EVALUATION OF HAIR DAMAGE BASED ON MEASUREMENTS OF LABILE PROTEIN

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Presented in part at the 50th Scientific Meeting of the Society of Cosmetic Chemists of Japan, Osaka, June 26, 2002.

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KEY WORDS:

labile protein, hair damage, evaluation, bleach, commercial product

SYNOPSIS

Most consumers have noted hair damage following coloring treatments. Proper evaluation of hair bleaching products must be performed using quantitative assessments of hair damage, though they are difficult, because of the slight fluctuations in hair composition. In the present study, we utilized a sensitive evaluation method for hair damage and found that the amount of soluble protein fraction extracted from hair under a reducing condition, termed labile protein, dramatically increased after bleaching. We measured the increase of labile protein to assess hair damage caused by bleaching and our results showed that labile protein levels were well correlated with hysteresis ratio, a sensitive index of tensile property changes. Further, the amounts of labile proteins differed by type of alkaline agent and protein hydrolysates used as supplements in the bleaching agents. We also found that labile protein levels in hair treated with various commercial products fluctuated despite their nearly equal bleaching effect. Our findings showed that labile protein level is a novel sensitive index that enables the selection of bleaching agent components in regards to their effect on hair damage.

INTRODUCTION

The hair colorant market in Japan has grown rapidly in the past 10 years, especially among younger generations. However, in questionnaire style investigations many consumers have expressed their dissatisfaction with apparent hair damage that occurs following permanent hair coloring and bleaching treatments [1, 2]. Thus, proper assessment of hair damage is an important requirement for designing permanent hair color and bleaching products that are acceptable.

A variety of methods have been used for the assessment of hair damage, including analyses of tensile property and chemical property changes [3], however, it is difficult to assess hair damage caused by hair bleaching, due to slight fluctuations in those properties. As a result, many studies have used excessive conditions to assess hair damage from bleaching [4]. We considered that an accurate and sensitive method for assessment of hair damage that is applicable under milder conditions similar to normal consumer usage is required.

We previously established a novel partial extraction method for hair protein (labile protein), which was shown to dramatically increase following bleaching treatments [5]. A proposed mechanism of labile protein accumulation is shown in **Figure 1**. Compared

to tensile strength, a popular index of hair damage, labile protein amounts are altered in more widely dynamic range [5]. We speculated that this index had the potential to assess hair damage caused by bleaching. In the present study, we found that labile protein levels were significantly altered by mild hair bleaching treatments that were similar to practical usage conditions. Furthermore, since our method was sensitive in its quantification of labile protein levels, we used it to assess variations of hair damage caused by modification of the bleaching agent compositions.

MATERIALS AND METHODS

Materials

Hair Samples

Hair strands 20 to 40 cm long were obtained from Japanese women who had not employed any chemical treatments, including permanent waving and bleaching. Hair tresses each weighing 1 g were made from these samples and then washed with a 0.2% sodium laureth sulfate solution.

Bleaching products

Four top-selling hair bleaching products in the Japanese market were chosen, and normal, hard, and extra-hard types of each brand were tested.

Methods

Bleaching treatments

All treatments were done at 30℃ with a liquid to fiber ratio of 1:1 for 30 minutes. A model bleaching lotion containing 3% hydrogen peroxide and 1.2% ammonia (0.7 M) was used for the standard experiments, however, for comparative study of an alkaline agent, the ammonia was substituted with 4.3% 2-amninoethanol (0.7 M). The bleaching contents of each commercial product were mixed according to the instruction manuals written by the supplier. After exhaustive rinsing, the bleached hair samples were dried at room temperature.

Measurement of labile protein levels

The amounts of labile proteins were measured according to the method described in our previous study [5]. Briefly, the hair samples were extracted in 200 mM of Tris containing 200 mM of 2-mercaptoetanol at 37℃ for 16 hours with a liquid to fiber ratio of 50:1. The protein extract was passed through a cellulose acetate filter to remove hair

fragments and then concentrated using a centrifugal filter device. The resultant labile protein fractions were subjected to a dye-binding assay for determining protein concentration.

