

Hair treatment products containing pigeon pea oil as chemical properties and anti-oxidant activity

Warongporn Rattanabun¹, Wannisa Keawbankrud^{1,*}, Supranee Pimpila¹, Kulaporn Roekudomsak¹, Chokchai Phocharoen¹, Petch Suthiporn², Surawit Nantakaratt³

¹Health Science and Aesthetic Program, Faculty of Science and Technology, Rajamangala University of Technology Krungthep, Bangkok, 10120 Thailand

²Division of Biology, Faculty of Science and Technology, Rajamangala University of Technology Krungthep, Bangkok, 10120 Thailand

³Division of Printing Technology, Faculty of Science and Technology, Rajamangala University of Technology Krungthep, Bangkok, 10120 Thailand

*Corresponding Author: Wannisa.k@mail.rmutk.ac.th

DOI: 10.55674/cs.v16i3.256828

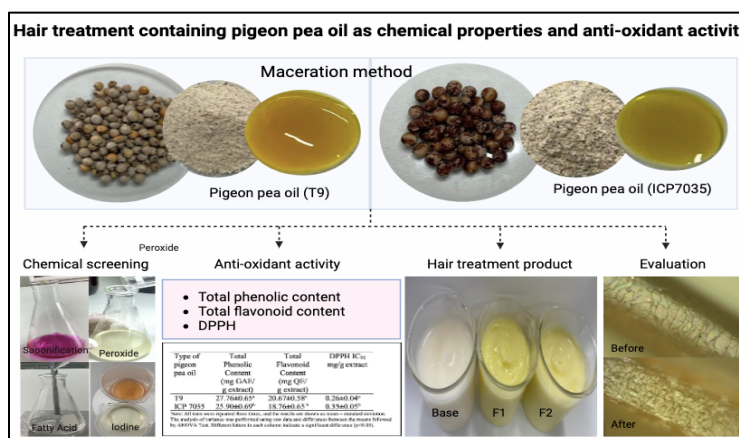
Received: 21 May 2024; Revised: 14 June 2024; Accepted: 4 July 2024; Available online: 1 September 2024

Abstract

The comparing small type (T9) and big type (ICP 7035), which were extracted by hexane maceration, this study looks at the properties of pigeon pea oil in hair treatment products. It finds that the percentage yield of big seed (ICP 7035) oil is higher than that of small seed (T9) oil, at 9.64 ± 0.10 and 9.32 ± 0.08 , respectively. A study on the chemical properties of the oil revealed values for saponification, acidity, iodine, and peroxide. In all trials, T9 outperformed ICP 7035 in terms of test results,

demonstrating the presence of fatty acids in the molecular components of triglycerides. Compared to ICP 7035, T9 had a lower molecule weight and a lower saturation level, making it less susceptible to oxidative lipid disorder and the tendency to deplete oil. The flavonoid content of T9 is higher than that of ICP 7035, equal to 27.76 ± 0.65 mg GAE g⁻¹ extract and 25.90 ± 0.69 mg GAE g⁻¹ extract, respectively. The flavonoid content of T9 is higher than that of ICP 7035, equal to 20.67 ± 0.58 mg QE g⁻¹ extract and 18.76 ± 0.65 mg QE g⁻¹ extract, respectively. The DPPH test showed that T9 was a stronger antioxidant than ICP 7035, with amounts of 0.26 ± 0.04 mg g⁻¹ of extract and 0.35 ± 0.05 mg g⁻¹, respectively. The development of hair care products revealed that a mixture containing the optimal dosage of T9 effectively ensured smooth hair lines and prevented microorganism contamination in all recipes.

