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"Effects of Fermentation on the Components of Noni Seed Oil and Its skincare Activity"

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1. Introduction

Noni (Morinda citrifolia L.), a tropical plant native to Polynesia, has long been valued for its medicinal and nutritional properties [1]. Noni seeds, a byproduct of the noni juice industry, are rich in bioactive compounds such as phytosterols, unsaturated fatty acids (e.g., linoleic acid), and antioxidants [2]. These components position noni seed oil (NSO) as a promising raw material for functional cosmetics. However, the limited bioavailability and stability of unmodified plant oils often hinder their efficacy in skincare applications. Fermentation, a biotechnological process, has been shown to enhance the bioactivity of natural products by altering molecular structures and enriching functional components [3]. For instance, fermented plant extracts have demonstrated improved immune modulation and skin barrier repair capabilities in atopic dermatitis models [4]. Fermentation, a controlled enzymatic process, offers a promising strategy to modify lipid structures and enhance functional components [5]. In this study, we applied fermentation protocol using immobilized lipase to optimize NSO, aiming to (1) analyze compositional changes induced by fermentation, (2) evaluate improvements in emulsification and absorption efficiency, and (3) validate the enhanced skincare efficacy of fermented noni seed oil (FNSO) in hydration, barrier repair, and soothing activity. By bridging the gap between traditional fermentation techniques and modern skincare science, this work provides a foundation for developing high-value cosmetic ingredients from sustainable agro-industrial byproducts.

2. Materials and Methods

2.1. Preparation of FNSO

Fresh Morinda citrifolia (noni) fruits were harvested from Sanya, Hainan Province, China. The preparation process of FNSO involves four stages: germination, lipase extraction, enzymatic fermentation, and separation. Fresh noni seeds are hydroponically germinated for 1-7 days. Germinated seeds (10-25 g) are homogenized at 3-7°C, pH-adjusted (5-7), filtered, and centrifuged sequentially to isolate crude oil supernatant. Neutral lipids are extracted via four cycles of diethyl ether mixing, followed by sodium sulfate washing and nitrogen evaporation to

obtain oleosomes, with oil composition analyzed by thin-layer chromatography (TLC). For fermentation, oleosomes are adjusted to pH 7-10, combined with emulsified gum arabic trioleate, deoxycholate, and sodium chloride at 35-43°C. Lipase activity is screened using p-nitrophenyl palmitate, and the highest-activity lipase is added to cold-pressed oil for 1-7 days. Finally, the obtained fermented oil was stored for further use.

2.2. Determination of the Total Amount and Composition of Sterols in FNSO and NSO by Gas Chromatography

To determine the sterol composition and content in FSNO and NSO, the method described in GB/T 25223-2010/ISO 12228:1999 was employed [6]. Briefly, 250 mg of sample was saponified with 5 mL KOH-ethanol solution under reflux for 15 min. The unsaponifiable matter was separated using an alumina column, eluted with ethanol (5 mL) and diethyl ether (30 mL), and concentrated. The sterols were further purified by thin-layer chromatography (TLC) on silica plates developed with a hexane-ether (1:1, v/v) mixture. The sterol band was scraped off, extracted with ether, and derivatized with N-methyl-N-trimethylsilyl-heptafluorobutyramide (MSHFBA) at 105°C for 15 min. Analysis was performed by gas chromatography (GC) using a SE-54 capillary column (50 m × 0.25 mm, 0.10 μm film thickness) with hydrogen as the carrier gas (36 cm/s, split ratio 1:20). The oven temperature was programmed from 240°C to 255°C at 4°C/min. Sterol identification was based on relative retention times, and quantification was achieved via peak area normalization and an internal standard (betulin) method.

2.3. Emulsification capability test

The FNSO and NSO were homogeneously mixed with water at a specified ratio. Following one hour of standing, the emulsification degree was visually evaluated, and the diameter of oil droplets was observed using a CX43 optical microscope (OLYMPUS).

2.4. In - vitro Antioxidant Potential Detection

Antioxidant capacities of FNSO and NSO were evaluated via DPPH assay. DPPH in ethanol shows purple color with strong absorbance at 517 nm; scavengers reduce absorbance proportionally to activity. FNSO was diluted in absolute ethanol. For each dilution, 500-μL aliquots were mixed with 0.2 mM DPPH (Ai), ethanol (Aj), and ethanol-DPPH (Ac). After 30-min dark incubation, OD at 517 nm was measured using a BioTek Synergy microplate reader to calculate scavenging rate.