Tensile properties (hysteresis ratio)

Tensile properties of single hair fibers were determined at 25℃ and 60% relative humidity using a texture analyzer (TA-XT2, Stable Micro Systems, U. K.). The hysteresis ratio [6] was calculated from the energy required to extend the fiber 20% and that regained after unloading.

Bleach index (ΔE)

The color indexes of the hair tresses were measured using a chromometer (CR300, Minolta Camera, Japan) and classified by light index (L), red index (a), and blue-yellow index (b). The bleach index was determined by calculating the differences of these indexes between untreated and bleached hair samples (i.e.; ΔL, Δa, Δb).

$$\text{Bleach index } (\Delta E) = \text{SQRT}\{(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2\}$$

Statistical analysis

Statistical analyses were performed using a computer program (EXSAS version 4.00, Arm, Japan).

RESULTS AND DISCUSSION

Labile protein level as an index of hair damage

Hair tresses were subjected to repetitive bleach treatments under the standard condition (see **Methods**), and then the amounts of labile proteins and hysteresis ratio were determined. Labile proteins levels rose as the number of bleach treatments increased, as shown in **Figure 2**, and there were significant differences between each treatment. We considered that our standard bleach procedure was mild and a near-practical treatment condition, therefore, our results indicated that hair damage induced by conventional bleaching could be analyzed using labile protein levels.

It has also been reported that hysteresis ratio is a sensitive parameter for the evaluation of tensile property changes and a useful index of hair damage [6]. In the present study, hysteresis ratio consistently decreased as the number of bleach treatments increased, as shown in **Figure 3**, and there was a significant difference between the

untreated and single treated groups. However, we did not find any significant differences between the single treated and double treated groups, or between the double treated and triple treated groups. These results suggest that labile protein level is a more sensitive parameter than hysteresis ratio (**Figure 2** vs. **Figure 3**).

The relationships between the amounts of labile proteins and hysteresis ratio results are shown in **Figure 4**, and a high correlation was found between them (R^2=0.95). This finding indicated that labile protein content could be used as an index of hair damage.

Effect of cosmetic product components on hair damage

1) Alkaline agents

Ammonia and 2-aminoethanol are alkaline agents typically used in commercial bleaching products. We analyzed the bleaching effect and compared labile protein levels in hair treated by a bleaching agent containing either ammonia or 2-aminoethanol (0.7 M each), and found that labile protein amounts in hair treated by that containing 2-aminoethanol were significantly greater than those treated by that containing ammonia (**Figure 5**). On the other hand, the mean bleach index in 2-aminoethanol treated hair tresses (ΔE =7.79±0.62) was lower than that of the ammonia treated hair tresses (ΔE

=6.79±0.63). These results indicate that ammonia gave a superior bleaching effect with less hair damage as compared to 2-aminoethanol.

2) Protein hydrolysate

It has been reported that protein hydrolysates are capable of reducing hair damage [7], therefore, we examined whether the hydrolysate samples listed in **Table I** had an effect on labile protein levels when used as supplements with the standard bleaching agents. The amounts of labile proteins and levels of bleach used are shown in **Figure 6**. Labile protein levels in hairs treated with a bleaching agent containing either hydrolyzed wheat protein or hydrolyzed soy protein were lower than those who received the control treatment. These results indicate that labile protein level is a sensitive index that can accurately represent the damage reducing effect of protein hydrolysates.

The addition of hydrolyzed wheat protein did not significantly alter the bleach index, whereas hydrolyzed soy protein decreased the index to some extent. Nevertheless, the effect of hydrolyzed soy protein on the amount of labile protein was much larger than its effect on the bleach index. These results indicate that protein hydrolysates have a larger effect on labile protein level than bleach index. Therefore, we considered that the use of protein hydrolysates is likely important for the reduction of hair damage.

