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Penetration of Vegetable Oils into Textured Hair Fibers: Integrating Molecular Matrix Assisted Laser Desorption Ioni-Zation Time-of-Flight Mass Spectroscopy (MALDI TOF/TOF MS) Analysis with Mechanical Measurements

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Abstract: The promotion of natural beauty has empowered women with textured hair to embrace their natural hair texture and opt for bleaching as a means of style variation. However, bleaching exacerbates the inherent fragility of this hair type, necessitating treatments to partially restore its mechanical properties. Vegetable oils, renowned for their strengthening properties, were evaluated regarding (I) their ability to penetrate both virgin and bleached textured hair using Matrix-Assisted Laser Desorption Ionization (MALDI) time-of-flight (TOF) analysis, and (II) their effects by way of tensile and fatigue tests. The MALDI–TOF results revealed groups of oil molecules in the cortical region of the bleached textured hair. The tensile test results, in turn, showed that the oil treatments were unable to alter the mechanical properties of the hair. Conversely, the fatigue test showed an increase in resistance in the virgin hair, most likely attributed to a lubrication effect in the outermost portions of the cortex and cuticles. In the bleached hair, a reduction in resistance was noted following the treatment with the oils. Comparative analysis with a previous study on straight hair by our group suggests that external molecules diffuse more homogeneously in straight hair than in textured hair. The unique cortical structure of textured hair creates two areas with distinct diffusion zones, resulting in the irregular distribution of external materials and different effects compared to straight hair.

Keywords: argan oil; avocado oil; coconut oil; textured hair; penetration of oils; MAILDI–TOF; mechanical measurements

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1. Introduction

With the increasing tendency towards promoting natural beauty, women with textured hair have begun to proudly embrace their curls and demand customized and efficient solutions from the cosmetics industry to address their challenges and meet their needs. Straightening treatments are becoming less popular in salons, while bleaching, once overlooked by these consumers, has now become an option for lightening and diversifying their appearance.

Bleaching is considered one of the most aggressive treatments for hair, as it exacerbates the hair fiber's already fragile state [1–5]. Textured hair, in particular, is well-known for its relative susceptibility to mechanical damage and is more prone to breakage, even with minimal amounts of applied force [6]. The presence of twists and the flattened structure of textured hair fibers leads to irregular mass distribution, creating areas of both greater and lesser accumulation, thus increasing susceptibility to breakage [6,7]. The diffusion of external materials into the cortical region of hair strands has been studied and shown to partially mitigate fragility, thereby improving hair resistance [8–15]. Vegetable oils play a significant role in these initiatives, as they are widely recognized for their ability to reduce dryness, provide nourishment, strengthen hair, offer pre-wash protection, control frizz, and repair split ends [16].

There are unique characteristics of textured hair morphology that dictate how molecules will diffuse into this hair type and, consequently, the benefits derived from these interactions. Textured hair shafts exhibit a distinctive appearance characterized by waves and twists that vary among individuals in terms of tightness. From a chemical standpoint, studies have shown that there are no significant differences in protein content between textured hair and Caucasian or Asian hair types [17–21]. The formation of curls is attributed to the arrangement of specific cortical cells, which form two distinct zones known as the paracortex and orthocortex [20–23]. The ratio between intermediate filament proteins (IFPs) and keratin-associated proteins (KAPs) determines the type of cortex that is formed [20]. Intermediate filaments (IFs) have a shorter, straight shape aligned with the hair axis, characterizing the paracortex when closer to the internal portion of the curls, and a longer, spiral shape characterizing the orthocortex when located in the external portion of the curls [24–27], indicating a bilateral distribution of cells in curly hair. The lipid content further distinguishes textured hair from other hair types, as demonstrated by Marti et al. [28] and Cruz et al. [29], who reported that this hair type presents higher lipid content, possibly due to the absorption of sebum from the surface.

The study of molecule penetration into hair requires utilizing a combination of techniques to ensure the accurate interpretation of diffusion events [30]. Matrix-Assisted Laser Desorption Ionization (MALDI) time-of-flight (TOF) was used to assess the diffusion process of vegetable oils, particularly coconut, argan, and avocado oil. This technique has long been used in biological fields to probe a variety of specimens, such as proteins, peptides, saccharides, and nucleotides [31]. In human hair, it is primarily applied to investigate the presence of drugs, commonly employed in forensic sciences. Cosmetic industries seek techniques capable of elucidating the mechanism of action of various molecules to study their effects and demonstrate their efficacy on substrates like skin and hair. Although secondary ion mass spectrometry (SIMS) has been more utilized in hair studies among mass spectrometry techniques [8,32,33] due to its resolution, MALDI–TOF allows for the investigation of a wider mass range of materials (1–500 kDa) compared to SIMS [31].

This study innovatively investigates the efficacy of vegetable oils on bleached textured hair using a combined approach of MALDI–TOF mass spectrometry for high-resolution penetration analysis and mechanical testing. This approach allows for the determination of oil–hair interactions within the complex structure of textured hair, providing a significant advancement over previous studies, which primarily focused on straight hair. The direct comparison to previously published data on straight hair further elucidates the influence of hair texture on product efficacy and highlights the need for texture-specific treatment formulations.

2. Materials and Methods

2.1. Triacylglycerols Compositions of the Vegetable Oils

The argan, avocado, and coconut oils (Symrise, Holzminden, Germany) were characterized based on their triacylglycerol distribution using a chromatograph (Agilent 6850 Series GC System, Santa Clara, CA, USA) following the method described in the Official Methods and Recommended Practices of the American Oil Chemists' Society [34]. Details of this analysis are reported in a previous study by our group [35].

Hair

Type IV textured hair [36], measuring 17 cm long, was purchased from International Hair Importers and Products, Ltd. (New York, NY, USA). Two-gram hair tresses were prepared using lamination adhesive. A 10% sodium lauryl ether sulfate (SLES) solution was applied to each tress at a ratio of 1.0 mL for every 10 g of hair, then massaged into the hair using fingers for 1 min and rinsed for another minute under tap water at 32 ± 2 °C, with a flow rate of 4.0 L/min. Subsequently, they were left to dry overnight under controlled conditions (22 ± 2 °C and 50 ± 5% relative humidity, RH). Four hair tresses were assigned to each of the vegetable oil treatments, and another four to the untreated group.

