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# Investigation of the *in vitro* effects of shea butter on skin barrier function and hydration using analytical chemistry methodologies

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**Abstract:** Shea butter is a natural moisturizer used to improve skin health. This study investigates its effects on skin barrier function, hydration, and lipid profile, using analytical chemistry methodologies. Trans-Epithelial Water Loss (TEWL), corneometry, impedance spectroscopy, and gas chromatography-mass spectrometry (GC-MS) were used to assess skin barrier function, hydration, and lipid profile after shea butter application. Results show that TEWL decreased by 37.8% after 24 hours ( $p < 0.01$ ), Skin hydration increased by 58% after 24 hours ( $p < 0.001$ ). Impedance spectroscopy showed a 33% increase in skin impedance, GC-MS analysis revealed a balanced fatty acid composition in shea butter, ceramide profiling showed six subclasses, with Ceramide 1 and 2 being the most abundant. The results demonstrate shea butter's efficacy in improving skin barrier function, hydration, and lipid profile. The rapid decrease in TEWL and increase in skin hydration suggest immediate effects on skin lipids. Shea butter's fatty acid composition contributes to its moisturizing and barrier-enhancing properties. This study provides evidence for the benefits of shea butter in maintaining healthy skin. Its natural moisturizing properties make it a valuable ingredient in skincare products.

**Keywords:** analytical chemistry, shea butter, skin barrier function, hydration, lipid profile, ceramides, fatty acids

## 1. Introduction

Shea butter, derived from the nut of the African shea tree (*Vitellaria paradoxa*), has been used for centuries in traditional African medicine and cosmetics for its moisturizing and protective properties. Nigeria, being one of the largest producers of shea butter, offers a unique opportunity to investigate its effects on skin health [1]. Nigeria produced 350,000 metric tons of shea nuts in 2020, accounting for approximately 20% of global production [2]. Nigeria's shea butter production was valued at USD 200 million in 2019 [3]. The country's shea butter industry is projected to grow at a CAGR of 10% from 2020 to 2025 [4]. Shea butter is a high-value shea nut fat used as an edible oil, antimicrobial and moisturizer in the food, pharmaceutical and cosmetic industries, respectively [5]. Various plant and seed extracts are utilized for skin care, cosmetics, and alternative skin therapy. One of the most used in Nigeria and many other countries in Sub-Saharan African is shea butter, a thick yellowish butter produced from the nuts of the Shea tree (*Vitellaria paradoxa*). Shea butter is a well-known multipurpose skin care item in many African countries, and it is believed to have several skin maintenance and healing properties. Shea butter has been shown to have both emollient and occlusive properties which enables it to trap moisture in the epidermal layers of the skin. Shea butter was found superior to mineral oil in preventing trans-epidermal water Loss (TEWL). Shea butter was recommended for repairing dry inflamed skin caused by dermatitis [6].

The global market for shea butter has experienced significant growth, driven by increasing demand for natural and organic products in the cosmetics and pharmaceutical industries [7]. The skin, the body's largest organ, serves as a vital barrier against external factors such as environmental stressors, pathogens, and toxins. Disruptions in the skin barrier can lead to various disorders, including dryness, irritation, inflammation, and accelerated aging. Hydration plays a crucial role in maintaining skin health, elasticity, and firmness [8].

Skin barrier dysfunction and dehydration are prevalent dermatological concerns in Nigeria, particularly in regions with high temperatures and humidity. The harsh climate and limited access to effective skincare products exacerbate these issues, leading to conditions such as dry skin, eczema, and acne. Shea butter's composition, rich in triglycerides, fatty acids, vitamins, and polyphenols, makes it an attractive ingredient for topical applications aimed at enhancing skin barrier function and hydration. Despite its widespread use, the scientific understanding of shea butter's effects on skin health remains incomplete. Current methods for evaluating skin barrier function and hydration are often subjective, invasive, or require complex instrumentation and the scientific understanding of shea butter's effects on skin barrier function and hydration is limited [9]. Several factors contribute to the complexity of shea butter's effects on skin such as the variability in shea butter composition-depending on factors like region, climate, and processing, diverse skin types and conditions-shea butter may interact differently with various skin types and conditions and lack of standardized analytical methods-to evaluate shea butter's effects on skin barrier function and hydration.

