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Valorizing Sicilian Grape Pomace for Sustainable Nanocosmetic Applications

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Abstract

(1) Background: Grape pomace (GP), a major by-product of winemaking, is a sustainable source of bioactive polyphenols with antioxidant, anti-inflammatory, and antimicrobial properties, although their instability limits cosmetic applications. This study aimed to valorize GP through a green extraction process and improve its incorporation and apparent stability in cosmetic formulations through SLN-based systems. (2) Methods: GP extracts were obtained using an eco-friendly extraction method and encapsulated using a *W/O/W double emulsion-solvent evaporation* technique; nanoparticles were characterized (size, polydispersity, zeta potential) and incorporated into cosmetic formulations compared with a blank and a formulation containing free extract. (3) Results: GP-SLNs exhibited suitable physicochemical properties and preserved antioxidant activity, as confirmed by DPPH and ORAC assays; SLN incorporation appeared to preliminarily improve the photostability profile of the formulation under UVA irradiation conditions; in vivo tests showed enhanced skin hydration and moderate occlusivity, while stability studies confirmed consistent color, odor, pH, and viscosity over 60 days; microbiological analyses demonstrated safety and concentration-dependent antimicrobial activity. (4) Conclusions: SLN encapsulation preserved GP bioactivity and improved formulation stability and performance, supporting its potential use in multifunctional cosmetic products.

Keywords: grape pomace; lipid nanoparticles; emulsion

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1. Introduction

Grape crops are one of the main extended agro-economic activities in the world, with more than 60 million tons produced globally every year [1]. Grapes can be consumed fresh in different ways, such as table fruit, juice, and raisins. Moreover, they are mainly used to produce wine, one of the most valued and consumed alcoholic beverages worldwide. Italy is the biggest wine producer in the world, with more than 8.5 million tons per year [2]. In particular, Sicily is a growing area for grape cultivation and winemaking. The most

important Sicilian cultivars of red wine grape are Nero d'Avola, Nerello Mascalese, Nerello Cappuccio, Frappato, and Cabernet Sauvignon [3,4].

However, wine processing generates large amounts of by-products, such as stems, leaves, seeds, peel, and wine lees, that are directed to compost or discard in open areas, potentially causing environmental problems [5]. Nowadays, considerable attention has been paid to the recovery of waste plant matrices as possible sources of functional compounds with health properties [6,7]. Studies have demonstrated that the main by-products of wine processing contain various bioactive molecules. Grape pomace (GP) is the most important by-product of winemaking, and it is considered to be a very abundant source for the extraction of several bioactive compounds, such as polyphenols [8]. The polyphenol content in grape pomace varies significantly depending on the grape variety, cultivation conditions, fruit ripeness, winemaking process, and extraction method; in particular, it has been shown that it is generally higher in red grape varieties compared to white grape varieties [9]. Phenolic compounds mainly contained in grape pomace can be classified, according to their structure, into (i) phenolic acids (hydroxybenzoic and hydroxycinnamic acids), (ii) flavonoids (flavan-3-ols, flavonols, anthocyanins, proanthocyanidins/condensed tannins), and (iii) stilbenoids (resveratrol) [8,10]. Several studies have reported the potential of phenolic compounds from grape pomace to pharmaceutical and cosmetic applications thanks to their antioxidant, anti-inflammatory, and antimicrobial properties [11–14] and protection from UVA damage thanks to procyanidins derived from grape seeds and skins which significantly promote gene expression and collagen deposition in human fibroblasts [15].

The benefic antioxidant and anti-inflammatory actions of GP are presented in the literature, and derive from a large variety of experiments in vitro and in vivo on grape pomace as a whole extract, but also different individual polyphenols that are contained in GP can modulate the endogenous pathway responsible in reducing oxidative stress and chronic inflammation, including that caused by acne vulgaris, a common multifactorial skin disorder that mainly affects adolescents' face, back, and chest, with a significant impact on the quality of life of these young patients [16]. The use of natural compounds, such as phenolic compounds isolated from grape pomace, may represent a valid alternative to the use of topical antibiotics which causes an increase in antibiotic resistance worldwide [17]. In fact, quercetin, tannins, and resveratrol have shown interesting anti-acne potential [18,19].

Moreover, the use of polyphenolic compounds derived from grape by-products as a source of functional ingredients for formulations targeting various fungal pathogens represents a promising field of research. In fact, several topical formulations with antifungal activity have already been patented for both human and veterinary applications [20].

Therefore, the development of a topical GP-based product, which represents a reservoir of all these bioactive compounds, could be a promising and sustainable strategy for the topical treatment and prevention of skin disorders. Although natural bioactive compounds possess several health properties, their therapeutic application is strongly compromised due to their low bioavailability, poor water solubility, and chemical instability. These limitations can be overcome by encapsulating these compounds into solid lipid nanoparticles (SLNs) [21,22], colloidal nanocarriers made from solid lipids and stabilized by surfactant with a mean diameter ranging from 50 and 1000 nm. SLNs have many benefits, such as ease of preparation, high-scale production, excellent physical stability, low cost, chemical versatility, no toxicity, and biodegradability of lipids. The encapsulation of natural compounds into these nanosystems has been reported to improve their solubility, stability, cell uptake, and therapeutic index [23].

Thus, the present work aimed to evaluate the health properties and chemical composition of grape pomace and formulate a topical GP-based product as a multifunctional agent. The novelty of this work lies in the development of a sustainable and innovative

cosmetic formulation that exploits the bioactive potential of grape pomace, enhances its beneficial effects on the skin, and may contribute to improving formulation stability and topical performance.

This study is part of a larger context of research and innovation, aimed at using reasonably natural resources to ensure that they are protected for the benefit of future generations, transforming waste into high value-added products to be reintroduced as raw material in a new production cycle according to the circular economy model, in line with the European Green Deal and the 2030 Agenda.

2. Materials and Methods

2.1. Materials

Grape pomace (GP) was collected after pressing from the Mascalese variety of pre-phylloxera vineyards, grown with organic methods, on the slopes of Mount Etna at 700 m (37°52′00″ N–15°01′41″ E) above sea level. Once collected, the grape pomace was stored at −20 °C until lyophilization. The lyophilized samples were stored at 4 °C for further analytical analyses.

2.2. Chemicals

All chemicals used were of analytical grade and utilized as received from commercial suppliers. HPLC-grade standard quercetin-3-*O*-glucoside, cyanidin-3-*O*-glucoside chloride, and malvidin-3-*O*-glucoside chloride were purchased from Extrasynthese (Genay Cedex, France). Quercetin, caffeic acid, catechin, and gallic acid were purchased from Sigma Aldrich (Milan, Italy). Acetonitrile, Folin–Ciocalteu reagent, formic acid (FA), methanol (MeOH), 96% ethanol (EtOH), 1,1-diphenyl-2-picrylhydrazyl free radical (DPPH$^{\bullet}$), and fluorescein sodium salt were sourced from Merck (Darmstadt, Germany). Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), 2,2-azobis(2-amidinopropane) dihydrochloride (AAPH), $NaNO_2$, and aluminum chloride were procured from Merck. All experiments were conducted using MilliQ water (Merck Life Science S.r.l., Milan, Italy). Hydrogenated lecithin (Lecinol S-10) was obtained from Nikko Chemical (Milan, Italy). Poloxamer 188 (Lutrol F68) was purchased by BASF Chem-Trade GmbH (Burgbernheim, Germany), while stearic acid and hydroxypropylmethyl cellulose (HPMC) were provided by Sigma-Aldrich (St. Louis, MO, USA). All excipients used in the cosmetic formulation, including emollients, emulsifiers, thickeners, humectants, stabilizers, preservatives, and fragrance components, were of cosmetic or pharmaceutical grade and purchased from certified suppliers. Lavandula angustifolia flower water was obtained from Chimpex Industriale S.p.A. (Caivano, Italy). Decyl cocoate, Glycerin, Cetyl ricinoleate, Sesamum indicum seed oil, Hydrogenated ethylhexyl olivate, Polyglyceryl-3 methylglucose distearate, Glyceryl stearate, Cetearyl alcohol, Pentylene glycol, Cellulose, Ricinus communis seed oil, Sodium gluconate, Cellulose gum, Vitis vinifera seed oil, Jojoba esters, Tocopherol, Helianthus annuus seed oil, Glyceryl caprylate, Sodium levulinate, Sodium anisate, Sodium lactate, Sodium PCA, Sodium hyaluronate, Hydrolyzed Sodium Hyaluronate, Phytosterols, Citric acid, Glycine, Fructose, Urea, Inositol, Lactic acid, Sodium benzoate, Glucose, and Potassium sorbate were purchased from ACEF S.R.L. (Piacenza, Italy). Parfum, Citrus limon peel oil, Mentha piperita oil, Rosmarinus officinalis leaf oil, and Melaleuca alternifolia leaf oil were obtained from L.R. Flavours & Fragrances Industries S.p.A. (Milan, Italy). Chondrus crispus powder, Ceratonia siliqua gum, Jojoba alcohol, and Propanediol were purchased from Safic Alcan (Milan, Italy). Water was produced using MilliQ instrument.

