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Raman spectroscopy and electronic microscopy structural studies of Caucasian and Afro human hair

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ABSTRACT

Human hair fibre is subjected to various structural modifications due to the application of chemicals such as dyes, shampoos and bleaches and/or physical procedures such as heating, and often more than one procedure is performed on the same hair. The present work aims to analyze the changes incurred in hair samples of two ethnic groups, namely Caucasian and Afro, before and after different treatments such as thermal, bleaching and straightening. In addition to observing the damage caused by each treatment separately, the study of samples that received all three treatments was carried out. For molecular structural characterization, the Raman vibrational spectroscopic technique was used and scanning electron microscopy (SEM) was used for morphological analysis of the hair fibres. This investigation has shown, through vibrational spectroscopy, that several important bonds have been modified, such as the S-S, C-S, C-C and S-O bonds as well as the secondary structures of proteins that have indergone changes in their conformation as a result of the treatment. Hair from the two ethnic groups showed small differences in relation to each applied treatment. Excessive heat generated a higher rate of Raman spectral band intensity changes when compared to the other treatments and it was observed that the action of several treatments on the same hair fibres resulted in even more pronounced structural changes. Finally, scanning electron microscopy showed that each treatment caused a different morphological deformation pattern on the capillary surface of the human hair.

1. Introduction

Hair is a natural biopolymer composed mainly of keratotic fibrous structural proteins and its structure is divided into three main regions: the cuticle, cortex and medulla. The cuticle is structurally amorphous and has colorless cells in the form of "scales" that overlap, forming 5 to 10 layers on the hair fibre. The main function of this cuticular matrix is to provide protecion against external aggressive attack. The cortex is responsible for the properties of mechanical strength and elasticity of the hair fibres and constitutes about 90% of the hair fibre [1, 2, 3, 4, 5, 6]; it has an elongated structure running parallel to the fibre direction, being composed of macrofibrils, each of which comprises microfibrils supported in an amorphous matrix. The microfibrils contain crystalline chains of an α-helical conformation, with low cystine content, whereas the amorphous matrix has a high cystine composition, providing a high content of disulfide bonds which form a cross-linked network. Another protein present in the fibre is β-keratin (in a β-sheet conformation) which is located mainly in the cuticle region [7, 8, 9, 10, 11]. Comprising only a small percentage of the fibrous capillary mass, the marrow is located in the centre of the fiber, and can be structurally continuous, fragmented or completely absent [9].

All hair types present several common characteristics, such as the chemical composition and molecular structure, where the proportion of the component amino acids is similar in different types. However, their tertiary structural shape can vary among different ethnic groups and the various types of hair are classified into three main groups: Oriental, Caucasian and Afro. The oriental type hair has a straight fibre, presenting its cross-section in a cylindrical format. The Caucasian hair, on the other hand, has a variation in the shape of its cross-section, generally following an oval shape, and leads to various forms of fibre between different individuals, being able to alternate from wavy to very curly in structure. On the other hand, the Afro type hair has a flattened and thin cross-section: it has a higher degree of irregularity in fibre diameter, tending to be very curly, and presents less resistance towards stretching, breaking more

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easily and having a lower water content in comparison to Caucasian hair [12, 13, 14, 15].

Over the years, analytical studies addressing hair structures have evolved significantly and vibrational spectroscopic techniques such as Raman and infrared have been increasingly used for such purposes [16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26]. Baddiel [16] analyzed the structure and reactions of hair keratin from infrared spectroscopy. Frushour and Koenig [17] were the pioneers in the use of Raman spectroscopy in keratin analysis, using vibrational spectroscopy for the structure of keratin present in wool. Early FT Raman and infrared studies of human skin [18] and human keratotic biopolymers [19] paved the way for establishing the spectral band assignemnts for human hair using long wavelength laser excitation. Wilson *et al.* [20] studied Raman spectroscopic of archaeological hair, demonstrated the capability of the technique for identifying extraneous material adsorbed in human hair from its burial environment. A later Raman spectroscopic study of historical grey hair specimens from Sir Isaac Newton and Robert Stephenson [21] which had been preserved in museum collections used these spectral band assignments to facilitate a comparison with modern hair for the identification of sites of potential degradative damage. Akhtar *et al.* [22] have used Raman spectroscopy to analyze untreated and discolored human hair and Kuzuhara [23] studied virgin hair treated with thioglycerol using Raman spectroscopic analysis whereas Zhang *et al.* [24] studied changes in the chemical and structural composition in hair fibers by infrared and Raman spectroscopic imaging.

Raman spectroscopy is a very applicable technique for the structural analysis of human hair since it is non-destructive of the specimen, it can be used *in situ* for the examination of the sample and does not require either chemical or mechanical pre-treatment of the sample. Previous Raman spectroscopic studies have been reported on archaeological and ancient hair stored in museum collections and the adoption of a near-infrared excitation wavelength is critical for the acquisition of spectral data, which is additionally advantageous for naturally depigmented grey or white hair as shown above. Concerns about hair appearance and its condition affect a large proportion of women and men in modern society and much time can be spent in cosmetic daily treatment and hair care over several ethnicities but little information is available about the chemical structure effects of certain applied procedures on each ethnic group.

In this work, Raman spectroscopy and scanning electron microscopy have been used to analyze hair strands of two ethnicities, namely Caucasian and Afro, before and after specific chemical and physical treatment have been applied to the hair. Through the observation of changes in the spectral intensity and wavenumber displacement of characteristic Raman bands, it will be possible to correlate changes in the capillary structure of the hair fibres for the Caucasian and Afro hair specimens. All physical changes will be monitored by scanning electronic microscopy to assess the damage to the hair fibres morphologically and, finally, to compare the information provided from both techniques to determine the effect of the different procedures or treatment upon the integrity of the hair fibres.

