Women over 80: longevity assessed from the perspective of the skin biophysics, metagenomics and lipidomics, before and after cosmetic intervention with Brazilian biodiversity ingredients

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Abstract

Our study aims to unravel the interplay between different skin parameters in women over 80, providing insights into a new skincare product containing Brazilian Biodiversity (BBD) ingredients. Brazilian women with a mean age of 83±2 years, Fitzpatrick II-V, had their skin evaluated for several skin biophysical parameters, metagenomics, and lipidomics. Afterward, a facial care product containing active ingredients from BBD was applied once a day for 28 days, and the same parameters were evaluated and compared. The results showed that aged skin was fragile, with reduced thickness and low dermal density. The microbiota profile showed a high alpha diversity, while the lipid composition presented a predominance of certain groups, such as free fatty acids. After 28 days of treatment, there was a significant increase in all biophysical parameters and stimulation of sebaceous lipids. No significant changes in the skin microbiota were observed. Our study paves the way for new solutions since the improvement in all skin parameters shows that the skin of people over 80 is still responsive to cosmetic treatments and that new products can be developed targeting skin longevity during aging.

Keywords: Longevity; skin; microbiome; lipidome; Brazilian biodiversity.

1. Introduction.

The increase in human life expectancy over recent decades, driven by significant medical and biological advances, is reshaping global demographics. By 2030, the number of individuals aged 60 and older will rise from 1 billion in 2020 to 1.4 billion, with those aged 80 and above expected to triple from 2020 to 2050, reaching 426 million [1].

Aging is accompanied by numerous physiological changes, particularly in the skin, leading to the development of cutaneous disorders [2]. These changes include alterations in the stratum corneum (SC) structure and lipid content due to reduced epidermal lipid synthesis [3], as well as compromised collagen and elastic fiber production [4, 5]. Consequently, these alterations result in a defective skin barrier function, impaired recovery, and increased transepidermal water loss (TEWL) [6].

Advancements in the understanding of the skin microbiome and lipidome dynamics during aging have highlighted significant interactions between the skin microbiota and key components of the skin barrier, such as keratinocytes and immune cells [3]. Profound shifts in these microbial populations are increasingly recognized for their impact on aging skin [7]. Concurrently, lipid composition, critical for cellular structure, signaling, and bioenergetics, exhibits distinct changes in expression patterns across different lipid subclasses during the aging process [8].

Despite the critical need to understand the intricate mechanisms underlying skin aging, especially in the elderly, the characteristics of the skin in women over 80 remain underexplored in scientific literature. Our study aims to unravel the complex interplay between skin biophysics, metagenomics, and lipid composition in women over 80 years of age. By doing so, we seek to provide valuable insights that could contribute to the development of a novel skincare product containing active ingredients from the BBD, ultimately enhancing skin health.

2. Materials and Methods.

2.1. Ethical compliance.

The clinical trials were conducted under Resolution 466/12 of the National Council of Health on Regulatory Guidelines and Standards for Research Involving Humans. Dermatological and ophthalmological monitoring was maintained throughout the protocol. The research protocol (number 63578922.9.0000.5514) received approval from the Institutional Ethics Committee, with second opinion numbers 5.675.918 and 5.729.344, and all subjects in this research had given their informed consent to participate.

2.2. Study population.

Subjects were women aged 83 ± 2 years, classified as Fitzpatrick skin phototypes II-V. Participants applied a cosmetic product containing BBD active ingredients once daily for 28 days. Analyses were conducted at baseline (D0), 7 days (D7), 14 days (D14), and 28 days (D28) post-application.

2.3. Skin biophysical parameters.

Long-term skin hydration was evaluated by randomly applying the product to the face. Measurements were obtained using a Corneometer (Courage+Khazaka Electronic GmbH, Germany) at D0, D28, and three days post-use (D28+3).

Transepidermal water loss (TEWL) was measured using the evaporimetry technique with a Tewameter (Courage+Khazaka Electronic GmbH, Germany) on the forearm. Measurements were taken at D0, D7, D14, and D28. Dermal density was evaluated at D0 and D28 using an Ultrascan UC 22MHz (Courage+Khazaka Electronic GmbH, Germany).

Skin pH was performed on the forearms at D0 and D28, and it was assessed with a Skin-pHmeter® probe coupled to a Multi Probe Adapter (Courage+Khazaka Electronic GmbH, Germany).

The significance of variations in skin biophysical parameters was assessed using the bimodal paired Student's t-test, with a 95% confidence interval. Statistical analyses were conducted using GraphPad Prism 8.00 software (GraphPad Software, USA). Values obtained at D0 were compared to those obtained after treatment.

