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Effect of rinse-off hair conditioner containing argan oil or camellia oil on the recovery of hair damaged by bleaching

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Abstract

Effect of hair conditioner formulated with Argan oil or Camellia oil was investigated on the protection of hair damaged by bleaching. Six different rinse-off type hair conditioners were made with the basic ingredients of hair conditioner and one of the following conditioning agent; Argan oil (AO), Camellia oil (CO), Palmitic acid (PA), Stearic acid (SA), Oleic acid (OA), and Linoleic acid (LA). L*, a*, b* color values and tensile strength, elongation were measured, and the amount of protein leak was examined using the Bradford Protein Assay. Statistical significance was tested using the SPSS statistical software. Although both AO and CO were effective in protecting the tensile properties of bleached hair, significant effects were observed with AO in enhancing the tensile strength and retaining the color of bleached hair. This might be due possibly to the difference in the composition of four major fatty acids in Argan oil and Camellia oil.

Keywords: Rinse-off hair conditioner, Argan oil, Camellia oil, Bleaching, Tensile strength

Introduction

Hairstyling is an important way of expressing one's body image, and techniques such as permanent wave, dyeing, or bleaching are often used for this purpose. These styling techniques are chemical treatments which impart changes in the hair protein structure, and repeated practice will inevitably cause serious damages to hair. It is reported that bleaching, or hair lightening, causes oxidative damage in hair resulting in the loss of protein and an alteration of physical characteristics (Grosvenor et al., 2018). Since healthy hair is an essential element for maintaining a beautiful body it is necessary to protect the damaged hair and for this reason hair conditioning products are widely used for daily haircare at home. Hair conditioner protects hair by decreasing friction, entanglement and minimizing frizz (Dias, 2015). Such protection is generally obtained by lubricating and coating the surface of hair, making the hair more hydrophobic, and by penetrating the hair, making the hair protein more intact (Dias, 2015; Kozubal et al., 2014; Rele & Mohile, 2003).

Hair conditioners are classified into rinse-off type or leave-on type depending on whether the product is applied during the shampooing process and rinsed off or it is applied after the shampooing process to be left on the hair (Kozubal et al., 2014). Rinse-off conditioners are sold in lotion or creme form while leave-on conditioners are usually sold in toner or spray form (Kozubal et al., 2014). Due to its short remaining time on hair the effect of a rinse-off hair conditioner on the protection of damaged hair is usually less than that of a leave-on hair conditioner (Kozubal et al., 2014). However, the rinse-off conditioner is still the most common type of hair conditioner used for daily haircare since it is easy to apply during shower (Kozubal et al., 2014).

A commercial hair conditioner usually contains lubricating substances such as polymers, oils, waxes, and cationic surfactants (Dias, 2015). With a growing interest in using natural ingredients in skin and hair cosmetic products it is now easy to find that many hair conditioners also have natural oils listed on their ingredient labels. Natural oil such as plant oil is told to fill the gaps between the cuticle cells and also to some degree fill the gaps between the cortical cells by penetrating into the hair (Dias, 2015). Dias (2015) suggested that applying oil to hair regularly would enhance lubrication effect and prevent the breakage of hair. Rele and Mohile (2003) who compared the effect of mineral oil, coconut oil, and sunflower oil on damaged hair reported that the loss of protein from hair was minimized by coconut oil because it was able to penetrate the hair and fill the gap to keep hair protein intact. Mineral oil and sunflower oil did not penetrate the hair but they were adsorbed to the surface of hair, enhancing shine and reducing friction (Rele & Mohile, 2003).

Argan oil and Camellia oil are two other types of plant oils often used in the hair conditioners available in the market (Lee, 2019; Shunatona, 2020). Argan oil, native to Morocco, is produced from the kernel of Argan tree (*Argania spinosa* L.) and due to its limited production it is known to be the most expensive edible oil in the world (Dias, 2015). Park and Bae (2016) who investigated the cosmetic products in the market reported that more consumers are using Argan oil containing haircare products (43.8%) than Argan oil containing skincare (36.0%) or bodycare (17.7%) products. Despite the popular commercial usage of Argan oil in haircare products, limited research has been done to examine the actual effect of Argan oil on damaged hair (Dias, 2015; Faria et al., 2013; Lee, 2019). Camellia oil is obtained from the seeds of Camellia tree (*Camellia japonica* L.) which is native to eastern and southern Asia including Korea ("Camellia", 2021; Choi et al., 2013). It was the common hair oil used by the ancestors of Korea and it is still widely used as the plant oil ingredient for haircare products (Choi et al., 2013). Choi et al. (2013) who tested the effect of Camellia oil on hair damaged by permanent wave found that the cuticle of permed hair treated with Camellia oil showed less looseness and crack compared to the cuticle of untreated hair. There tends to be more research on Camellia oil than Argan oil regarding their application as the ingredient for hair cosmetics. Nevertheless, research on Camellia oil in the area of hair cosmetics is still fewer. Considering the popularity of using Argan oil and Camellia oil in hair cosmetics, more research is needed to verify the specific effects of these oils on the protection of damaged hair.

Both Argan oil and Camellia oil consist of unsaturated and saturated fatty acids as their major chemical composition. Fatty acid with the highest composition in Argan oil and Camellia oil is oleic acid, followed by linoleic acid, palmitic acid, and stearic acid (Charrouf & Guillaume, 2008; Chung, 2010; Gharby et al., 2011; Khallouki et al., 2003; Ku & Lee, 2018; Monfalouti et al., 2010; Yuan et al., 2013; Zeng & Endo, 2019) (Table 1). Oleic acid is a monosaturated omega-9 fatty acid which has one double bond in the 9th carbon from the methyl end of the 18 carbon chain ("Oleic acid", 2021) (Fig. 1). Linoleic acid is a polysaturated omaga-6 fatty acid which has two double bonds in the 6th and 9th carbon from the methyl end of the 18 carbon chain ("Linoleic acid", 2021). Palmitic acid is a saturated fatty acid with no double bond in the 16 carbon chain ("Palmitic acid", 2021). Stearic acid is a saturated fatty acid with no double bond in the 18 carbon chain ("Stearic acid", 2021). Among the four fatty acids, the molecules of linoleic acid and oleic acid are bulky due to their double bonds and long carbon chain while palmitic acid is least bulky due to the shorter carbon chain and no double bonds. Although the types of major fatty acids in Argan oil and Camellia oil are the same, there is a difference in the composition of each fatty acid. Camellia oil (40.3 ~ 86.6%) contains higher composition of oleic acid than Argan oil (43.0 ~ 49.0%) and Argan oil (29.0 ~ 36.0%) contains higher composition of linoleic acid than Camellia oil (1.1 ~ 33.1%) (Table 1). Examining the effect of these fatty acids could broaden the understanding of protective effect of Argan oil or Camellia oil on damaged hair.

The purpose of this research was to investigate the effect of Argan oil and Camellia oil on the protection of hair damaged by bleaching. Effect of four major fatty acids of two plant oils on the protection of bleached hair was also investigated to further the understanding of the protective effect of two natural oils. For this purpose, a crème type rinse-off hair conditioner was formulated with one of each of the following ingredient- Argan oil, Camellia oil, oleic acid, linoleic acid, palmitic acid, stearic acid. Effect of protection was investigated in terms of surface characteristics, color values, tensile property, and loss of protein.