2.3. Grape Pomace Extraction

The GP powder was extracted using three methods: maceration with stirring (SE), ultrasound-assisted extraction (UAE), and microwave-assisted extraction (MAE). Two solvents were utilized: 96% ethanol (EtOH) and a mixture of ethanol and water (EtOH:H_2O, 60:40, *v*/*v*).

In each experiment, 0.4 g of GP was suspended in 1.4 mL of solvent and vortexed for 5 min at 415× *g*. The samples were then subjected to extraction under the conditions reported below:

- SE occurred by mixing (300 rpm) for 2 h at room temperature; the supernatant was removed and residue was extracted again for 2 h, and then for 24 h;
- UAE was performed with an ultrasonic bath (35 kHz, 380 W, Bandelin, Berlin, Germany) for an extraction time of 45 s; the supernatant was removed and the residue was extracted twice in the same conditions; the cumulative extraction time was 135 s;
- MAE was performed employing 1000 W power for 40 s with 10 s cycles; after supernatant removal, the extraction was repeated twice, for a total extraction time of 120 s.

The combined supernatant of each extraction was centrifuged at 7850× *g* for 15 min. The combined supernatants were dried and stored at −20 °C until further use. The extraction was repeated three times and was performed in triplicate.

2.4. HPLC-UV Characterization of GP-MAE

MAE extract (40.0 mg/mL) was injected onto an Agilent 1100 series HPLC-UV equipped with an autosampler and a DAD-detector (1220 series). The chromatography run was performed on Luna C-18 column (4.6 × 250 mm, 5 µm; Phenomenex, Milan, MI, Italy) with the following gradient elution of water + 1% HCOOH (solvent A) and CH_3CN + 1% HCOOH (solvent B): t_0 B = 5%, t_{10} B = 15%, t_{30} B = 25%, t_{35} B = 30%, t_{50} B = 55%, t_{55} B = 90%, t_{57} B = 100%, t_{63} B = 100%, t_{75} B = 5%. The system was eluted at 1.0 mL/min. The analyses were recorded at multiple wavelengths: 254 nm, 280 nm, 325 nm, 360 nm, and 530 nm [24]. In the same chromatographic conditions, several standards were injected and among these cyanidin-3-*O*-glucoside, caffeic acid, malvidin-3-*O*-glucoside, quercetin-3-*O*-glucoside, and quercetin were identified. All the identified compounds were quantified by external standard method employing reference standards for calibration curve acquisition. Quercetin (R^2 = 0.9991) and quercetin-3-*O*-glucoside (R^2 = 0.9970) were quantified at 280 nm, caffeic acid was quantified at 325 nm (R^2 = 0.9958), and the two anthocyanins (cyanidin-3-*O*-glucoside R^2 = 0.9979, malvidin-3-*O*-glucoside R^2 = 0.9994) at 350 nm.

2.5. Total Phenolic (TPC) and Flavonoid Contents (TFC) of the Extracts and Formulations

The TCP and TFC of the extracts (1 mg/mL in water: MeOH 50:50) were determined according to the procedure previously reported [25]. The TPC and TFC of the following formulations: skin cream (SC), 1% Grape Pomace-loaded skin cream (GP-SC), and Grape Pomace-loaded SLNs on skin cream (GP-SLNs-SC), were evaluated after preliminary dilution tests. Sample aliquots of the SC and GP-SC were diluted 1:30 in water and GP-SLNs-SC were diluted 1:20 in water. Then, the samples were centrifuged at 2500 rpm for 20 min. The supernatants were used for spectrophotometric analyses. Gallic acid (ranging from 40 to 800 µg/mL) was employed as the reference standard for TPC, whereas catechin (ranging from 18 to 350 µg/mL) was used for TFC. For TPC, the final results are reported as Gallic acid equivalent (GAE) expressed as mg GAE/g of extract. Analogously for TFC, the results are expressed as catechin equivalent (CAT) in mg CAT/g of extract and as µg CAT/g of formulation.

2.6. In Vitro Antioxidant Activity of Extracts and Formulations

The antioxidant activity (measured by DPPH and ORAC assays) of extracts (1 mg/mL) was determined as previously reported [26]. As shown in the next paragraph, skin cream (SC) and 1% GP-SC were assayed at different dilutions (1:30, 1:40, 1:50); whereas GP-SLNs-SC were assayed at 1:20, 1:30, 1:40 dilution factors. The samples were centrifuged at 2500 rpm for 20 min before being employed. For DPPH, aliquots of 20 µL of extracts and of the diluted formulations were added to 200 µL of a 0.19 M DPPH methanolic solution and homogenized. A blank was performed employing water: MeOH 50:50 instead of samples. Trolox solutions, ranging from 0.01 to 0.2 mg/mL, were assayed in the same conditions. The antioxidant activity was expressed in Trolox equivalents (mg TE/g extract or formulation) as the mean standard deviation (n = 3).

For the ORAC assay, a 61.2 nM fluorescein solution (prepared in 75 mM phosphate buffer, pH = 7.4; 200 µM) was pipetted in a 96-multiwell plate, then 25 µL of each sample was tested, or a standard solution was added, and finally, 25 µL of AAPH was added. Fluorescence was acquired for 1 h (λ_{ex}: 485; λ_{em}: 528) and the area under the curve was finally determined. A blank using phosphate buffer instead of the samples, base skin cream, or empty SLNs for skin cream instead of GP-SC and GP-SLNs-SC was included in the assay. The ORAC values were calculated referring to Trolox and assayed in the same conditions of the samples, with concentration ranging from 4×10^{-4} to 7×10^{-7} M. The results are expressed as of µmol TE/g of extract or of formulation.

2.7. Grape Pomace-Loaded SLNs Preparation (GP-SLNs)

GP-SLNs were prepared by *W/O/W double emulsion-solvent evaporation* method using stearic acid as matrix lipid (5 mg) [27]. The external aqueous phase consisted of hydroxypropylmethyl cellulose (50 mg), soy lecithin (50 mg), and Lutrol F68® (50 mg) dissolved in hot water (70 °C). Firstly, the internal aqueous phase containing GP (2 mg/mL), heated at 70 °C, was added dropwise to lipid phase composed of ethanol and stearic acid, previously melted at the same temperature. Then, the obtained pre-emulsion W/O was gently added into the external aqueous phase under stirring (1400 rpm for 8 min), and the mixture was homogenized by an ultrasonic device (UP 400 S, Dr. Hielscher GmbH, Stuttgart, Germany) for 10 min. Subsequently, the W/O/W nanoemulsion was subjected to thermal shock using an ice-bath and filtered. The residual ethanol was removed under vacuum.

2.8. Grape Pomace-Loaded SLNs Characterization (GP-SLNs)

The average size (Z-Ave) and polydispersity index (PDI) of GP-SLNs were measured using *Photon Correlation Spectroscopy* (PCS) with a Zetasizer Nano-ZS90 (Malvern Instruments Ltd., Worcestershire, UK), which features a solid-state laser with a nominal power of 4.5 mW and a maximum output of 5 mW at 670 nm. Measurements were based on the principle of dynamic light scattering and conducted at a scattering angle of 90° and a temperature of 20 ± 0.2 °C. The zeta potential (ZP), which indicates the stability of a dispersed system, was assessed using the *Electrophoretic Light Scattering* (ELS) technique by measuring the electrophoretic mobility of particles in dispersion or solution. For the analysis, 100 µL of the nanoformulation was diluted with 900 µL of distilled water, and measurements were carried out at 25 °C. Each value was recorded in triplicate.