2.2. Bleaching Process

The bleaching process took place for 30 min at 25 °C, using a mixture of commercially available 12% hydrogen peroxide emulsion (Yamá, Cotia, Brazil) and bleaching powder (Yamá, Cotia, Brazil) at a proportion of 1:2 (w/w). The resulting cream was applied to the hair at a ratio of 2.5 g of product per gram of hair. The tresses were then rinsed under running water (33 ± 3 °C and 4.0 L/min), cleaned with 10% SLES solution, and left to dry overnight under controlled conditions (22 ± 2 °C and 50 ± 5% RH).

2.3. Oil Application

The oils were applied directly to the tresses at a ratio of 1.0 g per gram of hair. The tresses were then individually wrapped in aluminum foil and left for 24 h at 25 °C before being washed with 10% SLES solution to remove excess oil from the fiber surface. The tresses were subsequently dried under controlled conditions (22 ± 2 °C and 50 ± 5% RH).

2.4. MALDI–TOF Analysis

The analyses were conducted using bleached hair tresses. Following the damaging process and oil application, they were subjected to a dialysis process to completely remove SLES solution residues, which could interfere with the matrices and oil structures in the spectra. The water used was changed every two hours for 12 h, followed by a resting period of 12 h, a process that was repeated until completing 48 h. The oils were applied to the tresses as described in the Oil Application section, then washed with a 5% SLES solution for 1 min and rinsed abundantly with running water. The samples underwent a final rinse with deionized water to ensure the removal of oil from the surface of the fibers, which could be carried into the cortex during the subsequent step of cutting the samples. They were then placed in an oven at 28 °C for 24 h to dry.

The fibers were cut along their length using an apparatus consisting of a polymeric block with a microtome blade at an angle of approximately 20° and an aluminum block containing grooves measuring between 40 and 50 µm, in which the hair fibers were positioned and attached using adhesive tape. Subsequently, the fibers were affixed to glass slides with a conductive coating of ITO (indium tin oxide), and the tips of the fibers were secured with Scotch® 419 aluminum tape (3M, BRL). A mixture of dihydroxybenzoic acid (DNB) and α-cyano-4-hydroxycinnamic acid (CHCA) matrices at a 3:1 ratio (m/m) with a concentration of 8.0 mg/mL in a 1:1 acetonitrile solution acidified with 0.1% trifluoroacetic acid (v/v) was applied to the slides using TM-Sprayer™ equipment (HTX Technologies, Houston, TX, USA) [37].

MALDI–TOF/TOF analyses were conducted using the Autoflex Mass Spectrometer maX® (Bruker Daltonics, Bremen, Germany), equipped with a Smartbeam-II laser operating at a wavelength of 355 nm. The laser power was set at 90%, with a focus diameter of 80 µm and a repetition rate of 2000 Hz. Spectra were obtained in positive ion mode using the reflector in the m/z 450–2000 region. The FlexControl software (Bruker Daltonics, Bremen, Germany) was used for acquisition, and data processing utilized flexAnalysis software (Bruker Daltonics, Bremen, Germany) in conjunction with the open-access MZmin software [37]. Ten measurements were taken on each fiber, totaling two measurements per treatment.

2.5. Tensile Test

The cross-sectional dimensions of 45 fibers from both the treated and untreated tresses were measured using an FDAS-770—Fiber Dimensional Analysis System (Dia-Stron, Andover, UK) and an LSM-6200—Laser Scan Micrometer (Mitutoyo, Kawasaki, Japan) to calculate the sectional areas of the fibers. The tensile test was conducted using an MTT-680—Miniature Tensile Tester (Dia-Stron, Andover, UK) with a constant stretch rate of 15 mm/min at 22 ± 2 °C. The fibers were soaked in water for one hour to equilibrate their humidity. The data were extracted with UvWin software (Dia-Stron, Andover, UK) and analyzed using one-way ANOVA, followed by Tukey's post-hoc HSD test with a 95% confidence interval. The analyses were performed using Microsoft Office Excel with XL STAT.

2.6. Fatigue Test

The cross-sectional dimensions of 50 fibers from both the treated and untreated tresses were measured using an FDAS-770—Fiber Dimensional Analysis System (Dia-Stron, Andover, UK) and an LSM-6200—Laser Scan Micrometer (Mitutoyo, Kawasaki, Japan) to calculate the sectional areas of the fibers. The fatigue test was conducted using a CYC-801—Cyclic Tester (Dia-Stron, Andover, UK) with a constant stress of 130 MPa and a stretch rate of 40 mm/min. Samples preparation, as well as testing, was conducted under controlled conditions (22 ± 2 °C and 50 ± 5% RH). Data analysis was performed using UvWin software (Dia-Stron, Andover, UK).

3. Results

3.1. Triacylglycerols Compositions of the Vegetable Oils

The vegetable oils were characterized based on their composition of triacylglycerols, following the Official Methods and Recommended Practices of the American Oil Chemists' Society [34]. The results have been reported in a previous publication [35].

3.2. MALDI–TOF/TOF

Matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI–TOF) is a technique based on the ionization of materials supported by a matrix that evaporates due to the conversion of laser energy into heat energy. In this process, the matrix, which is deposited over the analyte, carries its molecules, generating its ionization through ion or charge transfer [31]. These ionized agents are then separated according to their mass-to-charge ratio (m/z) using a time-to-flight (TOF) mass analyzer.

Figure 1 shows the spectra of the isolated oil, the treated hair, the untreated hair, and the matrix used in the sample. Argan, avocado, and coconut oil were identified by the letters A, B, and C, respectively. The generated ions and the ratios among them are unique to each material and provide the signature for their identification. The m/z values of the ions generated for each oil and the corresponding treated hair are shown in the spectra for each oil.

Table 1 shows the obtained values for m/z associated with their intensities for the three studied oils and for the not-treated hair. The highlighted intensities are present in high levels for the three oils within a narrow range of values for m/z, indicating that, although with slight deviations among them, it is probable that they are related to similar structures of the oils.

