UNIVERSIDADE ESTADUAL DE CAMPINAS
FACULDADE DE CIÊNCIAS FARMACÊUTICAS

CAROLINA BOTELHO LOURENÇO

AVALIAÇÃO DA EFICÁCIA DE TRATAMENTOS COSMÉTICOS EM CABELO HUMANO ATRAVÉS DA APLICAÇÃO DE TÉCNICAS SOFISTICADAS DE CARACTERIZAÇÃO DE MATERIAIS

CAMPINAS
2023

CAROLINA BOTELHO LOURENÇO

AVALIAÇÃO DA EFICÁCIA DE TRATAMENTOS COSMÉTICOS EM CABELO HUMANO ATRAVÉS DA APLICAÇÃO DE TÉCNICAS SOFISTICADAS DE CARACTERIZAÇÃO DE MATERIAIS

Tese apresentada à Faculdade de Ciências Farmacêuticas da Universidade Estadual de Campinas como parte dos requisitos exigidos para a obtenção do título de Doutora em Ciências, na área de Fármacos, Medicamentos e Insumos para a Saúde.

Orientadora: Profa. Dra. Priscila Gava Mazzola

ESTE TRABALHO CORRESPONDE À VERSÃO FINAL DA TESE DEFENDIDA PELA ALUNA CAROLINA BOTELHO LOURENÇO, ORIENTADA PELA PROFA. DRA. PRISCILA GAVA MAZZOLA.

CAMPINAS

2023

Ficha catalográfica
Universidade Estadual de Campinas
Biblioteca da Faculdade de Ciências Médicas
Maristella Soares dos Santos - CRB 8/8402

Lourenço, Carolina Botelho, 1983-
L934a Avaliação da eficácia de tratamentos cosméticos em cabelo humano através da aplicação de técnicas sofisticadas de caracterização de materiais / Carolina Botelho Lourenço. – Campinas, SP : [s.n.], 2023.

Orientador: Priscila Gava Mazzola.
Tese (doutorado) – Universidade Estadual de Campinas, Faculdade de Ciências Farmacêuticas.

1. Cabelo. 2. Testes mecânicos. 3. Espectroscopia Raman. 4. Cosméticos. 5. Espectrometria de massas por ionização e dessorção a laser assistida por matriz. I. Mazzola, Priscila Gava. II. Universidade Estadual de Campinas. Faculdade de Ciências Farmacêuticas. III. Título.

Informações Complementares

Título em outro idioma: Evaluation of the efficacy of cosmetic treatments on human hair through the application of sophisticated techniques for material characterization
Palavras-chave em inglês:
Hair
Mechanical tests
Raman spectroscopy
Cosmetics
Spectrometry, Mass, Matrix-assisted laser desorption-ionization
Área de concentração: Ciências Farmacêuticas: insumos farmacêuticos naturais, biotecnológicos e sintéticos
Titulação: Doutora em Ciências
Banca examinadora:
Priscila Gava Mazzola [Orientador]
Gislaine Ricci Leonardi
Guilherme Diniz Tavares
Paulo César Pires Rosa
Marlus Chorilli
Data de defesa: 07-02-2023
Programa de Pós-Graduação: Ciências Farmacêuticas

Identificação e informações acadêmicas do(a) aluno(a)
- ORCID do autor: https://orcid.org/0000-0002-1547-2225
- Currículo Lattes do autor: http://lattes.cnpq.br/2742353912883111

UNIVERSIDADE ESTADUAL DE CAMPINAS

FACULDADE DE CIÊNCIAS FARMACÊUTICAS

Autora: Carolina Botelho Lourenço

Orientadora: Profª Drª Priscila Gava Mazzola

Tese aprovada em 7 de fevereiro de 2023

Comissão Examinadora

Profª Drª Priscila Gava Mazzola

Profª Drª Gislaine Ricci Leonardi

Prof. Dr. Guilherme Diniz Tavares

Prof. Dr. Paulo César Pires Rosa

Prof. Dr. Marlus Chorilli

A ata de defesa com as respectivas assinaturas dos membros encontra-se no SIGA/Sistema de Fluxo de Dissertação/Tese e na Secretaria de Pós-Graduação da Faculdade de Ciências Farmacêuticas.

Campinas, 7 de fevereiro de 2023.

EPÍGRAFE

“A vida é sempre o resultado de nossa própria escolha”

(Emmanuel, Chico Xavier)

“Quem quer passar além do Bojador
Tem que passar além da dor.
Deus ao mar o perigo e o abismo deu,
Mas nele é que espelhou o céu.”

(Fernando Pessoa)

AGRADECIMENTOS

“Ninguém cruza nosso caminho por acaso e nós não entramos na vida de alguém sem nenhuma razão”. Este ensinamento de Chico Xavier esteve presente em toda a jornada, na concretização deste trabalho. A vida coloca em nosso caminho as pessoas e os recursos de que necessitamos em nossa evolução. Eu fui extremamente agraciada por um sem-número de recursos e pessoas que contribuíram imensamente para a chegada deste momento.

Meus agradecimentos começam pelos meus pais, que dedicaram suas vidas ao trabalho para que eu e meus irmãos pudéssemos ter a oportunidade de estudar. Em especial à minha mãe, que além do trabalho no banco, enfrentava uma rotina pesada de mãe de três mais o trabalho com os cuidados de casa. Mesmo assim, nunca nos faltou um lápis ou uma borracha para a escola. Também nunca nos faltou acolhimento, crédito e apoio incondicionais. Ela é a responsável primária pela finalização deste projeto.

Agradeço ao meu irmão Carlos Eduardo, que foi o segundo principal influenciador, depois dos meus pais, para o estudo. Ele foi a primeira pessoa que eu vi dedicar horas ao estudo e que eu vi obter sucesso através dele. E também veio dele o principal incentivo ao mestrado.

Agradeço à minha orientadora, Professora Dra. Priscila Gava Mazzola, por continuar me dando crédito, continuar apostando na minha proposta de tocar o trabalho da empresa e do projeto paralelamente e confiar que eu alcançaria os objetivos propostos. Ainda, agradeço porque além de orientadora, sempre vi nela uma amiga e um exemplo de mulher inteligente, ativa, dedicada, lutadora, competente e humana.

Agradeço ao meu antigo gestor, Adelino Nakano, que acreditou antes de mim que o doutorado podia ser o próximo degrau na minha trajetória profissional, e me deu a oportunidade e as condições necessárias para que eu iniciasse este projeto.

Agradeço a Symrise, representada pelos gestores Eder Ramos, Alencar Lima, Gerhard Schmaus e Imke Meyer, que entenderam a importância do meu desenvolvimento acadêmico e me permitiram a oportunidade de desenvolver este projeto.

Agradeço às minhas queridas colegas de trabalho Rebeca Gasparin e Fernanda Thomaz, parceiras deste e de outros trabalhos, que muito mais do que colegas, foram apoio, guia, suporte, orientação, contraponto, moderação, mediação e colaboração em todas as fases.

Agradeço ao meu marido, que segurou as pontas em casa e com nossa filha todas as vezes que precisei me ausentar para trabalhar neste projeto. Agradeço também por sempre ter me incentivado e me dado forças para continuar, por nunca ter diminuído minhas aspirações, por nunca ter desacreditado meus planos e sonhos, por estar comigo em todas as conquistas e seguir comigo a jornada da vida.

Agradeço a minha filha Alice, luz dos meus olhos, amor da minha vida, por quem toda a luta e todo o esforço se justificam, que tanto veio me ensinar, que me desafia todos os dias a buscar conhecimento sempre, que me inspira a ser exemplo, a constantemente ser melhor em tudo que faço, que me impulsiona a crescer como estudante, profissional, mãe e ser humano.

Agradeço aos familiares e amigos queridos, por estarem ao meu lado, por acreditarem na minha capacidade de realização, por respeitarem minhas limitações, pelas palavras amigas, pelas risadas e bons momentos que tornaram a jornada mais leve.

Agradeço a Jesus e aos espíritos iluminados cuja presença senti em todos os passos desta caminhada.

Por fim, agradeço a Deus, que tudo isso permitiu; que guiou os caminhos, que viabilizou os recursos, que me concedeu saúde, que caminhou comigo, me mostrou as soluções, compreendeu minhas falhas, me trouxe entendimento, escutou meu choro e acolheu minhas dores, me iluminou as ideias, me concedeu uma vida e um propósito.

RESUMO

Descolorir o cabelo é uma escolha muito popular para variar a aparência. Porém, o processo de oxidação provoca perda da resistência mecânica em todos os tipos de cabelos, e, particularmente para os cabelos texturizados, intensifica uma condição de fragilidade natural. A mitigação da condição de dano do cabelo requer a penetração de substâncias externas para estabilizar as ligações clivadas, recompor lipídios e proteínas, restaurar a hidrofobicidade e recuperar as propriedades mecânicas do cabelo. Os óleos vegetais, em geral, evidenciam um rol de características vantajosas muito desejadas pelos consumidores, dados os benefícios associados a esses tratamentos à fibra capilar. Os óleos de argan e abacate foram estudados em comparação com o óleo de coco, mais conhecido, quanto à sua capacidade de se difundir pelo córtex capilar e possíveis efeitos. A espectroscopia Raman e MALDI-TOF/TOF foram realizadas para investigar a capacidade de penetração desses três óleos em cabelos lisos e texturizados, respectivamente, nas condições virgem e descolorida. Além disso, testes de tração e fadiga foram feitos para investigar seu efeito, uma vez difundidos para o interior dos cabelos. Os óleos foram aplicados em quantidade suficiente diretamente nas mechas limpas e deixados por 24 horas a 25°C. Os resultados mostram que os três óleos se difundiram e interagiram com a região cortical do cabelo liso. Nos cabelos lisos virgens, os óleos de coco e abacate reforçaram a barreira hidrofóbica do complexo da membrana celular, evitando que a água perturbe intensamente as propriedades mecânicas. O óleo de argan, devido ao alto grau de insaturação de suas cadeias graxas, causa aumento a absorção de água, levando à perda da resistência do cabelo. Quando descoloridos, a hidrofilicidade do cabelo liso é elevada, o que determina maior afinidade com o óleo de argan. Consequentemente, a afinidade com a água também é elevada, levando ao aumento da fragilidade ao estresse mecânico. Nos cabelos virgens texturizados, o tratamento com os óleos não conseguiu alterar suas propriedades mecânicas. O teste de fadiga mostrou um aumento na resistência do cabelo provavelmente devido a um efeito de lubrificação das cutículas. Nos cabelos descoloridos texturizados, notou-se redução da resistência a partir do tratamento com os óleos. A difusão dos óleos vegetais no cabelo depende de características deste quando íntegro ou quimicamente danificado, bem como de sua morfologia, que influencia a cinética de difusão e determina a intensidade dos efeitos das moléculas externas. Por fim, a água é um fator impactante que afeta o resultado final de um tratamento cosmético.

Palavras-chave: cabelo, descoloração, óleo de argan, óleo de abacate, óleo de coco, propriedades mecânicas, força, cabelo texturizado, cosméticos.

ABSTRACT

Discoloring hair is a very popular choice to vary the style. However, the oxidation process provokes loss of the resistance in all types of hair, and particularly intensifies a condition of natural fragility of the textured hair. The mitigation of the damaged condition of the hair requires penetration of external substances to stabilize broken bonds sites, return lipids and proteins, restore hydrophobicity, and recover hair mechanical properties. Vegetable oils, in general, evidence a list of advantageous characteristics much desired by consumers, given the benefits associated with these treatments to the hair fiber. Argan and avocado oils were studied in comparison with better known coconut oil, regarding their ability to diffuse through the hair cortex and possible effects. Raman spectroscopy and MALDI-TOF/TOF were carried out to investigate the penetration ability of these three oils into the virgin and bleached straight and textured hair, respectively. Moreover, tensile and fatigue tests were done to investigate their effect once penetrated the hairs structures. The oils were applied in sufficient amount directly to the clean tresses and allowed for 24 h at 25 °C. Results show that the three oils can diffuse and interact with the cortical region of the straight hair. In the straight virgin hair, coconut and avocado oils reinforce the hydrophobic barrier of the cellular membrane complex, preventing water from intense perturbation of the mechanical properties. In turn, argan oil, due to the high degree of unsaturation of its fatty acid chains, increase water absorption, leading to loss in the hair resistance. When bleached, the hydrophilicity of the straight hair is raised, which determines more affinity for argan oil. Consequently, the affinity with the water also is elevated, leading to increased fragility to the mechanical stress. In the textured virgin hair, the tests showed that the treatment with the oils could not alter its mechanical properties. Fatigue test showed an increase in the hair resistance most probably resulting from a lubrication effect in the outermost portions of the cortex and cuticles. In the textured bleached hair, a reduction of resistance was noted from the treatment with the oils. The affinity of vegetable oils to the hair is dependent on its characteristics when integrate or chemically damaged as well as its morphology, which influences the diffusion kinetics and determines the intensity of the external molecules' effects. Finally, water is an impacting factor that affects the final results of a cosmetic treatment.

Keywords: hair, bleaching, argan oil, avocado oil, coconut oil, mechanical properties, strength, textured hair, cosmetics.

SUMÁRIO

1. INTRODUÇÃO

O cabelo / pelo tem função biológica de proteção contra fatores externos e termorregulação do corpo (1). Como os mamíferos organizam suas vidas em sociedades, o cabelo adicionalmente influencia as interações entre os indivíduos. Considerando que os seres humanos usam roupas e não vivem diretamente expostos às intempéries climáticas, a função de proteção e termorregulação do cabelo tornou-se secundária e ele acabou por assumir um papel mais importante no âmbito das relações sociais, o que por sua vez, num mundo movido por aceleradas mudanças como o atual, impulsiona consumidores a constantemente buscar aparências modernas e diferenciadas. Esta busca mobiliza a indústria cosmética a ter como um de seus mais importantes alvos os cuidados com as fibras capilares. O mercado global de produtos para o cabelo é de 75 milhões de euros cresceu incríveis 37% nos últimos 10 anos (2). No entanto, restam diversos temas ligados à ciência de cabelo ainda por serem estudados.

A capacidade de difusão de materiais na haste capilar está relacionada à eficácia dos tratamentos cosméticos. Mas, sua chegada às regiões internas dos fios pode promover benefícios ou prejuízos à estrutura. Em xampus, surfactantes de baixo peso molecular podem atingir o córtex e perturbar o arranjo das pontes de hidrogênio e ligações salinas (3). Os tratamentos para modificar a forma ou a cor do cabelo, como descoloração, permanentes, alisantes e tinturas, atingem o córtex e clivam as pontes dissulfeto, gerando aumento da sua hidrofilicidade, o que por sua vez leva a maiores taxas de intumescimento e maior difusão de materiais (4, 5). Por outro lado, os compostos condicionantes catiônicos, principais constituintes dos condicionadores, podem penetrar no córtex protegendo o cabelo da fadiga e estresses mecânicos (6). Os óleos saturados e monoinsaturados são capazes de penetrar no cabelo e impedir a entrada de água, reduzindo consequentemente os índices de intumescimento (7). Outros ingredientes presentes nas formulações capilares como substâncias proteicas também se difundem e proporcionam bons efeitos de finalização (8).

Os óleos vegetais são uma escolha popular para atender às necessidades de cabelos danificados, já que são associados a benefícios para o cabelo, como redução do ressecamento, hidratação, nutrição, fortalecimento, equilíbrio de sebo, aprimoramento de máscaras de tratamento, proteção pré-lavagem, controle de frizz, reparação de pontas duplas, e proteção contra sal e cloreto (9). Não apenas a expectativa de eficácia é um fator para o emprego de óleos vegetais em produtos capilares, mas também a necessidade de produtos renováveis e verdes justifica o crescente interesse da indústria por esses componentes.

As interações entre o cabelo e as moléculas externas são orientadas por sua estrutura, afinidade com o complexo da membrana celular, condição do cabelo e sua morfologia (10-16).

Existem particularidades nos cabelos liso e texturizado que influenciam o modo como os diferentes materiais se difundem nessas estruturas e consequentemente o resultado que se obtém dessas interações. Alguns tipos de óleos vegetais já foram estudados pela indústria cosmética, como girassol, oliva, farelo de arroz, gergelim, mostarda, coco, jojoba e amêndoa (13-16). Porém, existem alguns amplamente posicionados como agentes de cuidados com os cabelos, ainda carentes de estudos mais aprofundados sobre seus efeitos na estrutura interna do cabelo e possíveis benefícios, como é o caso dos óleos de argan e abacate.

O estudo da penetração de moléculas no cabelo requer métodos mais sofisticados que permitam a investigação dos eventos em nível molecular, como microscopia de alta resolução, espectroscopia, espectrometria, tomografia e outros (17-24), que frequentemente envolvem a laboriosa manipulação das amostras e/ou um alto custo de operação, reduzindo as chances de sua ampla aplicação no contexto industrial.

Diante do exposto, este estudo teve como objetivo discutir a penetração dos óleos de argan e abacate em comparação com o óleo de coco, mais conhecido, nas fibras capilares lisas e texturizadas, nas condições de integridade e quimicamente danificadas, utilizando uma combinação de técnicas tradicionais e modernas comumente empregadas na ciência de cabelos, particularmente espectroscopia Raman, MALDI-TOF, testes de tração e fadiga.

Para tanto, o presente trabalho encontra-se dividido da seguinte forma:

- Capítulo I - 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 (artigo publicado na *International Journal of Cosmetic Science*, *DOI*: 10.1111/ics.12683).
- Capítulo II - penetration profile of coconut, avocado and argan oils into Caucasian hair fibers.
- Capítulo III - Penetration of vegetal oils into textured hair fibers: a joint of MALDI-TOF analysis and mechanical measurements.

Desta forma, determinadas informações que constam em um capítulo poderão estar repetidas nos capítulos seguintes, assim como na discussão e na conclusão. As referências de cada artigo encontram-se ao final de cada capítulo, formatadas de acordo com as normas das revistas científicas a que os artigos foram/serão submetidos. Ao final do trabalho, há lista de referências usadas na introdução e discussão.

2. OBJETIVO

2.1. Objetivo Geral

Estudar a eficácia dos óleos vegetais de argan, abacate e coco em cabelos liso e texturizado com alguns níveis de dano (virgem e com descoloração), através de técnicas sofisticadas de caracterização de materiais, tais como espectroscopia Raman e tempo de voo de ionização por dessorção a laser assistida por matriz (MALDI-TOF) e técnicas mais tradicionais de estudos mecânicos em fibras proteicas como teste de tensão-deformação de teste cíclico de fadiga.

2.2. Objetivos Específicos

- Avaliar perfil de penetração de ativos cosméticos em cabelo liso e texturizado nas condições íntegro e quimicamente danificado.
- Avaliar efeito dos ativos cosméticos nas propriedades mecânicas dos fios em cabelo liso e texturizado nas condições íntegro e quimicamente danificado.
- Avaliar efeito dos ativos cosméticos na resistência e probabilidade de sobrevivência dos fios frente a estresse mecânico repetido em cabelo liso e texturizado nas condições íntegro e quimicamente danificado.

3. EXECUÇÃO

CAPÍTULO I. “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”

Carolina Botelho Lourenço, Ana Laura Masquetti Fava, Erica Mendes dos Santos, Lucas Malvezzi de Macedo, Louise Lacalendola Tundisi, Janaína Artem Ataide and Priscila Gava Mazzola

International Journal of Cosmetic Science, 43, 113–122, 2021.

DOI: 10.1111/ics.12683

ABSTRACT

Consumers are attracted by modern and different look, which motivates the search for novel hair treatments. Distinct treatments are able to penetrate the hair shaft. The entrance of materials in the cortex can either promote benefits or injuries. Different techniques have been explored to proper evaluate molecules penetration and the conditions under which it occurs. This article revised the techniques applied for this purpose. Microscopy provides clear and colorful images which collaborated with the elucidation of the diffusion pathways and the exact location of molecules, but requires laborious sample preparation that culminates with its destruction as cross sectioning is often demanded. Other techniques have been successfully tested with regard to the efficiency of the investigation, but most of them involve variable level of work and specific instrumentation for sample preparation. Spectroscopy, on the other hand, with several variant techniques available, has been largely employed for penetration studies due to the high level of accuracy, preservation of the samples and fast response.

Keywords: Hair; diffusion; penetration; microscopy; spectroscopy; hair cortex; hair substantivity.

1. INTRODUCTION

Hair fibers may be seen as a simple and fragile material. But over the past years, many scientists have employed a variety of techniques to bring up a high degree of detail about the morphology and functions of each component of the hair fiber. It is divided in three main structural components: cuticle, cortex and medulla.

The cuticle is the outermost part and presents a laminar structure, with the function of protecting the inner structures from heat and mechanical abrasion (Yamauchi and Yamauchi, 2018). Each scale is called a cuticular cell. The most external layer is covered by lipids, including the 18-methyl eicosanoic acid (18-MEA). Chemically, the cuticle is considered a resistant region, with less molecular organization than the cortex (Robbins, 1994). The cortex is found under cuticle scales and represents 70 to 90% of total fiber mass. It is composed of cellular structures in an intercellular matrix. Cortical cells comprise macrofibrils, which in turn comprise smaller protein filaments, called microfibrils. The matrix involving these structures is equally rich in proteins, known as keratin associated proteins (KAPs) or intermediate filament associated proteins (IFAPs) and concentrate the greatest amount of disulfide bonds of entire cortex () (Wortmann, 1993; Robbins, 2012). The medulla is reported as a long cylindrical and lean structure located in the fiber central region, with small bags where fragments of proteins are found (Yamauchi and Yamauchi, 2018).

The cell membrane complex, known as CMC, is responsible for the maintenance of hair structures together, working as cement. It is responsible for the most important pathway for penetration of materials into the fiber, representing the intercellular route. The transcellular route is also possible, though less likely to happen (Leeder and Rippon, 1983). The first one involves less crosslink regions while the second one occurs through high and low crosslink zones, throughout the cells. Both routes are viable depending on molecular size, solvent, temperature, pH and hair condition. However, organic molecules with very different characteristics of size and polarity have been shown to diffuse preferably via intercellular route, as small and hydrophilic species migrate through the transcellular way (Robbins, 1994).

