Article

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Exploring Skin Biometrics, Sensory Profiles and Rheology of a Multifunctional Photoprotective Formulation and Its Vegan Version

Karine Campos Nunes [1], Bruna Lendzion Alves [1], Rafaela Said dos Santos [1], Lennon Alonso Araújo [2], Rosângela Bergamasco [2], Marcos Luciano Bruschi [1], Tânia Ueda-Nakamura [1], Sueli de Oliveira Silva Lautenschlager [1] and Celso Vataru Nakamura [1,*]

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Abstract: Background: The cumulative exposure of UV radiation can result in dangerous consequences such as skin burns, photoaging, and skin cancer, hence the importance of using photoprotective formulations. Currently, the formulations seek to be more efficient, innovative and reach a wider audience. Many sunscreens are vegan, and have antioxidant substances to ensure additional photochemoprotective action. To evaluate biological, functional, and mechanical characteristics of them on the skin, biometric and rheologic methods can be used. **Objective:** Evaluate biophysical, rheological and sensorial parameters of Face Care Facial Moisturizing Cream® (P1) and a vegan formulation (P2) by in vitro and in vivo tests. **Methods:** A UV scan experiment was performed covering the range of 250 to 400 nm. Sun Protection Factor (SPF) was evaluated by Mansur method. Rheological profiles were obtained using a MARS II (Haake®) controlled shear stress and gradient rheometer. Biophysical parameters were analyzed: skin sebum content, hydration level, transepidermal water loss, erythema and melanin level, skin color, and skin pH. The acceptance profile of the formulations by the volunteers was determined using a 9-point hedonic scale and a 5-point purchase intention test. **Results:** The formulations effectively absorbed light covering the UV radiation spectrum from UVA to UVB. The SPF of P1 was 25.21 and of P2 was 12.10 by in vitro tests. They had also pseudoplastic and thixotropic behavior, it can contribute to better spreadability and to form a protective film. Biometric tests showed an increase in hydration and skin sebum, decreased erythema, and maintenance of skin pH after application of both formulations. The products also had similarly good acceptance and purchase intention by the volunteers. **Conclusion:** The comparation of a commercialized product and a vegan test version showed very similar rheological and great acceptance profiles. Therefore, the vegan formulation is a good alternative to reach a different market.

Keywords: Photoprotection; UV Radiation; Skin biometrics; Rheology; Vegan Products

Introduction

The skin is an important protection organ of the human body. It has a complex structure that is constantly renewed, acting as a barrier against various harmful agents [1]. The skin has different layers: the outermost layer is the epidermis and, just below this, there is the dermis. There is also a layer composed mainly of adipose tissue called the hypodermis [2].

Solar radiation can cause several types of damage to the organism depending on the duration and type of exposure [3]. Individuals' negligence regarding skin protection, coupled with excessive sun exposure, can contribute to the development of various skin pathologies. Inflammation, apoptosis and necrosis can occur following acute UV radiation exposure, which can lead to tissue

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damage. DNA damage can also occur, which leads to a higher probability of developing skin cancer [4,5].

The damage to cellular structures is mediated by oxidative stress that occurs with an exacerbated increase in reactive oxygen species (ROS), that is, very unstable and reactive molecules [4,6]. When this imbalance between oxidizing and antioxidant compounds occurs, there is oxidation of biomolecules and loss of their functions [7]. To combat these damages, the skin has an enzymatic antioxidant system, which includes enzymes such as superoxide dismutase, glutathione peroxidase, and catalase. Antioxidants are important to counteract effects caused by ROS [8–11].

Therefore, some studies have sought to incorporate antioxidants in cosmetic formulations, constituting a viable and effective alternative for greater photoprotection [12–15]. Sunscreens are formulations designed to specifically attenuate the effect of UV radiation on the skin by means of physical or chemical action resulting in radiation absorption, dispersion, or reflection mechanisms. The quality control of a photoprotective formulation is essential, and this depends not only on its sun protection factor (SPF), but also on its physical-chemical properties, stability and solubility [12,16].

Rheology is an effective instrument for the physical and behavior analysis of formulation flow, and there are different methods available for the evaluation of the stability of cosmetic products [17]. Furthermore, non-invasive in vivo methods have enabled a greater understanding of skin physiology [18,19] and these cutaneous biometrics can be used to assess the physicochemical properties of the skin and the behavior of a formulation within it. Such parameters that can be evaluated include skin hydration, pH, transepidermal water loss, melanin and erythema level, and temperature [20].

The quality profile of a product also covers the sensory aspect and the degree of acceptance of the product by the target audience [21,22]. Some consumers are looking for products that meet the ethical requirements of their lifestyle. There is concern on the part of such individuals regarding the impacts that products generate on the environment, in themes such as cruelty-free, sustainability, and vegan formulations [23,24]. Vegan formulations are pharmaceutical preparations whose composition and raw materials are not of animal origin nor have been tested on animals [25].

The present work evaluated the biophysical properties of two multifunctional formulations: Face Care Facial Moisturizing Cream® and the second formulation is a vegan test formulation. Additionally, a comparison was conducted between the two products regarding in vitro SPF, rheological profiles, and sensory analysis, encompassing male and female human volunteers.

Materials and Methods

The two formulations evaluated in this work were the commercialized Face Care Facial Moisturizing Cream (P1) with SPF 30 from the company PURIFIC PREMIUM® and a vegan formulation (P2) provided by the company Naturelle®. P1 and P2 are classified as multifunctional products and their compositions are shown in Table 1:

Table 1. Components of Face Care Facial Moisturizing Cream® (P1) and a vegan test formulation (P2) (INCI - International Nomenclature Cosmetic Ingredient).

