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"Advanced Lipidomics Analysis in Cosmetics: dive deeper into claim substantiation"

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1. Introduction

The skin is a complex and dynamic organ that serves as a primary barrier, immunological interface, and site of environmental interaction. Understanding its biochemical composition is key to advancing dermatological research, improving personalized skincare, and addressing skin-related diseases. While significant progress has been made in characterizing the skin surface lipidome, detailed molecular profiling of the stratum corneum (SC) remains underdeveloped. This limitation restricts our ability to understand early biochemical signals associated with barrier dysfunction, irritation, and subclinical inflammation. The SC, as the skin's outermost layer, plays a pivotal role in epidermal homeostasis, yet is often overlooked in favor of broader epidermal or whole-skin analyses.

Recent technological advances have transformed the potential of SC research. In particular, sequential tape stripping combined with high-resolution tandem mass spectrometry (HRMS/MS) allows for molecular analysis across discrete SC layers, capturing vertical gradients of lipid distribution and transformation[1]. This workflow, when coupled with robust bioinformatic tools and chromatographic separation, enables the detection and quantification of over 300 individual lipid species—including ceramides, fatty acids, sterols, and oxylipids—with precise chain-length and saturation resolution[2,3].

Building on this framework, a refined protocol that expands the use of SC lipidomics in both functional and mechanistic dermatological investigations has been developed. This approach is especially suited to cosmetic product evaluation and translational skin research, where high-resolution molecular data can provide evidence for both efficacy and tolerability.

For example, by monitoring the diffusion kinetics of topically applied ceramides across SC strata via LC-MS/MS, a quantitative formulation performance can be assessed. These data are complemented by measurements of exogenous and/or endogenous lipid remodeling, which provide insights into the product's capacity to restore lipid balance in barrier-compromised skin.
In addition to lipid structural profiling, the method is adapted for the detection of oxylipids. When detected in the superficial SC, it could serve both as biomarkers of bacterial presence and the

presence of oxidative stress on the skin. These insights allow for subclinical assessment of product-induced stress or protective effects.

Furthermore, we integrate analysis of urocanic acid (UCA)—a naturally occurring histidine metabolite in the SC—whose isomerization and degradation patterns are increasingly linked to inflammation and photodamage, offering a marker of inflammatory priming[4].

By providing stratified molecular evidence of cosmetic efficacy and subtle irritation, this protocol represents a robust, non-invasive alternative to skin biopsies[5]. Its adaptability enables broad application in product development, clinical research, and exposome-driven dermatology[6], contributing to a more nuanced and ethical understanding of skin physiology and perturbation.

In the context of recent advances in lipidomics, a multidimensional fingerprinting strategy was applied to improve lipid identification. This approach combines accurate mass measurements, MS/MS fragmentation patterns, and retention time alignment, enhancing the specificity and reproducibility of results. Data interpretation was supported by machine learning algorithms, enabling automated and precise lipid annotation across large datasets. The integration of these methodologies within the oloMAP platform provided a structured framework for lipid identification and quantification. High-resolution lipidomic profiling was performed using ultra-performance liquid chromatography coupled with quadrupole time-of-flight mass spectrometry (UPLC-Q-TOF-MS), offering comprehensive insights into the composition and dynamics of the skin lipidome.
This study aimed to investigate the penetration and early effects of a cosmetic formulation containing three types of Ceramides (NP, AP, and EOP) on the stratum corneum lipidome using high-resolution mass spectrometry-based lipidomic analysis and incorporating the oloMAP technology. The research focused on evaluating the formulation's penetration and efficacy in modulating stratum corneum lipid metabolism.

