higher SPF to provide more skin protection efficiency.

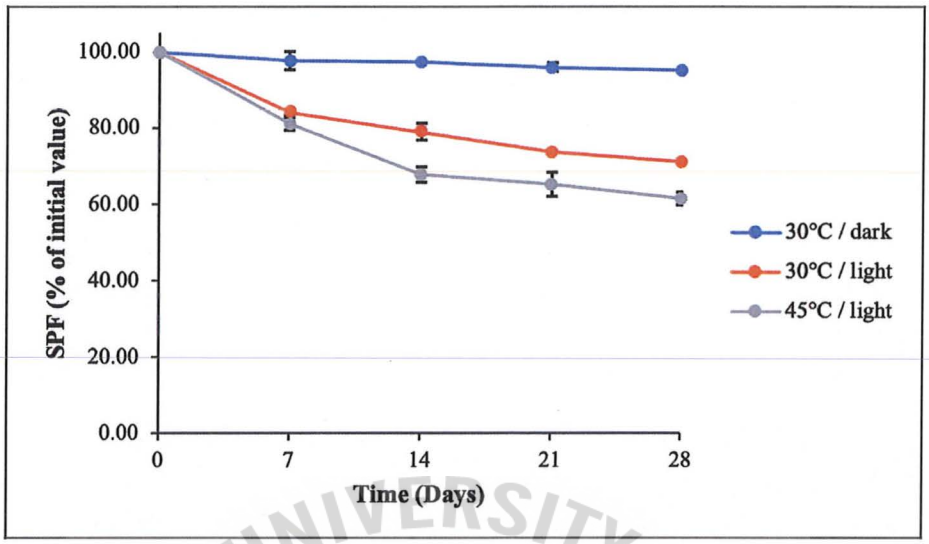

Figure 25: SPF variation of cosmetic emulsion during 28 days of incubation under accelerated conditions

To study the effect of temperature and light on the stability of VPSO cosmetic emulsion, the control condition, 30°C / dark, was set to compare with two of accelerated conditions which were 30°C / light and 45°C / light. Figure 25 showed the gradual decrease of SPF value during storage at 0, 7, 14, 21, and 28 days in the three different storage conditions. The results revealed that SPF of VPSO cosmetic emulsion was more stable in the 30°C / light storage condition, the SPF after kept 28 days was 95.31% of initial SPF value. Otherwise, SPF of VPSO cosmetic emulsion storage in the 30°C / light and 45°C / light conditions after 28 days were 71.20% and 61.60% of initial SPF value, respectively.

Considering an individual parameter, temperature, the degradation of PUFA was occurred by the effect of high temperature but increased in saturated fatty acid content (Abbas *et al.* 2017) which the carbon atoms are connected with single strong σ -bonds. Thus, it cannot absorb UVR and transmit most of the rays (Anil, *et al.* 2013). Therefore, decreasing SPF while kept in 45°C incubator occurred from the degradation of PUFA in VPSO, SPF values measured in 7 days interval were significantly decreased as shown in Table 29.

The sunscreen product can be classified into two types which are physical sunscreen which reflects or scatter UVR and chemical sunscreen which absorb UVR

and re-emitting chemical energy as heat or light (Saewan *et al.* 2013). Flavonoids in barley were used as a chemical UV absorber by the potential to absorb UVR. The cosmetic emulsion storage in 30°C / light showed the significantly decreased in SPF value compare with 30°C / dark in every 7 days interval during 28 days of storage as shown in Table 29. Therefore, the presence of light can degrade the potential of chemical UV-filters (Kockler *et al.* 2012).

Table 29. SPF of cosmetic emulsion during 7 days of incubation under accelerated conditions

Storage condition	SPF of cosmetic emulsion				
	Fresh [NS]	7 days	14 days	21 days	28 days
30°C / dark	8.75 ± 0.08 ^{A}	8.55 ± 0.27 ^{ABa}	8.52 ± 0.08 ^{ABa}	8.40 ± 0.13 ^{Ba}	8.34 ± 0.10 ^{Ba}
30°C / light	8.75 ± 0.08 ^{A}	7.39 ± 0.03 ^{Bb}	6.92 ± 0.16 ^{Cb}	6.44 ± 0.05 ^{Db}	6.23 ± 0.05 ^{Eb}
45°C / light	8.75 ± 0.08 ^{A}	7.09 ± 0.10 ^{Bb}	5.92 ± 0.18 ^{Cc}	5.71 ± 0.23 ^{Cc}	5.39 ± 0.16 ^{Dc}

Notes. A:E determine means in the same roll are not significantly different ($p>0.05$), a:c determine means in the same column are not significantly different ($p>0.05$)

The photodegradation of cosmetic emulsion was followed apparent first order kinetics and is described by the following equation (Couteau, *et al.* 2007):

$$\frac{SPF}{SPF_0} = e^{-kt} \quad \rightarrow \quad t_{1/2} = \frac{ln\ 2}{k}$$

where SPF and SPF_0 are the sun protection factor after and before incubated, respectively, and k is the apparent first order degradation rate constant.

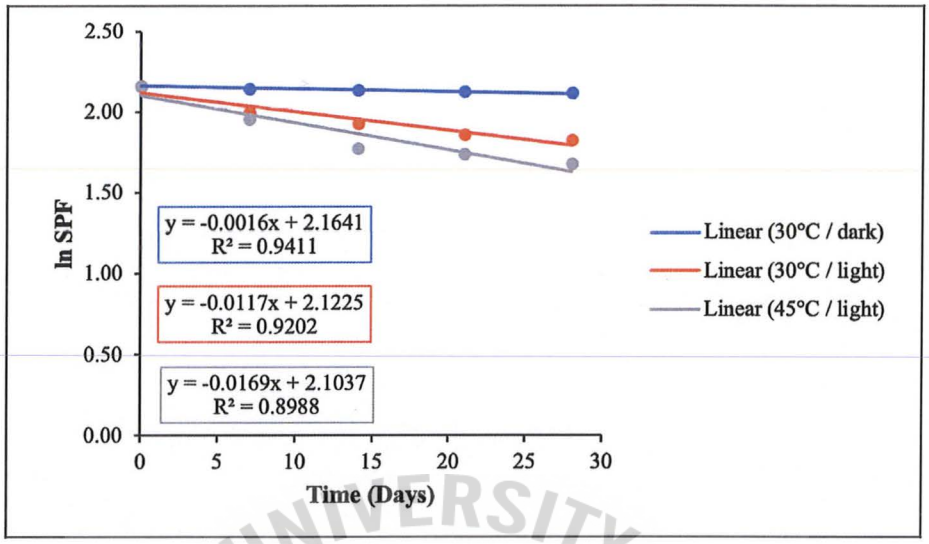

Figure 26: SPF degradation of cosmetics emulsion for first-order reaction.

According to Table 30, the half-life time ($t_{1/2}$) of SPF of cosmetic emulsion incubated in different storage conditions was calculated using degradation rate constant (k) that calculated from the slope of the line of SPF versus time (days). The cosmetic emulsion storage in 30°C / dark condition which mimics the storage condition of the product sold on the shelf in the market. It showed half-life time at 415.89 days or approximately 416 days which the product can storage in this condition for more than one year. The half-life time of control (30°C / dark condition) was calculated in the range that have not be changed. Thus, the error can be occurred by the short of storage time. The control should be kept until the degradation of SPF occurred.

