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Infrared Spectroscopy-Based Principal Component Analysis for Differentiation of Soaps Produced from Vegetable Oils and Animal Fats

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The soap-making process involves heating, encompassing various mechanisms such as oxidation and thermal reactions. These reactions can potentially modify the chemical structure of both animal and vegetable oils, making it challenging to trace their original sources in processed products. In this study, seven distinct bar soaps were produced using the saponification method, employing locally available commercial oils, including Canola Oil (LA), Coconut Oil (CN), Corn Oil (CO), Olive Oil (OV), Palm Oil (PO), Sunflower Oil (SF), and animal fat from Lard (LD). The chemical profiles of the raw lard and vegetable oils, as well as their respective soaps and the fats/oils extracted from the soap, were obtained using Attenuated Total Reflectance Fourier Transform Infrared (ATR-FTIR) spectroscopy, followed by analysis through a chemometric approach. Principal Components Analysis (PCA) was employed after pre-treatment of the FTIR dataset to discern their distribution in a score plot for determining clustering. For clustering lard from vegetable oils, the wavenumbers ranging from 1500 to 1000 cm^{-1} were selected in the PCA analysis. The extraction of fats/oils from the final product, coupled with the chemometric method, successfully demonstrated the differentiation of soaps produced from vegetable oils and animal fats commonly sold in the market.

Keywords: Lard; sodium fatty acid; saponification; chemometric; Fourier transform infrared spectroscopy

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The adulteration of pork, lard, and their derivatives in consumer products, including foods and cosmetics, poses a significant challenge to halal authority bodies and global food security. Product fraud often revolves around valuable ingredients with high nutritional content for human consumption, such as vegetable oils [1]. Lard, for instance, serves as an attractive substitute for the costly fats and oils frequently utilized in the production of consumer goods [2]. Consequently, verifying the presence of any illicit component, even in minute quantities, becomes imperative within consumer products.

Authenticity assessment is crucial for tracing unfamiliar ingredients' origins and combatting product fraud [3]. This assessment serves a dual purpose, as it establishes a product's authenticity and is critical in ensuring compliance with halal standards. Muslim consumers must exercise caution to avoid contaminated halal products containing questionable ingredients. This principle is highlighted in the narration attributed to Abu Abdullah Bin Nu'man B. Bashir (may Allah be pleased with him), who reported that he heard the Messenger of Allah, peace and blessings be upon him, saying, "and whoever falls into matters of ambiguity, he falls into illegal."

The physical and chemical characterization and the functional properties of oils largely depend on the variation and composition of triacylglycerol compounds [4]. Concurrently, compounds that contribute to sensory attributes encompass free fatty acids, tocopherols, phospholipids, sterols, monoacylglycerols, diacylglycerols, resins, pigments, and minor volatile compounds [4]. Many of these compounds have the potential to serve as 'fingerprints' in authenticity assessments for tracing adulteration.

Lard, known for its richness in saturated fatty acids, is readily accessible and cost-effective. High-quality lard is predominantly derived from the shoulder and back of the pig [6, 25]. Unsaturated Fatty Acids (UFA) found in high-quality lard comprise oleic (18:1), linoleic (18:2), and linolenic (18:3) acids.

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Meanwhile, Saturated Fatty Acids (SFA) include stearic (18:0) and palmitic (16:0) acids [5, 6]. UFA and SFA profiles of lard have been reported to resemble those of palm oil [7, 8]. In contrast, vegetable oils such as corn, canola, and sunflower are characterized by higher polyunsaturated acids and lower SFA content [9-12].

Furthermore, the chemical family of volatile compounds identified in lards includes sulphur, ketones, aldehydes, terpenes, and alcohols [5, 6]. Conversely, volatile aroma and flavour compounds like aldehydes, ketones, alcohols, and terpenes have been reported in sunflower, olive, and soybean oils [13]. Lard in consumer products is widely used for daily basics, including cooking, cleaning, and even fish feed production. Traditionally, vegetable oil is blended with lard for cooking, and this method has been suggested as beneficial for reducing body fat and other related diseases [9, 14]. Another potential use of blended lard with vegetable oil is soap production. Soap is one of the consumer products produced from the saponification process of oil fats with an alkali. The commonly used oils and fats in soap production include coconut oil, palm kernel oil, palm oil, sunflower oil, corn oil, and lard [15]. Based on chemical properties, soap is an anionic surface-active agent (surfactant) known as a salt of fatty acid. This process also produces glycerol as a side product.