The coincident values of m/z in the spectra of the oils treated hairs and absent in the not-treated hair indicate their presence in the hair cortex. While the data are qualitative, the consistency of analytical conditions, with identical MALDI–TOF analysis performed on a single plate, allows for the valid comparative assessment of signal intensities. The results show that argan oil was detected with greater intensity, followed by avocado oil and then by coconut oil, which was detected in smaller intensities in the bleached textured hair.

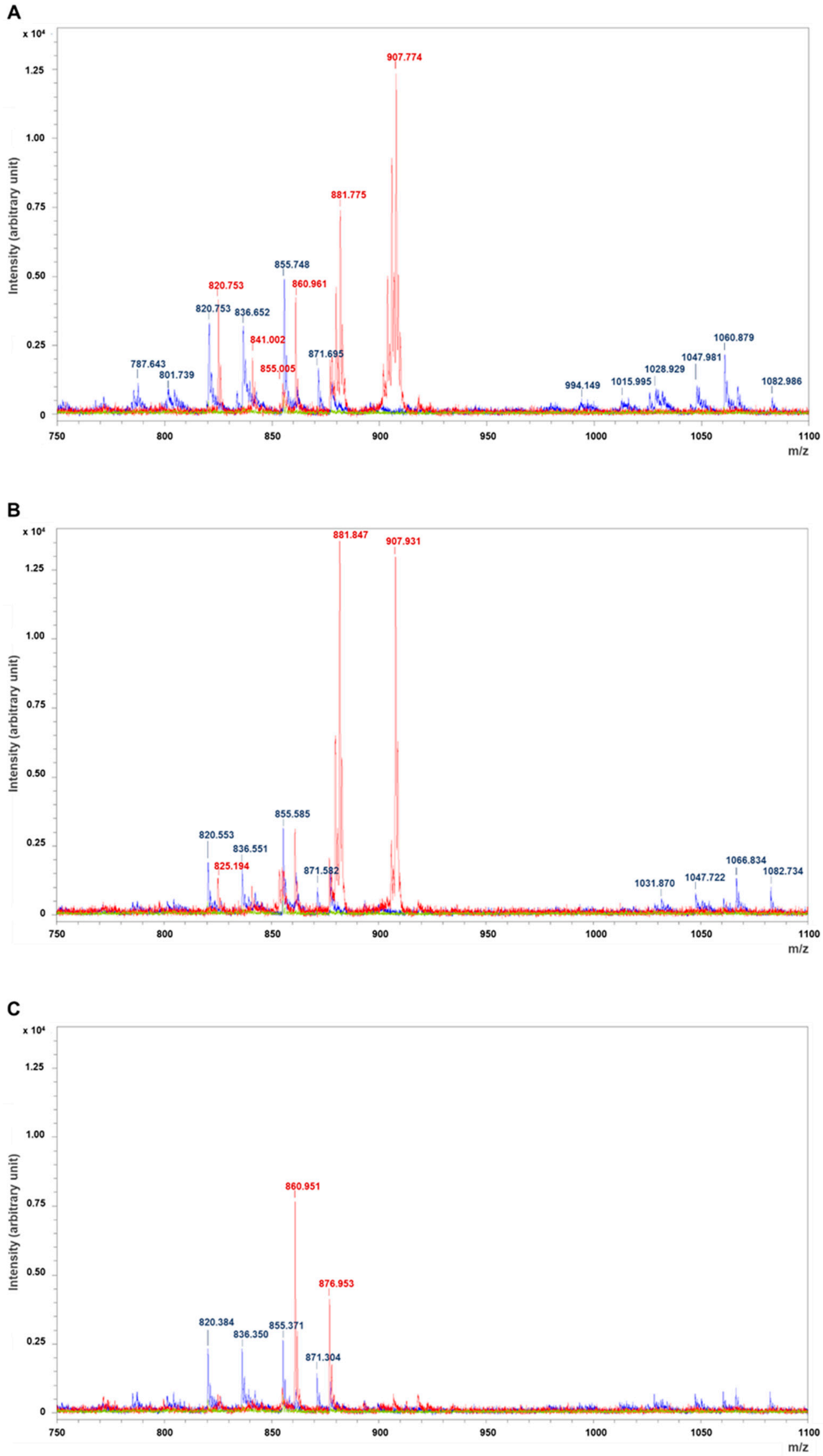

Figure 1. Spectra ranging from 750 to 1100 m/z, highlighting the identified peaks of the isolated oil, the oil-treated hair, the untreated hair, and the matrix. The red line corresponds to the peaks identified for the isolated oil, the blue line for the oil-treated hair, the green line for the untreated hair, and the black line for the matrix. (**A**)—spectrum of argan oil. (**B**)—spectrum of avocado oil. (**C**)—spectrum of coconut oil.

Table 1. Values for m/z and their respective intensities for the three studied oils and the untreated hair. The highlighted values of m/z are present with different intensities in the spectra of each oil, indicating similar structures. The intensities detected for these m/z values are not detectable in the untreated hair, leading to the conclusion that they express the presence of oils in the treated hairs.

m/z	Intensities of the Oils		
	Argan	Avocado	Coconut
801.739	943		
820.384			**2338**
820.553		**1914**	
820.753	**3311**		
836.35			**2334**
836.551		**1696**	
836.652	**3205**		
855.371			**2640**
855.585		**3160**	
855.748	**4924**		
861.585		1544	
871.304			**1436**
871.582		**1001**	
871.695	**1676**		
877.586		1762	
878.092	1180		
1010.583			166
1015.995	599		
1028.392			**691**
1028.929	**935**		
1031.87		590	
1047.406			**692**
1047.722		**791**	
1047.981	**1042**		
1060.516			770
1066.424			**913**
1066.834		**1325**	
1082.436			**770**
1082.734		**1013**	
1082.734	**617**		

3.3. Tensile Test

Figure 2 demonstrates the results obtained for Young's modulus (A) and break stress (B) during the wet tensile test. In the virgin hair, none of the treatments were capable of modifying Young's modulus or break stress in these fibers. However, in the bleached hair, although the treatments eventually led to similar levels of stiffness as the untreated hair, there was a noticeable difference in Young's modulus between the argan oil and the coconut oil-treated hairs. Argan oil showed a tendency to increase the stiffness of the textured hair, as Young's modulus increased by 3.40%, while coconut oil led the textured hair to become more flexible, reducing Young's modulus by 8.11% compared to argan oil. Avocado oil, on the other hand, significantly impacted the break stress of the textured bleached hair, increasing its resistance to breakage by 13.56%, while argan oil contributed with 8.79% and coconut oil with 4.35%.

