

Article

Upcycling Winery Waste into Functional Cosmetic Ingredient: Green Recovery of Squalene from Wine Lees as a Potential In Vitro Permeation Enhancer

Ela Hoti ¹, Lucrezia Di Nicolantonio ^{2,3}, Marco Zannotti ³, Rita Giovannetti ³, Stefano Ferraro ³,
Piera Di Martino ⁴ and Maria Rosa Gigliobianco ^{4,*}

¹ Department of Pharmacy, Faculty of Medicine, University of Medicine Tirana, ALB1005 Tirana, Albania; ela.hoti@umed.edu.al

² Recusol Srl, 62032 Camerino, Italy; lucrezia.dinicolantonio@unicam.it

³ Chemistry Interdisciplinary Project (ChIP), School of Science and Technology, Chemistry Division, University of Camerino, 62032 Camerino, Italy; marco.zannotti@unicam.it (M.Z.); rita.giovannetti@unicam.it (R.G.); stefano.ferraro@unicam.it (S.F.)

⁴ Department of Pharmacy, University "G. D'Annunzio" Chieti-Pescara, 66100 Chieti, Italy; piera.dimartino@unich.it

* Correspondence: maria.gigliobianco@unich.it

Abstract

Squalene and squalane are widely used cosmetic ingredients valued for their emollient properties and excellent skin compatibility, yet sustainable sourcing remains a challenge. This study presents an integrated and eco-friendly strategy for valorizing wine lees as a renewable source of squalene and converting it into stable, high-performance squalane. Squalene was efficiently recovered from yeast-rich winery waste through optimized ultrasound-assisted extraction, followed by chromatographic purification. Green catalytic hydrogenation using palladium supported on natural clay minerals enabled the selective conversion of squalene into squalane under mild conditions. The functional evaluation via in vitro transport studies across an artificial membrane, using quercetin as a poorly permeable model antioxidant, demonstrated enhanced permeation compared with conventional vehicles, while accelerated aging experiments further confirmed the superior oxidative stability of squalane relative to native squalene. Overall, this work provides a proof of concept for upcycling winery by-products into multifunctional cosmetic ingredients that combine sustainability, stability, and functional performance, supporting circular economy principles and the growing demand for ethically sourced raw materials in the cosmetic industry.

Keywords: squalene; wine lees; winery waste upcycling; sustainable cosmetics; green extraction; ultrasound-assisted extraction; penetration enhancers; quercetin



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1. Introduction

Squalene (C₃₀H₅₀) is a naturally occurring triterpene hydrocarbon found across animals, plants, and microorganisms, and serves as a key intermediate in sterol biosynthesis. In humans, squalene constitutes a major component of skin surface lipids, contributing to skin lubrication and providing antioxidant defense against environmental oxidative stress [1,2]. For decades, shark liver oil has been the principal industrial source of squalene; however, increasing ethical and environmental concerns regarding shark overexploitation have driven the search for sustainable alternative sources [1]. Vegetable oils, such as olive or amaranth oil, contain considerable amounts of squalene; however, plant-based production

is constrained by low squalene yield per unit biomass and long cultivation cycles, resulting in limited productivity at the industrial scale [3]. In contrast, microbial sources, including yeasts such as *Saccharomyces cerevisiae*, offer rapid growth, short production cycles, and opportunities for process intensification, making them attractive candidates for sustainable squalene recovery [3,4].

Wine lees, a by-product of winemaking, are particularly rich in *S. cerevisiae* cells and residual lipophilic compounds. Ultrasound-assisted extraction (UAE) has demonstrated the feasibility of recovering squalene from wine lees, highlighting their potential as a renewable feedstock for high-value ingredients [5–7]. Systematic reviews further emphasize the growing interest in green extraction technologies for wine lees valorization while also emphasizing the strong dependence of extraction efficiency on process parameters and matrix characteristics [8]. These findings support the need for optimized extraction protocols tailored to specific winery residues, such as those derived from Verdicchio di Matelica vinification.

Conventional extraction methods, including solvent maceration, Soxhlet extraction, and mechanical pressing, often require large amounts of solvent and long processing times, whereas green techniques—such as ultrasound-assisted extraction (UAE) [9], supercritical fluid extraction (SFE), and microwave-assisted extraction (MAE) [10]—are more efficient and environmentally friendly [9]. Non-polar solvents such as n-hexane are commonly used for selective squalene recovery, and purification using silica gel chromatography, TLC, or HPLC ensures high purity for industrial and cosmetic applications [11]. Among these approaches, the UAE is particularly advantageous for yeast-rich matrices, offering fast, scalable, and mild extraction conditions, supporting the methodology adopted in this study [12].

Native squalene, despite its valuable biological and cosmetic properties, is chemically unstable due to its six carbon–carbon double bonds and is highly susceptible to oxidative degradation, which limits its shelf life and formulation stability [13]. Consequently, squalene is commonly converted into its fully saturated and more stable derivative, squalane ($C_{30}H_{62}$), which retains the excellent skin compatibility, emollient, and moisturizing properties of squalene while offering superior oxidative stability [1,14]. Recent advances in green hydrogenation strategies, particularly palladium-based heterogeneous catalysts supported on natural clays, such as montmorillonite, sepiolite, and palygorskite, provide efficient, low-cost, and environmentally friendly alternatives for the selective hydrogenation of squalene under mild conditions [15,16].

Beyond their role as emollients, squalene and squalane can actively influence the dermal delivery of cosmetic actives. Otto et al. have reported that squalane-based formulations enhance the skin penetration of poorly permeable compounds, including polyphenols, compared with conventional vehicles [17]. These findings support the use of squalane not only as a sensory and stabilizing ingredient but also as a functional excipient capable of improving topical bioavailability. For the preliminary evaluation of permeation, artificial cell-free membranes combined with Franz diffusion cells were used. Such systems enable the comparison of formulation performance while reducing cost and ethical concerns. Recent studies have validated the use of cellulose-based membranes for ranking permeation behavior and investigating penetration-enhancing effects, provided that their limitations are appropriately acknowledged [18,19].

This work presents an innovative and cohesive strategy that advances the State-of-the-Art by integrating three essential components that are rarely considered together. First, an optimized ultrasound-assisted extraction (UAE) process is applied to a real, compositionally complex winery residue, addressing a critical gap highlighted in the recent literature regarding the strong dependence of extraction efficiency on matrix-specific parameters [6].

Second, a green hydrogenation process using palladium-based heterogeneous catalysts supported by natural clays, such as montmorillonite and sepiolite, converts the extracted squalene into squalane ($C_{30}H_{62}$), which is more stable. These materials offer substantial benefits over traditional catalytic systems in cost, environmental impact, and functionality under mild conditions; however, their application to squalene derived from complex waste matrices remains largely unexamined [20,21]. Third, this study goes beyond the focus on production and stabilization to show that both squalene and upcycled squalane can help cosmetic actives penetrate the skin [22]. Using quercetin as a model antioxidant that does not readily cross membranes, their performance is tested in vitro with Franz diffusion cells. This is a reliable and repeatable alternative to ex vivo systems. This multifunctional assessment signifies a notable progression beyond prior studies, which generally examine extraction, conversion, or application in isolation.

The approach proposed in this work shows that it is possible to create a circular and sustainable way to use wine lees by combining green extraction, eco-friendly catalytic upgrading, and functional testing that focuses on formulation. This work helps close the gap between recovering raw materials and using them, creating high-value, upcycled cosmetic ingredients that work better with a smaller environmental impact.

