

Influence of Ethnicity and Damage Levels on Fragrance Substantivity on Hair

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Synopsis

Product fragrance and performance are crucial for individuals choosing hair care products. Thus, fragrance raw materials should possess physicochemical characteristics that ensure good blooming upon product unpacking and during use and additionally exhibit good substantivity on hair fibers. To investigate the influence of fragrance raw material characteristics, hair damage level, and ethnicity on fragrance substantivity on hair fiber, hairs of different ethnicities were chemically and thermally damaged. Along with this, the level of damage was characterized by attenuated total reflectance Fourier-transform infrared spectroscopy, protein loss evaluation, scanning electron microscopy analysis, tensile test, and swelling test. The substantivity of each fragrance ingredient was evaluated for wet and dry hairs using gas chromatography coupled with mass spectroscopy. Our results indicated that the physicochemical properties of fragrance raw materials (mainly polarity) altered their availability within the shampoo formulation, as well as their substantivity on hair fibers. Furthermore, the substantivity of fragrance compounds was influenced by alterations in hair permeability and hydrophobicity caused by various damaging processes.

INTRODUCTION

The fragrance of hair care products is an important factor for consumers worldwide. Consumers expect their hair care products to impart a sensation of cleanliness and perfume their hair with a long-lasting scent (1–3). Thus, fragrances used in hair cosmetics should be derived from raw materials that exhibit physicochemical characteristics that ensure good blooming upon product unpacking and during use and also exhibit good substantivity on hair fibers.

The physicochemical characteristics of hair fibers can differ between hairs from different ethnicities and can also be altered by chemical, physical, or environmental agents and processes, which transform its naturally hydrophobic and slightly charged surface into a hydrophilic, polar, and negatively charged surface. These modifications change the reactivity of the hair toward external agents and lead to a decrease in the number of cuticular layers, formation of split ends and cracks, breakage and weakening, decreased

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shine, and the appearance of unhealthy hair (4–7). Therefore, the level of damage in the hair can also interfere with the fragrance substantivity on the fibers (8).

In this context, this study aims to improve the understanding of the influence of different factors (fragrance raw materials' [FRMs] physicochemical characteristics, hair damage level, and hair ethnicity) on the substantivity of FRMs on hair. Hence, we intend to provide information that can be used to deliver unique and personalized products within the hair care industry; this is regarded as a real consumer need rather than a trend.

MATERIALS AND METHODS

HAIR

Caucasian, Asian, and African virgin dark brown hair were purchased from International Hair Importers & Products (USA). Hair tresses (5 g, 17–20 cm long) were washed using a 10% sodium lauryl ether sulfate (SLES) aqueous solution (1 g/10 g of hair) for 1 min, rinsed for 1 min under tap water ($33 \pm 3^\circ\text{C}$, 4 L/min), and left to dry overnight under controlled conditions ($22 \pm 2^\circ\text{C}$ and $50 \pm 5\%$ relative humidity [RH]).

DAMAGE GROUPS

Tresses were divided into three damage groups (straightened hair, straightened + thermally damaged hair, and straightened + bleached hair) according to the applied damage procedure. Three tresses from each ethnicity were used for each group. Virgin hair tresses were used as the control.

HAIR DAMAGE PROCEDURES

Straightening. Tresses were straightened using an ammonium thioglycolate-based formulation (3.5 g/5 g of hair). The product was spread over each tress, and a comb was used to align the fibers; the applied product was left on for 25 min. Then, the tresses were rinsed with tap water ($33 \pm 3^\circ\text{C}$, 4 L/min) for 3 min. A hydrogen peroxide neutralizer was applied to each tress (2 mL). After 10 min, the neutralizer was rinsed off with tap water ($33 \pm 3^\circ\text{C}$, 4 L/min). The tresses were washed thrice with a 10% SLES aqueous solution and left to dry overnight under controlled conditions ($22 \pm 2^\circ\text{C}$ and $50 \pm 5\%$ RH).

Bleaching. Hair tresses were bleached using a mixture comprising of one part of commercial bleaching powder (Yamá, BRL) and two parts of hydrogen peroxide emulsion (12%, Yamá, BRL). The bleaching mixture was applied to the hair tresses (13 g/5 g of hair) and homogeneously spread over them. The tresses were individually wrapped in aluminum foil and kept in an oven at 25°C for 45 min. Then, they were rinsed under running water ($33 \pm 3^\circ\text{C}$, 4 L/min) until all the product residues were removed. The tresses were washed thrice times with 10% SLES aqueous solution and left to dry overnight under controlled conditions ($22 \pm 2^\circ\text{C}$ and $50 \pm 5\%$ RH).

