Method to Determine Plant Oil Compositions for Delivering Optimum Hair Benefits

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Abstract (Maximum of 200 words)

The use of plant oils in hair care is growing and the number of oils available is extensive. We developed a method to understand the detailed oil composition including diglycerides, triglycerides and unsaponifiables. The compositions are complex with typically more than 30 individual materials and are quite different between the oils. The aim of this work was to use these data to correlate oil compositions with oil physical properties and hair care benefits. Included in this study were four triglyceride oils (coconut, camellia oleifera, castor, rice bran and safflower seed) and one butter (shea).

Keywords: Hair Penetration, triglyceride oils, fatigue strength

Introduction.

Civilizations for centuries have used plant oils to enhance their beauty. In ancient East Asia seeds from the *Camellia oleifera* plant were crushed and pressed to release an oil that was a treasured commodity, especially in China. In Japan Geishas and their apprentices applied the Camellia seed oil to maintain their skin's perfection. Ancient Greeks and Egyptians both used olive oil for moisturizing skin and hair and in India the 5000-year-old science of Ayurveda used coconut oil in the ritual of hair oiling. All were harnessing the regenerative power of natural oils for hair, scalp and skin moisture and nourishment. Today we use these same oils across the Beauty industry and in 2022, a quarter of skin care launches and a half of hair care launches in North America contained at least one plant oil or butter[1]

Plant oils and butters are typically glycerol esters of fatty acids called triglycerides and most are low in saturated fats and high in saturated monounsaturated and polyunsaturated fats, i.e., each fatty acid chain has one or two double bonds. The nomenclature used counts the fatty acid chain length number of carbons and the number of double bonds. For example, C12:0 is a fatty acid with 12 carbons and no double bonds and C18:1 is a fatty acid with 18 carbons and one double bond. The ω nomenclature notes the double bond position from the last carbon in the fatty acid chain. These oils and butters are typically found in plant nuts or seeds and their purpose is to provide energy for the nut or seed to grow[2]. Each plant oil has a unique composition of different triglycerides with varying fatty acid chain lengths and degrees of saturation. Saturated fatty acids provide more energy to the growing plant than unsaturated fatty acids[3]. Due to this energy difference, depending on the location in which the plant is grown, there will be variability in the types of triglycerides present in the oils[4]. The triglycerides make up more than 95% of the oil composition but there may be a low level of di- and mono-glycerides (0-5%) as well as a low level of fatty acids (typically less than 1%) from triglyceride breakdown.

The exact composition of the mixture is important as it can impact its physiochemical properties and thus the skin or hair benefit properties including penetration. In this work we wanted to compare detailed composition of oils with their properties in order to make the right material choice while formulating for a hair care product. In order to do the analysis an efficient method was identified to identify and semi-quantify individual triglycerides using LC-MS/MS.

Materials & Methods

Hair Preparation

4 g, 20 cm Caucasian light brown untreated hair (i.e., no chemical treatment) and bleached hair were purchased from International Hair Importers & Products Inc. (Glendale, NY). For the bleached hair medium brown hair was submerged in a solution of 30% Hydrogen Peroxide (34% concentrated) and 14% ammonium hydroxide (6% concentrated) for four hours.

Materials

Propane-1,2,3-triyl tri(octadecenoate) (tristearin) and propane-1,2,3-triyl tri(docanoate) (trilaurin) were supplied by TCI Chemical (Tokyo, Japan). Propane-1,2,3-triyl tris(octanoate) (tricaprylin) was supplied by Sigma Aldrich (St. Louis, MO, USA), and propane-1,2,3-triyl tri((9Z)-octadec-9-enoate) (triolein) was supplied by MP Biomedicals (Irvine, CA, USA). Oils were sourced from various suppliers. Measurements were made with fresh oils to minimize oil oxidation.

Hair Treatment

Pure TAGs were heated to 75°C to ensure all TAGs were liquid and added to hair (1 g oil to 1 g hair), wrapped in foil, and left in oven at 75°C for 3 hours. Excess oil was removed with a paper towel and the hair was washed 3 times in hexane for 30 secs. Plant oils were heated to 40°C and added to hair (1 g oil to 1 g hair) and left in oven for 24 hrs at 40°C. Excess oil was removed with a paper towel and then the hair was washed 3 times in hexane for 30 secs. The leave-on treatment containing 2% of the oils was added at a dose of 0.05g/g hair to hair washed with a clarifying shampoo and then dried. The friction was measured by a sled friction method where a weight was drawn over the hair tress and the force measured by an instron. Five repeats were completed for each tress and three tresses were measured per treatment.