Methods

Materials

Virgin hair tress (VH, Bona Hair, Korea) with no previous chemical treatment was used in the experiment. Hair bleaching agent (S brand, Korea) was composed of ammonium persulfate as the base forming Pack 1 and 6% hydrogen peroxide (35%) as the oxidant forming Pack 2. A commercial shampoo (L brand, Korea) was used for shampooing. Argan oil, Camellia oil, and other basic ingredients for formulating the hair conditioner were supplied from a cosmetics ingredient supplier (W company, Korea). Oleic acid, linoleic acid, palmitic acid, and stearic acid were purchased from Sigma Aldrich (USA). Ethanol (70–75%, Daejung Chemical, Korea), methanol (J. T. Baker, USA), chloroform, urea, tris base, 2-mercaptoethanol, Bovin serum albumin (all from Sigma-Aldrich, USA), thiourea (Junsei Chemical, Japan), sodium hydroxide (OCI company, Korea), Bio-Rad protein assay dye (Bio-Rad Laboratories, USA) were used in the analysis of protein loss. Water used in the experiment was distilled by Human Corporation (Korea). A Whatman® Glass Microfiber Filter (No. 1822-110, Pore Size 1.2um) was used to filter hair during the protein analysis.

Table 1 Composition of fatty acids in Argan oil and Camellia oil reported in previous literatures

Fatty acids	Argan oil (% composition)					Camellia oil (% composition)				
	Khallouki et al. (2003)	Charrouf & Guillaume (2008)	Monfalouti et al. (2010)	Gharby et al. (2011)	Range	Chung (2010)	Yuan et al. (2013)	Ku & Lee (2018)	Zeng & Endo (2019)	Range
Oleic acid (C18:1)	45.0	43–49	43.0–49.1	43.0–49.0	43.0–49.1	40.3	70.3–86.2	67.6	86.6	40.3–86.6
Linoleic acid (C18:2)	35.0	29–36	29.3–36.0	29.3–36.0	29.3–36.0	33.1	3.2–17.1	1.1	8.3	1.1–33.1
Palmitic acid (C16:0)	16.5	11–15	11.5–15.0	11.5–15.0	11.5–16.5	14.5	7.0–13.8	17.9	8.1	7.0–17.9
Stearic acid (C18:0)	3.7	4–7	4.3–7.2	4.3–7.2	3.7–7.2	12.1	1.3–5.4	8.0	1.7	1.3–12.1

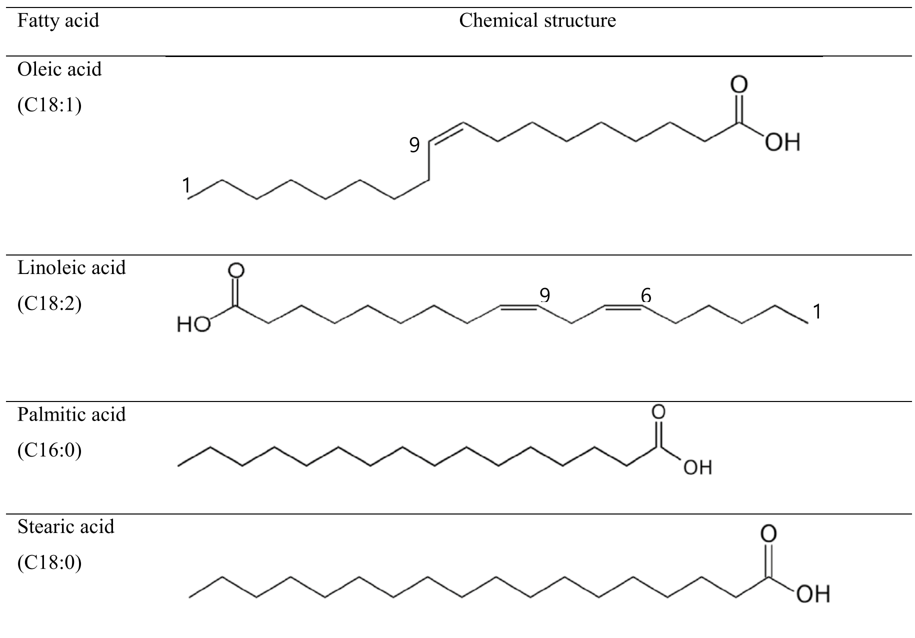


Fig. 1 Chemical structure of major fatty acids of Argan oil and Camellia oil ("Linoleic acid", 2021; Oleic acid", 2021; Palmitic acid", 2021; Stearic acid", 2021)

Sample preparation

Preparation of bleached hair (BH)

A 3 g each of Pack 1 (base) and Pack 2 (oxidant) of the bleaching agent was mixed well and the mixture was thoroughly brushed on VH. Treated hair was left at room temperature for 30 min. The hair was shampooed using a commercial shampoo and running tap water. The hair was blow-dried using a heat drier by letting the air outlet 10 cm apart from the hair and gently shaking the hair. The above process was repeated 4 times on each hair tress to prepare the bleached hair (BH). Three BH was prepared for each hair conditioner.

Preparation of hair conditioner

Six different hair conditioners- Argan oil (AO), Camellia oil (CO), Oleic acid (OA), Linoleic acid (LA), Palmitic acid (PA), and Stearic acid (SA) conditioners- were prepared following the methods of previous literature (Park & Bae, 2016; You & Kang, 2009). Hydroxyethylcellulose (1 g) was dissolved in water (172 g) at 75 ~ 80 °C. To this mixture cetearyl alcohol (10 g), glyceryl stearate (3 g), polysolbate 60 (2 g), distearyldimonium chloride (4 g), behynyl alcohol (2 g), and one of the hair conditioning agent (6 g) was added (Table 2). The mixture was dissolved at 75 ~ 80 °C, emulsified for 10 min, then cooled at 30 °C. Common but functional ingredients of commercial hair conditioner such as silicone, humectant, preservative, or fragrance were not added.

Table 2 Formulation of crème-type wash-off hair conditioners

Raw material	Content (g)	Content (%)	Function
Water	172.00	86.00	Solvent
Hydroxyethylcellulose	1.00	0.50	Viscosity increasing agent
Cetearyl alcohol	10.00	5.00	Surfactant, Emulsion stabilizer
Glyceryl stearate	3.00	1.50	Surfactant
Polysorbate 60	2.00	1.00	Surfactant
Distearyldimonium chloride	4.00	2.00	Surfactant
Behenyl alcohol	2.00	1.00	Emulsion stabilizer
(One of the following) Argan oil (AO), Camellia oil (CO), Oleic acid (OA), Linoleic acid (LA), Palmitic acid (PA), Stearic acid (SA)	6.00	3.00	Hair conditioning agent
Total	200.00	100.00	

Preparation of conditioner treated BH

Three BH were allotted for each hair conditioner treatment. For 21 days the process of 'shampooing → hair conditioner (2 g) treatment → leave at room temperature for 10 min → rinse with running tap water → blow dry' was repeated once a day. Blow dry was done using a 1000 W hair dryer for 10 min with the air nozzle of the drier approximately 10 cm away from the BH tress. During the drying process, the heat on contact with the hair tress was approximately 40 ~ 45 °C.

