IFSCC 2025 full paper (IFSCC2025-630)

"Understanding the smell of aging: Analysis of volatile organic compounds emitted on the bust area by young and aged Caucasian skin"

1. Introduction

In the cosmetic industry, understanding age-related changes on skin, body and mind is essential for developing targeted products and diagnostics. Humain skin continuously emits a complex mixture of volatile organic compounds (VOCs) shaped by metabolism, glandular secretions, microbiota and environmental factors. While diet and stress modulate these emissions, stable traits such as genetic, gender and age also influence odor profiles.

Over the past two decades, human olfaction has revealed that body odors convey biologically and socially relevant cues. Early studies suggested perceptual discrimination between male and female odors [1], though later critiques attributed these findings to differences in odor intensity or pleasantness. Nevertheless, evidence supports the existence of sex-specific volatile compounds [2] and variations in concentrations of non-volatile odor precursors in axillary sweat, such as fatty acids and thiols [3]. Age also alters body odor, notably through hormonal changes, as androgen levels decline with age, affecting aprocrine and sebaceous gland secretions [4]. Research has begun to elucidate the chemical profile associated with "old age odor", among the most studied age-related odorants is trans-2-nonenal, an unsaturated aldehyde predominantly found in individuals over 40 years of age, believed to result from the oxidative degradation of omega-7 unsaturated fatty acids in sebaceous lipids, and associated with a greasy, grassy scent often described as characteristic of "aged" body odor [5]. However, Gallagher et al. (2008) [6] did not replicate this finding. A possible explanation given by the authors is that the findings of Haze et al. might be a diet-linked phenomenon, which may not generalize to non-Japanese populations. Age-body odor markers identification remains debated.

Body odor analysis relies on diverse sampling strategies. Polydimethylsiloxane (PDMS) materials have proven effective for collecting sweat for gas chromatography-mass spectrometry (GC-MS) analysis [7]. The design of these patches can be customized to enhance sensitivity based on the sampling area and study requirements [8]. PDMS stir bars, along with other tools such as sorbent cylinders, SPME fibers, and cotton pads, each present strengths and limitations for VOCs collection from skin areas like the arms and underarms [9-11]. Despite technical advances, methodological heterogeneity and sample variability, such as sample contamination, lifestyle influences, and potential misrepresentation of skin VOCs, continue to challenge the accurate characterization of body and skin volatiles.

This study investigated the emission of age-related molecules in a Caucasian population across a broad age range (19 - 77 years), at the chest area, a region of particular relevance in the cosmetic and fragrance industries. By developing a tailored method for sampling human

body odor, using PDMS stir bars, this research aimed to deeper understand how body odor profiles may vary with aging.

2. Materials and Methods

2.1. Recruitment of Panelists

A total of 55 participants (mean age = 48.6 ± 17.9 year old (yo)) were recruited, comprising 27 men (mean age = 49.7±19.3 yo) and 28 women (mean age = 47.5 ± 16.3 yo).

Participants were categorized into three age groups: “Young”, “Middle-age”, “Elderly”. Final sample sizes and characteristics are provided in Table 1.

	Young (under 45yo)	Middle-age (between 45 to 65yo)	Elderly (over 65yo)
Men	N=10 Mean age = **27.1 ± 5.7** yo	N=9 Mean age = **54.8 ± 5.8** yo	N=8 Mean age = **72.1 ± 3.1** yo
Women	N=10 Mean age = **28 ± 4.6** yo	N=11 Mean age = **52.3 ± 4.5** yo	N=7 Mean age = **68 ± 1.7** yo
Total	N=20 Mean age = **27.5 ± 5.2** yo	N=20 Mean age = **53.4 ± 5.3** yo	N=15 Mean age = **70.2 ± 3.3** yo

Table 1. Characteristics for each age-group of participants. N=number of participants, yo=year old.

Participants were recruited based on specific inclusion criteria such as age, skin phototype 1 to 3, low omega-7 diet, field of activity. Eligible individuals were required to use the provided shower gel 48 hours prior to and on the day of the test. They had to wear a provided cotton t-shirt washed with a fragrance-free detergent on the test day and refrain from using any scented products, including deodorants and hair care items, for 48 hours before and on the test day. Participants were also instructed to avoid strong-smelling foods and alcohol during the same period. Non-smokers and individuals without allergies were preferred, and pregnant or breastfeeding women were excluded.

