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Ultraviolet Absorbing Efficacy and Photostability of Feruloylated Soybean Oil

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Abstract Feruloylated soy glycerides (FSG) are a natural-based, ultraviolet (UV) absorbing, antioxidant vegetable oil synthesized from the lipase-catalyzed transesterification of ethyl ferulate and soybean oil. Commercial broad spectrum UV absorbing formulations contain multiple UV absorbing compounds that absorb UV radiation in specific regions. The most commonly used compounds are Avobenzone (AVO, λ_{max} 356 nm) and Octinoxate (ONX, λ_{max} 310 nm), which absorb primarily UVA and UVB radiation, respectively. The FSG chromophore is chemically similar to ONX but has a λ_{max} of 328 nm, approximately the median λ_{max} of AVO and ONX. Equimolar mixtures of AVO-ONX and AVO-FSG, 50 μM:50 μM solutions in ethanol were compared to determine if FSG was fungible for ONX in total absorbance capacity, photostability when exposed to UV radiation and broad spectrum absorbance coverage before and after exposure to UV radiation. While it was determined that AVO-FSG mixtures possessed statistically indistinguishable total absorbance capacity compared to AVO-ONX solutions, AVO-FSG possessed slightly better photostability after 4 h of UV exposure, based on 95 % confidence interval comparisons from weighted regression equations. Substituting FSG for half of the ONX (e.g. 50 μM:25 μM:25 μM AVO-ONX-FSG) resulted in the best mixture with total absorbance capacity and photostability statistically equal to the AVO-ONX mixtures but with statistically superior broad spectrum UV absorbance compared to AVO-ONX and AVO-FSG mixtures. The natural, vegetable oil-based FSG can be substituted on an equimolar bases for ONX in mixtures with AVO to produce formulations with similar to superior efficacy.

Keywords Antioxidant · Avobenzone · Feruloyl soy glycerides · Octinoxate · Photostability · Ultraviolet absorbance

Introduction

Market research suggests that there is a growing demand for "Organic," "Natural," and "Naturally Derived" cosmeceutical and skin care ingredients in the personal care and beauty markets. The organic personal care and beauty markets were estimated to be US$11 BB in 2016 and are forecasted to double to US$20 – 22 BB by 2025. [1-3] Consequently, considerable effort has been made to research, identify, isolate, and assess the efficacy of natural, plant-derived extracts, compounds, and oils as bioactive ingredients in the personal and skin care industry. [4] The natural plant compounds and oils are used as bioactive antioxidants, ultraviolet (UV) absorbers, exfoliants, emulsifiers, and moisturizers in anti-aging, anti-wrinkling, rejuvenating, and broad spectrum UV protecting formulations. [5-8]

One category of plant-derived, natural-based ingredients that has gained interest in the personal care and beauty industries is feruloylated vegetable oils. First reported nearly two decades ago, the enzyme-catalyzed transesterification of the ethyl ester of ferulic acid and soybean oil resulted in feruloylated triacylglycerols, which combine the UV absorbing and antioxidant properties of ferulic acid with the hydrophobicity of vegetable oils. [9] Ferulic acid (a cinnamate) is ubiquitous throughout the plant kingdom, generally present as esters in the lignin and hemicelluloses of plant cell walls and in the suberin and waxy surfaces of leaves. Ferulic acid is also present esterified to phytosterols in grains such as rice and corn bran, and is a component of the human diet. Ferulic acid has limited use as a bio-based active in cosmetic and personal care formulations due to its water solubility and tendency to yellow when applied to the skin. These deficiencies are alleviated by the transesterification of the feruloyl group onto a vegetable oil triacylglycerol. [10]

The lipase-catalyzed transesterification of ethyl ferulate and vegetable oil has been described in a variety of solvents including, conventional organic solvents, ionic liquids, and supercritical carbon dioxide. [9,11-12] Over the years, studies have also focused on the optimization of the transesterification reaction by examining the transesterification efficiency of different ferulate ester substrates [13], deacylation of vegetable oils via gylcerolysis prior to the transesterification with ethyl ferulate [14], and response surface methodology to optimize such parameters as reaction temperature, time, and enzyme load. [15] The ideal solvent for the lipase-catalyzed transesterification of ethyl ferulate proved to be the vegetable oil itself as described in the backed-bed, enzymatic bioreactor transesterification using soybean oil at 60 °C as the mobile phase in the semi-continuous process. [16] It is this bench-scale bioreactor design and reaction parameters described therein using vegetable oil as the solvent that has been scaled to the 1 metric ton/year, modular, continuous, packed-bed, bioreactor production of feruloylated vegetable oil. [17-18]

