Assessing UV Damage and Antioxidant Influence on Human Hair Using a Combination of Spectroscopic, Thermal and Physical Measurements

He, Yingxia [1]*; Park, Kimun [1]; Edouard, Farahdia [1]; Horn, Alissa [1]; Bernard, Laure [2]

[1] Croda Inc., Plainsboro, New Jersey, United States

[2] Sederma, Le Perray en Yvelines, France

* He, Yingxia; 777 Scudders Mill Road, Bldg. 2, Suite 200, Plainsboro, NJ 08536, United States

Telephone: 302-530-7143, Email: yingxia.he@croda.com

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Abstract

UV destruction of human hair has been well documented in literatures. The impact on hair cuticle and cortex causes surface impairment and protein loss. Methodologies applied to evaluate UV damaged hair include Scanning Electron Microscopy, protein analysis, Electron Paramagnetic Resonance, Raman, Fourier Transform Infrared, and tensile measurement. The level of UV damage is directly linked to exposure dosage, hair type and condition, and preventing treatment, such as utilizing antioxidant. The aim of this study is to use a combined approach of Fluorescence Spectroscopy with DCFH assay to detect reactive oxygen species, FTIR to evaluate cystine oxidation, Differential Scanning Calorimetry to evaluate structural changes within hair cortex, tensile and cyclic fatigue methods to assess overall mechanical impact on hair. The study results confirm that different measurements can provide distinctive evidence on the damage effect of UV to human hair. The intensity of fluorescence from DCFH-DA (2'-7'-Dichlorodihydrofluorescein diacetate) assay reflects quantity of reactive oxygen species generated due to photo oxidation of hair; the damage can also be detected by FTIR using intensity of cysteic acid peak as a marker. DSC data validates that UV diminishes cross-link density of hair matrix, denaturation temperature

and enthalpy of UV damaged hair decreases as UV exposure dosage increases. Tensile and cyclic fatigue data are consistent with results from DCFH-DA, FTIR and DSC measurement. The collective impact and antioxidant protection effect from Hydrolyzed Cicer Seed Extract can be best verified with a combined approach of employing various spectroscopic and physical measurement techniques.

1. Introduction

Human hair is mainly constituted of protein, protein-bound sulfur, and lipid; components that are naturally labile to oxidative damage induced by photo energy and reactive oxygen species [1, 2]. Ultraviolet (UV, 200-400 nm) can provoke hair protein degradation, the photochemical process is known to oxidize the sulfur-sulfur bond within hair cortex, reducing hair strength and diminishing hair color [2, 3]. The proteins of hair cuticle can also be compromised by UVB (280-315 nm) and UVA (315–400 nm), the destruction causes hair to become dry, dull, stiff, and difficult to manage [4, 5]. While different approaches have been applied to study the degree of hair damage caused by UV irradiation [6-11], because the level of UV damage is linked to exposure dosage, hair condition, and preventing treatment, there are still unidentified characteristics remained to be discovered. In this study, we explored the combined approach of employing various spectroscopic and physical measurement techniques to study UV induced damage of medium brown hair after exposed to controlled dose of UVA, recurrent cycles of UV/Ozone, accumulative prolonged exposure of UVA/UVB, and the antioxidant protection effect of Hydrolyzed Cicer Seed Extract for the treated hair from both solution and hair conditioner applications.

2. Materials and Methods

- **Hair sample:**

European medium brown hair purchased from International Hair Importers, 8729 Myrtle Ave, Glendale, NY 11385.

- **Testing formulation**
    1) 15% Sodium Laureth Sulfate (SLES) solution

2) 0.25% and 1.0% active Hydrolyzed Cicer Seed Extract (HCSE) solution
3) Conditioner formulation listed in Table 1:

Table 1, Rinse-off hair conditioner formulation

Ingredients (wt.%)	**Placebo**	**HCSE-0.25**	**HCSE-1.00**
De-ionized Water	93.0	91.4	86.5
Behentrimonium Methosulfate (and) Cetyl Alcohol (and) Butylene Glycol	6.0	6.0	6.0
Phenoxyethanol (and) Ethylhexylglycerin	1.0	1.0	1.0
Hydrolyzed Cicer Seed Extract (15.39% active)	0.0	1.6	6.5