Keyword: Pigeon pea oil; Chemical properties; Anti-oxidant; Hair care products



1. Introduction

Pigeon pea seeds have been found to possess antioxidant properties, which can be beneficial in various applications, including hair care. The seeds contain a high amount of polyphenols, which are known to exhibit antioxidant activities. These antioxidants can help protect the hair from damage caused by free radicals, leading to healthier and more resilient [1]. Additionally, the antioxidant properties of pigeon pea seeds can also help to reduce oxidative stress and inflammation in the scalp, which can contribute to hair loss and other scalp issues. This makes pigeon pea seeds a promising natural ingredient for hair care products, particularly those aimed at promoting hair health and reducing oxidative damage [2]. Pigeon pea (*Cajanus cajan* L.) is a widely cultivated legume crop that plays a significant role in the diets of people in many parts of the world, particularly in Asia and Africa [3]. The seeds of pigeon pea are a rich source of protein, carbohydrates, and various essential vitamins and mineral. Pigeon pea seeds are commonly consumed in various forms, such as whole, split, or processed into flour for making breads, porridges, and other food products [4]. There are several types of pigeon pea seeds that differ in their size, color, and other characteristics. The most common types include the small, round, and white-colored seeds, the large, flat, and brown-colored seeds, and the speckled or mottled seeds. These different types of seeds may have varying nutritional compositions and culinary properties. Pigeon pea seeds are a versatile and valuable crop, providing a rich source of nutrients and various industrial applications. The seeds are rich in protein, fiber, and minerals, making them a staple food in many parts of the world [5]. In addition to their nutritional value, pigeon pea seeds have been used in traditional medicine and as a natural dye for textiles [6]. The seeds also have cosmetic applications, with their oil being used in skincare products due to its moisturizing and anti-inflammatory properties [7]. This diversity of uses highlights the importance of pigeon pea seeds in both human consumption and industrial applications.

2. Materials and Methods

Materials

The samples of *Cajanus cajan* L. from Tak province, Thailand. It shown in Fig. 1. The chemicals consisted of Ethanol (Absolute L), Iodine (Loba Chemie), Hexane (KemAus), Potassium Hydroxide (KemAus), Phenolphthalein (Qrec), Acetic Acid (RCI Labscan), DPPH (Sigma-Aldrich), Ascorbic acid (Chem-Supply), Hydrochloric Acid (RCI Labscan), Diethyl Ether (Qrec), Potassium Iodide (KemAus), Sodium thiosulfate (KemAus), Chloroform (RCI Labscan), Stearyl alcohol (MySkin Recipes), Glyceryl stearate (Chemipan), Sciwis Hair Condng Wax (Chemipan), Polyquaternium-44 (Chemipan), Cetrimonium Chloride (Chemipan), Propylene Glycol (Chemipan), 5-Bromo-5-Nitro-1,3-Dioxane (Sigma-Aldrich). The equipment consisted of UV-Vis Spectrophotometer (Jasco,V-730), Colorimeter (MiniScan 4000), Rotary Evaporators (Buchi, R300), Centrifuge (Ortoalresa, Biocen22R), Viscometer (Brookfield,DV2T), Microscope (VH-S1,Keyence VH-Z450), Colony Counter (Funke Gerber)

Preparation of extracts

The preparation of the extract begins with the introduction of two varieties of pigeon peas, consisting of a small (T9) and a large (ICP 7035) derivative from Tak province, as shown in Fig. 1, to be cleaned and baked at a temperature of 50 °C for 3 days. Then apply fine grinding and maceration with a hexane solvent [8]. Drying powder to solvent ratio (1:4) takes 7 days to grind and then filter with Whatman No. 1 paper. Remove the solvent with the rotary evaporator, then calculate the percent yield, and then analyze the oil composition [9].