2. Materials and Methods

2.1. Study Design and Ethical Approval

All study procedures were conducted in compliance with the ethical standards established by the Declaration of Helsinki and its subsequent revisions for cosmetic testing outlined in EU Regulation No. 1223/2009. As the test product is a commercially available cosmetic with an established safety profile and was used under normal conditions of use, the study was exempt from additional ethical approval requirements. Prior to inclusion in this study, the ceramide-based lotion had undergone regulatory safety assessment consistent with EU guidelines for marketed products.
This pilot study employed a controlled, intra-individual comparison design. Seven healthy female volunteers (aged 22–29 years) applied the commercially available ceramide-based lotion to a defined area of the volar forearm twice daily for 7 days. An adjacent, untreated site on the opposite forearm was used as an intra-individual control to allow direct comparison within each subject.
The use of a young, homogeneous cohort reduced confounding variables such as age-related skin variation. The 7-day application period was chosen to investigate early-stage lipidomic changes. The intra-individual control design minimized subject variability and controlled for potential confounders such as environmental exposure and application consistency. Subjects were enrolled after meeting strict inclusion criteria and providing written informed consent.

2.2. Participants

A pilot cohort of 7 healthy female volunteers aged 22–29 years was enrolled to evaluate the short-term penetration efficacy of a ceramide-rich skin lotion into the stratum corneum layers. The study was conducted over 7 days with twice-daily applications under controlled conditions. All participants were screened to confirm the absence of dermatological conditions, systemic diseases, or known allergies to cosmetic ingredients.

Protocol Overview
The study protocol included instrumental evaluations at baseline (T0) and after 7 days (T7) of product application. The cosmetic formulation was applied to the forearm twice daily (morning and evening) for 7 consecutive days. Participants were instructed to apply a standardized quantity (0.5 mg/cm^2) of the lotion uniformly across the test area, followed by gentle massage until complete absorption. Ten stratum corneum samples were consecutively obtained from the same spot via adhesive tape stripping (D-Squame®) at baseline (Day 0, T0) and following treatment (Day 7, T7) from the treated skin site. A corresponding untreated control site (Day 7, T7C)—an adjacent region on the opposite forearm (identical anatomical location, no product application)—was sampled at Day 7 as an intra-individual paired control, isolating the effects of the lotion by controlling for: Environmental/lifestyle factors (e.g., pollution, diet) and biological variability (e.g., participant-specific lipid profiles). Both treated and control sites were subjected to lipidomic analyses for comparative evaluation.

Ethics and Compliance
All participants provided written informed consent after receiving detailed explanations of the study's objectives and procedures. Volunteers were screened to exclude those with active skin conditions, known allergies to cosmetic ingredients, or recent use of topical products that could interfere with the study outcomes. No adverse events were reported during the study period.

2.3. Chemicals and reagents

Skin Lotion
The skin lotion selected for this study stands out due to its thoughtfully designed formulation, which harnesses the power of its star ingredients: three ceramides (Ceramide NP, AP, and EOP)—key players in restoring and maintaining the skin barrier. Beyond these lipid compounds, the product incorporates a plethora of bioactive ingredients, including tocopherol (vitamin E) for antioxidant protection, cholesterol and palmitic acid for barrier support, and amino acids like glycine, serine, and alanine for natural hydration. Even the inclusion of sodium hyaluronate and phytosphingosine further underscores its focus on hydration and barrier integrity.
What distinguishes this product—and justifies its selection over comparable alternatives—is its well-documented composition, characterized by a transparent and rational integration of evidence-based active compounds within a functionally optimized formulation: moisturizers (shea butter, glycerine) and stabilizing agents, ensuring both efficacy and user-friendly application. Below is the product's ingredient list, meticulously verified and presented in standard descending order of concentration per INCI (International Nomenclature of Cosmetic Ingredients) guidelines: Aqua (Water/Eau), Glycerine, Butyrospermum Parkii Butter (Shea Butter), Hydroxyethyl Urea, Isopropyl Palmitate, Propanediol, Cetearyl Alcohol, Octyldodecanol, C15-19 Alkane, Glycine, Stearic Acid, Ceramide Np, Ceramide Ap, Ceramide Eop, Sorbitan Tristearate, Sorbitan Oleate, Carbomer, Glyceryl Stearate, Dimethicone, Behentrimonium Methosulfate, Triethyl Citrate, Isohexadecane, Sodium Pca, Serine, Sodium Lauroyl Lactylate, Sodium Hydroxide, Myristic Acid, Sodium Hyaluronate, Cholesterol, Palmitic Acid, Alanine, Tocopherol, Hydroxyacetophenone,

