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Original Article

Effect of grape seed extract as a sunscreen booster

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Abstract

Grape seed extract (GSE) contains a high content of polyphenols that prevent the generation of reactive oxygen species which suggests an anti-aging action. The aims of this study were to investigate the antioxidant effects of GSE, ultraviolet (UV) and visible spectrums, and *in vitro* sunscreen efficacy. The results showed that GSE possessed DPPH free radical scavenging capacity at an IC_{50} of 33.17±1.87 µg/mL and ferric reducing power of 1 mg/mL. GSE was equivalent to 4.17±0.23 mM vitamin C and 0.73±0.04 mM Trolox. Moreover, 3% and 5% w/w GSE absorbed broad UV and blue light spectra to a sufficient extent. The sun protection factor (SPF) and persistent pigment darkening (PPD) increased to 5 units and 1 unit, respectively, after addition of 3% GSE to an over-the-counter brand product. In conclusion, GSE possessed antioxidant activity and boosted the SPF/PPD value of sunscreen product. Therefore, GSE can be a value-added component to sunscreen products.

Keywords: grape seed extract, antioxidant, sunscreen

1. Introduction

Over the last few decades, it has become common practice to add sunscreen agents of variable potency to cosmetics to protect the skin against premature aging and other significant adverse effects of ultraviolet (UV) radiation (Grune, 2008). UV radiation has a broad spectrum from 40 to 400 nm but of greatest concern are the ranges of UVB (290–320 nm) and UVA (320–400 nm).

The damaging effect of UV radiation on human health, in particular the development of skin cancer, is a serious concern and has been well addressed during the last 20 years. The awareness of effective ways to provide protection has spread recently to the wider population. The field of UV-protection has successfully moved forward in further investigations of skin damage by reactive oxygen species (ROS). Apart from UVA and UVB protection, visible (VIS) light, particularly blue light (400–495 nm), is one of high interest in the context of photodamage (Vandersee, Beyer, Lademann, & Darvin, 2015) with the need to overcome this hazard by the introduction of efficient photoprotective and chemopreventive strategies (Lim, Arellano-Mendoza, & Stengel, 2017).

Despite the differences between UV and VIS light, which is conditional, photosensitized production of ROS is possible in the entire visible range (Nakashima, Ohta, & Wolf, 2017). According to the free radical theory of premature skin aging, UV and blue light can produce ROS that may create conditions promoting the formation of prostaglandins and sunburn cells due to skin damage (Greene, 2001; Nakashima *et al.*, 2017). Although the skin has a variety of enzymatic and small molecular antioxidants that inhibit oxidative damage, excessive ROS generation can overwhelm the antioxidant defense capacity of the skin that may result in cell oxidative stress (Rinnerthaler, Bischof, Streubel, Trost, & Richter, 2015) and consequently in oxidative photodamage of the main skin biomolecules (Filip *et al.*, 2011).

Recently, a growing trend of incorporating plant extracts as the main source of antioxidants in sunscreen formulations may be beneficial against the effects of visible light (Lim *et al.*, 2017). Among the extracts of interest is grape seed (*Vitis vinifera*) extract (GSE) because it has various bioactive properties and powerful antioxidant activity (Saric & Sivamani, 2016). The primary components of GSE are proanthocyanidins that act as antioxidants that are well known as agents that reduce free radical-mediated damage in cells after

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UV and VIS light irradiation (Lim *et al.*, 2017; Rahman, 2007; Zhou & Raffoul, 2012).

Martincigh and Ollengo (2016) found that exposure of GSE to UV radiation increased the UV absorption capacity of the extract which gave a strong foundation for the assumption that a sunscreen formulation that contains GSE may provide protection against the damaging effects of UV. Hence, GSE was recommended as an additive to sunscreen products with a broad spectrum of protection (Greene, 2001).

In this study, the bioactive properties and antioxidant effects of GSE were investigated as well as UV and blue light protection. Studies on GSE as a sunscreen booster in sunscreen products were also carried out to determine the sun protection factor (SPF).

