cosmetics

MDPI

Article

Evaluating the Effect of Fresh and Aged Antioxidant Formulations in Skin Protection Against UV Damage

John Ivarsson [1,2], Patricia Brieva [3], Hina Choudhary [3] and Giuseppe Valacchi [2,4,5,*]

[1] Department of Food, Bioprocessing and Nutrition Sciences, Plants for Human Health Institute, NC Research Campus, NC State University, Kannapolis, NC 28081, USA; jcivarss@ncsu.edu
[2] Department of Animal Sciences, Plants for Human Health Institute, NC Research Campus, NC State University, Kannapolis, NC 28081, USA
[3] SkinCeuticals, New York, NY 07008, USA; patricia.brieva@loreal.com (P.B.); hina.choudhary@loreal.com (H.C.)
[4] Department of Environmental and Prevention Sciences, University of Ferrara, 44121 Ferrara, Italy
[5] Department of Food and Nutrition, Kyung Hee University, Seoul 02447, Republic of Korea
* Correspondence: gvalacc@ncsu.edu

Abstract

Introduction: Extrinsic skin damage is often a result of oxidative stress caused by exposure to environmental factors such as ultraviolet (UV) radiation, ozone (O_3), and various pollutants. As a result, topical antioxidants have been evaluated for their effectiveness in mitigating or reversing skin damage caused by environmental factors. Topical antioxidants containing a combination of l-ascorbic acid, tocopherol, and ferulic acid have significantly improved markers of skin health after exposure to environment-induced skin damage. However, research suggests that l-ascorbic acid and tocopherol tend to be relatively unstable, possibly affecting their efficacy against outdoor stressor damage. It has been shown that ferulic acid significantly improves the stability of both l-ascorbic acid and tocopherol, but its long-term stabilization effects on these antioxidants are relatively unknown. Material and Methods: This study evaluated the time-dependent effectiveness of a topical antioxidant mix containing 15% l-ascorbic acid, 1% tocopherol, and 0.5% ferulic acid (AOX) on UV-induced skin damage. Skin biopsies (12 mm, n = 60) were placed in a 6-well plate with medium and incubated at 37 °C and 5% CO_2 overnight. The day after, skin samples were pretreated with 10 µL of differently aged AOX (0-, 6-, 12-, and 36-month-old) and then exposed to different doses of UV light (100, 200, 400 mJ/cm^2) daily over four days. AOX formulations were stored in a cool, dry, and dark place at approximately 20–22 °C during the whole study. This study evaluated 4-hydroxynonenal (4-HNE) and 8-hydroxy-2′-deoxyguanosine (8-OHdG) as oxidative damage and skin DNA damage markers, Collagen1 and Filaggrin as skin structure, and IL-8 and Nrf2 as inflammatory and defensive response. Results: UV exposure significantly increased oxidative and inflammatory markers in human skin explants affecting also filaggrin and collagen levels. However, pre-treatment with the antioxidant formulation, particularly in its younger formulations (0-, 6-, and 12-month-old), significantly reduced the damaging effect of UV. Additionally, all antioxidant formulations effectively mitigated UV-induced damage across all doses. Conclusions: Our results indicate that pre-treatment with this formulation consistently reduces UV-induced oxidative damage and DNA damage in human skin explants, regardless of the formulation age and the discoloration state. Although effective, the protective capacity of aged formulations may be reduced only when extreme UV exposure is tested, a condition that is unlikely to occur under typical environmental conditions. These results support ferulic acid as a stabilization agent for topical antioxidant mixtures.

Academic Editor: Enzo Berardesca

Received: 16 June 2025
Revised: 4 August 2025
Accepted: 5 August 2025
Published: 7 August 2025

Citation: Ivarsson, J.; Brieva, P.; Choudhary, H.; Valacchi, G. Evaluating the Effect of Fresh and Aged Antioxidant Formulations in Skin Protection Against UV Damage. *Cosmetics* **2025**, *12*, 166. https://doi.org/10.3390/cosmetics12040166

Keywords: antioxidants; stability; photoaging; collagen

1. Introduction

The skin is the key barrier to an extensive array of exogenous stressors, warranting the use of topical cosmetic formulations to enhance the skin barrier, preserving its health [1]. The usage of topical cosmetics to enhance skin characteristics is well demonstrated and understood to be an optimal approach to skin health [2]. Indeed, consumers understand the importance of skin health, exhibited by the continued annual growth of the cosmetic market valued over USD 100 billion [3]. While the cosmetic market can include a variety of products from haircare to perfumes, skincare remains the leading category underlining the importance of cosmetic research [3]. One of the predominant mechanisms of cosmetic formulations is proactive protection from incoming exogenous stressors, which can drastically degrade essential structural proteins needed to retain ideal skin complexion, strength, and elasticity [4,5]. These formulations can comprise any number of thousands of ingredients with a total of 12,500 chemicals approved for usage in skincare products [6]. Among these ingredients, antioxidant compounds are among the most common; this is due to their crucial role in combating the formation of reactive oxygen species (ROS), key oxidative molecules that signal and perpetuate inflammatory processes [7,8].

Antioxidant skincare ingredients often include key vitamins with antioxidant capacity like vitamin C and E, as well as cofactors for antioxidant defense enzymes like zinc; antioxidants are not limited to vitamins and can also include other molecules with proven antioxidant activity like carotenoids and polyphenols [9–11]. A wide range of antioxidant compounds have been proven to effectively scavenge oxidative stress mediators (reactive oxygen species–ROS), therefore inhibiting the activation of inflammatory mechanisms [8,11]. This interaction is now defined as an OxInflammatory tissue responses as oxidative stress mediators can further trigger inflammation in a positive feedback cycle. [12]. Outside of ROS scavenging, antioxidant compound application has demonstrated promising enhancements in skin tone and hydration as well as reducing premature aging and wrinkle formation [13]. Studies have demonstrated the ROS activation of numerous inflammatory signaling pathways such as the activation of nuclear factor kappa-B (NF-κB), inflammasomes, mitogen-activated protein kinase; the pathways can stimulate the production of proinflammatory cytokines like interleukin 1 alpha (IL-1α) and interleukin 8 (IL-8) [14–16]. While antioxidant compounds are indisputability effective in reducing ROS and enhancing cutaneous characteristics, studies on unique antioxidants in controlled laboratory settings do not consider interactions with other cosmetic ingredients and the shelf-life of formulations [17]. Moreover, they fail to address how the daily use of formulations by the consumer could compromise the formulation's stability and reduce overall protective efficacy [18]. This concern, in the cosmetics industry, is partially addressed through approaches aimed to maintain the physicochemical stability of the compounds (i.e., specialized packaging, chemical preservatives, multifunctional ingredients) [18].

UV exposure has been demonstrated to be among the most aggressive and prevalent of the exogenous daily stresses encountered by the skin [5,19,20]. Through its interactions with both epidermis (UV-B/UV-C) and dermis (UV-A), UV radiation upregulates the production of ROS and stimulates the formation of DNA photolesions like pyrimidine dimers [21,22]. The abundance of ROS can overwhelm cutaneous defense, leading to degradation of important structural proteins and weakening skin health and appearance [23]. Extensive UV exposure has been definitively linked to cutaneous premature aging and an increased incidence of numerous skin ailments and cancers, such as basal cell carcinoma

and melanoma [24]. Exogenous skin aging from UV light, also known as photoaging, is characterized by clinical manifestations of deep wrinkles, hyperpigmentation and rough skin [25]. This underlines the importance of topical applications to supplement basal cutaneous defenses slowing the process photoaging.

In this study, we employed human skin biopsies to evaluate the ability of a cosmeceutical formulation comprising 15% l-ascorbic acid, 1% tocopherol, and 0.5% ferulic acid to counteract the damaging oxidative effects of UV exposure. To retain real-world conditions surrounding the storage and usage of cosmetic formulations, this study implements two models: a vertical testing of a sealed formulation across 0 to 36 months, and the comparison of closed formulation with one used daily for 6 months. The protective efficacy of these formulations was assessed through explant exposure to a variety of UV doses: 100, 200, and 400 mJ/cm^2 over four days. Doses were chosen to mimic a mild, everyday exposure (100 mJ/cm^2), a realistic exposure under strong sunlight (200 mJ/cm^2), and an intense exposure in high UV-index areas (400 mJ/cm^2) [26,27].

A variety of oxidative and inflammatory markers, such as 4-HNE protein adduct formation and cytokine release, were examined to understand the effects of the different cosmetic formulations on OxInflammatory protective efficacy. Additionally, to understand modulations to antioxidant defense as well as skin strength and elasticity, nuclear factor erythroid 2-related factor 2, hemeoxygenase-1, epidermal and dermal proteins were surveyed.

2. Materials and Methods

2.1. Tissue Culture and Treatments

Human Caucasian skin explants were obtained from elective abdominoplasties from 3 different donors after the approval of the Institutional Biosafety (IBC) Committee at NC State University [28]. Before biopsies, the explant was washed in phosphate-buffered saline supplemented with 100 U/mL Penicillin and 100 µg/mL Streptomycin. Afterwards, a 12 mm punch biopsy was used to extract biopsies from the explant, and the subcutaneous fat was removed. Biopsies were cultured in 6-well plates with 1 mL of 4.5 g/L high glucose Dulbecco's Modified Eagle Medium (DMEM). After overnight recovery in an incubator at 37 °C in 5% CO_2, tissues were pretreated with 10 µL, a topical antioxidant mix containing 15% l-ascorbic acid, 1% tocopherol, and 0.5% ferulic acid (AOX); in addition, the following ingredients were included in the commercially available serum: water, glycerin, aqueous glycol system, laureth-23, phenoxyethanol, and sodium hyaluronate. AOX components were stored at room temperature, in an amber color glass bottle, in a Post Consumer Recycle dropper with a glass pipette. The topical antioxidant mixes were kept in the sealed bottle for 0, 6, 12, and 36 months. Alternatively, to mimic consumer usage, another experiment was conducted using a fresh, 0-month-old (closed, C) antioxidant mix and a mix used daily over a period of 6 months (opened, O). Every day, the antioxidant mix was opened and 2 drops were taken with the bottle dispenser, to mimic the daily usage. After 6 months of usage, the experiments were performed. Concentration of the AOX components during the 6-month experiment's average was as follows: Ascorbic Acid 13.5 to 16.5%, a-tocopherol from 0.9 to 1.1%, and Ferulic acid from 0.45 to 0.55%.