P1	P2
Aqua (water)	*Aqua (water)*
Tribehenin PEG-20 esters	*Caprylic/capric triglyceride*
Theobroma Grandiflorum seed butter	*Titanium dioxide*
Tocopheryl acetate	*Hydrated silica*
C12-15 Alkyl benzoate	*Hydrogen dimethicone*
Diethylamino hidroxybenzoyl hexyl benzoate	*Aluminium hydroxide*
Ethylhexyl triazone	*Zinc oxide*
Algae extract	*Triethoxycaprylylsilane*
Bis-ethylhexyloxyphenol methoxyphenyl triazine	*Cetearyl olivate/sorbitan olivate*
Ethylhexyl methoxycinnamate	*Propanediol*
Titanium dioxide	*Coco-caprylate/caprate,*
Hydrated silica	*Polyglyceryl-10 pentastearate*

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Hydrogen dimethicone	*Behenyl alcohol*
Aluminium hydroxide	*Sodium stearoyl lactylate*
Dimethicone	*Squalene,*
Panthenol	*Hypnea musciformis extract*
Glycerin	*Gellidiela acerosa extract*
Disodium EDTA	*Cucumis sativus (cucumber) seed extract*
Acrylates/C10-30 alkyl acrylate crosspolymer	*Ammonium acryloyldimethyltaurate/vp copolymer*
Triethanolamine	*Phenoxyethanol*
Biosaccharide gum 4	*Ethylhexylglycerin*
Methylisothiazolinone	*Tocopheryl acetate*
Phenoxyethanol	*Sodium stearoyl glutamate*
Cyclomethicone	*Disodium EDTA*
Parfum (Fragrance)	*Parfum (fragrance)*
Xanthan gum	
Cyclopentasiloxane	
Dimethicone crosspolymer	
Glass butylphenyl methylpropional	
Alpha-isomethyl ionone	
Coumarin	
Hexyl cinnamal	
Linalool	

Evaluation of Formulation pH

To determine the pH of the formulations, they were diluted 10% (w/w) in water and three determinations were made for each sample, using a pH meter (Digimed®), previously calibrated with pH 4.00 and pH 6.86 buffers.

Absorbance Scan

For the UV scan experiment, samples P1 and P2 were diluted in absolute ethanol at a concentration of 100 µg/mL. A scan was then carried out, measuring the absorbance from 250 to 400 nm in UV-Vis equipment (Shimadzu, UV-1700).

SPF Determination

The Mansur equation was used to calculate the SPF in vitro [26]. The samples were diluted to 100 µg/mL in triplicate, and the absorbance of P1 and P2 was read between 290-320 nm at 5 nm intervals (Shimadzu, UV-1700). The SPF was calculated using the following equation:

$$SPF = CF \times \sum_{290}^{320} EE\ (\lambda) \times I\ (\lambda) \times Abs\ (\lambda) \times 2$$

where:

CF = correction factor (equal to 10);
EE (λ) = erythematogenic effect of radiation with wavelength λ;
I (λ) = intensity of sunlight at wavelength λ;
Abs (λ) = spectrophotometric reading of the absorbance of the sample solution at wavelength (λ);
2 = Dilution factor
The EE (λ) x I (λ) values are given in the supplementary material (Table S1).

Rheological Analysis

Continuous Flow Shear Rheometry

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Rheograms were generated by means of a gradient rheometer and controlled shear stress MARS II® (Haake®), in continuous flow mode, at temperatures of 4, 25, 34, and 40 ± 0.1 °C, with parallel cone-plate geometry of 35 mm in diameter, separated by a fixed distance of 0.052 mm. It was found that the formulations did not break at up to 2000 s^{-1} of shear gradient. Therefore, the measurements of the flow curves were taken with a variation of the shear rates from 0 to 2000 s^{-1}, in order to verify the behavior of the formulations submitted to such rates.

The upward and downward flow curves were calculated based on the Oswald de Waele equation (Power Law - Power Law), obtaining the *k* and *n* indices [27]:
$\tau = k.\ \gamma^n$, where τ is the shear stress (Pa), *k* is the consistency index [(Pa·s)n], γ is the shear rate (s^{-1}), and *n* is the flow behavior index (dimensionless).

In addition, the yield of each formulation was obtained using the Herschel-Buckley equation/model [28]: $\tau = \tau_0 + k.\ \gamma^n$,

where τ is the shear stress (Pa), τ0 is the yield stress (Pa), *k* is the consistency index [(Pas)n], γ is the rate of shear (s^{-1}), and *n* is the flow behavior index (dimensionless).

The hysteresis area was also obtained using the RheoWin 4.10.0000 program (Haake®) and the thixotropy coefficient (Kt) was calculated using the equation [29].

Oscillatory Rheometry

The samples were gently applied to the bottom plate, allowing a resting time of 1 min before each determination, and ensuring the minimum shear of the formulation [30]. After determining the linear viscoelastic region, the frequency scan analysis from 0.1 to 10.0 Hz was performed. Viscosity (n ′), tangent (tan), storage module (G′), and loss module (G″) were calculated using the RheoWin 4.10.0000 (Haake) software [27]. Three repetitions were made for each sample.

Evaluation of Formulation by Cutaneous Biometrics

Selection of Test Subjects

Inclusion Criteria

Fourteen healthy female and 14 healthy male volunteers between 18 and 60 years of age were recruited to the study. The individuals had skin phototypes I, II, and III based on the Fitzpatrick classification scale, which are the skin types more sensitive to UV radiation and thus greater sun protection is recommended [31]. Recruited individuals were also instructed to read the Free and Informed Commitment Term and sign it if they agreed to participate in the research. This study was approved by the Ethics Committee of the State University of Maringa, with the number of the report: 2.990.495.