2.5. Clinical efficacy research

2.5.1. Test sample preparation

Two facial creams with 2% FNSO and 2% NSO were prepared and the ingredients of the formula were listed in Table 1. Preparation Procedure: Heat Phase A to 85 °C and stir until uniformly mixed. Heat Phase B to 85 °C and stir until uniformly mixed. Cool Phase B to around 50 °C, add Phase C, and stir well for neutralization and thickening with the pH around 5.0. Add the pre-dissolved Phase D to the thickened Phase B, and then add the resulting mixture to Phase A, followed by homogenization at 4000 rpm for 3 minutes.

Table 1. Formula of facial cream

Phase	Ingredient	Concentration/%
	FNSO/NSO	2
A	Polyglycerol fatty acid ester	1.5
	Cetostearyl Alcohol	0.5
B	Deionized water	To 100
	Carbomer 940	0.2
C	10% NaOH solution	0.1
	Butanediol	3
D	P-hydroxyacetophenone	0.5
	Hexanediol	0.5

2.5.2. Study design

This study aims to comprehensively assess the efficacy of the 2% FNSO/NSO containing facial cream through a series of well-designed tests. The tests are divided into short-term (2-hour) and long-term (two-week) categories, covering aspects such as moisturization, transdermal absorption, soothing, and skin barrier repair. Thirty subjects (aged 18-60 years old) were selected. Test site: inner forearms; Test environment conditions: Temperature 20~22℃, humidity 40%~60%.

2.5.2.1. Transdermal Absorption Ability Test

Subjects were directed to apply the test products as follows: the 2% FNSO facial cream on one inner forearm and the 2% NSO facial cream on the other inner forearm. After the 2-hour application period, the water content of the stratum corneum was measured using the Multiprobe Moisture Meter D. Employing the capacitance method, the water content within the stratum corneum of the skin was measured. This measurement was carried out to assess the short-term transdermal absorption capacity of the samples.

2.5.2.2 Evaluation of Soothing Efficacy

A standardized skin irritation model was established by inducing physical barrier disruption via tape stripping on a 2x2 cm area of the volar forearm. Baseline measurements of transepidermal water loss (TEWL) and erythema index (EI) were recorded immediately post-irritation using a VapoMeter® (Delfin Technologies, Finland) and a SkinColorCatch® colormeter (Delfin Technologies, Finland), respectively. The colormeter quantified erythema through the a* value within the CIELAB color space, reflecting skin redness. Subsequently, the test formulation was applied to the damaged site, and post-treatment TEWL and EI values were measured after a 2-hour experimental cycle. The reduction in TEWL and EI post-application served as key indicators of the formulation's short-term soothing efficacy, aligning with validated dermatological protocols for irritant recovery assessment.

2.5.2.3. Long - lasting Moisturizing Test

On a 2×2 cm area of the inner forearm, apply the 2% FNSO/NSO facial cream. Over a two-week period, use the Multiprobe Moisture Meter D tissue water content measuring instrument from Delfin. By measuring the water content in the stratum corneum of the skin based on the capacitance method, the long-lasting moisturizing efficacy of the sample can be verified.

2.5.2.4. Skin Barrier Repair Efficacy Test

With the temperature controlled between 20°C - 22°C and the relative humidity at 40% - 60%, on a 2×2 cm area of the inner forearm, start by using tape stripping to cause physical damage and measure the erythema value. After the skin undergoes water loss, apply the 2% FNSO/NSO facial cream. During the two-week test cycle, use the VapoMeter and the Skin Color Catch (Delfin) to re-measure the erythema value. This is to evaluate the repair effect of the sample.

2.5.2.5. Statistical method

All tests were replicated in triplicate, and the results were presented as the mean ± standard deviation (SD) or the standard error of the mean (SEM). SPSS was used for statistical analysis and a normal distribution test was conducted on the test data. If the values were normally distributed, the T-test method was used for statistical analysis. If the test data were not normally distributed, the rank sum test was used for statistical analysis, and $p < 0.05$ was considered significant.