The interest of feruloylated vegetable oils in the personal care and health and beauty industries is as a naturally derived antioxidant and UV absorber. Feruloylated vegetable oils possess both UV absorbing capacity (λ_{max} 328) and antioxidative capacity, and a small, limited, independent laboratory clinical trial determined that feruloylated vegetable oils have an empirical benefit as an emulsifier and moisturizer in anti-wrinkling applications. The antioxidant capacity of the feruloylated vegetable oils as a free radical scavenger and lipid oxidation inhibitor has been well documented [19-22]; however, to date, the UV absorbing capacity, photostability, and broad spectrum absorbance capacity of the feruloylated vegetable oils have not been determined. This information is essential for the further adoption of the feruloylated vegetable oils as ingredients by the personal care and health and beauty industries.

Focusing on the potential of feruloylated vegetable oils as a UV protecting bioactive in formulations, it is helpful to provide a primer on the United States Food and Drug Administration's (FDA) and European Commission's (EC) definition of the UV spectrum as it pertains to active ingredients. The FDA and EC divide UV radiation into ultraviolet A (UVA) and B (UVB), and the FDA further subdivides the UV regions as follows, UVA I (400 – 340 nm), UVA II (340 – 320 nm) and UVB (320 – 290 nm). [23-24] To protect from solar UV irradiation a cosmetic, skin care, or personal care formulation must contain substances that block (e.g. TiO_2, ZnO) or absorb (e.g. Avobenzone, Octinoxate) the entire UVA and UVB spectrum. Solar radiation < 290 nm, ultraviolet C, is absorbed by stratospheric oxygen and forms ozone, which partially absorbs solar UVB radiation. [25] The immediate, acute consequence of unprotected exposure to UVB is inflammation of the skin, "sun burn," and reddening of the skin, erythema, while chronic, unprotected exposure to UVB has been correlated to increased incidents of skin cancers. [26-27] Chronic, unprotected UVA exposure has been shown to result in damage to DNA, proteins, and lipids and has been demonstrated to cause DNA lesions and mutations in human skin cells. [26] The FDA and EC categorize and regulate UV absorbing/blocking active ingredients as Category 1, Over-the-Counter drugs. [23-24]

Transition metal oxides provide sustained efficacy as physical blockers of both UVA and UVB in formulations; however, the natural opaqueness of these inorganic oxides is an undesirable characteristic often rejected by consumers. Research continues to micro-size (< 100 μm) and nano-size (< 100 nm) transition metal oxides to obtain formulations resulting in translucent topical applications. The effect of micro- and nano-sized transition metal oxide particles on UV attenuation, physicochemical characteristics, biological health, and the environment has yet to be determined. [28]

Organic, UV absorbing compounds possess heteroatom functional groups, aromatic rings, and unsaturated carbon chains, resulting in electron delocalization energies that correspond to electron transitions in the UVA and UVB regions. [29] Specific chemical structure classes (e.g. cinnamates, salicylates, benzophenones) of the organic UV absorbers correlate to narrow UV absorbing maxima, and multiple compounds must be used to provide broad spectrum protection in formulations. [30] A commonly used blend of UV absorbing compounds in commercial formulations is the benzophenone, Avobenzone (λ_{max} 356), and the cinnamate, Octinoxate (λ_{max} 310). Use of these and similar compounds is quite high and can consist up to 25 wt% or vol% of the retail formulation. [23-24] Concerns have been raised about potential adverse health and ecological effects of these commonly used biologically active organic compounds. [31-32] The estrogenic activity of several of the commercial UV absorbing organic compounds have been demonstrated *in vitro* and *in vivo*. [33] The bioaccumulation and environmental impact of these compounds has yet to be definitively determined, however, some studies suggest that these compounds are present in trace amounts in the central European water shed and are considered ubiquitous trace contaminants in aquatic systems. [31] These concerns have motivated efforts to develop natural, plant-derived extracts, compounds, and oils as benign, alternative, bioactive ingredients in the personal and skin care industry.

Feruloylated vegetable oils are a potential, natural-based alternative for some petroleum-based, commercially used UV absorbing organic compounds with the added benefit of possessing antioxidant capacity. Herein, we examine the UV absorbance capacity, photostability, and broad UV spectrum absorbance of feruloylated soybean oil and compared them to commercial UV absorbing compounds. Additionally, the feruloylated soybean oil's intrinsic antioxidant capacity was determined and compared to that of the commercial UV

absorbing compounds. The determination of the UV absorbing and photostabalizing efficacy of feruloylated vegetable oils compared to and used in conjunction with commercial UV absorbing compounds is crucial to the adoption of feruloylated vegetable oils as a natural, plant derived active ingredient in the personal care industry.

