Food Analytical Methods (2022) 15:1407–1417
https://doi.org/10.1007/s12161-021-02193-z

Determination of Mono-Oil Proportion in Blended Edible Vegetable Oil (BEVO) with Identical Fatty Acid Profile: a Case Study on Coconut-Palm Kernel Oil Discrimination

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Received: 1 September 2021 / Accepted: 7 December 2021 / Published online: 20 January 2022

Abstract
Blended edible vegetable oil (BEVO) is suggested as a convenient alternative to edible oil rotation to better balance saturated/unsaturated fatty acids rather than using single oil (mono-oil). Coconut and palm kernel blended oil (coco-PK BEVO) gains popularity in the market. However, the identical fatty acid profile of the coco-PK BEVO poses a challenge in determining the proportion of the mono-oil. Thus far, iodine value is the only method to find the presence of palm kernel oil in the coconut oil qualitatively. Heretofore, there are no quantitative methods available to find the proportion of coconut oil when blended with PK. Herein, by performing GC–MS analysis of coconut oil ($n = 34$) and palm kernel oil ($n = 121$) of different quality levels, a new method to determine the mono-oil proportion using C14:0/C18:1 ratio was proposed. The method was sensitive and robust to detect mono-oil proportions ranging from 20–80% coconut in PK with a mean bias of 2.6%. The method was validated by performing the Bland–Altman analysis. Overall, we suggest that the C14:0/C18:1 ratio could be an expedient parameter to identify the percentage of the mono-oil proportion in the coco-PK BEVO.

Keywords Coconut oil · Palm kernel oil · Blended oil · Gas chromatography · Mass spectrometry

Introduction

Oils are an essential dietary ingredient added in the food while cooking that enhances the flavor of food, induces satiety, and improves palatability (Yin et al., 2017). Oils are required for many physiological functions (Namiki, 1995), transportation of fat-soluble vitamins (German and Dillard, 2006), membrane structure (Clandinin et al., 1991), and many other functions. Oils serve as a major macronutrient source; fatty acids are the major component of the oils. Different fatty acids perform different functions. For instance, omega-3 fatty acids such as alpha-linoleic acid (ALA), docosahexaenoic acid (DHA), and eicosapentaenoic acid (EPA) play a role in the brain, nerve, and eye development in infants (Swanson et al., 2012). Omega-6 fatty acids such as linoleic acid (LA), arachidonic acid (ARA), gamma-linolenic (GLA), and conjugated linoleic acid (CLA) play an important role in regulating our genes, promoting immune health, and blood clotting (Silva et al., 2018). However, not all fatty acids are available in single oil. Nevertheless, a balanced omega-6/omega-3 ratio is important for health (Simopoulos, 2016). Therefore, to get the benefit of most of the available fatty acids, we have to diversify the use of various oils in various cooking applications. On the contrary, blended edible vegetable oils (BEVO) can improve the availability and consumption of diverse fatty acids. BEVO is made by mixing two or more edible vegetable oils to provide a wider range of fatty acids that would be not available in using single vegetable oils (mono-oils). BEVO has been suggested as a convenient alternative to edible oil rotation to better balance saturated/unsaturated fatty acids.

Commercialization of a product is in the purview of maximizing profit. Profitability is often associated with cost cutting during the manufacturing process by either navigating

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within the legal requirements or occasionally by performing illegal adulteration. The industry and regulators have to be vigilant for the quality and standards of the BEVO Recently, the Food Safety and Standards Authority of India (FSSAI), the apex body for food regulation in India, had triggered a massive surveillance drive to ensure the sale of safe blended vegetable oil (Maindola, 2019; Kumar, 2020). FSSAI permits blending of any two vegetable oils provided the percentage of either of the oil should not be less than 20%. However, the lack of efficient testing methods to identify the percentage proportion of individual mono-oils in the BEVO paralyzes FSSAI from efficiently enforcing the legal parameters. For instance, the blending of coconut oil (derived from the kernel of the coconut, *Cocos nucifera* L.) and palm kernel oil (derived from the kernel of the fruit of the oil palm, *Elaeis guineensis*) is advocated to have a nutritional advantage (Bhatnagar et al., 2009). The FSSAI provides a license for the sale of coco-PK BEVO in the ratio of 80% coconut oil with 20% PK. Of note, the cost of coconut oil is much higher than PK. Therefore, if a manufacturer obtains a license for coco-PK in the ratio 80:20 and packs the product in the 20:80 coco-PK ratios, there is no efficient method for FSSAI to detect this malpractice. In this work, we have addressed the challenges faced in identifying the mono-oil proportion when blending these two oils (coco-PK) in BEVO. Based on the results, we suggest that the C14:0/C18:1 ratio of coco-PK blended oils could be used to determine the percentage of mono-oil proportion in coconut-palm kernel blended oil. In addition, we present reference values for individual fatty acids in coconut oil and palm kernel oil using an MS detector. This is the first report providing a guideline to determine the proportion of mono-oil in coco-PK BEVO.

Materials and Methods

Chemicals and Reagents

Samples used in this study as pure mono-oils of coconut were provided by V.V.D and Sons Private Limited, Thoothukudi, Tamil Nadu, India. All the chemicals used in this study (unless otherwise specifically stated) were procured from Merck India, Mumbai. Water used in the study was of 18 MΩ resistivity obtained after due processing using Milli-Q® direct water purification system (Merck Millipore, Merck KGaA, Darmstadt, Germany). Fatty acid standards (Supelco 37 Component FAME Mix) were from Sigma-Aldrich, USA.

Sample Collection and Processing

Pure mono-oils of coconut ($n = 34$) and palm kernel oil ($n = 121$) were collected at a different time points during 2018–2021. Five hundred milliliters of samples were collected and used for analysis. We preprocessed the oil samples as described below. Briefly, 20 mg of the oil samples were weighed in a 10-ml glass test tube (Borosil, Grade I). The oil samples were esterified by adding 1 ml of 0.5 M methanol–NaOH at room temperature and mixed well by vortexing for a few seconds. Further, the samples were heated at 65 °C for 10 min with intermediate shaking. The mixture was then allowed to cool at room temperature. Further, to the mixture, 1 ml of deionized H_2O (18 MΩ resistivity) was added followed by the addition of 1 ml of hexane and the mixture was vortexed for 1–2 min. The mixture was then rested for 2–5 min at room temperature for layer separation. The upper hexane layer containing fatty acid methyl esters (FAME) was transferred to a clean and fresh GC vial and subjected to chromatographic separation followed by mass spectral detection as described below.

