# Bifidobacterium/Lactobacillus/Soybean Seed Extract Ferment Filtrate: A Novel, Efficient, and Gentle Bio-Penetration Enhancer

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## 1. Introduction

The efficient transdermal delivery of active cosmetic ingredients is critical for formulation efficacy, yet the skin's stratum corneum presents a natural barrier that limits compound penetration [1]. Traditional chemical permeation enhancers, such as ethanol and salicylic acid, improve absorption by disrupting stratum corneum structure; however, their clinical utility is constrained by dose-dependent irritation, barrier damage, and incompatibility with sensitive skin [2,3]. Consequently, developing safe and effective bio-based permeation systems has emerged as a pivotal challenge in cosmetic science.

Recent advancements have highlighted the potential of microbial co-fermentation products in permeation enhancement. Co-fermentation of probiotics (e.g., Lactobacillus, Bifidobacterium) with plant-derived substrates (e.g., soybean) yields bioactive metabolites, including organic acids and amino acids, which modulate stratum corneum properties without compromising barrier integrity [4]. For example, lactic acid—an iconic byproduct of lactic acid bacteria fermentation—exhibits AHA-like keratolytic effects but with superior skin tolerance, positioning it as a gentle alternative for sensitive formulations [5]. Despite these insights, the systematic evaluation of multi-strain ferment filtrates as "multifunctional permeation enhancers"—specifically their roles in balancing "stratum corneum regulation, permeation enhancement, and safety"—remains underdeveloped, necessitating rigorous scientific validation [6].

This study investigates a novel bioactive ferment filtrate: Bifidobacterium/Lactobacillus/Soybean Seed Extract Ferment Filtrate, enriched with organic acids (e.g., lactic acid) and amino acids via probiotic-plant co-fermentation. We hypothesize that this filtrate enhances transdermal delivery through a "non-destructive" mechanism, whereby natural metabolites gently optimize stratum corneum structure while preserving epidermal barrier function. The research addresses three interrelated objectives:

Mechanistic Characterization: Evaluate the keratolytic and barrier-preserving properties of the ferment filtrate using an ex vivo porcine skin model, contrasting its gentle action with traditional chemical enhancers.

Broad-Spectrum Permeation Efficacy: Validate its ability to enhance penetration of hydrophilic, lipophilic, and macromolecular active ingredients via Franz-cell diffusion and Raman spectroscopy, dissecting underlying transport mechanisms.

Clinical Translation: Assess the safety and anti-aging efficacy of formulations containing the ferment filtrate through human trials, focusing on skin tolerance and objective improvements in wrinkle parameters.

The ferment filtrate's core innovation lies in its "ternary mechanism" of stratum corneum regulation–permeation enhancement–barrier protection, achieved through synergistic

metabolites at low concentrations (0.32%–0.45%). By overcoming the efficacy-safety tradeoff of conventional enhancers, this bio-based system aligns with industry trends toward "gentle skincare" and "green chemistry," offering promising solutions for anti-aging and barrier-repair applications [7].

## 2. Materials and Methods

### 2.1 Experimental Materials

Bifidobacterium/Lactobacillus/Soybean Seed Extract Ferment Filtrate (registered by Guangzhou Yatsen Global Co., Ltd.,China, with the record number of National Cosmetic Ingredient Record No. 20230032.). Salicylic acid solution (provided by SHAANXI BIOCELL GENERAL TESTING Co., Ltd, China). Chemical penetration enhancer(BIS-ETHOXYDIGLYCOL CYCLOHEXANE 1,4-DICARBOXYLATE, purchased from NIPPON FINE CHEMICAL CO., LTD.). Hydroxypropyl Tetrahydropyrantriol (Pro-Xylane™，purchased from Tianjin Taipu Medicine Technology Development Co., Ltd., China). Pomegranate Flower Extract and Peptide Blend (consisting of Punica granatum flower extract and palmitoyl tripeptide-1, self - prepared in the laboratory of Guangzhou Jiyan Cosmetic Technology Co., Ltd., China),. Type III and XVII Collagen Complexes (self-prepared in the laboratory of Guangzhou Jiyan Cosmetic Technology Co., Ltd., China). Cream products (self-prepared in the laboratory of Guangzhou Jiyan Cosmetic Technology Co., Ltd., China). Porcine Skin-Franz Cell System model, Ex Vivo porcine skin Raman spectroscopy detection model (provided by SHAANXI BIOCELL GENERAL TESTING Co., Ltd, China).