2.4. Metagenomics.

Samples from the study population were sequenced using the Ion S5 Torrent System (Thermo Fisher Scientific). DNA was extracted with the MagMAX™ Microbiome Ultra Nucleic Acid Isolation Kit, following the manufacturer's instructions. The extracted DNA was then amplified and used as a template in a PCR to insert specific DNA barcodes for each sample at the DNA 5' end, creating the libraries. Amplicons for the V3-V4 regions of the 16S ribosomal RNA gene were generated using in-house protocols. PCR products were purified with magnetic beads (AMPure XP) followed by selection of the fragment. The size-selected pool was quantified, diluted, and loaded onto the sequencing chip (Ion 530 Chip) with the Ion Chef Instrument.

Standard data analysis tools [9] for denoising, chimera, and quality checks [within the R (v 4.3.1) statistical computing environment] were used to obtain taxonomic identification for microbiome profiling with RefSeq database. DADA2 methods were used to construct taxonomic feature tables from error-containing sequencing reads, chimeras were identified using the removeBimeraDenovo function, and taxonomy was assigned using DADA2's native naïve RDP Bayesian classifier against the RefSeq database. R vegan package [10] and Phyloseq package [11] were used to calculate bacteria diversity. Shannon diversity [12] was reported. Wilcoxon signed-rank test with a 95% confidence interval was used to identify group–associated microbial changes.

2.5. Lipidomics.

To evaluate the modulation of skin lipid composition, adhesive tapes from the equipment Corneofix F20 (Courage-Khazaka Electronic GmbH, Germany) and non-adhesive contact tapes from the equipment Sebun measurement cartridge SM810 (Courage-Khazaka Electronic GmbH, Germany), were collected from the forehead region of each subject. Afterward, High Resolution Mass Spectrometry (HR-MS) was performed for the quantification of epidermal lipids on the samples of adhesive tapes and Gas Chromatography Mass Spectrometry (GC-MS) was performed on the non-adhesive tapes samples for sebaceous lipids quantification.

Lipidomic data analysis was performed in the R environment using stats and agricolae packages. Wilcoxon signed-rank test with a 95% confidence interval was used to compare differences among groups.

3. Results.

3.1. Skin biophysical.

Application of the investigational product significantly enhanced skin hydration, as evidenced by increased capacitance values at D28 and D28+3 (Figure 1). Specifically, skin hydration improved by 32.8% at D28 and 26.1% at D28+3 compared to baseline, with all subjects exhibiting enhanced hydration.

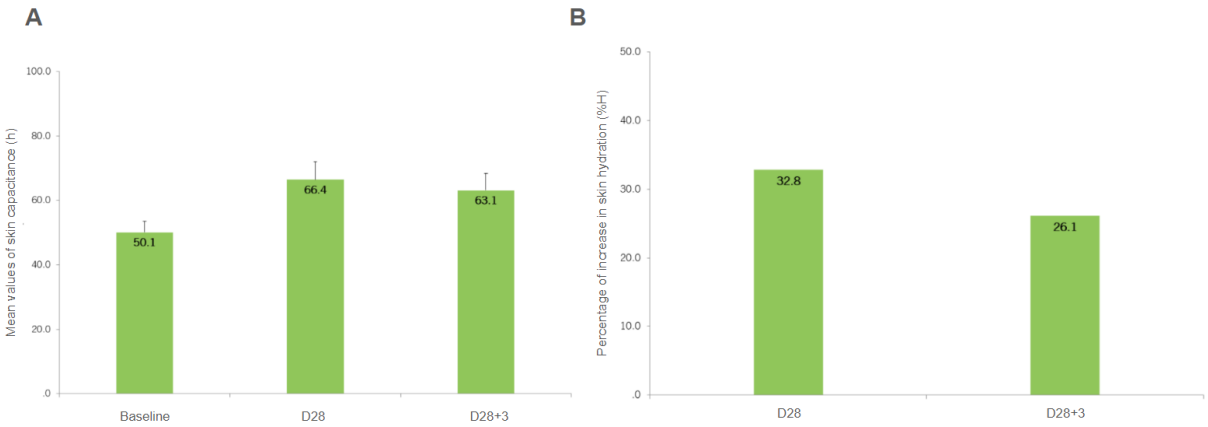

Figure 1. Long-term skin hydration. A) Mean values of skin capacitance at baseline (day 0), after 28 days of product use (D28), and after 3 days post-use (D28+3). B) Increased percentage of skin hydration at D28 and D28+3. Bar graphs represent the mean values of 11 subjects (n=11) evaluated before and after random application of the product to the face. Data were analyzed using bimodal paired Student's t-test ($p<0.0001$).