As seen in Figure 1, tissues were irradiated with 100, 200, and 400 mJ/cm^2 of UVA/UVB light using UVA/UVB Newport Oriel, Sol1ATM, 1600W, Xenon Lamp, UVC & AM0 filters after two 2 h of pretreatment at room temperature, circa 20–22 °C. During exposure, UV doses were monitored with a radiometer ILT2400 Hand-Held Light Meter Optometer (International Light Technologies, INC., Peabody, MA, USA). UV exposure was conducted daily for four days, totaling four UV exposures; 24 h after the last exposure, biopsies and cell culture media were collected for analysis.

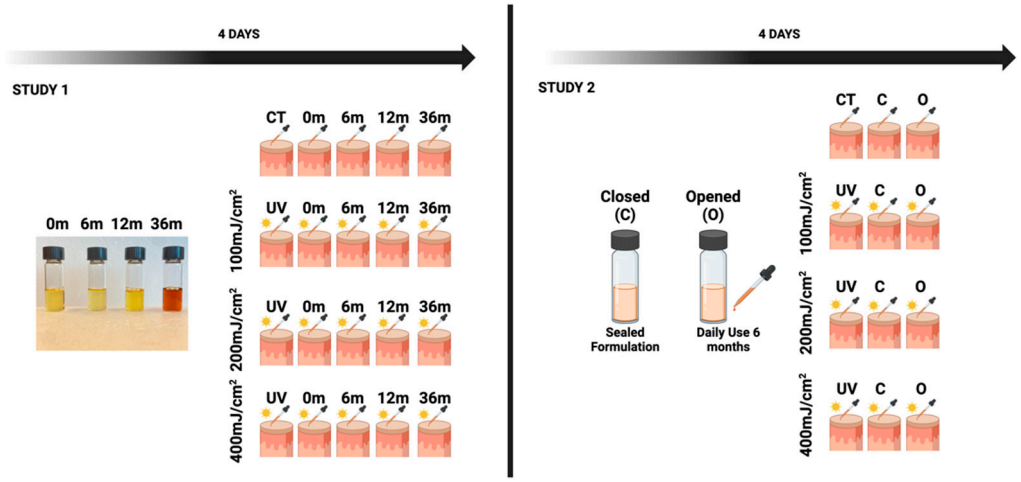

Figure 1. Treatment timeline and experimental conditions.

2.2. Skin Immunofluorescence

After the conclusion of experiments, 12 mm skin biopsies were collected and placed in cassettes containing 10% neutral buffered formalin at room temperature for 24 h and subsequently transferred into 70% ethanol for an additional 24 h. After, the cassettes were transferred into a Leica tissue processor (Leica Biosystems, Deer Park, IL, USA) to dehydrate the tissues using the following steps: 30 min ethanol (EtOH) 70%, 30 min EtOH 80%, 60 min EtOH 95%, 90 min EtOH 100%, 90 min xylene, overnight paraffin. Tissue samples were removed from the machine and embedded in paraffin using Shandon Histocentre. Samples were then sectioned using a microtome (Leica Biosystems, Deer Park, IL, USA). Slides were heated at 60 °C for 20 min and deparaffinized using xylene and subsequently rehydrated using decreasing alcohol gradient solutions (100%, 80%, 70%, 30% EtOH). Slides were placed in distilled water, and antigen retrieval was performed using citrate buffer (cat. C9999, Sigma-Aldrich, Merck Millipore, Burlington, MA, USA) (pH 6.0) in a 95 °C heated water bath for 10 min. Following antigen retrieval, samples were left at room temperature for approximately 20–30 min to cool, washed twice in PBS, and then blocked with 2% FBS in PBS for 45 min at room temperature. Tissues were then incubated overnight at 4 °C with primary antibodies: 8-OHdG (sc-393871, Santa Cruz Biotechnology Inc., Dallas, TX, USA), 4-HNE (AB5605, Millipore Sigma, Burlington, MA, USA), filaggrin (sc-66192, Santa Cruz Biotechnology Inc., Dallas, TX, USA), Collagen-1 (ab138492, Abcam, Cambridge, UK), and Nrf2 (sc-365949, Santa Cruz Biotechnology Inc., Dallas, TX, USA), HO-1 (PA5-27338, Invitrogen, Thermo-Fisher Scientific, Waltham, MA, USA) in 0.25% BSA in PBS. The next day, slides were washed three times in PBS for 5 min and incubated with corresponding fluorochrome-conjugated secondary antibodies (Alexa Fluor 568 A11057, Alexa Fluor 568 A11004, Alex Fluor A11008) at 1:500 dilution in 0.25% BSA/PBS) at room temperature for one hour. Next, the slides were washed another three times in PBS, and the nuclei were stained with DAPI (D1306, Invitrogen, Thermo-Fisher Scientific, Waltham, MA, USA), and examined with a Zeiss Z1 AxioObserver LSM10 confocal microscope at 40× magnification. Images were then quantified using ImageJ software 1.53a (Java 1.8.0_172, National Institutes of Health, Bethesda, MD, USA) [16].

2.3. Skin Protein Extraction and Western Blotting

Snap-frozen 12 mm human skin biopsies were homogenized in T-PER™ Tissue Protein Extraction Reagent (Thermo Fisher Scientific, Waltham, MA, USA) with 1% of phosphatase and protease inhibitor cocktails (Sigma, USA) at 1:20 (w/v) ratio with a specific amount of tissue (1 mg per 20 µL). Processed samples were placed in tubes with ceramic beads

and homogenized with a Precellys Evolution tissue homogenizer equipped with Cryolys Evolution cooling unit. Tubes were homogenized at 6500 rpm × three cycles with 30 s rests. Samples were then centrifuged at 10,000× *g* for 15 min at 4 °C, and the supernatant was subsequently collected. Protein extract was quantified using Quick Start Bradford protein assay (Bio-Rad, Hercules, CA, USA). Following quantification, equivalent amounts of proteins were loaded into 12% polyacrylamide SDS gels and separated by molecular size. Gels were electroblotted onto nitrocellulose membranes, washed briefly with water, and then stained with Ponceau for 5 min. After a five-minute wash with double distilled water, a ChemiDoc (BioRad, USA) was used to image the Ponceau stain for protein normalization. After imaging, membranes were washed with TBS-T, and blocked with EveryBlot Blocking Buffer (Bio-Rad, USA) for five minutes. Nitrocellulose membranes were then incubated at 4 °C overnight on rocker at 60 rpm with primary antibodies: 4-HNE (AB5605, Millipore Sigma, Burlington, MA, USA) and filaggrin (sc-66192, Santa Cruz Biotechnology Inc., Dallas, TX, USA) diluted in EveryBlot Blocking Buffer. The next day, membranes were washed three times for ten minutes with TBS-T and incubated with secondary antibody for one hour (170–6515, 170–6516, 1721037, BioRad, USA). Detection of chemiluminescence was conducted with ChemiDoc (BioRad, USA), and bands were quantified using ImageJ software 1.53a (Java 1.8.0_172, National Institutes of Health, Bethesda, MD, USA).

2.4. ELISA Assays

Cutaneous release of inflammatory cytokines IL-8 and IL-1α were determined in the media (2 mL) in which the biopsies were cultured Human IL-8 ELISA Kit (Invitrogen #KAC1301, Carlsbad, CA, USA) and Human IL-1 alpha ELISA Kit (Invitrogen #BMS243-2.) The absorbance was measured with a spectrophotometer with a filter indicated in kit instructions. The Gen5 2.0 software (BioTek, Agilent, Santa Clara, CA, USA) was used for detection. Tests were conducted according to the kit protocols— GraphPad Prism 10 Version 10.3.1 (464) Boston, MA, USA, (www.graphpad.com) was used to create a 5-parameter standard curve and to extrapolate data.

2.5. Statistical Analysis

Statistical analysis was performed using GraphPad Prism 10 Version 10.3.1 (464) Boston, Massachusetts, MA, USA (www.graphpad.com). Analyses performed include ordinary one-way ANOVA with subsequent Tukey multiple comparisons for each condition tested. Data on bar graphs are expressed as mean with standard deviation of the mean (SEM) obtained in three independent experiments. Statistical significance was considered at * $p <$ or equal to 0.05.

3. Results

3.1. Comparison Between Fresh and Aged AOX Formulations

3.1.1. Oxidative Damage—Lipid Peroxidation and DNA Oxidation

UV exposure is known to lead to the production of ROS, which can subsequently increase lipid peroxidation and DNA damage. As a proof of principle, the formation of 4-HNE protein adducts, an immediate byproduct of lipid peroxidation, and 8-OHdG, a production of DNA oxidation, was measured in human skin explants. Figure 2A demonstrates that, under our experimental conditions, exposure to 100, 200, and 400 mJ/cm^2 of UV radiation significantly induced the formation of 4-HNE protein adducts in the exposed skin 2-fold compared to the untreated control. All aged AOX formulations were able to significantly protect against damage across all UV doses except for 36-month aged AOX at 400 mJ/cm^2. Amongst the aged AOX formulations, fresh, 0-month AOX provided higher lipid peroxidation protection in high-dose UV (400 mJ/cm^2)

scenarios with a 70.42% difference compared to the UV 400 mJ/cm^2 explant; other AOX formulations aged 6 through 36 months exhibited similar a percentage of protection, around 20%, as depicted in Figure 2B. 4-HNE protein adduct levels were confirmed also via Western blot analysis (Figure 2C,D), with significant similar reductions in 4-HNE protein adduct formation among all the aged formulations in human skin biopsies irradiated with 200 mJ/cm^2.

