

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 #: 13675

Lot #: N251001AS & N251104E

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

Study Director: *Daniel Shill*

Principal Investigator: *Kayla Goodson*

Test Performed:

Cellular Renewal Study

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. Enzymes are frequently incorporated into topical formulations to aid these renewal processes due to their role as biological catalysts that accelerate specific biochemical reactions. However, when enzymes are used in solubilized form, they may be susceptible to denaturation, potentially resulting in diminished enzymatic activity over time or under stress conditions such as elevated temperature. Consequently, careful evaluation and monitoring of enzymatic activity are essential to confirm product functionality and to understand the extent of biological efficacy within a formulation.

Accordingly, a 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. Bromelain and Papain, proteases with known exfoliative and renewal activity, were also tested as comparative materials.

Study Principle

Derma 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. To understand changes in enzymatic activity over time, samples from time of manufacture (Lot #: N362204E), one month stability (Lot #: N251001A), and one-month heat-treated stability (Lot # N251001A) were used for test materials.

Materials

- A. **Equipment:** DermaLab Skin Combo (Skin Color Probe); Digital Camera
- B. **Products:** Dermal Dye Max™ (Alpine Valley Naturals); Base Cleanser (Cetaphil® Daily Facial Cleanser All Skin Types); Bromelain (comparative material); Papain (comparative material)
- C. **Software:** Excel Analysis ToolPak (Microsoft)

Table 1. Enzyme Test Materials with For Each Production Variable

1.0% Bromelain in Base Lotion	Time of Manufacture
	One Month Stability
	One Month Heat-Treated Stability
1.0% Papain in Base Lotion	Time of Manufacture
	One Month Stability
	One Month Heat-Treated Stability
10.0% ACB Modified Pomegranate Enzyme PF in Base Lotion	Time of Manufacture
	One Month Stability
	One Month Heat-Treated Stability

Methods

Fifteen volunteers between the ages of 20 and 35, who were known to be free of any skin pathologies with Fitzpatrick skin types I to III, participated in this study (Table 2).

Table 2. 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

Each production variable had its own set of five participants who were randomly selected for the respective variable. Six 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 five 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 3). The Untreated Dye Control received Dermal Dye Max™ but no treatment to demonstrate normal cellular renewal.

All lotion formulations and the Base Lotion were adjusted to a direct pH of 5.0 – 5.5 (Table 3). The Base Lotion utilized in this study was Cetaphil® Moisturizing Cream 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. 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 3. Descriptions of the Conditions and Treatments for each Skin Test Site for Each Production Variable

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 Lotion	Yes	Base Lotion	5.4
4	1.0% Bromelain	Yes	1.0% Bromelain in Base Lotion	5.4
5	1.0% Papain	Yes	1.0% Papain in Base Lotion	5.3
6	10.0% ACB Modified Pomegranate Enzyme PF	Yes	10.0% ACB Modified Pomegranate Enzyme PF in Base Lotion	5.4

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.

Percent change in Cumulative Cellular Renewal with respect to the Base Lotion was calculated for each test site, using the following equation:

$$\text{Percent Change (\%)} = \frac{\text{Condition}_{AUC} - \text{Base Lotion}_{AUC}}{\text{Base Lotion}_{AUC}} \times 100$$

Results

The data obtained met criteria for a valid study as the Comparative Skin Site, Untreated Dye Control, and Comparative Materials performed as anticipated for each production variable. For all production variables evaluated, 10.0% **ACB Modified Pomegranate Enzyme PF** demonstrated greater pigmentation reduction than 1.0% Bromelain and 1.0% Papain, with the highest cellular renewal activity observed in the time of manufacture test materials. Application of 10.0% **ACB Modified Pomegranate Enzyme PF** accelerated cellular renewal as pigmentation values returned to the baseline levels of the Comparative Skin Site after three days of application for each production variable.

