IFSCC 2025 full paper (IFSCC2025-1346)

"Fermented Berry-Derived Short-Chain Fatty Acids for Enhancing Skin Barrier Function and Alleviating Itch: An In Vitro Study"

Soo Ah Jeong [1], Jooho Park [2] Dong Wook Shin [3] and Beong Ou Lim [1,2,*]

[1] Human Bioscience Corp, Chungju, Korea, South; [2] Medicinal Biosciences, Konkuk,University, Chungju, Korea, South ; [3] Research Institute for Biomedical and Health Science, Konkuk University, Chungju, Korea, South

1. Introduction

Skin homeostasis is maintained by a delicate balance of immune, barrier, and microbial functions. Disruption of this balance due to inflammation, oxidative stress, or environmental insults can compromise barrier integrity and result in conditions such as dryness, irritation, and itch. Among the skin's defenses, short-chain fatty acids (SCFAs) have recently gained attention for their dual roles in modulating inflammation and reinforcing skin barrier function through immune signaling pathways and keratinocyte regulation[1,2].

SCFAs such as acetate, propionate, and butyrate are primarily known for their gut-derived origins, produced through microbial fermentation of dietary fibers[3]. However, emerging evidence suggests that topically or systemically delivered SCFAs can exert beneficial effects on skin cells, including anti-inflammatory, antioxidant, and barrier-protective actions. This has led to growing interest in the development of SCFA-enriched functional ingredients, especially those derived from natural, sustainable sources.

Fermented berries offer a promising platform for SCFA production. Rich in polyphenols, anthocyanins, and dietary fibers, berries such as blueberry serve as ideal substrates for microbial fermentation. When fermented with specific probiotic strains such as *Lactobacillus johnsonii*, *Lacticaseibacillus casei*, *Limosilactobacillus fermentum*, *Lactiplantibacillus plantarum*, and *Ligilactobacillus salivarius* they can yield high levels of SCFAs along with enhanced antioxidant capacity[4].

In this study, we developed a fermented blueberry-derived short-chain fatty acid complex (BBS) using *Lactiplantibacillus plantarum* under aerobic conditions. The aim was to evaluate the functional efficacy of this SCFA-rich postbiotic material in skin health applications. Specifically, we investigated its effects on cell viability, antioxidant protein expression, and the regulation of genes associated with skin barrier function and extracellular matrix integrity in HaCaT keratinocytes under oxidative stress. In addition, the SCFA content was quantified via HPLC to confirm its biochemical activity and relevance to the observed biological effects.

2. Materials and Methods

2.1. Preparation of Fermented Berry-Based SCFA Complex (BBS)

Fresh blueberries (*Vaccinium spp.*) were homogenized and subjected to fermentation using a single lactic acid bacterial strain*, Lactiplantibacillus plantarum*, under aerobic conditions. The fermentation was carried out in MRS broth at 37°C for 24 hours with shaking (150 rpm). After fermentation, the mixture was centrifuged at 8,000 ×g for 10 minutes to remove cell debris. The supernatant was collected, filtered, and concentrated using a food dehydrator at 50°C. The final fermented powder, rich in short-chain fatty acids (SCFAs), was designated as BBS (berry-based SCFA complex) and stored at −20°C until further use.