Thermal process. The tresses were flat-ironed 60 times (Nano Titanium, Babyliiss Pro, USA) at 230°C , with 30 min intervals between every 10 hot flat-iron passes. Each pass took 5 s on an average. The application force was constant throughout the tress length.

HAIR DAMAGE ASSESSMENT

Fourier-transform infrared spectroscopy (ATR-FTIR). One hair tress from each damage group was analyzed using an infrared spectrometer with Fourier-transform, using the accessory for attenuated total reflectance (ATR, Cary 630, Agilent Technologies, USA). For each analysis, 64 scans were performed with a resolution of 4 cm^{-1} , ranging from $4,000$ to 400 cm^{-1} .

Protein loss quantification. Hair fibers from each damage group were cut (0.5 to 1.0 cm long), 250 mg of this cut hair was placed in plastic tubes with a conical bottom, and 4 mL of deionized water was added to it. The samples were incubated at 45°C for 72 h and centrifuged twice a day at 1,500 rpm for 1 min. The quantification of the total amount proteins in the supernatant was performed using the BCA1 Kit (Bicinchoninic Acid Protein Assay Kit, Sigma-Aldrich, USA) following the manufacturer's instructions. The samples were analyzed using a UV-Vis spectrometer (Lambda 25, Perkin Elmer, USA) at 562 nm.

Tensile Test. Forty-five fibers from each group were separated, prepared for analysis, and subjected to diameter measurement on the automated platform FDAS770 (Fiber Dimensional Analysis System, Dia-Stron, UK), which is coupled with a laser scan micrometer (LSM-6200, Mitutoyo, JPN). The tensile test was performed on a MTT680 (Miniature Tensile Tester, Dia-Stron, UK) using a constant stretch rate of 15 mm/min under controlled environmental conditions ($22 \pm 2^\circ\text{C}$ and $50 \pm 5\%$ RH).

Swelling test. Thirty fibers from each group were separated, prepared for analysis, and subjected to a swelling test on the FDAS770 automated platform (Fiber Dimensional Analysis System, Dia-Stron, UK) with a DSM770 (Dynamic Swelling Module, Dia-Stron, UK) accessory. The diameters of the fibers were measured in the rotational mode for 200 s using a laser micrometer (LSM-6200, Mitutoyo, JPN) coupled to the FDAS770 platform, while the fibers were submerged in water.

Scanning electron microscopy (SEM). Three fibers from each group were separated and prepared for analysis by fixing 1 cm fragments with carbon tape. The samples were Au/Pd (80:20) coated by sputtering in a Bal-Tec MD020 instrument (Balzers, Liechtenstadt). The images were acquired using a Quanta FEG 250 (FEI, Thermo Fischer Scientific, USA) operating at 5 kV.

FRAGRANCE SUBSTANTIVITY TEST

Fragrance raw materials. Five FRMs with different physicochemical characteristics were selected (Table I).

Shampoo formulation. A mixture of the five FRMs at the same concentration (20% w/w) was prepared and applied at 0.5% (w/w) to a shampoo formulation (Table II).

Table I
Tested Fragrance Raw Materials

Fragrance raw material	Molecular weight (g/mol)	Polarity (log P)
Sulfurol	143.2	1.11
Geranyl acetate	196.3	4.48
Isoeugenol	164.2	2.65
Gamma Undecalactone	184.3	3.06
Tonalide	258.4	5.80

Table II
Shampoo Formulation

Component (INCI name)	Concentration (%)
Water	q.s.100.00
Disodium EDTA	0.05
Polyquaternium-10	0.20
Glycerin	1.00
Sodium Laureth Sulfate	29.63
Cocamidopropyl Betain	8.00
1,2-Hexanediol, Caprylyl Glycol	0.80
Trideceth-9, PEG-5 Isononanoate, Water	1.00
PEG-150 Distearate	0.50
Cocamide DEA	1.50
Citric Acid	q.s. pH 5.5–6.5
Sodium Chloride	0.15

Internal standard. Ethoxybenzene (0.1 mg/mL in dichloromethane) was used as an internal standard for the quantification of the FRMs.