2. Materials and Methods

2.1. Materials and Reagents

Wine lees were kindly supplied by Belisario Winery (Matelica, Italy) and derived from the 2022 vinification process of Verdicchio di Matelica. Upon collection, samples were stored at $-20\text{ }^{\circ}\text{C}$ until further processing. Quercetin hydrate ($\geq 95\%$) was purchased from Sigma-Aldrich (Steinheim, Germany). Squalene analytical standard was obtained from Merck KGaA (Darmstadt, Germany). Cyclohexane ($>99.5\%$) was supplied by Honeywell Riedel-de HaënTM (Charlotte, NC, USA), while ethyl acetate ($>99.5\%$), n-hexane, and acetonitrile were purchased from Sigma-Aldrich and Carlo Erba Reagents (Cornaredo, Italy), respectively.

For catalytic hydrogenation, palladium (II) nitrate hydrate was purchased from Alfa Aesar (Haverhill, MA, USA). Montmorillonite, sepiolite, and palygorskite clays were obtained from Sigma-Aldrich. Deionized water was produced using a G3 RO CUBIC-S2 demineralizer (Gamma 3, Castelveverde, Italy). All solvents and reagents were of analytical grade and used as received unless otherwise specified.

2.2. Ultrasound-Assisted Extraction of Squalene from Wine Lees

Fresh wine lees were lyophilized prior to extraction in order to eliminate residual water and improve contact between intracellular lipids and the apolar extraction solvent. Lyophilized lees (100 g) were suspended in 1 L of n-hexane and subjected to ultrasound-assisted extraction (UAE) using a probe-type sonicator (Q500, QSonica, Newton, CT, USA) equipped with a 25 mm titanium probe.

Sonication was performed at a fixed frequency of 20 kHz with the amplitude set at 97% of the maximum instrument power (500 W). A pulsed cycle (8 s on/2 s off) was applied to limit excessive heating, and the extraction vessel was immersed in an ice bath throughout the process. According to our preliminary evaluation, 29 min were selected as the extraction condition.

After sonication, samples were centrifuged at 5000 rpm for 10 min to separate the solid residue (Rotina 380R, Hettich, Tuttlingen, Germany). The organic phase was recovered, and the solvent was removed under reduced pressure at $40\text{ }^{\circ}\text{C}$ using a rotary evaporator (RV 8 PRO V-C, IKA, Staufen, Germany). Extracts were stored under a nitrogen atmosphere at $-20\text{ }^{\circ}\text{C}$ until further analysis.

Cell disruption induced by ultrasonic treatment was qualitatively assessed by optical microscopy (100× magnification) by comparing yeast cell morphology before and after sonication.

2.3. Quantification of Squalene by HPLC–DAD

Quantitative determination of squalene in lipid extracts was performed by high-performance liquid chromatography coupled with diode-array detection (HPLC–DAD) using an Agilent 1200 Series chromatographic system (Agilent Technologies, Santa Clara, CA, USA). Chromatographic separation was achieved on a C18 reversed-phase column (ALLTIMA, 150 mm × 4.6 mm, 5 µm particle size), maintained at 30 °C.

The mobile phase consisted of acetonitrile delivered in isocratic mode at a flow rate of 1.5 mL min^{−1}. Prior to analysis, a squalene analytical standard at 100 ppm was prepared in acetone and then diluted to different standard concentrations in acetonitrile, with no precipitation observed. Calibration curves were constructed using external standards at concentrations ranging from 0 to 50 ppm and showed linearity over the investigated range.

Freeze-dried extract (29.85 mg) was dissolved in 2 mL acetonitrile/acetone (80:20) and sonicated for 10 min to ensure complete solubilization. The sample was then filtered through 0.45 µm membrane filters to remove particulate matter and injected at a volume of 5 µL. The extract was diluted 100-fold in acetonitrile before HPLC analysis, and no precipitation was observed. Detection was carried out at 195 nm, corresponding to the maximum absorbance of squalene. Quantification was performed by external standard calibration, and results were expressed as milligrams of squalene per gram of dry wine lees.

2.4. Purification of Squalene by Column Chromatography and Thin-Layer Chromatography (TLC)

Crude lipid extracts obtained after ultrasound-assisted extraction were subjected to purification in order to isolate squalene from co-extracted lipophilic components. Column chromatography was performed using silica gel (SiO₂, 60 Å, 35–70 µm; Carlo Erba Reagents, Cornaredo, Italy) as the stationary phase. The column was packed using cyclohexane as the initial eluent. The lipid extract was dissolved in a minimal volume of cyclohexane and loaded onto the column. Elution was performed with a cyclohexane–ethyl acetate (95:5, *v/v*) mixture as the mobile phase.

Fractions were collected and monitored by thin-layer chromatography (TLC) to identify those containing squalene. TLC analysis was performed on silica gel 60 F₂₅₄ plates (Merck KGaA, Darmstadt, Germany) using cyclohexane/ethyl acetate (98:2, *v/v*) as the mobile phase. Plates were visualized under UV light (254 nm) and by iodine vapor exposure. Fractions showing a single spot with an R_f value corresponding to that of the squalene analytical standard were pooled, and the solvent was removed under reduced pressure. The fractions were collected sequentially (approximately 34 fractions in total), with an average volume of about 8–10 mL per fraction. Only fractions showing a single spot corresponding to squalene, with no additional detectable impurities, were collected and dried under reduced pressure to obtain purified squalene as a colorless, oily residue. Purified squalene was stored under a nitrogen atmosphere at −20 °C until subsequent hydrogenation and analysis.

2.5. Catalytic Hydrogenation of Squalene to Squalane

Catalytic hydrogenation of purified squalene was carried out using palladium supported on natural clay minerals as heterogeneous catalysts. Palladium-loaded clays were prepared by wet impregnation of montmorillonite, sepiolite, or palygorskite with an aqueous solution of palladium (II) nitrate hydrate, followed by drying at 80 °C and thermal treatment at 300 °C for 3 h under an air atmosphere to obtain the active Pd phase. Hydrogenation reactions were performed in a glass batch reactor equipped with magnetic stirring.

Purified squalene was dissolved in cyclohexane and transferred to the reactor, followed by the addition of the Pd-supported clay catalyst (substrate-to-catalyst ratio 100:1, *w/w*). The reactor was purged with nitrogen to remove residual air, then pressurized with hydrogen.

Reactions were conducted at 40 °C under a hydrogen pressure of 5 bar for 4 h. Upon completion, the reaction mixture was filtered to remove the solid catalyst, and the solvent was evaporated under reduced pressure. The obtained squalane was stored under nitrogen at −20 °C until further characterization and use in permeation studies. The progress and completion of the hydrogenation reaction were monitored by ¹H-NMR spectroscopy (Bruker Fourier 80, Bruker Italia, Milan, Italy) by following the disappearance of olefinic proton signals characteristic of squalene. Catalyst recyclability was preliminarily evaluated by recovering the solid catalyst by filtration, washing with cyclohexane, drying, and reusing it in subsequent hydrogenation cycles under identical reaction conditions.