Table 30. First-order rate constant of SPF degradation by photooxidation

Storage condition	Equation	$k \times 10^{-3}$ (day^{-1})	R^2	$t_{1/2}$ (day)
30°C / dark	$SPF/SPF_0 = e^{(-0.0017)t}$	1.67 ± 0.29	0.9411	415.89
30°C / light	$SPF/SPF_0 = e^{(-0.0116)t}$	11.63 ± 0.34	0.9202	59.58
45°C / light	$SPF/SPF_0 = e^{(-0.0170)t}$	17.00 ± 0.62	0.8988	40.77

2. Evaluation of physical characteristics of cosmetic emulsion

The physical characteristics including pH, color, texture analysis, and emulsion stability of cosmetic emulsion were examined under different storage conditions at 7 days interval during 28 days of storage time. The VPSO cosmetic emulsion storage at 30°C / dark condition was used as a control condition compared with 30°C / light and 45°C / light conditions. According to Table 31, the pH of the freshly prepared sample was in the alkaline region (pH 8.30) which differs from the commercial brand emulsion in the market that pH range from 4.5 to 6.5. This cosmetic emulsion can provide the benefit to the human with a skin disease in which the pH of the skin shifts to the acidic side (Dutta *et al.* 2018). The results showed that the pH values of VPSO cosmetic emulsions were stable in the storage at 30°C / dark and 30°C / light. However, the pH values were dropped slightly to 8.05 at 28 days of storage. Therefore, the light had no effect on the change of pH value that it did not cause the photooxidation of VPSO cosmetic emulsion, but the temperature can affect the pH in which the rancidity can be occurred by the increase of acidity (Oyelese *et al.* 2013). The color of the cosmetic emulsion was measured using the CIE L*a*b* system. L* value was decreased during the storage in which VPSO cosmetic emulsion storage at 45°C / light showed the significantly lowest compared with other conditions. Furthermore, a* and b* of all storage conditions also increased at the 28 days of storage. The difference in the color of the cosmetic emulsion was examined by ΔE* value. Storage the VPSO cosmetic emulsions at 30°C / dark and 30°C / light cannot notice the difference in color by the ΔE* values were between 0 and 1. In contrast, the color of cosmetic emulsion storage at 45°C / light showed a clear difference in color by the ΔE* value was equal to 4.53. Therefore, the temperature can affect the color of cosmetic emulsion which the color of the natural extracts i.e. roasted barely could degrade easier in thermal condition. The texture analysis of the cosmetic emulsion was performed by using the backward extrusion method. The graph of texture analysis was used to calculate the firmness and cohesiveness of cosmetic emulsions to evaluate the effect of storage conditions. The firmness was used to determine the force required to fully compress the product between thumb and forefinger (Savary *et al.* 2019).

Besides, the texture of the emulsion can break very easily with a low value of cohesiveness. The firmness and cohesiveness of each storage condition showed non-significantly different after 28 days of storage compared with a fresh prepared VPSO cosmetic emulsion. However, the firmness and cohesiveness of cosmetic emulsion showed significantly different among the different storage conditions. The emulsion stability of cosmetic emulsion was observed during the storage time. The results showed that emulsion stability of the cosmetic emulsions kept at 30°C / dark and 30° C / light were stable at the end of storage time. However, the accelerated condition of cometic emulsion, 45°C / light, showed the instability at 21 days of storage. The flocculation of emulsion can be occurred which the droplets stick to each other and present the separation of emulsion. It also enhances the creaming of emulsion by the large of flocs (Petsev, 2004). Therefore, the mix of emulsifiers of Tween 80 and Span 80 can maintain emulsion stability by providing the fixed number of required HLB values of the oil contained in the cosmetic emulsion.

Table 32. Physical characteristics of cosmetic emulsion under accelerated condition

Parameter	Storage condition	Average ± SD				
		Fresh [NS]	7 days	14 days	21 days	28 days
pH	30°C / dark [NS]	8.30 ± 0.03	8.30 ± 0.01 ^{a}	8.29 ± 0.02 ^{a}	8.28 ± 0.02 ^{a}	8.29 ± 0.04 ^{a}
	30°C / light [NS]	8.30 ± 0.03	8.29 ± 0.01 ^{a}	8.27 ± 0.01 ^{a}	8.27 ± 0.01 ^{a}	8.28 ± 0.06 ^{a}
	45°C / light	8.30 ± 0.03 ^{A}	8.11 ± 0.02 ^{Bb}	8.09 ± 0.02 ^{BCb}	8.07 ± 0.02 ^{BCb}	8.05 ± 0.03 ^{Cb}
L*	30°C / dark	68.58 ± 0.11 ^{A}	68.30 ± 0.08 ^{ABa}	68.09 ± 0.67 ^{ABa}	67.86 ± 0.20 ^{ABb}	68.05 ± 0.19 ^{Bb}
	30°C / light [NS]	68.58 ± 0.11	68.59 ± 0.27 ^{a}	68.39 ± 0.23 ^{a}	68.44 ± 0.15 ^{a}	68.80 ± 0.24 ^{a}
	45°C / light	68.58 ± 0.11 ^{A}	67.03 ± 0.06 ^{Bb}	67.08 ± 0.19 ^{Bb}	66.92 ± 0.38 ^{Bc}	65.02 ± 0.13 ^{Cc}
a*	30°C / dark	2.97 ± 0.01 ^{D}	3.15 ± 0.05 ^{Cb}	3.30 ± 0.08 ^{Ab}	3.19 ± 0.02 ^{BCb}	3.27 ± 0.07 ^{ABb}
	30°C / light	2.97 ± 0.01 ^{E}	3.19 ± 0.04 ^{Cb}	3.36 ± 0.03 ^{Ab}	3.09 ±0.03 ^{Dc}	3.26 ± 0.04 ^{Bb}
	45°C / light	2.97 ± 0.01 ^{D}	3.55 ± 0.02 ^{Ba}	3.47 ± 0.01 ^{Ba}	3.29 ± 0.02 ^{Ca}	3.99 ± 0.10 ^{Aa}
b*	30°C / dark	15.65 ± 0.02 ^{B}	16.07 ± 0.08 ^{Ab}	16.03 ± 0.37 ^{ABb}	15.94 ± 0.03 ^{ABb}	16.30 ± 0.26 ^{Ab}
	30°C / light	15.65 ± 0.02 ^{C}	15.89 ± 0.25 ^{BCb}	16.29 ± 0.14 ^{Aab}	15.64 ± 0.03 ^{Cc}	16.20 ± 0.30 ^{ABb}
	45°C / light	15.65 ± 0.02 ^{Ea}	17.44 ± 0.08 ^{Ba}	16.71 ±0.14 ^{Ca}	16.13 ± 0.09 ^{Da}	18.27 ± 0.31 ^{Aa}
ΔE*	30°C / dark	0.00 ± 0.00	0.54 ± 0.17	0.79 ± 0.73	0.81 ± 0.27	0.89 ± 0.39
	30°C / light	0.00 ± 0.00	0.46 ± 0.20	0.78 ± 0.14	0.26 ± 0.09	0.74 ± 0.19
	45°C / light	0.00 ± 0.00	2.44 ± 0.10	1.91 ± 0.19	1.76 ± 0.31	4.53 ± 0.37
Firmness	30°C / dark [NS]	21.36 ± 4.59	19.63 ± 2.20 ^{ab}	21.13 ± 5.18	18.89 ± 0.23 ^{b}	18.55 ± 0.20 ^{c}
(g)	30°C / light [NS]	21.36 ± 4.59	18.78 ± 0.23 ^{b}	19.32 ± 0.07	20.39 ± 0.58 ^{b}	19.82 ± 0.41 ^{b}
	45°C / light [NS]	21.36 ± 4.59	22.09 ± 0.47 ^{a}	21.78 ± 0.30	24.31 ± 1.85 ^{a}	22.24 ± 0.30 ^{a}
Cohesiveness	30°C / dark [NS]	11.71 ± 0.52	12.18 ± 1.25 ^{a}	12.06 ± 0.77	11.72 ± 0.13 ^{a}	11.64 ± 0.06 ^{a}
(g)	30°C / light	11.71 ± 0.52 ^{A}	1.75 ± 0.37 ^{ABab}	12.02 ± 0.18 ^{C}	12.14 ± 0.12 ^{BCb}	12.44 ± 0.30 ^{ABb}
	45°C / light	11.71 ± 0.52 ^{A}	12.44 ± 0.43 ^{ABb}	13.10 ± 2.77 ^{B}	13.44 ± 0.30 ^{ABc}	13.37 ± 0.06 ^{ABc}
Emulsion stability	30°C / dark	–	–	–	–	–
	30°C / light	–	–	–	–	–
	45°C / light	–	–	–	+	+