Caprylyl Glycol, Hydroxyethylpiperazine Ethane Sulfonic Acid, Trisodium Ethylenediamine Disuccinate, Xanthan Gum, Pentaerythrityl Tetra-Di-T-Butyl Hydroxyhydrocinnamate, Phytosphingosine, Acrylamide/Sodium Acryloyldimethyltaurate Copolymer, Acrylates/C10-30 Alkyl Acrylate Crosspolymer, Polysorbate 80, Benzoic Acid, Peg-100 Stearate, Peg-20 Methyl Glucose Sesquistearate. This carefully curated blend doesn't just check boxes for barrier repair—it reflects a modern understanding of barrier-repairing lipids, humectants, and skin-identical molecules, making it a robust choice for both clinical research and real-world use.

LC-MS reagents
UHPLC-MS-grade solvents (water, methanol, acetonitrile, and isopropanol), methyl tertbutyl ether (MTBE), and LC-MS-grade mobile phase modifiers (formic acid and ammonium formate were obtained from VWR International (Barcelona, Spain). The deuterated internal standard (SPLASH® LIPIDOMIX® Mass Spec) for LC-MS was purchased from Avanti Polar Lipids.

2.4. Sample collection and preparation

For the lipidomic analysis, samples of the stratum corneum were obtained from the cleansed forearm via a tape-stripping procedure using adhesive tape stripping (D-Squame® D101 + D500 Pressure Instrument) from the volunteers' dominant arm. Ten consecutive tape strips were applied to the same area, keeping all of them in 2 mL centrifuge tubes. All strips, representing the different layers of the stratum corneum, were collected and stored at −80°C for subsequent lipidomic analysis. Lipids were extracted from the strips using 275 µL cold methanol and shaken for 1 hour at 2000 rpm and 4 ºC. Subsequently, they were transferred to another centrifuge tube, and then a biphasic extraction was conducted by adding 275 µL of 10% MeOH and 1 mL of MTBE. Then, an aliquot of the organic phase was evaporated to dryness and reconstituted in MeOH. The samples were characterized by high-resolution mass spectrometry, using a 1290 Infinity II UHPLC (Agilent Technologies, CA, USA) coupled to a 7600 Zeno-TOF mass spectrometer (SCIEX, MA, USA). Lipids were separated at 65°C on a Waters Acquity Premier BEHC18 column (50 mm length x 2.1 mm id; 1.7 µm particle size) with a short chromatographic method (8 min). This methodology used (A) acetonitrile/H2O (60:40 v/v, with 10 mM ammonium formate and 0.1 % formic acid) and (B) isopropanol/acetonitrile (90:10 v/v, with 10 mM ammonium formate and 0.1 % formic acid) as A and B mobile phases, respectively. The mobile phase flow rate was set at 0.6 ml/min, and the injection volume was 8 µl. Following the separation, the flow was introduced by positive/negative mode electrospray ionization (ESI) into the mass spectrometer[7].

2.5. Data management and statistical analysis

The oloMAP platform was used for the bioinformatic processing and analysis of the lipidomics data. Compound identification was performed by matching experimental accurate mass, retention time, and tandem mass spectra against an in-house lipid database as MS-DIAL 5 in-silico lipid database. Validation and relative quantification utilized 69 deuterated standards belonging to 14 lipid classes, and unrelated lipid classes were normalized to the C16 Ceramide standard.