Detecting the presence of lard as a minor component in highly processed products presents a considerable challenge. Glycerol, a byproduct of the saponification process, cannot be distinguished as originating from an animal or plant source. The application of DNA-based technology to detect lard in soap or cosmetic products is not feasible [16] except for cases involving cosmetic products that utilize pig-derived collagen or placenta as anti-ageing compounds [17].

The soap-making process involves heating and encompasses various mechanisms, including oxidation and thermal reactions. These reactions can potentially modify the chemical structure of both animal and plant fats [18-19]. Fourier Transform Infrared (FTIR) spectroscopy, coupled with chemo-metrics, has gained widespread use as an analytical tool for detecting product adulteration, such as identifying lard adulteration in edible oils and porcine gelatine in capsules [20-22]. Peaks and shoulders observed in FTIR spectra can be attributed to distinct functional groups, effectively serving as 'fingerprint' tools for specific fats or oils. These spectra are then analyzed through chemometric techniques, such as Principal Component Analysis (PCA) [23-26]. Consequently, the combination of FTIR spectral data and PCA applied to both raw and processed fats of animal and plant origins can reveal their metabolite 'fingerprint.' This information can serve as a reference point for authenticity assessment, aiding in the detection of adulteration.

Moreover, the physicochemical analysis of soaps, including assessments of hardness, pH, moisture content, total fat matter, total alkali, and foam ability, can contribute to identifying the source of oils used in soap production [27]. The physico-chemical characteristics of soap are influenced by multiple factors, encompassing the strength and purity of the alkali, the type of oil utilized, and the completeness of the saponification process. Such characteristics encompass moisture content, Total Fat Matter (TFM), pH, free caustic alkalinity, and chloride percentage [28]. Results from previous studies indicate that oils with distinct properties are discernible from each other due to differences in fatty acid composition [29]. Consequently, the objectives of this study aimed to elucidate and compare the chemical profiles of fatty acids in i) raw lard and vegetable oils, ii) soap made from lard and vegetable oils, and iii) lard and vegetable oils extracted from the formulated soaps. The chemical profiles of these samples were determined using FTIR spectroscopy coupled with a chemometric approach. Concurrently, the physical properties of the soaps were evaluated using various parameters, such as pH, foam height, and hardness. Furthermore, this data has the potential to serve as certified reference material for evaluating the similarities and differences of soaps available in the market, thereby serving as an authentication procedure for regulatory bodies.

MATERIALS AND METHOD

Reagent and Sample Collection

All solvents, chemicals, and reagents used were of analytical grade and were purchased from Merck (Darmstadt, Germany). Seven (7) local commercial oils, such as Canola Oil (LA), Coconut Oil (CN), Corn Oil (CO), Olive Oil (OV), Palm Oil (PO), Sunflower Oil (SF), and Lard (LD) were purchased from the local market at Nilai, Negeri Sembilan, Malaysia.

Lard Extraction

Lard was extracted using the gravimetric method based on the procedure outlined [30]. Approximately 10 grams of lard were weighed and extracted with a mixture of chloroform and methanol in a 2:1 ratio. After dispersion, the mixture was agitated for 15-20 minutes in a shaker at room temperature. The mixture was then filtered using Whatman™ No. 1 filter paper (11 µm) to remove non-lard materials from the liquid phase. The liquid phase of chloroform/methanol was washed with 20 mL of a 0.9% w/v sodium chloride solution and left undisturbed for 30 minutes to allow the mixture to separate into two layers. The upper layer (methanol) was discarded, and the bottom layer (chloroform), containing the lipids, was retained in the flask. A small amount of anhydrous sodium sulphate was added to the flask to absorb any moisture. The chloroform layer was then filtered again and dried in a fume hood. A total of 2-3 grams of lipids were

successfully extracted and stored in a freezer at -4°C before FTIR analysis.

Soap Formulation

Lard or vegetable oils weighing 10.0 grams were placed in a beaker. A total of 12.5 mL of ethanol and 12.5 mL of deionized water were mixed with 5 grams of sodium hydroxide and stirred until dissolved. This solution was added to the vegetable oils or lard, placed on a hot plate at low heat, and stirred for 20 to 30 minutes. The mixture was cooled in a cold-water bath and stirred gently with an added saturated sodium chloride solution. The soap was filtered out using a Buchner funnel and filter paper. Finally, the soap was rinsed with diluted hydrochloric acid and deionized water, collected in a suitable container, and dried at room temperature. The different types of vegetable oils or lard were used to synthesize soaps, such as LA, CN, CO, OV, PO and SF with the composition of sodium hydroxide, water, oil or lard (1:5:2) respectively.

