Protein Quantification in hair fibers from different ethnic groups

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Abstract

Fiber protein loss is directly related to hair damage processes, such as brushing, dyeing, bleaching, among others. The more damaged the hair fiber, the greater the amount of protein lost from the hair. Natural and double bleached Caucasian, Asian and Curly hair tresses were evaluated on the study. A statistically significant protein loss was observed in hair damaged by double bleached in relation to each respective natural condition, in the 3 types of hair evaluated. Also, a reduction in the enthalpy of keratin degradation for Caucasian, Asian and Curly hair tresses. Thus, the new methodology used was able to measure with statistical significance the protein loss of tresses submitted to oxidative damage from double bleached. Data was corroborated by the Differential Scanning Calorimetry (DSC) technique already established in the evaluation of degradation of the protein constituent of the hair.

Key-words (3-5): Hair ethnicity, Protein Loss, DSC, Protein quantification, In vitro

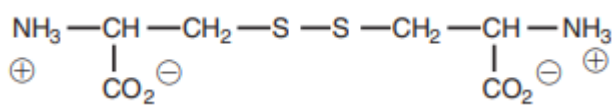
Introduction

Hair is basically made up of proteins (65 to 95%), some lipids, water, melanin and trace minerals. The most abundant protein in hair is keratin (mainly in the form of alpha-keratin), which provides resistance to the fiber and is found abundantly in the cortical matrix. Keratin is formed by cysteine sulfur chains, which form disulfide bridges, which are two sulfurs joined together by the sulfhydryl groups (-SH) of each cysteine, forming cross-links [1]. The keratin can be arranged both in curl onto helices (α- conformation) or a sheet stacked pattern (β-conformation) [2].

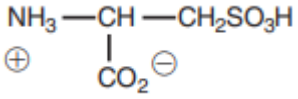
Hair from different ethnicities, such as Caucasian, African, and Asian, share the same chemical composition of proteins and amino acids that make up keratin. However, they differ in fiber structure. African hair has a more irregular diameter and an elliptical cross-section, featuring a curly shape with twists and less moisture content compared to Caucasian and Asian hair. Asian hair, on the other hand, has the largest diameter and a cylindrical cross-section. Caucasian hair falls between the two, with an intermediate diameter and a cylindrical cross-section [3].

Hair treatments, either chemical, such as coloring, bleaching and straightening, or physical, such as brushing, heat treatments with a hairdryer and straightening iron, among others, can damage the hair fiber structure. These treatments can promote keratin proteins loss from the hair, leading to a thinning process or even breakage. To measure the extent of damage caused to the hair fiber, the literature describes several methods for quantifying the amount of protein loss by the fiber [4].

The bleach process on hair using alkaline hydrogen peroxide or alkaline peroxide/persulfate causes a conversion of stable keratin resulting in lower cystine and conversion to cysteic acid. This conversion occurs primarily at the disulfide bonds and produces a large amount of unstable cysteic acid residues (e.g. sulfonic or sulfenic acids) [1].



Cystine



Cysteic Acid

Figure 1. Cystine and Cysteic acid formulas [1].

A simple and sensitive technique development for Sandhu and Robbins [5,6] to assess surface damage to hair submitted to different procedures: bleaching, permanent wave and acid-based oxidant treatments, using shaking hair in water and after measuring using a colorimetric detection.

Nowadays, the most used methods of protein loss quantification are spectrophotometric methods, like Lowry [7,8], Bradford [9,10] and the bicinchoninic acid (BCA) method [11].'

The Lowry reagent reacts with the amino groups of proteins, an alkaline solution containing copper. The colored complex formed is quantified spectrophotometrically, using by comparison with a calibration curve standard protein prepared with known concentrations, such as bovine serum albumin (BSA) or casein [7,8].

The chemical reaction involved in the protein quantification by Bradford is the interaction between Coomassie brilliant blue BG-250 dye (red) and the amino acid groups of hair or any other protein. In acid pH the Coomassie BG-250 dye reacts with protonated groups (NH3+) of amino acids and results in a blue complex. The proteins loss' quantity is proportional if the hair chemical procedure promotes more damage and the blue color is more pronounced and the maximum absorbance – at 595 nm – is observed by spectrophotometer [9,10,11].

In the BCA method, the stable blue complex bicinchoninic acid - cooper (II) reacts, in alkaline conditions, with cysteine residues reducing the cooper II to I and formation of a green complex (absorbance at 562 nm) [7, 12].