3. Results

3.1. Determination of the Total Amount and Composition of Sterols

As depicted in the Figure 1, notable changes occurred in the sterol content of Noni seed oil during fermentation. The total sterol content escalated from 239 mg/100g in NSO to 254 mg/100g in FNSO. Regarding specific sterol components, campesterol increased from 50.9 mg/100g to 51.6 mg/100g, stigmasterol from 40.8 mg/100g to 46.6 mg/100g, and sitosterol from 147 mg/100g to 156 mg/100g.

These compositional alterations are likely attributed to several factors. Firstly, during fermentation, microorganisms secrete enzymes such as lipases. These enzymes can catalyze lipid hydrolysis, releasing free fatty acids and sterols that were previously bound in complex lipid structures. This process may contribute to the increased availability and detection of sterols. Secondly, metabolic activities of fermenting microorganisms might trigger biochemical reactions that modify or synthesize sterol - related compounds. For example, they could introduce functional groups or alter the molecular structure of sterol precursors, facilitating the formation of more diverse and abundant sterol components. In summary, the fermentation process exerts a multi-faceted influence on the sterol composition of Noni seed oil, through enzyme-mediated reactions, microbial metabolism, and environmental changes.

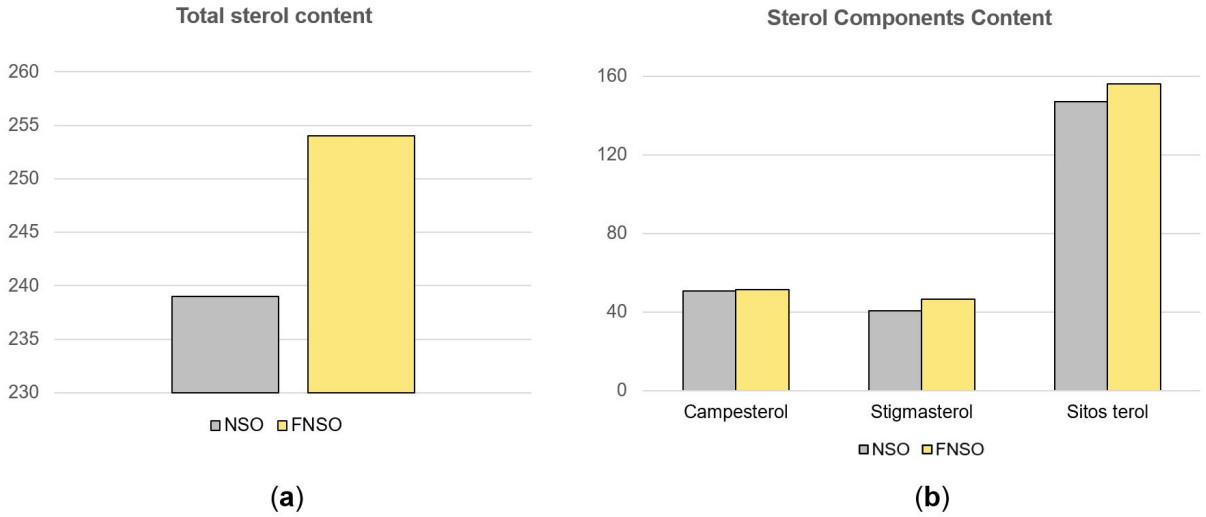

Figure 1. Comparison of total sterol content as well as the contents of specific sterol components (Campesterol, Stigmasterol, and Sitosterol) between Noni seed oil (NSO) and fermented Noni seed oil (FNSO).

3.2. Emulsification capability test

According to the results in Figure 2, FNSO demonstrates a broader emulsification range compared to NSO. In the “Tube” view, FNSO blends more extensively with water, forming a more uniformly dispersed mixture, which indicates its superior ability to create a wider - reaching emulsified system. When observed under the microscope, at both the “Microscope Center” and “Microscope Edge”, FNSO features smaller and more densely - packed particle sizes. In contrast, NSO shows relatively larger particles that are sparsely distributed. This difference implies that FNSO forms a finer and more stable emulsion, as smaller and densely - distributed particles contribute to better emulsion stability. During the fermentation process, long - chain fatty acids in NSO are broken down into short - chain fatty acids in FNSO. Long - chain fatty acids, due to their large molecular size and complex structure, possess poor hydrophilicity, impeding effective emulsification. In contrast, short - chain fatty acids have a smaller molecular size and increased polarity, which endows them with enhanced hydrophilicity. This improved hydrophilicity allows short - chain fatty acids to more efficiently reduce the surface tension at the oil - water interface when mixed with water. Moreover, short - chain fatty acids can align more readily at the oil - water interface, creating a more compact and stable interfacial film. This film effectively prevents the aggregation of oil droplets, resulting in the formation of an emulsion with smaller, densely - distributed particles and a wider emulsification range, thus enhancing the overall emulsifying performance of FNSO.

