

A simple and sensitive method using protein loss measurements to evaluate damage to human hair during combing

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Synopsis

A simple method to quantify hair damage during combing or brushing has been developed. The method involves collecting hair fragments that are chipped from hair during combing and quantitatively measuring the amount of protein using a colorimetric procedure capable of detecting as little as 5 μg of protein per ml. Using this procedure on hair tresses in the laboratory and on live heads (half-head tests), we were able to demonstrate significant differences in protein loss during post-shampoo combing of undamaged and chemically damaged hair previously treated with various shampoos/conditioners. The method is applicable to different types of hair tested, namely, Caucasian, Asian, Oriental and Negroid.

INTRODUCTION

The cuticle forms a protective barrier for each hair fiber. The cuticle governs the frictional properties of hair fibers and is also largely responsible for maintaining the structural integrity of hair. Many investigators have shown that the cuticle is gradually chipped, fragmented, and worn away by the abrasive action of combing, brushing, shampooing, and teasing of human hair (1–4). It is also known that gradual loss of cuticle layers can eventually lead to complete fibrillation of the fiber (4,5). Various investigators have used a number of techniques such as scanning electron microscopy [SEM (1,3,4)], combing measurements using an Instron Tensile Tester (4,6,7), frictional measurements (8), tensile measurements (9,10) and measurement of the sound or raspiness caused when combing (11) to evaluate hair damage due to combing and/or the conditioning effects of various products. Some of these techniques require expensive instrumentation (e.g., SEM), some are qualitative (11), and others lack sensitivity [tensile measurements (10)], simplicity (SEM), and/or the precision desired in an analytical technical for routine use to study surface damage.

Previously we published a simple technique to assess surface damage to hair caused by oxidative/reductive treatments such as bleaching and permanent waving (12). Since hair

is primarily a proteinaceous material, we hypothesized that measurements of the amount of protein abraded from hair could be used as an index to assess damage caused by abrasive actions. The method involves shaking hair in water (wet abrasion) and quantitatively measuring, via a colorimetric procedure, the amount of protein abraded/eroded from hair during shaking. During the course of these studies, it was observed that, unless the hair is very badly damaged and totally stripped of cuticle layers, the cuticle is the primary source of protein loss during abrasion (12). We have extended this work to show that protein loss measurements can also be used to quantify hair damage due to combing. We have demonstrated that this technique can also be used to measure hair damage on live heads (half-head tests) from combing. Furthermore, this technique can differentiate the conditioning/protective efficacy of current conditioning shampoos.

MATERIALS AND METHODS

Caucasian and Oriental Hair fibers used in these studies were commercial samples purchased from DeMeo Brothers, New York. Asian (Indian) and Negroid hair were taken from private individuals. All hair samples used in these studies, with the exception of Negroid hair, were approximately 9–11 inches long; the Negroid hair samples were ~5.5 to 7 inches in length. Using these hair samples, hair tresses, each weighing 3.0 g (1.0 g for Negroid hair), were prepared for various experiments. Reagents for protein estimation were obtained from Sigma Chemical Company, St. Louis, MO. All other reagents used were of analytical grade.

EVALUATION OF POST-SHAMPOO PROTEIN LOSS DURING COMBING

Laboratory studies using hair tresses. Laboratory studies to determine protein loss from hair during combing were conducted using a minimum of three tresses for each shampoo treatment as follows: The tresses were wet individually under running tap water (~105°F), pre-washed with SLES (20% @ 1 ml per tress, 0.5 ml for Negroid hair) and rinsed extensively for one minute under running tap water to remove any surfactant residue. Each tress was then shampooed individually, with the test product (1 ml per tress, 0.5 ml for Negroid hair) applied uniformly with a syringe, the hair gently rubbed between the fingers and occasionally on the palm of the hand to generate foam for a total of one minute and rinsed extensively for at least one minute to remove any shampoo residue. Finally, each tress was combed gently under running tap water to detangle the hair, and the excess water was squeezed out. To induce protein loss during combing, each tress was combed wet, by hand, using a fine-toothed nylon comb (20–22 teeth per linear inch) @ 50–100 strokes (always the same number of strokes in each test). During combing, the tress was held firmly in one hand and combed with the other hand, using a firm, smooth, vigorous combing action for the entire length of the tress and occasionally changing the side of the hair tress being combed. After every five strokes, the comb was dipped into a beaker containing 50 ml of distilled water, and after ten strokes, the tress was also dipped into the same water to recover loose/chipped protein fragments from hair. The water suspension from each tress was then tested for turbidity in a spectrophotometer at 600 nm and for protein concentration as described below. The data was statistically analyzed using an in-house computer program for one-factor analysis of variance (1-way ANOVA) to establish whether the observed differences between treatments are significant at 95% confidence level ($p = 0.05$).