Materials and methods

Hair tresses

Study groups: Caucasian, Asian and Curly hair tresses were used (natural and double bleached) – 2,5g / 25cm – 3 tresses of each hair type.

Protein loss quantification

The tresses were mechanically broken with the Ultra Turrax equipment, and the resultant fragments were placed in distillated water to release the loose keratins inside the fiber. The protein loss was quantified in the supernatant of the tresses solution with the aid of the Invitrogen Qubit® Protein and Differential Scanning Calorimetry (DSC) techniques.

The device used for Differential Scanning Calorimetry was the Hitachi High-Tech Sciences TA7000 Series Simultaneous Thermogravimetric Analyzer, STA7200. The analyses were made in triplicate with approximately 10 mg of chopped hair. The flow used was 100 mL/min of nitrogen gas with heating rate of 10°C/min. The analyses were made from 30°C to 300°C.

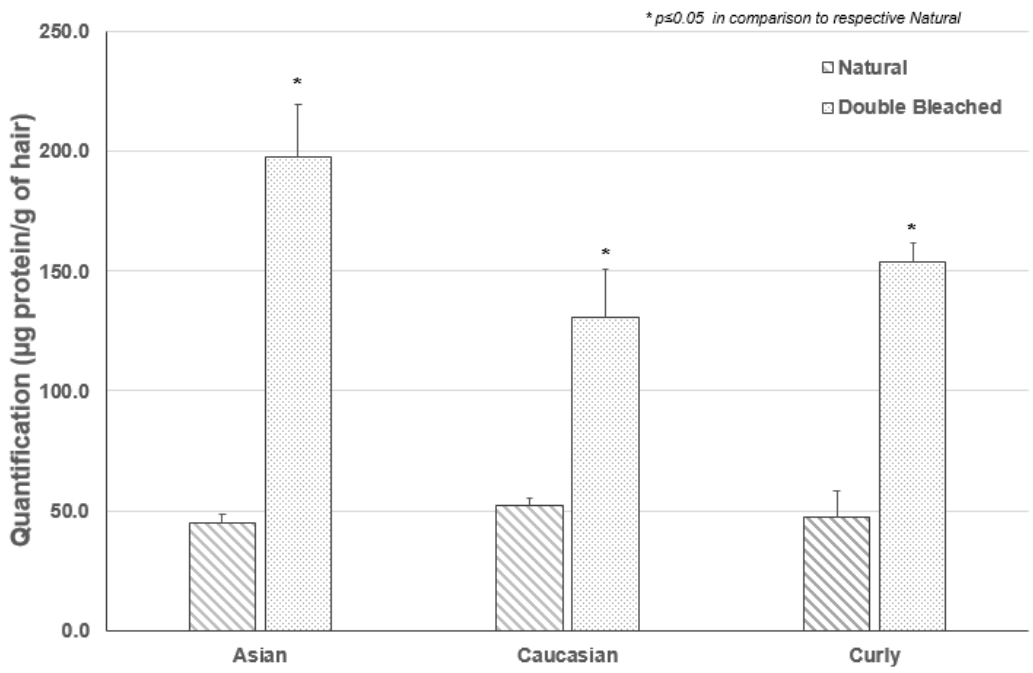
Statistics

Comparison Natural vs. Double bleached (each hair type): Student's t-test, bimodal, paired, considering a 95% confidence interval (p-value ≤0.05 for statistically significant differences). Statistical comparations were performed using the software GraphPad Prism, version 8.3.4.

Results

Graph 1 illustrates the mean values of protein quantification (µg protein / g of hair).

Graph 1. Results of Invitrogen Qubit®.