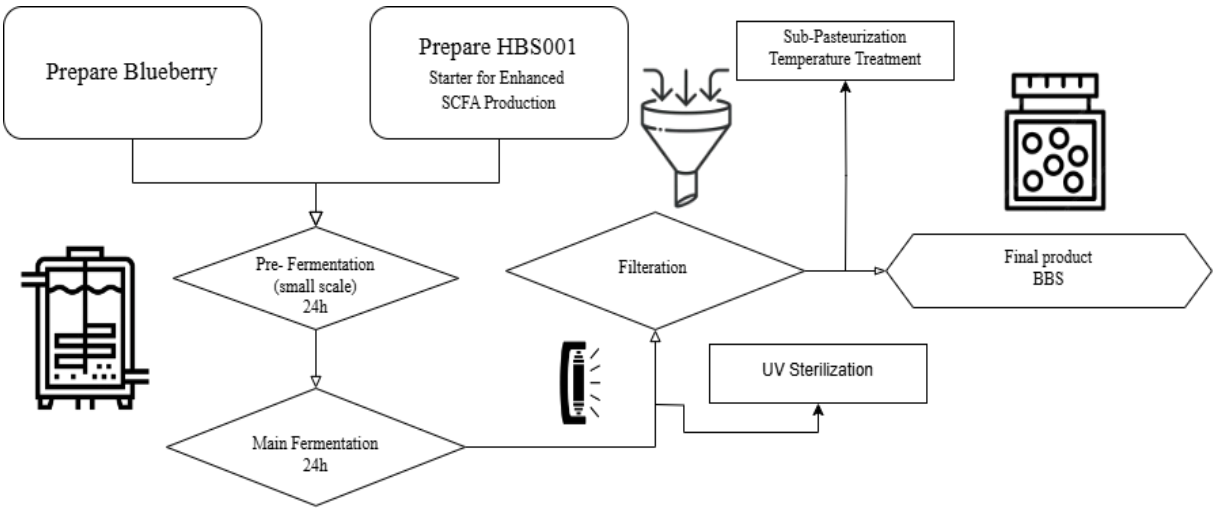

Figure 1. Overview of the Final Manufacturing Process

2. 2. Quantification of Short-Chain Fatty Acids (SCFAs)

The content of propionate in BBS was quantified using high-performance liquid chromatography (HPLC). Analysis was conducted using an Ascentis® Express C18 column (90 Å, 2.7 µm, 10 cm × 4.6 mm I.D., Sigma-Aldrich) with 0.01 N sulfuric acid as the mobile phase at a flow rate of 0.6 mL/min. The detection wavelength was set at 210 nm, and the column temperature was maintained at 40°C.

2.3. Cell Culture and MTT Assay

HaCaT human keratinocytes were maintained in DMEM supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin. Cells were seeded in 96-well plates at a density of 1×10^4 cells/well and incubated overnight at 37°C in a humidified atmosphere with 5% CO_2. BBS extract was applied at concentrations of 12.5, 25, 50, and 100 μg/mL for 24 hours. After treatment, 20 μL of MTT solution (5 mg/mL) was added to each well and incubated for 4 hours. The medium was then replaced with 150 μL of DMSO to solubilize formazan crystals, and absorbance was measured at 570 nm using a microplate reader.

2.4. Western Blot Analysis

To evaluate the protein-level modulation of skin barrier and inflammatory markers, Western blot analysis was performed. HaCaT cells were pretreated with 200 μM hydrogen peroxide (H_2O_2) to induce oxidative stress, followed by treatment with 100 μg/mL of BBS for 24 hours. Total protein was extracted using RIPA buffer containing protease and phosphatase inhibitors, and protein concentration was quantified using a Bradford Assay.
Equal amounts of protein (30 μg) were separated on SDS-PAGE and transferred onto PVDF membranes. Membranes were blocked in 5% BSA in TBS-T and incubated overnight at 4°C with a primary antibody against SOD2. GAPDH was used as the internal loading control. After washing, membranes were incubated with HRP-conjugated secondary antibodies for 1 hour at room temperature. Bands were visualized using enhanced chemiluminescence (ECL) reagents, and densitometry was performed using ImageJ software.

2.5. Quantitative Real-Time PCR (qRT-PCR)

HaCaT cells were treated with BBS (100 μg/mL) for 24 hours under oxidative stress conditions (H_2O_2, 200 μM). Total RNA was extracted using TRIzol reagent, and cDNA was synthesized using a reverse transcription kit. qRT-PCR was performed using SYBR Green Master Mix. Target genes included *FLG* (filaggrin), *COL1A1* (collagen type I alpha 1), and *MMP-9*. GAPDH was used as the reference gene. Relative gene expression was calculated using the ΔΔCt method.

2.6. Statistical Analysis

All experiments were conducted in triplicate. Data are expressed as mean ± standard deviation (SD). Statistical comparisons were made using one-way ANOVA followed by Tukey's post hoc test. Differences were considered statistically significant at $p < 0.05$.

