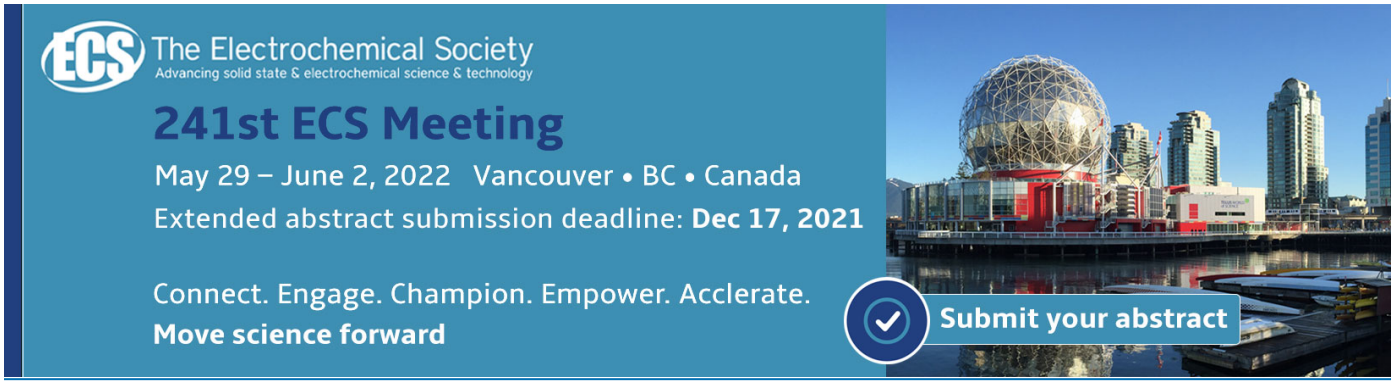
Evaluation of sunscreen cream incorporated with astaxanthin from *Haematococcus pluvialis* in different storage conditions



Evaluation of sunscreen cream incorporated with astaxanthin from *Haematococcus pluvialis* in different storage conditions

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Abstract. The use of sunscreen is highly recommended to protect the skin from ultraviolet radiation (UV) from the sun. Natural substances from natural source, such as astaxanthin, have been considered as potential sunscreen resources due to high UV ray absorption and antioxidant activity. However, substances originated from nature usually has low stability especially when incorporated into a mixture of ingredients with different physicochemical properties. Thus, this study aims to investigate the physicochemical properties of astaxanthin sunscreen cream from *Haematococcus pluvialis* extract at different storage conditions (25 °C, 4 °C, and 40 °C). The developed astaxanthin sunscreen cream formulations (F1=0%, F2=0.5% and F3=1.0% astaxanthin) were evaluated for SPF, pH and viscosity. Inclusion of astaxanthin in the sunscreen cream formulations significantly increase the SPF value at all storage conditions with higher SPF were observed at room temperature (25 °C). Astaxanthin significantly lower the pH of the base cream (F1) when stored at lower and higher temperatures (4 °C and 40 °C) but had no effect on pH at room temperature (25 °C). Viscosity was affected when stored at higher temperature (40 °C). This study shows that astaxanthin is an excellent natural UV filter that can improve the efficacy of the sunscreen and stable at room temperature (25 °C) with higher SPF observed and no significant change in pH and viscosity. In summary, the results will benefit the cosmetics industry and provide an alternative natural sunscreen cream to consumers.

1. Introduction

Human skin is the first physical protective barrier against various environmental factors that could harm the body. Skincare products such as sunscreen cream is specifically formulated to provide protection against detrimental effects of prolong UV exposure that could cause premature skin aging and also increase the risk of skin cancer [1]. It contains complex molecules of UV filters such as oxybenzone, octinoxate, octocrylene and 4-methylbenzylidene camphor that have been shown to affect aquatic environment such as causing coral reef bleaching, endangering aquatic life and disturbing the marine food web [2,3]. Zinc oxide and titanium dioxide are also commonly used in the sunscreen cream formulation due to their photostability, low photoallergy reaction rate and ability to provide opacity to reflect and absorb UV [1]. Although both compounds are considered as inert, some studies have shown that the nanoparticles of this compounds may cause systemic toxicity and induce DNA-damaging effects when exposed to UV [4,5].

