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Hair Protective Effect of Argan Oil (*Argania spinosa* Kernel Oil) and Cupuassu Butter (*Theobroma grandiflorum* Seed Butter) Post Treatment with Hair Dye

Pamella Mello Faria[1], Luciana Neves Camargo[1], Regina Siqueira Haddad Carvalho[1], Luis Antonio Paludetti[1], Maria Valéria Robles Velasco[2], Robson Miranda da Gama[1,3*]

[1]Pharmacy School, University of Santo Amaro, São Paulo, SP, Brazil; [2]School of Pharmaceutical Sciences of University of São Paulo, São Paulo, Brazil; [3]School of Pharmaceutical Sciences of Faculty of Medicine ABC, Santo André, Brazil.
Email: [*]rmdagama@gmail.com

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ABSTRACT

Hair coloring is widely used by women and men either to change their natural hair color or to delay the onset of gray hair. Oxidative dyes may damage the hair, since chemical and physical procedures are involved to alter the structure hair and consequently, alterations in its mechanical and of surface properties. One benefit of hair conditioners is to prevent flyaway hair, make the hair "shine", and protect the hair from further damage. In this research we analyzed the hair protective effect conditioner agents *Argania spinosa* kernel oil and/or *Theobroma grandiflorum* seed butter in hair care on Caucasian hair post treatment with hair dye. The hairs were submitted by quantifying protein loss. The samples were classified as: hair untreated (**I**); hair treated with a commercial oxidative ultra-blond hair dye (**II**); hair post treatment II and F1: Base hair care formulation (**III**), hair post treatment II and F2: Base hair care formulation containing 1.0% (w/w) *Argania spinosa* kernel oil (**IV**), hair post treatment II and F3: Base hair care formulation containing 1.0% (w/w) *Theobroma grandiflorum* seed butter (**V**) and hair post treatment II and F4: Base hair care formulation containing 0.5% (w/w) *Argania spinosa* kernel oil and 0.5% (w/w) *Theobroma grandiflorum* seed butter **(VI)**. For the protein loss, the results were: $\mathbf{II}^{A} = \mathbf{III}^{A} > \mathbf{I}^{B} = \mathbf{IV}^{B} = \mathbf{V}^{B} = \mathbf{VI}^{B}$. Results classified with different letters present statistically significant differents, for $\alpha = 5$, $p \leq 0.05$, $n = 6$. Based on the results, the incorporation of conditioners agents *Argania spinosa* kernel oil and/or *Theobroma grandiflorum* seed butter in base hair care formulation applied in *Caucasian* hair post treatment with hair dye decreased the damage caused to hair by the coloring process.

Keywords: Protein Loss; Damage Hair; *Argania spinosa* Kernel; *Theobroma grandiflorum*

1. Introduction

The human hair is composed of protein, lipid, water, melanin and trace elements. The main constituents of hair are of α-keratin, a group of proteins which account for 65% - 95% of hair weight. It is responsible for conferring mechanical properties such as elasticity, shape, strength and functionality [1].

The human hair presents three principal components: cuticle, cortex and medull which are spectively from outside to inside. The cuticle is composed of protein material and amorphous, and it is located in the outer portion of the hair fiber and consists of enucleate cells, translucent and flattened. Morphologically, the cuticle is composed of 6 to 8 cell layers overlapped in the longitudinal direction of the fiber. The overlapping cell adherence provides the physical properties of hair with reflection light and reduces the friction between the fibers being responsible for the properties of gloss and combing, respectively. Cosmetic treatments, such as conditioners, hair sprays, mousses and gels, alter the properties mentioned above because they are deposited on the cuticle layer. However dyes and straightening products due to the alkaline pH of the cuticle open up the layers for the active principles or dyes penetrate and act in the cortex, reducing the size or altering the color of hair. The cortex is a major constituent of the hair fiber (75%). Cortical cells are subdivided into macrofibrils formed per material interfilamentar amorphous rich sulfur and microfibrils

[*]Corresponding author.

arranged in α-helix, consisting of four protofibrils, and these two protofilaments, dimers possessing two α-keratin subunits. The α-keratin presented in the microfibrils determines the mechanical properties of fiber, such as strength and elasticity. In the same way as the cuticle, it has cells filled by cross links of cystine and others cells separated by the cell membrane complex (CMC). The medulla is a thin cylindrical layer at the center of the hair thread may or may not be present; it is presented only in terminal hair and its role is not clearly defined [1-3].