Separation of Fatty Acids by Gas Chromatography and Analysis Using Mass Spectrometry (MS) Coupled with GC

The samples were separated using an Agilent 7890B gas chromatography instrument and analyzed using an Agilent 5977B MSD detector (Agilent, USA) coupled to the GC. The instrument run conditions were as follows: For separation, 1 µl of the sample was injected with a split ratio of 50:1 using an Agilent autosampler into the sample inlet port which temperature was set at 120 °C (Ramya et al., 2021). The separation was held in HP-88 capillary column having 100 m length × 0.250 mm width and 0.20 µm film (Agilent J&W, USA) under the following conditions (David et al., 2005). Helium was used as the carrier gas at a constant flow rate of 1 ml min^{-1} (Prakash Shyam et al., 2021a; Balavigneswaran et al., 2020). The initial oven temperature was set at 120 °C with a hold time of 1 min followed by ramping the temperature to 175 °C at 10 °C/min ramp rate, at 175 °C. The instrument was kept on hold for 10 min. Again, the temperature was ramped to 210 ºC at 5 ºC/min ramp rate and kept on hold for 5 min (Ramya et al., 2019). Finally, the temperature was increased to 230 °C at 5 °C/min ramp rate and kept on hold for 5 min. For fatty acid analysis, the separated compounds were injected directly into the 5977B MSD detector where the compounds were fragmented using the electrospray ionization (EI) technique. The fragment ions were detected by

scanning the ion fragments (m/z) between m/z 50 and 500 (Prakash Shyam et al., 2021a, b; Rajkumar et al., 2018). The total ion chromatogram (TIC) was used to calculate the area sum percentage of individual compounds. The relative quantification of fatty acids was obtained by calculating the area under the curve (AUC) of a particular fatty acid peak in TIC relative to the total fatty acids present in the mixture. The solvent peak was excluded during the quantification process.

Quality Assurance (QA) and Quality Control (QC) Measures

Reagent (matrix) blank (RB) was prepared by an aliquot of analyte (oil) free matrix without the addition of internal standard and subjected to a similar extraction procedure. The RB was used to determine the absence of co-eluting peaks. Negative control (NC) was prepared by using an analyte-free matrix along with the addition of internal standards. The mixture was subjected to a similar FAME extraction procedure. This was injected immediately after the RB and is re-injected after the highest standard (100%) to determine the amount of analyte retained in the system from the prior injection (carryover). Positive controls (PC) were prepared by an aliquoting analyte-free matrix with the addition of internal standard and known amounts of analyte from the working standard solutions (20–80%). The mixture was subjected to the extraction procedure. The injection sequence was as follows: solvent wash, RC, NC: low standard (0%): standards ranging from 20–80%: high standard (100%), NC, RB, and PC. In addition, during this sequence, NC was injected (inserted) after every 5 samples, and PC was injected (inserted) after every 10 samples. The run ended with a PC injection. Care was taken to ensure that the retention time of the analyte and the internal standard peaks were within 2% of the retention time of the same compound in a PC sample run on the same day. The mass axis of the MS instrument was calibrated using Perfluorotributylamine (PFTBA) as the internal standard and then analyzed using a multi-component standard (Supelco 37-component FAME mix) that composed of the mixture of known concentration of fatty acids in dichloromethane (DCM) solvent. We were unable to resolve C20:3n3, C22:1n9, and C20:4n6 any further presentee in the Supelco 37-component FAME mix: those peaks were left unresolved since our matrix (coconut oil and PK) majorly consists of saturated fatty acid and our primary focus was C14/C18:1. There was a shift in the retention time (RT) between the Supelco 37-component FAME mix and test samples (FAME) because there were significant differences in the actual composition of fatty acids. Although retention time locking (RTL) can be employed, we had not used RTL to understand the actual influence of GC parameters.

Statistical Analysis

The data obtained from the experiments were statistically analyzed and presented graphically using Origin® 8.5 pro software, *R* programming, and MATLAB R2019a running on Windows® 7 platform. GraphPad Prism v6 was used to test the statistical differenceusing one-way ANOVA . The values were expressed as mean ± sd. Bland–Altman correlation analysis was performed (using MATLAB R2019a) for method validation.

Results

Fatty Acid Profiling of Coconut Oil and Palm Kernel Oil Using Gas Chromatography Coupled with Mass Spectrometry (GC–MS)

From the illustrative GC–MS total ion chromatogram (TIC) of coconut oil, palm kernel oil, and blended coconut-palm kernel oil (Fig. 1), it is well observed that both the coconut oil (Fig. 1a) and palm kernel oil (Fig. 1b) have the similar fatty acid profile. Notably, blending these two oils (coco-PK BEVO) shows a profile similar to that of coconut oil (Fig. 1c). Thus, at this point, from Fig. 1, it is clear that the proportion of the mono-oils cannot be determined in the BEVO admixture prepared by mixing these two oils owing to their identical fatty acid profiles. To address the problem, we started with analyzing the fatty acid profile of individual mono-oils viz coconut and PK oil. First, we calculated the percentage of occurrences of individual fatty acids relative to the total fatty acid present in coconut oil ($n = 34$) and palm kernel oil ($n = 121$) individually from the GC–MS chromatogram. We had tabulated the GC–MS reference range for the fatty acids present in coconut oil, palm kernel oil, and coco-PK blended oil (Table 1).

Second, we had analyzed the percentage occurrence of fatty acid relative to total fatty acids present in the mono-oils using a histogram and scatter plot. The histogram of coconut oil indicates that a majority of the fatty acids (exempting caproic (C6:0), stearic (C18:0), and linoleic C18:2) had a prominent mound in the center and tapering profile from the left to the right (Fig. 2a). Unimodal occurrences of the fatty acids present in the coconut oil were identified from the normal distribution curve peak. Of note, C6:0, C18:0, and C18:2 showed a left-skewed histogram with more gradual tapering towards the left side of the plot. Third, we used scatter plots to study the relationships between the fatty acids present in coconut oil. The dots in a scatter plot show the pattern of percentage