Experimental Procedures

Materials

2-Ethylhexyl-4-methoxycinnamate (Octinoxate, ONX), 4-tert-butyl-4'-methoxydibenzoylmethane (Avobenzone, AVO), 6-*O*-palmitoylascorbic acid (Ascorbyl Palmitate, ABP), and 2,2-diphenyl-1-picrylhydrazyl (DPPH) and all solvents were purchased from Sigma-Aldrich (St. Louis, MO, USA) and used as obtained.

Methods

Feruloyl Soy Glycerides (FSG)

The transesterification of soybean oil (SBO) with ethyl ferulate to produce feruloyl soy glycerides was conducted in a pilot-scale, continuous, packed-bed, enzymatic bioreactor as described previously. [17]

Irradiations

The protocol for irradiating the UV absorbing analytes was adapted from Huong et al. [34] Samples were irradiated using a Heraues Suntest CPS+ sun simulator with β-filter (Atlas Material Testing Solutions, Mount Prospect, IL, USA) calibrated by the manufacturer. The Suntest CPS+ sun simulator was cooled by a Neslab CFT-33 Refrigerated Recirculator (ThermoFisher Scientific, Waltham, MA, USA) set at 10 °C. The radiant flux at the sample surface was monitored using an International Light (Newburyport, MA, USA) IL 1400A Radiometer/Photometer fitted with International Light XRL340B 6134 (spectral range 326 –401 nm) and SEL240 (spectral range 265 – 332 nm) sensors calibrated by the manufacturer.

Samples of the UV absorbing analytes were prepared as 50 μM solutions in ethanol and dispensed into 3.5-mL, Teflon septum sealed, screw cap, quartz cuvettes (AdValue Technology, Tucson, AZ, USA). The cuvettes were threaded with Teflon tape for a tighter cap fit to prevent leaking and evaporation. The cuvettes were exposed horizontally in the Suntest CPS+ chamber to 32.5 ± 0.5 W/m^2 irradiation for up to 4 h (for relative comparison, the irradiance of the sun on June 8, 2017, 12:00 pm, a clear day in Peoria, IL, USA, was 34.4 ± 0.6 W/m^2). The Suntest CPS+ chamber temperature was 35.7 – 39.0 °C during the irradiations. Samples were weighed before and after irradiation to confirm that the samples did not leak or evaporate. Irradiations were conducted in triplicate.

UV Spectroscopy

Spectra were recorded on a Shimadzu (Shimadzu Scientific Instruments, Inc., Addison, IL, USA) UV-1280 UV-Vis Spectrophotometer in 1 cm path length, 3.5 mL volume quartz cuvettes

described above, with a scan range of 400 – 290 nm, scan speed of medium, and scan pitch of 1.0 nm. UV spectra were obtained at 5, 30, or 60 min intervals over the course of the irradiations.

Relative Absorbance

To compare the absorbance capacity of the UV absorbing analytes, the area under the UV spectra curves were determined using Eq. 1, where $A_{\int}$ is the integrated area under the UV absorbance, A, spectrum curves recorded from 290 – 400 nm. [30]

The integrated areas for each analyte over the course of an irradiation were normalized using Eq. 2, where A_N is the normalized area of the UV spectrum curve of the analyte relative to the area of the UV spectrum curve of the analyte at t = 0 min, $A_{\int 0}$.

To compare the broad spectrum UV absorbing capacity of the analytes, the ratio of the mean UVA to UVB absorbance, $A_{A/B}$, was calculated as suggested by Gaspar and Campos [35] and Diffey [36], using the current U.S. FDA and EU Commission definition of UVA (400 – 320 nm) and UVB radiation (320 – 290 nm), Eq. 3. [23-24]

$$A_{\int} = \int_{290}^{400} A(\lambda)\, d\lambda \quad (1)$$

$$A_N = A_{\int} / A_{\int 0} \quad (2)$$

$$A_{A/B} = \left(\int_{320}^{400} A(\lambda)\, d\lambda \,/\, \int_{320}^{400} d\lambda\right) / \left(\int_{290}^{320} A(\lambda)\, d\lambda \,/\, \int_{290}^{320} d\lambda\right) \quad (3)$$