LC-MS Analysis of Plant Oils

The studied oils were previously homogenized in their own packings (cans or plastic bottles) before sampling for LC-MS analysis. Oil aliquots of 5 mg were precisely weighted and diluted with isopropanol (IPA, Fisher Chemical A461212) at a concentration of 1 mg/ml. Vortex mixer was used for 30 sec. to produce a clear sample solution. Blank IPA was used as a background control. These solutions were stored in a -8°C fridge before testing.

An LC-MS method with reversed-phase chromatography conditions (using three solvents as mobile phase) was developed for detection of a wide range of TAGs and DAGs in the plant oils. LC-ESI-MS/MS analyses were performed on an UltiMate 3000 HPLC system (Thermo Scientific, USA) coupled to a photodiode array (PDA) detector and a Thermo Velos Pro Ion Trap (LC-MSn) mass spectrometer (Thermo Scientific, USA). An aliquot (5 µl) of each sample was injected directly onto a Phenomenex Luna C18(2) column (150 × 3.0 mm, 5 µm; Macclesfield, Cheshire, UK). Mobile phase of the analytical HPLC: A = MeOH; B = IPA; C = 10 mM NH_4HCO_2 in H_2O; Gradient program: 65–70% B (0–10 min), 70–90% B (10–20 min), 90% B (20–35 min), 90–65% B (35–40 min), solvent C was a constant 10% throughout the run. Column temperature was 50°C, and the tray temperature was kept at 15°C. All LC-MS grade solvents were purchased from Fisher Scientific UK Ltd. The buffer of 10 mM ammonium formate (in water) was prepared with chemical from Sigma (70221-25G-F).

Mass spectrometry detection was performed in both positive and negative ionization modes using the full scan and data dependent MS2 and MS3 acquisition methods. Total Ion Current (TIC) chromatograms were obtained over the range of 125-2000 m/z using a spray voltage of +3.0 kV and -2.5 kV for the positive and negative ionization modes, respectively. Additional parameters for the mass spectrometer include capillary temperature, 300°C; heater temperature, 300°C; sheath gas flow rate, 60; auxiliary gas flow rate, 20; automatic gain control (AGC) target, 3.0e4

(Full scan) and 1.0e4 (MSn); normalized collision energy for MSn, 35 eV; minimal signal required, 500 and isolation width, 4. nitrogen was used as the drying, nebulizer and fragmentation gas.

Triglyceride and Oil Penetration

For each sample ~0.1 g of hair was cut in 20-40 mm segments into vials (n=4). First the hair was extracted gently with hexane to remove the external oil The hexane extraction consists of extracting the hair with hexane two times (first with 10 ml then with 5 ml) with vortexing for 5 minutes each then discarding. Next the internal oil was extracted once using 6 ml of 2:1 mixture of chloroform:methanol with 10 mM dimethylhexylamine (DMHA) in the chlroforom and 1.0% formic acid in the methanol then twice with 8 ml of 1:1 chloroform:methanol also with 10 mM DMHA and 1.0% formic acid. Each extraction was heated for 30 mins at 65°C with the hair and then combined and the dried residue re-dissolved in 2 ml of the 2:1 chloroform:methanol. An aliquot of this was then analyzed by GC. The triglycerides were analyzed directly without hydrolysis or derivatization. Separation and quantitification of oil was measured by gas chromatography (GC) with flame ionization detection using a polydimethylsiloxane capillary column (2.5 m x 0.250 mm x 0.25 um) with hydrogen mobile phase at a 1.5 ml/ml flow rate with a 1 ul injection. The GC & FID was an Agilent 8890 type with the injector and detector at 350°C. The column oven was initially held at 150°C then ramped at 20°C/min to 210°C then at 35°C/min to 350°C and help for 7 min. The injection was a split type with a split ratio of 50:1. Tridecanoin was used as an internal standard with a calibration curve for the triglycerides with a linear fit. For the more complex oils coconut oil was used to calibrate for the coconut oil and triolein for the other oils with all the triglyceride peaks quantitated. Blank hair within a given experiment was analyzed and subtracted as background to determine only what additional triglyceride penetrated the hair.