Exclusion Criteria

Exclusion criteria were volunteers who were undergoing dermatological treatment, had an allergy to cosmetics, had endocrine or dermatological diseases, were smokers, and pregnant women [32]. Skin tones that were not type I, II, and III, or had the presence of sunburn, sun tanning, scarring, or active dermal lesions were also considered exclusion criteria.

Sample Application

The volunteers were received in a room with controlled temperature and humidity, and were instructed to wash their face with neutral soap before the measurements were taken. They then stayed in the test room for 20 min in order to adapt to the environmental conditions. Using a glove, the volunteers spread 180 grams of P1 on their right cheek area, until complete absorption. The same was done with P2, with application on the left side. The first measurements were made at time 0, with skin pH measurement, transepidermal water loss, skin sebum content, melanin content, erythema level, skin color, and hydration. Subsequently, new readings of the biometric parameters were made

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one and two hours after the application of the formulations. The negative control (NC) was a region of the skin to which formulation was not applied.

Determination of Cutaneous Sebum Content

The determination of the skin sebum content was performed using the Sebumeter® SM 815 cassette. The Sebumeter® SM 815 adhesive tape was placed in contact with the skin and, the surface of the main measurement area becomes transparent in the presence of grease/oiliness. Then, the tape was inserted into the opening of the device and the transparency was measured by a photocell. Light transmission represents the sebum content [33].

Assessment of Skin Hydration Level

The hydration level of the skin was measured using the Corneometer® probe, with the measurement of the capacitance of a dielectric medium. Changes in the dielectric constant due to the variation in the hydration of the skin surface can be measured in the precision measurement capacitor [34]. The device is capable of determining the water content of the superficial epidermal layers to a depth of 0.1 mm, and the values are expressed in arbitrary units (AU), where 1 AU corresponds to 0.2 to 0.9 mg of water per gram of stratum corneum [35].

Assessment of Transepidermal Water Loss (TEWL)

Using the Tewameter® probe, the percentage of water that evaporated on the skin surface was measured, since there is an increase in TEWL when the skin barrier is damaged [36]. When the capacity of the stratum corneum to retain water decreases, such as in the case of skin damage, there is an increase in the flow of water vapor and a consequent increase in the value of TEWL [20].

Assessment of Erythema Level and Melanin Content

The evaluation of the melanin content and the erythema level of the skin are based on absorption/reflection and were performed using the Mexameter® MX 18 probe. That probe emits 3 specific wavelengths of light and a receiver measures the light reflected by the skin. As the amount of light emitted is defined, the amount of light absorbed by the skin can be calculated. The melanin content was measured by specific wavelengths chosen to correspond to different rates of absorption by the pigments. Regarding the level of erythema, specific wavelengths were also used, corresponding to the peak spectral absorption of hemoglobin and to avoid other color influences (e.g., bilirubin) [33].

Skin Color Assessment

The skin color was assessed using the Skin-Colorimeter® CL 400 probe. The probe contains white LED light, arranged circularly to illuminate the skin in an even manner. The emitted light is spread in all directions, some parts go through the layers of the skin and some are reflected. The light reflected from the skin is then measured by the instrument [33].

Evaluation of Cutaneous pH

The pH measurement on the skin was made by the Skin-pH-meter® probe, which is based on a high-quality combined electrode. The glass H^+ ion sensitive electrode and the additional reference electrode are placed in a single reservoir [33].

Sensorial Analysis

A 9-point hedonic test was applied to perform the sensory analysis. The test was sample-blind, that is, the volunteers did not know which formulation was applied to their face. They also answered a test of intention to purchase the product, described by *Prudencio* et al. [37] and it is included in the supplementary material (Annex I).

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Statistical Analysis

All data were analyzed using the ANOVA test, considering $p < 0.05$ to be significant, followed by the Tukey test. Statistical analyzes were performed using the GraphPad Prism 5 software.

Results and Discussion

Absorbance Scan

Solar radiation can induce various types of damage to the skin, which varies depending on the duration and type of exposure [3]. The Earth receives a constant stream of light photons from the sun, including infrared light (780–5000 nm), visible light (400–780 nm), and ultraviolet (UV) light (290–400 nm). Regarding to UV range, it can be categorized based on wavelength into UVC (200–280 nm), UVB (280–320 nm), and UVA (320–400 nm) [4]. The scanning results showed that the two products analyzed were able to absorb light in the 250 to 400 nm range. This encompasses almost all range of the electromagnetic spectrum of UV radiation, including UVA and UVB (Figure 1).

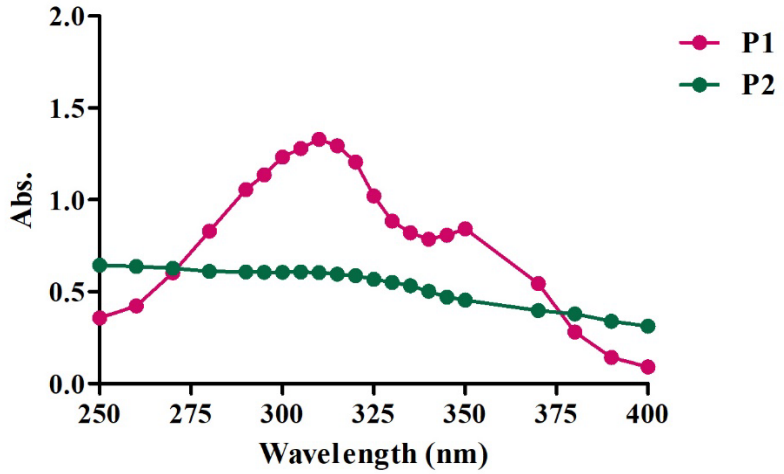

Figure 1. UV radiation scan covering 250 to 400 nm with P1 and P2 at a concentration of 100 µg/mL.