2.2. Odor collection

2.2.1. *In vitro* odor collection for sampling optimization

2.2.1.1. using cotton gauze pad and Headspace Sorptive Extraction (HSSE) method

Cotton gauze pads used were sourced from Urgo Laboratories (reference 0459, Lot 089030001). Initially, 2 mL of distilled water were introduced into a 70 mL glass vial, which was then spiked with varying concentrations of trans-2-nonenal (0 ng, 50 ng, 100 ng, 500 ng, 1000 ng). A gauze pad, cut in half, was positioned at the top of the vial, ensuring it remained in the headspace and did not contact the trans-2-nonenal solution (Figure 1.(a)). The vials were incubated at 37°C for 4 hours. In the subsequent step, the gauze pad was transferred to a new, empty 70 mL glass vial. A PDMS-coated magnetic stir bar (Twister®, Gerstel) was

affixed to the vial's cap using a magnet, with the side of the pad that had been exposed to the trans-2-nonenal solution facing upwards towards the magnetic stir bar (Figure 1.(b)). Extraction was performed in an oven at 37°C for 2 hours.

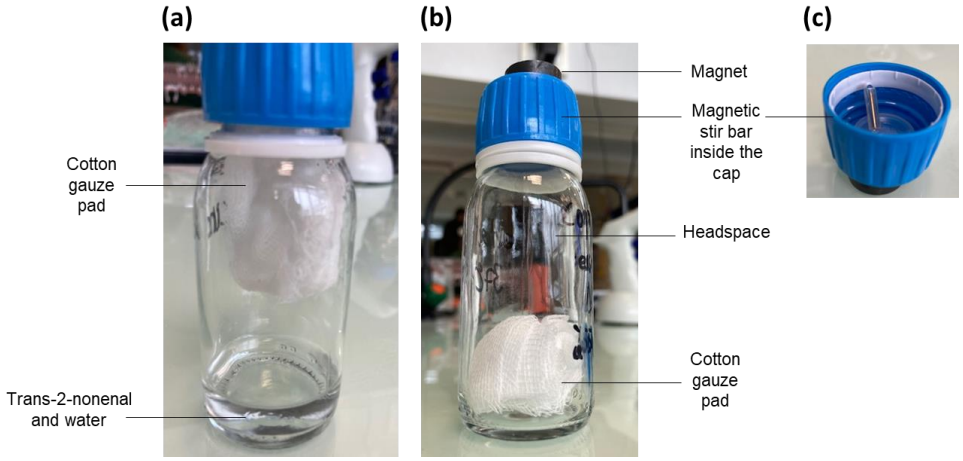

Figure 1. *In vitro* system for capturing trans-2-nonenal. (a) The initial phase involves using a cotton gauze pad; (b) The subsequent phase employs the Headspace Sorptive Extraction (HSSE) technique; (c) Zoom view of the cap interior, illustrating the positioning of the magnetic stir bar.

2.2.1.2. using Twister® medium

The initial step was repeated, replacing the gauze pad with a PDMS-coated magnetic stir bar (Twister®, Gerstel) (as on Figure 1.(c)), which was secured to the vial's cap using a magnet. The second step (HSSE) is not performed in this case, and the Twister® is analyzed directly by thermal desorption GC-ToF-MS.

2.2.2. Skin collection system for *In vivo* sampling of human body odor

In this study, a tailored made collection system to collect human odor samples was utilized. A system to position odors collection materials in contact with the skin on the chest area of human subjects and ensuring their remain in place for several hours. To achieve this, 3 magnetic stir bars, (Twister®; 10 mm,0.5 mm in film thickness, 24 µL PDMS phase volume, Gerstel) per subject were placed on the participant's chest, a few centimeters above the nipple line, and covered with an adhesive bandage (Labell, Leclerc selection – 6cmx5cm).
Participants were welcomed and asked to remove their cotton t-shirts to facilitate sample collection. Wearing clean nitril gloves, the lower part of the adhesive bandage was peeled-off and affixed to the skin of the participant's chest at the designated area. To prevent the transfer of compounds from the bandage material, a sheet of aluminum (6cmx3cm) was placed on the inner surface of the bandage. Then, using tweezers to ensure precise placement, the twisters were gently place at the base of the adhered bandage. After positioning the Twister®, the upper portion of the bandage was peeled off and pressed firmly on the skin to secure the twisters in place. To enhance the stability of the system, additional adhesive tape can be applied to the lateral sides and, if needed, above and below the bandage, taking care to accommodate any body hair or contours. It is essential that the system remains securely attached to

prevent any loss of the twisters during the sampling period. Once the system is in place, the participant may replace their cotton shirt and will be instructed to return in 4 hours for the removal and collection of the twisters.