Analysis of hair

Surface characteristics

Surface characteristics of hair were examined using a scanning electron microscope (SEM, 7001F, JEOL, Japan).

Color

A spectrocolorimeter (Color i5, X-rite, USA) was used to measure the color of hair using the D_{65} standard light and 10° standard observer. The measured values were L* for lightness, a* for redness (+) to greenness (−), b* for yellowness (+) to blueness (−), and ΔE* for the color difference between the control and the sample following the CIELAB color system ("CIELAB color space", 2021). ΔE* was calculated using Eq. 1 based on the CIELAB system ("Color difference", 2021).

$$\Delta E* = \left\{ (L*\text{sample} - L*\text{control})^2 + (a*\text{sample} - a*\text{control})^2 + (b*\text{sample} - b*\text{control})^2 \right\}^{1/2} \tag{1}$$

Tensile strength and elongation

From each hair tress, 4 ~ 5 strands of hair were randomly collected and each strand was mounted to the hair tensile tester (MPT-320, TMI, Korea) for measurement. For each measurement, the distance between the two jaws was 60 mm, and the scanning speed was 50 mm/min.

Protein leak

Protein was extracted from hair using the Shindai method and the extracted protein was quantified using the Bradford Protein Assay (Nakamura et al., 2002). Hair was washed with ethanol (70 ~ 75%) 3 times and blow dried. Dried hair was sliced to collect 0.02 g of powdery hair. Sliced hair was added to the mixed solvent of chloroform and methanol (2:1). The mixture was left in room temperature for 24 h to extract the lipid from hair, and then dried. Buffer solution was prepared by dissolving tris-base (0.302 g), thiourea (19.8 g), and urea (30.03 g) and by adding 2-mercapto ethanol to adjust the pH to 8.5. A 5 mL buffer solution was added to the hair and the mixture was left at 50 °C for 24 h. The mixture was centrifuged for 10 min, the supernatant was collected, and Bio-Rad dye was added to this extract. The absorbance of this mixture was measured using a spectrophotometer (Lambda 25, Perkin Elmer, USA) at 595 nm (λ_{max}). Standard graph was made by the UV–Vis absorbance of the mixed solution of Bovin Serum Albumin (BSA) and Bio-Rad dye at a series of concentration levels. The regression equation obtained from the standard graph was used to quantify the protein extracted from the hair samples.

Statistical analysis

Data were analyzed using the Student t-test and the Analysis of Variance (ANOVA) test and the Duncan's Multiple Range Test utilizing the SPSS statistical software (v. 25).

Results and discussion

Effect of bleaching on virgin hair

Surface characteristics

By visual inspection, the hair bleached 4 times (BH) showed frizzed appearance with less luster and alignment compared to the virgin hair (VH). When examined using the SEM, VH showed clear and flat cuticle layers without any crack whereas the cuticle layers of BH were severely damaged showing the swollen and flipped cuticle and some showing molten or exfoliated appearance (Fig. 2).

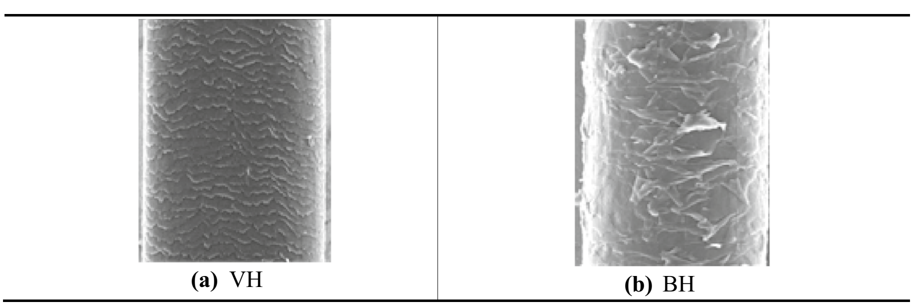


Fig. 2 SEM images of virgin hair (VH) and bleached hair (BH) (1000X)

Color

In color measurement L* value explains the lightness, where 0 represent the darkest value and 100 represent the lightest value (Table 3). Value of a* explains the greenness (−) to redness (+) with values ranging from − 128 to + 127, value of b* explains the blueness (−) to yellowness (+) with value ranging from − 128 to + 127 ("CIELAB color space", 2021). Number designate the magnitude and + or − sign designate the direction of color ("CIELAB color space", 2021). By bleaching VH turned yellow with a tint of red with mean ΔE* value of 43.70 and the significance level of $p < 0.05$ for L*, a*, and b*.

Hair has 2 types of melanin pigment, eumelanin which produces black to brown color and pheomelanin which produces yellow to red color ("Melanin in hair", n.d.). The two types of melanin pigment coexist in most hair and the color of hair is determined by the relative amount of these pigments ("Melanin in hair", n.d.). Black hair contains predominantly eumelanin whereas red hair contains predominantly pheomelanin ("Melanin in hair", n.d.). Melanin pigment exists in the cortex of hair which occupies 90% of the hair structure (Robbins, 2012). To bleach hair, the bleaching agent must pass through the cuticle layer and penetrate into the cortex so that the chemical can destroy the melanin pigment through oxidation (Wolfram et al., 1970). Cuticle of hair is made of layers of very thin, flat cells which resemble fish scales, and the scales are attached towards the root and are exposed towards the tip of hair. In a healthy hair, the scales would be flat, smooth, and closed (Schmidt, 2020). A bleaching agent is usually made of 2 packs- pack 1 containing alkali and pack 2 containing oxidizer- which are mixed together just before the application on hair. Upon application the alkali swells the hair, raising the cuticles to open up. This would allow the bleaching agent to pass through the cuticle and reach the cortex. In the cortex, the oxidizer which is mainly composed of hydrogen peroxide destroys melanin pigment by oxidation (Wolfram et al., 1970). During the bleaching process, pheomelanin is destroyed slowly since it is resistant to oxidizer whereas eumelanin is destroyed faster since is easily attacked by oxidizer (Kim et al., 2006). Therefore, when black hair is bleached the color of hair will become yellow with a tint of red color since a large amount of eumelanin is destroyed first while pheomelanin is still remaining (Kim et al., 2006).

Tensile strength and elongation

Bleaching resulted in a decrease in tensile strength (77.86%) and a slight increase in elongation (104.39%) relative to VH, but the statistical significance was not observed (Table 4). Result of the present investigation was in agreement with previous literatures which reported that hair decreased in tensile strength and increased in elongation as a result of bleaching (Kim et al., 2006; Min et al., 2011; Wolfram et al., 1970).

