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Determination of penetration and protection of fatty acids in bleached hair according to the fatty acid chain length and the application to understanding the protective effects of MCT oil and coconut oil

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Abstract

Hair treatments containing vegetable oils protect the damaged hair by surface coating and by penetrating in hair thereby filling the gaps caused by oxidative damage. Vegetable oil is composed mostly of medium-chain or long-chain fatty acids which exist as triglycerides. Although there are literatures which deal with the penetration of specific natural oils into hair there is a lack of research which provide the empirical data that can be used to explain the penetration of larger population of vegetable oils. This research was aimed to examine whether the chain length of fatty acid affect the penetration of fatty acid and protection in hair and also to apply the results on explaining the protective effect of Medium-Chain Triglyceride oil (MCT oil) and Coconut oil. Nine different hair treatments were formulated with three medium-chain fatty acids (C8:0, C10:0, C12:0), three long-chain fatty acids (C14:0, C16:0, C18:0), MCT oil, Coconut oil, and a blank. Composition of fatty acids of hair was examined by the Gas chromatography mass spectrometry. Penetration of fatty acid in hair was examined by the UV–VIS spectrophotometry. Protection of damaged hair was examined by the differences in color, tensile strength, thickness, and the concentration of protein leak of 21-day vs. 0-day measurements. Results of t-test indicated that the penetration and the protection of the medium-chain fatty acid was significantly better than those of the long-chain fatty acid, and that MCT oil and coconut oil were not significantly different.

Keywords: Hair, Bleached, Fatty acid, Penetration, Protection, Chain length, MCT oil, Coconut oil

Introduction

Hair damage can occur due to a variety of causes such as exposure to ultraviolet rays, chemical styling, stress, or daily grooming practices using electric hair stylers (Kim, 2022; Robbins, 2012). Repeated practice of chemical styling such as permanent wave, dyeing, or bleaching can result in rough appearance and texture along with the loss of luster, strength, and elasticity of hair (Robbins, 2012). The damage can develop into

thinning of hair and the scalp problems such as dandruff, erythema, or inflammation-the symptoms that can eventually lead to hair loss (Monselise et al., 2017; Robbins, 2012). Since hair is a major part of the body that is exposed to portray one's health and beauty, proper care is necessary to maintain the integrity of healthy hair.

Cuticle is the outermost layer of hair which is composed of overlapping flat cells (Robbins, 2012). Under the cuticle is the cortex which is made of spindle-shaped cortical cells (Robbins, 2012). Cell membrane complex is a cohesion component which fills the intercellular spaces and glues the cells of cuticle and cortex together (Robbins, 2012). Hair bleaching agents consist of alkaline substances and oxidizing agent such as hydrogen peroxide. Alkali swells the hair so that hydrogen peroxide can enter the cuticle layer and penetrate into the cortex of hair (Robbins, 2012; Wolfram et al., 1970). Hydrogen peroxide destroys melanin pigments to decolor hair and at the same time causes the oxidative degradation of hair protein (Wolfram et al., 1970). Oxidation of cuticle cells and the cell membrane complex leads to the fragmentation of cuticle cells and the creation of gaps which allow the penetration of unwanted aggressive chemicals (Robbins, 2012; Sarkar et al., 2017). Oxidation of cystine amino acid breaks the disulfide bond between the α-helix chains (Robbins, 2012; Wolfram et al., 1970). When this happens the slippage of the keratin chains occurs thereby decreasing the tensile strength and the structural integrity of hair (Robbins, 2012; Wolfram et al., 1970).

Vegetable oil can protect damaged hair first by forming a hydrophobic coating on the surface of hair and second by filling the gaps produced from oxidative damage as the oil penetrates into the cortex of hair (Dias, 2015; Gode et al., 2012; Keis et al., 2005; Lee & Ahn, 2022; Sarkar et al., 2017). Vegetable oils extracted from plant have been used and studied as raw materials for hair treatment formulations (Dias, 2015; Gode et al., 2012; Keis et al., 2005; Kim et al., 2021; Lee & Ahn, 2022; Min et al., 2013; Oh et al., 2014; Rele & Mohile, 2003; Sarkar et al., 2017). Vegetable oils are made up of fatty acids (> 95%) and other minor components (< 5%) (Vegetable oil, 2022).

The fatty acids exist in vegetable oils mostly as triglycerides (Vegetable oil, 2022). In each molecule of triglyceride, two or three fatty acid molecules are naturally linked to the hydroxyl groups of glycerol backbone (Keis et al., 2005). Triglycerides containing fatty acids with 6–12 carbon aliphatic chain are called the medium-chain triglycerides (MCT) and the triglycerides containing fatty acids with more than 12 carbon aliphatic chain are called the long-chain triglycerides (LCT) (St-Onge & Jones, 2002; Fatty acid, 2022). Fatty acids of MCT include caproic acid (C6:0), caprylic acid (C8:0), capric acid (C10:0), and lauric acid (C12:0), and the fatty acids of LCT include myristic acid (C14:0), palmitic acid (C16:0), stearic acid (C18:0), and more. (Fatty acid, 2022).

Among the many vegetable oils, coconut oil is unique in that its major constituent is saturated fatty acids and the large proportion of these saturated fatty acids is the medium-chain triglycerides (Handayani et al., 2019; Sarkar et al., 2017). Owing to the compositional characteristics, coconut oil is processed to make an even lighter structured oil product which is composed only of medium-chain triglycerides. This oil product is called the Medium-chain triglyceride oil and in short, MCT oil (Bach & Babayan, 1982). MCT oil is generally composed of either 100% caprylic acid (C8:0) or 100% capric acid (C10:0) or a mixture of the two fatty acids (Brown & Link, 2020). MCT oil is popular as the dietary source for its fast absorption after intake owing to its small molecular

size (Bach & Babayan, 1982; Healthessentials, 2021). Recently, MCT oil has gained attention as the raw material for skin cosmetics and launched its way as the raw material for hair cosmetics (Resnick, 2022). As one of the active ingredients of hair cosmetics it is expected that the MCT oil can provide the protection of damaged hair through its fast penetrable characteristics. However, it is difficult to find an empirical data which provide the evidence on the protective mechanism of MCT oil on damaged hair.

While a number of research discussed about the effect of penetration of vegetable oils into hair (Keis et al., 2005; Lee & Ahn, 2022; Rele & Mohile, 2003) there has been only a few research efforts which provided the empirical data (Gamez-Garcia, 2009; Gode et al., 2012; Hornby et al., 2005; Ruetsch et al., 2001). Ruetsch et. al. (2001) used the time-of-flight secondary ion mass spectrometry to find that coconut oil penetrated into the center of hair while mineral oil showed no evidence of penetration (Ruetsch et al., 2001). Using the same method Hornby et. al. (2005) found that polyunsaturated oils did not penetrate the hair while mono-unsaturated oils penetrated readily. Using the radiolabeling method, Gode et. al. (2012) found that tritiated coconut oil penetrated the hair by 14.5–26.3% by weight of hair (Gode et al., 2012). While previous literatures provided valuable information on the penetration of some vegetable oils, investigation was restricted to one or several specific vegetable oils, limiting the generalization of the results to a larger population of vegetable oils. Since a wide variety of fatty acids exist in different vegetable oils and the fatty acids themselves differ greatly by the length of aliphatic chain and the degree of saturation, a more generalizable experimental protocol seems necessary to understand the penetration behavior of vegetable oils in general.

The purpose of this research was to examine whether the length of aliphatic chain affect the penetration of fatty acid into hair and the resulting hair protection performance. For this purpose, six saturated fatty acids were selected as the target of investigation and a cream-type wash-off hair treatment was formulated for each fatty acid. The fatty acid composition of hair was examined using the gas chromatography mass spectrometry. The extent of penetration of fatty acid into hair was examined by the maximum absorbance data of UV–VIS spectrophotometry. Hair protection effect of fatty acid was examined in regard to the color, tensile strength, thickness, and protein leak characteristics after treatment. The results of fatty acids were used to understand the protective effects of MCT oil and coconut oil. Some earlier studies applied pure oil and longer treatment time (such as overnight) on hair. In this study the fatty acids and the vegetable oils were applied on hair in the form of cream-type hair treatments formulated with these materials (3% by weight of the hair treatment). And much shorter treatment time (10 min) was applied to simulate close to real-life situation. To our knowledge, this study is the first attempt to introduce the quantitative results on the penetration of individual fatty acids into hair and their protective effects.

