Comparative Study for Hair Protection Effect of Hair Essence Prepared Using Human Hair Keratin

Lee Soonhee · Bae Giyeon · Park Doohyun · Kim Sungnam*  
Doctor's Course, Dept. of Beauty Art, Seokyeong University  
Doctor's Course, Dept. of Beauty Art, Seokyeong University  
Professor, Dept. of Chemical and Biological Engineering, Seokyeong University  
Professor, Dept. of Beauty Art, Seokyeong University*

Abstract

This study was performed to quantitatively and qualitatively estimate the effect of keratin essence on hair protection against physicochemical damage.

Damaged hairs were obtained from an early thirty woman who dyed her hair two times and did digital permanent treatment of her hair two times. The damaged hairs were divided into four experimental groups, which are the control hair (CH) group without additional beauty treatment, the damaged hair (DH) group by additional dyeing treatment, basic essence–treated hair (BEH) group, and keratin essence–treated hair (KEH) groups according to the research goal.

The protection effect of keratin essence against the physicochemical damage was quantitatively compared by difference of chrominance measured using a color difference meter and qualitatively compared by difference of outer morphological structure images pictured using scanning electron microscopy (SEM). The brightness and yellowish blue color of KEH were relatively lower but the reddish blue color was relatively higher than other groups of test hairs. Cuticle structure of the previously DH was irregularly deformed and more strongly deformed or partially broken by additional dyeing treatment. On the other hand, the gaps between cuticle scales of the DH were reformed by treatment with basic essence and reformed and filled by treatment with keratin essence in comparison with the DH group.

Conclusively, the keratin essence was effective to protect hair structure against the structural damage induced by the dyeing–treatment, by which the coloring efficiency is thought to be improved.

Key Words : Damaged Hair, Hair Essence, Human Hair Keratin, Hair Protection
I. Introduction

Dyeing and permanent of hair are essential beauty activities together with makeup for beautiful appearance and completion of harmony that the contemporary people seek. As hair is damaged physically and chemically in the process of hairdressing, it causes a result that deteriorates effect of beauty or that goes against the contemporary concept of beauty.

Natural hair may be damaged by environmental factors such as dryness, humidity, temperature change and physical contact and be deteriorated by artificial chemical agents such as detergent, dyeing agent and permanent agent considerably. Specifically, hair may be thinner by sublation, specifically cuticle’s detachment, incision and rupture. And the elasticity of hair is deteriorated by protein denaturalization inside, denaturalization of mesenchyme and humidity loss.\(^1\)

Various hair care products for the prevention and reduction of hair damage by natural or environmental factors are sold which have moisturizing, coating and filler function. If hair damage is minimized and products are applied to damaged hair, it may possible to restore hair structure in a limited way. Thanks to the development of chemical synthetic technology, economic, excellent and convenient chemical property is made for hair control. However, any sensitive users may be vulnerable for skin diseases and allergies.\(^2\) Therefore, it needs continuous research and development for hair care products without side effects or less side effects reflecting physical property and preferences of users.

Generally property used for food and composing living organism and body are very unlike to cause side effect which is good for biological affinity and environmental protection. Such is called natural material which is used for hair care extensively. Herbs like deer antler, licorice, iris, dong quai, mugwort are reported to have excellent performance in function and satisfaction of users.\(^3\) W.J.Choi (2010) confirmed the improvement of scalp and hair care by applying kiwi extract. Besides, worm, lugworm, bacteria (Bacillus sp.) extracted property, viscosity material and enzyme are also reported effective for the hair care improvement. (H.S.Kim, 2010; S.E.Yoo, 2010; S.S.Lee, 2011). Theoretically, raw materials used for hair care products might be very effective if it wraps damaged part or lost part of the cuticle surface. Accordingly, it must be water soluble and disperse and must be attachable stably with structural affinity with lost part. And, it is necessary to have biological affinity. In this sense keratin hydrolysis is the most effective as it has the same structure of hair. It is followed by collagenhydrolysis and elastine hydrolysis. However, if products with protein hydrolysis are used, protein is released outside easily which results in not satisfying expected effect.\(^4\)

This study extracted, refined and took water soluble process keratin that had the highest gravity of hair compositions and maintains structural stability. By using this, keratin essence was produced. To evaluate effect of essence for hair care, it is necessary to compare tolerance against chemical denaturant (dyeing and permanent) and positive effect on beauty that has an effect on the restriction of morphological change of surface structure and hair structure. The change of hair structure by dyeing or permanent can only be found by electron microscopic observation of cuticle surface as scanning electron microscope is not available to observe cross-section of hair.\(^5\)
Accordingly, it needs to analyze keratin essence effect using a chromatograph in a quantitative manner as well as a qualitative structural comparison using a microscope. In order to compare essence effect more clearly, partially damaged hair was used three times, and the level of damage of hair and the level of restoration were compared through additional dyed hair treatments.

