

Tradename: ACB Modified Pomegranate Enzyme PF

Code: 20440PF

CAS #: 84961-57-9 & 1686112-10-6 (or) 84775-94-0 (or) 9015-54-7

Test Request Form #: 9564

Lot #: 8755200

Sponsor: *Active Concepts, LLC; 107 Technology Drive Lincolnton, NC 28092*

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Principal Investigator: *Jennifer Goodman*

Test Performed:

Cellular Renewal Study: Wash-Off

Introduction

As the largest human organ, the skin's integrity is critical to properly function as a physical barrier and maintenance of a healthy appearance for aesthetics. The epidermis constantly undergoes major self-renewal as the superlayer of cells are lost by desquamation and replaced by cells in the basal layers. The constant replacement of cells mitigates the negative long-term effects of ultraviolet light damage and harmful agents (chemicals, pollutants, etc.) on the skin. Aiding in the processes of cellular renewal can improve the skin's physical appearance as well as function as a protective barrier.

Accordingly, a wash-off cellular renewal study was conducted to evaluate the ability of **ACB Modified Pomegranate Enzyme PF** to accelerate skin cell replacement by assessing changes in pigmentation.

Study Principle

Dermal Dye Max™ (active ingredient: dihydroxyacetone (DHA) / glycerone) is applied to the skin and creates artificially high pigmented skin. The controls and test materials are applied to the artificially pigmented areas and pigmentation is measured overtime. The artificially pigmented areas provide a model to assess cellular renewal by measuring pigmentation with decreases in pigmentation representing cellular renewal.

Materials

- A. Equipment:** DermaLab Skin Combo (Skin Color Probe); Digital Camera
- B. Products:** Dermal Dye Max™ (Alpine Valley Naturals); Base Cleanser (Cetaphil® Gentle Cleanser for All Skin Types); Glycolic Acid (positive control)
- C. Software:** Excel Analysis ToolPak (Microsoft)

Methods

Ten volunteers between the ages of 20 and 45, who were known to be free of any skin pathologies with Fitzpatrick skin types I to III, participated in this study (Table 1).

Table 1. The Fitzpatrick Classification of Skin Types Chart¹

Fitzpatrick Skin Type Descriptions*	
Skin Type	Description
I	Always burns, never tans
II	Burns easily, tans minimally
III	Burns moderately, tans to light brown
IV	Burns minimally, tans to moderate brown
V	Rarely burns, tans to dark
VI	Never burns, least sensitive to changes

*Adapted from The Surgeon General's Call to Action to Prevent Skin Cancer

Five test sites were identified on the volar forearm of participants. The first test site was identified as the Comparative Skin Site, and no dye nor treatment were applied to this site to demonstrate normal pigmentation readings. Dermal Dye Max™ was applied to the remaining four test sites and allowed to develop for 24 hours prior to baseline readings. After dye development and prior to initial treatment application, baseline DermaLab pigmentation index readings were taken for all five identified sites. The skin test site conditions and treatments are described below (Table 2). The Untreated Dye Control received Dermal Dye Max™ but no treatment to demonstrate normal cellular renewal.

All cleanser formulations and the Base Lotion were adjusted to a direct pH of 4.3-4.8 (Table 2). The Base Cleanser utilized in this study was Cetaphil® Gentle Cleanser for All Skin Types. Approximately 0.2 g of each treatment was applied to three 2 cm x 2 cm respective locations on the volar forearm. After each treatment was applied, each test site was thoroughly rinsed with warm water and patted dry with a paper towel. Pigmentation readings and images were taken every 24 hours until the active test site returned to baseline. After each daily reading, treatment of each respective test site was performed following the same parameters listed above.

Table 2. Descriptions of the Conditions and Treatments for each Skin Test Site

Skin Test Site	Condition	Dermal Dye Max™ Application?	Treatment / Test Material Application Description	Lotion pH
1	Comparative Skin Site	No	None	N/A
2	Untreated Dye Control	Yes	None	N/A
3	Base Cleanser	Yes	Base Cleanser + Wash-off	4.4
4	5.0% Glycolic Acid	Yes	5.0% Glycolic Acid in Base Cleanser + Wash-Off	4.7
5	5.0% ACB Modified Pomegranate Enzyme PF	Yes	5.0% ACB Modified Pomegranate Enzyme PF in Base Cleanser + Wash-Off	4.6

The percent change of Pigmentation values was calculated for each test site at every timepoint relative to Baseline values, using the following equation:

$$\text{Percent Change (\%)} = \frac{\text{Pigmentation Index}_{\text{Day}} - \text{Pigmentation Index}_{\text{Baseline}}}{\text{Pigmentation Index}_{\text{Baseline}}} \times 100$$

