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Yellowing and bleaching of grey hair caused by photo and thermal degradation

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ABSTRACT

Yellowing is an undesirable phenomenon that is common in people with white and grey hair. Because white hair has no melanin, the pigment responsible for hair colour, the effects of photodegradation are more visible in this type of hair. The origin of yellowing and its relation to photodegradation processes are not properly established, and many questions remain open in this field. In this work, the photodegradation of grey hair was investigated as a function of the wavelength of incident radiation, and its ultrastructure was determined, always comparing the results obtained for the white and black fibres present in grey hair with the results of white wool.

The results presented herein indicate that the photobehaviour of grey hair irradiated with a mercury lamp or with solar radiation is dependent on the wavelength range of the incident radiation and on the initial shade of yellow in the sample. Two types of grey hair were used: (1) blended grey hair (more yellow) and (2) grey hair from a single-donor (less yellow). After exposure to a full-spectrum mercury lamp for 200 h, the blended white hair turned less yellow (the yellow–blue difference, Db^* becomes negative, $Db^* = -6$), whereas the white hair from the single-donor turned slightly yellower ($Db^* = 2$). In contrast, VIS + IR irradiation resulted in bleaching in both types of hair, whereas a thermal treatment (at 81 °C) caused yellowing of both types of hair, resulting in a $Db^* = 3$ for blended white hair and $Db^* = 9$ for single-donor hair. The identity of the yellow chromophores was investigated by UV–Vis spectroscopy. The results obtained with this technique were contradictory, however, and it was not possible to obtain a simple correlation between the sample shade of yellow and the absorption spectra. In addition, the results are discussed in terms of the morphology differences between the pigmented and non-pigmented parts of grey hair, the yellowing and bleaching effects of grey hair, and the occurrence of dark-follow reactions.

1. Introduction

Research on protein photochemistry is important for understanding the degradation processes that occur as a consequence of light incidence on hair and for developing strategies to avoid such reactions. Solar radiation that reaches the earth's surface consists of three wavelength ranges: ultraviolet (UV), visible (Vis) and infrared (IR). Natural and synthetic polymers are susceptible to the damaging effects of sunlight. Although the UV range represents only 4–6% of the total spectrum of solar radiation, the energy associated with UV radiation is enough to break covalent bonds present in organic compounds [1,2]. Free radicals can be formed in these cleaving reactions, which will favour the occurrence of other free radical reactions, including protein damaging reactions [3].

It is well documented that hair, which is mainly composed of proteins (65–95% in weight) [4], is vulnerable to photodamaging [5,6]. However, the current literature about hair photodegradation is not conclusive with respect to white hair colour changes and the type of chromophores formed. Melanins, proteins and lipids are damaged after solar irradiation, resulting in complex photochemical reactions [7–9]. The main consequence of this process is colour modification [6,10] that depends on the natural colour of the hair and on the radiation range of exposure.

Hair proteins absorb radiation between 200 and 350 nm. Aromatic amino acids such as tryptophan, tyrosine and phenylalanine and the sulphur-containing amino acids cystine and methionine are UV-absorber chromophores that are present in keratin [4]. Melanins also absorb UV-radiation and are degraded or bleached

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during the process [11]. There are two types of melanins: the eumelanins (brown–black pigments) and the pheomelanins (red pigments) [12]. It is known that melanins, which are present in pigmented hair, prevent to some extent the colour change of hair due to light exposure. [13,14]. The absence of melanins in white hair is the reason why white hair is normally more prone to display the effects of photodegradation during light exposure than dark hair [10].

According to most of studies in this area, photodegradation results in yellowing of white hair and also wool after sun exposure [15,16]. Materials such as wood and paper of natural origin, and also synthetic polymers such as polyethylene are also known to become yellower after sun exposure [17]. A previous study by this research group [10], however, found a less predictable behaviour. White hair became less yellow when exposed to full-spectrum solar light but yellower when exposed to heat (at 50 °C). The photobleaching effect was also reported in wool exposed to visible (Vis) radiation, whereas yellowing occurred under UV exposure [18–20]. In the case of wool, the ratio of photobleaching to photoyellowing is directly related to the initial colour shade.

Published works concerning the presence of yellow chromophores on free amino acids after exposure to sun radiation have identified significant yellowing in tryptophan, tyrosine, histidine, phenylalanine, and cystine in solutions [21]. Tryptophan and tyrosine, in particular, are considered to be involved in photoyellowing, as the photo oxidation products of these amino acids were detected by fluorescence spectroscopy of wool after solar irradiation [21]. Our previous study with hair and free amino acids irradiated with a mercury lamp indicates that although a decrease in the tryptophan content of hair after lamp irradiation is observed, a direct correlation with hair yellowness cannot be achieved [10]. A limitation of the studies using free amino acids is that they may present a different behaviour when comparing the amino acids linked in the keratin structure. To overcome this problem, experiments based on the irradiation of wool fibres with a xenon lamp followed by the characterisation of the products of protein photodegradation were performed by Bryson and collaborators. Using a quasi-proteomic approach, these authors concluded that the photoproducts mainly originated from tryptophan and tyrosine [21].

The present paper contributes to the comprehension of the photoyellowing and photobleaching behaviour in white hair. Two types of hair were evaluated: blended grey hair and grey hair from a single-donor. These are composed of totally black fibres, totally white fibres or fibres that were partially white and partially black. The white regions of these two hair types were yellow before irradiation with UV, Vis and IR and a strong dependence of the result of the photodegradation process on the initial hair colour was noticed. Photoyellowing or photobleaching took place in each case, depending on the initial colour shadow and on the wavelength of incident light.

The morphology of untreated grey hair was also analysed by transmission electron microscopy to investigate the pre-existing differences present in white and black hair before photodegradation. Single partially black and partially white fibres from grey hair were used, so that the effect of genetic variability would not influence the final results. To our knowledge, this is the first study detailing the ultrastructure of a region of white hair in comparison with the morphology of a black region in the same hair fibre.

2. Experimental

2.1. Samples

Blended grey hair was purchased from De Meo Brothers Inc. (New York, USA). Grey hair was also collected from a male volunteer with no history of chemical treatments (called grey hair from single-donor along the text). Grey hair is a mixture of fibres with some that are totally white and totally black and some fibres that are partially white and partially black. White fibres were separated by hand, and samples weighing 0.5 g were used.

Prior to the experiments, the hair samples were washed with a 2.0% w/w sodium lauryl sulphate aqueous solution, according to the following steps: (1) hand-washing with 1 mL of the solution for 1 min, (2) rinsing with tap water at 40 °C for 30 s, (3) repetition of step (1) and (2) but with rinsing for 2 min, and (4) combing using a polypropylene comb. Then, the samples were dried at room temperature and stored in plastic bags.