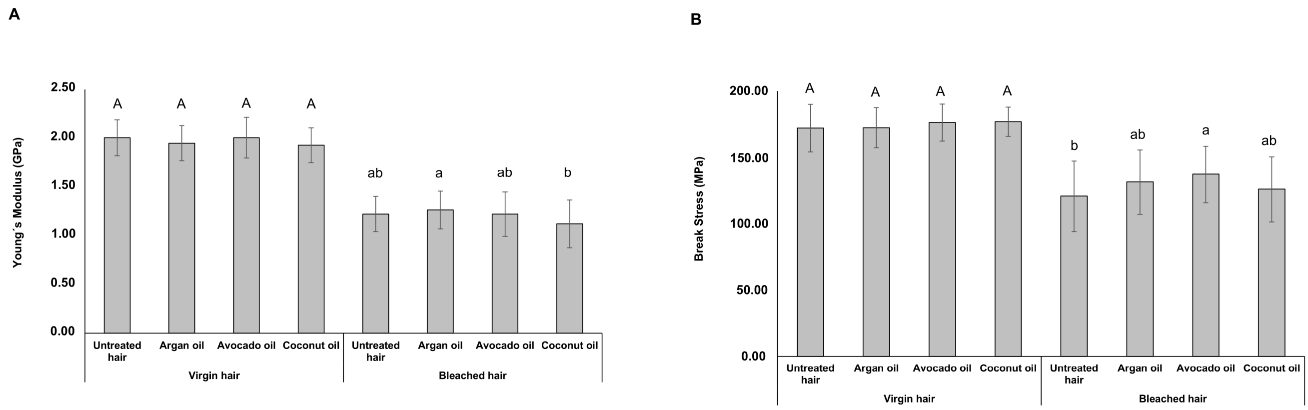

Figure 2. Mechanical properties obtained from the wet tensile test for the virgin and bleached textured hair with and without the oil treatments. (**A**)—Young's modulus. (**B**)—break stress. Identical letters indicate $p > 0.05$, while different letters indicate $p < 0.05$.

3.4. Fatigue Test

The fatigue test is used in a more pragmatic manner compared to the tensile test, as it enables the investigation of the mechanical resistance of hair to low loads applied repeatedly. This simulation reflects the daily routine of consumers, who continuously subject their hair to combing and grooming stresses. The test identifies weaker fibers due to prior damage that fail with minimal repetition of the loads, generating a survival probability curve for the fibers. In this context, we presented the survival probability of the studied fiber population, along with two additional parameters related to data distribution, as it follows a Weibull distribution. Alpha (α) represents the cycle (number of pulls) at which 63.2% of the fibers have already ruptured, while beta (β) characterizes the shape of the distribution and indicates the tendency for the premature rupture of the fibers [38].

The fatigue test curves are shown in Figure 3. In the virgin hair (Figure 3A; Table 2), argan oil had a greater impact than the other two oils. Table 2 shows the values of the α parameters for the oils and the untreated hair. As the load applications progressed, avocado oil stood out with an increase in the hair's resistance, leading to a 26% rise in α from its initial value. Coconut oil had a similar impact on the α parameter, with an increase of 24%. Argan oil caused a dramatic decrease in both the survival probability (SP) and α values, with the latter decreasing by 73%.

Table 2. Characteristic life (α) and shape parameter (β) obtained from the fatigue test for the virgin and bleached hair with and without the oil treatments.

Treatment	Characteristic Life (α)		Shape Parameter (β)	
	Virgin	Bleached	Virgin	Bleached
Not treated hair	5487	2502	0.84	0.68
Argan oil	1430	1146	0.79	0.94
Avocado oil	6953	1803	1.06	0.68
Coconut oil	6804	2249	0.67	0.67

In the bleached hair (Figure 3B; Table 2), the performance of the oils did not indicate an improvement in SP. Nevertheless, argan oil showed a greater influence, with a reduction in the α parameter of 54%. Coconut oil demonstrated less influence, with the α parameter varying by only 10%. Avocado oil exhibited a similar curve profile, but a variation in the α parameter of 28%.