2.6. In Vitro Permeation Studies Using Franz Diffusion Cells

The equilibrium solubility of quercetin was first determined by adding an excess of powder to 50 mL of water, glycerin, squalene, or squalane and maintaining the system at room temperature (20 ± 0.5 °C) under continuous stirring in an incubator (Velp Scientifica, FTC 90E, Usmate, Italy). The equilibrium solubility was assumed to have been reached when the standard deviation of three subsequent measurements was smaller than 1%. Upon reaching equilibrium, generally after 24 h, aliquots were taken, filtered through a regenerated cellulose filter syringe (0.45 µm pore size; Filalbet, Rossello, Barcelona, Spain), and the filtrate concentration was determined by HPLC. Assays were performed in triplicate.

In vitro permeation experiments were performed using vertical Franz diffusion cells (PermeGear Inc., Hellertown, PA, USA) with an effective diffusion area of 1.77 cm² and a receptor chamber volume of 12 mL. A regenerated cellulose membrane (PermeaPlain®, 25 µm thickness) was used as an artificial barrier to simulate passive diffusion. Prior to experiments, membranes were hydrated in phosphate-buffered saline (PBS, pH 7.4) for 30 min. The receptor compartment was filled with PBS containing 20% (*v/v*) ethanol to ensure sink conditions for quercetin, maintained at 32 ± 0.5 °C, and continuously stirred at 600 rpm.

Quercetin was selected as a poorly permeable model antioxidant. Donor formulations consisted of quercetin (0.1%, *w/w*) dissolved or dispersed in (i) water (control), (ii) squalene, or (iii) squalane obtained from catalytic hydrogenation. An equal volume (500 µL) of each formulation was applied to the donor compartment at the beginning of the experiment.

At predetermined time intervals (1, 2, 4, 6, and 24 h), aliquots (500 µL) were withdrawn from the receptor compartment and immediately replaced with fresh receptor medium maintained at the same temperature. Samples were filtered through 0.45 µm membrane filters and analyzed by HPLC–DAD under the chromatographic conditions described in Section 2.3.

Cumulative permeated amounts of quercetin were calculated as a function of time and normalized to the diffusion area. All experiments were performed in triplicate, and results were expressed as mean ± standard deviation.

The permeated quercetin as a function of diffusion time was fitted to Higuchi's Equation (1):

$$\frac{Q_t}{Q_0} = k \times t^{1/2} \quad (1)$$

where Q_t/Q_0 is the fraction of permeation of the drug, and the constant k expresses the drug diffusion rate, also related to the interaction between the drug and the vehicle.

Statistical analysis of derived permeation parameters (Higuchi slope, apparent diffusion rate, and cumulative transported amount at the final time point) was performed using Origin software (version 8.5). One-way ANOVA followed by Bonferroni's post hoc test was applied to compare formulations. A p -value < 0.05 was considered statistically significant.

2.7. Oxidative Stability Assessment

The oxidative stability of purified squalene and upcycled squalane was evaluated under accelerated aging conditions in order to assess the effect of hydrogenation on resistance to oxidative degradation. Samples (2 mL) were placed in open glass vials and stored at 40 °C under ambient air for up to 7 days.

At predetermined time points (0 and 7 days), samples were analyzed by HPLC–DAD under the conditions described in Section 2.3. Changes in the chromatographic profile and variations in the main peak area were monitored as indicators of oxidative degradation. All analyses were performed in triplicate, and results were expressed as a percentage of residual compound relative to the initial value.

3. Results and Discussion

3.1. Ultrasound-Assisted Extraction Results of Squalene from Wine Lees

Optical microscopy observations (Figure 1) qualitatively confirmed the strong mechanical effect of ultrasound on *Saccharomyces cerevisiae* cells, revealing extensive cell wall disruption after sonication when compared to untreated samples. Cavitation-induced shear forces are known to promote cell lysis and enhance the release of intracellular lipophilic compounds, particularly non-polar metabolites such as squalene [23,24]. These results align with previous reports demonstrating UAE's effectiveness in improving lipid recovery from yeast-rich matrices and winery by-products [5,6,25].

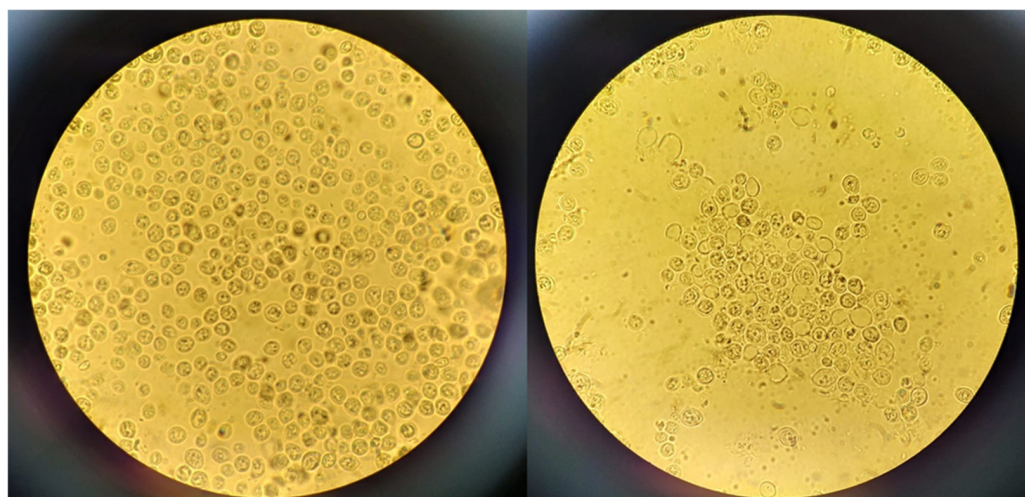


Figure 1. Optical microscope images (100×) of yeast cells before and after the sonication for the squalene extraction. Cell lysis, confirmed by cell wall breakdown and cellular aggregation, allows the release of squalene into the extracellular environment.

The use of n-hexane as an extraction solvent proved effective for selectively recovering non-polar lipids while minimizing co-extraction of polar components. Compared with conventional maceration approaches, UAE significantly reduced extraction time while maintaining high recovery efficiency, consistent with principles of process intensification and green extraction [8,26]. Notably, lyophilized lees further enhanced extraction efficiency by improving solvent accessibility and reducing mass-transfer limitations, as previously observed for microbial lipid [27].

Overall, these results confirm that the UAE represents a robust and scalable strategy for valorizing winery waste streams, enabling the efficient recovery of squalene under mild conditions. The established extraction protocol provides a solid foundation for subsequent purification and catalytic conversion steps, while remaining completely compatible with industrial sustainability requirements.

3.2. Quantification and Purification of Squalene

Qualitative and quantitative HPLC–DAD analysis confirmed the presence of squalene as a major non-polar component in the lipid extracts obtained from wine lees following ultrasound-assisted extraction. The HPLC–DAD chromatogram of the optimized extract, diluted 100-fold, and analyzed using acetonitrile as the mobile phase is shown in Figure 2. The most intense peak, attributed to squalene, appears at a retention time of 14.3 min, consistent with previous studies [11,28]. The chromatographic method provided good peak resolution and reproducibility, allowing reliable quantification of squalene in complex lipid matrices.

