IFSCC 2025 full paper (IFSCC2025-662)

M. luteus Mediates Skin Anti-aging Effects of Saccharomyces Rice Ferment Filtrate through Microbiome And Metabolite Modulation

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1. Introduction

The skin, the body's largest and most complex organ, serves as the first line of defense against the external environment. It performs essential functions including barrier protection, sensory perception, temperature regulation, immune defense, and metabolic activities. Among all organs, the skin displays the most visible signs of aging, which result from both intrinsic and extrinsic factors. Intrinsic aging is a genetically driven process associated with thinning, dryness, fine lines, and reduced elasticity, while extrinsic aging arises from environmental stressors such as UV exposure, pollution, and smoking, leading to deeper wrinkles, sagging, and pigmentation [1,2]. Skin aging, beyond aesthetics, involves increased fragility, barrier dysfunction, and higher risk of skin disorders [3,4].

With a rapidly aging global population—expected to reach 2.1 billion people over the age of 60 by 2050 [5]—effective strategies to mitigate skin aging are increasingly important. Current interventions include antioxidants that neutralize reactive oxygen species (ROS), hormone replacement therapy (HRT) to enhance collagen and hydration, and topicals like hyaluronic acid, retinoids, and niacinamide, which have demonstrated clinical efficacy [2,3,6].

Recently, yeast-derived ingredients have gained attention in anti-aging skincare. Among these, yeast fermentation filtrate—rich in small bioactive molecules such as amino acids, peptides, nucleotides, and vitamins—has shown promise as a multifunctional skin-conditioning agent. Studies suggest its benefits in improving skin appearance and delaying aging [7]. Yeast fermentation filtrate is now widely incorporated into cosmetic formulations; however, the mechanisms underlying its anti-aging effects remain poorly understood.

In this study, we explored the anti-aging potential of Saccharomyces rice ferment filtrate (RFF) and its microbiome-related mechanisms. A total of 83 healthy volunteers were enrolled in a 12-week randomized controlled trial comparing RFF-containing and non-RFF skincare products. The RFF group showed significantly enhanced skin hydration and elasticity. Notably, Micrococcus luteus abundance increased selectively in the RFF group and was positively correlated with uracil levels and negatively with isocitric acid levels—changes not observed in the control group. These findings suggest that *M. luteus* mediates the anti-aging effects of RFF

through modulation of the skin microbiome and metabolome, supporting its potential role as a functional strain in skincare formulations.

2. Materials and Methods

2.1 Subject Recruitment and Acquisition of Skin Physiological Parameters

Eighty-three healthy subjects were recruited and randomly assigned to the test and control groups at a 2:1 ratio. Both groups received facial skin interventions for 12 weeks. Clinical skin parameters were evaluated at four time points: before the intervention, and at 4, 8, and 12 weeks after the start of the intervention. Specifically, skin moisture content was measured using a Corneometer; transepidermal water loss (TEWL) was measured using a Tewameter; skin elasticity was assessed using a Cutometer® MPA580; and skin density and thickness were measured using the Ultrascan UC22 skin ultrasound system.

2.2 Sample Collection

Facial skin samples were collected using cotton swabs. Briefly, sterile swabs were rinsed with 50 mM Tris buffer (containing 1 mM EDTA [pH 8.0] and 0.5% Tween-20) and rubbed on a ~2 × 2 cm^2 area of skin at least 50 times. The samples were then centrifuged at 13,000 rpm for 10 minutes at 4℃. The supernatant was stored at –80℃ for metabolite detection and analysis, while the pellet was used for skin metagenomic sequencing.

2.3 DNA Extraction and Library Preparation

Genomic DNA was extracted from treated skin samples using the QIAamp PowerSkin Pro DNA Kit (QIAGEN, USA, 51804). DNA concentration was measured with a Qubit 3.0 fluorometer using the Qubit dsDNA HS Assay Kit (Thermo Fisher, USA), and integrity was confirmed by 1% agarose gel electrophoresis. For library construction, 300 ng of high-quality DNA was enzymatically fragmented using the KAPA Frag Enzyme and Buffer (KAPA Biosystems, USA, KK8514) at 37°C for 10 min, followed by end-repair and A-tailing in the same tube. After a 30 min incubation at 65°C, adapters were ligated at 20°C for 15 min. Post-ligation cleanup and size selection were performed with VAHTS DNA Clean Beads (Vazyme, China). Following PCR amplification and purification, library quality was assessed by (a) size distribution using the Agilent 2100 Bioanalyzer with the High Sensitivity DNA Kit (Agilent, USA), and (b) quantification using the StepOnePlus RT-PCR System (Thermo Fisher, USA).