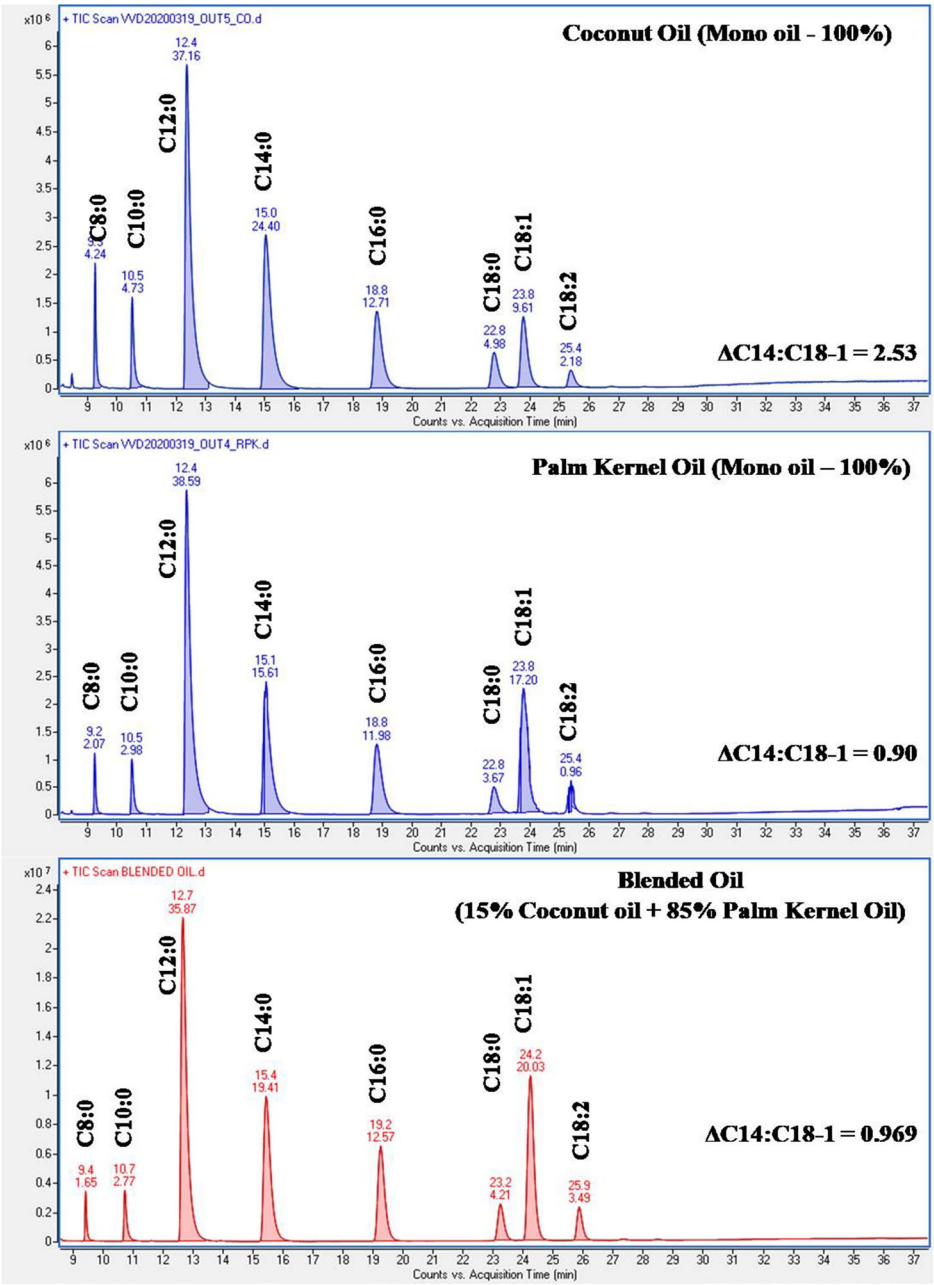

Fig. 1 Illustrative GC–MS total ion chromatogram (TIC) of the fatty esters obtained from (a) coconut oil (b) palm kernel oil (c) blended coconut and palm kernel oil (coconut 15% and palm kernel oil 85%)

proportion of fatty acid when the data are taken as a whole. Figure 2b shows the scatter plot of the occurrences and the correlation of individual fatty acid to other fatty acids present in the coconut oil ($n = 34$). Among the fatty acids present in the coconut oil, caproic acid (C6:0) had a strong, positive, and linear relationship with caprylic (C8:0) and capric acid (C10:0). The other fatty acids were not much correlated to caproic acid while palmitic acid (C16:0) exhibited a strong positive correlation with stearic (C18:0), oleic (C18:1), and linoleic (C18:2) acid. Interestingly, lauric acid (C12:0) showed a moderate, negative linear correlation with palmitic (C16:0), stearic (C18.0), oleic (C18:1), and linoleic (C18:2) acid. Lauric acid and myristic acid were not correlated significantly. On the contrary, myristic acid exhibited minimal correlation to palmitic (C16:0), stearic (C18:0), oleic (C18:1), and linoleic (C18:2) acid.

Similar to coconut oil, we studied the palm kernel mono-oil viz (a) calculated the percentage of occurrences of individual fatty acids relative to the total fatty acid present in palm kernel oil ($n = 121$) using GC–MS chromatogram (Table 1), (b) studied the percentage occurrences of various fatty acids relative to total fatty acid present in palm kernel oil using a histogram (Fig. 3a), and (c) performed the

Table 1 Percentage occurrences of various fatty acids present in coconut oil ($n=121$), palm kernel oil ($n=121$), and BEVO (coconut oil 80% + palm kernel oil 20%) ($n=6$) relative to the total fatty acids detected by the GC–MS analysis

Fatty acid	Coconut oil			Palm kernel oil			BEVO (coconut oil 80% + Palm kernel oil 20%)		
	Mean		s.d	Mean		s.d	Mean		s.d
C6:0	0.29	±	0.10	0.08	±	0.15	0.131	±	±0.01
C8:0	5.49	±	0.66	2.38	±	0.91	3.667	±	±0.31
C10:0	5.47	±	0.55	3.17	±	0.72	4.641	±	±0.05
C12:0	38.23	±	1.77	36.55	±	3.69	38.25	±	±0.27
C14:0	23.19	±	0.98	18.55	±	1.55	22.632	±	±0.15
C16:0	11.78	±	0.63	12.10	±	1.18	12.238	±	±0.18
C18.0	4.24	±	0.26	3.75	±	0.71	4.299	±	±0.22
C18:1	9.05	±	0.60	20.09	±	2.55	11.867	±	±0.22
C18:2	1.94	±	0.20	3.36	±	1.62	2.275	±	±0.04

correlation of various fatty acids among themselves present in palm kernel oil using a scatter plot (Fig. 3b). The analysis of PK histogram showed a prominent mound in the center with an exception to caproic (C6:0) acid. Unimodal occurrences of the fatty acids were identified from the normal distribution curve peak similar to coconut oil. Caproic acid (C6:0) alone showed a right-skewed histo-profile (Fig. 3a). The scatter plot was used to study the correlation of individual fatty acids to other fatty acids present in the palm kernel oil ($n=121$). Results indicate a strong, positive, and linear correlation of C8:0 with C10:0. C12:0 had a perfect negative linear correlation with C18:0. The other fatty acids had either weak correlation to each other or not correlated (Fig. 3b). From the data, it is apparent that both coconut oil (Fig. 4a) and palm kernel oil (Fig. 4b) have a similar fatty acid profile and their discrimination by conventional method is least possible.

Comparison of the Fatty Acid Profile of Coconut and Palm Kernel Oil by Cluster Analysis and GC-MS

To observe any possible variation in the profile of coconut and palm kernel oil, we subjected the complete set of data for the cluster analysis (coconut oil: $n=36$ and palm kernel oil: $n=121$). The objective is to organize the observations (fatty acid occurrences) that are close together and separate them into groups. The results of cluster analysis were graphically represented as a heat map for coconut oil in Fig. 4c and palm kernel oil in Fig. 4d. We assumed that the fatty acid from the coconut oil will cluster into one group and the fatty acids from the palm kernel will cluster into a distinct group. However, the data clustered with the overlapping fatty acids derived from the coconut and palm kernel oil. Very few samples were distributed between the bottom and top quartile and there is neither a major deviation in the fatty acid profile among coconut and palm kernel oil nor there were any groupings of fatty acids. To address this issue and to differentiate coconut oil and palm kernel oil by GC–MS analysis, we employed a workaround using the ratio between myristic acid (C14:0) to Oleic acid (C18:1). We found out that the ratio of C14:0/C18:1 for coconut oil was 2.57 ± 0.23. Similarly, the ratio between C14:0 and C18:1 for palm kernel oil was 0.9 ± 0.14. Hence, the C14:0 to C18:1 ratio may yield the direct discrimination of coconut and palm kernel mono-oils.