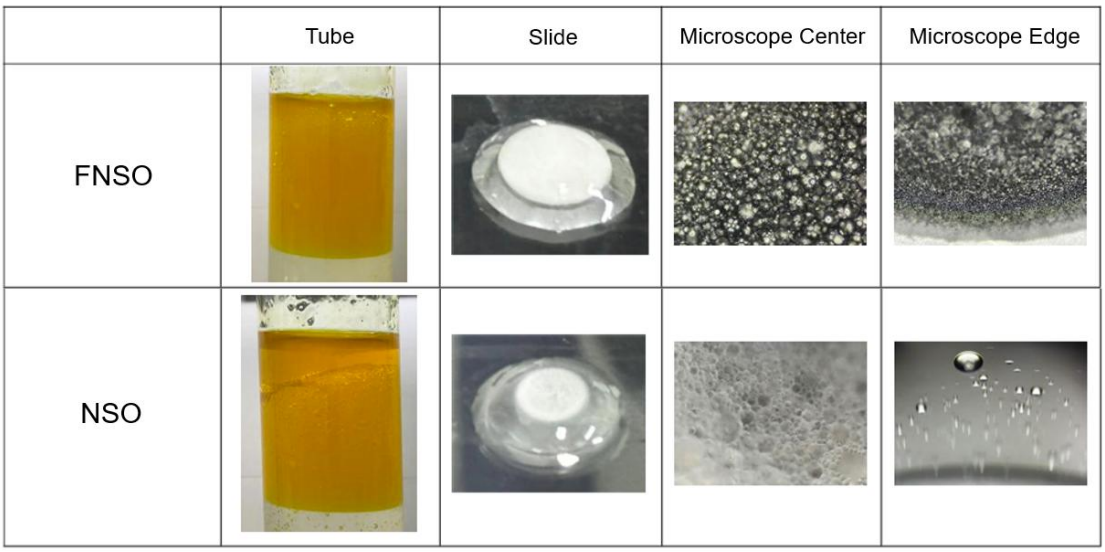

Figure 2. Comparison of the emulsification capability between Noni seed oil (NSO) and fermented Noni seed oil (FNSO).

3.3. Antioxidant activity of FNSO and NSO

As shown in Table 2, the DPPH radical scavenging rates of 2% FNSO and 2% NSO are between 85% and 90%. The antioxidant capacity of the fermented group is slightly higher than that of the unfermented group.

Table 2. DPPH Radical - Scavenging Percentage of 2% FNSO and 2% NSO

Samples	Percentage of DPPH radical scavenged
2%FNSO	89.94%
2%NSO	88.67%

3.4. Skin absorption activity of 2% FNSO and 2% NSO samples

Figures 3(a) and 3(b) show changes in epidermal and dermal moisture during a short - term hydration test for the 2% fermented noni seed oil (FNSO), 2% unfermented noni seed oil (NSO), and control groups.

Epidermal Moisture: Ten minutes post - application, FNSO achieved a peak epidermal moisture improvement rate of 10.18%, maintaining elevated levels of 7.68% and 6.96% at 30 and 60 minutes, respectively. This pattern underscores FNSO's rapid epidermal uptake and its capacity for sustained hydration. Conversely, NSO reached a peak of 4.38% at 10 minutes, followed by a decline to 0.58% at 30 minutes and a further drop to - 4.09% at 60 minutes, indicative of water loss and subpar epidermal absorbency. Dermal Moisture: FNSO exhibited an initial 2.50% increase in dermal moisture at 10 minutes, signaling efficient dermal absorption. NSO, too, showed an initial 2.50% increase, yet this was followed by a decrease to - 5.80% at 30 minutes and - 7.49% at 60 minutes, highlighting its unstable dermal absorption capabilities. Overall, during the test, the 2% FNSO group outperformed the 2% NSO group in enhancing epidermal and dermal moisture. FNSO has better skin absorbability and is more effective at hydrating and retaining skin moisture.

