Influence of grape seed oil on sun protection factor in sunscreen formulations: a study using Central Composite Design approach

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This study aimed to evaluate the influence of grape seed oil on sun protection factor of sunscreen emulsified formulations containing butyl methoxydibenzoylmethane (avobenzone) and octyl methoxycinnamate developed by phase inversion temperature employing a Central Composite Design. Seventeen emulsions containing different amounts of grape seed oil, avobenzone and octyl methoxycinnamate were obtained according to experimental design. In vitro sun protection factor was determined by spectrophotometric method. As expected, formulations with the highest concentrations of ultraviolet filters, avobenzone and octyl methoxycinnamate, resulted in the highest sun protection factor values, while the lowest values were obtained by formulations containing concentrations of these filters below the central point. In the tested concentrations, the variable octyl methoxycinnamate combined with grape seed oil showed to linearly increase sun protection factor in the formulations. The combination of grape seed oil with octyl methoxycinnamate enhance in vitro sun protection factor. The use of natural antioxidant compounds, such as grape seed oil, is a viable strategy to improve the effectiveness of sunscreens and to protect the human skin against ultraviolet-induced damage.

Introduction

Excessive exposure to solar radiation is associated with many dermatological alterations. Ultraviolet (UV) radiation exposure causes many effects, including erythema, pigment darkening, delayed tanning, epidermal hyperplasia, photoaging, immunosuppression, photocarcinogenesis and exacerbation of photodermatoses (1). Other deleterious effects are deoxyribonucleic acid (DNA) damage, protein degradation and mutations caused by reactive oxygen species (ROS) including free radicals (2). Therefore, sun protective measures are primordial (3). Sunscreens are products developed to protect the skin from the deleterious effects of UV radiation. Its efficacy is expressed by the sun protection factor (SPF), which is calculated by the ratio of the UV energy required to produce a minimal erythematogenic effect to protected and unprotected skin (4).

The active ingredients of the sunscreens are the UV filters that can absorb the UV radiation in the range of UVA (400–320 nm) and/or UVB (320–280 nm) (5). They are classified into organic and inorganic UV filters based on chemical composition and mechanism of action (6). Sunscreens formulations are usually comprised of one or more UV filters, this combination increases the protection giving broad-spectrum sunscreens (5), but it is known that UV filters do not afford 100% protection to the skin (7). Nowadays is recommended the topical application of UV filters and antioxidants to minimize UV damage of the

skin, most of UVA-induced damage is mainly mediated by ROS generated after UV exposure. Natural antioxidants mainly polyphenols and carotenoids, have been used in cosmetic products due to their efficacy in reducing free radical generation and thereby decreasing skin photodamage (7). In addition to bioactivity, natural products are, in general, not harmful for humans, not expensive (4).

Furthermore, the development of products with large number of components of plant origin has been highlighted in the cosmetic field (8, 9). There is a structural similarity among the active compounds of the natural products with photoprotective activity and the synthetic UV filters (10).

Natural products with polyphenols and flavonoids have been studied as UV absorbers, due to their structural similarity to organic UV filters. They have a lower cost and many of them have demonstrated antioxidant activity (11). Some vegetable oils are currently being promoted as a safe alternative to commercial sunscreens (12).

The grape seed is a by-product generated during winemaking. Using grape seed as a raw material contributes to a sustainable development of products, as well as to the recycling and reuse of grape pomace. Since the grape seed oil (GSO) is rich in bioactive compounds it has great potential application in pharmaceutical and cosmetic industry (13). This oil present high content of monounsaturated fatty acids (90%), such as linoleic acid (58-78% w/w), oleic acid (12-28% w/w) and antioxidants such as tannins, phenolic compounds and tocopherol

(vitamin E) (14, 15) which are capable of improving UV protection and epidermal antioxidant activity responsible for premature aging and photoaging (16).

Chu et al. (2021) (16) published a review article about the use of seed oils in sunscreen formulations, and the studies demonstrated that GSO inhibits lipid oxidation and ROS-induced damage and has anti-inflammation properties. However, the effect of GSO on the SPF is not described.

The aim of this study was to evaluate by Central Composite Design (CCD) the influence of GSO in the SPF value of sunscreens emulsions containing organic UV filters such as Avobenzone (AVO) and Octyl methoxycinnamate (OMC) by spectrophotometric method.