- **Testing instrument**
  1) Xenon Weather-Ometer CI3000+, Atlas, Mount Prospect, IL, USA.
  2) NO2/NO/O3 generator Model 713, 2B Technologies, Boulder, CO, USA.
  3) Automatic Tensile Tester MTT690, Dia-Stron, Andover SP10 5NY, UK.
  4) Cyclic Tester CYC802, Dia-Stron, Andover SP10 5NY, UK.
  5) Differential Scanning Calorimeter (DSC) Q250, TA Instrument, DE, USA.
  6) FTIR spotlight System 400, PerkinElmer, Inc., Waltham, MA, USA.
- **Experimental protocol**
  1) DCFH assay method assessing UVA-induced hair oxidation damage

     A. Hair leave-on treatment:

     Hair was treated with a ratio of 400mg of hair for 8mL of DI-water, 0.25% or 1.0% active HCSE DI-water solution. These formulations are considered as leave-on. After 10 minutes, hair was spread on absorbent paper until dry.

     B. Hair rinse-off conditioner treatment:

     Hair tress was prepared and wet using tap water. Each tress was treated with an equal amount of conditioner placebo, 0.25% or 1.0% active HCSE as listed in Table 1, which was applied homogeneously along the hair. After 10 minutes, hair tresses were rinsed under tap water. Hair tresses were dried naturally without using hair dryer.

C. DCFH-DA assay and UVA exposure:

Quantification of reactive oxygen species (ROS) in response to oxidative stress can be measured using the assay 2'-7'-dichlorofluorescine (DCFH). This assay turns to its highly fluorescent 2′,7′-dichlorofluoresceine analogue upon oxidation. The fluorescence intensity is proportional to the amount of ROS. Treated hair tresses were UVA-irradiated (10J/cm2 in total). Right after irradiation, DCFH was added to the hair tresses for 30 minutes at 37°C. Fluorescence was recorded with a microplate reader.

2) FTIR method evaluating UV/Ozone induced hair damage and tensile testing

A. Treatment and UV/Ozone exposure protocol:

Testing hair was washed with 15% SLES, blow dried, divided into three groups, and proceeded with their respective treatments: DI-water, 0.25% and 1.0% active HCSE solution for one hour, then measured surface FTIR spectrum of each group as before pollution FTIR baseline. After obtaining FTIR of the treated hair sample, hair was spread into a thin layer in the pollution chamber, exposed to UV/ozone for 2 hours (UV radiation: 0.24J/cm^2.hr, 540ppb Ozone). The treatment and pollution procedure were repeated for 12 cycles, and UV/Ozone polluted hair was assessed again with FTIR.

B. Tensile testing condition:

For each treatment group, randomly selected 50 fibers of before and after 12 cycles of treatment/pollution were tested with tensile tester under controlled 50% relative humidity, tensile break stress and break extension were compared to assess the impact of UV/Ozone exposure to hair physical property.

3) Cyclic fatigue and DSC methods measuring UVA/UVB induced hair damage

A. Treatment protocol:

Testing hair was first cleaned with standard shampoo, then conditioned with respective testing conditioners in Table 1 for 10 minutes, then hair tress was rinse-off with tap water for 30 seconds, air dried. After treatment, hair tress samples were assessed with cyclic fatigue and DSC for before UV damage condition, then exposed to UV (18J/cm^2·hr, mixed UVA/UVB, 6 hours per cycle). The

treatment/UV exposure protocol was repeated for 20 cycles, hair was assessed after each 5 cycles to quantify the damage.

B. Cyclic fatigue testing condition:

For each treatment group, randomly selected 50 fibers, before and after 20 cycles of treatment/UV exposure, were tested with cyclic fatigue tester under constant strain and controlled 50% relative humidity.

C. DSC testing condition:

Denaturation temperature (T_d) and denaturation enthalpy (ΔH_d) of hair samples with different treatments were determined using 5-7mg of powder hair sample in water [9]. Temperature range: 50-190^{o}C; heating rate: 10^{o}C/min.

3. Results

1) UVA induced hair damage and protection effect of HCSE antioxidant

After hair was treated with respective formulations and subjected to UVA (10J/cm^2) irradiation. DCFH assay was used to assess the damage level and antioxidant protection effect of hair treated with leave-on solution and rinse-off conditioners. The DCFH assay results are listed in Tables 2 and 3.