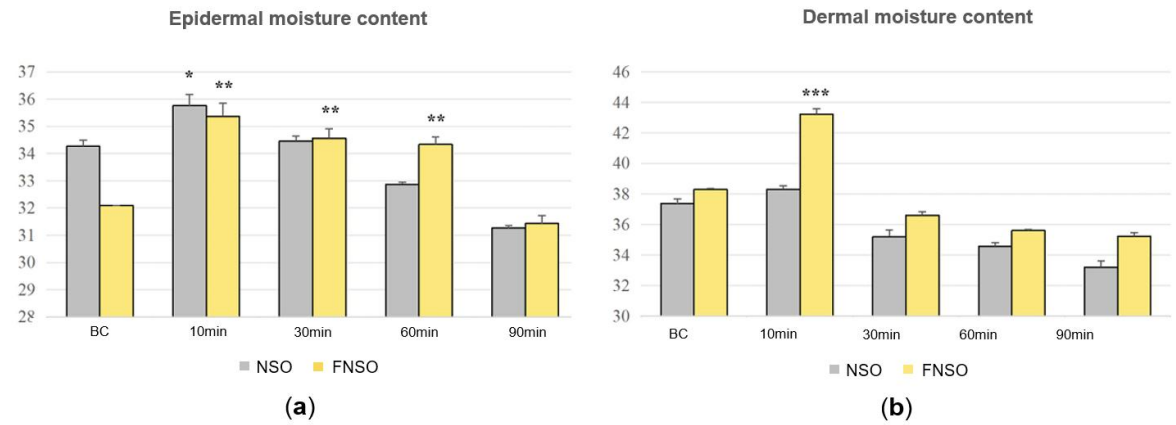

Figure 3. Comparison of the epidermal and dermal moisture content and their improvement rates between 2% FNSO and NSO samples at different time points.

3.5. Skin soothing activity of 2% FNSO and 2% NSO samples

Figure 4 shows the improvement rates of transepidermal water loss (TEWL) and reduction rates of erythema over time for the 2% unfermented noni seed oil (NSO) and 2% fermented noni seed oil (FNSO) groups.

Transepidermal Water Loss: In Figure 4(a), 10 minutes post - treatment, FNSO had a 9.26% improvement rate compared to NSO's 7.91%. At 90 minutes, FNSO's rate rose to 31.48% versus NSO's 19.77%. FNSO outperformed NSO at all time points, indicating its greater and increasing efficacy in reducing TEWL.

Erythema Reduction: In Figure 4(b), 10 minutes after treatment, FNSO's reduction rate was 6.12% and NSO's was 2.08%. At 90 minutes, FNSO reached 59.18% compared to NSO's 43.75%. FNSO significantly outperformed NSO at all time points, showing stronger erythema - relieving ability.

Overall, FNSO outperforms NSO in improving TEWL and reducing erythema, highlighting its superior soothing efficacy.