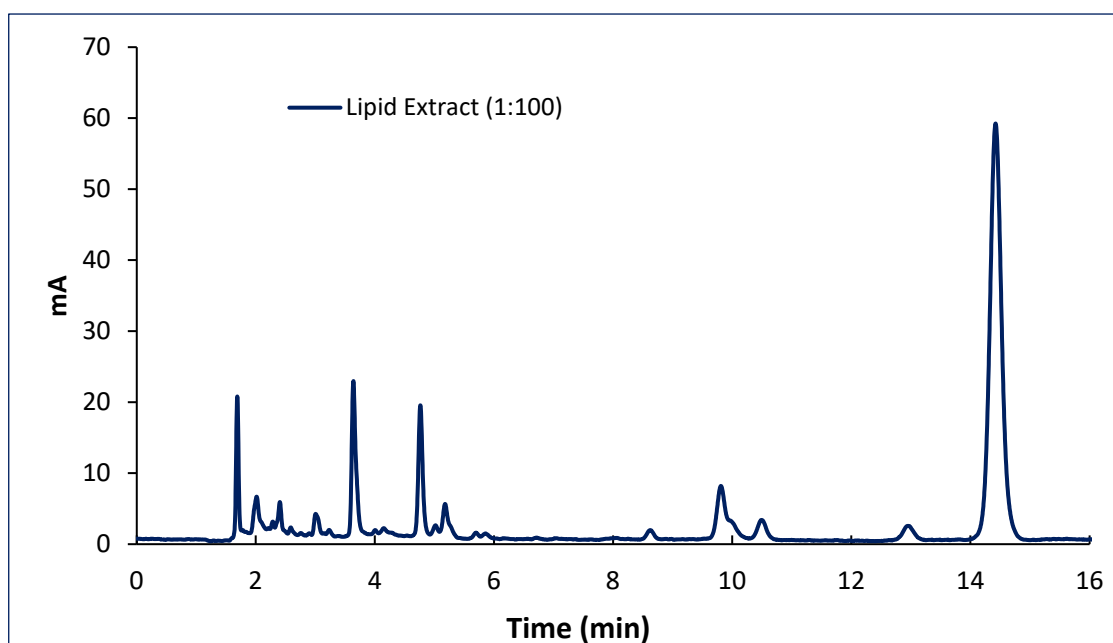


Figure 2. HPLC–DAD chromatogram of the lipid extract, under optimized extraction conditions.

To confirm the presence of squalene, the lipid extract was spiked with 5 ppm of a squalene analytical standard and subsequently analyzed by HPLC–DAD using the same chromatographic conditions. As shown in Figure 3, spiking resulted in a marked increase in the peak at a retention time of 14.3 min. Moreover, both the retention time and the UV–Vis absorption spectrum of the compound in the extract were consistent with those of the analytical standard, thereby confirming the identity of squalene.

Once the presence of squalene had been confirmed, a calibration curve was constructed.

The calibration curve was established using a squalene analytical standard over the concentration range of 0–50 ppm. The HPLC–DAD chromatograms of squalene standards at different concentrations are shown in Figure 4a. The resulting calibration curve exhibited excellent linearity, as demonstrated by the linear equation $f(x) = mx + q$, where $y = 24.279x + 0.0035$, with a linear regression coefficient (R^2) of 0.9998 (Figure 4b).

HPLC–DAD analysis of the lipid extract diluted 100-fold yielded a squalene concentration of $33.6421 \text{ mg L}^{-1}$. After correction for the dilution factor, this value corresponds to an actual squalene concentration of $3364.21 \text{ mg L}^{-1}$ in the original lipid extract. As described

in Section 2.3, 29.85 mg of the lyophilized lipid extract was dissolved in 2 mL of solvent, corresponding to a total squalene amount of 6.73 mg. Based on this value, the squalene content of the lyophilized extract was calculated to be 22.54% (w/w).

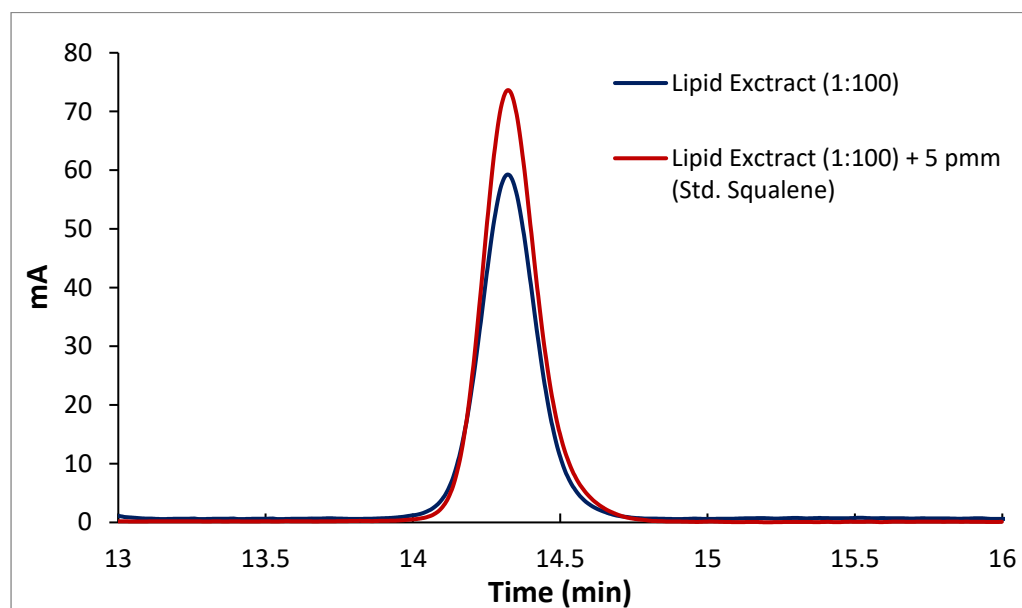


Figure 3. Squalene identification peak obtained by spiking 5 ppm of squalene standard into the lipid extract.

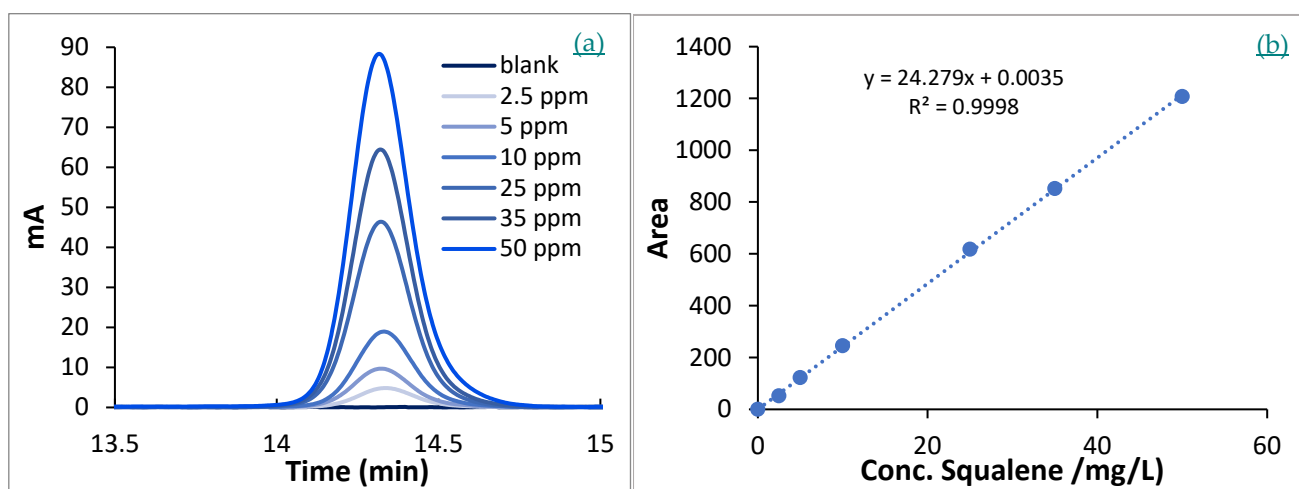


Figure 4. (a) HPLC-DAD chromatogram of squalene standard at different concentrations and (b) calibration curve of squalene in the range 0–50 ppm.