2.4 Metagenome Sequencing

Whole-genome shotgun sequencing was performed on the Illumina NovaSeq 6000 platform (Illumina, USA) to generate 150 bp paired-end reads. Trimmomatic v0.39 was used to preprocess raw reads and obtain clean data [8]. Clean reads were aligned to the human reference genome (GRCh38) using Bowtie2 to filter out host-derived reads [9]. Taxonomic profiling was performed using MetaPhlAn4, while functional profiling of metabolic pathways was performed using HUMAnN3 [10].

2.5 Bioinformatics Analysis

For microbiome data, alpha diversity was calculated using the diversity function in the vegan package, and Bray–Curtis distances were computed with vegdist. Principal coordinates analysis (PCoA) was performed using the dudi.pco function in ade4. Differential taxa before and after treatment were identified using the rank_biserial function from the effectsize package to

compute effect sizes and p-values. To identify microbes affected by RFF, pre- and post-treatment abundance changes were compared within each group using the same method. PAM clustering based on genus-level Jaccard distances identified two distinct skin types. For metabolomic data, missing values were imputed using KNN from the VIM package, and centered log-ratio transformation was applied to 173 metabolites. PCA was performed using dudi.pca from ade4. Metabolites significantly influenced by RFF were identified using the same statistical approach as for microbiome data. Associations among microbiome, metabolome, and skin physiological parameters were assessed using the envfit function in vegan. All correlations were calculated using Spearman's method via the correlate function in ggcor.

2.6 Statistical Analysis

Statistical analyses were performed using R software version 4.3.1 (R Foundation for Statistical Computing, Vienna, Austria). A significance threshold of $P < 0.05$ was considered statistically significant.

3. Results

3.1 RFF-Containing Toner Significantly Enhances Skin Hydration and Elasticity

The overall study design is shown in Figure 1A, and no significant difference in age was observed between the two groups (Figure 1B). Significant improvements from baseline in skin hydration, TEWL, elasticity, wrinkle index, and smoothness were observed in both the RFF and non-RFF groups, indicating that both formulations enhanced skin condition (Figure 1C). Self-assessment results were consistent with instrumental findings (Figure 1D). Notably, compared to the non-RFF group, the RFF group showed significantly greater improvements in skin hydration, TEWL, total elasticity, and firmness, suggesting superior efficacy of the RFF-containing toner in enhancing skin hydration and elasticity (Figure 2A, B). Correlation analysis showed no significant association between age and changes in objective indicators in either group, supporting the suitability of both toners across a wide age range (Figure 2C). In contrast, subjective improvements in the non-RFF group correlated positively with age, suggesting greater perceived benefits of the RFF-containing toner among older users (Figure 2D).

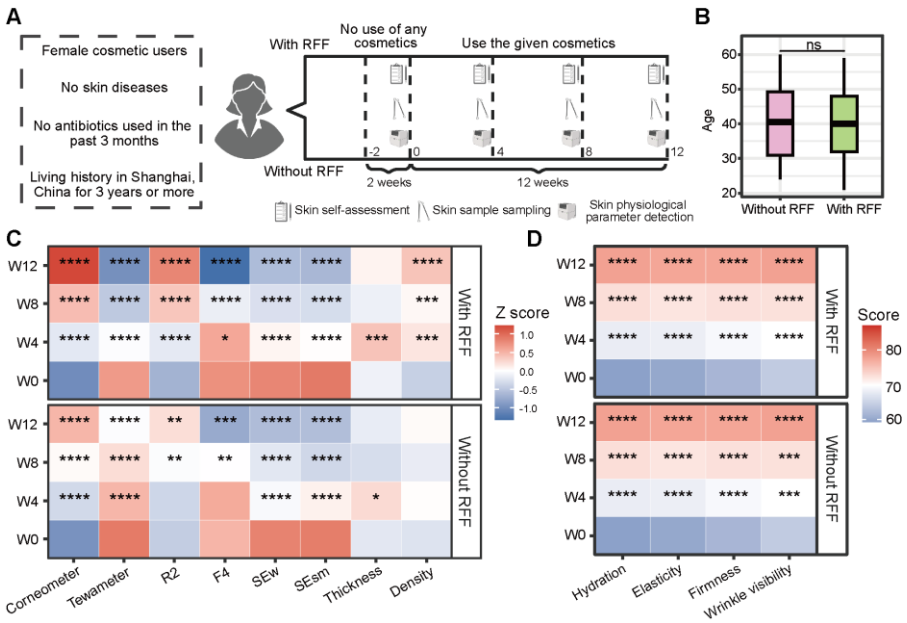

Figure 1. Effects of lotion on the condition of the skin. (A) Schematic diagram of the intervention study. (B) Age comparison between the Without RFF group and the With RFF group.