All of the Merino wool used in the work was untreated and came from a single sheep. The wool closer to the sheep's body (more exposed to the body heat) was yellower, whereas the wool at the tips (farther from the body and more exposed to the sun) was less yellow. Samples were separated into yellow or white, cleaned with ethyl ether in a Soxhlet extractor for 8 h, dried at room temperature and stored in plastic bags.

2.2. Measurements of fibre diameter

The diameters of 35 partially white and partially black fibres from blended and single-donor grey hair were measured using two distinct devices: (1) a light microscope and (2) a micrometer. In both cases, the measurements were carried out in six distinct points in each fibre: three points on the white area and another three points on the black area. The diameter results, in each area, are thus an average of the three measurements in the 35 fibres (total of 105 values). When using the micrometer, the measuring points were randomly distributed along the white and the black areas of the fibre. In the case of the light microscope, images from the white and black areas of the fibre were obtained at distances of 2 cm, 4 cm and 6 cm far from the white–black boundary. The images were then imported to the Axio Vision SE Rel 4.9.1 Software (Carl Zeiss Company, Germany) and the diameters were measured.

2.3. Characterisation of the radiation sources

Direct sunlight and a mercury vapour lamp (OSRAM HQL 125 W, São Paulo, Brazil) were used as radiation sources. The lamp has an emission spectrum with strong lines at 367 nm (UV) and 406 nm, 438 nm, 548 nm and 580 nm (visible light), Fig. 1, in addition to emitting very low infrared (IR) radiation. Thus, it is very different from the continuous solar spectrum. The overall procedure for irradiation with the mercury vapour lamp is described elsewhere [6]. Measurements of light intensity from all of the sources were carried out with a radiometer (PMA 2100, Solar Light Co., USA), considering the dose incident on the samples. The distance source-sample and source-radiometer were the same. The intensity of sunlight was measured at noon in Campinas, Brazil (22°53′ S; 47°04′ W). The values of radiation intensity obtained for the mercury vapour lamp were 1.5 W m^{-2} (UVB), 26.0 W m^{-2} (UVA) and 70.0 W m^{-2} (Vis + IR), and for sunlight, they were 2.4 W m^{-2} (UVB), 32.0 W m^{-2} (UVA) and 658.0 W m^{-2} (Vis + IR).

The exposure times were calculated such that the daily doses of UV radiation from the lamp and sun were comparable. The intensities of visible radiation could not be equalised because the intensity emitted by the lamp is less than half of the sun emission.

2.4. Exposure to artificial radiation

One of the following exposure conditions were applied to the hair and wool samples: (1) exposure to Vis and IR only, using a common borosilicate glass (50 × 40 × 0.4 cm) covered with a polyester film to block the UV from the lamp full-spectrum and (2)

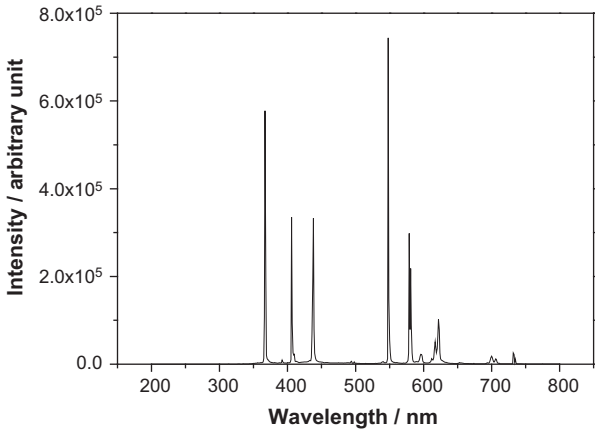

Fig. 1. Emission spectrum of the mercury vapour lamp 125 W.

exposure to the lamp full-spectrum (UV, Vis and IR) for periods of 8 h, followed by a period of 16 h in the dark (inside a laboratory cabinet). A variation of method 2 was used for white hair samples, including a washing step (as previously described) between the irradiation and the period in the dark. White hair samples were stored in the dark for 1 year after all cycles of irradiation.

In general, the irradiation time of the hair and wool samples by the mercury vapour lamp was more than 200 h. Irradiation was carried on inside a fume hood, as described elsewhere [6], so that the experimental conditions could be controlled. Irradiated samples were regularly rotated during the process. The temperature and the relative humidity inside the fume hood were monitored daily and kept under average values of 28 ± 2 °C and 35 ± 9%, respectively. The temperature data indicate that the mercury lamp emitted very low IR radiation.

2.5. Exposure to solar radiation

Samples were exposed to sunlight from 10 am to 3 pm (period of highest UV intensity), only on sunny days in Campinas, Brazil. One of the following exposure conditions was used: (1) exposure to Vis and IR only, using the same borosilicate glass described in Section 2.4 and (2) exposure to solar full-spectrum (UV, Vis and IR). The total exposition time in both conditions was 50 h. Samples were also regularly rotated during solar irradiation, and the average values of local temperature and relative humidity were 28 ± 2 °C and 56 ± 14%, respectively.

2.6. Exposure to thermal treatment

The effect of thermal treatment was verified by keeping the hair samples inside an oven at 53 ± 5 °C or 81 ± 6 °C. At both temperatures, an exposure time of 10 h was applied, followed by a period of 14 h in the dark. At 81 °C, an alternative procedure was carried out, including a washing step (as previously described) between the heat exposure and the period in the dark. After all cycles of exposure, the samples were stored in the dark for 1 year.

2.7. Bleaching treatment

Grey hair (white and black areas) underwent bleaching with a hydrogen peroxide solution (4% v/v, pH = 9.5) for 2.5 h. The solution consisted of 15% (w/w) of ammonium persulphate, 15% (w/w) of a hydrogen peroxide solution (29 wt.% in water) and 70% of distillate water. The pH was reached using a sodium hydroxide solution. After the bleaching treatment, samples were washed with the solution of sodium lauryl sulphate, as previously described.

2.8. Measurements of colour changes

Changes of sample colour were measured by diffuse reflectance spectrophotometry (DRS), using a GretagMacbeth Colour-eye® 2180UV device (New York, USA). The method of analysis has been previously described [22]. Briefly, the spectra provide values of coordinates L^* (colour lightness), a^* (redness, if positive or greenness, if negative) and b^* (yellowness, if positive or blueness, if negative) from the CIELAB system of equations. The values before the thermal treatment or irradiation are indicated by the index (i) from initial and the values after these procedures by the index (f) from final.