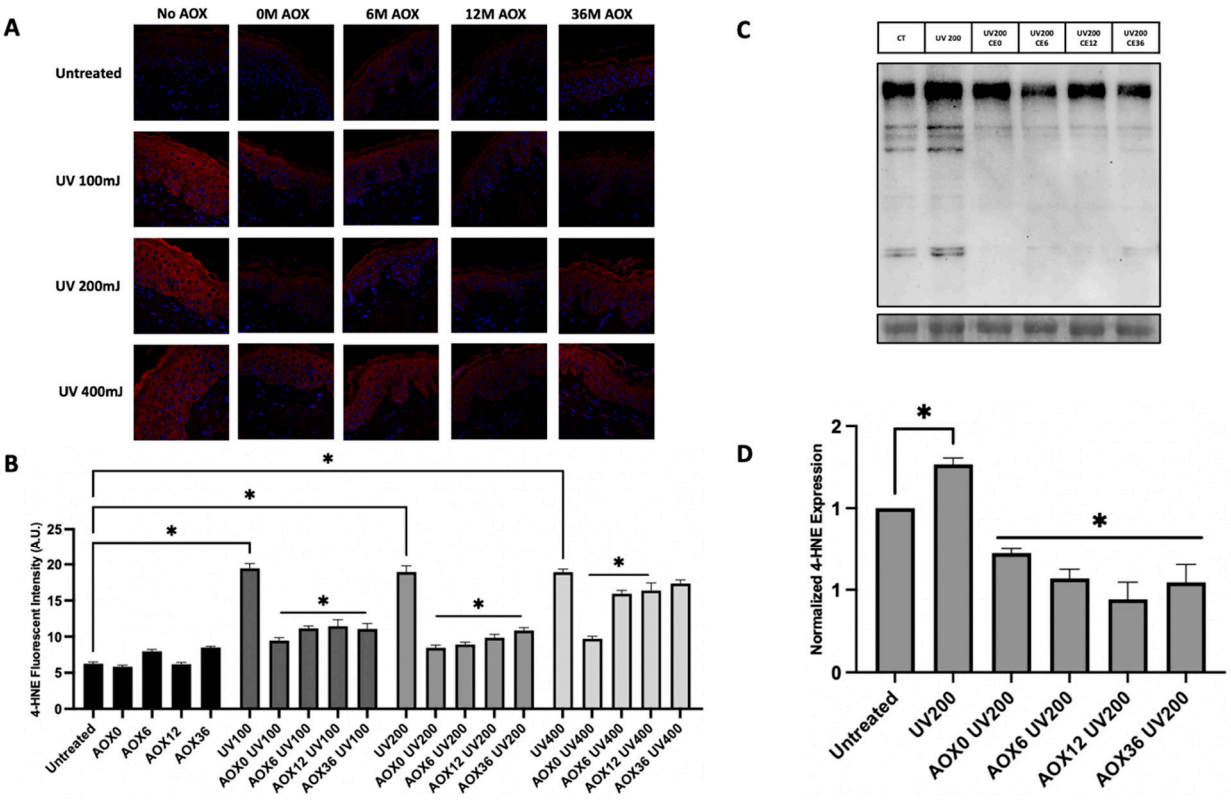

Figure 2. Formation of 4-HNE protein adducts in human skin explants treated with varying doses of UV light following pre-treatment with different aged AOX formulations. (**A**) Representative images of immunofluorescence staining for 4-HNE and (**B**) ImageJ quantification of fluorescent signal. (**C**) Representative Western blot image of 4-HNE (top band) and ponceau staining (bottom band) and (**D**) normalized ImageJ quantification of Western blot bands. Data analysis consisted of one-way ANOVA followed by Tukey's post hoc comparison test. Data are the results of the averages of at least three different experiments, * $p < 0.05$. Line with * demonstrates significance to respective UV dose (e.g., UV100 to AOX0 UV100).

As depicted in Figure 3A,B, cutaneous exposure to 200 mJ/cm^2 of UV manifested the highest DNA oxidation among all the tested doses, with a 31.04% increase compared to the control. Exposure to 100 and 400 mJ/cm^2 exhibited a slight insignificant increase compared to the control with 22.96% and 7.87% increases, respectively. Pretreatment with the AOX formulations was effective in reducing cytoplasmic 8-OHdG presence induced through all doses of UV irradiation and exhibiting some effect in basal conditions. Exposure to 400 mJ/cm^2 of UV radiation, a high-dose condition, did not result in a statistically significant reduction in DNA oxidation with the 36-month-old AOX formulation.

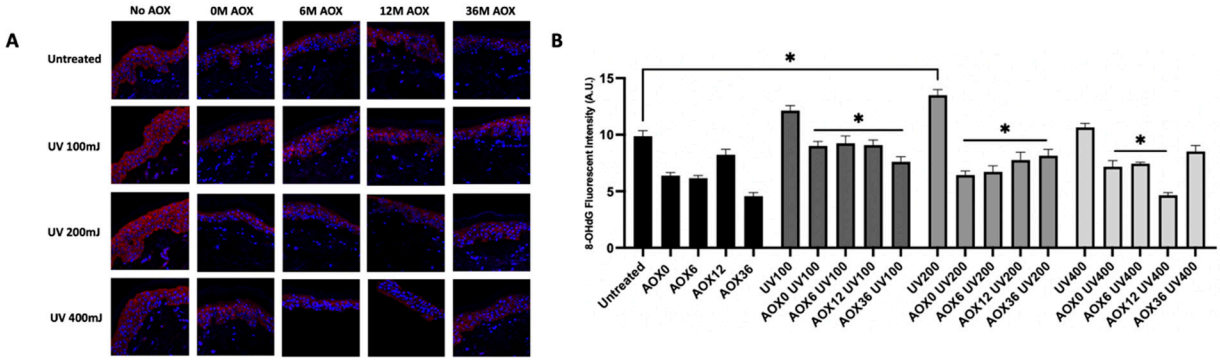

Figure 3. 8-OHdG formation in human skin explants treated with varying doses of UV light following pre-treatment with different aged AOX formulations. (**A**) Representative images of immunofluorescence staining for 8-OHdG and (**B**) ImageJ quantification of fluorescent signal. Data analysis consisted of one-way ANOVA followed by Tukey's post hoc comparison test. Data are the results of the averages of at least three different experiments, * $p < 0.05$. Line with * demonstrates significance to respective control (e.g., UV100 to AOX0 UV100).

3.1.2. Cutaneous Antioxidant and Inflammatory Response

To assess the ability of the AOX formulations to activate tissue cellular antioxidant defense, the activation levels of the transcription Nrf2 and the protein levels of HO-1 were investigated. In Figure 4A,B, significant nuclear translocation of Nrf2 was seen in irradiated cutaneous biopsies with all the UV doses (from 100 to 400 mJ/cm^2). AOX formulations pretreatment exhibited lower nuclear Nrf2 compared to their untreated counterparts, as demonstrated in the images in Figure 4A. The benefits were best demonstrated in 100 mJ/cm^2 irradiated biopsies with an average 90% difference in AOX pretreated biopsies when compared to the UV control. Additionally, as seen in Figure 4C, UV exposure significantly increases HO-1 epidermal protein expression with 75.07%, 90.60% and 56.8% increases across 100, 200, and 400 mJ/cm^2 doses, respectively. Similarly to 4-HNE and 8-OHdG, 36-month aged formulations exhibited reduced efficacy against high-dose UV exposure upregulation of HO-1.

As shown in Figure 5A, four days of UV irradiation caused a significant release of IL-8 in the tissue culture as it concerns 100 and 200 mJ/cm^2 doses. The highest upregulation was seen with 100 mJ/cm^2 (+26.3% respect to the control). A significant anti-inflammatory effect was exhibited across all aged AOX formulations through reduced cutaneous release of IL-8. AOX pretreatment generated an approximate 150% difference between their respective untreated and irradiated controls.

3.1.3. Photoaging and Skin Barrier

Significant ROS formation leading to lipid peroxidation and DNA oxidation can induce cutaneous structural changes damaging barrier properties and function. To demonstrate the efficacy of AOX formulations to protect and prevent epidermal and dermal proteins from UV damage, filaggrin and collagen-1 levels were measured. Figure 6A displays the significant, dose-dependent degradation of filaggrin, a key epidermal barrier protein. Human biopsy exposure to UV induced significant filaggrin degradation with the highest percentage difference of 101.02% between 400 mJ/cm^2 and the untreated control, as depicted in Figure 6B. Aged AOX formulations demonstrated a similar protective ability from UV degradation, independently of their ages, exhibiting significant retainment of filaggrin levels across all UV doses. Notably, AOX treatment increases filaggrin expression compared to basal untreated condition. These data are confirmed through Western blot analysis, as shown in Figure 6C,D.