Bleaching negatively affects tensile strength of hair since the alkaline agent and the oxidizer destroy not only melanin pigment but also cystine amino acid which is responsible for maintaining the strength of hair through numerous disulfide crosslinks (Robbins, 2012). By oxidation, cystine is destroyed mostly into 2 molecules of cysteic acid and some to taurine or β-alanine (Wolfram et al., 1970). Wolfram et al. (1970) who analyzed the protein extracted from hair before and after bleaching reported that less cystine and

Table 3 Color values of virgin hair (VH) and bleached hair (BH) and the results of *t*-test

Sample	N	L*				a*				b*				ΔE*	
		Mean	SE	*t*	*p*	Mean	SE	*t*	*p*	Mean	SE	*t*	*p*	Mean	SE
Virgin hair	3	18.90	0.25	− 9.233	.011	2.23	0.16	− 10.740	.000	2.00	0.23	− 25.794	.000	0	0
Bleached hair	3	49.30	3.28			8.99	0.60			23.79	0.81			43.70	2.80

Table 4 Tensile strength and elongation of virgin hair (VH) and bleached hair (BH) and the results of *t* test

Sample	N	Tensile strength (MPa)					Elongation (%)				
		Mean	SE	% Change	*t*	*p*	Mean	SE	% Change	*t*	*p*
VH	3	365.03	38.22	100.00	1.009	.370	41.67	4.76	100.00	− .298	.785
BH	3	284.20	70.43	77.86			43.50	0	104.39		

[*] Number of samples for elongation measurement of bleached hair (BH) was 2

more cysteic acid was detected in bleached hair. It is highly probable that the destruction of cystine amino acid was the predominant cause of the decrease in tensile strength of bleached hair. It is probable that the slight increase in elongation was also caused by the destruction of cystine crosslink- the breakage which would enable easier slipping of the α-helix chains allowing hair to extend more. Kim et al. (2006) suggested that the decrease in tensile strength along with the increase in elongation of hair after repeated bleaching was due to the exposure of relatively softer cortex as a result of damage in the cuticle. It is likely that the destruction of cystine especially in the cuticle layer was critical for the measured tensile values.

Protein leak

To examine whether bleaching increased the leaking of protein from hair, protein was extracted from VH and BH using the Shindai method and the amount of extracted protein was determined using the Bradford Protein Assay (Nakamura et al., 2002). For the quantitative analysis of leaked protein, Bovine Serum Albumin (BSA) was prepared into 0.625 ~ 10.000 μg/mL standard solutions and Bio-Rad dye was added to each. Standard graph was prepared using the UV–Vis absorbance of the standard solutions at 595 nm and the regression equation of $y = 0.0334x + 0.238$ ($R^2 = 0.9951$) was obtained (Table 5). Amount of protein extracted from hair was calculated using the above regression equation. Result showed that the protein extracted from BH was 110.76% of the protein extracted from VH suggesting that bleaching increased the protein leaking from hair (Table 6). However, similar to the tensile values, the difference in protein leak between VH and BH was not statistically significant.

Higher amount of protein leak suggests that the cuticle layer is opened up and the cell membrane complex (CMC) which protects the hair structure by filling the pores between the cells of cuticle and the cortex is partially destroyed (Robbins, 2012). This would allow the protein of hair to be damaged by chemicals such as cosmetic substances and leak through the pore openings (Robbins, 2012). Hydrogen peroxide reacts much faster with melanin than with keratin of hair (Wolfram et al., 1970). This would account for the relatively small increase of protein leak in BH compared to VH. Wolfram et al. (1970) suggested that the reaction of hydrogen peroxide on hair keratin was mostly limited to cystine amino acid based on the fact that the amount of cysteic acid in the protein extracted from bleached hair quadrupled that of the protein extracted from untreated hair. High alkaline condition of bleaching which swells and opens up the cuticle layer would allow bleaching agent to pass through the cuticle, and as a result protein of cell membrane complex and also the matrix protein of

Table 5 Standard graph of Bovine Serum Albumin (BSA) and Bio-Rad dye

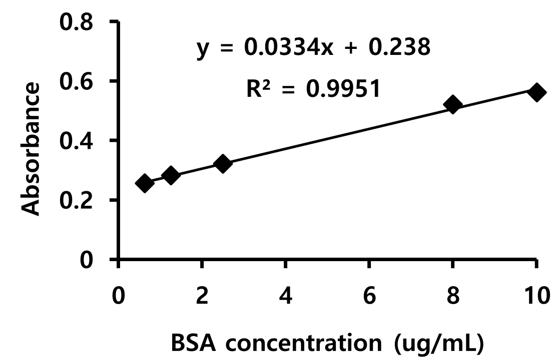
BSA (μg/mL)	Absorbance (λ_{max}: 595 nm)	Standard graph
0.625	0.2547	
1.250	0.2819	
2.500	0.3205	
8.000	0.5207	
10.000	0.5606	

Table 6 Amount of protein leak from virgin hair and bleached hair and the results of *t*-test

Sample	N	Amount of protein leak (μg/mL)				
		Mean	SE	% Change	*t*	*p*
Virgin hair	3	9.10	0.26	100.00	− 2.043	.111
Bleached hair	3	10.08	0.40	110.76		

Table 7 Composition of fatty acids in Argan oil and Camellia oil used in this study

	Argania Spinosa Kernel Oil (% Composition)	Camellia Japonica Seed Oil (% Composition)
Oleic acid (C18:1)	40.0	74.0 ~ 87.0
Linoleic acid (C18:2)	31.0	5.0 ~ 12.0
Palmitic acid (C16:0)	14.0	8.0 ~ 10.0
Stearic acid (C18:0)	3.1	1.5 ~ 3.5

[*] Data provided by the suppliers of Argan oil and Camellia oil used in this study

cortex, which are more susceptible than the α-helix protein, could be oxidized into smaller molecules which could leak by cosmetic treatments such as shampooing, permanent wave, etc. (Grosvenor et al., 2018; Rele & Mohile, 2003).

The effect of bleaching reported in this research was based on the usage of a specific brand of the bleaching agent chosen for this study. The degree of damage due to bleaching may vary by the composition of the bleaching agent and by different hair bleach product due to the difference in the alkalinity, concentration of hydrogen peroxide, or the use of different type of oxidizer. However, such difference might lead to the difference in the degrees of damage and not on the basic principle of hair damage caused by bleaching.

Effect of hair conditioner containing Argan oil or Camellia oil on bleached hair

Fatty acid compositions of Argan oil and Camellia oil

Table 7 shows the fatty acid compositions of Argan oil and Camellia oil that were used in this study to formulate the crème type rinse-off hair conditioners AO and CO. The data were provided by the supplier of Argan oil and Camellia oil based on their test results. The percent composition of oleic acid in Argan oil was 40.0% which was slightly lower than the literature values (Charrouf & Guillaume, 2008; Gharby et al., 2011; Khallouki et al., 2003; Monfalouti et al., 2010). The percent concentration of oleic acid in Camellia oil was low (5.0 ~ 12.0%) although the value was within the range of literature values (Chung, 2010; Ku & Lee, 2018; Yuan et al., 2013; Zeng & Endo, 2019). Compositions of other fatty acids in Argan oil and Camellia oil well fell into the range of literature values. Total percent concentration of oleic acid and linoleic acid, which are bulky molecules with 18 carbon chain and 1 or 2 double bonds, was 71.0% in Argan oil whereas it was higher than 87.0% in Camellia oil (oleic acid:

74.0 ~ 87.0%, linoleic acid: 5.0 ~ 12.0%). The fatty acid compositions of Argan oil and Camellia oil suggest that the molecules of Argan oil are less bulky than the molecules of Camellia oil. Therefore it is possible that the hair conditioner formulated with Argan oil may penetrate better into the hair structure than the hair conditioner formulated with Camellia oil.