Baseline skin pH values were consistent between the product-treated and control forearms, confirming initial homogeneity. The application of the product maintained natural skin pH levels at D28, with no significant differences observed compared to the control (Figure 2).

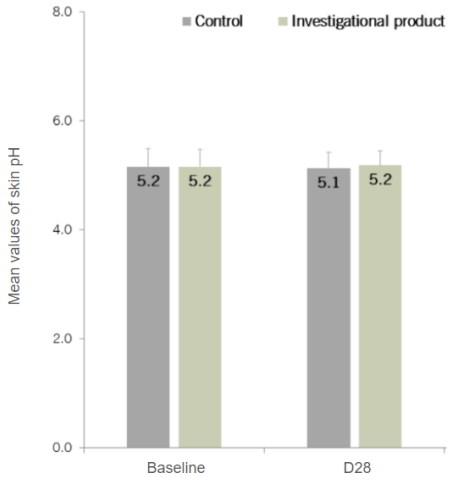

Figure 2. Mean values of skin pH. The measurements were obtained on the forearms at baseline (D0) and after 28 days of product use (D28). Bar graphs represent the mean values of 11 subjects (n=11). Data were analyzed using bimodal paired Student's t-test. No statistical differences were found among the groups.

TEWL values decreased at all time points assessed (Figure 3A), indicating that the product effectively strengthened the cutaneous barrier. Notably, cutaneous barrier enhancement was 7.4% at D7, 11.9% at D14, and 16.4% at D28, with 100% of subjects showing improvement from D7 onwards (Figure 3B).

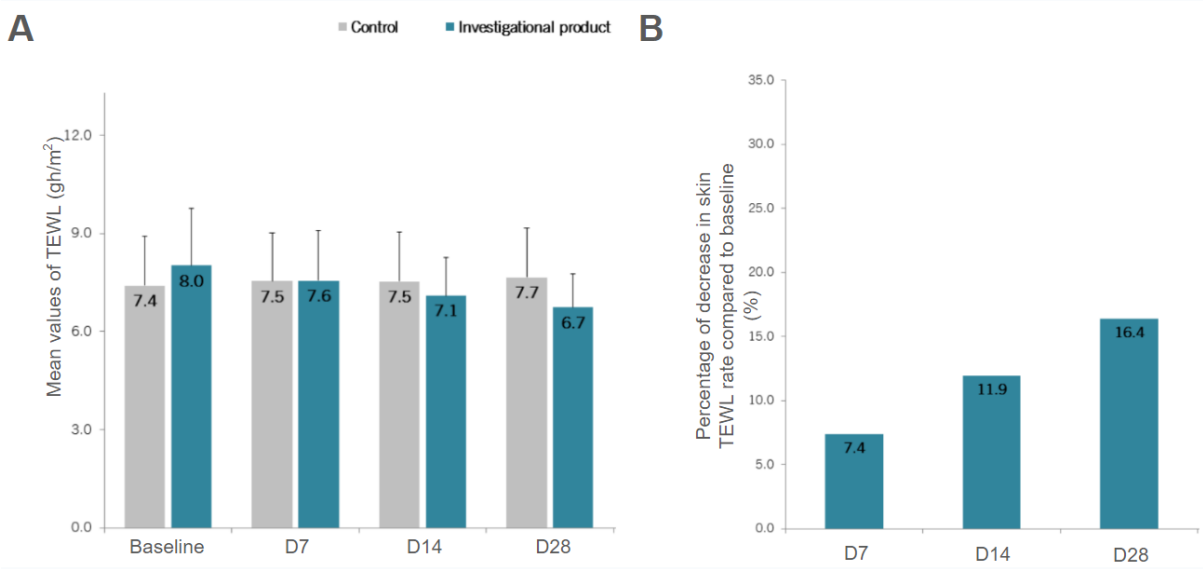

Figure 3. Transepidermal water loss (TEWL) evaluation. A) Mean values of TEWL at baseline (D0), after 7 days (D7), 14 days (D14), and 28 days (D28) of product use. Control group was represented by grey bars; treated group was represented by blue bars. B) Percentage of decrease in skin TEWL rate compared to D0 after D7, D14, and D28. Bar graphs represent the mean values of 8 subjects (n=8) evaluated before and after random application of the product on the forearms. Data were analyzed using bimodal paired Student's t-test ($p<0.05$).