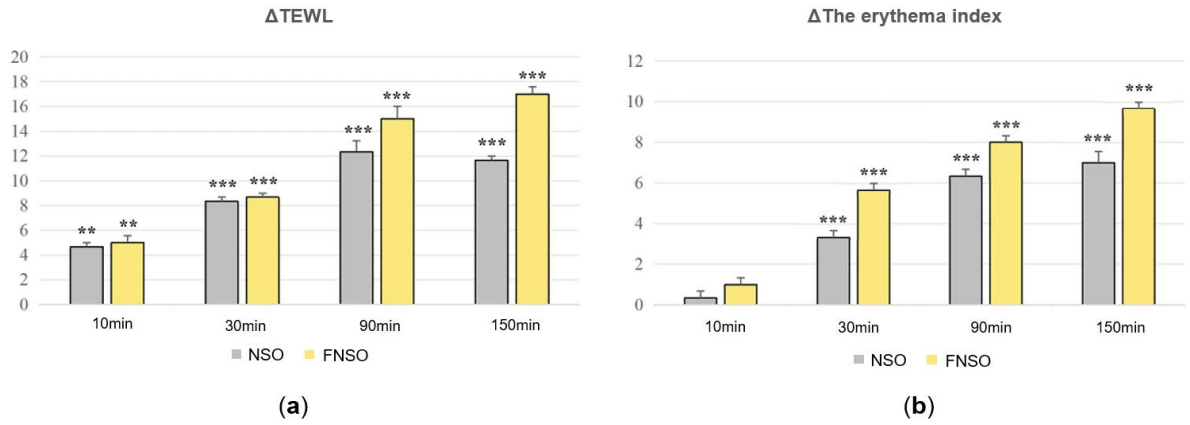

Figure 4. Comparison of the changes in transepidermal water loss (ΔTEWL) and erythema index (EI) between 2% FNSO and 2% NSO samples at different time points.

3.6. Long-term Moisturizing Properties of 2% FNSO and 2% NSO samples

As shown in Figure 5, the results demonstrate that the 2% FNSO has a significant positive effect on both epidermal and dermal moisture content, with a 7.90% increase in epidermal moisture content and a 2.44% increase in dermal moisture content after two weeks compared to the control group. In contrast, the 2% NSO shows no significant impact on either epidermal or dermal moisture content, highlighting the superior long-acting moisturization ability of theFNSO.

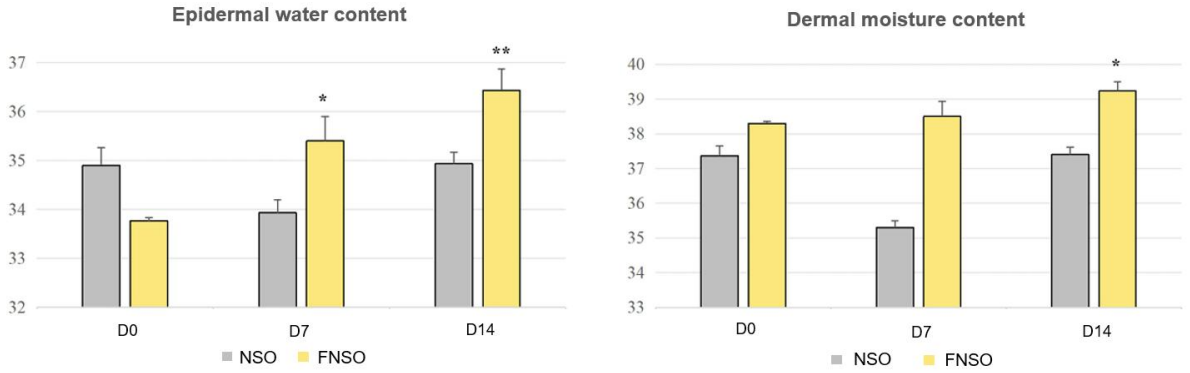

Figure 5. Comparison of epidermal and dermal moisture content and their improvement rates between 2% FNSO and 2% NSO samples at different time points (D0, D7, D14).

3.7. Long-term skin barrier repair properties of 2% FNSO and 2% NSO samples

Figure 6 displays the results for transepidermal water loss (TEWL) and erythema values. As shown in Figure 6(a), in comparison to the model group (MG), both the 2% fermented noni seed oil (FNSO) and non-fermented noni seed oil (NSO) groups manifested significant decreases in TEWL. Specifically, on Day 7 (D7), the TEWL improvement rates of the NSO and FNSO groups were 60.00% and 65.80%, respectively. By Day 14 (D14), these rates escalated to 75.65% and 80.80%, respectively.

Analogously, Figure 6(b) reveals that when compared with the MG, both the FNSO and NSO groups demonstrated a reduction in erythema values, with FNSO outperforming NSO. On D7, the erythema reduction rates were 55.56% for the NSO group and 69.39% for the FNSO group. By D14, these rates had increased to 83.33% and 87.76%, respectively.

In summary, both FNSO and NSO effectively reduced TEWL and erythema, with FNSO showing a more substantial effect at each time point examined.