An alternative safer UV filters are in need to ameliorate the harmful effects of UV to the skin and natural antioxidant such as polyphenols with UV absorptive or photoprotective properties can be a suitable

candidate. Natural antioxidants have the ability to exert their photoprotective effect by absorbing the UV energy reducing the formation of free radicals generated by UV-induced oxidation reaction [6,7]. Lignin, silymarin, plant extracts and marine sources are excellent natural UV blockers with antioxidant properties that are able to increase the Sun Protective Factor (SPF) values of formulated sunscreen cream [8]. Natural sources from the marine such as microalgae produces mycosporine-like amino acids (MMAs), carotenoids, scytonemin and flavonoids that function to reduce environmental stress causes by UV [9]. These compounds could prevent sunburn in humans with photosensitive skin and low melanin level [8].

Astaxanthin or commonly known as 3, 3′-dihydroxy-b,b-carotene- 4, 4′-dione belongs to the family of Xanthophylls, the oxygenated derivatives of carotenoid that is naturally synthesized by bacteria, microalgae, and yeasts [10]. Astaxanthin is widely used in nutraceuticals, cosmetics, food and aquaculture industries for its health benefits bioactivities such as antioxidant, anti-inflammatory and antimicrobial [11,12]. The antioxidant activity of astaxanthin is 65 times more powerful than vitamin C, 54 times stronger than β-carotene and 100 times more potent than α-tocopherol [12]. Among the commercially important microalgae, *Haematococcus pluvialis* has been shown to contain the richest source of astaxanthin [13]. *H. pluvialis* produces very large amount of astaxanthin when exposed to stressful climatic conditions such as excessive light, high salinity and poor nutrients availability[14]. The "red biomass" of astaxanthin is usually harvested through centrifugation process, where the hematocyst containing astaxanthin are separated from the water through passive settling and later concentrated with centrifugation [12]. This present study utilized astaxanthin from *H. pluvialis* in sunscreen cream formulation to provide a safer and environmentally friendly sunscreen options in the market. Thus, the objective of this study was to evaluate the physicochemical properties of sunscreen cream incorporated with astaxanthin from *H. pluvialis*.

2. Materials and Methods

2.1 Materials

H. pluvalis powder (10%) was purchased from Herbertviginia, China and of cosmetic grade; stearic acid, liquid paraffin, cetyl alcohol, cetearyl alcohol and ceteareth-20, glycerin, triethanolamine, Euxyl-PE9010 and fragrance (Ungerer) were of cosmetic grade; ethanol was purchased from Sigma Aldrich and deionized water was obtained from water filtration system.

2.2 Sunscreen cream preparation

The sunscreen formulation consists of water and oil phases. The water phase; glycerin (2 g), TEA (1 g), deionized water (80.5 g), Euxyl- PE9010 (0.3 g) and fragrance (0.1 g) were weighed using an analytical weighing balance and placed in a beaker. The oil phase; cetearyl alcohol and ceteareth-20 (8 g), stearic acid (4 g), cetyl alcohol (2 g) and liquid paraffin (2 g) were weighed and placed in a beaker. Later, the water and oil phases were heated up to 80 °C in a separate beaker using a hot plate stirrer until the oil phase fully melted. The oil phase was added to the water phase while maintaining the temperature at 80 °C. Then, the sunscreen cream formulation was homogenized using a homogenizer at 9000 rpm for 15 minutes until a homogenous emulsion was formed. The emulsion was stored at temperature not exceeding 37 °C until further tests.

2.3 Sunscreen cream formulation

Previous formulation was modified to prepare three sunscreen formulations as shown in Table 1[15]. F1 represented base sunscreen cream with no astaxanthin. While, F2 dan F3 represented sunscreen cream formulation with 0.5% and 1.0% of astaxanthin respectively. Each of the sunscreen sample was prepared in triplicates.

Table 1. Composition of sunscreen cream.