Consumers are attracted by modern and different look, which motivates the search for hair treatments. Distinct treatments are able to access the internal portions of the hair. The entrance of materials in the cortex can either promote benefits or injuries. Low molecular weight surfactant in shampoos, for example, can reach the cortex and perturb the arrangement of hydrogen bonds and salt linkages (Gode et al., 2012). Treatments for modifying hair shape or color such as bleaching, perming treatments, hair straighteners and hair dyes also reach the cortex and cause large damage, frequently of deep extension (Tate

et al., 1993; Ruetsch et al., 2003; Nawa et al., 2013). Disulfide bonds, responsible for the integrity of hair entire constitution, are broken increasing its hydrophilicity, which in turn leads to higher swelling rates and increased diffusion of materials (Ruetsch et al., 2003; Grosvenor et al., 2018). On the other hand, cationic conditioning compounds, main constituent of conditioners, can penetrate the cortex protecting the hair from fatigue stress and contributing to moisture retention (Ruetsch and Kamath, 2005). Saturated and monounsaturated oils are also able to penetrate the hair and prevent water ingress, consequently reducing the swelling rates (Keis et al., 2005). Other ingredients present in hair formulations, like protein substances, also act in moistness and delivers good finishing effects (Silva et al., 2007).

Due to its dual character, penetration of materials into hair fibers has been a topic of interest for many scientists over the years. Various techniques have been studied to proper evaluate molecules penetration and the conditions under which it occurs. These comprehend microscopy techniques (optical microscopy, fluorescence microscopy, electronic microscopy), different techniques involving spectroscopy and spectrometry (spectrophotometer and microspectrophotometer; optical, fluorescence and ultraviolet spectroscopy; FTIR-AR spectroscopy; Raman spectroscopy; secondary ion mass spectroscopy – nanoscale and time-of-flight - and mass spectrometry), radiolabeling, chromatography, extraction techniques among others. This review aims to concentrate the different methods once applied for this purpose, discuss about advantages and disadvantages of each one and point those with most relevance to our understanding of hair science.

2. MICROSCOPY

Since 1800’s, microscopes became widely available and an important tool for scientific discoveries, allowing observation of structures, physiology, and functions of small objects (Lippincott-Schwartz, 2010). Modern microscopes have been developed through the years, and new techniques have been described (Quarles, 2008). Some of them have been largely employed in hair science to elucidate its structure and associated phenomena like the penetration of materials, as optical methods allow non-destructive probing of chemical structures in the hair (Zimmerley et al., 2009). The microscopy can be used to assess the diffusion of compounds, often by seeing how much it penetrates, before and after an exposure, or to analyze the hair damage.

The study of penetration by microscopic methods involves the tracking of molecules or dyes. Literature brings fluorescent dyes (or compounds linked to one), naked eye visual dyes, UV visible dyes and molecules that can be distinguished from the hair by

chemical reactions (used in electron microscopy). In addition to molecules tracking, procedure implicates sample fixation either in some kind of resin or glycerol, or the using of optimal cutting temperature.

2.1 Optical Microscopy

Optical microscopy is a simple form of amplifying objects or samples and it is broadly used in scientific field. The method relies on the light diffraction emitted in a dark chamber and adjusted by lenses, as a form to amplify the sample size (Egerton, 2005). For optical microscopy, dyes or pigments that can be seen without excitation neither chemical reactions are required.

Using Orange II (acid dye) to color keratin fibers at low temperature, two different treatments applied to human hair were compared with optical microscopy: polyethyleneimine (PEI) solution (as a counter ion reagent) with and without urea. Urea in the solutions behaved as penetration enhancer, making PEI penetration deeper and faster. PEI in turn exerted counter ionization on Orange II, improving hair coloring due to a better permeation (Kuzuhara, 2004).

2.2. Fluorescence Microscopy

2.2.1 Conventional fluorescence microscopy

Fluorescence microscopy relies on short wavelength's light emission, characterized as fluorescence, specifically by the object in study (Lichtman and Conchello, 2005). To use a fluorescence microscope, a fluorescent dye needs to be present isolated or linked with the compound of interest. After sample preparation, microscope needs to be set to the absorption and emission wavelengths of dye. It allows the observation of the layer around hair cuticle and the depth of diffusion of dye in cross-sections.

Fluorescence microscopy was used to compare permeation of two synthesized peptides with 17 amino acids each, together with the effect of hair bleaching. The peptides were covalently linked by the N-terminal to fluorescent dye 5(6)-TAMRA (5(6)-carboxytetramethylrhodamine, succinimidyl ester) to be observed. Silva et al. (2007) confirmed that oxidation increases protein permeation once it negatively charges hair surface. Peptide permeation in hair occurs mainly due to electrostatic complementarities, and thus charge localization at a peptide structure is extremely important.

An alternative treatment for over-bleached hair by promoting the grafting of two cysteine-containing peptides onto hair was tested using fluorescent dye (5(6)-TAMRA)

linked peptides to analyze hair penetration. The use of the peptides contributed to reduce hair damage caused by over-bleach. After bleaching the physicochemical properties of the fiber (i.e. surface charge, porosity) and its integrity changed, and chemically damaged hair presented higher permeation of peptides into hair cortex (Fernandes and Cavaco-Paulo, 2012).

Rhodamine B is a widely used dye in fluorescence microscopy and was used to test the influence of a treatment on its diffusion to hair after bleaching, photo bleaching and abrading (Silva and Joekes, 2005). Sideris et al. (2008) also used rhodamine B to study its and octadecylrhodamine B diffusion pathway into wool fibers, comparing these two dyes in the same fiber.

A dye diffusion technique was developed aiming to investigate aliphatic alcohols penetration into hair and other keratin fibers. Nile red, eosin Y and rhodamine B were used as dyes. Hair fibers were kept at 60 °C for 2 hours in dye solution in t-butanol prior to fluorescence microscopy examination. Nile red completely penetrated in cortex intracellular regions with similar staining behavior in different hair samples, while hair fibers were evenly stained by eosin Y, indicating complete dye penetration through fiber cortex. The authors attributed these findings to complete penetration of t-butanol in hair fiber cortex, and different dyes affinity, where Nile red showed more affinity to intracellular components of cortex. Rhodamine B remained preferentially in hair cuticle. Differently from Nile red, rhodamine B is positively charged, and authors believed that electrostatic interactions were responsible for its staining pattern (Jurdana and Leaver, 1992).

2.2.2. Confocal fluorescence microscopy

Confocal microscopy has an important ability to acquire images that are in focus selected depths, a process known as optical sectioning. Images are acquired point-by-point and reconstructed by a computer, which allows 3D reconstructions of topologically complex objects (Anderson, 2007).

Using laser scanning confocal microscopy, different oils (rice bran oil, refined til oil and light liquid paraffin oil) and formulations containing those oils plus excipients were evaluated, after Nile red labelling. Results showed that mineral oils demonstrated better penetration through hair fiber than vegetable oils, and formulations combining oils and excipients significantly influenced oil permeation (Srivastav et al., 2019).

2.2.3. Electronic microscopy

Electron microscopy requires the material to have an electron density to show contrast with the fiber. The electrons particles emit a wave assignment, which permits the detection of composts by changing the photon energy and increasing their acceleration. That modification creates brightness or darkness for each component, which is identified by microscopy. There are many types of electron microscopy: transmission electron microscope, serial-section electron microscopy, scanning electron microscopy, reflection electron microscopy, scanning transmission electron microscopy and scanning tunneling microscopy (Egerton, 2005). It is important to take into account the temperature created inside the microscope in some techniques, as transmission electron microscopy (TEM), to maintain the sample intact. If there is any possibility of damaging the sample by the heat, cryo-TEM is required.

In order to compare the diffusion capacity of a colored silica nanoparticle in virgin and bleached hair, scanning electron microscopy (SEM) was used. Images showed holes in the bleached hair and the nanoparticles in cross sections samples. This technique, along with others used, could enable an understanding of the best conditions for hair permeation (Sampaio et al., 2011), as SEM technique is largely applied to analyze surfaces.

Aqueous silver nitrate was used to immerse hair fiber for 10 min at room temperature. Sodium hydroxide was added to the solution to make it alkaline in order to precipitate silver hydroxide within the fiber for TEM investigation. TEM provides a closer image than SEM, allowing to differentiate the hair structures and offering to researches a more accurate view (Gummer, 2001).

Staining choice is a crucial step for TEM. To allow the usage for human hair, a modification to reduction-osmication method previously described for TEM of wool cortex was proposed (Orwin et al., 1984). This modified stain was compared to other alternative stains as in bloc silver nitrate and section stains based on uranyl acetate and lead citrate, phosphotungstic acid, potassium permanganate, ammoniacal silver nitrate and some combinations of those stains. Different methods elucidate different ultrastructural features of human hair and in different resolutions. Sometimes the stain method provides better contrast but it demands intensive resources and extra time, not being suitable for large sample throughput (Harland et al., 2011).

3. SPECTROSCOPIC TECHNIQUES

Spectroscopic methods are based on the electromagnetic radiation emitted by atoms or molecules in the return to their ground state of energy, after its absorption characterized by an excited state. The type of energetic transition will determine the region of electromagnetic spectrum. For example, the nuclear magnetic resonance transitions

(NMR), which correspond to wavelengths in the radio wave region of the spectrum, are those with the smallest gap between energy levels, and electronic transitions in the ultraviolet-visible (UV-Vis) region have the largest energy gap between transition levels. These techniques are most considered to identify molecules and elucidate their structure (Anderson, Bendell and Groundwater, 2004). It was possible to localize literature applying spectroscopic methods since 1950s showing the vast use of these techniques in hair science.

3.1. Optical and Fluorescence Spectroscopy

The fluorescence spectroscopy is based on the detection of the fluorescence irradiated from a sample, which is related to concentration of compound under evaluation. After detection with photomultiplier tubes, the fluorescence is quantified by a specific electronic device (Lakowicz, 2006). Optical spectroscopy comprises UV-Vis spectroscopies. In order to detect silicones and hydrocarbons penetration in hair after shampoos, X-rays fluorescence and optical spectroscopy were employed by Haake et al. (2007). The X-ray fluorescence showed no conclusive results as silicone signals from the hair are unknown. Optical spectroscopy could detect the amount of dimethiconol absorbed by hair, after shampoo treatment. Even after 3 times shampooing, about half of silicone amount remained in the hair.

In the work of Deppert et al. (1991), authors proposed to add isothiuronium moiety to cationic surfactants and dye products to test the hypothesis of increased duration of these treatments on hair. Dyes and surfactants were prepared and exposed to hair fibers, during adsorption experiment. The substantivity was evaluated by UV-spectroscopy. According to the results, the addition of isothiuronium group contributed to raise these materials affinity for hair, leading to increased penetration, possibly because of enhanced electrostatic interactions, change in solubility or formation of disulfide bonds with hair keratin (Deppert et al., 1991).

3.2. Induced Couple Plasma Optical Emission Spectroscopy (ICP-OES)

The purpose of Haake et al. (2007) was to evaluate silicone absorption residue in human hair by induced couple plasma optical emission spectroscopy (ICP-OES), from application of two different shampoo formulations, after removability assays with sodium laureth sulfate. A great amount of dimethiconol was absorbed and about 50% of this content remained in the hair after washing. In a second evaluation, silicone accentuated presence

was detected in the hair treated with Asiatic shampoo (3.5 times higher) (Haake et al., 2007).

Inductively coupled plasma atomic emission spectroscopy (ICP-AES) was employed to quantify metals of hard water in hair (Evans et al., 2011). Virgin dark brown Caucasian swatches were submitted to oxidative damage by hydrogen peroxide, washed with hard water and dipped in alkaline solution and divided according to damage level. Calcium and Magnesium presented affinity for hair possibly due to the fact that oxidative substances and alkaline pH promoted more anionic sites, which facilitated their bonds even when water hardness levels were low. Results shown that calcium affinity for hair was higher when compared to magnesium and increased with damage level. This finding was attributed to the fact that calcium has larger ionic radius, which leads to smaller hydrated radius than magnesium, and thus calcium could be more easily absorbed to anions (Evans et al., 2011).

3.3. Fourier Transformed Infrared Spectroscopy (FT-IR)

Fourier Transformed Infrared – Attenuated Total Reflectance (FTIR-ATR) spectroscopy detects the materials molecular vibration identified by absorption bands that can be related to functional groups present in the molecule (Griffiths and De Haseth, 1986; Colthup et al., 1990; Berthomieu and Hienerwadel, 2009). The infrared light is reflected in the crystal of ATR, where sample is placed. Each reflection generates a wave that examines the sample (Iwaki et al., 2002; Berthomieu and Hienerwadel, 2009).

The study of Itou et al. (2006) analyzed Chinese hair-set durability in high humidity by organic acids, using near-infrared spectroscopy (NIR). Hair samples were subjected to malic acid and control treatments for 20, 40 or 60 minutes, and compared with untreated samples. The results of NIR mapping showed NH and amide II stretching with three different intensities, suggesting malic acid formed more hydrogen bonds with hair proteins than untreated and control groups. This information collaborated to conclude that malic acid penetrated into hair fiber and occupied sites that once was occupied by water, improving hair-set durability against high humidity (Itou et al., 2006).

Human hair bleaching process impact was evaluated by FT-IR microspectroscopy imaging (Ryu et al., 2016). Black virgin hair was submitted to bleach treatment with 6% of hydrogen peroxide from 30 to 120 minutes. The differences in the amount of amide I and II were described, being reported higher contents in the cortex. Hydrogen peroxide penetrated the hair cortex due to keratin cuticle opening and oxidized disulfide bonds (amide I), releasing by-products, especially cystine-monoxide which increased with bleaching time. Samples physical appearance analysis showed square format of hair cross sections (Ryu et al., 2016).

3.4. Raman spectroscopy

The inelastic and elastic scattered light is a phenomenon resultant from light incidence in a sample. Raman technique is based on inelastic scattering using light collision to change energy level and energy frequency of molecules (Ozaki, 1988; Browne and Mcgarvey, 2007) Clark 2013. A research performed by Kuzuhara (2011) used Raman to study penetration of amino acids, peptides and hydrolyzed egg white protein into virgin white human hair after 1h and 16h at 50 °C of incubation. The most hydrophobic amino acids, especially those with an aromatic ring, showed a higher level of penetration compared to those of a more hydrophilic character, even after 16 h. The egg white protein has a content rich in beta chains and it was possible to observe an increase of this content in the cortex. In addition, egg protein content along the depths is practically constant, showing that the presence of hydrolyzed protein is dispersed in structures and can reach greater depths.

Raman technique was used to evaluate thioglycerol reduction profile in permanent waving process (Kuzuhara, 2018). Chinese hair samples were dipped into thioglycerol solution (pH 9.0) adjusted with ammonia and monoethanolamine, 1:13 ratio hair to solution. The amount of gauche-gauche-gauche and gauche-gauche-trans conformations in hair decreased due to the change into trans-gauche-trans. This change could be explained by disconnection and rearrangement of disulfide groups which led to thioglycerol penetration into cortex. The study also compared thioglycerol with thioglycolic acid diffusion profile and effect on hair, and conclude that mechanism of thioglycolic acid is related only to -SS- disconnection and cause more damage to hair (Kuzuhara, 2018).

3.5. Secondary Ion Mass Spectrometry (SIMS)

Secondary ion mass spectrometry (SIMS) involves striking of samples by ions. Numerous collisions occur and allow secondary ion species formation (positive, negative, and neutral) which can be analyzed (Walker, 2017). The diffusion of chromophores groups formed by oxidative hair coloring was studied using nanoscale secondary ion mass spectrometry (NanoSIMS) and isotope-labeled oxidative dye. The test was performed in black, white and bleached hair using two types of deuterium dyes. Results showed that deuterium ion was found inside melanin granules in black hair and ion absence due to the lack of melanin in white hair (Kojima et al., 2013).

Another study using this technique analyzed deuterium resorcinol, deuterium salicylic acid, pentadecyl-d31 alcohol and pentadecanoic-d29 acid partition in hair fibers (Marsh et al., 2019) The authors studied lipophilicity (LogP) and distribution of labeled active

compounds isotopes by the ratio of 2H/1H. Resorcinol and salicylic acid showed low LogP and high 2H/1H ratio that contributed to cortex permeation. Medulla and cuticle are rich in lipid constituents that make diffusion easier even with low 2H/1H ratio. Pentadecyl alcohol and pentadecanoic acid showed high LogP and were more present in the medulla, nuclear remnants and cuticle layers. The cortex showed low presence of these molecules (Marsh et al., 2019).

Palm oil penetration into hair was investigated by Kojima et al. (2012) using time-of-flight secondary ion mass spectrometry technique (TOF-SIMS). According to Brunelle et al. (2005) and Saleem and Galla (2010), TOF-SIMS is a technique where the particles, after ionization, are accelerated into a "flight tube" and their mass is determined by measuring the exact time at which they reach the detector, resulting in high resolution and precise detection. Black human hair was treated with bleaching and permanent waving and then treated with hydrogenated palm oil to recover the damage. TOF-SIMS images showed that the tripalmitin, a constituent present in palm oil, permeated into hair cuticle and outer cortex of damaged hair (Kojima et al., 2012).

The evaluation of cationic conditioning compounds penetration into brown European hair by TOF-SIMS was proposed by Ruetsch and Kamath (2005). Hair fibers were treated with polyquaternium-10 (PQ-10) and cetyl trimethyl ammonium bromide (CETAB), which are high and low molecular weight compounds, respectively. After TOF-SIMS analysis of positive and negative charge ions, CETAB was detected from 10 µm depth to the whole cross section of the fiber, but PQ-10 could not be identified because of its non-unique fragments. Fatigue resistance was used to monitor hair properties and was positively influenced by CETAB penetration (Ruetsch and Kamath, 2005).

4. SPECTROPHOTOMETRY

The amount of light absorbed by an analyte permit its identification and/or quantification of chemical constituents on it. This is the principle of spectrophotometer analysis (Burgess, 2017). The technique is divided into three groups, according to wavelength range: ultraviolet (190-380 nm), visible (380-750 nm) and near infrared (800-2500 nm) (Worsfold and Zagatto, 2019).

Rhodamine B penetration into bleached, photo bleached and abraded hair fibers was analyzed using spectrophotometer by Silva and Joekes (2005). The diffusion of rhodamine B after the saturated limit decreased from 1.04 x 10-4 mol L-1 to 58-74% of this value. Two different kinetics absorption, in cuticle and cortex, were showed explaining higher penetration in cuticle and lower penetration in cortex. These results led to understanding that rhodamine B penetrated by intracellular path (Silva and Joekes, 2005).

Microspectrophotometer and dyeing techniques were combined to analyze the diffusion of thioglycolic acid, thiolactic acid and L-cysteine into virgin white human hair (Kuzuhara, 2004; Kuzuhara and Hori, 2004). Electrostatic interactions were important to study cysteine penetration, but no evidence of it was found in cortex when hair was submitted to pH 9.0. On the other hand, thioglycolic and thiolactic acids had their penetration increased when treatment time and pH were also increased (Kuzuhara and Hori, 2004). Another study of Kuzuhara and Hori (2005) using the same technique showed the penetration profile of polyethyleneimine into hair, and in pH 11 it showed a penetration profile independent of concentration (Kuzuhara and Hori, 2005).

5. CHROMATOGRAPHY

Chromatography is a separation method, using a column which includes a stationary and a mobile phase which passes through the stationary phase, leading to separation of analytes present in a sample, by affinity with one of the phases, which increases or reduces the retention time (Touchstone and Dobbins, 1992). Literature reports the use of different types of chromatography to study molecules penetration into hair fibers.

Gel permeation chromatography (GPC) and gel filtration chromatography (GFC) were performed to detect cationic polymers adsorption on hair. Authors could not relate polymers substantivity with their molecular weight nor with cationic charge percentage (Blanco et al., 1997).

In the work of Mintz et al. (1991), the GPC and GFC techniques were also used to evaluate collagen peptide hydroxyproline substantivity on hair, based on its molecular weight distribution. Determination of specific molecular weights peptides were done using fluorescamine method, which is a reaction between the fluorescamine reagent with the primary amino groups of proteins, resulting in a fluorescent derivate (Udenfriend et al., 1972). Both methods contributed with the identification and determination of peptide present in hair samples.

6. RADIOLABELLING

In radiolabeling method, a substance is marked with a radioactive element allowing its further quantification (Gode et al., 2012). For example, coconut oil was marked with tritium and its penetration into hair strains studied after embedding for one and six hours (Gode et al., 2012). Oil amount that penetrated the hair ranged from 14.5% to 26.3% in mass, evidencing efficiency of this method to distinguish substance deposited on the surface and absorbed by the fiber (Gode et al., 2012).

Another study with radiolabeling incorporated different proportions of 14C wheat amino acid in commercial base of shampoo and conditioner. Approximately 100 mg of normal brown European hair were treated once, three and five times with the formulations. The wheat amino acids in shampoo was found mostly inside the hair rather than on its surface, showing good penetration with 95.7% to 99.5% of substantive wheat amino acids (0.15% active) inside the hair and the greater the number of treatments, the greater the penetration. With conditioner, the penetration was of 95.0% to 99.0%, and similar to the shampoo, the more treatments the greater the penetration percentage (Jones and Chahal, 1997).

7. AUTORADIOGRAPHY

Autoradiography was the first molecular technique developed for localization of radioactive substances loaded with radioisotopes in biological samples. It is a very sensitive technique that can be used in cells, organelles, tissues and biomolecules. Autoradiography principle is the ability of the radioactive substance to expose the photographic film by ionization (Solon et al., 2010). Through this technique it was possible to visualize the penetration of fatty alcohol into the hair, where the gel network shampoo formulated with stearyl alcohol was radiolabeled with 14C and applied to the hair for one and five washing cycles. The hair was compared to control under an optical microscope to analyze the penetration of radiolabeled stearyl alcohol through presence of black dots in image. This technique permitted to observe penetration of stearyl alcohol, with cycle with 5 washes showing greater color intensity than cycle with one wash (Marsh et al., 2017).

8. EXTRACTION METHODS

Penetration and deposition of fatty alcohol in the hair in a quantitative way was carried out through a method of differential extraction. Virgin and chemically altered hair were treated with 16 cycles of cetyl (short chain) and stearyl (long chain) alcohols. The fatty alcohol from the surface was removed using hexane. The remaining fatty alcohol from inside the hair was removed using chloroform and methanol. Penetration and deposition were greater in virgin hair than in chemically treated hair, which may be explained by hydrophilic nature of chemically treated hair (Marsh et al., 2017).