Table 2, DCFH assay results of leave-on solution treated hair

Hair treatment	No UVA, Fluorescence*	10J/cm^2 UVA, Fluorescence*	%Fluorescence change vs. no UVA	%Fluorescence reduction vs. untreated
Untreated hair	15402, +/- 1852	38094, +/- 726	147	Reference
0.25% HCSE solution	8511, +/- 371	15069, +/- 1682	77	-70**
1.0% HCSE solution	7514, +/- 31	13152, +/- 773	75	-72**

*: Arbitrary fluorescence units; **: difference is significant.

Table 3, DCFH assay results of rinse-off conditioner treated hair

Hair treatment, conditioner	no UVA, Fluorescence*	10J/cm^2 UVA, Fluorescence*	%Fluorescence change vs. no UVA	%Fluorescence reduction vs. placebo
Placebo treated hair	10925, +/- 600	48307, +/- 14055	342	Reference
HCSE-0.25 treated hair	10799, +/- 2293	47160, +/- 7313	337	-5**
HCSE-1.00 treated hair	6030, +/- 816	24119, +/- 4259	300	-42***

*: Arbitrary fluorescence units; **: difference is not significant; ***: difference is significant.

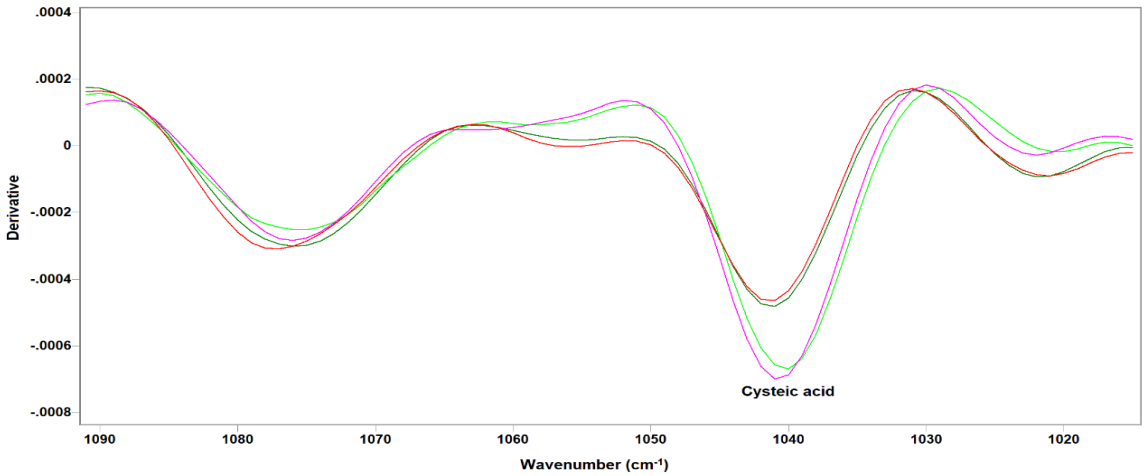
DCFH data in Tables 2 and 3 indicated that the antioxidant Hydrolyzed Cicer Seed Extract reduced the ROS quantity of hair from UVA exposure. When applied as leave-on solution treatment, the protection effect is significant at both 0.25% and 1.0% active use level, and the fluorescence percentage reduction is 70% or more versus that of the untreated hair.

While applied as rinse-off conditioner treatment, hypothetically due to the rinse-off of the formulation, only 1.0% Hydrolyzed Cicer Seed Extract conditioner showed antioxidant protection effect to the treated hair, significant 42% fluorescence reduction was observed in comparison with that of placebo treated hair.

2) UV/Ozone induced hair damage and protection effect of HCSE antioxidant

In this study, the UV/Ozone exposure is a mixed irradiation of UVA/UVB combined with laboratory generated Ozone to mimic oxidative stress impacted to hair from environmental pollution. Up on completion of 12 cycles of treatment and pollution, FTIR spectra and tensile property of hair were measured, the results were illustrated in Figures 1, 2, and Tables 4, 5 and 6.