Materials	Percentage (% w/w)		
	F1	F2	F3
Oil phase			
Cetearyl alcohol and ceteareth-20	8	8	8
Stearic acid	4	4	4
Cetyl alcohol	2	2	2
Liquid paraffin	2	2	2
Water phase			
Glycerin	2	2	2
Triethanolamine (TEA)	1	1	1
Deionized water	ad. 100	ad.100	ad.100
Marshmallow vanilla Fragrance	0.1	0.1	0.1
Euxyl-PE9010	0.3	0.3	0.3
Astaxanthin powder	-	0.5	1
Total (%)	100	100	100

2.4 Physicochemical properties

2.4.1 SPF

The formulated sunscreen cream (0.4 g) was weighed and dissolved in 25 mL of ethanol. The dissolved sunscreen sample was pipetted (2 mL) into quartz cuvette Shimadzu. Ethanol was used as a blank. The absorbance reading was measured at wavelength ranging from of 290-320 nm, with 5 nm interval using a UV-VIS spectrophotometry (Shimadzu). The value of EE x I is as described by Pratama et al., (2019). The SPF value was calculated using the formula below:

$$SPF = CF \times \sum EE(\lambda) \times I(\lambda) \times abs(\lambda) \quad (1)$$

Where,

CF= correction factor; EE = spectrum of erytermal effects; I= spectrum of intensity from the sun and Abs =absorbance of the sample

2.4.2 pH

Each of the prepared sunscreen creams was weighed (0.5g) and dissolved in 50 mL distilled water with on a hot plate. The pH of the solution was measured using a pH meter (LAQUA TWIN pH-11) and was recorded. Measurements were taken in triplicates.

2.4.3 Viscosity

Each of the formulated sunscreen creams (F1, F2 and F3) was weighed (30 g) and placed in a viscometer container. The cream was ensured to touch the viscometer electrode and the readings from the viscometer were recorded. All measurements were taken in triplicate.

2.5 Accelerated stability study

The accelerated stability was performed at different storage conditions (25 °C ± 2), (4 °C ± 2), and (40 °C ± 2). The physicochemical properties of the sunscreen creams (SPF, pH and viscosity) were observed and

recorded at day 1, 7, 10 or 11 and 14. All data were recorded in triplicate. For comparison, data from day 1 and day 14 were analyzed.

2.6 Statistical Analysis
Analysis of variance (ANOVA) was performed in Graphpad Prism 9 and $p \leq 0.05$ was considered as significant.

3. Results and Discussion

3.1 SPF
The formulation showed significant difference in SPF between formulations (F1< F2 <F3) for all the storage conditions (25 °C, 4 °C and 40 °C), as shown in Figure 1. However, similar formulations showed no changes when compared at day 1 and day 14. The results suggest that astaxanthin at 0.5% (F2) and 1% (F3) significantly improve SPF value of the sunscreen cream. Astaxanthin pigment produces by *H. pluvialis* is a natural sunscreen pigment. This pigment provides protection against photooxidative stress that could cause critical damage to its cells and interfere with its cellular functions through the generation of free radical species [11,14]. Similarly, when exposed to UV skin cells also produce reactive free radical, thus application of sunscreen is required to ameliorate the effect of UV on the skin [6]. Sunscreen activity is usually measured using Sun Protective Factor (SPF), and a product with higher SPF provides more protection against the hazardous effects of the UV [16]. The observed results were consistent with increasing concentrations, where F3 (1% astaxanthin) had higher SPF than F2 (0.5% astaxanthin). However, in this experiment storage conditions had no effect on the formulations, when compared to day 1 and day 14. Higher SPF was observed in all sunscreen creams stored at room temperature suggesting the formulation was stable at 25 °C.