P1 showed an absorption peak at 310 nm, which corresponds to UVB radiation. P2, on the other hand, did not show much variation in absorbance, which remained present throughout the range analyzed. It is well known that, in the event of an emission of UV light, sunscreens can protect the skin by absorbing the radiation, attenuating its effects on the skin [12].

SPF Determination

SPF is an interesting tool for evaluating the effectiveness of multifunctional products. In vitro techniques for evaluating SPF have been developed and standardized, offering lower cost and labor than in vivo ones [22]. The technique described by Mansur [26] was used and the results were favorable for both products. P1 obtained an SPF of 25.21 ± 1.09, while P2's result was 12.10 ± 0.43.

P1 has some chemical filters that can absorb UV radiation, such as *Diethylamino hydroxybenzoyl hexyl benzoate*, an organic filter. The composition of P2 has more components and physical filters, such as zinc oxide and titanium dioxide, which act to reflect solar radiation. To complement UV protection, P2 contains antioxidant components (Table 1) [9].

Despite P2 achieving a lower SPF compared to P1, studies state that products with an SPF of 10 can absorb 90% of erythomatous radiation [16]. P1 is marketed as SPF 30 (by in vivo tests), while P2 has not yet undergone in vivo testing. It is important to highlight that in vitro SPF results can differ from those obtained *in vivo*. Studies show that the in vivo SPF for some formulations already on the market are higher than that achieved in vitro using the Mansur method [61].

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Rheological Analysis

Continuous Flow Shear Rheometry

Rheological studies are tools to characterize cosmetic formulations and analyze their behavior under different conditions, obtaining characteristics such as spreadability on the skin and sensory aspects [17]. Our results showed that both formulations are non-Newtonian fluids with pseudoplastic behavior ($n < 1$) (Table 2); the viscosity decreased as the shear rate increased, a characteristic that can be observed in Figure 2 (A and B). There was also a decrease in viscosity with increasing temperature, a characteristic that is reported in the literature for sunscreens [38,39].

Table 2. – Results of the hysteresis areas, *k*, *n*, and τ_0, at temperatures of 4, 25, 34, and 40 °C for P1 and P2. Means with the same letter are significantly different comparing P1 with P2 ($p < 0.05$) according to the one-way ANOVA with Tukey test.

Table .	k (Pa.s)	*n* (dimensionless)	τ_0 (Pa)	Hysteresis area (Pa/s)
			P1	
4	^{c}35.08 ± 1.41	0.27 ± 0.01	^{g}36.50 ± 2.57	^{a}89097.50 ± 9034.23
25	^{d}14.66 ± 0.95	0.37 ± 0.00	17.37 ± 2.47	12934.00 ± 832.60
34	^{e}12.29 ± 0.46	0.35 ± 0.02	17.80 ± 2.82	^{b}30046.67 ± 2832.85
40	^{f}13.88 ± 1.48	0.30 ± 0.02	^{h}11.82 ± 3.04	57483.33 ± 6890.91
			P2	
4	^{c}41.25 ± 2.68	0.26 ± 0.00	^{g}15.45 ± 4.56	^{a}105525.00 ± 1951.71
25	^{d}22.67 ± 0.47	0.32 ± 0.00	22.73 ± 0.81	26993.33 ± 12.79
34	^{e}20.95 ± 1.31	0.33 ± 0.00	18.07 ± 3.31	^{b}61085.00 ± 2990.85
40	^{f}24.38 ± 5.13	0.28 ± 0.01	^{h}2.93 ± 0.85	66178.00 ± 13671.29

The rheograms were better adjusted in the Herschel-Bulkley model, that is, the formulations started to flow after an initial shear stress (τ_0) and, later, they flowed with the increase in the shear rate. Thixotropy consists of a gradual reduction in viscosity under shear stress followed by a recovery of the structure when the stress is stopped [17,40]. It was observed that the shear gradient increased until reaching its maximum value (2000 s^{-1}) and, subsequently, the process was reversed by decreasing the gradient and generating the two curves [40].

The rheological profiles of both formulations showed the presence of a hysteresis area, mainly at the extremes of temperature (4 °C and 40 °C) (Figure 2). P2 had a significantly larger area of hysteresis at temperatures of 4 and 34 °C than P1 (Table 2). The presence of a hysteresis area is an interesting finding, since it contributes to the release of the fragrance and the composition's assets [41]. The increase in the hysteresis area in photoprotective formulations may be related to the presence of emollients and emulsifiers, which alter the rheological behavior, causing a desirable effect for the formulation [12,42].