The amount of squalene recovered after UAE was comparable to or exceeded values previously reported for wine lees and yeast-derived matrices processed using conventional extraction techniques, highlighting the effectiveness of the optimized protocol adopted in this study [5–7,25]. The use of HPLC–DAD, rather than gravimetric lipid determination alone, enabled selective quantification of squalene and prevented overestimation due to co-extracted lipids, as emphasized in recent analytical studies on yeast-derived triterpenes [29].

Purification via silica gel column chromatography efficiently isolated squalene from accompanying lipophilic components, as confirmed by thin-layer chromatography. The mass of the purified squalene, obtained after chromatographic purification, was approximately 1.76 g, corresponding to a recovery yield of 88.25% relative to the loaded fraction. No additional purification steps were required after column chromatography, as TLC analysis

confirmed the absence of detectable co-eluting impurities. This purified fraction was used directly in subsequent catalytic hydrogenation experiments. TLC analysis revealed a single spot corresponding to the squalene standard, indicating high purity after chromatographic separation. The selected eluent system allowed effective separation while minimizing solvent consumption, in agreement with previously reported purification strategies for highly unsaturated hydrocarbons [30].

The combination of quantitative HPLC analysis and chromatographic purification is a critical step for downstream applications, particularly catalytic hydrogenation, where impurities may adversely affect catalyst activity and selectivity [31]. Moreover, obtaining highly purified squalene is essential for cosmetic applications, as impurities may compromise product stability, safety, and sensory performance. Overall, these results demonstrate that the integrated extraction–purification workflow developed here enables the recovery of squalene with high purity and analytical control, an ideal starting point for green conversion into squalane.

3.3. Catalytic Hydrogenation Results of Squalene to Squalane

Purified squalene was successfully converted into squalane via catalytic hydrogenation using palladium supported on natural clay minerals as heterogeneous catalysts. Under the mild reaction conditions adopted (40 °C, 5 bar H₂), complete hydrogenation of the six carbon–carbon double bonds was achieved without the formation of detectable by-products, confirming the high selectivity of the catalytic system. The disappearance of unsaturated species was evidenced by chromatographic analysis, which showed no squalene-related signals upon completion of the reaction. Complete hydrogenation was further confirmed by ¹H-NMR spectroscopy, which showed the disappearance of the characteristic olefinic proton resonances of squalene, indicating full saturation of carbon–carbon double bonds (Figures 5 and 6). The disappearance of unsaturated species was also evidenced by chromatographic analysis, which showed the absence of squalene-related signals after reaction completion.

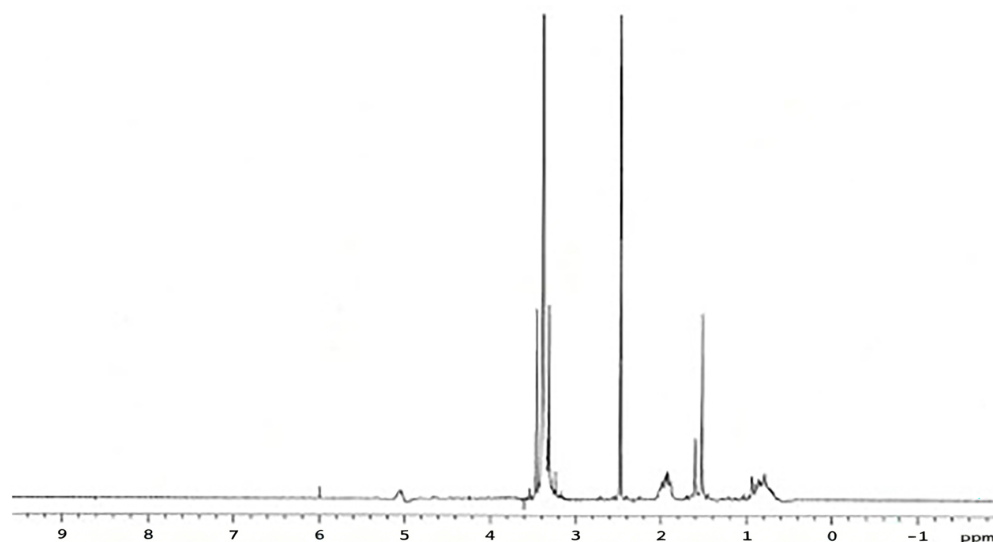


Figure 5. ¹H-NMR of squalene after purification.

The use of Pd-supported clays represents a strategic alternative to conventional hydrogenation catalysts, combining high catalytic efficiency with reduced environmental impact. Natural clays such as montmorillonite, sepiolite, and palygorskite offer high surface area, low cost, and good metal dispersion, which contribute to enhanced catalytic performance under relatively mild conditions [15,16,32]. Previous studies have demonstrated that clay-

supported palladium catalysts are particularly effective for the selective hydrogenation of polyunsaturated substrates, thereby minimizing over-reduction or side reactions commonly observed with more aggressive catalytic systems [33].

Compared to traditional hydrogenation approaches that rely on high-pressure reactors or noble-metal catalysts supported on synthetic oxides, the strategy adopted here insignificantly reduces energy input while maintaining high conversion efficiency. This is particularly relevant for cosmetic and personal care applications, where process sustainability and traceability are increasingly important [34]. Moreover, the heterogeneous nature of the catalyst facilitates its separation from the reaction mixture and enables preliminary catalyst recycling, further supporting a green chemistry approach.

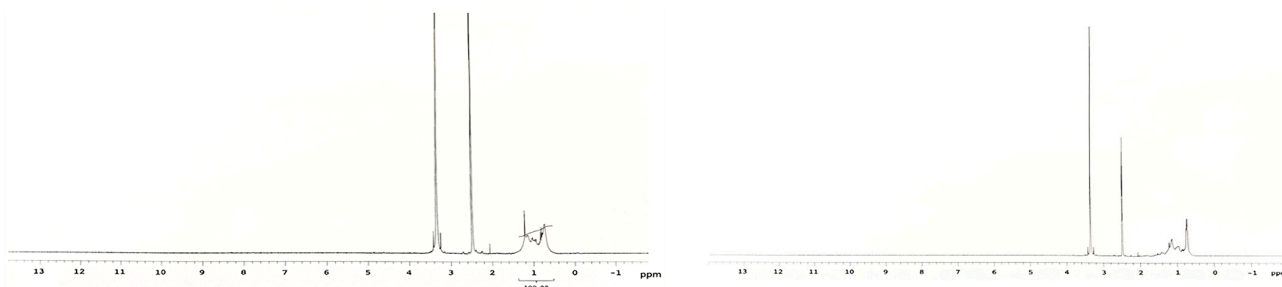


Figure 6. ^1H -NMR of hydrogenated squalene using montmorillonite-metal and palygorskite-metal. The hydrogenation in squalene is confirmed by the disappearance of the signals at 5 ppm, characteristic of the vinyl protons of squalene, and by the signals between 0.5 and 2 ppm, characteristic of the aliphatic structure of squalene. Signals at 2.5 and 3.2 ppm correspond to DMSO and water, respectively.