3. Results & Discussion

To assess the penetration and functional impact of the ceramide-rich formulation, comprehensive lipidomic profiling was conducted at baseline (T0) and after 7 days of treatment (T7). A total of 969 individual lipid species were detected across all samples, spanning major lipid classes including ceramides, free fatty acids, sterols, and minor bioactive lipids.

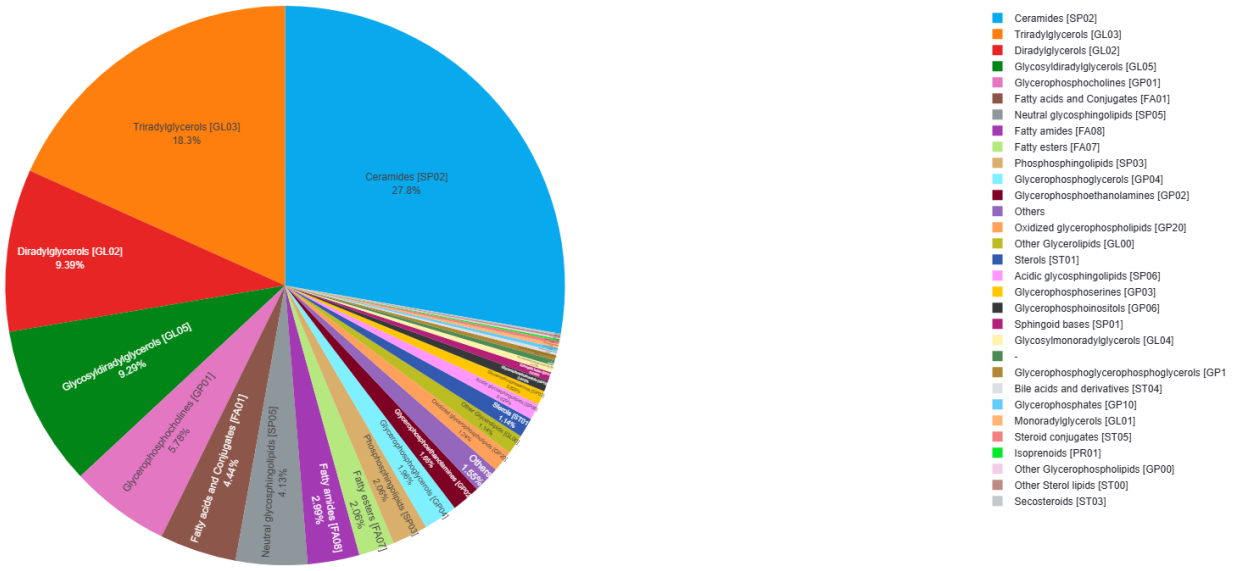

Figure 1. *Pie chart of Lipid Annotation distribution.*

3.1. Hierarchical Clustering Analysis of Lipid Categories

Hierarchical clustering of lipid-category abundances showed that the T7-treated samples (T7 cream in the figure) formed a distinct cluster, clearly separated from both the T0 and T7 control baselines; this segregation was primarily driven by sphingolipid and glycerophospholipid remodelling. On the other hand, T0 and T7 Control remained more closely related, suggesting minimal lipid profile drift without treatment.