9. NUCLEAR REACTION ANALYSIS

Nuclear reaction analysis (NRA) is a technique that assesses hydrogen content in a material using an ion beam from an accelerator with energies in the range of 1 to 10 MeV. Hydrogen present in the sample has an unique quantitative signature, provided by the products of nuclear resonance reaction with the ion beam (Benka, 2011; Becker and Rogalla, 2016).

Undamaged and damaged hairs were studied to determine the permeation of surfactants. Virgin and bleached hair samples from European origin were immersed in an aqueous solution of non-ionic surfactant for 1 h at 20 °C and cut with a microtome in slices of 30 µm. Ion beam analysis was performed with surfactant undergoing to a substitution reaction with deuterium, which formed a 2-D map of deuterium and carbon that permitted to visualize surfactants position and concentration in the hair fiber with an optical microscope (Jenneson et al., 1997). Damaged fiber showed a higher surfactant concentration in central region, related to cortex and medulla. On the other hand, in undamaged fiber, surfactant was more concentrated in fiber's exterior region, related to cuticle. Additionally, it was possible to observe that damaged fiber had three times surfactant amount of undamaged fiber (Jenneson et al., 1997).

10. MOLECULAR MODELING

Molecular modeling is a compilation of computer-based techniques that allows evaluation, visualization and manipulation of molecular structures and reactions, and dependent properties of these structures in three dimensions. This technique can be applied from simple molecules to large proteins and nanostructures (Pimentel et al., 2013). Morel et al. (2008) reanalyzed data already published on fluorescent dyes to refine results and characterize molecular movement of dyes in the process of diffusion into hair fiber.

Permeation distance within hair fibers for virgin hair ranged from 2 to 41 µm while in permanent hair this distance was 8 to 40 µm. In virgin hair, size limit for dye to pass through cuticle and reach the cortex was 11.8 Å. However, in permed hair this value increased dramatically, which can be related to the damage that hair suffered. Chemical treatments damage hair cuticle and cortex, leaving them with pores, which facilitates penetration of larger dyes (Morel et al., 2008).

11. OPTICAL COHERENCE TOMOGRAPHY

In 1991, a technique called optical coherence tomography was developed, capable of generating images of tomographic structure of living tissue (Tsugita and Iwai, 2014). This technique was developed with primary colors of visible band, red, green and blue, using a LED light source to simultaneously assess hair appearance and depth of penetration of dye after dyeing without sectioning the hair. With tomographic image of dyed hair, a relationship between dyes' wavelength and their penetration into hair was found. Therefore, results obtained with dyes in visible light region corroborate the characteristics of light reflected in optical coherence tomography images at each wavelength (Tsugita and Iwai, 2014).

12. COLLOID TITRATION

Colloidal titration is a method based on changing a solution color according to the blue o-toluidine indicator, which occurs with the first excess of potassium polyvinylsulfat (KPVS) titrant. An anionic polymer, KPVS, was added to analyte solution containing a cationic polymer, forming a preferential complex. After complete cationic analyte complexation, titrant excess begins to interact with blue o-toluidine indicator, resulting in a color change from blue to violet. Polymer amount present in the solution was determined according to titration curve and its point of equivalence. Polymer uptake by hair can be indirectly determined by the difference in treatment solution concentration before and after hair immersion. Hair dipping solution used in this test contained 0.1% polyquaternium-10 (Hutter et al., 1991). Cationic polymer adsorption was proportional to damage degree caused by discoloration. Under mild bleaching conditions (30 minutes at 32 °C) average adsorption was 2.6 ± 0.1 mg/g. In more severe conditions (four hours at 40 ° C) this result was greater, with an adsorption of 9.2 ± 0.3 mg/g. In addition, the higher the concentration of Polyquaternium-10 solution and the contact time between hair and polymer, the greater the uptake (Hutter et al., 1991).

13. PROTEIN DETERMINATION

Protein determination is largely employed for hair into genetic field, mostly to identify patterns with forensic goals (Budowle and Acton, 1981; Maxfield and Babbie, 2014; Mason et al., 2019). In cosmetic filed, protein determination is used for measurement of hair damage (Sandhu and Robbins, 1993) and products action (Rele and Mohile, 1999). The objective is to determine the amount of protein extracted from hair after certain damage and

quantify them by spectrophotometric methods. Among the techniques to detect total protein are Lowry, Biureto, Bradford, Smith, ultraviolet absorption and others (Gornall et al., 1949; Lowry et al., 1951; Bradford, 1976; Smith et al., 1985; Stoscheck, 1990; Talman and Boughner, 1995).

Rele and Mohile (1999) investigated coconut oil effect as a protector against damage and based their studies on protein loss detection as a hair damage parameter. They used Lowry method (Folin-Ciocalteu), which involves a complex formation in alkaline solution (Lowry et al., 1951; Dietzen, 2018). Protein loss in samples without coconut oil was observed, especially in curly hair, after washing process. Coconut oil has a hydrophobic composition, forming a coat on hair fiber and inhibiting water penetration. Furthermore, a small amount of oil may have penetrated into the hair, which contributed to reduce fibers intumescence and curving (Rele and Mohile, 1999).

14. DYNAMIC ELECTROKINETIC

This method measures the streaming potential of materials by linking plugs to hair fibers and using electrolyte solutions in a constant ionic strength at a specific pH. Hair fibers are added into a streaming potential cell and their streaming potential is measured as test solution passes through the cell under pressure (Jachowicz et al., 1993). The method allowed determination of changes in ionic character of fibers, permitting the observation of molecules desorption kinetics, and enabling the assessment of colloids affinity to fibers.

Anionic or cationic surfactants, or cationic polymers were applied to hair and their interactions were studied using this technique. Ammonium and sodium sulfates slowly bond to hair surface, while triethanolammonium dodecyl sulfate and diethanolammonium dodecyl sulfate were rapidly deposited. For cationic surfactant, streaming potential was gradually reduced from the surface, but the maintenance of conductivity in the plug suggested that there was more penetration than deposition in the fiber. Finally, cationic polymers were unable to penetrate, probably not only because of their large molecular weight but also due to strong bonds that polymers create to fibers surface, which reduces the variation in streaming potential (Jachowicz et al., 1993).

15. CONCLUSIONS

Harmful or beneficial, the penetration of materials into the hair ensures the success of cosmetic treatments. The first situation is the case of the bleaching, perming waving, straighteners and colorants. It is well established that these procedures cause extensive damage throughout the fibers, affecting permanently all the structures of the hair.

However, to deliver the purpose of these treatments, the substances involved must reach the inner portions of the fiber whether to eliminate the melanin granules, or to cleavage covalent bonds and permit shape manipulation, or to deliver pigments that make the desired look feasible. The second situation is often associated with the recovery of the damage caused by the first one. The cortex also must be reached to stabilize sites resultant from broken bonds, replenish mass, lost due to leaching of lipids and proteins, return hydrophobicity to hair and ultimately recover some important properties as resistance and texture.

A large list of techniques is available not only to provide information about the diffusion processes of materials but also to consent the understanding of the mechanism of actions of products. Over the past 45 years (interval of time analyzed by our group for this review), the developed techniques allowed improvement in quality of detection and precision of quantification. One of the most relevant techniques in these studies is microscopy. The oldest references considered in this work are from 1990's. Since then many variants were successfully utilized to provide clear and colorful images which collaborated with the elucidation of the diffusion pathways and the exact location of molecules. These methods frequently involve dyes to be tracked, which in turn require reaction of the materials with the dyes. These modifications interfere in the interaction of substances with the hair. Additionally, microscopy requires laborious sample preparation, demanding the cross section of the fibers, which requires proper instrumentation and implicates the destruction of samples.

Several other techniques have been reported in literature with the advantage of precision and less laborious preparation than microscopy. Most of them, however, require some work for sample preparation or reaction with markers to allow for more accurate analysis and investigation of the penetration events. These are the cases of the chromatography, radiolabeling, autoradiography, extraction methods, colloid titration, protein determination and other cited.

Spectroscopy, on the other hand, applied in many distinct variants, proved to be a very efficient tool, bringing precise information about molecules behavior, permitting the elucidation of the mechanism of action, and preserving the samples as generally they involve relatively low energy radiation for short periods of time, returning faster responses in comparison with microscopy. Generally, it does not require sample preparation besides the application of the material of interest on hair, neither additional instrumentation as it can be run directly on the fiber after the treatments. As it does not demand addition of other markers, the interaction of materials with hair is not affected by the technique, permitting the elucidation of the events with less interference. The analysis of the available papers

showed the increasing use of such methods for the elucidation of penetration events but also it has been used to investigate structure and study damage in hair science.

DECLARATION OF COMPETING INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

The authors acknowledge Symrise AG, FAPESP (grant number 2018/06475-4 and 2017/10728-2) and CAPES agencies for the financial support of this study.

16. REFERENCES

1. Yamauchi, A. and Yamauchi, K. New Aspects of the Structure of Human Scalp Hair-II: Tubular Structure and Material Flow Property of the Medulla. Journal of cosmetic science. 69(1):19-33 (2018).
2. Robbins, C.R. Chemical and Physical Behavior of Human Hair. 5th ed. New York, NY: Springer-Verlag Berlin Heidelberg (2012).
3. Wortmann, F.-J. and Deutz, H. Characterizing keratins using high-pressure differential scanning calorimetry (HPDSC). Journal of Applied Polymer Science. 48(1):137-50 (1993).
4. Leeder, J.D. and Rippon, J.A. Some observations on the dyeing of wool from aqueous formic acid. J Soc Dyers Col. 99:64-5 (1983).
5. Gode, V., Bhalla, N., Shirhatti, V., Mhaskar, S. and Kamath, Y. Quantitative measurement of the penetration of coconut oil into human hair using radiolabeled coconut oil. Journal of cosmetic science. 63(1):27-31 (2012).
6. Tate, M.L., Kamath, Y.K. and Ruetsch, S.B. Quantification and prevention of hair damage. J Soc Cosmet Chem. 44:347-71 (1993).
7. Ruetsch, S.B., Yang, B. and Kamath, Y.K. Chemical and photo-oxidative hair damage studied by dye diffusion and electrophoresis. Journal of cosmetic science. 54(4):379-94 (2003).
8. Nawa, T., Kawaguchi, A., Kitano, H., Yamamoto, T., Fujinami, S., Asao, N., et al. Alkaline peroxide treatment induces acquired unruly hair by apparently affecting distinct macrofibrils. Journal of cosmetic science. 64(4):261-71 (2013).
9. Grosvenor, A.J., Deb-Choudhury, S., Middlewood, P.G., Thomas, A., Lee, E., Vernon, J.A., et al. The physical and chemical disruption of human hair after bleaching -

studies by transmission electron microscopy and redox proteomics. Int J Cosmet Sci. 40(6):536-48 (2018).

10. Ruetsch, S.B. and Kamath, Y.K. Penetration of cationic conditioning compounds into hair fibers: a TOF-SIMS approach. Journal of cosmetic science. 56(5):323-30 (2005).

11. Keis, K., Persaud, D., Kamath, Y.K. and Rele, A.S. Investigation of penetration abilities of various oils into human hair fibers. Journal of cosmetic science. 56(5):283-95 (2005).

12. Silva, C.J., Vasconcelos, A. and Cavaco-Paulo, A. Peptide structure: Its effect on penetration into human hair. Journal of cosmetic science. 58(4):339-46 (2007).

13. Lippincott-Schwartz, J. Microscopy. Current Protocols in Cell Biology. 47(1):4.0.1-4.0.6 (2010).

14. Quarles, J. Microscopy and Imaging. Current Protocols in Microbiology. 11(1):2.0.1-2.0.2 (2008).

15. Zimmerley, M., Lin, C.-Y., Oertel, D.C., Marsh, J.M., Ward, J.L. and Potma, E.O. Quantitative detection of chemical compounds in human hair with coherent anti-Stokes Raman scattering microscopy. J Biomed Opt. 14(4):044019- (2009).

16. Egerton, R.F. Physical principles of electron microscopy: Springer (2005).

17. Kuzuhara, A. Influence of urea on the coloring ability of a low-temperature coloring method of keratin fibers using polyethyleneimine. Journal of Applied Polymer Science. 91(6):3827-34 (2004).

18. Lichtman, J.W. and Conchello, J.-A. Fluorescence microscopy. Nature Methods. 2(12):910-9 (2005).

19. Fernandes, M. and Cavaco-Paulo, A. Protein disulphide isomerase-mediated grafting of cysteine-containing peptides onto over-bleached hair. Biocatalysis and Biotransformation. 30(1):10-9 (2012).

20. Silva, A.L.d.S. and Joekes, L. Rhodamine B diffusion in hair as a probe for structural integrity. Colloids and surfaces B, Biointerfaces. 40(1):19-24 (2005).

21. Sideris, V., Holt, L. and Leaver, I. A microscopical study of the pathway of diffusion of rhodamine B and octadecyl-rhodamine B into wool fibres. Journal of the Society of Dyers and Colourists. 106:131-5 (2008).

22. Jurdana, L.E. and Leaver, I.H. Penetration of Alcohols into Wool and Hair as Studied by Fluorescence Microscopy. Textile Research Journal. 62(8):463-8 (1992).

23. Anderson, S.I. 11 - Characterisation using imaging techniques. In: Tissue Engineering Using Ceramics and Polymers. (A.R. Boccaccini, J.E. Gough, ed.^eds), p.^pp. 226-47. Woodhead Publishing, (2007).

24. Srivastav, A., Dandekar, P. and Jain, R. Penetration study of oils and its formulations into the human hair using confocal microscopy. Journal of Cosmetic Dermatology. 18(6):1947-54 (2019).

25. Sampaio, S., Maia, F. and Gomes, J.R. Diffusion of coloured silica nanoparticles into human hair. Coloration Technology. 127(1):55-61 (2011).

26. Gummer, C.L. Elucidating penetration pathways into the hair fiber using novel microscopic techniques. Journal of cosmetic science. 52(5):265-80 (2001).

27. Orwin, D.F.G., Woods, J.L. and Ranford, S.L. Cortical Cell Types and their Distribution in Wool Fibres. Australian Journal of Biological Sciences. 37(4):237-56 (1984).

28. Harland, D.P., Vernon, J.A., Walls, R.J. and Woods, J.L. Transmission electron microscopy staining methods for the cortex of human hair: a modified osmium method and comparison with other stains. Journal of Microscopy. 243(2):184-96 (2011).

29. Anderson, R.J., Bendell, D.J. and Groundwater, P.W. General principles. In: Organic Spectroscopic Analysis. (E.W. Abel, ed.^eds), p.^pp. 1-6. The Royal Society of Chemistry, (2004).

30. Lakowicz, J.R. Principles of Fluorescence Spectroscopy. Boston, MA: Springer (2006).

31. Haake, H.M., Lagrené, H., Brands, A., Eisfeld, W. and Melchior, D. Determination of the substantivity of emollients to human hair. Journal of cosmetic science. 58(4):443-50 (2007).

32. Deppert, T.M. and Murphy, B.P. Substantivity of dyes and surfactants containing isothiuroniurn groups to hair. J Soc Cosmet Chem. 42:1-17 (1991).

33. Twyman, R.M. ATOMIC EMISSION SPECTROMETRY | Principles and Instrumentation. In: Encyclopedia of Analytical Science (Second Edition). (P. Worsfold, A. Townshend, C. Poole, ed.^eds), p.^pp. 190-8. Elsevier, Oxford (2005).

34. Mermet, J.M. ATOMIC EMISSION SPECTROMETRY | Inductively Coupled Plasma. In: Encyclopedia of Analytical Science (Second Edition). (P. Worsfold, A. Townshend, C. Poole, ed.^eds), p.^pp. 210-5. Elsevier, Oxford (2005).

35. Wilschefski, S.C. and Baxter, M.R. Inductively Coupled Plasma Mass Spectrometry: Introduction to Analytical Aspects. Clin Biochem Rev. 40(3):115-33 (2019).

36. Jarvis, I. and Jarvis, K.E. Inductively coupled plasma-atomic emission spectrometry in exploration geochemistry. Journal of Geochemical Exploration. 44(1):139-200 (1992).

37. Evans, A.O., Marsh, J.M. and Wickett, R.R. The uptake of water hardness metals by human hair. Journal of cosmetic science. 62(4):383-91 (2011).

38. Colthup, N.B., Daly, L.H. and Wiberley, S.E. Introduction to Infrared and Raman Spectroscopy (Third Edition). San Diego: Academic Press (1990).

39. Griffiths, P. and De Haseth, J. Attenuated Total Reflectance Spectrometry. In: Fourier Transform Infrared Spectrometryed.^eds), p.^pp. 191-4. Wiley New York, (1986).
40. Berthomieu, C. and Hienerwadel, R. Fourier transform infrared (FTIR) spectroscopy. Photosynthesis research. 101(2-3):157-70 (2009).
41. Iwaki, M., Andrianambinintsoa, S., Rich, P. and Breton, J. Attenuated total reflection Fourier transform infrared spectroscopy of redox transitions in photosynthetic reaction centers: comparison of perfusion- and light-induced difference spectra. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy. 58(7):1523-33 (2002).
42. Itou, T., Nojiri, M., Ootsuka, Y. and Nakamura, K. Study of the interaction between hair protein and organic acid that improves hair-set durability by near-infrared spectroscopy. Journal of cosmetic science. 57(2):139-51 (2006).
43. Ryu, S., Jang, W., Yu, S.-i., Lee, B.-h., Kwon, O.-S. and Shin, K. FT-IR Microspectroscopic Imaging of Cross-Sectioned Human Hair during a Bleaching Process. Journal of Cosmetics, Dermatological Sciences and Applications. 06:181-90 (2016).
44. Ozaki, Y. Medical Application of Raman Spectroscopy. Applied Spectroscopy Reviews. 24(3-4):259-312 (1988).
45. Browne, W.R. and McGarvey, J.J. The Raman effect and its application to electronic spectroscopies in metal-centered species: Techniques and investigations in ground and excited states. Coordination Chemistry Reviews. 251(3):454-73 (2007).
46. Kuzuhara, A. Raman Spectroscopic Analysis of L-Phenylalanine and Hydrolyzed Eggwhite Protein Penetration into Keratin Fibers. Journal of Applied Polymer Science. 122 (2011).
47. Kuzuhara, A. A Raman spectroscopic investigation of the mechanism of the reduction in hair with thioglycerol and the accompanying disulphide conformational changes. International Journal of Cosmetic Science. 40(1):34-43 (2018).
48. Walker, A.V. Secondary Ion Mass Spectrometry. In: Encyclopedia of Spectroscopy and Spectrometry (Third Edition). (J.C. Lindon, G.E. Tranter, D.W. Koppenaal., ed.^eds), p.^pp. 44-9. Academic Press, Oxford (2017).
49. Kojima, T., Yamada, H., Yamamoto, T., Matsushita, Y. and Fukushima, K. Dyeing regions of oxidative hair dyes in human hair investigated by nanoscale secondary ion mass spectrometry. Colloids and Surfaces B: Biointerfaces. 106:140-4 (2013).
50. Marsh, J.M., Huang, S., Whitaker, S., Guagliardo, P., Lucas, R.L., Arca, H.C., et al. High-resolution visualization of cosmetic active compounds in hair using nanoscale secondary ion mass spectrometry. Colloids and surfaces B, Biointerfaces. 174:563-8 (2019).
51. Kojima, T., Tsuji, S., Niwa, M., Saito, K., Matsushita, Y. and Fukushima, K. Distribution Analysis of Triglyceride Having Repair Effect on Damaged Human Hair by

TOF-SIMS. International Journal of Polymer Analysis and Characterization. 17(1):21-8 (2012).

52. Brunelle, A., Touboul, D. and Laprévote, O. Biological tissue imaging with time-of-flight secondary ion mass spectrometry and cluster ion sources. Journal of Mass Spectrometry. 40(8):985-99 (2005).

53. Saleem, M. and Galla, H.-J. Surface view of the lateral organization of lipids and proteins in lung surfactant model systems—A ToF-SIMS approach. Biochimica et Biophysica Acta (BBA) - Biomembranes. 1798(4):730-40 (2010).

54. Burgess, C. Chapter 1. The Basis for Good Spectrophotometric UV–Visible Measurements. In: UV-Visible Spectrophotometry of Water and Wastewater (Second Edition). (O. Thomas, C. Burgess, ed.^eds), p.^pp. 1-35. Elsevier, (2017).

55. Worsfold, P.J. and Zagatto, E.A.G. Spectrophotometry: overview. In: Encyclopedia of analytical science. (P. Worsfold, A. Townshend, C. Poole, M. Miró, ed.^eds), p.^pp. Elsevier, (2019).

56. Kuzuhara, A. and Hori, T. Diffusion behavior of reducing agents into keratin fibers using microspectrophotometry. Journal of Applied Polymer Science. 94(3):1131-8 (2004).

57. Kuzuhara, A. and Hori, T. Diffusion behavior of poly(ethylene imine) into keratin fibers using microspectrophotometry. Journal of Applied Polymer Science. 97(1):65-71 (2005).

58. Touchstone, J.C. and Dobbins, M.F. Practice of Thin Layer Chromatography (1992).

59. Blanco, B., Durost, B. and Myers, R. Gel permeation chromatography: An effective method of quantifying the adsorption of cationic polymers by bleached hair. Journal of the Society of Cosmetic Chemists. 48(3):127-31 (1997).

60. Mintz, G.R., Reinhart, G.M. and Lent, B. Relationship between collagen hydrolysate molecular weight and peptide substantivity to hair. J Soc Cosmet Chem. 42:35-44 (1991).

61. Udenfriend, S., Stein, S., Böhlen, P., Dairman, W., Leimgruber, W. and Weigele, M. Fluorescamine: A Reagent for Assay of Amino Acids, Peptides, Proteins, and Primary Amines in the Picomole Range. Science (New York, NY). 178(4063):871 (1972).

62. Jones, R.T. and Chahal, S. The use of radiolabelling techniques to measure substantivity to, and penetration into, hair of protein hydrolysates. In: ed.^eds), p.^pp., (1997).

63. Solon, E.G., Schweitzer, A., Stoeckli, M. and Prideaux, B. Autoradiography, MALDI-MS, and SIMS-MS imaging in pharmaceutical discovery and development. AAPS J. 12(1):11-26 (2010).