Figure 1, Second derivative spectra from average FTIR spectra (cysteic acid region)



Placebo: untreated hair, no damage

Placebo: 24hr. pollution hair

1.0% HCSE: no damage

1.0% HCSE: 24hr. pollution hair

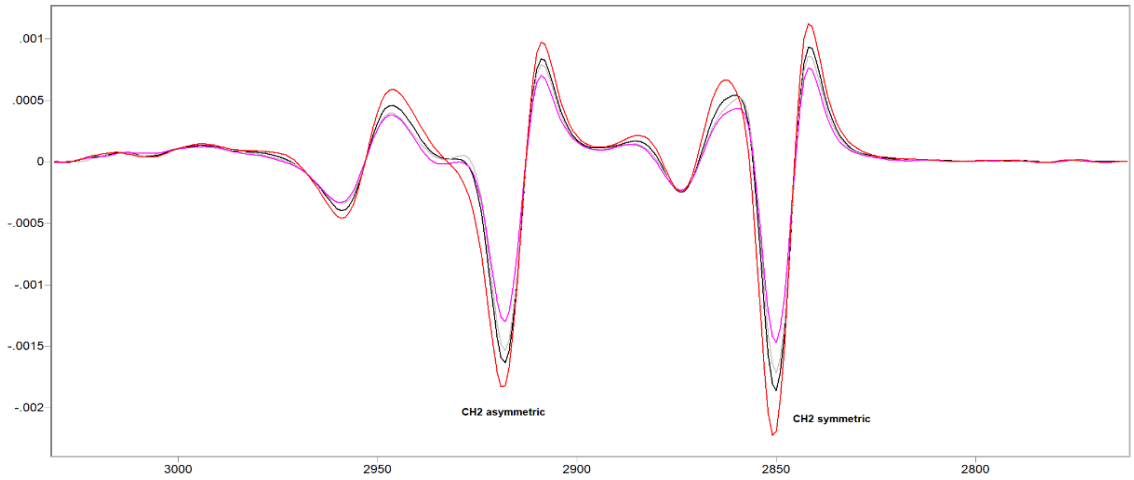
Table 4, Analysis of second derivative cysteic acid peak height

Hair treatment	Mean cysteic acid peak height*		Cysteic acid %peak height	
	no UV/Ozone pollution	24hr. UV/Ozone pollution	Change vs. no pollution	Reduction vs. Placebo
Placebo, DI-water treated hair	5.50E-04	8.46E-04	53.82	Reference
1.0% HCSE solution treated hair	5.69E-04	7.98E-04	40.25	-13.57**

*: mean of 30 values; **: difference is significant.

Figure 1 displayed the second derivative FTIR spectra of hair samples in cysteic acid peak region (1040 cm^{-1}) [12]. Cysteic acid peak intensities of hair treated with and without antioxidant from the secondary derivative spectra are listed in Table 4. The data shows that 13.57% more cysteine was oxidized to cysteic acid from the unprotected hair, compared to that of HCSE antioxidant protected hair. The data proves that antioxidant can reduce UV/Ozone induced cysteine oxidation.

Figure 2, Second derivative spectra from average FTIR spectra (CH2 region)



Placebo: untreated hair, no damage

Placebo: 24hr. pollution hair

1.0% HCSE: no damage

1.0% HCSE: 24hr. pollution hair

Table 5, Analysis of second derivative CH2 peak height

Hair treatment	Mean CH2 asymmetric peak height*		%CH2 peak height	
	no UV/Ozone pollution	24hr. UV/Ozone pollution	Change vs. no pollution	Change vs. Placebo
Placebo, DI-water treated hair	2.74E-03	1.94E-03	-29.20	Reference
1.0% HCSE solution treated hair	2.47E-03	2.24E-03	-9.31	19.89
Hair treatment	Mean CH2 symmetric peak height*		%CH2 peak height	
	no UV/Ozone pollution	24hr. UV/Ozone pollution	Change vs. no pollution	Change vs. Placebo
Placebo, DI-water treated hair	3.12E-03	2.09E-03	-33.01	Reference
1.0%HCSE solution treated hair	2.71E-03	2.43E-03	-10.33	22.68

*: mean of 30 values.

Second derivative FTIR spectra of CH2 region (2855 and 2925 cm^{-1}) representing lipid amount [10] is shown in Figure 2, peak intensities of CH2 symmetric and asymmetric stretches are listed in Table 5. The data suggests that more CH2 peak intensity reduction was observed for the unprotected hair.