Estimation of hair protein. Protein concentration in a given solution containing hair protein was determined as previously described (12). This procedure is a modification of the Lowry Method (13), one of the most widely used techniques for protein measurement in biological samples. Briefly, each sample was well shaken by hand, and 1 ml of the turbid liquid was pipetted directly from the beaker and added to a 16×125 -mm tube containing 0.1 ml of 5N NaOH (for samples suspected to contain protein in high concentration, 0.5 ml of the sample was used and mixed with 0.5 ml of 1 N NaOH). The contents of the tube were mixed well and allowed to sit at room temperature for 30 minutes to solubilize the suspended protein fragments. At the end of the incubation period, 1 ml of alkaline Cu-carbonate solution was added and the samples incubated at room temperature for 20 minutes. At the end of the incubation period, 3 ml of Folin-phenol solution was added to each tube and the sample was vortexed immediately. The samples were further incubated for 40 minutes and the absorbance determined in a spectrophotometer at a wavelength of 750 nm. Protein concentration in each sample was then determined from a standard curve (protein conc.; $\mu\text{g/ml} = \text{slope} \times \text{absorbance} - \text{intercept on Y axis}$; this value was then used to calculate the total cuticular protein loss expressed as total protein recovered, mg/g hair) prepared separately using crystalline bovine serum albumin as a standard and assayed under conditions identical to the test samples. A control (water blank) was always run with each assay, and the absorbance value for each test sample was adjusted for the blank. Using this procedure, we were able to determine as little as 5 micrograms of hair protein per ml in a given solution. The sensitivity of the method can, however, be increased to as little as 1 μg of protein with reasonable precision by certain modifications such as reducing the volumes of sample and reagents and the use of smaller tubes for reaction and microcuvettes for absorbance readings.

COMBING STUDIES TO EVALUATE THE PROTECTION EFFICACY OF A SHAMPOO

Limited studies were also done to determine the protection efficacy of certain shampoos using the laboratory tress test routinely used for such evaluations. For such studies, hair tresses (three for each product) were pre-washed with a 5% solution of SLES (1 ml/tress), shampooed with the test product @ 1 ml/tress, rinsed thoroughly with tap water (105°C), and gently combed to remove snags. Wet tresses were then randomly arranged and evaluated by a panel of at least six judges. Judges were asked to rank the tresses for ease of combing from best to worst and also to rate each tress on a scale of 1 (worst) to 10 (best). Tresses were re-wet between evaluations. The data was then statistically analyzed using an in-house computer program of the Friedman test, a nonparametric procedure for multiple comparisons for ranking (14), and one-way ANOVA for rating.

HALF-HEAD TESTS

Half-head tests provide a side-by-side comparison of the relative efficacy of any two test products under "real-life" conditions. Each half-head test was a double-blind, randomized block experiment using 40 healthy, adult, female subjects with 16–24-inch-long hair and was carried out as follows: The panelist's hair was wetted with warm water (~105°F, 150 ppm water hardness) and parted down the middle from the forehead to the nape of the neck. To remove any dirt or grease, hair on each side of the head was

pre-washed using 10 ml of a non-conditioning shampoo and rinsed well with warm water. Subsequently, 10 ml of a given test product was applied evenly to one side of the head, a lather worked up, and the hair rinsed well with warm water to remove all shampoo residue. The above process was repeated in a similar manner on the other side of the head using the second test product. To avoid inherent differences in damage from the left to the right side, test products were randomized within each subject (all panelists previously assigned odd subject numbers received a given test product on the left side, while those with even numbers received the same product on the right side). At the end of the shampooing process, hair on each side of the head was combed the entire length using a fine-toothed comb designated for each side. After ten combing strokes, both the comb and hair were dipped into a large stainless steel or glass bowl, ~9 inches in diameter, 1-liter capacity, containing 200 ml of distilled water, to recover loose cuticle fragments from the hair. The excess water from the hair was squeezed out into the dish. The above process was repeated for a total of 100 strokes, alternating between the left and right side of the head. Following completion of the combing process, the solution from each side was analyzed for protein concentration (as described above) to determine total protein released from each side of the head during combing. The data was then statistically analyzed [Wilcoxon signed rank test (15)] to evaluate the difference in treatment effects.