2.9. Study Design and Preparation of Formulation

A total of three cosmetic preparations with and without grape pomace lyophilized extract were prepared. The cosmetic product was prepared following the GMP (Good manufacturing practices) ISO 22716:2007 [28], by mixing different ingredients with the following percentages (m/m):

The product was divided into three aliquots: one was used as a blank (SC), one was added with grape pomace extract (GP-SC) in a concentration of 1%, and one with the nanoparticle extract (GP-SLNs-SC) at the same concentration.

Samples of 100 mL for each product were poured into sterilized glass containers for subsequent analysis.

2.10. Stability Evaluation on Formulation

With the aim to maintain the set of chemical–physical, organoleptic, microbiological, and functional characteristics that give it the claimed activity, the stability of the cosmetic emulsion was evaluated.

Some of the samples were stored at room temperature for stability testing and the other samples were stored at different temperatures for cycle testing.

The related organoleptic properties, pH, phase separation, viscosity changes, and microbial growth were evaluated immediately after preparation and up to 60 days.

For the evaluation of the organoleptic properties, a sensorial analysis was conducted; in particular, a visual analysis of the appearance, color, and odor to understand the compactness of the emulsion, gloss/opacity, and presence or absence of lumps. The determination of the color was carried out in a room colored in absolute white, with a warm light lamp and always at the same height and distance as the sample. The analysis was performed at a constant room temperature of 22 $\pm$ 1 °C to ensure the stability of the emulsion and the reproducibility of the olfactory profile. Also, the olfactory experience of smell was evaluated in terms of pleasantness.

For the pH analyses, a bench-top pH meter (XS pH 8+ DHS Basic—Giorgio Bormac srl) was used, properly calibrated with three buffer solutions (pH 4, 7 and 10), and the temperature was set at 20 $\pm$ 2 °C during the test. In particular, the pH of the formulations was first evaluated immediately after preparation (T_0) in order to assess their initial values and to verify whether adjustments needed to be made and then monitored at 24 h (T_{24}) and at 30 and 60 days (T_{30}, T_{60}) to evaluate the stability of the formulation over the time.

For the evaluation of viscosity, a rotational viscometer (MyrVR 3000—Viscotech Hyspania, S.L., Tarragona, Spain) was used and the result was a direct reading of the viscosity value in mPas. Using the R5 spindle, the rheological behavior of SC, GP-SC, and GP-SLNs-SC was evaluated by measuring the viscosity on increasing shear rates (viscometry test). Specifically, five shear rates were applied to study the effect of shear rate on viscosity: 2, 4, 5, 6, and 10 rpm (rotation per min). Every measurement was taken three times and a mean value was calculated. The test was effectuated at T_{24}, T_{30}, T_{60} to compare the rheological behavior.

To predict the phenomenon of creaming or phase separation, a sign of instability of the emulsions, the mechanical shock test was evaluated using an electric centrifuge (TD4C, 4000 rpm). An aliquot of each formulation test was centrifuged for 30 min at 3000 rpm in order to verify the stability of the oil-in-water (O/W) emulsion.

The stability test was carried out 24 h after production (T_{24}), to minimize the influence of production stress on the formulation. The study included an intermediate assessment at 30 days (T_{30}) and continued up to a maximum of 60 days (T_{60}).

The cycle testing was evaluated to understand the capacity of accelerating instability phenomena in order to identify their resistance that represents an excellent index of stability. In particular, samples were subjected to three freeze–thaw cycling between −10 °C and 25 °C. All measurements were performed in triplicates.

2.11. Antimicrobial Activity

After the production process, parts of the products were collected and analyzed to check their microbiological quality and consequently to avoid degradation phenomena.

The microbiological control was carried out according to the EU Cosmetic Regulation (EC) No 1223/2009 [29] and ISO methods.

Specifically, the Total Aerobic Microbial Count (TAMC) and Total Yeast and Mold Count (TYMC) were measured. Tryptic Soy Agar (TSA) and Sabouraud Dextrose Agar (SDA) were used. Incubation was carried out at 37 ± 2 °C for 24 h for bacterial samples and 72 h for fungal samples. For mucous membranes, the eye area, and children under three years of age, the acceptable limit is $<10^2$ CFU/g and $<10^3$ CFU/g for other cosmetic products.

The absence of pathogens (*Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*) was performed using Mannitol Salt Agar, Cetrimide Agar, McConkey Agar; pathogens must be absent in 1 g of product for cosmetic safety compliance.

Before the analysis, 1 g of each product was emulsified with a quantity of sterile neutralizing agent, Polysorbate 80, stirring mechanically by vortexing, for the neutralization of antimicrobial agents and preservatives. The samples were diluted (1:10) in Sodium Chloride Peptone Solution and 0.1 mL of the primary suspension was distributed on the surface of the specific media and the plates (two Petri dishes for each test) were incubated at the specific temperatures. To ensure the safety of the process, plates with only 0.1 mL of the previous diluent were prepared and incubated at the same time, in the absence of the cosmetic product (control plate). The results were expressed as CFU per gram of sample.

2.12. Time–Kill Assay

The antimicrobial efficacy was evaluated by comparing the three previously described formulations: the blank cream (SC), the extract-loaded cream (GP-SC), and the SLN-encapsulated extract cream (GP-SLNs-SC). In this experimental design, the SC formulation serves as a dual control for both the standard preservative system and the lipidic matrix, allowing to isolate the specific biological contribution of the grape pomace extract from the background effect of the vehicle.

Especially, time–kill assay was performed to evaluate the antimicrobial efficacy of the grape pomace extract-based cosmetic formulation against two representative bacterial strains associated with skin microbiota and opportunistic infections: *Staphylococcus aureus* ATCC 6538 and *Pseudomonas aeruginosa* ATCC 9027. Bacterial suspensions were cultured in Tryptic Soy Broth (TSB) at 37 °C until reaching the mid-logarithmic phase (~1×10^8 CFU/mL), and subsequently diluted to obtain a working inoculum of ~1×10^7 CFU/mL.

Samples (minimum, intermediate and maximum concentrations) were prepared in sterile tubes by mixing 0.1 mL of the working inoculum with 1, 2, and 4 g of formulation and maintained at 20 ± 2 °C. Aliquots (100 µL) were obtained at 0, 1, 2, 4, 8, and 24 h, serially diluted in neutralizing buffer, and plated in triplicate on TSA. Plates were incubated at 37 °C for 24–48 h before counting surviving colonies. Results were expressed as log10 CFU/g of product. The test substance was considered bactericidal when it produced a reduction of at least 3 log10 CFU relative to the initial inoculum.

2.13. Photostability Evaluation Under UVA Exposure

A UVA stability assay was conducted to assess the resistance of three cosmetic formulations to photoinduced degradation: (i) the blank formulation (without extract; SC), (ii) the formulation containing the crude extract (GP-SC), and (iii) the formulation containing grape pomace extract-loaded lipid nanoparticles (GP-SLNs-SC). The test was designed to

compare the protective capacity of lipid nanoparticles in preventing extract degradation under UVA exposure, simulating prolonged solar irradiation.

For each sample, 600 µL was placed in a well of a 24-well plate, covered with a quartz slide, and positioned on a brass block kept on ice during UVA exposure. Samples were irradiated from above for 15 min using a commercial UVA solar lamp, corresponding to an incident dose of 275 kJ/m^2 (approximately equivalent to ~90 min of solar exposure). After irradiation, 200 µL of each sample was collected and extracted with 1 mL of ethyl acetate by vortexing for 2 min, followed by brief centrifugation to separate the organic from the aqueous phase. The UV/Vis spectra of the organic phase were recorded using a Varian Cary 50 UV spectrophotometer (Agilent Technologies Italia S.p.A., Milan, Italy), with ethyl acetate as the control.