64. Marsh, J.M., Brown, M.A., Felts, T.J., Hutton, H.D., Vatter, M.L., Whitaker, S., et al. Gel network shampoo formulation and hair health benefits. Int J Cosmet Sci. 39(5):543-9 (2017).

65. Becker, H.-W. and Rogalla, D. Nuclear Reaction Analysis. In: ed.^eds), p.^pp. 315-36, (2016).

66. Benka, O. Nuclear Reaction Analysis (NRA). In: Surface and Thin Film Analysis. (G. Friedbacher, H. Bubert, ed.^eds), p.^pp. 229-36, Germany (2011).

67. Jenneson, P.M., Clough, A.S., Keddie, J.L., Lu, J.R. and Meredith, P. Non-ionic surfactant concentration profiles in undamaged and damaged hair fibres determined by scanning ion beam nuclear reaction analysis. Nuclear Instruments and Methods in Physics Research Section B: Beam Interactions with Materials and Atoms. 132(4):697-703 (1997).

68. Pimentel, A.S., Guimarães, C.R.W. and Miller, Y. Molecular Modeling: Advancements and Applications. Journal of Chemistry. 2013:875478 (2013).

69. Morel, O., Christie, R., Greaves, A. and Morgan, K. Enhanced model for the diffusivity of a dye molecule into human hair fibre based on molecular modeling techniques. Coloration Technology. 124:301-9 (2008).

70. Tsugita, T. and Iwai, T. Optical coherence tomography using images of hair structure and dyes penetrating into the hair. Skin research and technology : official journal of International Society for Bioengineering and the Skin (ISBS) [and] International Society for Digital Imaging of Skin (ISDIS) [and] International Society for Skin Imaging (ISSI). 20(4):389-98 (2014).

71. Hutter, J.M., Clarke, M.T., Just, E.K., Lichtin, J.L. and Sakr, A. Colloid titration' A method to quantify the adsorption of cationic polymer by bleached hair. Journal of The Society of Cosmetic Chemists. 42:87-96 (1991).

72. Budowle, B. and Acton, R.T. A technique for the detection of variable electrophoretic patterns of hair proteins. Electrophoresis. 2(5-6):333-4 (1981).

73. Maxfield, M.G. and Babbie, E.R. Research methods for criminal justice and criminology: CT: Cengage Learning (2014).

74. Mason, K.E., Paul, P.H., Chu, F., Anex, D.S. and Hart, B.R. Development of a protein-based human identification capability from a single hair. Journal of forensic sciences. 64(4):1152-9 (2019).

75. Sandhu, S.S. and Robbins, C.R. A simple and sensitive technique, based on protein loss measurements, to assess surface damage to human hair. Journal-Society of Cosmetic Chemists. 44:163- (1993).

76. Rele, A.S. and Mohile, R. Effect of coconut oil on prevention of hair damage. Part I. Journal of cosmetic science. 50(6):327-39 (1999).

77. Bradford, N. A rapid and sensitive method for the quantitation microgram quantities of a protein isolated from red cell membranes. Analytical biochemistry. 72:248-54 (1976).

78. Gornall, A.G., Bardawill, C.J. and David, M.M. Determination of serum proteins by means of the biuret reaction. Journal of biological chemistry. 177(2):751-66 (1949).

79. Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. Protein measurement with the Folin phenol reagent. J Biol Chem. 193(1):265-75 (1951).

80. Smith, P.K., Krohn, R.I., Hermanson, G.T., Mallia, A.K., Gartner, F.H., Provenzano, M.D., et al. Measurement of protein using bicinchoninic acid. Analytical biochemistry. 150(1):76-85 (1985).

81. Stoscheck, C. Methods in Enzymology. In: ed.^eds), p.^pp. 50-68. Academic Press, Inc.: San Diego, CA, (1990).

82. Talman, E.A. and Boughner, D.R. Glutaraldehyde fixation alters the internal shear properties of porcine aortic heart valve tissue. The Annals of thoracic surgery. 60:S369-S73 (1995).

83. Dietzen, D.J. Amino acids, peptides, and proteins. In: Principles and Applications of Molecular Diagnosticsed.^eds), p.^pp. 345-80. Elsevier, (2018).

84. Jachowicz, J., Maxey, S. and Williams, C. Sorption/desorption of ions by dynamic electrokinetic and permeability analysis of fiber plugs. Langmuir. 9(11):3085-92 (1993).

CAPÍTULO II. "IMPACT OF HAIR DAMAGE IN THE PENETRATION PROFILE OF COCONUT, AVOCADO AND ARGAN OILS INTO CAUCASIAN HAIR FIBERS"

Carolina Botelho Lourenço, Rebeca Mantuan Gasparin, Fernanda Malanconi Thomaz, Gustavo Carlos da Silva, Airton Abrahao Martin, Ana Claudia Paiva-Santos, Priscila Gava Mazzola

Por ser submetido

ABSTRACT

The mitigation of the damaged condition of the hair requires the application and penetration of substances to stabilize broken bonds sites, return lipids and proteins, restore hydrophobicity, and recover hair mechanical properties. Vegetable oils, in general, evidence a list of advantageous characteristics much desired by consumers, given the benefits associated with these treatments to the hair fiber. Coconut is a very popular oil in the hair care market and has been largely studied for its ability to diffuse through the hair cortex. Avocado and argan oils still lack further studies on their effects on the internal structure of the hair and their possible benefits. Tensile test, fatigue test and Raman spectroscopy were carried out to investigate the interaction of these three oils with virgin and bleached Caucasian hair. The oils were applied in sufficient amount directly to the tresses and allowed for 24 h at 25 °C. Results show that the three oils can diffuse and interact with the cortical region of the hair. Their effect in the mechanical properties is dependent on the level of damage and humidity conditions. In the virgin hair, coconut and avocado oils reinforce the hydrophobic barrier of the cellular membrane complex, preventing water from intense perturbation of the mechanical properties, leading to increase of the stiffness and break stress. In turn, argan oil, due to the high degree of unsaturation of its fatty acid chains, increase water absorption, leading to loss in the hair resistance. When bleached, the hydrophilicity of the hair is raised, which determines more affinity for argan oil. Consequently, the affinity with the water also is elevated, leading to increased fragility to the mechanical stress. The vegetable oils are not always beneficial for hair care. Their specific chemical characteristics and the hair conditions will influence the final results and should be taken into consideration for hair care developments.

Keywords: argan oil, avocado oil, coconut oil, hair damage, penetration of oils, Caucasian hair, Raman spectroscopy.

1. INTRODUCTION

The diffusion capacity of materials into the hair shaft is related to the efficacy of cosmetic treatments. Chemical treatments commonly used such as bleaching, straightening, and coloring, once reaching the cortex, dramatically modify its structure, breaking covalent bonds, oxidizing lipids, and degrading keratin and other associated proteins (1-5). The entrance of external molecules is the only way to extenuate the damage caused to the structure, neutralizing the generated charged species, restoring hydrophobicity, and partially rescuing mechanical properties (6-8).

The employment of vegetable oils for hair care increased considerably in past years. This fact may be associated with the search of green and renewable sources of ingredients by consumers, which mobilized the cosmetic industry to offer alternatives to fulfil this demand. Another factor is that vegetables oils have been linked to hair benefits that go far beyond the classic combination of lubrication and shine. Reduction of dryness, moisturization, nourishment, strengthening, sebum balance, enhancement of treatment masks, pre-wash protection, frizz control, split end repair, and salt and chloride protection, are among the benefits associated with the use of vegetable oils in hair care (9).

Oils and hair have been investigated by the scientific community who pointed out that their interactions are guided by the affinity of molecules with the cellular membrane complex (CMC), the intercellular cement that keeps the cuticle cells and cortex united, by molecular structure and size (10-17). In addition, there is the influence of the condition of the hair, which may drastically alter the affinities with materials in general. Hence, it is speculative to theoretically determine the behavior of a molecule when applied to the hair.

The study of the effects of vegetable oils in hair, particularly regarding their ability of penetration, has a pragmatic value for cosmetic industries. Many studies have already been conducted, most of them focusing on the following oils: sunflower, olive, rice bran, sesame, mustard, coconut, jojoba, and almond (13-16). Some of the most popular oils for hair care, such as avocado and argan oils, lack further studies on their effects on internal structure of the hair and possible benefits.

Many techniques have been employed to study the penetration properties of molecules in hair (18). More sophisticated methods of analysis are often needed to investigate the molecular interactions that drive the material diffusion into the hair structures, as spectroscopy, spectrometry, tomography, and others (18-26). However, these techniques frequently involve the laborious manipulation of the samples and/or a high cost of operation which reduces the chances of applying it widely in the industrial context.

Considering the aforementioned, this study aimed at evaluating the penetration of argan and avocado oils in comparison with better known coconut oil into hair fibers, using a combination of traditional and modern techniques commonly employed in hair science, particularly Raman spectroscopy, tensile and fatigue tests (19-22, 27-30).

2. MATERIALS & METHODS

2.1. Vegetable oils

Three vegetable oils commonly used in hair care, namely argan, avocado, and coconut oils (Symrise AG, DE), were selected for the further experiments.

2.2. Distribution of fatty acids (by gas chromatography)

Samples were analyzed using a chromatograph (Shimadzu 2010, JAP) with a Flame Ionization Detector (FID). The analyzes were performed in Stabiwax (30 m x 0.25 mm i.d., 0.25 µm film thickness) capillary column. The flow rate of the carrier gas (He) was 1.30 mL/min. The oven temperature was set as follows: 40 °C for 1 min, and further increasing up to 250 °C with a heating rate at 5 °C/min, maintaining the final temperature for 20 min.

2.2. Hair

Natural white hair tresses (25 g, 10 cm wide, and 25 cm long) were purchased from International Hair Importers & Products (New York, NY, USA). These tresses were cut into 2 g fragments, cleaned with a 10% sodium lauryl ether sulfate solution (SLESS), and left to dry overnight under controlled conditions (22 ± 2 °C and 50 ± 5% relative humidity (RH)). Four hair tresses were separated for each vegetable oil treatment group, and another four for the untreated groups.

2.3. Bleaching process

Half of the tresses from each group were subjected to a bleaching process (30 min at 25°C) with a mixture 1:2 (w/w) of commercially available 12% hydrogen peroxide emulsion (Yamá, BRL) and bleaching powder (Yamá, BRL), in a ratio of 2.5 g of product per gram of hair. After the bleaching process, the tresses were rinsed under running water (33 ± 3 °C and 4 L/min), washed with 10% SLESS, and left to dry overnight at controlled conditions (22 ± 2°C and 50 ± 5% RH).

2.4. Vegetable oil application

Each vegetable oil was applied directly to the tresses (1 g/g of hair) for each treatment group, which were then individually folded in aluminum foils and allowed to rest for 24 h at 25 °C before being cleaned with 10% SLESS to remove the excess of oil from the fibers' surface. After washing, the tresses were dried under controlled conditions (22 ± 2 °C and 50 ± 5% RH).

2.5. Tensile test

Forty-five fibers were randomly collected from each group of treatment. Their cross-sectional dimensions were measured using a FDAS-770 - Fiber Dimensional Analysis System (Dia-Stron, UK) and a LSM-6200 – laser scan micrometer (Mitutoyo, JPN). The obtained areas were used to calculate the stress to which the fibers were subjected during the tensile test, which was performed using a MTT-680 – Miniature Tensile Tester (Dia-Stron, UK), with a constant stretch rate (15 mm/min) and under controlled temperature (22 ± 2 °C). The fibers were then soaked in water for one hour at the equipment's carousel (wet tensile). Elastic modulus and break stress parameters were obtained from UvWin software (Dia-Stron, UK). The data was analyzed using one-way ANOVA, followed by post-hoc Tukey HSD test, at 95% confidence interval ($P \leq 0.05$). The analyses were performed using Microsoft Office Excel with XL STAT.

2.6. Fatigue test

Using a FDAS-770 - Fiber Dimensional Analysis System (Dia-Stron, UK) and a LSM-6200 - laser scan micrometer (Mitutoyo, JPN), the cross-sectional dimensions of 50 fibers, randomly collected from each group, were measured. The obtained areas were used to calculate the stress to which the fibers were subjected during the fatigue test, which was performed using a CYC-801 – Cyclic Tester (Dia-Stron, UK), with a constant stress of 130 MPa and a stretch rate of 40 mm/min. The data analysis was carried out using a UvWin software (Dia-Stron, UK).

2.7. Raman spectroscopy analysis

Six fibers from each group were randomly collected and individually positioned over the Raman system lens of 40x magnification. Three points of each fiber were analyzed using a Confocal Raman - Model 3510 Skin Composition Analyzer (Rivers Diagnostics®) coupled to a solid-state laser excitation, operating at 785 nm with the power of 20 ± 2 mW, with a Charge Coupled Device (CCD) detector. The spectra acquisition was performed over a spectral range of 1800 to 400 cm^{-1}. The final spectrum is a combination of the ones obtained for each treatment.

3. RESULTS

3.1. Fatty acid distribution of the vegetable oils

Vegetable oils' samples were esterified to obtain the methyl esters of fatty acids, and their identification was performed by comparing the retention times observed in the analysis of a standard. Table 1 shows the results of the fatty acid distribution of the analyzed oils (argan, avocado and coconut).

Table 1. Fatty acid distribution of the vegetable oils obtained by gas chromatography (GC), evidencing the percentage of normalized area that indicates the relative distribution of compounds in the samples. Results are expressed as mean and mean deviation of two determinations.

Fatty acids	Argan oil (%)	Avocado oil (%)	Coconut oil (%)
C8:0	-	-	4.2 (±0.1)
C10:0	-	-	4.3 (±0.1)
C12:0	-	-	44.4 (±0.1)
C14:0	0.15 (±0.01)	0.04 (±0.01)	20.9 (±0.1)
C16:0	13.3 (±0.1)	17.5 (±0.1)	11.9 (±0.1)
C16:1	0.11 (±0.01)	7.7 (±0.1)	0.02 (±0.01)
C18:0	4.4 (±0.1)	0.46 (±0.01)	3.9 (±0.1)
C18:1	46.6 (±0.1)	62.6 (±0.1)	8.3 (±0.1)
C18:2	35.1 (±0.1)	11.6 (±0.1)	1.7 (±0.1)
C20:0	0.23 (±0.02)	0.06 (±0.01)	0.12 (±0.01)
C22:0	0.07 (±0.01)	-	0.03 (±0.01)
C24:0	0.03 (±0.01)	0.02 (±0.01)	0.06 (±0.01)

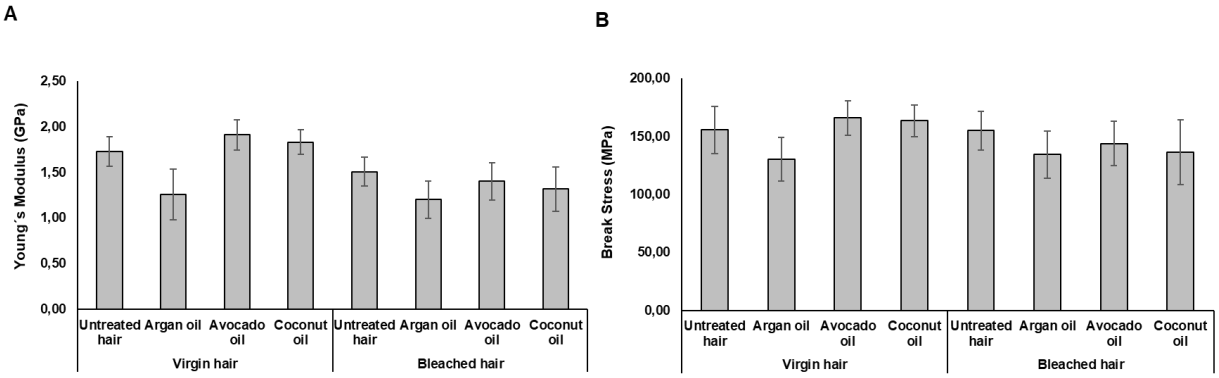
Argan oil presents a high concentration of fatty acids with 18 carbons (almost 81%). This amount is split in the presence of one or two unsaturation, the latter being more relevant. In comparison with saturated chains, the presence of double bonds increases the polarity and adds spatial volume to the molecules. Avocado oil presents a distribution containing 73% of long chain fatty acids (C18), but also a significant presence (approx. 24%) of structures of lower molecular weight relative structures (C16). There is the presence of unsaturated molecules, as in argan oil, but in a lower proportion, and monounsaturated compounds are more present than in argan oil. In general, the distribution of fatty acids in avocado oil is balanced with long and short carbon chains in balanced amounts. Regarding coconut oil, saturated molecules represent more than 85% of its composition in fatty acids, most of which present chains with 12 to 16 carbons. The lack of

unsaturation in these molecules makes them a hydrophobic mixture of components. However, the greater concentration of shorter chains diminishes the hydrophobicity of coconut oil compared to other oils.

3.2. Tensile test

Figure 1 shows the impact of the oils treatments in (A) the Yong´s modulus and (B) the break stress for virgin and bleached hair fibers. For virgin, hair became more elastic (decrease in Young´s modulus) with argan oil and less elastic (increase in Young´s modulus) using the coconut and avocado oils. A similar variation of break stress occurred. For the bleached state, the treatment with the oils led to the reduction of the evaluated mechanical parameters, with argan oil being the treatment with the most important impact.

Figure 1. Mechanical properties of the hair obtained from the tensile test for the virgin and bleached hair with and without the applications of the oils. A – Young´s modulus, representing the elastic behavior of the fibers. B – Break Stress, representing the maximum stress at the rupture moment. The capital and small letters represent the Tukey's analysis of the differences among the treatments with a confidence interval of 95% for the virgin hair and bleached hair, respectively, where the same letters mean $p>0.05$ and different letters mean $p<0.05$.



3.3. Fatigue test

The fatigue test evaluates the failure tendency of hair fibers submitted to repeated mechanical stresses (cycles) within their elastic behavior zone. The generated data do not follow a normal distribution. Instead, the data fit the Weibull distribution, described by alpha (α) and beta (β) parameters. Alpha represents the cycle in which 63.5% of the fibers have already ruptured and expresses the resistance of the fibers to fracture. Beta, in turn, represents the shape of data distribution and, in the fatigue test, is related to

the prematurity of fiber rupture, which is related to the number of cracks cumulated on its surface (27).

Figure 2 and Table 2 show the survival probability curves, over the cycles of mechanical stress, obtained for each group and their values for α and β parameters, respectively. For the bleached hair, the treatment with avocado and coconut oils caused an increase in α of 50% and 109% respectively, with a decrease in β. Meanwhile, argan oil treatment provoked a reduction of both α and β values, with α varying 57% in comparison with the not treated hair. For the virgin hair, on the contrary, the results obtained go in a different direction, showing a reduction of α of 25% and 29% for the avocado and coconut oils respectively, and increase of β for all the tested oils, in comparison to the results obtained for not treated hair. Alpha parameter for the argan oil treated hair was decreased in 68%, even greater variation than the bleached hair treated with argan oil.

Figure 2. Survival probability curves from the fatigue test for virgin (A) and bleached (B) hair with and without oil treatments.

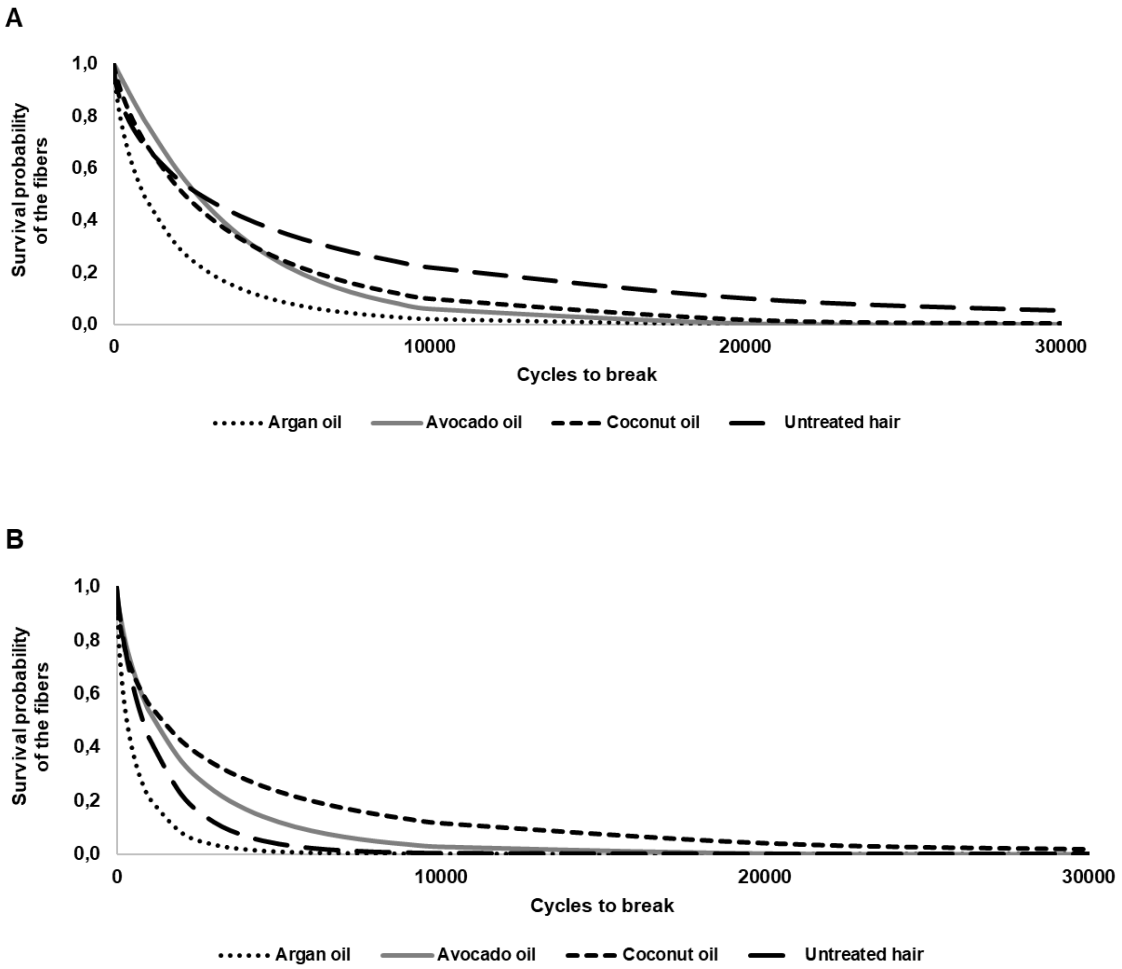


Table 2. Characteristic life (α) and shape parameter (β) obtained from the fatigue test for virgin and bleached hair with and without oil treatments s.