Experimental section

Chemicals and reagents

Ethyl alcohol 99.5ºGL was provided by Jalles Machado (Brazil); grape seed oil was obtained from Campestre (Brazil); octyl methoxycinnamate was purchased from All Chemistry® (Brazil); polyethylene glycol 40 (PEG-40) hydrogenated castor oil and sorbitan monooleate were provided by La Belle® (Brazil); and butyl methoxydibezoylmethane (Avobenzone) was purchased from Fagron (Brazil).

Formulations development

Emulsions were obtained by Phase Inversion Temperature (PIT) method (17) and were composed of PEG-40 hydrogenated castor oil (6.2%); sorbitan monooleate (3.8%); GSO, AVO and OMC and distilled water *quantum satis* (q.s.) to complete 30g of each formulation. The GSO and UV filters quantity (%) was determined according to the Central Composite Design (CCD). The blank was prepared with grape seed oil (10%); PEG-40 hydrogenated castor oil (6.2%); sorbitan monooleate (3.8%); and distilled water q.s. 100%. To prepare each formulation, the oil phase (OP) (oil and surfactants) and the aqueous phase 1 (AP1) were weighed separately and in the same total amount. A second amount of water (aqueous phase 2 – AP2) was also weighed and kept apart to complete the formulation.

OP and AP1 were heated to 70ºC and AP1 was poured onto OP slowly under manual stirring. After the emulsification process, samples were returned to manual stirring under heating, until the phase inversion temperature (approximately 90ºC) was reached. Then, emulsions were removed from heating and AP2 (at room temperature) was poured onto them under mechanical stirring (400 rpm). The mechanical agitation was maintained until complete emulsion cooling (Figure 1).
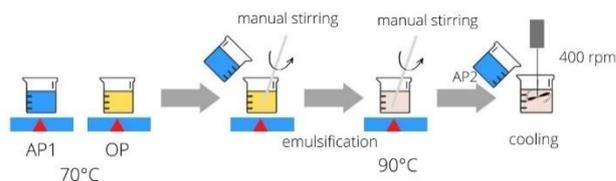

Figure 1. Schematic illustration of the emulsification by phase inversion temperature (PIT) method used to obtain the formulations

In this study, the concentration of of grape seed oil (GSO), avobenzone (AVO) and octyl methoxycinnamate (OMC) were used as variables to determine whether or not they would influence SPF activity *in vitro*.

These three variables were evaluated in a Central Composite Design in five levels (upper limit (+1), lower limit (-1), axial points (+α and -α) and central points (0)), as described in Table 1. The value for alpha (1.67) was calculated to achieve rotability in the design.

Table 1. Levels decoded of independent variables used in central composite design.

Variables	Concentration (%)				
	-α (-1.67)	-1	0	+1	+α (-1.67)
GSO[1]	1.65	5	10	15	18.35
OMC[2]	0.825	2.5	5	7.5	9.175
AVO[3]	1.33	2	3	4	5.67

[1]Grape seed oil, [2]Octyl methoxycinnamate, [3]Avobenzone.

Seventeen formulations were prepared according to experimental design. Formulations 5, 10 and 17 are triplicate of central point (Table 2). Variables coding for the Central Composite Design are shown in Table 1. Other details regarding experiments are shown in Table 2.

In vitro SPF determination

A spectrophotometric method was used to analyze the *in vitro* SPF value according to Mansur et al. (1986) (18). Absorbance values for each formulation was determined in triplicate at a final concentration of 0.2 μg/mL using ethanol 99.5°GL as solvent in a Shimadzu UV-2600 spectrophotometer (11, 18).

The absorbance was determined by scanning in the range of 220 to 400 nm, speed of 240 nm/min, low slit opening (1.0) and sampling interval of 1 nm.

The *in vitro* SPF determination, equation (1) and the correlation between the erythemogenic effect (EE) and the radiation intensity at each wavelength (EE × I) were adjusted according to Sayre (19).

$$\text{Spectrophotometric SPF} = CF \sum_{290}^{320} EE(\lambda)\, I(\lambda)\, abs(\lambda) \quad (1)$$

The correction factor (CF) =10, EE (λ) is the erythemogenic effect of radiation on wavelength λ, I (λ) is the intensity of solar light with wavelength λ, and abs (λ) is the spectrophotometric absorbance value of a solution of the test substance at wavelength λ (19).

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Table 2. Design of experiments obtained by Central Composite Design.