Notes. NS refer to no significant different (p>0.05), A:E determine means in the same roll are not significantly different (p>0.05), a:c determine means in the same column are not significantly different (p>0.05), L* = CIE lightness coordinate, a* = CIE red (+) / green (−) colour attribute, b* = yellow (+) / blue (−) colour attribute, Emulsion stability (+) indicated separation and (−) indicated no separation.

Figure 27: Emulsion stability cosmetic emulsion.

CONCLUSIONS

In conclusion, the accelerated storage conditions were used to evaluate the thermal degradation and photooxidation on the SPF and physical characteristics of the cosmetic emulsion. Virgin perilla seed oil and roasted barley provided the potential to absorb UVR which recommended to use as the natural UV absorbers. The SPF of the cosmetic emulsion can be affected by both thermal degradation and photooxidation which remained 71.2% and 61.6% of initial SPF value at the end of storage time. Furthermore, the thermal degradation caused the change of pH, color, and emulsion stability that occurred during the storage time in which the rancidity of oil can be generated and the color can be noticed the difference compare with the fresh sample. The phase separation occurred at 21 days of storage at 45°C / light condition Moreover, the texture analysis of each storage condition showed non-significantly different during storage of 28 days.

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CHAPTER VI
CONCLUSIONS

Roasted barley and six natural oils including virgin coconut, virgin avocado, virgin olive, virgin sweet almond, virgin perilla seed oil, and *Camellia oleifera* seed oil exhibited the UV absorption spectrum profile, especially in the UVB region. Virgin perilla seed oil (VPSO) was reported to carry the strongly UVB absorption which the SPF was the highest. Roasted barley has been shown a scavenging activity that prevents the oxidation and it also provided the UV absorber according to its UV spectroscopy. Consequently, VPSO and roasted barley could be recommended as a natural UV absorber which flavonoids in roasted barely and high contents of polyunsaturated fatty acids of VPSO provide the ability to absorb UVR. The formulation of cosmetic emulsion from the organic chemical absorber and natural plant extract enhanced the board range SPF as found in the combined of oxybenzone and roasted barley extract due to the different regions of their UV absorbing capability. The pH of VPSO cosmetic emulsion was ranged in alkaline which has an efficiency to treat some skin diseases that pH of skin shift in acidic. Moreover, VPSO was a very effective occlusive moisturizer because it provided the forming of protective film to prevent the skin from losing moisture. Six cycles of heating-cooling stability tests affected the SPF and physical characteristics including pH and color of VPSO cosmetic emulsion. Phase separation was not observed after 6 cycles of incubation which mixed emulsifiers, Tween 80 and Span 80, assisted in the stabilization of the emulsion. The formulation containing the combined roasted barley and oxybenzone showed the resistance on SPF reduction since SPF was not significantly decreased. Effect of thermal degradation and photooxidation on the SPF and physical characteristics of the cosmetic emulsion were evaluated under accelerated condition, the SPF of the cosmetic emulsion was affected by both thermal degradation and photooxidation which remained 71.2% and 61.6% of initial SPF value at the end of

storage time. Furthermore, the effect of temperature was showed on the pH, color, and emulsion stability of cosmetic emulsion at the end of storage time. The phase separation occurred at 21 days of storage at 45°C / light. Moreover, the texture analysis of each storage condition showed non-significantly different during storage of 28 days.

RECOMMENDATIONS FOR FURTHER RESEARCH

1. The rheological measuring techniques should be applied to the cosmetic emulsion.
2. The microbiological testing should be examined on the cosmetic emulsion for both gram positive and gram negative bacteria.

APPENDIX A
FOOD INNOVATION ASIA CONFERENCE 2020

THE 22nd FOOD INNOVATION ASIA CONFERENCE 2020

"INNOVATION FOR FUTURE FOOD AND NUTRITION SECURITY"

18 – 19 JUNE 2020

Bangkok, Thailand

The 22nd Food Innovation Asia Conference 2020

June 18-19, 2020

June 18, 2020

Session 1: Opening remark and Keynote Speeches MC: *Dr. Nopparat Plucktaveesak, Thammasat University*	
10.00-10.15	**Opening remark** by *Assistant Professor Dr. Anadi Nitithamyong, President of FoSTAT/* *Associcate Professor Dr. Anuchita Moongngarm, Dean of Faculty of Technology, Mahasarakam University* *Assistant Professor Dr. Nuttanont Hongwarittorrn, Dean of Faculty of Scince and Technology, Thammasat Univeristy/* *Dr. Narinthorn Boonbrahm, Dean of Faculty of Agriculture, Ubon Ratchatani University/*
10.15-10.30	**Keynote speech 1**: The Global Food Supply System: The Massive Challenges (and Opportunities) Caused by COVID-19 by *Professor Chris Elliott, The Institute for Global Food Security (IGFS) Queen's University Belfast, Northern Ireland*
10.35-10.50	**Keynote speech 2**: Future Perspectives for Active Ageing by *Professor Weon-Sun Shin, Department of Food and Nutrition, College of Human Ecology, Hanyang University, Korea*
10.55-11.10	**Keynote speech 3**: Bacteriocin-Producing Lactic Acid Bacteria: Their Production, Application and Future Research Trend for Food Industry By *Assistant Professor Takeshi Zendo, Department of Bioscience and Biotechnology, Faculty of Agriculture, Kyushu University, Japan*
11.15-11.30	**Keynote speech 4**: Biodiversity for Food Security: The Mekong Fish Perspective By *Professor Tuantong Jutagate, Ubon Ratchathani University, Thailand*

June 18, 2020

Session 2: Oral presentation of Division A (Food Chemistry, Nutrition, and Analysis) Chair: *Asst. Prof. Dr. Utai Klinkesorn, Kasetsart University* Co-Chair: *Assoc. Prof. Dr. Sirithon Siriamornpun, Mahasarahkham University*	
13.05 – 13.15	**O5-AB**: Pepsinogens and Pepsins from Lizardfish (*Saurida micropectoralis*) Stomach: Purification and Some Biochemical Properties ***By Sakonwat Kuepethkaew**, Thaksin University*
13.15-13.25	**O15-AB**: Gelation of Threadfin Bream (*Nemipterus* spp.) Surimi with Various NaCl Contents under High Intensity Ultrasound ***By Ling Tang,** Suranaree University of Technology*