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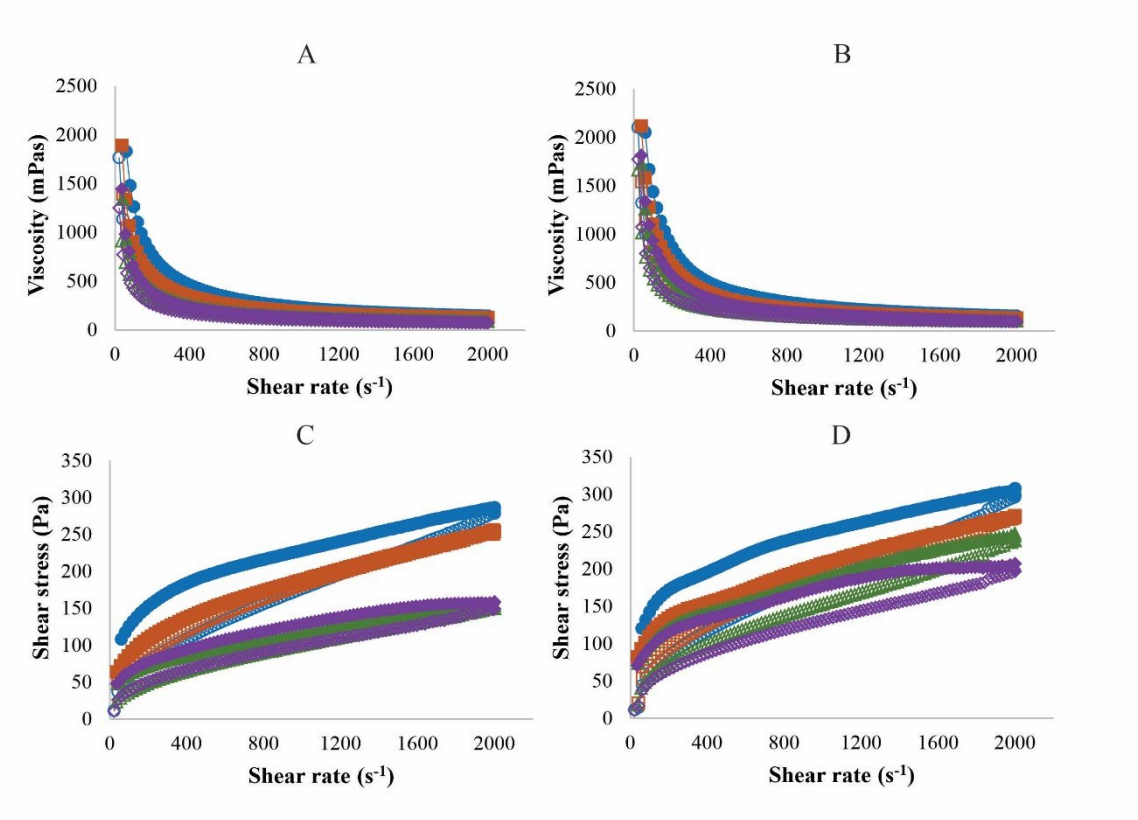

Figure 2. Rheological behavior of P1 and P2. Viscosity as a function of the shear rate, flow diagram of P1 (A) and P2 (B). Shear stress as a function of the shear rate, flow diagram of P1 (C) and P2 (D). The analyses were performed at temperatures of 4 °C (●), 25 °C (■), 34 °C (▲), and 40 °C (♦). The closed symbol represents the forward curve and the open symbol represents the return curve. Each rheogram is the average of at least 3 replicates with a variation coefficient of less than 10%.

Regarding the consistency index values (*k*), Table 2 shows that there was a significant difference ($p < 0.05$) comparing the values between P1 and P2 at each temperature. Since *k* is related to the degree of resistance of the fluid to the flow [17], it can be inferred that P2 is more consistent than P1 due its higher consistency index. The lowest temperature generated a higher *k* value in both products, which can be explained by the fact that the temperature influences the consistency of the formulations [43]. However, there was no significant variation in the *k* value between the values at 25, 34, and 40 ºC. This fact demonstrates the possible stability with the gradual increase in temperature.

Oscillatory Rheometry

Many emulsions have viscoelastic properties that can be affected by oscillatory frequency and temperature [44]. With P1 and P2, the increase in oscillatory frequency raised G′ mainly at the highest temperature (Figure 3). The formulations presented a G′ (elastic modulus) greater than G″ (loss modulus), confirming the characteristics of a viscoelastic system [17,45] (Figure 3).

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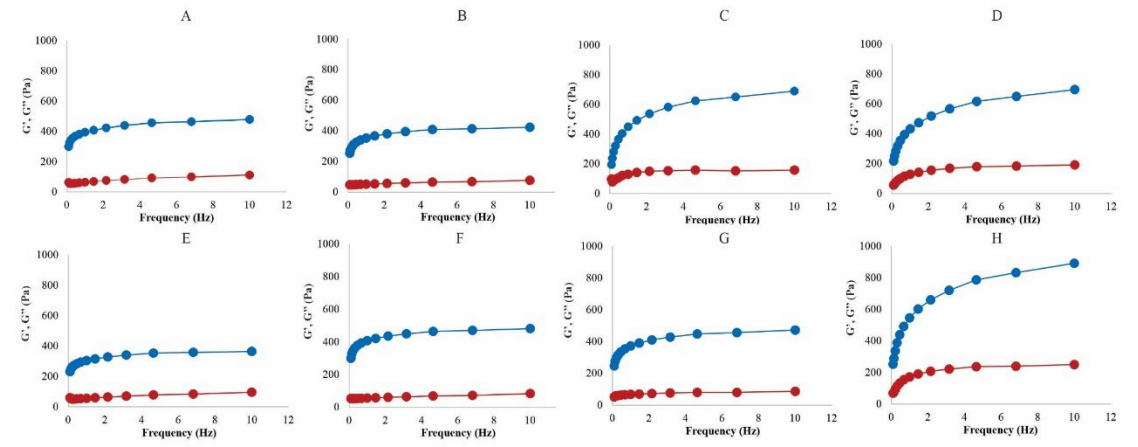

Figure 3. Storage module G '(●) and loss module G''(●) depending on the frequency of P1 at temperatures of 4 °C (A), 25 °C (B), 34 °C (C), and 40 °C (D), and P2 at temperatures of 4 °C (E), 25 °C (F), 34 °C (G), and 40 °C (H). Each rheogram is the average of at least 3 replicates with a variation coefficient of less than 10%. Tangent δ, on the other hand, remained relatively constant with an increase in frequency, but P1 had the tangent values higher for higher temperatures (34 and 40 °C). Both products showed tangent δ values and less than 1, at all temperatures studied, indicating that the viscoelasticity of the formulations was also maintained (supplementary material, Figure S1).

Studies with photoprotective formulations demonstrated a viscoelastic profile, that is, they had a predominant elastic behavior [38,46], with hysteresis or thixotropy area. These features are suitable for this kind of formulation since it facilitates the application, indicates the reversible variation of the viscosity with the time, and increases the stability [12]. It is very important to analyze the rheological profiles of the formulations in order to modulate the desired sensory properties and also to analyze the quality and stability of products [17].