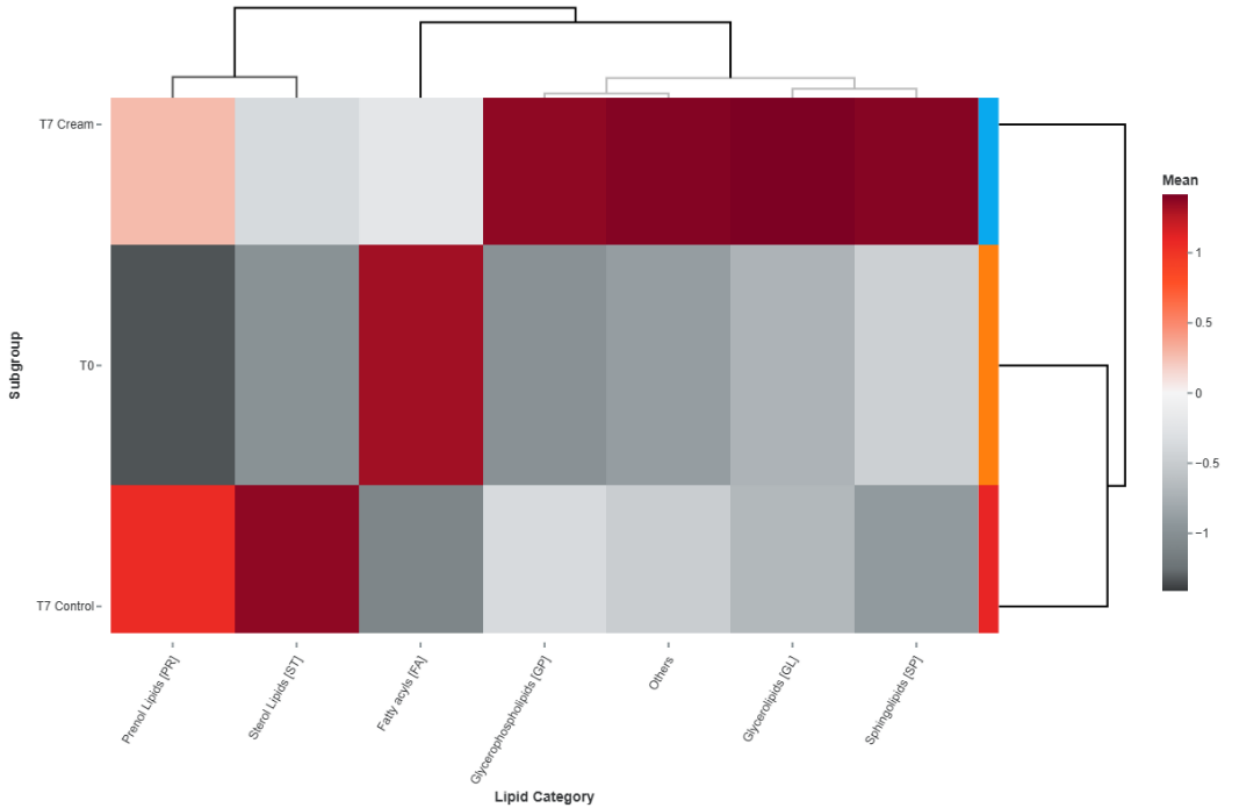

Figure 2. *Hierarchical clustering of lipid categories showing distinct separation between baseline (T0), untreated controls after 7 days (T7 Control), and treated sites after 7 days (T7 Cream).*

Fluctuations in prenol lipids—particularly Vitamin A Ester 19:4—observed in both T7 Control and T7 Treated groups suggest an endogenous adaptive response rather than a direct effect of the cosmetic formulation. Several factors may contribute to this phenomenon, with ultraviolet (UV) exposure being the most likely. Notably, the treated group may exhibit enhanced photoprotection compared to baseline and untreated controls, possibly due to the barrier-reinforcing effects of the lotion. Additional contributing factors could include oxidative stress or a diet rich in vitamin A or carotenoids[8,9]. As for Sterol Lipids, variations in this class (e.g., ST 27:1;O) are consistent with homeostatic epidermal turnover and lipid remodelling associated with the repair of external mechanical disruption[10]. Thus, the clustering results reflect a combination of treatment-induced effects and natural skin adaptation mechanisms.

3.2. Ceramides Analysis

The cumulative abundance of long-chain ceramides (chain length ≥ C34) in the stratum corneum was notably increased after 7 days of product application compared to both baseline (T0) and untreated sites (T7 Control). In addition, the use of the skin lotion also has an impact on the shorter-chained ceramides.