13.25-13.35	**O57- AB**: Purification, Identification and Characterization of Antioxidant Peptides from Tilapia (*Oreochromis niloticus*) Protein Hydrolysate ***By Xiaogang Zhang,*** *Suranaree University of Technology*
13.35-13.45	**O66-AP**: Antioxidant Properties of Mulberry Leaf using Ultrasound-assisted Extraction ***By Supasit Insang, Chulalongkorn University***
13.45-13.55	**O104- AB**: Chemical and Functional Properties of Banana Flour (Kluai Namwa) at Different Ripening Stage ***By Nur-asikin Masaesa-I,*** *Prince of Songkla University*
13.55-14.05	**O127-AJ**: Investigation of Gydrolyzed Ceramide in Thai Color Rice (*Oryza sativa* L.) and By-products ***By Chawin Paosila,*** *Kasetsart University*
14.05-14.15	**O67-AP**: Effect of Extraction Conditions on Bioactive Compounds of Barley (*Hordeum vulgare* L.) and Sun Protection Factor (SPF) Determination of Barley-virgin Oil Based Cosmetic Emulsion ***By Pongsakorn Vithayanon,*** *Assumption University*
14.15-14.25	**O17-AP**: Separation and Characterization of Fat, Protein and Chitosan from Eri Silkworm Pupae (*Philosamia ricini*) ***By Thanapon Pattanasatian,*** *King Mongkut's University of Technology Thonburi*
14.25-14.35	**O51-AJ:** Effect of Extraction Solvent on the Total Phenolic Content, Carotenoid and Antioxidant Activity of Australian Chia Seed (*Salvia hispanica* L.) Oil ***By Izzreen Ishak,*** *National University of Malaysia*

June 18, 2020

Session 3: Oral presentation of Division B (Food Processing and Engineering) Chair: *Dr.Jatuporn Aroonkamonsri, Rajamangala University of Technology Tawan-ok* Co-Chair: *Dr.Krittiya Khuenpet, Thammasat University*	
14.35-14.45	**OB111**: Effect of Preparation Methods on *B*-Cyclodextrin Encapsulated Holy Basil (*Ocimum Sanctum* Linn.) Essential Oil Properties ***by Wantanee Noichinda,*** *Chulalongkorn University*
14.45-14.55	**OB53**: Optimization of Pectin Extraction from Green Mature *Garcinia atroviridis* Rind Using Response Surface Methodology ***By Gerry Renaldi,*** *Prince of Songkla University*
14.55-15.05	**OB70**: Subcritical Ethanol Extraction of Oil from Coconut Meal ***by Thussanee Plangklang,*** *Silpakorn University*
15.05-15.15	**OB140**: Production of D-tagatose from D-galactose by Subcritical Aqueous Ethanol ***by Neeranuch Milasing****, Silpakorn University*

15.15-15.25	**OB81**: Optimization of Hydrothermal Technique for the Production of Nanocellulose from Bamboo Shoot Shell **by *Kanjana Manamoongmongkol***, *King Mongkut's Institute of Technology Ladkrabang*
15.25-15.35	**OB83**: Effects of High-pressure Processing on Textural Properties of Threadfin Bream Surimi Gel ***by Boodasayapuk Buasakchai,*** *Thammasat University*
15.35-15.45	**OB139**: Effects of Pasteurization and High Pressure Processing on Quality of Sweetened Condensed Rice-Cereal Milk ***by Rawiporn Polpued***, *Thammasat University*

June 18, 2020

Session 6: Oral Competition Chair: *Assoc.Prof. Dr. Jirawat Yongsawatdigul, Suranaree University of Technology* Co-Chair: *Asst. Prof. Dr. Wiriya Onsa-ard, Ubon Ratchathani University and Dr.Teerarat Likitwattanasade, Mahidol University*	
9.10-9.20	**OC22**: Development of Silken Tofu Coagulated by Glucono-delta-lactone for Consumers with Mastication and Swallowing Problems ***By Thanakorn Wongprasert,*** *Chulalongkorn University*
9.25-9.35	**OD85:** Chemical Characterization and Antibacterial Activity Against Foodborne Pathogens of Biosurfactant from Aureobasidium Melanogenum ***By Vipawan Jandee,*** *Thammasat University*
9.40-9.50	**OA51**: Effect of Extraction Solvent on the Total Phenolic Content, Carotenoid and Antioxidant Activity of Australian Chia Seed *(Salvia hispanica L.)* Oil *By **Izzreen Ishak**, National University of Malaysia*
9.55-10.05	**OC122**: Comparison of Date Palm Syrup Made from Premature Fruit Drop, Fresh and Dried Date Pulp ***By Kittanan Burapalit***, *Assumption University*
10.10 -10.20	**OD24**: Bioinformatic Tools and In-Vitro Analysis for Screening of Potential Bacillus Probiotic Used as a Food Supplement ***By Gauri Khullar***, *Chulalongkorn University*
15.05-15.20	**ORAL and POSTER Awards PRESENTATION**

June 19, 2020

Session 7: Poster Competition Chair: *Asst. Prof. Dr. Orn-in Prachaiyo, Naresuan University* Co-Chair: *Asst. Prof. Dr. Panchaporn Tadpitchayangkun Promchote, Ubon Ratchatani Univeristy*	
10.35-10.45	**P9-AB**: Influence of Ultrasound to the Activity and Conformational Changes of Purified Pepsin from the Stomach of Lizardfish (*Sauridamicropectoralis*) ***by Sakonwat- Kuepethkaew,*** *Thaksin University*
10.50-11.00	**P75-EB**: Development of Bioplastic Active Packaging to Inhibit Fungal Growth in Bakery Product Using Cast-Extrusion ***by Atcharawan Srisa,*** *Kasetsart University*
11.05-11.15	**P95-EP**: Effect of Polybutylene Succinate Film Incorporated with Vanillin Against *Escherichia coli* and *Staphylococcus aureus* ***by Natticha Leaktong****, Kasetsart University*
11.20-11.30	**P141-EB**: Preparation of Specific Antibody-Conjugated Ferromagnetic Nanoparticles for the Detection of *Campylobacter jejuni* in Immunomagnetic Separation (IMS) System ***by Pattarapong Wenbap****, King Mongkut's University of Technology Thonburi*
15.05-15.20	**ORAL and POSTER Awards PRESENTATION**

June 19, 2020

Session 4: Oral presentation of Division C (Food Product Development, Sensory, and Consumer Research) Chair: *Dr. Aussama Soontrunnarudrungsri, Kasetsart University* Co-Chair: *Asst. Prof. Dr. Manatchaya Sungsri-in, Mahasarakham University*	
13.05-13.15	**OC65**: Bread Quality as Affected by Sorghum and Breadfruit Flours as Wheat Substitutes ***By Fahrunnisa Adzqia****, Kasetsart University*
13.15-13.25	**OC99**: Effect of Red Jasmine Brown Rice Flour on Quality of Gluten-Free Noodles ***By Patomporn Waewkum****, Ubon Ratchathani University*
13.25-13.35	**OC22**: Development of Silken Tofu Coagulated by Glucono-Delta-Lactone for Consumers with Mastication and Swallowing Problems ***By Thanakorn Wongprasert****, Chulalongkorn University*
13.35-13.45	**OC122**: Comparison of Date Palm Syrup Made from Premature Fruit Drop, Fresh and Dried Date Pulp ***By Kittanan Burapalit****, Assumption University*