From these, the colour difference parameters could be calculated: DL* (lightness difference: $L_f^* - L_i^*$, lighter if positive, darker if negative), Da* (red–green difference: $a_f^* - a_i^*$ redder if positive, greener if negative), Db* ($b_f^* - b_i^*$ yellow–blue difference: yellower if positive, bluer if negative) and DE* (total colour difference: $[(DL^*)^2 + (Da^*)^2 + (Db^*)^2]^{1/2}$).

Experiments were done with the same sample region and turning of the hair sample holder in the instrument. Ten measurements were performed in each of the two replicates of the samples. An internal reference was established by measuring the samples before heating or irradiation (control samples). Human hair and wool exhibit inherent colour heterogeneity, which limits the precise determination of the colour. Thus, taking into account the standard deviation of the colour difference parameters, they were considered significant if DE* > 1.5, DL* > 1.0, Da* > 0.3 and Db* > 0.8 for hair and DE* > 3.5, DL* > 2.5, Da* > 1.0 and Db* > 2.0 for wool.

2.9. UV–Vis spectroscopy

A spectrophotometer (Hewlett–Packard model 8452) was used for UV–VIS absorbance measurements. A 0.01 % (w/w) stock solution of samples before and after heating or irradiation was prepared in NaOH. Hair samples were left to be solubilised for 24 h and then diluted 80 times before analysis. Measurements were carried out four times and were very reproducible.

2.10. Transmission electron microscopy (TEM)

Hair fibres were fixed in the dark with a 2% (v/v) solution of OsO_4 (Sigma) in a 0.1 mol L^{-1} sodium cacodilate buffer (pH = 7) for 4 h. Then, they were dehydrated with ethanol solutions of increasing concentration (from 50 to 100% v/v) and propylene oxide. Dehydrated samples were embedded in Spurr resin (formulation with 0.2 g of catalyst) for 5 days and then transferred to moulds and cured at 70 °C for 24 h [23]. The samples were cut using ultra-thin sections and stained with 2% (w/v) uranyl acetate solution for 1 h and then with 1% (w/v) lead citrate solution for 15 min. [24]. TEM micrographs were obtained using a Philips CM 200 microscope (USP-São Paulo) operating at 160 kV.

3. Results

3.1. Morphology of grey hair

Grey hair consists of a mixture of totally white fibres, totally black fibres and fibres that are partially black and partially white. To investigate the differences in the ultrastructure between the white and black areas of blended grey hair, transmission electron microscopy (TEM) images were obtained. In Figs. 2 and 3, there are micrographs from one grey hair fibre that is partially black

and partially white. Fig. 2 shows micrographs of the cortex region of the black area on the grey hair. Many melanin granules can be observed in the black hair, such as the one represented by the dark area in Fig. 2(a). In some cases, the formation of internal aggregates is observed in these granules and characterised by small grains of variable grey levels, as shown inside the melanin granule in Fig. 2(b). Crystalline domains can also be detected within some granules, as indicated by the arrow in Fig. 2(c).

Fig. 3 shows micrographs of the cortex region from a white area of the blended grey hair. The cortex of the white hair area presents many nuclear remnant structures, as indicated by arrows in Fig. 3(a). A few melanin domains persist on these white hair samples (Fig. 3(b)). In some cases, the melanin globules presented a fragmented structure formed by small grains, as shown in Fig. 3(c).

There is controversy in the literature about the diameter of white and black hair. In some studies, the diameter is reported to be different, whereas in others, it is not significantly different [25,15]. To evaluate this point, the diameters of 35 fibres of partially black and partially white grey hair were measured using a micrometer and light microscopy images. Table 1 shows the diameters obtained using both techniques for white and black areas from blended and single-donor grey hairs. Comparing the results obtained with these two techniques, it can be seen that the diameters of white and black areas are very similar between the blended or single-donor hair. Based on these results, it can be concluded that the main difference between the white and the black areas of grey hair lies in the amount of melanin in the fibres and not in the thickness of the fibres. It is noticeable that the values measured with the micrometer are always smaller than those obtained by light microscopy. This may be an indication that the micrometer, even with all of the care taken during the experiments, may be pressing the fibres to some extent, resulting in a smaller diameter measurement than the true value. Thus, this instrument may not be useful for measuring hair diameter.

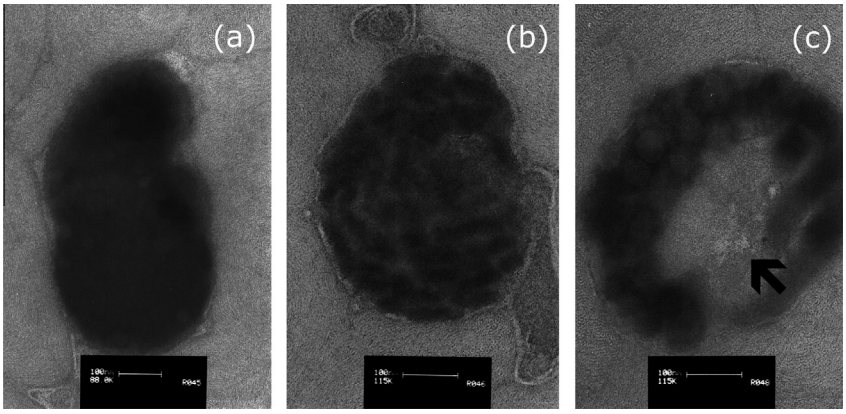

Fig. 2. TEM micrographs of ultra-thin sections of black areas from blended grey hair showing: (a) a typical melanin granules; (b) smaller aggregates formed inside the granule; and (c) a crystalline region inside a melanin granule (indicated by the arrow). (Scale bar: 100 nm).

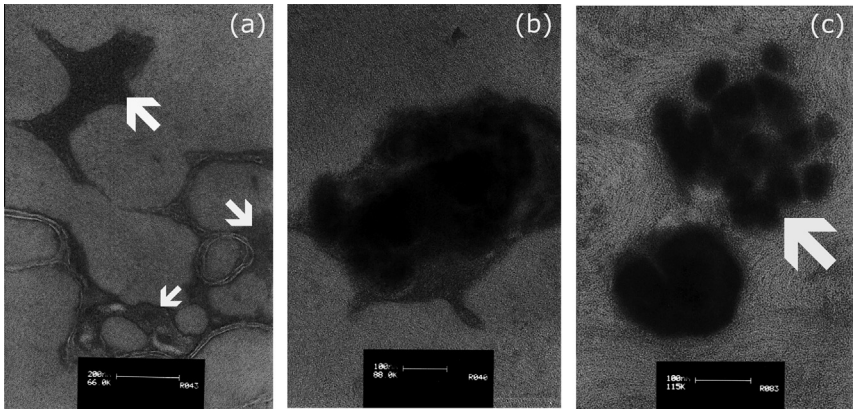

Fig. 3. TEM micrographs of ultra-thin sections of white areas from blended grey hair showing: (a) nuclear remnant structures (dark areas indicated by the arrows); (b) an example of melanin granules that can still be found in white hair; and (c) fragmented melanin (indicated by the arrow). ((a) scale bar: 200 nm and (b) and (c) scale bar: 100 nm).