2. Materials and methods

2.1. Samples

White Caucasian hair and White Afro hair samples were used from female donors. It should be noted that the samples are taken of "virgin" hair, that is, they have not undergone any recorded prior chemical or physical treatment. All specimens were collected in the region near the nape of the neck. As sampling, a total of 52 hair strands were used, where 13 received termal treatment, another 13 the bleaching treatment, 13 were straightened and 13 received the total treatment, composed by all the individual methods. For each one of the samples, we have obtained the Ramnan spectra bedore and after the treatment, in order to evaluate the changes caused by.

The reason for working with white hair specimens is due primarily to the minimisation of fluorescence emission at the laser excitation wavelength, since white hair is deficient in fluorescent melanin granules, as well as the prevention of potential sample deterioration through laser absorption and thermal effects [27].

2.2. Materials

It has been used for hair treatment: Straightening iron (200 °C); a product based on linear sodium alkyl benzene sulfonate for washing; ammonium persulfate and hydrogen peroxide 40% v/v for decolorizing hair specimens; straightening product based on ammonium thioglycolate. All these materials are commercially available.

2.3. Instrumentation

The Raman spectra were obtained using a Bruker SENTERRA spectrometer, solid state laser excitation operating at a wavelength of 785 nm and a CCD detector. The measurements were made with resolution of 3–5 cm^{-1}, aperture of 25 × 1000 μm and over a spectral range of 1780 to 390 cm^{-1}. The laser power at the exit of the laser was 50 mW and 10 accumulations were made with an acquisition time of 50 s each. These parameters were previously adjusted to obtain the best signal-to-noise ratio without altering the physical and chemical integrity of the samples. The spectra obtained were treated with OPUS 7.0 and Origin 8 software from Bruker and a baseline correction was applied.

SEM measurements were performed in a scanning electron microscope combined with an Energy dispersive X-ray spectroscopy (EDS) Hitachi model TM 3000 compact instrument, with a smoothing magnification of 15x up to 30,000x (digital zoom: 2x and 4x), which allows topographic images to be obtained with a large depth of focus. No sample preparation was required for the analyses, and each one of the samples were fixed in a specific metallic sampler from the SEM equipment.

2.4. Hair treatment

Thirteen hair strands were used for each treatment (thermal, discoloration, relaxation and total) for both Caucasian and Afro hair specimens. The replicates were fixed on glass slides by means of double-sided tape at their ends and the interrogated analytical region of each strand was always determined close to the root of the hair.

Cleaning of the hair: All hair fibres were moistened with water then received the uniform application of the linear sodium benzene sulfonate solution based product for degreasing, being rubbed gently with the fingers for a period of 1 min. After this process the fibres were washed with running water and finally with distilled water and dried in the open air.

Heat treatment: The fibres received the application of heat directly from the Straightening iron at 200 °C over the entire length of the hair shaft, a process which was repeated 10 times. In this work, a flat iron was selected for use at 200 °C, since most of the Brazilian hair salons used the same board in this condition as part of the progressive hair straightening process.

Bleaching treatment: Bleaching powder was added to hydrogen peroxide in the ratio of 1: 2, this mixture was applied throughout the length of the hair shaft for 40 min. The hair fibres were then washed with tap water for 2 min and then with distilled water.

Relaxation treatment: Hair fibres were treated with the relaxing/straightening (reducer) product for 45 min, subsequently the hair shafts were washed with water and then hydrogen peroxide (oxidant) was applied throughout the length of each shaft for 10 min. After these procedures, the hair shafts were washed with tap water for 2 min and then with distilled water.

Total treatment: In order to observe the damage caused in the same hair specimen by the use of several procedures, the total treatment was applied to some hair specimens, namely the application of the three

individual treatments described above following cleaning: relaxation, discoloration and heating.

3. Results and discussion

3.1. Characterization and comparison of Caucasian and Afro hair shafts by Raman spectroscopy

Analytical Raman spectroscopy is appropriate for the investigation of the influence of chemical and physical processes on the hair fibre since it not only provides chemical information on the individual groups and bonds but it is also capable of characterizing the secondary structures of proteins [28].

3.1.1. Natural white hair

In Fig. 1, panel A shows the Raman spectrum of the virgin white hair strand specimen for the spectral range of 3170–360 cm^{-1}. The vibrational assignment of the enlarged region corresponds to the asymmetric and symmetric stretches of the CH_3 group, at 2955 and 2930 cm^{-1}, respectively, and at 2876 cm^{-1} the symmetrical stretching mode of the CH_2 group is depicted [18]. Panel B presents the average of 52 spectra for the Caucasian ethnicity (shown in black) and the average of 52 spectra for the Afro ethnicity (shown in red), being the Raman spectra of each one of the samples exatly the same, which ensures the reproducibility of Raman data of the samples; the spectra are stackplotted with baseline adjustment for presentation purposes. The 1750-450 cm^{-1} wavenumber range is the fundamental region analytically as it is rich in vibrational information, showing the main bands that undergo modification resulting from the treatment procedures. As hair is a very complex matrix, attempts at characterization and interpretation of the spectral data were made based on previous studies (see Table 1) [8,22,23,29,30].