June 19, 2020

Session 5: Oral presentation of Division D (Food Microbiology, Food Biotechnology, Fermentation) Chair: *Assoc. Prof. Dr. Pravate Tuitemwong, King Mongkut's University of Technology Thonburi* Co-Chair: *Asst. Prof. Dr. Awanwee Petchkongkaew, Thammasat University*	
14.05-14.15	**OD24**: Bioinformatic Tools and *in-vitro* Analysis for Screening of Potential *Bacillus* Probiotic Used as a Food Supplement ***by Gauri Khullar**, Chulalongkorn University*
14.15-14.25	**OD100**: The Effect of Pineapple Waste Extracts on Quality of Probiotic Yogurt ***by Sreymom Hun**, Mae Fah Luang University*
14.25-14.35	**OD120**: Effect of *Saccharomyces* sp. No.9 on Physicochemical Characteristics of Aged Roselle Wine and Its Consumer Preference ***by Kotchakorn Sereephantwong**, Assumption University*
14.35-14.45	**OD108**: Development of Innovative Amazake from Portuguese Chestnuts Using Controlled Fermentation Conditions ***by Marisa Santos**, Universidade de Lisboa*
14.45-14.55	**OD98**: Improved Survival of *Candida tropicalis* TISTR 5922 Starter Culture with Difference Form ***by Natwaran Nukrohwad**, Prince of Songkla University*
14.55-15.05	**OD85**: Chemical Characterization and Antibacterial Activity Against Foodborne Pathogens of Biosurfactant from *Aureobasidium melanogenum* ***by Vipawan Jandee**, Thammasat University*
15.05-15.20	**ORAL and POSTER Awards PRESENTATION**

"INNOVATION FOR FUTURE FOOD AND NUTRITION SECURITY"

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Effect of Extraction Conditions on Bioactive Compounds of Barley (*Hordeum vulgare* L.) and Sun Protection Factor (SPF) Determination of Barley-Virgin Oil Based Cosmetic Emulsion

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ABSTRACT: Barley (*Hordeum vulgare* L.) is the main source of several bioactive compounds e.g. phenolic compounds, flavonoids that could absorb the ultraviolet (UV) light. The virgin oils also contain photo-protective compounds that absorb UV radiation. This study aimed to analyze the effect of barley extraction condition on the total phenolic content, total flavonoid content and antioxidant activity, furthermore, the sun protection factor (SPF) of extracted barley and virgin oils were determined. The oil in water (o/w) cosmetic emulsion was formulated to study the efficiency of virgin oil types which could aid the physical sunscreen, benzophenone-3, to enhance the SPF. The occlusion factor indicated the prevention of dehydration of water from the skin. Extracted barley at 45°C without microwave preheating revealed the highest total phenolic content (19.62±1.35 mg GAE/g) whereas 45°C extraction of 2 minutes microwave preheating showed the highest total flavonoid content (430.92±1.91 mg QE/g). Radical scavenging DPPH reported the optimum extraction temperature at 45°C which microwave preheating 5 minutes showed the highest the half-maximal inhibitory concentration value (IC_{50}) at 6.23±0.41 mg TE/g. The sun protection factor (SPF) of 10% (w/v) of extracted barley and virgin avocado oil (VAO) were 6.12±0.05 and 8.71±0.37 respectively, whereas virgin coconut oil and virgin olive oil were 2.00±0.08 and 0.71±0.01 respectively. The SPF obtained from cosmetic emulsions E1, E4, and E6 have higher SPF, above SPF of 5.5, when compared to E2, E3, E5, E7, and E8. The SPF ranged from 4.31 to 5.64. Virgin oils significantly enhanced the benzophenone-3 to exhibit UV absorption. The pH was in the range of alkaline cosmetics which designated to people with psoriasis therapy. All formulae showed occlusion factor varied from 41.81 to 75.40 % within 48 hrs.

Keyword: Barley, virgin oil, cosmetic emulsion, sun protection factor, occlusion factor

INTRODUCTION

Barley (*Hordeum vulgare* L.) contains high content of phenolic compounds e.g. flavonoids, phenolic acids, diterpenes, and tannins which have a high anti-oxidative activity that can be reduced the risk of coronary heart diseases, cancers, and the aging processes [1]. Furthermore, flavonoids are phytochemical compounds which protect against UV radiation by UVB absorbing compounds [2]. During to the barley roasting process, the Melanoidins are synthesized by Maillard reactions. The Maillard reaction products (MRPs) may be considered as the

active compounds which show antioxidant activity that can delay some types of cell damage and promote anti-aging actions [3].

The emulsions are systems which two immiscible liquids can be mixed together. There are several types of emulsions e.g. oil-in-water (o/w), water-in-oil (w/o), oil-in-water-in-oil (o/w/o), water-in-oil-in-water (w/o/w), etc. The disperse phase is the phase that liquid droplets are dispersed in another liquid medium. To mix two immiscible liquids together, the emulsifier should be mixed into the solution. To maintain stability longer, the emulsifier should be adjusted in the optimum hydrophilic-lipophilic balance (HLB) value [4].

Cosmetics and personal cares market have a trend to use natural compounds as the main ingredient for improving the skin appearance, moisturizing to provide long-lasting hydration, reducing wrinkle, etc. Dryness and dehydration of skin occur when skin could not retain sufficient moisture. Regarding to the frequent bathing, aging, living in the dry air or some skin diseases, those can be the cause of the loss of moisture. The cosmetic emulsion is one of the famous products that can be used to maintain moisture in the skin. One factor that people buy and use this product are the benefits and natural ingredients in the cosmetic emulsion.

Sun protection factor (SPF) is used to determine the efficiency of the sunscreen which the high value of the SPF might be determined that the sunscreen can protect the skin from the solar radiation. The harmful from sun light radiation can be divided into 3 regions with are UVA (320 to 400 nm), UVB (290 to 320 nm) and UVC (200 to 290 nm). Furthermore, the UVA radiation can penetrate into the deeper layer of the skin when compared to the UVB. It can generate the aging of the skin. The UVB radiation is not completely filtered by the ozone layer which the skin can be damaged due to sunburn. The UVC is filtered by the atmosphere [5].

The virgin oils are the oils that extract without heating process which the vitamins, minerals, and bioactive compounds will not lose from the oils. Virgin coconut oil (VCO), virgin olive oil (VOO), and virgin avocado oil (VAO) contain high amounts of vitamins, antioxidants, minerals, medium chain fatty acids, taste, fragrance. Furthermore, the high amount of anti-oxidative of VCO, VOO, and VAO can prevent skin damage and reduce the appearance of wrinkles. In addition, the sun protection factor (SPF) of herbal oils were reported that contain the efficiency of UVB (290 to 320 nm) protection [6].

This study aimed to analyze the bioactive compounds presenting in the roasted barley grain and the effect of microwave-heat conditions on the radical scavenging activity. Moreover, in vitro spectrophotometric method was used to determine SPF of extracted barley solutions, virgin oils and cosmetics emulsion formulated with extracted barley and virgin oil.