There is a need for a systematic, analytical chemistry-based approach to investigate shea butter's impact on skin barrier function and hydration.

This study aims to investigate the in-vitro effects of shea butter on skin barrier function and hydration using analytical chemistry methodologies, providing valuable insights into its potential benefits and mechanisms of action.

Hence the Objectives of this work are as follows:

- 1) Develop and optimize an in vitro analytical methodology to assess shea butter's effects on skin barrier function and hydration.
- 2) Investigate the chemical composition of shea butter and its interactions with skin lipids.
- 3) Evaluate the impact of shea butter on skin hydration and barrier function using biochemical and biophysical assays.
- 4) Validation of an in vitro analytical methodology for evaluating natural moisturizers.

Expected Outcomes are as follows:

- 1) Improved understanding of shea butter's effects on skin barrier function and hydration.
- 2) Evidence-based recommendations for shea butter usage in skincare.
- 3) Contributions to the development of effective skincare products for Nigerian population.

## 2. Materials and Methodology

This study employs a combination of advanced analytical techniques to evaluate the effects of shea butter on skin barrier function and hydration. The methodologies include:

- Gas Chromatography–Mass Spectrometry (GC–MS) for analyzing the chemical composition of shea butter.
- High-Performance Liquid Chromatography (HPLC) for profiling skin lipids.
- Fourier Transform Infrared Spectroscopy (FTIR) to assess changes in skin barrier function.
- Transepidermal Water Loss (TEWL) measurements to evaluate skin hydration.

The materials and instruments used in this study include:

- Extracted shea butter
- Porcine skin and human skin equivalents (HSEs)
- Tewameter<sup>®</sup> TM 300 (Courage + Khazaka)
- Corneometer<sup>®</sup> CM 825
- Conductance Meter SCM 500
- HPLC-MS system (Agilent 12000 series)

### 2.1. Shea butter extraction

40g of the grounded shea nut particles was placed in a handkerchief and placed in the thimble of the soxhlet extractor. 150ml of n-hexane was added and placed in an electro heating mantle set at 70°C (below the boiling point of the n-hexane). The extraction was carried out batch by batch at a time of 60mins and 80mins [7]. The shea butter was melted at 50-60°C and filtered through a 0.2-0.5  $\mu\text{m}$  filter to remove impurities, then centrifuged by spinning the filtered shea butter at 3,000-5,000 rpm 15 minutes to separate further impurities, cooled to 30°C for 24 hours and the shea butter was then filtered through a 0.5  $\mu\text{m}$  filter [10].

## 2.2. Trans-epithelial water loss (TEWL) analysis

Using the porcine skin and using Tewameter TM 300 (Courage+Khazaka) and a control substance phosphate-buffered saline. The porcine skin sample was acclimated to room temperature 25°C and humidity (50%), the porcine skin samples was cleaned with phosphate-buffered saline and dried with lint-free tissue. The base-line TEWL before treatment was measured, and the treated porcine skin treated with 20 mg/cm<sup>2</sup> surfaceskin and the TEWL value at g/m<sup>2</sup>/h was recorded at interval as shown in the results table at a temperature of 25°C and 45% humidity and at skin surface temperature of 32°C [11].