Changes in skin physiological parameters (C) and Skin questionnaire (D) at three time points relative to baseline (W0) in both groups. ns p>0.05, * p<0.05, ** p<0.01, *** p<0.001, **** p<0.0001.

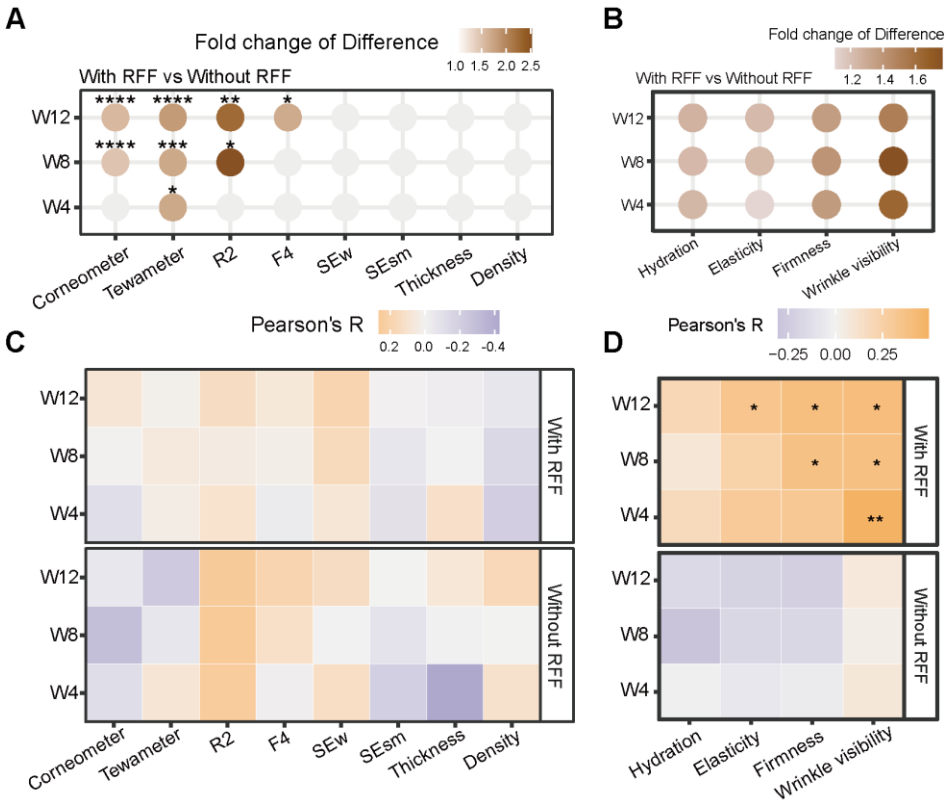

Figure 2. The difference in the effect on the skin of lotions with and without RFF. Differences in the degree of improvement of skin physiological parameters (A) and skin questionnaires (B) between the two groups. Correlation analyses between age and the degree of improvement of skin physiological parameters (C) and skin questionnaires (D) in both groups. * p<0.05, ** p<0.01, *** p<0.001, **** p<0.0001.

3.2 Selective enrichment of M. luteus by RFF-containing skincare products

At baseline, the skin microbiome showed no significant differences in alpha or beta diversity between the RFF and non-RFF groups (Figure 3A, B). After the intervention, both groups exhibited significant changes in alpha and beta diversity, with similar extents of change between groups (Figure 3C,D). Microbial community structure was significantly or marginally associated with hydration, firmness, and density (Figure 3E), indicating a link between microbiome alterations and improvements in skin physiological parameters. Further analysis identified 21 microbial species whose abundance changed significantly in either group, including *Tepidiphilus succinatimandens*, *Staphylococcus epidermidis*, *Cutibacterium acnes*, and *M. luteus*, all of which significantly increased in abundance (Figure 4A). Comparison of abundance changes (W12–W0) between groups revealed that only *M. luteus* showed a significant difference, increasing exclusively in the RFF group (Figure 4A, B). This increase was significantly associated with transepidermal water loss and showed trends towards correlations with skin hydration, elasticity, and texture (Figure 4C).