If the ratio of C14:0/C18:1 was 2.57 ± 0.23, then the oil understudy is coconut oil; on the contrary, if the ratio of C14:0/C18:1 was 0.9 ± 0.14, then the oil may be palm kernel oil. Thus far, iodine value (WIJS) is the only gold standard for the discrimination of coconut and palm kernel oil; the coconut oil iodine value ranges from 6–11 (Young, 1983) (FSSAI statutory limit is 7.5–9), whereas, for palm kernel oil, the iodine value ranges from 13–23 (Young, 1983) (FSSAI statutory limit is 10–23). Other than iodine value, Polenske value (PV) may yield marginal information about the nature of oil. The PV ranges from 13–18 and 8–12 for coconut oil and palm kernel oil, respectively (Young, 1983). Therefore, C14:0/C18:1 could be an additional advantage in the discrimination of oil by the GC–MS method without the need for conventional titration experiments. However, if the oils were blended as in the case of coco-PK BEVO, then will the C14:0/C18:1 ratio help discriminate the mono-oil proportion in BEVO remains an open question.

Determination of Mono-Oil Proportion in the Coco-PK BEVO Using C14:0/C18:1 ratio and Method Validation Using Bland–Altman Analysis

To understand the change in the C14:0/C18:1 ratio in BEVO, we performed the blending of coconut oil with palm kernel oil to yield coco-PK BEVO at various proportions. The samples were subjected to GC–MS analysis to find their fatty acid proportion including C14:0 and C18:1. The ratio between C14:0 and C18:1 was calculated and a standard graph was plotted using polynomial regression

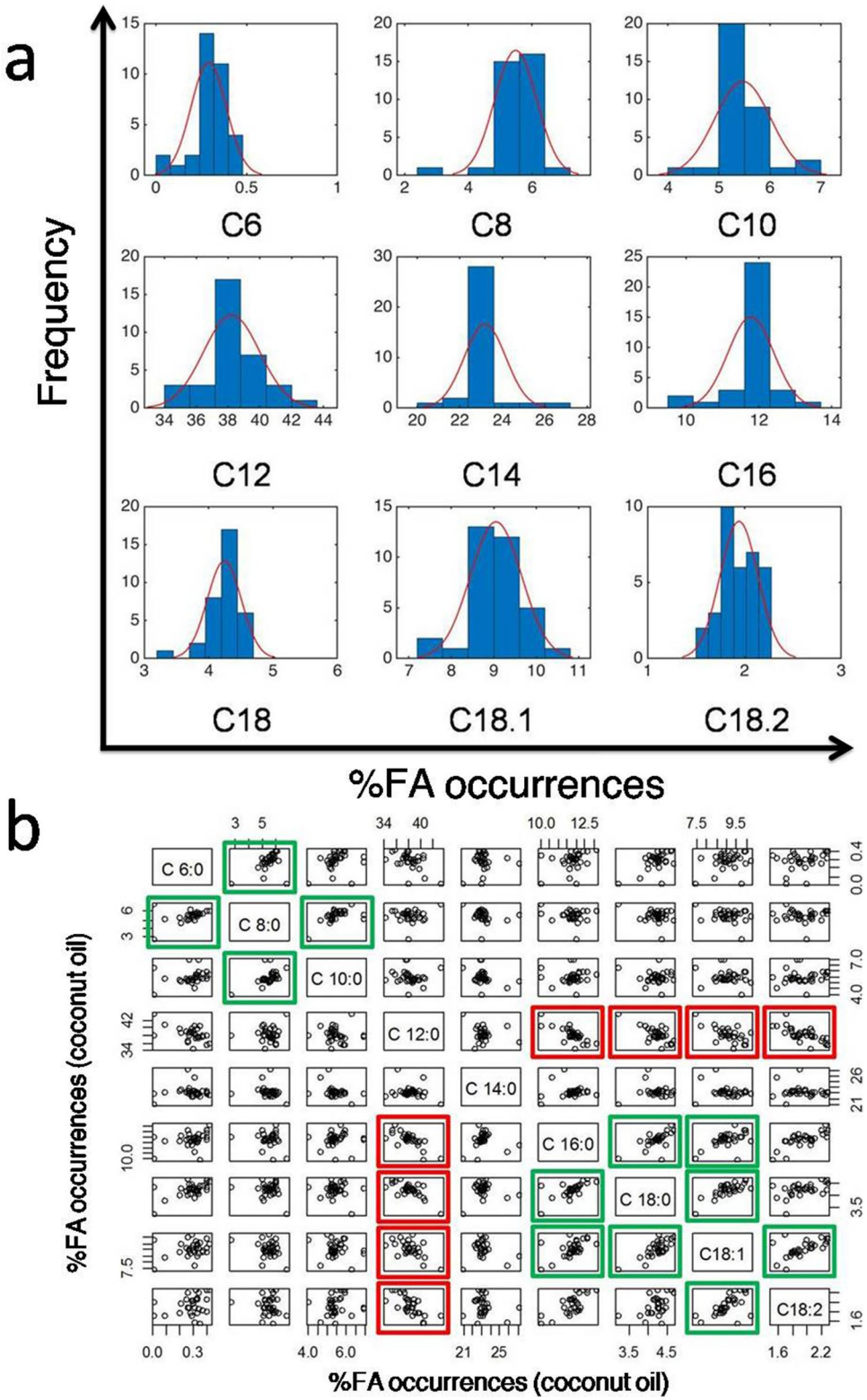

Fig. 2 **a** Histogram of the occurrences of various fatty acids present in coconut oil. **b** Scatter plot of the occurrences of various fatty acids detected by GC–MS analysis and their correlation to other fatty acids present in the coconut oil ($n = 34$)

model ($R^2 = 0.98$) and linear regression model ($R^2 = 0.86$) (Fig. 4e). The coefficient of determination ($R^2 = 0.98$) for polynomial model fits very well, whereas the coefficient of determination for liner model is appreciable ($R^2 = 0.86$) (Ellick et al., 2021). For the testing purpose, BEVO test samples were prepared and blinded to the analyst performing the GC–MS. The analyst determined the mono-oil proportion in the BEVO using the standard graph. We then compared the