Assessment of hair damage after treatment with commercial bleaching products

Next, labile protein levels in hair treated with the commercial bleaching products were determined by simultaneously examining the bleach index values of each sample. As shown in **Figure 7**, there was a weak correlation between labile protein level and bleaching index, as the stronger bleaching type products tended to cause a greater increase in labile protein, though some of the plots were far from the correlation line. Since labile protein levels were found to be significantly affected by the type of alkaline agent and by supplements, these differences were likely caused by the different components in each bleaching product.

CONCLUSION

We were able to quantitatively evaluate hair damage caused by commercial hair bleaching products by measurement of labile protein levels. Further, this hair damage index is also considered applicable for the screening of cosmetic materials used to prevent hair damage.

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TABLE

Table I: Protein hydrolysate

	Component	Average Molecular Weight
Silk	Hydrolyzed silk protein	850
Wool	Hydrolyzed wool keratin	400
Wheat	Hydrolyzed wheat protein	3500
Soy	Hydrolyzed soy protein	700

FIGURES

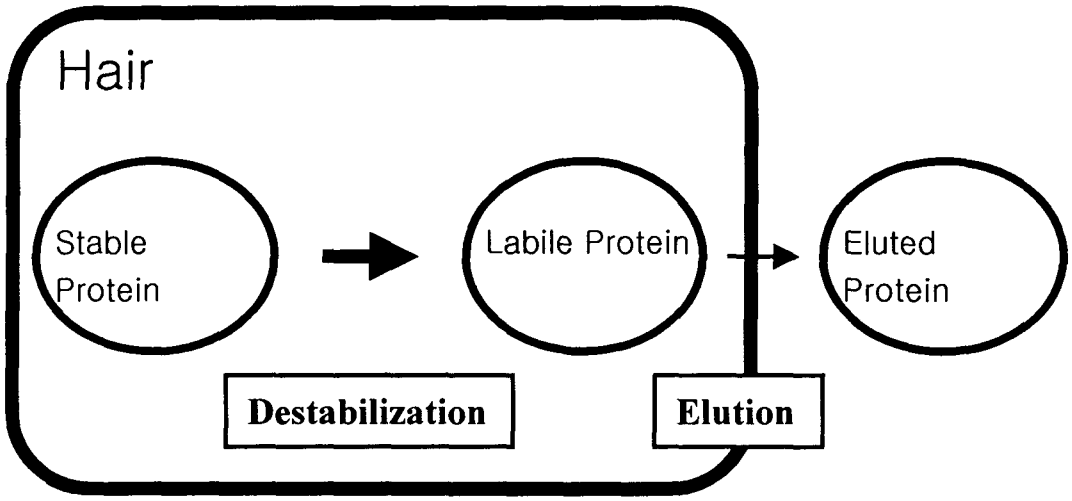

Figure 1: Hypothetical process of molecular or environmental modifications of soluble proteins in bleached hair [5]. Following bleaching, soluble proteins in a stable state are transformed into a labile state. However, nearly all soluble proteins are retained in hair, because the amount of protein converted is much larger than the amount of protein eluted. The resultant labile proteins were isolated under a partial extraction (mild reducing) condition.

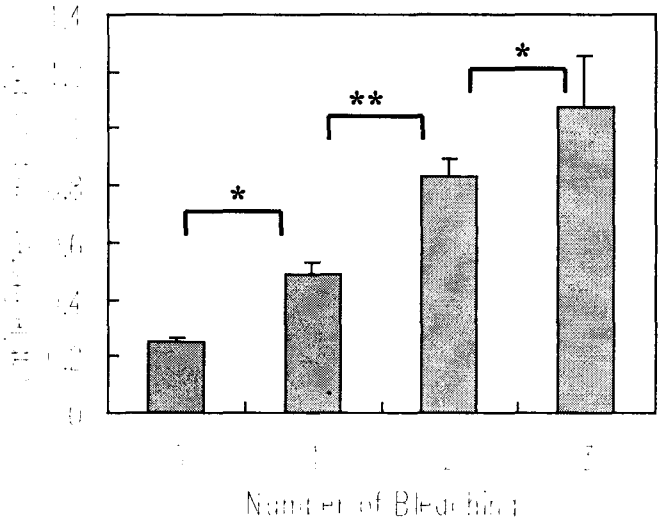

Figure 2: Increase in labile protein levels following bleach treatments. Error bars indicate standard derivation from 3 or 4 experiments. Statistical significance was analyzed using a Tuky test. * $p<0.05$, ** $p<0.01$