2. Materials and Methods

2.1 Grape seed extraction

Dried grape seeds were crushed in a FitzMill® Comminutor (The Fitzpatrick Company, USA) into a fine powder. One part of the ground grape seed powder was macerated in 3 parts of 95% ethanol for 1 week. Then, the filtrate was collected and concentrated using a rotary vacuum evaporator at 40 °C. The residue filtrate was dried in a hot air oven at 45 °C for 2-3 days until constant weight was obtained. The percent yield of dry extract was calculated from the powder of grape seeds. The experiment was performed in triplicate.

2.2 Determination of total phenolic content

The concentration of phenolics in GSE was determined by the spectrophotometric method. An ethanol solution of GSE at different concentrations (1-100 µg/mL) was used in the analysis. The reaction mixture was prepared by mixing 0.3 mL of the ethanol solution of GSE, 1.5 mL of 10% (w/v) Folin-Ciocalteu's reagent, and 1.2 mL of 7.5% (w/v) sodium carbonate were prepared in centrifuge tube. The sample mixture was left in the dark place for 2.5 h. The absorbance was measured using spectrophotometer at 760 nm. The same procedure was repeated for the standard solution of gallic acid. Based on the measured absorbance, the concentration of phenolics was calculated (mg/mL) from the constructed calibration line. The content of phenolics in the extracts was expressed in terms of gallic acid equivalent (mg GAE/g DW) (Singleton, Orthofer, & Lamuela-Raventós, 1999). The standard curve equation: $y = 0.0011x - 0.0027$, $r^2=0.98189$.

2.3 Determination of total anthocyanin content

Anthocyanin pigment content was determined using the pH-differential method of Giusti and Wrolstad (2005). GSE solution (10 µL) was mixed with 150 µL of 0.2% (w/v) potassium chloride buffer solution (pH 1.0), and 10 µL of GSE solution was mixed with 3% (w/v) sodium acetate buffer solution (pH 4.5). Both solutions were left for 15 min. The absorbance of each solution was measured at 510 nm and 700 nm (Pukdee, Kumar, Chaiwut, & Sripisut, 2016). The absorbance (A) of the diluted sample was calculated as follows: A = (Aλ vis-max – A 700) pH 1.0 – (Aλ vis-max – A 700) pH 4.5. Monomeric anthocyanin concentration was calculated using this equation:

$$\textit{Total Monomeric Anthocyanin} = A \times MW \times DF \times 1000 / \varepsilon \times 1$$

The total monomeric anthocyanin content was calculated as cyanidin-3-glucoside equivalents using the extinction coefficient of 26,900 L cm^{-1} mg^{-1}, molecular weight (MW) of 449.2 g mol-1, and the appropriate dilution factor (DF) (Lee, Durst, & Wrolstad, 2005).

2.4 Bioactivity and UV protection activities of GSE

2.4.1 Antioxidant activity

1) DPPH radical scavenging assay

GSE at different concentrations (1-100 µg/mL) and the positive control agents including, vitamin E acetate, Trolox and butylatedhydroxy-toluene (BHT) in the range of 1-1000 µg/mL were prepared by a two-fold dilution with ethanol (1:1) in 96-well plates. An amount of 50 µL of 1,1-diphenyl-2-picrylhydrazyl (DPPH) (0.1 mM) was added into each well except the blank well contained GSE and ethanol and incubated at room temperature for 30 min before measuring the absorbance at 517 nm. The antioxidant index (%) and IC_{50} were then calculated (Brand-Williams, Cuvelier, & Berset, 1995).