Methods

Materials

Virgin black hair tresses without any prior chemical treatment were purchased from Chunhyesa (Korea). A commercial hair bleach containing powder-type alkaline agent and cream-type oxidizing agent composed of 6% hydrogen peroxide (35%) were used. MCT oil and coconut oil were each purchased from Tradithbio (Korea) and Honest

Korea (Korea). Table 1 shows the composition of fatty acids in MCT oil and coconut oil used in the study. Caprylic acid, capric acid, lauric acid, myristic acid, palmitic acid, and stearic acid were purchased from Sigma-Aldrich (Korea). Distilled water (Joylife, Korea), hydroxyethylcellulose (Lunaon, Korea), cetearyl alcohol (Cakesoap, Korea), Olivem 800 (Cakesoap, Korea), and Arlacel 83 (Cosnet, Korea) were purchased for the formulation of hair treatments. Chloroform, 2-mercapto ethanol, urea, trizma-base, Bovine serum albumin purchased from Sigma-Aldrich (Korea), ethyl alcohol (94.5%) and methyl alcohol purchased from Daejung (Korea). Thiourea (Junsei Chemical, Japan), Bio-Rad protein assay dye (Bio-Rad laboratories, USA), and glass microfiber filter (diameter 110 mm, Whatman, UK) were used in the analysis of protein leak.

Experimental

Preparation of bleached hair (BH)

A 5 g of virgin hair (VH in the following) cut to approximately 15 cm was attached at the root end with a glue gun. 10 pieces of VH tresses were prepared by this method. Alkaline agent and the oxidizing agent of the hair bleach were mixed by 1:3 ratio and the mixture was spread evenly on the 10 VH pieces. Treated hair tresses were left at room temperature for 30 min and then washed with shampoo under running water. Remaining water was completely dried using a hair dryer. This process was repeated 5 times to produce the bleached hair (BH in the following) tresses for the study.

Preparation of wash-off type hair treatment

The 9 different cream-type wash-off hair treatments, 8 of which contain one among the 8 conditioning agents- MCT oil, coconut oil, caprylic acid (C8:0), capric acid (C10:0), lauric acid (C12:0), myristic acid (C14:0), palmitic acid (C16:0), or stearic acid (C18:0)- and a blank hair treatment were prepared in reference to Kim et. al. (2021). Aqueous phase (phase A) was prepared by adding hydroxyethyl cellulose to distilled water. The contents of phase A were dissolved by heating at 80 °C for 20 min using a stirring hot plate. Oil phase (phase B) was prepared by adding cetearyl alcohol, Arlacel 83, Olivem 800, and one of the 8 treatment agents. The oil phase of blank was prepared without the treatment agent. The contents of phase B were dissolved by heating at 80 °C for 20 min using a stirring hot plate. Phases A and B were mixed and emulsified for 10 min at 3500 rpm using the homogenizing

Table 1 Fatty acid composition of MCT oil and coconut oil used in the study

Oil	Fatty acid	Lipid number (C:D)	Molecular formula	Molar mass (g/mol)	Content (%)
MCT oil	Caprylic acid	C8:0	$C_8H_{24}O_2$	144.21	60
	Capric acid	C10:0	$C_{10}H_{26}O_2$	172.26	40
Coconut oil	Caprylic acid	C8:0	$C_8H_{24}O_2$	144.21	8
	Capric acid	C10:0	$C_{10}H_{26}O_2$	172.26	6
	Lauric acid	C12:0	$C_{12}H_{28}O_2$	200.31	48
	Myristic acid	C14:0	$C_{14}H_{30}O_2$	228.37	18
	Palmitic acid	C16:0	$C_{16}H_{32}O_2$	256.40	9
	Stearic acid	C18:0	$C_{18}H_{36}O_2$	284.48	3
	Oleic acid	C18:1	$C_{18}H_{34}O_2$	282.47	6

Lipid number—(C) number of carbons and (D) double bonds in the aliphatic chain

mixer (Mark II 2.5, Primix, Japan). The content was cooled at 30 °C to complete 200 g each of 9 different cream-type wash-off hair treatments. The 9 hair treatments prepared as above were crème in color and had the similar thickness all of which could be pumped for application. The composition of 9 different wash-off type hair treatments prepared in the study are shown in Table 2.

Application of hair treatment to BH

Nine hair treatments were each applied on different piece of BH for 21 days. For the nine pieces, the process of 'application of hair treatment (2 g) → leave at room temperature for 10 min → wash using lukewarm water → dry using a hair dryer' was repeated daily for 21 days. Same washing process was applied to a hair piece without the application of hair treatment. The temperature of the hair dryer was about 40–45 °C and the distance between the air nozzle and the hair was about 10 cm.

Examination of protective effects

Measurement of color

Color of hair tress was measured using the Color i5 (X-rite, USA) spectrophotometer which was operated with the Color iQC software (v. 9.2.6). The color values were obtained based on the CIE 1976 L*a*b* (CIELAB) color space using D_{65} CIE standard illuminant and 10° standard observer view angle (ISO/CIE, 2019). Measurements were made in the reflectance mode of Color i5 with specular component included (SCI) and in the wavelength range of 360–750 nm. Aperture size of 6 mm was used throughout the measurement. Color measurements were made on the whole piece of hair tress by holding the hair tress on the root end and aligning the bulk of hair strands so that the tress completely cover the vertical aperture of the spectrophotometer. Hair tress was maintained vertically with complete coverage of the aperture by the sample arm device which automatically held the sample with a snapping motion. The color values L^*, a^*, b^* were obtained from the Color iQC software. The mean values of color difference ΔL^*, Δa^*, Δb^*, and ΔE^*_{ab} were calculated using the Excel software using the color difference equation of CIE 1976 L*a*b color space (ISO/CIE, 2019) (Eq. 1). Following the CIE 1976 L*a*b* color space L^*_0, a^*_0, b^*_0 were the values of the reference and L^*_1, a^*_1, b^*_1 were the values of the samples. Here, the reference was 0 day color value of each hair and the sample was 21 day color value of the each corresponding hair.

$$\Delta L^* = L^*_1 - L^*_0$$

$$\Delta a^* = a^*_1 - a^*_0$$

$$\Delta b^* = b^*_1 - b^*_0$$

$$\Delta E^*_{ab} = \left[\left(L^*_1 - L^*_0\right)^2 + \left(a^*_1 - a^*_0\right)^2 + \left(b^*_1 - b^*_0\right)^2 \right]^{1/2} \quad (1)$$

Table 2 Formulation of 10 different wash-off type hair treatments

Type	Material	Content function	Hair treatment																	
			Blank		C8		C10		C12		C14		C16		C18		MCT		Coconut	
			(%)	(g)	(%)	(g)	(%)	(g)	(%)	(g)	(%)	(g)	(%)	(g)	(%)	(g)	(%)	(g)	(%)	(g)
A (water phase)	Distilled water	Solvent	90	180	87	174	87	174	87	174	87	174	87	174	87	174	87	174	87	174
	Hydroxy-ethylcellulose	Viscosity	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2
B (oil phase)	Caprylic acid (C8:0)	Conditioning agent	–	–	3	6	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	Capric acid (C10:0)		–	–	–	–	3	6	–	–	–	–	–	–	–	–	–	–	–	–
	Lauric acid (C12:0)		–	–	–	–	–	–	3	6	–	–	–	–	–	–	–	–	–	–
	Myristic acid (C14:0)		–	–	–	–	–	–	–	–	3	6	–	–	–	–	–	–	–	–
	Palmitic acid (C16:0)		–	–	–	–	–	–	–	–	–	–	3	6	–	–	–	–	–	–
	Stearic acid (C18:0)		–	–	–	–	–	–	–	–	–	–	–	–	3	6	–	–	–	–
	MCT oil		–	–	–	–	–	–	–	–	–	–	–	–	–	–	3	6	–	–
	Coconut oil		–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	3	6
	Cetearyl alcohol	Emulsion stabilizer	5	10	5	10	5	10	5	10	5	10	5	10	5	10	5	10	5	10
	Arlacel 83	Surfactant (nonionic)	2	4	2	4	2	4	2	4	2	4	2	4	2	4	2	4	2	4
	Olivem 800	Surfactant (nonionic)	2	4	2	4	2	4	2	4	2	4	2	4	2	4	2	4	2	4
Total			100	200	100	200	100	200	100	200	100	200	100	200	100	200	100	200	100	200

Measurement of tensile strength

Tensile strength (MPa) of hair was measured using the MPT-320 model tensile tester (TMA, Korea). Measurements were made under 50 mm/min speed, 0.1 N/s load condition. Thickness of hair was set as 0.08 mm. Each measurement was made using a single strand of hair randomly collected from the sample hair tress. Five strands were measured for each hair tress. Tensile test was carried out in reference to ASTM D2256/D2256M-10 (Standard test method for tensile properties of yarns by the single-strand method) (ASTM International, 2015) and Kunchi et. al. (2018).

Measurement of thickness

Thickness of single strand of hair was measured using a constant load type Digital thickness gauge (Hanwonsoway, Korea). The presser foot was 9.5 mm in diameter, measuring range was 16 mm, with 0.001 mm precision. The thickness measurement was carried out in reference to KS K ISO 5084 (Korea Standards Association, 2017) and Kim et. al. (2021).