## 2.3. Corneometry assessment

Corneometry assessment of the hydration of the Shea butter treated porcineskin using Corneometer CM 825. The porcine skin samples was acclimated to room temperature , 25°C and humidity and current humidity was taken, the porcine skin sam-ples was clean with phosphate-buffered saline and dried with lint-free tissue. The baseline Corneometry before treatment was measured, and the 20 mg/cm<sup>2</sup> treated porcine skin hydration values at room temperatutre25oC, and the current humidity, at 32°Cwere recorded at interval as shown in the results tables. Thepercentage change in hydration was calculated based on the formula shown below:((Hydration post-treatment - Hydration baseline) / Hydration baseline) × 100.

## 2.4. Analysis of impedance spectroscopy

The skin barrier function was Evaluated using skin Conductance Meter (SCM) 500. The porcine skin samples was acclimated to room temperature (25°C) and the current humidity was taken, the porcine skin samples was clean with phosphate-buffered saline and dried with lint-free tissue. The baseline skin barrier function of the por-cine skin before treatment was measured, and the shea butter treated porcine (at 20 mg/cm<sup>2</sup>) skin was also measured using the Skin Conductance Meter (SCM) 500, the conductances (at (μS/cm) were recorded at room temperature (25OC) and at the cur-rent roomhumidity, and at the skin surface temperature of 32°C. The percentage change in conductances were calculated as shown below: ((Conductance post-treatment - Conductance baseline) / Conductance baseline) × 100.

## 2.5. Quantification of ceramide levels and other skin lipids in porcine skin using HPLC-MS

The porcine skin sample was cleaned and dried, and the lipids was extracted using methanol which evaporated later and Agilent 1260 Infinity II was used to determine the Ceramide and other lipids following the [11] standard operation procedure.

## 2.6. GC-MS

Analyze fatty acid composition of shea butter: 2g of the shea butter was melted in a water bath set to 40°C this was transferred to a 10 mL centrifuge tube and 3 mL of hexane was added to dissolve the shea butter and this was vortexed for 2 minutes and later the mixture was centrifuged at 3000 rpm for 5 minutes [11]. Gas Chromatograph coupled with Mass Spec-trometer- as lightly polar capillary column (DB-23) was used, the oven temperature program were 100°C (held for 2 minutes) at initial, at 10°C/min ramp rate and a final temperature of 250°C (held for 5 minutes). Helium at flow rate of1mL/min injected at 1 μL and electron ionization was used. Fatty acid percentages using internal standard was calculate and the results was com-pared with reference standards National Institute of Standards and Technology (NIST) [10].

## 3. Results and discussion

### 3.1. Trans-epithelial water loss (TEWL)

Table 1 shows the TEWL measurements, results demonstrate a significant reduction in Trans-Epithelial Water Loss (TEWL) after application of shea butter, indicating improved skin barrier function and hydration [12,13]. The rapid decrease in TEWL within the first 2 hours suggests immediate effects of shea butter on skin lipids.

The baseline TEWL was 8.2 g/m<sup>2</sup>/h and Post-shea butter application TEWL was 5.1 g/m<sup>2</sup>/h (37.8% reduction). Paired t-test: p < 0.01 (significant difference between baseline and all time points).

Shea butter's fatty acids, including oleic, stearic, and linoleic acids, interact with skin lipids, forming a more hydrated and ordered lipid matrix [14]. This interaction enhances skin barrier function, reducing TEWL and leading to increased skin hydration [8].

Similar TEWL reductions (25-40%) have been reported in studies using other natural moisturizers, such as coconut oil and olive oil [12,13]. These findings support the efficacy of shea butter as a natural moisturizer for improving skin barrier function and hydration.

**Table 1.** Results for TEWL

Time Point	Mean TEWL $\pm$ SD (g/m <sup>2</sup> /h)	% Change
1 hr	7.5 $\pm$ 1.2	8.5%
2 hr	6.9 $\pm$ 1.1	16.0%
4 hr	6.3 $\pm$ 0.9	23.2%
8 hr	5.8 $\pm$ 0.8	29.3%
12 hr	5.4 $\pm$ 0.7	34.1%
24 hr	5.1 $\pm$ 0.6	37.8%

### 3.2. Skin Hydration analysis

Table 2 shows the results for the Skin Hydration analysis, the results demonstrate a significant increase in skin hydration after application of shea butter, indicating improved skin moisturization and hydration [15]. This increase in skin hydration is attributed to the humectant properties of shea butter.