Hair coloring is widely used by women and men either to change their natural hair color, and to delay the onset of gray hair, or to donate new pigments to gray hair [3]. The oxidative dyes are formed by two components, in cosmetic base as emulsions or gels, which are mixed and provide the coloration by chemical reactions in alkaline and/or oxidant medium, in the cuticle and cortex of the hair fiber. Oxidative dyes may damage the hair, since chemical and physical (exposure of cortex because of damage of cuticle) procedures are involved to alter the hair color [4,5].

When exposed, the hair fiber, with the adverse environmental conditions as solar radiation, wind, wet, pollution and the daily care routines and/or cosmetic treatments including permanents and dyes among others can present damages in its structure and, consequently, alterations are in its mechanical and of surface properties. Damaged hair can appear cloudy, dry, rough, fragile and/or dull [5].

The primary function of hair conditioners is to make the hair easier to comb due to reduction in antistatic property to the hair. Secondary benefits such as preventing flyaway hair, making the hair "shine", and protecting the hair from further damage are also important functions to hair conditioners. Substances that perform this function are usually silicones, polyquats, cationic surfactant, hydrolyzed proteins, fatty alcohols, fatty esters, vegetable oils, mineral oils, or humectants [6,7].

Rele and Mohile [8] established the superiority of the protective effect of coconut oil on hair damage in grooming processes when it is used as a pre-wash conditioner as compared to mineral oil and other vegetable oils such as sunflower oil. It not only has a protective effect on undamaged hair but also on chemically treated hair, UV-treated hair, and hair treated with boiling water (*i.e.*, hair in water at 100˚C for 2 hr). The ability of coconut oil to penetrate into hair cuticle and cortex seems to be responsible for this effect. In general, saturated and monounsaturated oils penetrate into the hair because of a compact molecular structure and the polar head group of the triglyceride molecules that constitute these oils [9].

Argan oil is prepared from the fruits of argan trees (*Argania spinosa* (L.) Skeels) following a multistep process. The argan tree (*Argania spinosa* (L.) Skeels; Sapotaceae) is a slow-growing tree exclusively endemic to the barren lands of southwest Morocco. The argan oil is constitute of acylglycerols, including 95% of triacylglycerols, constitute 99% of extract. The remaining 4% are composed of monoacylglycerols (0.27% - 0.65%), diacylglycerols (0.68 - 1.53), and free fatty acids (1.1% - 2.0%) [10].

Fat from seeds of *Theobroma grandiflorum* (cupuassu) has been particularly investigated because of its increasing demand as a new fruit crop. There is an increasing market for natural products, and the Brazilian *Theobroma* species could be used as alternative source of special fats. The percentage composition of fatty acids in the fat of cupuassu is 58.13%, 39.19% and 2.61% respectively for saturated, monounsaturated and polyunsaturated fatty acids [11].

In this research we analyzed the hair protective effect conditioner agents *Argania spinosa* kernel oil and/or *Theobroma grandiflorum* seed butter in hair care on Caucasian hair post treatment with hair dye. The hairs were submitted by quantifying protein loss.

2. Materials and Methods

2.1. Hair Samples

Caucasian virgin dark brown hair tresses of 20.0 cm in length, purchased from Bella Hair® (Brazil), were used. Each hair strand was washed for 30 s with 15.0% (w/v) sodium lauryl sulphate to remove impurities. All were wetted with warm distilled water (37.0˚C ± 2.0˚C) constant flow of 240.0 mL·min^{-1} for 1 min and the excess of water was first removed by passing the tresses three times between the fingers and then they were dried on paper towel, for 12 h dried at room temperature (22.0˚C ± 1.0˚C) and relative humidity (RH 60% ± 5%) prior to the analysis [12].