Lard and Vegetable Oils Extraction from Soap

The extraction of lard and vegetable oils from the soap was conducted using the TFM content method. Fatty acids from the soap could be separated after mixing with mineral acid. Approximately 5 g of powdered soap was diluted in 75 mL distilled water and heated. Consequently, 10 mL of 15% sulfuric acid was added to the soap solution while heating until a clear solution was obtained. The solution was then left for about 30 minutes, resulting in the formation of two separate layers. The upper layer containing the fatty acids was filtered using filter paper and transferred to a petri dish. The layer was allowed to cool and dry in the fume hood before being stored in a glass bottle for further analysis.

FTIR Analysis

A Perkin Elmer Spectrum 100 instrument equipped with a Deuterated Triglycine Sulphate (DTGS) detector, a KBr/Germanium beam splitter, and connected to Spectrum operating system software (Ver. 1.1) was employed for FTIR data collection. The obtained oil-fat and soap samples were placed on a multibounce plate in direct contact with an Attenuated Total Reflectance (ATR) crystal. FTIR spectra were recorded over 16 scans with a resolution of 4 cm^{-1} in the range of 4000–650 cm^{-1}. The background of the air spectrum was subtracted from the overall spectra. The ATR plate was meticulously cleaned with methanol and dried using soft tissue before being used for the next sample. The cleanliness was confirmed by collecting a background spectrum and comparing it to the previous one. These spectra were recorded as transmittance values at each data point.

Principle Component Analysis (PCA)

The analysis was divided into three sections, encompassing: i) analysis of the raw dataset for lard and vegetable oils, ii) analysis of the dataset for soap made from lard and vegetable oils, and iii) analysis of the dataset for lard and vegetable oils extracted from formulated soap. The spectra from the triplicate lard and vegetable oil data were exported to Unscrambler software (version 10.2; CAMO Software, Oslo, Norway). The acquired FTIR spectra were transformed into ASCII format and pre-processed using the normalized method, which retained the original data wavelengths while automatically characterizing wavelength absorbance. Subsequently, the Savitzky-Golay Second Derivative (SG2D) was applied to mitigate both additive and multiplicative effects in the spectra. The obtained spectra from each dataset were pre-processed before undergoing PCA.

Physicochemical Analyses on Soaps

The physicochemical parameters of the soap samples, including pH, foam height, and hardness, were determined based on the official American Oil Chemists' Society (AOCS) methods with some modifications.

pH

The pH of the soap samples was determined using the official AOCS method G 7-56. Approximately 5 mL of distilled water was added to a 25 mL beaker and heated to a temperature of 70 to 80°C. The heated water was poured onto 5 g of the soap sample with a few added boiling chips. A thermometer was inserted into the mixture, and the temperature was slowly raised to 95°C while stirring the solution with a glass rod. Once the sample reached the specified temperature, the beaker was removed from the heat and left to stand until the phases separated. The aqueous layer was subsequently drawn off and transferred to a 10 mL beaker using a suitable pipette. The pH of the aqueous solution was determined to the nearest 0.1 unit at 25°C [31].

Foam Height

To determine foam formation, approximately 2 grams of the soap sample were placed in a 500 mL measuring cylinder, and distilled water was added to reach the 100 mL mark. The resulting solution was vigorously shaken for 2 minutes (in an up-and-down motion, taking care to prevent spills). Subsequently, the measuring cylinder was allowed to stand for approximately 10 minutes. The foam height was measured from the 100 mL mark on the cylinder to the level of the foam and recorded as the foam capacity [31].

Hardness Test

A TA Hdplus texture analyzer (Stable Micro System Ltd., Surrey, UK) with a load cell of 500 N was

employed to measure the hardness of the soap. The hardness value was measured using a stainless-steel P/2:2 diameter needle cylinder probe. Hardness was reported as the maximum penetrating force (in N) required for the needle to penetrate through 30% strain of the soap sample at a test speed of 0.50 mm/sec.