Sample	Characteristic Life (α)		Shape Parameter (β)	
	virgin	bleached	virgin	bleached
Untreated hair	4974	1235	0.60	0.85
Argan oil	1569	526	0.73	0.70
Avocado oil	3720	1858	1.05	0.76
Coconut oil	3503	2585	0.80	0.57

3.4. Raman spectroscopy

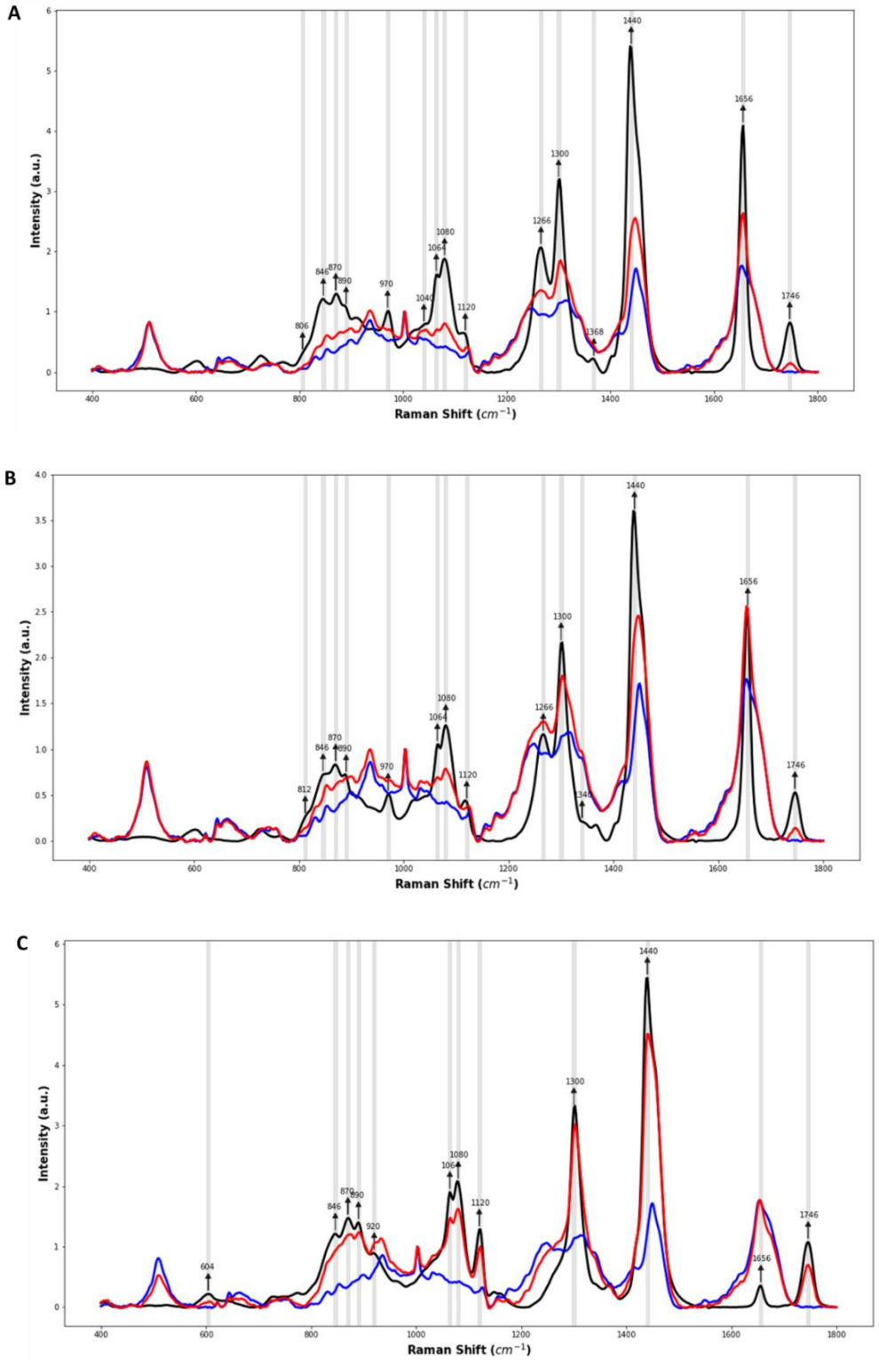
Raman spectroscopy is a technique where the vibrational energy of molecules is changed by the collision of photons from inelastic scattering of light, which causes a modification in their frequency (31). Each functional group constituting the biomolecules vibrates and scatters light at specific frequencies, allowing the respective identification in a matrix.

Considering that hair fibers may present an average cross-sectional dimension of 80 to 100 µm, the characteristic spectra of each isolated oil, of the untreated and treated virgin hair were obtained every 4 µm depth from the cuticles to a depth of approximately 50 µm to cover the depth from the surface to the central zone of the fibers. The spectra were superimposed to determine the bands of interest, defined as those present in the spectra of the treated hair and oils, but absent in the spectrum of untreated hair

Figure 3 - A, B and C show the spectra of the argan, avocado and coconut oils respectively, pointing to the bands identified as of interest. The band at 1064 cm^{-1} appears with relevant intensity in the three oils and consequently in the hair treated with them, but with less intensity in the untreated hair. Thus, for this study, this band was used as a reference for penetration analyses. Once the penetration profiles were determined for each measurement, the average penetration profile was obtained (Figure 4) for the virgin and bleached hair. In both hair states, the coconut oil was identified at a higher intensity in depths beyond 30 µm. Argan and avocado oils were present in lower intensities than coconut oil. The penetration profile of the virgin hair features high intensities of avocado oil up to depths of approximately 25 µm and of coconut oil up to 50 µm. Argan oil presents high intensity at depths of 0 to 5 µm, but its presence is small. The oils generally appear with higher penetration intensities in bleached hair than in virgin hair, most likely because of the facilitated routes, a consequence of the oxidation process to which this hair was subjected.

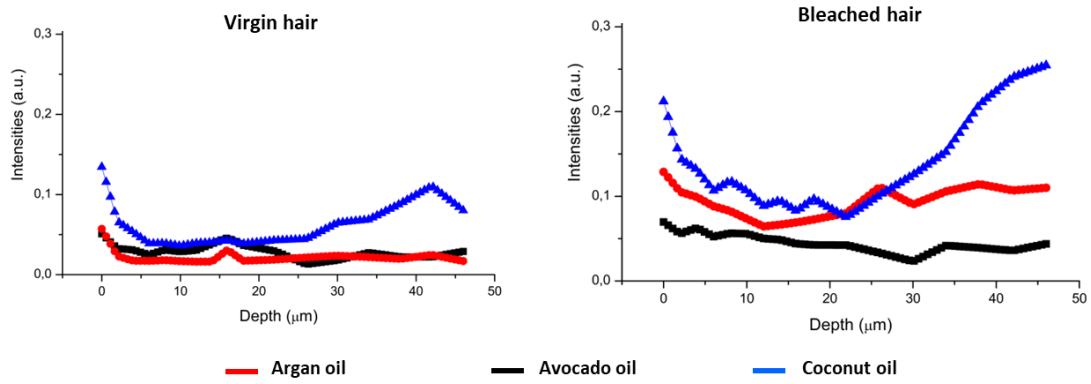
Coconut diffused at higher intensities in this hair type, showing an accumulation at depths of 30 to 50 µm. Argan oil could diffuse in higher amounts than in virgin hair, while avocado oil has little presence in deeper locations.

Figure 3. Spectra of the isolated oil with its respective treated hair and untreated virgin hair for the (A) argan oil, (B) avocado oil, and (C) coconut oil, for the determination of the bands



of interest, defined as those present in the oils and in the treated hairs, but absent in the untreated hair. The grey lines highlight the bands of interest for each oil.

Figure 4. Penetration profile of the argan, avocado and coconut oils in virgin (left) and bleached hair (right) as a function of the depth in the cortical region, measured from the hair surface up to the central area of the cross-section of the fibers.



4. DISCUSSION

Vegetable oils are constituted by a distribution of different fatty acids. These molecules present a long carbon, hydrophobic chain, and a hydrophilic carboxylic acid head. The length of the carbon-saturated chains may vary the hydrophobic character of these species. In the middle of the carbon chain, unsaturation may occur, which generates negative poles due to the concentration of electrons in this molecular zone, providing the molecule with hydrophilic sites. in addition, unsaturation is responsible for enlarging the spatial volume of structures, as they generate folding points in the molecule that increases the three-dimensional occupied area.

These differences in the fatty acids composition of the oils lead to different affinities with the structure of the hair. But as the hair structure is changed by its damage condition, it is expected that also the affinity with these oils will be impacted according to the hair damage level.

Robbins and Crawford (32, 33) concluded from their experiments that the variations in the mechanical properties of the hair are a consequence of alterations in the cortex, without the influence of the cuticles. Based on this, changes in the parameters of

the tensile test on virgin and bleached hair treated with the oils may indicate that they were able to interact with the hair cortex, leading to the conclusion that they could penetrate the hair fibers.

In the fatigue test, the alpha parameter expresses the resistance of the fibers to fracture. Thus, the higher the alpha values for a given group of fibers, the more they will resist the applied stresses. The beta parameter is related to the prematurity of fiber rupture, which is related to the number of cumulated cracks on its surface. Therefore, the lower the beta value, the more premature ruptures are observed, and it can be concluded that this given group of fibers has more presence of cracks on its surface (27).

It is a known fact that the humidity condition plays a vital role in the mechanical properties of the hair. Despite this, the wet-state tensile test seems to increase the sensitivity of the method, as reported by Evans (30). Hair was firstly exposed to the oils and then to the humidity in the tensile test (100%) and fatigue test (50%). Thus, the penetration of oils into hair will be discussed taking into account the influence of water on the mechanical properties.

Virgin hair presents an intact CMC, which favors the diffusion of nonpolar and compact molecules, such as coconut fatty acids. Avocado and argan oils present long carbon chains and unsaturation as a major part of their composition, which hinders the diffusion of these molecules in the hair, and a minor but not negligible portion formed by shorter saturated carbon chains, 25.2% and 17.7%, respectively. Young´s modulus was increased for hair treated with avocado and coconut oils, while decreased for hair treated with argan oil. The same profile was observed for the break stress variations. Due to that, the α parameter obtained in the fatigue test is expected to follow Young´s modulus and also increase, elevating SP, as the hair becomes stiffer and more resistant to the stress. The results of the fatigue test showed that the three oils caused a reduction in the α parameter and in the SP, while raising the β values. With these findings for α and SP, it is reasonable to conclude that the difference in humidity conditions of the two tests affected the results.

The compositions of avocado and coconut oils have less unsaturation and, consequently, smaller molecular volumes, which favored their deeper diffusion into the virgin hair cortex. Argan oil could also diffuse, as evidenced by the results, but remained between the cuticles layers and in the outer zones of the cortical region thanks to its larger molecular volume. The penetration profile of this oil identified higher intensities of 0 to 5 µm. Given its higher degree of unsaturation and because it could not reach the cortex in depth and in large amounts, the argan oil-treated hair cortex remained less hydrophobic in comparison with the cortex of hair treated with avocado and coconut oil. In the tensile test, these differences in the hydrophobicity of the cortices penetrated by the oils determined their relationship with the added water. The presence of argan oil, rich in unsaturation, led

to an increased affinity to the added water, which generated an increase in the plasticizing effect in this sample. As a result, the Young´s modulus and break stress were reduced. The avocado and coconut-treated hairs repelled water more intensely, as their cortices were more hydrophobic, leading to an increase in Young´s modules and, consequently, of the break stress.

In the relative humidity of 50%, where the fatigue test occurred, the relationship of the hair with the water is less pronounced. We conjecture that the observed results show more clearly the effects of the oils on the hair structural molecules, as water is not so relevant for the interpretation of the results. Marsh et al. (34) pointed out that the presence of external materials inside the fiber can interfere with molecular interactions in the cortex. Having penetrated through the intact CMC of the virgin hair, avocado and coconut oils probably established hydrophobic interactions with the keratin chains and the matrix proteins. These interactions generate competition for the inter and intramolecular interactions of the matrix and keratin chains (29). The mechanical stress resistance supported in part by these interactions is decreased, leading to lower breaking force, and consequently lower survival probability. Beta (β) increased with the oils treatments, demonstrating that the propagation of flaws in the hair surface tends to reduce with the presence of the oils.

In the bleached hair, the oxidation provoked by the bleaching process cleavages bonds and changes the chemical character of the structural molecules of the hair. The cuticular barrier can be partially reduced with the loss of scales, or cracked, making the way shorter and facilitating molecules transportation. The bleached hair, therefore, has a greater affinity with polar substances. Parameters from the tensile test showed that the effect of the treatment with avocado was different from argan and coconut treatments. The latter ones diminished the Young´s modulus and the break stress, while the treatment with avocado did not. In this type of hair, the CMC has been partially degraded and there is the presence of charged species in the cortical area. The affinity of this cortex for non-polar materials is diminished. The penetration profile of these oils in the bleached hair points that coconut and argan oil could diffuse in higher amounts through the cortex than avocado oil. The argan fatty acids, richer in unsaturation than the other two oils, possessed an advantage for diffusion due to their polarity. Coconut oil, whose composition presents fatty acids with more polarity in comparison to avocado oil, also presented more affinity with bleached hair than avocado oil. Avocado oil, then, had a disadvantage for diffusion in the bleached hair. Although coconut and argan could diffuse more, the highly hydrophilic condition of the hair could not be significantly changed by the presence of the oils. Moreover, the results suggest that the presence of argan oil intensified the hair’s affinity with the water, when compared to coconut and avocado oils, culminating in a more

pronounced humidity effect, which led to the reduction of the mechanical parameters. The higher amount of combined non-polar fatty acids and the lower level of unsaturation in avocado oil compared to the other two, may have led it to concentrate more on the hair surface, being more intensely removed during the pre-test washing.

Evans (28) observed that lubrication at the hair surface impacts in reduction of the grooming forces, and consequent reduction of the fatiguing forces, meaning α and β should increase with the surface lubrication effect. In the dry-state fatigue test, avocado and coconut increased α, meaning they contributed to increase hair resistance to the breakage. Given the low presence of polar groups in their majority fatty acids chains, the hair affinity with these oils was reduced. It is probable that they were retained in the outer zones of the cortical region and in the cuticular layers, forming a more hydrophobic belt, retarding the absorbance of the humidity from the environment. These findings are in accordance with those obtained by Keis et al. (35) in their vapor adsorption experiments, in which a similar effect was observed due to a clogging effect caused by the treatment with oils, decelerating the water dissipation in the hair. Argan oil, on the contrary, lower the value of α, increasing hair fragility to mechanical stress. We conjecture that the presence of the argan oil on the cortex, as well as in tensile test, contributed to the increase in the absorbance of humidity from the environment. The β parameter shows that the treatments lead to an increase in the propagation of cracks in the hair surface, showing the oils could not protect the hair from premature breakage, an aftereffect of the aggressive bleaching process.

The diffusion pathway of materials in the hair cortex has been a topic of interest for many scientists (36-38). These works showed that the most preferred route seems to be the intercellular route, where materials surround the cells of the cuticle and the cortex, through the CMC and non-crystalline regions. Hornby et al. (11) added that the low-sulfur, non-keratinous environs swell easier than the highly cross-linked regions, accommodating better the presence of diffusing molecules. The same was pointed out by Malinauskyte at al. (8), which evidenced that low molecular weight species allocate in these regions, where they establish ionic interactions with the matrix proteins.

5. CONCLUSION

The penetration of cosmetic agents in hair is a strategy to recover part of the properties of the hair fibers, lost in chemical treatments. Vegetable oils, in general, gather a list of relevant and much more convenient characteristics for the cosmetic industry, such as relative ease of obtention, green process, sustainable label, safety and efficiency, and

for consumers, such as the associated benefits of sensory, lubrication, strength, resistance and many others. Among the oils employed for hair care, avocado and argan oils have not yet been studied in depth. Coconut oil, on the other hand, is known for its penetration ability and interactions with the hair cortex, being selected as a reference for our study. We concluded that the three oils are constituted of fatty acids capable of penetrating and interacting with the hair cortex. The chemical character of the hair, when intact or damaged, will determine the interactions with the different materials, increasing or decreasing their affinity with the internal structure, leading to different effects. Once penetrated in the hair, they will modify the way the hair interacts with the water and, consequently, how water will affect the mechanical properties of the hair structure. Avocado and coconut oils will interact more with the CMC lipid route intensifying the hydrophobic character of virgin hair and preventing water from diffusing through the structure. On bleached hair, their effect will be insufficient to protect the hair from humidity and recover the mechanical properties. Argan oil, on the contrary, despite having penetrated the hair, acted by increasing the hair's affinity for water instead of reducing it, leading to the expansion of the hair's affinity for water and consequently making it more fragile to rupture.

CONFLICTS OF INTEREST

The authors declare that there is no conflict of interest.

6. REFERENCES

1. Tate, M.L., Kamath, Y.K. and Ruetsch, S.B. Quantification and prevention of hair damage. Journal of the Society of Cosmetic Chemists. 44:347-71 (1993).

2. F.-J. Wortmann, C. Springob, and G. Sendelbach, Investigations of Cosmetically Treated Human Hair by Differential Scanning Calorimetry in Water, Journal of Cosmetic Science. 53, 219-228 (2002).

3. F.-J. Wortmann, G. Sendelbach, and C. Popescu, Fundamental DSC Investigations of A-Keratinous Materials as Basis for the Interpretation of Specific Effects of Chemical, Cosmetic Treatments on Human Hair, Journal of Cosmetic Science., 58, 311-317 (2007).

4. T. Fujii, Y. Ito, T. Watanabe, and T. Kawasoe, Effects of Oxidative Treatments on Human Hair Keratin Films, Journal of Cosmetic Science., 63, 15-25 (2012).

5. Grosvenor, A.J., Deb-Choudhury, S., Middlewood, P.G., Thomas, A., Lee, E., Vernon, J.A., et al. The physical and chemical disruption of human hair after bleaching - studies by transmission electron microscopy and redox proteomics. International Journal of Cosmetic Science. 40(6):536-48 (2018).

6. Ruetsch, S. B., and Kamath, Y. K. Penetration of cationic conditioning compounds into hair fibers: A TOF-SIMS approach. .Journal of Cosmetic Science. 56:323-330 (2005).

7. 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 and Surfaces B: Biointerfaces. 174:563-568 (2019).

8. Malinauskyte, E., Shrestha, R., Cornwell, P. A., Gourion-Arsiquaud, S. and Hindley M. Penetration of different molecular weight hydrolysed keratins into hair fibres and their effects on the physical properties of textured hair. International Journal of Cosmetic Science. 1–12 (2020).

9. Manoel, I. Manual dos cabelos: o poder os óleos. Sao Paulo, São Paulo, Brazil. Laços (2003).

10. Gode, V., Bhalla, N., Shirhatti, V., Mhaskar, S. and Kamath, Y. Quantitative measurement of the penetration of coconut oil into human hair using radiolabeled coconut oil. Journal of Cosmetic Science. 63(1):27-31 (2012).

11. Hornby, S. B., Appa, Y., Ruetsch, S., and Kamath, Y. Mapping penetration of cosmetic compounds into hair fibers using time-of-flight secondary ion mass spectroscopy (TOF-SIMS). IFSCC Magazine. 8(2): 99-104 (2005).

12. Rele, A.S. and Mohile, R. Effect of coconut oil on prevention of hair damage. Part I. Journal of Cosmetic Science. 50(6):327-39 (1999).

13. Ruetsch, S. B., Kamath, Y., Rele, A. S. and Mohile, R. B. Secondary ion mass spectrometric investigation of penetration of coconut and mineral oils into human hair fibers: relevance to hair damage. Journal of Cosmetic Science. 52:169-184 (2001).

14. Srivastav, A., Dandekar, P. and Jain, R. Penetration study of oils and its formulations into the human hair using confocal microscopy. Journal of Cosmetic Dermatology. 18(6):1947-54 (2019).

15. Leite, M. G. A., Campos, P. M. B. G M. Development and efficacy evaluation of hair care formulations containing vegetable oils ans silicone. International Journal of Phytocosmetics and Natural Ingredients. 5-9 (2018).

16. Keis, K., Persaud, D., Kamath, Y.K. and Rele, A.S. Investigation of penetration abilities of various oils into human hair fibers. Journal of Cosmetic Science. 56(5):283-95 (2005).

17. Fregonesi, A., Scanavez, C., Santos, L., Oliveira, A., Roesler, R., Escudeiro, C., Moncayo, P., Sanctis, D. and Gesztesi, J. L. Brazilian oils and butters: the effecr of different fatty acid chain composition on human hair physiochemical properties. Journal of Cosmetic Science. 60:273-280 (2009).

18. Lourenco, C. B., Fava, A. L. M., Santos, E. M., Macedo, L. M., Tundisi, L. L., Ataide, J. A. and 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. International Journal of Cosmetic Science. 43:113–122 (2021).

19. Zimmerley, M., Lin, C.-Y., Oertel, D. C., Marsh, J. M., Ward, J. L. and Potma, E. O. Quantitative detection of chemical compounds in human hair with coherent anti-Stokes Raman scattering microscopy. Journal of Biomedical Optics. 14(4):044019- (2009).

20. Colthup, N.B., Daly, L.H. and Wiberley, S.E. Introduction to Infrared and Raman Spectroscopy (Third Edition). San Diego: Academic Press (1990).

21. Kuzuhara, A. Raman Spectroscopic Analysis of L-Phenylalanine and Hydrolyzed Eggwhite Protein Penetration into Keratin Fibers. Journal of Applied Polymer Science. 122 (2011).

22. Kuzuhara, A. A Raman spectroscopic investigation of the mechanism of the reduction in hair with thioglycerol and the accompanying disulphide conformational changes. International Journal of Cosmetic Science. 40(1):34-43 (2018).