II Experiment material and method

1. Experiment material

1) Keratin extracting hair

300g hair of women in their twenties and thirties with healthy body and without chemical treatment was collected for the keratin extract. Vocation, residence, and educational level of the hair-offering women were not considered but gender and health condition were thoroughly considered to establish purpose for collection of healthy and long hairs.

2) Keratin extract and essence production material

Chloroform, Methanol, Thiourea, Tris-HCl, Urea, Mercaptoethanol, Ammonium sulfate, Glycerol, Tween 80 and Ethanol were from Sigma-Aldrich (USA).

3) Catapult film

For catapult film for protein refinement, a product that penetrated less than 10,000 dalton of old protein base, was used (Spectra/Por, USA).

4) Protein measurement material

For measuring dyeing reaction of protein, Bradford Coloring Reagent (Bradford, USA) was used and 0.1% (w/v) of calf serum protein solution (BSA, BioRad, USA) was used as a standard material.

5) Test device

Ultrasonic oscillator (400W, Vibra cell™, Sonic & Materials, USA), high speed centrifuge (20,000 rpm max., Hanil Science, Seoul) and refined electronic scale (SHEN-ZHEN ACCT, KB-2000, CHINA) were used for separating refinement and quantitative measurement of protein. Other scanning electron microscope photo was taken at SNU analysis center, and the color difference was measured at the special institution.

2. Experiment method

1) Hair keratin extract

To remove fatty ingredient in cuticle of hair, it was input in cleansing solution 5 liter that mixed chloroform and methanol in 2:1 and sealed and stored for 24 hours at the room temperature. It was dried at 50℃ and cleansing solution was completely evaporated. Then, it was input extract solution of 3 liter that contained 25mM Tris-HCl (pH 8.5), 2.6M thiourea, 5M urea and 5% 2- mercaptoethanol and cultivated for 3 days at 50℃. The remaining solution was treated in centrifugal process for 30 minutes at 15,000xg and 20℃ to remove insoluble impurities. The solution was cooled down at 3℃ and protein was precipitated by adding 6M ammonium sulfate. All chemical materials used for extracting, cohesion, and refinement of keratin were purchased at Sigma-Aldrich (USA). Solution containing protein was taken centrifugal
process at 5,000xg, and 4°C for 40 minutes. The precipitated protein was input into the 2nd distilled water of X5,000 volume in catapult film(Spectra/Por, cut-off 10,000 dalton, USA), which was preserved 4~5 days in the refrigerator to substitute ammonium sulfate with distilled water. The protein content in the keratin solution without ammonium sulfate was measured using Bradford coloring reagent(BioRad, USA) and calf serum protein standard solution(BSA, BioRad, USA).  

2) Basic essence and keratin essence production

Refined water 100mL, olive oil 10mL and tween #80 5mL mixed solution was treated using ultrasonic oscillator (400W, Vibra cellTM, Sonic & Materials, USA) for 60 minutes and added heat at 60°C and homogenized for 30 minutes at 2000 rpm using homogeneity mixer to obtain emulsified olive oil. Emulsified olive oil 100mL was added by lactobacillus exopolysaccharide 1g, pine extract 1g, glycerin 1g, ethanol 2g, and keratin solution 2mL to produce keratin essence. Basic essence was produced in the same manner without adding keratin to compare effect of keratin.