2.14. Occlusion Factor

The occlusive properties of cosmetic formulation were assessed using the occlusion factor (F), as described by Wissing et al. [30]. For this experiment, glass beakers (100 mL) filled with 50 mL of distilled water were used and covered with a semi-permeable cellulose membrane (molecular weight cut-off: 12,000–14,000 Da). Two conditions were prepared: (i) test beakers, in which 1 g of the three different cream formulations was evenly spread on the membrane surface, and (ii) control beakers, in which the membrane remained uncovered. All beakers were stored at 37 °C, for 24 h. The occlusion factor (F) was calculated as

$$F = ((A - B)/A) \times 100$$

where A is the water loss from the control (g) and B is the water loss from the sample covered with the formulations (g). All experiments were performed in triplicate and results expressed as mean ± standard deviation (SD).

2.15. Short-Term Test

The short-term in vivo evaluation was performed to assess the tolerability and protective properties of the grape pomace extract-based facial cream [31].

The study was conducted in accordance with the principles of the Declaration of Helsinki: the volunteers were recruited after completing a health questionnaire. After they were fully informed on the nature of the study and on the procedures involved, they gave their written consent. The participants did not suffer from any ailment and were not on any medication at the time of the study. Exclusion criteria included active dermatological disorders, systemic treatments interfering with skin physiology, and recent use of topical drugs or cosmetic products in the test area.

In particular, ten healthy volunteers (aged 25–60 years) with comedone-prone but otherwise healthy skin were recruited.

All subjects remained with their forearms uncovered for 30 min to allow the skin to acclimate to the temperature and relative humidity of the testing room (T: 22 °C; RH: 50 ± 5%). If, after the acclimatization phase, at least two hydration or elasticity values differed markedly (greater than ±5 units), additional time was allowed before proceeding with the measurements.

A standardized amount of cream (200 mg) was applied to a 2 cm^2 area of the volar forearm. Control sites included (i) the vehicle cream without grape pomace extract and (ii) untreated skin. A single application was performed. Skin hydration was measured at baseline (T_0) and at 15, 30, and 60 min after application (T_{15}, T_{30}, T_{60}) using a MoistureMeterSC (Delfin Technologies, Kuopio, Finland). Any potential side effects were also monitored.

2.16. Data Analysis

All the findings were expressed as mean ± standard deviation (SD), as achieved from experiments performed at least in triplicate. Tukey's test was employed to denote differences between samples, defined as statistically significant when p-values were lower than 0.05. Data analysis was performed with OriginPro 2024 software (Northampton, MA, USA).

3. Results and Discussion

3.1. Extraction

Three extraction techniques were evaluated as an initial step in obtaining the bioactive constituents of grape pomace with high phenolic content, flavonoid content, and antioxidant activity. Due to the polar nature of polyphenols, they can be extracted with high effectiveness in polar protic media such as hydroalcoholic solutions. Several researchers have used water/alcohol mixtures for the extraction of grape by-products because water can promote better mass transfer by molecular diffusion as well as high cell tissue permeability. It is reported that ethanol/water mixtures afforded the highest recovery of phenolics compared to acetone or methanol/water mixtures [32]. For example, the extraction of catechins and procyanidins was more efficient with the use of ethanol/water mixtures [33]. It is noteworthy that ethanol is cheaper and GRAS (Generally Recognized As Safe, according FDA) and is regularly employed in the case of successive food applications. Conventional maceration with SE was compared to green technologies, such as MAE and UAE. These last techniques offer significant advantages, including environmental friendliness, efficient extraction, and the preservation of bioactive components. They require short extraction time (min) at a controlled temperature and consume minimal energy [34]. Furthermore, in the case of the MAE process, the microwave field can positively affect the dipole rotation of polar solvents, causing a greater energy transfer to the matrix, the cell destruction, and improving the solubilization of target compounds in the solvent [35]. On the other hand, UAE has been reported as a more effective tool for phenolic recovery as sonication simultaneously enhances the hydration and the penetration of solvent into the matrix trough cavitation phenomena, facilitating the mass transfer of solutes to the extraction solvent [33]. For each extraction procedure, two solvents were used: 96% ethanol (EtOH) and a mixture of EtOH:H_2O (60:40, v/v) [36]. SE, UAE, and MAE were carried out according to literature conditions for similar extracts [37,38]. The experimental details are summarized in Table 1 and detailed in Materials and Methods.

Table 1. Extraction of GP with several procedures, TPC, TFC, and antiradical activity (DPPH and ORAC assays).

Exp	Method	Solvent	Yield%	TPC (mg GAE/g)	TFC (mg CAT/g)	DPPH (mg TE/g)	ORAC (μmol TE/g ± SD)
1	SE	EtOH:H_2O	23.64%	127.7 ± 11.5 ^{d}	238.9 ± 5.1 ^{a,b}	0.502 ± 0.031 ^{c}	1347.3 ± 124.2 ^{b,c}
2	SE	EtOH	23.6%	109.8 ± 11.2 ^{d}	266.4 ± 37.7 ^{a,b}	0.448 ± 0.086 ^{c}	1036.4 ± 74.5 ^{c}
3	UAE	EtOH:H_2O	20.9%	188.2 ± 0.6 ^{b}	277.4 ± 17.5 ^{a}	0.684 ± 0.085 ^{a,b}	1693.2 ± 129.0 ^{b}
4	UAE	EtOH	21.1%	124.3 ± 9.2 ^{d}	217.2 ± 22.8 ^{a,b}	0.559 ± 0.022 ^{b,c}	1572.6 ± 143.7 ^{b}
5	MAE	EtOH:H_2O	22.8%	218.1 ± 14.2 ^{a}	280.6 ± 14.5 ^{a}	0.809 ± 0.046 ^{a}	2597.3 ± 197.2 ^{a}
6	MAE	EtOH	19.6%	159.0 ± 1.8 ^{c}	204.5 ± 11.4 ^{b}	0.585 ± 0.018 ^{b,c}	1520.8 ± 100.1 ^{b}

All the results reported are mean ± SD (n = 3); $^{a–d}$ different letters in each column indicate statistical differences at $p < 0.05$ (Tukey test).

According to the results of total phenolic content (TPC), all the extractions occurring with EtOH:H_2O (60:40. v/v) gave higher recovery in phenolics than extractions performed with EtOH alone. This is in agreement with the literature as water–ethanol solvent mixtures

are more effective in the extraction for the higher compound diffusion rate than a single solvent [39]. Among the procedures employed, MAE proved the most effective technique in the recovery of phenolic compounds (TPC = 218.1 mg GAE/g) including flavonoids (TFC = 280.6 mg CAT/g). It is known that MAE applied to grape pomace proved to recover a greater amount of antioxidants in less time in respect to other extraction methods [40]. Álvarez et al. obtained 57% of improvement in the extraction of polyphenols from grape pomace employing MAE [41].

3.2. HPLC-UV Characterization of PG-MAE Extract

Due to the polar properties of phenolics present in grape pomace, the C18-HPLC-UV technique is the most injected to HPLC-UV and subjected to quali-quantitative analysis. Standards of previously identified constituents of grape pomace were injected in the same conditions in order to verify their content. Caffeic acid, quercetin, and quercetin-3-*O*-glucoside, as well as anthocyanins cyanidin-3-*O*-glucoside and malvidin-3-*O*-glucoside, were identified in the extract (Figure 1). The results were in agreement with previously reported findings on grape pomace [10,42].