### 2.2 Experimental Methods

### 2.2.1 Ex Vivo Keratolytic Activity Evaluation

Porcine skin preparation: Thawed porcine skin was cleaned and secured between the donor and receptor chambers of a vertical Franz diffusion cell with the stratum corneum facing the donor chamber and the dermis in contact with the receptor fluid. The receptor chamber was filled with 7.0 mL of PBS (receptor solution), and 1.5 mL of PBS was added to displace air bubbles, ensuring complete adhesion between the dermis and receptor fluid. Sample application: Test samples (50 µL), including PBS buffer (control group), 0.45% bioactive fermented filtrate, and 10% salicylic acid (SA, experimental groups), were applied in a radial pattern from the center to the edge onto the skin surface (exposed area: 3.14 cm²). Each group had 3 replicates (n=3), incubated in a 32±1°C water bath. Keratinocyte removal and collection: After 30 minutes of incubation, the treated skin surface was gently rubbed with gloved fingertips for 2 minutes to loosen shed keratinocytes. Subsequently, 0.5 mL of 0.1% Triton X-100 solution was added to the donor chamber to rinse off detached cells, and the rinsate containing shed keratinocytes was transferred to a 1.5 mL centrifuge tube. The tube was centrifuged at 15,000 rpm for 10 minutes, the supernatant was discarded, and the pellet was resuspended in 0.5 mL deionized water for subsequent analysis. Total protein quantification: To the resuspended cell pellet, 25 µL of 12 M NaOH was added, and the mixture was boiled in a water bath for 30 minutes to lyse keratinocytes. After cooling, 25 µL of 12 M HCl was added to neutralize the lysate. Total protein content was measured using a BCA protein assay kit according to the manufacturer's instructions. The total protein content in the lysate was proportional to the number of shed keratinocytes—higher protein levels indicated more exfoliated keratinocytes, reflecting stronger keratolytic efficacy of the sample.

### 2.2.2 Transdermal Permeation Study via Porcine Skin-Franz Cell System

The transdermal permeability of samples was evaluated using a porcine skin-Franz cell system. Porcine skin stored at -20°C was first thawed at room temperature, rinsed with PBS buffer, and fixed in the Franz diffusion cell with the stratum corneum facing the donor compartment and the dermal side in contact with the receptor solution. The receptor chamber was filled with 7 mL of PBS receiving solution, and an additional 1 mL was added to eliminate air bubbles, ensuring complete contact between the dermis and the solution. Test

formulations (50 μL) were applied radially from the center to the edge of the skin surface (exposed area: 3.14 cm²), with three replicates per group (n=3). The receptor chamber was maintained at 32 ± 1°C via a circulating water jacket, and a magnetic stirrer was used at 300 rpm to ensure uniform mixing of the solution.

At 1, 2, 4, 8, and 24 hours post-application, 2 mL of receptor fluid was withdrawn from the sampling port and replaced with an equal volume of fresh PBS. Collected samples were stored in 2 mL centrifuge tubes for high-performance liquid chromatography (HPLC) analysis. After 24 hours, the skin surface was rinsed five times with 1 mL acetonitrile, and the   (wash solution) was collected for analysis. The skin within the donor chamber was excised, minced, and sonicated in 5 mL acetonitrile; the supernatant was collected for further processing. Receptor fluid samples from all time points were combined for quantitative analysis.

Data Analysis

Cumulative permeation amount (Q): Calculated by integrating the concentration of permeated samples in the receptor fluid over time, considering the volume of the receptor chamber (V = 7 mL) and the replacement volume ($V_0$ = 2 mL) at each sampling point.

Diffusion percentage (P): Defined as $P=Q/P_0\times100\%$, where $P_0$ represents the total amount of sample applied to the donor chamber.