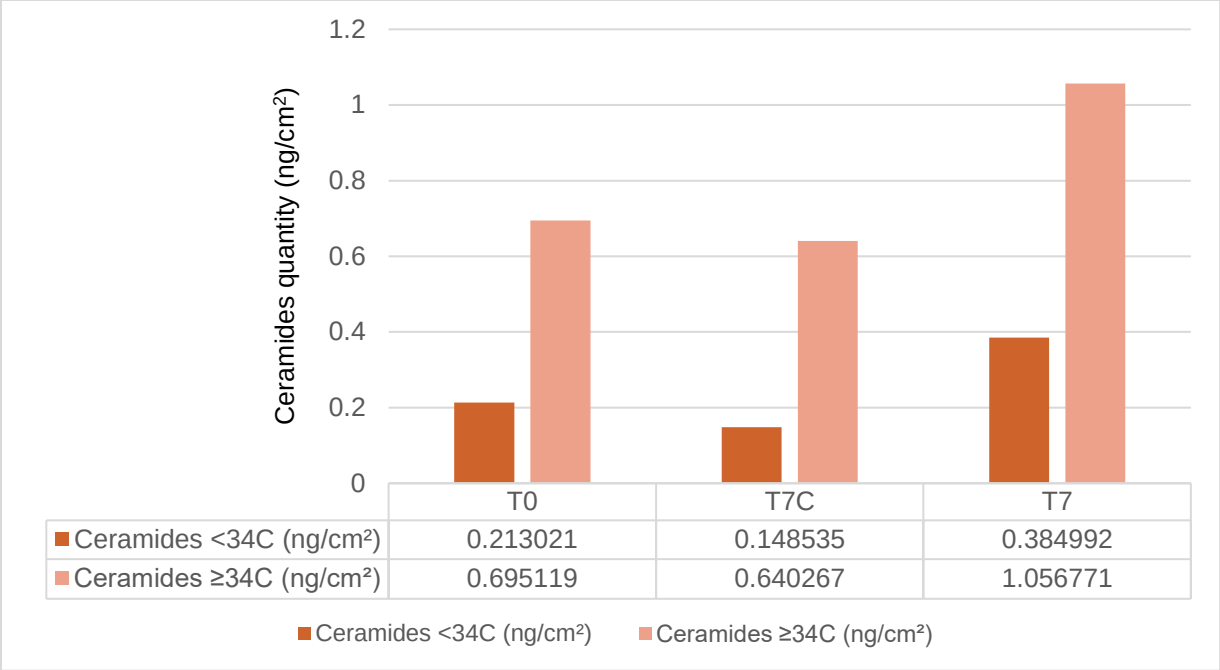

Figure 3. *Quantitative analysis of long-chain ceramides (≥ C34) at baseline, T7 Control, and T7 Treated sites. A significant increase in long-chain ceramides is observed after treatment.*

Because higher levels of long-chain ceramides (C > 34) are known to improve the lamellar organization of the stratum corneum—thereby strengthening the skin barrier, reducing transepidermal water loss, and enhancing hydration[11,12]—our results suggest that the topical ceramide formulation replenishes essential barrier lipids and may actively reinforce epidermal barrier function.

3.3. Penetration Depth and Molecular Remodelling

3.3.1. Penetration Depth Assessment

For the assessment of the penetration depth of the cosmetic lotion, the ingredient list was evaluated. As the exact quantitative composition is not disclosed, the most abundant exogenous ingredients were considered as suitable penetration markers. Among them, caprylic/capric triglyceride (TG 8:0_8:0_10:0) was identified as the optimal choice. According to Article 19 of Regulation (EC) No 1223/2009 on cosmetic products, cosmetic ingredients must be listed in descending order of concentration, allowing the identification of principal components based on their order. Consequently, caprylic/capric triglyceride, listed among the first ingredients, was assumed to be a major component. Moreover, given that it belongs to a lipid class readily detectable via lipidomics, it was selected as a suitable molecular tracer.