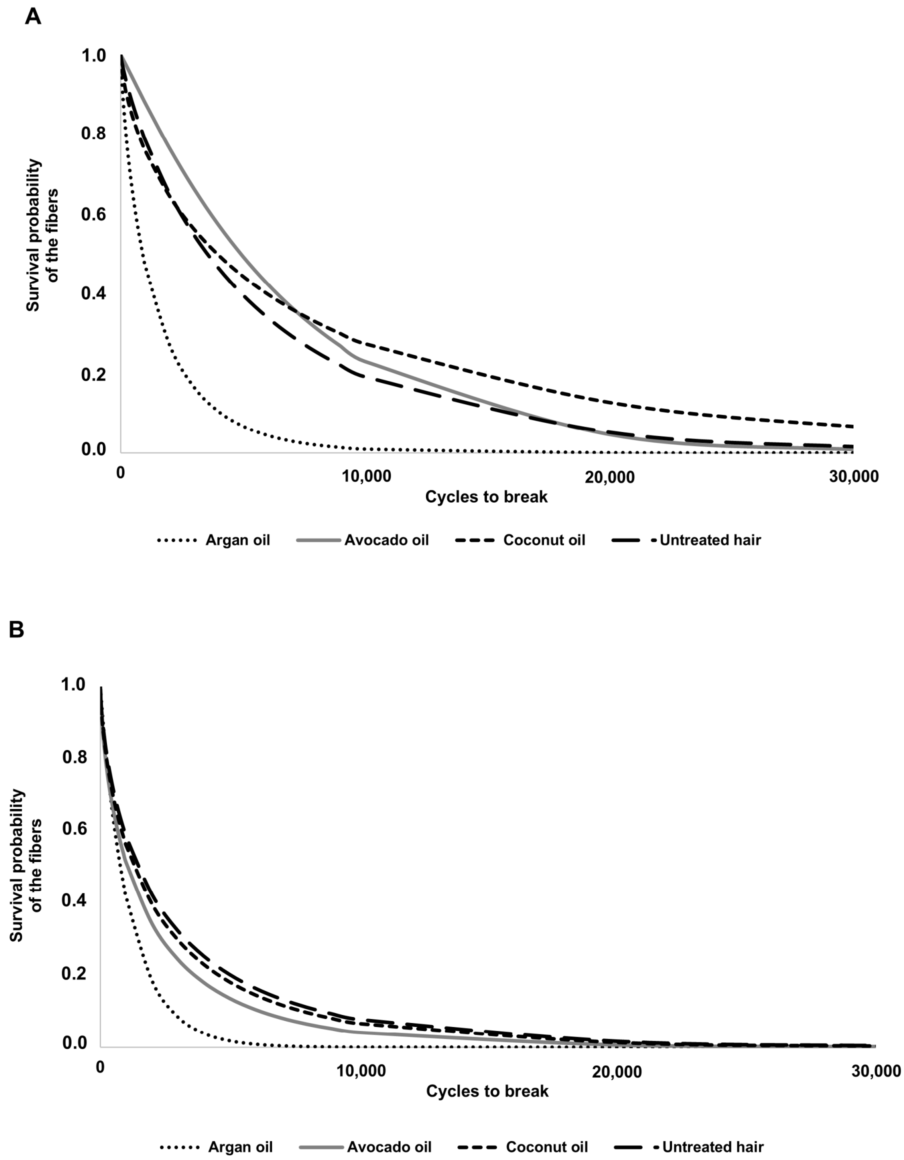

Figure 3. Survival probability results from the fatigue test for the virgin (**A**) and bleached (**B**) hair with and without the oil treatments.

The β parameter decreased with the treatments using argan and coconut oil, while it increased with the treatment using avocado oil in the virgin hair. In the bleached hair, there were no variations in the β parameter with the treatments using coconut and avocado oil, but the argan oil treatment resulted in an increase in β.

Survival data were analyzed using the Kaplan–Meier method. Differences between them were evaluated using the log-rank test. A statistically significant difference was observed between argan oil treatment and the other studied treatments (log-rank test, $p < 0.05$) for both the virgin and bleached conditions.

4. Discussion

In previous work, our group investigated the distribution of triacylglycerols in coconut, argan, and avocado oil [35]. More than 80% of the composition of coconut oil is composed of saturated molecules with chain lengths of up to 40 carbons. Argan oil, on the other hand, aside from consisting of unsaturated molecules, is rich in longer carbon chains, with more than 90% containing 52 carbons or more. These characteristics provide argan oil with a more hydrophilic character, as unsaturation sites are areas of electron concentration that facilitate interactions with water. Meanwhile, more than 90% of the triacylglycerols present in avocado oil consist of more than 52 carbons. This oil presents more unsaturation than coconut oil molecules, but less than argan oil molecules, denoting an intermediate character among the three. These aspects determine how these oils interact with the hair in both the virgin and bleached conditions, given the important chemical changes that occur during the bleaching process. Aside from the damage condition, the morphology of the hair also plays a significant role in the kinetics of diffusion of materials.

The tensile test assesses the parameters of Young's modulus and break stress. Young's modulus characterizes the stiffness of the fibers and is associated with the weak interactions at the molecular level of keratin, which contribute to the elastic behavior of the fiber within extensions of 0 to 5%. Break stress reflects the maximum stress that the fiber can withstand before rupture. Variations in these parameters are a consequence of molecular interactions with keratin and matrix proteins, thus indicating that a given material has reached the cortical portion [20]. Ruptures in the tensile test often occur at extensions exceeding 30% strain, which does not accurately represent the situation to which consumers' hairs are exposed daily. As a result, the fatigue test was developed to allow for the evaluation of the resistance of hair fibers to mechanical stresses with low load levels, but repeated multiple times to simulate their regular routine [38].

The fatigue test is a method used to test a single fiber, where the fiber is repeatedly pulled within its elastic zone with a constant stress or strain. This process causes the formation of small cracks on the hair surface, and the accumulation of stresses leads to their propagation along the fiber until it eventually fails [38].

In virgin hair, the CMC remains intact and is recognized as the preferred pathway for the diffusion of molecules, especially the hydrophobic ones found in oils [39–41]. Our group previously reported [35] that the penetration of these oils into Caucasian straight hair led to an increase in both tensile parameters, Young's modulus and break stress. This increase was attributed to the enhancement of hydrophobicity in the hair cortices, which hindered water absorption. However, the same effect was not observed in textured hair. The results from the wet tensile test clearly showed that the oils could not modify Young's modulus or break stress in this hair type. Despite the identical test conditions, the results varied significantly, suggesting that hair type plays a crucial role in explaining these findings. Textured hair is considered weaker than straight hair, primarily due to the irregularity of its shape, resulting in areas with varying mass accumulation along the twists, thereby increasing its fragility to breakage [20,21,38]. This hair type breaks approximately 10 times faster than straight hair [6]. The addition of water in the test was expected to induce a plasticizing effect, disrupting internal hydrogen bonds and salt ionic interactions, thus leading to a reduction in the tensile test parameters. It was expected that once the oils penetrated the hair, a similar effect to that observed in straight hair would occur. However, the results from the tensile test indicated that the oils could not diffuse efficiently into the textured hair cortex.

When subjected to the fatigue test, the virgin hair showed a positive impact on the α parameter, indicating an improvement in hair resistance with the treatments using avocado and coconut oil, whereas argan oil led to a significant decrease in hair resistance. In our previous study with straight hair [35], we observed a reduction in the α parameter with the absorption of avocado and coconut oil, suggesting competition for interaction sites with keratin and matrix proteins. Despite this, Young's modulus and the break stress of these samples remained unaffected by the presence of the oils, leading us to conclude that there was no deep penetration, but rather an accumulation of the oils in the superficial layers of the hair, which generated a lubricant effect, diminishing the fatigue process of the fibers, thus decreasing their likelihood of fracturing [42]. Furthermore, the presence of argan oil in the outer layers of the hair seemed to increase its affinity to water due to its more polar nature resulting from strong unsaturation, thereby intensifying the plasticizing effect. A similar effect was also observed in straight hair [35].

