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Detection of coconut oil adulteration with palm oil through NMR spectroscopic method

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SUMMARY: Coconut oil is a costly commodity in the food and traditional medicinal sectors and its adulteration with cheap palm oil is a serious issue. The present study evaluates the application of ^{1}H NMR spectroscopy to authenticate coconut oil, and to monitor its adulteration with the cheap palm oil substitute. Various parameters such as average chain length (14.25), saponification index (244.66 mg KOH/100 g), molecular weight (652.12), iodine value (8.27 mg/100 g), peroxide value (0.02 meqO_2/kg) and percentage of unsaturation (7.81%) were calculated through the NMR technique, and were found to be in concurrence with the values obtained from wet lab experiments. The extent of palm oil adulteration can be detected through NMR by evaluating the chemical shift values for olefinic protons. The findings have a significant impact on both the food and traditional medicine sectors, as NMR spectroscopy can replace the conventional wet lab methods as a reliable and precise method for analysis.

KEYWORDS: *Adulteration; Coconut oil; NMR; Palm oil.*

RESUMEN*: Detección de la adulteración del aceite de coco con aceite de palma mediante espectroscopía de RMN.* El aceite de coco es un producto costoso en los sectores alimentario y de medicina tradicional y su adulteración con un sustituto barato como el aceite de palma es un problema grave. El presente estudio evalúa la aplicación de la espectroscopía de RMN ^{1}H para autenticar el aceite de coco y monitorear su adulteración con el sustituto barato de aceite de palma. Diversos parámetros como longitud media de cadena (14,25), índice de saponificación (244,66 mg KOH/100 g), peso molecular (652,12), índice de yodo (8,27 mg/100 g), índice de peróxido (0,02 meqO_2/kg) y porcentaje de insaturación (7,81%), se calcularon mediante la técnica de RMN y se encontró que coincidían con los valores obtenidos en pruebas de laboratorio. El alcance de la adulteración con aceite de palma se puede detectar mediante RMN evaluando los valores de desplazamiento químico de los protones olefínicos. El hallazgo tiene un impacto significativo, tanto en el sector alimentario, como en el de la medicina tradicional, ya que la espectroscopia de RMN puede reemplazar los métodos convencionales de laboratorio, como un método fiable y preciso para el análisis.

PALABRAS CLAVE: *Aceite de coco; Aceite de palma; Adulteración; RMN.*

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1. INTRODUCTION

Coconut oil is widely consumed as a food ingredient worldwide, especially in Asia and south India. The oil is also used in cosmetic and indigenous medicinal systems like Ayurveda and Sidha (Joshi *et al*., 2020). It is a rich source of the saturated fatty acids lauric and myristic acid (Widianingrum *et al*., 2019). Lauric acid forms the active and digestible monolaurin in the human body, and recently coconut oil and monolaurin have been investigated in managing COVID-19 (Maruyama *et al*., 2014; Angeles-Agdeppa *et al*., 2021). Saturated fatty acids are associated with the risk of cardiovascular disease; however, coconut oil contains medium-chain saturated fatty acids which are readily absorbed by the portal vein and play a greater role as a source of energy than in cholesterol synthesis (Sacks *et al*., 2017). Also, coconut oil is regarded as a super-food as it improves memory, promotes heart health, strengthens immunity, and possesses wound healing, antiviral, antibacterial, and anti-inflammatory properties (Joshi *et al*., 2020; Peedikayil *et al*., 2016; Chen *et al*., 2022). Coconut oil is expensive compared to other common oils and this often leads to adulteration, and palm oil is the most widely used adulterant due to its low price and easy availability (Alkan *et al*., 2012). Physicochemical properties are significant in the purity checking and monitoring of adulteration in oils, and ^{1}H NMR spectroscopy has been used as an efficient tool for the characterization of oils (Siudem *et al*., 2022). A recent work from our group has investigated the application of NMR for the identification of fatty acid constituents and evaluation of physicochemical parameters of *Garcinia gummi-gutta* seed oil (Priya Rani *et al*., 2022). The food sector, especially the edible oil sector, demands rapid, precise and reliable alternatives to the existing wet lab methods, and the present study elaborates the application of ^{1}H NMR spectroscopy in the authentication of coconut oil and in the detection of adulteration of coconut oil with palm oil.