23. Kojima, T., Tsuji, S., Niwa, M., Saito, K., Matsushita, Y. and Fukushima, K. Distribution Analysis of Triglyceride Having Repair Effect on Damaged Human Hair by TOF-SIMS. International Journal of Polymer Analysis and Characterization. 17(1):21-8 (2012).

24. Brunelle, A., Touboul, D. and Laprévote, O. Biological tissue imaging with time-of-flight secondary ion mass spectrometry and cluster ion sources. Journal of Mass Spectrometry. 40(8):985-99 (2005).

25. Saleem, M. and Galla, H.-J. Surface view of the lateral organization of lipids and proteins in lung surfactant model systems—A ToF-SIMS approach. Biochimica et Biophysica Acta (BBA) - Biomembranes. 1798(4):730-40 (2010).

26. Tsugita, T. and Iwai, T. Optical coherence tomography using images of hair structure and dyes penetrating into the hair. Skin research and technology. Official Journal of International Society for Bioengineering and the Skin (ISBS) [and] International Society for Digital Imaging of Skin (ISDIS) [and] International Society for Skin Imaging (ISSI). 20(4):389-98 (2014).

27. Evans, T. Fatigue testing of hair – a statystical approach to hair breakage. Journal of Cosmetic Science. 60:599-616 (2009).

28. Evans, T. An unifying theory for visualizing the causes of hair breakage and subsequent strategies for mitigation. Journal of Cosmetic Science. 68:137-140 (2017).

29. Wortmann, F.-J and Zahnt, H. The Stress Strain Curve of alpha-Keratin Fibers and the Structure of Intermediate Filaments. Textile Research Journal. 64(12): 737-743 (1994).
30. Evans, T. Measuring hair strength – Part 1: Stress-Strain Curves. Cosmetics and Toiletries. 128(8):591-594 (2013).
31. Raman, C. V., Krishnan, K. S. A new type of Secondary Radiation. Nature. 121:501 (1928).
32. Robbins, C. R., Crawford, R. Cuticle damage and the tensile properties of human hair. Journal of the Society of Cosmetic Chemistry. 42:59-67 (1991).
33. Robbins, C.R. Chemical and Physical Behavior of Human Hair. 5th ed. New York, NY: Springer-Verlag Berlin Heidelberg (2012).
34. Marsh, J. M., Clarke, C. J., Meinert, M. and Dahlgren, R. M. Investigations of cosmetic treatments on high-pressure differential scanning calorimetry. Journal of Cosmetic Science. 58:319-327 (2007).
35. K. Keis, C. L. Huemmer and Y. Kamath. Effect of oil films on moisture vapor absorption on human hair. Journal of Cosmetic Science. 58:135-145 (2007).
36. Potsch, L. and Moeller, M. R., On Pathways for Small Molecules into and Out of Human Hair Fibers. Journal of Forensic Sciences. 41(1):121-125 (1996).
37. A. Kelch, S. Wessel, T. Will, U. Hintze, R. Wepf and R. Wiesendanger, Penetration Pathways of Fluorescent Dyes in Human Hair Fibres Investigated by Scanning Near-Field Optical Microscopy, Journal of Microscopy. 200, pp. 179-186 (2000).
38. Gummer, C. L. Elucidating penetration pathways into the hair fiber using novel microscopic techniques. Journal of Cosmetic Science. 52:265-280 (2001).

CAPÍTULO III. "PENETRATION OF VEGETAL OILS INTO TEXTURED HAIR FIBERS: A JOINT OF MOLECULAR MALDI-TOF ANALYSIS AND MECHANICAL MEASUREMENTS"

Carolina Botelho Lourenço, Rebeca Mantuan Gasparin, Fernanda Malanconi Thomaz, Valéria Longo Parsekian, Ana Claudia Paiva-Santos, Priscila Gava Mazzola

Por ser submetido

ABSTRACT

The valorization of the naturality in beauty has encouraged textured hair women to assume their original hair and use the bleaching as a choice to vary the style. However, bleaching intensifies a condition of natural fragility of this type of hair, which requires treatments that contribute to partially recover its mechanical properties. Vegetable oils, popularly associated with strengthening, were studied regarding (I) their ability to penetrate this virgin and bleached hair type with Matrix Assisted Laser Desorption Ionization (MALDI) time-of-flight (TOF) and (II) their effects using tensile and fatigue tests. MALDI-TOF results show that groups of molecules of the oils were identified in the cortical region of the bleached textured hair. However, tensile test results show that the treatment with the oils could not alter its mechanical properties. Fatigue test showed an increase in the virgin hair resistance most probably resulting from a lubrication effect in the outermost portions of the cortex and cuticles. In the bleached hair, a reduction of resistance was noted from the treatment with the oils. The differences of diffusion of the textured hair in comparison with the Caucasian hair, studied in previous work of our group, indicate the external molecules diffuse more homogeneously in the latter one than in the first one. The unique cortical settlement of the textured hair imposes two areas with different diffusion zones that determines the irregularity of occupancy of the external materials, leading to effect others than those observed for the Caucasian hair.

Key words: argan oil, avocado oil, coconut oil, textured hair, penetration of oils, Caucasian hair.

1. INTRODUCTION

With the growing tendency to value the naturalness of beauty, women with textured hair began to proudly assume their curls and demand from the cosmetic industry customized and efficient solutions to alleviate their difficulties and address their needs. Straightening has lost room in salons and the bleaching process, that was previously little considered by these consumers, has become an option to lighten and vary the look.

Bleaching is one of the most aggressive processes into which the hair can be subjected, generating an intensification of the known fragile condition of the fibers (1-5). Textured hair is known for its relative fragility to face mechanical aggression and is prone to breakage with lower levels of applied force (6). Due to the presence of twists and flattened configuration of the fibers, there is an irregular mass distribution, forming points of greater and lesser accumulation, which consequently create areas of greater susceptibility to breakage (6,7).

The diffusion of external materials to the cortical region of the strands has been studied and can partially reverse the fragility, improving hair resistance (8-14). Vegetable oils have a wide space in these initiatives as they are popularly associated with reduction of dryness, nourishment, strengthen, pre-wash protection, frizz control and split end repair (15).

There are particularities of textured hair morphology that orient uniquely the mode how the molecules will diffuse into this type of hair and consequently the benefits that comes from these interactions. This hair shaft possesses a characteristic appearance formed by waves and twists that varies in different individuals according to their tightness. Chemically, studies showed that no significant differences occur regarding the protein content of the hair in comparison to the Caucasian and Asian hair types (16-20). The reason behind the curls formation relies on the disposal of distinct cortical cells, forming two differentiated zones so called paracortex and orthocortex (19-22). The ratio between the intermediate filament protein (IF's) and the keratin associated protein (KAP's) determine the type of cortex to be formed (19). The intermediate filaments (IFs) presented shorter straight shape, aligned to the hair axis, characterizing the paracortex, when closer to the internal portion of the curls and longer, spiral shape, characterizing the orthocortex, when in the external portion of the curls (23-26), evidencing a bilateral distribution of the cells in the curly hair type. The lipid content completes the differences pointed between textured hair and the others, as demonstrated by Cruz et al. and Marli et al. (27, 28) who reported that this type of hair presents higher lipid content than the

others suggesting the possibility that it comes from the absorption of sebum of the surface.

The study of the penetration of molecules into the hair requires the employment of a combination of techniques to ensure the correct interpretation of the diffusion events (29). Matrix Assisted Laser Desorption Ionization (MALDI) time-of-flight (TOF) was used to study the diffusion process of vegetable oils, namely coconut, argan and avocado oils. This technique has been used for a long time in biological fields where it allows the probe of a diversity of specimens, such as proteins, peptides, saccharides, and nucleotides (30). In the human hair, it is most applied in the investigation of the presence of drugs, most employed in forensic sciences. The cosmetic industries search techniques capable of elucidating the mechanism of action of a variety of molecules to study their effect and prove their efficacy on the substrates, skin and hair. Although among the mass spectrometry techniques, secondary ion mass spectrometry (SIMS) has been more utilized in hair studies (8, 31, 37) due to its resolution, MALDI-TOF permits the investigation of wider mass range materials (1-500 kDa) than SIMS (30).

The goal of this work is the understanding of the penetration of argan, avocado and coconut oil in textured hair type at the molecular level and its effects in the macroscopic properties of the virgin and bleached states.

2. MATERIALS & METHODS

2.1. Distribution of fatty acids

Argan, avocado, and coconut oils (Symrise AG, DE) were characterized using a chromatograph (Shimadzu 2010, JAP) with a Flame Ionization Detector (FID). Details of this analysis is reported in our previous work (32).

2.2. Hair

Textured hair type IV (Loussouarn et. al (33)) and 17 cm long was purchased from International Hair Importers & Products, Ltd (New York, NY, USA). Two-gram tresses were prepared using lamination adhesive (Iberê, BRL), cleaned with 10% sodium lauryl ether sulfate solution (SLESS), and left to dry overnight under controlled conditions (22 ± 2 °C and 50 ± 5% relative humidity [RH]). Four hair tresses were separated for each of the vegetable oil treatments and another four for the untreated group.

2.3. Bleaching process

The bleaching process occurred for 30 min at 25°C, using a mixture 1:2 (w/w) of commercially available 12% hydrogen peroxide emulsion (Yamá, BRL) and bleaching powder (Yamá, BRL). The cream formed was applied to hair following the ratio of 2.5 g of product per gram of hair. The tresses were, then, rinsed under running water (33±3 °C and 4 L/min), cleaned with 10% SLESS, and left to dry overnight at controlled conditions (22±2°C and 50±5% RH).

2.4. Oil application

The oils were applied directly to the tresses at the proportion of 1 g per gram of hair. The tresses were then individually folded in aluminum foils and allowed for 24 h at 25 °C before the washing with 10% SLESS to remove the excess of oil from the fibers surface. The tresses were dried under controlled conditions (22±2 °C and 50±5% RH).

2.5. MALDI-TOF analysis

Analyzes were carried out in the bleached hair tresses. After the damaging process and oil application, they were submitted to a dialysis process for complete removal of SLESS residues, which may coincide with the matrices and oils structures in the spectra. The water used was changed every two hours for 12 hours, followed by a resting time of 12 hours and repetition of the process until completing 48 hours. The oils

were applied to the tresses according to the described in Oil Application section and then washed with a 5% LESS solution for 1 minute and rinsed with running water in abundance. The samples underwent a final rinse with deionized water to ensure the removal of oil from the surface of the fibers, which could be dragged to the cortex during the subsequent step of cutting the samples, and then were placed in an oven at 28°C for 24 hours 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°C and an aluminum block with grooves between 40 and 50 μm, in which the hair fibers were positioned and attached to with adhesive tape. Then, the fibers were fixed on glass slides with conductive coating ITO (indium tin oxide) with Scotch® 419 aluminum tape (3M, Brazil) fixing the fibers tips. A mixture of dihydroxybenzoic acid (DNB) and α-cyano-4-hydroxycinnamic acid (CHCA) matrices in a 3:1 ratio (m/m) with a concentration of 8 mg/mL of 1:1 acetonitrile solution acidified with 0.1% trifluoroacetic acid (v/v) was applied to the slide using TM-Sprayer™ equipment (HTX Technologies).

MALDI-TOF/TOF analyzes were carried out in the Autoflex Mass Spectrometer maX® (Bruker Daltonics, Bremen, Germany), with Smartbeam-II laser with a wavelength of 355 nm, with laser power at 90%, focus diameter at 80 μm and repetition rate at 2000 Hz. The spectra were obtained in the positive ion mode, using the reflector in the region of m/z 450-2000. The FlexControl software (Bruker Daltonics) was used for acquisition and flexAnalysis software (Bruker Daltonics) added to the open access MZmin software for data processing. Ten measurements were made in each fiber, in a total of two per treatment.

2.6. Tensile test

The cross-sectional dimensions of 45 fibers from the treated and not treated tresses were measured using a FDAS-770 - Fiber Dimensional Analysis System (Dia-Stron, UK) and a LSM-6200 – laser scan micrometer (Mitutoyo, JPN) to calculate the sectional areas of the fibers. The tensile test was performed using a MTT-680 – Miniature Tensile Tester (Dia-Stron, 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 the fibers humidity. The data was extracted from UvWin software (Dia-Stron, UK) and analysed using one-way ANOVA, followed by post-hoc Tukey HSD test, at 95% confidence interval. The analyses were performed using Microsoft Office Excel with XL STAT.

2.7. Fatigue test

The cross-sectional dimensions of 50 fibers from the treated and not treated tresses were measured using a FDAS-770 - Fiber Dimensional Analysis System (Dia-Stron, UK) and a LSM-6200 – laser scan micrometer (Mitutoyo, JPN) to calculate the sectional areas of the fibers. The fatigue test was performed using a CYC-801 – Cyclic Tester (Dia-Stron, UK), with a constant stress of 130 MPa and a stretch rate of 40 mm/min. The data analysis was performed using a UvWin software (Dia-Stron, UK).

3. RESULTS

3.1. Fatty acid distribution of the oils

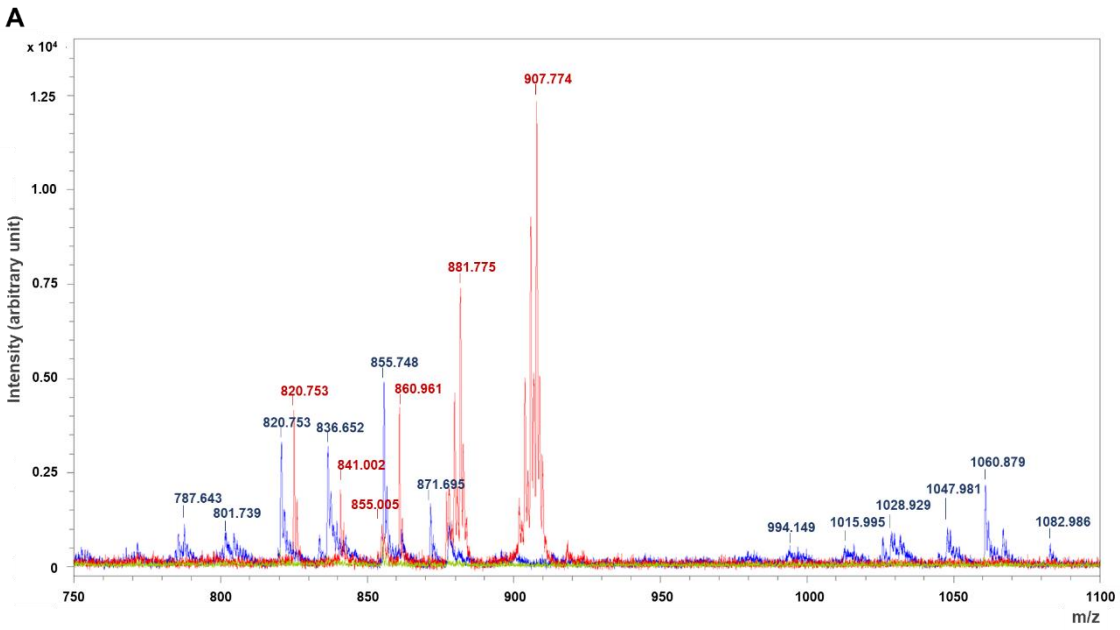
Our group employed esterification to obtain the methyl esters of the fatty acids of the three oils. The different retention times were used to identify the molecules in comparison with a standard and finally obtain the fatty acid distribution of the oils. Results have been reported in previous publication (32).

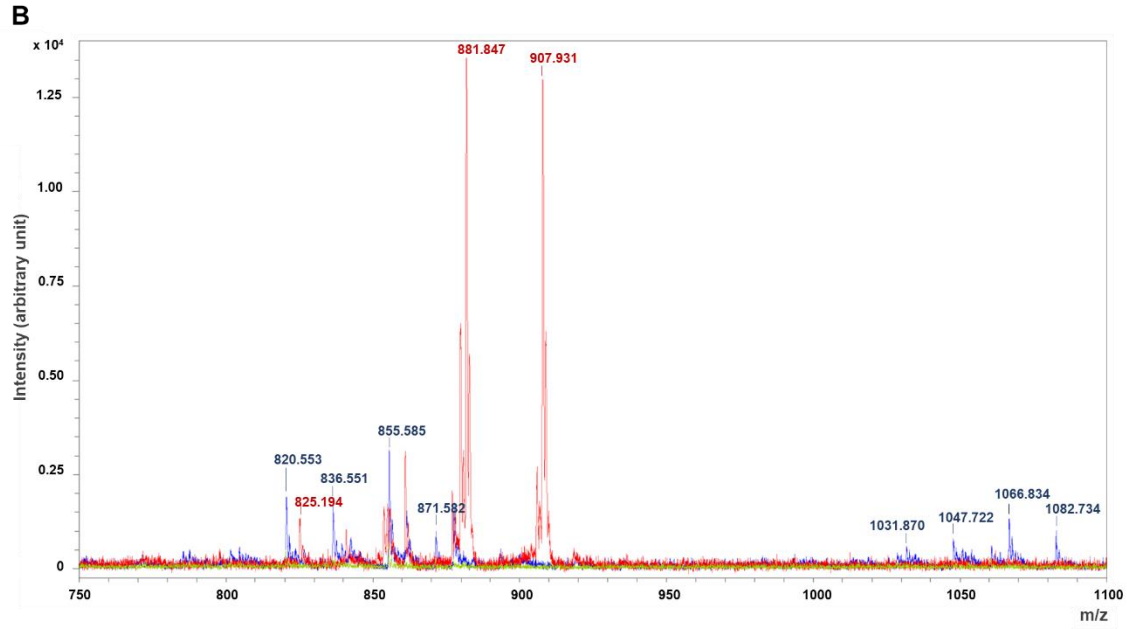
3.2. MALDI-TOF/TOF

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 from the conversion of a laser energy into heat energy. In this process, the matrix, which is deposited over the analyte, carries its molecules generating its ionization by ion or charge transference (30). These ionized agents are then separated based on their mass-to-charge ratio (m/z), with the usage of a time-to-flight (TOF) mass analyzer.

Figure 1 shows the spectra of the isolated oil, the treated hair, the not treated hair and the matrix used in the sample. Argan, avocado and coconut oils were identified by letters A, B and C, respectively. The generated ions as well as the ratio among the ions are specific for each material and provide the signature for its identification. The m/z values of the ions generated for each oil and the respective treated hair are shown in the spectra for each oil.

Figure 1. Spectra from 750 to 1100 m/z with identified peaks of the isolated oil, the oil treated hair, the untreated hair and the matrix. The red line represents the peaks found for the isolated oil. The blue one represents the oil treated hair. The green one sets for the untreated hair and finally the black one represents the matrix. A - spectrum of the argan oil. B - spectrum of the avocado oil. C - spectrum of the coconut oil.





C

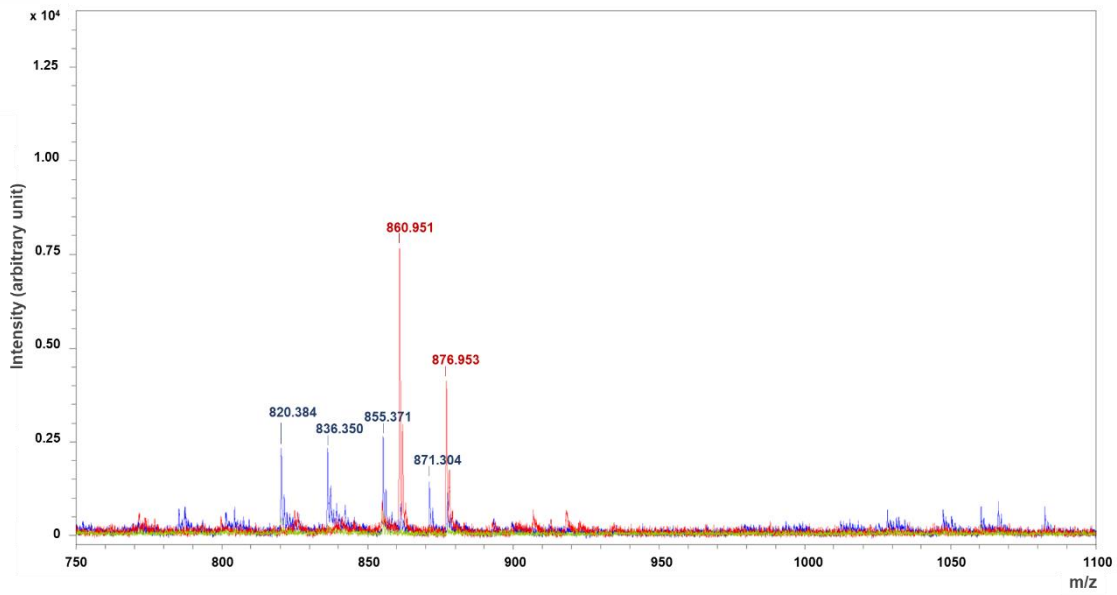


Table 1 shows the obtained values for m/z associated with their intensities, for the three studied oils and for the not treated hair. The highlighted intensities are present in high levels for the three oils within a narrow range of values for m/z, indicating that, although with slight deviations among 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 and the registered intensities provide an estimate of their relative quantity in the cortical zone. The results show the argan oil was detected with greater intensity, followed by the avocado oil and then by coconut oil, which was detected in smaller intensities in the bleached textured hair.

Table 1. Values for m/z and their respective intensities for the three studied oils and for the not treated 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 values of m/z are not detectable in the not treated hair, leading to the conclusion that they express the oils presence in the treated hairs.