After drying, the tresses were treated with a comercial oxidative ultra-blond hair dye (Niely®), color 10.0, composed by: aqua, cetearyl alcohol, propylene glycol, deceth-3, laureth-12, ammonium hydroxide, oleth-30, hexadimetrine chloride, lauric acid, glycol distearate, polyquaternium-22, ethanolamine, silica dimethyl salylate, CI 77881, 2,4-diamophenoxyethanol HCl, *p*-aminophenol, *m*-aminophenol, ascorbic acid, sodium metabissulfite, *p*-phenilenodiamine, pentasodiumpentetate, carbomer, dimethicone, resorcinol and parfum (fragrance). The dye was mixed with hydrogen peroxide (30 vol) in a ratio of 1:1 (w/w) and applied to hair tresses in a ratio of 1:1 (w/w). The reaction occurred for 40 min. After this period, the tresses were washed according to the methodology described, and left to dry at room temperature.

2.2. Hair Care Formulations Development

A hair care formulation was prepared according to the composition in **Table 1**.

Conditioner agents were incorporated into the formulation, according to **Table 2**. The pH was adjusted to a 4.5 value. Hair care formulations (**Table 2**) were applied to the hair tresses, in a 1:0.5 (hair:formulation) ratio, before each assay for protein quantification. The applying procedure was performed with gentle movements assuring that the product distribution was uniform.

The emulsion base hair care formulation was prepared for conventional method in which oil and aqueous phases are heated at 70.0˚C - 75.0˚C and the aqueous phase are added to the oil phase, gradually and continuously, with stirred until 45˚C, when the conditioning agents were added in the base hair care formulation in accordance with **Table 2**.

The samples were classified as: hair untreated (**I**); hair treated with a commercial oxidative ultra-blond hair dye (**II**); hair post treatment II and F1: Base hair care formulation (**III**), hair post treatment II and F2: Base hair care formulation containing 1.0% (w/w) *Argania spinosa* kernel oil (**IV**), hair post treatment II and F3: Base hair care formulation containing 1.0% (w/w) *Theobroma grandiflorum* seed butter (**V**) and hair post treatment II and F4: Base hair care formulation containing 0.5% (w/w) *Argania spinosa* kernel oil and 0.5% (w/w) *Theobroma grandiflorum* seed butter **(VI)**.

Table 1. Qualitative and quantitative composition of base hair care formulation.

INCI[a] component	Proportion (% w/w)
Oil phase	
Cetearyl alcohol (Mapric®)	5.00
Cetearyl alcohol (and) behentrimonium methosulfate (Mapric®)	2.00
Cocoamide DEA (Mapric®)	1.00
BHT(Mapric®)	0.05
Aqueous phase	
Propylene glycol(Mapric®)	1.00
PEG-12 dimethicone (Dow Corning®)	1.00
Cetrimonium chloride (Mapric®)	5.00
Phenoxyethanol (and) methylparaben (and) ethylparaben (and) butylparaben (and) isobutylparaben (Croda®)	0.30
Dissodium EDTA (Mapric®)	0.05
Citric acid (Mapric®)	q.s. pH 4.5
Aqua	84.60

Legend: INCI[a]: International Nomenclature of Cosmetic Ingredient.

Table 2. Proportion of conditioner agents *Argania spinosa* kernel oil and/or *Theobroma grandiflorum* seed butter in hair care formulations.

INCI[a] component	Proportion of components incorporated (% w/w)			
	F1	F2	F3	F4
Argania spinosa kernel oil (Beraca®)	-	1.0	-	0.5
Theobroma grandiflorum seed butter (Croda®)	-	-	1.0	0.5

Legend: F1: Base hair care formulation; F2: Base formulation containing 1.0% (w/w) *Argania spinosa* kernel oil; F3: Base formulation containing 1.0% (w/w) *Theobroma grandiflorum* seed butter; F4: Base formulation containing 0.5% (w/w) *Argania spinosa* kernel oil and 0.5% (w/w) *Theobroma grandiflorum* seed butter; (–) not added. INCI[a]: International Nomenclature of Cosmetic Ingredient.