Analysis of protein leak

Hair tress was washed 3 times using ethyl alcohol (94.5%) and then cut into 0.1–0.2 cm length. 0.02 g of cut hair was placed in a conical tube. A mixed solution of 40 mL of chloroform and 20 mL of methyl alcohol (2:1 v/v) was prepared and 4 mL of this solution was added to the above conical tube. The tube was left at room temperature for 24 h to remove the external lipid from hair. Buffer solution was prepared by mixing 0.30 g of trizma-base, 19.80 g of thiourea, 30.03 g of urea, and 90 mL of distilled water. The contents were dissolved on the stirring hot plate for 1 h. 5 mL of 2-mercapto ethanol was added to the mixture and the pH of the buffer solution was adjusted to 8.5. In each conical tube containing hair, 5 mL of the buffer solution was added. The tubes were placed in an oven at 50 °C for 24 h then were centrifuged for 10 min to complete the protein extraction. Bio-Rad dye (Coomassie brilliant blue G-250) was added to the protein extraction and the absorbance of the solution was examined at 595 nm (λ_{max}) using the UV–VIS spectrophotometer (Lambda 25, Perkin Elmer, USA). Standard protein solutions (3–9 μg/mL) were prepared by mixing bovine serum albumin (BSA) and the Bio-Rad dye. The absorbance of the standard protein solutions were measured using the UV–VIS spectrophotometer at 380–700 nm. The standard calibration curve of BSA was generated using the absorbance values of the standard solutions at the maximum absorbance wavelength (595 nm).

Examination of surface characteristics

Surface characteristics of hair was examined using the scanning electron microcopy (FE-SEM 7001F, JEOL, Japan) at 1000 magnification.

Analysis of penetration of fatty acid into hair

0.2 g of cut hair was immersed in the delipidizing solution for 24 h for the extraction of lipid from hair. The solution was prepared by mixing chloroform/methanol in 2:1 v/v ratio in reference to Nakamura et. al. (2002) and Song et. al. (2020). The

absorbance of the hair lipid extract in 190–700 nm was observed using the UV–VIS spectrophotometer (Lambda 25, Perkin Elmer, USA). The absorbance of the lipid extract of each sample at the maximum absorbance wavelength (256 nm) was recorded. Standard solutions of caprylic acid, capric acid, lauric acid, myristic acid, palmitic acid, and stearic acid were each made by dissolving 1000 mg of fatty acid standard in the mixed solution of chloroform/methanol (2:1 v/v) to prepare 20 mL of the stock solution. The solutions were diluted into 3.12, 6.25, 12.5, 25, and 50 mg/mL and were observed using the UV–VIS spectrophotometer to obtain the maximum absorbance data at 256 nm. Calibration curves were prepared for each standard fatty acid and the corresponding regression equations were generated.

Analysis of fatty acid composition of hair

Composition of fatty acids contained in hair was analyzed using the gas chromatography mass spectrometry (GC–MS) based on Garcés and Mancha (1993). The GC instrument was Agilent 7890A (Agilent, USA) with DB-23 (120 mm × 0.25 mm × 0.25 μm) column and a flame ionization detector (FID). Detection temperature was 250 °C, the carrier gases were H_2 (35 mL/min), air (350 mL/min), and He (10 mL/min). Oven temperature was programmed with initial temperature 80 °C with 1.5 min hold, Ramp 1 was 110 °C by 30 °C/min with 2.0 min hold, Ramp 2 was 200 °C by 15 °C/min with 8.0 min hold, Ramp 3 was 215 °C by 1 °C/min with 8.0 min hold, and finally Ramp 4 was 250 °C by 2 °C/min with 3.0 min hold. Supelco 37 Component FAME Mix (Supelco, USA) was used for the fatty acid standard. Pentadecanoic acid (Sigma-Aldrich) was used for the internal standard. 40 mg of each BH was cut into small pieces, freeze-dried, and powdered. 2 mL of the sample and methylation mixture (methanol: benzene: 2,2-dimethoxy-propane: sulfuric acid = 39:20:5:2) and 1 mL of heptane was added to a 4 mL vial with Teflon-lined-cap. The vial was left at 80 °C for 2 h to extract the lipid from hair. The extract was cooled in room temperature after which the supernatant was injected to the inlet of GC with the injection volume of 1 μL. The retention time of each fatty acid in the Supelco 37 Component FAME Mix was used to confirm each fatty acid detected from the extract. Concentration of fatty acid was calculated using method by Garcés and Mancha (1993).

Statistical analysis

Collected data were analyzed using the SPSS statistical package (v. 2.1). Independent sample *t*-test was used to compare the differences in the mean of two independent groups of hair. The statistical significance of each test was verified at $\alpha = 0.05$.

Definition of sample labels

BH treated with 6 fatty acid hair treatments which were formulated with caprylic acid (C8:0), capric acid (C10:0), lauric acid (C12:0), myristic acid (C14:0), palmitic acid (C16:0), or stearic acid (C18:0) were labeled C8BH, C10BH, C12BH, C14BH, C16BH, and C18BH, respectively. BH treated with the hair treatment formulated with MCT oil or coconut oil were labeled MCT and Coconut. BH treated with the hair treatment formulated with only the basic ingredient was labeled BBH. One BH received daily washing and drying only without the application of hair treatment and this sample was labeled WBH. C8BH, C10BH, C12BH were grouped into the medium-chain fatty acid group

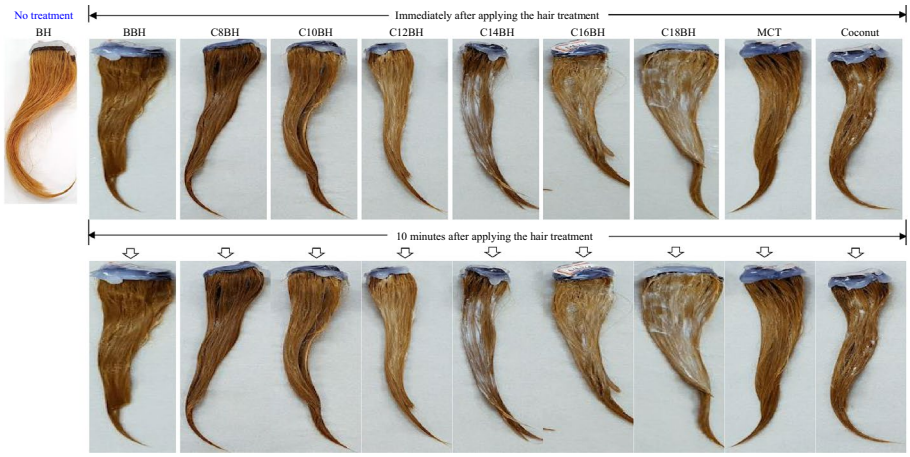

Fig. 1 Appearance of bleached hair (BH) after the application of hair treatments. BH was untreated (0-day) and the photos of BBH–Coconut were taken during the 21th day of treatment

(MFBH) and C14BH, C16BH, C18BH were grouped into the long-chain fatty acid group (LFBH) for the statistical analysis.

Results and Discussion

Visual appearance of BH before and after hair treatment application

The photographs of BH immediately after and 10 min after the application of hair treatments are shown in Fig. 1. The photos were taken during the 21-day application of the hair treatment. In BBH and the MFBH group (especially in C8BH and C10BH), the hair treatments disappeared as soon as they were applied on the hair, suggesting that most or all of the hair treatments were absorbed. In C12BH, some hair treatment remained on the surface of hair until the end of the treatment time. In LFBH group (C14BH, C16BH, C18BH) the hair treatments became cakey and there was less or no sign of disappearing as they were applied. Especially in C14BH and C18BH a great deal of hair treatment remained on the surface of hair with cakey appearance until the treatment time was over. The fact that there was no trace of hair treatment in BBH after the application of blank treatment confirmed that the basic substances added to the hair treatment formulation did not cause the mal-absorptivity of LFBH. The visual inspection suggested that overall medium-chain fatty acids tended to penetrate the hair better than long-chain fatty acids with some deviation such as lauric acid or palmitic acid. The appearance of MCT immediately after and 10 min after the application of hair treatment was similar to C8BH or C10BH and Coconut was similar to C12BH. Since all 9 hair treatments showed similar thickness of a cream-type hair treatment it is highly probable that the above results were due to the difference in the fatty acids contained in the hair treatment.