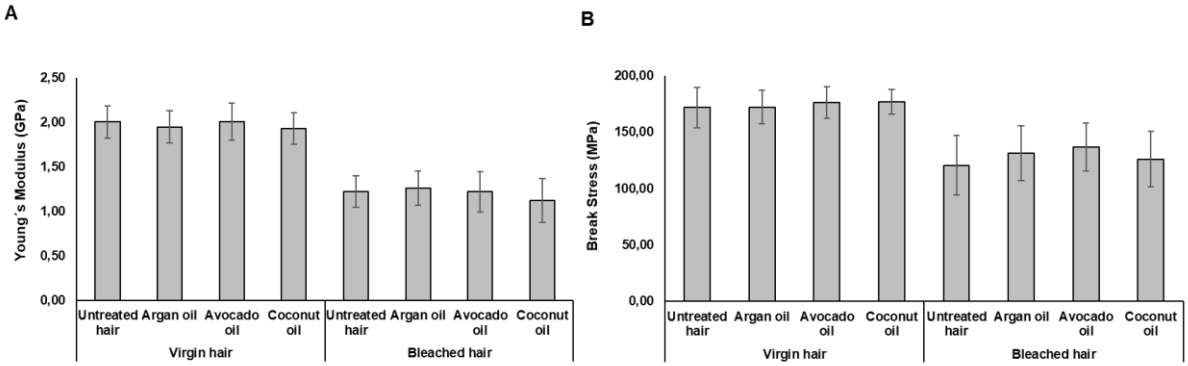
m/z	Intensities of the oils		
	Argan	Avocado	Coconut
801,739	943		
820,384			2338
820,553		1914	
820,753	3311		
836,35			2334
836,551		1696	
836,652	3205		
855,371			2640
855,585		3160	
855,748	4924		
861,585		1544	
871,304			1436
871,582		1001	
871,695	1676		
877,586		1762	
878,092	1180		
1010,583			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 4 demonstrates the results obtained for the Young´s modulus (A) and break stress (B) for the wet tensile test. For the virgin hair, none of the treatments could modify the Young´s modulus neither the break stress of these fibers. For the bleached hair, although the treatments eventually led to the same level of stiffness of the not treated hair, it is possible to note the difference between the argan oil and the coconut oil treated hairs. Argan oil showed a tendency of increasing of the stiffness of the textured hair as Young´s modulus increased while coconut led textured hair to become more elastic in the comparison of the two treatments. Avocado oil, differently, significantly

impacted the break stress of textured bleached hair, elevating the resistance of the hair to breakage.

Figure 4. Mechanical properties obtained from the wet Tensile Test for the virgin and bleached textured hair with and without the treatment with the oils. A – Young´s Modulus. B – Break Stress. Same letters mean p>0.05. Different letters mean p<0.05.



3.4. Fatigue Test

Fatigue test is employed in a more pragmatic approach in relation to the Tensile test as it permits the investigation of the mechanical resistance of the hair to low loads, but applied repeatedly, to simulate the daily routine of consumers, who submit the fibers to combing and grooming constantly with such level of stresses. It leads fibers weaker due to previous damage to failure with the little repetition of the loads, generating a curve of the survival probability (SP) of the fibers.

Fatigue test curves are shown in Figure 5. For the virgin hair (Figure 5A; table 2), argan oil showed greater impact than the other two oils. Table 2 shows the values of α parameters for the oils and untreated hair. Through the advance of the load applications, avocado oil highlighted with the increase of the resistance of the hair, leading α to raise around 26% of its initial value. Coconut oil impacted α parameter similarly, with an increase of 24%. Argan oil provoked the dramatically decrease of both SP and α parameter, the latter to diminished 73%.

In the bleached hair (Figure 5B; table 2), the performance of the oils did not point improvement of SP. Yet, argan oil showed greater influence with reduction in α parameter of 54%. Coconut oil demonstrated less influence with α varying 10% only. Avocado oil presented similar profile of the curve but a variation in α of 28%.

Beta parameter was reduced with the treatment with argan and coconut oils while raised for the one with avocado oil, in the virgin hair. In the bleached hair, there

was no variations in beta parameter with the coconut and avocado oils treatments, but argan oil treatment impacted in the increase of β.

Figure 5. Survival probability results from the Fatigue Test for the virgin (A) and bleached (B) hair with and without treatment with the oils.

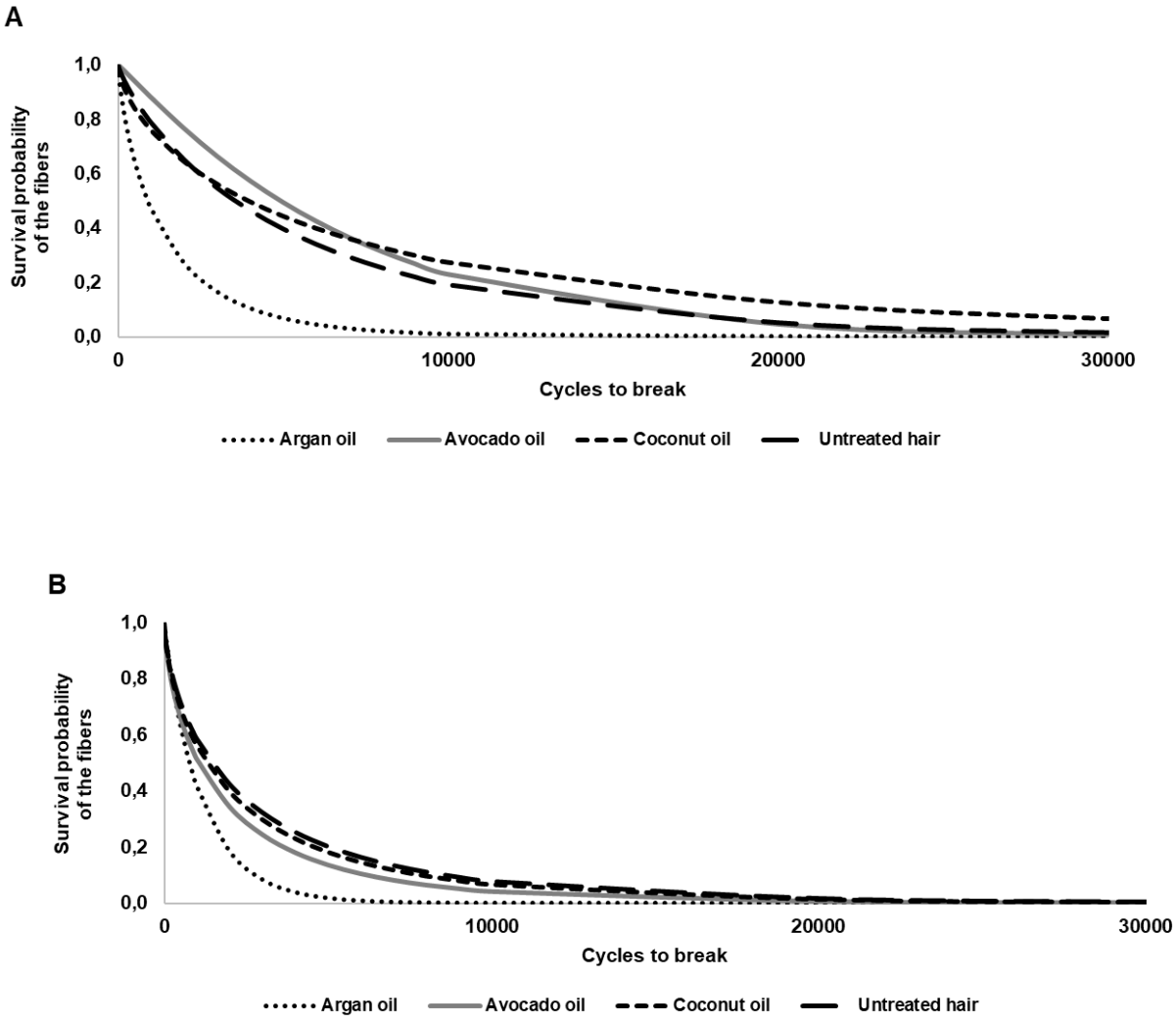


Table 2. Characteristic life (α) and shape parameter (β) obtained from the Fatigue Test for the virgin and bleached hair with and without treatments with the oils.

Treatment	Characteristic Life (α)		Shape Parameter (β)	
	virgin	bleached	virgin	bleached
Untreated 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

4. **DISCUSSION**

In previous work, our group investigated the fatty acid distribution of coconut, argan and avocado oils (32). Saturated molecules with chain length up to 16 carbons represent more than 85% of coconut oil fatty acids composition. Argan oil presents a composition rich in longer carbon chain fatty acids, above 80% of 18 carbons, with one or two unsaturation in most of its molecules. These characteristics lead oils to acquire a more hydrophilic character as the unsaturation are sites of electron concentration and the relative shorter carbon chain favors the interactions with water. Avocado oil, diversely, contains more than 70% of fatty acids with 18 carbon and more than 20% of molecules with 16 carbons. It presents more unsaturation than coconut oil fatty acids, but less than argan oil molecules, presenting an intermediate character among the oils. These aspects determine how these oils will interact with the hair in both virgin and bleached condition, due to the important chemical changes that occurs in its structure during the bleaching process. Besides the damage condition, the morphology of the hair also plays an important role in the kinetics of diffusion of materials.

The tensile test generates the parameters Young´s modulus and break stress. The first one expresses the stiffness of the fibers and is associated with the weak interactions at the molecular level of keratin, which are responsible for the elastic behavior of the fiber in extensions of 0 to 5%. Break stress reflects the maximum stress the fiber can resist before the rupture. Variations in these parameters are consequence of molecular interactions with the keratin and matrix proteins, thus reflecting that a given material reached the cortical portion (19). Ruptures in tensile test often happens at extensions above 30% strain, which do not represent the situation the consumers' hairs are exposed daily. Due to that, fatigue test was developed to permit the evaluation of resistance of the hair fibers to mechanical stresses with low load levels but repeated several times to mimic their regularly routine (34).

Fatigue test is a single fiber test method where the fiber is pulled within its elastic zone repeated times with a constant stress or strain. This process provokes the formation of small cracks on hair surface and the accumulation of stresses lead to their propagation along the fiber which eventually fails. The survival probability of the studied fiber population is presented as well as two additional parameters related to the data distribution, as it does not follow the normal distribution. Alpha (α) represents the cycle (number of pulls) in which 63.5% of the fibers have already ruptured while beta (β) is related to the shape of the distribution and expresses the tendency for premature rupture of the fibers (34).

In the virgin hair, the CMC remains integrate and is recognized as the preferred pathway for diffusion of molecules, especially the hydrophobic ones as the oil fatty acids (35-38). Our group reported previously (32) that the penetration of these oils in the Caucasian hair implied increase in both tensile parameters Young's modulus and break stress, as consequence of the reinforcement of the hydrophobicity of these cortices which prevented water from being absorbed. The same was not evidenced in the textured hair. Wet-tensile test results clearly show that the oils could not modify Young's modulus and break stress of this hair. The condition of the tests was the same, but the results were significantly diverse leading to the conclusion that the type of hair is determinant to explain these findings. Textured hair is classified as weaker than the Caucasian hair, thanks to the irregularity of its shape which presents area of greatest and least accumulation of mass along the twists, increasing its fragility to the breakage (19, 20, 33) and making it to break 10 times faster than Caucasian hair (6). The water added to the test was expected to induce a plasticizing effect, perturbing internal hydrogen bonds and salt ionic interactions, and causing reduction in the tensile test parameters. Having the oils penetrated the hair, a similar effect to that observed for Caucasian hair was expected. The obtained results for the tensile test indicated that the oils could not diffuse efficiently into the textured cortex.

In fatigue testing of the virgin hair, α was positively impacted showing the improvement of hair resistance for the treatments with avocado and coconut oils whereas argan oil led the hair resistance to decrease dramatically. In the work done with the Caucasian hair (32), we observed the reduction in α parameter with the absorption of avocado and coconut oils and conjectured that they competed for the interaction sites of the keratin and matrix proteins, creating new bonds with the external materials. Taking into consideration that the Young's modulus and the break stress of these samples were not modified by the presence of the oils, we conclude that there was not deep penetration, but an accumulation of the oils in the hair superficial layers, which generated a lubricant effect, that led to the reduction of the fatiguing process of the fibers,

diminishing their fracture (39). Furthermore, the presence of argan oil in the outermost layers of the hair seems to have increased its affinity to the water thanks to its more polar character due to the strong unsaturation presence, which intensified the plasticizer effect. A similar effect was verified for the Caucasian hair as well (32).

The clear differences of oil absorption by Caucasian and textured virgin hairs evidenced for avocado and coconut oil treatments may find elucidation in the types of cortical cells and their distribution within the cortical area of these fibers. The orthocortex is a region of the cortical zone characterized by low presence of matrix (amorphous material) in relation to the intermediate filaments (IFs, crystalline material). Therefore, the lower level of cross-links between matrix and IFs leaves this area with lower density and more prone to molecules diffusion and swelling than the paracortex. The paracortex, on the other hand, is a region of the cortex where there is more matrix in relation to IFs, forming a greater degree of cross-links, which configures it as a high-density area and less favorable to molecules movement (24, 25). These two different zones are present in both hair types, Caucasian and textured hair. What seems to be determining the oils penetration variations is how these zones are settled in the cortical region. In the Caucasian hair, the orthocortex is located right below the cuticles, in the outermost portion of the cortex, surrounding the right-below paracortex, in a ring format. In the textured hair type, the orthocortex and the paracortex cells are disposed in a two-sided way, with orthocortex concentrated in the external portion of the curl and the paracortex, in the internal portion (24, 25). Hornby et al. (38) reported that the diffusion coefficient of the orthocortex area is approximately one order of magnitude greater than the one of the paracortex. Taking these configurations into account, the molecules to penetrate the Caucasian hair will have orthocortex as primarily region to diffuse along the whole perimeter of the fibers, after trespassing the cuticular barrier and may spread throughout the entire orthocortical ring. Molecules to penetrate the textured hair type will have orthocortex in approximately half of the perimeter of the fibers and paracortex in the other half, which imposes a hurdle to diffuse.

The damage caused by the bleaching process is widely known, involving loss of lipid and protein content, disulfide bonds cleavage and charge generation throughout the hair structure, which alters drastically the hair chemical character from more hydrophobic, in the virgin state, to more hydrophilic in the bleached state (1-5). The affinity with external materials are oriented by these changes in this state of the hair. MALDI-TOF analysis resulted in the visualization of argan oil belonging components with more intensity than that of avocado and coconut oils. Coconut oil was the least identified within the bleached hair whilst avocado oil presence was average. The presence of argan and coconut oils in the hair structure did not alter its Young’s modulus, neither the

break stress, indicating their diffusion into the hair was not sufficient to create new interactions that could lead to the partial recover of the mechanical properties or returning partially the hair hydrophobicity to keep humidity apart. Avocado oil also did not impact the Young's modulus but did increased the hair break stress. It is more likely that it is an effect of returning part of the lost hydrophobicity, protecting the hair from water absorption, similar to the one reported by Keis et al. (40), who verified an obstruction effect caused by the treatment with oils, retarding the water uptake by the hair.

Fatigue test results for the bleached hair show that the oils demonstrated a contrary effect of that found for the virgin hair, with the reduction of the α parameter and SP. The impact in α parameter followed the intensity of the oils found in the cortex of the hair by MALDI-TOF in which argan presented higher intensity and higher impact in α; avocado, intermediate intensity and average impact in α, and finally coconut oil with less intensity and less impact in α parameter. This strongly suggests that these variations are directly related to the presence of the oils in the hair. Having the oils penetrated the cortex of the bleached hair, they were not capable of stabilizing sufficiently the charges and establish interactions to increase mechanical resistance. Moreover, the tensile test results indicate they did not make difference in the relationship of the hair with the added water, as all samples were similarly affected by the humidity.

Once more the configuration of the textured hair cortex seems to have been responsible to orient the oils diffusion and determine the detected differences. Given the distinct diffusion coefficient of the orthocortex and paracortex and their diverse capacities of swelling and accommodating external molecules, the oils diffused into the hair and may have acquired an irregular occupancy, probably most accumulated in the orthocortex than in the paracortex, creating a gradient of hydrophobicity inside the hair. The irregular dissemination of the oils could not establish a homogeneous protection against humidity, that once interacting with the hair, caused the plasticizer effect as much as in the not treated hair, as observed in the tensile test results. Argan oil, as in the virgin hair and in the tests with Caucasian hair (32), increased the hair affinity with the water in the fatigue test, generating greater loss of resistance for the treated hair. Avocado oil, curiously, may have played a similar role, reinforcing the hypothesis of the irregular distribution within the hair as its protector effect once observed in the tensile test, was not identified in the fatigue results. Beta parameter was not changed by the presence of coconut and avocado oils, but increased with argan oil treatment, which is unexpected as α diminished. We concluded that Beta parameter is not so important for the analysis of the penetration of the oils.

5. CONCLUSION

The diffusion of materials in hair is a strategy to increase the resistance of the fragile textured hair as well as treat the damage from the oxidation aggression. Vegetable oils are much relevant in this context as they are popularly associated with hair benefits, including strengthening. Argan, avocado and coconut oils have been studied regarding their ability to penetrate the bleached textured hair structure using MALDI-TOF and furthermore increase hair resistance to the mechanical stress using tensile and fatigue tests. The results from MALDI-TOF analysis showed the oils could be identified in the hair cortex, with argan oil belonging components with grater intensities and coconut oil with the least intensities identified inside the hair. However, their effect, evidenced by tensile and fatigue tests, do not indicate relevant influence in the mechanical parameters of the virgin and bleached states of the textured hair. In comparison with the Caucasian results, obtained in previous work of our group, it was observed that avocado and coconut oils not only penetrated but also influenced positively the tensile parameters of the virgin hair and contributed to protect the bleached hair from the humidity. In Raman spectroscopy, the results showed that the oils were able to penetrate deep in the cortex, of both hair states. For the textured hair, on the contrary, the same was not evidenced. We conjecture hypothesize that the reason to these differences relies on the distinct configuration of the cortices of these hair types. The unique arrangement of the orthocortex and paracortex with low and high densities, respectively, disposed in a two-sided way promotes an irregular diffusion with diverse kinetics in the two sides, which leads the external molecules to occupy the cortex heterogeneously. This irregularity in the external molecules spreading reduces the efficiency of the effect of these molecules. In the Caucasian hair, in which the distribution of the orthocortex and paracortex is in a ring format, with the first one circling the second one, the occupancy tends to be more homogeneously, as well as their effect.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

6. REFERENCES

1. Tate, M.L., Kamath, Y.K. and Ruetsch, S.B. Quantification and prevention of hair damage. *J Soc Cosmet Chem.* 44:347-71 (1993).

2. F.-J. Wortmann, C. Springob, and G. Sendelbach, Investigations of Cosmetically Treated Human Hair by Differential Scanning Calorimetry in Water, J. Cosmet. Sci., 53, 219-228 (2002).

3. F.-J. Wortmann, G. Sendelbach, and C. Popescu, 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., 58, 311-317 (2007).

4. T. Fujii, Y. Ito, T. Watanabe, and T. Kawasoe, Effects of Oxidative Treatments on Human Hair Keratin Films, J. Cosmet. Sci., 63, 15-25 (2012).

5. Grosvenor, A.J., Deb-Choudhury, S., Middlewood, P.G., Thomas, A., Lee, E., Vernon, J.A., et al. The physical and chemical disruption of human hair after bleaching - studies by transmission electron microscopy and redox proteomics. Int J Cosmet Sci. 40(6):536-48 (2018).

6. Cornwell, P., Malinauskyte, E. Defying damage. Cosmetics and Toiletries. 135(2):20-29 (2020).

7. Bryant, H., Porter, C. and Yang, G. Curly hair: measured differences and contributions to breakage. International Journal of Dermatology. 5:8-11 (2012).

8. Ruetsch, S. B., and Kamath, Y. K. Penetration of cationic conditioning compounds into hair fibers: A TOF-SIMS approach. .Journal of cosmetic science. 56:323-330 (2005).

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 and Surfaces B: Biointerfaces. 174:563-568 (2019).

10. Malinauskyte, E., Shrestha, R., Cornwell, P. A., Gourion-Arsiquaud, S. and Hindley M. Penetration of different molecular weight hydrolysed keratins into hair fibres and their effects on the physical properties of textured hair. International Journal of Cosmetic Science. 1–12 (2020).

11. Rele, A.S. and Mohile, R. Effect of coconut oil on prevention of hair damage. Part I. Journal of cosmetic science. 50(6):327-39 (1999).

12. Ruetsch, S. B., Kamath, Y., Rele, A. S. and Mohile, R. B. Secondary ion mass spectrometric investigation of penetration of coconut and mineral oils into human hair fibers: relevance to hair damage. Journal of cosmetic science. 52:169-184 (2001).

13. Srivastav, A., Dandekar, P. and Jain, R. Penetration study of oils and its formulations into the human hair using confocal microscopy. Journal of Cosmetic Dermatology. 18(6):1947-54 (2019).

14. Keis, K., Persaud, D., Kamath, Y.K. and Rele, A.S. Investigation of penetration abilities of various oils into human hair fibers. Journal of cosmetic science. 56(5):283-95 (2005).

15. Manoel, I. Manual dos cabelos: o poder os óleos. São Paulo, Brasil. Laços (2003).

16. Wolfram, L. J. Human hair: a unique physicochemical composite. Journal of the American Academy of Dermatology. 48(6):106-14 (2003).

17. Dawber, R. Hair: its structure and response to cosmetic preparations. Clinics in Dermatology. 14(1):105–12 (1996).

18. 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. and Rice, R. H. Human hair shaft proteomic profiling: Individual differences, site specificity and cuticle analysis. Peer Journal. 2(e506):1-17 (2014).

19. Robbins, C.R. Chemical and Physical Behavior of Human Hair. 5th ed. New York, NY: Springer-Verlag Berlin Heidelberg (2012).

20. Aguh, C., Okoye, G. A. Fundamentals of Ethnic Hair, the dermatologist's perspective. Springer International Publishing Switzerland (2017).

21. Yang, F. C., Zhang, Y., Rheinstadter, M.C. The structure of people's hair. Peer Journal. 2e619 (2014).

22. Khumalo NP, Stone J, Gumedze F, McGrath E, Ngwanya MR, de Berker D. 'Relaxers' damage hair: evidence from amino acid analysis. Journal of the American Academy of Dermatology. 62(3):402-8 (2010).

23. Bryson, W. G., Harland, D. P., Caldwell, J. P., Vernon, J. A., Walls, R. J., Woods, J. L., Nagase, S., Itou, T. and Koike, K. Cortical cell types and intermediate filament arrangements correlate with fiber curvature in Japanese human hair. Journal of Structural Biology. 166(1):46-58 (2009).

24. Thibaut, S., Barbarat, P., Leroy, F. and Bernard, B. A. Human hair keratin network and curvature. International Journal of Dermatology. 46(1):7-10 (2007).

25. 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. Journal of Structural Biology. 155(3):438-444 (2006).