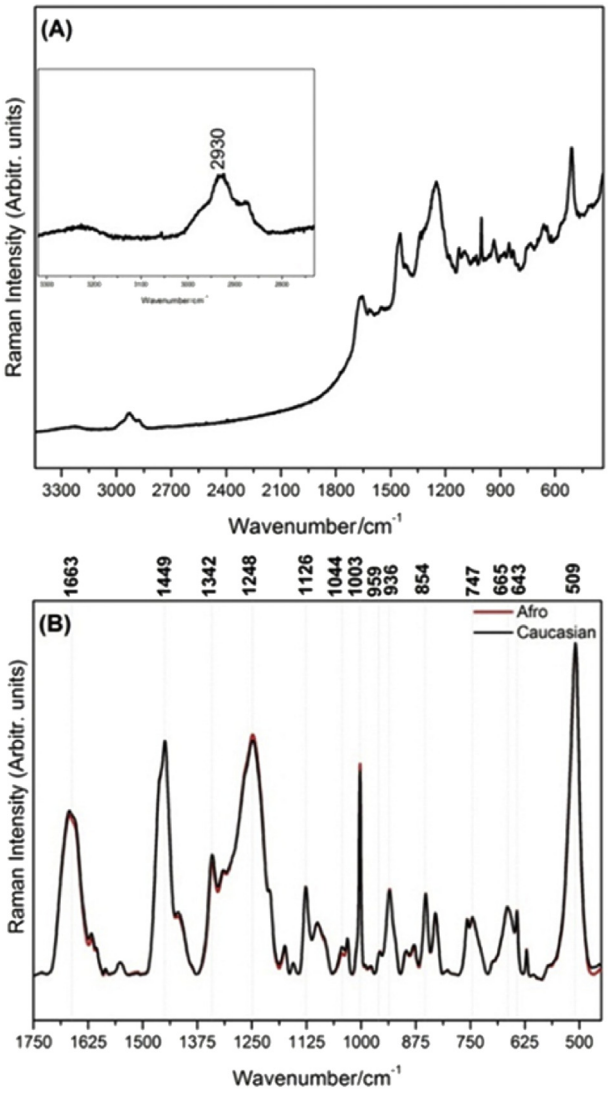

Fig. 1. Raman spectra of natural white hair. Panel (A): spectral range between 3170-360 cm^{-1}. Panel (B): stackplotted spectra of the two ethnic groups, Caucasian (shown in black) and Afro (shown in red), identifying the main bands, in the spectral range of 1750–450 cm^{-1}, with baseline adjustment.

In order to observe possible vibrational differences between the spectra of natural Afro and natural Caucasian hair strands, a spectral average of 52 spectra taken for specimens from the two ethnicities (Fig. 1: panel B) was carried out. There were no significant differences observed in the intensity or wavenumber shifts between the two ethnicities, demonstrating through Raman spectroscopy that the chemical characteristics of the two ethnicities are practically identical.

All spectra presented below were subjected to a baseline adjustment and were normalized to the intensity of the C-H band at 1449 cm^{-1}, as it was shown to be a band that is not influenced by the individual treatment carried out on the hair specimens [23].

3.1.2. Treatments

3.1.2.1. Heat treatment and bleaching treatment. In this investigation, the same tratments were applied to the samples from both etnies, in order to observe if each one of then (Caucasian and Afro) would behave, as well in Figs. 2 and 3 the overlay of the Raman spectra, before and after the treatments, should give some different spectral response. Fig. 2 shows the average Raman spectra (from measurements on 13 strands each) of the superimposed hair speciemns from the two ethnic groups, Caucasian and Afro, before and after being submitted to two types of treatment, namely thermal (panel A) and discoloration (panel B).

Panel A of Fig. 2 shows the Raman spectra of hair from the two

Table 1
– Tentative vibrational assignments (in cm^{-1}) for the bands observed in the Raman spectra of natural white hair samples.

Tentative Assignment	$\nu(cm^{-1})$
ν_{as} CH_3	2955
ν_s CH_3	2930
ν_s CH_2	2876
Amide I	1663
Tyr e Trp	1617
Trp	1550
δ_s CH_2	1449
ν_s CH_2, Trp	1342**
δ_s $C_{(\alpha)}$ – H	1317**
Amide $III_{(desordenada)}$	1248
Tyr, Phe	1210**
Tyr	1176**
ν_s C-N	1126
ν_s S-O	1044
Phe	1003,1030*
δ_{as} CH_2	959*
ν_s C-$C_{(\alpha)}$ skeleton	936**
Trp	884
Tyr	854
Trp	750
ν_s C-S	665
Tyr, C-S	643
Cys S-S stretch g-g, g-g-g	509
ν_s S-S	490

* Vibrational mode present only in cuticle.
** Vibrational mode present only in cortex; ν_s, symmetrical stretching; ν_{as}, antysymmetrical stretching; δ_s, in plane angular deformation; δ_{as}, in plane asymmetrical angular deformation. Aminoacids: tyrosine (Tyr), tryptophan (Trp), phenylalanine (Phe).

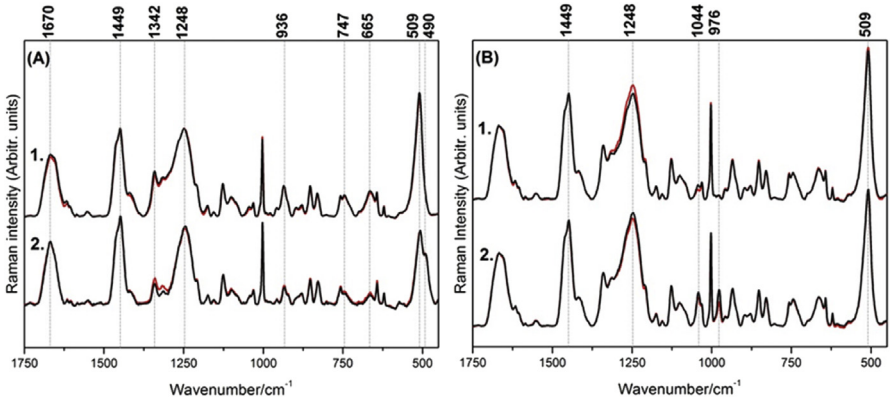

Fig. 2. Stackplotted Raman vibrational spectra of hair strands of the two ethnic groups, Caucasian (shown in black) and Afro (shown in red). Panel (A): 1. Spectra of Caucasian and Afro-natural hair. 2. Spectra of Caucasian and Afro hair after heat treatment. Panel (B): 1. Spectra of Caucasian and Afro natural hair. 2. Spectra of Caucasian and Afro hair after discoloration treatment.