3. Results

1.1. SCFA Composition of BBS

The content of short-chain fatty acids (SCFAs) in the BBS (berry-based SCFA complex) extract was determined using high-performance liquid chromatography (HPLC). Among the SCFAs

analyzed, propionate was the predominant compound, with a clear retention time at 10.847 minutes. Its concentration was quantified as 1.858 ± 0.186 mg/mL, reflecting consistent SCFA production with approximately 10% variability across replicates. These findings confirm that aerobic fermentation of berry extracts using *Lactiplantibacillus plantarum* effectively generates bioactive metabolites, with propionate being a major product of microbial conversion. The presence of this SCFA supports the functional potential of BBS in modulating inflammation and reinforcing skin barrier function.

Table 1. This is a table. Tables should be positioned within the main text, close to their first citation

Sample	Ret.Time	Propionate content
BBS	10.847	1.858 ± 0.186 mg/mL

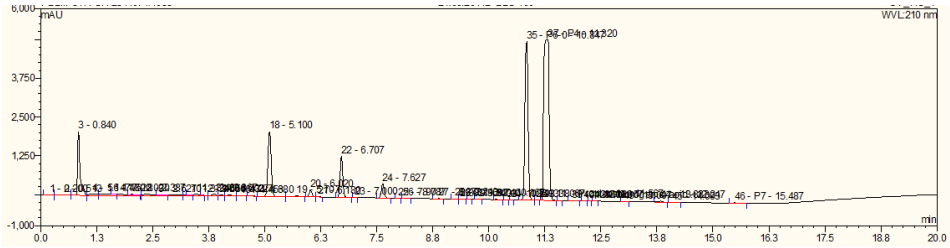

Figure 2. Representative HPLC chromatogram of the BBS (berry-based SCFA complex) extract at 210 nm.

1.2. Cell Viability and Antioxidant Protein Expression in HaCaT Cells Treated with BBS

To evaluate the biocompatibility of the BBS extract, an MTT assay was performed on HaCaT human keratinocytes. Cells were treated with BBS at concentrations of 12.5, 25, 50, and 100 µg/mL for 24 hours. As shown in Figure 3A, cell viability remained above 90% across all concentrations, indicating that BBS exhibited no cytotoxicity and was well-tolerated by skin keratinocytes.

To further assess the antioxidant effect of BBS at the protein level, the expression of SOD2 (superoxide dismutase 2) was analyzed by Western blot following oxidative stress induction using 200 µM H_2O_2. As shown in Figure 3B, BBS treatment markedly increased SOD2 expression compared to the H_2O_2-only treated control group. Densitometric analysis normalized to GAPDH confirmed a statistically significant upregulation of SOD2 in the BBS-treated group ($p < 0.05$), suggesting that BBS confers cellular protection by enhancing endogenous antioxidant defense mechanisms.

Together, these results demonstrate that BBS is non-cytotoxic and capable of stimulating antioxidant enzyme expression, thereby supporting its potential as a functional cosmetic ingredient for oxidative stress defense.

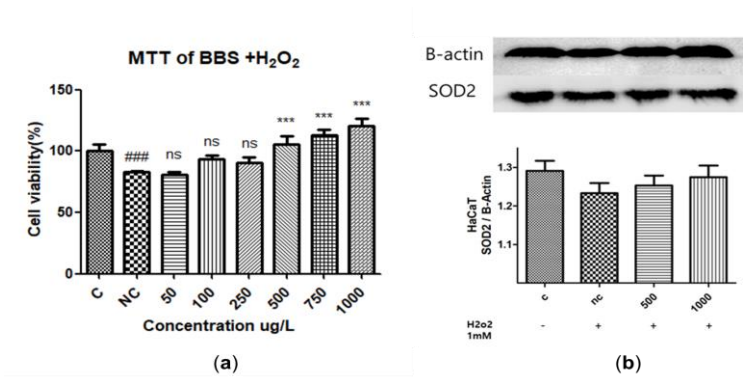

Figure 3. Representative HPLC chromatogram of the BBS (berry-based SCFA complex) extract at 210 nm.