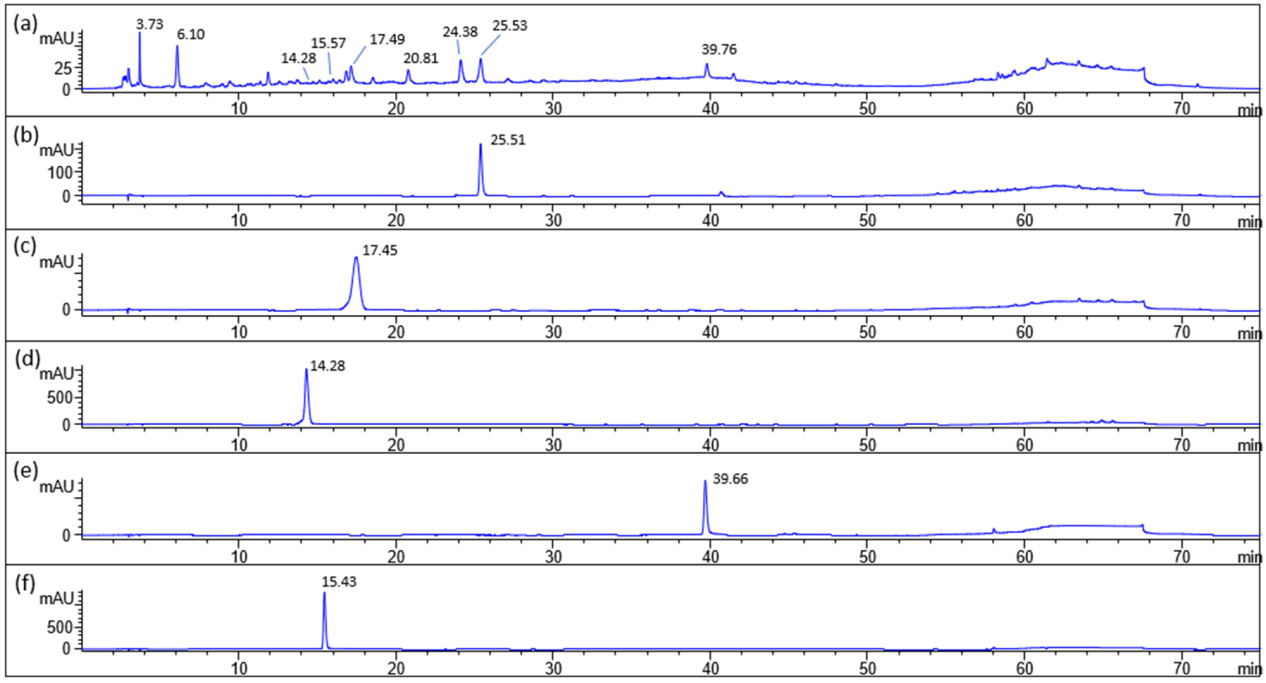


Figure 1. HPLC-UV chromatograms (280 nm) of (**a**) GP-MAE, (**b**) quercetin-3-*O*-glucoside, (**c**) malvidin-3-*O*-glucoside, (**d**) cyanidin-3-*O*-glucoside, (**e**) quercetin, (**f**) caffeic acid.

The concentration of the different constituents depends on the extractive method as well as on the cultivar under evaluation. For example, quercetin-3-*O*-glucoside is the most abundant compound identified in PG-MAE (472.9 ± 12.4 mg/Kg); the same trend was observed by V. Amico et al. on grape pomace extract from Nerello Mascalese obtained by classical extraction with MeOH (179 mg/Kg) [10]. This comparison suggested that MAE extraction, developed in this work, is more effective in the recovery of quercetin-3-*O*-glucoside and other phenolics including cyanidin-3-*O*-glucoside (11.2 mg/Kg in this work versus 6.99 mg/Kg) and quercetin (178.7 mg/Kg in this work versus 15.30 mg/Kg). Similarly, Sciortino et al. quantified a comparable amount of quercetin in grape pomace from Perricone (178.60 mg/Kg) and Syrah (170.23 mg/Kg), and this was lower in Nero d'Avola (51.34 mg/Kg) by extraction with microwave diffusion and gravity [42].

3.3. GP-SLNs Characterization

The formulation of GP-SLNs was carried out using the *W/O/W double emulsion-solvent evaporation* technique, where stearic acid was employed as the lipid phase and Lutrol F68® (Poloxamer 188) as the surfactant to stabilize the system. Despite the inherent instability of GP, this approach demonstrated both reliability and reproducibility in producing the nanoparticles. In detail, PCS measurements revealed that the nanoparticles exhibited a particle size of 142.7 ± 5.162 nm, with a size distribution of 0.221 ± 0.015, indicating good homogeneity. Furthermore, the zeta potential value of −19.9 ± 0.056 mV confirmed the stability of the nanodispersion, suggesting a physically homogeneous nanodispersion immediately after preparation.

3.4. Antioxidant Activity of Skin Cream and SLNs-Skin Cream Formulations

The evaluation of TPC, TFC, and antioxidant activity (DPPH and ORAC) previously performed on the extract (Table 2) was also conducted on the blank skin cream (SC), 1% Grape Pomace-loaded skin cream (GP-SC) and Grape Pomace-loaded SLNs on skin cream (GP-SLNs-SC) (Table 3).

Table 2. Composition of cosmetic preparations.

Ingredient	Percentage % (m/m)
Aqua	60.22
Lavandula angustifolia flower water	5
Decyl cocoate	5
Glycerin	5.09
Cetyl ricinoleate	4
Sesamum indicum (sesame) seed oil	3
Hydrogenated ethylhexyl olivate	2.28
Polyglyceril-3 methylglucose distearate	3
Glyceril stearate	1.5
Cetearyl alcohol	1.5
Pentylene glycol	1
Cellulose	2
Ricinus communis seed oil	0.6
Sodium gluconate	0.2
Cellulose gum	0.16
Vitis vinifera (grape) seed oil	0.5
Jojoba esters	0.13
Tocopherol	0.50012
Heliantus annuus seed oil	0.15
Glyceryl caprylate	0.3
Parfum	0.2
Sodium hyaluronate	0.1
Hydrolyzed Sodium Hyaluronate	0.1
Mentha piperita leaf extract	0.17
Sodium levulinate	0.3
Sodium anisate	0.12
Salicylic acid	0.5
Sodium lactate	0.46
Sodium PCA	0.46
Phytosterols	0.12
Chondrus crispus powder	0.04
Ceratonia siliqua gum	0.04
Jojoba alcohol	0.06
Propanediol	0.01

Table 2. *Cont.*

Ingredient	Percentage % (m/m)
Citric acid	0.04
Niacinamide	0.02
Glycine	0.02
Fructose	0.02
Urea	0.012
Inositol	0.02
Lactic acid	0.02
Glucose	0.009
Sodium benzoate	0.008
Potassium sorbate	0.008
Citrus limon peel oil	0.003
Mentha piperita oil	0.003
Rosmarinus officinalis leaf oil	0.003
Melaleuca alternifolia leaf oil	0.003

Table 3. TPC, TFC, and antiradical activity (DPPH and ORAC assays) of formulations.

Sample	TPC (μg GAE/g ± SD)	TFC (μg CAT/g ± SD)	DPPH (mg TE/g ± SD)	ORAC (μmol TE/g ± SD)
SC	51.39 ± 0.85 ^{c}	187.34 ± 2.84 ^{b}	0.25 ± 0.001 ^{b}	32.51 ± 0.48 ^{b}
GP-SC	245.93 ± 1.50 ^{a}	278.70 ± 10.49 ^{a}	0.33 ± 0.02 ^{a}	85.21 ± 2.73 ^{a}
GP-SLNs-SC	216.60 ± 1.68 ^{b}	269.02 ± 13.21 ^{a}	0.36 ± 0.01 ^{a}	83.59 ± 1.38 ^{a}

All the results reported are mean ± SD (n = 3); different letters in each column indicate statistical differences at $p < 0.05$ (Tukey test).

SC was used as a blank in the evaluation of formulated GP-SC and GP-SLNs-SC. The base formulation contains 0.50012% of tocopherol and 0.5% of grapeseed oil. Globally, the values (Table 4), lower than those acquired for the extract (entry 5, Table 2), are consistent with the inclusion of 1% of the extract.

Table 4. Compounds identified by HPLC-UV on GP-MAE extract.

t_R (min)	Identification	mg/Kg of Extract
14.28	Cyanidin-3-*O*-glucoside	11.2 ± 0.4 ^{c}
15.43	Caffeic acid	25.7 ± 1.6 ^{c}
17.45	Malvidin-3-*O*-glucoside	27.4 ± 2.1 ^{c}
25.51	Quercetin-3-*O*-glucoside	472.9 ± 12.4 ^{a}
39.66	Quercetin	178.7 ± 9.4 ^{b}

Different letters indicate statistical differences at $p < 0.05$ (Tukey test).