3) Preparation and treatment of sample hair

The damaged hair in this study was collected from a woman in her 30s who took twice dyeing and 1 digital permanent. When the damaged level was checked visibly, it had no difference from natural hair. Hair used for sample was collected between 10~15cm from hair root from occipital region. The collected hair was measured in 2g with an electronic scale (SHENZHEN ACCT, KB-2000, CHINA), and the bottom 1 cm was fixed with silicon, cleansed with lukewarm water and dried at room temperature. The dried mobile was classified into 4 including control group, drying treatment group, basic essence treatment group and keratin essence treatment group. Cleansed and dried damaged hair was used as <control hair (CH) group>. 1 additional dyeing treatment was carried to damaged hair which was designated as <damaged hair (DH) group>. Meanwhile, before additional dyeing treatment, basic essence and keratin essence 1mL were spread to cleansed and dried hair 2g evenly and dried with hot wind dryer (80°C for 5 minutes. Then dyeing was carried out to classify <basic essence-treated hair (BEH) group> and <keratin essence-treated hair (KEH) group>. In the preparation of <BEH group> and <KEH group>, dyeing treatment was carried out by spreading Wella Company’s red series dyeing agent 8/45(1 sort of ingredient) and H₂O₂ 6% 2 sorts of ingredient (oxidizer)in 1:1. It was left for 30 minutes at room temperature and cleansed with neutral shampoo and dried naturally.

4) Chromaticity measurement of hair

The chromaticity of hair sample in 4 groups was injected with CIE D65, 10°, d/0 TYP E using chromatograph (Minolta 3700d, Japan), and the value was measured in L*(Lightness), a*(Redness), b*(Yellowness) and ΔE*ab. The chromaticity of damaged hair by dyeing treatment was measured and compared based on values of lightness(L*), redness(a*), and yellowness(b*). ΔE*ab was calculated based on difference of L*, a*, and b* between virgin hair and test groups of hairs that are CH, DH, BEH, and KEH groups. L* value was marked between 0 and 100. 0 is closer to black and 100 means white. 'a' assembly is based on green and red axes. +a is closer to red, and -a is closer to...
green. ‘b’ assembly is based on yellow and green axes. +b is closer to yellow, and −b is
closer to blue. By increasing or decreasing a
value or b value, chromaticity increases or
decreases to clear or blur color. And, when a
value and b value get closer to 0, it becomes
colorless.\(^7\) \(\triangle E^{ab}\) is the difference of color if
the difference of yellowness or redness is huge,
the value becomes large. Then, it can be a
criteria to determine color homogeneity between
test groups.

5) Observation of morphological change of
cuticle

Each hair samples in 4 groups were
vacuuming coating for 5 minutes so that silver
ion could be attached to surface regularly using
ion sputtering device (SCD005, BAL-TEC,
Germany) by fixing sample treatment plate (silver
fasten). Then, scanning electron microscope
(SUPRA\(^\text{TM}\) 55VP; ZEISS, Germany) was observed
by X2000 and X5000.

6) Hair protection effect of essence and
comparative study method

The photo and color of electron microscope
of control group was used as the standard of
damaged hair. The damaged hair group used
increased damaged hair by dyeing as standard
hair. To compare protection effect on the
damaged hair during the beauty process, and
the level of additional damage between basic
essence and keratin essence, essence was
treated to hair before dyeing.

III Test result and consideration

1. Characteristics of keratin extracted
from hair

The content of keratin solution in 300g hair
was about 500mL and protein content was 1.7%
over 10mg/mL. Such low extract efficiency could
be solved by using short crushing of hair as
keratin was extracted from cross-section of hair.
This study used collected hair as it stands, the
extract efficiency was very low. The keratin
molecular amount by recalculation of relative
movement rate by electric movement was about
60,000–70,000.

2. Effect of essence on hair color
expression

While hair color is a useful method to check
effect of dyeing, it does not provide information
to compare dyeing effect definitely. Accordingly,
measurement value of lightness(L⁎), redness(a⁎),
and yellowness(b⁎) for 4 test groups that are
<CH>, <DH>, <BEH>, and <KEH> was
compared and evaluated in reference with
<standard chromaticity value of virgin hair color>
as shown in Table 1. Both a⁎ and b⁎ values of
virgin hairs (color level 4) used as a criterion of
hair chromaticity were significantly lower than
those of 4 test hair groups. It is reasonable,
considering that the virgin hair was not dyeing
but CH, DH, BEH, and KEH were dyed with
redness series one or two times. Hair–dyeing
treatment is not a factor to influence lightness
(L⁎) variation but definitely change redness (a⁎)
and yellowness (b⁎) of hair. Accordingly,
relatively lower a⁎ value and high b⁎ value of
CH than DH, BEH, and KEH may be caused by
dyeing effect but not by damage degree of
hairs. As the value by chromaticity measurement
may vary due to structural difference of hair surface, it must be considered. If the increase of $a^*$ value it could be determined as the increase of red color appearance but could not be accepted as the increase of damage degree. As in Table 1 $a^*$ value of BEH and KEH was relatively reduced compared with DH but increased compared with CH, it may be caused by the effect of surface structure of DH.