1.3. Expression of Skin Barrier and ECM-Related Genes in HaCaT Cells

To investigate the protective effects of BBS on oxidative stress–induced damage in skin keratinocytes, we evaluated the expression of genes associated with skin barrier function (FLG), extracellular matrix integrity (COL1A1), and matrix degradation (MMP-9) in HaCaT cells. Oxidative stress was induced using hydrogen peroxide (200 μM H_2O_2), and cells were treated with BBS at concentrations of 500 and 1000. As shown in Figure 3, exposure to H_2O_2(NC group) led to a significant downregulation of FLG and COL1A1 expression levels compared with the untreated control group ($p < 0.001$), while MMP-9 expression was significantly upregulated ($p < 0.001$), indicating disruption of both barrier and structural homeostasis.

BBS treatment effectively reversed these alterations. Specifically:

- *FLG* mRNA levels were significantly increased by BBS treatment at both 500 and 1000 μg/mL compared to the NC group (**$p < 0.001$), restoring barrier-related gene expression.
- *COL1A1* expression, which encodes a primary component of dermal collagen, was significantly elevated at both concentrations of BBS (**$p < 0.001$ at 500 μg/mL; *$p < 0.01$ at 1000 μg/mL), suggesting restoration of ECM integrity.
- *MMP-9* expression, which was upregulated by oxidative stress, was significantly suppressed by BBS at 500 μg/mL (*$p < 0.01$), indicating an anti-inflammatory and matrix-preserving effect.

Taken together, these results demonstrate that BBS exerts protective effects on skin cells under oxidative conditions by upregulating key genes involved in barrier maintenance and collagen synthesis, while concurrently downregulating enzymes responsible for matrix degradation.

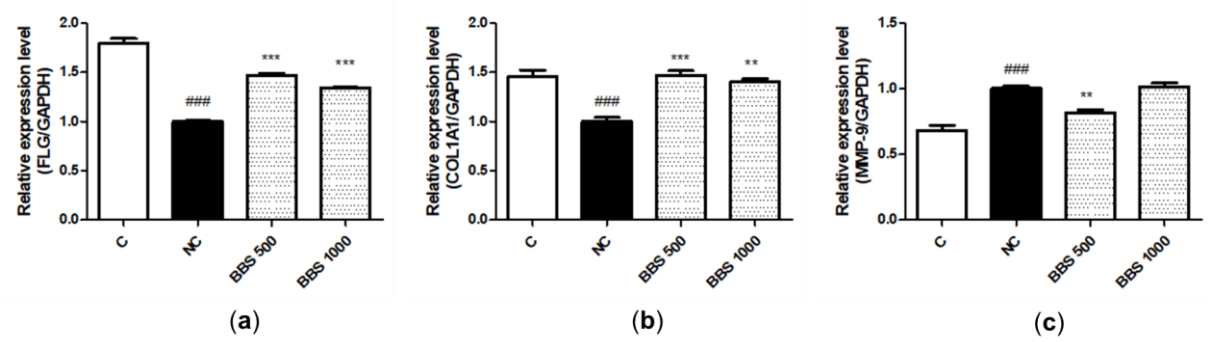

Figure 4. Effect of BBS treatment on the mRNA expression of *FLG*, *COL1A1*, and *MMP-9* in HaCaT cells exposed to oxidative stress (200 μM H_2O_2). C: untreated control; NC: H_2O_2-treated negative control; BBS 500/1000: BBS treatment at respective concentrations. Values are expressed as mean ± SD (n = 3). ###$p < 0.001$ vs. C; *$p < 0.01$, **$p < 0.001$ vs. NC.

4. Discussion

This study investigated the functional potential of a fermented berry-derived short-chain fatty acid (SCFA) complex, BBS, for enhancing skin barrier function and mitigating oxidative stress–induced damage in keratinocytes. The findings demonstrated that aerobic fermentation of blueberries using *Lactiplantibacillus plantarum* yielded a bioactive extract rich in propionate (1.858 ± 0.186 mg/mL), a key SCFA known to exert anti-inflammatory and barrier-supportive effects in epithelial tissues[5].

Notably, the BBS extract maintained a high level of biocompatibility, as evidenced by the MTT assay showing no cytotoxicity at concentrations up to 100. Beyond safety, BBS treatment significantly upregulated SOD2 protein expression, indicating its capacity to induce endogenous antioxidant defense mechanisms. This finding is consistent with previous studies reporting SCFA-mediated modulation of oxidative stress pathways through upregulation of mitochondrial antioxidant enzymes such as SOD2.