Initial dermal density values did not significantly differ between the product application site and control. However, at D28, dermal density significantly increased by 5.1% at the product application site compared to the baseline (Figure 4A). A notable 83% of subjects experienced improved dermal density after 28 days of product use, reflecting significant enhancements in dermal density and functional structure (Figure 4B).

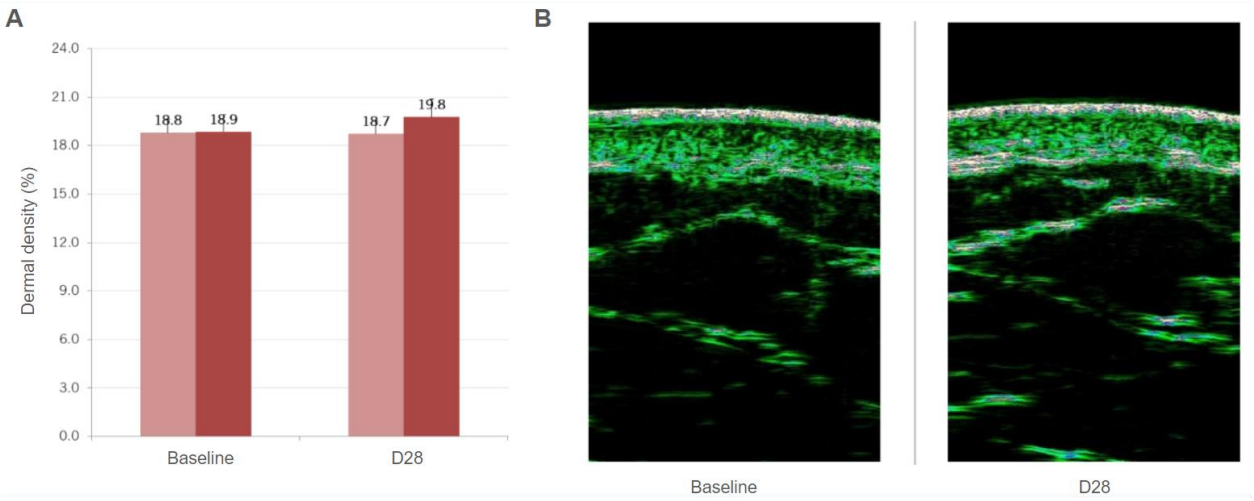

Figure 4. Assessment of skin density via image analysis obtained using ultrasound probe. (A) Mean values of dermal density at baseline (D0) and after 28 days (D28) of product use. Control group was represented by light red bars; treated group was represented by red bars. B) Representative High-frequency Ultrasound image obtained from subject P5 at D0 and D28. Bar graphs represent the mean values of 18 subjects (n=18) evaluated before and after random application of the product on the forearms. Data were analyzed using bimodal paired Student's t-test ($p<0.05$).

3.2. Metagenomics.

The microbiome of volunteers' faces was characterized using an amplicon sequencing technique focused on bacteria (16S V3-V4 marker). From this analysis, the relative abundance of bacterial genera in each sample was obtained.

Initially (Figure 5A), subject groups showed a predominance of *Cutibacterium* and *Staphylococcus* microorganisms. After 28 days of treatment (Figure 5B), this predominance remained. *Pseudomonas*, *Corynebacterium*, *Enhydrobacter* and *Streptococcus* microorganisms increased or decreased in very small quantities. *Burkholderia* levels decreased. Differential abundance of *Cutibacterium* spp. and *Staphylococcus* spp. between groups A and B was not observed (ANCOM-BC + FDR adjust ($p<0.05$)).