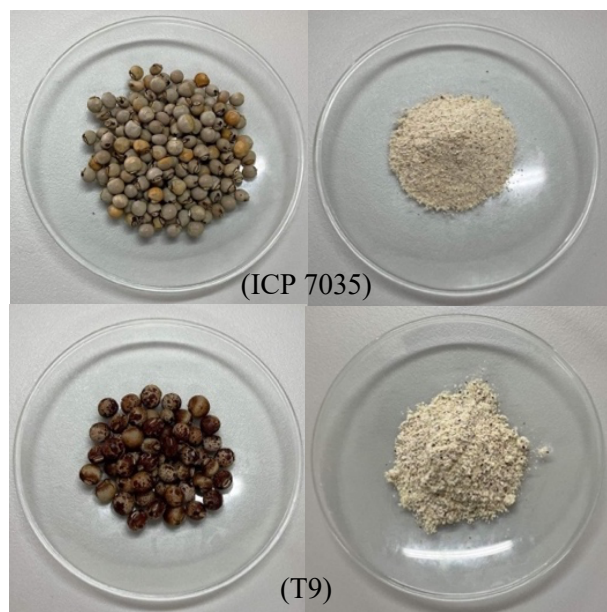


Fig. 1 The seeds of pigeon peas

Testing of chemical properties

Detection of Saponification: The oil sample weighed two grams and was placed in a volumetric flask. After that, 25 mL of 1.0 N alcoholic KOH was pipetted into the mixture and left to drain for roughly a minute. For full saponification, a condenser was attached to the flask, and the mixture sample was boiled steadily but gently for 45 minutes. After cooling the flask and the condenser slightly but not enough to create a gel, the condenser's interior was cleaned with roughly 1 mL of distilled water. After disconnecting the condenser, 1 milliliter of phenolphthalein indicator was added. 0.5N hydrochloric acid (HCl) was added to the solution to titrate it until the pink color barely persisted [10].

Detection of Iodine value: A 1 g oil sample by weight. To make sure the sample was completely dissolved, 15 mL of carbon tetrachloride were added and swirled. Next, using a pipette, 25 mL of Wijs solution was added to the flask holding the sample. To make sure there was thorough mixing, the flask was stopped and agitated. After that, the sample was left at room temperature for half an hour in the dark. After taking the flask out of storage, 150 mL of distilled water and 20 mL of a 10% potassium iodide (KI) solution were added.

0.1N thiosulfate ($\text{Na}_2\text{S}_2\text{O}_3$) solution was added to the mixture gradually while being shaken vigorously and continuously until the yellow color almost completely vanished. Starch indicator solution (1.5 mL) was added [10].

Detection of Fatty acid value: By titrating it against potassium hydroxide (KOH) and using phenolphthalein as an indicator, the amount of free fatty acids is estimated. The acid value is the milligrams of KOH needed to neutralize one gram of the sample's worth of free fatty acids. It is stated as the equivalent of oleic acid (octadec-9-enoic acid). In a 250 mL conical flask, dissolve 10g of oil in 50 mL of the neutral solvent (25 mL of ether plus 25 mL of 95% ethanol and 1 mL of phenolphthalein solution neutralized with 0.1M KOH). After that, add three to four drops of phenolphthalein indicator and shake to combine. The material was titrated against 0.1M KOH until a fifteen-second-long pink hue was achieved [10].

Detection of Peroxide value: 20 mL of solvent mixture, 1 g of powdered KI, and 1 gram of oil were added to the tube after it had been weighed. I placed the tube in the boiling water and allowed it to boil vigorously for a maximum of thirty seconds. After that, swiftly transfer the contents to a conical flask holding 20 milliliters of a 5% KI solution. Wash the tube twice, collecting the water each time in a 25 mL conical flask. 0.002M $\text{Na}_2\text{S}_2\text{O}_3$ solution was titrated to a light yellow color. added an additional 0.5 mL of starch, gave it a good shake, and then cautiously titrated it one more time until the blue hue vanished [10].

Total phenolic content

Folin-Ciocalteu method, using gallic acid as a standard. Add a 200 mL sample extract. Fill the folin-ciocalteu's reagent, then add the sodium carbonate (Na_2CO_3) solution at a 7.5% concentration of 2 mL. Mix the mixture together and fill in 3 mL of distilled water. Mix it together. Keep it in a dark place at room temperature for 2 hours, then measure the absorption of light at a 750 nm wavelength. Repeat all three experiments and bring the results to a standard graph. Then compare the results of the test with the standard graph [11]. Results are in milligrams per gram of extract.