From a formulation perspective, converting squalene into squalane is essential to improve oxidative stability and shelf-life without compromising biocompatibility or sensory properties. Squalane obtained through this upcycling route retains the excellent skin affinity of squalene while overcoming its intrinsic susceptibility to oxidation, as widely reported in the cosmetic literature [1,14,35]. Together, these results, combining purified winery-derived squalene with clay-palladium catalysts, provide an efficient, selective, and environmentally conscious route to squalane, fully aligned with circular economy principles and cosmetic industry requirements.

3.4. *In Vitro* Transport Enhancement Across an Artificial Membrane by Squalene and Squalane

The functional performance of upcycled winery-derived squalene and its squalane derivative was finally assessed through *in vitro* permeation studies using quercetin as a poorly permeable model antioxidant. Quercetin is characterized by low aqueous solubility and limited passive diffusion across biological barriers, making it a suitable probe for evaluating the penetration-enhancing potential of cosmetic excipients [36]. We preliminarily tested quercetin solubility in different media, and the results agreed with the scientific literature (0.003 ± 0.001 and 0.050 ± 0.007 mg mL $^{-1}$ at 25 °C in water and glycerin, respectively). Then, a solubility of 3.650 ± 0.011 and 4.012 ± 0.213 mg mL $^{-1}$ at 25 °C in squalene and squalane, respectively, was determined, confirming a very high solubility of quercetin in these media.

The *in vitro* permeation studies demonstrated that both squalene- and squalane-based formulations increased the cumulative amount of quercetin permeated through the artificial membrane compared to the control, as shown by the permeation profiles over time (Figure 7). This finding confirms that both vehicles can enhance the apparent diffusion of a poorly permeable antioxidant across an artificial membrane under controlled *in vitro* conditions.

Analysis of cumulative permeation data and corresponding Higuchi plots revealed comparable diffusion kinetics for the two systems, with squalane-based formulations displaying slightly higher permeation slopes and calculated diffusion rates, particularly at longer diffusion times. These differences, while consistent across replicates, remain moderate and should therefore be interpreted as quantitative rather than qualitative enhancements of permeation performance.

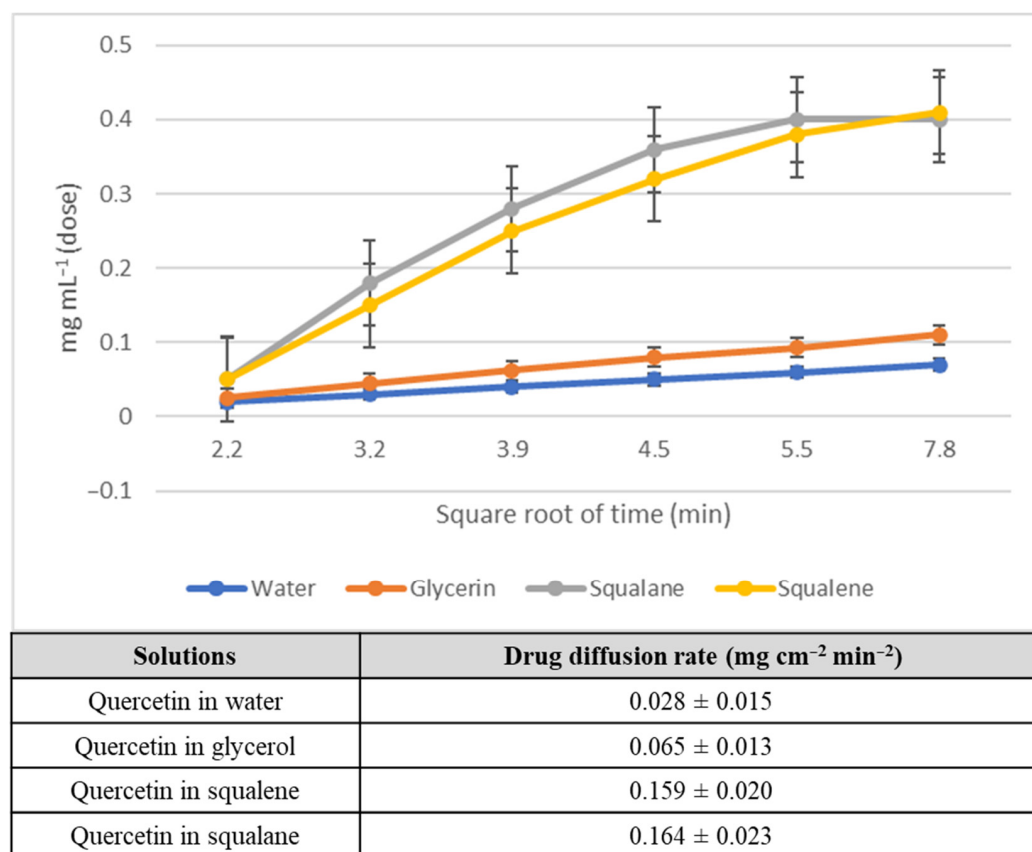


Figure 7. Permeated quercetin as a function of the diffusion time fitted to Higuchi's equation and drug diffusion rates of quercetin derived from Higuchi's equation.

The distribution of quercetin among the different Franz cell compartments further supports this interpretation. As illustrated in Figure 8, the increased amount of quercetin detected in the receptor compartment for squalane-based formulations is not accompanied by a disproportionate accumulation within the membrane itself, indicating that the observed enhancement is primarily associated with improved solute transport across the artificial membrane rather than increased membrane retention. Taken together, the analysis of permeation kinetics (Figure 7) and compartmental distribution (Figure 8) suggests that the slightly superior performance of squalane is mainly driven by vehicle-dependent physicochemical factors, such as its ability to maintain quercetin in a solubilized, diffusible state at the membrane interface. In particular, squalane and squalene formulations reached cumulative transport amounts of approximately 0.40 mg/mL at the final time point (7.8 min), whereas water and glycerin remained below 0.10 mg/mL. Squalane and squalene formulations exhibited significantly higher values compared to water and glycerin ($p < 0.01$), while no statistically significant difference was observed between squalane and squalene ($p > 0.05$).

Given the artificial nature of the membrane employed, the observed differences should be attributed to vehicle-dependent physicochemical factors, such as solute solubilization,

partitioning, and diffusion behavior, rather than to any modulation of biological barrier properties. Accordingly, these results should be interpreted as a comparative assessment of transport efficiency across an artificial membrane rather than as evidence of enhanced skin permeation. Overall, the use of PermeaPlain® membranes provides a reproducible ranking of formulation performance while minimizing biological variability. Although this model does not mimic the skin barrier, it serves as a robust and suitable screening tool for early-stage formulation studies to compare penetration-enhancing vehicles before validation on skin-mimicking or biological membranes [18,19,37].