RESULTS AND DISCUSSION

PRELIMINARY STUDIES ON PROTEIN LOSS FROM HAIR—EFFECT OF COMBING

Studies were conducted to determine whether measurements of protein loss from hair can be used to evaluate hair damage due to combing. For these studies, hair tresses using undamaged Oriental hair were prepared and shampooed in triplicate with a leading brand 2-in-1 conditioning shampoo (shampoo A). For comparison, hair tresses from the same batch were also shampooed in a similar manner with a prototype 2-in-1 conditioning shampoo (shampoo B). Following shampooing, each tress was combed (wet) for a total of 200 combing strokes, and the turbid solution was analyzed for protein concentration at the end of 25, 50, 100, and 200 combing strokes. Samples containing cuticular material were also tested for turbidity, which measures the degree of opacity of a suspension. However, as previously reported (12), turbidity measurements are not always a true indicator of hair damage. Results of these studies are summarized in Table I. As shown, protein loss from hair increased progressively relative to the number of combing strokes for both shampoos tested. Statistical analysis of the data showed that the relationship between the amount of protein loss vs the number of combing strokes is highly significant ($p = 0.0001$) and that tress-to-tress variability of the measurements (standard error) for a given treatment is rather low. Furthermore, the data also show that regardless of the number of strokes, protein loss from hair, shampooed with shampoo B is significantly less ($p = 0.05$) than that from hair shampooed with shampoo A. This suggests that shampoo B is more effective in protecting hair against combing damage as compared to shampoo A and that this protection was not lost during repeated combing. In separate studies, rank combing scores comparing these two shampoos in triplicate using multiple combers showed that hair washed with shampoo B did comb significantly more easily than hair washed with shampoo A, and as mentioned above,

Table I
Effect of Post-Shampoo Combing on Protein Loss From Oriental Hair

Treatment	Turbidity (Optical density at 600 nm)		Protein loss (mg/gm Hair*)			
	Number of combing strokes		Number of combing strokes			
	100	200	25	50	100	200
Shampoo A (2-in-1)						
Tress 1	0.178	0.315	0.243	0.376	0.824	1.614
Tress 2	0.189	0.371	0.219	0.464	0.949	2.133
Tress 3	0.199	0.442	0.215	0.461	1.058	2.149
Av.	0.189	0.376	0.226 ± .01	0.433 ± .03	0.944 ± .07	1.965 ± .17
Shampoo B (2-in-1)						
Tress 1	0.042	0.123	0.115	0.269	0.511	1.096
Tress 2	0.067	0.181	0.101	0.269	0.502	1.238
Tress 3	0.093	0.202	0.139	0.333	0.623	1.514
Av.	0.067	0.169	0.118 ± .01	0.290 ± .02	0.545 ± .04	1.283 ± .12

* Three-gm tresses, prepared by using a commercial Oriental hair sample ~10 inches long, were prewashed with 1 cc of 20% SLES, followed by shampooing with the test product @ 1 cc/tress. Each tress was then combed for a total of 200 combing strokes, and the abraded cuticular material was collected in a glass beaker containing 50 ml of distilled water. The turbid water solution was analyzed for protein concentration at the end of 25, 50, 100, and 200 combing strokes (see Materials and Methods section for details). The observed differences in protein loss for shampoo A vs shampoo B are significant at the $p = 0.05$ level for all combing strokes.

shampoo B did also provide less protein loss. This is usually, but not always, in agreement with easier combing and less protein loss. However, these comparisons are not part of this paper for proprietary reasons.

To further validate the test methodology, another experiment was conducted using Asian hair shampooed with three different shampoos identified as shampoos A, B, and C; shampoos A and B were the same as tested above. These studies were conducted by a different operator. As shown in Table II, protein loss from hair shampooed with shampoo B is significantly less as compared to hair shampooed with shampoo A or shampoo C. Additional studies showed that the results are reproducible from day to day, although the level of measured damage, that is absolute quantitative values for protein loss, may vary slightly from operator to operator. The above studies clearly support our hypothesis that protein loss measurements can provide meaningful results in studying hair damage during combing.

PROTEIN LOSS FROM HAIR DURING COMBING AFTER TREATMENT WITH DIFFERENT SHAMPOOS AND CONDITIONERS

To explore whether we could detect significant differences in protein loss due to combing from hair shampooed with various shampoos, hair tresses (European hair) were shampooed with five different shampoos, identified as shampoos 1 through 5. Among the five shampoos tested, the first three were leading-brand commercial products,

Table II
Effect of Post-Shampoo Combing on Protein Loss From Asian Hair

	Turbidity (O.D. at 600 nm)	Protein loss (mg/gm Hair @ 50 combing strokes*)
Shampoo A (2-in-1)		
Tress 1	0.097	0.341
Tress 2	0.067	0.281
Tress 3	0.071	0.337
Av.	0.078	0.319 ± .02
Shampoo B (2-in-1)		
Tress 1	0.007	0.108
Tress 1	0.006	0.104
Tress 3	0.003	0.108
Av.	0.013	0.107 ± .01
Shampoo C (2-in-1)		
Tress 1	0.054	0.184
Tress 2	0.053	0.191
Tress 3	0.062	0.191
Av.	0.056	0.189 ± .01

* For experimental details, see footnote in Table I. Observed differences in protein loss among the three shampoos tested are significant at the $p = 0.05$ level.

whereas the last two were prototype formulations prepared in our laboratory. Shampoo 1 was a non-conditioning shampoo primarily containing a mixture of ALS/ALES (19% AI). It has been used for most of our studies as a standard shampoo to often compare results from study to study. All other shampoos tested were 2-in-1-type conditioning shampoos containing the following conditioning ingredients:

Shampoo 1	None
Shampoo 2	Dimethicone
Shampoo 3	Dimethicone, guar hydroxypropyltrimonium chloride
Shampoo 4	Dimethicone, guar hydroxypropyltrimonium chloride, and polyquaternium 10
Shampoo 5	Dimethicone, guar hydroxypropyltrimonium chloride, polyquaternium 10, polyquaternium 6, and polyquaternium 16

After shampooing, each tress was combed for a total of 100 strokes, and the water solution was analyzed for protein concentration. Results of these experiments are summarized in Figure 1. As shown, total protein loss was the highest from hair shampooed with the non-conditioning shampoo (shampoo 1). It is not surprising to see that, among the five shampoos tested, hair shampooed with the non-conditioning shampoo, namely shampoo 1, experienced greater protein loss during combing compared to hair treated with the conditioning shampoos. Even among the various conditioning shampoos tested, significant differences ($p = 0.05$) in hair damage could be detected using this method, illustrating the sensitivity and the utility of the method.

This work was further extended to study the effect of hair conditioners on protein loss during combing. In this experiment the protection efficacy of three leading-brand U.S. conditioners was compared to that of the conditioning shampoo 5, previously found to

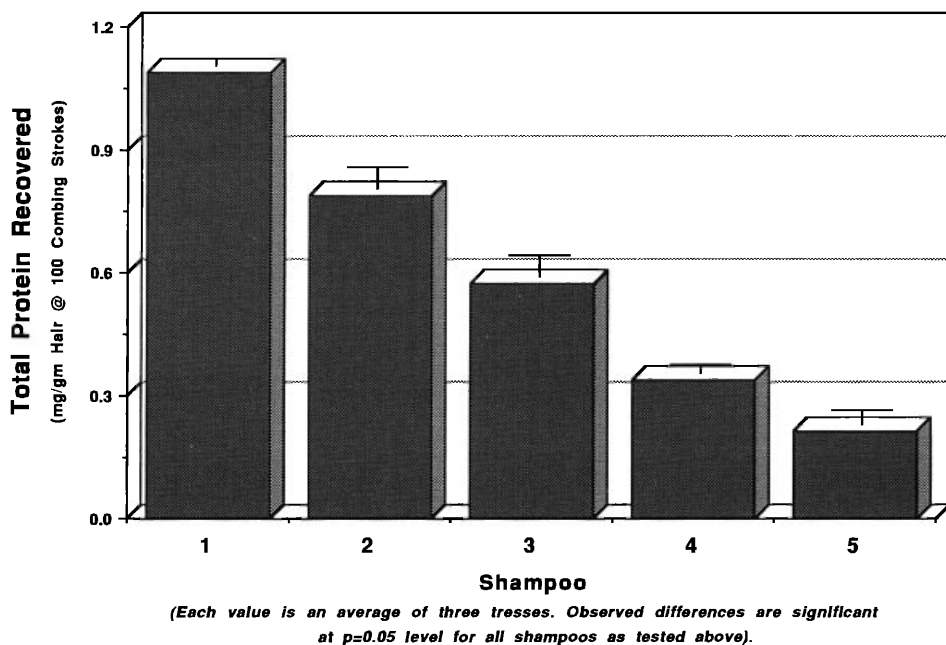


Figure 1. Effect of various shampoos on post-shampoo protein loss from European hair during combing.

provide superior hair protection during combing as shown above. For such studies, multiple hair tresses were shampooed either with the conditioning shampoo (shampoo 5) or with a non-conditioning shampoo (for normal hair), followed by conditioner