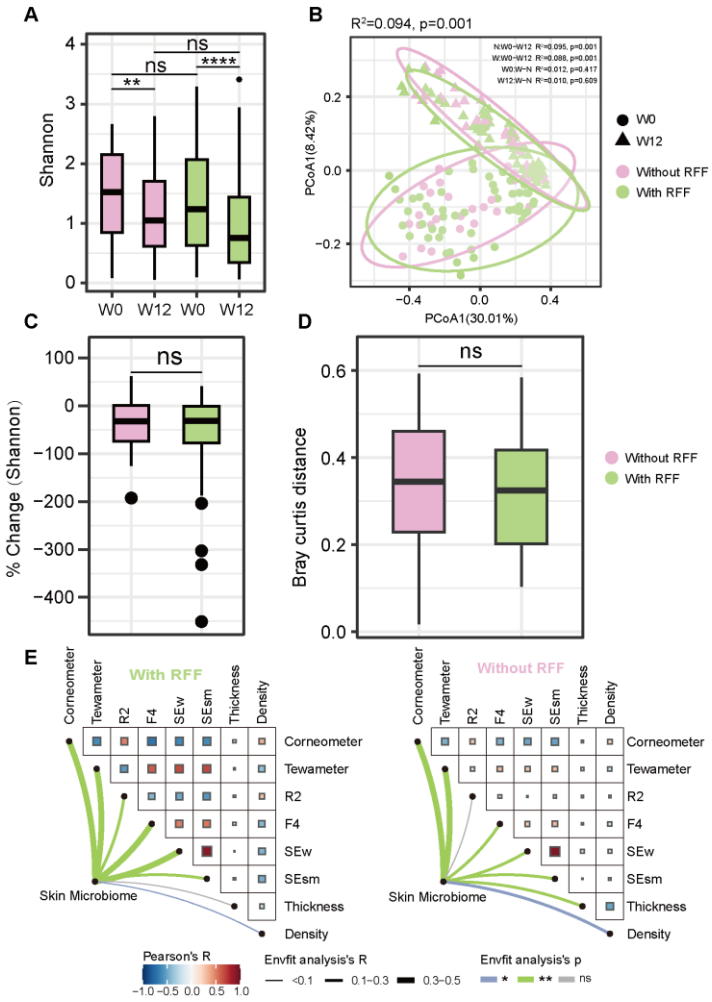

Figure 3. Skincare products containing RFF modulate the skin microbiota. (A) Alpha diversity of the skin microbiota before and after intervention. (B) Changes in beta diversity before and after intervention. (C) Percentage change in alpha diversity in the With RFF and Without RFF groups. (D) Microbiota similarity between the With RFF and Without RFF groups. (E) Correlation between skin microbiota and skin physiological parameters. ns p>0.05, * p<0.05, ** p<0.01, *** p<0.001, **** p<0.0001.

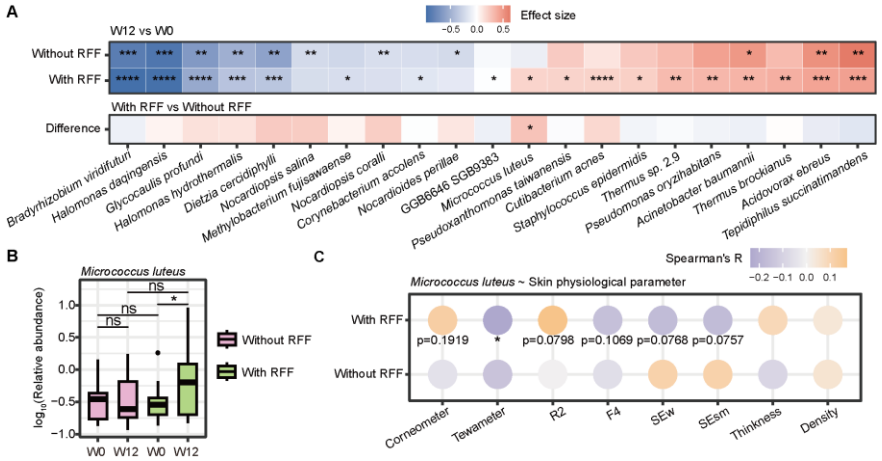

Figure 4. Skincare products containing RFF specifically increase the abundance of M. luteus. (A) Microbial species showing significant changes following intervention. (C) Changes in the abundance of *M. luteus*. (C) Correlation between *M. luteus* and skin physiological parameters. ns p>0.05, * p<0.05, ** p<0.01, *** p<0.001, **** p<0.0001.