Figure 3, Break stress of undamaged vs. UV/Ozone damaged fiber samples

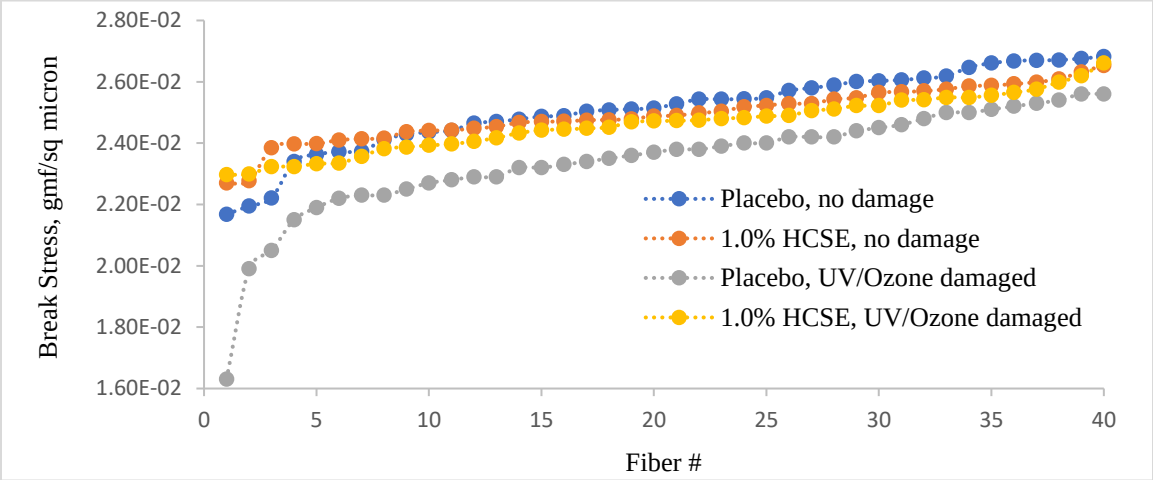
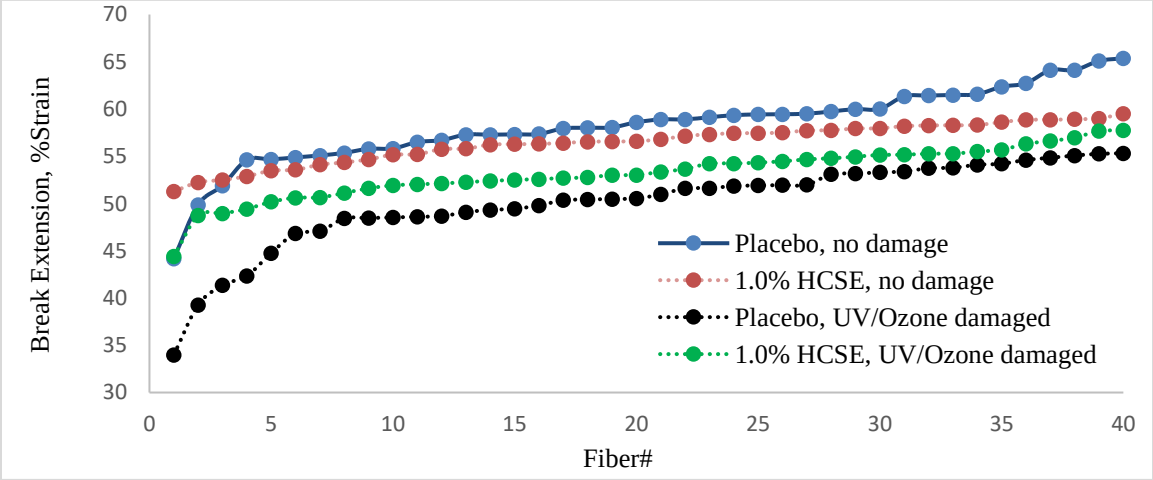


Figure 4, Break extension of undamaged vs. UV/Ozone damaged fiber samples



UV/Ozone damaged hair fibers were evaluated with tensile testing at 50% humidity, break stress and break extension of fiber samples were sorted from the lowest to the highest values and then graphed versus fiber sample in Figures 3 and 4. The data shows that HCSE antioxidant protected hair has longer break extension and higher break stress throughout all tested populations.