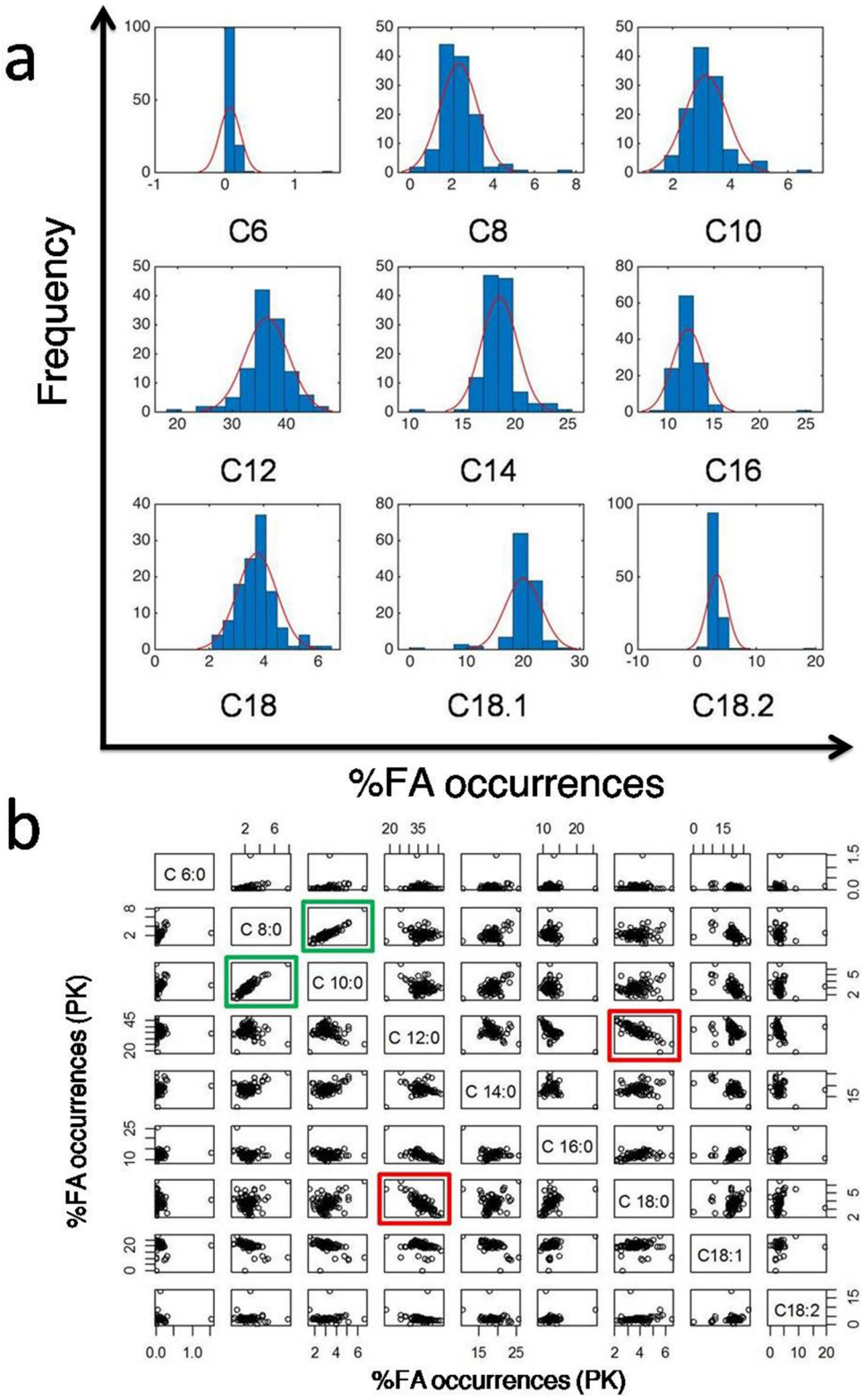

Fig. 3 **a** Histogram of the occurrences of various fatty acids present in palm kernel oil. **b** Scatter plot of the occurrences of various fatty acids detected by GC–MS analysis and their correlation to other fatty acids present in the palm kernel oil ($n = 121$)

actual values against the experimentally determined values using a correlation plot (Fig. 4e). The samples within the range of 30, 40, 50, 60, 70, and 80% coconut oil in BEVO were detected with a near accuracy. However, the sample population that had coconut oil below 20% and greater than 80% deviated significantly from the actual value. Of note, as per the FSSAI regulations, blending of two edible oils is permitted, provided the proportion by weight of edible