Sample preparation. Six tresses (0.5 g/20 cm long) from each group were washed for 1 min with the shampoo formulation containing the FRMs (50 mg/0.5 g of hair) and rinsed under tap water ($30 \pm 3^\circ\text{C}$) for 30 s. Three of these tresses (T0 samples) were immediately placed in 10 mL glass flasks with lids containing 2 mL of dichloromethane. The other three tresses (T24 h sample) were subjected to the same process after drying under controlled conditions ($22 \pm 2^\circ\text{C}$ and $50 \pm 5\%$ RH) for 24 hours. After 24 hours of extraction, the hair was removed from the flasks, and 100 μL of the internal standard solution was added to each flask. The samples were manually homogenized, dried with anhydrous sodium sulfate (Nuclear, CAQ, BRL) and filtered through a 0.2 μm PTFE filter (ValuPrep™ 25 mm, PALL Life Sciences, USA) to 3 mL chromatography vials.

Response factor. Six tresses (0.5 g/17–20 cm long) from each group were washed with the shampoo formulation without the FRMs (50 mg/0.5 g hair) for 1 min and rinsed under tap water ($30 \pm 3^\circ\text{C}$) for 30 s. Each tress was placed in a 10 mL glass flask with a lid, with 2 mL of dichloromethane, and 100 μL of a solution of the five raw materials in dichloromethane at 0.1 mg/mL each. This procedure was performed immediately after washing (T0 samples) or after drying under controlled conditions ($22 \pm 2^\circ\text{C}$ and $50 \pm 5\%$ RH) for 24 hours (T24 h samples). After 24 hours of extraction, the hair was removed from the flasks, and 100 μL of the internal standard solution was added to each flask. The samples were manually homogenized, dried with anhydrous sodium sulfate (Nuclear, CAQ, BRL), and filtered through a 0.2 μm PTFE filter (ValuPrep™ 25 mm, PALL Life Sciences, USA) to 3 mL chromatography vials.

GC-MS analysis. The samples were injected in a gas chromatograph (6890N, Agilent Technologies, USA) with an automatic injector (Multi-Purpose Sampler 2, Gerstel, USA) coupled to a mass detector (Mass Selective Detector 5973, MSD 5973; Agilent Technologies, USA). A ZB-1MS column (60 m \times 0.25 μm \times 250 μm) was used for component separation. The injection mode was set to the splitless mode. The oven temperature program was adjusted to heat up to 50°C , hold for 8 min at this temperature, and then heat up to 240°C . The samples were analyzed in the selected ion monitoring mode after choosing three appropriate mass/load ratios for each raw material at the time of the assembly of the method in the equipment software (ChemStation, Agilent Technologies, USA).

Substantivity calculation. FRM substantivity was calculated using Equation 1.

$$\text{Substantivity}(\%) = \frac{\text{Std weight} * \text{FRM peak area}}{\text{Std peak area} * \text{RF} * \text{FRM weight}} * 100 \quad \text{Equation 1}$$

RESULTS AND DISCUSSION

HAIR DAMAGE ASSESSMENT

The results obtained for the damage characterization of hair tresses are summarized in Table III.

In the comparison among the spectra obtained for virgin hair, the most important differences were observed in hair tresses that were straightened plus bleached for all the evaluated ethnicities. For these samples, hair oxidation caused by the oxidative agents present in this type of treatment was evidenced by the increased intensity of the band centered at $1,043 \text{ cm}^{-1}$ (assigned to $-\text{S}=\text{O}$ symmetric stretch), and the widening of the band at $1,177\text{--}1,233 \text{ cm}^{-1}$ (characteristic of sulfonate and thiosulfate groups and portions of cysteic acid) (9–11).

From the stress–strain curves obtained in the tensile test, we evaluated two important parameters: elastic modulus and break stress. In general, straightening followed by thermal damage was the process that generated the most severe changes in hair structure. However, all the evaluated damage processes resulted in an increase in the hair elastic modulus and a decrease in the break stress, thereby increasing its stiffness and fragility in comparison to virgin hair.

For all evaluated ethnicities, the straightened and thermally damaged hair lost the highest amount of protein among the damage groups. As expected, virgin hair showed extremely low values of protein loss, which was statistically smaller than those of the other groups in all three ethnicities.

