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Cosmetic and amino acid analysis of the effects of lye and no-lye relaxer treatment on adult black female South African hair

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Synopsis

Surveys indicate that many South African women use relaxers to straighten their hair for cosmetic reasons, which can damage the hair and scalp. The objective of this study was to assess the effects of treating hair with two types of relaxers: Product A (a lye relaxer, sodium hydroxide base) and Product B (a no-lye relaxer, guanidine hydroxide base). Five adult black female South African subjects were used for the study that was divided into two parts. The first part used a half-head study design in a clinical study in which the researcher and the subjects visually assessed various hair quality parameters before and after relaxer treatment. Product B was assessed to perform better (p = 0.032) than Product A in terms of hair straightening. The second part of the study involved hair amino acid analysis by reversed-phase high performance liquid chromatography. There was a decrease in the amount of cystine [Median (range) g/100 g hair] after treatment with both Product A [7.8 (2.5–9.9), p = 0.086] and Product B [4.0 (2.9–4.8), p = 0.005] compared to before treatment [9.1 (6.4–11.9)]; this decrease was greater (p = 0.085) for Product B. Reduction in cystine content was consistent with increased straightness. Product B (the no-lye relaxer) was found to be more effective and safer to use.

INTRODUCTION

The use of chemical straightening products is said to put African American women in particular at high risk for various "traumatic" alopecia, or hair loss, and other adverse hair and scalp reactions. Due to the characteristic curly nature of African American hair, straightening the hair using chemical treatments allows for greater manageability and flexibility in styling (1). The most common chemical hair straighteners are termed "relaxers." There are two types of relaxers: lye relaxers [sodium hydroxide (Na^+OH^-) base] and no-lye relaxers [guanidine hydroxide ($HNC(NH_2)NH_3^+OH^-$) base]. The size of the

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relaxer market in South Africa, with a predominantly black female clientele, is approximately R400 ($50) million per annum (2). The durability of the shape of hair is attributable to cross-linking disulphide bonds (C-S-S-C) in the amino acid cystine (Cys), which makes up to *ca*. 18% of human hair (3–5); using a relaxer to break these bonds in curly hair allows the hair to be straightened.

The Photobiology Laboratory at Medunsa Campus, University of Limpopo, has been involved in relaxer efficacy work with multinationals for the past 10 years, but that work is subject to confidentiality agreements. According to the Laboratory's experience (6), South African origin hair in different individuals behaves differently with the same cosmetic treatment, performed at the same time points, using the same procedure. Subjects of the same type of hair (e.g., coarse hair) may be treated with the same cosmetic treatment, e.g., a relaxer, and the results may not be the same. In some subjects, the resultant hair is straighter, shinier, and silkier than in others; hence, there is a need to determine the effect of different types of hair relaxers on the surface characteristics of South African origin hair.

There appears to be relatively few research publications in scientific journals on this type of work. Only one study (7) on the effect of relaxers on South African hair could be found in the scientific literature. The study reported changes in amino acid composition but did not study lye and no-lye relaxers separately. There is a need to contribute to the existing body of knowledge on the effect of different types of relaxers on the biochemical composition of South African hair, which may be expected to be not too dissimilar to African American hair. The aim of this study was to elucidate the cosmetic and biochemical effects of lye and no-lye relaxer treatment on African hair by visual assessment and amino acid analysis, respectively.

EXPERIMENTAL

The study was divided into two parts. The first was a clinical study involving the visual assessment of hair appearance, and the second was the amino acid analysis of hair by reversed-phase liquid chromatography (RP-HPLC).

CLINICAL STUDY

Subjects, samples, and test products. Five South African subjects were enrolled for the study. They were adult black females with at least 4 cm of virgin hair and in good general health. Ethical clearance for the use of human participants for the study was obtained from the Medunsa Research Ethics Committee of the University of Limpopo. All the subjects voluntarily completed a Health Questionnaire form and signed a Subject Information and Consent form. Strands of hair were sampled from each subject before and after relaxer treatment. Two types of hair relaxers manufactured by AMKA Products (Pty) Ltd (Pretoria, SA) were tested in the study. These were Product A: "PC Super relaxer," a lye relaxer (sodium hydroxide base) and Product B: "PC No-lye relaxer," a no-lye relaxer (guanidine hydroxide base); both were "Perfect Choice®" (Pretoria, SA) products. A neutralizing shampoo and a normal shampoo from the same manufacturer were also used.