The clear differences in oil absorption observed between straight and textured virgin hairs, particularly evident in the treatments using avocado and coconut oil, can be attributed to the types of cortical cells and their distribution within the cortical area of these fibers. The orthocortex, a region of the cortical zone characterized by a low presence of matrix (amorphous material) compared to intermediate filaments (IFs, crystalline material), exhibits fewer cross-links between the matrix and IFs. This results in a lower density in the orthocortex, making it more susceptible to molecule diffusion and swelling than the paracortex. The paracortex, on the other hand, is a region that contains more matrix compared to IFs, leading to a greater degree of cross-links and forming a high-density area that is less favorable to molecule movement [25,26]. These two different zones are present in both hair types, straight and textured hair. The variation in oil penetration seems to depend on how these zones are arranged in the cortical region. In straight hair, the orthocortex is located directly below the cuticles, in the outermost portion of the cortex, surrounding the right-below paracortex in a ring format. In textured hair, the orthocortex and paracortex cells are arranged bilaterally, with the orthocortex concentrated in the external portion of the curl, and the paracortex in the internal portion [25,26]. Hornby et al. [33] reported that the diffusion coefficient of the orthocortex area is approximately one order of magnitude greater than that of the paracortex. Considering these configurations, molecules that penetrate straight hair will primarily diffuse through the orthocortex along the entire perimeter of the fibers, after passing through the cuticular barrier, and may spread throughout the entire orthocortical ring. Conversely, molecules that penetrate textured hair will encounter the orthocortex in roughly half of the perimeter and the paracortex in the other half, posing a diffusion obstacle.

The damage caused by the bleaching process is well-documented and involves the loss of lipid and protein content, the cleavage of disulfide bonds, and the generation of charges throughout the hair structure. These changes drastically alter the hair's chemical character from being more hydrophobic in its virgin state to becoming more hydrophilic after bleaching [1–5]. The affinity with external materials is influenced by these changes in the hair's state. MALDI–TOF analysis revealed that the components of argan oil were visualized with greater intensity compared to avocado and coconut oil. Coconut oil showed the least presence in the bleached hair, while avocado oil's presence was moderate. The presence of argan and coconut oil in the hair structure did not affect its Young's modulus or break stress, indicating that their diffusion into the hair was not sufficient to create new interactions that could partially restore the mechanical properties or return the hair's hydrophobicity to repel moisture. The avocado oil also did not impact Young's modulus, but increased the hair's break stress. This effect is more likely due to the partial restoration of lost hydrophobicity, protecting the hair from water absorption. This protective effect is similar to what was described by Keis et al. [43], who observed an obstruction effect caused by oil treatments, which delayed the water uptake by the hair.

The fatigue test results for the bleached hair showed that the oils promoted an opposite effect to what was observed for the virgin hair, with a reduction in the α parameter and SP. The impact on the α parameter correlated with the intensity of the oils detected in the hair cortex via MALDI–TOF analysis. Argan oil presented higher intensity and had a greater impact on the α parameter; avocado oil, with intermediate intensity, had a moderate impact on α; and coconut oil, with lower intensity, had less impact on the α parameter. This strongly suggests that these variations are directly associated with the presence of the oils in the hair. Despite penetrating the cortex of the bleached hair, the oils were unable to sufficiently stabilize the charges and establish interactions to enhance mechanical resistance. Moreover, the tensile test results indicate that the oils did not affect the hair's response to the added water, as all samples were similarly affected by humidity.

Once again, the arrangement of the textured hair cortex seems to have influenced the diffusion of the oils and contributed to the observed differences. Due to the distinct diffusion coefficients of the orthocortex and paracortex, along with their varying abilities to swell and accommodate external molecules, the oils diffused into the hair and may have acquired an irregular distribution, likely accumulating more in the orthocortex than in the paracortex, creating a gradient of hydrophobicity within the hair. The irregular dispersion of the oils could not establish consistent protection against humidity, resulting in a plasticizing effect when interacting with the hair, as observed in the tensile test results. Similar to its effects on virgin and straight hair in previous tests [35], argan oil increased the hair's affinity with water during the fatigue test, leading to a greater loss of resistance in the treated hair. Interestingly, avocado oil may have had a similar protective effect due to its irregular distribution within the hair, as observed in the tensile test, but this effect was not evident in the fatigue test results. The β parameter remained unchanged with the presence of coconut and avocado oil, but increased with argan oil treatment, which was unexpected given the decrease in α. We concluded that the β parameter may not be as crucial for analyzing oil penetration.

While Marsh et al. [44] found that oil treatments improved single-fiber fatigue strength, our findings showed that oil treatment did not significantly improve tensile strength in textured hair. The contrasting findings highlight the complexity of hair–oil interactions and the importance of considering hair type in these studies.

5. Conclusions

The diffusion of materials into hair is a strategy aimed at enhancing the resistance of fragile textured hair and addressing damage caused by oxidative stress. Vegetable oils play a significant role in this regard, as they are commonly associated with hair benefits such as strengthening. Argan, avocado, and coconut oil have been studied regarding their ability to penetrate the structure of bleached textured hair using MALDI–TOF analysis, and additionally to enhance hair resistance to mechanical stress through tensile and fatigue tests.

The results of the MALDI–TOF analysis revealed the presence of these oils in the hair cortex, with argan oil components showing greater intensities and coconut oil exhibiting the least intensity among the identified oils inside the hair. However, their effect, as evidenced by the tensile and fatigue tests, did not significantly influence the mechanical parameters of either the virgin or bleached states of the textured hair. In comparison with previous results from our group's work on straight hair, it was observed that avocado and coconut oil not only penetrated, but also positively influenced the tensile parameters of the virgin hair and contributed to protecting the bleached hair from humidity. The Raman spectroscopy results indicated that the oils were able to penetrate deep into the cortex in both hair states.