Evaluation of Formulations by Cutaneous Biometrics

Determination of Cutaneous Sebum Content

It is extremely important to study and develop safe, effective photoprotective formulations that meet consumer demand. Therefore, the choice of raw materials and components must be made carefully, as they influence the biophysical parameters as well as the acceptance of the product by the consumer [37,38]. At time 0, the skin sebum content of the volunteers was low for both sexes (Figures 4A and 4G). However, that may be a result of the volunteers first washing their faces with neutral soap 20 min before the administration of the formulations.

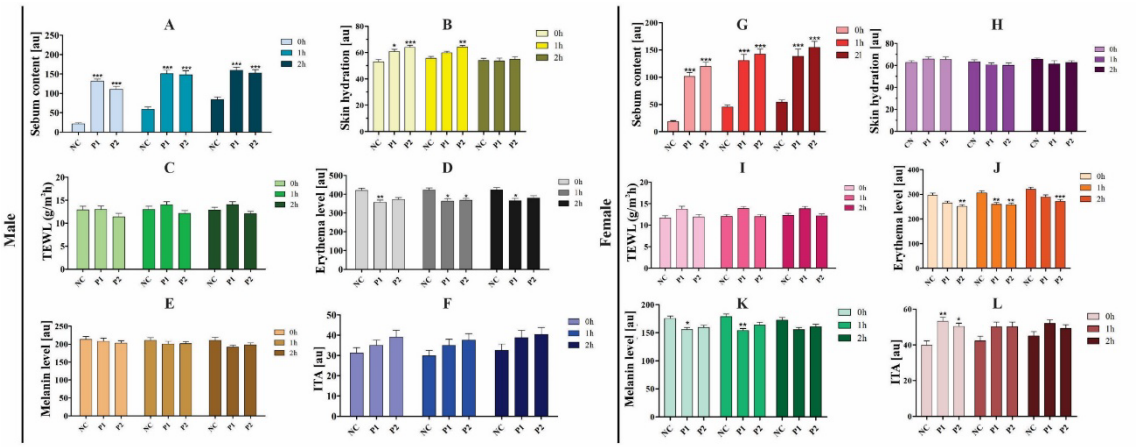

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Figure 4. Skin sebum content assessment in male (A) and female (G) individuals, skin hydration for male (B) and female (H), transepidermal water loss for male (C) and female (I), skin erythema for male (D) and female (J), melanin level for male (E) and female (K), and skin color in male (F) and female (L) (MPA 9, Courage-Khazaka) at time 0, time 1 (one hour after application), and time 2 (two hours after application). (NC) negative control, (P1) product 1, (P2) product 2. * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$ indicate a significant difference compared with NC, according to one-way ANOVA with Tukey test.

Studies have proven that, even when there is a disturbance in the hydrolipidic film, normal skin is able to restore the skin sebum content in approximately two hours [35]. That event can be observed in our study (Figure 4A and 4G). It also appeared that there could be differences between the sexes, perhaps due to different skin characteristics, since the secretions excreted by the sebaceous glands are under the influence of androgenic hormones [47].

The two formulations showed excellent results in terms of skin sebum content, and there were no significant differences between them at the evaluated times. Comparing the area of skin where the formulations were applied with the negative controls (NC; skin without product application), a significant difference was observed with $p < 0.001$. From the moment of application, the products were able to raise the sebaceous content to over 100, which restored the skin to its normal characteristic in both sexes. Even after two hours, the sebum content did not exceed 180, revealing that the formulations did not leave the skin oily, but only restored its normal condition. In short, the sebum excreted by the sebaceous glands, along with the moist components excreted with sweat, form a hydrolipidic film, protecting the skin from dryness [48,49]. The sebum content is essential for the health of the skin, since it has an emollient function and maintains the appropriate level of humidity in the stratum corneum [47].

Assessment of Skin Hydration Level

Regarding to hydration, there were not many significant differences in women, who had sufficiently hydrated skin (Figure 4H). At time 0, men exhibited lower skin hydration levels compared to women, in which they have a hydration level of 50 (AU), whereas female skin started the tests at 60 (AU). These sex-related differences may also be linked to the use of moisturizers and sunscreens [39], as the majority of male participants in this study reported not using skincare products daily. For males, at time 0, the two products caused a significant increase in hydration when compared to the NC, reaching (60 AU). After 1 hour, only P2 resulted in significantly higher hydration values compared to NC at $p < 0.01$ (Figure 4B).

An increase in skin hydration is a desirable factor for skin care formulations. It can occur due to the presence of hydration-promoting components present in both products. In P1, for example, there is the presence of panthenol, dimethicone, glycerin, and triethanolamine alkyl acrylate crosspolymer, emollient components, moisturizers that contribute to skin softness. P2 has caprylate/caprate, squalene, in addition to others, that act as emollient and moisturizing components.

There are reports in the literature of treatments for many pathologies related to skin such as sunburn, as well as scaly or dry skin, through photoprotective formulations [50]. Although there was a significant increase in skin hydration in males only, all results were higher than 50 (AU), which implies sufficiently hydrated skin. The skin hydration level and the barrier function are essential for a hydrated, healthy- and good-looking skin [51].

Assessment of Transepidermal Water Loss

Transepidermal water loss (TEWL), a biophysical parameter, refers to the stratum corneum's ability to prevent uncontrolled water evaporation from the skin layers [52]. According to the table specified by the manufacturer, values of 10-15 (AU) reveal a healthy skin condition of individuals. That result could be seen in both males (Figure 4C) and females (Figure 4I). In other words, in both sexes, with and without the presence of formulations, values below 15 were obtained, showing that the individuals were healthy in relation to this parameter.