3.3 RFF-containing skincare products alter the skin metabolome by increasing uracil and reducing isocitric acid

As shown in Figure 5A, metabolomic profiles were distinctly separated between W0 and W12, whereas no clear separation was observed between the With RFF and Without RFF groups, consistent with the microbiome findings.The skin metabolome was significantly correlated with hydration, TEWL, elasticity, and surface texture (Figure 5B), indicating its close association with skin physiological improvements. Among 173 identified metabolites, 164 showed significant changes after the intervention in both groups. Comparative analysis of post-intervention changes (vs. W0) revealed 27 metabolites with significantly different alterations between groups (Figure 5C), among which Capric acid, Uracil, Betaine, and Isocitric acid differed at least at two time points. In the With RFF group, Uracil and Isocitric acid were significantly correlated with multiple skin parameters, whereas such correlations were absent in the Without RFF group (Figure 5D). Notably, *M. luteus* showed a trend of association with both metabolites(Figure 5E). These findings suggest that while both toners modulated the skin metabolome, the RFF-containing formulation induced a greater increase in Uracil and a more pronounced decrease in Isocitric acid.

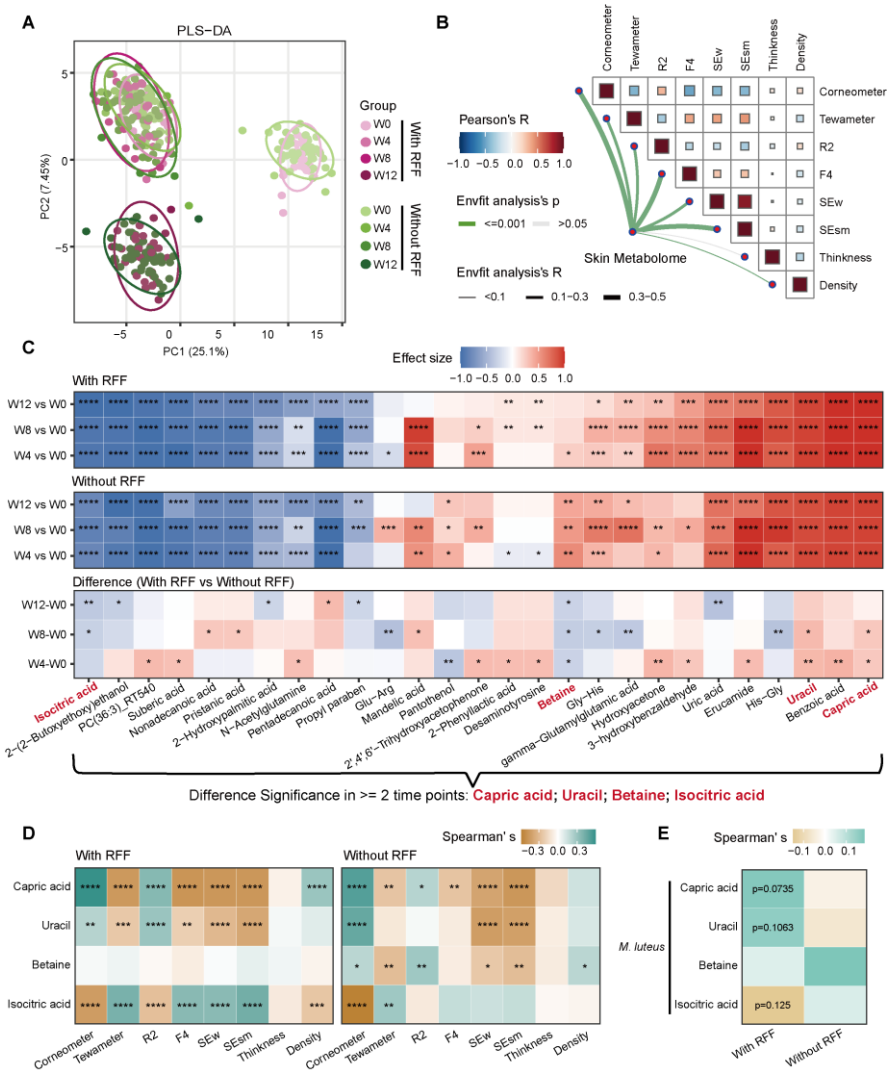

Figure 5. Metabolomic changes associated with RFF-containing skincare. (A) Partial least squares discriminant analysis (PLS-DA) of skin metabolites across four time points. (B) Corre-