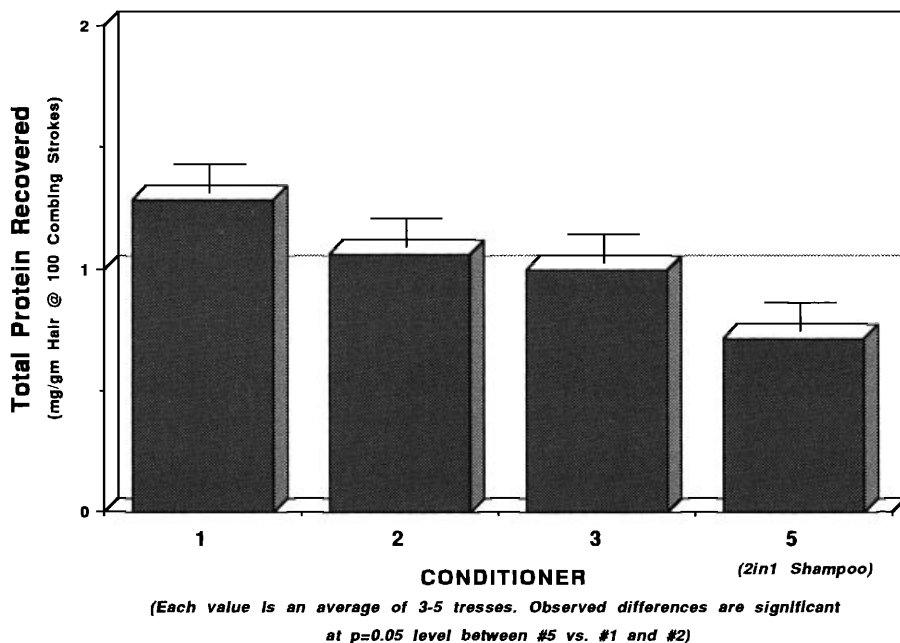


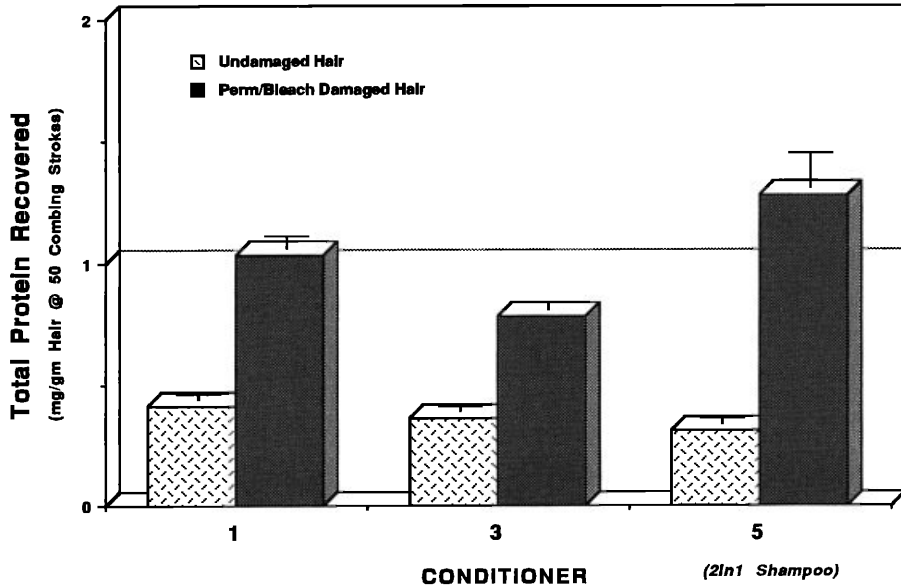
Figure 2. Effect of various conditioners on post-shampoo protein loss from European hair during combing.

application @ 1 cc/tress). Results (Figure 2) show that protein loss during combing was significantly less ($p = 0.05$) from hair treated with conditioning shampoo 5 vs conditioner 1 or 2; the differences between shampoos 5 and 3 are, however, not statistically significant. Furthermore, the differences in protein loss among the three conditioners tested (shampoos 1, 2, and 3) are also not significant. This suggests that these three conditioners are very similar with respect to hair protection efficacy.

EFFECT OF COSMETIC TREATMENTS ON PROTEIN LOSS FROM HAIR DURING COMBING

Damage to hair from cosmetic treatments is well documented (1,3,16). As the hair weathers or is physically or chemically damaged (perming/bleaching), the scales begin to lift and partially detach from the fiber surface (3,16). It has been shown that chemically damaging treatments such as permanent waves and bleach treatments increase interfiber friction and make hair more difficult to comb (8). We previously reported that the permed/bleached hair surface is more susceptible than undamaged hair to protein loss when such hair is shaken in water (12). Experiments were designed to test whether we could demonstrate greater protein loss due to combing from chemically damaged hair as compared to undamaged hair. To pursue our goal, two separate experiments were performed in which European hair tresses, prepared from a single bundle of hair, were tested for protein loss due to combing before and after chemical treatment. Damage to hair was induced under controlled laboratory conditions by perming followed by bleaching, using commercially available products as previously described (12). Undamaged and damaged hair were conditioned in the manner described above by using two leading-brand conditioners (identified as conditioners 1 and 3, Figure 2) and a 2-in-1 conditioning shampoo (shampoo 5). The treated tresses were then subjected to protein loss measurements by the proposed methodology. Results of these experiments are summarized in Figure 3.

As shown in the figure, protein loss during combing is significantly greater ($p = 0.05$) from the permed/bleached hair as compared to the same hair before the chemical treatments. This suggests that chemical treatments make hair more susceptible to damage from combing. These results support our previous observations on protein loss from shaking chemically damaged hair in water (12). Furthermore, statistical analysis of the data also show that, on undamaged hair, the protein loss from hair treated with test product 5 is significantly less ($p = 0.05$) as compared to test product 1, a leading-brand conditioner; the differences in protein loss between 5 vs 3 and 1 vs 3 were not significant. However, when the hair was chemically damaged, the efficacy of these three conditioners to protect hair against combing damage changed. Protein loss measurements suggest that following chemical damage, the superiority of product 5 over product 1 to protect hair against combing damage was lost, whereas product 3 was now found to provide significantly better protection over both 5 and 1. Therefore, these studies suggest that a conditioner that is found to show superior efficacy on undamaged hair may not provide the same protection against combing damage if the hair is chemically damaged by cosmetic treatments such as permanent waving and bleaching. It is well known that chemical damage to hair changes the surface properties. It is therefore conceivable that changes in the surface properties of hair due to chemical treatments not only make hair more susceptible to protein loss during combing but also change its substantivity toward different conditioners and the subsequent conditioning/protective effects.