Procedure. The clinical study was conducted according to internationally recognized Good Clinical Practice Guidelines (8). This was a randomized, single-blind, half-head study. Subjects received a baseline treatment of hair wash with a normal shampoo. Three days later, the hair was parted centrally from brow to nape of the neck by the researcher who then carried out a half-head (either left side or right side) application of the relaxers according to a randomization list. The no-lye relaxer was applied immediately after mixing.

The relaxer was left on until the subject complained of scalp tingling (up to 20 min) and then it was washed off using the neutralizing shampoo. Approximately 50 strands of hair were sampled from each side treated with Product A (the lye relaxer) and from the side treated with Product B (the no-lye relaxer) of each subject. Subjects used six parameters to assess the quality of their own hair before and after relaxer treatment. These were length (cm), damage (5 = not damaged; 1 = extremely damaged), straightness (4 = very straight; 1 = not straight), softness (3 = very soft; 1= not soft), shininess (3 = very shiny; 1 = not shiny), and volume (3 = thin; 1 = thick). The researcher also assessed these six parameters for each subject, together with split ends (3 = low; 1 = high), dryness (4 = oily; 1 = very dry), and wash-off time (min). The parameters were ranked in such a way that the higher the number the better the performance. Data were collected on the day of the treatment using questionnaires for researcher and subject self-assessments. The Wilcoxon rank sum test for nonparametric data was used to test observed differences between groups for statistical significance.

AMINO ACID ANALYSIS BY RP-HPLC

Instrumentation. A Waters Breeze HPLC system (Waters, Millipore Corp., Milford, MA) was used for the amino acid analysis. The system included a workstation for performing pre-column hydrolysis and derivatization. Empower 3 Chromatography Data Software was used to process all data.

Reagents. Acetonitrile, triethylamine, sodium acetate, glacial acetic acid, hydrochloric acid, phenylisothiocyanate (PITC), ethanol, L-α-amino-*n*-butyric acid (AAB), and L-cystine were obtained from Sigma-Aldrich (Johannesburg, SA). Amino acid standard H from Pierce (Prod no. 20088) was obtained from Waters (Milford, MA) through the local Suppliers, Separations (Johannesburg, SA). It is a quantitative mixture of 17 free amino acids, each supplied at a concentration of 2.5 µmol/ml of 0.1 N HCl, for use as a high-purity calibration standard for HPLC analysis of protein hydrolysates. It includes L-Alanine, L-Arginine, L-Aspartic acid, L-Cystine, L-Glutamic acid, L-Glycine, L-Histidine, L-Isoleucine, L-Leucine, L-Lysine, L-Methionine, L-Phenylalanine, L-Proline, L-Serine, L-Threonine, L-Tyrosine, and L-Valine. Ultrapure water was supplied by a Milli-Q purification system (Millipore, Bedford, MA).

Sample Preparation. Hydrolysis: Stock solutions containing 1.0 mg/ml were prepared by dissolving 10mg hair samples (in duplicate) in 10 ml of 0.1 M HCl. Volumes corresponding to 50 µg sample were dried under vacuum and hydrolysed with acid vapours from 200 µl of 6 M HCl at 110°C for 24 h.

Derivatization: Pre-column derivatization of the amino acids with a chromophore was carried out to enable UV absorbance detection. A modified Pico-Tag method (9–12) was used for derivatization (and analysis). As much as 50 µl solution of PITC in ethanol was

added to the dry hydrolysates and the mixtures were allowed to react in the sealed vacuum vial for 20 min at room temperature to produce phenylthiocarbamyl amino acids. The standard H was also loaded and derivatized.

