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Optical coherence tomography to evaluate the effects of oxidative hair dye on the fiber

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Background/purpose: Oxidative hair dyes can damage the hair, since these chemical procedures are involved to change the fiber structure and therefore changes in their mechanical and surface properties. Evaluate and compare the effect of the two colors of oxidative hair dye emulsions on Caucasian hair. This research analyzed the Dark brown hair untreated (**I**); Dark brown hair treated with light brown dye (**II**); Dark brown hair treated with light blond dye (**III**); Light blond hair untreated (**IV**); Light blond hair treated with light brown dye (**V**); Light blond hair treated with light blond dye (**VI**) on Caucasian hair.
Methods: The hair samples were submitted to breaking strength, color, and optical coherence tomography (OCT) analysis.
Results: For the breaking strength assay no presented statistically significant differences between treatments. The parameters of color and brightness can differ in some hair dye formulations, but also the hair type can respond differently. The OCT images of the sample **I** and **IV** was possible observed, clearly *Medulla* and *Cortex*, which was not observed clearly after treatment with both oxidative hair dye colors.
Conclusion: Based on the results, the oxidative hair dyes increased alteration in color and ultrastructure of hair.

Key words: hair dye – color – breaking strength – optical coherence tomography

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THE KNOWLEDGE of the anatomy and physiology of the hair facilitates understanding the action mechanisms of hair cosmetics. The hair thread has a highly organized, cylindrical structure, formed by mostly keratinized inert cells, which follow a precise and pre-defined design (1).

The hair shaft has three main structures: *Cuticle*, *Cortex*, and *Medulla*, from outside to inside, respectively. As a matter of course, all contribute to the appearance of the shaft by affecting its structure and shape as well as features such as light absorption, reflection, and refraction (2).

The *Cuticle* is made up of approximately 8–11 layers of cuticle cells, which closely overlap in the distal direction of the thread, depending on the type, condition, and length of the hair. Its role is to protect the shaft against environmental and chemical damages. The dependent on adherence and orientation of the cuticle scales are responsible for brightness; resistance to combing and surface properties (2).

Cortex is the layer located below the cuticle; it is the most important layer. It occupies the main bulk of the shaft and largely determines the shape, texture, and strength of the hair due to its crystallized α-keratin fibrils' organization by numerous series of long, spindle-shaped cells that are closely packed together and thus forming a fibrous mass. In this layer, the melanin and the artificial hair dyes are located (2).

The *Medulla*, another component of hair fiber, may not be present, and its role is not clearly defined but it is characterized by the presence of air spaces that are formed by the shrinking of medulla cells during differentiation. Sometimes, the cells are present inflated with air; more frequently, it occurs intercellularly (2, 3).

Hair color is determined by the presence or absence of several types of melanins deposited in the cortex of the hair fiber. Melanins are usually divided into two groups: *eumelanins* from brown to black, and *pheomelanins* coloring from yellow to red. The variability in staining the hair fiber is related to the proportions of the two kinds of melanins, which differ only in the color

but also in the chemical composition and physical arrangement of granules into the fiber (4).

Hair melanin photochemistry provide protection to proteins present in the hair fiber, particularly with the ultraviolet (UV) radiation of short wavelength, acting as sunscreens, and it dissipates it as heat or other kind of energy. However, in this process, the pigments are degraded or discolored (5).

Oxidative hair dye are the most used products to change the color of the hair. Oxidative hair dye are formed by highly reactive molecules that react in a highly alkaline and oxidizing environment, yielding colored polymers remain deposited on the hair cortex, and the color should remain unchanged to several washing procedures (6).

Optical coherence tomography (OCT) applies the principles of low-length coherence interferometry in which the coherence features of photons directly reflected from a structure that carry real high-resolution tomography images of biological tissues. The OCT is capable of producing internal sectional images with high resolution of internal microstructures from living tissue (7, 8).

Nowadays, OCT also caused significant impact in Dermatology as this technique can produce images, where it is possible to identify the different skin structures, and thus can evaluate the morphological changes in skin with the formulations as: melanoma, anti-aging, or antiwrinkle (7). The OCT image is based mainly on an optical property of sample, such as the backscattering coefficient and refraction index variation. The false color in the image represents the backscattering coefficient, whereas the white color represents high-scattering coefficient, and black color represents the low-scattering coefficient (7, 8).

In this research, we analyzed the effect of oxidative hair dye oil in water (O/A) emulsions on mechanical resistance, color, and morphological changes of human hair characterized with the imaging method called OCT.