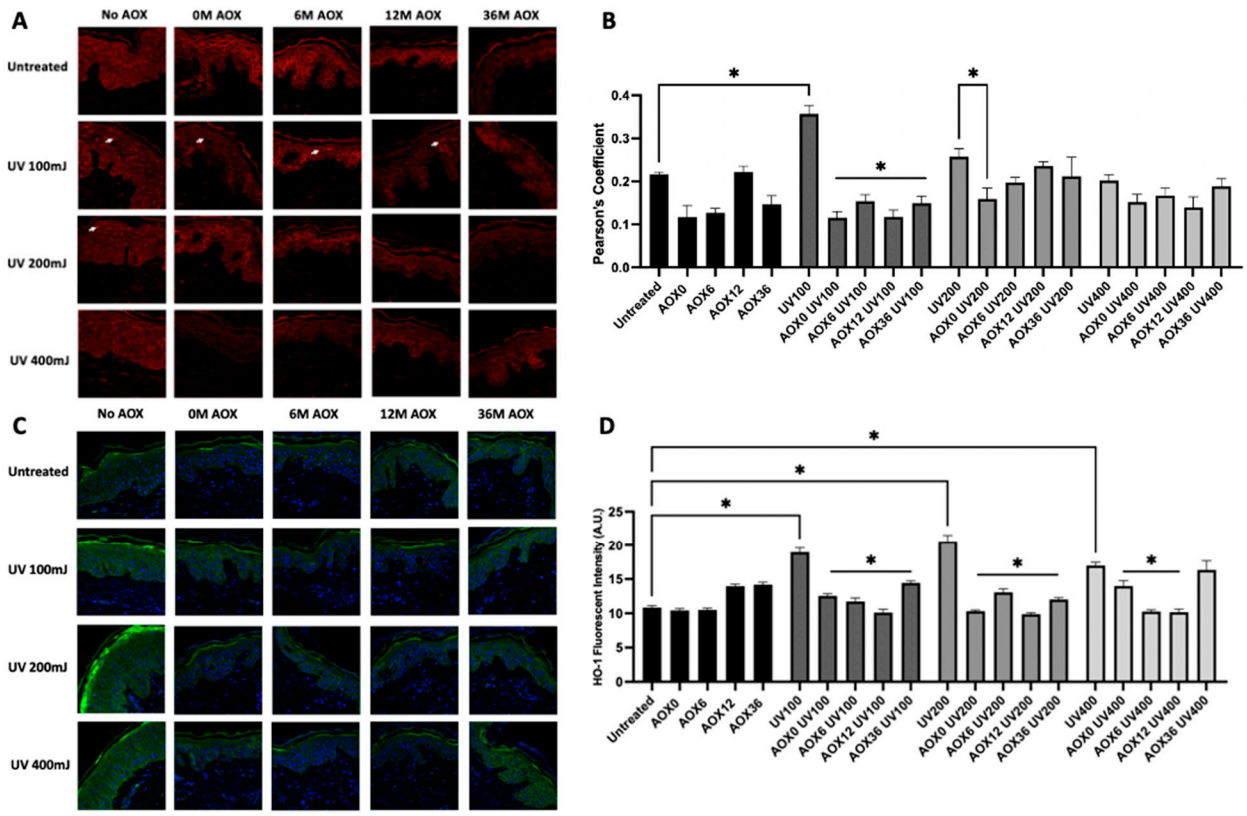

Figure 4. Antioxidant and anti-inflammatory capacity of aged AOX formulations in human skin explants treated with varying doses of UV light following pre-treatment with different aged formulations. Representative images of immunofluorescence staining for (**A**) Nrf2 and (**B**) ImageJ nuclear translocation quantification—Pearson's coeff. (**C**) HO-1 and (**D**) ImageJ quantification of fluorescent signal. Data analysis consisted of one-way ANOVA followed by Tukey's post hoc comparison test. Data are the results of the averages of at least three different experiments, * $p < 0.05$. Line with * demonstrates significance to respective control (e.g., UV100 to AOX0 UV100). White arrows mark highlight nuclei with and without Nrf2.

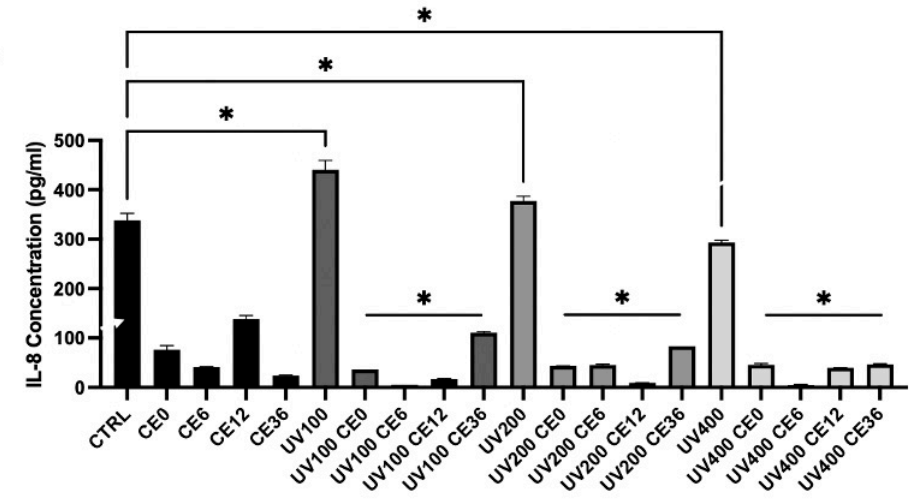

Figure 5. Inflammatory cytokine release in human skin explants treated with varying doses of UV light following pre-treatment with different aged formulations. IL-8 concentration (pg/mL) in culture media. Data analysis consisted of one-way ANOVA followed by Tukey's post hoc comparison test. Data are the results of the averages of at least three different experiments, * $p < 0.05$. Line with * demonstrates significance to respective control (e.g., UV100 to AOX0 UV100).

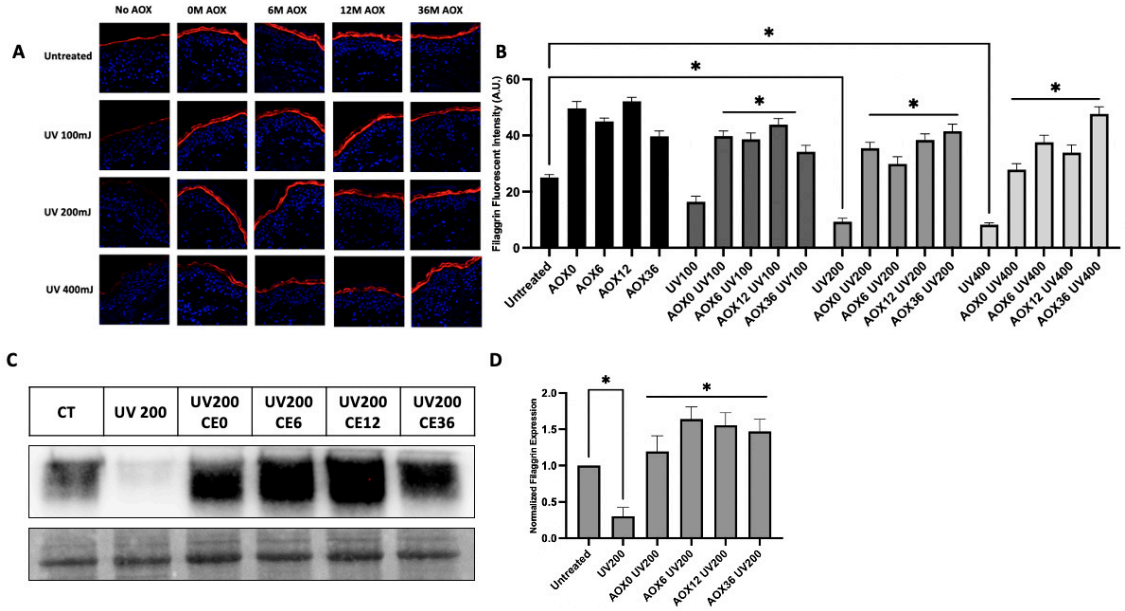

Figure 6. Filaggrin protein expression in human skin explants treated with varying doses of UV light following pre-treatment with different aged formulations. Representative images of immunofluorescence staining for (**A**) filaggrin and (**B**) ImageJ quantification of fluorescent signal. (**C**) Representative Western blot image of filaggrin (top band) and ponceau staining (bottom band) and (**D**) normalized ImageJ quantification of Western blot bands. Data analysis consisted of one-way ANOVA followed by Tukey's post hoc comparison test. Data are the results of the averages of at least three different experiments, * $p < 0.05$. Line with * demonstrates significance to respective control (e.g., UV100 to AOX0 UV100).

Collagen-1 is a key structural protein in the extracellular matrix (ECM) whose degradation has been associated with extrinsic premature aging. Indeed, as seen in Figure 7A,B, significant collagen degradation was detected across all UV doses with a 68.7% average degradation compared to the control. Aged formulations demonstrated a significant protective ability against incoming UV irradiation. Notably, the fresh AOX formulation exhibited an enhanced protective ability compared to other ages, particularly at UV doses 100 and 400 mJ/cm^2.

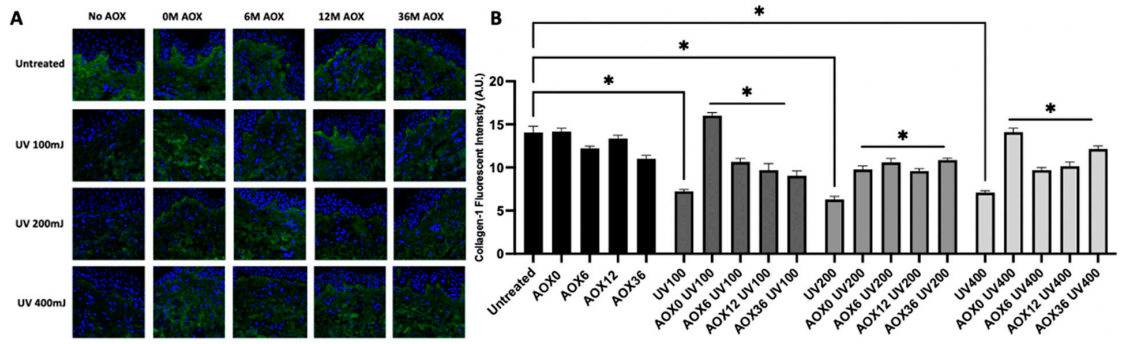

Figure 7. Collagen-1 protein expression in human skin explants treated with varying doses of UV light following pre-treatment with different aged formulations. Representative images of immunofluorescence staining for (**A**) collagen-1 and (**B**) ImageJ quantification of fluorescent signal. Data analysis consisted of one-way ANOVA followed by Tukey's post hoc comparison test. Data are the results of the averages of at least three different experiments, * $p < 0.05$. Line with * demonstrates significance to respective control (e.g., UV100 to AOX0 UV100).