Analysis of samples. A Pico-Tag column (Waters, Millipore Corp., Milford, MA): C-18 bonded phase, reversed- phase mode, 3.9 × 150 mm, silica particle size 4 µm, pore size 60 Å, was used for the separations. The column temperature was controlled at 45 ± 1°C. The sample injection volume and flow rate were 20 µl and 1.5 ml/min, respectively. The solvent system consisted of two eluents: A, 50 mM sodium acetate aqueous buffer pH 5.5 and B, 60:40 acetonitrile:water. The program was run using a gradient of A and B with an initial 12% B and ending with 53% B after 10min. All the amino acids eluted in less than 10 min. After this, a wash-up step was programmed to 100% B so that any residual sample components would be cleaned from the column. Analytes were detected at 254 nm using a UV absorbance detector. A calibration file generated from analysis of the standard H was used to determine the amino acid content of the samples. Peak areas were used for quantitation.

Method validation. Samples were analyzed in duplicate to obtain mean values. AAB was used as an internal standard to minimize between-runs variability. The accuracy of the method was checked by spiking samples with a standard cystine solution and determining the percentage recoveries.

RESULTS AND DISCUSSION

CLINICAL STUDY

There was no erythema or any other adverse event observed on any subject during the treatments used in the study. The relaxer wash-off times are shown in Table I.

Although the mean wash-off time was longer for the no-lye relaxer, the difference (p = 0.069) was found not to be statistically significant ($p > 0.05$).

Each subject assessed the quality of their own hair in terms of six parameters: length, damage, straightness, softness, shininess, and volume. The self-assessment results are summarized in Table II.

Table III represents a summary of the results of the researcher assessment of nine hair quality parameters. These were the six assessed by the subjects, together with split ends, dryness, and wash-off time.

Table I
Wash-off Times (Min)

Subject no.	Product A (Lye)	Product B (No-lye)	p-value
1	15	15	
2	8	9	
3	20	20	
4	9	10	
5	15	17	0.690

Table II
Subject Self-Assessment of Hair Quality Parameters (Mean Values, $n = 5$)

		After treatment with hair relaxer	
Parameters	Before treatment	Product A (Lye)	Product B (No-lye
Length (cm)	8.8	10.0	9.8
Damage	4.8	5.0	5.0
Straightness	3.0	3.4	3.6
Softness	2.0	2.6	2.8
Shininess	2.4	2.6	2.6
Volume	1.2	1.8	1.4

A comparison of Tables II and III indicates that Product B (the no-lye relaxer) was assessed to perform better than Product A (the lye relaxer) with regard to straightness, softness, shininess, and dryness. The researcher and subjects assessed the two relaxer types to perform similarly with regard to damage but did not agree on the relative performance with regard to volume and length.

The data for the researcher assessment of hair straightness are shown in Table IV.

The results of the statistical analysis of the data for the researcher assessed straightness are shown in Table V. It can be seen that there was a statistically significant ($p < 0.05$) increase in straightness after treatment with both the lye ($p = 0.016$) and the no-lye ($p = 0.008$) relaxers, and that the increase was significantly greater ($p = 0.032$) for the no-lye relaxer. Differences in relaxer performance observed by the researcher and subjects with regard to other hair quality parameters such as softness, shininess, and dryness were found not to be statistically significant ($p > 0.05$).

Shaw (13) reported that no-lye relaxers tend to cause less irritation and more drying on the hair and scalp and leave the hair with less sheen compared to lye relaxers. The finding of this study appears to be in agreement with respect to the no-lye relaxer causing less

Table III
Researcher Assessment of Hair Quality Parameters (Mean Values, $n = 5$)

		After treatment	
Parameters	Before treatment	Product A (Lye)	Product B (No-lye)
Length (cm)	8.8	9.7	9.9
Damage	4.4	5	5.0
Straightness	1.6	3	4.0
Softness	1.2	2.2	2.8
Shininess	1.2	2.0	2.8
Volume	1.4	1.8	2.2
Dryness	2.0	2.0	2.4
Split ends	2.6	2.6	2.8

Table IV
Researcher Assessment of Hair Straightness

		After treatment	
Subject no.	Before treatment	Product A (Lye)	Product B (No-lye)
1	1	3	4
2	1	2	4
3	2	3	4
4	2	3	4
5	2	4	4
Mean	1.6	3.0	4.0

irritation, as evidenced by the longer mean wash-off time (14.2 vs. 13.4 min). However, the no-lye relaxer (Product B) tested in this study was found to produce less drying (2.4 vs. 2.0) and more shininess (2.8 vs. 2.0). It was also found to produce fewer split ends (2.8 vs. 2.6) than the lye relaxer (Product A).