Material and Methods

Hair dyes formulations

Oxidative hair dyes of two colors (Light Blond Dye and Light Brown Dye) were prepared, differing only in the pigments used. The hair dyes were prepared with pigments obtained from Les Colorants Wackherr (LCW™, São Paulo, Brazil).

Qualitative composition for Light Brown Dye formulation comprised (International Nomenclature of Cosmetics Ingredients/INCI): *cetearyl alcohol (and) dicetyl phosphate (and) ceteth-10 phosphate; cetearyl alcohol; caprylic/capric triglyceride; BHT; propylene glycol; tetrasodium EDTA; sodium metabisulfite; aqua; ammonium hydroxide and pigments (m-aminophenol (MAP), p-phenylenediamine (PPD), 2,4-diaminophenoxyethanol (2,4-DAPE), resorcinol (RCN), t-butyl hydroquinone (TBQ), erythorbic acid).* For Light Blond Dye formulation, *cetearyl alcohol (and) dicetyl phosphate (and) ceteth-10 phosphate; cetearyl alcohol; caprylic/capric triglyceride; BHT; propylene glycol; tetrasodium EDTA; sodium metabisulfite; aqua; ammonium hydroxide and pigments (p-aminophenol (PAP), 4-amino-2-hydroxytoluene (AHT), resorcinol (RCN), t-butyl hydroquinone (TBQ), erythorbic acid)* were used.

Preparation of oxidative hair dyes

The oily and aqueous phases of the emulsion formulations were added in different steel beakers. The oily and aqueous phases were heated to 70.0 ± 5.0°C on heating plates and the oily phase was added to the aqueous phase until obtaining a homogenous emulsion by stirring (9, 10). Two different emulsions were prepared, differing only in the pigments used, as already described, thus resulting in the formulations: Light Blond Dye and Light Brown Dye.

Preparation of tresses

Caucasian virgin light blond and dark brown hair tresses of 20.0 cm in length and weighing approximately 2.0 g each purchased from Bella Hair™ (São Paulo, Brazil) were used.

Each hair tress was first washed for 30 s with 15.0% (w/v) sodium lauryl sulfate to remove impurities. All were wetted with warm (37.0 ± 5.0°C) distilled water with a constant flow of 240.0 mL/min for 1 min and then were dried at room temperature (22.0 ± 1.0°C) with relative humidity (RH 60 ± 5%) for 12 h before to the analysis (9, 10).

Application of hair dye

For the treatment, 1.5 g of each dye formulation plus 1.5 g of emulsion commercial (LBS™) containing 20.0% (v/v) hydrogen peroxide was

applied on the tresses and it was let for 40 min (9, 10). The samples were then washed as described previously. The samples were classified into six groups: *Group I* – Dark brown hair untreated; *Group II* – Dark brown hair treated with light brown dye; *Group III* – Dark brown hair treated with light blond dye; *Group IV* – Light blond hair untreated; *Group V* – Light blond hair treated with light brown dye; *Group VI* – Light blond hair treated with light blond dye.

Mechanical properties

The analysis of breaking strength was performed in Texturometer TAXT2 Analyzer™ model, operating at clutch speed traction of 300 mm/min, distance of 80 mm, 25.0 kg load, and sensitivity of 0.49 N. Fifteen fibers of tresses from each treatment measuring 10.0 cm each were used for the tests. Their diameters were measured with micrometer Mitutoyo™, at three points (root, middle portion, and tip), and the mean value was used to calculate the total area of the hair fiber (11).

Color changes

Color measurements were performed by the Hunter Miniscan Labs™ XE Plus (CIELAB – Universal Software v. 4.01). The measures were performed with five replicates, using the middle portion of the tresses. Hunter L-a-b parameters were measured. The color shifts of the samples were determined using Eq. (1). The equipment provides the color parameters based on three vectors: ΔL* is the difference in brightness (with positive values standing for clearer and negative values meaning darker), Δa* defines the difference in color-coordinated green–red (being positive if the hair shows redness and negative for greenness), Δb* represents the difference in color-coordinated blue–yellow (with yellowness presented by positive numbers and blueness by negative). All these color parameters can be summarized in ΔE that indicates the total difference in color (5).

$$\Delta E = [(L_0 - L_s)^2 + (a_0 - a_s)^2 + (b_0 - b_s)^2]^{1/2} \quad (1)$$

Legend: L_0, a_0, and b_0 are the values of hair untreated sample and L_s, a_s, and b_s are the values of hair treated sample.