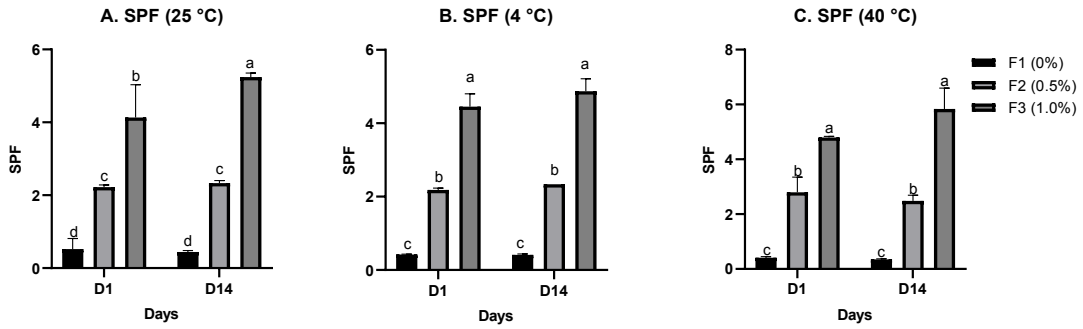

Figure 1. Comparison of SPF for sunscreen cream incorporated with astaxanthin; F1 (0%), F2 (0.5%) and F3 (1.0%) between day 1 and day 14 at different storage conditions (25 °C, 4 °C and 40 °C). Data represent mean ± SD of triplicates. Different alphabets indicate significant difference at $p<0.05$.

3.1.1 pH
Most skincare products are formulated to achieve "pH balance" ranging from 4 (acidic) to 7 (neutral) since the skin pH is naturally slightly acidic [17]. In this present study, the pH of the base cream (F1) was slightly higher than the desired pH, but it was still acceptable since the normal acidic skin pH has been shown to shift to higher pH with aging [18]. Moreover, the skin tends to equalize to its normal pH within hour of pH change due to any products application. Room temperature (25 °C) had no effect on the pH of F1, F2 and F3 but at lower pH (4 °C) when compared with day 1 and day 14, as shown in Figure 2A. However, both formulations containing astaxanthin (F2 and F3) had significantly lower pH than F1 at day 1 and day 14, as shown in Figure 2B. Similar trend was observed at storage condition of 40 °C, except the pH of F1 became significantly lower at day 14 as compared with day 1, as shown in Figure 2C. The overall results suggest that astaxanthin may have influence the pH of the sunscreen cream to become lower than

control (F1) when stored at lower (4 °C) and higher temperature (40 °C). The observed results could be explained by the nature of astaxanthin that is resistant to extreme pH [19,20], and stable at lower pH ranging from 3.5 to 6.5 [21].

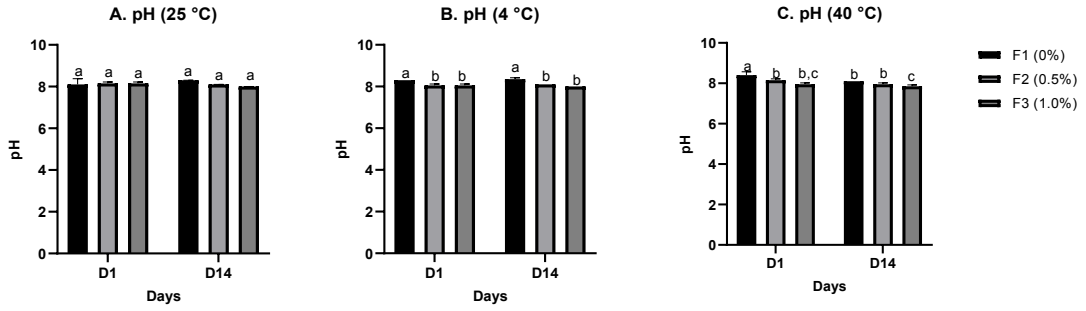

Figure 2. Figure shows the comparison of pH for sunscreen cream incorporated with astaxanthin; F1 (0%), F2 (0.5%) and F3 (1.0%) between day 1 and 14 at different storage conditions (25 °C, 4 °C and 40 °C). Data represent mean ± SD of triplicates. Different alphabets indicate significant difference at $p<0.05$.

3.1.2 Viscosity

Viscosity measures the resistance of a fluid to flow, thus higher viscosity causes higher friction for a moving fluid. Viscosity of a product is very critical to its quality since it will affect the texture especially the smoothness and the spreadability of the sunscreen cream. At room (25 °C) and lower (4 °C) temperatures, F1 and F2 showed lower viscosity than control (F1), but the results were not statistically significant, as shown in Figure 3A and 3B. However, F1 showed significantly higher viscosity at day 14 at higher temperature (40 °C), as shown in Figure 3C. Similar trends of higher viscosity were observed for F2 and F3 at day 14 as compared to day 1, but the data was not statistically significant. Higher viscosity indicated that the formulations were resistant to flow and the results can be explained by the moisture reduction due to high temperature [22]. Water content in all formulation would lose since higher temperature caused water to evaporate, and thus would increase the viscosity of all the sunscreen creams.