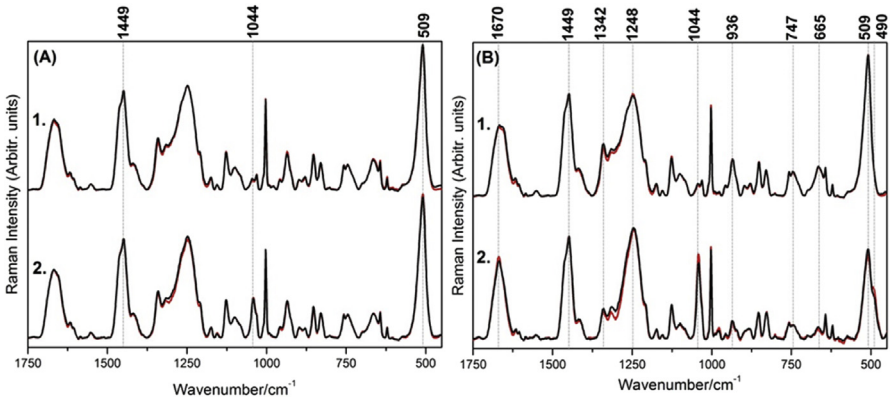

Fig. 3. Stackplot of the Raman spectra of hair strands of the two ethnic groups, Caucasian (shown in black) and Afro (shown in red). Panel (A): 1. Spectra of Caucasian and Afro-natural hair. 2. Spectra of Caucasian and afro hair after relaxation treatment. Panel (B): 1. Spectra of Caucasian and Afro natural hair. 2. Spectra of Caucasian and Afro hair after total treatment.

ethnicities: number 1 contains the spectra of natural Caucasian hair (shown in black) and natural Afro hair (shown in red); it can be clearly seen that there is no difference between them. Number 2 shows the spectra of the thermally treated Caucasian (shown in black) and Afro hair (shown in red), and again it is noted that the hair specimens from the two ethnicities are showing the same pattern of modifications after the heat treatment. The spectral analysis shows that the band at 509 cm^{-1}, assigned to the S-S stretch of the gauche-gauche-gauche conformation (GGG) of the CSSC linkage of cysteine, suffered a decrease in its band intensity and a shoulder is observed at at 490 cm^{-1} which can be assigned to strained conformations of the CSSC linkage [27]. There were also decreases observed in the intensities of the bands at 665 (CS bond), 747 (CS bond), 936 (CC bond), 1248 (Amide III mode), 1342 (CH_2 bend and tryptophan, Trp) and the band at 1663 shifted to 1670 cm^{-1}, which has been assigned to the vibrational mode associated with the β-sheet and/or random coil forms of the protein amide groups [29].

Exposure of the fibre to excessive heat has therefore caused the thermal decomposition of the protein material [31], leading to the appeareence of a yellowish color due to this thermal decomposition, which leads us to consider that part of the disulfide bridge (SS bond), the CS and CC bonds of the polypeptide backbone and the Amide III (related to the secondary protein structure) bonds were broken. It can be inferred that permanent damage was hence caused to the hair, since these are important bonds of the α-keratin protein, which provide shape, resistance and flexibility, among other characteristics of the fibre [5].

Panel B of Fig. 2 shows the Raman spectra of the two ethnicities, number 1 is the spectrum of the natural Caucasian hair (shown in black) and the natural Afro hair (shown in red), which presents a slightly higher intensity for the Amide III band (1248 cm^{-1}), attributed to the greater presence of a random protein coil structure than in Caucasian hair. The spectrum #2 shows the spectra of discolored Caucasian (shown in black) and Afro (shown in red) hair; it has been noticed that the Amide III band in the Caucasian hair specimen spectrum exhibited an increase in intensity, whereas in contrast the Afro hair had a decrease in the intensity of this band after the discoloration treatment. Another important issue is the appearence of a band at 976 cm^{-1} of high intensity for Caucasian hair, which could be assigned to residues from cystine, and the band at 1044 cm^{-1}, due to the S-O stretching, also has suffered an increase in intensity.

Hair bleaching occurs through the oxidation of the melanin granules present in the cortex, however, due to the reaction conditions required for the destruction of the pigments, secondary reactions involving the proteins occur simultaneously. Since hair contains a large percentage of oxidizable clusters (eg. disulfide bonds) then the degradation of capillary proteins also occurs during discoloration [5]. The decolorizing treatment caused changes in certain spectral regions, such as the appearance of the band at 976 cm^{-1}, and can be attributed to certain cystine oxides, such as monoxide (R-SO-SR), dioxide (R-SO_2-SR) or the disulphide trioxide (R-SO_2-SOR), which are lower stability intermediates between cystine and cysteic acid. The band at 1044 cm^{-1} is related to the S-O symmetrical stretch belonging to the amino acid cysteic acid (R-SO_3H) [29, 32]. Cysteic acid is one of the constituent amino acids of α-keratin, but its content can be intensified through photochemical oxidation due to exposure of hair to natural (sun) or artificial sources of light radiation.

Another way of causing the formation of this residue of cysteic acid is the action of a decolorant in the fibre, where cysteine residues are converted into cysteic acid residues; this occurs because the reaction of the oxidizing agents with the proteins of the capillary fibre happens first in the disulfide bond, generating the cysteic acid residue [33].

The presence of the Amide III mode at 1248 cm^{-1} is attributed to the creation of a random protein coil structure; an increase in the intensity of this band is related to the modification of the α-helix secondary structure to an amorphous conformation, because when the protein is damaged, it converts into different protein chains, such as β-sheet and random coil, where these changes affect the structure of the hydrogen bonds that stabilize the helical structure [34]. This type of structural modification, caused by different treatments, has also been observed in investigations performed by Liu *et al.* [35] and Kuzuhara [36]. Thus, excessive bleaching treatment may leave the embrittled fibres more susceptible to breakage [3].