Optical coherence tomography analysis

An OCT system (OCP930SR™; Thorlabs, Newton, NJ, USA), working at 930.0 ± 5.0 nm with 100.0 ± 5.0 nm FWHM and an optical power of 2 mW, axial resolution of 6.2 mm in air, and maximum image depth of 1.6 mm, was used to generate cross-sectional images of standard Caucasian hair physically fixed in a sample holder capable of maintaining the fiber straight, without natural distortions. Ten samples of *Caucasian hair* were measured transversally at three different positions/distances along the samples: 10 mm from root, middle position (equidistant point between the root and the end), and 10 mm from the end (8).

Statistical analysis

The data were analyzed for homogeneity of variance by Hartley test. Possible significant differences in the results were analyzed by one-way ANOVA and the differences between treatments were identified by Tukey's test ($\alpha = 0.05$).

Results and Discussion

The oxidative hair dye in fiber causes the formation of indo-dyes, form colorless precursors with low molecular weight (p-aminophenol and *p*-phenylenediamine), by oxidation with hydrogen peroxide under alkaline conditions. At this point, the precursors react with the couplers as *m*-aminophenols (*m*-aminophenol, 4-amino-2-hydroxytoluene, and 2,4-diaminophenoxyethanol) and *m*-diamines (resorcinol) which react to each other in strongly alkaline/oxidizing environment, yielding colored polymers (6, 9, 12).

During the coloration process, hair dyes provide the opening of the cuticle due to the alkaline pH of the process, and optimizing the absorption of the colorants present in the formulation into the cortex. This mechanism reduces the softness, brightness, and difficult to comb of hair are the attributes of healthy and conditioned hair (6, 9, 10).

Breaking strength analyses

Breaking strength of hair samples for: *Group I* – Dark brown hair untreated; *Group II* – Dark brown hair treated with light brown dye; *Group III* – Dark brown hair treated with light blond

dye; *Group IV* – Light blond hair untreated; *Group V* – Light blond hair treated with light brown dye; *Group VI* – Light blond hair treated with light blond dye are shown in Fig. 1.

When a load is applied, the hair fiber stretches in proportion to this force and results in elongation of about 2% of its original length (elastic property). The hair then stretches quickly, about 25–30% in length, with a moderate increase in load (plastic property). Keeping the value of the constant force applied, the fibers stretch proportionally to the load until rupture occurs (1). The lower the value of breaking strength more damaged is the cortex.

The analyses of the breaking strength from the *Caucasian* hair tresses before and after the application of oxidative hair dye emulsions (Fig. 1) showed that there was no statistical difference among all treatment analyzed.

That data confirmed results obtained by Robbins and Crawford (13) who described that the oxidative process of hair fibers produced extensive damage throughout several cuticle layers but, no detectable changes in the tensile properties are detectable, confirmed the results obtained in this research.

Color changes

The perception of color is subjective, and therefore it is important to use analytical methods to allow discrete measurements to be taken. One model existing to measure color is developed and proposed in 1976 by Commision Internationale L'Eclairage, CIELAB, or CIE L* a* b*, which measures color on three axes broadly linear human perception. Standard L*, a*, b* measurements were collected where L* refers to the lightness on a scale of 0–100, a* denotes the red-green color range (positive value denotes higher red) and b* represents the yellow-blue color range (positive value denotes higher yellow) (14).

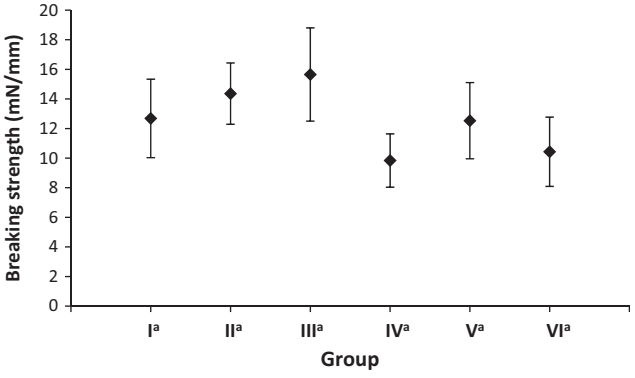

Fig. 1. Breaking strength (mN/mm) from Caucasian hair tresses before and after application of the oxidative dye hair emulsions. Group I: Dark brown hair untreated; Group II: Dark brown hair treated with light brown dye; Group III: Dark brown hair treated with light blond dye; Group IV: Light blond hair untreated; Group V: Light blond hair treated with light brown dye; Group VI: Light blond hair treated with light blond dye. Results classified with different letters present statistically significant different, for $\alpha = 5$, $P \leq 0.05$, $n = 15$.