RESULTS AND DISCUSSION

FTIR Results

Sample of lard and oils before and after the saponification process were analyzed using FTIR spectroscopy in the mid-infrared region (4000–650 cm^{-1}). FTIR spectroscopy is an ideal technique for the analysis of fats and oils because they are essentially single-component systems of Triglycerides (TGs). It can be applied directly to their neat form on an ATR crystal or passed through a flow cell [32].

The importance of infrared spectroscopy for qualitative analysis originates from the wealth of information obtained and the possibility of assigning specific absorption bands related to functional groups. In fats and oils, many of the peaks and shoulders in the spectrum can be attributed to specific functional groups [22]. The representative spectra obtained from lard, vegetable oils, and sodium fatty acids for each sample are shown in Figures 1, 2, and 3.

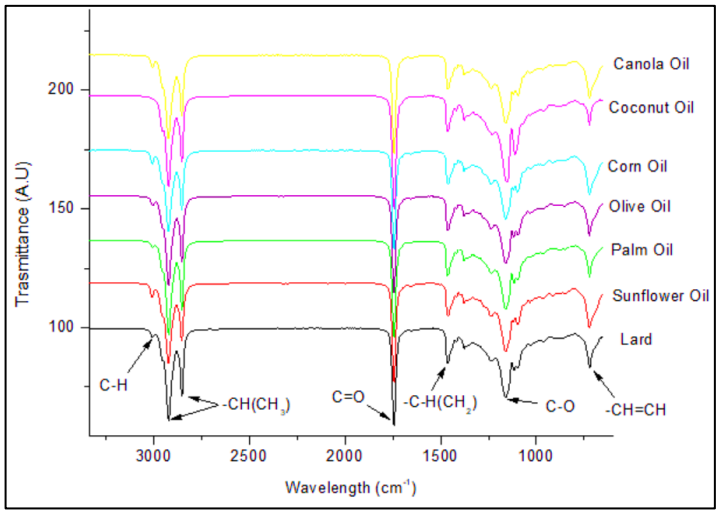

Figure. 1: FTIR spectra of raw lard and vegetable oils.

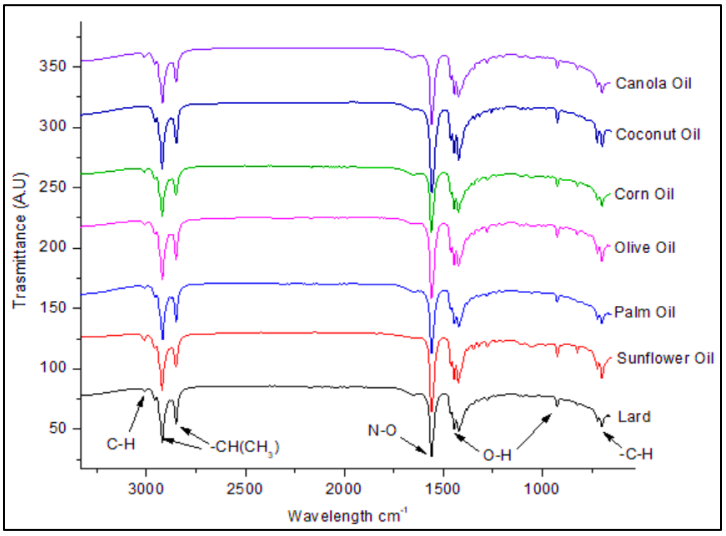

Figure 2: FTIR spectra of soap made from lard and vegetable oils.

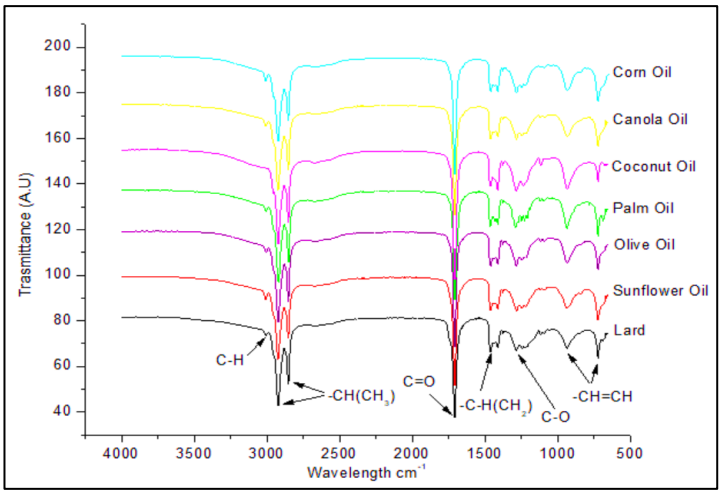

Figure 3: FTIR spectra of lard and vegetable oils from the extraction of formulated soap.