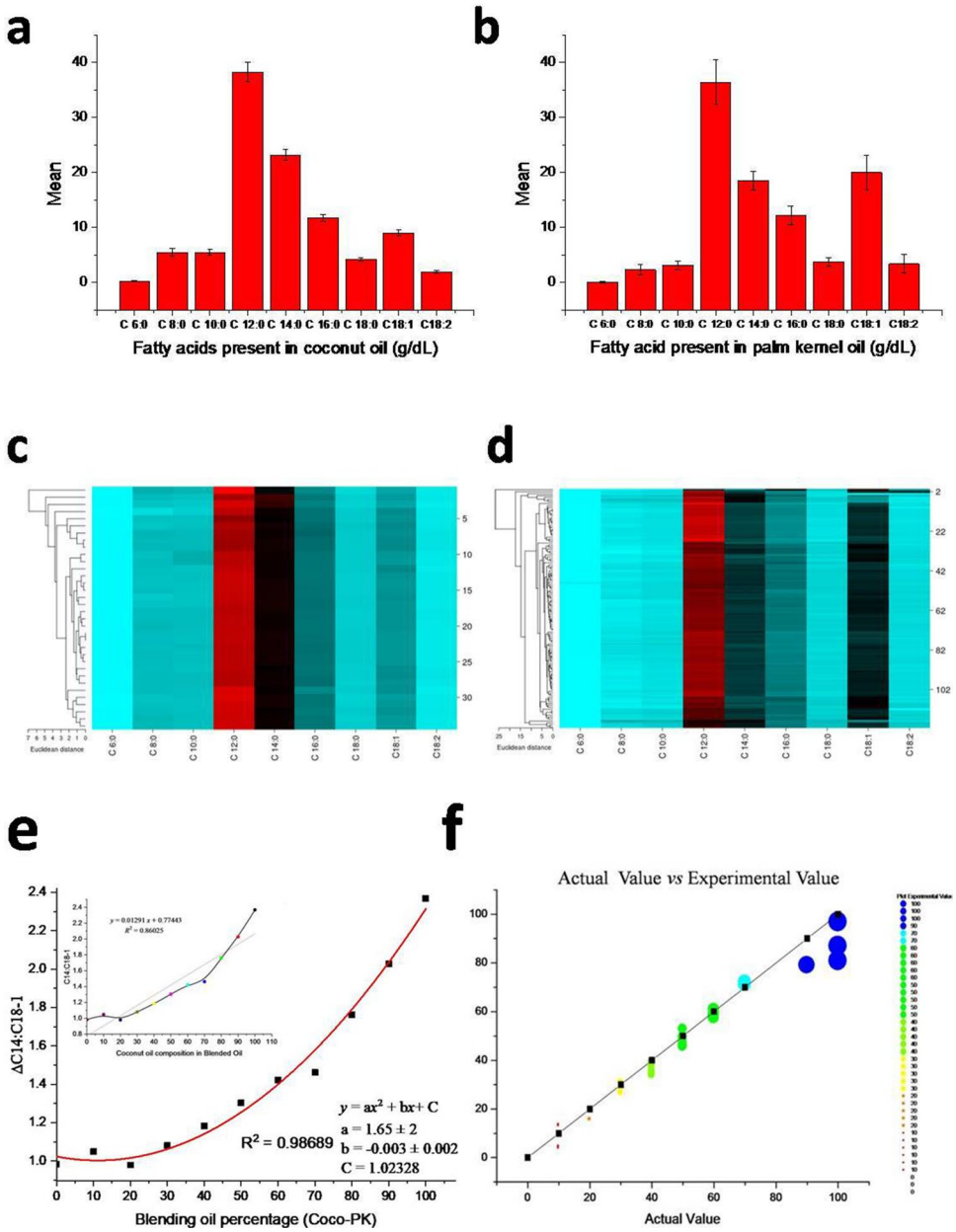

Fig. 4 **a** Bar diagram showing mean values of individual fatty acids present in coconut oil. **b** Bar diagram showing mean values of individual fatty acids present in palm kernel oil. **c** Heat Map showing the Euclidian distance after correlation in coconut oil, matrix alignment $n \times p$, keeping fatty acid position (p) unaltered. **d** Heat Map showing the euclidian distance after correlation in palm kernel oil, matrix alignment m x p, keeping fatty acid position (p) unaltered. **e** Calibration plot of C14:0/C18:1 for determination of mono-oil proportions in coconut-palm kernel BEVO [X-axis — the percentage of coconut oil in BEVO (coconut-palm kernel blended edible vegetable oil), Y-axis — ratio between C14:0 and C18:1 obtained by measuring the area sum percent of C14 and C18-1 using GC-MS ($\boldsymbol{n} = 9$, values are expressed as mean; standard deviation was calculated but not included for calculating the ratio)]. **e**. Standard graph plotting actual values of coconut oil in BEVO **vs** experimentally calculated value of coconut mono-oil using the C14:0/C18:1 standard curve using polynomial regression model ($\boldsymbol{R}^2 = 0.98$) (inset: linear regression model, $\boldsymbol{R}^2 = 0.86$). **f**. Correlation plot showing the actual value **vs** experimental value of coconut oil proportion value in coco-PK blended oil

vegetable oil used in the blending process is not less than 20 percent. Therefore, the present method is considered suitable for determining the mono-oil proportion in coco-PK BEVO.

Validation of a new measurement method for an application requires comparison with gold standard techniques. Since the gold standard method is not available for determining the mono-oil proportion in coco-PK BEVO, herein, we used known proportions of coco-PK BEVO as a reference value. We performed GC–MS analysis for the blended samples (blinded to analyst) ($n = 39$) in triplicates and determined the proportion of mono-oil using the experimental (C14/18:1 ratio) method. Results indicate a very good agreement of results between actual and experimental values (Fig. 5a). Further, we quantified the difference between measurements using the Bland–Altman method (Bunce, 2009). The Bland–Altman plot showed the mean bias ($\pm\alpha$) between actual and experimental results for coco-PK percentage determination as 2.60 ($\pm$ 1.96), and the limits of agreement (LOA) were 8.58 and − 3.38 (Fig. 5b). LOA estimates the interval within which a proportion of the differences between the two methods lies. The LOA includes both systematic (bias) and random error (precision), thus providing a useful measurement for comparing the likely differences between individual results measured by two methods (Martin Bland and Altman, 1986). In our case, one is the actual value (used instead of the gold standard) and the other is the experimentally determined value using C14:0/C18:1 ratio. The mean bias

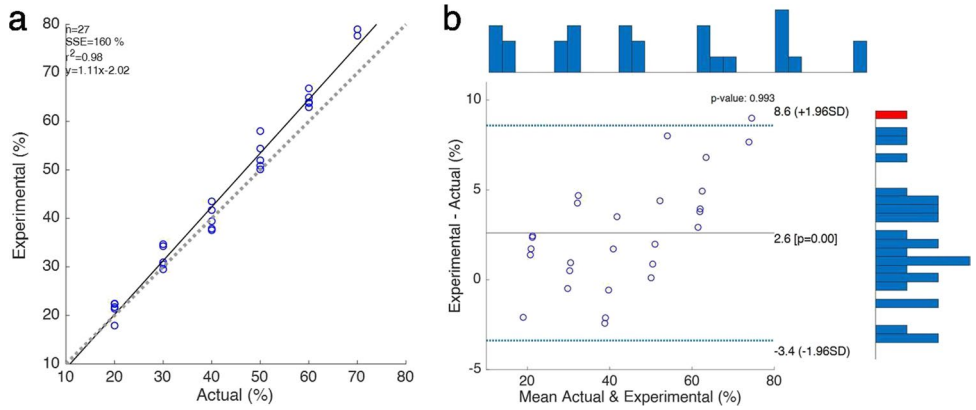

Fig. 5 Mono-oil determination in coco-PK BEVO using C14:0/C18:1 method validation. **a** Correlation of coconut oil proportion actual *vs* experimentally determined using C14:0/C18:1 ratio. **b** Bland–Altman plots of coconut mono-oil measurements. The horizontal black line shows the mean of the differences (=bias) between the two methods (mean bias = 2.6%), and the doted blue horizontal lines show the upper and lower 95% limits of agreement (=bias ± 1.96 × SD) (LOA were 8.6 and − 3.4%). R^2 (the goodness of fit) = 0.98 indicates the robustness of the method

of 2.58% indicates the robustness of the method. Thus, we suggest that C14:0/C18:1 ratio can be employed in the routine analysis to detect the proportion of coconut oil in coco-PK BEVO.

Discussion

The prevention of premature mortality is thought to be achievable by an improvement in dietary quality at a global level (Wang et al., 2019). In-country like India, the major contributors to the low score in the alternate healthy eating index (AHEI score = 38.2; global mean = 50; global maximum = 94) are majorly due to the low consumption of fruit, vegetables, whole grains, and ω–3 fatty acids as well as high *trans* fat intakes (Damerau et al., 2020). The low AHEI index entails the need for the decisive alignment of agricultural policy with nutrition policy at the global and country levels (Wang et al., 2019). In this context, FSSAI has set the limit to the industries to reduce the levels of trans fats to < 2% by 2022 in hydrogenated vegetable oils. In addition, BEVO has been suggested as a convenient alternative to edible oil rotation to better balance saturated/unsaturated fatty acids than a single oil. However, in terms of consumer acceptability, BEVO should have improved sensorial/culinary attributes, a better frying medium, and improved shelf life. Many countries, including India, Kenya, Canada, Russia, and Pakistan, have developed standards and guidelines for BEVO. Nevertheless, there are several challenges in enforcing the standards and guidelines prescribed.