Permeation rate (Kp ): Determined by linear regression of cumulative permeation amount (Y-axis) against time (X-axis), with the slope of the regression equation representing Kp (unit: $\mu g\cdot cm^{-2}\cdot h^{-1}$).

### 2.2.3 Ex Vivo Porcine Skin Confocal Raman Spectroscopy Analysis

Ex vivo porcine skin stored at -20℃ was cut into 3.5 cm×3.5 cm, rinsed with PBS buffer for 15 s and dried. It was stretched and fixed between the donor and receptor chambers of the Franz diffusion cell. 8.0 mL PBS buffer was added to the receptor chamber, air removed by tapping to ensure full dermal - solution contact. A 30 - μL sample was radially distributed from the skin center, then the donor chamber was sealed. The diffusion cell was placed in a TK - 12D transdermal diffuser, stirred at 300 rpm, maintained in a (32±1)℃ water bath with no bubbles.

Post - experiment, 1 mL PBS was pipetted to wash the skin surface 5 times. The skin center was trimmed to 1 cm×1 cm, dried, placed in an EP tube for frozen sectioning (16 - μm thickness), then mounted on a quartz gold - plated slide. Before detection, a silicon wafer calibrated the confocal Raman spectrometer. The slide was on the sample stage; under a microscope, a site with no dermal separation, no damage, and clear structure was selected for single - spectrum measurement. A rectangular scan range centered on a point with prominent characteristic peak and low noise obtained the Raman dataset. Witec software performed spectral selection, cosmic - ray removal, polynomial baseline fitting, and Savitzky - Golay smoothing. Univariate analysis extracted component info, and pseudo - color images were reconstructed based on characteristic - peak intensity (intensity - color correlation). Finally, Origin software analyzed single - spectrum data, drew multi - point Raman spectra of the full skin layer and large - range pseudo - color maps, completing semi - quantitative analysis

### 2.2.4 HET-CAM Assay for Irritation Evaluation

In the experiment, Specific Pathogen Free (SPF) - grade White Leghorn chicken embryos were used. After being incubated until 9 days old, the eggshell was removed to expose the white egg membrane, and the inner membrane was eliminated to ensure the vascular membrane remained intact. The chicken embryo was fixed in the HET - CAM device. Following marking the air - chamber position, part of the eggshell was stripped off.

Under controlled culture conditions, 0.3 mL of the test sample was directly dropped onto the surface of the chicken embryo chorioallantoic membrane (CAM). Reactions of CAM—including hemorrhage, coagulation, and vascular lysis—were observed and recorded, noting both the time of occurrence and degree. The Irritation Score (IS) method was

employed to calculate the irritation score, predicting the sample's classification and evaluating its ocular irritation accordingly.

Post - experiment, the sample was rinsed away with normal saline, and CAM reactions were observed within approximately 30 seconds after rinsing. Through these procedures, the safety and potential irritation of the sample were assessed.

### 2.2.5 Clinical Efficacy and Safety Assessment

Thirty-three healthy female volunteers aged 35–57 years were recruited, excluding those who were pregnant/lactating, had a history of skin inflammation, or had undergone medical aesthetic treatments in the past 3 months, and they applied a test sample containing 0.45% bioactive ferment filtrate twice daily (morning and evening) to the face and neck for 28 consecutive days while avoiding other functional skincare products. Facial images were captured at baseline (D0) and after 28 days (D28) using the Canfield PRIMOS-CR 3D imaging system in a controlled environment (21±1°C, 50±10% humidity), with the system software analyzing changes in parameters such as the number, length, volume, and depth of under-eye wrinkles to quantify anti-aging effects. Safety evaluation involved dermatologists visually inspecting subjects at D0 and D28 for adverse reactions like erythema and papules, recording their location, severity, and association with the sample, while subjects logged subjective discomfort in daily records. Statistical analysis included descriptive statistics (mean, standard deviation, change rate) for skin parameters such as wrinkle data, followed by normality testing: paired t-tests were used for normally distributed data to compare within-group differences, non-normally distributed data were analyzed via Wilcoxon rank-sum tests, and subject self-assessment satisfaction results were tested for significance using binomial analysis, with a p-value < 0.05 considered statistically significant. This study evaluates the practical effects of the sample on improving skin wrinkles and safety through standardized procedures, providing clinical evidence for its application in anti-aging skincare products.