Table 1. Functional group and mode of vibration between raw lard and vegetable oils (A), soap from lard and vegetable oils (B), and lard and vegetable oils extracted from soap (C) are shown in Figures 1, 2, and 3.

Lard and vegetable oils (A) are shown in the FTIR spectrum in Figure 1.

Frequencies (cm^{-1})	Frequencies (cm^{-1})
3009-3005	3009-3005
2920 and 2854	2920 and 2854
1744-1742	1744-1742
1463-1461	1463-1461
1242-1234	1242-1234
1159 and 1116	1159 and 1116
960	960
720	720

Soap made from lard and vegetable oils (B) is shown in the FTIR spectrum Figure 2.

Frequencies (cm^{-1})	Frequencies (cm^{-1})
3014-3008	3014-3008
2957-2954	2957-2954
2920 and 2850	2920 and 2850
1559-1557	1559-1557
1462, 1445 and 1442	1462, 1445 and 1442
924	924
720 and 697	720 and 697

Lard and vegetable oils extracted from soaps (C) are shown in the FTIR spectrum in Figure 3.

Frequencies (cm^{-1})	Frequencies (cm^{-1})
3005-3007	3005-3007
2922 and 2851	2922 and 2851
1707-1705	1707-1705
1460-1463	1460-1463
1412-1411	1412-1411
1290-1280	1290-1280
1115-1117	1115-1117
937-933	937-933
721	721

Overall, the FTIR spectra exhibited differences between raw lard and oils (Figure 1), soap made from lards and oils (Figure 2), and lard and oils extracted from the soap (Figure 3). Figure 1 displays spectra measured between 4000 and 650 cm^{-1}, characterized by several peaks related to their functional groups. The spectra of raw lard and oils underwent changes in their profiles upon saponification, primarily due to the

presence of N-O stretching and O-H bending. Additionally, the trans- and cis-CH=CH bending that appear in the raw lard and oils spectra are absent in the soap's spectra, which are expected to be located at 960 and 720 cm^{-1}, respectively. The soap spectra also reveal O-H bending and -C≡C-H: C-H bending, noticeable at 924, 720, and 697 cm^{-1}, respectively (Table 1).

A comparison of the FTIR spectra between raw lard and oils (Figure 1) and lard and oils extracted from soaps (Figure 3) showed similarities in the presence of functional groups and fingerprint regions, respectively.

Chemometric Assessment using PCA Exploratory Analysis

A total of 63 FTIR spectra for each dataset of i) lard and vegetable oils, ii) soap made from lard and vegetable oils, and iii) lard and vegetable oils extracted from formulated soap were collected and measured using fingerprint regions ranging from 1500 cm^{-1} to 1000 cm^{-1}. Each dataset was pre-treated using multiplicative Scattering Correction and Savitzky-Golay Second Derivative (SG2D) to correct the baseline issue before proceeding with PCA. The first two Principal Components (PCs) explained 97% of the total variance for the raw lard and vegetable oil samples. Examination of the PC1 versus PC2 score plot reveals a distinct separation of the three sample groups: sunflower oil, corn oil, and canola oil in one group; olive oil, palm oil, and lard in another; and coconut oil, which is completely isolated from the others (Figure 4).

This observation suggests that lard and vegetable oils can be differentiated based on their intrinsic properties and characteristics. Notably, olive and palm oil are clustered together with lard in a single group. However, lard and palm oil can be distinguished based on the percentage of sn-2 C16 fatty acids, which is higher in lard than palm oil [7, 33].