Cumulative Cellular Renewal ACB Modified Pomegranate Enzyme PF

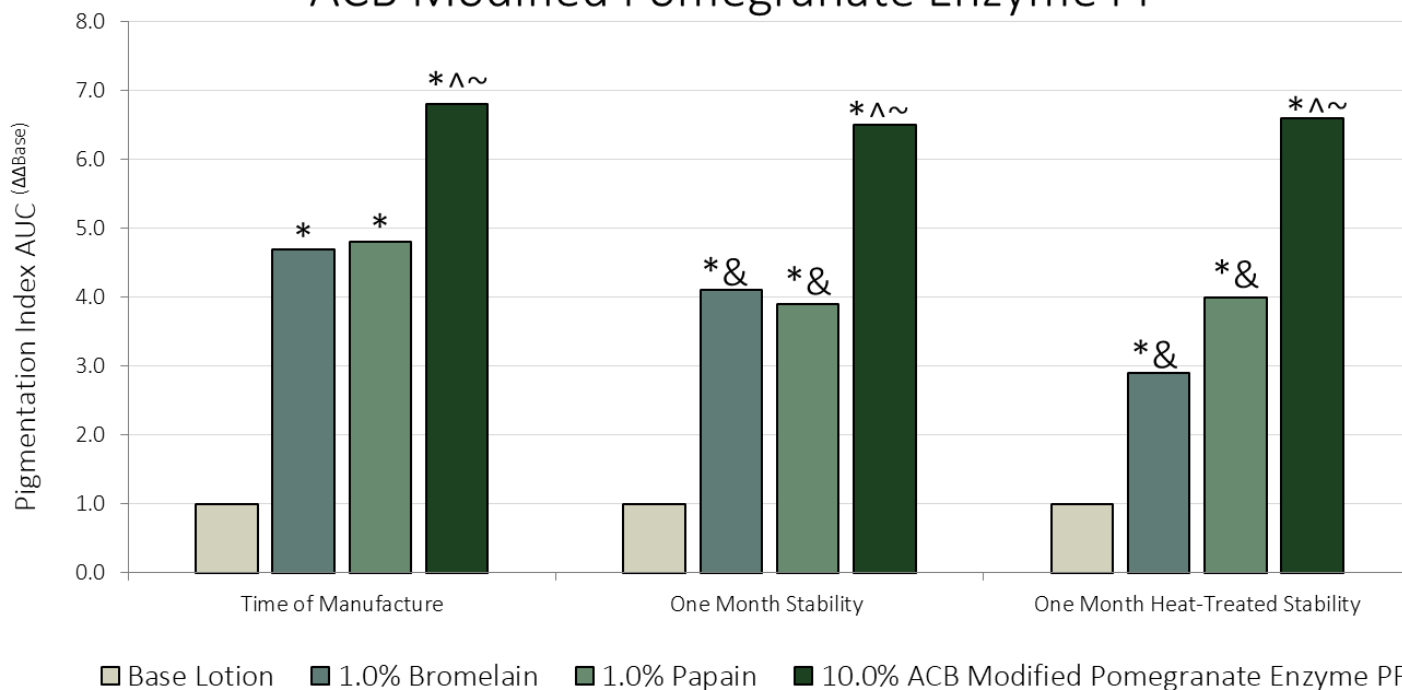


Figure 1. Cumulative Cellular Renewal in Pigmentation Relative to the Untreated Dye Control for Each Production Variable. * indicates significance ($p \leq 0.05$) compared to Base Lotion. ^ indicates significance ($p \leq 0.05$) compared to 1.0% Bromelain. ~ indicates significance ($p \leq 0.05$) compared to 1.0% Papain. & indicates significance ($p \leq 0.05$) within condition compared to Time of Manufacture and One Month Stability Production Variables.

Table 4. Results from One-way ANOVA Analysis of Cumulative Cellular Renewal for Production Variable. * indicates significance ($p \leq 0.05$) compared to Base Lotion. ^ indicates significance ($p \leq 0.05$) compared to 1.0% Bromelain. ~ indicates significance ($p \leq 0.05$) compared to 1.0% Papain.