As can be observed from Table 3, the basic formulation (SC) gave positive results on all the assays and this can be explained looking at the complex composition of cream, which includes 0.5% tocopherol and 0.5% grapeseed oil. However, it is noteworthy that the addition of 1% grape pomace extract (GP-SC and GP-SLNs-SC) led to a significant and marked increase in all antioxidant parameters compared to the SC base ($p < 0.05$). Both the formulations have shown comparable results as TPC (GP-SC 245.93 μg GAE/g; GP-SLNs-SC 216.60 μg GAE/g) and TFC (GP-SC 278.70 μg CAT/g; GP-SLNs-SC 269.02 μg CAT/g). Analogously, the antioxidant behavior of the two preparations in scavenging DPPH (GP-SC 0.33 mg TE/g; GP-SLNs-SC 0.36 mg TE/g) and fluorescein radicals (ORAC; GP-SC 85.21 μmol TE/g; GP-SLNs-SC 83.59 μmol TE/g) was found to be statistically superior to the blank SC, demonstrating that the bioactive polyphenols remain fully functional even when encapsulated in the SLN matrix. This confirms that the lipidic matrix of SLNs acts

as an inert delivery system that does not quench the radical scavenging activity of the phytocomplex. A similar trend has been reported in commercial creams containing 5% of an hydroalcoholic extract from seeds of Vitis vinifera Marcelan [43].

3.5. Stability Evaluation on Formulation

The results demonstrate that cosmetic formulations containing grape pomace extract, both in free form and encapsulated in SLNs, maintained organoleptic and chemical stability over long-term storage.

Regarding the organoleptic parameters, the control formulation SC retained a constant white appearance, indicating good visual stability. Both GP-SC and GP-SLNs-SC exhibited a stable pinkish coloration from 24 h onward, suggesting chromatic stability of the phenolic compounds. In all formulations the characteristic fragrance of the base product was maintained without perceptible changes, indicating aromatic stability and the absence of oxidative or degradative processes.

The mechanical shock test performed on a centrifuge showed no sign of emulsion instability. No creaming, phase separation, or coalescence phenomena were observed. Therefore, the emulsion was found to be stable in the mechanical shock test.

3.5.1. pH Determination

The pH of the formulations was first evaluated at T_0 and then monitored at T_{24}, T_{30}, and T_{60}, as reported in Table 5.

Table 5. pH values of the three formulations at T_0, T_{24}, T_{30}, T_{60}. Time points: T_0 = baseline; T_{24} = 24 h; T_{30} = 30 days; T_{60} = 60 days.

pH	T_0	T_{24}	T_{30}	T_{60}
SC	5.75 ± 0.02	5.82 ± 0.01	5.84 ± 0.01	5.84 ± 0.01
GP-SC	4.99 ± 0.01	4.87 ± 0.01	4.88 ± 0.01	4.88 ± 0.01
GP-SLNs-SC	5.35 ± 0.01	5.20 ± 0.02	5.22 ± 0.01	5.27 ± 0.01

Every value was calculated as the mean (±SD) of three sample measurements (n = 3).

The SC formulation showed stable pH values, increasing slightly from 5.75 at T_0 to 5.84 at both 30 and 60 days, confirming its chemical stability in the absence of extract. In contrast, GP-SC exhibited lower values already at T_0 (4.99), which further decreased to 4.87 at 24 h and remained constant thereafter. This trend reflects the intrinsic acidity of polyphenols and confirms that the extract is the main factor responsible for the lower pH of the formulation.

GP-SLNs-SC presented an intermediate behavior: after an initial drop at 24 h (5.20), a slight but progressive increase was observed (5.22 at 30 days and 5.27 at 60 days). These variations may reflect the overall physicochemical equilibrium of the formulation during storage. Nevertheless, all formulations maintained pH values within the physiological range suitable for topical application.

3.5.2. Viscosity Determination

Viscosity profiles at different shear rates (rpm) over 24 h, 30 days, and 60 days are summarized in Figure 2. All samples exhibited pseudoplastic behavior, with viscosity decreasing as shear rate increased. Over time, SC formulation showed a mild variation during storage, with an initial decrease at 30 days followed by recovery at 60 days. After 60 days, the SC formulation showed a partial recovery, displaying values close to the baseline despite the time-dependent rheological variations observed during storage.

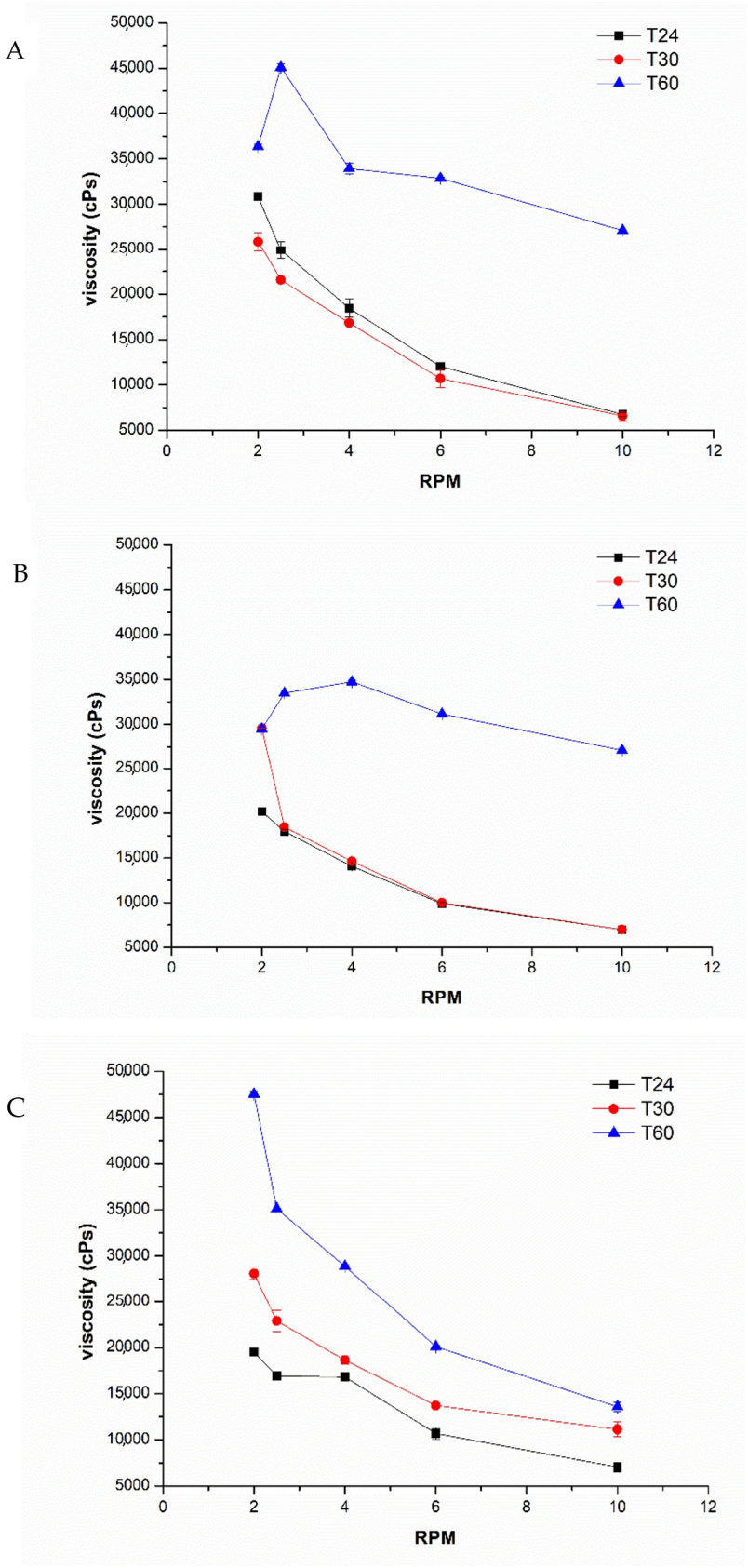


Figure 2. Viscosity profiles overtime of (**A**) SC, (**B**) GP-SC, and (**C**) GP-SLNs-SC formulations. Values are expressed as the mean (±SD) of sample replicates (n = 3). Different symbols indicate the differences among the viscosity values on the products ($p < 0.05$). Time points: T_0 = baseline; T_{24} = 24 h; T_{30} = 30 days; T_{60} = 60 days.