lation between skin metabolome and physiological parameters. (C) Metabolites showing significant pre-post intervention changes between the With RFF and Without RFF groups. Top and middle panels: metabolite changes relative to baseline (W0); red indicates an increase, blue a decrease. Bottom panel: between-group comparisons of metabolite deltas; red indicates higher values in the With RFF group, blue in the Without RFF group. (D) Correlations of four representative metabolites with skin physiological parameters. (E) Associations between these metabolites and *M. luteus.* * p<0.05, ** p<0.01, *** p<0.001, **** p<0.0001.

3.4 RFF-Containing Toner Improves Skin Parameters Consistently Across Skin Types

To assess whether baseline skin microbiota composition influences skincare efficacy, we classified skin types based on a microbiome enterotype-like approach. All 162 samples were grouped into two distinct types. LEfSe analysis revealed that Skin Type 1 was enriched with genera such as Nocardiopsis, Halomonas, and Moraxella, while Skin Type 2 was dominated by Cutibacterium (Figure 6A, B). The distribution of skin types at baseline was comparable between the RFF and non-RFF groups, and post-intervention shifts in skin type occurred at similar rates in both groups (Figure 6C). We then evaluated treatment efficacy across skin types. In both groups, individuals with either skin type showed significant improvements in hydration, transepidermal water loss, elasticity, and surface texture following the intervention (Figure 6D). No significant differences were observed between skin types in the magnitude of improvement (Figure 6E), suggesting that RFF-containing toner exerts consistent skin benefits across diverse microbiome profiles.

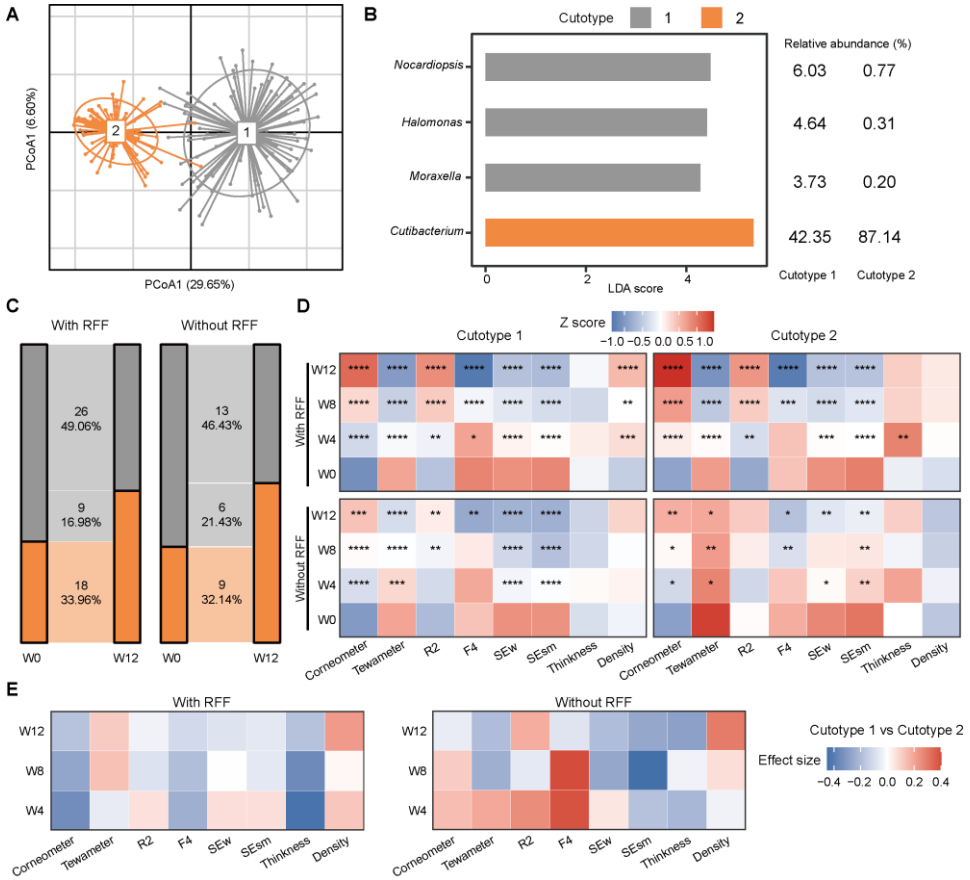

Figure 6. Lotion containing RFF improves skin conditions consistently across different skin types. (A) Skin type classification. (B) Bacterial genera showing significant differences between