The wide range of natural hair colors is determined by the total amount of melanin pigments (eumelanin or pheomelanin or the mixture of both) present in the cortex of the hair fiber (4). The function assigned to the melanin granules is the effective light absorption protecting against damage hair, dark hair containing significantly more melanin than light hair. Table 1 show the *Color and Brightness* parameters, while Fig. 2 show ΔE that indicates the total difference in color data for virgin *Caucasian* hair samples (light blond or *light* brown hair), before and after treated with oxidative hair dye light or brown color.

The light blond dye contained with precursor (*p*-aminophenol) and couplers (resorcinol and 4-amino-2-hydroxytoluene) react and development a light blond color in the hair (15). Meanwhile, the light brown dye containing the precursor *p*-phenylenediamine and the couplers *m*-aminophenol; resorcinol and 2,4-diaminophenoxyethanol development a brownish color depend of oxidative reaction (15).

These results can be explained by the chemical composition of hair dyes. According Gama et al. (10) the final color of hair dye is obtained

TABLE 1. Color and Brightness parameters data for virgin Caucasian hair samples (light blond or light brown hair), before and after the treatment with oxidative hair dye light or brown color

	Color and brightness parameters data		
Group	L*	a*	b*
I	15.15 ± 0.32^{a}	-0.48 ± 0.13^{a}	5.39 ± 0.19^{a}
II	16.37 ± 0.49^{a}	-1.42 ± 0.11^{b}	4.78 ± 0.22^{a}
III	19.93 ± 0.13^{b}	3.96 ± 0.37^{c}	9.59 ± 0.43^{b}
IV	68.04 ± 0.19^{d}	5.80 ± 0.33^{d}	37.02 ± 0.36^{c}
V	16.50 ± 0.19^{a}	-0.72 ± 0.15^{a}	5.89 ± 0.37^{a}
VI	61.51 ± 0.19^{c}	12.96 ± 0.18^{e}	35.69 ± 0.37^{c}

Group I: Dark brown hair untreated; Group II: Dark brown hair treated with light brown dye; Group III: Dark brown hair treated with light blond dye; Group IV: Light blond hair untreated; Group V: Light blond hair treated with light brown dye; Group VI: Light blond hair treated with light blond dye. Results classified with different letters present statistically significant different, for $\alpha = 5$, $P \leq 0.05$, $n = 15$.

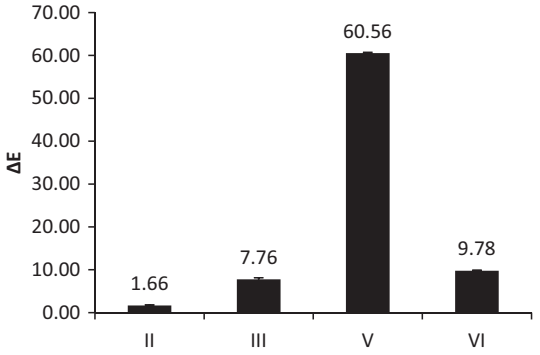

Fig. 2. ΔE indicates the total difference in color data for virgin Caucasian hair samples (light blond or light brown hair), before and after the treatmentwith oxidative hair dye light or brown color. Group II: Dark brown hair treated with light brown dye; Group III: Dark brown hair treated with light blond dye; Group V: Light blond hair treated with light brown dye; Group VI: Light blond hair treated with light blond dye.

after the reaction between the precursor and the couplers in a strong alkaline and oxidant medium. In this study, the alkalinizing agent used was ammonium hydroxide. According to this study, the light brown dye had a lower percentage of ammonium hydroxide than the light blond dye.

The high percentage of ammonium hydroxide in the light blond dye and the presence of the precursor (*p*-aminophenol) and couplers (resorcinol and 4-amino-2-hydroxytoluene) causes the reaction of them and it leads to the development of light purple color, but when it is not completed, the final color is slightly yellow to brown color (15). These could explain the increased positive values for Brightness (L*); red-green color (a*) and yellow-blue color (b*) for virgin dark brown hair, after all treatments with this hair dye (Table 1), possibly by oxidation of melanin. Meanwhile, for virgin light blond hair the results after treatments (Table 1) were negatives values for L*; positives value for a* and the most varied results for b*.