Total flavonoid content

Flavonoid concentration analysis. 100 μL of sample extract. Put it in the tube. Fill 1.25 mL of distilled water with the sodium nitrate (NaNO_2) solution, 75 μL . Add an aluminum chloride solution (AlCl_3) at 150 μL . Add 500 μL of sodium hydroxide (NaOH). Use catechins at concentrations of 5, 10, 20, 50, and 100 $\mu\text{g mL}^{-1}$ as a standard measurement of light absorption at wavelengths of 510 nanometers. Add a standard graph, compare the results with the standard graph, and report them in units of catechin milligrams per 1 gram of extract [12].

Antioxidant activity by DPPH assay

After making a solution of DPPH (0.039 g of 100 mL in methanol), it was left in the dark for 30 minutes. In order to prepare serial dilutions in the concentration range of 1000, 500, 250, 125, 62.5, and 31.05 mg mL^{-1} , the extract stock solution (1 mg mL^{-1}) was also prepared in methanol. 3 mL of DPPH solution was combined with about 0.1 mL of each working dilution, and the mixture was incubated for 30 minutes at 25 °C. At 517 nm, the mixture's absorbance was finally measured with a UV spectrophotometer. The standard ascorbic acid treatment followed the same protocol. The following formula was used to calculate the %DPPH scavenging potential [13].

Oil colour measurement

Oil color measurement requires a colorimeter (HunterLab, MiniScan EZ) by selecting Setup. No average should be set to Ill, Obs at D65 and 10° (the light source used in the color meter), and the color scale should be selected as $L^*a^*b^*$. Save the tested value three times.

The development of a hair care formulation

Treatment development hair care product consists of 3 recipes using pigeon pea oil (T9), pigeon pea oil (ICP 7035), and a non-recipient, as shown in Table 1. The mixing method of the hair care product starts with Phase A. Mix it together, and then heat it at 75 °C until it melts. Then take Phase B. Mix it together, and then heat it at 75 °C until it melts. Then bring phase A to blend. Phase B. Make a cool down at 45 °C. Finally, add the Phase C mix together.

Evaluate the performance of hair treatment products.

The washing the hair with shampoo, then rinse thoroughly. After that, apply the hair treatment product, formula 1 and formula 2, for 30 minutes. Then, dry the hair by blowing cold air for 5 minutes. Finally, compare the hair care products by observing the hair texture, shine, and smoothness of the hair structure before and after using the products using a zoom microscope with a VH-S1 stand from Keyence VH-Z450.

Table 1 The recipes for hair treatment products.

Phase	Ingredient	Base (%w w ⁻¹)	F1 (%w w ⁻¹)	F2 (%w w ⁻¹)	Function
A	Stearyl alcohol	0.5	0.5	0.5	Thickener
A	Pigeon pea Oil (T9)	-	2	1	Emollient
A	Pigeon pea Oil (ICP 7035)	-	1	2	Emollient
A	Glyceryl stearate	0.5	0.5	0.5	Emulsifier
A	Sciwis hair condensing wax	7	7	7	Emulsifier
B	Polyquat-ernium-44	5	5	5	Conditioning agent
B	Cetrimonium chloride	7	7	7	Surfactant
B	DI Water	79	76	76	Solvent Preservative
C	Microcare PHC	1	1	1	
	Total	100	100	100	

Note : F1 = Formula 1 , F2 = Formula 2

The microbial content of the hair care product

A bacterial dosage test [14-15] uses a sterile technique in every phase of the test. The bacteria are diluted in a series of dilution procedures, and the samples are fed into a fertilized feed dish. The fertilized dishes are then buried in an incubator, and the number of bacterial colonies is counted using a colony counter. The results and the quantity are recorded.

Statistical analysis

The mean \pm standard deviation is used to present the results, which were all measured and administered in triplicate. One-way analysis of variance (ANOVA) and Duncan's multiple range tests with a p-value < 0.05 were used to analyze the data.