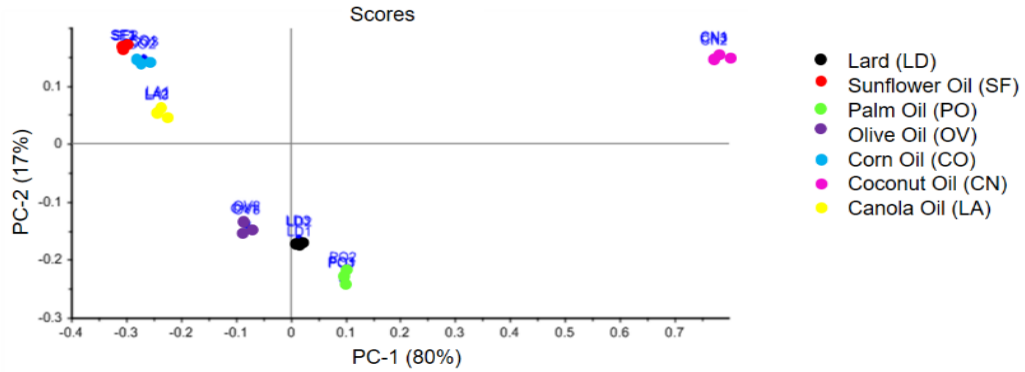

Figure 4. PC-1 and PC-2 score plots on raw lard (LD) and vegetable oils (Sunflower Oil, SF; Palm Oil, PO; Olive Oil, OV; Corn Oil, CO; Coconut Oil, CN; and Canola Oil, LA) data using FTIR spectra.

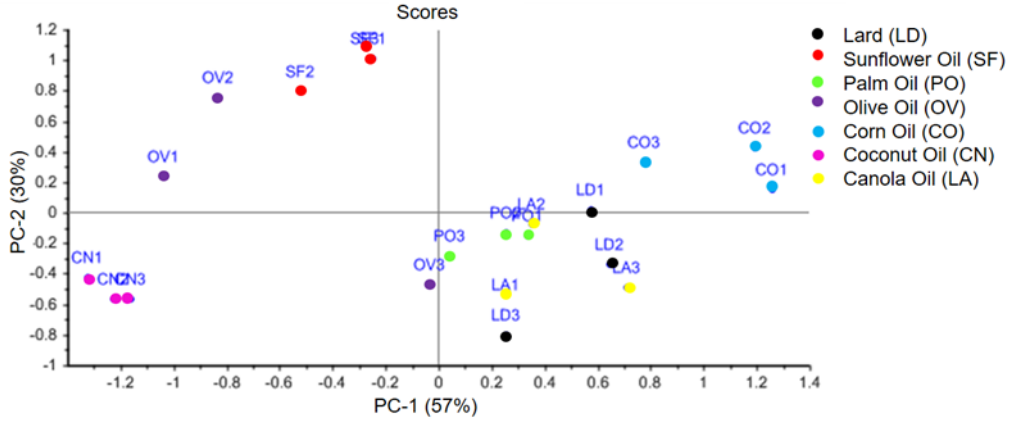

Figure 5. PC-1 and PC-2 score plots on soaps made from lard (LD) and vegetable oils (Sunflower Oil, SF; Palm Oil, PO; Olive Oil, OV; Corn Oil, CO; Coconut Oil, CN; and Canola Oil, LA) using FTIR spectra.

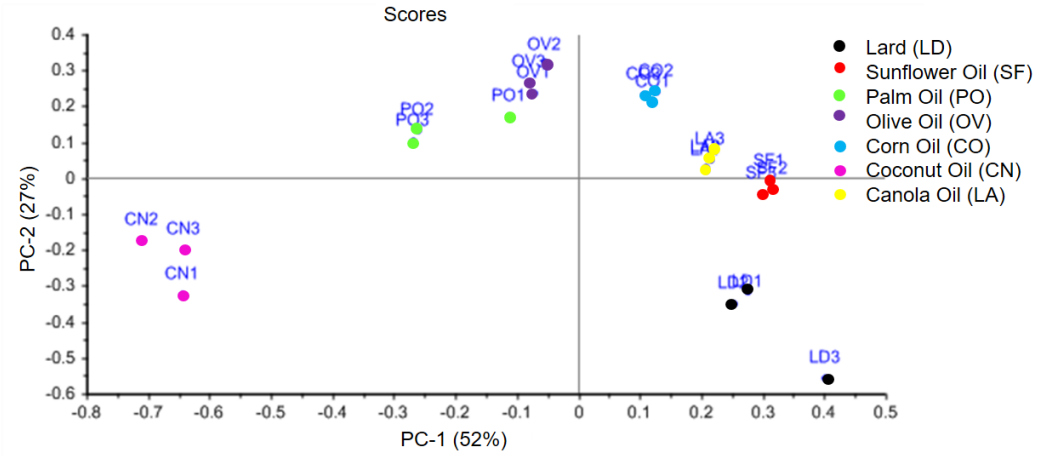

Figure 6. PC-1 and PC-2 score plots on lard (LD) and vegetable oils (Sunflower Oil, SF; Palm Oil, PO; Olive Oil, OV; Corn Oil, CO; Coconut Oil, CN; and Canola Oil, LA) extracted from formulated soap using FTIR spectra.