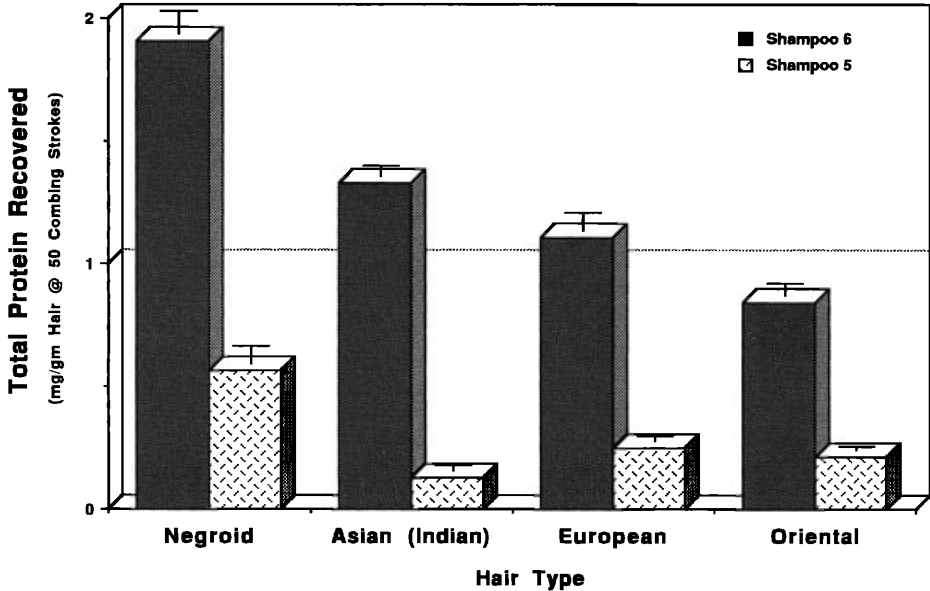


(Each value is an average of three tresses. Observed differences are significant at $p=0.05$ level).

Figure 3. Protein loss from undamaged and perm/bleach-damaged hair during combing.

PROTEIN LOSS FROM VARIOUS HAIR TYPES DURING COMBING

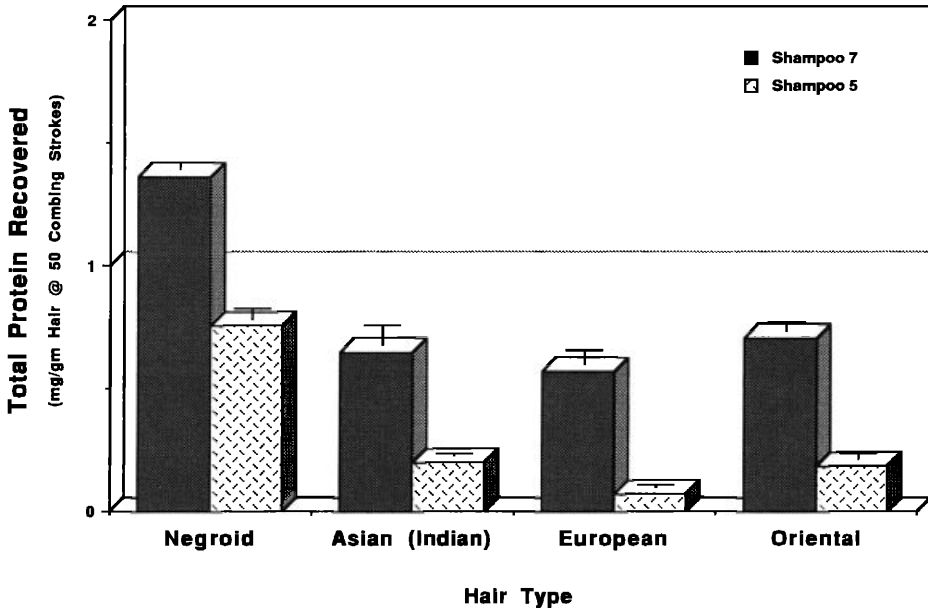
It is known that hair type influences combing ease. The fiber properties that influence ease of combing are curvature, friction, stiffness, diameter, length, and cohesion. An increase in fiber curvature (as in curly hair), friction, or static charge (dry hair) will make hair more difficult to comb, whereas an increase in stiffness, diameter, and cohesion will make hair easier to comb (17). To test whether protein loss measurements can provide meaningful results for different types of hair, three different shampoos were tested for post-shampoo protein loss due to combing. Four different hair types were used, namely, Caucasian, Asian (Indian), Oriental, and Negroid. The shampoos tested include shampoo 5 (previously evaluated 2-in-1 prototype conditioning shampoo) vs a leading brand baby shampoo (shampoo 6), and shampoo 5 vs a leading brand 2-in-1 conditioning shampoo (shampoo 7). Results of these studies are summarized in Figures 4 and 5. As shown, protein loss due to combing was found to be significantly lower ($p = 0.05$) from hair treated with shampoo 5 as compared to both shampoos 6 and 7, regardless of the hair type tested. Limited combing studies on shampoos 5 and 7 also supported these observations. Wet-combing experiments showed that hair shampooed with shampoo 5 was significantly easier ($p = 0.05$) to comb than hair shampooed with shampoo 7 [the wet-combing scores for shampoo 5 vs shampoo 7 were 7.85 and 6.64 for virgin Oriental hair (a higher combing score implies easier combing; see Materials and Methods), 7.5 and 6.6 for permed Oriental hair, and 6.61 and 3.94 for European bleached hair]. Although wet-combing studies on shampoo 5 vs shampoo 6 were not conducted, one would expect similar results because shampoo 6 is a non-conditioning shampoo and therefore would not condition hair any better than shampoo 7, which, as mentioned above, is a conditioning shampoo. In fact, one would expect that between these two shampoos (6 and 7), hair shampooed with shampoo 6 will be more difficult to comb. As