3.2. Comparison of Closed and Opened AOX Formulations

3.2.1. ROS Defense—Lipid Peroxidation and DNA Integrity

In the follow-up study, the different efficacies of opened AOX formulation and sealed AOX formulation were compared in terms of cutaneous 4-HNE protein adducts and 8-OHdG levels after different doses of UV exposure. UV irradiation significantly increased both 4-HNE and 8-OHdG levels at all doses. As shown in Figure 8A, both closed and opened AOX formulations were able to prevent 4HNE protein adducts formation after the exposure to the different doses of UV from 100 to 400 mJ/cm^2 (23.1%, 14.4%, 17.4% difference, respectively, as seen in Figure 8B). As it is exhibited in Figure 8C, the highest DNA oxidation was seen with 200 mJ/cm^2 (94.16% difference to control demonstrated in Figure 8D). AOX formulations significantly prevented DNA oxidation for incoming UV doses; comparatively, no particular formulation demonstrated an enhanced preventative ability. UV upregulation of 4-HNE and the protective capacity of both closed and opened AOX formulations were confirmed through Western blot, as depicted in Figure 8E, where an evident decrease in UV-induced 4-HNE protein adduct formation was observed.

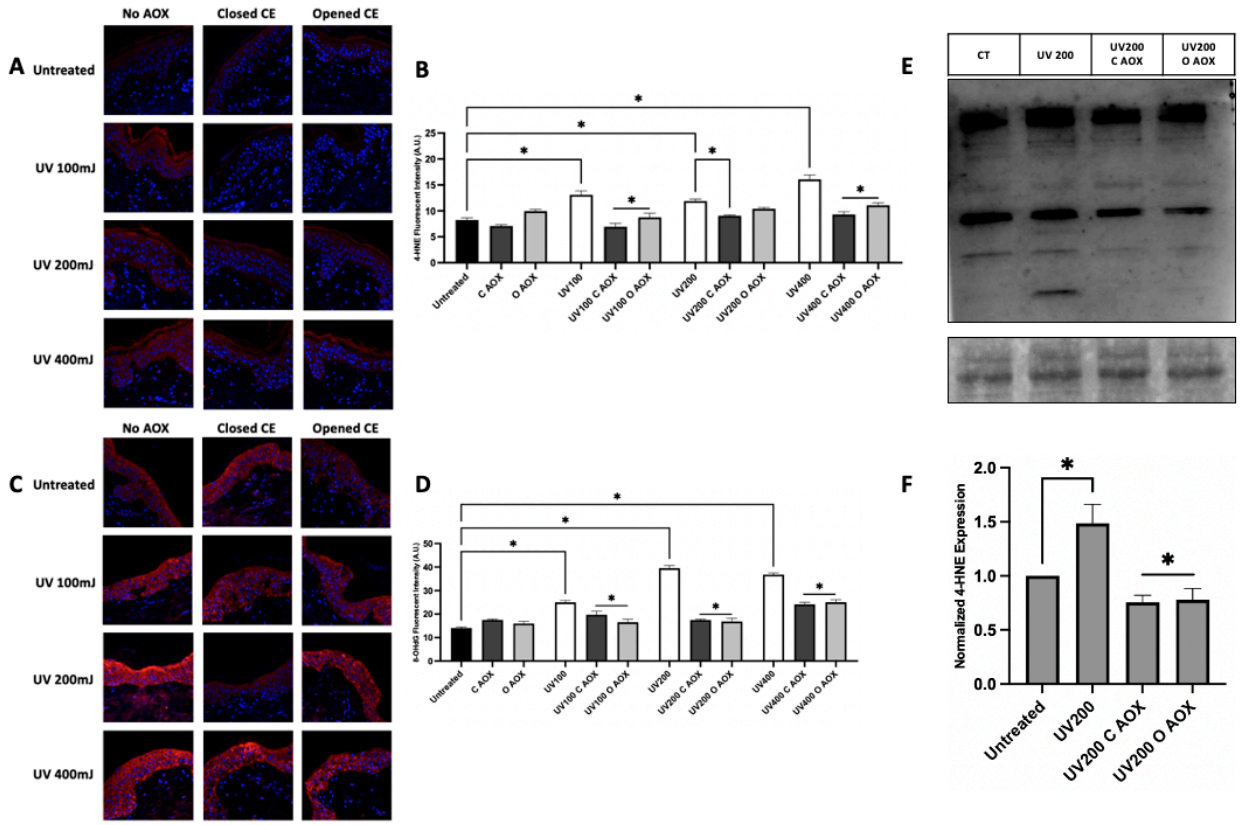

Figure 8. Formation of 4-HNE protein adducts and 8-OHdG in human skin explants treated with varying doses of UV light following pre-treatment with closed and opened AOX formulations. (**A**) Representative images of immunofluorescence staining for 4-HNE and (**B**) ImageJ quantification of fluorescent signal. (**C**) Representative images of immunofluorescence staining for 8-OHdG and (**D**) ImageJ quantification of fluorescent signal. (**E**) Representative Western blot image of 4-HNE (top band) and ponceau staining (bottom band) and (**F**) normalized ImageJ quantification of Western blot bands. Data analysis consisted of one-way ANOVA followed by Tukey's post hoc comparison test. Data are the results of the averages of at least three different experiments, * $p < 0.05$. Line with * demonstrates significance to respective UV dose (e.g., UV100 to AOX0 UV100).

3.2.2. Cutaneous Antioxidant and Inflammatory Response

To explore antioxidant and anti-inflammatory capacity of opened AOX formulations, Nrf2 activation and HO-1 protein levels were investigated. In Figure 9A,B, significant nuclear translocation of Nrf2 was exhibited in cutaneous biopsies irradiated with 100 and 200 mJ/cm^2 compared to the control. A partial, insignificant increase was seen in 400 mJ/cm^2 with a 58.55% increase in nuclear Nrf2. Both closed and opened AOX formulations exhibited lower nuclear Nrf2 compared to 100 mJ/cm^2 irradiated biopsies with a 35.29% and 37.58% percent decrease, respectively. Significant differences in Nrf2 activation between UV and AOX-treated groups were not detected at 200 and 400 mJ/cm^2; however, a partial decrease can be seen at 200 mJ/cm^2. In addition, in Figure 9C,D, UV exposure significantly increases epidermal protein expression of HO-1. Closed and opened AOX formulations exhibited similar stabilizing effects across all doses, inhibiting the UV upregulation of HO-1 protein levels.

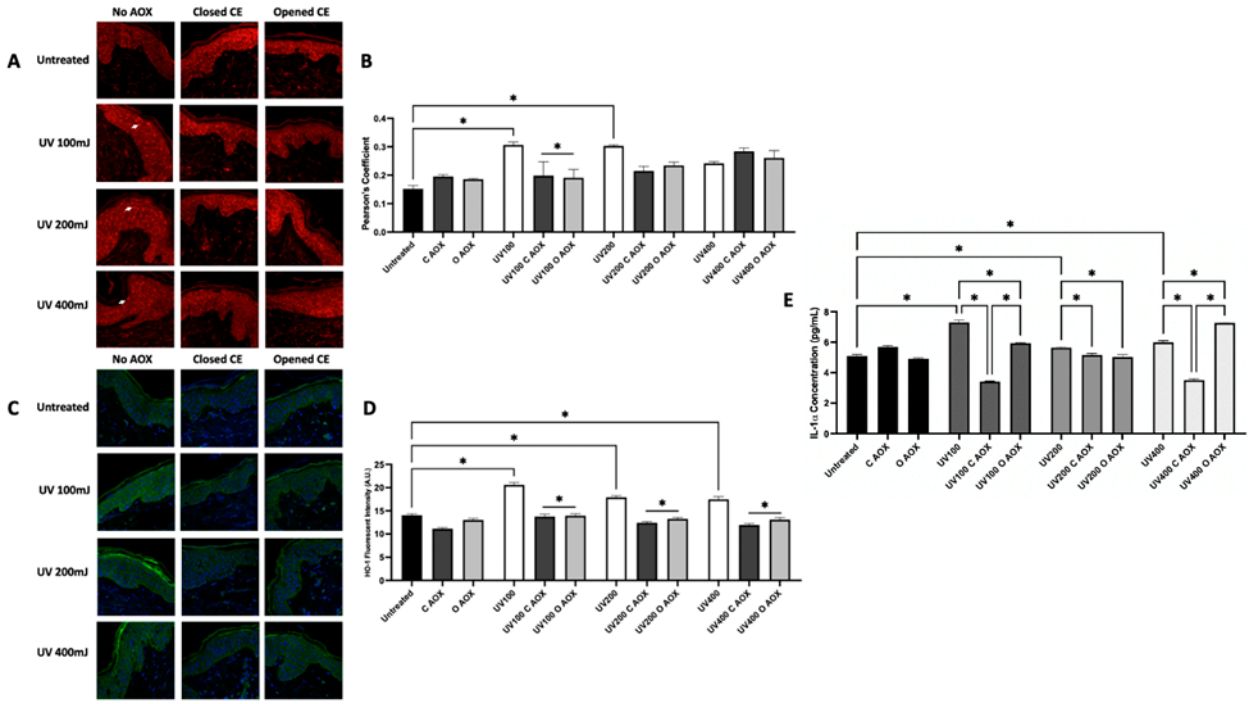

Figure 9. Antioxidant and anti-inflammatory capacity of aged AOX formulations in human skin explants treated with varying doses of UV light following pre-treatment with closed and opened AOX formulations. Representative images of immunofluorescence staining for (**A**) Nrf2 and (**B**) ImageJ nuclear translocation quantification—Pearson's coeff. (**C**) HO-1 and (**D**) ImageJ quantification of fluorescent signal. (**E**) Released IL-1α concentration (pg/mL) in culture media. Data analysis consisted of one-way ANOVA followed by Tukey's post hoc comparison test. Data are the results of the averages of at least three different experiments, * $p < 0.05$. Line with * demonstrates significance to respective UV dose (e.g., UV100 to AOX0 UV100). White arrows mark highlight nuclei with and without Nrf2.

The release of IL-1α cytokines was investigated to survey the ability of consumer usage to modulate the anti-inflammatory effect of AOX formulations. As shown in Figure 9E, UV exposure was able to induce a significant release of the inflammatory cytokine IL-1α across all the doses. The highest release of IL-1α was seen at 100 mJ/cm^2 with a 35% percent difference compared to the control. Exposure to 200 and 400 mJ/cm^2 exhibited 10% and 15% increases in IL-1α release. Nonetheless, the AOX formulations demonstrated significant reductions in IL-1α after UV exposure across all doses. Closed formulations exhibited a significant anti-inflammatory effect compared to opened/used AOX formula-

tions, particularly with 100 and 400 mJ/cm^2 of UV exposure. Notably, the opened AOX formulation was unable to prevent the UV induction of IL-1α.