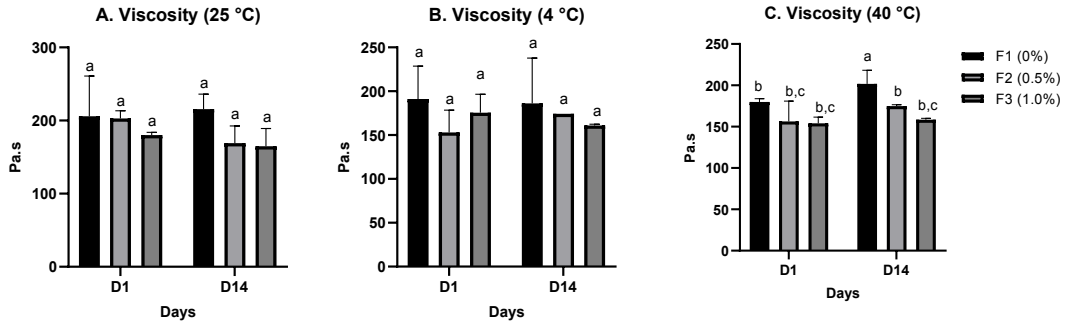

Figure 3. Figure shows the comparison of viscosity for sunscreen cream incorporated with astaxanthin; F1 (0%), F2 (0.5%) and F3 (1.0%) between day 1 and 14 at different storage conditions. Data represent mean ± SD of triplicates. Different alphabets indicate significant difference at $p<0.05$.

4. Conclusion

As a conclusion, inclusion of astaxanthin in the sunscreen cream formulations would significantly increase the SPF value at all storage conditions with higher SPF was observed at room temperature, suggesting that higher protection would be provided against UV when the sunscreen creams were stored at 25 °C.

Astaxanthin would lower the pH of the base cream (F1) when stored at extreme temperatures (4 °C and 40 °C) but had no effect on pH at room temperature (25 °C). Meanwhile, viscosity was affected when stored at higher temperature (40 °C) due to water loss. This study shows that astaxanthin is an excellent natural UV filter that can improve the efficacy of the sunscreen and stable at room temperature (25 °C) with higher SPF observed and no significant change in pH and viscosity.

Acknowledgement
Authors would like to thank Fakulti Industri Asas Tani, Universiti Malaysia Kelantan that provides the facilities where this study was conducted.