Surface characteristics

To examine whether the rinse-off conditioner containing Argan oil (AO) or Camellia oil (CO) had effect on the protection of bleached hair, comparisons were made between the BH shampooed for up to 21 days without the hair conditioner treatment and the BH shampooed for up to 21 days with the hair conditioner treatment. Visual inspection showed that the BH treated with AO and CO became more lustrous and softer than the BH shampooed without the conditioning treatment. Cuticle of BH treated with the conditioner became less swollen, less flipped, and less opened up than the cuticle of BH not treated with the conditioner (Fig. 3). By 21 days of conditioning treatment, the surface of hair became smooth, cuticles were flat, tightly covering the surface of hair.

Color

After 21 days of treatment BH shampooed only and BH treated with CO slightly decreased in L* value compared to the control sample indicating that the sample slightly became darker (Table 8). However, the L* value of BH treated with AO was similar to that of the control sample. Similar phenomenon was observed with the b* value- BH

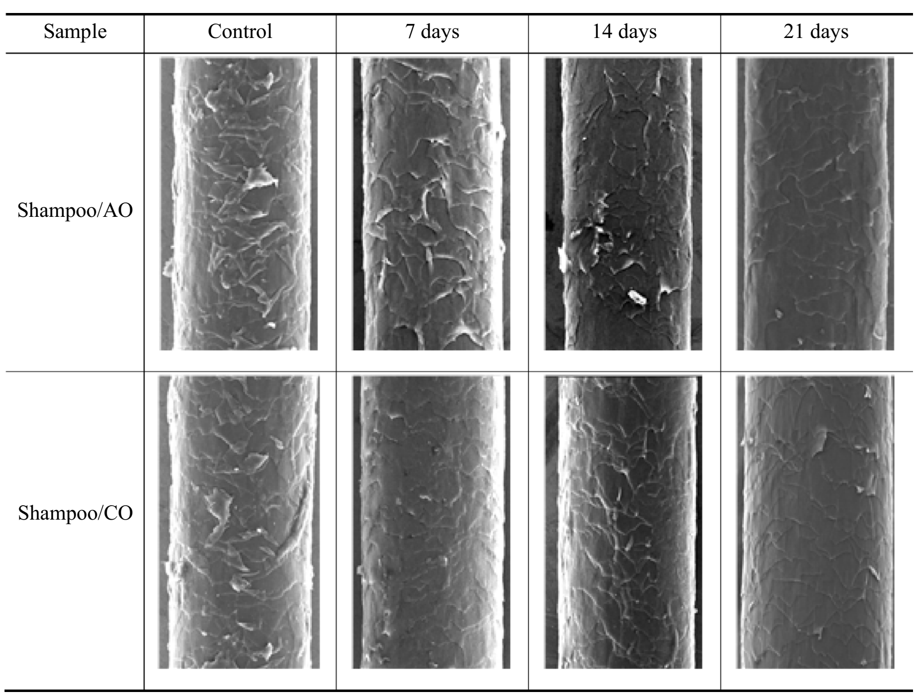


Fig. 3 SEM images of bleached hair shampooed with or without the hair conditioner containing Argan oil (AO) or Camellia oil (CO) (1000X)

Table 8 Color values of bleached hair shampooed with or without the treatment of hair conditioner containing Argan oil (AO) or Camellia oil (CO)

Sample	Treatment	N	L*		a*		b*		ΔE*	
			Mean	SE	Mean	SE	Mean	SE	Mean	SE
Shampoo only	Control	3	49.30	3.28	8.99	0.60	23.79	0.81	0	0
	21 days	3	47.28	2.72	7.95	0.50	22.34	0.91	4.87	0.49
Shampoo/AO	Control	3	45.40	1.15	9.17	0.53	22.84	1.61	0	0
	21 days	3	45.59	1.05	8.04	0.13	21.99	0.10	2.06	0.17
Shampoo/CO	Control	3	45.57	1.46	9.37	0.48	23.01	0.31	0	0
	21 days	3	43.57	2.05	7.71	0.48	20.37	0.43	4.26	0.64

Table 9 Tensile strength and elongation of bleached hair shampooed with or without the use of hair conditioner containing Argan oil (AO) or Camellia oil (CO)

Sample	Treatment	N	Tensile strength (MPa)			Elongation (%)		
			Mean	SE	% Change	Mean	SE	% Change
Shampoo only	Control	3	284.20	70.43	100.00	43.50	0	100.00
	21 days	4	211.82	40.29	74.53	30.87	9.61	70.98
Shampoo/AO	Control	3	251.63	74.78	100.00	42.00	16.04	100.00
	21 days	5	427.34	44.92	169.82	40.40	0.95	96.19
Shampoo/CO	Control	3	279.12	37.74	100.00	32.16	12.99	100.00
	21 days	5	390.52	33.63	139.91	44.00	2.17	137.50

shampooed only and BH treated with CO decreased in b* value but the b* value of BH shampooed with AO showed only minor decrease. By 21 days of shampooing ΔE* value of BH shampooed only was 4.87 and that of BH shampooed with CO was 4.26 whereas the ΔE* of BH treated with AO was 2.06. The result suggested that Argan oil was more effective than Camellia oil in retaining the color of bleached effect. It is possible that one of the reason is because the molecules of Argan oil is less bulky and therefore they can penetrate the hair more easily than Camellia oil. Oil molecules penetrating the hair could fill the gaps between the protein structure of hair and possibly minimize the loss of melanin pigment. Color retention of bleached hair during daily shampooing is important since if the color is held for longer period of days the bleaching interval would be longer and this would allow less damage to hair.

Tensile strength and elongation

Tensile strength of BH shampooed without the hair conditioner decreased gradually and after 21 days of shampooing tensile strength became 74.53% of the control (Table 9). Elongation also decreased to 70.98% of the control after 21 days of shampooing. On the other hand, BH which was treated with AO or CO showed a rather increase in tensile strength by 169.82% and 139.91% of the control. The results suggested that using hair conditioner during shampooing may recover or even enhance the tensile strength of bleached hair. By 21 days of treatment, elongation of BH treated with AO and CO were 96.19% and 137.50% of the control sample. Use of CO resulted in a significant increase in the elongation of BH whereas the elongation of BH treated with AO showed a slight decrease.

Results suggest that AO was more effective in protecting the tensile strength of bleached hair than CO. As an ingredient of hair conditioner, oil can fill the gaps between the cuticle cells, some penetrating through the cuticle cells by diffusion (Dias, 2015; Keis et al., 2005). Oil that is composed of saturated fatty acid and shorter, straight chain can diffuse into hair more easily than those made of unsaturated fatty acids with longer chain that spread out due to the presence of double bond (Keis et al., 2005). Higher protection of Argan oil on the tensile strength of hair could be due to the better penetration and diffusion of Argan oil through the cuticle cells than Camellia oil due to the less bulky fatty acid molecules. Significant increase of elongation in BH treated with Camellia oil supports the above premise since elongation is expected to increase when there are more gaps between the cuticle and the cortex cells.