For system removal, participants were again asked to remove their cotton t-shirts. Clean gloves were worn, and the upper section of the system was carefully detached from the skin. Twisters were retrieved using clean tweezers, and individually stored in vials. Each vial was securely closed and stored at 4°C for preservation. The system was then fully removed from the participant, who then redressed.

2.3. Analytical parameters

2.3.1. Thermal desoprtion

Twisters® were introduced in a Thermal Desorption Unit (TDU, Gerstel). The TDU initial temperature was set at 40°C and increased at 120°C/s until 250°C (hold 8 min) under a pure Helium (99.999%) flow. VOCs were trapped in a Cooled Injector System (CIS, Gerstel) equipped with a cylindric glass liner filled with Tenax TA. The CIS initial temperature was set at -10 °C (0.1 min) and increased at 12°C/s until 300°C (hold 5 min).

2.3.2. GC-ToF-MS analysis

Samples were analyzed using GC (7890B, Agilent Technologies) coupled to a Time of Flight - Mass Spectrometer (ToF-MS) (Pegasus BT-ToF-MS, Leco), equipped with a Multi-Purpose Sampler (MPS, Gerstel). Chromatographic separation was carried out via a polar capillary column (DB-WAX, Agilent Technologies) with the following dimensions: 30 m length x 0.25 mm internal diameter x 0.25 µm film thickness. The GC oven temperature program started at 40°C (held for 2 min), followed by a temperature ramp of 5°C/min up to 250°C (held for 5 min). Ultrapure grade helium was utilized as carrier gas at 1,8 mL/min. The transfer line and ion source were set at 250°C. The MS acquisition was performed using EI ionization mode at 70 eV, in full scan mode, in the mass m/z range from 30 to 400. The extraction frequency was set to 30 kHz, with an acquisition rate of 5 spectra/second.

2.3.3. Data treatment

Data collected were processed using MassTwist® (Twistaroma) software. VOCs were identified by comparing experimental mass spectra with the NIST database (version 2017) and retention indexes with literature data.

For trans-2-Nonenal, to increase sensitivity, a targeted identification method was carried out using ChromaToF® (Leco) software. The ion 70.05 was selected as the quantifier ion, and ions 57.04 and 83.06 as qualifiers. To confirm the compound identification, the peaks area ratio of the qualifier/quantifier was verified with a tolerance of ± 20 % compared to the analytical standard. To normalize the data, peak area values were centered and reduced using the Autoscaling method via MetaboAnalyst 6.0 software. An analysis of variance (ANOVA) was performed to compare the results obtained for the three age groups (“Young”, “Middle-age” and “Elderly” – Table 1.).

3. Results

3.1. *In vitro* sampling optimization

This first segment of the study aimed to identify an effective capture system for human skin odor molecules, with a particular emphasis on trans-2-nonenal, a molecule previously recognized for its role in age-related skin odor mechanisms. To achieve this, two distinct in vitro methods for odor capture were established and subsequently compared, using first cotton gauze pads capture coupled with HSSE or using PDMS stir bars collection system.
The use of cotton gauze pads to collect trans-2-nonenal by headspace with the in vitro protocol developed in this study allows its detection in concentrations ≥ 500 ng (Figure 2 (a)). In contrast, the use of PDMS magnetic stir bars allows the detection of trans-2-nonenal at lower concentrations ≥ 50 ng (Figure 2(b)).

Although cotton gauzes are easier to handle to collect body odors, PDMS stir bars appear to be more efficient to increase the analytical sensitivity.