2) Ferric reducing antioxidant power (FRAP) assay

Samples for FRAP assay were prepared by mixing GSE at different concentrations (1-100 µg/mL) with 0.2 M phosphate buffer (pH 6.6) and 1% (w/v) potassium hexanocyanoferrate in a tube and incubated at 50 °C for 20 min. The reaction was stopped by adding 10% (w/v) trichloroacetic acid and left for 10 min followed by centrifugation at 1000 rpm for 10 min. The supernatant (30 µL) was mixed with 160 µL of distilled water and freshly prepared 10 µL of 0.1% (w/v) ferric chloride solution in a 96-well plate. The plate was incubated at room temperature for 10 min. Trolox (0.1 M) and Vitamin C (0.1 M) standard solutions were diluted with ethanol to make concentrations of 0.2 mM to 10 mM. The absorbance was measured at 700 nm (Manmohan, 2011).

2.4.2 UV absorption spectrum

Solutions of GSE in ethanol were prepared in various concentrations of 1%, 3%, and 5% (w/v). The absorbance spectrums of samples were measured in the range of 200-800 nm using a Shimadzu UV-1650PC spectrophotometer.

2.5 Formulation of cream base containing GSE

2.5.1 Factors affecting stability and appearance of cream base formulations

Development of oil in water cream base formulations were formulated by varying the types of emulsifiers including nonionic emulsifiers, e.g., cetomacrogol 1000 and

Tween 80 mixed with Span 80 and also an anionic emulsifier (triethanolamine stearate) (Table 1).

2.5.2 Method of preparation of cream base formulations

Water and oil phase ingredients (Table 1) were weighed in the separate beakers. The procedure was separated into three parts. The water phase ingredients that included Tween 80, triethanolamine, glycerin, and water were added to the first beaker and heated in water bath to 75 °C. The second beaker was prepared for the forming of a gel that contained either Carbopol 940 and triethanolamine or xanthan gum. The oil phase containing mineral oil, cetomacrogol 1000, stearic acid, and Span 80 were heated in a water bath to 70 °C. The water phase mixture was poured into the oil phase mixture and vigorously stirred to form the primary emulsion until the temperature reached 45 °C. The gel mixture was then slowly added to the primary emulsion. A preservative was finally added to the mixture.

2.5.3 Evaluation of cream base formulations

Texture profiles, viscosity, and pH of each cream base formulation were tested before and after heat cool cycling (6 cycles) for 12 h at 25 °C and 4 °C. The pH of each formulation was measured by a pH-meter (Testo 206-pH2, versatile pocket-size pH/°C meter) before and after the heat/cool cycles.

1) Texture profile analysis

The physical properties of each formulation were determined by a texture analyzer (Model TA-XT*Plus*, Stable Micro Systems, Surrey, UK) at 25 °C according to Jones, Woolfson and Brown (1997). The same amount (50 g) of each sample from before and after the heat/cool cycles was weighed in a glass jar (diameter of 3 cm and height of 7 cm) for texture analysis.

A stainless steel probe of 1 cm in diameter (P/0.5R) was compressed twice into the sample at the speed of 6 mm/s to a depth 1.5 cm with a delay period of 15 s between two compressions. Data was analyzed by XTRA Dimension software package of the instrument used.

The texture profile analysis results for hardness, cohesiveness, and adhesiveness were obtained by evaluation of the load and displacement at predetermined points on the texture profile analysis curve (Figure 1). Data for hardness was obtained from the column named "Force 1", adhesiveness (A3) from the column named "Area F-T 3:4", and cohesiveness was calculated by the formula *A2/A1 = Cohesiveness*, where "Area F-T 4:6" (A2) is the area under the curve of the second peak and "Area F-T 1:3" (A1) or compressibility, is the area under the curve of the first peak.

2) Evaluation of rheological properties

Viscosity of the formulations was measured at 25 °C by a Brookfield viscometer (Model DV-III+ Programmable Rheometer, Stoughton, MA, USA) that was placed on

Table 1. Ingredients of the prepared base formulations.