MATERIAL AND METHODS

The roasted barley grains (Edo Baku, Japan), virgin coconut oil (King Island, Thailand), virgin olive oil (Filippo Berio, Italy), virgin avocado oil (Olivado, New Zealand) were obtained by local store.

Sample preparation

The roasted barley was pre-heated by microwave (800 watts) under three heating condition; (1) no heating, (2) 2 min heating, and (3) 5 min heating. The roasted barley 100 grams was blended for 15 sec then the ground roasted barley was poured into the container and kept in a dry place at room temperature.

Sample extraction

The bioactive compounds of ground roasted barley using a method modified from Omwamba *et al.* 2013 and L Hęś *et al.* 2014 [3,7]. It was extracted using distilled water at the ratio of 1:10 (w/v) at 25°C, 45°C, and 90°C with 200 rpm for 10 min and leave at room temperature 20 min for complete extraction. The extracted solution was lyophilized by freeze drying then kept in dry place.

Total phenolic content

Total phenolic content (TPC) of the barley extracts was determined by Folin-Ciocalteu spectrophotometric method modified from Šimić *et al.* 2017 and Oh, *et al.* 2015 [8,9]. The lyophilized barley was dissolved in distilled water in the concentration of 2 mg/ml. Sample (500 µl) was added into the test tubes followed by 1 ml of 10% (v/v) Folin-Ciocalteu's reagent and 1 ml of 7.5% (w/v) sodium carbonate solution. The tube was allowed to stand for 30 min, then measured the absorbance at 765 nm. TPC was expressed as gallic acid equivalents (GAE) in mg/g material. The calibration curve for gallic acid was y=0.0177x (R^2=0.9922) where y is the absorbance and x is the concentration of gallic acid in µg/ml.

Total flavonoid content

Total flavonoid content (TFC) of the barley extracts was determined by aluminum chloride method modified from Oh *et al.* 2015 and Rebaya *et al.* 2014 [9,10]. The 2 mg/ml of lyophilized barley was prepared in distilled water. Sample (500 µl) was added into the test tubes followed by 3 ml of distilled water and 150 µl of 5% (w/v) sodium nitrite. The tubes were allowed to stand for 5 min, then added 150 µl of 10% (w/v) aluminium chloride. The mixture was incubated for 5 min at room temperature, then added 1 ml of 1M sodium hydroxide. The reaction mixture was then incubated for 30 min at room temperature to complete the reaction. The absorbance was measured at 510 nm. TFC was expressed as quercetin equivalents (QE) in mg/g material. The calibration curve for quercetin was y=0.001x (R^2=0.9978) where y is the absorbance and x is the concentration of quercetin in µg/ml.

Radical scavenging activity assays

The antioxidant activity was determined by DPPH assay modified from Šimić *et al.* 2017; Oh *et al.* 2015; Rebaya *et al.* 2014 [8,9,10]. Different dilutions of sample (0.5, 1, and 2 mg/ml) were added by 2 ml of 2,2-diphenyl-1-picrylhydrazyl. Absorbance was measured at 517 nm after 30 min incubated at room temperature without light. Radical scavenging ability was calculated as IC_{50} and expressed as TEAC in mg Trolox/g sample as follows:

$$TEAC\ (mg\ TE/g) = \frac{IC_{50\ (Trolox)}}{IC_{50\ (sample)}}$$

The IC_{50} of Trolox used for calculation of TEAC was 83.78 mg/ml.

SPF Determination

The sun protection factor (SPF) was determined by UV-spectrophotometer using a method modified from Dutra *et al.* 2004 [11]. The 1.0 g of all samples were weighed, transferred into a 100 ml volumetric flask, then diluted to volume with ethanol and ultrasonication for 5 min. The samples were filtered through cotton and rejected the ten first ml. A 5.0 ml of sample was transferred into a 50 ml volumetric flask and diluted to volume with ethanol. Then, a 5.0 ml of sample was transferred into a 25 ml volumetric flask and diluted to volume with ethanol. The absorbance values were measured in the range of 290 to 320 nm (5-nm intervals), using ethanol as a blank. Each measurement was

determinations three times. The SPF of the samples were calculated using the Mansur equation [12] as follows:

$$SPF = CF \times \sum_{320}^{290} EE(\lambda) \times I(\lambda) \times Abs(\lambda)$$

where Correction Factor (CF)=10, EE= erythemal effect spectrum, I=solar intensity spectrum, and Abs=absorbance of the sample. The values of EE × I are constants, which were determined and are shown in Table 1 [13].

Table 1 Normalized product function used in the calculation of SPF

Wavelength (λ nm)	EE×I (normalized)
290	0.0150
295	0.0817
300	0.2874
305	0.3278
310	0.1864
315	0.0839
320	0.0180
Total	1

Emulsification process

Cosmetics emulsion was done by mixing well of aqueous phase and oily phase shown in Table 2. The oily phase was slowly dropped into the aqueous phase with 1000 rpm using magnetic stirrer (VELP, Italy) until obtain the homogeneous o/w emulsion. The emulsion was continually agitated for 15 min then kept for further analysis.

***In vitro* occlusion test**

The occlusion factor of emulsion was determined using a method modified from Teeranachaideekul *et al.* 2008 and López *et al.* 2015 [14,15]. The beakers (100 ml) were filled with 50 ml of water, covered with Whatman® filter paper grade 42 (surface area = 15.9 cm^2). Samples (200 mg) was spread on the filter surface, using petroleum jelly (Vasaline) as a positive control. The beakers were stored at 32°C and weighed at 4, 6, 24, and 48 hr. The occlusion factor (F) was calculated using the following equation:

$$F = \left(\frac{A - B}{A}\right) \times 100$$

where A refers to the water loss without a sample (reference) and B is the water loss with a sample. An F value of 0 indicates that no occlusive effect compared to the reference. On the other hand, an F value of 100 indicates maximum occlusiveness.

pH measurement

The pH of the cosmetic emulsion was measured in triplicate with a pH meter (HANNA instruments, Thailand). The sample was transferred into the beaker and the pH meter probe was immersed into the container.

Color measurement

The color value of O/W cosmetic emulsion was measured with Miniscan EZ-4500L spectrophotometer (Hunter Lab Co. Ltd, US). CIE-L*a*b* system, D 65°/10° standard light source (outdoor daylight), 45°/0° angle of illumination/observer. The cosmetic emulsion was poured into the petri dish. All the sample were performed in three replications.

Statistical analysis

All experiments were conducted in three replications and statistical analysis is accomplished using ANOVA with Duncan's multiple range tests by the R Statistics (R version 2.15.3) [16]. Different at $p \leq 0.05$ was considered to be significant level.