Fatigue Measurements

Fibers were cut for fatigue strength measurements from the center of hair tress (60mm) and washed with hexane (rinsed in 50ml hexane for 30 secs). The control was also washed with hexane. It was then crimped at 30 mm using a Dia-stron Auto-Assembly System (AAS 1600) (Andover, Hampshire, UK). The average cross-sectional area along each fiber, was analyzed using a Dia-Stron Fiber Dimensional Analysis System (FDAS 770), which incorporates a Mitutoyo laser micrometer (LSM-6200) (Malborough, MA, USA). The average cross-sectional area was calculated from three diameter measurement points along each 30mm crimped fiber. The average cross-sectional values for each of the fibers were then used to set the Dia-Stron Cyclic Tester (CYC801) in controlled stress mode. Stress used was 140MPa with a speed 40mm/s. Data were analyzed by Weibull statistical tools (JMP Pro 12.1.0, SAS Cary, NC). For each data set, fit with the Weibull Distribution was confirmed. Fibers with break cycles less than ten

were omitted from the analysis due to premature breakage. In most cases this was between 2-4 fibers. 100 fibers per sample were measured. All measurements were made at a relative humidity of 50% and temperature of 23°C and fibers equilibrated for 24 hrs minimum before testing.

Results & Discussion

Table I show the oils investigated for this study and their measured physical properties. Shea and coconut are both solid at room temperature but the remaining oils are liquid. Rice bran, camellia and safflower have similar viscosities but castor oil has a higher viscosity.

Oil	Solid/Liquid	Melting Point (˚C)	Viscosity at shear rate 10-1sec (Pa.s)
Coconut	Solid	24	-
Shea	Solid	~40	-
Castor	Liquid	-	0.770
Rice Bran	Liquid	-	0.085
Camellia Oleifera	Liquid	-	0.083
Safflower Seed	Liquid	-	0.086

Table I – Oils used for this study

The oil and butter compositions were measured using an LC-MS/MS technique to determine the detailed di- and triglyceride components. The methodology identified involved separation of each individual triglyceride and then fragmentation into free fatty acids. See Figure 1 which shows the fragmentation pattern for 1-palmitoyl-2-oleoyl-3-linoleoyl-glycerol as an example of the process of triglyceride identification.

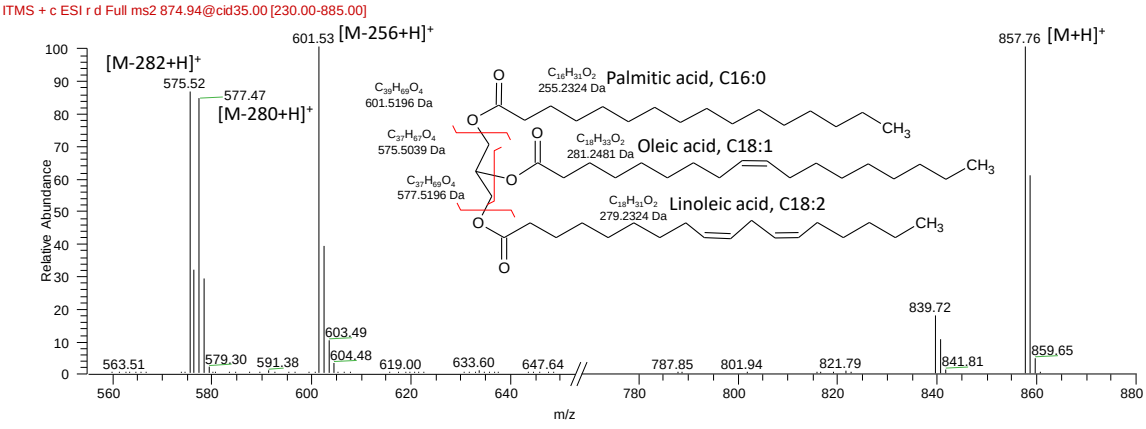
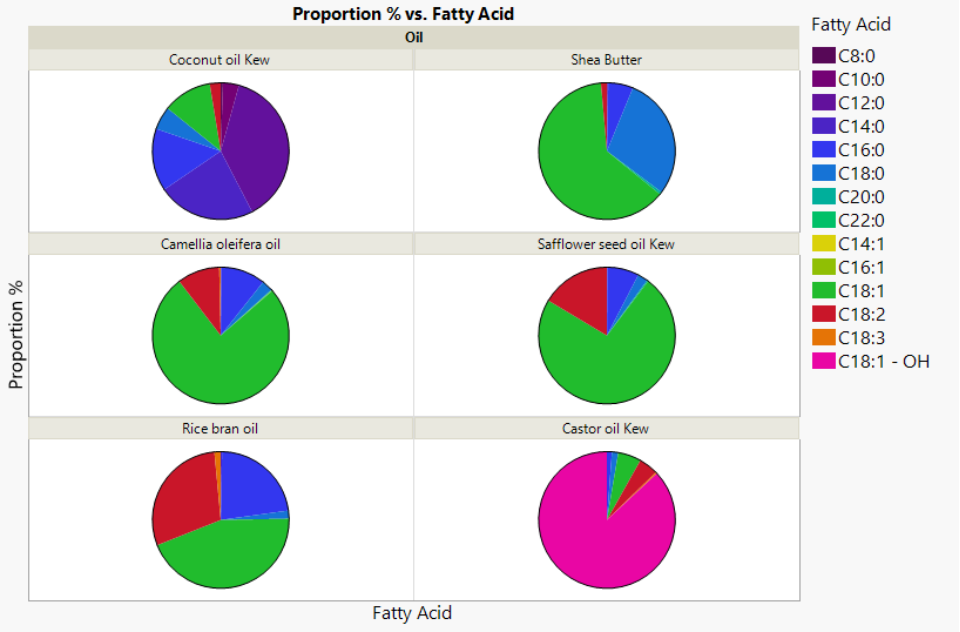