Baseline skin hydration was 32.4%, and Post-shea butter application skin hydration was 51.2% (58% increase).

Shea butter's humectant properties, particularly its fatty acids (such as the oleic, stearic, and linoleic acids), attract and retain water in the skin, enhancing skin hydration [16]. Improved skin hydration subsequently enhances skin elasticity, firmness, and overall appearance [17].

**Table 2.** Corneometry (Skin hydration)

Time Point	Mean Hydration $\pm$ SD (AU)	% Change
1 hr	38.5 $\pm$ 6.1	+18.8%
2 hr	42.1 $\pm$ 6.9	+30.1%
4 hr	45.6 $\pm$ 7.4	+40.7%
8 hr	48.3 $\pm$ 8.1	+49.1%
12 hr	50.2 $\pm$ 8.5	+55.1%
24 hr	52.5 $\pm$ 9.2	+62.3%

### 3.3. The impedance spectroscopy

The Impedance Spectroscopy shows a baseline skin impedance of 2.4  $K\omega$ , Post-shea butter application skin impedance was 3.2  $K\Omega$  (33% increase). The Impedance Spectroscopy results using Conductance Meter (SCM) 500 demonstrate a significant increase in skin impedance after application of shea butter. The 33% increase in skin impedance suggests improved skin barrier function, enhancing skin health and appearance [13], enhanced skin hydration which improves skin elasticity and firmness [12] and reduced skin permeability which minimizes the risk of skin irritation and allergic reactions [18].

Similar impedance increases (25-40%) reported in studies using other natural moisturizers (such as coconut oil, olive oil) [14,19].

### 3.4. Quantification of ceramide levels

Table 3 shows the results for quantification of ceramide levels. The results show a diverse ceramide profile in porcine skin, with six subclasses detected. Ceramide 1 (CER[NS]) and Ceramide 2 (CER[NP]) are the most abundant, comprising approximately 53% of total ceramides. This distribution is consistent with previous studies on human and porcine skin [20] and [21] compared the ceramide profiles of human and porcine skin and found similarities in the distribution of ceramide subclasses. The concentrations of individual ceramide subclasses vary significantly, ranging from 0.45  $\mu\text{g}/\text{mg}$  skin (Ceramide 6) to 2.45  $\mu\text{g}/\text{mg}$  skin (Ceramide 1). These differences may reflect distinct biological functions and interactions with other skin lipids.

**Table 3.** Results for quantification of ceramide levels

Ceramide Subclass	Concentration ( $\mu\text{g}/\text{mg}$ skin)	Standard Deviation
Ceramide 1 (CER[NS])	$2.45 \pm 0.35$	0.15
Ceramide 2 (CER[NP])	$1.83 \pm 0.27$	0.12
Ceramide 3 (CER[AS])	$1.42 \pm 0.23$	0.10
Ceramide 4 (CER[AP])	$0.95 \pm 0.19$	0.08
Ceramide 5 (CER[EOS])	$0.63 \pm 0.15$	0.06
Ceramide 6 (CER[EOP])	$0.45 \pm 0.12$	0.05

### 3.5. Other skin lipids

Table 4 shows the results for other Skin Lipids. Cholesterol is the most abundant lipid ( $14.2 \mu\text{g}/\text{mg}$  skin), followed by triglycerides ( $10.5 \mu\text{g}/\text{mg}$  skin) and phospholipids ( $6.3 \mu\text{g}/\text{mg}$  skin). Squalene and fatty acids are present in lower concentrations.

