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A novel ex vivo test to assess UV-induced oxidation of human sebum and its protective effects

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1. Introduction

Human sebum is composed of triglycerides, free fatty acids, wax esters, squalene, cholesterol, and diglycerides, with squalene accounting for approximately 10–15% of the total lipid content. Among these components, squalene functions as a natural emollient that helps maintain skin hydration and protects the skin from oxidative damage [1,2].

However, due to its unsaturated structure, squalene is highly susceptible to oxidation triggered by external factors such as ultraviolet (UV) radiation and tobacco smoke. Oxidized squalene has been shown to have detrimental effects on the skin, acting as an irritant and a comedogenic substance. Furthermore, it is known to exacerbate acne lesions and promote inflammatory responses [3,4]. Therefore, inhibiting the oxidation of squalene is considered crucial for maintaining skin health and preventing skin disorders. In this context, the development of cosmetic formulations containing antioxidants has been actively pursued to enhance the skin's defense against various external stressors.

In this study, we aimed to establish an *ex vivo*-based evaluation method that enables the rapid and convenient quantification of squalene oxidation products in sebum, thereby allowing for the assessment of the antioxidant efficacy of the test product. Additionally, we sought to analyze changes in sebum squalene oxidation following the application of a formulation containing vitamin C and to evaluate its effectiveness in improving skin condition.

2. Materials and Methods

The study was conducted in accordance with the Ethical Principles for Medical Research Involving Human Subjects outlined in the Declaration of Helsinki [5] (IRB approval number: P2501-7653). A total of 34 healthy female adults (ages 30–49 years, mean age 42.853 ± 5.456 years) participated in the study. The test product used in this study was a serum formulation (Amorepacific, Korea) containing 40% vitamin C. Participants applied the test product to their entire face once daily for 28 consecutive days.

Sebum samples were non-invasively collected from the facial skin using Sebutape(S100, CuDerm Corp., USA). To induce oxidation, the collected samples were irradiated with ultraviolet (UV) light and subsequently analyzed using desorption electrospray ionization mass

spectrometry (DESI-MS). The experiments were performed on a Waters Xevo G2-XS Quadrupole Time-of-Flight (QTOF) mass spectrometry system (Waters Corporation, USA). The degree of oxidation was assessed by quantifying the ratio of squalene to squalene peroxide levels (SqOOH ratio), and imaging was performed to evaluate the antioxidant efficacy of the product. Skin brightness and tone evenness were assessed by analyzing facial images collected using the VISIA-CR system(Canfield Imaging Systems, USA), while protein carbonylation levels were evaluated using corneocyte samples collected from the facial skin before and after product application.

3. Results

This study confirmed that exposure to light including ultraviolet (UV) radiation can induce the oxidation of squalene in sebum. Using DESI-MS analysis, the oxidation status was assessed by calculating the ratio of the intensity of squalene peroxide to that of squalene.

Following UV irradiation, the SqOOH ratio significantly increased compared to the negative control group. In contrast, sebum collected from areas treated with the test product showed a significantly lower level of squalene oxidation even after UV exposure compared to untreated areas (Figure 1). The oxidation inhibition rate, calculated based on the data below, was 37.49%, demonstrating the antioxidant efficacy of the test formulation.

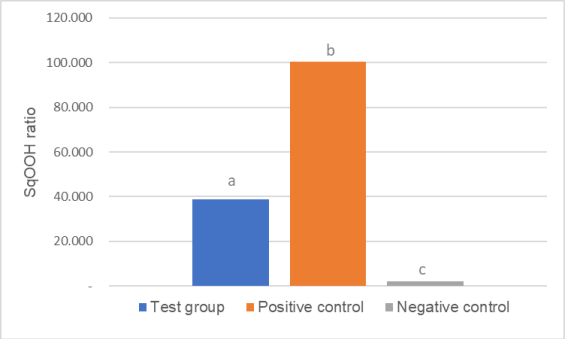


Figure 1. SqOOH ratio in sebum across different groups. The test group represents areas where the product was applied, the positive control refers to areas with no product application and UV exposure, and the negative control refers to areas with no product application and no UV exposure. Bars labeled with different letters (a, b, c) are significantly different from each other (p<0.05).