Microscopic view of BH before and after hair treatment application

The results of scanning electron microscopy (SEM) of BH before the treatment (0 day) and after 21 days of hair treatment application is shown in Fig. 2. The surface of BH samples before they were treated with the hair treatment looked severely damaged. Outermost layer of cuticle cells were completely destroyed and peeled, exposing the

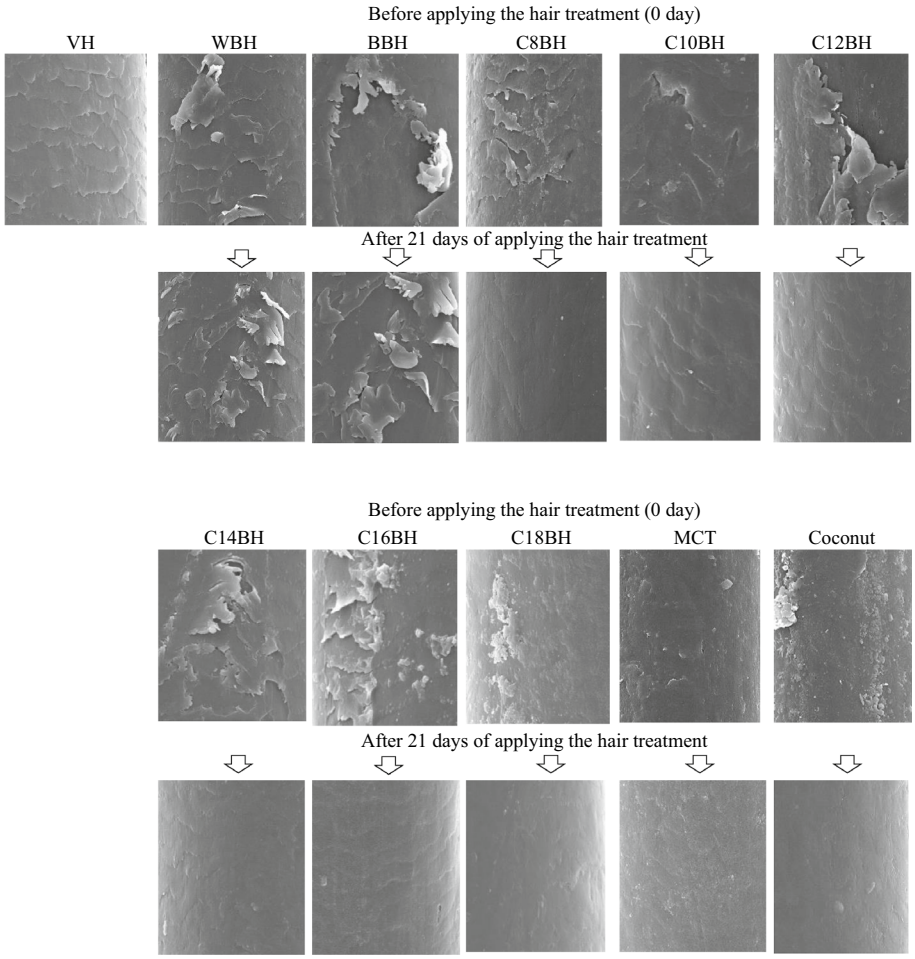

Fig. 2 SEM images of untreated virgin hair (VH) and bleached hair (BH) before treatment and after 21 days of applying the hair treatments

underneath cuticle layer (BBH–C14BH). The edge of cuticle cells were cracked and raised (C16BH, C18BH). Such surface characteristics suggest that the oxidative treatment caused breakage of cystine bond which led to the structural deformation and breakdown (Wolfram et al., 1970). After treating the hair with 9 types of hair treatments, a dramatic change occurred in BH treated with the fatty acid hair treatments, MCT and Coconut hair treatments. The edge of the outermost cuticle cells were tightly adhering to the underneath layer, the surface of the cuticle layers became smooth and showed a coated appearance. Similar appearance was observed in MCT and Coconut both before the treatment and after 21 days of treatment.

Fatty acid composition of hair

GC–MS analysis was conducted on BH after 21 days of treatment and also on the untreated VH and BH to analyze the composition and the concentration of fatty acids in hair. Figure 3 shows the concentration of the six fatty acids of interest in each hair sample. Each BH treated with the specific fatty acid hair treatment had the highest concentration of corresponding fatty acid among all samples. C10BH and C8BH

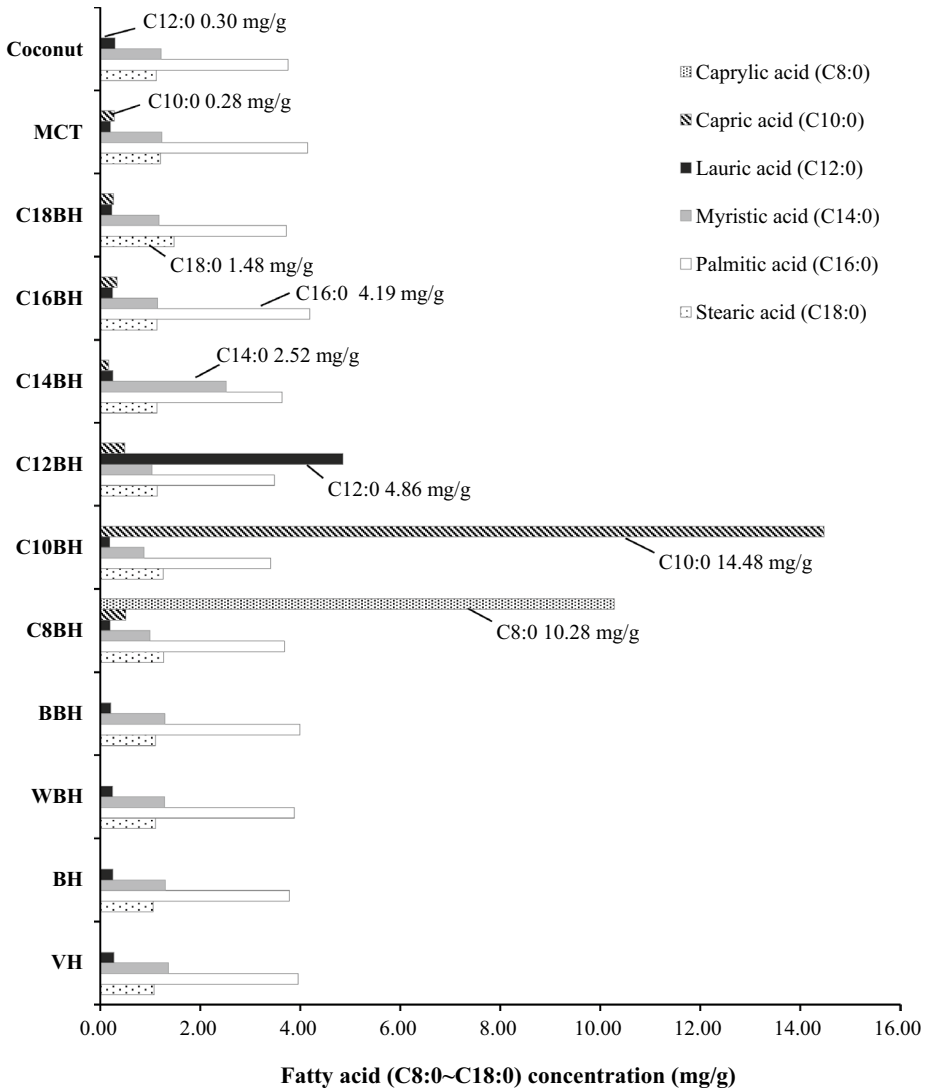

Fig. 3 Result of GC–MS analysis on the C8:0–C18:0 fatty acid concentration of hair samples. VH, BH were untreated and WBH–Coconut were after 21 days of treatment

showed new and significantly high concentration of capric acid (C10:0) (14.48 mg/g) and caprylic acid (C8:0) (10.28 mg/g) respectively. In C12BH–C18BH the increase in the concentration of fatty acid corresponded to the fatty acid added to the hair treatment formulation. However the rate of increase was much smaller than C10BH and C8BH. The fatty acid concentration of MCT and Coconut seemed similar to VH, BH, WBH, or BBH. The concentration of medium-chain triglycerides did not reflect the composition of MCT oil contained in the hair treatment which was 60% caprylic acid and 40% capric acid. In fact, caprylic acid was not detected at all and the concentration of capric acid was only 0.28 mg/g which was lower than C8BH (0.51 mg/g), C10BH (14.18 mg/g), C12BH (0.49 mg/g), and C16BH (0.33 mg/g). Such difference in C8BH and C10BH vs. MCT might be due to the fact that the fatty acids in MCT oil is present in the form of triglyceride where the fatty acids are bound to the glycerol

backbone (Kail et al., 2012). On the other hand, the fatty acid hair treatments contain free fatty acids (Kail et al., 2012).

In all samples except C12BH, the concentration of palmitic acid (C16:0) was the highest among all the fatty acids. Its concentration range across the samples was 3.41–4.14 mg/g (excluding C16BH) which was about 3–16 times higher than other fatty acids detected from hair. Wertz and Downing (1988) reported that 41% of the fatty acid inherent in human hair was 18-methyl eicosanoic acid ($C_{21}H_{42}O_2$) (40.5%) followed by palmitic acid (C16:0) (18.3%), stearic acid (C18:0) (7.0%), oleic acid (C18:1) (4%), and others. In the present GC–MS analysis eicosanoic acid (C20:0) was detected at t_R 31.26 min but 18-methyl eicosanoic acid, the eicosanoic acid (C20:0) with a methyl group at the No. 18 carbon, was not directly detected. In our analysis palmitic acid (C16:0) showed the highest concentration in hair. The difference between the present result and the literature value could be due to the difference in the method of sample preparation such as the extraction medium, time, or the derivatization (Kail et al., 2012).