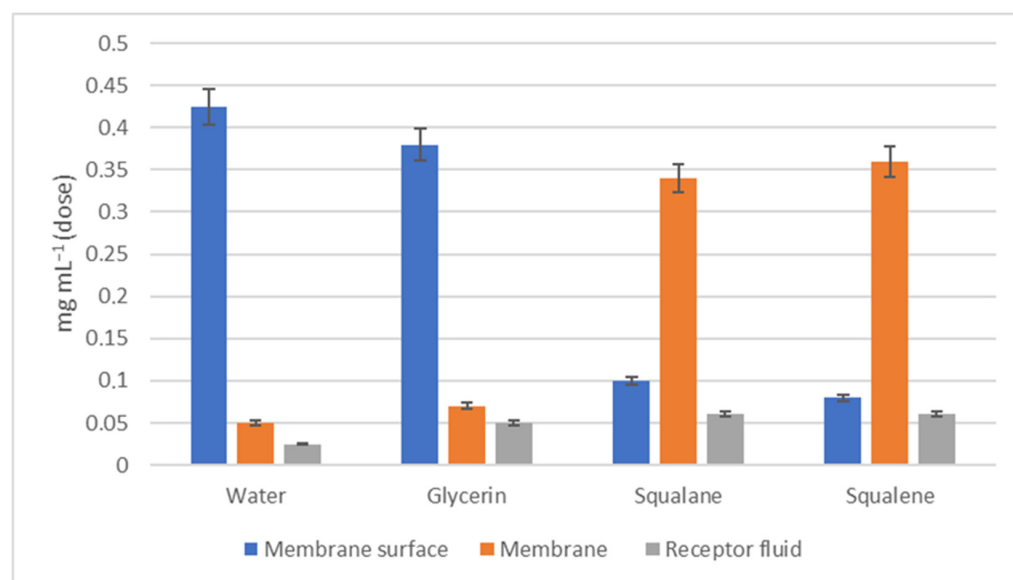


Figure 8. Distribution of quercetin in the three compartments of Franz's cells according to the dissolution medium.

3.5. Oxidative Stability of Wine-Lees-Derived Squalene and Squalane

The oxidative stability of purified squalene and winery-derived squalane was investigated under accelerated aging conditions to evaluate the functional impact of the hydrogenation step. After 7 days of exposure to air at 40 °C, squalene exhibited a marked decrease in the main chromatographic peak area, accompanied by the appearance of additional minor peaks attributable to oxidation products. In contrast, squalane showed minimal variation in its chromatographic profile, indicating a substantially higher resistance to oxidative degradation.

These results align with the well-documented susceptibility of polyunsaturated triterpenes to autoxidation and confirm that saturation of carbon–carbon double bonds via hydrogenation is a highly effective strategy for improving chemical stability [13,14,35]. The improved oxidative stability of squalane is particularly relevant for cosmetic formulations, where exposure to oxygen, light, and elevated temperatures during storage can compromise product quality and safety.

From a formulation perspective, improved oxidative stability translates into extended shelf-life, reduced antioxidant requirements, and more predictable performance over time. Several studies have highlighted the importance of using fully saturated emollients to mitigate oxidative degradation in cosmetic products, especially in minimalist or preservative-reduced formulations [38,39]. In this context, the upcycled squalane developed here provides a clear functional advantage over native squalene, reinforcing its potential as a sustainable cosmetic ingredient.

4. Conclusions

This study establishes the feasibility of an integrated and sustainable strategy for valorizing wine lees as a renewable source of high-value cosmetic ingredients. By combining ultrasound-assisted extraction, chromatographic purification, and green catalytic hydrogenation, squalene was efficiently recovered from winery waste and converted into its more stable derivative, squalane, using palladium supported on natural clay minerals.

The extraction protocol enabled effective recovery of squalene from yeast-rich wine lees under mild conditions. Meanwhile, HPLC-based quantification and purification ensured suitable analytical control and purity for downstream processing. The hydrogenation step was shown to be highly selective and efficient under low-temperature and low-pressure conditions, highlighting the potential of clay-supported palladium catalysts as environmentally conscious alternatives to conventional systems.

Beyond chemical conversion, the functional relevance of winery-derived squalane was demonstrated through in vitro permeation studies using quercetin as a challenging model antioxidant. Compared to conventional vehicles, squalane promoted enhanced permeation across an artificial membrane, supporting its role not only as an emollient but also as a functional penetration-enhancing excipient. Accelerated-aging experiments further confirmed squalane's superior oxidative stability relative to native squalene, a critical requirement for the stability and shelf life of cosmetic formulations.

Overall, this work provides a proof-of-concept for upcycling winery by-products into multifunctional, sustainable, and stable cosmetic ingredients, offering a scalable framework for the development of next-generation sustainable formulations.

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References

1. Smith, T.J. Squalene: Potential chemopreventive agent. *Expert Opin. Investig. Drugs* **2000**, *9*, 1841–1848. [[CrossRef](#)]
2. Huang, Z.R.; Lin, Y.K.; Fang, J.Y. Biological and pharmacological activities of squalene and related compounds: Potential uses in cosmetic dermatology. *Molecules* **2009**, *14*, 540–554. [[CrossRef](#)] [[PubMed](#)]
3. Spanova, M.; Daum, G. Squalene—biochemistry, molecular biology, process biotechnology, and applications. *Eur. J. Lipid Sci. Technol.* **2011**, *113*, 1299–1320. [[CrossRef](#)]
4. Rude, M.A.; Schirmer, A. New microbial fuels: A biotech perspective. *Curr. Opin. Microbiol.* **2009**, *12*, 274–281. [[CrossRef](#)]
5. Tao, Y.; Wu, D.; Zhang, Q.-A.; Sun, D.-W. Ultrasound-assisted extraction of phenolics from wine lees: Modeling, optimization and stability of extracts during storage. *Ultrason. Sonochem.* **2014**, *21*, 706–715. [[CrossRef](#)] [[PubMed](#)]
6. Naziri, E.; Mantzouridou, F.; Tsimidou, M.Z. Recovery of squalene from wine lees using ultrasound assisted extraction—a feasibility study. *J. Agric. Food Chem.* **2012**, *60*, 9195–9201. [[CrossRef](#)] [[PubMed](#)]