3.1.2.2. Relaxation treatment and total treatment. The results of the action of the relaxation process and total treatment on the capillary structure are shown in Fig. 3. Panels A and B of Fig. 3 show the overlapping Raman spectra of the two hair specimen ethnicities, before and after the relaxation and total treatments, respectively. In panel A, the number 1 spectrum shows the spectrum of natural Caucasian hair (shown as black) and natural Afro hair (shown as red), and there is no difference between them. The number 2 spectrum shows the effect of treating the specimen with the relaxation procedure, with the same pattern of modification being observed in the stackplotted spectra. The band that undergoes the most significant modification was observed at 1044 cm^{-1}, assigned to the S-O symmetrical stretch belonging to the amino acid cysteic acid.

Analysing the number 1 spectra in panel B (Fig. 3) there is no difference to be seen between the two hair specimen ethnicities before total treatment. The number 2 spectral plots present modifications to several bands in the spectra of the two ethnic specimen groups as a consequence of the total treatment, with the same type of modification being observed. The band at 509 cm^{-1}, assigned to the SS bond stretching of the CSSC gauche-gauche-gauche conformation (GGG), exhibits a decrease in its intensity, and at 490 cm^{-1} a new shoulder appears, which has been assigned to strained conformations, similar to that observed for the spectra of heat treated hair (Fig. 2). Several other differences in intensity can be observed for other vibrational modes, such as C-S (665 cm^{-1}), C-S (747 cm^{-1}), C-C (936 cm^{-1}), CH_2 and Trp (1342 cm^{-1}), where the intensity has decreased; some bands present an increase in intensity, such as those at 1044 cm^{-1} (S-O) and the vibration at 1248 cm^{-1} (related to the Amide III mode).

When analysing the spectra shown in panel B it is possible to observe changes in several regions of the vibrational spectrum of the two hair specimen ethnicities. The SS, CS and CC bands showed a noticeable decrease in their intensities, which can be explained by the drastic action of the applied chemical and physical treatments, mainly modifying the main structure of the polypeptide skeleton, α-keratin, and generating the weakening and wear of the specimen capillary properties. However, the bands related to the S-O bond (1044 cm^{-1}) and the Amide III vibration (1248 cm^{-1}) exhibited an increase in their intensities, caused by the formation of cysteic acid residues and the random coil conformation,

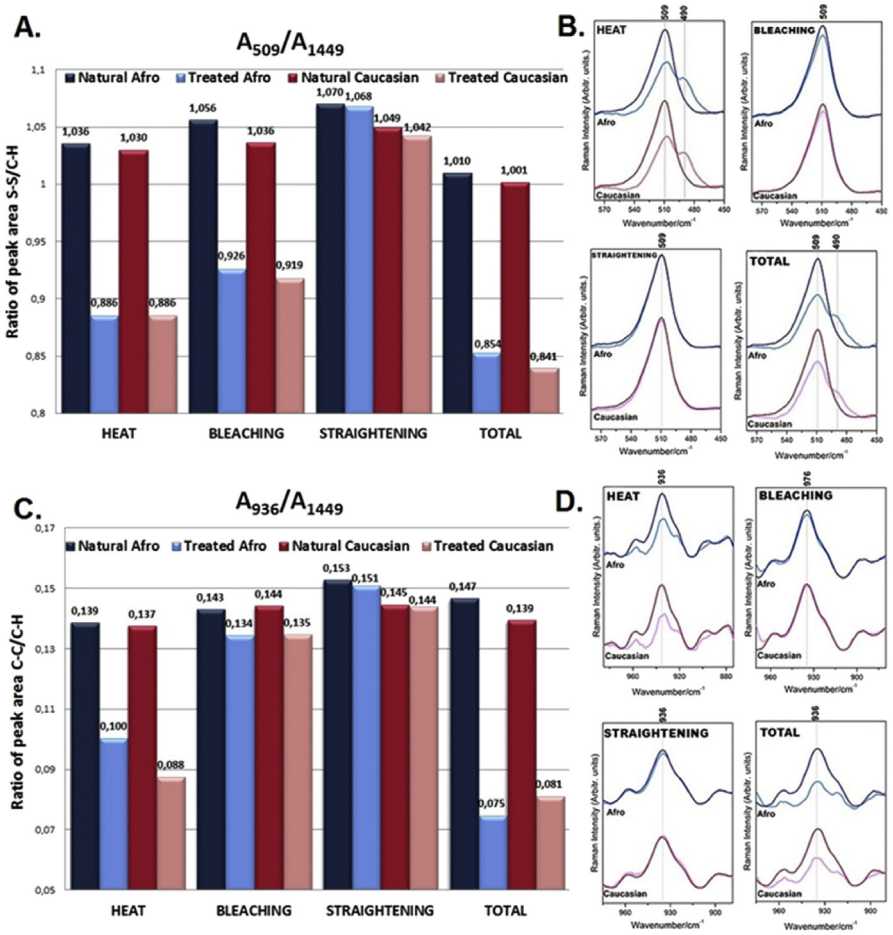

Fig. 4. A. Histogram of the S-S band area ratio to the C-H band for all treatments. B. Raman spectra of the S-S band (509 cm^{-1}) of the virgin hair samples (shown in dark blue and dark pink) and treated (shown in light blue and light pink). C. Histogram of the C-C band area ratio to the C-H band for all treatments. D. Raman spectra of the C-C band (936 cm^{-1}) of the virgin hair samples (shown in dark blue and dark pink) and treated (shown in light blue and light pink).

respectively. The amide I mode (1663 cm^{-1}) had a small increase in its intensity and shifted to 1671 cm^{-1}, which can be understood as an increase in the content of secondary structures of the β-sheet and/or random coil type [29].