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A reduction in TEWL is observed when there is the application of occlusive components, which contribute to avoid water evaporation [52]. In the formulation, it is possible to observe the presence of components such as polyglyceryl-10 pentaestearate, capric/caprylic triglyceride, and berrenyl alcohol, components already reported in multifunctional formulations that can fulfill the emollient and occlusive function. The use of multifunctional formulations for daily use becomes an excellent alternative for protection against solar radiation [22], since exposure to solar radiation through UV rays generates several negative consequences on the skin [4].

Assessment of Erythema Level and Melanin Content

Erythema, the redness caused by the vasodilation of cutaneous capillaries, is just one of the consequences of UV exposure in the skin [53]. The level of skin erythema was analyzed when administering the two test products. These products were not irritating to the skin of the volunteers at the time analyzed. Furthermore, in both sexes, there was a significant decrease in the level of erythema following treatment (Figures 4D and 4J).

It has been proven that plant extracts containing polyphenols have high antioxidant and anti-inflammatory activity, being able to also reduce erythema [54,55]. P1 has *Theobroma grandiflorum* in its composition, a species known for its high content of phenolic compounds. In addition, P1 also contains seaweed and coumarin extract, known for their antioxidant and stimulating action [56,57]. The vegan formulation (P2) has in its constitution extracts of two red algae *Hypnea musciformis* and *Gelidiela acerosa*, and cucumber extract (*Cucumis sativus*), active in the scavenging of free radicals, besides relieving the skin of cutaneous irritations [58].

The skin phototype can be associated with the melanin content. Melanin determines the color of our hair and skin, and provides protection against UV radiation. The Fitzpatrick skin phototype describes different skin tones, photosensitivity, and response to tanning [59]. The volunteers had phototypes I, II, and III, with melanin values as expected, since the melanin level did not exceed 250 (AU) in both sexes (Figures 4E and 4K).

In the female volunteers, a significant decrease in the level of melanin was observed between zero and one hour with the application of P1 (Figure 4K). Interestingly, according to the questionnaire in this study, most women reported using sunscreen daily. There are reports in the literature that the prolonged use of photoprotective formulations were able to reduce the melanin content in patients with hyperpigmentation [32]. Another study proved that the administration of sunscreens containing antioxidants reduces the pigmentation of the skin and decreased the degradation of collagen in the dermis [39].

Skin Color Assessment

The study also showed some results about the skin color of the volunteers. The men presented individual typology angles (ITAs) from 30º to 40º (AU), revealing an intermediate skin color. The women obtained ITAs from 40º to 55º (AU), featuring a white skin (Figures 4F and 4L). Individuals with type I, II, and III may have lighter skin, and that claim was confirmed by analyzing the results of the ITAs of both sexes.

Formulation pH and Skin pH

It is important to maintain the appropriate skin pH [1], and the analysis and study of the cutaneous pH parameter can assist in the interpretation of skin conditions. Furthermore, it elucidates the action of topical formulations, as well as the effectiveness of active substances [27]. The pH *(in vitro)* of both studied formulations remained in a neutral range. P1 had a pH of 7.48 ± 0.13 and P2 was pH 7.40 ± 0.12, using a pH meter (Digimed®). The application of the formulations did not generate significant changes in the pH value of the volunteers' faces *(in vivo)*, which was approximately 5.5, compatible with the pH of the skin.

Sensorial Analysis

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A sensory analysis was carried out on a 9-point hedonic scale, representing a scale with 9 categories, ranging from "I liked it very much" to "I disliked it very much". Through this scale, opinions of the volunteers in relation to the products can be verified [60]. For the hedonic test, the acceptance index was given in percentage, and the parameters investigated were: appearance, fragrance, texture, and sensation on the skin.

The results of P1 (Figure 5A) showed that most volunteers answered "like very much" for the four parameters analyzed. For this formulation, "dislike extremely" was not selected by any individual for any of the parameters. For the fragrance, a small percentage answered "dislike very much". There was also a small percentage of people who were indifferent to the parameters, answering "I neither like nor dislike". The vast majority of the results were between "Like extremely and like slighty".

For P2 (Figure 5B), the result of the sensory analysis was similar to P1, in which most responses were positive. The appearance, texture, and sensation parameters on the skin obtained a greater number of "like very much" in P1 than in P2. P2 did not get any "dislike very much" and "dislike extremely" for the four parameters, a positive point to be taken into account. As in P1, most volunteers responded that they liked P2.

The purchase intention graph (Figure 5C) revealed that most volunteers would probably buy both formulations (more than 50%). P2 did not obtain any votes for "Certainly would buy" and "Certainly would not buy". Approximately 17% of volunteers would probably not buy P2, and approximately 4% would certainly not buy P1. Finally, almost 20% of volunteers would certainly buy P1.