Penetration of fatty acids in hair

In order to examine whether the detection of certain fatty acid in hair was due to the penetration of hair treatments, samples of C8BH–C18BH were tested to examine the actual penetration of fatty acid into hair. For the test, lipid was extracted from hair after all the surface remaining hair treatment was washed from the hair. To examine the penetration of caprylic acid for example the extent of penetration was deduced by comparing the concentration of caprylic acid (C8:0) in the 0-day sample of C8BH versus 21-day sample of C8BH. The same strategy was used to examine the concentration of capric acid (C10:0) in C10BH, lauric acid (C12:0) in C12BH, and so on. According to the Beer-Lambert law, the absorbance of a solution is directly proportional to the concentration of the absorbing species within the solution (Bhanvase & Barai, 2021). Based on the Beer-Lambert law the concentration of each fatty acid in the treated BH was determined by the absorbance of the lipid extract at the maximum absorption wavelength. The solutions of six standard fatty acids diluted into 3.12–50.00 mg/mL concentrations were examined using the UV–VIS spectrophotometer to determine the λ_{max} and to obtain the absorbance data. Using the absorbance data, calibration curve was made for each fatty acid standard and the corresponding regression equation was generated.

The maximum absorbance of the six reference standards occurred at 256 ± 2 nm with only slight deviation in the absorbance values within 254–258 nm range. Based on the results and following Czauderna and Kowalczyk (2002) 256 nm was used as the λ_{max} for the standards and the samples. Figure 4 shows the absorbance spectra of the lipid extracted from C8BH–C18BH before the treatment (0 day) and after 21 days of hair treatment application. After 21 days of applying the hair treatment all samples of C8BH–C18BH showed an increase in their absorbance at λ_{max} compared to 0 day. The mean absorbance values of 0-day and 21-day measurement of C8BH–C18BH are shown in Table 3. The greatest change in fatty acid concentration was observed in C10BH followed by C8BH and the smallest change was observed in C16BH. C12BH which belongs to the medium-chain fatty acid group showed the change in concentration of fatty acid slightly smaller than those of C18BH and C14BH indicating that not all samples in MFBH group showed higher penetration of fatty acid than LFBH group. Change in the

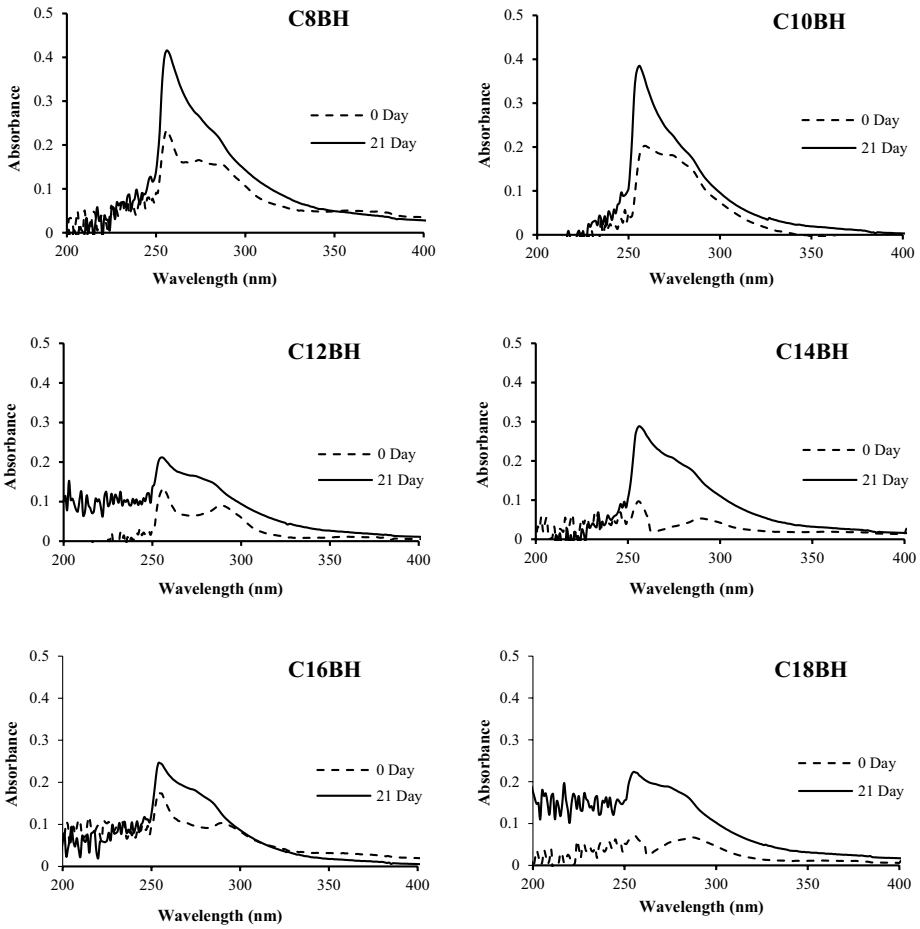

Fig. 4 UV absorbance spectrum (λ_{max}: 256 nm) of bleached hair treated with six fatty acid hair treatments before (0 day) and after 21 days of application

Table 3 Concentration of fatty acids in BH after 21 days of fatty acid hair treatment

Group	Hair	N	0 Day (A)		21 Day (B)		B–A concentration (mg/mL)	SE	Fatty acid
			Mean absorbance (A)	SE	Mean absorbance (B)	SE			
MFBH	C8BH	3	0.2342	0.0162	0.4156	0.0027	31.83	3.3214	Caprylic
	C10BH	3	0.1817	0.0027	0.3851	0.0100	41.64	1.9806	Capric
	C12BH	3	0.1349	0.0174	0.2109	0.0051	15.60	2.7482	Lauric
LFBH	C14BH	3	0.0967	0.0009	0.2884	0.0031	17.53	0.3561	Myristic
	C16BH	3	0.1728	0.0145	0.2427	0.0170	5.01	0.2814	Palmitic
	C18BH	3	0.0702	0.0115	0.2218	0.0112	17.27	2.7737	Stearic

concentration of palmitic acid (C16:0) in C16BH was comparatively much lower than other samples in LFBH group. Six samples were grouped into MFBH group and LFBH group and the change in the concentration of fatty acid was compared between the two groups using the independent sample *t*-test (Table 4). The result showed that the mean penetration of fatty acid in MFBH (31.098 mg/mL) was significantly different and higher

Table 4 Result of independent *t*-test on the change of concentration of fatty acids in the MFBH group and the LFBH group after 21 days of hair treatment application

Variable	Group	N	Mean (mg/mL)	SE	*t*	*p*
Fatty acid concentration	MFBH	9	30.39	4.1219	3.435	0.005
	LFBH	9	14.17	2.3051		

Table 5 Color values of bleached hair (BH) before and after 21 days of applying different hair treatments

Group	Hair	Day	N	*L**	*a**	*b**	Δ*L**	Δ*a**	Δ*b**	ΔE^*_{ab}
WBH	WBH	0	3	38.23	10.69	22.39	–	–	–	0
		21	3	41.93	11.76	26.31	3.69	1.07	3.92	5.52
BBH	BBH	0	3	36.68	11.86	22.42		–	–	–
		21	3	40.84	11.07	23.36	4.16	−0.78	0.93	4.38
MFBH	C8BH	0	3	42.23	11.58	25.94	–	–	–	–
		21	3	41.81	10.91	24.98	−0.42	−0.67	−0.96	1.83
	C10BH	0	3	42.56	12.29	27.17	–	–	–	–
		21	3	42.28	11.26	25.85	−0.28	−1.03	−1.32	2.14
	C12BH	0	3	41.89	11.06	24.94	–	–	–	–
		21	3	42.54	10.93	26.08	0.65	−0.13	1.14	1.49
LFBH	C14BH	0	3	44.41	11.66	26.25	–	–	–	–
		21	3	40.61	11.13	23.99	−3.80	−0.53	−2.26	4.45
	C16BH	0	3	48.27	11.76	29.07	–	–	–	–
		21	3	46.23	10.47	26.63	−2.04	−1.29	−2.44	3.47
	C18BH	0	3	50.57	9.74	27.05	–	–	–	–
		21	3	46.64	11.63	28.11	−3.93	1.89	1.06	4.68
MCT	MCT	0	3	40.91	11.83	24.74	–	–	–	0
		21	3	41.72	11.06	25.02	0.81	−0.77	0.27	1.50
Coconut	Coconut	0	3	41.54	10.99	24.85	–	–	–	0
		21	3	37.93	11.30	23.27	−3.61	0.31	−1.58	3.98

than that of LFBH (15.073 mg/mL) at $\alpha = 0.05$ ($t = 3.435$, $p = 0.005$). Such result verifies that the significantly higher concentration of caprylic acid (C8:0) in C8BH and capric acid (C10:0) in C10BH, and relatively high concentration of lauric acid (C12:0) in C12BH which was examined by GC–MS was due to the actual penetration of fatty acid hair treatment in these hair samples. The results of GC–MS and the results of UV–VIS spectrophotometry together strongly suggested that the concentration of fatty acid increased after 21 days of hair treatment application and that the increase was due to the actual penetration of fatty acid agent contained in the hair treatment.