RESULTS AND DISCUSSION

Chemical profiles of extracted barley

Roasted barley grained obtained from a local store was treated in microwave 800 watts for 0, 2 and 5 min then barley was subjected to be extracted in distilled water which water-soluble bioactive compounds in the barley grain were obtained [17]. The extraction temperatures were varied at 25°C, 45°C, and 90°C to observe the effect of temperature at low, medium and high temperature on the bioactive compounds of roasted barley. The lyophilization barley was done by using the freeze-drying method to preserve the barley extracts and to prepare the concentration of barley used for the determination of bioactive compounds. Phenolic compounds and flavonoids are the plant secondary metabolites that provide the antioxidant activity. These compounds contain aromatic ring and hydroxyl group which acts as a reducing agent that provided antioxidant activity [18]. According to the results (Table 3), Barley-IV provided the highest TPC value (19.62±1.35 mg GAE/g). Flavonoids are a group of natural products from plant secondary metabolites. It has phenolic structures and can be found in several kinds of fruits, vegetables, grains, bark, roots, stems, flowers, tea, and wine [19]. Barley-V showed the highest TFC value (430.92±1.91 mg QE/g). The preheat had a positive effect on the TFC value because of the release of change of glucosides or bound phenolics into free phenolic derivatives which provided the higher TFC value [20], whereas the TPC value was not affected by the preheating. Sulaiman *et al.* reported that the optimum temperature for extracting phenolic compounds and flavonoids were at 60°C to 70°C [21]. At room temperature (25°C), the bioactive compounds might not release out from the lyophilized barley because the extraction temperature was not enough to weaken the linkage. On the other hand, boiling temperature (90°C) was considered as overheating condition since several bioactive compounds might be degraded. Thus, the barley extracted at 25°C and 90°C provided lower antioxidant activity (IC_{50}) than barley extracted at 45°C. According to the results (Table 3), Barley-VI showed the significantly highest IC_{50} value at 6.23±0.41 mg/ml. The roasted barley grain with 5 min microwave-heating might provide high antioxidant activity because the heating process might enhancement naturally occurring compounds e.g. Maillard reaction products that provided antioxidant activity [20]. Furthermore, the TPC and TFC value from table 3 also showed that the higher TPC and TFC provided more antioxidant activity.

SPF of barley extract and virgin oils

The scanning wavelength of the 10% w/v xtracted barley and all virgin oils (figure 1) were shown that it can absorb the UVA (320 to 400 nm) and UVB (290 to 320 nm) which it can be used as a natural sunscreen agent in cosmetic emulsion.

The SPF of extracted barley and all virgin oils was determined. The VCO contain 92% (w/w) of saturated fatty acid (SFA), 6% (w/w) of monounsaturated fatty acid (MUFA), and 2% (w/w) of polyunsaturated fatty acid (PUFA). The VOO contain 14% (w/w) of SFA, 83% (w/w) of MUFA, and 3% (w/w) of PUFA. The VAO contain 12% (w/w) of SFA, 71% (w/w) of MUFA, and 13% (w/w) of PUFA [22].

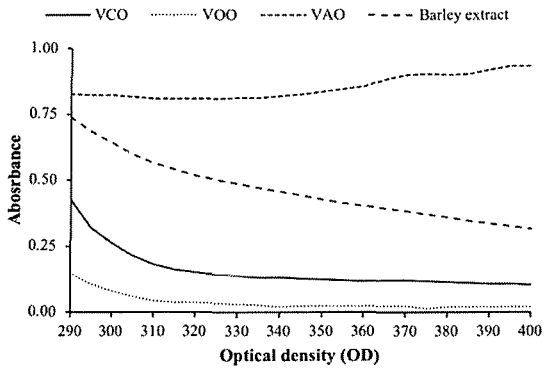

Figure 1 Scanning wavelength of virgin oils and extracted barley

The SFA has no double bond between the carbon atoms of the fatty acid chain which shows lower efficiency of UV absorption. In contrast to unsaturated fat, one or more double bonds are found in the fatty acid chain. The double covalent bond is weaker than a single bond, so oils containing unsaturated fatty acids as the main part are able to absorb UV light. PUFA contains more than one double bond that provides the photoprotective effect on the oil. The higher PUFA content, the higher UV absorption value. [23]. According to Table 4, VAO revealed a significantly high in SPF value when compared with the other sample by the highest amount of PUFA. In contrast, the VCO contains high SFA but low unsaturated fat. It should show lower UV absorption. Anil *et al.* 2013 reported that the VCO presence of a small number of nonbonding electrons which provide some higher absorptions in UV radiation [23].

Table 2 The formulae of cosmetic emulsion

Ingredient	**Cosmetic emulsion (% w/w)**							
	E1	E2	E3	E4	E5	E6	E7	E8
Oily phase								
Virgin coconut oil	4	6	6	-	12	-	-	-
Virgin olive oil	4	6	-	6	-	12	-	-
Virgin avocado oil	4	-	6	6	-	-	12	-
Mineral oil	-	-	-	-	-	-	-	12
Benzophenone-3	6	6	6	6	6	6	6	6
Bee wax	5	5	5	5	5	5	5	5
Stearic acid	3	3	3	3	3	3	3	3
Vitamin E-acetate	2	2	2	2	2	2	2	2
Aqueous phase								
Tween 80	3.06	3.11	3.11	2.95	3.27	2.95	2.95	3.91
Span 80	4.94	4.89	4.89	5.05	4.73	5.05	5.05	4.09
Glycerin	4	4	4	4	4	4	4	4
Triethanolamine	2	2	2	2	2	2	2	2
Aloe vera extract	5	5	5	5	5	5	5	5
Xantan gum	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Preservative	1	1	1	1	1	1	1	1
Fragrance	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8
Barley extract	5	5	5	5	5	5	5	5
Distilled water	46	46	46	46	46	46	46	46

SPF of barley-virgin oil based cosmetic emulsion

To develop the o/w cosmetic emulsion, barley and virgin oils were used to study their photo-protective efficiency by formulate 8 different formulae of emulsion (Table 2). The barley extract (10% w/v) was used to enhance the SPF value in the cosmetic emulsion. In addition, the organic sunscreens are involved with the aromatic ring which in roasted barley containing the flavonoids that help to absorb UVB radiation [2]. The fixed ratio of barley extract (10% w/v) and synthetic UV absorber, benzophenone-3, was used to formulate cosmetic emulsions, only the amount of virgin oil was varied in order to examine the effect of natural oil on the SPF value. The results in table 5 showed that the SPF values found for E1-E7, containing virgin oil, were in between 4.97 to 5.64 whereas E8, containing mineral oil, was 4.31. The formulae contained virgin oil as a photo-protective ingredient exhibited the higher SPF significantly than mineral oil. According to this result, it was suggested that virgin oils could enhance and aids benzophenone-3 to promote the higher SPF approximately up to 23.58% compared with mineral oil. Furthermore, o/w emulsion formulae containing VOO (E1, E2, E4 and E6) showed SPF value above 5.55. Even though the SPF determination of only VOO indicated SPF value of 0.71 which was the lowest SPF while compared with other, the higher SPF of these o/w cosmetic emulsions might be resulted from the VOO had reaction with benzophenone-3 or other ingredients as emulsifier or extraction solvent that caused it showed the higher SPF than other formulae. The chemical reaction among the VOO with other ingredients might be taken in the formulated emulsion which could be resulting in the increase of SPF. Thus, this should be further examined. The pH of cosmetic emulsions was in the range of alkaline (pH 7.94-8.68) which could be designated for people with psoriasis therapy that the pH of the skin shifts to the acidic side [24]. The color measurement reported that color of the cosmetic emulsions contained VAO was dark yellow which was the resulting of color of VAO itself.