3.2. Calculation of the bandmarker areas

The calculation of the ratios of the important band areas in the Raman spectra of the hair strands was performed on the basis of comparison with the C-H band at 1449 cm^{-1}, as this band is not influenced by any of the performed treatments [36]. The relative content of disulphide bonds was determined using the ratio of the SS band areas (calculated from a baseline between 580 and 465 cm^{-1}) and the CH bond (calculated from a baseline between 1500 and 1375 cm^{-1}). The structural C-C content was compared using the ratio of the C-C bands (calculated from a baseline between 953 and 910 cm^{-1}) and the C-H band. The cysteic acid residue content of the samples was compared using the ratio of the areas of the S-O bands (calculated from a baseline between 1066 and 1020 cm^{-1}) and the same C-H deformation band. In this sense, the disordered secondary structure content was compared by estimating the ratio of the areas of the amide III band (calculated from a baseline between 1327 and 1190 cm^{-1}) and the C-H deformation band. The results were expressed in histograms for a better visual comparison of the differences between the virgin and the treated hair samples.

Fig. 4 allows a better visualization of the changes in the S-S and C-C bands after the applied treatments. From the histogram of panel A it is noticed that the two hair specimen ethnicities gave practically the same result with respect to each procedure applied. Excessive heat and total treatment were the procedures that caused the greater decrease in band intensity at 509 cm^{-1}, which can be observed in the panel B spectra and histogram A. The discolored samples from both hair specimen ethnicities also showed decreases in the intensity of the same band. The relaxation treatment did not cause any significant change. In panels C and D it is observed that the thermal and total treatment procedures were those which generated the most significant changes in the C-C bonds, since the intensity of this band decreased considerably in the spectra of the two hair specimen ethnicities (panel D). The Caucasian hair showed, as seen in histogram C, a greater damage after the heat treatment and Afro hair after the total treatment. The discoloration treatment caused a small decrease of this band intensity in the two hair specimen ethnicities to an equal extent and the relaxation procedure did not cause any significant modification. The C-C bonds are part of the polypeptide backbone and is of paramount importance for the protein structure but the high temperature generated significant rupture of these bonds, leading to irreversible damage to the hair fibre.

The ratio of the SO band area to the CH band área is demonstrated in the panel A histogram and the SO band Raman spectra in panel B of Fig. 5, where only the spectrum of the heat treated sample did not show considerable changes in the intensity of this SO band for both the Afro and Caucasian ethnicities, thus suggesting that there was no formation of cysteic acid residues with the application of heat. However, the discoloration and relaxation treatments (and consequently the total treatment)

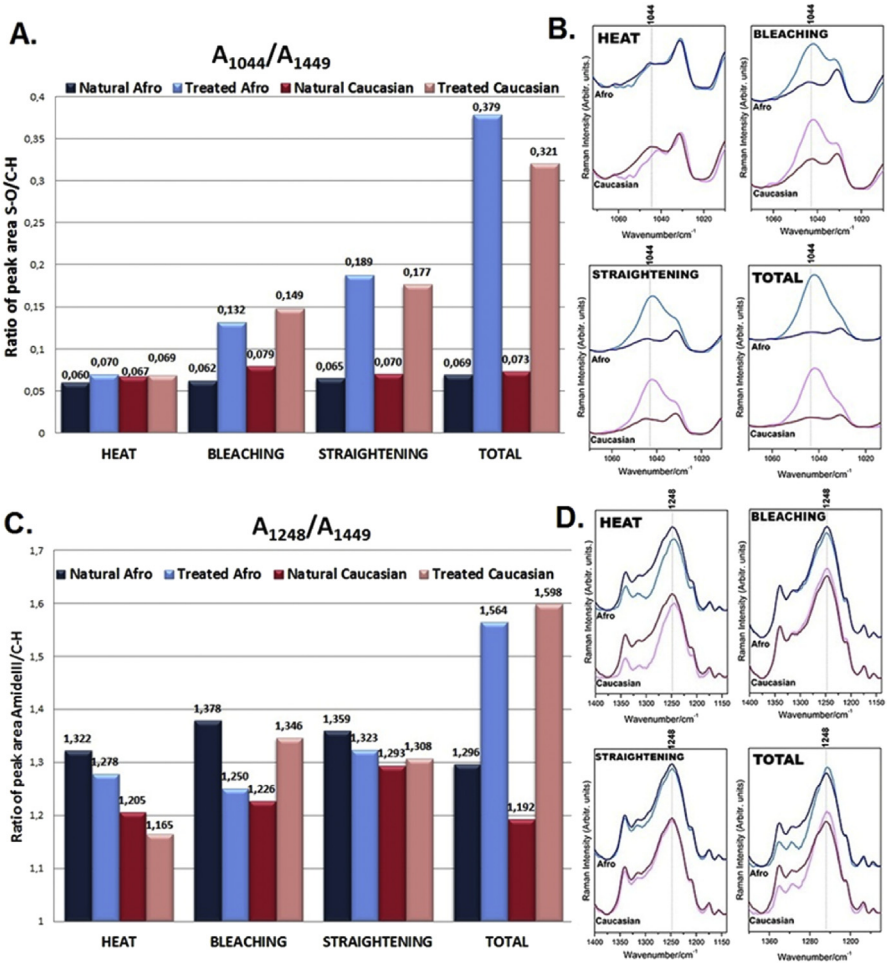

Fig. 5. A. Histogram of the S-O band area ratio to the C-H band for all treatments. B. Raman spectra of the S-O band (1044 cm^{-1}) of the virgin hair samples (shown in dark blue and dark pink) and treated (shown in light blue and light pink). C. Histogram of the ratio of the Amide III band area to the C-H band for all treatments. D. Raman spectra of the Amide III band (1248 cm^{-1}) of the virgin hair samples (shown in dark blue and dark pink) and treated (shown in light blue and light pink).