Protective effects of different treatments

Color

Color measurements of the hair samples of WBH–Coconut are shown in Table 5. Based on the CIE 1976 L*a*b* color space L^* value represent the lightness which range from 0 (black) to 100 (white), a^* value represent green (−) to red (+), and b^* value represent blue (−) to yellow (+) (ISO/CIE, 2019). In ΔL^*, Δa^*, and Δb^* the sign (+, −) designate the direction of change and the magnitude of color change is explained by the absolute

values of ΔL^*, Δa^*, and Δb^*. In the color change issue of bleached hair more concern is on the magnitude of color change. Therefore, the amount of color change was interpreted in the following based on the absolute values of ΔL^*, Δa^* and Δb^* and not their mathematical values. Lower absolute values of ΔL^*, Δa^*, and Δb^* and the total color difference value of ΔE^*_{ab} were preferable since it means that there was less color change in bleached hair after the application of hair treatment. Less color change of bleached hair could lengthen the bleaching interval which thereby reduce the damage caused by repeated bleaching (Lee & Ahn, 2022).

The color of virgin black hair (L^*: 19.27, a^*: 2.38, b^*: 2.22) became lighter, redder, and yellower after bleaching. After 21 days the greatest color difference value (ΔE^*_{ab}) was observed in WBH (Table 5). Next was C18BH which showed even higher ΔE^*_{ab} than BBH. Overall, the hair samples treated with the fatty acid hair treatments became darker and lost redness and yellowness. Exceptions were observed in C12BH, C18BH, C12BH and C18BH which showed positive values for each color component. When the samples of C8BH–C18BH were grouped into MFBH and LFBH, MFBH showed a lower range of ΔE^*_{ab} (1.24–1.70) than the LFBH (3.43–4.49) (Fig. 5). MCT became lighter, lost redness and increased in yellowness. Coconut became darker, slightly redder and lost yellowness. ΔE^*_{ab} of Coconut was greater than that of MCT. ΔE^*_{ab} of MCT was comparable to those of MFBH while ΔE^*_{ab} of Coconut was comparable to those of LFBH.

Tensile strength

Tensile strength of BH treated with different hair treatments were measured and the results are shown in Table 6. Tensile strength of VH (253.90 MPa) decreased dramatically by bleaching as observed by the values of the 0 day samples (147.86–178.20 MPa). After 21 days of hair treatment application, tensile strength of WBH and BBH decreased from the 0 day value with greater decrease observed in WBH than BBH. All samples of MFBH and LFBH increased in tensile strength with a higher percent increase shown in MFBH (123.14–132.26%) compared to LFBH

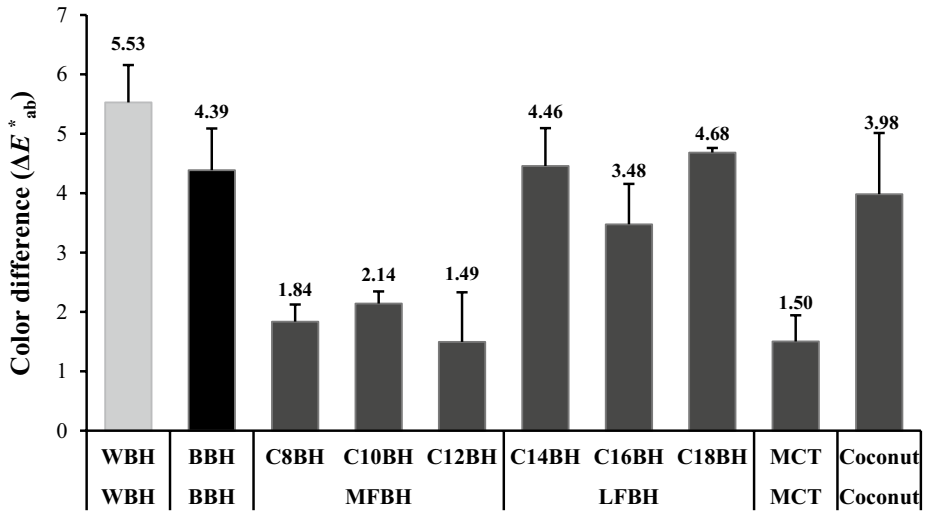

Fig. 5 Color difference (ΔE^*_{ab}) of bleached hair (BH) after 21 days of applying different hair treatments

Table 6 Tensile strength of bleached hair (BH) before and after 21 days of applying different hair treatments

Group	Hair	Days	N	Tensile strength (MPa)		
				MPa	SE	%
WBH	WBH	0	5	166.42	5.8615	100.00
		21	5	147.72	9.8250	88.76
BBH	BBH	0	5	148.00	11.7706	100.00
		21	5	141.80	10.0996	95.81
MFBH	C8BH	0	5	147.86	6.5425	100.00
		21	5	195.56	12.3932	132.26
	C10BH	0	5	158.88	18.4576	100.00
		21	5	197.06	15.6490	124.03
	C12BH	0	5	157.36	10.5185	100.00
		21	5	193.78	11.1187	123.14
LFBH	C14BH	0	5	168.52	10.0798	100.00
		21	5	188.46	9.0606	111.83
	C16BH	0	5	171.06	8.7824	100.00
		21	5	186.64	10.4872	109.11
	C18BH	0	5	178.20	10.0003	100.00
		21	5	187.90	9.0766	105.44
MCT	MCT	0	5	179.62	8.7471	100.00
		21	5	231.56	10.5284	128.92
Coconut	Coconut	0	5	167.94	9.1951	100.00
		21	5	212.32	4.7967	126.42

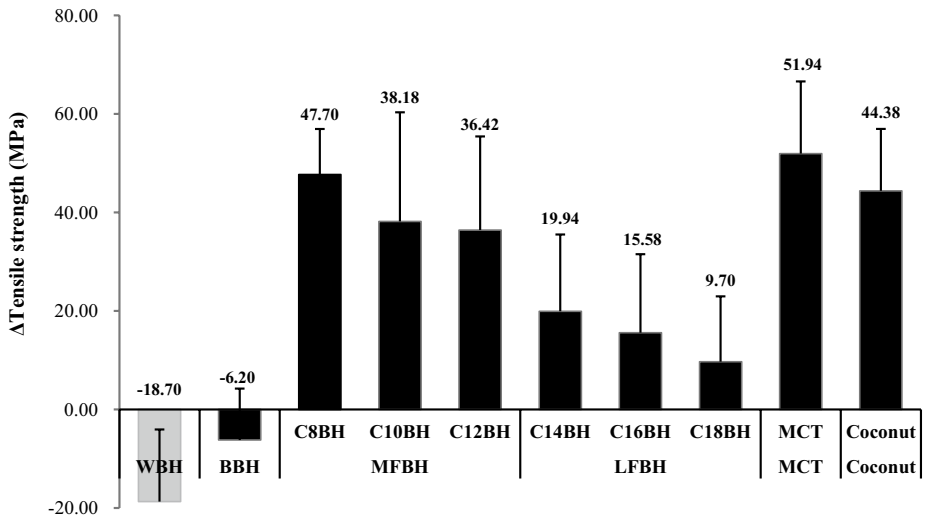

Fig. 6 Comparison of the tensile strength difference of 21-day vs. 0-day among bleached hair (BH) treated with different hair treatments

(105.44–111.83%). The order of percent increase was by C18BH < C16BH < C14BH < C12BH < C10BH < C8BH which suggested that there might be a relationship between the length of aliphatic chain and the recovery of tensile strength (Fig. 6). MCT and Coconut increased in its tensile strength by similar percent increase as observed in

MFBH and the tensile strength of MCT after 21 days of treatment was especially closer to that of VH.

Thickness

The thickness of untreated (0 day) BH was in the range of 0.0467–0.0563 mm which was thinner than that of VH (0.0608 mm) (Table 7). By 21 days of hair treatment application WBH and BBH decreased in thickness with higher percent decrease observed in WBH than BBH. All samples of MFBH and LFBH increased in thickness with the highest increase observed in C8BH and the lowest in C16BH. Although the differences were small, the increase of thickness was in the order of C16BH < C18BH < C14BH < C12BH < C10BH < C8BH with the samples of MFBH showing the higher increase in thickness. MCT showed the percent increase in thickness comparable to C8BH, and Coconut showed the percent increase comparable to C14BH and C12BH. The result suggested that MCT oil had better effect on the thickness of hair than coconut oil, and washing alone or using the hair treatment without the conditioning ingredient had negative effect on the thickness protection of hair.