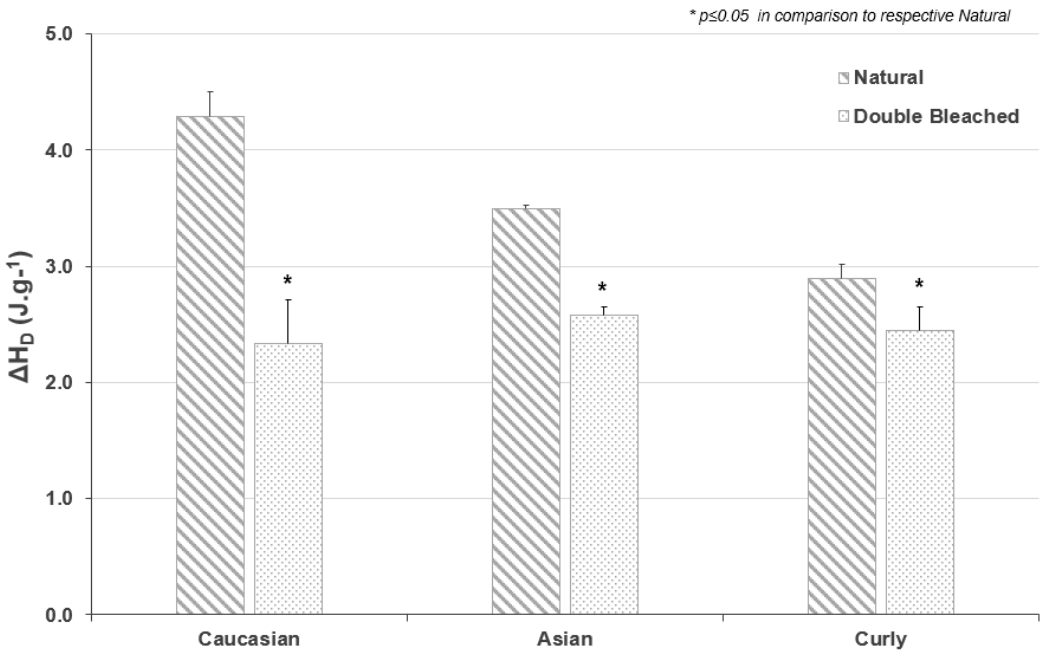


Protein quantification of the study groups. Mean ± standard deviation.

In the study, a statistically significant protein loss was observed in hair damaged by double bleached compared to each respective natural condition, in the 3 types of hair evaluated: Natural Asian hair showed (45.05±3.50) µg protein/g of hair versus (197.65±22.01) µg protein/g of double bleached Asian hair (p value <0.0001). Natural Caucasian hair showed (51.09±3.35) µg protein/g of hair versus (130.75±20.13) µg protein/g of double bleached condition (p value 0.0005). Natural Curly/African hair showed (47.15±11.32) µg protein/g of hair versus (153.85±7.89) µg protein/g of double bleached (p value <0.0001).

Graphs 2 and **3** illustrate the mean values of denaturation enthalpies and temperature of keratin of the analyses performed in the temperature range between 30°C and 300°C.

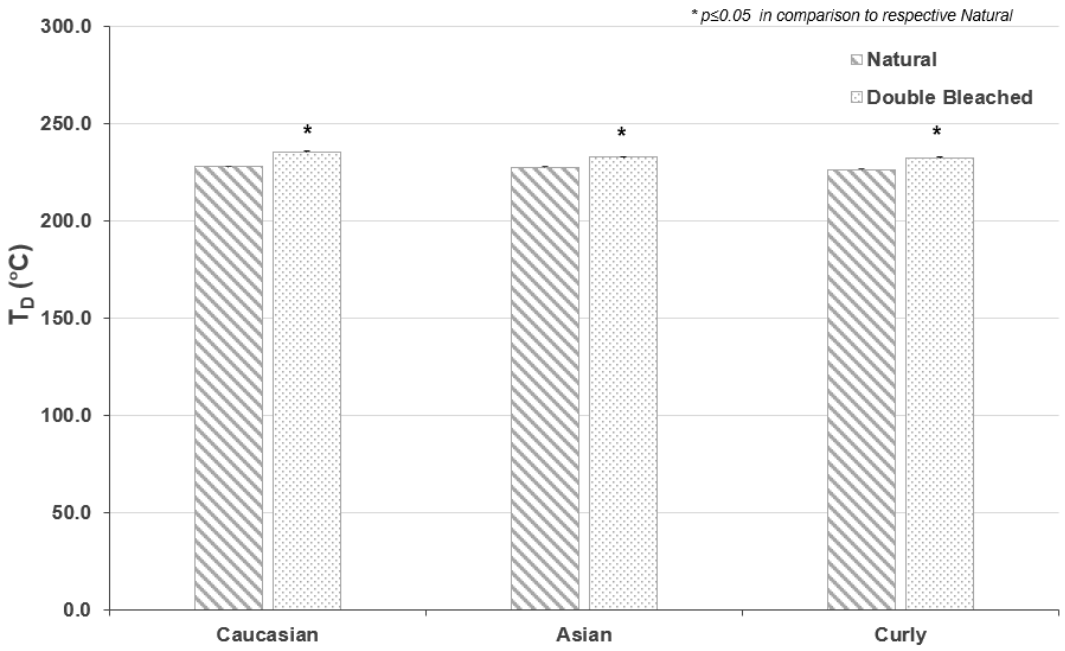
Graph 2. Results of DCS analysis - Enthalpy.