Table III
Summary of Damage Characterization Results

Damage group	Ethnicity	FTIR–ATR	Tensile		Protein loss (mg/g of hair)	Swelling (%)
			elastic modulus (MPa)	Break stress (MPa)		
Straightened	African	No alteration	+6%***	−7%***	+740%***	+38%***
	Asian	No alteration	+4%*	−3%*	+1,059%***	+39%***
	Caucasian	No alteration	+12%***	−5%***	+967%***	+35%**
Straightened + thermally damaged	African	No alteration	+31%***	−12%***	+1,851%***	−19%*
	Asian	No alteration	+29%***	−6%***	+2,651%***	−43%***
	Caucasian	No alteration	+37%***	−2%	+2,500%***	−35%***
Straightened + bleached	African	Oxidation	+28%***	−4%*	+920%***	+120%***
	Asian	Oxidation	+27%***	−4%**	+1,068%***	+161%***
	Caucasian	Oxidation	+30%***	−3%*	+736%***	+99%***

*** $p < 0.001$, ** $p < 0.01$, and * $p < 0.05$ versus virgin hair from same ethnicity.

The swelling test results made it possible to gain a better understanding of the variation in hair fiber permeability to water after the different damage processes. The greatest variations in the fiber diameter occurred immediately after immersion in water, especially in the first minute of the test. After 120 s under water, a plateau formation was observed in the swelling curves, with the exception of the ones taken from the straightened and thermally damaged hairs. In this case, the percentage of diameter change grew almost linearly with time (Figure 1), possibly indicating that the swelling process continued even after 200 s and that these hair fibers may present higher values of diameter variation after long periods of water immersion. The profiles observed for the different damage groups were highly similar when comparing different ethnicities.

After 200 s, the tresses that were straightened and straightened and bleached exhibited statistically larger diameter variations than those observed for the virgin hair. This increase may be related to the internal and external changes in these fibers caused by chemical treatments, such as lipid and protein oxidation and cuticle lixiviation (12,13), which results in a decrease in hair hydrophobicity and an increase in its water permeability. The straightened and bleached hair group presented the largest increase in fiber diameter after this period, exhibiting a water permeability that was at least twice as high as that observed for virgin hair.

However, the hair fibers that were subjected to straightening followed by thermal damage showed decreased swelling rates and final diameter variations (during the evaluation period) compared with the virgin fibers. This fact may be correlated with the fusion of the cuticles that may occur after strong thermal processes (14), which can decrease the water permeability of these fibers by occluding the water entrance paths.

The damage caused by the different procedures was also evident in the SEM images (Figure 2), where cuticle lixiviation, fractures, holes, and exposed cortex were observed; these observations are in accordance with the results obtained from the instrumental tests detailed above.

FRAGRANCE SUBSTANTIVITY TEST

The substantivities of the five chosen FRMs were evaluated for wet (T0) and dry hairs (T24 h). From the general results (Table IV), it was observed that there was a difference in the substantivity values of wet and dry hairs, with greater substantivity values obtained for wet hair as compared to dry hair. Since FRMs are small and volatile compounds, it was expected that a portion of the initial amount of fragrance retained on the wet substrate would be lost to the environment during the drying process. Polarity also influenced FRM elimination, with the most polar substances being dragged by the water during the drying process, thus exhibiting large differences in substantivities of wet and dry hairs.

The greatest decays in absolute FRM substantivity values of wet and dry conditions were observed for sulfurol, and the smallest decays were observed for gamma undecalactone (Table V). Among the tested FRMs, sulfurol was the most volatile and the most polar substance, which explains why it was eliminated during the drying process. In contrast, gamma undecalactone presented lower volatility and polarity than sulfurol, showing better retention (or a long-lasting effect) on hair fibers.

Among the FRMs, sulfurol presented the highest substantivity on hair fibers. An exception to this behavior was in the group of hairs that were straightened and bleached in dry conditions.