(Each value is an average of three tresses. Observed differences are significant at $p=0.05$ level).

Figure 4. Post-shampoo protein loss from various hair types during combing.

expected, protein loss was the highest from Negroid hair (curliest) for any given shampoo among the three products tested (see figures). As mentioned earlier, an increase in fiber curvature makes hair more difficult to comb. Obviously, one would experience more difficulty in combing curly hair such as Negroid hair among the four hair types



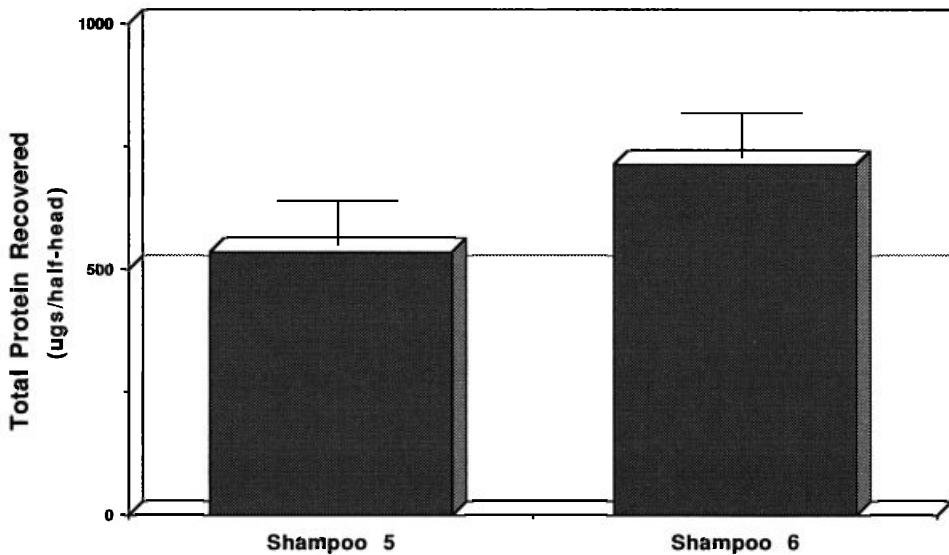
(Each value is an average of three tresses. Observed differences are significant at $p=0.05$ level).

Figure 5. Post-shampoo protein loss from various hair types during combing.

tested, and combing ease is a contributing factor to protein loss in this experiment. These results clearly show that the proposed methodology can be applied to different types of hair to study post-shampoo hair damage protection during combing.