3) Accumulative UV induced hair damage and protection effect of HCSE antioxidant

Figure 5, Denaturation temperature (Td, ºC) change vs. UV exposure time

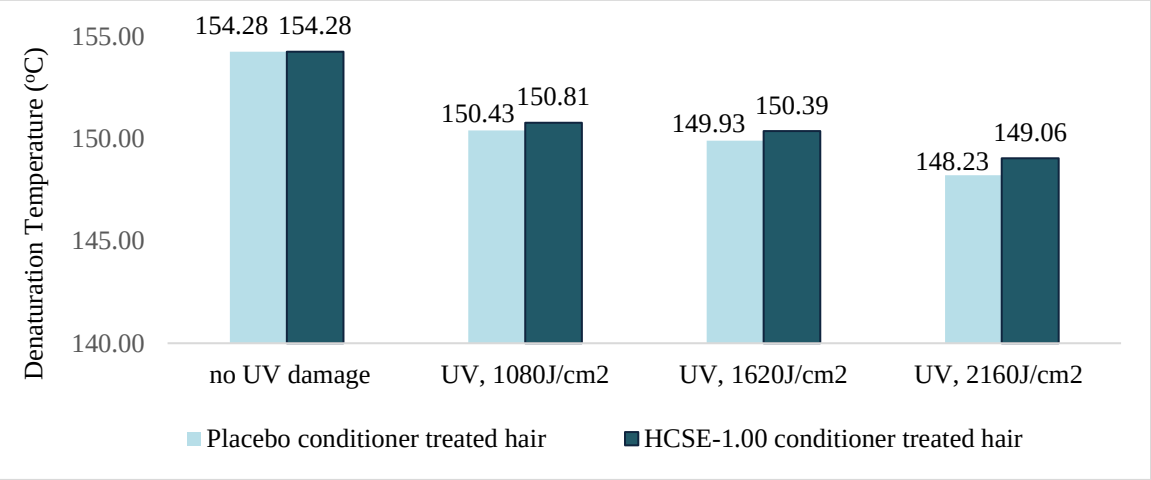
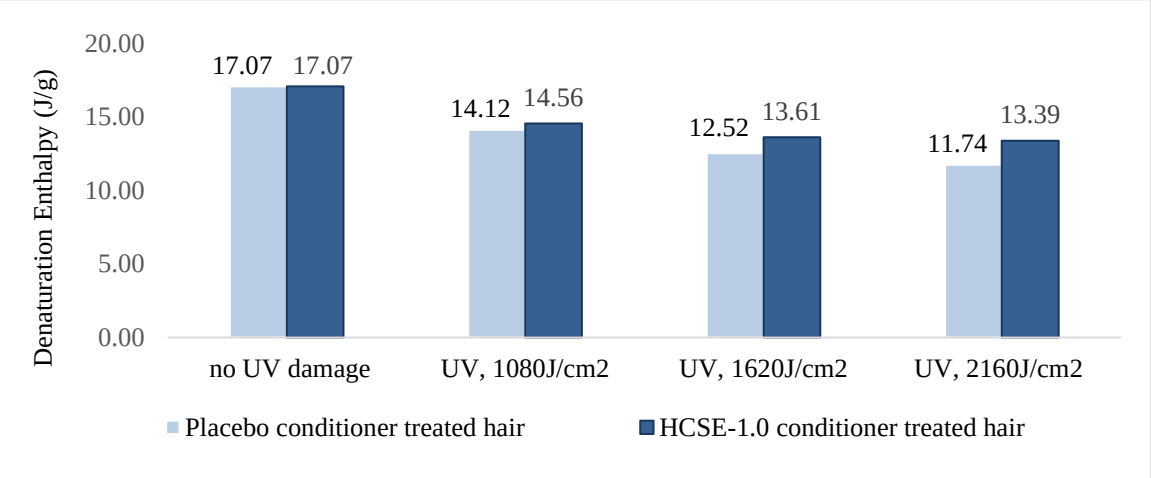