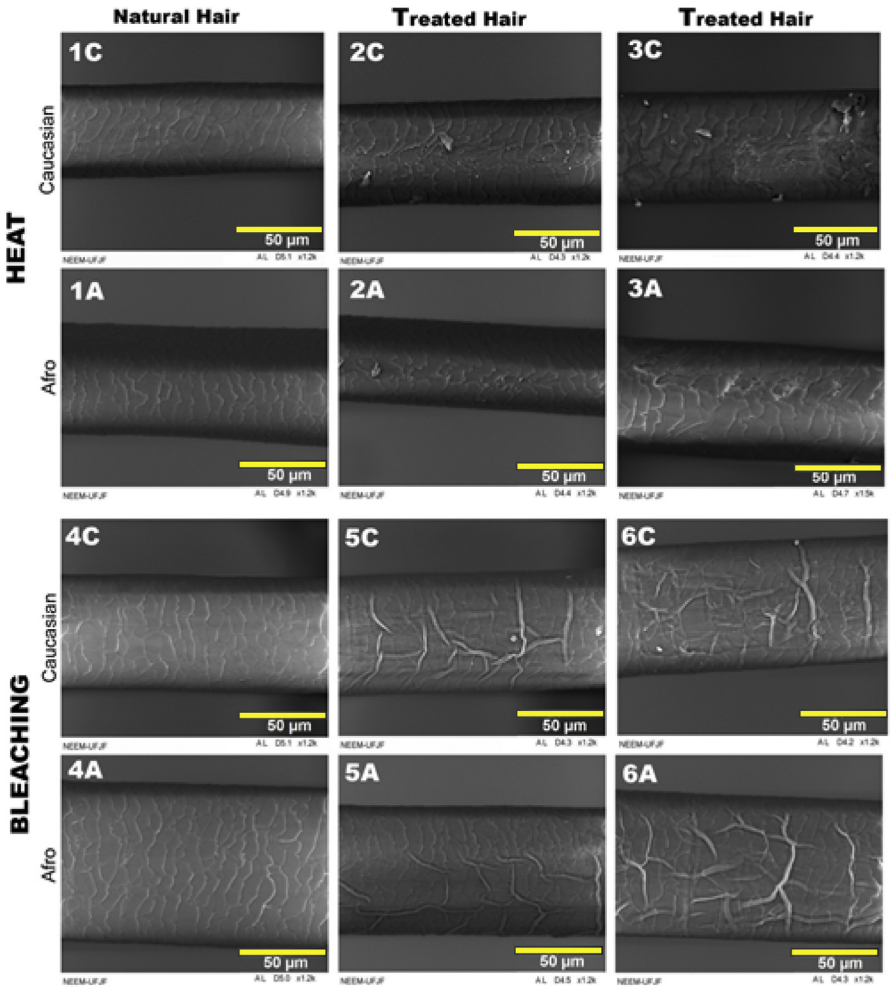

Fig. 6. Scanning electron microscopy of white and afro hair: Natural (1A, 1C, 4A, 4C) with heat treatment (2A, 2C, 3A, 3C) and bleaching treatment (5A, 5C, 6A, 6C).

resulted in the formation of these residues in the hair fibre and the hair specimen ethnicities responded to these treatments with small diferences observed: the discoloration treatment caused in the Afro and Caucasian hair an increase of the same intensity in the SO band, whereas the relaxation and total treatments produced a greater intensity of the SO band in the Afro hair specimen. The amide III band, shown in panel D, is associated with the random coil structure, i.e. the secondary structure of proteins. In panel C it can be seen that the amide III band in the Afro natural hair specimen is slightly larger in intensity than in the natural Caucasian hair specimen, leading to the conclusion that Afro hair has a higher content of random coil protein structures than the Caucasian hair. The heat treatment caused a small decrease in the intensity of the Amide III band in the hair speciemns from the two ethnicities, but in contrast the discoloration treatment caused a decrease in intensity of this band in Afro hair and in Caucasian hair the reverse occurred, i.e. the Amide III band has had an increase of intensity. The same effect occurred in the relaxation treatment but to a lesser extent. Since the total treatment generated an increase in the intensity of the amide III band in the two ethnicities, being more intense in the Caucasian hair, this increase can be related to the modifications in the secondary α-helix structure, leading to an increase in the content of the random coil conformation.

3.3. Scanning electronic microscopy

To investigate the morphological changes in the capillary structure of the hair specimens, scanning electron microscopy (SEM) was used. Fig. 6 presents images of the natural Caucasian and Afro hair and their morphological changes after the applications of the thermal and bleaching treatments. In the images of natural hair (1A, 1C, 4A, 4C) it is possible to observe the cuticles closed and with regular contour, without any visible presence of deterioration in the structure. The heat treated hair fibres, shown in 2C and 3C for Caucasian speciemns and 2A and 3A for the Afro specimens, suffered cuticular damages with similar characteristics, since the cuticles showed similar longitudinal modifications, as if they had been removed and/or broken. The action of the bleaching solution for 40 min on the hair fibre (shown in the images 5C and 6C for Caucasian and 5A and 6A for Afro specimens) leads to the appearance of 'striations' on the surface of the fibre and to a decrease in the definition of the scales, probably generated by the solubilization of proteins present in the cuticle [3].

Fig. 7 shows the results of the relaxation and total treatments on the specimens and it is possible to observe in the healthy hair images (1A, 1C, 4A, 4C) healthy hair with sealed cuticles with no deformations visible in their morphologies. After the processes of reduction and oxidation (images 2A, 2C, 3A and 3C), both constituents of relaxation, there appear to be some lesions in the fibrous structure which become manifest as irregularities, cuticle detachment in some areas and small undulations in its extension, possibly caused by the initial solubilization process of the protein material.