The GP-SC formulation, however, consistently exhibited lower viscosity compared to the control at all time points, in agreement with the structural influence of the extract. Interestingly, GP-SC samples showed a progressive increase in viscosity over time, reaching their maximum values at T_{60}. A similar pattern was observed for the GP-SLNs-SC formulation, which combined the effect of the extract with the lipid matrix, producing an even more pronounced low-shear viscosity peak at 2.5 rpm. This behavior may reflect the complex rheological properties of the formulation system, likely influenced by the combined contribution of formulation components, including thickening agents and dispersed phases. Similar non-Newtonian and shear-thinning behaviors are commonly observed in semisolid topical formulations. These findings indicate that extract incorporation influenced the rheological profile of the formulation, contributing to time-dependent viscosity variations during storage, indicating time-dependent changes in formulation viscosity during storage.

From a practical perspective, the selective increase in viscosity at low shear rates may influence the spreadability characteristics of the formulation during application. Nevertheless, the preservation of shear-thinning behavior at higher shear rates indicates that all formulations remained suitable for topical application throughout the stability test period. As previously reported by Calienni et al. [44], the non-proportionality between shear rate and shear stress is characteristic of non-Newtonian behavior, and pseudoplastic fluids are fluids whose viscosity decreases with an increase in shear rate (shear thinning) [45]. As expected for topical products, a pseudoplastic behavior was observed in all formulations, which is considered advantageous for topical spreading and application [46].

3.5.3. Cycle Testing

The impact of thermal stress on the formulations was evaluated through three consecutive freeze–thaw cycles. No changes in visual appearance, color, or odor were detected in any formulation, indicating that the organoleptic properties were preserved under extreme temperature fluctuations (Table 6).

Table 6. Physicochemical stability evaluation of formulations after freeze–thaw cycling.

Formulation	Color/ Odor Changes	Phase Separation	pH Stability	Viscosity Changes After Freeze–Thaw	Stability Assessment
SC	No detectable changes	Absent	Maintained within skin-compatible range	Slight decrease in viscosity	Physically stable
GP-SC	No detectable changes	Absent	Maintained within skin-compatible range	Moderate rheological variation	Physically stable
GP-SLNs-SC	No detectable changes	Absent	Maintained within skin-compatible range	Moderate rheological variation after thermal stress	Physically stable

The pH values remained substantially stable, with only minor deviations from baseline exhibiting a slight increase and indicating the chemical stability of formulations despite thermal cycling.

After cycle testing, viscosity measurements, however, revealed a reduction in all formulations, compared to a trend of long-term storage data (30 and 60 days), where viscosity increased progressively, especially in the extract-containing formulation, indicative of structural consolidation over time. This decrease may reflect changes in the rheological organization of the formulation under repeated temperature stress.

Differences observed under freeze–thaw cycles versus long-term ambient storage suggest that intermittent thermal stress transiently alters the colloidal structure, in accordance with Bernal-Chávez et al. 2023 who found that the composition of the solution and its physicochemical properties can be drastically different from the initial ones, including

potential changes in pH, ionic strengths, osmotic pressure, or viscosity, depending on the nature of the polymer [47]. Similar variations in physicochemical properties after freeze–thaw cycles have been reported in complex semisolid systems.

Moreover, this is consistent with the literature showing that plant extracts can interact with emulsion matrices to modulate rheological behavior [44].

3.6. Microbiological Control

At the end of the manufacturing process, samples of the formulations subjected to microbiological quality control, showed no detectable bacterial or fungal growth, thereby confirming compliance with microbiological safety requirements and demonstrating the robustness of the manufacturing process.

Moreover, all formulations remained microbiologically stable throughout the storage period (30 days and 60 days). No bacterial or fungal colonies were detected in total viable counts, and selective testing confirmed the absence of *S. aureus*, *E. coli*, and *P. aeruginosa*. The lack of detectable growth indicates that neither primary contamination during manufacturing nor secondary proliferation during storage occurred.

Importantly, these findings suggest that the preservative system alone, together with the intrinsic antimicrobial activity of grape pomace polyphenols, provided effective protection. While the blank formulation (SC) relies on a standard and 'green-oriented' preservative system (including salicylic acid, sodium benzoate, potassium sorbate, sodium levulinate, and sodium anisate) for basic stability, the significant contribution of GP extract is evidenced by the enhanced robustness of the loaded formulations. Unlike traditional cytotoxic agents, these modern preservative systems base their efficacy on complex mechanisms, such as cytoplasmic acidification and interference with microbial membrane potentials, which are highly dependent on the surfactant matrix and pH. In an aqueous matrix characterized by the presence of non-ionic surfactants, the antimicrobial efficacy is often governed by the partitioning between the continuous phase and the micellar phase. The incorporation of GP polyphenols may provide a fundamental synergistic effect in this environment; while some preservatives can undergo micellar sequestration—a thermodynamically favored phenomenon that reduces their free bioavailable fraction—the bioactive compounds from grape pomace can function as additional membrane-disrupting agents or permeation enhancers. The absence of even low-level contamination over 60 days supports the robustness of the formulations and highlights their suitability for long-term stability studies.

The consistency of negative results across multiple formulations also suggests that the incorporation of grape pomace extract did not compromise microbial safety; rather, it may have contributed synergistically to the inhibitory profile, in accordance with previous studies have investigated the antimicrobial properties of grape pomace extracts and their ability to inhibit various pathogenic properties. Grape pomace phenolic compounds have shown the ability to inhibit the activities of enzymes and proteins found in bacteria [48].

Sateriale et al. reported significant antibacterial activity of grape pomace extracts, with minimum inhibitory concentrations (MICs) as low as 0.312 mg/mL against *E. coli* and 0.625 mg/mL against *S. aureus* [49].

These data collectively demonstrate that the formulations meet the microbiological quality standards required for topical cosmetic products and can be considered safe for intended use.

3.7. Time–Kill Assay of the Cosmetic Product Against Staphylococcus aureus and Pseudomonas aeruginosa

The antimicrobial activity of the formulations was confirmed through time–kill analysis in GP-SC and GP-SLNs-SC against *Staphylococcus aureus* (initial inoculum

1.5×10^7 CFU/mL) and *Pseudomonas aeruginosa* (initial inoculum 4×10^7 CFU/mL). Distinct concentration and species dependent effects were observed.

As established in the experimental setup, the comparison between the loaded formulations and the SC control was fundamental to deconvolve the antimicrobial effects.

While the SC base provides the necessary microbiological stability through its standard preservative system, the significant dose-dependent reduction in S. aureus viability observed only in GP-SC and GP-SLNs-SC unequivocally attributes the bactericidal effect to the grape pomace polyphenols. This confirms that the SLN matrix, whose components are already represented in the SC matrix, functions as an inert delivery system.

For *S. aureus*, growth was detected only at the lowest tested concentration after extended incubation (~10^4 CFU/mL), while increasing concentrations of the formulation resulted in a progressive reduction of viable cells. This dose–response trend indicates a bactericidal effect mediated by the encapsulated grape pomace extract, which enhanced the antimicrobial profile of the base formulation. Notably, since the concentration of standard preservatives remained constant across all tested samples, the observed dose-dependent reduction in viable cells can be unequivocally attributed to the increasing concentration of the GP extract, rather than the cream base alone.

In contrast, *P. aeruginosa* was completely inhibited under all tested conditions, with no detectable colonies at any concentration or incubation time. This suggests that the extract exerts a more potent and rapid bactericidal action against Gram-negative strains, likely due to differences in cell wall susceptibility to polyphenolic interactions.

Overall, the formulations demonstrated effective antimicrobial activity at concentrations compatible with topical application. These results highlight the dual role of the encapsulated grape pomace extract: not only as a bioactive cosmetic ingredient but also as a functional agent contributing to product preservation and potentially supporting microbiome balance by inhibiting the overgrowth of opportunistic pathogens such as S. aureus and P. aeruginosa. Finally, the consistent activity observed in GP-SLNs-SC confirms that the lipidic scaffold of SLNs—whose components are already represented in the SC matrix—acts as an inert delivery system that does not interfere with the intrinsic antimicrobial activity of the phytocomplex.