Protein leak

Amount of protein leak in the BH shampooed without the conditioning treatment increased significantly by 136.50% of the control after 21 days of shampooing (Table 10). Amount of protein leak from the BH treated with AO slightly decreased by 98.16% of that of control and the amount of protein leak from the BH treated with CO slightly increased by 102.58% after 21 days of shampooing. The results implied that using AO or CO during shampooing, opposed to not using conditioner, would decrease the damage of protein in bleached hair and it is possible that AO can provide a slightly better effect than CO. This result was in agreement with the results on tensile properties which suggested the penetration of Argan oil into hair filling the gaps to keep the protein structure of hair intact.

Statistical significance

One-way Analysis of Variance (ANOVA) test was conducted to see whether there were statistical significances on the differences between the measurements of control and 21-day sample among the BH shampooed with or without the use of AO or CO (Table 11). Following the CIELAB color system ΔL^*, Δa^*, and Δb^* values were each calculated as (L^*_{sample}-$L^*_{control}$), (a^*_{sample}-$a^*_{control}$), and (b^*_{sample}-$b^*_{control}$) and the absolute values of the calculated results were used in the statistical analysis

Table 10 Amount of protein leaking from bleached hair shampooed with or without the use of hair conditioner containing Argan oil (AO) or Camellia oil (CO)

Sample	Treatment	N	Amount of protein leak (μg/mL)		
			Mean	SE	% Change
Shampoo only	Control	3	10.08	0.40	100.00
	21 days	3	13.76	0.96	136.50
Shampoo/AO	Control	3	9.26	0.31	100.00
	21 days	3	9.09	2.23	98.16
Shampoo/CO	Control	3	9.30	0.10	100.00
	21 days	3	9.54	1.66	102.58

Table 11 Analysis of Variance test on the change of physical characteristics of bleached hair after 21 days of shampoo with or without the use of Argan oil (AO) or Camellia oil (CO) conditioners

Physical characteristics		Sample	N	After 21 days			
				Mean	Duncan	F	*p*
Color	ΔL*	Shampoo only	3	4.30	b	7.385	.024*
		Shampoo/AO	3	1.44	a		
		Shampoo/CO	3	2.72	ab		
	Δa*	Shampoo only	3	1.04	a	0.667	.548
		Shampoo/AO	3	1.12	a		
		Shampoo/CO	3	1.66	a		
	Δb*	Shampoo only	3	1.55	a	2.748	.142
		Shampoo/AO	3	0.85	a		
		Shampoo/CO	3	2.63	a		
	ΔE*	Shampoo only	3	4.87	b	9.560	.014*
		Shampoo/AO	3	2.06	a		
		Shampoo/CO	3	4.26	b		
% Change relative to Control	Tenacity	Shampoo only	4	74.53	a	9.837	.004**
		Shampoo/AO	5	169.42	b		
		Shampoo/CO	5	139.91	b		
	Elongation	Shampoo only	4	70.97	a	8.038	.007**
		Shampoo/AO	5	96.19	a		
		Shampoo/CO	5	136.81	b		
	Protein leak	Shampoo only	3	136.40	a	1.320	.335
		Shampoo/AO	3	98.15	a		
		Shampoo/CO	3	102.86	a		

("Color difference", 2021). After 21 days of shampooing BH shampooed only and BH treated with AO or CO showed significant F statistics in ΔL* ($p = 0.024$) and ΔE* ($p = 0.014$) and the tenacity ($p = 0.004$) and elongation ($p = 0.007$). Duncan's Multiple Range Test revealed that for ΔL* value the hair treated with AO ((ΔL* = 1.44) was significantly different from BH shampooed only. Similar result was observed with ΔE* value where the hair treated with AO (ΔE* = 2.06) was significantly different from BH shampooed only and from BH treated with CO as well. For tenacity the BH shampooed only was significantly different from the hair treated with AO or CO. The results imply that the color of bleached hair may be better retained when treated with the hair conditioner containing AO, and the strength of bleached hair may be enhanced by using the hair conditioner containing either AO or CO, with a higher protection provided by AO.

Effect of hair conditioner containing fatty acid on bleached hair

Surface characteristics

After 21 days of shampooing, BH tresses which were treated with four types of fatty acid conditioners were less rough and frizzy and more lustrous and smooth compared to the bleached hair which was shampooed only. Cuticle layers became less swollen and less opened up, and looked smoother (Fig. 4). Based on the observation by Scanning electron microscopy, BH treated with PA showed the most recovery whereas the BH treated with OA showed the least recovery from the control sample.

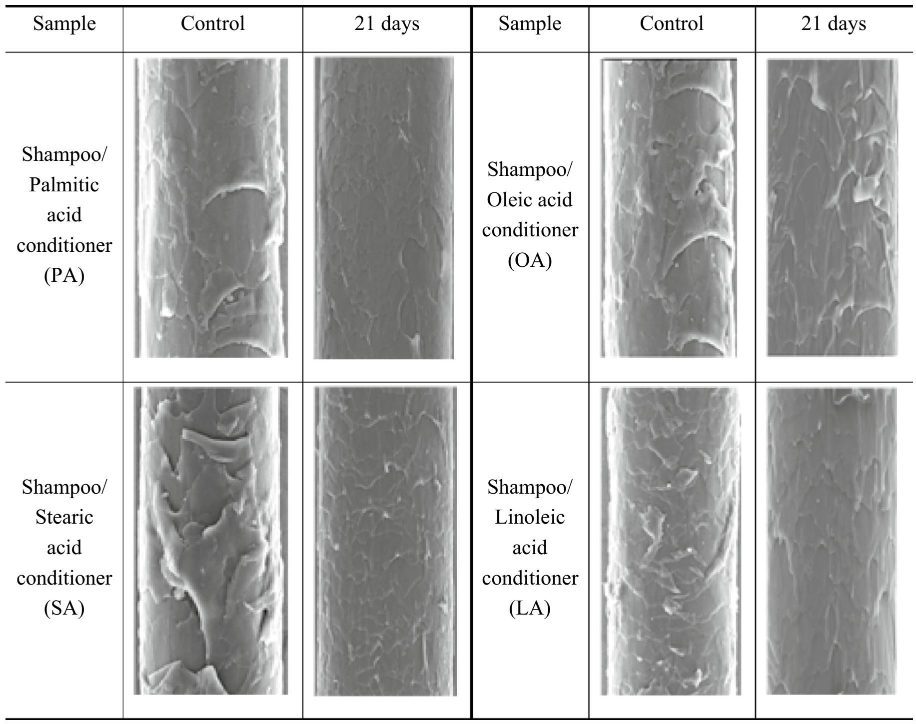


Fig. 4 SEM images of bleached hair treated with hair conditioner containing four types of fatty acids

Color

Sample which best retained the bleached color was the hair treated with PA ($\Delta E^* = 3.35$) and the highest color change was observed in the hair treated with OA ($\Delta E^* = 4.40$) (Table 12). But this difference between the lowest and the highest ΔE^* value was only by 1.05 which was in the range of ΔE^* not conceivable by human eyes (Schuessler, n.d.). T-test on the ΔE^* values between BH shampooed only and BH shampooed with each of the four fatty acid conditioners showed no significant difference while the p value of PA ($p = 0.051$) indicated a meaningful difference (Table 13).