Formulation ingredients	F1	F2	F3	F4	F5	F6	F7	F8
Mineral oil (O)	36.07	36.07	30	30	16.07	16.07	10	10
Cetomacrogol 1000 (O)	-	-	-	10	-	-	-	10
Stearic acid (O)	-	-	10	-	-	-	10	-
Span 80 (O)	3.93	3.93	-	-	3.93	3.93	-	-
Tween 80 (W)	3.07	3.07	-	-	3.07	3.07	-	-
Triethanolamine (W)	-	0.4	1	0.4	-	0.4	1	0.4
Carbopol 940 (W)	-	0.5	-	0.5	-	0.5	-	0.5
Xanthan (W)	0.5	-	0.5	-	0.5	-	0.5	-
Glycerin (W)	5	5	5	5	5	5	5	5
Propylene Glycol, Diazolidinyl Urea, Methylparaben, Propylparaben (W)	1	1	1	1	1	1	1	1
DI water qs to (W)	100	100	100	100	100	100	100	100

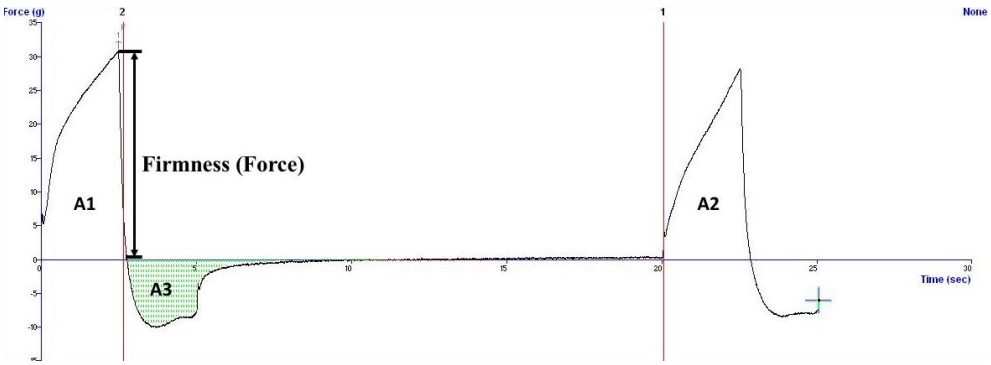

Figure 1. Texture profile analysis curve.

a Helipath™ Stand and connected with a TF-Spindle No. 95. The rheometer was set at 1.0 rpm. At least three measurements of the apparent viscosity expressed in centipoise (cps) were recorded for each formulation every 30 s.

2.6 Determination of sunscreen effect of creams containing GSE

2.6.1 SPF/PA standard curve

A thin layer of an over-the-counter brand sunscreen product was evenly spread on a cell model made from a transparent plastic sticker (1x2 cm) attached to 3M-transpore tape (1.2x3 cm). Each sample of the sunscreen product that was spread on the transpore tape was weighted to 2.0-2.2 mg by an analytical balance previously attached to the solid fusion board. The cells were left for about 10 min to dry. The 3M-transpore tape was attached to a black fusion plastic board (1.2x4 cm) with a rectangular shape cut in the middle. The SPF and PPD values were carried out using UV transmission spectroscopy by Shimadzu UV-1650PC spectrophotometer at wavelengths 290-400nm. Values of transmittance were used to calculate SPF/PPD of counter-brand products and construct the calibration curve for estimation of SPF/PPD properties of the designed cream base formulation containing GSE (Khunkitti *et al.*, 2014).

In vitro SPF was calculated using the equation suggested by Diffey and Robson (1989):

$$\text{In vitro SPF} = \frac{\Sigma_{290}^{400} E(\lambda)\in(\lambda)}{\Sigma_{290}^{400} E(\lambda)\in(\lambda)/T(\lambda)} \text{In vitro SPF} = \frac{\Sigma_{290}^{400} E(\lambda)\in(\lambda)}{\Sigma_{290}^{400} E(\lambda)\in(\lambda)/T(\lambda)},$$

where E(λ) is the spectral irradiance of the used light spectrum at wavelength λ nm, ∈(λ) is the erythemal action spectrum at wavelength λ nm corresponding to the International Commission on Illumination (CIE) publication (McKinlay & Diffey, 1987), and T(λ) is the spectral transmittance of the sunscreen.