## 3. Results

### 3.1 Keratinolytic Activity Evaluation

The keratolytic efficacy of the bioactive ferment filtrate was evaluated via ex vivo porcine skin assay, quantifying exfoliated keratinocytes through total protein content analysis. As summarized in Table 1 and Figure 1a, the blank control group (BC, PBS) exhibited a baseline protein concentration of 0.305±0.004mg/mL. The positive control group (10% salicylic acid, SA) showed a significant increase to 0.496±0.003mg/mL(62.6% enhancement vs. BC, $p < 0.01$) confirming the keratolytic activity of traditional chemical acids. Notably, the 0.45% ferment filtrate group achieved a protein concentration of 0.721±0.006mg/mL, representing a 136.4% increase over BC ($p < 0.01$) and surpassing the SA group by 45.4%. These results demonstrate that the multi-strain ferment filtrate promotes keratinocyte exfoliation at low concentrations more effectively than single-component chemical enhancers, aligning with its "gentle yet efficient" action profile.

**Table 1.** Total Protein Content of Detached Keratinocytes

| Group | Mean(mg/mL) | Standard Deviation (SD) | P-value | Enhancement rate %, vs BC |
|---|---|---|---|---|
| BC | 0.305 | 0.004 | / | / |
| A-1: 10% Salicylic Acid | 0.496 | 0.003 | P<0.01,** | 62.62 |
| A-2: Bioactive Ferment Filtrate | 0.721 | 0.006 | P<0.01,** | 136.39 |

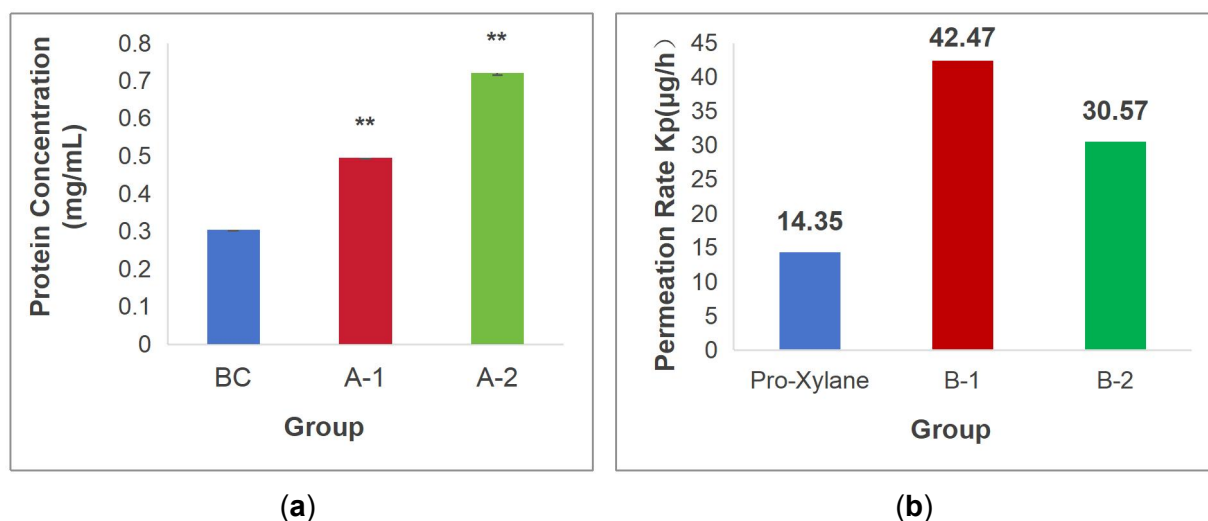(**a**)                                                            (**b**)

**Figure 1.** Panel a shows the detection results of total protein content in detached corneocytes. Panel b shows the results of Pro-Xylane permeation rate.