Figure 1. MS2 spectra of the positive ion 874.94 $[M+NH4]^+$ of POL (1-palmitoyl-2-oleoyl-3-linoleoyl-glycerol, $C_{55}H_{100}O_6$, 856.75199 Da) demonstrates the fragmentation pattern used for identification of TAG

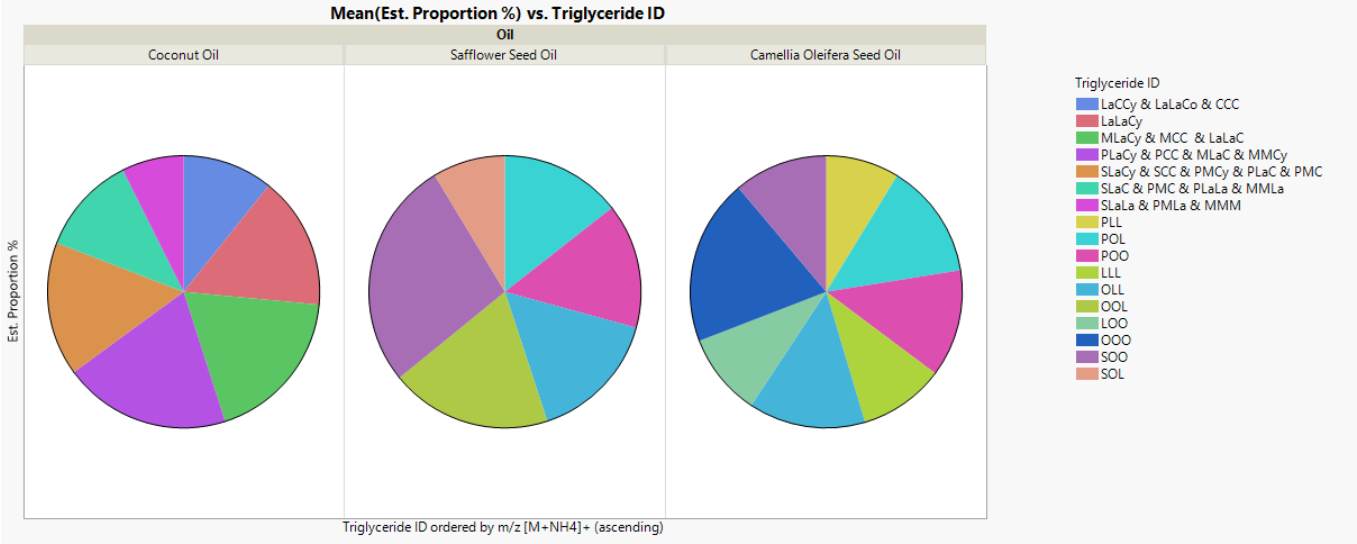
The complete triglyceride compositions of all oils were measured and more than 60 individual triglycerides were measured. Shown in Figure 2 are fatty acid levels for oils and shea butter showing the diversity of compositions of these oils. The free fatty acid plot shows *Camellia oleifera* oil and safflower seed oil have very similar fatty acid compositions. Coconut has mainly short chain saturated fatty acids that are responsible for the solid nature of coconut oil; saturated triglycerides have higher melting points than unsaturated ones due to superior packing between fatty acid chains. Shea butter also has a relatively high level of saturated fatty acids (C18:0 and C16:0) which have higher melting points. The camellia and safflower oils have very similar fatty acid compositions with predominantly C18:0 fatty acids and rice bran oil is a blend of C18:1 and C18:2 triglycerides. Castor oil is an outlier as it has high levels of ricinoleic acid which is oleic acid with a hydroxy group in the C12 position. This hydroxy group can form hydrogen bonds between the triglycerides caused the higher viscosity measured for castor oil.