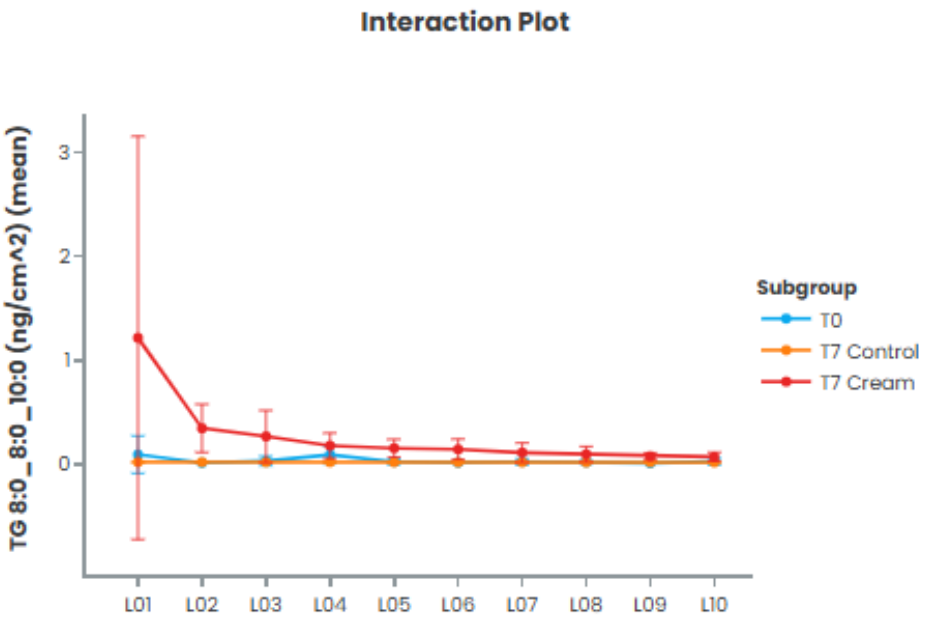

Figure 4. *Distribution of caprylic/capric TG levels across stratum corneum layers, demonstrating progressive decrease with depth.*

Our results show a 60% decrease in caprylic/capric triglyceride signal intensity was observed over the first five SC layers, indicating a steep diffusion gradient. Beyond the fifth layer, the decline became more gradual, although detectable traces of the lotion persisted up to the 10th layer. This data supports that product action remains mainly confined to the superficial and intermediate strata of the SC.

Alongside endogenous lipid remodelling, several metabolites detected in this study suggest microbial contributions to the stratum corneum (SC) molecular landscape in a comparative analysis between deeper SC layer 10 and superficial layer 2 at Day 7, a significant downregulation of these bacterial-origin lipid species was revealed. Specifically, the identification of oxidized digalactosyldiacylglycerols (DGDG O-21:0_16:0) and monogalactosyldiacylglycerols (MGDG O-19:0_24:5), along with diacylglycerol-based glycolipids (DGGA 9:0_13:1, DGGA 8:0_14:1), reveals a distinct microbial lipidomic fingerprint within the SC[13,14].

The decision to focus on layer 2 rather than layer 1 for surface-level analyses was based on established methodological considerations. Previous studies have shown that the outermost tape (layer 1) is highly vulnerable to contamination by environmental pollutants, particulate matter, and transient exogenous microbes from the exposome[15,16]. In contrast, the second tape provides a more stable, reproducible, and endogenous molecular snapshot, minimizing external confounders without losing relevance to surface skin physiology. Together, these findings align with emerging evidence that bacterial lipidomics plays a significant role in shaping the biochemical environment of the skin barrier[17].

3.3. Urocanic Acid Levels across SC Layers

Urocanic acid (UCA) distribution was stratified by stratum corneum (SC) depth, revealing a progressive increase in concentration with deeper SC layers across all groups. Notably, layer L1 was excluded from comparative analysis due to its high variability, attributed to greater exposure to external environmental factors.