### 3.2 Permeation Enhancement of Diverse Active Ingredients

This study employed an ex - vivo porcine skin model in combination with Franz - cell diffusion experiments and confocal Raman spectroscopy imaging technology. Quantitative analyses were respectively performed on hydrophilic components (Hydroxypropyl Tetrahydropyrantriol), lipophilic complexes (pomegranate flower extract and peptide blend), and macromolecular collagens (Composition of Collagen Type III and Collagen Type XVII).

### 3.2.1 Hydrophilic Component Permeation Efficiency Significantly Enhanced

Using Hydroxypropyl Tetrahydropyrantriol (Pro-Xylane™, a representative hydrophilic ingredient) as the target analyte, this study compared the permeation kinetics of a 10.5% Pro-Xylane™ solution with and without 0.32% fermented filtrate at multiple time-points (1, 2, 4, 8, 24 hours), quantifying permeation flux (Q, μg) and apparent permeation rate (Kp, μg/h) via regression analysis of cumulative permeation data. As shown in Table 2 and Figure 1-b, the control group (single Pro-Xylane™) exhibited progressive permeation over 24 hours, with a cumulative permeation amount of 376.08 μg and a Kp of 14.35 μg/h. In contrast, the experimental group containing Pro-Xylane™ and 0.32% fermented filtrate displayed significantly accelerated permeation at all time-points: the 24-hour cumulative permeation amount increased to 1025.78 μg, with a Kp of 42.47 μg/h—2.96-fold that of the control group, representing a 196% increase. The chemical penetration enhancer (bis-diethoxydiglycol cyclohexane 1,4-dicarboxylate) achieved a permeation rate 2.13-fold that of the control group, with a 113% increase.

### 3.2.2 Lipophilic Complex Permeation Depth and Distribution

For the lipophilic pomegranate flower extract/peptide blend, confocal Raman spectroscopy revealed significant improvements in permeation depth and skin retention. After 4 hours of treatment with 0.45% ferment filtrate, the total integrated Raman intensity (1039 $cm^{-1}$, characteristic of lipophilic components) in the epidermis and dermis increased by 38.5% compared to the control group ($p < 0.01$, Figure 2a-b). Pseudocolor imaging showed that the lipophilic complex not only penetrated deeper into the basal layer but also exhibited more uniform distribution in the dermal-epidermal junction, whereas the control group remained primarily in the upper stratum corneum. This indicates that the ferment filtrate modulates lipid bilayers to facilitate lipophilic compound transport, overcoming the superficial retention limitation of traditional systems.

**Table 2.** Permeation Kinetics of Pro-Xylane™ with/without Ferment Filtrate

| Group | Q(μg) | | | | | Permeation Rate | |
|---|---|---|---|---|---|---|---|
| | 1h | 2h | 4h | 8h | 24h | Regression Equation | Kp(μg/h） |
| 10.5% Pro-Xylane | 14.84 | 67.14 | 134.8 | 143.11 | 376.08 | y=14.346x+35.298 | 14.35 |
| B-1：10.5%Pro-Xylane and 0.32%Bioactive Ferment Filtrate | 36.07 | 97.65 | 188.14 | 306.31 | 1025.78 | y=42.467x-0.4552 | 42.47 |
| B-2：10.5%Pro-Xylane and 0.32%Chemical penetration enhancer | 26.23 | 66.57 | 118.52 | 189.36 | 733.64 | y=30.572x-11.594 | 30.57 |