In Figure 1, the hair on the left side of the head of Subject no 2 is clearly seen to be straighter than that on the right side. The left side was treated with Product B (no-lye) and the right side with Product A (lye). The straightness of the hair on the left side compared to the right corroborates the results in Tables II and III wherein the researcher and the subjects observed better performance for Product B (no-lye) in terms of straightness.

AMINO ACID ANALYSIS BY RP-HPLC

Figure 2 shows the chromatograms obtained for the amino acid standard H and an untreated hair sample from Subject no.1. It can be seen that in both cases the amino acids (including the AAB internal standard) were separated with good resolution and reproducible retention times.

Figure 3 shows the chromatograms obtained after treatment of hair samples from Subject no.1 with Product A (lye relaxer) (right side of head) and Product B (no-lye relaxer) (left side of head). The wash-off time for each relaxer was 15 min.

A comparison of the cystine peaks in Figures 2 and 3 clearly shows that hair treatment with the lye and no-lye relaxers both led to a decrease in the cystine content, with the decrease more pronounced for the latter. High cystine recovery values in the range

Table V
Statistical Analysis of Researcher Assessed Straightness Data

Group (n = 5)	Groups compared	p-value
Before treatment (BT	BT vs. L	0.016
After lye treatment (L)	BT vs. N	0.008
After no-lye treatment (N)	L vs. N	0.032

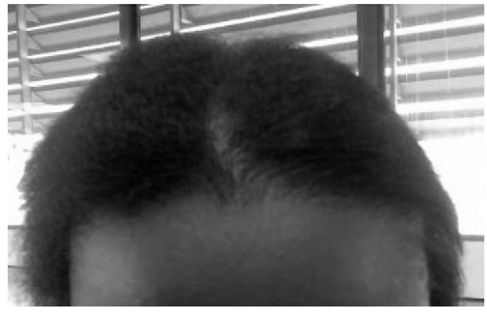

Figure 1. Subject no. 2, hair parted into two sections. After treatment of the right side with Product A (lye relaxer) and the left side with Product B (no-lye relaxer).

90–105% were obtained in the spiking experiment, which was deemed to be validation of the quantitative data. The data for the cystine content (g/100 g hair) are shown in Table VI.