***In vitro* occlusion test**

The occlusion factor (F) indicates the occlusive of the emulsion which used to maintain the moisture in the skin. Petroleum jelly, vaseline brand, (R) was use as a positive reference by the high potential of occlusive. VCO, VOO, and VAO can function as occlusive because of their saturated and unsaturated fatty acid contents. Thus, this occlusive property provides the forming of the barrier that blocks water from evaporation. Furthermore, oils also have an emollient property that can replace natural skin oils [25]. According to the result (figure 2), Vaseline showed the highest occlusion factor which the water loss from the system only 0.7% at 48 hr. Furthermore, the cosmetic emulsion contained mineral oil (E8) had significantly high F value (2.36% water loss at 48 hr.) when compared with other formulae which mineral oil and petrolatum are two of the most effective occlusive ingredients. The water loss from the cosmetic emulsion formulae E1-E7 range from 2.86% to 3.20% which slightly different from the reference and E8 at 48 hr.

Table 4 SPF of 10% w/v barley and virgin oils

Sample	SPF
Barley extract	6.12 ± 0.05^{b}
Virgin coconut oil	2.00 ± 0.08^{c}
Virgin olive oil	0.71 ± 0.01^{d}
Virgin avocado oil	8.71 ± 0.37^{a}

Note. mean ± SD, means with the same letter in the same column are not significantly different ($p \geq 0.05$)

Table 3 Chemical profiles of extracted barley using different extraction temperature

Sample	Temperature (°C)	Microwave heat (min)	Chemical profiles of extracted barley		
			TPC (mg GAE/g)	TFC (mg QE/g)	TEAC (mg TE/g)
I	25	0	11.52 ± 0.17 ^{e}	387.08 ± 0.80 ^{f}	4.69 ± 0.42 ^{b}
II		2	12.54 ± 0.24 ^{d}	402.67 ± 4.00 ^{d}	3.96 ± 0.45 ^{c}
III		5	13.00 ± 0.17 ^{cd}	378.00 ± 1.05 ^{g}	4.39 ± 0.24 ^{bc}
IV	45	0	19.62 ± 1.35 ^{a}	403.33 ± 3.50 ^{d}	5.64 ± 0.18 ^{a}
V		2	14.75 ± 1.17 ^{b}	430.92 ± 1.91 ^{a}	4.50 ± 0.13 ^{bc}
VI		5	14.76 ± 0.42 ^{b}	394.58 ± 3.54 ^{e}	6.23 ± 0.41 ^{a}
VII	90	0	13.49 ± 0.14 ^{c}	406.67 ± 1.97 ^{c}	4.01 ± 0.09 ^{c}
VIII		2	12.92 ± 0.29 ^{cd}	417.17 ± 1.69 ^{b}	3.89 ± 0.09 ^{c}
IX		5	11.72 ± 0.54 ^{e}	387.42 ± 2.50 ^{f}	3.92 ± 0.14 ^{c}

Note. mean ± SD, means with the same letter in the same column are not significantly different ($p \geq 0.05$)

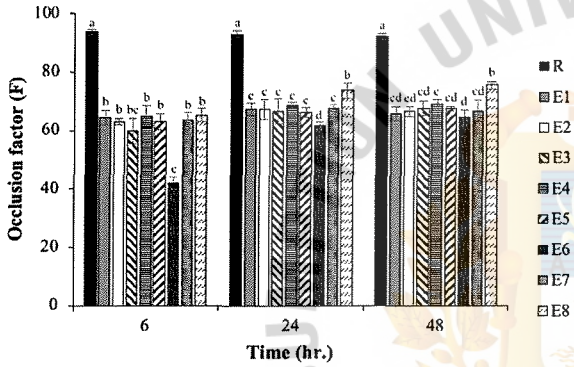

Figure 2 Occlusion factor of cosmetic emulsion

CONCLUSION

In this study, it can be concluded that the extraction temperature had affected the bioactive compounds of barley extract which extracted at 45°C provided the highest TPC, TFC and antioxidant activity. Therefore, 5 minutes of microwave preheating showed the highest antioxidant activity (IC_{50} value). The *in vitro* UV spectrophotometric method revealed that the barley had photo-protective compounds which absorb the UVB radiation. Besides, all virgin oils also provided SPF value, VAO represented the most effective of sunscreen protection. Barley and virgin oil could be recommended as a natural UV absorption to be effective in preventing skin cancer, sunburn, and wrinkle reduction. The barley-virgin oil based cosmetic emulsion exhibited the occlusion factor that prevents dehydration.

Table 5 SPF, pH, and color of cosmetic emulsion

Cosmetic emulsion	SPF	pH	Color of cosmetic emulsion		
			L*	a*	b*
E1	5.58 ± 0.05 ^{ab}	7.94 ± 0.01 ^{g}	73.50 ± 0.12 ^{b}	-0.24 ± 0.03 ^{d}	18.36 ± 0.24 ^{c}
E2	5.55 ± 0.02 ^{b}	8.39 ± 0.01 ^{f}	74.66 ± 0.12 ^{a}	0.09 ± 0.05 ^{ab}	13.78 ± 0.09 ^{e}
E3	5.30 ± 0.06 ^{c}	8.43 ± 0.02 ^{e}	73.58 ± 0.51 ^{b}	-0.69 ± 0.16 ^{e}	19.72 ± 0.09 ^{b}
E4	5.57 ± 0.02 ^{ab}	8.45 ± 0.01 ^{d}	74.39 ± 0.19 ^{a}	-0.17 ± 0.02 ^{cd}	19.75 ± 0.29 ^{b}
E5	4.97 ± 0.02 ^{e}	8.68 ± 0.01 ^{a}	70.57 ± 0.20 ^{d}	-0.30 ± 0.10 ^{d}	11.02 ± 0.02 ^{g}
E6	5.64 ± 0.07 ^{a}	8.61 ± 0.02 ^{b}	68.88 ± 0.08 ^{f}	0.25 ± 0.14 ^{a}	16.99 ± 0.19 ^{d}
E7	5.18 ± 0.04 ^{d}	8.67 ± 0.01 ^{a}	69.58 ± 0.23 ^{e}	-1.00 ± 0.12 ^{f}	24.65 ± 0.32 ^{a}
E8	4.31 ± 0.01 ^{f}	8.58 ± 0.02 ^{c}	71.81 ± 0.16 ^{c}	-0.03 ± 0.09 ^{bc}	12.37 ± 0.08 ^{f}

Note. mean ± SD, means with the same letter in the same column are not significantly different ($p \geq 0.05$)

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APPENDIX B
STATISTICAL ANALYSIS

Example of statistical analysis

1. Statistical analysis (RCBD)

```
> attach(Dataset)
> RCBD<-aov(y~trt+rep,data=Dataset)
> summary(RCBD)
```

Table . The ANOVA table for SPF of herb oils.

Source	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Treatment	5	221.01	44.20	1546.338	4.21e-14 ***
Replication	2	0.03	0.02	0.583	0.576
Residuals	10	0.29	0.03	-	-

Note. Significant codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

2. Statistical analysis (Duncan test)

```
> library(agricolae)
> attach(Dataset)
> model<-aov(y~trt, data=Dataset)
> comparison<-duncan.test(model,"trt",main="y dealt with different trt")
> duncan.test(model,"trt",alpha=0.05,console=TRUE)
```

Table . Duncan's multiple range test for SPF of herb oils.

Group	Treatments	Means
a	VPSO	9.517
b	VAO	8.71
c	VSAO	5.077
d	VOO	2.06
d	VCO	2.003
e	COSO	0.22

Note. Means with the same letter are not significantly different (p>0.05)