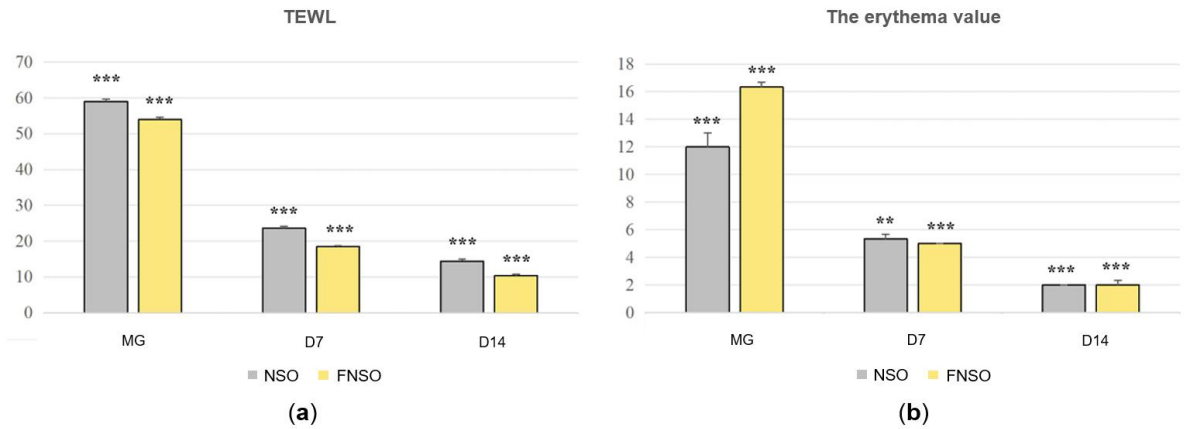

Figure 6. Comparison of transepidermal water loss (TEWL) and erythema values between NSO and FNSO samples at different time points (D7, D14).

4. Discussion

The fermentation process induced multifaceted modifications in noni seed oil (NSO), significantly enhancing its composition and functionality for skincare applications. Gas chromatography analysis revealed a 6.28% increase in total phytosterols post-fermentation (Figure 1), with notable elevations in stigmasterol (+14.2%) and sitosterol (+6.1%). Phytosterols are critical for reinforcing the skin barrier by integrating into lipid bilayers [7], which aligns with FNSO's ability to reduce transepidermal water loss (TEWL) by 31.48% and erythema by 59.18% within 90 minutes (Figure 4). This effect likely stems from phytosterols' capacity to stabilize ceramide synthesis and suppress inflammatory mediators [8].

Enzymatic hydrolysis during fermentation reduced fatty acid molecular weights, as evidenced by smaller oil droplet sizes (Figure 2) and enhanced emulsification stability. Smaller fatty acids penetrate the stratum corneum more effectively [9], explaining FNSO's rapid hydration effects: epidermal moisture content increased by 10.18% within 10 minutes, while unfermented NSO showed negligible improvement (Figure 3).

The 89.94% DPPH radical scavenging rate of FNSO (vs. 88.67% for NSO) highlights its antioxidant capacity (Table 2). This enhancement may arise from fermentation-induced release of phenolic compounds or synergistic interactions between phytosterols and hydrolyzed fatty acids. Clinically, FNSO's long-term moisturizing efficacy—7.90% increase in epidermal hydration after two weeks (Figure 5)—correlates with its ability to form a stable lipid film and reducing water evaporation.

Crucially, FNSO's 80.80% reduction in TEWL and 87.76% decrease in erythema after 14 days (Figure 6) underscores its dual role in barrier repair and anti-inflammatory action. These outcomes are mechanistically linked to the synergistic effects of phytosterols (barrier reinforcement and anti-irritation) and low-molecular fatty acids (rapid penetration).

5. Conclusion

Fermentation transforms noni seed oil into a high-performance skincare ingredient by: (1) Enriching phytosterols (239→254 mg/100g), which stabilize the skin barrier and reduce inflammation. (2) Reducing fatty acid molecular weights, improving emulsification, absorption, and hydration retention. (3) Enhancing antioxidant activity through enzymatic release of bioactive metabolites.

FNSO's rapid transdermal absorption, sustained moisturization, and exceptional barrier repair efficacy position it as a sustainable alternative to conventional oils. Its ability to address both clinical dryness and inflammation makes it particularly suited for sensitive or compromised skin formulations. Future studies should explore long-term safety profiles and optimize fermentation protocols to further enhance bioactive yields.

6. References

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