3.2.3. Photoaging and Skin Barrier

Similar to the aged experiment, four-day UV irradiation induced significant degradation of both filaggrin and collagen-1. As seen in Figure 10A, compared to the control, 100, 200, and 400 mJ/cm^2 caused significant degradation of filaggrin. There was a 60.54% (100 mJ/cm^2), 57.18% (200 mJ/cm^2), and 63.98% (400 mJ/cm^2) difference between UV-irradiated samples and the control (Figure 10B). These data were confirmed via Western blot assay, as depicted in Figure 10C–F, which confirmed significant filaggrin loss in skin biopsies exposed to 200 mJ/cm^2 of UV radiation. Both closed and opened formulations exhibited a protective effect (Figure 10).

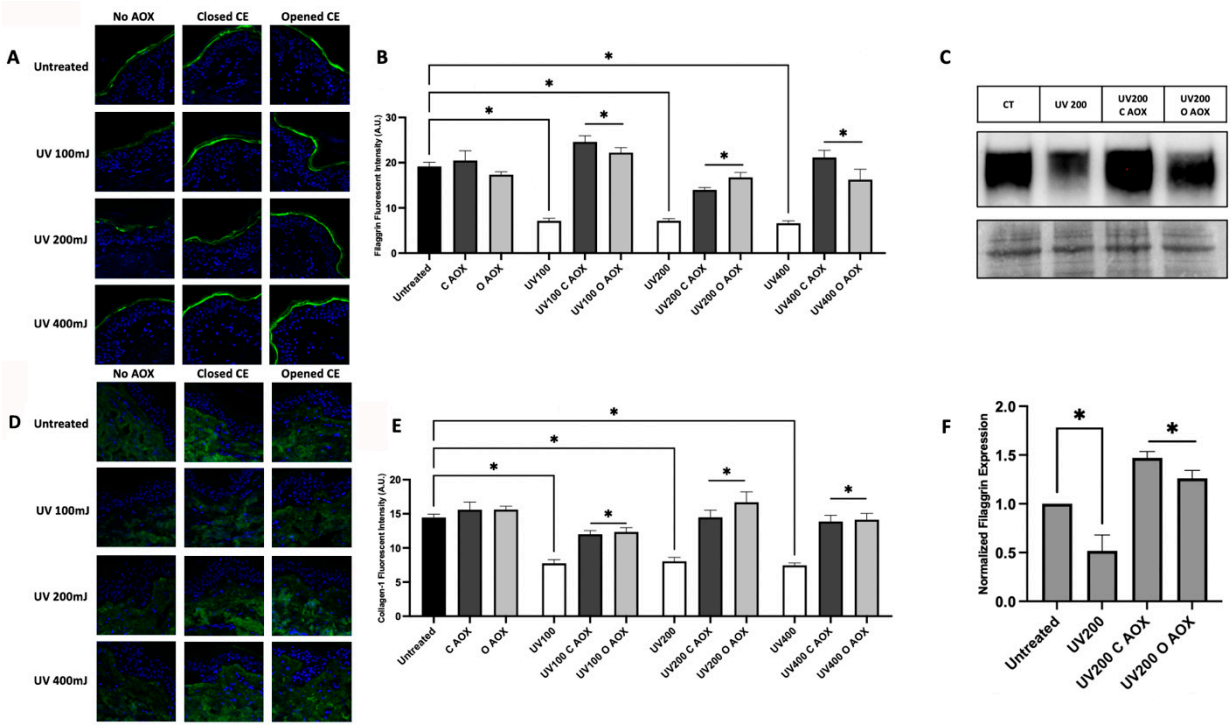

Figure 10. Filaggrin and collagen-1 protein expression in human skin explants treated with varying doses of UV light following pre-treatment with closed and opened AOX formulations. (**A**) Representative images of immunofluorescence staining for filaggrin and (**B**) ImageJ quantification of fluorescent signal. (**C**) Representative Western blot image of filaggrin (top band) and ponceau staining (bottom band) and (**D**) normalized ImageJ quantification of Western blot bands. (**E**) Representative images of immunofluorescence staining for collagen-1 and (**F**) ImageJ quantification of fluorescent signal. Data analysis consisted of one-way ANOVA followed by Tukey's post hoc comparison test. Data are the results of the averages of at least three different experiments, * $p < 0.05$. Line with * demonstrates significance to respective control (e.g., UV100 to UV100 C AOX).

Across all doses of the four-day UV irradiation, significant and comparable collagen-1 decrease was detected with 46.49% (100 mJ/cm^2), 44.46% (200 mJ/cm^2), and 48.44% (400 mJ/cm^2) (as seen in Figure 10E,F). Pre-treatment with AOX formulations exhibited a significant protective effect from UV damage. Closed and opened AOX formulations had similar defensive effects on cutaneous structure with no significant difference in filaggrin and collagen-1 between cutaneous biopsies pre-treated with the closed and opened AOX formulations.

4. Discussion

The application of cosmetic formations to enhance skin features and health is a key approach for slowing and preventing premature aging [29]. Exposure to exogenous stressors like UV radiation are accelerators to the cutaneous aging process, and thus a predominant facet and target of cosmetic topical defense [30,31].

The use of both vitamin C and E to provide cutaneous benefits has been substantially established. Topical administration of vitamin C has been demonstrated to protect against photoaging through the neutralization of oxidative stress, providing a variety of cutaneous benefits such as reduction in wrinkling and erythema induction [32]. Furthermore, vitamin C can restore vitamin E levels through recycling oxidized vitamin E back to its active, antioxidized form, allowing for the continued scavenging of ROS [33,34]. Similar to vitamin C, vitamin E can protect cutaneous structures as a ROS scavenger; in particular, through its incorporation into membranes, vitamin E can limit lipid peroxidation [35,36]. The combination of these two vitamins has been demonstrated to provide enhanced UV photoprotection compared to the application of one alone [9]. Additionally, combinations of vitamins help retain their chemical stability, lowering their degradation rate [37]. The addition of ferulic acid to vitamin C and E formulations has been demonstrated to provide significant stabilization, improving its protective capacity against UV radiation [38]. Additionally, ferulic acid has been shown to provide cutaneous benefits, itself improving skin hydration and melanin in people with photoaging [39].

Cosmetic products can undergo alterations on the shelf and during usage by the consumer; this can cause the degradation or oxidation of critical bioactive ingredients present in the formation, which could account for desired biological effects [40]. Our study demonstrates how an antioxidant mix, sealed and aged over time, and an opened mix, undergoing daily usage, can affect the protective efficacy from UV oxidative stress and inflammation.

The principal way UV induces its deleterious effects is through the generation of ROS, which can severely impact skin physiological homeostasis, leading to inflammation and degradation of key structural proteins [41]. For example, cutaneous exposure to UV can increase oxidative DNA damage, causing the upregulation of 8-OHdG [42]. Additionally, through interactions with ROS like hydroxy radicals, lipid peroxidation can occur, creating byproducts like 4-HNE, which can modify and stimulate protein dysfunction [43–45]. UV irradiation demonstrated significant oxidative effects across all doses 100 mJ/cm^2–400 mJ/cm^2 compared to the control; this was exhibited by the increased formation of 4-HNE protein adducts and significant oxidation of DNA evidenced through 8-OHdG formation. Aged and used topical antioxidant formulations proved resilient in their protective capacity from oxidative stress except in a high-dose UV exposure environment. The 36-month formulation exhibited a slight reduced ability to protect from UV formation of 4-HNE and 8-OHdG. Understandably, at the high UV dosage of 400 mJ/cm^2, 6-month and 12-month aged AOX formulations provided less protective effect than fresh (0-month) formulation, although all effects remained statistically significant. This could plausibly be attributed to increased formulation oxidation and diminished ROS scavenging capacity. In fact, in manufactured samples, ascorbic acid concentration decreased from 15% to 13.6% over six months of real-time use. A further reduction to approximately 12% was observed in extremely aged samples equivalent to 3 years (internal L'Oreal analytical data on file). Nonetheless, fresh and aged AOX formulations provided significant, comparatively protective effects at 100–200 mJ/cm^2 of UV exposure.

Using a standard erythemal dose (SED) of 10 mJ/cm^2 and UV index, our UV exposures can be extrapolated to a real-world exposure. Given approximately 10 SED/hour can be achieved at an UV index of 10, a 100 mJ/cm^2 exposure is equivalent to one hour in a

strong, UV index 10, sunlight [46]. This scales accordingly to the 200 and 400 mJ/cm^2 doses with 2 and 4 h of direct exposure needed to meet 20 and 40 SEDs in UV index 10 sunlight [46–48]. That being said, cutaneous responses of an individual can highly vary as minimal erythemal doses (MEDs) vary between Fitzpatrick skin types [49].

UV modulates skin barrier proteins through the upregulation of matrix metalloproteinases, which degrade key structural fibers such as collagen-1 [50]. Collagen-1 comprises 80–85% of the ECM, and is thus a critical protein for healthy skin structure [51]. Indeed, collagen-1 was downregulated across a four-day exposure to all UV doses 100 mJ/cm^2, 200 mJ/cm^2, and 400 mJ/cm^2. Both aged and opened AOX formulations demonstrated significant protective effects on collagen-1 fibers, suggesting fresh cosmetic products containing antioxidants are not necessary to provide protective benefits to structural proteins. Filaggrin, a key structural protein of the stratum corneum (epidermal layer) necessary for optimal barrier function, can suppress filaggrin expression and mRNA expression of profilaggrin [52–54]. Similar to the protective effects seen with collagen-1, aged and used AOX formulations demonstrated the significant protection of filaggrin.