Table 1
Diameters of white and black areas of grey hair measured using a micrometer and an optical microscope. A total of 35 hair fibres were analysed.

Measurement instrument	Blended hair		Single-donor hair	
	Black area (μm)	White area (μm)	Black area (μm)	White area (μm)
Micrometer	54 ± 7	55 ± 6	71 ± 6	71 ± 5
Light microscopy	75 ± 12	72 ± 13	86 ± 11	91 ± 14

3.2. Photoyellowing and photobleaching of grey hair and wool

The following results were collected with the aim of improving our knowledge about the effects of ultraviolet (UV), visible (Vis) and infrared radiation (IR) on hair by allowing a comparison between the white and black parts of grey hair and also with wool. Because the main recognised photodegradation effect on hair is a colour change [6,10], special attention was given to this feature during the work.

Colour changes were measured in white and black fibres from the blended and single-donor grey hair so that the behaviour of the pigmented (black) and non-pigmented (white) areas could be compared before and after irradiation. Fig. 4 shows the changes in the yellowness of the white areas of a hair after exposure to the full-spectrum mercury vapour lamp (UV + Vis + IR) or to filtered radiation (Vis + IR). Samples submitted to the full-spectrum irradiation were irradiated for 8 h and then left in the dark for 16 h.

The white hair used in this work had a high luminosity, with initial values of $L_i^* > 70$, and little green or red contributions to the colour, with values close to zero in the coordinate a^* (blended hair $a_i^* = 0.1$ and single-donor hair $a_i^* = -0.7$). However, both hairs were initially yellow, with the blended hair being yellower ($b_i^* \cong 18$–21) than the single-donor hair ($b_i^* \cong 9$–10), as can be observed in Fig. 4 for the zero accumulated dose. Therefore, the two hair types had yellow chromophores before treatment, but the black parts of the hair had fewer yellow chromophores, as indicated by the low b^* values in Fig. 4 ($b_i^* \cong 2$–3).

The white hair from the blended sample (white squares in Fig. 4) became significantly less yellow after irradiation ($b_f^* = 15$, $Db^* = -5.9$), confirming the behaviour previously observed by this research group [10]. The single-donor hair, which was initially less yellow (white diamonds in Fig. 4, with initial $b_i^* = 9$), turned slightly yellower after irradiation ($b_f^* = 10.5$, $Db^* = 1.5$).

Thus, the initial colour shade of the white hair directly affects the final colour acquired after irradiation. That is, whereas yellowed hair bleaches, hair that is less yellow may undergo photoyellowing. These results agree with previously published works on wool, but these findings are new in the context of hair science.

The results presented in Fig. 4 were obtained using white hair samples that were washed only before irradiation. As previously described, a second set of grey hair samples was washed twice (before irradiation and also between the irradiation and the period in the dark). Washing was carried out to deactivate free radicals and to stop any degradation reaction that could occur during the dark period. This additional washing step, however, did not result in any significant difference in the yellowing behaviour compared to hair washed only once. As the results obtained for both samples coincide, only the results for samples washed once are shown in Fig. 4.

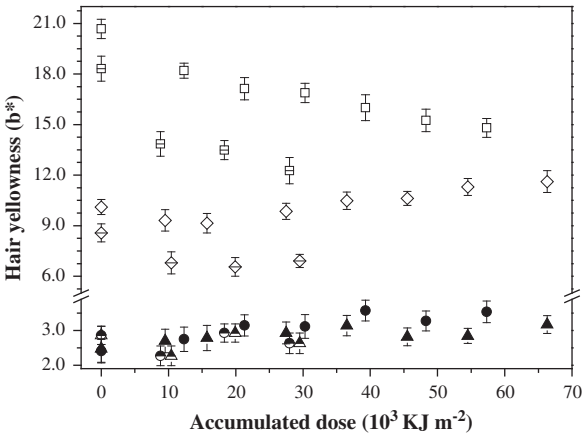

Fig. 4. Hair yellowness (b^*) of grey hair (white and black fibres) after exposure to a mercury vapour lamp. Irradiation with full-spectrum (UV + Vis + IR): (□): blended white hair; (◇): single-donor white hair; (●) blended black hair; (▲): single-donor black hair; irradiation with filtered radiation (Vis + IR): (⊟): blended white hair; (◈): single-donor white hair; (◓) blended black hair; (◭): single-donor black hair. Hair fibres were measured in duplicate, and 10 colour measurements were done for each sample.

White hair irradiated with filtered radiation (Vis + IR) displayed more pronounced photobleaching (white squares and diamonds with horizontal fill in Fig. 4) compared with the hair undergoing full-spectrum lamp irradiation. The colour difference parameters are $Db^* = -6.3$ for the blended hair and $Db^* = -1.7$ for the single-donor hair, indicating that Vis light causes photobleaching in white hair independently of the initial yellowness of the fibres.

In the case of black hair, both the blended hair and the single-donor hair (black circle and black triangle in Fig. 4) became yellower after irradiation using the full-spectrum mercury lamp, which is consistent with the fact that they present similar b_i^* values. The calculated values of Db^* are $Db^* = 1.2$ for blended hair and $Db^* = 0.8$ for single-donor hair. In the case of black hair under filtered radiation (Vis + IR), no significant difference was observed in the b^* parameter of blended hair or single-donor hair, $Db^* = -0.1$ (circle and triangle with upper half black in Fig. 4). The pigmented hairs differently than the white hairs (non-pigmented) have initial colour shade very similar. It is not possible to verify if, as in white hair, the initial yellow shades of the black hairs are directly affecting the final colour acquired after irradiation.

The mercury vapour lamp has a discontinuous emission spectrum, which is different from the continuous solar spectrum. Table 2 shows the values of hair yellowness (b^*) after solar irradiation (filtered or full-spectrum) for the white fibres of grey hair.

The results acquired under solar irradiation agree with the results obtained under mercury lamp irradiation in all aspects. It is possible to observe in Table 2 that white fibres from blended hair photobleach in all cases ($Db^* < 0$), but photobleaching was more pronounced with filtered radiation ($Db^* = -6.3$). Conversely, white hair from a single-donor became slightly yellower when exposed to full-spectrum irradiation ($Db^* = 1.1$) but less yellow when exposed to filtered radiation ($Db^* = -1.9$).