Ceramides, cholesterol, and triglycerides play crucial roles in maintaining skin barrier integrity. The presence of ceramides, cholesterol, and fatty acids suggests a natural moisturizing mechanism. Imbalances in ceramide and lipid profiles may contribute to skin conditions like dryness, irritation, and inflammation.

**Table 4.** Results for other skin lipids

Lipid Class	Concentration ( $\mu\text{g}/\text{mg}$ skin)	Standard Deviation
Cholesterol	$14.2 \pm 2.1$	0.9
Triglycerides	$10.5 \pm 1.8$	0.7
Phospholipids	$6.3 \pm 1.2$	0.5
Squalene	$2.1 \pm 0.5$	0.2
Fatty Acids	$1.9 \pm 0.4$	0.2

### 3.6. GC-MS shea butter analysis

Table 5 Shows the GC-MS Shea Butter Analysis, Shea butter's fatty acid composition reveals a balanced mix of saturated and unsaturated fatty acids. This composition likely contributes to its moisturizing and barrier-enhancing properties. Comparison to Literature Values indicates the following

- 1) Oleic acid: 40-50% (range reported in literature) [8,22].
- 2) Stearic acid: 20-30% (range reported in literature) [1,8].
- 3) Linoleic acid: 5-10% (range reported in literature) [22,23].

**Table 5.** Shows the GC-MS shea butter analysis

S/N	Fatty Acid	Percentage	Acid Type
1	Oleic acid (C18:1)	43.2%	mainly cis-9 octadecenoic acid
2	Stearic acid (C18:0)	24.5%	octadecanoic acid
3	Linoleic acid (C18:2)	8.4%	cis-9,12 octadecadienoic acid

## 4. Conclusion

In conclusion, this study provides robust evidence of the beneficial effects of shea butter on skin barrier function and hydration, using analytical chemistry methodologies. The significant reduction in Trans-Epithelial Water Loss (TEWL) by 37.8% and increase in skin hydration by 58% within 24 hours demonstrate shea butter's efficacy in improving skin health. The rapid decrease in TEWL and increase in skin hydration suggest immediate effects on skin lipids, indicating shea butter's potential to rapidly restore skin barrier function.

The impedance spectroscopy results showing a 33% increase in skin impedance further support shea butter's barrier-enhancing properties. The GC-MS analysis revealed a balanced fatty acid composition, with oleic, stearic, and linoleic acids being the major constituents. Ceramide profiling identified six subclasses, with Ceramide 1 and 2 being the most abundant, indicating shea butter's potential to modulate ceramide levels and improve skin barrier function.

These findings suggest that shea butter's natural moisturizing properties make it a valuable ingredient in skincare products, particularly for individuals with dry or compromised skin. The study's results are

consistent with previous research on the benefits of natural moisturizers in maintaining skin health. Overall, this study demonstrates the potential of shea butter as a natural, effective, and safe moisturizer for improving skin barrier function and hydration. The findings have implications for the development of skincare products and highlight the importance of natural ingredients in maintaining healthy skin.

Recommendations for future studies include investigating shea butter's effects on specific skin conditions, such as eczema or psoriasis, and exploring its potential synergies with other natural moisturizers.

## 5. Abbreviations

TEWL- Trans-Epithelial Water Loss

GC-MS-Gas Chromatography-Mass Spectrometry

FTIR-Fourier Transform Infrared Spectroscopy

HSE- Human skin equivalents

TM - Tewameter

SCM- Conductance Meter

HPLC-MS- HPLC-MS is High-Performance Liquid Chromatography-Mass Spectrometry

SCM- Skin Conductance Meter NIST- National Institute of Standards and Technology

**Conflicts of Interest:** Institute of Public Analyst of Nigeria (IPAN) an arm of Federal Ministry of Health in Nigeria is much interested in the results of this research, IPAN is an arm of the government who register, supervise, regulate and enforce Private Laboratories in Nigeria.

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