7. Rani, J.; Indrajeet; Rautela, A.; Kumar, S. Chapter 4—Biovalorization of winery industry waste to produce value-added products. In *Biovalorisation of Wastes to Renewable Chemicals and Biofuels*; Krishnaraj Rathinam, N., Sani, R.K., Eds.; Elsevier: Amsterdam, The Netherlands, 2020; pp. 63–85.
8. Barba, F.J.; Zhu, Z.; Koubaa, M.; Sant'Ana, A.S.; Orlie, V. Green alternative methods for the extraction of antioxidant bioactive compounds from winery wastes and by-products: A review. *Trends Food Sci. Technol.* **2016**, *49*, 96–109. [[CrossRef](#)]
9. Shen, L.; Pang, S.; Zhong, M.; Sun, Y.; Qayum, A.; Liu, Y.; Rashid, A.; Xu, B.; Liang, Q.; Ma, H.; et al. A comprehensive review of ultrasonic assisted extraction (UAE) for bioactive components: Principles, advantages, equipment, and combined technologies. *Ultrason. Sonochem.* **2023**, *101*, 106646. [[CrossRef](#)]
10. Tsevdou, M.; Ntzymani, A.; Katsouli, M.; Dimopoulos, G.; Tsimogiannis, D.; Taoukis, P. Comparative Study of Microwave, Pulsed Electric Fields, and High Pressure Processing on the Extraction of Antioxidants from Olive Pomace. *Molecules* **2024**, *29*, 2303. [[CrossRef](#)]
11. Lu, H.-T.; Jiang, Y.; Chen, F. Determination of Squalene Using High-Performance Liquid Chromatography with Diode Array Detection. *Chromatographia* **2004**, *59*, 367–371. [[CrossRef](#)]
12. Cheng, Y.; Fei, T.; Liu, Y.; Chen, S.; Wang, Z.; Han, Y.; Wang, L.; Li, C. Ultrasound-Assisted Extraction of Squalene and 2-Acetyl-1-Pyrroline from Pandan Leaf: The Effects of Drying Methods and Extraction Conditions. *Foods* **2024**, *13*, 4010. [[CrossRef](#)] [[PubMed](#)]
13. Frankel, E.N. Lipid oxidation. *Prog. Lipid Res.* **1980**, *19*, 1–22. [[CrossRef](#)] [[PubMed](#)]
14. Choe, E.; Min, D.B. Mechanisms of Antioxidants in the Oxidation of Foods. *Compr. Rev. Food Sci. Food Saf.* **2009**, *8*, 345–358. [[CrossRef](#)]
15. Medina Ruiz, M.E.; Maderuelo-Solera, R.; Jiménez-Gómez, C.P.; Moreno-Tost, R.; Malpartida, I.; García-Sancho, C.; Cecilia, J.A.; Len, C.; Mérida-Robles, J.M.; Maireles-Torres, P. Supported Palladium Catalysts for the Selective Hydrogenation of Furfural with Polymethylhydrosiloxane. *ACS Sustain. Chem. Eng.* **2024**, *12*, 14910–14920. [[CrossRef](#)]
16. Fan, W.K.; Tahir, M. Structured clay minerals-based nanomaterials for sustainable photo/thermal carbon dioxide conversion to cleaner fuels: A critical review. *Sci. Total Environ.* **2022**, *845*, 157206. [[CrossRef](#)]
17. Ng, K.W. Penetration Enhancement of Topical Formulations. *Pharmaceutics* **2018**, *10*, 51. [[CrossRef](#)] [[PubMed](#)]
18. Haq, A.; Dorrani, M.; Goodyear, B.; Joshi, V.; Michniak-Kohn, B. Membrane properties for permeability testing: Skin versus synthetic membranes. *Int. J. Pharm.* **2018**, *539*, 58–64. [[CrossRef](#)]
19. Neupane, R.; Boddu, S.H.S.; Renukuntla, J.; Babu, R.J.; Tiwari, A.K. Alternatives to Biological Skin in Permeation Studies: Current Trends and Possibilities. *Pharmaceutics* **2020**, *12*, 152. [[CrossRef](#)]
20. Gupta, U.; Krishnapriya, R.; Sharma, R.K. A Sustainable Palladium-Intercalated Montmorillonite Clay Catalytic System for Imine Hydrogenation under Mild Conditions. *ChemPlusChem* **2021**, *86*, 540–548. [[CrossRef](#)]
21. Pandarus, V.; Ciriminna, R.; Béland, F.; Pagliaro, M.; Kaliaguine, S. Solvent-Free Chemoselective Hydrogenation of Squalene to Squalane. *ACS Omega* **2017**, *2*, 3989–3996. [[CrossRef](#)]
22. Oliveira, A.L.S.; Valente, D.; Moreira, H.R.; Pintado, M.; Costa, P. Effect of squalane-based emulsion on polyphenols skin penetration: Ex vivo skin study. *Colloids Surf. B Biointerfaces* **2022**, *218*, 112779. [[CrossRef](#)] [[PubMed](#)]
23. Mason, T.J.; Lorimer, J.P. General Principles. In *Applied Sonochemistry*; Wiley: New York, NY, USA, 2002; pp. 25–74.
24. Kumar, K.; Srivastav, S.; Sharanagat, V.S. Ultrasound assisted extraction (UAE) of bioactive compounds from fruit and vegetable processing by-products: A review. *Ultrason. Sonochem.* **2021**, *70*, 105325. [[CrossRef](#)]
25. Perez-Torrado, R.; Gamero, E.; Gómez-Pastor, R.; Garre, E.; Aranda, A.; Matallana, E. Yeast biomass, an optimised product with myriad applications in the food industry. *Trends Food Sci. Technol.* **2015**, *46*, 167–175. [[CrossRef](#)]
26. Chemat, F.; Vian, M.A.; Cravotto, G. Green Extraction of Natural Products: Concept and Principles. *Int. J. Mol. Sci.* **2012**, *13*, 8615–8627. [[CrossRef](#)] [[PubMed](#)]
27. Ratledge, C.; Wynn, J.P. The biochemistry and molecular biology of lipid accumulation in oleaginous microorganisms. *Adv. Appl. Microbiol.* **2002**, *51*, 1–52. [[CrossRef](#)]
28. Bavisetty, S.C.; Narayan, B. An improved RP-HPLC method for simultaneous analyses of squalene and cholesterol especially in aquatic foods. *J. Food Sci. Technol.* **2015**, *52*, 6083–6089. [[CrossRef](#)]
29. Waksmondzka-Hajnos, M.; Sherma, J. *High Performance Liquid Chromatography in Phytochemical Analysis*; CRC Press: Boca Raton, FL, USA, 2010.
30. Stahl, E. Thin Layer Chromatography. A Laboratory Handbook, Second Edition, Edited by E. Stahl, 1041 pp., 244 Figs., Springer-Verlag. Berlin. Heidelberg. New York 1969, Preis: 128.-DM. *Fette Seifen Anstrichm.* **1971**, *73*, 736. [[CrossRef](#)]
31. Abdussalam-Mohammed, W.; Ali, A.Q.; Errayes, A. Green chemistry: Principles, applications, and disadvantages. *Chem. Methodol.* **2020**, *4*, 408–423. [[CrossRef](#)]
32. Fatimah, I.; Fadillah, G.; Yanti, I.; Doong, R.-a. Clay-Supported Metal Oxide Nanoparticles in Catalytic Advanced Oxidation Processes: A Review. *Nanomaterials* **2022**, *12*, 825. [[CrossRef](#)] [[PubMed](#)]
33. Nishimura, S. *Handbook of Heterogeneous Catalytic Hydrogenation for Organic Synthesis*; Wiley: New York, NY, USA, 2001.

34. Martins, A.M.; Silva, A.T.; Marto, J.M. Advancing Cosmetic Sustainability: Upcycling for a Circular Product Life Cycle. *Sustainability* **2025**, *17*, 5738. [[CrossRef](#)]
35. Eudier, F.; Savary, G.; Grisel, M.; Picard, C. Skin surface physico-chemistry: Characteristics, methods of measurement, influencing factors and future developments. *Adv. Colloid Interface Sci.* **2019**, *264*, 11–27. [[CrossRef](#)] [[PubMed](#)]
36. Casagrande, R.; Georgetti, S.R.; Verri, W.A., Jr.; Borin, M.F.; Lopez, R.F.; Fonseca, M.J. In vitro evaluation of quercetin cutaneous absorption from topical formulations and its functional stability by antioxidant activity. *Int. J. Pharm.* **2007**, *328*, 183–190. [[CrossRef](#)] [[PubMed](#)]
37. Jenner, J. OECD Test Guideline 428—A Method for In Vitro Percutaneous Absorption Measurement? In *Topical Drug Bioavailability, Bioequivalence, and Penetration*; Shah, V.P., Maibach, H.I., Jenner, J., Eds.; Springer: New York, NY, USA, 2014; pp. 381–387.
38. Guaratini, T.; Gianeti, M.D.; Campos, P.M.B.G.M. Stability of cosmetic formulations containing esters of Vitamins E and A: Chemical and physical aspects. *Int. J. Pharm.* **2006**, *327*, 12–16. [[CrossRef](#)] [[PubMed](#)]
39. Petkovic, A.; Jakovljevic, V.; Tomovic, M.; Jeremic, J.; Ristic, G.; Bradic, J. Improving Oxidative Stability of Cosmetic Emulsions with Plant Extracts: Current Status and Potential. *J. Cosmet. Sci.* **2021**, *72*, 189–199.

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