N[1]	Grape seed oil		OMC[3]		Avobenzone	
	Code	%[2]	Code	%[2]	Code	%[2]
1	-1	5.0	-1	2.5	-1	2.0
2	-1	5.0	1	7.5	1	4.0
3	1	15.0	-1	2.5	1	4.0
4	1	15.0	1	7.5	-1	2.0
5	0	10.0	0	5.0	0	3.0
6	-1	5.0	-1	2.5	1	4.0
7	-1	5.0	1	7.5	-1	2.0
8	1	15.0	-1	2.5	-1	2.0
9	1	15.0	1	7.5	1	4.0
10	0	10.0	0	5.0	0	3.0
11	-1.67	1.65	0	5.0	0	3.0
12	1.67	18.35	0	5.0	0	3.0
13	0	10.0	-1.67	0.825	0	3.0
14	0	10.0	1.67	9.175	0	3.0
15	0	10.0	0	5.0	-1.67	1.33
16	0	10.0	0	5.0	1.67	5.67
17	0	10.0	0	5.0	0	3.0
B[4]	-	10.0	-		-	

[1]Number of experiments, [2]Quantity in formulation, [3]Octyl methoxycinnamate, [4]Blank formulation.

Statistical analysis

All data were analyzed by ANOVA, using $p < 0.05$ to determine significant differences among groups, using software STATISTICA 7.0 (Stat Soft. Inc., Tulsa, OK, USA).

Results and Discussion

Formulations development and in vitro SPF determination

According to the experimental design, using the Central Composite Design, seventeen formulations (Figure 2) were prepared containing different concentrations of the variables grape seed oil, octyl methoxycinnamate and avobenzone, with triplicates of the central point.

The results of the *in vitro* SPF determination are shown in Table 3. A blank formulation was also produced, without the filters, the obtained SPF value (0.22), was not significant, demonstrating that GSO alone does not have significant photoprotective properties.

Ácsová et al. (2021) (12) studied the *in vitro* SPF of 14 vegetable oils by the Mansur's method and *in vivo* SPF by the International Organization for Standardization (ISO) method. The resulting *in vitro* SPF values were very low, except for tamanu seed oil that presented a certain UVB absorption capacity (SPF value 4.1). The remaining oils analyzed (carrot seed, coconut, raspberry seed, rosehip seed, and wheat germ) exhibited very low SPF values (2.5, 1.2, 2.6, 2.6, and 2.8).
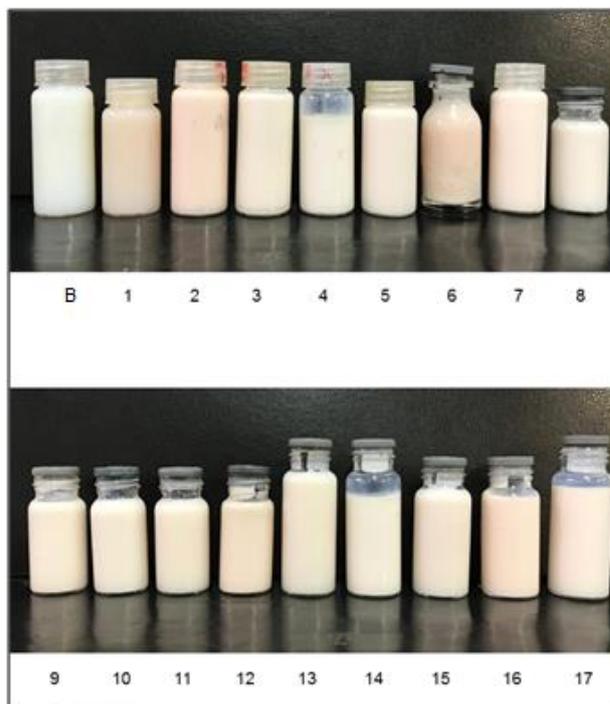

Figure 2. Formulations developed according to the Central Composite Design. B: Blank formulation.

Table 3. Results of the *in vitro* SPF of the formulations.