ΔH values of keratin denaturation of the study groups. Mean ± standard deviation.

The doubled bleached hair showed a significant reduction in the enthalpy of keratin degradation than natural hair of same ethnicity. Natural Asian hair showed (3.50±0.02) J/g of hair versus (2.58±0.07) J/g of double bleached Asian hair (p value <0.0001). Natural Caucasian hair showed (4.29±0.21) J/g of hair versus (2.34±0.37) J/g of double bleached condition (p value = 0.0014). Natural Curly/African hair showed (2.89±0.13) J/g of hair versus (2.44±0.21) J/g of double bleached (p value = 0.0341).

Graph 3. Results of DCS analysis - Temperature.



T values of keratin denaturation of the study groups. Mean ± standard deviation.

The keratin degradation temperature showed a statistically significant increase in the types of hair studied. Natural Asian hair showed (227.55±0.37) °C of hair versus (232.67±0.46) °C double bleached Asian hair (p value = 0.0001). Natural Caucasian hair showed (227.76±0.38) °C of hair versus (235.50±0.39) °C of double bleached condition (p value <0.0001). Natural Curly/African hair showed (226.26±0.38) °C of hair versus (232.09±0.77) °C of double bleached (p value = 0.0003).

Discussion

The double bleached process induces an oxidation breaking bonds, cleaving the S-S bonds and forming unstable residues, changing the structural and chemical properties in relation to natural hair state. Those reactions and changes affect the cuticular barrier and promote alteration in the cortex [1, 13].

In its natural state, hair maintains a network of strong covalent bonds, particularly the disulfide (S-S) bonds, which contribute significantly to its structural stability and integrity of the hair fiber [1,14]. When hair undergoes processes of double bleaching, these covalent bonds are disrupted, leading to a significant loss of protein content [15]. The results from the study highlight a profound disparity in protein loss between natural hair and hair subjected to double bleaching across diverse ethnicities – Caucasian, African, and Curly [16].

The impact of double bleaching on hair penetrates the hair cuticle, oxidizing and breaking down the S-S bonds within the cortex. This disruption weakens the structural integrity of the hair fiber, rendering it more susceptible to protein loss [17,18].

Moreover, the observed increase in protein quantification in double-bleached hair further corroborates the extent of structural damage incurred. This heightened protein loss can be attributed to the severe disruption of the hair's protein matrix, leading to the release of keratin fragments into the surrounding environment [19].

Importantly, the disparities in protein loss among different ethnicities underscore the diverse structural characteristics of hair across populations [20]. While all hair types are susceptible to damage from chemical treatments, variations in hair morphology and composition may influence the extent of protein loss observed.

The decrease of keratin enthalpy denaturation of observed across the three different ethnicities, in hair damaged by double bleaching compared to natural hair, signifies a profound alteration in the cross-linkage arrangement of keratin causes by double bleaching process, which induce significant structural changes in covalent bonds of S-S [14]. This happens because the bleach can induce structural modifications in keratin proteins, causing oxidative damage and a disruption of hydrogen bonds and disulfide bridges within the keratin matrix [16].

Finally, the results from the study reveal a significant increase of keratin temperature denaturation in double-bleached hair compared to its natural counterpart across diverse ethnicities – Asian, Caucasian, and Curly/African. Furthermore, the observed increased in

the temperature of keratin denaturation in double-bleached hair suggests that protein matrix alteration results that β conformation was first affected than the crystalline conformation.

Conclusion

The findings from the study on the impact of double bleaching on hair reveal significant alterations in both structural and chemical properties, particularly in relation to the disruption of covalent bonds, such as the crucial disulfide (S-S) bonds. These disruptions weaken the structural integrity of the hair fiber, making it more susceptible to protein loss. Moreover, the study highlights pronounced differences in protein loss among the conditions natural and double bleached of the three ethnicities.

The observed reduction in enthalpy of denaturation of keratin in double-bleached hair compared to its natural counterpart further emphasizes the profound structural changes induced by bleaching. These changes indicate compromised stability and thermal degradation susceptibility of the protein matrix.

Overall, the study underscores the importance of understanding the diverse structural characteristics of hair across different populations and the significant impact of chemical treatments like double bleaching

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