The ability of UV to induce inflammation is well documented; this is primarily done through ROS modulation of gene expression upregulating inflammatory cytokines [55,56]. In this study, we gauged the release of two proinflammatory cytokines, IL-8 and IL-1α, to determine the anti-inflammatory efficacy of aged and used formulations. Aged AOX formulations from 0 months to 36 months provided pronounced downregulation of IL-8 in response to all UV doses. No age demonstrated additional anti-inflammatory capacity compared to others. On the other hand, between closed and opened (6-month daily usage) formulations, the closed AOX formulation demonstrated significant anti-inflammatory effects, suggesting reduced biostability with consumer usage. Additional experiments should be conducted surrounding the biostability of other antioxidant ingredients in cosmetic formulations to underline compositions of effective formulations against exogenous stressors.

Across UV exposure doses, most markers demonstrated similar reductive effects on cutaneous structural proteins filaggrin and collagen-1 exhibiting the aggressive nature of UV photoaging. Similarly, all doses induced increases in 4-HNE and 8-OHdG formation; notably, 200 mJ/cm^2 exhibited the highest DNA damage across both experiments. The lack of dose-dependency in DNA damage could be due to enhanced antioxidant mechanisms triggered over the four-day exposure period with 400 mJ/cm^2 or a limitation of our detection approach. The ability of the AOX formulation to prevent Nrf2 nuclear translocation clearly indicates its potential to prevent the formation of an oxidative environment. It is possible that this effect is a consequence of the AOX formulation activating Nrf2 at earlier timepoints, but this was not possible to detect in our study.

Through simulating both seal-stored conditions across multiple, long timepoints and daily consumer use (opened 6-month formulation), the present study effectively mirrors real-world product usage. Additionally, the assessment of numerous biomarkers in ex vivo human skin biopsies provides physiologically relevant and comprehensive data on skin protection. Some limitations of our study could be represented by the use of only skin from donor phototypes 2 and 3, which are more susceptible to photoaging but could limit the extrapolation of the results across diverse skin phototypes. In addition, the use of skin explants, although considered as one of the best models to study skin responses in a laboratory, presents some physiological limitations that need to be taken into consideration when extrapolating the results to real life. For instance, the lack of blood flow and the peculiar history of each donor can affect the results. On the other hand, considering that our experimental approach was limited to topical application, we believe that the lack of

blood flow did not affect our results due also to the short time of culture of the skin biopsies (4 days).

5. Conclusions

Our study demonstrates the long-term biostability of an antioxidant formulation containing 15% l-ascorbic acid, 1% tocopherol, and 0.5% ferulic acid and its ability to protect cutaneous structures from oxidative damage and premature aging induced from a variety of intensities of UV exposure. Aged AOX formulations (ages 0 to 36 months), as well as formulations used daily over 6 months (with only a decrease of circa 10% of the antioxidant content), demonstrated significant suppression of lipid peroxidation and DNA oxidation exhibited by decreases in 4-HNE and 8-OHdG. Downstream inflammatory processes were generally inhibited by aged and used AOX formulations with significant downregulation of cytokines IL-8 and IL-1α. Importantly, UV-induced degradation of critical epidermal and dermal structural proteins was effectively conserved, indicated by the preservation of filaggrin and collagen-1 underscoring the protective effects of the formulation on cutaneous structure. These results suggest that the tested formulation offer long-term shelf-life and are stable during extended consumer use; furthermore, our experiments highlight the general need to evaluate the formulation efficacy after long-term storage or consumer use.

Author Contributions: Conceptualization, G.V., P.B., and H.C.; methodology, G.V., P.B., H.C. and J.I.;; software, J.I.; validation, G.V. and J.I.; formal analysis, J.I.; investigation, J.I.; resources, G.V.; data curation, J.I.; writing—original draft preparation, J.I.; writing—review and editing, J.I. and G.V.; visualization, J.I.; supervision, G.V.; project administration, G.V.; funding acquisition, G.V. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Acknowledgments: Authors thank Skinceuticals for supporting the study.

Conflicts of Interest: The authors Patricia Brieva and Hina Choudhary are employees of L'Oréal USA. The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest. The funders had no role in the design of the study; in the collection, analyses or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

References

1. Passeron, T.; Zouboulis, C.C.; Tan, J.; Andersen, M.L.; Katta, R.; Lyu, X.; Aguilar, L.; Kerob, D.; Morita, A.; Krutmann, J.; et al. Adult Skin Acute Stress Responses to Short-term Environmental and Internal Aggression from Exposome Factors. *J. Eur. Acad. Dermatol. Venereol.* **2021**, *35*, 1963–1975. [CrossRef]
2. Griffiths, T.W.; Watson, R.E.B.; Langton, A.K. Skin Ageing and Topical Rejuvenation Strategies. *Br. J. Dermatol.* **2023**, *189*, i17–i23. [CrossRef]
3. Mondello, A.; Salomone, R.; Mondello, G. Exploring Circular Economy in the Cosmetic Industry: Insights from a Literature Review. *Environ. Impact Assess. Rev.* **2024**, *105*, 107443. [CrossRef]
4. Ferrara, F.; Woodby, B.; Pecorelli, A.; Schiavone, M.L.; Pambianchi, E.; Messano, N.; Therrien, J.-P.; Choudhary, H.; Valacchi, G. Additive Effect of Combined Pollutants to UV Induced Skin OxInflammation Damage. Evaluating the Protective Topical Application of a Cosmeceutical Mixture Formulation. *Redox Biol.* **2020**, *34*, 101481. [CrossRef] [PubMed]
5. Parrado, C.; Mercado-Saenz, S.; Perez-Davo, A.; Gilaberte, Y.; Gonzalez, S.; Juarranz, A. Environmental Stressors on Skin Aging. Mechanistic Insights. *Front. Pharmacol.* **2019**, *10*, 759. [CrossRef]
6. Goyal, N.; Jerold, F. Biocosmetics: Technological Advances and Future Outlook. *Environ. Sci. Pollut. Res.* **2023**, *30*, 25148–25169. [CrossRef]

7. Revolutionizing Cosmetic Ingredients: Harnessing the Power of Antioxidants, Probiotics, Plant Extracts, and Peptides in Personal and Skin Care Products. Available online: https://www.researchgate.net/publication/384008413_Revolutionizing_Cosmetic_Ingredients_Harnessing_the_Power_of_Antioxidants_Probiotics_Plant_Extracts_and_Peptides_in_Personal_and_Skin_Care_Products (accessed on 7 February 2025).
8. Dong, Y.; Wang, Z. ROS-Scavenging Materials for Skin Wound Healing: Advancements and Applications. *Front. Bioeng. Biotechnol.* **2023**, *11*, 1304835. [CrossRef]
9. Lin, J.Y.; Selim, M.A.; Shea, C.R.; Grichnik, J.M.; Omar, M.M.; Monteiro-Riviere, N.A.; Pinnell, S.R. UV Photoprotection by Combination Topical Antioxidants Vitamin C and Vitamin E. *J. Am. Acad. Dermatol.* **2003**, *48*, 866–874. [CrossRef] [PubMed]
10. Gupta, M.; Mahajan, V.K.; Mehta, K.S.; Chauhan, P.S. Zinc Therapy in Dermatology: A Review. *Dermatol. Res. Pract.* **2014**, *2014*, 709152. [CrossRef]
11. Sun, M.; Deng, Y.; Cao, X.; Xiao, L.; Ding, Q.; Luo, F.; Huang, P.; Gao, Y.; Liu, M.; Zhao, H. Effects of Natural Polyphenols on Skin and Hair Health: A Review. *Molecules* **2022**, *27*, 7832. [CrossRef]
12. Valacchi, G.; Virgili, F.; Cervellati, C.; Pecorelli, A. OxInflammation: From subclinical condition to pathological biomarker. *Front. Physiol.* **2018**, *9*, 858. [CrossRef]
13. Masaki, H. Role of Antioxidants in the Skin: Anti-Aging Effects. *J. Dermatol. Sci.* **2010**, *58*, 85–90. [CrossRef]
14. Lingappan, K. NF-κB in Oxidative Stress. *Curr. Opin. Toxicol.* **2018**, *7*, 81–86. [CrossRef] [PubMed]
15. Liu, T.; Zhang, L.; Joo, D.; Sun, S.-C. NF-κB Signaling in Inflammation. *Signal Transduct. Target. Ther.* **2017**, *2*, 17023. [CrossRef]
16. Ivarsson, J.; Ferrara, F.; Vallese, A.; Guiotto, A.; Colella, S.; Pecorelli, A.; Valacchi, G. Comparison of Pollutant Effects on Cutaneous Inflammasomes Activation. *Int. J. Mol. Sci.* **2023**, *24*, 16674. [CrossRef] [PubMed]
17. Aboul-Enein, H.Y.; Kruk, I.; Kładna, A.; Lichszteld, K.; Michalska, T. Scavenging Effects of Phenolic Compounds on Reactive Oxygen Species. *Biopolymers* **2007**, *86*, 222–230. [CrossRef]
18. Halla, N.; Fernandes, I.P.; Heleno, S.A.; Costa, P.; Boucherit-Otmani, Z.; Boucherit, K.; Rodrigues, A.E.; Ferreira, I.C.; Barreiro, M.F. Cosmetics Preservation: A Review on Present Strategies. *Molecules* **2018**, *23*, 1571. [CrossRef]
19. Tang, X.; Yang, T.; Yu, D.; Xiong, H.; Zhang, S. Current Insights and Future Perspectives of Ultraviolet Radiation (UV) Exposure: Friends and Foes to the Skin and beyond the Skin. *Environ. Int.* **2024**, *185*, 108535. [CrossRef] [PubMed]
20. D'Orazio, J.; Jarrett, S.; Amaro-Ortiz, A.; Scott, T. UV Radiation and the Skin. *Int. J. Mol. Sci.* **2013**, *14*, 12222–12248. [CrossRef]
21. Rastogi, R.P.; Richa, N.; Kumar, A.; Tyagi, M.B.; Sinha, R.P. Molecular Mechanisms of Ultraviolet Radiation-Induced DNA Damage and Repair. *J. Nucleic Acids* **2010**, *2010*, 592980. [CrossRef]
22. Kciuk, M.; Marciniak, B.; Mojzych, M.; Kontek, R. Focus on UV-Induced DNA Damage and Repair—Disease Relevance and Protective Strategies. *Int. J. Mol. Sci.* **2020**, *21*, 7264. [CrossRef]
23. Jaffri, J.M. Reactive Oxygen Species and Antioxidant System in Selected Skin Disorders. *Malays. J. Med. Sci.* **2023**, *30*, 7–20. [CrossRef]
24. Merin, K.A.; Shaji, M.; Kameswaran, R. A Review on Sun Exposure and Skin Diseases. *Indian J. Dermatol.* **2022**, *67*, 625. [CrossRef]
25. Chen, X.; Yang, C.; Jiang, G. Research Progress on Skin Photoaging and Oxidative Stress. *Adv. Dermatol. Allergol.* **2021**, *38*, 931–936. [CrossRef] [PubMed]
26. Zhao, H.C.; Xiao, T.; Chen, Y.J. Ultraviolet induced skin inflammation. *Int. J. Dermatol. Venereol.* **2021**, *4*, 229–235. [CrossRef]
27. Humans, I.W.G. On the E. of C.R. to solar and ultraviolet radiation. In *Radiation*; International Agency for Research on Cancer: Lyon, France, 2012.
28. Ferrara, F.; Bondi, A.; Pula, W.; Contado, C.; Baldisserotto, A.; Manfredini, S.; Boldrini, P.; Sguizzato, M.; Montesi, L.; Benedusi, M.; et al. Ethosomes for Curcumin and Piperine Cutaneous Delivery to Prevent Environmental-Stressor-Induced Skin Damage. *Antioxidants* **2024**, *13*, 91. [CrossRef] [PubMed]
29. Ganceviciene, R.; Liakou, A.I.; Theodoridis, A.; Makrantonaki, E.; Zouboulis, C.C. Skin Anti-Aging Strategies. *Derm.-Endocrinol.* **2012**, *4*, 308–319. [CrossRef] [PubMed]
30. Rinnerthaler, M.; Bischof, J.; Streubel, M.K.; Trost, A.; Richter, K. Oxidative Stress in Aging Human Skin. *Biomolecules* **2015**, *5*, 545–589. [CrossRef]
31. Farris, P.K.; Valacchi, G. Ultraviolet Light Protection: Is It Really Enough? *Antioxidants* **2022**, *11*, 1484. [CrossRef]
32. Al-Niaimi, F.; Chiang, N.Y.Z. Topical Vitamin C and the Skin: Mechanisms of Action and Clinical Applications. *J. Clin. Aesthetic Dermatol.* **2017**, *10*, 14–17.
33. Pullar, J.M.; Carr, A.C.; Vissers, M.C.M. The Roles of Vitamin C in Skin Health. *Nutrients* **2017**, *9*, 866. [CrossRef]
34. Packer, L.; Valacchi, G. Antioxidants and the Response of Skin to Oxidative Stress: Vitamin E as a Key Indicator. *Ski. Pharmacol. Physiol.* **2002**, *15*, 282–290. [CrossRef] [PubMed]
35. Keen, M.A.; Hassan, I. Vitamin E in Dermatology. *Indian Dermatol. Online J.* **2016**, *7*, 311–315. [CrossRef] [PubMed]
36. Vitamin E in Human Skin: Functionality and Topical Products. Available online: https://www.researchgate.net/publication/352470124_Vitamin_E_in_Human_Skin_Functionality_and_Topical_Products (accessed on 7 February 2025).