In vitro UVA-PF was calculated using equation suggested by Ferrero, Pissavini, Marguerie and Zastrow (2002):

$$\text{In vitro UVA-PF} = \frac{\Sigma_{320}^{400} \Delta\lambda}{\Sigma_{320}^{400} T_\lambda \cdot \Delta\lambda)} = \frac{1}{T_m} \text{In vitro UVA-PF} = \frac{\Sigma_{320}^{400} \Delta\lambda}{\Sigma_{320}^{400} T_\lambda \cdot \Delta\lambda)} = \frac{1}{T_m},$$

where T_m is the arithmetic mean of the transmittance data in the UVA range. Evaluation of UVA-PF data was performed according to classification of the Japan Cosmetic Industry Association into four categories: UVA-PF < 2 – no protection against UVA; 2-4 (PA+) – protection against UVA; 4-8 (PA++) – significant protection against UVA; and ≥ 8 (PA+++) – the highest protection against (UVA) (Herzog *et al.*, 2002).

2.6.2 Estimation of SPF/PPD of GSE in a prepared base formulation and over-the-counter brand product

GSE was added to the prepared base formulation for further development of a formulation that requires the addition of sunscreen agents in the concentration that was found to be effective in protection from UVA and UVB radiation, and over-the-counter brand products that already contained sunscreen agents. The SPF/PPD was measured for both the base cream formulations and over-the-counter brand product with GSE. The SPF/PPD over-the-counter brand product with the GSE was also compared to the over-the-counter brand product without the GSE.

2.7 Statistical analysis

All experiments were performed in triplicate with three independent experiments. The results are expressed as mean±SD. The statistical analysis was performed using the Statistical Package for Social Sciences (SPSS version 17.0, SPSS Inc., Chicago, IL, USA). The statistical differences among GSE, Vitamin E acetate, and Trolox were analyzed with ANOVA and Tukey test was used for multiple comparisons. A P value less than 0.05 indicated statistical significance.

3. Results and Discussion

3.1 Determination of GSE yield, total phenolic content, and total monomeric anthocyanin content

The yield of solid residue after extraction and evaporation of the dried grape seeds was 2.47±0.185 g. The total phenolic content contained in 1 mg/mL of GSE was equivalent to 205.1±14.024 mg GAE/g DW. The total anthocyanin content of GSE was 7.44±0.58 mg/g DW (Table 2).

Table 2. Characteristics of grape seed extract.

GSE Content	Amount
Yields	12.33±0.925 w/w%
Total phenolic content	205.1±14.024*mg GAE/g DW
Total monomeric anthocyanin content	7.44±0.586mg/g DW

*Each value is the average of three measurements ± standard deviation

3.2 Bioactivity and UV protection activities of GSE

3.2.1 Antioxidant activity

1) DPPH radical scavenging assay and FRAP

Figure 2 shows that the largest capacity to neutralize DPPH radicals (IC_{50}) was found for Trolox and Vitamin E acetate, at the concentrations of 6.08 µg/mL and 12.23 µg/mL, respectively, followed by GSE having an inhibitory concentration of 33.17 µg/mL. The minimal capacity to inhibit DPPH radicals was BHT with an IC_{50} of 206.81 µg/mL. In the reducing power assay, antioxidant compounds convert the oxidation form of iron (Fe^{3+}) in ferric chloride to ferrous (Fe^{2+}). The results of this research showed that the reducing power of 1 mg/mL GSE was equivalent to 4.17±0.23 mM of vitamin C and 0.73±0.04 mM of Trolox (Table 3).