However, the PCA analysis of the soaps reveals that each sample is scattered away from its replicates and among other samples (Figure 5). This indicates an inconsistency in the metabolite fingerprints among replicates. The first two PCs, which account for 87% of the variance, clearly discriminate between lard and sunflower oil, coconut oil, and corn oil. However, distinguishing lard-based soaps from canola and palm oil is less pronounced. In contrast, olive oil soaps were scattered across three replications.

To confirm the distinct separation of lard from the cluster of vegetable oils, lard, and oils from the soap products were extracted. The PCA plot in Figure 6 demonstrates that the first two PCs, explaining 79% of the variance, effectively discriminate lard from the vegetable oils extracted from the soaps (Figure 6). This result suggests that lard and vegetable oils can be effectively distinguished again based on their initial properties and characteristics.

Physicochemical Analysis of Different Types of Soaps

Soap production begins with the saponification reaction of fats/oils with sodium hydroxide. This study utilized seven types of fats/oils, namely sunflower oil, olive oil, palm oil, corn oil, canola oil, coconut oil, and lard, for soap production. Subsequently, all the produced soaps underwent evaluations for hardness, pH, and foam ability.

Hardness of Soaps

The results presented in Table 2 reveal that olive oil soap exhibits the highest hardness, while sunflower oil soap demonstrates the lowest hardness. A statistical analysis using one-way analysis of variance (ANOVA) indicates that the hardness of soaps made from Lard is comparable to those made from coconut, canola, and corn oils and is significantly different (P value=0.000) from soaps made from olive, palm, and sunflower oils.

Ultimate pH

The pH of soap derived from various vegetable oils (sunflower, olive, palm, corn, and canola) and soap made from lard is observed to have an average pH value of 13, except for soap made from coconut, which shows a pH value of 12. The higher pH values in these soaps can be attributed to incomplete hydrolysis resulting from the saponification process. Excess fat or oil can be added to mitigate the harshness of the soap. However, it is important to note that the elevated pH values indicate that the analyzed soaps may be corrosive to the skin. In general, soap tends to be alkaline in aqueous solution. These alkaline properties are a barrier against bacteria and viruses, neutralizing the body's protective acid mantle. Healthy skin typically has a pH of 5.4 to 5.9 [34, 35].

Foam Height

Regarding foam height, soap made from lard shows lower foaming ability, similar to soap made from olive oil, palm oil, and corn oil (Table 2). The highest foam height value was observed in sunflower, canola, and coconut soaps.

Table 2. Hardness, pH, and foam height of different types of soaps.

Type of soap	Hardness	pH	Foam
Sunflower	225.45±14.08^{d}	13.26±0.02^{b}	8.03±0.06^{a}
Olive	2289.94±89.10^{a}	13.71±0.07^{a}	2.53±0.06^{b}
Palm	1970.74±301.67^{a}	13.27±0.07^{b}	2.10±0.17^{b}
Corn	344.79±25.22^{c,d}	13.31±0.04^{b}	2.00±0.00^{b}
Canola	775.56±56.77^{b}	13.12±0.04^{c}	7.67±0.58^{a}
Coconut	1041.54±216.49^{b}	12.52±0.07^{d}	7.97±0.06^{a}
Lard	715.59±13.10^{b,c}	13.40±0.02^{b}	2.50±0.00^{b}

Note: Values are from an average of three determinations ± standard deviation. Different alphabets within the same column differ significantly ($P<0.05$).

CONCLUSION

PCA analysis has successfully evaluated the similarities and differences between soaps made from different fats/oils. Note that extraction of fat/oils from the final products, coupled with the chemometric method, effectively demonstrated the ability to distinguish lard (animal fats) obtained from the soaps made from vegetable oils commonly sold in the market. This study highlights the efficacy of using PCA exploratory analysis as a significant method for identifying lard-based products, contributing to eradicating adulterated products. Based on the physicochemical analysis conducted in this study, it is apparent that soap types can be classified according to their distinctive physicochemical attributes. The analysis of various soaps, derived from seven distinct fats and oils, has illuminated variations in soap hardness, pH levels, and foamability.

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