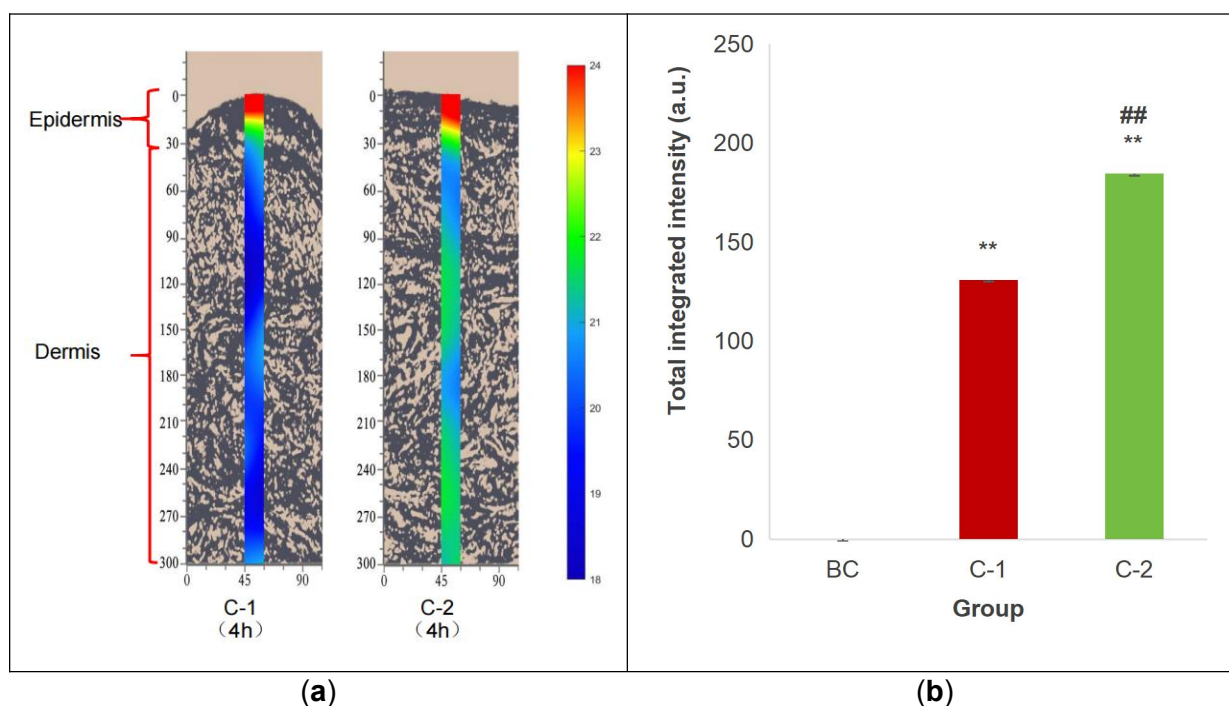(**a**)                                        (**b**)

**Figure 2.** Panel a shows the reconstructed Raman pseudocolor map (1039 cm⁻¹) after large-area scanning. Panel b shows the total integrated intensity plot (1039 cm⁻¹). Here, C-1 represents the 2.67% pomegranate flower extract and peptide blend group, and C-2 represents the group containing 2.67% pomegranate flower extract and peptide blend combined with 0.45% Bioactive Ferment Filtrate.