2.3. Assay for Protein Quantification

This assay was based on the reduction of the Folin reagent by protein previously treated by copper in alkalinemedium. A copper atom bonds to four residuals of amino acid. This complex reduces the Folin reagent, becoming the solution blue. In this work, the Lowry method modified by Peterson was used [13,14].

Samples of 0.10 g, in 15.0 mL of distilled water, were sonicated (Ultrasonic Clean® 1600 unique) for 40 min [15]. Protein loss was determined from 2.0 mL **supernatant aliquots**, which were added to 2.0 mL of **Reagent A**, and waited for 10 minutes for the reaction occurs. After this time it was added 1.0 mL of **Reagent B**, waited for 30 minutes, in the dark conditions in order to complete the reaction [16,17].

In a first step, the hair protein and the secondary standard Bovine Serum Albumin (BSA) one react with **Reagent A** withcupric ions (Cu^{2+}), in alkaline medium with sodium hydroxide and sodium carbonate (buffer with pH about 10.0). In this stage, sodium potassium tartarate is also used to avoid copper precipitation and, this way, increasing the solution stability. It is believed that the complexation of cupric ions (Cu^{2+}) with peptide bonds leads it to the reduction to cuprous ions (Cu^{+}). Ten minutes are waited for the reaction processing. The production of cuprous ions is followed by the reduction of Folin reagent (**Reagent B**) in the second stage of the assay, which turns the solution blue. Wait for 30 minutes in order for the reaction occurs [13,14].

Quantification was performed in Micronal® B-542UV-Visible spectrophotometer with a 1 cm quartz cuvette at 750.0 nm. Analytical curve was obtained with BSA, and used distilled water as blank. The calculation equation of the analytical curve was made by linear regression using the least squares method and calculating the linear correlation coefficient. The Equation (1) used to calculate the results. This method previously validated by Gama [18].

$$y = 0.0028x + 0.0402 \quad \text{with } R^2 = 0.992 \qquad (1)$$

2.4. Statistical Analyses

Possible significant differences in the results were analyzed by Kruskal-Wallis and the differences between treatments were identified by Student-Newman-Keulstest ($\alpha = 0.05$).

3. Results

Protein loss albumin equivalent of hair samples for: untreated (**I**); treated with a commercial oxidative ultra-blond hair dye (**II**); post treatment II and F1: Base hair care formulation (**III**), post treatment II and F2: Base hair care formulation containing 1.0% (w/w) *Argania spinosa* kernel oil (**IV**), post treatment II and F3: Base hair care formulation containing 1.0% (w/w) *Theobroma grandiflorum* seed butter (**V**) and post treatment II and F4: Base hair care formulation containing 0.5% (w/w) *Argania spinosa* kernel oil and 0.5% (w/w) *Theobroma grandiflorum* seed butter **(VI)** are shown in **Figure 1**.

4. Discussion

During the coloration process, the hair dyes provide the opening of the cuticle, and optimizing the absorption of the colorants into the cortex. This mechanism reduces the softness, brightness and difficult to comb of hair are the attributes of healthy hair [4].