Therefore, exposing white hair to daylight results in both photobleaching and photoyellowing processes occurring concurrently, and thus, the initial colour of the white hair influences the observed colour change.

To study another structure formed by keratin, non-pigmented wool was subjected to the same irradiation conditions as non-pigmented hair. Wool is known to present photoyellowing after exposure to UV radiation, as previously reported in literature [18,26,20]. Table 3 shows the results obtained for virgin wool after exposure to sun or to mercury vapour lamp sources. The wool that is considered to be white has b^* values between 12 and 14, whereas the yellow wool has a b^* value in the range from 23 to 25.

Wool photobleaches in all cases, but yellower wool photobleaches in a more pronounced way. It is important to highlight that wool is initially yellower than hair in general, and the photobleaching process is in agreement with the results obtained for the yellowing of white hair.

To further investigate the reason why the white hair became yellow, the influence of thermal degradation was evaluated. Fig. 5 shows the values of hair yellowness (b^*) of white hair after exposure to heat at 81 °C as a function of exposure time. Hair samples that were washed once (before heat exposure) and samples that were washed twice (before exposure and between exposure and the period in the dark) were evaluated.

At this temperature (81 °C), blended and single-donor white hair washed by both methods turned significantly yellower, as indicated by the increasing b^* values in Fig. 5. This effect is more

Table 2
Hair yellowness (b^*) of grey hair (white fibres) after exposure to solar full-spectrum (accumulated dose of 179 MJ m^{-2}) or to filtered radiation (Vis + IR) (accumulated dose of 164 MJ m^{-2}). Hair fibres were measured in duplicate, and 10 colour measurements were done on each sample.

Hair	Treatment	b^* initial	b^* final	Db*
Blended	Full-spectrum	14.9 ± 0.6	11.4 ± 0.7	−3.3 ± 0.7
	Filtered radiation	14.6 ± 0.8	8.3 ± 0.5	−6.3 ± 0.5
Single-donor	Full-spectrum	7.3 ± 0.8	8.6 ± 0.5	1.1 ± 0.7
	Filtered radiation	8.0 ± 1.0	6.3 ± 0.6	−1.9 ± 0.5

Table 3
Yellowness (b^*) of virgin wool (white and yellow areas) after exposure to solar full-spectrum (accumulated dose of 179 MJ m^{-2}) or filtered radiation (Vis + IR) (accumulated dose of 164 MJ m^{-2}) or mercury vapour lamp full-spectrum (accumulated dose of 163 MJ m^{-2}) or filtered radiation (Vis + IR) (accumulated dose of 117 MJ m^{-2}). Wool fibres were measured in duplicate, and 10 colour measurements were done for each sample.

Wool	Source	Treatment	b^* initial	b^* final	Db*
White	Sun	Full-spectrum	13 ± 2	12 ± 1	−1 ± 1
		Filtered radiation	13 ± 2	10 ± 2	−3 ± 2
White	Mercury lamp	Full-spectrum	14 ± 2	10 ± 1	−3 ± 1
		Filtered radiation	12 ± 1	9 ± 1	−3 ± 1
Yellow	Sun	Full-spectrum	24 ± 2	15 ± 2	−9 ± 2
		Filtered radiation	23 ± 2	13 ± 2	−10 ± 2
Yellow	Mercury lamp	Full-spectrum	24 ± 2	12 ± 1	−12 ± 1
		Filtered radiation	25 ± 2	12 ± 2	−14 ± 2

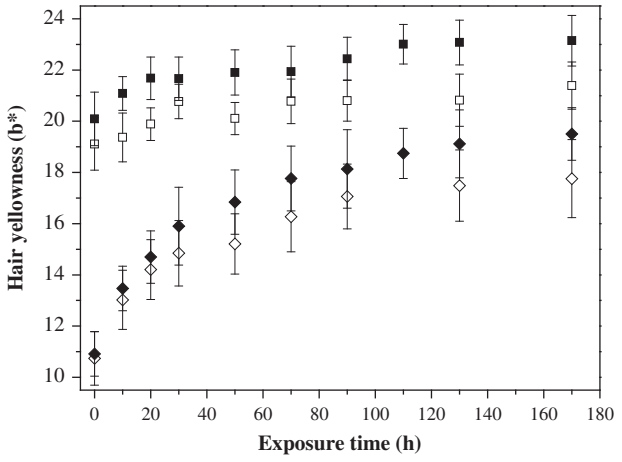

Fig. 5. Hair yellowness (b^*) of white hair after exposure to heat at 81 °C as a function of time of exposure. (■): blended hair washed once; (□): blended hair washed twice; (♦): single-donor hair washed once; (◇): single-donor hair washed twice. Hair fibres were measured in duplicate, and 10 colour measurements were done on each sample.

pronounced in the single-donor hair (Db* = 8.6) than in the blended hair (Db* = 3.0). The two methods of washing did not result in significant differences between the samples in terms of the yellowing behaviour.

3.3. Colour change for white hair in the dark-follow reactions

A previous paper reports that wool undergoes a photoyellowing process when stored in the dark [27]. According to the author, wool bleaches after exposure to full-spectrum solar radiation and then recovers the original yellower colour after being stored in the dark for a period. This behaviour is attributed to the formation of free radicals during the irradiation period that continue acting in the dark period [27]. To investigate this effect, samples of white fibres from grey hair were irradiated and then left in the dark for 1 year; their Db* values re-evaluated after the dark period. The hair samples were irradiated with a mercury lamp (full-spectrum, during more than 200 h) and submitted to thermal treatment in an oven (at 81 °C, for 170 h). The results are shown in Table 4.

White hair irradiated with the vapour lamp (full-spectrum) displays small and positive Db* values, indicating a slight yellowing of these samples independently of the initial colour shade of the hair (blended or single-donor hair). This effect is more pronounced in samples washed only before irradiation than in samples washed before and after irradiation (compare the two first lines in Table 4).

The samples thermally treated (oven at 81 °C) displayed diverse behaviour after being stored in the dark. Heat resulted in yellowing of the hair samples, as was previously shown in Fig. 5. However, after the period of 1 year in the dark, the thermally treated samples that were washed only before exposure became slightly bleached (Db* < 0), and the samples washed before and after heat exposure displayed b^* values that were almost unchanged (last two lines of Table 4). This behaviour was the same for both blended and single-donor hair.

3.4. Chromophore investigation

As an approach to investigate the yellow chromophores, changes in the UV–Vis spectra of hair solutions caused by photo and thermal treatments were studied. White hair and wool with different shades of yellow were solubilised in NaOH solution, and their spectra were recorded. Very similar UV–Vis spectra were obtained for white hair (blended or from a single-donor) and wool (yellow or white). Thus, in Fig. 6, only the spectra obtained for the blended hair solution are presented.