3. Results and Discussion

The pigeon pea extraction

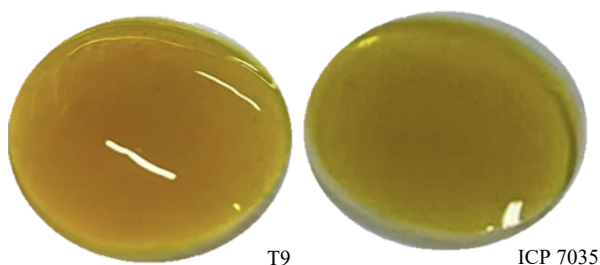


Fig. 2 Pigeon pea oils

The pigeon pea extraction results showed that the physical characteristics of the two oils are stiff and sticky. The smell is a slightly shiny, yellow-brown color, as shown in Fig. 2. The percentage yield of big seed (ICP 7035) is higher than small seed (T9) oil, equal to 9.64 ± 0.10 and 9.32 ± 0.08 , respectively. In line with previously journal as smaller pigeon pea seeds (milled) had higher larger seeds [16].

The chemical properties of the pigeon pea oil *The saponification test*

A study of the number of milligrams of potassium hydroxide using a complete hydrolysis of 1 gram of fat or oil was done on soap and glycerol. The saponification values are used to indicate the molecular size or weight of the fatty acid that is a component of the triglyceride molecule. The test found that the oil contained in the small seed (T9) had a higher saponification value than the large seed (ICP 7035), as shown in Table 3. In conclusion, the high saponification value of T9 indicates that the fatty acid is a component of the triglyceride, which has a very low molecular weight, resulting in many molecules per unit of weight that are better than ICP 7035. The value of test is in standard [17].

The result of the fatty acid value test

One gram of fat or oil contains a neutral hydroxide to produce free fatty acids. The test results showed that small seeds (T9) are less acidic than large seeds (ICP 7035), as shown in Table 3, where the acidity of both types of oils is not more than 4 mg of the standard value. This may indicate a tendency toward depletion of fat and oil. Both oils are less susceptible to

unwanted odors, as well as easy oxidation reactions. The small seed (T9) is more effective than the large seed (ICP 7035).

The result of the iodine value test

The measurement of the degree of unsaturation, or the number of double bonds in the molecules of fatty acids that are composed of the fat, or pigeon pea oil, in which the oil contains a highly unsaturated fatty acid that has many pairs of bonds. The double bond absorbs the iodine, indicating a high iodine content. This indicates that the oil exhibits a high degree of instauration, leading to an oxidative reaction or interruption due to a simple oxidation reaction. The iodine ratio is in the range of 67–85, and the large seed (ICP 7035) ratio has a drop-down of 8 mL of iodine solution, which equals the iodine ratio in the 85–103 range, as shown in Table 3. Both types have values that do not exceed 120, which is acceptable in small industries and communities.

The result of the peroxide value test

The ratio used to measure the rate of the lipid oxidation reaction, which causes rancidity, indicates the degradation of oils and fats. High indicates that the oil or fat is highly antioxidant resistant by pyrocatechol violet (P.V.) [18]. The fatty tissue is oxidized into hydroperoxides, which can then decompose into other compounds. The trial found that small seed (T9) has a lower peroxide value than large seed (ICP 7035). Table 3 concludes that T9 is less likely to resist the reaction than ICP 7035.

Total phenolic content

An aromatic ring and a hydroxyl group directly link to form the family of chemical compounds known as phenolic compounds. Table 2 also displays the phenolic levels, as determined by the Folin-Ciocalteu assay. The phenolic content was highest in T9 (27.76 ± 0.65 mg GAE g⁻¹ extract). The concentration was lowest in ICP 7035, measuring between 25.90 and 0.69 mg GAE g⁻¹ extract. This experiment aligns with the previous paper, given that seeds are the primary source of phenolic compounds. In a statistical comparison of the phenolic value between T9 and ICP 7035, it was found that both types ($P < 0.05$) In line

with previous research, pigeon pea is a rich source of phenolic compounds [19].

Total flavonoid content

The aluminum chloride colorimetric assay tested the total flavonoid content. In the experiment, it was highest in T9 (20.67 ± 0.58 mg QE g⁻¹ extract) and lowest in ICP 7035 (18.76 ± 0.65 mg QE g⁻¹ extract). This experiment aligns with the previous paper, revealing the highest abundance of flavonoid compounds in seeds. The statistical comparison revealed that both T9 and ICP 7035 had flavonoid values ($P < 0.05$). In line with previous research, pigeon peas are a rich source of flavonoid compounds [20].