Experimental Design and Statistical Analysis

The absorbance values calculated from Eq. 1-3, $A_{\int}$, A_N, and $A_{A/B}$, for each irradiation experiment were analyzed using weighted regression on mean values ($n = 3$) of $A_{\int}$, A_N, and $A_{A/B}$ over time with a standard weighting factor of 1/variance. The three experimentally determined absorbance variables were $Y = A_{\int}$, $Y = A_N$, and $Y = A_{A/B}$. The X-variable of time was measured at 0, 30, 60, 90, 120, 150, 180, 210, and 240 min. Nine irradiance experiments were statistically analyzed for significant differences in $A_{\int}$ and A_N, AVO, ONX, FSG, AVO-SBO, AVO-ONX, AVO-FSG, AVO-ONX-FSG, AVO-ABP, and ONX-FSG. Seven irradiance experiments were statistically analyzed for significant differences in $A_{A/B}$, AVO, ONX, FSG, AVO-ONX, AVO-FSG, AVO-ONX-FSG, and AVO-ABP. 95% confidence intervals on predicted values from weighted regressions were used to compare the UV absorbing compounds within an experiment at each time period. TableCurve 2D ver. 5.01 (copyright © SYSTAT Software Inc., 2002) was used for all weighted regression equations and confidence intervals. The experimental mean and standard error values are presented for $A_{\int}$ (**Fig**. **4** – **6**, *top panels*), A_N, (**Fig**. **4** – **6**, *bottom panels*) and $A_{A/B}$ (**Fig. 8**). Mean values within a single time period (e.g. 0, 30, 60 min) sharing the same letter are not significantly different based on overlap of 95% confidence intervals of predicted $Y = A_{\int}$, $Y = A_N$, and $Y = A_{A/B}$ values from weighted regression equations (see *Supplementary Material* **Tables S2, S4, and S6**).

Free Radical Scavenging Capacity

The free radical scavenging capacity was measured using a previously described DPPH antioxidant assay with the modification where DPPH spontaneously forms free radicals in ethanol solutions. [20,37-38] The DPPH assays were conducted in triplicate.

Results and Discussion

UV Absorbing Compounds

To obtain broad UV spectrum absorbance multiple compounds are used in formulations. Two of the most common compounds used commercially are AVO and ONX, also known as octyl methoxycinnamate (**Fig. 1**). AVO is a dibenzoylmethane derivative used at up to 6 wt% in formulations and absorbs nearly the entire UVA II and UVA I radiation spectrum (**Fig. 2**). [24] AVO is oil soluble and is not absorbed by the skin as other UVA organic filters are (e.g. oxybenzone). [39] AVO undergoes a keto-enol tautomerization and exists mostly as the enol form which is responsible for AVO's λ_{max} at 356 nm. [39] ONX is a benzylcinnamate with a methoxy group in the *para*-position that is used up to 10 wt% in formulations (**Fig. 1**). [24] The ethylhexyl group lowers ONX's water solubility compared to other benzylcinnamates and promotes solubility in lipophilic-based creams and lotions. ONX absorbs mostly in the UVB region (**Fig. 2**) with a λ_{max} at 310 nm, corresponding to an electronic transition that delocalizes an electron across the entire benzoyl structure from the methoxy group to the carbonyl group. [29]

FSG are a covalent combination of the natural plant component, ferulic acid, and soybean oil. The enzymatic transesterification of ethyl ferulate and soybean oil results in a mixture of

molecular species, the most prevalent being a triacylglycerol with one feruloyl moiety and two soybean oil fatty acid groups (**Fig. 1**). [40] Another minor, triacylglycerol species possesses two feruloyl moieties and a single fatty acid chain. There are also minor diacylglycerol species with two feruloyl moieties and no fatty acid chains and diacylglycerol species with one feruloyl moiety and one fatty acid chain. The balance of the product mixture is unreacted soybean oil tri, di, and monoacylgycerides and unreacted ethyl ferulate. [40] The concentration of the feruloylated species in the mixture, [Total Ferulates], was determined to be 930 mM. [17] The chemical structure of the FSG chromophore, ferulic acid, is very similar to that of the ONX chromophore, *p*-methoxycinnamate (**Fig. 2**). FSG presumably undergoes a similar electron delocalization as ONX; however, ferulic acid possesses a methoxy group in the *meta*-position and a hydroxyl group in the *para*-position resulting in an electronic transition that corresponds to an energy that is red shifted by 18 nm to a λ_{max} 328 nm compared to ONX (**Fig. 2**).