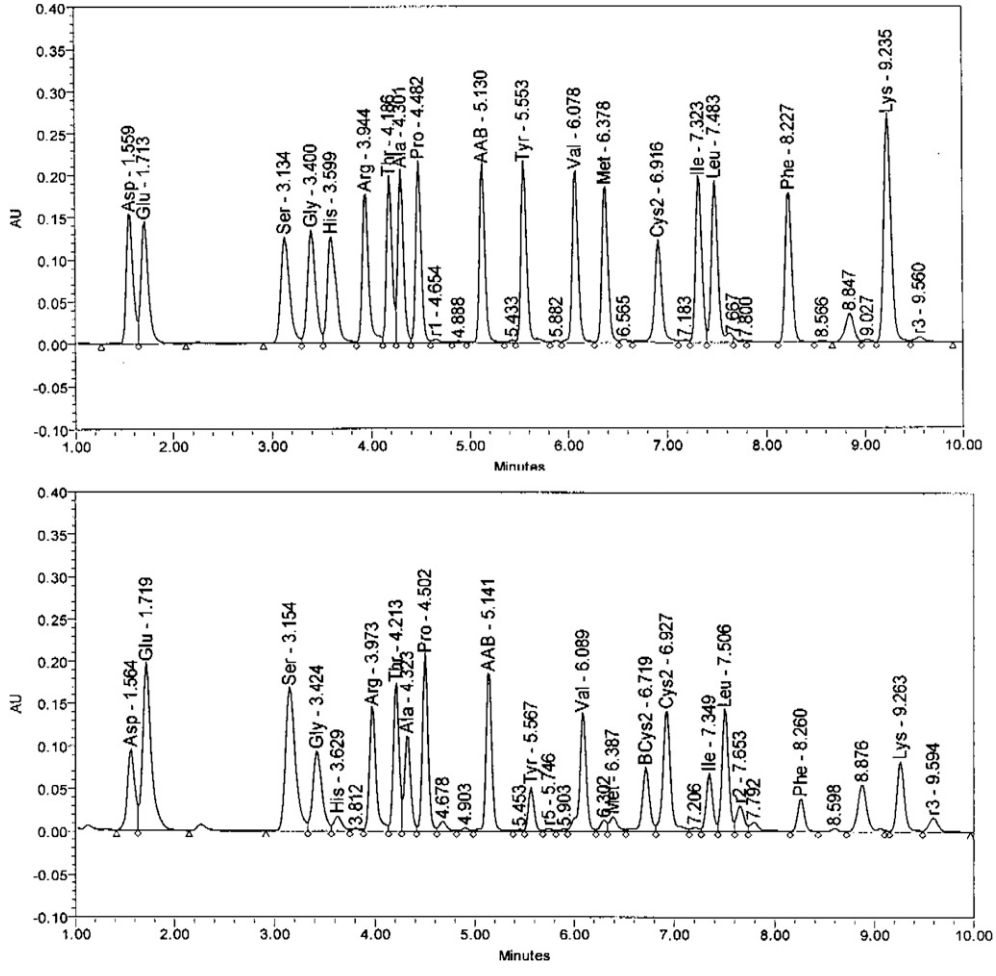

Figure 2. Chromatograms for the amino acid standard H (top) and untreated hair for Subject no. 1 (bottom).