	Base Lotion vs 1.0% Bromelain	Base Lotion Vs 1.0% Papain	Base Lotion vs 10.0% ACB Modified Pomegranate Enzyme PF	1.0% Bromelain vs 1.0% Papain	1.0% Bromelain vs 10.0% ACB Modified Pomegranate Enzyme PF	1.0% Papain vs 10.0% ACB Modified Pomegranate Enzyme PF
Time of Manufacture	0.042*	0.040*	0.034*	0.518	0.037^	0.032~
One Month Stability	0.038*	0.043*	0.027*	0.345	0.029^	0.036~
One Month Heat-Treated Stability	0.042*	0.048*	0.041*	0.233	0.029^	0.024~











Time of Manufacture					
Baseline					
After Three Days of Application					
	Untreated Control	Base Lotion	1.0% Bromelain	1.0% Papain	10.0% ACB Modified Pomegranate Enzyme PF

Image 1. Participant Images of Each Test Patch at Baseline (Top) and After Three Days (Bottom) for Time of Manufacture Results.




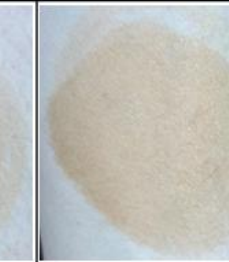




One Month Stability					
Baseline					
After Three Days of Application					
	Untreated Control	Base Lotion	1.0% Bromelain	1.0% Papain	10.0% ACB Modified Pomegranate Enzyme PF

Image 2. Participant Images of Each Test Patch at Baseline (Top) and After Three Days (Bottom) for One Month Stability Results.











One Month Heat-Treated Stability					
Baseline					
After Three Days of Application					
	Untreated Control	Base Lotion	1.0% Bromelain	1.0% Papain	10.0% ACB Modified Pomegranate Enzyme PF

Image 3. Participant Images of Each Test Patch at Baseline (Top) and After Three Days (Bottom) for One Month Heat-Treated Stability Results.

Discussion

The ability of **ACB Modified Pomegranate Enzyme PF** to accelerate skin cellular renewal was assessed through changes in pigmentation across multiple production variables. As shown in Figure 1, the Base Lotion test sites for each production variable did not exhibit significant changes in pigmentation. However, 1.0% Bromelain and 1.0% Papain produced significant cumulative increases in cellular renewal relative to the Base Lotion across each production variable (Figure 1; Table 4). Furthermore, 1.0% Bromelain and 1.0% Papain significantly reduced cumulative cellular renewal after one month stability and one month heat-treated stability, compared to both time of manufacture production variables (Figure 1; Table 4). Notably, 10.0% **ACB Modified Pomegranate Enzyme PF** exhibited significantly greater cumulative cellular renewal, within condition, compared to the Base Lotion and both protease comparative materials for each production variable (Figure 1). Examining images of the skin test sites at Baseline and after three days of application, 10.0% **ACB Modified Pomegranate Enzyme PF** visually outperformed the Base Lotion and both protease comparative materials in terms of reducing pigmentation in each production variable (Images 1, 2, 3). Overall, 10.0% **ACB Modified Pomegranate Enzyme PF** consistently produced the greatest pigmentation reduction compared with 1.0% Bromelain and 1.0% Papain, indicating superior enzymatic activity relative to both proteases over time and under prolonged exposure to elevated temperatures (Figure 1). In contrast, the time of manufacture production variable resulted in the largest increase in cumulative cellular renewal across all test materials, suggesting enzymatic activity is maximal at the time of manufacture and progressively declines with extended exposure to elevated temperatures (Figure 1).

These results indicate **ACB Modified Pomegranate Enzyme PF** enhances cellular renewal across different production variables 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 with a healthier and more vibrant skin as well as function as a protective barrier to help reverse the signs of aging.

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/>