Theoretically, surface of tough or bent hair reflects red light (relative low energy ray) and is highly plausible to absorb short wave blue light (relative high energy ray). Accordingly, effect of hair color expression by basic essence and keratin essence was desirable to compare with control group excepting for damaged hair. Lightness ($L^*$) is a value to indicate lightness of color which was reverse proportionate against light absorption, and proportionate with the reflection level of light. It can be directly affected by arrangement of hair surface cuticle scale and specific material on surface. It is same with results of relative reduction of light by essence treatment. It is also because by the difference of density between scale surface and essence as hair cuticle scale was added by essence material that absorbed relatively more light. $\Delta E^{*ab}$ value could not be a criteria to determine hair dyeing effect as it is square value of lightness ($L^*$), redness ($a^*$) and yellowness ($b^*$), it shows the level of realization of colors and therefore can be information to determine color expression stability of dyeing agent. Accordingly, the treatment of basic essence and keratin essence has high possibility of reflection of blue light with high energy as it reduces bent of hair and filling gap.

According to experimental results, lightness, redness and yellowness were relatively reduced by treating damaged hair with essences. Essence treatment does not affect dyeing effect but essence treatment affects hair surface cuticle structure that made differences in light absorption and reflection. In reality, ingredients of essence on hair surface (fat, EPS, keratin, pine extract, etc) penetrate cuticle that does not have any harmful operation, but it has no possibility of deformation by chemical reaction. Therefore, the relevancy between essence treatment and dyeing hair effect could not be ignored. Such result was demonstrated by the fact that the variation of redness and yellowness value of hairs used for experiment may be a major factor to change $\Delta E^{*ab}$ value because lightness of virgin hair, CH, DH, BEH, and KEH was not meaningfully different. The $\Delta E^{*ab}$ value for BEH and KEH was a little lower than that for DH, which was caused by a little increase of redness but a little decrease of yellowness by essence treatment. A little decrease of $\Delta E^{*ab}$ value for BEH and KEH by essence treatment in comparison with that for CH may be an index to evaluate essence effect for improvement of hair structure but not for increase of dyeing effect. Such result seems to be caused by the reduction of diffused reflection by vertical cross-section of cuticle scale and by the reduction of bent part of surface by filling gap between cuticle scales.

3. Protection effect of essence on hair surface structure

Generally, healthy hair cuticle is 10~15% of hair ingredient. If this content increases, hair becomes stable and has good gloss with increase resistance against humidity and friction. Cuticle layer is thin and transparent plate phase cell which is overlapped on hair surface regularly reflecting light in a constant direction that gives glossy to hair and protects hair from external stimulation. In addition, it refrain evaporation of
humidity (about 15%) in hair root to prevent dryness of hair. However, hair with long growing period is vulnerable for damage increase by environmental change, physical and chemical damage by beauty action and physical friction in life. Therefore, it increases diffused reflection of light with structural denaturalization by scale rupture or partial detachment. Lightness increases near to hair surface, but gloss, elasticity and tensile strength could be reduced by regular reflection of light. Such level of loss is related to lightness ($L^*$) of hair measured by chromatograph and lightness increases level of damage proportionally. Accordingly, structural deformation by cuticle detachment and partial rupture of cuticle could fill gap between cuticle scales and make even surface by attaching to cuticle scale. Thus, it enabled to make masking temporarily. As shown in Table 2, CH C and D showed gap between scales by detachment. The level of damage was deepened by repetitive dyeing, and as in Table 2, DH A and B and Table 2, DH C and D detachment and ruptured cuticle increased more. Such result was proportionate to the differences with lightness (Table 1).