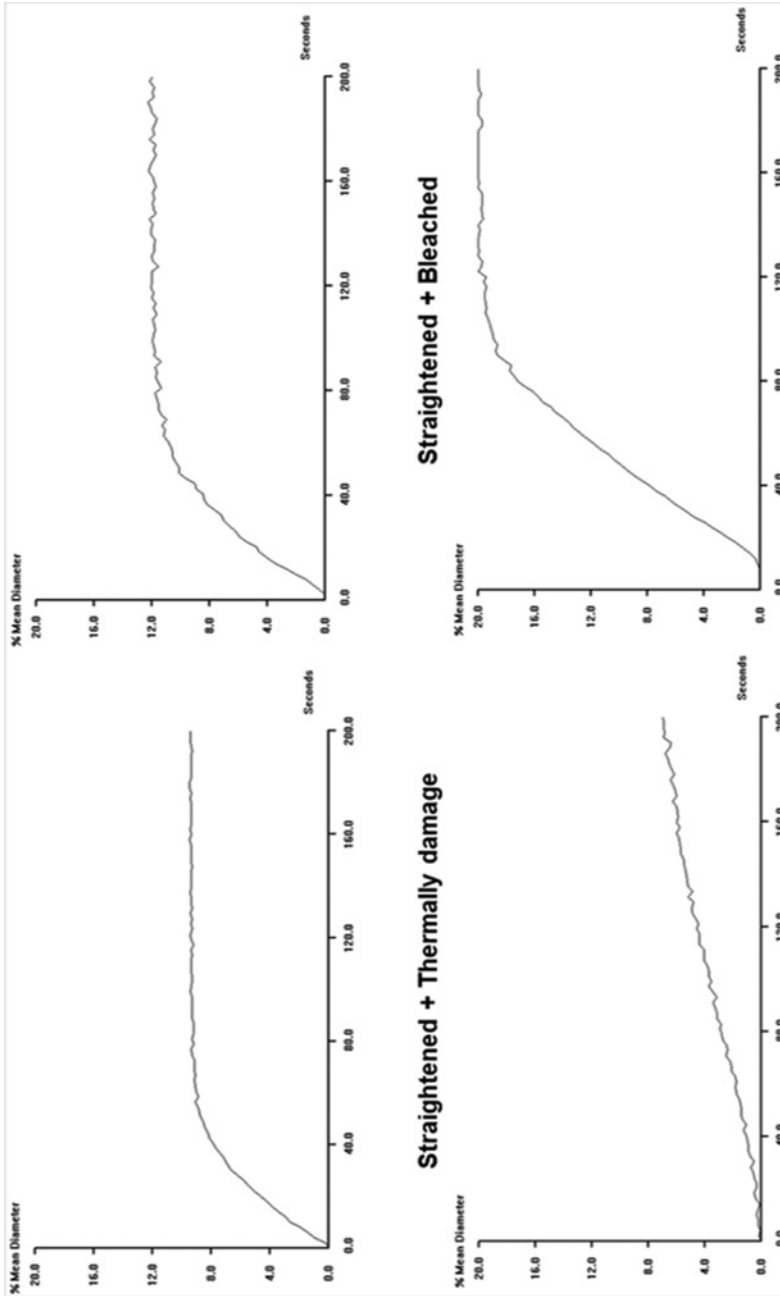


Figure 1. Swelling profiles of African virgin and damaged hairs.

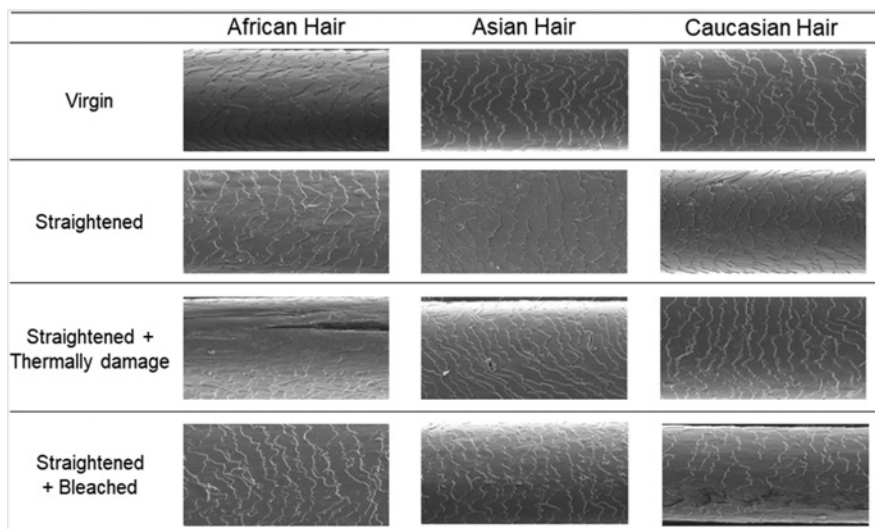


Figure 2. SEM images of hair fiber from different ethnicities and damage groups (magnification: $\times 2,000$).