According to the results of hair color of the light brown dye (Table 1) as previously mentioned, this product was composed of precursor (*p*-phenylenediamine) and couplers (*m*-aminophenol; resorcinol, and 2,4-diaminophenoxyethanol) and the result of their reaction was the brownish color (15) that could explain the increased negative values for brightness (L*), red-green color (a*), and yellow-blue color (b*) for virgin dark brown hair, and also for virgin light blond hair after all treatments with this hair dye.

In Fig. 2, the values of ΔE for virgin *light* blond or *light* brown *Caucasian* hair samples (*III* and *VI*) treated with oxidative hair dye light color increased in a way the oxidative of melanin (4) and deposition of the intermediate compound formed as already explained (12), while in the group (*II* and *V*) submitted to treatment with *light* brown hair has a similar increase due to the deposition of the intermediate compound formed as already explained.

Morphological changes of human hair characterized by OCT

Optical coherence tomography is a new morphological method for non-invasive investigation of the hair (15). The OCT images of the cross-sections of the generated hair fibers were analyzed and compared based on the change in displayed colors, which represent the backscattering coefficient and refraction index variation in different structures present on the hair. The transversal OCT images displayed using a map of false color to grayscale of hair samples in the middle position are shown in Fig. 3.

In the OCT images, it was possible to identify the main structures of the hair fiber (Fig. 3); the lighter observed in some central region images corresponding to the *medulla* of the hair fiber, while the surrounding region corresponds to the *cortex*. The *cuticle* was rarely seen in these images due to its thickness, which generally corresponds to about 6 μm, less than the maximum resolution of the OCT images generated by the system used (8).

When analyzed Fig. 3(I) Dark brown hair untreated and Fig. 3(IV) Light blond hair untreated was possible observed, clearly *Medulla* and *Cortex.* Can say that after the treatment with the oxidative dyes scattering coefficients and refractive indexes increased, compared to the same before the treatment Fig. 3, which is evidenced by the lightening of sectional OCT images generated, since these lighter colored images correspond to higher refractive index and light scattering coefficients. After the treatments, the ultrastructure of the hair was possibly observed, with less sharpness of *Medulla* and *Cortex* Fig. 3 (II, III, V and VI). This effect may be related to alteration of the ΔE values that indicates the total difference in color data for virgin *Caucasian* hair samples

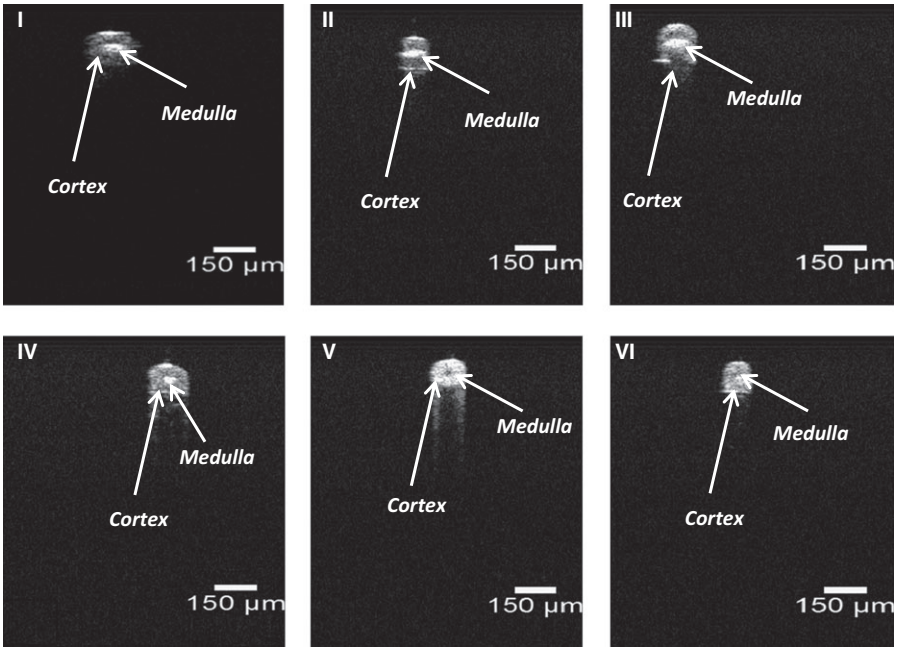

Fig. 3. Optical coherence tomography images of Caucasian hair samples of a cross-section fiber in a transversal mode show the main hair structures: Medulla and Cortex. Group I: Dark brown hair untreated; Group II: Dark brown hair treated with light brown dye; Group III: Dark brown hair treated with light blond dye; Group IV: Light blond hair untreated; Group V: Light blond hair treated with light brown dye; Group VI: Light blond hair treated with light blond dye.