For instance, coconut oil has a very high content of lauric acid (42–54 g/dL, determined using GC-FID) and tocopherol content (29 mg/kg) (Bhatnagar et al., 2009). Of note, the medium-chain fatty acids of coconut oil are easily absorbed into the body. Consuming virgin coconut oil daily increases 'good' cholesterol (high density lipoprotein, HDL) level in blood (Chinwong et al., 2017). Thus, coconut oil improves blood circulation by maintaining a good LDL (low density lipoprotein) to HDL and TG (triglyceride) to HDL ratio, blends of coconut oil with other vegetable oils were strongly suggested to maximize the health benefit (Hernandez, 2016). Furthermore, coco-PK BEVO has improved sensorial/culinary attributes. Interestingly, PK has a fatty acid profile almost similar to the coconut oil. It is currently unclear, how two oils with identical fatty acid profiles have different health benefits. The health effects of the major component lauric acid (C12.0) are still being investigated at large. Commercially, blending PK with coconut oil would have a cost advantage, since the cost of PK is much lower than the coconut oil. Therefore, in BEVO presenting coco-PK at 80–20 proportions, consumers and vigilant agencies should ensure the mono-oil proportions to ensure the consumer benefit. Thus far, the iodine value is the only method available to discriminate coconut oil and PK. However, the determination of iodine value is time-consuming and susceptible to error owing to the manual titration method, inter-lab testing solution, and strength variation. Moreover, the iodine value cannot yield the exact percentage of mono-oil proportion in BEVO. Alternatively, GC–MS could be used to calculate the individual percentage proportion of mono-oil in the blended oil. Nonetheless, the GC–MS approach would be feasible only if a mono-oil in the blended oil mixture has the presence of a unique fatty acid that could be separated by a chromatographic method. For example, safflower seed oil shows 67.8–83.2 g/dL C18:2 (linoleic acid), whereas palm stearin has only 3.0–10.0 g/dL. On the other hand, palm stearin shows 48.0–74.0 g/dL C16:0 (palmitic acid), whereas safflower seed oil has C16:0 in the range of 5.3–8.0 g/dL.

When safflower seed oil and palm stearin are blended, the percentage increase in the C16:0 can be used to monitor the percentage of palm oil (mono) in the blended oil. If the BEVO had two mono-oils with identical fatty acid proportions such as coco-PK, then the method of detection will become challenging. For example, if a manufacturer markets a coco-PK BEVO in 80% coconut and 20% PK proportion, how would the consumer or the consumer protection agencies such as FSSAI can ensure that the packaged BEVO is indeed 80% coconut and 20% PK? The lack of an efficient testing method to determine the proportion of mono-oils is a major limitation. In that case, the C14:18–1 ratio method described in this manuscript can be used to determine the mono-oil proportion in coco-PK BEVO. Previous researches have explored different techniques that include the use of triacylglycerols for the quantification of olive oil in blends with vegetable oils using high-performance liquid chromatography (HPLC) coupled to a charged aerosol detector (CAD) (De La Mata-Espinosa et al., 2011), FTIR-ATR, Raman spectroscopy (Jiménez-Carvelo et al., 2017), and three-dimensional fluorescence spectra using a cluster analysis coupled with Quasi-Monte Carlo integral approach (Xu et al., 2016). Of note, FTIR spectroscopy combined with an attenuated total reflectance (ATR) and chemometric software has emerged as rapid non-destructive and reliable techniques for the authentication analysis. Although the technique is most widely used for the determination of authenticity and adulteration purpose, it is more challenging in the determination of mono-oil proportion in samples with identical fatty acid fingerprints such as coco-PK BEVO. In addition, it has to be noted that FTIR and Raman spectra in a matrix containing multiple analytes are complex; therefore, chemometrics (a sophisticated statistical technique) has to be used for quantitative analysis purposes. Conversely, C14/C18:1 ratio obtained by GC–MS or GC-FID instruments can be readily employed. Similarly, the samples are destructed during the testing process; hence, online monitoring is not feasible. However, this method is pitched mostly for the vigilant agencies, testing laboratories, and edible oil manufacturing industries, especially for those procuring oil from external providers in a blended state and repacking them under a brand name. Hence, the benefits oust the cost factor.

Conclusion

To conclude, we have provided the reference range of fatty acid occurrences in coconut oil and palm kernel oil relative to the total fatty acid content. Further, we have provided a procedure to identify the blending proportions of coconut oil in coco-PK BEVO using the C14:0/C18:1 ratio value obtained from GC–MS. The C14:0/C18:1 ratio obtained by GC–MS may be used in conjunction with iodine and Polenske value obtained from titration experiments to vigil the actual proportion of coconut oil and palm kernel oil in coco-PK BEVO that are commercially sold.

Acknowledgements V. Ramya greatly acknowledges the Department of Science and Technology–Science and Engineering Research Board (DST–SERB) India, Confederation of Indian Industry (CII), and V.V.D & Sons Private Limited, Thoothukudi, Tamil Nadu for DST (SERB)-CII Prime Minister Research Fellowship (PMRF/April-2018). VR sincerely thanks her DC members Dr. S. Achiraman, Department of Environmental Biotechnology, and Dr. S. Shanmugapriya, Department of Biomedical Science, Bharathidasan University for their continuous support, encouragement, and guidance during the course of this research. VR would like to show her gratitude to Dr. R. Thirumurugan, Professor and Head, Department of Animal Science, Dr. K. Emmanuvel Rajan, Professor, Department of Animal Science, and Dr. Mahesh Kandasamy, UGC Assistant Professor (under FRP), Department of Animal Science, Bharathidasan University for their continuous support, encouragement, and guidance during the course of this research. VR acknowledges the moral support and help rendered by her fellow lab mates Ms. Sathiya-Priya Krishnamoorthy and Mr. Vignesh Kalimuthu. The authors greatly acknowledge the instrument facilities at Department of Animal Science established by Department of Science and Technology, Government of India, under DST-Promotion of University Research and Scientific Excellence (PURSE) Scheme-Phase II, Rashtriya Uchchatar Shiksha Abhiyan (RUSA)-2.O.

Author Contribution VR, KPS, and BK designed the experiment. VR was the lead investigator. VR and KPS performed the experiments, collected the data, performed the data analysis. VR, KPS, and BK interpreted the results. BK supervised the project, provided critical suggestions and channelized the research direction. All the authors contributed in writing the manuscript and shaping the research.

Funding V. Ramya is a recipient of Prime Minister Fellowship through DST(SERB)-CII Prime Minister's Fellowship Scheme for Doctoral Research (April 2018–Batch 8). The financial support is provided by the DST (SERB), India and V.V.D & Sons Private Limited, Thoothukudi, Tamil Nadu through Confederation of Indian Industry (CII).

Data Availability The data that support the findings of this study are available from the corresponding author (BK) upon request.

Declarations

Ethics Approval Ethical approval not applicable

Consent to Participate Informed consent not applicable

Conflict of Interest Mrs. Venkatesan Ramya is a recipient of DST (SERB)-CII Prime Minister Research Fellowship award (April 2017). The financial support was provided by the DST (SERB), India and V.V.D & Sons Private Limited, Thoothukudi, India. V.V.D & Sons Private Limited is one of the leading coconut oil manufacturer companies in India.