The photostability studies of FSG's influence on AVO and ONX were conducted in solutions of ethanol. It should be noted that solvent polarity has a significant effect on the λ_{max} and extinction coefficient, ε (L M^{-1} cm^{-1}), of the UV absorbing compounds. ONX showed a $\Delta\lambda_{max}$ = 23 nm in solvents ranging in polarity from mineral oil (λ_{max} 289 nm) to 70% aqueous ethanol (λ_{max} 312nm). [41] AVO possessed a lower $\Delta\lambda_{max}$ = 6 nm in solvents ranging in polarity from hexane (λ_{max} 550 nm) to ethanol (λ_{max} 556 nm). [42] For convenience of solubility of the FSG component species, AVO, and ONX the photostabilty studies herein were conducted in ethanol solutions. The ε of AVO (λ_{max} 556 nm) and ONX (λ_{max} 310 nm) have been reported as 34,000 and 23,300 – 24,000 L M^{-1} cm^{-1}, respectively, in ethanol solutions.[41-42] The ε of FSG (λ_{max} 328 nm) was measured in ethanol as the slope of the linear regression of FSG absorbance vs. FSG concentrations ranging from 10 – 100 μM and determined to be 7,900 ± 500 L M^{-1} cm^{-1}

(n = 3). The difference in extinction coefficients of the UV absorbers in ethanol solutions is illustrated by their different absorbance intensities at their respective λ_{max} (**Fig. 2**).

Photostability of the UV Absorbing Compounds

Solutions of AVO, ONX, and FSG were exposed to UV radiation and their absorbance spectra (290 – 400 nm) were measured periodically over 4 h. All three UV absorbing analytes showed a loss of UV absorbing capacity over time. **Fig. 3** shows the spectra of a FSG (50 μM) solution in ethanol over 4 h of UV irradiation. It is qualitatively evident from the loss of absorbance intensity at FSG's λ_{max} 328 nm that FSG loses UV absorbance capacity over the course of the irradiation. Control experiments were conducted to eliminate temperature as a contributing factor to the photodegradation and loss of absorbance capacity of the UV absorbing analytes. The temperature at the sample surface of the Suntest CPS+ sun simulator ranged from 35.7 to 39.0 °C. Solutions of AVO, ONX, and FSG (50 mM) in ethanol were incubated at 40 °C in the dark and UV spectra of the solutions were measured every 30 min for 4 h (data not shown). The ethanol solutions of AVO, ONX, and FSG exhibited no loss of absorbance intensity; therefore, it was concluded that neither ethanol nor temperature contributed to the analytes' degradation and loss of absorbance capacity.

To quantify the loss of FSG absorbance capacity and for relative comparison to AVO and ONX absorbance capacities, the area of the absorbance curves were integrated (**Eq. 1**) and the integrated areas were normalized at time points over the course of the 4 h irradiation relative to the initial area of the absorbance curve at t = 0 (**Eq. 2**). As discussed above, the UV absorbing chromophores of FSG and ONX are structurally similar (**Fig. 1**). The loss of absorbance

capacity of ONX solutions in ethanol upon exposure to UV radiation has been studied. [34,43] The aliphatic C=C bond in ONX and FSG exist mostly in the *trans* form as depicted in **Fig. 1**. [44] Exposure of ONX solutions to UV radiation results in the photoisomerization of the aliphatic C=C from the *trans* to a *cis* conformation. [34,39,43-44] The *cis*-ONX has an ε approximately half that of the *trans*-ONX, explaining the loss of UVB absorbance capacity. [43] **Fig. 3** shows the loss of FSG absorbance capacity over time during UV irradiation, and the FSG chromophore is presumed to undergo a similar *trans* to *cis* photoisomerization. At t = 0 and 30 min, FSG and ONX have the same relative absorbance capacity, A_J (**Fig. 4** *top pannel*), although FSG absorbs primarily in the UVA II region (**Fig. 2**). After UV irradiation both FSG and ONX lost ~ 25 % absorbance capacity after 30 min as illustrated by the normalized absorption capacity, A_N, in **Fig. 4** *bottom panel*. After 4 h of irradiation ONX had lost 29 % of its absorbance capacity and FSG had lost 33 % of its absorbance capacity. The initial rapid ~ 25 % loss of absorbance capacity followed by a prolonged photostationary state and relatively stable absorbance capacity is typical for solutions of ONX. [39,43] The similarity in chemical structure of the FSG chromophore to ONX (**Fig. 1**) and statistically indistinguishable loss of relative absorbance capacity over time upon UV irradiation (**Fig. 4** *bottom panel*) demonstrated that FSG functions similarly to ONX.