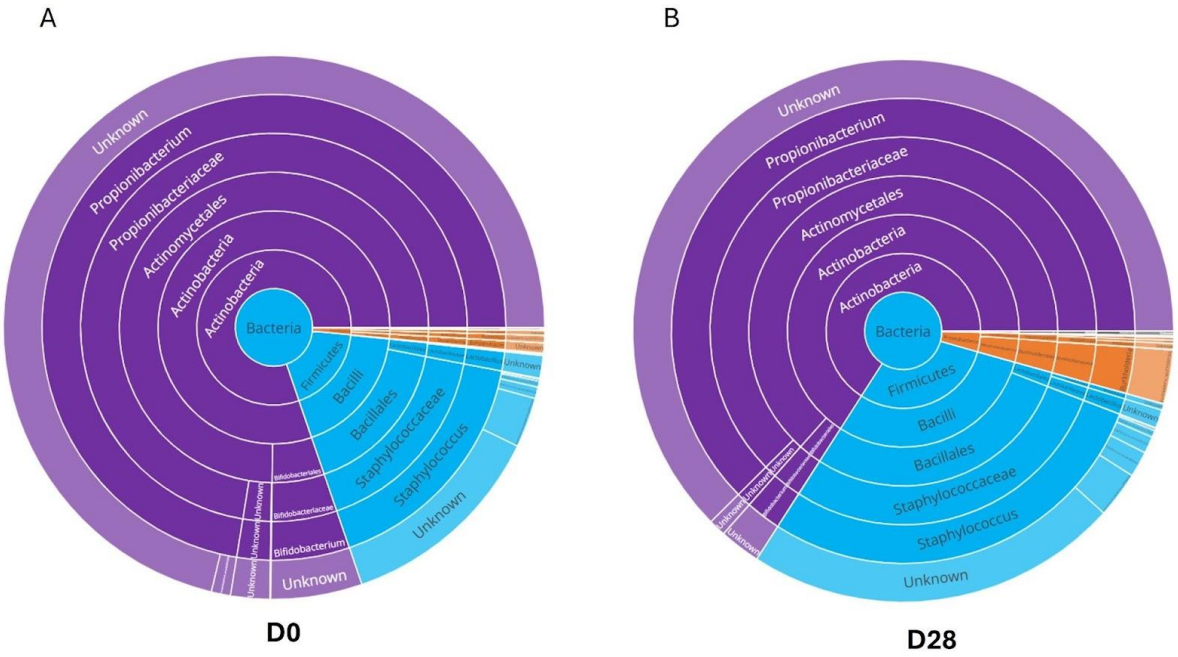

Figure 5. Relative abundance of bacteria. (A) Relative abundance of volunteers before using the product and (B) relative abundance of volunteers after 28 days using the product. 21 subjects (n=21) evaluated before and after random application of the product to the face.

The microbiota profile showed a high alpha diversity measured by the Shannon index (Figure 6) that did not change significantly according to Wilcoxon signed rank exact test. Beta diversity remained stable, showing no significant changes based on Bray-Curtis dissimilarity, as well as unweighted and weighted UniFrac distances (Adonis). Therefore, skin microbiota did not show significant changes.

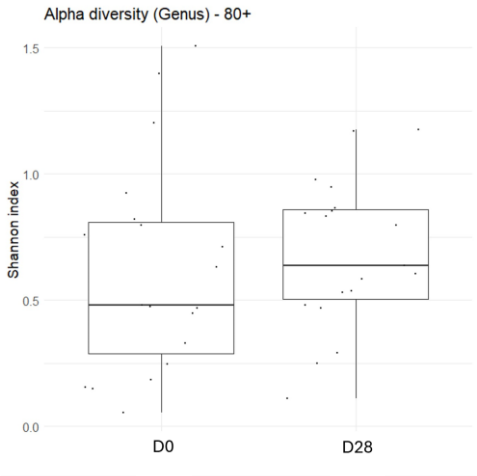

Figure 6. Alpha diversity between D0 (before product use) and D28 (after 28 days of product daily application). No statistical significance was observed between times (Wilcoxon signed rank exact test). 21 subjects (n=21) evaluated before and after random application of the product to the face.

3.3. Lipidomics.

The epidermal lipids profile was shown to be composed mainly of glycerides and the classes observed include fatty acids, ester, alcohol, squalene, sphingomyelin, ceramides, and sphingolipids ceramide precursors. Figure 7 presents a barplot that compares the epidermal lipid profiles of skin samples collected at D0 and D28.