Figure 6, Denaturation enthalpy (J/g) change vs. UV exposure time

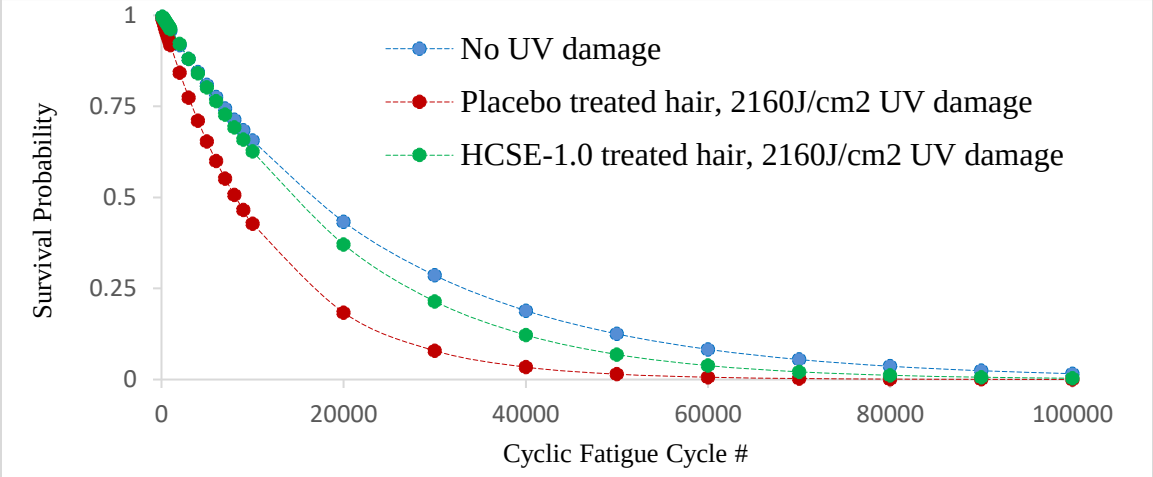


After hair samples were repetitively treated and exposed to different UV dose, hair samples were subjected to DSC test, the denaturation temperature end enthalpy are shown in Figures 5 and 6. The data indicates that HCSE antioxidant protected hair retained better hair integrity evidenced by both higher denaturation temperature and greater enthalpy vs. those of treated with placebo.

Table 6, Cyclic fatigue break cycles of hair before and after UV damage

Treatment	Break cycles, before UV damage	Break cycles, 2160J/cm^2 UV damage	%Cycle change after UV damage
Placebo conditioner treated hair	26769	13214	-50.6
HCSE-1.00 conditioner treated hair	25140	21903	-12.9

Figure 7, Weibull survival probability of undamaged and UV damaged hair



Following completion of 2160 J/cm^2 UV exposure, hair fibers were evaluated by cyclic fatigue testing under 50% humidity, the results are listed in Table 6. The data discloses that HCSE antioxidant protected hair reserved superior fatigue strength versus that of placebo treated hair, 37.7% cyclic fatigue improvement was achieved. Weibull analysis graphed in Figure 7 also suggests that antioxidant protected hair has higher survival probability in comparison with that of unprotected hair treated with placebo formulation.

5. Discussion

1) UVA induced hair damage and DCFH results

2'-7'-Dichlorodihydrofluorescein diacetate (DCFH-DA) is one of the most widely used techniques to measure hydroxyl, peroxyl and other ROS activity in cells [13], but hardly applied to measure ROS activity of UV exposed hair. In this study, we employed the method to assess the amount of ROS generated after applied 10J/cm^2 UVA irradiation to medium brown hair samples, the DCF quantity revealed in Tables 2 and 3 suggests the increase of ROS for both untreated hair, and hair treated with different concentration of Hydrolyzed Cicer Seed Extract (HCSE). The results confirm that UVA exposure indeed provoked generation of ROS in hair samples, and the application of antioxidant like HCSE can reduce the production of ROS from UVA irradiated hair. The antioxidant protection efficacy of HCSE is not dosage dependent from a leave-on solution application, as both 0.25% and 1.0% solution treated hair resulted similar fluorescence intensity after UVA irradiation. This is different while applied from a rinse-off conditioner, where we can observe a dose dependency. 0.25% HCSE in rinse-off conditioner formulation did not provide similar effect as 1% HCSE in the same condition. The results can be attributed to the different interaction and deposition mechanism of the formulation with hair surface, in addition to the rinsed off procedure that reduced the amount of HCSE on hair surface.

Hydrolyzed Cicer Seed has been studied for its antioxidant composition and structure cultivated from different sources [14, 15]. The use of pea peptide hydrolysate as an antioxidant agent in a cosmetic composition was also claimed in EP 2 588 075 B1 [16]. However, to our knowledge, this study is the first to explore the antioxidant effect of HCSE against UVA induced hair damage with DCFH method. The study results prove that applying HCSE on hair surface can reduce the generation of ROS from UVA exposed hair in comparison to that of unprotected hair.