Conversely, this was not observed in the case of textured hair. We hypothesize that these differences stem from the distinct configuration of the cortices in these hair types. The unique arrangement of the orthocortex and paracortex, with low and high densities, respectively, disposed bilaterally, leads to irregular diffusion with varying kinetics on each side. This results in external molecules occupying the cortex heterogeneously, thereby reducing the efficiency of their effects. In contrast, straight hair, with its orthocortex and paracortex distributed in a ring format, where the former encircles the latter, tends to have a more homogeneous occupancy and effect.

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References

1. Tate, M.L.; Kamath, Y.K.; Ruetsch, S.B. Quantification and prevention of hair damage. *J. Soc. Cosmet. Chem.* **1993**, *44*, 347–371.
2. Wortmann, F.-J.; Springob, C.; Sendelbach, G. Investigations of Cosmetically Treated Human Hair by Differential Scanning Calorimetry in Water. *J. Cosmet. Sci.* **2002**, *53*, 219–228. [PubMed]
3. Wortmann, F.-J.; Sendelbach, G.; Popescu, C. Fundamental DSC Investigations of A-Keratinous Materials as Basis for the Interpretation of Specific Effects of Chemical, Cosmetic Treatments on Human Hair. *J. Cosmet. Sci.* **2007**, *58*, 311–317.
4. Fujii, T.; Ito, Y.; Watanabe, T.; Kawasoe, T. Effects of Oxidative Treatments on Human Hair Keratin Films. *J. Cosmet. Sci.* **2012**, *63*, 15–25.
5. Grosvenor, A.J.; Deb-Choudhury, S.; Middlewood, P.G.; Thomas, A.; Lee, E.; Vernon, J.A.; Woods, J.L.; Taylor, C.; Bell, F.I.; Clerens, S. The physical and chemical disruption of human hair after bleaching—Studies by transmission electron microscopy and redox proteomics. *Int. J. Cosmet. Sci.* **2018**, *40*, 536–548. [CrossRef]
6. Cornwell, P.; Malinauskyte, E. Defying damage. *Cosmet. Toilet.* **2020**, *135*, 20–29.
7. Bryant, H.; Porter, C.; Yang, G. Curly hair: Measured differences and contributions to breakage. *Int. J. Dermatol.* **2012**, *5*, 8–11. [CrossRef]
8. Ruetsch, S.B.; Kamath, Y.K. Penetration of cationic conditioning compounds into hair fibers: A TOF-SIMS approach. *J. Cosmet. Sci.* **2005**, *56*, 323–330. [CrossRef]
9. Marsh, J.M.; Huangb, S.; Whitakera, S.; Guagliardob, P.; Lucasa, R.L.; Arcaa, H.C.; Jiangb, H. High-resolution visualization of cosmetic active compounds in hair using nanoscale secondary ion mass spectrometry. *Colloids Surf. B Biointerfaces* **2019**, *174*, 563–568. [CrossRef] [PubMed]
10. Malinauskyte, E.; Shrestha, R.; Cornwell, P.A.; Gourion-Arsiquaud, S.; Hindley, M. Penetration of different molecular weights hydrolysed keratins into hair fibres and their effects on the physical properties of textured hair. *Int. J. Cosmet. Sci.* **2020**, *43*, 26–37. [CrossRef]
11. Rele, A.S.; Mohile, R. Effect of coconut oil on prevention of hair damage. *Part I. J. Cosmet. Sci.* **1999**, *50*, 327–339.
12. Rele, A.S.; Mohile, R. Effect of mineral oil, sunflower oil, and coconut oil on prevention of hair damage. *J. Cosmet. Sci.* **2003**, *54*, 175–192. [PubMed]
13. Ruetsch, S.B.; Kamath, Y.; Rele, A.S.; Mohile, R.B. Secondary ion mass spectrometric investigation of penetration of coconut and mineral oils into human hair fibers: Relevance to hair damage. *J. Cosmet. Sci.* **2001**, *52*, 169–184. [PubMed]
14. Srivastav, A.; Dandekar, P.; Jain, R. Penetration study of oils and its formulations into the human hair using confocal microscopy. *J. Cosmet. Dermatol.* **2019**, *18*, 1947–1954. [CrossRef]
15. Keis, K.; Persaud, D.; Kamath, Y.K.; Rele, A.S. Investigation of penetration abilities of various oils into human hair fibers. *J. Cosmet. Sci.* **2005**, *56*, 283–295. [CrossRef]
16. Manoel, I. *Manual dos Cabelos: O Poder dos Óleos*; Laços: Sao Paulo, Brazil, 2003.
17. Wolfram, L.J. Human hair: A unique physicochemical composite. *J. Am. Acad. Dermatol.* **2003**, *48*, 106–114. [CrossRef]
18. Dawber, R. Hair: Its structure and response to cosmetic preparations. *Clin. Dermatol.* **1996**, *14*, 105–112. [CrossRef] [PubMed]
19. Laatsch, C.N.; Durbin-Johnson, B.P.; Rocke, D.M.; Mukwana, S.; Newland, A.B.; Flagler, M.J.; Davis, M.G.; Eigenheer, R.A.; Phinney, B.S.; Rice, R.H. Human hair shaft proteomic profiling: Individual differences, site specificity and cuticle analysis. *Peer J.* **2014**, *2*, e506. [CrossRef]
20. Robbins, C.R. *Chemical and Physical Behavior of Human Hair*, 5th ed.; Springer: Berlin/Heidelberg, Germany; New York, NY, USA, 2012.
21. Aguh, C.; Okoye, G.A. *Fundamentals of Ethnic Hair, the Dermatologist's Perspective*; Springer International Publishing: Cham, Switzerland, 2017.
22. Yang, F.C.; Zhang, Y.; Rheinstadter, M.C. The structure of people's hair. *Peer J.* **2014**, *2*, e619. [CrossRef]
23. Khumalo, N.P.; Stone, J.; Gumedze, F.; McGrath, E.; Ngwanya, M.R.; Berker, D. 'Relaxers' damage hair: Evidence from amino acid analysis. *J. Am. Acad. Dermatol.* **2010**, *62*, 402–408. [CrossRef]
24. Bryson, W.G.; Harland, D.P.; Caldwell, J.P.; Vernon, J.A.; Walls, R.J.; Woods, J.L.; Nagase, S.; Itou, T.; Koike, K. Cortical cell types and intermediate filament arrangements correlate with fiber curvature in Japanese human hair. *J. Struct. Biol.* **2009**, *166*, 46–58. [CrossRef] [PubMed]
25. Thibaut, S.; Barbarat, P.; Leroy, F.; Bernard, B.A. Human hair keratin network and curvature. *Int. J. Dermatol.* **2007**, *46*, 7–10. [CrossRef]