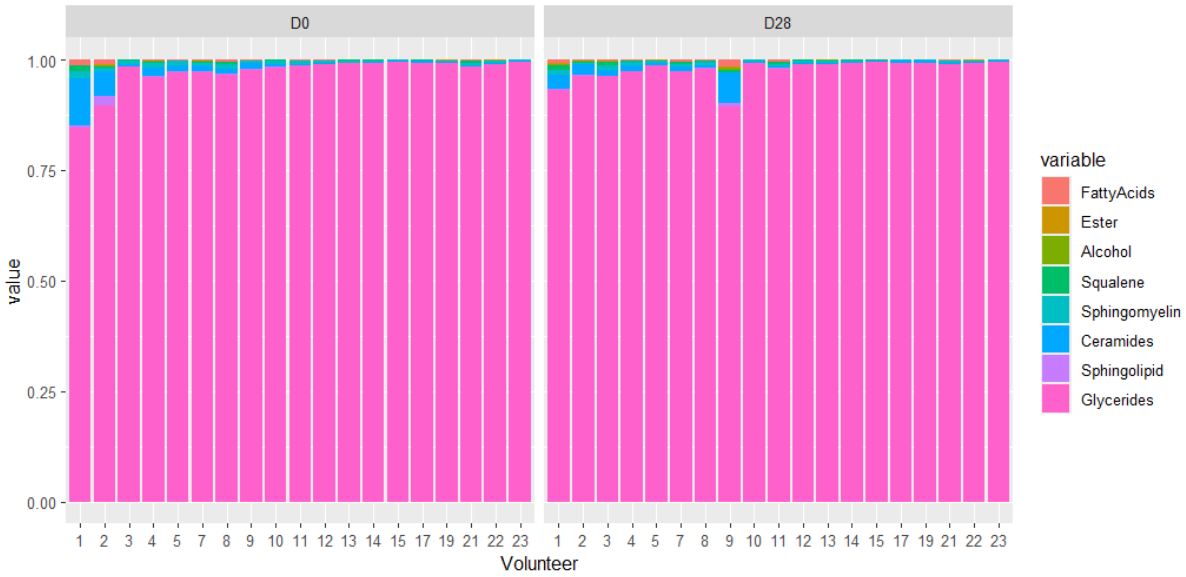

Figure 7. Barplot of epidermal lipids profile, at baseline (D0) and after 28 days of product use (D28). The x-axis represents the different lipid classes, and the y-axis shows the relative abundance of each lipid class. 19 subjects (n=19) evaluated before and after random application of the product to the face.

Overall, the barplot suggests that the product did not induce substantial alterations in the lipid profile of epidermal lipids after 28 days of use. The Wilcoxon sum rank test revealed significant changes between groups D0 and D28. There was an increase of isopropyl palmitate ($p < 0.01$) and a reduction of C12 Ceramide-1-Phosphate (d18:1/12:0) ($p < 0.01$).

The lipidomics for the sebaceous area resulted in a profile composition characterized by the presence of fatty acids, squalene, amide, ester, and alcohol molecules as shown in Figure 8.

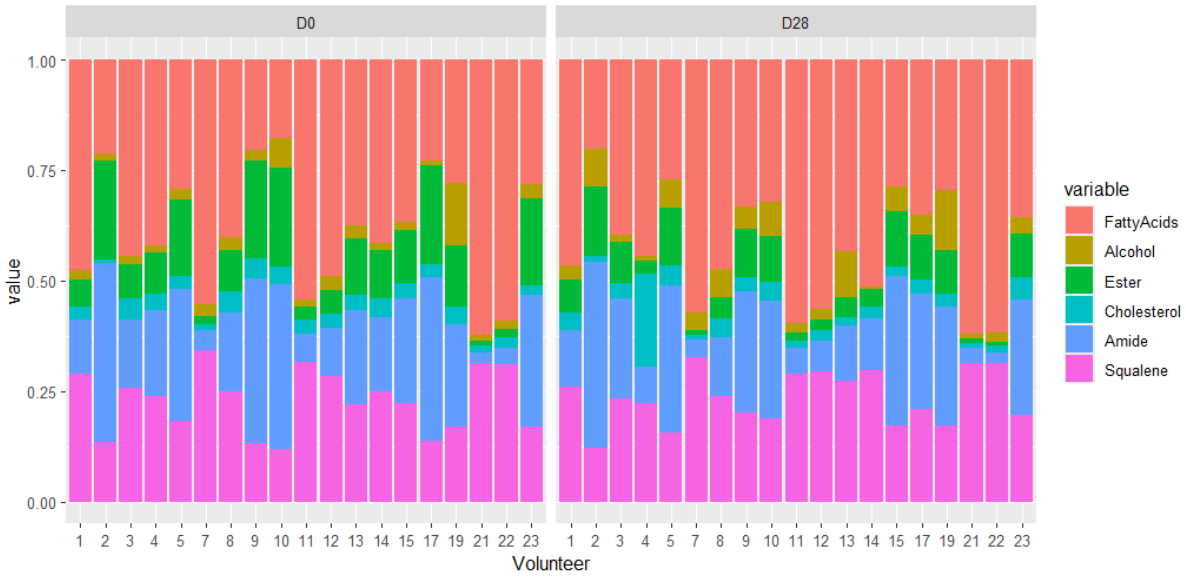

Figure 8. Barplot of sebaceous lipids profile, at baseline (D0) and after 28 days of product use (D28). The x-axis represents the different lipid classes, and the y-axis shows the relative abundance of each lipid class. 19 subjects (n=19) evaluated before and after random application of the product to the face.