Meanwhile, when permanent dyeing was carried out by pre-processing of damaged hair with basic essence, as shown in Table 2, BEH A and B and Table 2, BEH C and D it filled gap between cuticle scales even though it was unstable. It was because the high molecules like fat or EPS was absorbed and dried. Such result was related to the reduction of lightness of hair with basic essence. Such result seems to be caused by the reduction of diffused reflection by vertical cross-section of cuticle scale and by the reduction of bent part of surface by filling gap between cuticle scales. Keratin is structural
protein and has a function to keep cortex-cuticle complex by being attached to cuticle scale.\textsuperscript{10} Such function of cuticle protein was made by hydrophobic property physio-chemical reaction generated by gravity between molecules having ionic operation, hydrogen bond reaction and giant molecule.\textsuperscript{11} Accordingly, when hair surface was treated with cuticle contained essence, keratin with high affinity with cuticle internal structure could be attached to gap between cuticle scales. As shown in <Table 2, KEH A and B> and <Table 2, KEH C and D> surface structures of test group and control group treated with keratin essence were different. It was because keratin filled gap between cuticle scales perfectly. Such phenomenon could effectively restrain light diffused reflection from vertical cross-section and increase regular reflection of cuticle scale. Thus, while lightness reduces, gloss increases. And, it affects light absorption and reflection of hair surface and increases redness (a*) and decreases yellowness (b*) in comparison with control groups but decreases both redness (a*) and yellowness (b*) in comparison with damaged hair groups <Table 1>. As the use of hair essence aimed to extend structural stability of hair and minimize hair damage\textsuperscript{12}, it is sufficient to have 1 or 2 days effect. Accordingly while applying keratin on hair, keratin protects hair structure and minimizes damage level. Accordingly, it is necessary to apply hair keratin to various hair care products based on positive results.

\textbf{IV. Conclusion and suggestion}

This study produced essence using keratin that was extracted and refined from human hair chemically. To evaluate its efficiency, surface structure’s morphological character change, tolerance of chemical denaturant and influence of beauty effect were compared quantitatively and qualitatively.

Hair keratin used in this study was about 60,000–70,000 molecules with water soluble. While it can be attached to hair surface stably, it may not be easily reduced during shampooing and rinsing based on the molecular weight of protein. Thus, it is considered to be proper as a material for hair essence.

When basic essence without keratin was applied to highly damaged hair, the hair loss was restricted, and keratin-contained essence treatment could resist chemical damage of hair and contribute to structural stability of damaged hair.

When damaged hair and repetitive dyed hair were treated with basic essence and keratin essence, lightness\textsuperscript{(L*)} and yellowness\textsuperscript{(b*)} were reduced and redness\textsuperscript{(a*)} increased in comparison with CH groups but both yellowness\textsuperscript{(b*)} and redness\textsuperscript{(a*)} decreased in comparison with DH groups. Thus, the cuticle gap of damaged hair is proportionate with the restoration of essence basic ingredient and keratin protein.

As such, human hair extracted and refined water soluble keratin had hair protection effect and filling effect of damaged part. It is recommended to verify effect of coloration of hair and protection effect by controlling keratin contents in essence further.

The solubilized keratin protein extracted from human hairs may be more structurally familiar with the inside part of human hairs than proteins originated from other sources because composition of amino acids of the solubilized keratin is similar highly or same to natural keratin.
Table 2: SEM Image of The Control Hairs (CH), Damaged Hairs (DH), Basic Essence-Treated Hairs (BEH), and Keratin Essence-Treated Hairs (KEH)

<table>
<thead>
<tr>
<th>CH A ×2,000</th>
<th>CH B ×2,000</th>
<th>CH C ×5,000</th>
<th>CH D ×5,000</th>
</tr>
</thead>
<tbody>
<tr>
<td>DH A ×2,000</td>
<td>DH B ×2,000</td>
<td>DH C ×5,000</td>
<td>DH D ×5,000</td>
</tr>
<tr>
<td>BEH A ×2,000</td>
<td>BEH B ×2,000</td>
<td>BEH C ×5,000</td>
<td>BEH D ×5,000</td>
</tr>
<tr>
<td>KEH A ×2,000</td>
<td>KEH B ×2,000</td>
<td>KEH C ×5,000</td>
<td>KEH D ×5,000</td>
</tr>
</tbody>
</table>
in human hairs. The interaction between natural keratin and solubilized keratin under molecular level is a subject to be studied for development of cosmetics with effect of beauty therapy.

Therapeutic effect of cosmetics for skin and hair may be generate or increased by employment of the natural compounds like keratin, collagen, and chitosan. Accordingly, research and development of cosmetics using polymeric compounds originated from animals and plants are required to be enlarged by majors of cosmetology, chemistry, and biology.

Reference


7) Lee Sookyong(2007), "The Epidermal Change and Degree of Discoloration of Hair according to Hair Detergent after the Operation of Acid Hairdye", Hannam University Graduate School of Social Culture Department of Cosmetic Beauty master’s degree dissertation, p.16.


Received (Jun. 4, 2013)
Accepted (Jun. 28, 2013)