It can be seen in Fig. 6 that the solution of white hair irradiated with a mercury lamp (full-spectrum) presented the most intense absorption around 320 nm in the UV spectrum. Besides, the spectra of untreated white hair and white hair exposed to filtered radiation (Vis + IR) and to heat are quite similar. Considering the colour of these samples presented in Figs. 4 and 5, it can be observed that it is not possible to correlate the UV–Vis spectra obtained by hydrolysis in NaOH with the yellow chromophores formed in hair by irradiation or heat incidence. White hair with different yellow shades displays the same spectral behaviour, considering the absorbance in the UV–Vis region. While samples that presented a

Table 4
Hair yellowness difference (Db*) of grey hair (white fibres) after exposure to a mercury vapour lamp (full-spectrum) or to heat (oven at 81 °C) and storage in the dark for 1 year. Two sets of samples are presented: samples washed only before exposure and samples washed before and after exposure. The hair fibres were measured in duplicate, and 10 colour measurements were done for each sample.

Exposure	Hair	Washed before treatment Db*	Washed before and after treatment Db*
Full-spectrum (202 h)	Blended	+1.5 ± 0.8	+0.9 ± 0.9
	Single-donor	+3.0 ± 0.6	+1.2 ± 0.8
Heat (170 h)	Blended	−1.5 ± 0.7	0.0 ± 1.0
	Single-donor	−1.9 ± 1.0	+0.3 ± 0.7

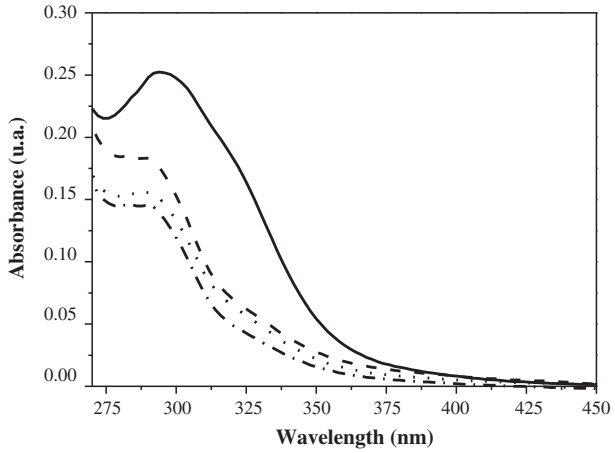

Fig. 6. UV–Vis spectra of white fibre from grey hair (blended) in NaOH solutions: (– · –): non-irradiated, b_i^* = 16.0; (— —): after irradiation with a mercury lamp (full-spectrum), b_f^* = 11.1; (· · ·): after irradiation with a mercury lamp (Vis + IR), b^* = 8.3; (– – –): after heat exposure (at 81 °C), b^* = 17.5. Exposure time in all cases: 264 h.

lighter shade of yellow before alkali dissolution became yellower after it (for example, white hair irradiated with full spectrum). This is probably caused by the formation or destruction of yellow chromophores on alkaline hydrolysis due to reactions involving cystine from keratin [28]. White hair exposed to full-spectrum (UV + Vis + IR) light also presented a shoulder at ~320 nm, which is correlated to yellow chromophores in literature and attributed to the photoproducts from the amino acid tryptophan [18]. In our results, however, the presence of this shoulder should not indicate the same feature, considering the limitations of the technique mentioned above.

3.4.1. Hair morphology after bleaching

The hair bleaching treatment using H_2O_2 was compared to the hair photodegradation, as both processes are considered oxidation reactions [4]. Bleaching is more aggressive than photodegradation and is expected to cause greater apparent damage to the hair structure. To verify this, white and black fibres from grey hair were subjected to oxidation using H_2O_2 at pH = 9.5.

In the case of white hair, it was possible to observe that the bleaching treatment resulted in fibres that were less yellow, as expected. These results indicate that yellow chromophores are being destroyed inside the structure of white hair. In addition, chemical bleaching always results in less yellow fibres, independent of the initial hair shade. Thus, chemical bleaching produces different results than the photobleaching process (Section 3.2). Considering the colour coordinates in black hair, it was possible to observe that black hair became yellower after bleaching, just as in the photodegradation process.

Fig. 7 shows micrographs of the black areas of bleached black hair (blended and single-donor hair). Melanin granules are replaced by holes that formed inside the cortex (C) and also holes were seen in the endocuticle (E), as indicated by the arrows in Fig. 7(a). Fig. 7(b) and (c) shows the resin inside the hole, indicating that these holes are not caused by sample preparation for TEM.

Fig. 8 shows micrographs of bleached white hair. There are no holes inside the cortex, which is consistent with the fact that these samples present almost no melanin granules to be replaced by holes as a consequence of bleaching. The microfibrils became more transversally oriented when compared with untreated hair. The transversal sections of the hair fibres display extensive regions of parallel microfibrils, as can be observed in the white circles inside the dark region (matrix) in Fig. 8(c).

4. Discussion

The results concerning the colour changes occurring on grey hair as a consequence of irradiation with a mercury lamp or sunlight indicate distinct behaviour depending on the wavelength range of the incident radiation and on the initial colour shade of the hair sample, as shown in Fig. 4. When white hair with an initially yellower shade (blended hair, $b^* \cong 18–21$) is exposed to full-spectrum radiation, photobleaching occurs. Conversely, when a white hair that is less yellow (single-donor, $b^* \cong 9–10$) is exposed to the same radiation, discrete photoyellowing occurs. Thus, the incidence of full-spectrum irradiation (UV + VIS + IR) appears to initiate concurrent photoprocesses, which can result in bleaching or yellowing of the white hair. In addition, the initial colour shade of the white hair directly affects the final colour acquired after irradiation. This behaviour does not depend on the source of the full-spectrum radiation (mercury lamp or solar).

A previous study by this research group [10] revealed that irradiation with full-spectrum irradiation from a lamp results in bleaching of white hair. This behaviour is not in agreement with the literature regarding colour changes in white hair after undergoing irradiation with full-spectrum radiation [15], which instead indicates the yellowing of these fibres.

Yellowing was also observed in the present work after thermal degradation at 81 °C. This behaviour is independent of the initial shade of the white hair. A possible reason for the yellow colour observed in white hair is more intense after thermal degradation than photo degradation is the place that treatment achieves inside the fibre. UV radiation only penetrates to a cuticle region, hair surface. On the other hand, thermal degradation may occur throughout the cortex and cuticle from fibre, hair surface and bulk.