Unlike the rapid, initial loss of absorbance capacity exhibited by FSG and ONX, solutions of AVO steadily declined in absorbance capacity over time upon UV irradiation (**Fig. 4** *bottom panel*). AVO had a much higher relative absorbance capacity, A_J, compared to FSG and ONX as 50 μM solutions in ethanol. After 30 min of UV irradiation AVO had lost only 10 % of absorbance capacity compared to FSG and ONX, ~ 25 % (**Fig4.** *bottom panel*). After 4 h of irradiation AVO had lost 23 % of its absorbance capacity, which was determined to be

statistically different than the ~ 30 % loss exhibited by FSG and ONX. The AVO absorbance at λ_{max} 556 nm is attributed to a π to π^* transition of the molecular structure's enol form. [45] Upon exposure to UV irradiation, it is believed that AVO undergoes an enol-keto tautomerization to the keto form which has a λ_{max} 245, resulting in AVO's loss of absorbance capacity in the UVAI region. [45] This enol-keto tautomerization is reversible in acetonitrile where AVO regains a portion of its UVA absorbance capacity when left in the dark; however, AVO's loss of UVA I absorbance capacity in ethanol and hexane is irreversible, suggesting that the keto tautomer may be unstable and degrade in these solvents. [42] The 23 % loss of AVO absorbance capacity observed in this study is similar to that determined in other AVO photostability studies, ~ 20 %, after 4 h of UV irradiation. [42]

While FSG primarily absorbed radiation in the UVA II region compared to ONX, which absorbed primarily in the UVB region, the relative absorbance capacity and relative photostability of FSG was the same as ONX. This result encouraged further investigation into whether FSG was fungible with ONX in mixed solutions with AVO.

Photostability of Mixtures of the UV Absorbing Compounds

As discussed previously, to provide broad spectrum UV protection formulations must contain mixtures of UVA absorbing and UVB absorbing compounds. The most commonly used combination is AVO and ONX. We wanted to determine if FSG provided equal absorbance capacity and photostability as ONX when formulated with AVO. An equimolar mixture of AVO and ONX (AVO-ONX) had a UV absorbance capacity (A_J 147) about 50 % greater compared to AVO (A_J 97) and about 220 % greater compared to ONX (A_J 45), **Fig. 4** and **5**, *top panels*. It was

evident that the total absorbance capacity of the AVO-ONX mixture was additive of the individual absorbance capacities of AVO and ONX. Replacing the ONX with FSG resulted in an equimolar mixture of AVO-FSG with a total absorbance capacity of A_J 141, which was statistically indistinguishable from AVO-ONX (**Fig. 5**, *top panel*). To show that the residual SBO in the FSG did not contribute to the absorbance capacity of AVO-FSG mixtures, an AVO-SBO mixture was tested as a control. The control AVO-SBO mixture possessed an A_J 97, the same as AVO, proving that the SBO does not possesses a UV absorbance or blocking capacity at the concentrations tested (**Fig. 4** and **6**, *top panels*). Additionally, the palmitoyl ester of vitamin C, ABP, has been shown to photostabalize AVO when exposed to UV radiation (discussed below). [35] A control mixture of AVO-ABP showed, however, that ABP does not contribute any absorbance capacity to AVO-ABP mixtures (**Fig. 4** and **6**, *top panels*). Based on relative total absorbance capacities, FSG was fungible for ONX in equimolar solutions with AVO.

The photostability (A_N) of AVO-ONX (50 μM:50 μM) solutions in ethanol was determined over 4 h of UV irradiation (**Fig. 5**, *bottom panel*). The AVO-ONX mixture lost 15 % absorbance capacity after 30 min of UV irradiation followed by photostabalization for the next 3.5 h (**Fig. 5**, *bottom panel*). The AVO-ONX mixture was more stable than either AVO (ΔA_N -23 %) or ONX (ΔA_N -29 %), individually, after 4 h of UV irradiation (**Fig. 4** and **5**, *bottom panels*). While AVO and ONX have unique photodegradation mechanisms when irradiated with UV, discussed above, it has been suggested that mixtures of AVO and ONX undergo photo-induced cycloaddition to form photoadducts that stabilize the chromophores relative to the individual filters. [30,46] Thus, the photostability of mixtures of the UV absorbing compounds is not predictable based on the photostability of the individual compounds. It was not certain,

despite FSG's similar structure, absorbance capacity, and photostability to ONX, if AVO-FSG mixtures would perform similarly to AVO-ONX mixtures.