Protein leak

Analysis of concentration of protein extractable from hair is one way to determine the degree of hair damage and the possible amount of protein leak from the damaged hair (França-Stefoni et al., 2015; Joo & Lim, 2015; Lee & Ahn, 2022; Park & Ahn, 2022; Rele & Mohile, 2003). More protein would be extracted from hair if the hair has become porous

Table 7 Thickness of bleached hair (BH) before and after 21 days of applying different hair treatments

Group	Hair	Days	N	Thickness (mm)		
				Thickness (mm)	SE	%
WBH	WBH	0	10	0.0563	0.0030	100.00
		21	10	0.0530	0.0025	94.14
BBH	BBH	0	10	0.0536	0.0024	100.00
		21	10	0.0520	0.0021	97.01
MFBH	C8BH	0	10	0.0471	0.0030	100.00
		21	10	0.0497	0.0028	105.52
	C10BH	0	10	0.0483	0.0040	100.00
		21	10	0.0505	0.0039	104.55
	C12BH	0	10	0.0467	0.0033	100.00
		21	10	0.0480	0.0032	102.78
LFBH	C14BH	0	10	0.0508	0.0046	100.00
		21	10	0.0520	0.0044	102.36
	C16BH	0	10	0.0502	0.0035	100.00
		21	10	0.0513	0.0035	102.19
	C18BH	0	10	0.0483	0.0037	100.00
		21	10	0.0494	0.0036	102.28
MCT	MCT	0	10	0.0545	0.0026	100.00
		21	10	0.0574	0.0024	105.32
Coconut	Coconut	0	10	0.0556	0.0023	100.00
		21	10	0.0570	0.0024	102.52

due to chemical damage (França-Stefoni et al., 2015; Lee & Ahn, 2022; Park & Ahn, 2022; Rele & Mohile, 2003). Different methods are used for the extraction and quantification of protein from hair. Methods include the Lowry method which use the Folin–Ciocalteau reagent (Rele & Mohile, 2003), BCA method which use the bicinchoninic acid (BCA) (Dias et al., 2008; França-Stefoni et al., 2015), alkaline method which use the NaOH solution (Joo & Lim, 2015), and Bradford protein assay which use the Coomassie Brilliant Blue to bind the hair protein (Lee & Ahn, 2022; Park & Ahn, 2022; Silva et al., 2004). Among these methods the Bradford protein assay is known to be a rapid and sensitive method which can eliminate the interference of surfactants or lipids which may be present in hair (Silva et al., 2004).

In this research protein was extracted from hair using the Shindai method and the extracted protein was quantified using the Bradford protein assay. The extracted protein was reacted with Bio-Rad dye to produce a clear blue solution which showed maximum absorbance at 595 nm by the UV–VIS spectrophotometry (Bradford, 1976). Standard calibration curve was made by the maximum absorbance values of known concentrations (3, 4, 5, 7, 9 μg/mL) of bovine serum albumin (BSA) reacted with Bio-Rad dye (Bradford, 1976). The concentration of extracted protein was quantified by substituting the maximum absorbance of the extracted protein in the y value of the regression equation generated from the standard calibration curve which was $y = 0.025x + 0.204$ ($R^2 = 0.9973$).

The concentration of protein extracted from VH was 3.13 μg/mL while the concentration increased to 3.46–6.17 μg/mL after bleaching (Table 8). The data indicated

Table 8 Concentration of protein extracted from bleached hair (BH) before and after 21 days of applying different hair treatments

Group	Hair	Days	N	Protein concentration (protein leak)		
				Conc. (μg/mL)	SE	%
WBH	WBH	0	3	3.92	0.1674	100.00
		21	3	5.83	0.0576	148.72
BBH	BBH	0	3	4.28	0.2727	100.00
		21	3	5.47	0.0288	127.80
MFBH	C8BH	0	3	6.17	0.0053	100.00
		21	3	4.74	0.0345	76.82
	C10BH	0	3	5.92	0.2217	100.00
		21	3	5.33	0.0100	90.03
	C12BH	0	3	5.74	0.3979	100.00
		21	3	4.96	0.0201	86.41
LFBH	C14BH	0	3	4.05	0.0854	100.00
		21	3	3.44	0.0122	84.93
	C16BH	0	3	3.89	0.0183	100.00
		21	3	3.47	0.0074	89.20
	C18BH	0	3	3.46	0.0139	100.00
		21	3	3.40	0.0179	98.26
MCT	MCT	0	3	7.34	0.2199	100.00
		21	3	6.50	0.0312	88.55
Coconut	Coconut	0	3	4.61	0.2155	100.00
		21	3	4.10	0.0890	88.93

that the hair became porous due to oxidative treatment and that such damage caused the increase in protein leak after bleaching (Hill et al., 2014). After 21 days of hair treatment application, the concentration of protein extracted from BH decreased in all samples of MFBH and LFBH with the lowest concentration observed in C8BH. C8BH in fact decreased in protein concentration by 76.8% of the 0 day value which was much lower than C18BH which showed a 98.3% concentration of the 0 day value. MCT and Coconut showed a decrease in protein concentration and the percent decrease of the two samples were similar. Protein concentration increased significantly in WBH followed by BBH after 21 days. Washing with water alone or applying the hair treatment without the conditioning agent had a negative effect on preserving protein of hair. The result implied that applying the hair treatments formulated with fatty acid, MCT oil, or Coconut oil on bleached hair could decrease the protein leaking from hair.

Statistical significance of the results on protective effects

Significant difference was observed between VH and BH in all the protective effects. Independent *t*-test was conducted between any two hair groups among MFBH, LFBH, MCT, and Coconut on the results of ΔE^*_{ab}, ΔTensile strength, ΔThickness, and the ΔProtein leak (Table 9). ΔTensile strength, ΔThickness, and the ΔProtein leak were calculated by subtracting the 0-day value from the corresponding 21-day value. Between MFBH and LFBH groups the results of all four protective effects were significantly different at $\alpha = 0.05$. The MFBH group showed lower color difference, higher increase in tensile strength and thickness, and higher decrease in protein leak compared to the LFBH group. The *t*-tests on MCT vs. Coconut, MFBH vs. MCT, and LFBH vs. Coconut showed that the two comparison groups were not significantly different at $\alpha = 0.05$ in all protective effects. However, the *t*-tests on LFBH vs. MCT showed that LFBH and MCT were significantly different at $\alpha = 0.05$ in all protective effects. MCT showed lower color difference, higher increase in tensile strength and thickness, and higher decrease in protein leak compared to the LFBH group. The *t*-tests on MFBH vs Coconut showed that the two groups were not significantly different at $\alpha = 0.05$ in tensile strength, thickness, and protein leak. But there was a significant different in the ΔE^*_{ab} values between MFBH and Coconut, the MFBH group showing a lower color difference than Coconut.

Independent *t*-tests were conducted between any two individual hair samples of WBH–Coconut and the significance of the *t* value was verified at $\alpha = 0.05$ (Table 10). WBH and BBH were not significantly different in any of the protective effects. For the thickness and the protein leak, all samples of C8BH–Coconut were significantly different from WBH or BBH. However, such significance was not observed in ΔE^*_{ab} or ΔTensile strength. For ΔE^*_{ab} WBH was significantly different from C8BH, C10BH, C12BH, MCT, and BBH was significantly different from C8BH, C10BH, MCT. For ΔTensile strength WBH and BBH were both different from C8BH, MCT, Coconut. Among BH treated with fatty acid hair treatments significant differences were found especially in the protein leak of C8BH and most of the fatty acid treated hair, MCT, Coconut. For the tensile strength and the thickness there were no significant differences in any two samples among the hair samples of C8BH–C18BH.

Table 9 Results of independent *t*-test on the protective effects of hair groups (α = 0.05)