There is no variation in the relative composition of sebaceous lipids. However, significant differences were observed in the absolute profile of lipids. In Figure 9 the boxplot provides a concise and informative summary of the distribution of lipids class concentration in each group.

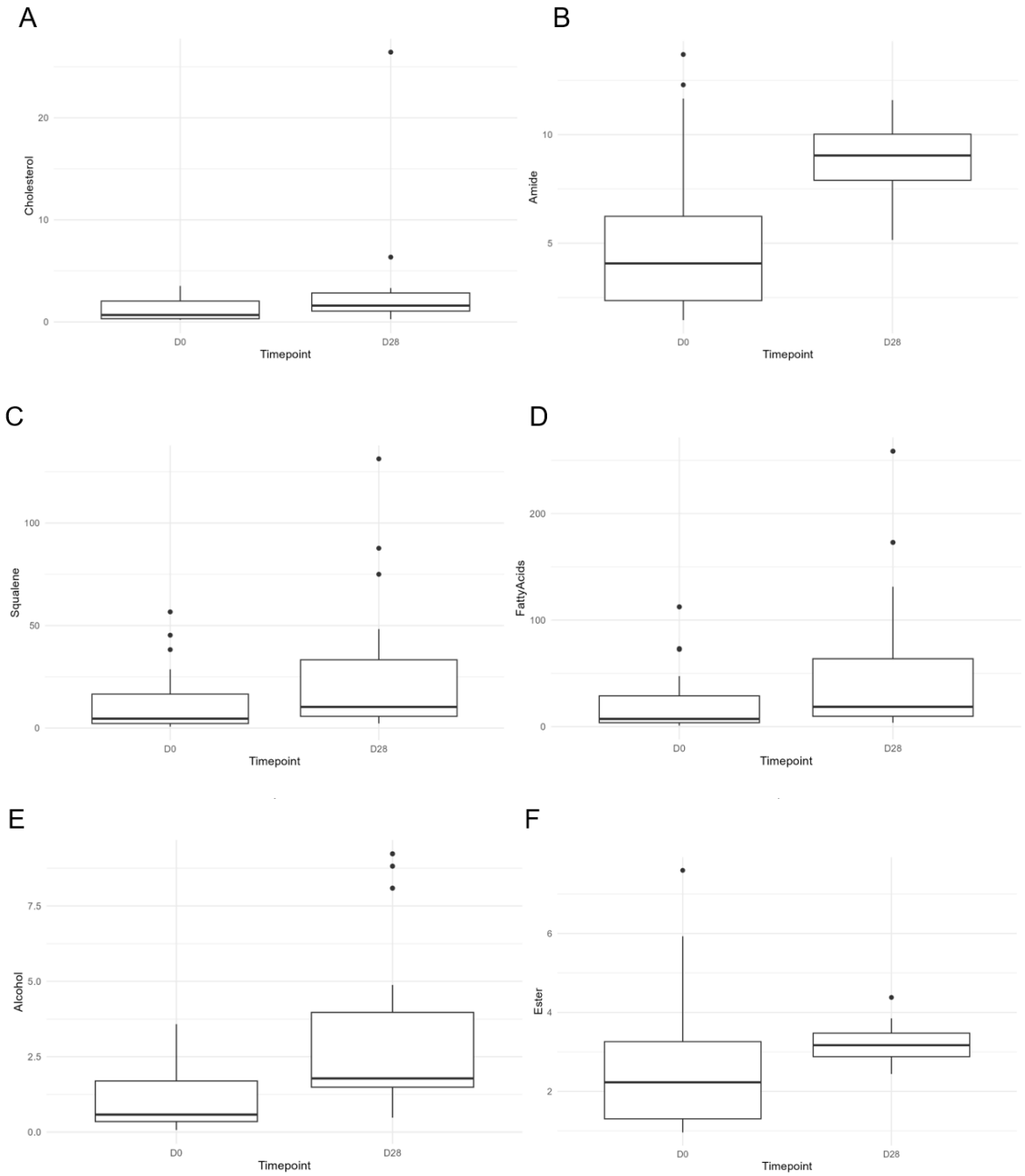

Figure 9. Boxplot representing the concentrations (ppb) of different sebaceous lipids in group D0 and D28. This includes (A) cholesterol, (B) amide, (C) squalene, (D) fatty acids, (E) alcohols, and (F) esters. 19 subjects (n=19) evaluated before and after random application of the product to the face.