References

[1] Burnett M E and Wang S Q 2011 Current sunscreen controversies: a critical review *Photodermatol. Photoimmunol. Photomed.* **27** 58–67
[2] Lee S H, Xiong J Q, Ru S, Patil S M, Kurade M B, Govindwar S P, Oh S E and Jeon B H 2020 Toxicity of benzophenone-3 and its biodegradation in a freshwater microalga Scenedesmus obliquus *J. Hazard. Mater.* **389** 122149
[3] Tsui M M P, Leung H W, Kwan B K Y, Ng K, Yamashita N, Taniyasu S, Lam P K S and Murphy M B 2015 Occurrence, distribution and ecological risk assessment of multiple classes of UV filters in marine sediments in Hong Kong and Japan *J. Hazard. Mater.* **292** 180–7
[4] Dufour E K, Kumaravel T, Nohynek G J, Kirkland D and Toutain H 2006 Clastogenicity, photo clastogenicity or pseudo-photo-clastogenicity: Genotoxic effects of zinc oxide in the dark, in pre-irradiated or simultaneously irradiated Chinese hamster ovary cells *Mutat. Res. - Genet. Toxicol. Environ. Mutagen.* **607** 215–24
[5] Hidaka H, Kobayashi H, Koike T, Sato T and Serpone N 2006 DNA Damage Photoinduced by Cosmetic Pigments and Sunscreen Agents under Solar Exposure and Artificial UV Illumination *J. Oleo Sci.* 55 249–61
[6] Lephart E D 2016 Skin aging and oxidative stress: Equol's anti-aging effects via biochemical and molecular mechanisms *Ageing Res. Rev.* **31** 36–54
[7] Ahsanuddin S, Lam M and Baron E D 2016 Skin aging and oxidative stress *AIMS Mol. Sci.* **3** 187 95
[8] He H, Li A, Li S, Tang J, Li L and Xiong L 2021 Natural components in sunscreens: Topical formulations with sun protection factor (SPF) Biomed. *Pharmacother.* **134** 1–11
[9] Singh R, Parihar P, Singh M, Bajguz A, Kumar J, Singh S, Singh V P and Prasad S M 2017 Uncovering potential applications of cyanobacteria and algal metabolites in biology, agriculture and medicine: Current status and future prospects *Front. Microbiol.* **8** 1–37
[10] Kawasaki S, Yamazaki K, Nishikata T, Ishige T, Toyoshima H and Miyata A 2020 Photooxidative stress-inducible orange and pink water-soluble astaxanthin-binding proteins in eukaryotic microalga *Commun. Biol.* **3** 1-9
[11] Mularczyk M, Michalak I and Krzysztof M 2020 Astaxanthin and other Nutrients from *Haematococcus pluvialis*-Multifunctional applications *Mar. Drugs* **10** 1–22
[12] Shah M M R, Liang Y, Cheng J J and Daroch M 2016 Astaxanthin-producing green microalga *Haematococcus pluvialis*: From single cell to high value commercial products *Front. Plant Sci.* **7** 1–28
[13] Rammuni M N, Ariyadasa T U, Nimarshana P H V and Attalage R A 2019 Comparative assessment on the extraction of carotenoids from microalgal sources: Astaxanthin from *H. pluvialis* and β-carotene from *D. salina Food Chem.* **277** 128–34
[14] Domínguez-Bocanegra A R, Guerrero Legarreta I, Martinez Jeronimo F and Tomasini Campocosio A 2004 Influence of environmental and nutritional factors in the production of astaxanthin from *Haematococcus pluvialis Bioresour. Technol.* **92** 209–14
[15] Pratama G, Yanuarti R, Ilhamdy A F and Suhana M P 2019 Formulation of sunscreen cream from *Eucheuma cottonii* and *Kaempferia galanga* (Zingiberaceae) *Earth Environ. Sci.* **278** 1–6
[16] Gaikwad M and Kale S 2011 Formulation and in vitro evaluation for sun protection factor of

Moringa oliefera Lam. (Family-Moringaceae) oil sunscreen cream *Int. J. Pharm. Pharm. Sci.* **3** 371–5

[17] Mojumdar E H and Sparr E 2020 The effect of pH and salt on the molecular structure and dynamics of the skin *Colloids Surfaces B Biointerfaces* 1–9

[18] Zouboulis C C, Elewa R, Ottaviani M, Fluhr J, Picardo M, Bernois A, Heusèle C and Camera E 2020 Age in fluences the skin reaction pattern to mechanical stress and its repair level through skin care products *Mech. Ageing Dev.* 1–8

[19] Ahmed F, Li Y, Fanning K, Netzel M and Schenk P M 2015 Effect of drying, storage temperature and air exposure on astaxanthin stability from *Haematococcus pluvialis Food Res. Int.* **74** 231–6

[20] Meléndez-Martínez A J, Britton G, Vicario I M and Heredia F J 2007 Relationship between the colour and the chemical structure of carotenoid pigments *Food Chem.* **101** 1145–50

[21] Hu Z C, Zheng Y G, Wang Z and Shen Y C 2006 pH control strategy in astaxanthin fermentation bioprocess by *Xanthophyllomyces dendrorhous* Enzyme *Microb. Technol.* **39** 586–90

[22] Farah M A, Oliveira R C, Caldas J N and Rajagopal K 2005 Viscosity of water-in-oil emulsions: Variation with temperature and water volume fraction *J. Pet. Sci. Eng.* **48** 169–84