2. MATERIALS AND METHODS

2.1. Sample preparation

Authentic samples of coconut oil and palm oil were procured from oil mills in Thiruvananthapuram, Kerala. For the preparation of blends (5-50% v/v), varying concentrations of coconut and palm oils were mixed and the resulting blends were homogenized at 80 ºC.

2.2. ^{1}H NMR analysis

For ^{1}H NMR analysis, $CDCl_3$ was used as the solvent along with TMS (Tetramethyl silane) as the internal standard. The spectra were taken using Bruker Avance III HD, 400 MHz spectrometer, at 30° pulse. The integral values for the peaks obtained from ^{1}H NMR analysis were used for the evaluation of various physicochemical parameters. For the authentication of coconut oil, various physico-chemical parameters such as average chain length, saponification value, molecular weight, iodine value, peroxide value and percentage unsaturation were calculated in triplicate (n = 3) from ^{1}H NMR as described previously (Skiera *et al*., 2012; Siudem *et al*., 2022).

2.3. Determination of physico-chemical parameters using the ^{1}H NMR spectroscopic method

^{1}H NMR values for oils and fats depict specific resonances for the protons present in the structural patterns of triglycerides, especially in the methylene groups (-CH_2), double bonds (-CH=CH-) and in the terminal methyl groups (-CH_3) (Ivanova *et al*., 2022). Average chain length, which is determined by adding 'one' to the total integral values corresponds to the characteristic peaks (δ 0.89, 1.30, 1.60, 2.00, 2.30, 2.80, 4.30, 5.26 and 5.36) in the ^{1}H NMR spectrum. These chemical shift values correspond to unsatuarated protons as well as protons of methylene groups of the fatty acyl chains. Saponification value (SV) is the number of milligrams of potassium hydroxide required to saponify 1 g of the sample completely (Reda *et al*., 2007). Saponification value can also be calculated from ^{1}H NMR values using the equation (Carvalho Dos *et al*., 2018):

$$SV = - 0.2385(M_w) + 398.42$$

where the value 398.42 is the average mass of the fatty acids.

Molecular weight (Mw) represents the weight corresponding to the fatty acid-derived triglycerides. While calculating the molecular weight of TAG (Triacyl glycerol), the terminal –CH_3 and glycerol entity

along with the carbonyl group of the fatty chain are given a fixed molecular weight, while the number of -CH_2- and –CH=CH- may vary. Molecular weight can be calculated using the equation:

$$M_w = 119.70 + 7.036T + 5.983V$$

where, T represents the total number of hydrogen and V represents the peaks of ^{1}H NMR spectrum assigned to unsaturated fatty acids. T value was obtained by the sum of integral values of hydrogen in the ^{1}H NMR spectrum (A-I) divided by an area of a single proton (PA).

$$PA = G/4$$

where, G = the sum of two hydrogen peaks attached to methylene glycerol carbons at δ 4.3 and 4.1 ppm.

$$T = (A + B + C + D + E + F + G + H + I)/PA$$

The peak area of olefinic hydrogen at δ 5.4 ppm (I) and 5.3 ppm (H) were used to obtain V by the equation:

$$V = [(H + I) – PA]/PA$$

Iodine value gives the number of grams of iodine absorbed per 100 g of sample, and gives the average amount of unsaturation (Reda *et al*., 2007),

$$IV = (126.91 \times 100 \times V)/M_w$$

As a milliequivalent of iodine per 1 kg of sample, peroxide value is determined by adding potassium iodide under specific conditions (Skiera *et al*., 2012). Peroxide value gives the extent of the oxidative deterioration of the oil sample. It corresponds to the sum of the integral values at δ 2.6 to 2.9 and δ 5.1 to 5.6 divided by the integral values at δ 0.6 to 2.5. Percentage unsaturation corresponds to the signals at δ 2.0, 2.8 and 5.4 ppm which are due to the -CH_2CH=CH-CH_2=CHCH_2CH= and -CH=CH- respectively of the fatty chain group of the triglyceride.

3. RESULTS AND DISCUSSION

The physicochemical parameters play an important role in assessing the quality and authenticity of oils and oil-derived value-added products. The conventional wet lab methods for physico-chemical evaluations are time consuming, destructive and require a large sample size, while ^{1}H NMR has emerged as a standardized and validated method for the determination of physico-chemical parameters (Skiera *et al*., 2012; Siudem *et al*., 2022). The ^{1}H NMR- derived (Figure 1) physicochemical parameters of coconut oil, and the mixture with palm oil are given in Table 1.