This case, gamma undecalactone showed better retention for the three studied ethnicities. In the case of sulfurol, straightening followed by bleaching contributed the most to its decay during hair drying, exhibiting the highest values of relative difference among all ethnicities.

The high observed substantivity of sulfurol on hair in this experiment can be explained by its distribution over the surfactant system used as vehicle (a shampoo formulation). In general, highly polar molecules, such as sulfurol, present greater affinity for the polar external phase of the surfactant system than for the internal one, thereby exhibiting a higher impact on the shampoo odor than the more nonpolar molecules, which are probably located inside the micelles (15,16). The molecules present in the external phase of the formulation may also tend to favorably interact with the substrate rather than the more nonpolar FRMs.

Another fact that corroborates this explanation is that the substantivities of sulfurol on hair, mainly on wet hair, can be correlated well with the swelling results observed for the different damage groups. The greater the swelling observed, the greater the sulfurol substantivity was. An exception was the straightened and bleached hair, which showed the highest values of swelling but lowest values of substantivity for sulfurol among the damage groups (Table VI). The substantivity of sulfurol on straightened and bleached hair was influenced by its polarity but also by the damage extent of these hair fibers, which led to decreased hydrophobicity, increased porosity, increased permeability, and, consequently, increased exchange with the external environment. In this scenario, substances with high polarity, such as sulfurol, may be easily dragged by water in and out of the fiber, resulting in the smallest substantivities for sulfurol on T24 h.

Among the damage groups, straightened hair presented the highest substantivity values on hair (mainly wet) for almost all the FRMs and ethnicities. We associated this behavior with the intermediate level of damage observed in this damage group. Although it also presented decreased hydrophobicity, charged sites, and increased permeability, these alterations occurred at a lower intensity when compared with the hair that was straightened

Table IV
Summary of FRM Substantivities

Damage group	Average Substantivity (%)											
	Sulfurol		Geranyl acetate		Isoeugenol		Gamma undecalactone		Tonalide			
	T0	T24	T0	T24	T0	T24	T0	T24	T0	T24		
African	Virgin	12.728±1.711	3.371±0.396	2.907±0.390	0.904±0.137	3.486±0.948	0.756±0.044	2.968±0.168	2.240±0.127	4.101±0.767	2.179±0.220	
	Straightened	23.081±2.285	4.407±0.616	4.452±0.407	1.289±0.101	5.088±1.876	1.655±0.516	6.414±1.803	3.205±0.132	4.663±0.597	2.339±0.189	
	Straightened + thermally damaged	12.374±2.210	4.145±1.419	2.482±0.284	1.135±0.130	1.763±1.731	2.162±0.280	4.370±0.619	2.816±0.936	3.622±0.452	1.991±0.352	
Asian	Straightened + bleached	10.609±1.276	0.987±0.451	2.599±0.452	1.457±0.168	4.604±0.750	0.379±0.202	4.872±0.560	3.548±0.431	2.356±0.518	1.461±0.136	
	Virgin	17.010±0.981	5.444±0.248	5.224±0.710	1.401±0.191	3.385±1.548	1.145±0.037	4.613±1.248	2.800±0.249	5.321±0.605	2.068±0.231	
	Straightened	22.494±5.361	9.333±0.376	5.575±1.422	1.721±0.195	5.633±1.823	3.264±0.693	5.891±0.787	4.532±0.424	5.560±1.535	2.906±0.277	
Caucasian	Straightened + thermally damaged	13.864±2.296	6.682±1.137	3.686±0.863	1.383±0.133	3.459±0.890	2.465±0.507	4.076±1.211	3.561±0.674	3.280±1.200	2.008±0.338	
	Straightened + bleached	13.580±0.390	2.989±0.892	4.476±0.631	2.114±0.271	1.934±0.753	3.014±0.856	5.954±1.460	4.163±0.258	2.904±0.534	2.159±0.078	
	Virgin	16.562±0.979	8.055±1.097	3.434±0.428	1.972±0.200	2.419±0.746	2.280±0.321	3.485±1.038	3.266±0.992	5.745±0.169	4.232±0.256	
Caucasian	Straightened	19.818±3.718	9.038±1.825	4.388±1.770	3.216±1.844	6.369±0.916	5.223±1.806	6.333±0.474	3.138±1.580	5.684±0.251	4.143±1.258	
	Straightened + thermally damaged	13.284±4.307	6.907±1.108	2.680±0.247	2.094±0.340	3.446±0.753	3.303±0.417	4.508±0.745	4.176±0.647	5.546±0.446	3.136±0.423	
	Straightened + bleached	12.801±0.991	1.185±0.303	2.989±0.374	1.382±0.172	5.310±0.498	1.464±0.176	4.604±1.007	3.615±0.607	3.085±0.071	1.820±0.030	