Figure 9 presents a general increase of sebaceous lipids in group D28. The Wilcoxon sum rank test revealed significant changes in cholesterol, amide, squalene, and fatty acids ($p<0.01$). A significant elevation in the levels of eight fatty acid molecules was observed.

4. Discussion.

Skin hydration peaks around the age of 40 and declines in subsequent decades, primarily due to a reduction in natural moisturizers within the epidermis [13]. This reduction leads to decreased water content in the stratum corneum, impairing enzymatic processes and resulting in dry skin [14]. Our study demonstrates significant improvement in skin hydration across all subjects following 28 days of treatment. The cosmetic product used, enriched with BBD with a high concentration of medium-chain fatty acids, has been shown in previous research to activate pathways associated with skin barrier function and hydration (data not shown), likely contributing to these positive results.

Aged skin exhibits elevated surface pH levels compared to younger skin, particularly in individuals over 70 [15, 16], which may delay permeability barrier recovery [2]. In our study, the pH levels of volunteers remained consistent at 5.2 after 28 days of product use. Given that optimal skin enzymatic activity occurs at a pH of around 5 [2], the product did not adversely affect enzymatic functions in the skin, maintaining a suitable environment for these processes.

Epidermal and dermal thinning, coupled with increased cutaneous stiffness, heightens the risk of shear-type injuries such as skin tears and pressure ulcers, with prevalence rates among those over 60 years ranging from 4% to 30% [17]. Disruptions in stratum corneum lipid composition led to impaired skin barrier functions and elevated TEWL [18]. Although TEWL varies with age, studies indicate that its baseline rates on body sites are often lower in aged skin compared to younger skin [2, 19, 20]. Dermal density also decreases significantly with age, with collagen content in individuals aged 80 and above being 75% lower than in young adults [4]. Chronic sun exposure further exacerbates skin aging, replacing the upper dermis with abnormal elastic fibers and altering collagen fiber organization [21].

Our data corroborate these findings, showing decreased TEWL values and dermal density in volunteers over 80 years old. Notably, after 7 days of treatment, improvements in barrier strength were evident, with significant enhancements in dermal density observed after

28 days. Despite the inherent fragility of aged skin, the subjects responded well to the treatment, as indicated by high responsiveness levels throughout the study.

Our research suggests that the product formulation is compatible with the elderly microbiome. This is because no significant changes were observed in the skin microbiota, indicating that the product is suitable for use by the elderly and does not disrupt their microbiome. This action leads to the product formulation being recognized as microbiome friendly.

Maintaining a balanced skin lipid composition is essential for preserving barrier integrity. Previous research has demonstrated that the aged SC exhibits a reduction of over 30% in total lipid content, with a significant decrease in cholesterol synthesis [22, 23]. Additionally, Hornburg et al. (2023) [8] reported an increase in saturated and monounsaturated fatty acids in older individuals, accompanied by a reduction in polyunsaturated fatty acids, which can be an indicative profile of dyslipidemia and inflammation. The increased stimulation of sebaceous lipids observed in our study may be related to the improved state of the skin barrier function.

Cutibacterium acnes and *Corynebacterium* spp., produce lipases that break down triglycerides in sebum to release free fatty acids. Free fatty acids maintain the acidic surface pH of the skin, which dictates the chemical barrier [24, 25].

Howard and colleagues examined the behavior of facial human skin microbiota concerning factors such as aging and lipid metabolism across different age groups, with the maximum age being 74 years [7]. Our study corroborates and complements these findings by examining an older population aged 83±2 years, which have been studied only in an incipient manner worldwide and finding that their facial microbiota remains diverse.

6. Conclusion.

As the global demographic shifts towards an older population, understanding the biological underpinnings of aging becomes increasingly urgent and essential. A longer life opens new opportunities to enhance the quality of life and redefine health standards, catalyzing transformative advancements in skincare for the elderly.

Our findings contribute to the understanding of the intricate relationship between skin biophysics, microbiome dynamics, and lipid composition of this understudied age group of women over 80 years old. Notably, the use of a cosmetic product containing BBD active ingredients, demonstrated enhanced skin health, proving that, despite old age, the subjects are responsive to topical treatment.

These results underscore the potential of the BBD in improving skin hydration and barrier function, even in elderly individuals with compromised skin integrity. Further research should explore the long-term benefits and underlying mechanisms of this treatment to optimize skin health in aging populations. Our research not only addresses a gap in the scientific literature but also paves the way for the development of innovative skincare products incorporating natural ingredients, designed to enhance skin health and resilience in the elderly population.

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Conflict of Interest Statement.

NONE.

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