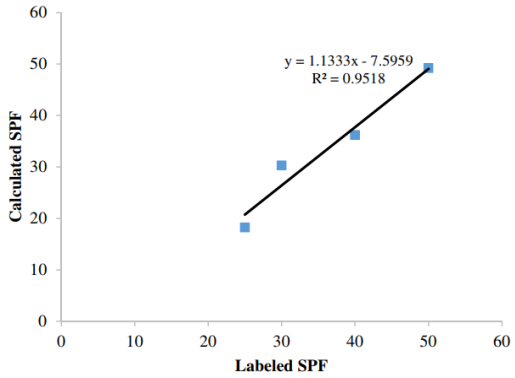

Figure 2. Calibration between labeled sun protection factor (SPF) and calculated SPF of the tested over-the-counter brand products. Correlation coefficient (r^2)=0.9518.

Table 3. Antioxidant activities of grape seed extract.

Tested compound	DPPH	FRAP	
	IC_{50} (µg/mL)	Equivalence to Vitamin C (mM)	Equivalence to Trolox (mM)
Grape seed extract	33.17±1.87*	0.179±0.010**	1.031±0.057**
BHT	206.81±1.03		
Trolox	6.08±0.71		
Vitamin E	12.23±1.33		

3.2.2 UV absorption spectrum

Plants constantly undergo sun exposure and consequently develop their own protection from UV light (Hamblin & Huang, 2013). The UV-visible spectrum of GSE in all of the tested concentrations showed absorbance in both the UVB and UVA ranges (290-400 nm) and covered a sufficient extent of the spectrum of blue light (400-495 nm) (Price, 1994). The GSE at all test concentrations absorbed UVB spectrum (290-320 nm) to the same extent, whereas 3% and 5% (w/v) GSE more effectively absorbed UVA (320-400 nm) and blue light (400-495 nm) than the 1% (w/v) GSE (Figure 3).

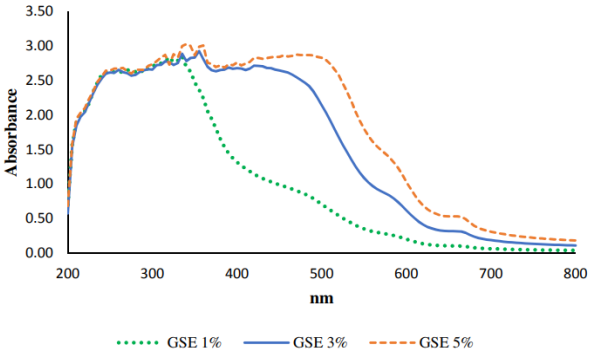

Figure 3. UV-visible spectrum of 1%, 3%, and 5% grape seed extract (GSE). Dotted green line represents 1% GSE; solid blue line represents 3% GSE; dashed orange line represents 5% GSE.

3.3 Formulation of base cream containing GSE

3.3.1 SPF/PPD standard curve

The estimated SPF values of four different over-the-counter brand products were used to construct a calibration curve (Figure 2). The relationship between the labeled SPF and the calculated SPF appeared to correlate well (r^2=0.9518). As a result, the SPF values of formulations in this study can be used for further testing and development of sunscreen products.

1) Factors affecting SPF/PPD values

Formulations F1 and F2 had the highest oil:water phase volume ratio of 40:60 and F5 with xanthan gum as the natural thickening agent were found to be unstable. Formulations F4, F6, and F8 had a smooth texture due to the presence of the synthetic high-molecular-weight polymer of acrylic acid (Carbopol 940) as the thickening agent. TEA stearate was the soap emulsifier in formulations F3 and F7 which made those textures sticky and greasy. Moreover, the higher oil: water phase volume ratio of 40:60 of formulation F3 created a thicker cream base compared to F7 which used one-third the amount of mineral oil. The other formulations were stable.