Tensile strength and elongation

LA showed a dramatic (221.06%) increase in tensile strength after 21 days of treatment followed by PA (137.27%) and SA (133.97%) (Table 14). The lowest tensile strength was observed with the BH treated with OA (105.00%). T-test on the tensile strength value was conducted to examine whether there were statistical significances on the difference between the BH shampooed only and the BH shampooed with any of the fatty acid conditioners (Table 15). Significant differences were observed in the BH treated with PA ($p = 0.017$), SA ($p = 0.035$), and LA ($p = 0.018$) indicating that tensile strength increased significantly after 21 days of shampooing with PA, SA, and LA conditioners. One- way Analysis of Variance test indicated that the four groups were significantly different ($p = 0.017$) in their change of tensile strength values from the control sample (Table 16). Duncan post-hoc test showed that LA was different from the other 3 fatty acid conditioners indicating that the tensile strength increase was the greatest. This could be a meaningful result since Argan oil has higher composition of linoleic acid than Camellia

Table 12 Color values of bleached hair after shampoo with or without the use of hair conditioner containing four types of fatty acids

Sample	Treatment	N	L*		a*		b*		ΔE*	
			Mean	SE	Mean	SE	Mean	SE	Mean	SE
Shampoo only	Control	3	49.30	3.28	8.99	0.60	23.79	0.81	0	0
	21 days	3	47.28	2.72	7.95	0.50	22.34	0.91	4.87	0.49
Shampoo/PA	Control	3	48.25	0.57	9.19	0.23	23.22	0.49	0	0
	21 days	3	48.18	1.61	7.16	0.19	22.36	0.66	3.35	0.24
Shampoo/SA	Control	3	53.10	2.31	8.17	0.26	25.23	1.09	0	0
	21 days	3	51.32	1.89	7.26	0.75	23.38	0.35	3.55	0.30
Shampoo/OA	Control	3	49.97	0.62	8.75	0.10	23.12	0.08	0	0
	21 days	3	43.10	0.85	8.63	0.20	21.15	0.67	4.40	0.97
Shampoo/LA	Control	3	47.89	1.71	9.37	0.42	24.59	0.25	0	0
	21 days	3	47.41	2.38	8.32	0.45	22.75	0.70	3.75	1.28

Table 13 T-test comparison of color change after 21 days of treatment between BH shampooed only and BH shampooed with fatty acid conditioners

Variable		Sample	N	After 21 days			
				Mean	SE	*t*	*p*
Color	ΔE*	Shampoo only	3	4.87	0.49	2.767	.051
		Shampoo/PA	3	3.35	0.24		
		Shampoo only	3	4.87	0.49	2.280	.085
		Shampoo/SA	3	3.55	0.30		
		Shampoo only	3	4.87	0.49	.431	.689
		Shampoo/OA	3	4.40	0.97		
		Shampoo only	3	4.87	0.49	.809	.464
		Shampoo/LA	3	3.75	1.28		

Table 14 Tensile strength and elongation of bleached hair after shampoo with or without the use of hair conditioner containing four types of fatty acids

Sample	Treatment	N	Tensile strength (MPa)			Elongation (%)		
			Mean	SE	% Change	Mean	SE	% Change
Shampoo only	Control	3	284.20	70.43	100.00	43.50	0	100.00
	21 days	4	211.82	40.29	74.53	30.87	9.61	70.98
Shampoo/PA	Control	2	277.70	11.70	100.00	37.50	4.00	100.00
	21 days	5	381.20	38.97	137.27	40.30	2.96	107.46
Shampoo/SA	Control	3	308.33	149.75	100.00	39.33	3.60	100.00
	21 days	5	413.08	51.93	133.97	34.70	6.82	88.22
Shampoo/OA	Control	3	336.31	101.47	100.00	41.00	2.46	100.00
	21 days	5	353.14	33.59	105.00	39.70	3.05	96.82
Shampoo/LA	Control	3	179.72	62.45	100.00	42.83	4.26	100.00
	21 days	4	397.30	77.71	221.06	35.37	7.99	82.58

oil. The result presents plausible basis that the significant increase of tenacity in BH by AO was possibly due to the presence of higher composition of linoleic acid in Argan oil compared to Camellia oil.

Table 15 *t-test* comparison of tensile strength and elongation after 21 days of treatment between BH shampooed only and BH shampooed with fatty acid conditioners

Variable		Sample	N	After 21 days			
				Mean	SE	*t*	*p*
% Change relative to Control	Tenacity	Shampoo only	4	74.53	14.17	− 3.104	.017*
		Shampoo/PA	5	137.27	14.03		
		Shampoo only	4	74.53	14.17	− 2.607	.035*
		Shampoo/SA	5	133.97	16.84		
		Shampoo only	4	74.53	14.17	− 1.810	.113
		Shampoo/OA	5	105.00	9.98		
		Shampoo only	4	74.53	14.17	− 3.220	.018*
		Shampoo/LA	4	221.06	43.24		

Table 16 Result of Analysis of Variance test among the four types of fatty acid conditioners on the change of tensile strength and elongation after 21 days of treatment

Variable		Sample	N	Mean	ANOVA		
					Duncan	F	*p*
% Change relative to Control	Tenacity	Shampoo/PA	5	137.27	a	4.705	.017*
		Shampoo/SA	5	133.97	a		
		Shampoo/OA	5	105.00	a		
		Shampoo/LA	4	221.06	b		

Protein leak

Least amount of protein leak was observed in BH treated with OA (93.35%) followed by the hair treated with SA (95.03%), and PA (98.02%), but the differences were very small (Table 17). Considering that BH shampooed without the hair conditioner showed the leak of protein that was 136.50% of the control, hair conditioners of PA, SA, OA, and LA all seemed to show the effect of decreasing protein leak compared to not using the conditioner. However, the T-test between the BH shampooed alone and the BH shampooed with any one of the four fatty acid conditioners showed no significant results, with SA ($p = 0.070$) showing a meaningful p value (Table 18).

Effect of shampooing on bleached hair

Untreated VH and untreated BH were each compared with the 21-day shampooed sample to see the effect of shampooing on the physical characteristics of hair (Table 19). Shampooing did not cause a significant change in VH and BH in regard to color, elongation (data not shown) or tensile strength. However, significant difference ($p = 0.025$) was observed in the amount of protein leak between BH and BH shampooed for 21 days. The result implied that shampooing alone would not cause a notable damage to virgin hair but when the hair is bleached shampooing alone would cause damage to the protein structure of hair leading to an increase in protein leak. However, since the data presented here is restricted to the results obtained by the specific shampoo that was used in this research, further research using shampoos with different compositions would be necessary in the future investigation.