2) UV/Ozone induced hair damage and evaluation results of FTIR and tensile

In this study, medium brown hair was exposed to the combination of UV and Ozone to mimic environmental pollution, and to investigate the protection benefit of HCSE against both photo and oxidative induced hair destruction. After completion of repeated 12 cycles of treatment and UV/Ozone exposure, hair samples with and without HCSE protection were evaluated with FTIR

technique to detect hair internal chemical structure change. Second derivative spectra of cysteic acid shown in Figure 1, and cysteic acid peak (1040 cm^{-1}) intensity listed in Table 4 indicate apparent 13.57% more peak intensity of placebo formulation treated hair after UV/Ozone exposure, in comparison to that of HCSE treated hair. The result agrees with findings from previous literatures; photooxidation of cysteine protein to cysteic acid can be used as a measure of hair oxidative damage [1, 5, 6, 12]. FTIR spectra and peak intensity of CH2 region (2855 cm^{-1} and 2925 cm^{-1}) were displayed in Figure 2 and revealed in Table 5. After 12 cycles of treatment and UV/Ozone exposure, both symmetric and asymmetric stretches of CH2 region decreased versus that of before exposure, the CH2 peak intensity reduction for the unprotected hair is more than that of HCSE protected hair. The CH2 region was known to represent the lipid amount [10]; FTIR data suggests that UV/Ozone exposure also caused degradation of hair lipid, which could also benefit from application of HCSE antioxidant treatment.

Besides assessing hair internal chemical structure alteration with FTIR, hair samples before and after UV/Ozone exposure were also evaluated for physical hair strength with single fiber tensile testing method. Fiber break stress and break extension measured under 50% relative humidity were sorted from the lowest to the highest value of the tested population and graphed against the fiber sample in Figures 3 and 4. Experimental results show that oxidative degradation of hair not only diminished the internal protein structure of hair, but the damage also weakened hair physical strength indicated by lower break stress and less break extension for the exposed hair. The data proves that antioxidant protection effect of HCSE can assist in preserving tensile strength of the treated hair. Although hair treated with HCSE also decreased tensile break stress and extension after UV/Ozone exposure, the level of decline is significantly less compared with the hair treated with placebo without antioxidant.

3) <u>Accumulative UV induced hair damage measured with DSC and cyclic fatigue</u>

To better understand prolonged UV damage effect to hair and how antioxidant can improve the condition, medium brown hair was evaluated with DSC method after being subjected to a total of 2160J/cm^2 dose of UV, with repeated application of rinse-off conditioner treatment after each 108J/cm^2 UV exposure. The denaturation temperature and enthalpy data of hair with different treatment were revealed in Figures 5 and 6. The results indicate a proportional relation of hair

damage as the factor of increased UV dosage. As UV exposure dose increased, hair denaturation temperature and enthalpy further decreased. The data suggests that more UV exposure indeed causes more hair damage detected by DSC method. Hair protected with HCSE conditioner continued to display higher denaturation temperature and enthalpy, suggesting that hair protected from HCSE conditioner retained better cross-link density of the matrix and structural integrity of the α-helical material in the intermediate filaments of the hair [9, 18].

Physical property of prolonged UV exposed hair was also investigated with single fiber cyclic fatigue test under 50% relative humidity. Cycles to break and Weibull survival probability were uncovered in Table 6 and Figure 7. The cyclic fatigue data supports the results from thermal analysis of the hair, preserved internal hair structure from HCSE protection also exhibited better hair strength, and improved survival probability toward mechanical breakage.

6. Conclusion

The study results confirm that different instrumental measurements can provide distinct insight on the damage effect of UV to human hair. The intensity of fluorescence from DCFH-DA assay reflects quantity of reactive oxygen species generated due to photo oxidation of hair proteins; the oxidative damage of hair internal structure can be detected by FTIR using intensity of cysteic acid peak as indicator. DSC data validates that UV diminishes cross-link density of hair matrix, denaturation temperature and enthalpy of UV damaged hair decreases as exposed UV dosage increases. Tensile and cyclic fatigue data are consistent with results from DCFH-DA, FTIR and DSC measurements, which indicates that the internal structure damage of hair weakens the natural strength of hair, the collective impact and antioxidant protection effect can be best verified with a combined approach of employing various spectroscopic and physical measurement techniques.

Experimental data proves that Hydrolyzed Cicer Seed Extract (HCSE) is effective on protecting the hair from oxidative degradation induced by UV/Ozone and prolonged UV exposure. The difference of the internal hair structure and physical strength of the protected hair versus unprotected hair can be characterized by FTIR, DSC, tensile and cyclic fatigue measurements.

7. Acknowledgments

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8. Conflict of Interest Statement

None

9. References

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