Table V
Substantivity Differences Between T0 and T24h

Ethnic group	Group of damage	Sulfurol		Gamma undecalactone	
		Absolute difference	Relative difference (%)	Absolute difference	Relative difference (%)
African	Virgin	9.358	73.516	0.727	24.500
	Straightened	18.674	80.905	3.209	50.024
	Straightened + Thermal Damaged	8.228	66.498	1.554	35.569
	Straightened + Bleached	9.622	90.694	1.323	27.163
Asian	Virgin	11.565	67.992	1.813	39.298
	Straightened	13.160	58.507	1.359	23.075
	Straightened + Thermal Damaged	7.182	51.802	0.515	12.642
	Straightened + Bleached	10.591	77.991	1.790	30.071
Caucasian	Virgin	8.508	51.368	0.219	6.281
	Straightened	10.781	54.396	3.196	50.455
	Straightened + Thermal Damaged	6.377	48.004	0.331	7.348
	Straightened + Bleached	11.616	90.742	0.988	21.468

Bold numbers represent significant differences between substantivities in T0 and T24 ($p < 0.05$).

and bleached or to the group with hair that was straightened and thermally damaged. This implies that a certain level of damage contributed to a higher retention of fragrance on hair fibers.

Considering the influence of ethnicity on the fragrance substantivity on hair, it was cumbersome to infer a global tendency as compared to the other factors. However, among the three ethnicities, on dry conditions, Caucasian hair presented the highest substantivities for virgin, straightened, and straightened and thermally damaged groups, while Asian hair showed similar behavior for straightened and bleached hair for all the tested FRMs.

Table VI
Substantivity of Sulfurol Versus Hair Swelling

Ethnic group	Group of damage	Substantivity of sulfurol at T0 (%)	Diameter variation (%)
African	Virgin	12.728 ± 1.711	9.07 ± 1.30
	Straightened	23.081 ± 2.285	12.55 ± 2.20
	Straightened + Thermal Damaged	12.374 ± 2.210	7.33 ± 1.76
	Straightened + Bleached	10.609 ± 1.276	19.98 ± 3.62
Asian	Virgin	17.010 ± 0.981	8.85 ± 1.01
	Straightened	22.494 ± 5.361	12.32 ± 2.53
	Straightened + Thermal Damaged	13.864 ± 2.296	5.02 ± 1.98
	Straightened + Bleached	13.580 ± 0.390	23.09 ± 6.30
Caucasian	Virgin	16.562 ± 0.979	9.18 ± 1.26
	Straightened	19.818 ± 3.718	12.35 ± 1.97
	Straightened + Thermal Damaged	13.284 ± 4.307	6.01 ± 1.38
	Straightened + Bleached	12.801 ± 0.991	18.30 ± 2.42

CONCLUSIONS

All the evaluated factors showed some degree of influence on the FRMs substantivity on hair. The polarity of FRMs can affect their distribution in surfactant systems, which are generally used in cosmetics for hair care, such as shampoo formulations, influencing their availability and interaction with the hair fibers.

The level of hair damage was also an important factor influencing FRMs substantivity on hair, with high damage levels resulting in smaller sulfurol substantivities on hair fibers, and intermediate levels of damage resulting in the highest substantivities observed for all FRMs apart from ethnicity.

The influence of hair ethnicity on FRM substantivity was more relevant for dry hair than wet hair, with Caucasian or Asian hair presenting the highest substantivities in this condition depending on the damage group.

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