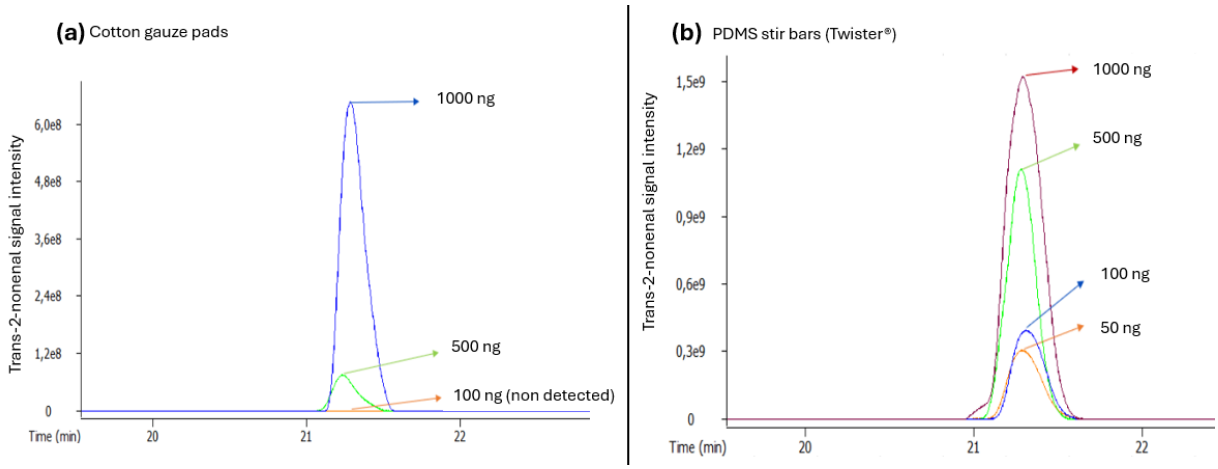

Figure 2. GC-ToF-MS chromatograms overlapped showing the trans-2-nonenal signal intensity (70.05 m/z) in spiked water at 50 ng , 100, 500 and 1000 ng, obtained for the two experimental setup: (a) sampling with cotton gauze pads followed by HSSE extraction (N= 2 per concentration); (b) direct sampling with PDMS magnetic stir bars (N= 2 per concentration).

3.2. *In vivo* skin VOC's analysis

As second phase, this study aimed to analyze the molecules emitted by the skin *in vivo*, specifically examining age-related variations within the carefully selected panel. Initially, the investigation focused on trans-2-nonenal. This targeted approach was later broadened to include a more extensive, untargeted analysis of the skin's VOCs, allowing a comprehensive understanding of the age-related changes in skin odor profiles.

3.2.1. Targeted analysis of trans-2-nonenal emission

No significant difference was observed in the peak areas of trans-2-nonenal across age groups (“Young”: 2.07E+05 (± 1.14E+05), “Middel-age”: 2.45E+05 (± 0.89E+05), “Elderly”: 1.80E+05 (± 2.88E+05); Figure 3); The results obtained in this study do not support the hypothesis that the concentration of trans-2-nonenal emitted at the skin surface increases after the age of 45 in humans.

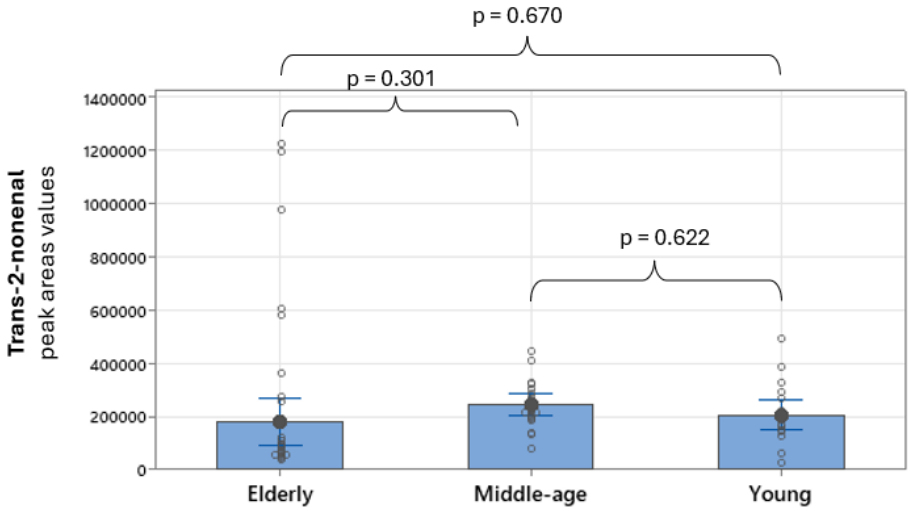

Figure 3. Boxplots illustrating the average trans-2-nonenal peak areas values obtained for the three age-groups: “Elderly” (N= 15), “Middle-age” (N= 20), “Young” (N= 18). The white dots represent individual data. Group means were compared using ANOVA, with Fisher's test for post-hoc analysis. Error bars represent a 95 % confidence interval.