2) Evaluation of cream formulations

The evaluations of the texture and viscosity stability of the eight cream formulations before and after the heat/cool cycles are shown in Table 4. All of the tested formulations were in the appropriate range for human skin and had slight changes after the heat/cool cycles. The pH was directly dependent on the presence of particular excipients in the formulations. F1 and F5 had nearly the same pH values at 6.23 and 6.33, respectively, and both of them had Tween 80 and Span 80 in the formulations. Lower acidic pH values (2.5-4.0) were found in formulations F2, F4, F6, and F8 which contained Carbopol 940 (Rowe, Sheskey, & Weller, 2003). The presence of TEA stearate resulted in the highest pH values in formulations F3 (7.57) and F7 (7.55) (Zhu *et al.*, 2007). According to Schulman and Cockbain (1940b), emulsifying agents with the same fatty acid composition or the same hydrocarbon chain length provide emulsions with higher stability. For instance, Tween 80 and Span 80 together are supposed to form more stable emulsions than Tween 80 and Span 20 together. However, the partition of some ingredients into or out of the oil phase was sometimes found to affect the forming of an interfacial film. For example, F1, F2, and F5 formulations had phase separation (breaking) before and after the heat/cool cycles unlike F6 which contained the combination of Tween 80 and Span 80. The other formulations were stable before and after the heat/cool cycles. Therefore, it should be kept in mind that the stability of the emulsion prepared using a pair of emulsifying agents with the same fatty acid may not be predictable (Viyoch, Klinthong, & Siripaisal, 2003).

The parameters used in a texture profile analysis are applicable for preliminary sensory characteristics and they are important for development of topical sunscreen formulations (Jones, Lawlor, & Woolfson, 2002). The effectiveness of

sunscreen products depends not only on the sensory characteristics influencing consumer's compliance but also skin affinity which is higher to creams than lotions (Moraes, Arêas, & Velasco, 2017; Limsuwan & Amnuikit, 2017). Appropriate viscosity values of sunscreen formulations improve the adhesiveness and spreading efficacy and are directly related to SPF values (Amnuaikit & Boonme, 2013).

Evaluation of the rheological properties showed that formulations F8 and F3 had the highest viscosity. Formulation F6 had the lowest viscosity with a minor reduction (<10%) after the heat/cool cycles among other stable formulations.

Evaluation of the texture profile showed that formulations F3 and F4 that contained higher amounts of mineral oil had greater hardness and lower adhesiveness. However, the stability changes of the F3 and F4 creams after the heat/cool cycles moved in different directions. In formulation F3, the hardness doubled and the adhesiveness reduced, whereas formulation F4 had a 10% reduction in hardness and the adhesiveness increased. The hardness and adhesiveness of formulations F7 and F8 were lower than formulations F3 and F4. Moreover, the profiles of formulation F7 behaved in the same way as F4, while F3 shared a similar direction in profile changes as F8 before and after the heat/cool cycles. Formulation F6 had the most prominent increase in hardness after the heat/cool cycles with a 50% reduction in adhesiveness while maintaining a pleasant appearance and smooth texture that was achieved due to the combination of the nonionic surfactants (Tween 80, Span 80) and thickening agent (Carbopol 940) (Garrett, 1965; Viyoch *et al.,* 2003).

The cohesiveness of all tested formulations was in the range of 0.8102 to 1.0457 and 0.813 to 1.012, before and after the heat/cool cycles, respectively, and did not exceed a 10% change.

Even though formulation F6 had the highest change in hardness, it was chosen for further development of a sun product based on the appropriate pH range, good stability before and after the heat/cool cycles, and the aesthetically pleasant appearance with a white colour base and a light and smooth lotion type texture.

3.4 Estimation of SPF/PPD of GSE in the prepared base formulations and over-the-counter brand product

In this study, GSE had pronounced free radical scavenging ability and reducing power activity. These findings gave credence to the notion that GSE can be applied topically to prevent aging (Hamblin & Huang, 2013). In addition to protective actions against the formation of ROS, antioxidants could also increase UV absorption of sunscreen agents (Galanakis, Tsatalas, & Galanakis, 2018). For instance, Martinsigh and Ollengo (2016) demonstrated that the coverage of two UVB-filters was broader after the addition of GSE, and the spectra of a UVA filter with avobenzone and GSE was extended to the visible region. However, Table 5 shows that 3% (w/w) of GSE added to the stable F6 possessed very low UV protection and less PPD while adding 3% (w/w) GSE into an over-the-counter brand sunscreen significantly boosted the SPF value up to at least 5 units and PPD up to 1 unit. Although the SPF and PPD values increased in the combination of the prepared base creams supplemented with 3% GSE, the protection from UV radiation did not reach a sufficient level. This result indicated that even though the GSE can absorb the UVA, UVB, and blue light spectrum, the extract cannot be used as a single agent in sun protection products. However, GSE was able to boost the SPF/PPD

Table 4. Evaluation of texture and viscosity stability of cream-based formulations before and after the heat/cool cycles.