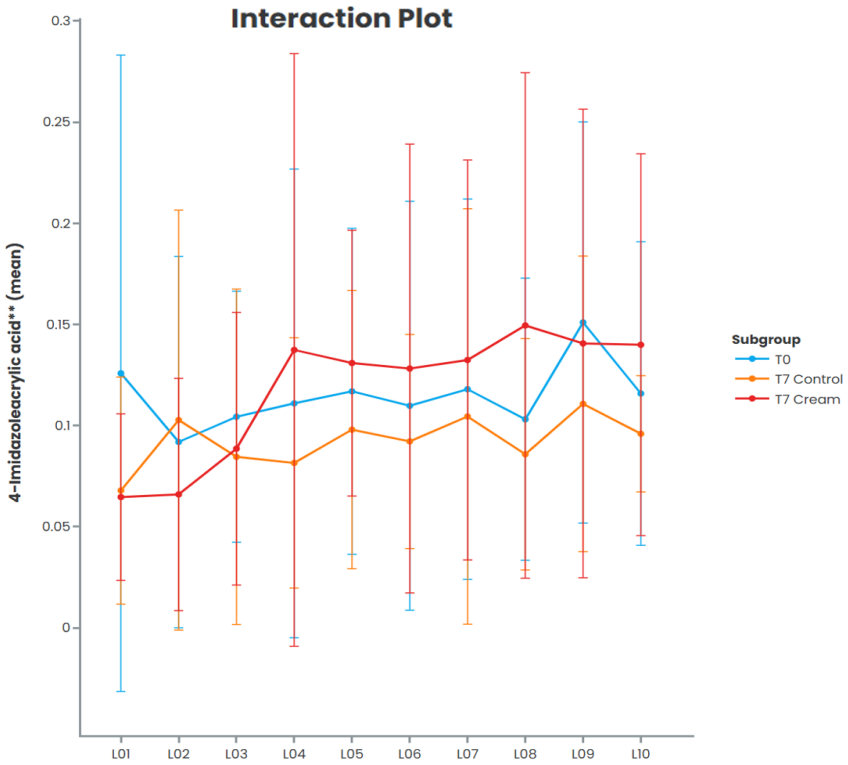

Figure 5. *Distribution of urocanic acid levels across stratum corneum layers, demonstrating progressive increase with depth and further enhancement following treatment.*

After the treatment, a twofold increase in UCA levels was observed in deeper SC layers (L09–L10) compared to baseline. This increase is interpreted as a sign of metabolic priming rather than inflammation, as UCA acts as a natural photoprotective filter and modulator of oxidative stress responses[18]. Higher UCA levels suggest enhanced epidermal readiness to defend against environmental inputs without triggering full immune activation. Importantly, no signs of barrier disruption or clinical irritation were observed, reinforcing the hypothesis that UCA accumulation represents a protective, beneficial response. Thus, UCA elevation complements the lipidomic findings, illustrating a coordinated skin fortification at both structural and biochemical levels following treatment.

4. Conclusion

This study highlights that a ceramide-rich formulation enhances stratum corneum (SC) lipid composition after 7 days of topical application, promoting a considerable increase in long-chain ceramides (≥C34) already described in the literature[19] to be able to improve barrier function and hydration. Penetration analysis confirmed that the product's activity was mainly restricted to superficial and intermediate SC layers, ensuring targeted efficacy. Besides structural lipid remodeling, treatment induced a reduction in bacterial-origin lipid species in upper SC layers, suggesting a subtle modulation of the skin microbiome. Additionally, elevated urocanic acid (UCA) levels in deeper SC layers indicate metabolic priming toward enhanced photoprotection and oxidative stress resilience, without signs of irritation.

The combined use of stratified tape stripping, high-resolution mass spectrometry, and the oloMAP platform provided a sensitive, non-invasive method to capture these early molecular changes. This approach offers a valuable alternative to skin biopsies, enabling detailed, ethical evaluation of cosmetic interventions and contributing to a refined understanding of skin barrier modulation.

5. References

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