### 3.2.3 Macromolecular Collagen Transdermal Delivery

The ferment filtrate demonstrated exceptional efficacy in enhancing the penetration of large molecular weight components, such as Type III/XVII collagen complexes. Raman spectroscopy at 1397 cm$^{-1}$ (collagen-specific peak) showed a 63.6% increase in dermal signal intensity after 4 hours of treatment with 0.45% ferment filtrate ($p < 0.01$ Figure 3a-b). Unlike chemical enhancers that disrupt tight junctions, the ferment filtrate likely widens intercorneocyte gaps via amino acid-ceramide interactions, creating temporary channels for macromolecules without compromising barrier integrity. This non-destructive mechanism represents a breakthrough in delivering collagen-based actives, which are typically restricted by their molecular size.
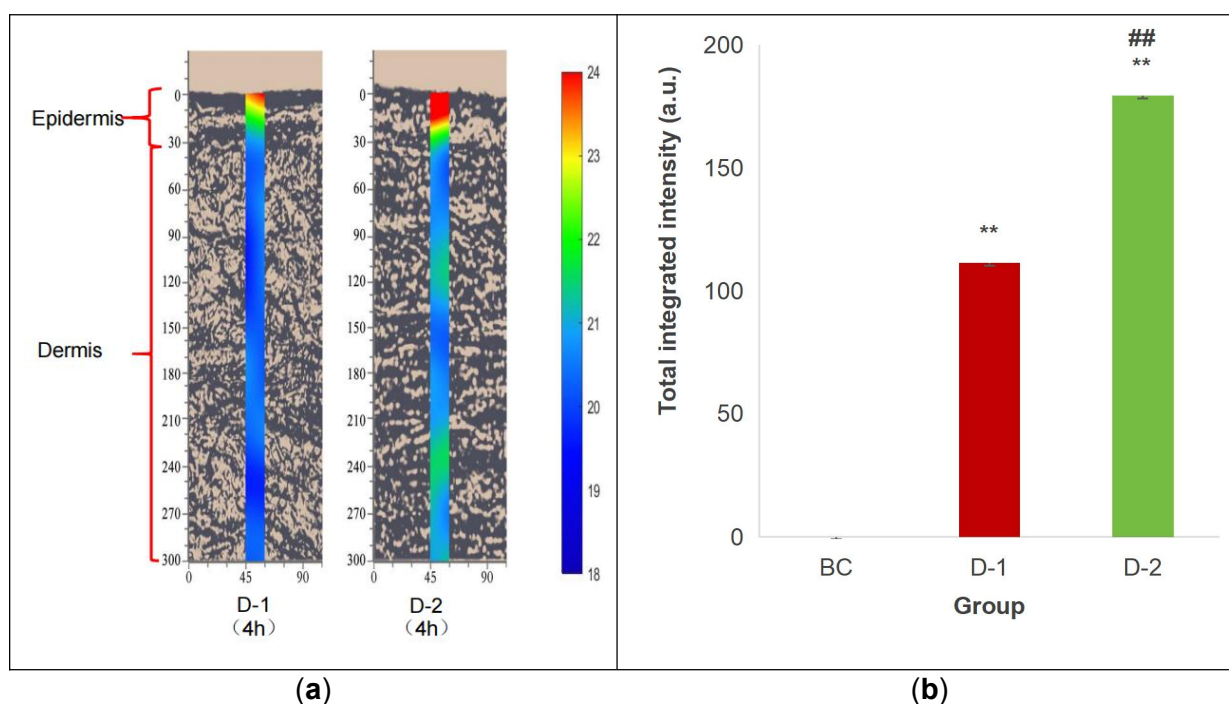


(a)                                                                 (b)

**Figure 3:** Panel a shows the reconstructed Raman pseudocolor image after large - area scanning (1397 cm$^{-1}$). Panel b shows the total integrated intensity result graph (1397 cm$^{-1}$). Here, D-1 represents the 0.022% Type III and Type XVII collagen group, and D-2 represents the group containing 0.022% Type III and Type XVII collagen and 0.45% Bioactive Ferment Filtrate.

### 3.3 Safety Evaluation via HET-CAM Assay

According to the criteria specified in SN/T 2329-2009, the safety of the sample was verified via the Hen's Egg Chorioallantoic Membrane (HET-CAM) assay. Results showed that the irritation score (IS) of the 0.45% fermented filtrate group was 0.00, classifying it as non-irritating. In contrast, salicylic acid at a 1% concentration exhibited a high IS score of 12.68, indicating strong irritation/corrosiveness.

### 3.4 Clinical Efficacy of Anti-Aging Formulation

The changes in the number and depth of skin wrinkles before and 28 days after using the cream sample containing 0.45% Bioactive Ferment Filtrate were evaluated. As shown in Table 3, among them, the change rate of the number of skin wrinkles before using the sample even reached - 33.33%, with a p - value < 0.001. Statistical analysis showed a significant difference, indicating a significant reduction in the number of skin wrinkles. The safety evaluation results showed that the safety was normal throughout the study, and no sample - related adverse events occurred.