Skin Type 1 and Skin Type 2, with LDA scores greater than 4. (C) Changes in skin types and their proportions before and after lotion intervention. (D) Changes in skin physiological parameters after intervention compared to W0 in Skin Type 1 and Skin Type 2; red indicates an increase, blue indicates a decrease. (E) Comparison of changes in skin physiological parameters before and after intervention between Skin Type 1 and Skin Type 2; red indicates higher changes in Skin Type 1, blue indicates higher changes in Skin Type 2. No significant differences were observed. * $p<0.05$, ** $p<0.01$, *** $p<0.001$, **** $p<0.0001$.

4. Discussion

Skin aging has been a major focus of cosmetic and dermatological research, as the skin serves as a visible indicator of aging and plays a key role in social interactions. With growing consumer interest, the global market for natural and organic skincare products was valued at USD 13.2 billion in 2018 and continues to expand rapidly [11]. Current anti-aging strategies include UV protection, topical agents, and injectable fillers [12]. Rice Ferment Filtrate (RFF), a fermentation-derived ingredient rich in amino acids, polysaccharides, and minerals, is widely recognized for its moisturizing and skin-repairing properties [13]. Formulations containing fermentation filtrates have been shown to improve hydration, reduce wrinkles, and enhance periorbital skin elasticity [7]. In this study, RFF-containing skincare products significantly improved facial skin parameters, particularly hydration and elasticity, after 12 weeks of use compared to formulations without RFF.

Advancing age is strongly associated with the progression of skin aging, primarily due to the age-related decline in collagen synthesis and accumulation, which compromises dermal structure and accelerates wrinkle formation [14]. Compared with young dermal collagen, collagen fibrils in aged skin are rougher and more rigid, with physical properties that change progressively over time [15]. Interestingly, some studies also suggest that aged skin displays better barrier repair and hydration capacity than younger skin [16].The effects of skincare products may thus be influenced by age. We assessed the correlation between changes in physiological skin parameters and age before and after product use, and found no significant correlations. This suggests that the efficacy of the skincare product is not influenced by age—at least within the studied age range of 21 to 60 years.

Our study demonstrated that topical application of skincare products significantly modulated the skin microbiome, altering both alpha and beta diversity, consistent with previous finding [17]. Notably, RFF selectively increased the abundance of *Micrococcus*, particularly *M. luteus.* Extracellular vesicles (EVs) derived from *M. luteus* have been reported to upregulate hsa-miR-4517 expression in airway epithelial cells, thereby suppressing IL-1β production, inhibiting ILC3 activation, and reducing neutrophil recruitment, ultimately alleviating asthma symptoms [18]. In dermatological studies, M. luteus Q24 improved key skin parameters and modulated the skin microbiome in healthy adults [19], while the culture supernatant of *M. luteus YM-4* upregulated genes associated with skin hydration, hyaluronic acid synthesis, barrier function, and cell proliferation [20]. Metabolomic analysis revealed elevated uracil and reduced isocitrate levels in the RFF-treated group. Uracil has been reported to inhibit type I procollagen degradation and MMP expression, thereby mitigating collagen disorganization and oxidative stress, while also promoting DNA synthesis and skin cell regeneration [21]. These results suggest that the skin benefits of RFF may be partly mediated through modulation of key metabolites, such as uracil.

5. Conclusion

In conclusion, the results of this study demonstrate that the RFF skincare product significantly outperforms products without RFF in improving skin hydration and elasticity, and is effective across a broad age range from 20 to 60 years old. RFF exerts its beneficial effects by modulating the skin microbiome—particularly by increasing the abundance of *M. luteus*—and by altering associated metabolite levels, such as elevating uracil and reducing isocitrate. These findings further support the potential of *M. luteus* in anti-aging skincare and provide scientific evidence for the application of RFF as a novel anti-aging skincare ingredient.

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