The photostability (A_N) of equimolar AVO-FSG (50 μM:50 μM) solutions in ethanol was determined over 4 h of UV irradiation (**Fig. 5**, *bottom panel*). The AVO-FSG solution lost 10 % of its absorbance capacity after 30 min of UV irradiation. The AVO-FSG performed slightly better, retaining ~ 5 % more absorbance capacity than the AVO-ONX, at the 30 min interval (**Fig. 5**, *bottom panel*) and photostabalized over the next 3.5 h at ΔA_N -13 %. Over the course of the 4 h UV irradiation the AVO-FSG mixture was statistically slightly more photostable (ΔA_N -13 %) than the AVO-ONX mixture (ΔA_N -17 %). Mixtures of AVO-ONX-FSG (50 μM:25 μM: 25 μM) were also tested where half of the ONX was replaced with FSG on a molar basis. The ternary mixture possessed a statistically slightly higher total absorbance capacity, A_J 156, relative to the statistically equivalent AVO-ONX, A_J 147, and AVO-FSG, A_J 141, binary mixtures (**Fig. 5**, *top panel*). The ternary AVO-ONX-FSG mixture lost 13 % of its absorbance capacity after 30 min of UV irradiation and remained photostabilized at ΔA_N -15 % for the next 3.5 h of irradiation (**Fig. 5**, *bottom panel*). Statistical analysis determined that the photostability of the ternary mixture (ΔA_N -15 %) over 4 h of UV irradiation was equivalent to the AVO-ONX (ΔA_N -17 %) mixture, which both performed statistically slightly worse than the AVO-FSG (ΔA_N -13 %). Overall, all three equimolar mixtures examined possessed very similar total absorbance capacities, A_J, and photostabilities, A_N, confirming that FSG was a fungible replacement for ONX in binary and ternary mixtures with AVO.

An advantage that FSG possesses over ONX as a component of binary mixtures with AVO is that FSG has been demonstrated to possess antioxidant properties as a free radical scavenger. [17] **Fig. 7** shows the free radical, DPPH*, scavenging capacity of the UV absorbing

compounds examined in this study and ABP. It is clear that neither AVO nor ONX possessed antioxidant free radical scavenging capacities. FSG performed as a rapid free radical scavenger as defined by Huang et al [37], 50 % reduction in DPPH* concentration in 5 min, and ABP was ~ 100x stronger antioxidant than FSG at the concentrations tested. The non ester form of ABP, vitamin C, has been shown to provide photostability to AVO in thin films. [35] Equimolar AVO-ABP (50 μM:50 μM) solutions in ethanol possessed statistically equivalent total absorbance capacities, $A_{\int}$ 101, relative to AVO solutions, $A_{\int}$ 97 (**Fig. 4** and **6**, *top panel*s), but significantly less total absorbance capacity than AVO-FSG mixtures, $A_{\int}$ 141 (**Fig. 5** and **6**, *top panel*s). As previously demonstrated in thin films, ABP provided photostabilization to AVO. Equimolar AVO-ABP (50 μM:50 μM) solutions in ethanol lost 16 % absorbance capacity after 4 h of UV irradiation compared to AVO, ΔA_N -23 %. The AVO-ABP mixtures possessed a slightly lower photostability than the AVO-FSG mixtures, ΔA_N -13 %, after 4 h of UV exposure. As a replacement for ONX in binary mixtures with AVO, FSG possessed antioxidant free radical scavenging capacity that ONX did not. FSG also had the advantage of providing additional total absorbance capacity to binary mixtures with AVO and possessed slightly higher photostability in mixtures with AVO compared to AVO mixtures with the antioxidant, ABP.

Broad Spectrum Absorbance Capacity of the UV Absorbing Compounds

The relative broad spectrum absorbance capacities of the UV absorbing compounds examined in this study were determined as the ratio of the mean UVA to the mean UVB absorbance, $A_{A/B}$, as described in the literature (Eq. 3). [30,35-36] The theoretical value of $A_{A/B}$ = 1.00 represented the ideal broad spectrum absorbance capacity where there was equal absorbance of the UVA

region and UVB region with each region weighted for total wavelength. It should be noted that the relative mean UVA to the mean UVB absorbance ratios determined in this study are not the same as the *in vitro* measurements required by the U.S. FDA to determine a critical wavelength of $\geq$ 370 nm to legally label a sunscreen as "Broad Spectrum." [24]