Table 17 Amount of protein leaking from bleached hair shampooed with or without the use of hair conditioner containing four types of fatty acids

Sample	Treatment	N	Amount of protein leak (μg/mL)		
			Mean	SE	% Change
Shampoo only	Control	3	10.08	0.40	100.00
	21 days	3	13.76	0.96	136.50
Shampoo/PA	Control	3	9.10	0.26	100.00
	21 days	3	8.92	1.55	98.02
Shampoo/SA	Control	3	10.08	0..40	100.00
	21 days	3	9.58	1.39	95.03
Shampoo/OA	Control	2	9.93	0.65	100.00
	21 days	3	9.27	1.87	93.35
Shampoo/PA	Control	3	9.30	0.10	100.00
	21 days	3	9.62	1.14	103.44

Table 18 *t-test* comparison of the amount of protein leak after 21 days of treatment between BH shampooed only and BH shampooed with fatty acid conditioners

Variable		Sample	N	After 21 days			
				Mean	SE	*t*	*p*
% Change relative to Control	Protein leak	Shampoo only Shampoo/PA	3 3	136.40 98.04	9.57 17.05	1.962	.121
		Shampoo only Shampoo/SA	3 3	136.40 94.98	9.57 13.84	2.461	.070
		Shampoo only Shampoo/OA	3 3	136.40 93.33	9.57 18.87	2.036	.112
		Shampoo only Shampoo/LA	3 3	136.40 103.41	9.57 12.28	2.118	.102

Table 19 Effect of shampooing on virgin hair and bleached hair

Characteristics	Sample	N	Mean	SE	*t*	*p*
Tenacity (MPa)	VH	3	365.03	38.22	0.677	.535
	VH shampoo only 21 days	3	335.40	21.55		
	BH	3	284.20	70.43	0.955	.383
	BH shampoo only 21 days	4	211.82	40.29		
Protein leak (μg/mL)	VH	3	9.10	0.26	− 1.266	.274
	VH shampoo only 21 days	3	10.24	0.85		
	BH	3	10.08	0.40	− 3.512	.025*
	BH shampoo only 21 days	3	13.76	0.96		

Virgin hair vs. bleached hair treated with hair conditioner

Physical characteristics of BH treated with hair conditioners were compared with those of VH to examine how bleached hair shampooed with or without the use of conditioners was different from virgin hair (Table 20). Comparisons were made only on tensile strength and protein leak. A significant difference was observed between the 21-day

Table 20 Comparison of physical characteristics between virgin hair (VH) and bleached hair (BH) shampooed with or without the use of Argan oil (AO) or Camellia oil (CO) conditioner

Characteristics	Sample	N	Mean	SE	*t*	*p*
Tenacity (MPa)	VH	3	365.03	38.22	2.668	.044*
	BH shampoo only 21 days	4	211.82	40.29		
	VH	3	365.03	38.22	− 0.943	.382
	BH shampoo/AO 21 days	5	427.34	44.92		
	Virgin hair	3	365.03	38.22	− 0.482	.647
	BH shampoo/CO 21 days	5	390.52	33.63		
Protein leak (µg/mL)	VH	3	9.10	0.26	− 4.649	.010*
	BH shampoo only 21 days	3	13.76	0.96		
	VH	3	9.10	0.26	0.005	.996
	BH shampoo/AO 21 days	3	9.09	2.23		
	VH	3	9.10	0.26	− 0.263	.805
	BH shampoo/CO 21 days	3	9.54	1.66		

shampooed bleached hair and the virgin hair in tensile strength ($p=0.044$) and protein leak ($p=0.010$). This result was interesting especially since no significant differences were found between VH and BH in tensile strength ($p=0.370$) and protein leak ($p=0.111$) when BH was not shampooed (Tables 5 and 7). The result suggests that when bleached hair is subject to daily shampooing, more damage would occur and the deviation from the original physical characteristics of virgin hair would be intensified to a notable degree. T-tests between VH and the BH treated with AO or VH treated with CO indicated no significant differences in the tensile strength and protein leak. Although statistical significances were not observed, the tensile strength of BH treated with AO or CO was even higher than that of virgin hair. The results suggest that when bleached hair is shampooed daily without the use of hair conditioner, a significant damage would occur. But if shampooing is accompanied by conditioning with Argan oil or Camellia oil conditioner hair could recover from damage and would be able to enhance the strength to the level before bleaching the hair.

Conclusions

This research was aimed to investigate the effect of using crème type rinse-off hair conditioner formulated with Argan oil and Camellia oil on the hair damaged by bleaching. Experiment was also carried out to examine the individual effect of four fatty acids comprising Argan oil and Camellia oil by formulating the rinse-off hair conditioners using the fatty acids and treating them on bleached hair.

Bleaching turned the color of black hair yellow with a tint of red color, and resulted in a severe damage of cuticle layer, decrease in tensile strength, and an increase in protein leak. The crème type rinse-off hair conditioners formulated with Argan oil or Camellia oil were effective for protecting the hair damaged by bleaching. In bleached hair, shampooing alone caused serious decrease in tensile strength and also caused serious leaking of protein. However, the results showed that by using the hair conditioner containing either Argan oil or Camellia oil during shampooing the bleached hair recovered from damage, enhanced in the tensile strength, and decreased in the protein leak up to the level before bleaching.

While the protection was observed by both oil types, the hair conditioner containing Argan oil exhibited better retention of bleached color, higher increase in tensile strength, and a slightly better protection from protein leak compared to the hair conditioner containing Camellia oil. Better retention of bleached color by Argan oil conditioner is especially an important factor in the realm of hair styling. Longer retention of bleached color would allow fewer number of bleaching and this would present a considerable benefit both physically and economically for those people who prefer to maintain the bleaching hair style. Although many factors would contribute to the higher protection provided by Argan oil conditioner over Camellia oil conditioner, one of the reason could be due to the difference in the fatty acid compositions of the two oil types.

When the effects of individual fatty acids were examined the hair conditioner formulated with palmitic acid seemed to show the best effect in recovering the surface characteristics of hair and the best retention of bleached color. On the other hand, the hair conditioner formulated with oleic acid showed the least recovery of surface characteristics and the least retention of bleached color. The hair conditioner formulated with linoleic acid exhibited a dramatic increase in tensile strength followed by the hair conditioner formulated with palmitic acid and stearic acid. The results on the fatty acid conditioners explained the protective effect of each fatty acid in the concentration level utilized in this experiment. For full understanding of the principles behind the current findings, a research which focus on the physical and chemical mechanism of the protection by fatty acids seems necessary in the future. At this point, it is only possible to conjecture that the higher concentration of linoleic acid and also higher concentration of palmitic acid in the Argan oil compared to the Camellia oil used in this study might have contributed to the higher protection provided by the Argan oil conditioner.

If daily shampooing of bleached hair does not accompany the use of a hair conditioner, more damage would occur and the physical characteristics of hair would severely deviate from that of the virgin hair. Since hair conditioner is applied not only on the hair but simultaneously on the scalp, future research seems necessary on the effect of the two oil types on the scalp during the conditioning treatment. It would be also necessary to examine whether the effect of conditioner containing Argan oil and Camellia oil would be warranted regardless of the type and composition of the shampoo used. This research provided empirical results which could verify the use of Argan oil or Camellia oil as an effective ingredient for the crème type rinse-off hair conditioner.

Authors' contributions

The data presented in this manuscript is part of the Master's thesis of SH. The research design, laboratory experiments, and data analysis of this research were conducted while she was a Master's student in the Department of Cosmetic Science and Management, Graduate School, Incheon National University, Korea. SH designed the research, conducted all the experiments, collected and analyzed the data. CS was the thesis advisor of SH, and together they contributed in the research design and manuscript preparation. CS conducted data analyses, and was a major contributor in writing the manuscript. All authors read and approved the final manuscript.

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Availability of data and material
The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

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

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