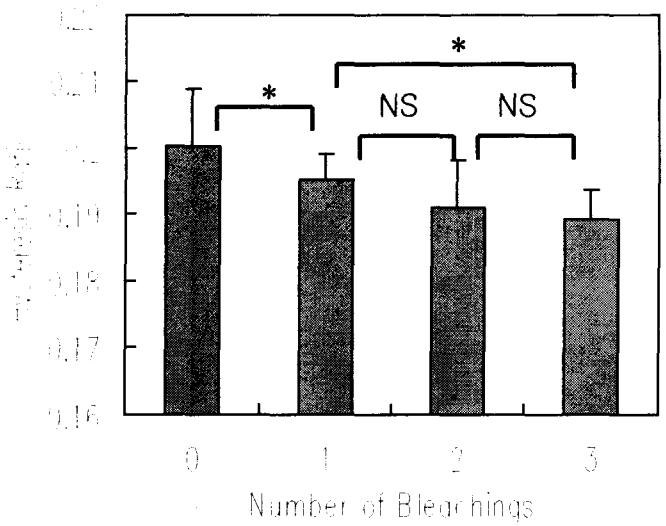

Figure 3: Decrease in hysteresis ratio following bleaching treatments. Error bars indicate standard derivation of the values of 20 hair fibers. Statistical significance was analyzed using a Tuky test. ^{NS} $p>0.05$, * $p<0.05$

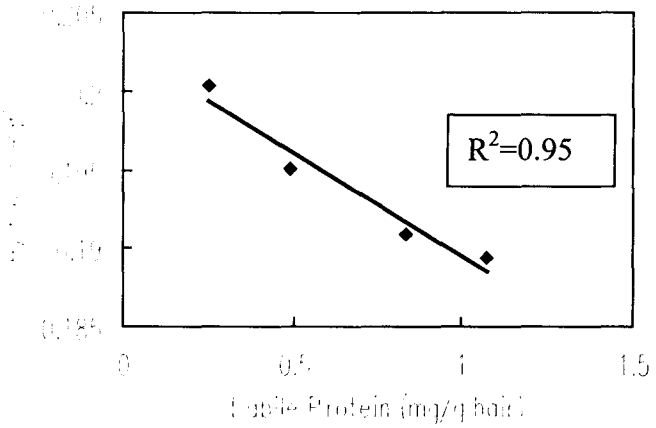

Figure 4: Correlation between labile protein amount and hysteresis ratio.