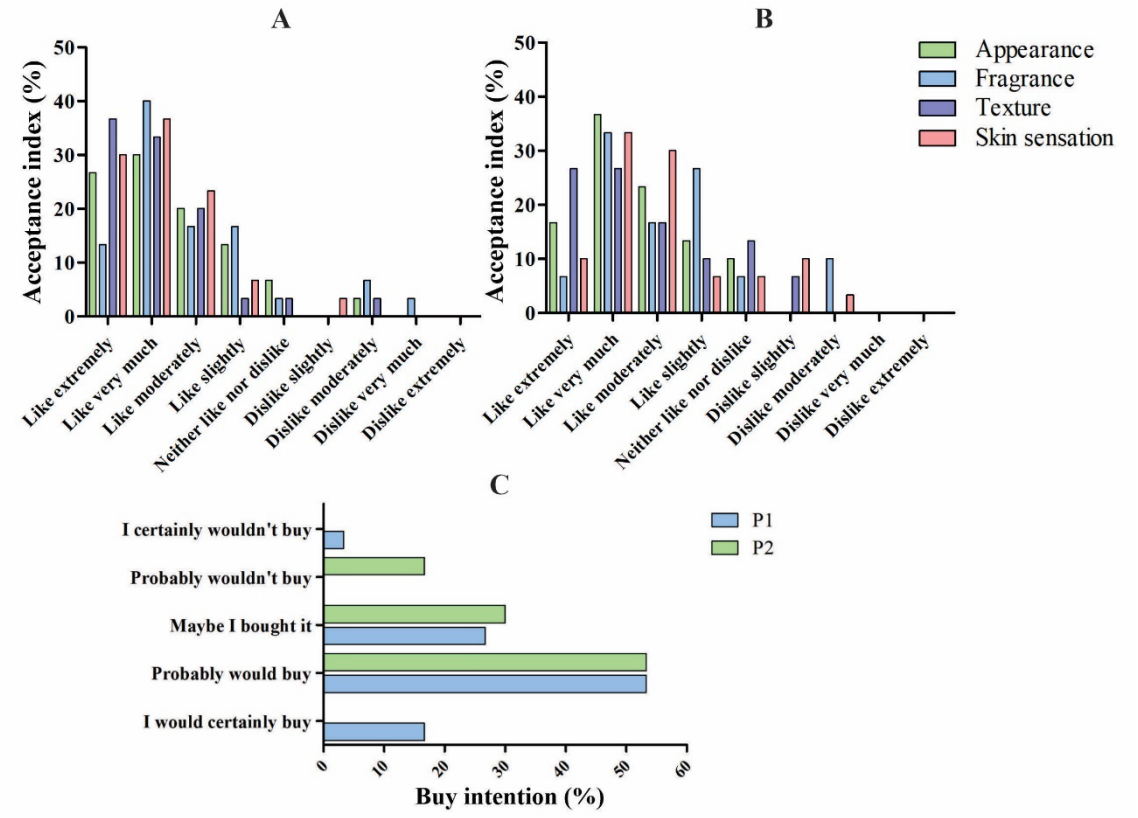

Figure 5. Acceptance index (%) using the adopted parameters: appearance, fragrance, texture, and skin sensation for (A) P1 and (B) P2. 5-point purchase intention graph (%) for P1 and P2 (C).

Studies have already been reported using this type of affective test to verify the acceptance of photoprotective formulations, in addition to applying a 7-point purchase intention test, and there was a good acceptance profile [37]. Determining the tactile characteristics of cosmetic products through sensory analysis is of great importance, as it generates additional improvements that could

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be made to achieve consumer acceptance [38]. In addition, there are few reports in the literature of the sensory analysis of vegan photoprotective multifunctional formulations, making this study relevant to expanding knowledge among different audiences in the cosmetic market.

Conclusion

The study evaluated biophysical, rheological and sensorial parameters of Face Care Facial Moisturizing Cream® (P1) and a vegan formulation (P2) by in vitro and in vivo tests. The formulations are photoprotective, presenting a SPF in vitro higher than 10. They increased the cutaneous sebum content, which can form an emulsion with water, playing a role in maintaining the hydration of the skin surface. There was an increase in hydration, maintenance of cutaneous pH, and reduction of erythema. In addition, the formulations had very similar rheological profiles, exhibiting pseudoplastic and thixotropic behavior, important for the dispersion of the present assets and to form a protective film. The sensory analysis showed a promising result for both products, which obtained great purchase intention scores by the participating volunteers (Figure 6). The vegan formulation presents itself as a viable alternative to access a distinct market.

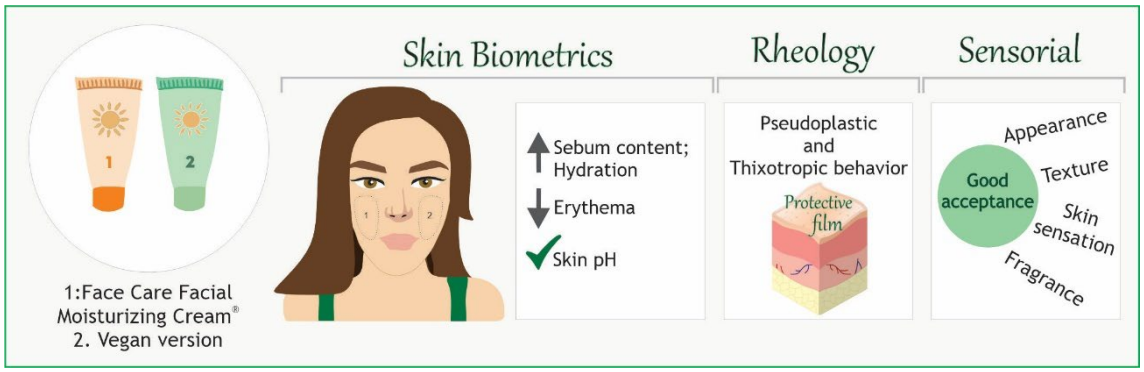

Figure 6. Graphical abstract demonstrating a multifunctional photoprotective formulation (P1) and its vegan version (P2) on the human skin. They had an SPF higher than 10, by in vitro tests. They also proved to be beneficial to the skin, with an increase in hydration, cutaneous sebum, and a reduction of erythema. Moreover, the sensory analysis showed a promising result for both products, great purchase intention scores by the participating volunteers.

Author Contributions: K.C.N. and C.V.N. conceptualized the project. K.C.N. and B.L.A. conducted the experimental work. M.L.B. and R.S.S. contributed to result interpretation. Data curation and graphics were made by K.C.N. and L.A.A. The initial draft was written by K.C.N. Funding acquisition was managed by R.B., S.O.S.L., T.U.N., and C.V.N. Project administration and review were conducted by C.V.N. All authors collectively analyzed the findings and contributed to shaping the final manuscript.

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