In the present study, the chain length value and percentage unsaturation of coconut oil increases

Table 1. ^{1}H NMR-derived physico-chemical and molecular characteristics of coconut oil, palm oil and blended samples (n = 3)

Parameters	Blend sample ratio (%)						
	Coconut oil	CO:PO (95:5)	CO:PO (90:10)	CO:PO (75:25)	CO:PO (50:50)	Palm oil	Wet lab results for CO
Average chain length	14.25±0.59	15.09±0.23	15.46±0.46	15.86± 0.40	16.77±0.28	20.05±0.41	12.3-13.3 (Crowther, 2008)
Saponification index (g KOH/kg oil)	244.66±0.67	240.50±0.51	236.44±0.40	229.53±0.48	220.47±0.39	191.91±0.37	250-260 (APCC, 2009)
Molecular weight	652.12±0.49	671.05±0.39	686.91±0.42	716.24±0.61	754.67±0.59	875.78±0.49	680 (Alander, 2004)
Iodine value (g/100 kg)	8.27±0.28	12.18±0.49	13.16±0.36	19.49±0.27	32.37±0.51	57.82±0.41	4-11 (APCC, 2009)
Peroxide value (meqO_2/kg)	0.02±0.01	0.02±0.01	0.02±0.01	0.03±0.02	0.04±0.01	0.06±0.01	<3 (APCC, 2009)
Percentage unsaturation	7.81±0.52	15.23±0.47	17.10 ±0.52	22.50 ±0.48	35.52 ±0.39	66.51±0.43	9 (APCC, 2009)

[a]CO: Coconut Oil; PO: Palm Oil

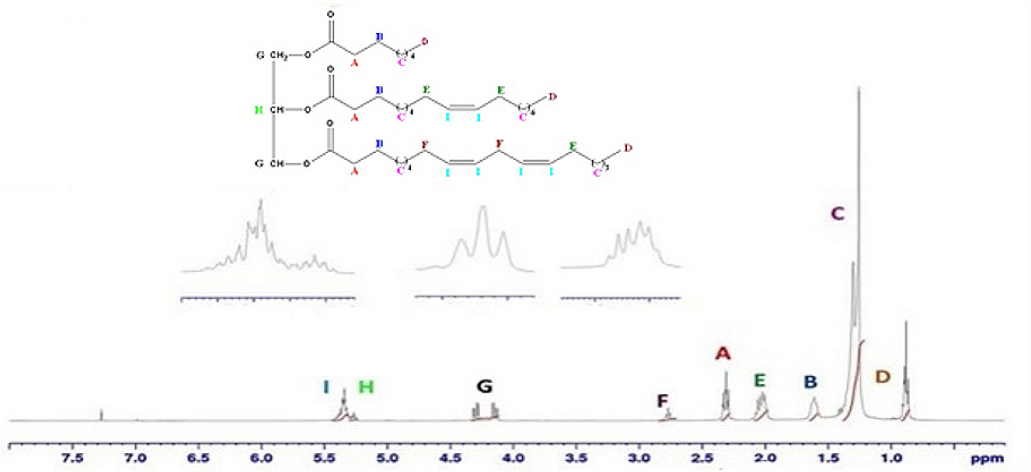

Figure 1. ^{1}HNMR spectrum of a triglyceride with saturated and unsaturated fatty acids. The protons present in different chemical shift values are depicted as denoted by A-I. The unsaturated protons are depicted as E, F and I in the spectrum.

upon adding palm oil. Coconut oil possessed an exceptional saponification value of 244.66 g KOH/kg, due to the presence of lauric (12:0) and myristic (14:0) acids, and the addition of palm oil led to significant decrease in saponification value. Therefore, the saponification value could be used as a reliable parameter for detecting adulteration in coconut oil with palm oil. Iodine value was determined to identify the extent of fat unsaturation, which varies with the type and proportion of unsaturated fatty acid present in the oil. The iodine value for pure coconut oil was 8.27 g/kg, and the addition of palm oil to coconut oil caused a gradual and significant increase in iodine value, and a higher iodine value can be taken as an index of adulteration of coconut oil with palm oil. The percentage value of unsaturation also increases with the increase in blending of palm oil with coconut oil (7.81% – 66.51%). Pure coconut oil had lower peroxide value (0.02 meqO_2/kg) than that of pure palm oil (0.06 meqO_2/kg), indicating the oxidative stability of coconut oil. The adulteration of pure coconut oil with palm oil caused a significant increase in peroxide value of the mixture, due to the high content of unsaturated fatty acids in palm oil (Table 1). The physico-chemical parameters of coconut oil were found to be well in agreement with the values provided by APCC (Alander, 2004).