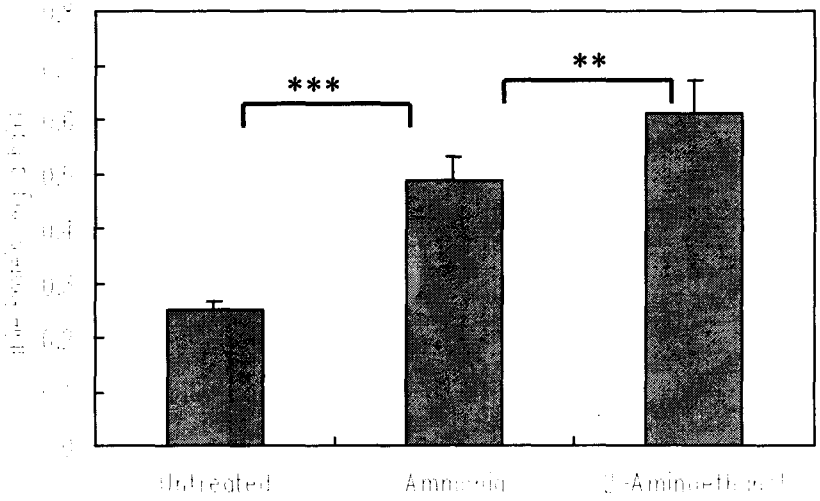

Figure 5: Comparison between ammonia and 2-aminoethanol. Error bars indicate standard derivation in 4 experiments. Statistical significance was analyzed using a Tuky test. ** $p<0.01$, *** $p<0.001$

A: Labile Protein **B: Bleach Index**

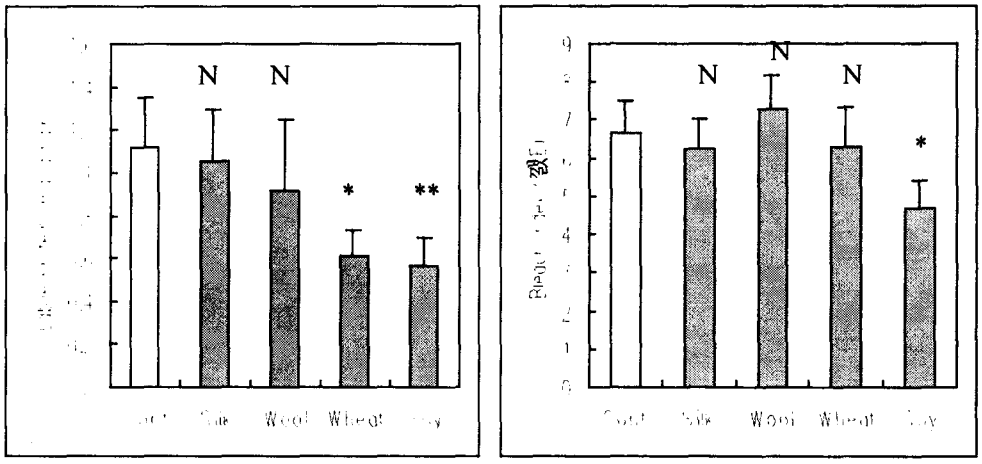

Figure 6: Effect of protein hydrolysate. Each of the protein hydrolysates corresponding to a dry weight of 2% was added to the standard bleach lotion. Error bars indicate standard derivation in 4 experiments. Statistical significance was analyzed using a Dunnett test. * $p<0.05$, ** $p<0.01$, ^{NS} $p>0.05$

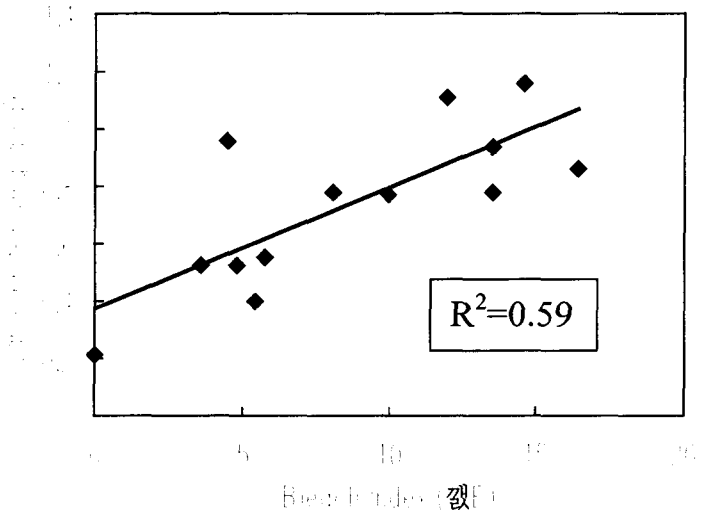

Figure 7: Correlation between labile protein amounts and bleach indexes in hair tresses treated with commercial hair bleaches. The mean values of 4 experiments are plotted.