Cumulative Cellular Renewal, represented by the Pigmentation Index values and relative to the Untreated Dye Control, was calculated as area under the curve (AUC) by using the following equation:

$$AUC = \frac{1}{2} \sum_{i=0}^{n-1} (t_{i+1} - t_i) (\text{Pigmentation Index}_i + \text{Pigmentation Index}_{i+1})$$

For pigmentation measurements *Pigmentation Index*₁ and *Pigmentation Index*₂ at times *t*₁ and *t*₂, the AUC between those two time points is equivalent to the product of difference in time and the average of the two Pigmentation measurements. Provided Pigmentation Index values decreased over time AUC was calculated as an inverse and presented as a positive value to demonstrate the amount of cellular relative to the Untreated Dye Control.

Results

The data obtained from this study met criteria for a valid study as the Comparative Skin Site, Untreated Dye Control, and Glycolic Acid performed as anticipated. Application of % **ACB Modified Pomegranate Enzyme PF** accelerated cellular renewal as pigmentation values returned to the baseline levels of the Comparative Skin Site after four days.

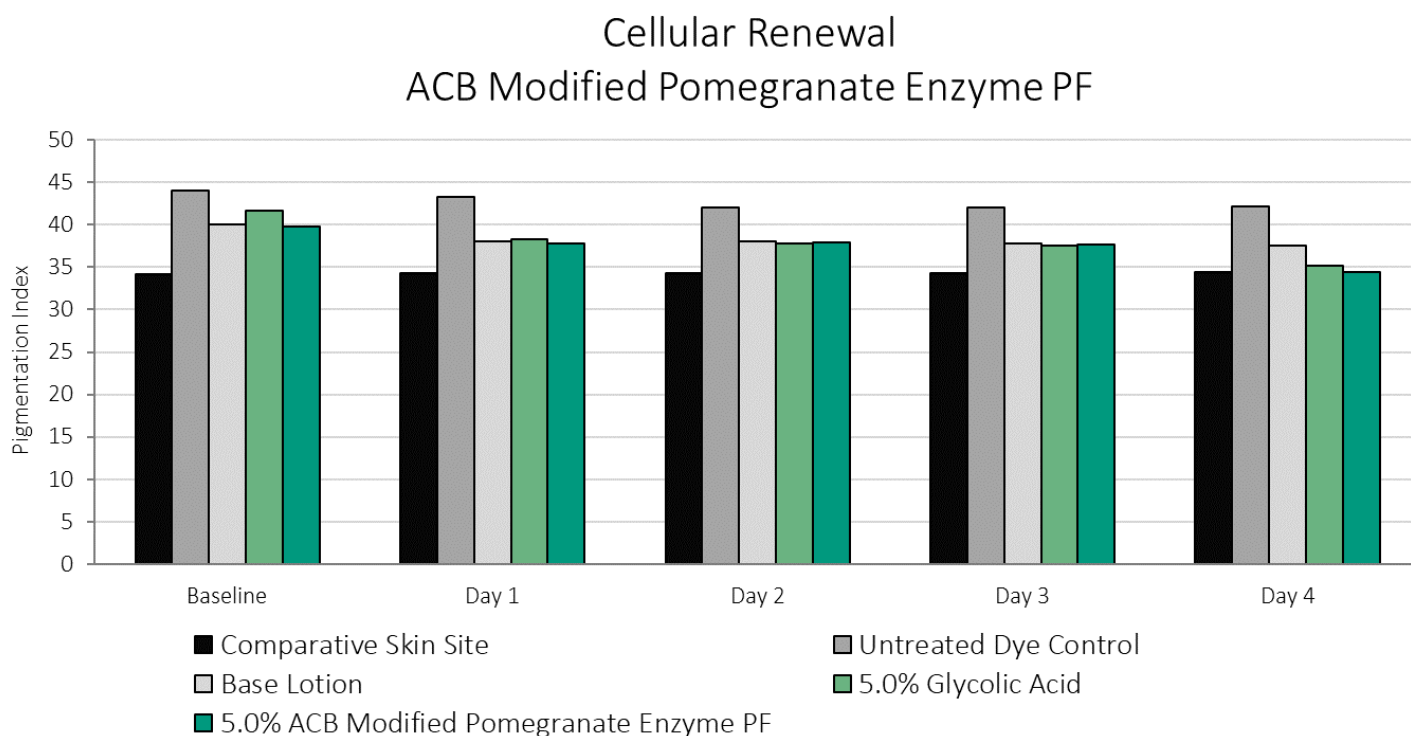


Figure 1. Cellular Renewal of Pigmentation Index Values Overtime

Table 3. Results from T-test Analyses of Pigmentation Index Values from Baseline to Day Four