Antioxidant activity

Tests with the DPPH radical-scavenging method showed that T9 oil had an IC₅₀ lower than ICP 7035 of 0.26 ± 0.04 and 0.35 ± 0.05 mg g⁻¹ of extract, respectively. As a result, T9 oils were more likely to inhibit oxidation than large oils, which is consistent with the study. In the statistical comparison of the IC₅₀ value between T9 and ICP 7035, it was found that both types ($p < 0.05$) had a 95% confidence interval. The antioxidant effects of T9 and ICP 7035 were about the same in all tests, with a p-value of less than 0.05 and a 95% confidence interval. The phenolic, flavonoid, and DPPH values demonstrated this. As shown in Table 2, The experiment revealed a significant positive correlation between the total phenolic content (TPC) and total flavonoid content (TFC) of pigeon pea oil (T9). The results of TPC, TFC, and antioxidant activity indicated that pigeon pea oil (T9) had better antioxidant activity than pigeon pea oil (ICP 7035), a statistically non-significant difference [20].

Colour measurement

The combination of two types of pigeon pea oil, small seed (T9) and large seed (ICP 7035), is used for oil color measurements using a colorimeter (Miniscan EZ) equipment employing the International Commission on Illumination (CIE) color differential values, which comprise three distinct color variables: L*, a*, and b*, where the oil of small seed (T9) has values of L*, a*, and b* equaling 61.91 ± 0.14 , -1.70 ± 0.04 , and 14.83 ± 0.12 ,

respectively. In the larger seed combination (ICP 7035), the values of L*, a*, and b* equal 62.60 ± 0.28 , 0.95 ± 0.08 , and -3.63 ± 11.25 , respectively, so the color of T9 is darker yellow than ICP 7035 due to the seeds being darker, the oil produced is darker in color.

Development and stability of product

In the development of the hair care product, there were 3 receipts, with differences in the dose ratio of base (non-oil), Formula 1 (ratio of T9 and ICP 7035 1:2), and Formula 2 (ratio of T9 and ICP 7035 2:1), respectively. Found that the physical characteristics of the base are white, no odour, Formula 1 is dark yellow, has a gentle smell of oil, and Formula 2 is light yellow, as shown in Fig. 3. The stability test consists of colour, smell, appearance, separation, heating and cooling cycle testing, and pH as re-prepared. After a period of 4 weeks, consisting of physical and chemical characteristics, all formulas good stability with no change. And when testing the performance of the product, which in the formula 1

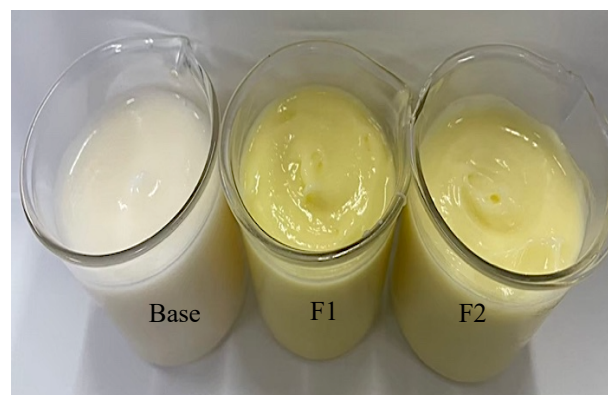


Fig. 3 Hair treatment products

Base = No add pigeon pea oil

F1 = The ratio of T9 and ICP 7035 (1:2)

F2 = The ratio of T9 and ICP 7035 (1:2)

Table 2 The result of antioxidant activity.