Formulation	pH		Viscosity		Stability*		Hardness (g)		Cohesiveness		Adhesiveness (g.sec)	
	Before	After	Before	After	Before	After	Before	After	Before	After	Before	After
F1	6.23	6.80	67720.00	22729.50	X	X	0.513	1.046	0.878	1.012	1.994	2.856
F2	5.86	6.04	31868.00	147156.00	X	X	0.862	0.948	0.9484	0.939	2.840	1.829
F3	7.57	7.23	245338.00	Too thick	✓	✓	28.401	53.996	0.8300	0.666	-22.638	-58.008
F4	5.82	5.92	Too thick	Too thick	✓	✓	32.500	29.755	1.0457	0.980	-28.774	-18.902
F5	6.33	6.65	42647.00	40538.00	X	X	0.390	0.576	0.8474	0.867	2.034	2.711
F6	5.93	6.12	100056.50	96073.33	✓	✓	-0.817	10.202	0.900	0.947	-2.636	-4.172
F7	7.55	7.34	236434.00	190740.50	✓	✓	18.122	14.687	0.8102	0.813	-13.405	-10.273
F8	5.86	6.09	Too thick	319619.00	✓	✓	25.727	26.876	0.9851	0.965	-17.040	-19.841

*Stability before and after heat/cool cycles/ X – not stable formulations underwent phase separation (breaking); ✓– stable formulations.

Table 5. SPF/PPD factors of sunscreen base with the addition of 3% and 5% GSE and over-the-counter brand product alone and with 3% GSE.

Factor	Base	Base + GSE 3%	Counter-brand sunscreen	Counter-brand sunscreen + GSE 3%
SPF	1.13±0.08 [1]	1.29±0.12	18.22±3.53*	23.16±0.75*
PPD	1.034±0.06	1.19±0.07	6.84 (2+)±3.57*	9.50 (3+)±1.20*

[1]Each value is the average of four measurements ± standard deviation *P<0.05
SPF = sun protection factor, PPD = persistent pigment darkening, *GSE = grape seed extract*

value of a sunscreen product. Along with these findings, Limsuwan and Amnuikit (2017) reported an increase of SPF value after incorporating a 1% (w/w) of grape seed extract in sunscreen lotion that contained organic and inorganic UV filters. The SPF boosting and photo stabilization effects of antioxidants in combination with formulations contained in sunscreen agents have been reported in several studies (Afonso *et al.*, 2014; Galanakis *et al.,* 2018). Earlier studies by Ramos *et al.* (1996) explained that the increase of SPF values by the synergism of natural polyphenols and sunscreen agents was due to the structural analogy. These findings confirmed the assumption that incorporation of grape seed extract in the formulations that contained sunscreen agents is likely to enhance the protection from UV radiation and also reduce the use of additives that boost the UV absorption efficacy (Anitha, 2012; Shaath, 2007).

4. Conclusions

The combination of a prepared cream base and grape seed extract did not provide sufficient protection from UV radiation. However, the addition of grape seed extract to an over-the-counter brand product could boost the SPF and PPD values of a sunscreen formulation. The incorporation of GSE in the formulations that contained sunscreens is likely to enhance the protection from ultraviolet and blue light radiation and also reduce the use of additives designed to boost UV absorption efficacy. However, it would be worth formulating sunscreen formulations containing GSE as a sunscreen booster in a cream and using a standard method of SPF/PPD testing to confirm this screening method.

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