	Comparative Skin Site	Untreated Dye Control	Base Lotion	5.0% Glycolic Acid	5.0% ACB Modified Pomegranate Enzyme PF
P-value	0.070	0.444	0.051	0.012	0.031

Table 4. Results from T-test Analyses of Pigmentation Index Values After Four Days of Application

	Untreated Dye Control vs Base Lotion	Untreated Dye Control vs 5.0% Glycolic Acid	Untreated Dye Control vs 5.0% ACB Modified Pomegranate Enzyme PF	Base Lotion vs 5.0% Glycolic Acid	Base Lotion vs 5.0% ACB Modified Pomegranate Enzyme PF	5.0% Glycolic Acid vs 5.0% ACB Modified Pomegranate Enzyme PF
P-value	0.094	0.026	0.024	0.068	0.042	0.234

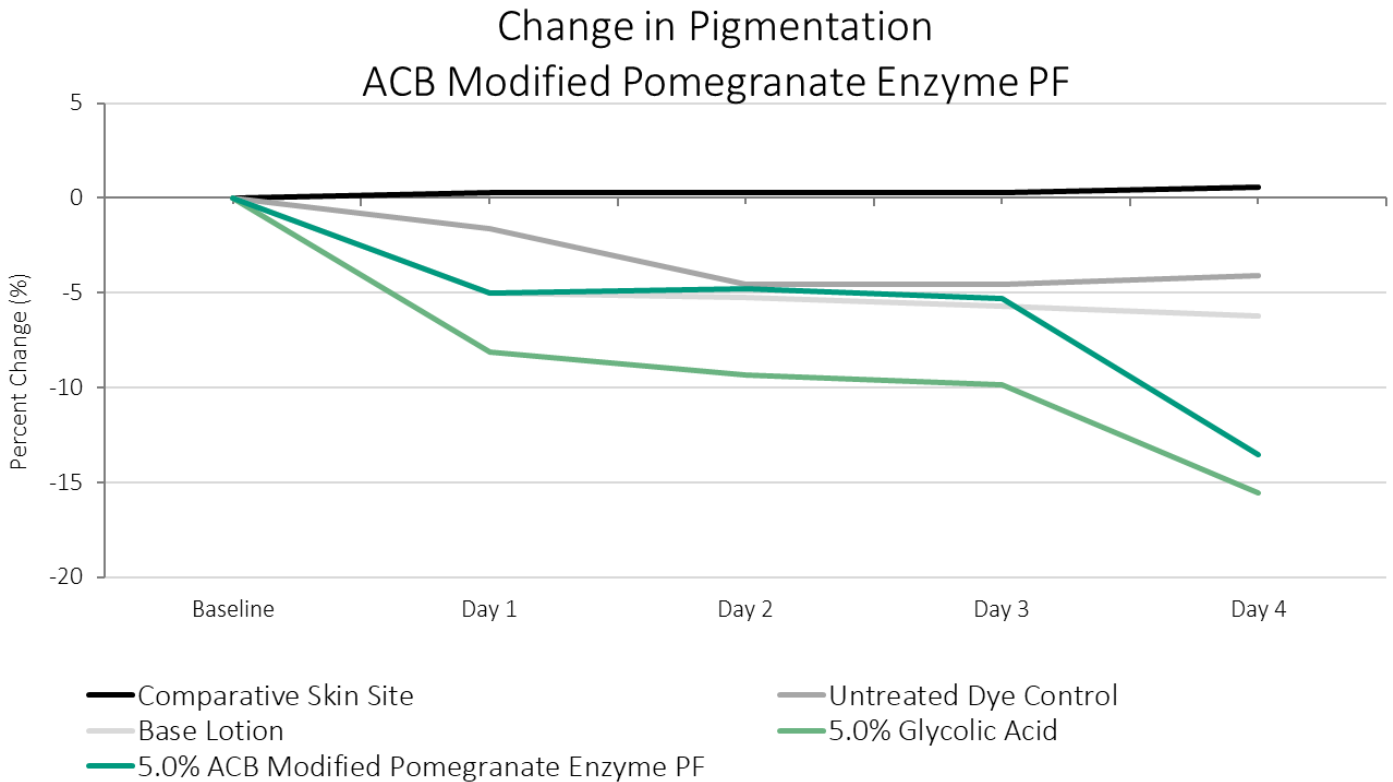


Figure 2. Percent Change in Pigmentation Relative to Baseline Readings

Cumulative Cellular Renewal ACB Modified Pomegranate Enzyme PF

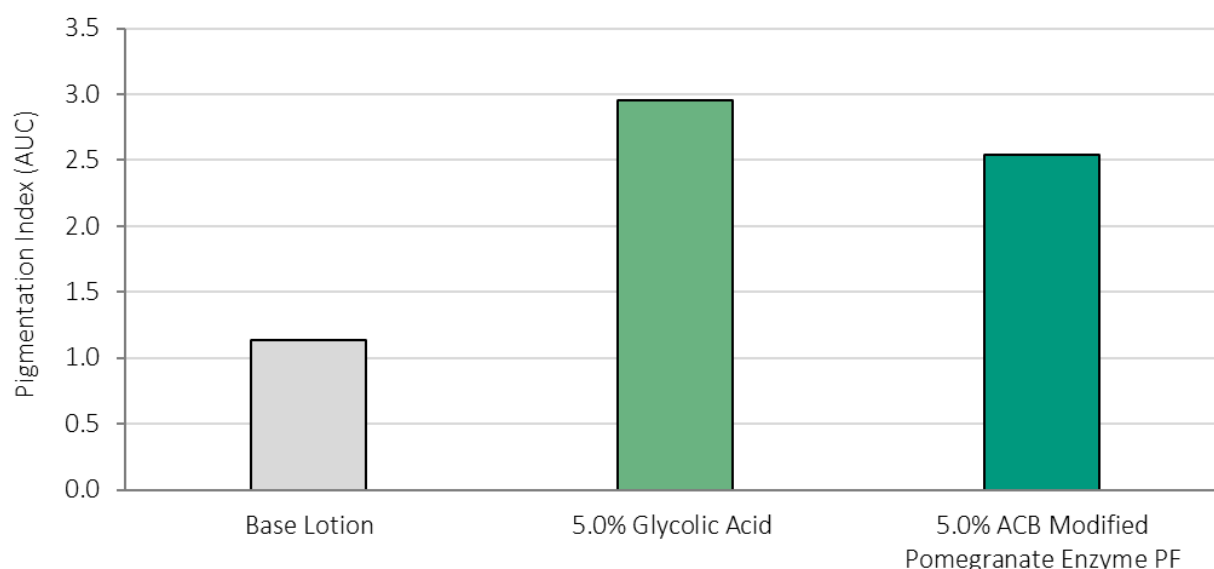


Figure 3. Cumulative Cellular Renewal in Pigmentation Relative to the Untreated Dye Control

Table 5. Results from One-way ANOVA Analysis of Cumulative Cellular Renewal

	Base Lotion vs 5.0% Glycolic Acid	Base Lotion vs 5.0% ACB Modified Pomegranate Enzyme PF	5.0% Glycolic Acid vs 5.0% ACB Modified Pomegranate Enzyme PF
P-value	0.035	0.001	0.196

Discussion

The ability of **ACB Modified Pomegranate Enzyme PF** to accelerate skin cellular renewal was assessed through a daily wash-off study measuring changes in pigmentation. As shown in Figure 1 and 2, the Dermal Dye Max™ artificially augmented pigmentation values at baseline measurements. After four days, the Untreated Dye Control demonstrated a 