Type of pigeon pea oil	Total Phenolic Content (mg GAE g ⁻¹ extract)	Total Flavonoid Content (mg QE g ⁻¹ extract)	DPPH IC ₅₀ mg g ⁻¹ extract
T9	27.76 ± 0.65 ^a	20.67 ± 0.58 ^a	0.26 ± 0.04 ^a
ICP 7035	25.90 ± 0.69 ^b	18.76 ± 0.65 ^b	0.35 ± 0.05 ^b

Note: All tests were repeated three times, and the results are shown as mean ± standard deviation. The analysis of variance was performed using raw data and differences between the means followed by ANOVA Test. Different letters in each column indicate a significant difference ($p < 0.05$).

Table 3 The result of the chemical properties of pigeon pea oil.

Parameters	Appearance	Results of Test		
		T9	ICP 7035	Standard value
Saponification	Pink disappeared	188.29 ± 0.61	187.35 ± 0.20	187 - 196
Fatty acid	Pink disappeared	0.39 ± 0.01	0.40 ± 0.01	< 4 mg
Iodine	Iodine color	67 - 85	85 - 103	<120
Peroxide value	Purple disappeared	0.10 ± 0.02	0.11 ± 0.06	0 - 12

Total microbial count test

The total microbial count test of microbial contamination using a colony counter (Funke Gerber) determined the value of the microorganism intake (CFU g⁻¹) from the introduction of three hair care products. The test found that the hair care product received the base formula (non-oiled), Formula 1 (T9 mixed to ICP 7035, ratio 1:2), and Formula 2 (T9 mixed to ICP 7035, ratio 2:1), respectively. Detected microbials were less than 4.0×10^4 , so it was concluded that all three hair care products received no microbial contamination because they did not exceed the standard.

Hair structure assessment test

The hair treatment product test results consist of two formulas, which include base and pigeon pea oil in a 2:1 ratio, both before and after application. The results showed that before using the product, the hair was dry, rough,

pointed, dried, and had little hair grinding, but after using the product for 4 weeks, pigeon pea oil in a ratio of T9 to ICP 7035 (2:1) became softer and more effective than the base formula. The comparison between the hair before and after using the pigeon pea formula, as depicted in Fig. 4, indicates that pigeon pea oil is the most effective. This hair care product is suitable for use in the cosmetic industry.

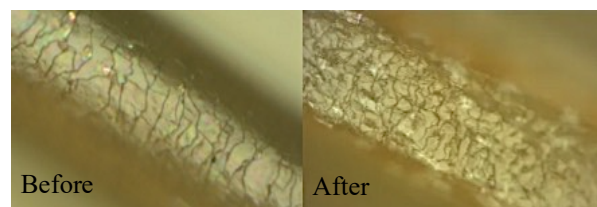


Fig. 4 The comparison of hair before and after using a hair treatment product containing pigeon pea oil in a ratio of T9 to ICP 7035 (2:1). (1000x)

4. Conclusion

A comparison of the oil and antioxidant properties of two pigeon pea varieties, T9 and ICP 7035, revealed that ICP 7035 yielded a higher percentage than T9. The oil properties of T9, including saponification, acidity, iodine, and peroxide values, were better than those of ICP 7035 in all tests. This suggests that T9 is less susceptible to oxidation reactions, which can lead to oil degradation. Additionally, T9 exhibited better antioxidant activity than ICP 7035. When measuring the Lab* color value of the oil, it was found that the darker yellow color of T9 resulted in more hair-care products that made hair smoother and glossier. Furthermore, primary colonial testing did not detect microbial contamination in any of the hair care products. Therefore, pigeon pea oil from T9 is a specific oil that can be effectively used in the cosmetics industry, hair maintenance, or other applications.

5. Acknowledgement

The study was supported by the Health Science and Aesthetic Program at Rajamangala University of Technology Krungthep in Bangkok, Thailand.