In the context of skin structure preservation, BBS treatment under oxidative stress conditions led to a marked restoration of FLG and COL1A1 mRNA expression, both of which are crucial for maintaining epidermal barrier integrity and dermal extracellular matrix (ECM) stability, respectively. These genes are often suppressed under inflammatory or oxidative conditions, contributing to skin dryness, fragility, and premature aging. Conversely, MMP-9, a matrix-degrading enzyme upregulated in response to ROS and inflammation, was significantly downregulated by BBS, supporting its anti-degradative and protective roles.

Collectively, these data suggest that the SCFA-rich BBS extract acts via dual mechanisms—enhancing the expression of barrier and structural proteins, while inhibiting inflammatory mediators—to protect skin cells from oxidative damage. This is aligned with the growing body of evidence supporting the skin benefits of postbiotics and SCFAs derived from microbial fermentation.

Future research should explore the in vivo efficacy of BBS in clinical or animal models of dermatitis, as well as its application in complex formulations for topical use. Investigating the signaling pathways involved in SCFA-induced gene regulation (e.g., Nrf2, GPR41/43) would further elucidate the mechanistic basis of its effects. In addition, expanding the fermentation system to include other probiotic strains or berry species may enhance its functional spectrum and product versatility.

5. Conclusion

This study demonstrated that a fermented berry-derived short-chain fatty acid complex (BBS), produced using *Lactiplantibacillus plantarum*, possesses strong potential as a multifunctional skin health ingredient. BBS was non-cytotoxic and enhanced endogenous antioxidant defense via SOD2 upregulation in keratinocytes exposed to oxidative stress. In addition, BBS significantly restored the expression of *FLG* and *COL1A1*, while suppressing *MMP-9*, thereby reinforcing skin barrier function and extracellular matrix integrity.

These findings suggest that BBS offers a promising postbiotic approach to protecting skin from oxidative damage and inflammation, with potential applications in anti-aging and skin barrier–strengthening cosmetic formulations. Further in vivo validation and formulation studies will support its translation into clinical and commercial use.

6. Acknowledgments

This research was supported by a grant of the Korea Health Technology R&D Project through the Korea Health Industry Development Institute (KHIDI), funded by the Ministry of Health & Welfare, Republic of Korea (grant number : RS-2024-00394432).

7. References

[1] Leemon N, Kamath GS, Sasikumar G, Latheef F, Panicker SP. Cutaneous homeostasis: a balancing cross-talk between epidermal stem cell pool and regulatory pathways. Stem Cells and Signaling Pathways, Elsevier; 2024, p. 67–85. https://doi.org/10.1016/B978-0-443-18800-8.00006-X.

[2] Utsunomiya A, Chino T, Utsunomiya N, Luong VH, Tokuriki A, Naganuma T, et al. Homeostatic Function of Dermokine in the Skin Barrier and Inflammation. Journal of Investigative Dermatology 2020;140:838-849.e9. https://doi.org/10.1016/j.jid.2019.09.011.

[3] Facchin S, Bertin L, Bonazzi E, Lorenzon G, De Barba C, Barberio B, et al. Short-Chain Fatty Acids and Human Health: From Metabolic Pathways to Current Therapeutic Implications. Life 2024;14:559. https://doi.org/10.3390/life14050559.

[4] Cheng Y, Wu T, Chu X, Tang S, Cao W, Liang F, et al. Fermented blueberry pomace with antioxidant properties improves fecal microbiota community structure and short chain fatty acids production in an in vitro mode. LWT 2020;125:109260. https://doi.org/10.1016/j.lwt.2020.109260.

[5] Trompette A, Pernot J, Perdijk O, Alqahtani RAA, Domingo JS, Camacho-Muñoz D, et al. Gut-derived short-chain fatty acids modulate skin barrier integrity by promoting keratinocyte

metabolism and differentiation. Mucosal Immunol 2022;15:908–26. https://doi.org/10.1038/s41385-022-00524-9.