37. Gianeti, M.D.; Gaspar, L.R.; de Camargo Júnior, F.B.; Berardo Gonçalves Maia Campos, P.M. Benefits of Combinations of Vitamin A, C and E Derivatives in the Stability of Cosmetic Formulations. *Molecules* **2012**, *17*, 2219–2230. [CrossRef]
38. Murray, J.C.; Burch, J.A.; Streilein, R.D.; Iannacchione, M.A.; Hall, R.P.; Pinnell, S.R. A Topical Antioxidant Solution Containing Vitamins C and E Stabilized by Ferulic Acid Provides Protection for Human Skin against Damage Caused by Ultraviolet Irradiation. *J. Am. Acad. Dermatol.* **2008**, *59*, 418–425. [CrossRef]
39. Zduńska-Pęciak, K.; Dębowska, R.; Kołodziejczak, A.; Rotsztejn, H. Ferulic Acid—A Novel Topical Agent in Reducing Signs of Photoaging. *Dermatol. Ther.* **2022**, *35*, e15543. [CrossRef] [PubMed]
40. Choi, E.; Maeng, S.J.; Yun, S.; Yu, H.; Shin, J.-S.; Yun, J.-Y. The Degeneration of Skin Cosmetics and the Structural Changes of the Chemical Components as an Indicator of Product Shelf Life. *J. Ind. Eng. Chem.* **2021**, *100*, 317–323. [CrossRef]
41. Salminen, A.; Kaarniranta, K.; Kauppinen, A. Photoaging: UV Radiation-Induced Inflammation and Immunosuppression Accelerate the Aging Process in the Skin. *Inflamm. Res.* **2022**, *71*, 817–831. [CrossRef]
42. Kato, M.; Iida, M.; Goto, Y.; Kondo, T.; Yajima, I. Sunlight Exposure–Mediated DNA Damage in Young Adults. *Cancer Epidemiol. Biomark. Prev.* **2011**, *20*, 1622–1628. [CrossRef]
43. Su, L.J.; Zhang, J.H.; Gomez, H.; Murugan, R.; Hong, X.; Xu, D.; Jiang, F.; Peng, Z.Y. Reactive Oxygen Species-Induced Lipid Peroxidation in Apoptosis, Autophagy, and Ferroptosis. *Oxid. Med. Cell Longev.* **2019**, *2019*, 5080843. [CrossRef]
44. Larroque-Cardoso, P.; Camaré, C.; Nadal-Wollbold, F.; Grazide, M.H.; Pucelle, M.; Garoby-Salom, S.; Bogdanowicz, P.; Josse, G.; Schmitt, A.M.; Uchida, K.; et al. Elastin Modification by 4-Hydroxynonenal in Hairless Mice Exposed to UV-A. Role in Photoaging and Actinic Elastosis. *J. Investig. Dermatol.* **2015**, *135*, 1873–1881. [CrossRef]
45. Pecorelli, A.; Woodby, B.; Prieux, R.; Valacchi, G. Involvement of 4-Hydroxy-2-Nonenal in Pollution-Induced Skin Damage. *BioFactors* **2019**, *45*, 536–547. [CrossRef]
46. International Agency for Research on Cancer (Ed.) *Exposure to Artificial UV Radiation and Skin Cancer*; IARC Working Group Reports; Reprint ed.; International Agency for Research on Cancer: Lyon, France, 2008; ISBN 978-92-832-2441-9.
47. Rigel, E.G.; Lebwohl, M.; Rigel, A.C.; Rigel, D.S. Daily UVB Exposure Levels in High-School Students Measured with Digital Dosimeters. *J. Am. Acad. Dermatol.* **2003**, *49*, 1112–1114. [CrossRef]
48. Jung, H.Y.; Shin, J.C.; Park, S.M.; Kim, N.R.; Kwak, W.; Choi, B.H. Pinus Densiflora Extract Protects Human Skin Fibroblasts against UVB-Induced Photoaging by Inhibiting the Expression of MMPs and Increasing Type I Procollagen Expression. *Toxicol. Rep.* **2014**, *1*, 658–666. [CrossRef]
49. Valbuena, M.C.; Villanueva, J.N.; Vanegas, G.S. Minimal Erythema Dose: Correlation with Fitzpatrick Skin Type and Concordance Between Methods of Erythema Assessment in a Patient Sample in Colombia. *Actas Dermosifiliogr. (Engl. Ed.)* **2020**, *111*, 390–397. [CrossRef]
50. Kim, D.J.; Iwasaki, A.; Chien, A.L.; Kang, S. UVB-Mediated DNA Damage Induces Matrix Metalloproteinases to Promote Photoaging in an AhR- and SP1-Dependent Manner. *JCI Insight* **2022**, *7*, e156344. [CrossRef] [PubMed]
51. Amirrah, I.N.; Lokanathan, Y.; Zulkiflee, I.; Wee, M.F.M.R.; Motta, A.; Fauzi, M.B. A Comprehensive Review on Collagen Type I Development of Biomaterials for Tissue Engineering: From Biosynthesis to Bioscaffold. *Biomedicines* **2022**, *10*, 2307. [CrossRef] [PubMed]
52. Mildner, M.; Jin, J.; Eckhart, L.; Kezic, S.; Gruber, F.; Barresi, C.; Stremnitzer, C.; Buchberger, M.; Mlitz, V.; Ballaun, C.; et al. Knockdown of Filaggrin Impairs Diffusion Barrier Function and Increases UV Sensitivity in a Human Skin Model. *J. Investig. Dermatol.* **2010**, *130*, 2286–2294. [CrossRef]
53. Sandilands, A.; Sutherland, C.; Irvine, A.D.; McLean, W.H.I. Filaggrin in the Frontline: Role in Skin Barrier Function and Disease. *J. Cell Sci.* **2009**, *122*, 1285–1294. [CrossRef]
54. Simonsen, S.; Thyssen, J.; Heegaard, S.; Kezic, S.; Skov, L. Expression of Filaggrin and Its Degradation Products in Human Skin Following Erythemal Doses of Ultraviolet B Irradiation. *Acta Derm.-Venereol.* **2017**, *97*, 797–801. [CrossRef]
55. Hruza, L.L.; Pentland, A.P. Mechanisms of UV-Induced Inflammation. *J. Investig. Dermatol.* **1993**, *100*, 35S–41S. [CrossRef] [PubMed]
56. Gratz, I.K.; Kofler, B. UV Irradiation-Induced Inflammation, What Is the Trigger? *Exp. Dermatol.* **2015**, *24*, 916–917. [CrossRef] [PubMed]