References

Balavigneswaran CK, Venkatesan R, Shyam Karuppiah P, Kumar G, Paliwal P, Krishnamurthy S, Kadalmani B, Kumar Mahto S, Misra N (2020) Silica Release from Silane Cross-Linked Gelatin Based Hybrid Scaffold Affects Cell Proliferation. ACS Applied Bio Materials 3(1):197–207. https://doi.org/10.1021/acsabm.9b00680

Bhatnagar AS, Prasanth Kumar P, Hemavathy J, Gopala Krishna A (2009) Fatty acid composition, oxidative stability, and radical scavenging activity of vegetable oil blends with coconut oil. J Am Oil Chem Soc 86(10):991–999

Bunce C (2009) Correlation, agreement, and Bland-Altman analysis: statistical analysis of method comparison studies. Am J Ophthalmol 148(1):4–6

Chinwong S, Chinwong D, Mangklabruks A (2017) Daily Consumption of Virgin Coconut Oil Increases High-Density Lipoprotein Cholesterol Levels in Healthy Volunteers: A Randomized Crossover Trial. Evid. Based Complement Alternat Med 2017:7251562. https://doi.org/10.1155/2017/7251562

Clandinin M, Cheema S, Field C, Garg M, Venkatraman J, Clandinin T (1991) Dietary fat: exogenous determination of membrane structure and cell function. FASEB J 5(13):2761–2769

Damerau K, Davis KF, Godde C, Herrero M, Springmann M, Bhupathiraju SN et al (2020) India has natural resource capacity to achieve nutrition security, reduce health risks and improve environmental sustainability. Nat Food 1(10):631–639. https://doi.org/10.1038/s43016-020-00157-w

David F, Sandra P, Vickers AK (2005) Column selection for the analysis of fatty acid methyl esters. Food analysis application. Palo Alto, CA: Agilent Technologies, 19: 19

De La Mata-Espinosa P, Bosque-Sendra J, Bro R, Cuadros-Rodríguez L (2011) Olive oil quantification of edible vegetable oil blends using triacylglycerols chromatographic fingerprints and chemometric tools. Talanta 85(1):177–182

Ellick S, Wicking C, Hancock T, Whitmarsh S, Arthur CJ, Gates PJ (2022) Chemometric approachesto resolving base oil mixtures. Rapid Commun Mass Spectrom 36(1):e9214. https://doi.org/10.1002/rcm.9214

German JB, Dillard CJ (2006) Composition, structure and absorption of milk lipids: a source of energy, fat-soluble nutrients and bioactive molecules. Crit Rev Food Sci Nutr 46(1):57–92

Hernandez EM (2016) Specialty oils: functional and nutraceutical properties. In TAB Sanders (ed) In Woodhead Publishing Series in Food Science, Technology and Nutrition, Functional Dietary Lipids (pp. 69–101). Woodhead Publishing. https://doi.org/10.1016/B978-1-78242-247-1.00004-1

Jiménez-Carvelo AM, Osorio MT, Koidis A, González-Casado A, Cuadros-Rodríguez L (2017) Chemometric classification and quantification of olive oil in blends with any edible vegetable oils using FTIR-ATR and Raman spectroscopy. LWT 86:174–184

Kumar S (2020). Enforcement and surveillance drive to ensure availability of blended edible vegetable oil (BEVO) with AGMARK certification. FSSAI Bulletin, New Delhi, India., 1–3. https://www.fssai.gov.in/upload/advisories/2020/06/5ef9ae16065c9Letter_BEVO_AGMARK_29_06_2020.pdf. Accessed 01 June 2021

Maindola A (2019) FSSAI aiming to reduce hydrogenated trans fats in Indian food to 2%. FSSAI Bulletin, New Delhi, India., 1–2. https://www.fssai.gov.in/upload/media/FSSAI_News_Transfat_FNB_03_01_2019.pdf

Martin Bland J, Altman D (1986) Statistical methods for assessing agreement between two methods of clinical measurement. Lancet 327(8476):307–310. https://doi.org/10.1016/S0140-6736(86)90837-8

Namiki M (1995) The chemistry and physiological functions of sesame. Food Rev Intl 11(2):281–329

Prakash Shyam K, Rajkumar P, Ramya V, Sivabalan S, Kings AJ, Monisha Miriam LR (2021a) Exopolysaccharide production by optimized medium using novel marine Enterobacter cloacae MBB8 isolate and its antioxidant potential. Carbohydrate Polymer Technologies and Applications 2:100070. https://doi.org/10.1016/j.carpta.2021.100070

Prakash Shyam K, Rajkumar P, Ramya V, Monisha Miriam LR (2021b) Biorefining Polyvinyl Alcohol (PVA) by Enterobacter cloacae and its Polyhydroxy Butyrate (PHB) Production Ability. Industrial Biotechnology 17:92–99. https://doi.org/10.1089/ind.2020.0039

Rajkumar P, Venkatesan R, Sasikumar S, Ramprasath T, Shyam Karuppiah P, Ramu A, Sadasivam Selvam G (2018) Characterization of agarolytic enzymes of Arthrobacter spp. AG-1 for the whole cell conversion of agar into 36-anhydro-α- l -galactose in one pot. Process Biochem, pp 6952–63. https://doi.org/10.1016/j.procbio.2018.03.017

Ramya V, Madhu-Bala V, Prakash-Shyam K, Gowdhami B, Sathiya-Priya K, Vignesh K, Vani B, Kadalmani B (2021) Cytotoxic activity of Indigofera aspalathoides (Vahl.) extracts in cervical cancer (HeLa) cells: Ascorbic acid adjuvant treatment enhances the activity.Phytomedicine Plus 1(4)100142. https://doi.org/10.1016/j.phyplu.2021.100142

Ramya V, Prakash Shyam K, Arumugam G, Kadalmani B (2019) Antifungal Activity by Inhibiting Ergosterol Biosynthesis in Aspergillus niger ATCC 16888. Proceedings of the National Academy of Sciences, India Section B: Biological Sciences 89:173–184. https://doi.org/10.1007/s40011-017-0930-4

Silva JR, Burger B, Kühl CMC, Candreva T, Dos Anjos MBP, Rodrigues HG (2018) Wound healing and omega-6 fatty acids: from inflammation to repair. Mediators Inflamm 2018:2503950–2503950. https://doi.org/10.1155/2018/2503950

Simopoulos AP (2016) An increase in the omega-6/omega-3 fatty acid ratio increases the risk for obesity. Nutrients 8(3):128–128. https://doi.org/10.3390/nu8030128

Swanson D, Block R, Mousa SA (2012) Omega-3 fatty acids EPA and DHA: health benefits throughout life. Adv Nutr 3(1):1–7

Wang DD, Li Y, Afshin A, Springmann M, Mozaffarian D, Stampfer MJ et al (2019) Global improvement in dietary quality could lead to substantial reduction in premature death. J Nutr 149(6):1065–1074. https://doi.org/10.1093/jn/nxz010

Xu J, Liu X-F, Wang Y-T (2016) A detection method of vegetable oils in edible blended oil based on three-dimensional fluorescence spectroscopy technique. Food Chem 212:72–77

Yin W, Hewson L, Linforth R, Taylor M, Fisk ID (2017) Effects of aroma and taste, independently or in combination, on appetite sensation and subsequent food intake. Appetite 114:265–274

Young FVK (1983) Palm kernel and coconut oils: analytical characteristics, process technology and uses. J Am Oil Chem Soc 60(2):374–379. https://doi.org/10.1007/BF02543521