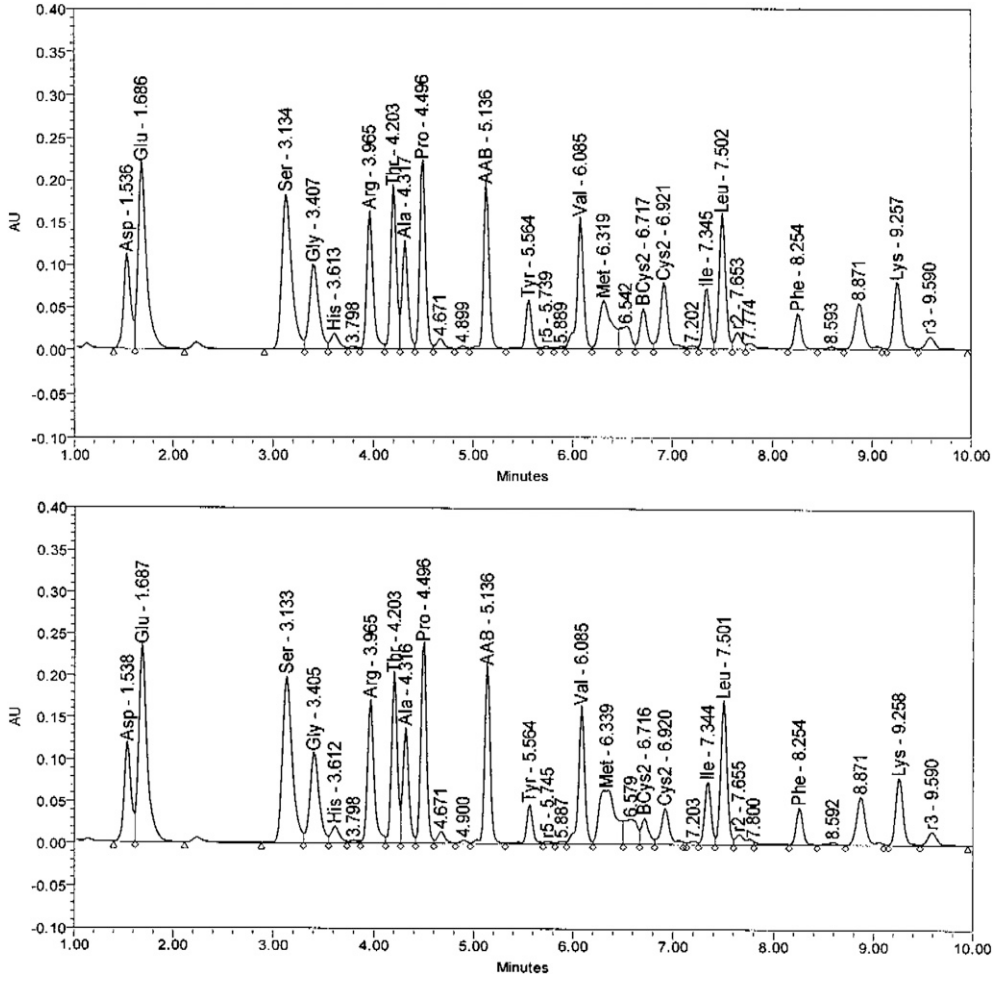

Figure 3. Chromatograms for hair samples from Subject no. 1 after treatment with Product A (lye relaxer) (top) and Product B (no-lye relaxer) (bottom).

The cystine content of the untreated hair decreased from 9.1 [6.7–11.9] to 7.8 [2.5–9.9] and 4.0 [2.9–4.8], after treatment with the lye relaxer and the no-lye relaxer, respectively. A paired student's *t*-test was used to calculate *p*-values to determine whether these observed decreases were statistically significant, and at what confidence levels (CL). The results are shown in Table VII.

The decreases in the cystine content were found to be statistically significant ($p < 0.1$): at the 90% CL ($p = 0.086$) and 99% CL ($p = 0.005$), respectively, for the lye and no-lye treatments compared to the virgin hair. Compared to the lye treatment, the decrease in the cystine content was significantly greater (at the 90% CL, $p = 0.085$) for the no-lye treatment. Interestingly, this trend was observed even in the cases of Subject no. 1 and Subject no. 3 with the same wash-off times for each relaxer type. Khumalo *et al.* (7), also reported a decrease in cystine content of hair of South African origin after relaxer treatment; however, they did not study lye and no-lye relaxers separately. They also reported a decrease for citruline and arginine and an increase for glutamine.

Table VI
Cystine Content (g/100 g hair) of the Hair Samples (Mean Values, $n = 2$)

		After treatment	
Subject no.	Before treatment	Product A (Lye)	Product B (No-lye)
1	11.9	7.8	3.9
2	10.7	8.5	4.8
3	9.1	9.9	4
4	8.7	2.5	2.9
5	6.7	4.9	4.6
Median [range]	9.1 [6.7–11.9]	7.8 [2.5–9.9]	4.0 [2.9–4.8]

A similar analysis of the data for lysine content found that there were statistically significant ($p < 0.10$) decreases after relaxer treatment: 2.0 [1.5–2.3], $p = 0.082$ and 2.0 [1.5–2.2], $p = 0.036$ for the lye and no-lye, respectively, compared to the virgin hair [2.1 (2.0–2.6)]. However, the difference between the decreases for the two relaxer types was not statistically significant ($p = 0.920$). Interestingly, lysine has been reported to be used as a treatment for hair loss (14). Changes observed for the other 15 amino acids analyzed in this study were found not to be statistically significant ($p > 0.10$) for either relaxer treatment.

CONCLUSIONS

The no-lye relaxer performed better than the lye relaxer with regard to hair straightness. The former also appeared to perform better overall with regard to other hair quality parameters such as dryness, softness, and shininess in addition to appearing to be milder on the scalp. There was consistency between the findings of the two parts of the study: increased straightness was accompanied by decreased cystine content. More research needs to be done on the no-lye relaxer because of its overall better clinical performance. The authors intend to repeat the study with a larger group of subjects.

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Table VII
Statistical Analysis of Cystine Data

Group ($n = 5$)	Groups compared	p-value	CL (%)
Before treatment (BT	BT vs. L	0.086 ($p < 0.1$	90
After lye treatment (L)	BT vs. N	0.005 ($p < 0.01$)	99
After no-lye treatment (N)	L vs. N	0.085 ($p < 0.1$)	90

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