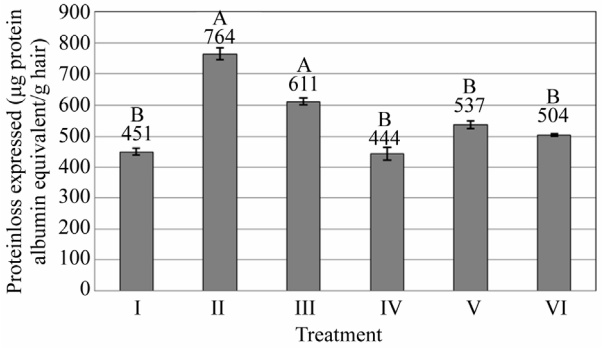

Figure 1. Protein loss albumin equivalent from Caucasian hair tresses before and after application of the oxidative dye hair emulsions either with or without conditioners agents (Protein loss was expressed in µg protein albumin equivalent/g hair). Legend: untreated hair (I); hair treated with a commercial oxidative ultra-blond hair dye (II); hair post treatment II and F1: Base hair care formulation (III), hair post treatment II and F2: Base hair care formulation containing 1.0% (w/w) *Argania spinosa* kernel oil (IV), hair post treatment II and F3: Base hair care formulation containing 1.0% (w/w) *Theobroma grandiflorum* seed butter (V) and hair post treatment II and F4: Base hair care formulation containing 0.5% (w/w) *Argania spinosa* kernel oil and 0.5% (w/w) *Theobroma grandiflorum* seed butter (VI). Results classified with different letters presents statistically significant differents, for $\alpha = 5$, $p < 0.05$, $n = 6$.

Hair fibers are constituted mainly by protein. The study of the damage hair shaft can evolve the quantification of protein loss after the coloring process on hair tresses and one option is the Lowry method modified by Peterson. The higher the greater the damage to cuticle protein loss compared to virgin hair [5].

The analyses of the protein loss from Caucasian hair tresses before and after application of the oxidative dye hair emulsions either with or without conditioner agents (**Figure 1**) showed difference in total protein loss between undamaged hair (**I treatment**) and hair damage after coloring process with (**II treatment**). The results confirmed by Robbins and Crawford [19] described that oxidative process of hair fibers produce extensive damage throughout several cuticle layers.

In this study, we observed (**Figure 1**) that using conditioners hair (**III**, **IV**, **V** and **VI treatments**) has a positive effect on reducing of protein loss in hair post treated with a commercial oxidative ultra-blond hair dye. The effectiveness of (**III treatment**) are expected, since the base hair care formulation contains a cationic compounds (cetrimonium chloride and behentrimonium methosulfate), which is substantive to hair and adsorbs on hair surface following a charge-driven mechanism [6,7] and silicone (PEG-12 dimethicone) that involved their adsorption to the hair fiber, because of its hydrophobic characteristics that reduce the intermolecular forces and surface tension leading to the formation of a hydrophobic film other the cuticle [20].

The addition of *Argania spinosa* kernel oil and/or *Theobroma grandiflorum* seed butter in the base hair care formulation (**IV**, **V** and **VI treatments**) statistically reduced the protein loss when compared to just base formulation (**III treatment**). This difference in results could arise from the composition of each of these oils. Keis *et al.* [9] have compared the ability of different oils (mineral oil, sunflower oil, and coconut oil) to penetrate into hair fibers, showing that their affinity to hair fiber depends on various factors, such as oil polarity, chain saturation and molecular weight.

Rele and Mohile [8] have compared the ability of different oils (mineral oil, sunflower oil and coconut oil) which were used as a pre-wash conditioner to prevention of hair damage measured by protein loss post chemically treated hair. Coconut oil, being a triglyceride of lauric acid (principal fatty acid), has a high affinity for hair proteins and it is able to penetrate inside the hair shaft because of its low molecular weight and straight linear chain.

The two main fatty acids found in *Argania spinosa* kernel oil acylglycerols are oleic acid (46% - 48%) and

linoleic acid (31% - 35%), a monounsaturated and diunsaturated fatty acid, respectively [10]. However the major fatty acids presented in *Theobroma grandiflorum* seed butter have: saturatedfatty acid (palmitic acid (11.25%), stearic acid (38.09%), arachidonic acid (7.97%)), monounsaturated fatty acid (oleic acid (38.79%)), and diunsaturated fatty acid (linoleic acid (2.39%) [11].

Introduction of this hydrophobic component reduces the swelling propensity of the cuticle, which limits the upward curving of the surface cuticle. This reduces the chipping away of the cuticle cells which reduces protein loss, as observed in this work.

Considering the assays performed quantification of protein loss the conditioner agents *Argania spinosa* kernel oil and *Theobroma grandiflorum* seed butter allowed a decrease in protein loss albumin equivalent when added separately (**IV or V treatment**) or together (**VI treatment**) in base hair care formulation applied in *Caucasian* hair post treatment with hair dye decreased the damage caused to hair by the coloring process.

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