Comparison	Protection	Group	N	Mean	SE	*t*	*p*
MFBH vs. LFBH	ΔE^*_{ab}	MFBH	9	1.8235	0.2782	− 5.542	< 0.001
		LFBH	9	4.2053	0.3277		
	ΔTensile strength (MPa)	MFBH	15	40.7667	9.5389	2.055	0.049
		LFBH	15	15.0733	8.0832		
	ΔThickness (mm)	MFBH	30	0.0020	0.0003	2.439	0.018
		LFBH	30	0.0011	0.0002		
	ΔProtein leak (μg/mL)	MFBH	9	− 0.9311	0.1857	− 2.786	0.013
		LFBH	9	− 0.3640	0.0835		
MCT vs. coconut	ΔE^*_{ab}	MCT	3	1.5015	0.4416	− 2.214	0.091
		Coconut	3	3.9828	1.0320		
	ΔTensile strength (MPa)	MCT	5	51.9400	14.6536	0.392	0.706
		Coconut	5	44.3800	12.5725		
	ΔThickness (mm)	MCT	10	0.0029	0.0006	1.346	0.195
		Coconut	10	0.0015	0.0008		
	ΔProtein leak (μg/mL)	MCT	3	− 0.8400	0.1975	− 1.416	0.230
		Coconut	3	− 0.5080	0.1265		
MFBH vs. MCT	ΔE^*_{ab}	MFBH	9	1.8235	0.2782	0.588	0.569
		MCT	3	1.5015	0.4416		
	ΔTensile strength (MPa)	MFBH	15	40.7667	9.5390	− 0.600	0.556
		MCT	5	51.9400	14.6536		
	ΔThickness (mm)	MFBH	30	0.0020	0.0003	− 1.371	0.178
		MCT	10	0.0029	0.0006		
	ΔProtein leak (μg/mL)	MFBH	9	− 0.9311	0.1857	− 0.262	0.798
		MCT	3	− 0.8400	0.1975		
MFBH vs. coconut	ΔE^*_{ab}	MFBH	9	1.8235	0.8345	− 2.964	0.014
		Coconut	3	3.9828	1.0302		
	ΔTensile strength (MPa)	MFBH	15	40.7667	9.5390	− 0.199	0.845
		Coconut	5	44.3800	12.5725		
	ΔThickness (mm)	MFBH	30	0.0020	0.0003	0.757	0.454
		Coconut	10	0.0015	0.0008		
	ΔProtein leak (μg/mL)	MFBH	9	− 0.9311	0.1857	− 1.250	0.240
		Coconut	3	− 0.5080	0.1265		
LFBH vs. MCT	ΔE^*_{ab}	LFBH	9	4.2053	0.3277	4.299	0.002
		MCT	3	1.5015	0.4416		
	ΔTensile strength (MPa)	LFBH	15	15.0733	8.0832	− 2.257	0.037
		MCT	5	51.9400	14.6536		
	ΔThickness (mm)	LFBH	30	0.0011	0.0002	− 2.684	0.021
		MCT	10	0.0029	0.0006		
	ΔProtein leak (μg/mL)	LFBH	9	− 0.3640	0.0835	2.633	0.025
		MCT	3	− 0.8400	0.1975		
LFBH vs. coconut	ΔE^*_{ab}	LFBH	9	4.2053	0.3277	0.281	0.784
		Coconut	3	3.9828	1.0302		
	ΔTensile strength (MPa)	LFBH	15	15.0733	8.0832	− 1.853	0.080
		Coconut	5	44.3800	12.5725		
	ΔThickness (mm)	LFBH	30	0.0011	0.0002	− 0.426	0.679
		Coconut	10	0.0015	0.0008		
	ΔProtein leak (μg/mL)	LFBH	9	− 0.3640	0.0835	0.884	0.398
		Coconut	3	− 0.5080	0.1265		

Table 10 *t* values of independent *t*-tests on the protective effects between samples (α = 0.05)

Protection	Hair	WBH	BBH	C8BH	C10BH	C12BH	C14BH	C16BH	C18BH
ΔE^*_{ab}	BBH	1.209							
	C8BH	5.328**	3.359*						
	C10BH	5.116**	3.071*	−0.860					
	C12BH	3.854*	2.649	0.386	0.751				
	C14BH	1.194	−0.074	−3.743*	−3.458*	−2.817*			
	C16BH	2.214	0.932	−2.218	−1.879	−1.838	1.052		
	C18BH	1.330	−0.420	−9.524**	−11.630***	−3.799*	−0.353	−1.765	
	MCT	5.234**	3.477*	0.634	1.312	−0.008	3.809*	2.433	7.098**
	Coconut	1.280	0.324	−2.007	−1.754	−1.876	0.391	−0.411	0.678
ΔTensile strength (MPa)	BBH	−0.695							
	C8BH	−3.835**	−3.866**						
	C10BH	−2.143	−1.813	0.397					
	C12BH	−2.297	−1.965	0.534	0.060				
	C14BH	−1.807	−1.394	1.532	0.674	0.671			
	C16BH	−1.584	−1.143	1.743	0.828	0.840	0.196		
	C18BH	−1.438	−0.942	2.351	1.103	1.153	0.500	0.284	
	MCT	−3.410**	−3.231*	−0.245	−0.518	−0.647	−1.496	−1.679	−2.137
	Coconut	−3.269*	−3.095*	0.213	−0.243	−0.349	−1.221	−1.418	−1.898
ΔThickness (mm)	BBH	−1.844							
	C8BH	−6.317***	−4.607***						
	C10BH	−6.437***	−4.578***	0.474					
	C12BH	−6.420***	−4.220**	1.849	1.516				
	C14BH	−6.458***	−4.200**	2.049	1.756	0.305			
	C16BH	−5.259***	−3.326**	1.818	1.498	0.352	0.184		
	C18BH	−5.734***	−3.648**	1.987	1.682	0.435	0.234	0.000	
	MCT	−6.792***	−5.056***	−0.332	−0.854	−2.371*	−2.599*	−2.246*	−2.470*
	Coconut	−4.496***	−2.957**	1.039	0.708	−0.229	−0.350	−0.411	−0.437
ΔProtein leak (μg/mL)	BBH	2.364							
	C8BH	25.000**	9.558**						
	C10BH	10.053**	5.173**	−3.871*					
	C12BH	6.175**	3.974*	−1.562	0.400				
	C14BH	16.853***	6.391**	−9.460**	0.088	−0.394			
	C16BH	17.857***	5.917**	−21.762***	−0.795	−0.858	−2.335		
	C18BH	15.282***	4.630*	−31.891***	−2.455	−1.711	−6.847**	−11.191***	
	MCT	11.688***	6.064**	−2.922*	0.863	0.139	1.087	2.116	3.912*
	Coconut	13.443***	5.687**	−6.956*	−0.328	−0.617	−0.683	0.692	3.473*

The significance of the t-test is shown through asterisks; *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$

Conclusions

In this research, the penetration of fatty acids into hair was examined according to their chain length. And the protective effects of the hair treatments formulated with the caprylic acid, capric acid, lauric acid, myristic acid, palmitic acid, stearic acid, MCT oil, and coconut oil were examined in regard to the color, tensile strength, thickness, and the protein leak. The GC–MS analysis of fatty acid composition of hair indicated that higher concentration of caprylic acid (C8:0), capric acid (C10:0), and lauric acid (C12:0) was contained in bleached hair which was treated by the corresponding hair treatments. The UV–VIS analysis on the concentration of fatty acid in

each bleached hair strongly suggested that the fatty acid detected from the GC–MS analysis was due to the penetration of the treatment agent especially in the case of caprylic acid or capric acid. The statistical comparison between the medium-chain fatty acid group and the long-chain fatty acid group on the results of UV–VIS analysis confirmed that the penetration of medium-chain fatty acids in bleached hair was significantly different from and higher in amount than that of the long-chain fatty acids.

Oxidation by bleaching caused severe damages to the cuticle structure of bleached hair such as complete exposure of inner layers and cracked and raised cuticle structures. After applying the hair treatment containing six fatty acids, MCT oil, and coconut oil bleached hair recovered from the damage caused by bleaching. Applying these hair treatments on bleached hair provided significantly different and better protection than washing the hair alone or treating the hair with the hair treatment with no conditioning agent. The statistical comparison indicated that the protective effect of the medium-chain fatty acid group was significantly different from and better than the long-chain fatty acid group. The evidence of protective effect was shown by the retention of bleached color, increase in tensile strength and thickness, and the decrease in the amount of protein leak. Statistically, there was no significant difference between the MCT oil and the coconut oil on the protective effects. The protective effects of coconut oil was similar to both the medium-chain fatty acid and the long-chain fatty acid groups. MCT oil, however, was similar to the medium-chain fatty acid group but it was significantly difference from and better than the long-chain fatty acid group in all protective effects.

The present results strongly suggested that the fatty acids with medium chain length has better penetration ability than the fatty acids with longer chain length. And that better protection of damaged hair is possible with better oil penetration. Among the six fatty acids examined in this study capric acid (C10:0) followed by caprylic acid (C8:0) showed the highest amount of penetration into hair. And the hair treatment formulated with caprylic acid showed significantly higher decrease in protein leak than any of the hair treatments. MCT oil would provide protection comparable to those of medium-chain fatty acids as an active ingredient of hair treatment. Coconut oil can also provide the protection comparable to those of medium-chain fatty acids or MCT oil. This research was the first attempt which investigated the penetration of individual fatty acids into hair and their protective effects.

Author contributions

The data presented in this manuscript came from the doctoral dissertation of SH. SH designed the research, conducted all the experiments, collected and analyzed the data. CS conducted part of the data analysis and was the major contributor in writing the manuscript. Both authors read and approved the final manuscript.

Author information

Suhwan Kim received the Ph.D. degree from the Graduate School of Incheon National University in 2022. The design of this research, laboratory experiments, and data analysis of this research were all carried out by Suhwan Kim as part of his doctoral dissertation. Cheunsoon Ahn is a professor in the Department of Cosmetic Science and Management and also the professor in the Department of Fashion Industry of Incheon National University, Korea. She was the thesis advisor of Suhwan Kim and made major contribution in writing this manuscript.

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Availability of data and materials

The datasets used and analyzed during the current study are 1 available from the corresponding author on reasonable request.

Declarations

Competing interests
The authors declare that they have no competing interests.

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