The $A_{A/B}$ values of the three UV absorbing compounds in ethanol solutions are shown in **Fig. 8** *top panel*. AVO with a λ_{max} 356 (**Fig. 2**) absorbed much more strongly in the UVA I and II regions and had an $A_{A/B}$ of 2.63 while ONX (λ_{max} 310) which absorbs much more strongly in the UVB region (**Fig. 2**) had an $A_{A/B}$ of 0.11. FSG (λ_{max} 328) absorbed most strongly in the UVA II region (**Fig. 2**), and at equimolar concentrations to AVO and ONX had an $A_{A/B}$ of 0.53. Over the course of 4 h of UV irradiation the $A_{A/B}$ value of AVO decreased to 2.17 ($\Delta A_{A/B}$ -0.46), showing that the AVO's loss of absorbance capacity upon UV exposure occurs mostly in the UVA region. ONX and FSG showed no significant increase or decrease of their relative mean UVA to the mean UVB absorbance ratios, $\Delta A_{A/B}$ -0.01 and $\Delta A_{A/B}$ -0.01, respectively. This suggested that ONX's and FSG's loss of absorbance capacity over the course of 4 h of UV irradiation was equally distributed across their UVB and UVA absorbance spectra. The $A_{A/B}$ values of the UV absorbing compounds before and after UV irradiation—none close to $A_{A/B}$ = 1.00—confirmed that the UV absorbing compounds cannot be used individually to provide adequate broad spectrum absorbance.

Equimolar solutions of AVO-ONX (50 μM:50 μM) in ethanol had an initial $A_{A/B}$ of 0.78 before exposure to UV radiation (**Fig. 8** *bottom panel*). This $A_{A/B}$ value was similar to values measured for thin films of formulations containing AVO and ONX with the addition of the UVB absorbing compound octocrylene (172 mM:60 mM: 193 mM), $A_{A/B}$ 0.74. [30] Comparatively, substituting FSG for ONX, equimolar solutions of AVO-FSG (50 μM:50 μM) in ethanol had an

$A_{A/B}$ of 1.42 before exposure to UV radiation. As could be surmised from the UV spectra of the individual compounds (**Fig. 2**), the $A_{A/B}$ values confirmed that mixtures of AVO-ONX had more complete absorption coverage of the UVB region and lacked strong coverage of the UVA II region of the spectrum; thus, an $A_{A/B} < 1.00$. Substituting FSG for ONX was expected to result in a mixture with stronger absorption coverage in the UVA region but weaker coverage of the UVB region, which was evidenced by an $A_{A/B} > 1.00$. Examining the λ_{max} of the individual compounds (**Fig. 2**), it was hypothesized that partially substituting FSG for ONX would result in a mixture with superior broad spectrum coverage. Ethanol solutions of AVO-ONX-FSG (50 μM:25 μM:25 μM) where half of the ONX was substituted with FSG resulted in $A_{A/B}$ values closest to unity, $A_{A/B}$ 1.04, proving that in the AVO-ONX-FSG mixtures FSG absorbed radiation in the UVA II gap that existed in mixtures of AVO-ONX. Over 4 h of UV irradiation, the AVO-ONX and AVO-FSG mixtures gained 0.10 and 0.03 units to finish with $A_{A/B}$ 0.88 and 1.47, respectively (**Fig. 8**). The AVO-ONX-FSG mixture had an $A_{A/B}$ 1.14, a gain of 0.10 units, after the UV irradiation. The increase in $A_{A/B}$ over the course of the UV exposure was expected based on the more pronounced loss of absorption capacity of the UVB absorbers, ONX and FSG, than the UVA absorber, AVO, over the 4 h of UV irradiation, discussed above. Overall, mixtures of AVO-ONX-FSG provided the best initial broad spectrum absorbance coverage and its broad spectrum absorbance was slightly higher in the UVA region, by the same amount as AVO-ONX solutions, after 4 h of UV irradiation.

Conclusion

The efficacy of FSG as a UVB absorber was determined and compared to the efficacy of the commercial UVB absorber, ONX, in mixtures with the commercial UVA absorber, AVO. The total absorbance capacity, photostability, and broad spectrum coverage were determined for the mixtures and statistically compared. On a molar basis, FSG was fungible with ONX, providing statistically indistinguishable total absorbance capacity and photostability in mixtures with AVO over the course of 4 h of UV irradiation. FSG absorbed more strongly in the UVA II region compared to ONX and mixtures with half the ONX substituted with FSG in mixtures with AVO provided the best broad spectrum absorbance before and after 4 h of UV irradiation. The use of FSG in mixtures with AVO provided the additional benefit of intrinsic antioxidant capacitance that the commercial UV absorbers lack.

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