26. Kajiura, Y.; Watanabe, S.; Itou, T.; Nakamura, K.; Iida, A.; Inoue, K.; Yagi, N.; Shinohara, Y.; Amemiya, Y. Structural analysis of human hair single fibers by scanning microbeam SAXS. *J. Struct. Biol.* **2006**, *155*, 438–444. [CrossRef] [PubMed]
27. Harland, D.P.; Vernon, J.A.; Woods, J.L.; Nagase, S.; Itou, T.; Koike, K.; Scobie, D.A.; Grosvenor, A.J.; Dyer, J.M.; Clerens, S. Intrinsic curvature in wool fibers is determined by the relative length of orthocortical and paracortical cells. *J. Exp. Biol.* **2018**, *221*, jeb172312. [CrossRef]
28. Marti, M.; Barba, C.; Manich, A.M.; Rubio, L.; Alonso, C.; Coderch, L.T. The influence of hair lipids in ethnic hair properties. *Int. J. Cosmet. Sci.* **2016**, *38*, 77–84. [CrossRef] [PubMed]
29. Cruz, C.F.; Fernandes, M.M.; Gomes, A.C.; Coderch, L.; Marti, M.; Mendez, S.; Gales, L.; Azoia, N.G.; Shimanovich, U.; Cavaco-Paulo, A. Keratins and lipids in ethnic hair. *Int. J. Cosmet. Sci.* **2013**, *35*, 244–249. [CrossRef]
30. Lourenço, C.B.; Fava, A.L.M.; Santos, E.M.; Macedo, L.M.; Tundisi, L.L.; Ataide, J.A.; Mazzola, P.G. Brief descriptions of the principles of prominent methods used to study the penetration of materials into human hair and a review of examples of their use. *Int. J. Cosmet. Sci.* **2021**, *43*, 113–122. [CrossRef] [PubMed]
31. Philipsen, M.H.; Haxen, E.R.; Manaprasertsak, A.; Malmberg, P.; Hammarlund, E.U. Mapping the chemistry of hair strands by mass spectrometry imaging—A review. *Molecules* **2021**, *26*, 7522. [CrossRef]
32. Harvey, A.; Carr, C.M. Time of flight secondary ion mass spectrometry (TOF-SIMS) analysis of the application of a cationic conditioner to clean hair. *J. Cosmet. Sci.* **2004**, *55*, 265–279.
33. Hornby, S.B.; Appa, Y.; Ruetsch, S.; Kamath, Y. Mapping penetration of cosmetic compounds into hair fibers using time-of-flight secondary ion mass spectroscopy (TOF-SIMS). *IFSCC Mag.* **2005**, *8*, 99–104.
34. American Oil Chemists' Society. *Official Methods and Recommended Practices of the American Oil Chemists' Society*, 5th ed.; American Oil Chemists' Society: Champaign, IL, USA, 2009.
35. Lourenço, C.B.; Gasparin, R.M.; Thomaz, F.M.; Silva, G.C.; Martin, A.A.; Santos, A.C.P.; Mazzola, P.G. Impact of hair damage on the penetration profile of coconut, avocado, and argan oils into Caucasian hair fibers. *Cosmetics* **2024**, *11*, 64. [CrossRef]
36. Loussouarn, G.; Garcel, A.L.; Lozano, I.; Collaudin, C.; Porter, C.; Panhard, S.; Saint-Léger, D.; Mettrie, R. Worldwide diversity of hair curliness: A new method of assessment. *Int. J. Dermatol.* **2007**, *46*, 2–6. [CrossRef] [PubMed]
37. Sartori, T.P. Development of Hair Analysis Methods by MALDI-TOF-MS and MALDI-MSI and Evaluation of Cosmetic Treatments. Ph.D. Thesis, Sao Paulo University, Sao Carlos, Brazil, 2024.
38. Evans, T. Fatigue testing of hair—A statystical approach to hair breakage. *J. Cosmet. Sci.* **2009**, *60*, 599–616. [PubMed]
39. Potsch, L.; Moeller, M.R. On Pathways for Small Molecules into and Out of Human Hair Fibers. *J. Forensic Sci.* **1996**, *41*, 21–125. [CrossRef]
40. Kelch, A.; Wessel, S.; Will, T.; Hintze, U.; Wepf, R.; Wiesendanger, R. Penetration Pathways of Fluorescent Dyes in Human Hair Fibres Investigated by Scanning Near-Field Optical Microscopy. *J. Microsc.* **2000**, *200*, 179–186. [CrossRef]
41. Gummer, C.L. Elucidating penetration pathways into the hair fiber using novel microscopic techniques. *J. Cosmet. Sci.* **2001**, *52*, 265–280.
42. Evans, T. An unifying theory for visualizing the causes of hair breakage and subsequent strategies for mitigation. *J. Cosmet. Sci.* **2017**, *68*, 137–140.
43. Keis, K.; Huemmer, C.L.; Kamath, Y. Effect of oil films on moisture vapor absorption on human hair. *J. Cosmet. Sci.* **2007**, *58*, 135–145. [CrossRef]
44. Marsh, J.; Whitaker, S.; Felts, T.; Cowans, C.; Gupta, S.; Masirevic, S.; Fang, R.; Simmonds, M.S.J.; Chen, G.; Jiang, H. Penetration of oils into hair. *Int. J. Cosmet. Sci.* **2024**, *46*, 905–917. [CrossRef]