6. References

- [1] L.S. Kristiani, F. M. Rajendra, S. Ariviani, Antioxidant capacity of pigeon pea (*Cajanus cajan* L.) sprouts elicited using NaCl with various elicitation time, Mater. Sci. Eng. 56 (2019) 1 – 6.
- [2] Y.S. Lai, W.H. Hsu, J.J. Huang, S.C. Wu, Antioxidant and anti-inflammatory effects of pigeon pea (*Cajanus cajan* L.) extracts on hydrogen peroxide- and lipopolysaccharide-treated RAW264.7 macrophages, Food Funct. 3 (2012) 1294 – 1301.
- [3] K.B. Saxena, Genetic improvement of pigeon pea – A review, Trop. Plant. Biol. 1 (2008) 159 – 178.
- [4] K.B. Saxena, N. Nadarajan, Prospects of pigeon pea hybrids in Indian agriculture. Electron. J. Plant. Breed. 1 (2010) 1107 – 1117.
- [5] B. Abebe, The Dietary Use of Pigeon Pea for Human and Animal Diets, Sci. World 2022 (2022) 1 – 12.
- [6] G. K. Rakesh, Pharmacology and Phytochemicals activities of *Cajanus cajan* : Review, Emerg. Technol. Innov. Res. 8 (2021) 307 – 319.
- [7] D. Tungmunthum, C. Hano, Cosmetic potential of *Cajanus cajan* (L.) : botanical data, traditional uses, phytochemistry and biological activities, Cosmetics. 7 (2020) 1 – 12.
- [8] P. Watoo, P. Waraporn, T. Hiroyuki, J. Kanchalee, W. Sakchai, I. Kornkanok, Comparison of various extraction methods of *Bacopa monnieri*, NU. J. 15 (2007) 29 – 34.
- [9] K. Nattapong, L. Thunnop, Effects of spray- drying temperatures on powder properties and antioxidant activities of Encapsulated anthocyanins from black glutinous rice bran, Nat. Life. Sci. Commun. 23 (2023) 1 – 19.
- [10] A.O.C.S., Official methods and recommended practices of the american oil chemists society, Am. Oil Chem. Soc. (1993) 3489 – 61826.
- [11] V.L. Singleton, R. Orthofer, R.M. Lamuela Raventós, Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent, Method Enzymol J. 299 (1999) 152 – 178.
- [12] Z. Jia, M. Tang, J. Wu, The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals, Food Chem. J. 64 (1999) 555 – 559.
- [13] W. Brand-Williams, M.E. Cuvelier, C. Berset, Use of a free radical method to evaluate antioxidant activity, Food Sci. Technol. J. 28 (1995) 25 – 30.
- [14] M.E. Allen, R.A. Gyure, An undergraduate laboratory activity demonstrating bacteriophage specificity, Microbiol. Biol. Educ. 14 (2013) 84 – 92.
- [15] A. Ben David, C.E. Davidson, Estimation method for serial dilution experiments, Microbiol. Methods. 107 (2014) 214 – 221.
- [16] K. Kalay, S.C. Moses, K. Ashok, K. Devesh, U. Abhishek, Physical properties of pigeon pea grains at different moisture content, Int. J. Pure App. Biosci. 5 (2017) 556 – 562.
- [17] S.O. Aruna, Chemical composition analysis of palm oils in ijebu-ode, ogun state, Afr. J. Chem. Educ. 13 (2023) 91 – 111.
- [18] J. Flieger, W. Flieger, J. Baj, R. Maciejewski, Antioxidants: classification, natural sources, activity/capacity measurements and usefulness for the synthesis of nanoparticles, Materials. 14 (2021) 1 – 54.
- [19] A.S. Gerrano, A. Moalafi, H.A. Seepe, S. Amoo, H. Shimelis, Nutritional and phytochemical compositions and their interrelationship in succulent pods of pigeon pea (*Cajanus cajan* L.), Heliyon. 8 (2022) 1 – 7.
- [20] R. Adilson, P. Locali, B. Adeline, B.C. Claire, R.T. Sebastião, S.J. Véronique, R.N. Vânia, Pigeon pea, An emerging source of plant-based proteins, ACS Food Sci. Technol. 3 (2023) 1777 – 1799.
- [21] N. Wu, K. Fu, Y.J. Fu, Y.G. Zu, F.R. Chang, Y.H. Chen, X.L. Liu, Y. Kong, W. Liu, C.B. Gu, Antioxidant activities of extracts and main components of pigeon pea *Cajanus cajan* (L.) leaves, Molecules. 14 (2009) 1032 – 1043.