**Table 3** Clinical Anti-Aging Efficacy Results (n=33)

| Evaluation Parameter | Evaluated Sample | | | |
|---|---|---|---|---|
| | Before Use | After Use | Change Rate | P - value |
| Skin Wrinkle Depth | 51 | 47 | -7.84% | 0.013 |
| Skin Wrinkle Number | 63 | 42 | -33.33% | <0.001 |
| Skin Wrinkle Mention | 1.82 | 1.54 | -15.38% | <0.001 |
| Skin Wrinkle Area | 35 | 29.02 | -17.09% | <0.001 |
| Skin Wrinkle Length | 69 | 58 | -15.94 | <0.001 |
| Skin Wrinkle Ra Value | 19.1 | 17.1 | -10.47 | 0.004 |

## 4. Discussion

This study systematically reveals the unique advantages of the fermented filtrate of Bifidobacterium/Lactobacillus/soybean seed extract (hereinafter referred to as "Bioactive Ferment Filtrate") as a bio-penetration enhancer. It constructs a ternary mechanism of "stratum corneum regulation - permeation enhancement - barrier protection (safety)" through natural metabolites (such as organic acids and amino acids) produced by multi-strain synergistic fermentation, achieving a balance between efficient transdermal delivery and safety at low concentrations of 0.32% - 0.45%. Metabolomic analysis shows that lactic acid in the fermented filtrate softens the lipids of the stratum corneum through an AHA-like mechanism [8], yet it is free from the irritation of traditional chemical penetration enhancers (irritation score [IS] of 10% salicylic acid is 12.68, while that of the fermented filtrate is 0). Amino acids may temporarily widen the intercellular spaces of keratinocytes by competitively binding to the polar head groups of ceramides [9], creating penetration channels for macromolecules such as Type III/XVII collagen complexes (with a 63.6% increase in dermal signal intensity, $p < 0.01$), and avoiding barrier damage caused by the disruption of tight junction proteins by substances like ethanol.

This "non-destructive penetration enhancement" mechanism endows the fermented filtrate with both high keratin exfoliation efficiency (a 136.4% increase, $p < 0.01$) and the ability to enhance the penetration of multiple types of ingredients. Specifically, it can improve the transdermal efficiency of hydrophilic ingredients (with a 196% increase in the permeability of Pro-Xylane™), lipophilic ingredients (with a 38.5% increase in the penetration of pomegranate flower extract), and macromolecular components (with a 63.6% increase in collagen signal intensity) (Figures 2 - 3). It breaks through the bottleneck of efficacy and safety of chemical penetration enhancers and provides new research evidence and ideas for the systematic evaluation of multi-strain fermented filtrates.

In terms of application value, the low-concentration efficiency of the fermented filtrate (0.45% outperforms the effect of 10% salicylic acid) and its compatibility with natural ingredients are in line with the trends of Clean Beauty and "microecological skincare". It especially provides an innovative approach for anti-aging products for sensitive skin. Its clinical verification in anti-wrinkle creams (with a 33.33% reduction in the number of wrinkles, $p < 0.001$) marks the feasibility of technology transfer. However, this study still has the following limitations: the impact of long-term use on the skin microecology has not been evaluated, and the interaction pathways between amino acids and active ingredients need to be clarified through molecular dynamics or fluorescence labeling techniques. Future research can combine delivery systems such as liposomes to explore and optimize application research in other areas facing transdermal efficiency issues, such as whitening or anti-photoaging.

## 5. Conclusion

This study systematically reveals for the first time the core value of the fermented filtrate of Bifidobacterium/Lactobacillus/soybean seed extract as a bio-penetration enhancer. Through natural metabolites produced by multi-strain synergistic fermentation, it constructs a ternary balance mechanism of "stratum corneum regulation - permeation enhancement - barrier protection", achieving dual breakthroughs in transdermal delivery efficacy and safety at extremely low concentrations. Experiments have confirmed that the fermented filtrate can not only gently optimize the structure of the stratum corneum through organic acids and widen the cell gaps through amino acids, creating non-destructive penetration paths for hydrophilic, lipophilic, and macromolecular active ingredients, but also break through the limitations of traditional chemical penetration enhancers that rely on barrier damage. Clinical verification shows its significant effect on wrinkle improvement and zero irritation.

As a bio-penetration enhancer with the triple functions of "dissolution - penetration enhancement - repair", its low-concentration efficiency and compatibility with natural ingredients provide an innovative paradigm for sensitive skin care and Clean Beauty technologies, demonstrating the feasibility from mechanism innovation to industrial transformation. Future research can focus on the synergy between skin microecology regulation and delivery systems to further expand its application potential in a broader range of efficacy fields.

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