Further, the adulteration of coconut oil using palm oil was detected through variation in peak area of characteristic ^{1}H NMR signals that arise due to blending with palm oil. The ^{1}H NMR peaks E, F and I of the triglyceride (Figure 1) are characteristic of unsaturated triglycerides. The extent of unsaturation in palm oil is about 9 times higher than that of coconut oil and this feature can be explored for detecting the presence of palm oil in coconut oil (Figure 2). The peak areas at δ 5.4, 2.8 and 2.0 ppm in the ^{1}H NMR data represent unsaturation in the TAG moiety in palm oil (–CH=CH-, =CHCH_2CH= and –CH_2CH=CHCH_2- respectively). The non-destructive NMR technique offers advantages over traditional wet lab experiments by providing rapid and accurate analysis of key pa-

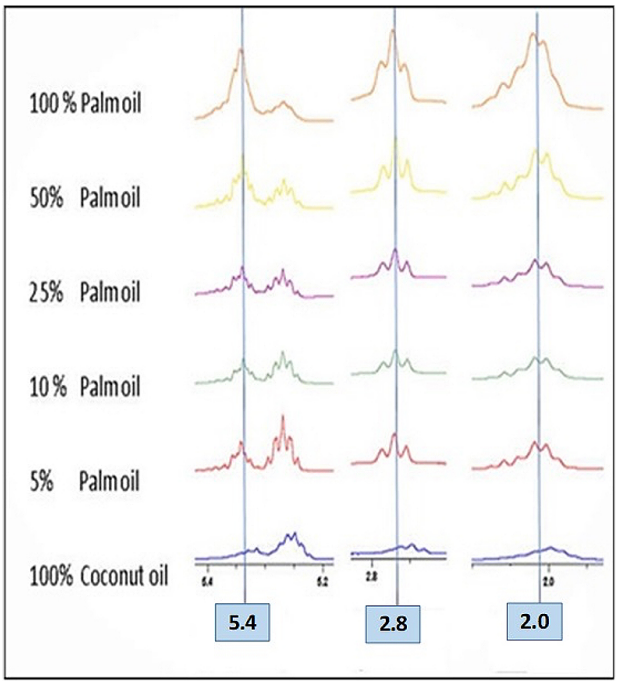

Figure 2. Comparative plot of ^{1}H NMR signals at δ 5.4, 2.8 and 2.0 ppm (unsaturated protons) of pure coconut oil with blended samples of coconut oil and palm oil at different ratios.

rameters such as average chain length, saponification index, molecular weight, iodine value, peroxide value, and percentage of unsaturation. Furthermore, the ability to discern the chemical shift values of olefinic protons enables precise determination of the extent of palm oil adulteration.

4. CONCLUSIONS

The authentication of oil represents one of the most challenging analytical problems in the food sector due to the complexity of lipid components in the oil. Though coconut oil is widely consumed, literature reports on authentication of coconut oil are scarce, except those based on conventional wet lab methods. The present work highlights ^{1}H NMR as a rapid and reliable analytical tool for the authentication of coconut oil as well as for the detection of adulteration of coconut oil using palm oil. The findings are of relevance to the food sector, especially in the context of the emerging international market for coconut oil. The results highlight the potential of NMR spectroscopy as an efficient tool for safeguarding the authenticity and purity of coconut oil across various domains, including food and traditional medicinal applications.

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DECLARATION OF COMPETING INTEREST

The authors of this article declare that they have no financial, professional or personal conflicts of interest that could have inappropriately influenced this work.

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AUTHORSHIP CONTRIBUTION STATEMENT

M. R. Gokul Raj: Conceptualization, Formal analysis, Investigation, Methodology; M. Priya Rani: Conceptualization, Formal analysis, Investigation, Methodology, Funding acquisition, Writing – original draft, review & editing; K. B. Rameshkumar: Investigation, Project administration, Writing – review & editing.

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