(light blond or *light* brown hair), before and after the treatment with oxidative hair dye light or brown color Fig. 2.

The signal generated by the OCT results were analyzed before and after treatment with the Light Blond Dye and Light Brown Dye, indicating that the technique has the advantage of possible analysis of the same hair fibers throughout the treatment process, or before and after application of oxidative hair dye. Thus, the analysis of the images allows for comparison of backscattering coefficient and refraction index variation in different structures present on the hair samples, of the same samples, allowing the comparison and reducing possible errors (8).

Conclusion

During the coloring processing of hair, the dye formulations provide excessive cuticle opening and it can cause, besides the color change, modification in the parameters such as brightness and not in the hair strength. It was found that we can clearly identify the main structures of a hair fiber like the cuticle, the cortex, and the medulla. In particular, we have shown that the backscattering coefficient for hair fiber is different before and after a chemical treatment. After the treatment, it was not possible to clearly identify the hair structures. The decrease in the refraction index difference between adjacent structures could explain this behavior.

References

1. Velasco MVR, Sá-Dias TC, Freitas AZ, Vieira-Junior ND, Pinto CASO, Kaneko TM, Baby AR. Hair fiber characteristics and methods to evaluate hair physical and mechanical properties. Braz J Pharm Sci 2009; 45: 153–162.
2. Lai-Cheong JE, Mcgrath JA. Structure and function of skin, hair and nails. Medicine 2009; 37: 223–226.
3. Wagner RC, Kiyohara PK, Silveira M, Joekes I. Electron microscopic observation of human hair medulla. J Microsc 2007; 226: 54–63.
4. Tobin DJ. Human hair pigmentation – biological aspects. Int J Cosmet Sci 2008; 30: 233–257.
5. Nogueira ACS, Joekes I. Hair color changes damage caused by ultraviolet radiation. J Photochem Photobiol B Biol 2004; 74: 109–117.
6. França SA, Dario MF, Esteves VB, Baby AR, Velasco MVR. Types of hair dye and their mechanisms of action. Cosmet 2015; 2: 110–126.
7. Freitas AZ, Amaral MM, Raele MP. Optical coherence tomography: development and applications. In: Duarte FJ, ed. Laser pulse phenomena and applications, Chapter 20. Rijeka, Croatia: InTech; 2010: 409–432.
8. Velasco MVR, Baby AR, Sarruf FD, Kaneko TM, Samad RE, Vieira ND

Jr, Freitas AZ. Prospective ultramorphological characterization of human hair by optical coherence tomography. Skin Res Technol 2009; 15: 440–443.
9. Gama RM, Balogh TS, França S, Sá-Dias TC, Bedin V, Baby AR, Velasco MVR. Thermal analysis of hair treated with oxidative hair dye under influence of conditioners agents. J Therm Anal Calorim 2011; 106: 399–405.
10. Gama RM, Balogh TS, França S, Sá-Dias TC, Bedin V, Consiglieri VO, Kaneko TM, Baby AR, Velasco MVR. Evaluation of hair damage after treatments with light brown and light blond permanent dyes: hair protein loss determination. Braz J Pharm Sci 2009;45 (Suppl. 1): 39.
11. Dario MF, Pahl R, Castro JR, Lima FS, Kaneko NTM, Pinto CASO, Baby AR, Velasco MVR. Efficacy of *Punica granatum* L. hydroalcoholic extract on properties of dyed hair exposed to UVA radiation. J Photochem Photobiol B Biol 2013; 120: 142–147.
12. Corbett JF. An historical review of the use of dye precursors in the formulation of commercial oxidation hair dyes. Dyes Pigm 1999;41: 127–136.
13. Robbins CR, Crawford RJ. Cuticle damage and the tensile properties of human hair. J Soc Cosmet Chem 1991; 42: 49–58.
14. Gulrajani ML. Colour measurement: principles, advances and industrial applications. London: Woodhead Publishing; 2010: 310.
15. Corbett JF. Chemistry of hair colorant processes – science as an aid to formulation and development. J Soc Cosmet Chem 1984; 35: 297–310.

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