3.2.2. Untargeted screening

An untargeted screening was performed to identity other potentials age-related biomarkers. More than 2000 VOCs were detected, and 422 compounds were identified.

The comparison among the three age groups revealed that the average peak areas of Acetoin is significantly increased in the Elderly group, with the following values: “Young”: 4.62E+06 (± 4.24E+06), “Middel-age”: 1.29E+07 (± 1.20E+07), “Elderly”: 2.07E+07 (± 1.70E+07) (Figure 4). No significant difference (ANOVA p-value > 0.05) was found between “Middle-age” and “Young” groups (Figure 4).

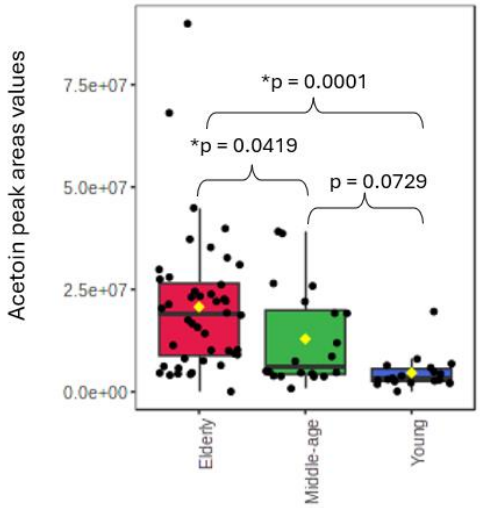

Figure 4. Boxplots illustrating the values obtained for Acetoin (xx m/z) are presented for the three age groups: Elderly" (N=15), "Middle-age" (N=20), "Young" (N=18). Black dots represent individual data, while the yellow dot refers to the average value for each group. The line within each box indicates the interquartile median (IQR), encompassing the middle half of the data. Whiskers extend from the box to show the distribution of data using IQR. Group means were compared using ANOVA, with Fisher's test for post-hoc analysis.

The average peak area values of four volatile fatty acids (Table 2) - Butanoic acid (Figure 5 (a)), Octanoic acid (Figure 5 (b)), Nonanoic acid (Figure 5 (c)) and Decanoic acid (Figure 5 (d)) – were also found to be significantly higher in the Elderly group compared to both the Middle-age and Young groups.

Group	Peak areas values	Butanoic acid	Octanoic acid	Nonanoic acid	Decanoic acid
Elderly	**Average**	5.03E+06	1.01E+07	5.76E+06	6.83E+06
	± Standard Deviation	2.83E+06	6.82E+06	4.46E+06	3.69E+06
Middle-age	**Average**	1.58E+06	1.92E+06	4.35E+05	1.25E+06
	± Standard Deviation	5.86E+05	1.18E+06	4.00E+05	1.30E+06
Young	**Average**	1.69E+06	1.80E+06	1.61E+06	1.37E+06
	± Standard Deviation	1.60E+06	1.19E+06	1.55E+06	1.13E+06

Table 2. Average peak area values of the four identified volatile fatty acids for each age-group of participants