McMullen and Jachowicz [29] investigated the effects of thermal treatments on white hair induced by curling irons (100–170 °C). Fluorescence analyses of white hair after thermal exposition indicate that the amount of tryptophan decreased in the samples in such a way that only 20% of the initial tryptophan was detected in the hair after 30 min of thermal exposure at 160 °C. These authors propose that this is caused by a free radical mechanism.

Crawford [30] published a study about the quantity of moisture absorbed by hair after being exposed to a hair dryer (50–110 °C).

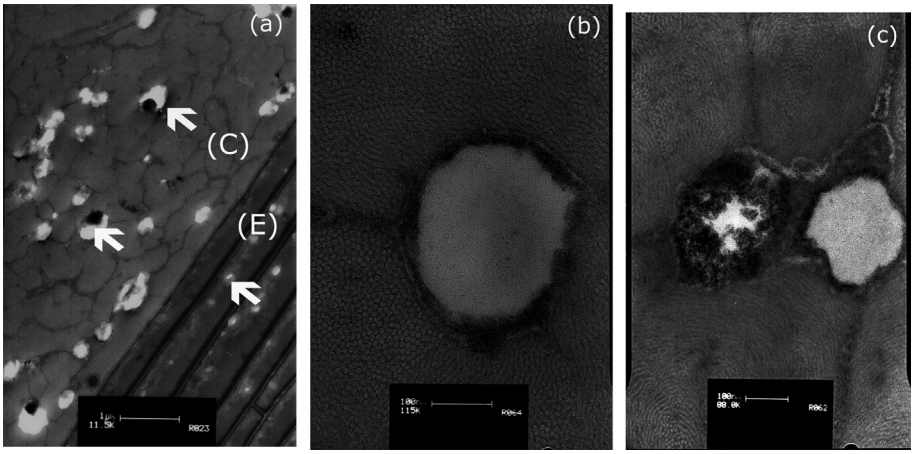

Fig. 7. TEM micrographs of ultra-thin sections of black areas from bleached grey hair, showing (a) holes in the cortex (C) and in the endocuticle (E) of blended hair, (b) and (c) melanin granules replaced by holes in single-donor hair. (Scale bar = 1000 nm in (a) and scale bar = 100 nm in (b) and (c)).

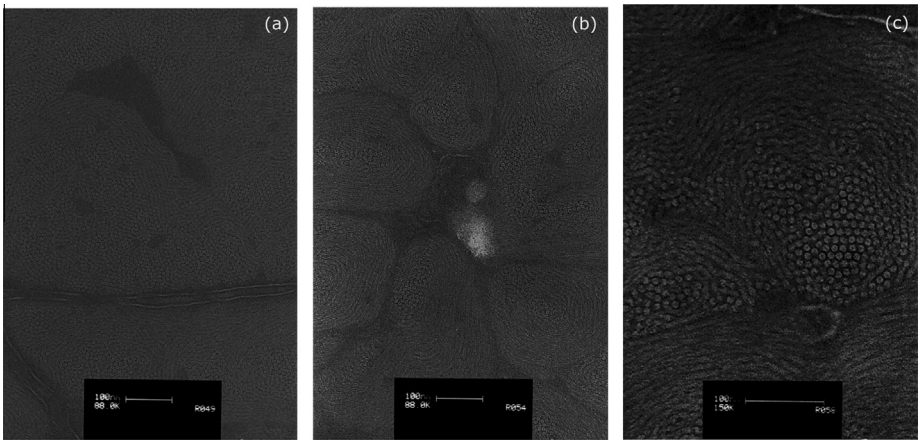

Fig. 8. TEM micrographs of ultra-thin sections of bleached blended white hair from grey hair. (Scale bar: 100 nm).

After drying, hair samples were placed under an atmosphere of 55% relative humidity at 22 °C, and the amount of water reabsorbed was evaluated. The results indicate a reduction in the amount of moisture reabsorbed compared with that of hair without treatment. The author suggests that structural changes occur in hair under thermal exposure, changing its polarity and resulting in a reduced amount of moisture absorbed.

However, in hair literature, no information is available to explain the problem of yellowing due to thermal degradation. In the case of wool, it is known that heat has a damaging effect on proteins [31]. Dyer reported an interesting result, which was obtained using the proteomic technique, concerning modifications of wool amino acid residues after heating at 90 °C. The photodegraded products are derived from tryptophan and tyrosine and are comparable to those resulting from UV protein damage. The author suggested that heating alone is sufficient to produce free radical reactions. Also, in wool literature Duffield and Lewis [32] suggest that the yellow chromophores formed after thermal treatments came from Maillard reactions. Maillard is a reaction of condensation between amino (cysteine, tryptophan, N terminal amino) and carboxyl groups leading to highly coloured Schiff's bases. It is observed that either acetylation of amino groups or esterification of the carboxyl groups in wool reduced the degree of thermal yellowing products. Both, free radical mechanisms and Maillard reactions may occur. In Maillard reaction, free radicals can be formed like intermediates.

In the case of white hair irradiated with filtered radiation (Vis + IR) from a mercury lamp, only photobleaching was observed in all of the samples, irrespective of the initial yellowness of the hair fibres. In addition, this photobleaching is more pronounced when the radiation is filtered to remove UV radiation (Vis + IR remaining) than when full-spectrum irradiation is used. This bleaching effect is well-established in the literature and is assigned to the destruction of the coloured chromophores that absorb in this region by VIS light, resulting in bleaching [33,34].

The photobehaviour of white hair irradiated with solar radiation is the same as that already observed under the mercury lamp. Solar radiation contains wavelengths comprising UV, Vis and IR regions but is formed by a continuum spectrum, different from the discrete emission lines from the mercury lamp. The exposure of white hair to the full solar spectrum results in both yellowing and bleaching effects depending on the initial hair colour shade. The effect of filtered radiation (IR + VIS) on white hair is photobleaching in all of the samples, independent of the source. These results indicate that the responsible source of the white hair photoyellowing is most likely the UV radiation or the interaction between UV and IR radiation. VIS radiation, on the other hand, promotes only hair bleaching. Therefore, when white hair undergoes full-spectrum irradiation, UV (or UV + IR) pulls the photodegradation process towards yellowing, whereas the VIS fraction of the spectrum directs the process towards bleaching. The final result on the white hair colour will depend on the initial shade of colour present before irradiation.

In the case of black fibres from grey hair, the photodegrading effects are much more subtle than in non-pigmented white hair,

as indicated in Fig. 4. Both the blended hair and the single-donor hair became slightly yellower after full-spectrum irradiation, and no significant difference was observed with VIS + IR irradiation.