Key for Fatty Acids
Cy = caprylic acid (C8:0)
C = capric acid (10:0)
La = Lauric acid (C12:0)
M = myristic acid (14:0)
P = Palmitic Acid (C16:0)
S = Stearic Acid (C18:0)
O = Oleic Acid (C18:1)
L = Linoleic Acid (C18:2)
A = Arachidic Acid (20:0)
B = Behenic Acid (22:0)
Rn = Ricinoleic Acid (C18:1-OH)

Figure 2 – Fatty acid composition of triglyceride oils

The full triglyceride compositions (Figure 3) show the added complexity of these oils when the full composition is considered. For simplicity only the triglycerides above 5% are shown but over 50 triglycerides were identified. Also not shown here are lower levels of diglycerides. The other major materials found exclusively in shea butter were the unsaponifiables (Lupeol cinnamate, Butyrospermol cinnamate and α-Amyrin cinnamate) which contributed ~5% to the total shea butter composition.



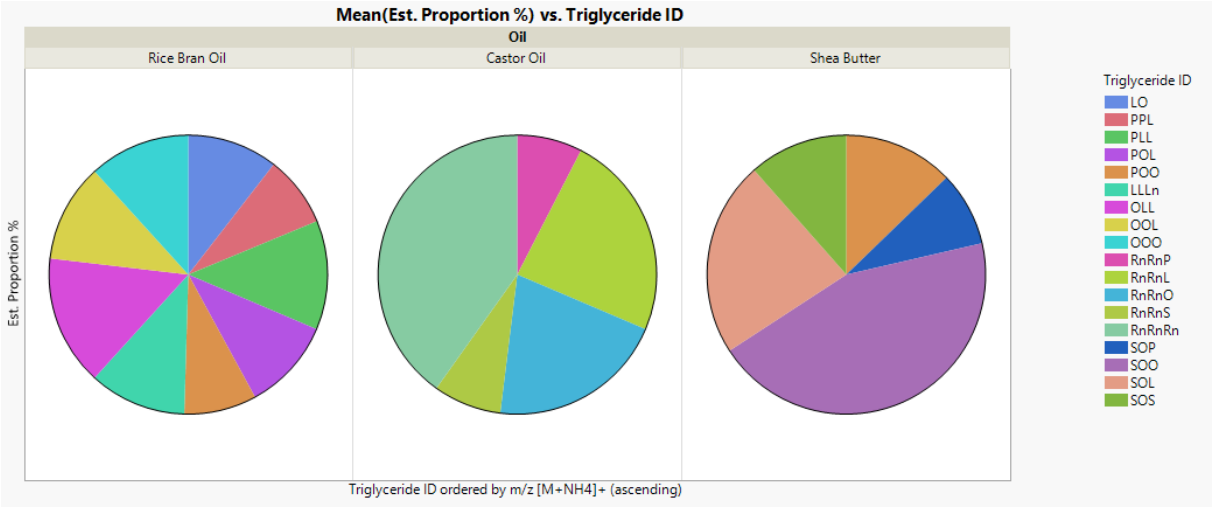


Figure 3 – Triglyceride composition of oils

There is a correlation between viscosity and oil composition indicating the importance of both chain length and degree of unsaturated to the oil physiochemical properties. Higher chain lengths and lower saturation increases viscosity. For oils with predominantly C18:1 and C18:2 fatty acids there were subtle differences in viscosity that can be correlated with triglyceride chain conformation.

The triglyceride composition impacts the oil performance including conditioning performance and penetration into hair. Figure 4 shows conditioning performance of oils when added to a leave on treatment at 2% levels. The measurement is a sled friction method where the force to move an object sliding over hair is measured with an instron. The lowest friction is coconut oil which although a solid at room temperature is likely fluid at the surface interface and thus has the lowest viscosity of all the oils due its shorter chain length triglycerides. The highest friction

is with the solid shea butter and higher viscosity castor oil. Viscosity is a key contributor to friction, especially in the hydrodynamic range of the Stribeck curve which appears to correlate with our friction data.