Furthermore, in evaluations without additional UV irradiation, the SqOOH ratio in natural sebum significantly decreased after 28 days of product use compared to baseline, indicating a sustained antioxidant protective effect (p<0.05, Figure 2).

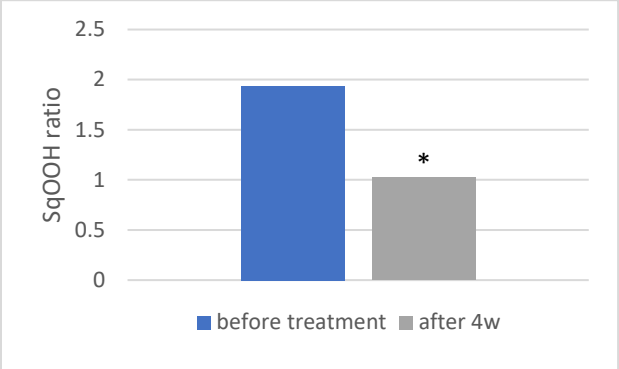
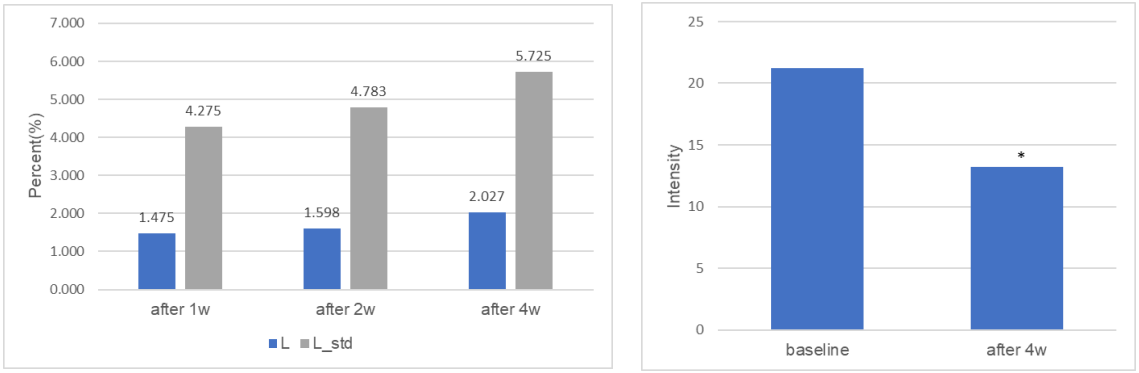


Figure 2. Comparison of the SqOOH ratio in sebum before and after 4 weeks of product use.

Analysis of skin brightness and tone evenness revealed improvements of 2.027% and 5.725%, respectively, after 28 days of product application. In addition, the level of protein carbonylation in corneocyte samples collected from the face also showed a significant decreasing trend after product use (Figure 3).



(**a**) (**b**)

Figure 3: (a) Percentage improvement in skin brightness and brightness evenness compared to baseline. (b) Changes in protein carbonylation levels in the stratum corneum after 4 weeks of product use.

4. Discussion & Conclusion

This study established an *ex vivo*-based evaluation method for the rapid and convenient quantification of oxidation levels in skin sebum and the assessment of the antioxidant efficacy of cosmetic formulations. In particular, using collected sebum to induce oxidation externally allowed for the rapid evaluation of the product's oxidation-inhibitory capacity. However, as this method relies on ex vivo sebum samples, it may not fully replicate the oxidative processes occurring on the skin surface *in vivo*. Nonetheless, the observed reduction in squalene oxidation levels after 28 days of product use under natural conditions suggests that the product may exert antioxidant effects directly on the skin surface, partially addressing this limitation. Future studies should further evaluate *in vivo* oxidative markers and clinical skin changes over extended periods.

Moreover, considering the high concentration of pure vitamin C, a potent antioxidant, in this formulation, it is reasonable to attribute the observed antioxidative effects at least in part to its activity. Oxidative stress increases levels of oxidative byproducts in the skin, leading to oxidative damage to proteins and changes in skin tone. The test product was shown to inhibit the

generation of oxidative products induced by UV exposure, reduce protein carbonylation in the stratum corneum, and improve skin brightness and tone evenness. Collectively, these findings support the potential of the product as an effective solution for maintaining skin health over the long term.

5. References

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