3.8. Photostability of Cosmetic Formulations Under UVA Exposure

The photochemical stability of three cosmetic formulations (SC, GP-SC, and GP-SLNs-SC) was assessed by UV–Vis spectroscopy at 300 nm before and after UVA exposure (275 kJ/m^2, 15 min) (Figure 3).

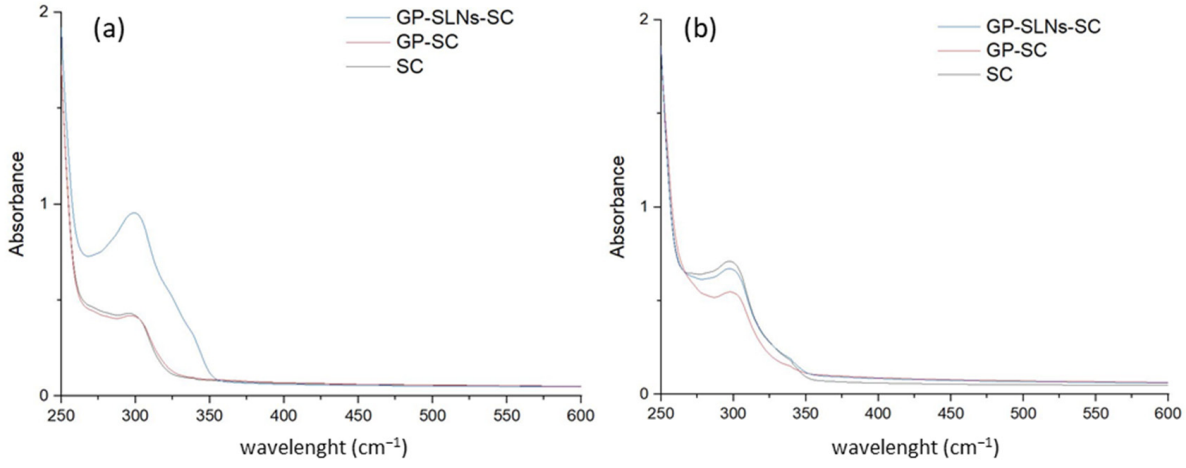


Figure 3. UV profiles of SC, GP-SC, and GP-SLNs-SC formulations before (**a**) and after (**b**) UVA exposure.

Before irradiation, the GP-SLNs-SC formulation showed significantly higher absorbance (0.95) compared to SC (0.43) and GP-SC (0.41), reflecting the higher concentration of UVA-absorbing polyphenols preserved through encapsulation. Following UVA exposure, absorbance increased by 65% in SC and 31% in GP-SC, likely due to formation of photodegradation products or matrix alterations. In contrast, GP-SLNs-SC exhibited a 29% decrease in absorbance, indicating reduced photochemical transformation, and thus, a greater apparent photostability of the active compounds. The observed behavior is consistent with previous studies reporting a protective role of SLNs against photodegradation of encapsulated compounds [50].

These results suggest that the free grape pomace extract is susceptible to photooxidation, which may compromise the stability and efficacy of phenolic constituents. Based on these preliminary results, the encapsulation within SLNs may contribute to reducing photodegradation phenomena. These results support the potential of SLN encapsulation to enhance the functional performance and longevity of plant-derived actives in topical applications. Moreover, this strategy represents a promising 'green' alternative to conventional UV filters, which often suffer from photoinstability and environmental concerns.

3.9. Occlusion Factor

The results of the occlusion factor (OF) showed that the SC formulation exhibited the highest OF, with a value of 18.28%, consistent with its emollient base and film-forming properties. The addition of grape pomace extract in free form (GP-SC) slightly reduced the occlusion factor (16.30%), likely due to the presence of polyphenolic compounds with antioxidant and mildly astringent properties, which may interfere with lipid film formation on the skin surface.

The GP-SLNs-SC formulation showed a slightly lower value of 14.33% (Table 7).

Table 7. OF values for the three tested formulations at 37 °C for 24 h.

Formulation	Occlusion Factor (%)
SC	18.28
GP-SC	16.30
GP-SLNs-SC	14.33

Nevertheless, all values fall within the medium occlusivity range and are consistent with the literature data, which report OF values above 10% as potentially relevant for skin hydration. Although higher values (e.g., ~25%) are sometimes classified as low but significant occlusivity, the present results suggest a potential contribution to the reduction of transepidermal water loss (TEWL).

These findings suggest that the incorporation of grape pomace extract does not markedly compromise the formulation's occlusive performance and that both blank and extract-containing creams may contribute to skin barrier reinforcement and hydration.

Indeed, as shown in different scientific studies, SLNs reduce occlusivity while enhancing skin delivery and stability of active compounds. Moreover, nanoparticles may modulate the release of entrapped natural bioactives by adjusting the formulation factors and nanoparticles' properties, to potentially modulate the release profile of entrapped natural bioactives depending on formulation characteristics and nanoparticle properties.

Improved topical performance has been associated with small particle size, film formation, and occlusive properties. Thus, by increasing skin hydration and protection, the penetration of the nanoparticles loading natural bioactives is improved, consequently, increasing their anti-inflammatory, antioxidant, and antimicrobial properties [51].

3.10. Short-Term Test

The study involved non-invasive cosmetic evaluations on healthy volunteers and was conducted in accordance with the Declaration of Helsinki after obtaining written informed consent from all participants.

Both the blank and extract-containing formulations increased skin hydration, with the SLN-based formulation (GP-SLNs-SC) showing the most pronounced effect. Instrumental measurements demonstrated that the encapsulated grape pomace extract significantly enhanced skin hydration, with the most pronounced effect observed 60 min after application, compared with the blank formulation. Our findings are consistent with the work of Pavlou et al. [52], who demonstrated that SLN and NLC formulations form a hydrophobic film on the skin surface, effectively reducing transepidermal water loss and improving hydration, especially in vulnerable skin.

These results demonstrate that SLN encapsulation enhances the immediate and sustained hydration performance of the extract compared with the blank formulation, suggesting a short-term improvement in skin hydration and water retention. However, longer-term studies with repeated applications are needed to confirm sustained clinical benefits. The findings support the potential of encapsulated grape pomace polyphenols as functional ingredients for prolonged skin moisturization in topical applications.

4. Conclusions

This study demonstrates that grape pomace, a by-product of the winemaking industry, can be effectively valorized to obtain a polyphenol-rich extract with multiple bioactivities, including antioxidant, soothing, and purifying properties, using a green and sustainable extraction process. The incorporation of this extract into cosmetic formulations, particularly through encapsulation in solid lipid nanoparticles (SLNs), appeared to improve its photostability, preserved its functional bioactivity, and improved critical formulation characteristics, such as skin hydration, occlusivity, and rheological behavior.

The SLN-based formulation (GP-SLNs-SC) exhibited long-term chemical and microbiological stability, maintained organoleptic acceptability, and provided a short-term hydrating effect, demonstrating that nanocarrier systems may help protect sensitive plant-derived compounds. Furthermore, the time–kill assay confirmed a species- and concentration-dependent antimicrobial activity, highlighting the additional role of grape pomace polyphenols in supporting skin microbiome balance and product preservation.

Overall, this work successfully achieved its dual objectives: (i) valorizing agro-industrial waste in line with circular economy principles, contributing to the sustainable use of natural resources, and (ii) developing a functional, safe, and skin-protective cosmetic product aligned with current trends in green and ethical cosmetics. The findings support the potential of grape pomace polyphenols as multifunctional cosmetic ingredients and underscore the value of combining bioactive plant extracts with nanocarrier technology to create eco-friendly, high-performance formulations.

Future studies should focus on in vivo assessments of long-term skin benefits, consumer acceptability, optimization of SLN formulations, and further investigation of delivery behavior and release properties. Such investigations would further strengthen the translational potential of agro-industrial by-products in sustainable cosmetic development.

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Institutional Review Board Statement: Ethical review and approval were waived for this study, since the studies were conducted to evaluate the hedonistic aspects, the compliance and the acceptability of the cosmetic products by volunteers in comparison with a product suggested by OMS. No invasive techniques were applied.

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The data used to support the findings of this study are available from the corresponding author upon request.

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Conflicts of Interest: The authors declare no conflicts of interest.

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