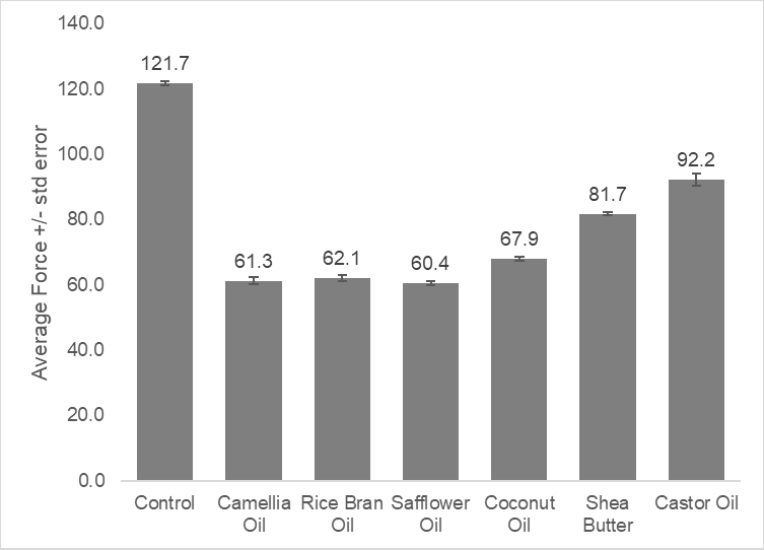


Figure 4 – Friction for hair treated with treatment & oil at 2%

The triglyceride composition also impacts penetration into hair. Figure 5 shows penetration of individual triglycerides into virgin untreated hair and Figure 6 shows penetration of selected triglycerides into the same hair. As expected, penetrate than saturated ones. Even though safflower seed and *Camellia oleifera* oils have different triglyceride compositions the penetration is very similar correlating with the fact that the overall chain length is very similar.

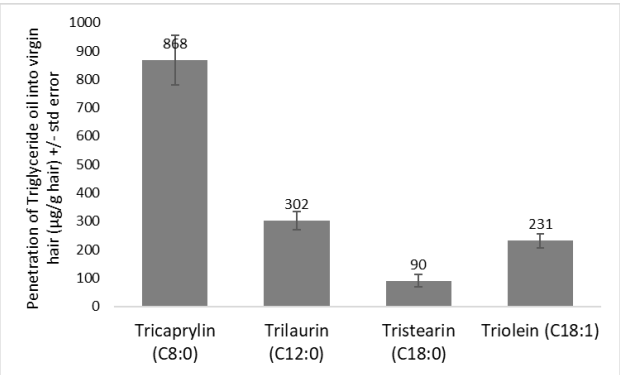


Figure 5 – Triglyceride penetration into hair

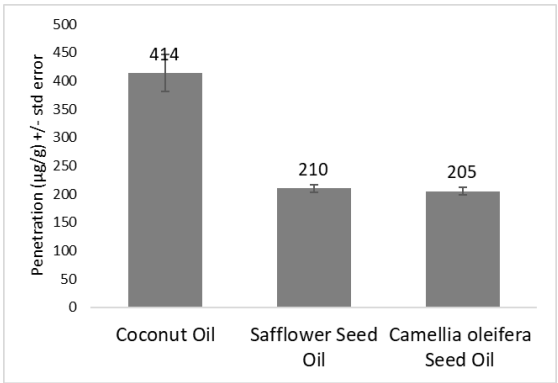


Figure 6– Oil penetration into hair

One benefit of penetrated oil is the increase in fatigue strength of the single fibers. Previous work has shown the triglycerides penetrate into the lipid-rich cell membrane complex (CMC) of hair[5] and can 'rebuild' the CMC. Table II shows that all the oils tested give a fatigue strength benefit.

Oil Details	α-value (number of cycles for 63% of fibers to break
Control	3620
Coconut oil	7011*
Camellia oleifera oil	5914*
Safflower seed oil	6465*

*Significant to >99% significance vs control hair. No significant differences between oils (N = 100 fibers)

Table II – Fatigue data for bleached hair treated with plant oils

Conclusion

A method was developed to measure individual triglyceride compositions of several oils and butters. These data showed the complexity of oils that gave more detailed information that measuring free fatty acid concentrations. The compositions can be correlated with the physical properties of the oils and their performance to deliver conditioning and strength benefits.

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Conflict of Interest

None of the authors have any conflict of interest.

R**eferences**

[1] GNPD search results for 2022, NA. Skin categories included were Face Cleansers and Face/Neck Care. Hair categories included were conditioners, shampoos, and hair styling.

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