26. Harland, D. P., Vernon, J. A., Woods, J. L., Nagase, S., Itou, T., Koike, K., Scobie, D. A., Grosvenor, A. J., Dyer, J. M. and Clerens, S. Intrinsic curvature in wool fibers is determined by the relative length of orthocortical and paracortical cells. Journal of Experimental Biology. 221 (2018).

27. Marti, M., Barba, C., Manich, A. M., Rubio, L., Alonso, C., and Coderch, L. T. The influence of hair lipids in ethnic hair properties. International Journal of Cosmetic Science. 38:77-84 (2016).

28. Cruz, C. F., Fernandes, M. M., Gomes, A. C., Coderch, L., Marti, M., Mendez, S., Gales, L., Azoia, N. G., Shimanovich, U. and Cavaco-Paulo, A. Keratins and lipids in ethnic hair. International Journal of Cosmetic Science. 35:244-249 (2013).

29. Lourenco, C. B., Fava, A. L. M., Santos, E. M., Macedo, L. M., Tundisi, L. L., Ataide, J. A. and 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. International Journal of Cosmetic Science. 43:113–122 (2021).

30. Philipsen, M. H., Haxen, E. R., Manaprasertsak, A., Malmberg, P. and Hammarlund, E. U. Mapping the chemistry of hair strands by mass spectrometry imaging – a review. Molecules. 26(7522): 2-20 (2021).

31. Harvey, A. and Carr, C. M. Time of flight secondary ion mass spectrometry (TOF-SIMS) analysis of the application of a cationic conditioner to clean hair. Journal of Cosmetic Science. 55:265-279 (2004).

32. 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 in the penetration profile of coconut, avocado and argan oils into caucasian hair fibers. Unpublished data. (2022).

33. Loussouarn, G., Garcel, A. L., Lozano, I., Collaudin, C., Porter, C., Panhard, S., Saint-Léger, D. and Mettrie, R. Worldwide diversity of hair curliness: A new method of assessment. International Journal of Dermatology. 46(1):2-6 (2007).

34. Evans, T. Fatigue testing of hair – a statystical approach to hair breakage. Journal of cosmetic science. 60:599-616 (2009).

35. Potsch, L. and Moeller, M. R., On Pathways for Small Molecules into and Out of Human Hair Fibers. Journal of Forensic Sciences. 41(1):121-125 (1996).

36. Kelch, A., Wessel, S., Will, T., Hintze, U., Wepf, R. and Wiesendanger, R. Penetration Pathways of Fluorescent Dyes in Human Hair Fibres Investigated by Scanning Near-Field Optical Microscopy. Journal of Microscopy. 200:179-186 (2000).

37. Gummer, C. L. Elucidating penetration pathways into the hair fiber using novel microscopic techniques. Journal of cosmetic science. 52:265-280 (2001).

38. Hornby, S. B., Appa, Y., Ruetsch, S., and Kamath, Y. Mapping penetration of cosmetic compounds into hair fibers using time-of-flight secondary ion mass spectroscopy (TOF-SIMS). IFSCC Magazine. 8(2): 99-104 (2005).

39. Evans, T. An unifying theory for visualizing the causes of hair breakage and subsequent strategies for mitigation. Journal of cosmetic science. 68:137-140 (2017).

40. K. Keis, C. L. Huemmer and Y. Kamath. Effect of oil films on moisture vapor absorption on human hair. Journal of cosmetic science. 58:135-145 (2007).

4. DISCUSSÃO GERAL

Os resultados obtidos por espectroscopia Raman em cabelos lisos e por MALDI-TOF/TOF em cabelos texturizados confirmam que as moléculas dos óleos de fato se difundiram para a região cortical das fibras avaliadas.

Os óleos vegetais de coco, abacate e argan apresentam grandes diferenças com relação a sua distribuição graxa e caráter químico, como colocado no capítulo II desta tese. Este capítulo também trouxe que o caráter químico dos cabelos nas condições íntegra e danificada determina a afinidade de sua estrutura com os diversos materiais presentes em produtos para cuidado dos cabelos, em linha com achados da literatura (10-17). Mais do que isso, o efeito dos ativos cosméticos nos cabelos é dependente das interações químicas que cada material estabelece com a estrutura (10, 25). A umidade é um terceiro fator na análise dos possíveis efeitos dos cosméticos (26, 27), especialmente no caso de óleos vegetais, uma vez que ela tanto estabelece interações com as estruturas químicas do cabelo, levando a alterações nas propriedades mecânicas dos fios, quanto com os ácidos graxos dos óleos vegetais, gerando aumento ou diminuição de sua afinidade com a estrutura capilar, uma vez que estão presentes no córtex dos fios. Por fim, a morfologia do cabelo também desempenha um papel importante na cinética de difusão dos materiais (10).

A difusão dos óleos vegetais ocorreu de forma mais intensa nos cabelos descoloridos do que nos cabelos virgens, para os dois tipos estudados, liso e texturizado, especialmente para o óleo de argan. O dano da descoloração altera o caráter químico das moléculas estruturais do cabelo, gerando maior afinidade pelas substâncias polares (28-31). Adicionalmente, a barreira cuticular pode ser parcialmente removida com a perda de escamas, o que torna o caminho de difusão mais curto, facilitando o transporte de moléculas (10). Porém, o interesse cosmético da penetração de óleos na estrutura é a reposição de massa lipídica, perdida com os danos químicos e das lavagens, e recuperação parcial da força das fibras, aumentando sua resistência frente ao estresse mecânico das escovações e atritos diários.

Neste contexto, o óleo de argan não se apresentou como uma opção eficaz na entrega destes benefícios. No capítulo II vemos que ele apresenta composição rica em ácidos graxos de cadeia carbônica mais longa, acima de 80% de 18 carbonos, com uma ou duas insaturações na maioria de suas moléculas. Essas características levam esse óleo a adquirir um caráter mais hidrofílico do que o de abacate, pois as insaturações são locais de concentração de elétrons. Os outros 20% de sua composição são formados por cadeias carbônicas mais curtas, o que também contribui para o caráter hidrofílico de sua estrutura.

Ao se difundir pelas regiões mais periféricas da porção cortical das fibras, o óleo de argan aumentou a afinidade do cabelo pela água, que ao penetrar o córtex, perturbou as ligações de hidrogênio e iônicas inter e intramoleculares das cadeias laterais da queratina e da matriz. A resistência ao estresse mecânico suportada em parte por essas interações foi diminuída, levando a uma menor força de ruptura e, consequentemente, menor probabilidade de sobrevivência dos fios (32).

Nos cabelos íntegros, em que o CMC se encontra intacto, a difusão de moléculas apolares, como os ácidos graxos do abacate, é favorecida. A hidrofobicidade aumentada do córtex repeliu a água de forma mais intensa, levando a um aumento da rigidez dos fios e, consequentemente, da força de ruptura. Este efeito, porém, não foi observado para os cabelos íntegros texturizados.

As diferenças de absorção evidenciadas para os tratamentos com óleos de abacate e coco por cabelos virgens lisos e texturizados podem encontrar elucidação nos tipos de células corticais e na sua distribuição dentro da área cortical dessas fibras. As sub-regiões corticais ortocórtex e paracórtex apresentam características distintas em relação à densidade de matriz e ligações cruzadas inter e intramoleculares (33). A relação matriz / filamentos é menor para a primeira e maior para a segunda, definindo áreas de maior e menor densidade, o que, por sua vez, definem áreas de maior e menor facilidade à mobilidade de substâncias, respectivamente (34, 35). Nos cabelos lisos, a posição destas áreas é de forma anelar, com o paracórtex mais adentro e o ortocórtex mais externo, circundando o paracórtex, de modo que todo o perímetro externo da fibra lisa apresenta maior facilidade à difusão de moléculas (33). No cabelo texturizado, existe concentração do ortocórtex na região convexa dos cachos e do paracórtex na região côncava (33-35), configurando uma distribuição bilateral. Assim, cerca de metade do perímetro destas fibras apresenta maior dificuldade ao transporte de moléculas.

Estas diferenças anatômicas nos arranjos das regiões corticais dos cabelos explicam por que, sob as mesmas condições, os últimos foram menos impactados pelos tratamentos do que os primeiros. Os cabelos texturizados descoloridos sofreram danos semelhantes aos dos cabelos lisos e tiveram a difusão de moléculas externas facilitada. Os resultados dos testes de tensão e de fadiga indicam, porém, que não houve aumento de resistência mecânica, o que sugere que os óleos podem ter se espalhado de forma irregular no córtex, provavelmente mais acumulada no ortocórtex do que no paracórtex. Este fato pode ter gerado um gradiente de hidrofobicidade dentro do cabelo, levando a uma proteção insuficiente contra a umidade, que, ao ser absorvida, causou o efeito plastificante tanto quanto nos cabelos não tratados.

5. CONCLUSÃO

A penetração de agentes cosméticos nos cabelos é uma estratégia para aumentar a resistência dos cabelos texturizados, naturalmente mais frágeis, e recuperar parte das propriedades das fibras capilares danificadas, perdidas em tratamentos químicos. Os óleos vegetais, em geral, reúnem uma lista de características relevantes e muito convenientes para a indústria cosmética, como relativa facilidade de obtenção, processo verde, rótulo sustentável, segurança e eficiência, e para os consumidores, como os benefícios associados de sensorialidade, lubrificação, força, resistência e muitos outros. Entre os óleos empregados para o cuidado do cabelo, os óleos de abacate e argan ainda não foram estudados a fundo. O óleo de coco, para o qual existe mais informação disponível, foi utilizado como referência em nosso estudo.

Uma vez difundidos para o interior do cabelo, estes óleos irão modificar a forma como o cabelo interage com a água e, consequentemente, como a água afetará as propriedades mecânicas da estrutura capilar. Os óleos de abacate e coco intensificam o caráter hidrofóbico do cabelo virgem, evitando que a água se difunda através da estrutura. Nos cabelos descoloridos, seu efeito é insuficiente para proteger os cabelos da umidade e recuperar as propriedades mecânicas. O óleo de argan, ao contrário, apesar de ter penetrado no cabelo, agiu aumentando sua afinidade pela água ao invés de reduzi-la, levando ao aumento da absorção de água e consequentemente tornando-o mais frágil à ruptura.

Para os cabelos texturizados, os resultados apontam uma cinética de difusão diferenciada para os mesmos óleos nas mesmas condições de dano. A razão para essas diferenças depende da configuração distinta dos córtices desses tipos de cabelo. O arranjo único do ortocórtex e do paracórtex com baixas e altas densidades, respectivamente, dispostos de forma bilateral, promove uma difusão irregular com cinética diversa nos dois lados, o que leva as moléculas externas a ocuparem o córtex de forma heterogênea. Essa irregularidade no espalhamento das moléculas externas reduz a eficiência de seus efeitos.

De forma geral, os óleos vegetais são capazes de difundir para a região cortical dos cabelos lisos e texturizados, quando as condições que favorecem esta difusão estão presentes. No entanto, deve-se haver adequado estudo de todos os fatores influentes para a correta indicação destes tratamentos aos cabelos, de modo a obter não apenas difusão de moléculas para o interior das fibras, mas principalmente benefícios aos cabelos e vantagens aos consumidores destes produtos.

6. REFERÊNCIAS

1. Bilgen Erdoğan, Anatomy and Physiology of Hair. Intech Open (2017).
2. Euromonitor value report, 2021 data, *Current prices*, € fixed rate. (2022).
3. Gode, V., Bhalla, N., Shirhatti, V., Mhaskar, S. and Kamath, Y. Quantitative measurement of the penetration of coconut oil into human hair using radiolabeled coconut oil. Journal of Cosmetic Science. 63(1):27-31 (2012).
4. Ruetsch, S.B., Yang, B. and Kamath, Y.K. Chemical and photo-oxidative hair damage studied by dye diffusion and electrophoresis. Journal of cosmetic science. 54(4):379-94 (2003).
5. Grosvenor, A.J., Deb-Choudhury, S., Middlewood, P.G., Thomas, A., Lee, E., Vernon, J.A., et al. The physical and chemical disruption of human hair after bleaching - studies by transmission electron microscopy and redox proteomics. International Journal of Cosmetic Science. 40(6):536-48 (2018).
6. Ruetsch, S. B., and Kamath, Y. K. Penetration of cationic conditioning compounds into hair fibers: A TOF-SIMS approach. .Journal of Cosmetic Science. 56:323-330 (2005).
7. Keis, K., Persaud, D., Kamath, Y.K. and Rele, A.S. Investigation of penetration abilities of various oils into human hair fibers. Journal of Cosmetic Science. 56(5):283-95 (2005).
8. Silva, C.J., Vasconcelos, A. and Cavaco-Paulo, A. Peptide structure: Its effect on penetration into human hair. Journal of cosmetic science. 58(4):339-46 (2007).
9. Manoel, I. Manual dos cabelos: o poder os óleos. Sao Paulo, São Paulo, Brazil. Laços (2003).
10. Hornby, S. B., Appa, Y., Ruetsch, S., and Kamath, Y. Mapping penetration of cosmetic compounds into hair fibers using time-of-flight secondary ion mass spectroscopy (TOF-SIMS). IFSCC Magazine. 8(2): 99-104 (2005).
11. Rele, A.S. and Mohile, R. Effect of coconut oil on prevention of hair damage. Part I. Journal of Cosmetic Science. 50(6):327-39 (1999).
12. Ruetsch, S. B., Kamath, Y., Rele, A. S. and Mohile, R. B. Secondary ion mass spectrometric investigation of penetration of coconut and mineral oils into human hair fibers: relevance to hair damage. Journal of Cosmetic Science. 52:169-184 (2001).
13. Srivastav, A., Dandekar, P. and Jain, R. Penetration study of oils and its formulations into the human hair using confocal microscopy. Journal of Cosmetic Dermatology. 18(6):1947-54 (2019).

14. Leite, M. G. A., Campos, P. M. B. G M. Development and efficacy evaluation of hair care formulations containing vegetable oils ans silicone. International Journal of Phytocosmetics and Natural Ingredients. 5-9 (2018).

15. Keis, K., Persaud, D., Kamath, Y.K. and Rele, A.S. Investigation of penetration abilities of various oils into human hair fibers. Journal of Cosmetic Science. 56(5):283-95 (2005).

16. Fregonesi, A., Scanavez, C., Santos, L., Oliveira, A., Roesler, R., Escudeiro, C., Moncayo, P., Sanctis, D. and Gesztesi, J. L. Brazilian oils and butters: the effecr of different fatty acid chain composition on human hair physiochemical properties. Journal of Cosmetic Science. 60:273-280 (2009).

17. Lourenco, C. B., Fava, A. L. M., Santos, E. M., Macedo, L. M., Tundisi, L. L., Ataide, J. A. and 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. International Journal of Cosmetic Science. 43:113–122 (2021).

18. Zimmerley, M., Lin, C.-Y., Oertel, D.C., Marsh, J.M., Ward, J.L. and Potma, E.O. Quantitative detection of chemical compounds in human hair with coherent anti-Stokes Raman scattering microscopy. J Biomed Opt. 14(4):044019- (2009).Colthup et al., 1990;

19. Kuzuhara, A. Raman Spectroscopic Analysis of L-Phenylalanine and Hydrolyzed Eggwhite Protein Penetration into Keratin Fibers. Journal of Applied Polymer Science. 122 (2011).

20. Kuzuhara, A. A Raman spectroscopic investigation of the mechanism of the reduction in hair with thioglycerol and the accompanying disulphide conformational changes. International Journal of Cosmetic Science. 40(1):34-43 (2018).

21. Kojima, T., Tsuji, S., Niwa, M., Saito, K., Matsushita, Y. and Fukushima, K. Distribution Analysis of Triglyceride Having Repair Effect on Damaged Human Hair by TOF-SIMS. International Journal of Polymer Analysis and Characterization. 17(1):21-8 (2012).;

22. Brunelle, A., Touboul, D. and Laprévote, O. Biological tissue imaging with time-of-flight secondary ion mass spectrometry and cluster ion sources. Journal of Mass Spectrometry. 40(8):985-99 (2005);

23. Saleem, M. and Galla, H.-J. Surface view of the lateral organization of lipids and proteins in lung surfactant model systems—A ToF-SIMS approach. Biochimica et Biophysica Acta (BBA) - Biomembranes. 1798(4):730-40 (2010);

24. Tsugita, T. and Iwai, T. Optical coherence tomography using images of hair structure and dyes penetrating into the hair. Skin research and technology: official journal of International Society for Bioengineering and the Skin (ISBS) [and] International Society for Digital Imaging of Skin (ISDIS) [and] International Society for Skin Imaging (ISSI). 20(4):389-98 (2014).

25. Malinauskyte, E., Shrestha, R., Cornwell, P. A., Gourion-Arsiquaud, S. and Hindley M. Penetration of different molecular weight hydrolysed keratins into hair fibres and their effects on the physical properties of textured hair. International Journal of Cosmetic Science. 1–12 (2020).

26. Evans, T. Fatigue testing of hair – a statystical approach to hair breakage. Journal of Cosmetic Science. 60:599-616 (2009).

27. Evans, T. Measuring hair strength – Part 1: Stress-Strain Curves. Cosmetics and Toiletries. 128(8):591-594 (2013).

28. Tate, M.L., Kamath, Y.K. and Ruetsch, S.B. Quantification and prevention of hair damage. Journal of the Society of Cosmetic Chemists. 44:347-71 (1993).

29. F.-J. Wortmann, C. Springob, and G. Sendelbach, Investigations of Cosmetically Treated Human Hair by Differential Scanning Calorimetry in Water, Journal of Cosmetic Science. 53, 219-228 (2002).

30. F.-J. Wortmann, G. Sendelbach, and C. Popescu, Fundamental DSC Investigations of A-Keratinous Materials as Basis for the Interpretation of Specific Effects of Chemical, Cosmetic Treatments on Human Hair, Journal of Cosmetic Science., 58, 311-317 (2007).

31. T. Fujii, Y. Ito, T. Watanabe, and T. Kawasoe, Effects of Oxidative Treatments on Human Hair Keratin Films, Journal of Cosmetic Science., 63, 15-25 (2012).

32. Wortmann, F.-J and Zahnt, H. The Stress Strain Curve of alpha-Keratin Fibers and the Structure of Intermediate Filaments. Textile Research Journal. 64(12): 737-743 (1994).

33. Robbins, C.R. Chemical and Physical Behavior of Human Hair. 5th ed. New York, NY: Springer-Verlag Berlin Heidelberg (2012).

34. Thibaut, S., Barbarat, P., Leroy, F. and Bernard, B. A. Human hair keratin network and curvature. International Journal of Dermatology. 46(1):7-10 (2007).

35. 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. Journal of Structural Biology. 155(3):438-444 (2006).

7. ANEXOS

Autorização da editora John Wiley & Sons A/S para que o artigo *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* integre esta tese.

RE: FW: Re: Paper published (Int. J. of Cosmetic Sci)

TT Trier-Mork, Thomas <ttm@wiley.co

Responder | Responder a Todos | Encaminhar

Para Carolina Lourenco

Cc Priscila Gava Mazzola

qua 11/01/2023 05:37

Política de Retenção Sym-Pol-Ret-Exchange-10Y (10 anos) Expira 08/01/2033

Você respondeu esta mensagem em 11/01/2023 10:31.

CAUTION: This email originated from outside the organization. Do not click links or open attachments unless you recognize the sender and know the content is safe.

Dear Carolina

Yes, this is the document.

Best regards
Thomas Trier-Mork

Thomas Trier-Mork
Senior Journals Publishing Manager
Health Sciences
ttm@wiley.com

John Wiley & Sons A/S
Holbergsgade 14, 2tv
1057 Copenhagen K
Denmark

www.wiley.com

WILEY

From: Carolina Lourenco <carolina.lourenco@symrise.com>
Sent: 10. januar 2023 22:46
To: Trier-Mork, Thomas <ttm@wiley.com>
Cc: Priscila Gava Mazzola <pmazzola@fcf.unicamp.br>
Subject: RES: FW: Re: Paper published (Int. J. of Cosmetic Sci)

This is an external email.

Dear Mr. Trier-Mork,

Thank you for your assistance.

I have followed your instructions and have been driven to this web page: https://s100.copyright.com/AppDispatchServlet

In it, I was asked to fill some information about where I will use the article.

Finally, I followed some process similar to a purchase process and received the below message.

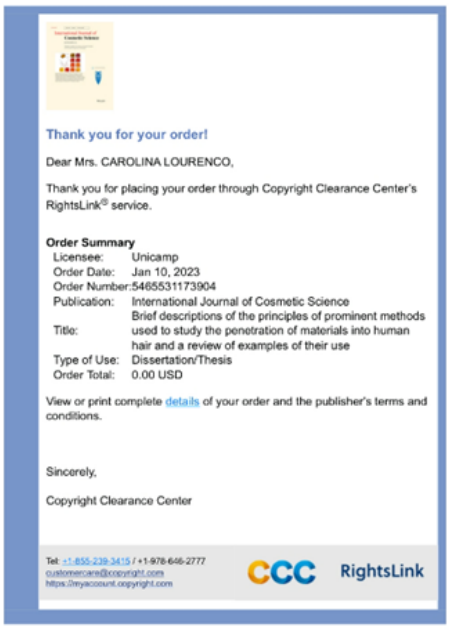
Order Completed

Thank you for your order.

This Agreement between Unicamp -- CAROLINA LOURENCO ("You") and John Wiley and Sons ("John Wiley and Sons") consists of your license details and the terms and conditions provided by John Wiley and Sons and Copyright Clearance Center.

By e-mail, I received the following:

Thank you for your order with RightsLink / John Wiley and Sons



Thank you for your order!

Dear Mrs. CAROLINA LOURENCO,

Thank you for placing your order through Copyright Clearance Center's RightsLink® service.

Order Summary

Licensee:	Unicamp
Order Date:	Jan 10, 2023
Order Number:	5465531173904
Publication:	International Journal of Cosmetic Science
Title:	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
Type of Use:	Dissertation/Thesis
Order Total:	0.00 USD

View or print complete details of your order and the publisher's terms and conditions.

Sincerely,

Copyright Clearance Center

Tel: +1-855-239-3415 / +1-978-646-2777
customercare@copyright.com
https://myaccount.copyright.com

CCC RightsLink

Could you inform us if this is the document that allows us to include the article in the thesis?

Thank you in advance,

Our kind regards,

Carolina B. Lourenço