N[1]	GSO[2] (%)	OMC[3] (%)	AVO[4] (%)	SPF[5] $X^6 \pm S^7$(RSD[8] %)
1	5	2.5	2	5.08 ± 0.44 (8.71)
2	5	7.5	4	17.54 ± 1.64 (9.34)
3	15	2.5	4	8.38 ± 0.17 (2.09)
4	15	7.5	2	28.06 ± 0.50 (1.78)
5 (CP[9])	10	5	3	14.55 ± 0.75 (5.13)
6	5	2.5	4	11.26 ± 0.05 (0.45)
7	5	7.5	2	12.70 ± 0.11 (0.83)
8	15	2.5	2	8.54 ± 0.24 (2.83)
9	15	7.5	4	38.45 ± 0.36 (0.94)
10 (CP[9])	10	5	3	10.75 ± 0.60 (5.51)
11	1.7	5	3	17.26 ± 0.37 (2.13)
12	18.4	5	3	15.73 ± 0.44 (2.82)
13	10	0.8	3	3.33 ± 0.05 (1.61)
14	10	9.2	3	30.17 ± 0.79 (2.63)
15	10	5	1.3	14.59 ± 0.83 (5.72)
16	10	5	5.7	20.62 ± 1.29 (6.25)
17 (CP[9])	10	5	3	11.61 ± 1.53 (13.17)
B[10]	10	-	-	0.22 ± 0.064 (29.02)

[1]Number of experiments, [2]Grape seed oil, [3]Octyl methoxycinnamate, [4]Avobenzone, [5]Sun protection factor, [6]Average (n=3), [7]Standard deviation, [8]Relative standard deviation, [9]Central Point, [10]Blank formulation.

In general, it is observed that the increase in the concentration of the components promotes an increase in the SPF value. Formulation number 1 that contained the components at the lowest concentrations presented SPF value of 5.08 while formulation number 9 with the

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components at the highest concentrations resulted in the highest SPF value (38.45).

The contribution of each component or the interaction between them to the SPF score can be determined by applying a mathematical model considering the experimental design used in this study. Formulations 5, 10 and 17 were triplicates of the central point and are important to obtain confidence intervals and to verify the validity of the obtained model. The following polynomial model (Equation 2) was used:

$$Y = b_0 + b_1x_1 + b_2x_2 + b_3x_3 + b_{11}x_1^2 + b_{22}x_2^2 + b_{33}x_3^2 + b_{12}x_1x_2 + b_{13}x_1x_3 + b_{23}x_2x_3 \quad (2)$$

Where, Y represents SPF and x_1, x_2 and x_3 are, respectively, the variables GSO, OMC and AVO. Table 3 shows the combinations of all studied variables, and the SPF results from each formulation.

The results of the statistical analysis for the SPF are shown in the Pareto chart (Figure 3). For the experiments, p values were considered significant in a confidence interval of 95%. The OMC variable and the linear interaction between OMC and GSO were significant ($p < 0.05$).
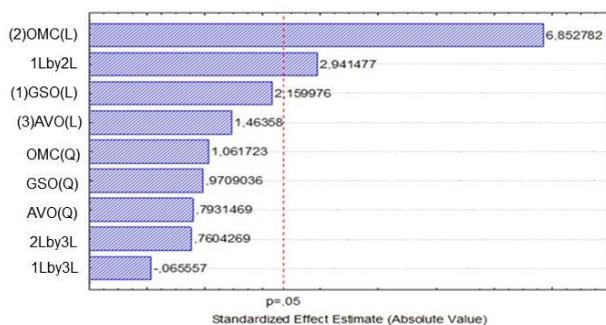

Figure 3. Pareto chart of standardized effects for SPF response. (2)OMC = Octyl methoxycinnamate , L= Linear, (1)GSO = Grape seed oil, (3)AVO = Avobenzone, Q = quadratic

According to Table 5, the calculated F value (23.11) is higher than the reference F value (19.43), indicating that the model is adjusted and influences the SPF linearly (20). The function that follows shows this influence through the value of R^2 obtained:

12.93 + 15.91 · OMC (L) + 8.921 · GSO (L) · OMC (L), $R^2 = 0,90$

The obtained R^2 value (0.90) indicates that the equation is valid and able to descript approximately 90% of the variation.

The three-dimensional response surface graph demonstrates the influence of the concentration of OMC and GSO on the SPF, which was obtained as a response (Figure 4).

Table 5. Variance Analysis (ANOVA) for the variables OMC and interaction between OMC and GSO.

Source of variation	SS[1]	DG[2]	MS[3]	Calculated F value	Reference F value
Regression	1038.10	2	519.05	23.11	19.43
Residue	314.42	14	22.46	-	-
Total	1352.52	16	84.53	-	-

[1]Sum of Squares, [2]Degrees of freedom, [3]Mean Square.