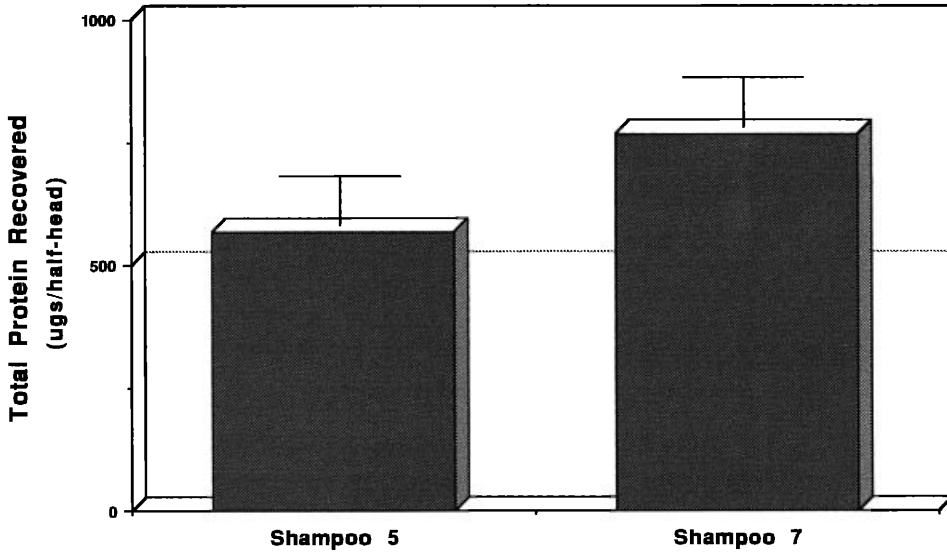
HALF-HEAD TESTS

The above laboratory studies have shown that shampoo 5 provides superior hair protection as compared to shampoos 6 and 7. To determine whether the laboratory findings on protein loss during combing are reflected under “real-life” conditions, these same test products were evaluated for hair protection efficacy in half-head tests using the proposed methodology described in the experimental section. As mentioned earlier, such tests provide a side-by-side comparison of the relative efficacy of any two of the test products. Two separate half-head tests were conducted to compare the hair protection efficacy of shampoo 5 vs shampoo 6 and shampoo 5 vs shampoo 7. Each study was a double-blind experiment using 40 healthy, adult, female subjects with 16–24-inch-long hair (see Materials and Methods for details). These studies were carried out in collaboration with an independent beauty salon. Protein analysis tests were done by an independent clinical laboratory using our protocol. The test results are summarized in Figures 6 and 7. As shown, protein loss from hair shampooed with shampoo 5 was found to be significantly less ($p = 0.05$) than from hair shampooed with shampoo 6 (Figure 6). Similar observations were made with respect to shampoo 5 vs shampoo 7 (Figure 7). Although the main objective of the clinical protocol was to determine protein loss from hair during combing, the beauticians observed that in almost all cases the hair on the side sham-



(Each value is an average of forty subjects. Observed differences are significant at $p=0.05$ level)

Figure 6. Half-head test: Post-shampoo protein loss during combing.



(Each value is an average of thirty-nine subjects. Observed differences are significant at $p=0.05$ level).

Figure 7. Half-head test: Post-shampoo protein loss during combing.

pooned with test product 5 was easier to comb as compared to the side shampooed with either test product 6 or test product 7. Because each study was double-blind, the beautician (or the panelist) did not know which product was used on which side. These clinical studies on "live heads" directly confirm our laboratory findings, verifying that the laboratory results do confirm the real-life practical damage protection offered by the protein loss measurements.

CONCLUSIONS

This paper describes a simple and sensitive technique to quantify damage to hair during combing. This method involves collecting cuticular protein fragments abraded from hair during combing, and quantitatively measuring the amount of protein by a colorimetric procedure capable of detecting as little as 5 µg of protein per ml. The proposed methodology is particularly useful in assessing the protection efficacy of various shampoos and conditioners in protecting hair against damage due to abrasion during combing. It can also be used to study the effect of chemical treatments such as permanent waving and bleaching on hair during combing. Using this procedure in the laboratory and in half-head tests, we were able to demonstrate significant differences ($p = 0.05$) in total protein loss induced by combing hair treated with different shampoos and conditioners.

Laboratory studies show that among the five different shampoos tested, protein loss was the highest from hair shampooed with a non-conditioning shampoo, followed by the conditioning shampoo treatments. Additionally, even among the various conditioning shampoos tested, significant differences in protein loss were observed. This suggests that post-shampoo cuticle protection differs among shampoos. Furthermore, hair shampooed

with one of the conditioning shampoos (shampoo 5) showed significantly less protein loss during combing relative to all three leading-brand "stand-alone" conditioners tested. However, no significant differences in protein loss were observed in the conditioner-treated hair, suggesting that these conditioners are very similar with respect to hair protection during combing. Additional studies using three different shampoos on four different hair types, namely, Caucasian, Asian, Oriental, and Negroid hair, show that the methodology can be applied to assess post-shampoo hair damage during combing regardless of the hair type. Finally, studies also show a significant increase in protein loss from hair during combing following chemical treatments, namely, permanent waving and bleaching. This suggests that chemically damaged hair is more susceptible to further damage from combing. Furthermore, the data indicate that chemical damage to hair not only makes hair more susceptible to combing damage but presumably also changes its substantivity towards different conditioners.

Half-head studies, which provide a side-by-side comparison of the relative efficacy of two of the test products under real-life conditions, also showed significant differences in protein loss during combing from hair shampooed with the different shampoos. These clinical studies directly confirm our laboratory findings, suggesting that laboratory results from protein loss measurements can reflect "real-life" damage to hair during combing.

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