Another interesting aspect investigated in the present work concerns the results obtained with white hair kept in the dark for a period of 1 year after the irradiation cycles. White hair samples subjected to full-spectrum irradiation underwent reactions causing yellowing during the period in the dark. On the contrary, when the same samples were kept in the dark period after thermal degradation at 81 °C, they underwent reactions causing bleaching.

In addition, the dark-follow reactions were susceptible to washing procedures between the exposure and the following steps. In samples that were washed twice (before exposure and between exposure and the period in the dark), the colours after the period in the dark remained very close to the colours right after irradiation. This occurs because the free radicals generated from hair proteins after photo or thermal degradation are unstable in the presence of water [35]. For this reason, the hair washed twice and then kept in the dark conserved their colour parameters close to the values obtained after irradiation. Conversely, hair fibres that were washed only one time (before exposure) do not have their free radicals disabled after exposure, and they keep reacting during the one-year period in the dark. The action of free radicals during a dark period was also reported by Launer [27,36] for white wool. Yellowing was observed on wool exposed to sun radiation and then kept in the dark for a period.

In general, the dark-follow reactions studied in the present article indicate that the photodegradation process initiates a reaction mechanism that turns white hair yellower. Conversely, thermal degradation induces a reaction mechanism that causes white hair bleaching. In both cases, water has an important influence in the deactivation of free radicals, causing these reversion reactions to be slower.

Our experiments to investigate the chromophore identity by UV–Vis spectroscopy (Fig. 6) indicated that it is not possible to obtain a quantitative or qualitative analysis of the yellow chromophores using this technique. Opposite results concerning sample colours before and after dissolution in NaOH are observed, indicating the occurrence of parallel reactions that alter the amount of yellow chromophores in solution. The solutions of untreated white hair and white wool, irradiated with Vis + IR and undergoing heat treatment (at 81 °C) display very similar absorption curves, despite the different yellow shades of these samples before alkali hydrolysis. On the other hand samples that were less yellow before alkali dissolution, such as white hair irradiated with full spectrum, can undergo yellowing after the process. The absorption differences may be associated not only to the difficulty of solubilising hair and wool to prepare the solutions [37], but also to keratin reactions involving cystine residues [28].

In the wool literature [18] the same behaviour for the UV–Vis absorption spectra is reported for wool samples after full-spectrum irradiation. The same shoulder at 320 nm also appears on the spectra of wool solutions. This shoulder at 320 nm is related to the photoyellowing chromophores from wool. The yellowing effect in wool has been extensively studied, and many photoyellowing products have been proposed to elucidate this problem [38–40].

In our results, the shoulder at 320 nm cannot be assigned to the yellow chromophores formed by UV exposure, because the blended white hair and the wool samples, where the absorption shoulder appears, become less yellow after full-spectrum irradiation. In addition, the shoulder does not appear in the samples that become more yellow with the heat treatments.

A series of simple experiments were conducted to investigate the nature of the yellow chromophores from white hair (not shown in the results). The first consisted of trying to extract the yellow chromophores from white hair using organic solvents of different polarities and a Soxhlet extractor for 24 h. The chromophores could not be extracted from the white hair using this method, as they are strongly linked with the hair structures.

In a second experiment, white hair was immersed in different solvents of distinct refraction indexes, and the colour change was analysed visually. If the observed colour was just a physical colour, its structure should change when the sample is immersed in different solvents. No changes in the colour were observed in white hair, indicating that the yellow colour is not related to physical structure organisation.

The third experiment consisted of immersing white hair in buffer solutions with different pH values (4, 7 and 10) and analysing the possible colour change visually for a long period (more than 6 months). The yellowed white hair did not change its initial colour after immersion in different buffers, thus demonstrating that the chromophores are not caused by tautomerism (hydrogen transfer) reactions.

The fact that these chromophores cannot be extracted by solvents indicates that they are oxidised amino acid residues, which is in agreement with the results found in the literature [2]. Analysing the ultrastructure of the white (non-pigmented) and black (pigmented) areas of the grey hair (Figs. 2 and 3), a clear difference in the melanin distribution is observed. In the literature, the presence or absence of melanin is the main difference between black and white hair [4,25]. Images from melanin granules in dark hair were obtained by different research groups using transmission electron microscopy [41,42,4] and are very similar to the micrographs presented here. Conversely, images from the few remaining melanin granules in the white hair are presented here for the first time. To our knowledge, these results have not previously been reported in the literature. Fig. 3 shows nuclear remnant structures and fragmented granules of melanin that indicate the degraded aspect of the remaining melanin granules in white hair.

In addition, the diameters of hair fibres were analysed on white and black areas of grey hair, as presented in Table 1. The measurements were done on fibres that were partially black and partially white, but no significant differences could be identified in the diameter of the two areas. By analysing the works previously published in the literature, it is possible to notice that this is a subject of conflicting opinions. Robbins [4] affirms that one of the remarkable changes that occurs in hair with age is that it becomes thinner. Hollfeller [25], on the contrary, affirms that the non-pigmented hair has a significantly larger diameter when compared with pigmented hair. Gao [15] also compared the difference in the diameter of pigmented and non-pigmented hair and concluded that there was no significant difference in the measured values.

Our results agree with those of the last author, as we could find no difference between the black and white areas from grey hair in diameter. The main morphological difference between them is the number and the distribution of melanin granules. In white areas, there are only a few granules or the total absence of them, whereas in pigmented hair, melanin granules are abundant.

When white hair is treated with H_2O_2 at pH = 9.5, the yellow chromophores are destroyed, and the bleaching effect can be noticed on the hair fibres. The ultrastructure of white hair after treatment with H_2O_2 shows that there are no holes inside the cortex, as we shown in Fig. 8. Black hair presents holes instead of melanin granules after the H_2O_2 treatment, as shown in Fig. 7. These results indicate that the melanin granules are destroyed after H_2O_2 treatment, and the remnant granules are not responsible for the yellow chromophores in white hair.

5. Conclusions

The results presented herein indicate that photobleaching and photoyellowing occur simultaneously on white hair when it is

irradiated with a mercury lamp or with sunlight full spectrum. Whereas VIS radiation is more prone to promote fibre bleaching, UV and heat tend to induce hair yellowing. The final colour acquired after irradiation is strongly dependent on the initial colour shade of the hair. The occurrence of dark-follow reactions were also observed on samples treated and then stored for 1 year in the dark. These dark-follow reactions are favoured by free radicals formed during the exposure process and may be retarded or suppressed by including an extra washing step between the exposure and the period of storage in the dark. Finally, the main difference detected between the black and the white areas of grey hair remains in the distribution of melanin granules. No differences in the diameter of the pigmented and of the non-pigmented areas were identified.

Acknowledgements

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