The effect on the SPF parameter can be visualized by the difference in SPF values among the different formulations (Table 3), as observed in formulations 1 and 6 with SPF values of 5.08 and 11.26, respectively, in which the concentrations of OMC and GSO are below their respective concentrations at the three central points. Also, in relation to the OMC and GSO interaction, formulations 4 and 9 (with SPF values of 28.06 and 38.45, respectively) were prepared with concentrations of OMC and GSO above the concentrations at the central points, presenting high SPF values.

Araújo et al. (2020) (11) determined the FPS by Mansur method of a mixture containing OMC and octocrylene, both at 10% (w/v) in chloroform and resulted in SPF 12. Additionally, Santos et al. (2018) (21) studied gel, cream and lotion formulations containing AVO (5%) and OMC (10%), higher concentrations than the ones we studied and resulted in SPF values of 18.2, 17.1, and 16.5, respectively by the Mansur method, using ethanol as solvent, lower SPF results than our findings, corroborating the positive interaction between OMC and GSO.
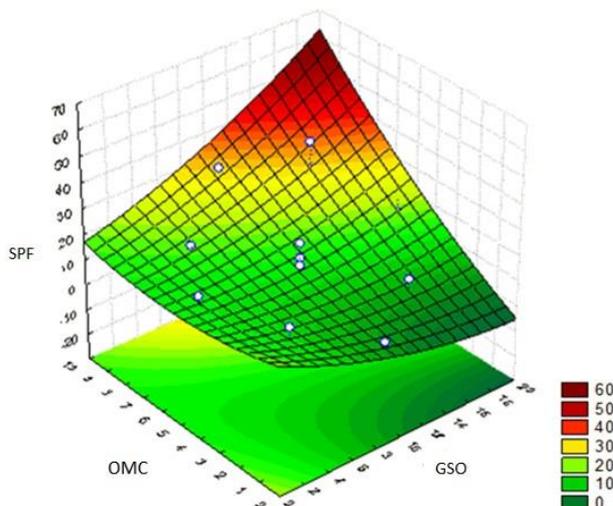

Figure 4. Response surface plots demonstrating the influence of independent variables Octyl methoxycinnamate (OMC) and Grape seed oil (GSO) on the sun protection factor (SPF).

By assessing the influence of variable amounts of OMC alone, it was possible to identify fluctuations in the SPF as a function of their concentration as shown in formulations 3, 8 and 13 (SPF values of 8.38, 8.54 and 3.33, respectively), when the concentration of SPF is below the central point value. Meanwhile, in formulations 2 and 14 (SPF 17.54 and 30.17, respectively) OMC concentration is above the threshold (Table 3).

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There are several *in vivo* and *in vitro* methods to determine SPF from oils and formulations. Among them, we can mention The Mansur method which is based on UV absorption from diluted solutions (11,18). Kaur et al. (2010) (22) have studied the SPF of many fixed and volatile oils such as rose oil (0.248) and olive oil (7.549) using a simple, fast and reliable UV spectrophotometric method capable of establishing SPF especially in screen formulation, giving important information before its *in vivo* determination.

Many studies have evaluated the use of natural products as aids in skin photoprotection. Chiari-Andreo et al. (2020) (23) presented a review regarding the photoprotection properties of phytochemical and secondary metabolites from plants. They possess the ability to absorb UVA and UVB rays as well the chemical sunscreen compound, besides antioxidant and anti-inflammatory effects.

An advantage to the use of GSO in cosmetic formulations is that natural products commonly have fewer undesirable effects when compared to synthetic ones (4). Furthermore, the oil contains compounds with great antioxidant activity (16).

The combination of antioxidant activity of the GSO components with UVB filter octyl methoxycinnamate in emulsified formulations represents a viable strategy to protect the human skin against UV-induced damage. This idea is attractive due to the fact that the sunscreen formulation would present two layers of protection instead of one. The first, a passive protection, offered by UV filters, which act by absorbing and reflecting UV rays. A second affording an active protection, which occurs by elimination of ROS, by increasing the natural antioxidant reserves of the human body.

The topical use of antioxidants contributes to prevent skin injuries induced by UV radiation (24). Thus, there is a synergism between the OMC and GSO that influences the SPF positively.

Conclusions

This study showed the influence of a vegetable oil, GSO, on SPF of sunscreen formulations containing organic UV filters. The combination between the GSO and OMC showed great potential to develop formulations with high UV protection and antioxidant properties so a multifunctional cosmetic formulation could be developed in future studies.

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Conflict of interest

The authors declare that there is no conflict of interests regarding the publication of this article.

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