4% reduction in pigmentation indicating normal cellular renewal after daily application and wash-off (Figures 1, 2; Tables 3, 4). Similarly, the Base Lotion exhibited a 6% decrease in pigmentation demonstrating a slight increase in normal cellular renewal (Figures 1, 2; Tables 3, 4). After four days of application and wash-off, 5.0% Glycolic Acid induced a 16% decrease in pigmentation as expected (Figures 1, 2; Tables 3, 4). Additionally, 5.0% **ACB Modified Pomegranate Enzyme PF** elicited a 14% reduction in pigmentation and performed similar to the Positive Control (Figures 1, 2; Tables 3, 4).

Similar results are shown when the data is displayed to illustrate the collective effect of each treatment when the Untreated Dye Control pigmentation values are accounted for. As shown in Figure 3, 5.0% Glycolic Acid and 5.0% **ACB Modified Pomegranate Enzyme PF** elicited similar changes in cumulative cellular renewal (Table 5).

Taken together, these results indicate **ACB Modified Pomegranate Enzyme PF** enhances cellular renewal when added to personal care applications at recommended use levels. Collectively, **ACB Modified Pomegranate Enzyme PF** accelerates the processes of cellular renewal which can improve the skin's physical appearance as well as function as a protective barrier.

References

1. Sharma AN, Patel BC. Laser Fitzpatrick Skin Type Recommendations. [Updated 2022 Mar 9]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2022 Jan-. Available from: <https://www.ncbi.nlm.nih.gov/books/NBK557626/>