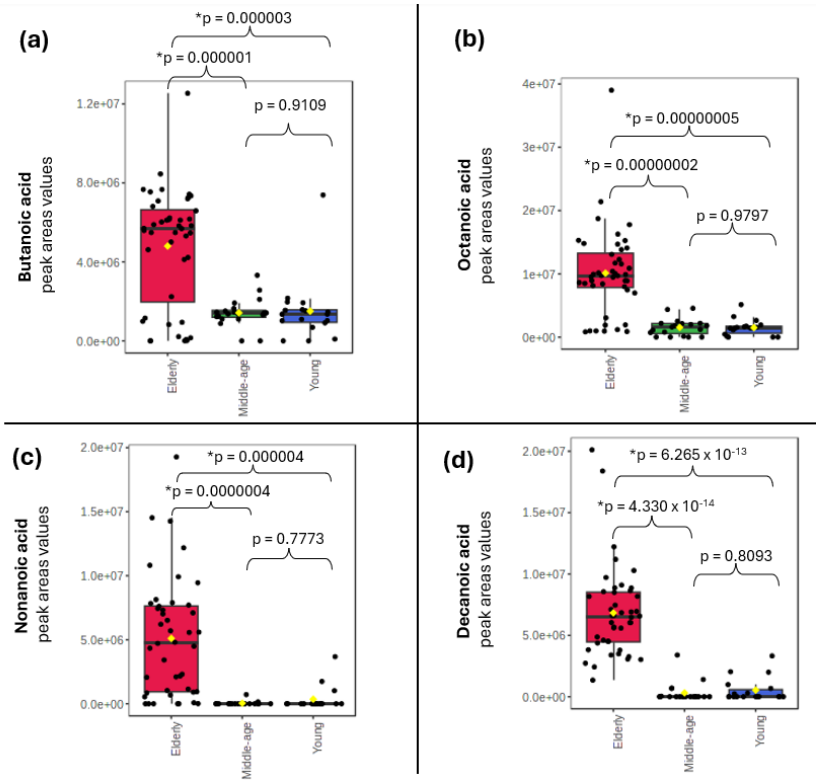

Figure 5. Boxplots showing peak area values obtained for the four volatile fatty acids: (a) Butanoic acid, (b) Octanoic acid, (c) Nonanoic acid, and (d) Decanoic acid) in the three age groups. Black dots represent individual data, while the yellow dot refers to the average value for each group. The line within each box indicates the interquartile median (IQR), encompassing the middle half of the data. Whiskers extend from the box to show the distribution of data using IQR. Group means were compared using ANOVA, with Fisher's test for post-hoc analysis.

4. Discussion

This study aimed to deepen the understanding of age-related changes in human odor profiles, using an optimized sampling method with PDMS stir bars and initially focusing on trans-2-nonenal. The results, obtained in a Caucasian population, do not support a significant increase of trans-2-nonenal with age, contrasting with previous findings in Asian population [5]. However, this aligns with the conclusions of Gallagher et al. (2008) [6], who did not observe a specific age-related trans-2-nonenal production.

However, the untargeted approach revealed a significant increase of several compounds in the “Elderly” group, particularly acetoin and four volatile fatty acids (butanoic, octanoic, nonanoic, and decanoic acids). These findings suggest that body odor changes in body odor associated with aging may not be only linked to trans-2-nonenal, but also to broader alterations in metabolism, such as the skin lipid metabolism.

Recent research indicates that the skin microbiome faces significant age-related changes, including an increase in skin bacterial diversity and a decrease in abundance of bacteria such as *Lactobacillus* and *Cutibacterium acnes* [12]. Concurrently, aging is associated with changes in sebaceous secretions, including a relative change in fatty acids. The interaction between these altered lipid profiles and the aging microbiota could thus contribute to the production of different metabolites or potential volatile fatty acids —such as butanoic, octanoic,

nonanoic, and decanoic acids—identified in this study. These compounds are known to emit sour or rancid odors commonly associated with aging.

Additionally, the significant increase in acetoin levels among “Elderly” group might indicate enhanced metabolic activity of certain fermentative bacteria such as *Staphylococcus epidermidis* or *Staphylococcus aureus*, which are known to produce this compound [13]. Acetoin has a sweet, buttery scent that could contribute to the overall change in body odor perception with aging.

These findings support the idea that age-related changes in body odor are probably not due to a single biomarker but rather from a complex interaction of skin metabolism and microbiome modifications, and their interrelated effects. They also highlight the importance of adopting a more integrative approach to studying “aging odors,” combining VOCs analysis, lipid profiling, and microbiome characterization.

Finally, this study demonstrates the effectiveness of PDMS stir bars for human skin VOCs sampling under controlled conditions, while emphasizing the importance of developing sensitive and reproducible analytical methods to capture the complexity of the human volatolome.

5. Conclusion

This study highlights the changes in skin-emitted volatile compounds that occur with age, particularly the increase in acetoin and malodorous fatty acids, potentially due to changes in skin mechanisms and microbiota composition. These findings emphasize the importance of targeting skin lipid balance and microbiome modulation in the development of age-specific cosmetic products. This is especially relevant in the field of skincare formulation, where understanding the unique needs of mature consumers can lead to more effective and appealing products. By addressing these factors, the cosmetic industry can better answer the evolving preferences and requirements of an aging population.

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