The results of the application of the three treatments (relaxation, discoloration and heat) on the same hair fibres are shown in Figs. 5A, 5C, 6A and 6C. These microscopic analyses exhibit hair strands with irregular contours, detached and deformed cuticles, with possible damage of the cortical material. The explanation for such effects can be explained, for

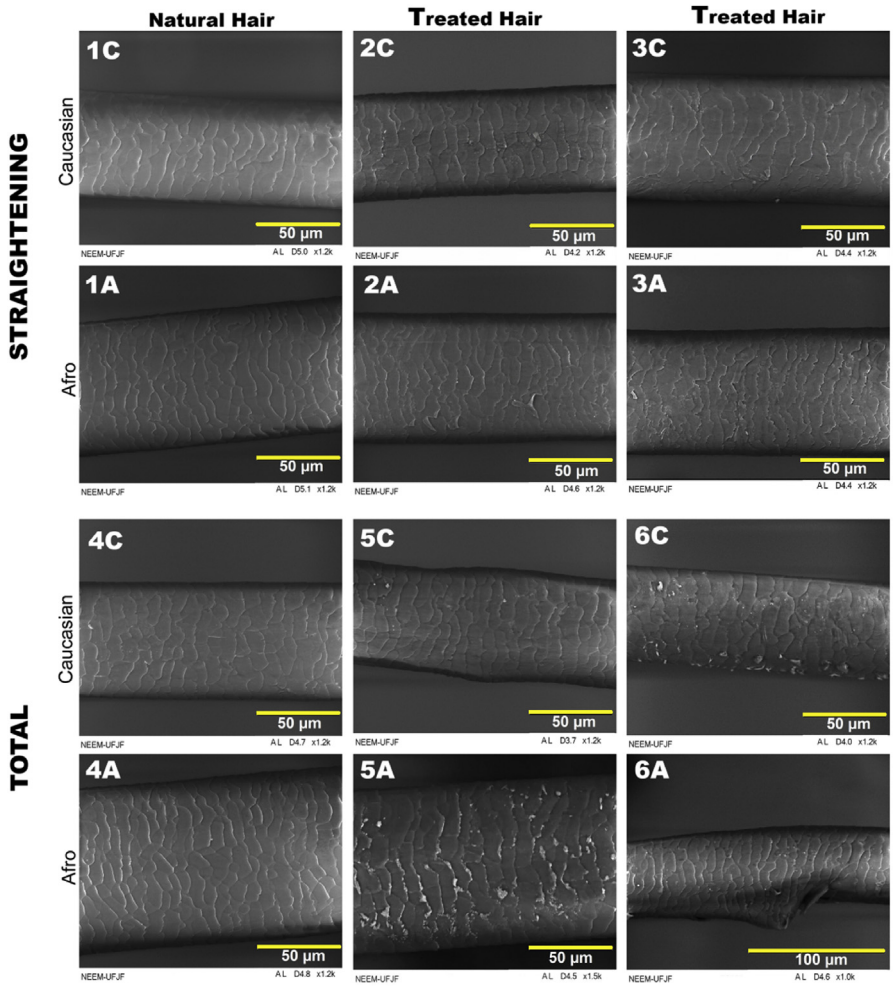

Fig. 7. Scanning electron microscopy of white and afro hair: Natural (1A, 1C, 4A, 4C), with heat treatment (2A, 2C, 3A, 3C) and bleaching treatment (5A, 5C, 6A, 6C).

example by the increased rupture of the hydrogen bonds occurring with increased pH which causes a dilation in the capillary caused mainly by urea and other amides. This process generates axial folds created by extreme dilation and then rapid heat dehydration. These folds are created due to a contraction in the different cuticle layers due to the leaching of solubilized protein matter [3].

To summarise, this work indicates that a severe modification in the hair structure results from a concerted action of different hair treatment, independent of the type of the human hair for those we have investigated here, and that the joint use of both heat and discoloration procedures causes major damage to the hair structure.

4. Conclusions

Caucasian and Afro hair specimens were analysed by Raman spectroscopy before and after thermal treatment, discoloration and relaxation. The natural untreated samples of the two ethnic groups did not present any significant spectral differences between them, except for the slightly higher intensity of the Amide III band in the Raman spectra of Afro hair, demonstrating a higher content of random coil structure in this ethnic group. The breakdown of disulfide bonds, as demonstrated by the decrease in S-S band intensity, was observed in almost all treatments, where the two ethnicities reacted in similar ways according to each treatment applied. The bands suffered a greater decrease of intensity after application of the thermal and total treatment, and the relaxation treatment practically did not modify the S-S band at all. The C-C band was also affected more significantly by the thermal and total treatment. Caucasian hair showed a lower intensity of this C-C band after heat treatment and Afro hair after the total treatment. The increase in the intensity of the S-O band was caused by the discoloration and relaxation treatments, which led to a higher S-O band intensity after the total treatment, and there was evidence that Afro hair had a more significant modification of this band. The heat treatment did not present any changes in the intensity of this band. The intensity of the amide III band decreased in the Afro hair after the thermal treatment, discoloration and relaxation treatment, and showed an increase after the total treatment. Caucasian hair only showed a decrease in the intensity of this band after the heat Treatment and all other treatments caused an increase in the intensity of the Amide III band.

Scanning electron microscopy images showed changes in the cuticular surface, where each treatment followed a pattern of deformation, and these results are in a perfect agreement with the Raman data. The heat treatment showed longitudinal modifications, as if the cuticle layers had been removed or broken; the discolouration caused the appearance of 'striations' on the surface of the hair along with the diminution of the cuticular definition; the relaxation treatment caused the detachment of cuticles in some areas and small undulations in the extension of the fibre, and the total treatment caused the formation of fibres with an irregular contour, detached cuticles and with axial folds.

Through the results obtained in this work it was possible to infer that the treatments that most modified the capillary structure were the thermal and total treatments, and that the two hair specimen ethnicities had small spectral differences which varied according to the treatment to which they had been subjected.

Declarations

Author contribution statement

Jordana Santos: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Howell Edwards: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Luiz Fernando Cappa de Oliveira: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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