QUALITY ASSESSMENT OF COCONUT OIL AND DETECTION OF ADULTERATION

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QUALITY ASSESSMENT OF COCONUT OIL AND DETECTION OF ADULTERATION

by

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(2018-12-007)

THESIS

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KERALA, INDIA

2020

DECLARATION

I, hereby declare that this thesis entitled **"QUALITY ASSESSMENT OF COCONUT OIL AND DETECTION OF ADULTERATION"** is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

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LIST OF ABBREVIATIONS AND SYMBOLS USED

%	Per cent
<	Less than
≤	Less than or equal to
°C	Degree Celsius
µm	micrometre
amu	Atomic mass unit
AOAC	Association of Official Analytical Chemists
AOCS	American Oil Chemists' Society
APCC	Asian and Pacific Coconut Community
B:C	Benefit : Cost
CAC	Codex Alimentarius Commission
Ca-O	Canola oil
CD	Critical difference
cfu	Colony forming units
cm	Centimetre
cm^{-1}	Per centimeter
CPKO	Crude palm kernel oil
CV	Coefficient of variation
DA	Discriminant analysis
EMB	Eosine methylene blue
et al.	And others

eV	Electron volt
FA	Fatty acid
FAME	Fatty acid methyl esters
FAO	Food and Agriculture Organization
FFA	Free fatty acid
FID	Flame Ionization Detection
Fig.	Figure
FSSAI	Food Safety and Standards Authority of India
FTIR	Fourier Transform Infra Red
g	Gram
GC	Gas chromatography
GCMS	Gas Chromatography Mass Spectrometry
GOI	Government of India
ICAR	Indian Council of Agricultural Research
IR	Infrared
ISO	International Organisation for Standardization
IV	Iodine value
KAU	Kerala Agricultural University
Kg	Kilo gram
KOH	Potassium hydroxide
m	Metre
MG	Monoglyceride
mg	Milligram
min.	Minutes

ml	millilitre
MOAH	Mineral oil aromatic hydrocarbons
MOSH	Mineral oil saturated hydrocarbon
MPN	Most probable number
MUFA	Monounsaturated fatty acid
N	Normality
NaCl	Sodium chloride
NAFDAC	National Agency for Food and Drug Administration Control
NIST	National Institute of Standards and Technology
No.	Number
PC	Principal component
PCA	Principal component analysis
PCR	Principal Component Regression
PE	Poly ethylene
PKO	Palm kernel oil
PLS	Partial least square
PUFA	Polyunsaturated fatty acid
RBDCNO	Refined bleached deodourized coconut oil
RNA	Ribonucleic acid
SE	Standard Error
SFA	Saturated fatty acid
Sl.	Serial

SN	Saponification number
SV	Saponification value
TLC	Thin layer chromatography
UV	Ultra Violet
VCO	Virgin coconut oil

INTRODUCTION

1. INTRODUCTION

The edible oil production in India was 10.06 million tonnes with an import of 14.92 million tonnes during 2018-19 while the total edible oil available for domestic consumption was 24.23 million tonnes (Commodity Profile of Edible Oil for September – 2019). Because of the greater demand of oil in national and international market adulteration in high price oil with low price oil is a major issue.

Adulteration in coconut oil is becoming common nowadays. The common adulterant in coconut oil is liquid paraffin or mineral oil and palm oil (Libish *et al*., 2011). The adulteration of fats and oils is not easy to detect when the adulterant has a composition near to that of the original oil. Palm kernel oil among oils is the closest to coconut oil in terms of fatty acid saturation level. It blends easily with coconut oil and price is nearly 60 per cent of that of coconut oil thus making mixing perfect and the process profitable. But coconut oil adulterated with mineral oil is bad for health. Hence an effective and efficient method for detecting the adulterant is required.

The standards for coconut oil are put forward by FSSAI and Codex Alimentarius. The common physical and chemical characterization along with fatty acid composition may reveal the adulteration. The accuracy up to which the physical and chemical characterization can detect the adulteration is not clear. Moreover, many of the physical and chemical parameters of coconut oil and palm kernel oil fall almost in the same range. Hence a more sophisticated analysis is needed for identifying the adulterant if the adulterant is palm kernel oil. However mineral oil in coconut oil can be identified by thin layer chromatography. Gas chromatography- mass spectroscopy and Fourier-transform infrared (FTIR) spectroscopy are some of the advanced techniques used to detect adulterants in coconut oil.

FTIR technique depends on the fact that majority of molecules will absorb light in the infra-red region of the electromagnetic spectrum. This absorption represents the characteristic bonds present in the molecule. Information regarding the

chemical composition of the sample can be acquired by the infrared spectroscopic technique. The frequency range will be measured as wave numbers (Ramaiah *et al.,* 2017). The infrared absorption bands will identify the molecular components and structure. So it will be easy to identify the adulterants. GCMS technique gives information on individual fatty acid composition in oil. Hence an experiment was formulated to identify the adulteration in coconut oil by mixing different percentage of palm kernel oil and mineral oil. The experiment was meant to explore the efficiency of different physical and chemical characters, thin layer chromatography, GCMS and FTIR spectroscopy for the detection of adulterant. The physical and chemical characterization, thin layer chromatography and GCMS are time consuming and laborious processes while FTIR is said to be an efficient and easy method. Hence an experiment was designed to assess the quality parameters of coconut oil and to identify an easy and efficient method to detect the adulterants in coconut oil.

The study entitled "Quality assessment of coconut oil and detection of adulteration" was conducted at the Department of Plantation Crops and Spices, College of Agriculture, Vellayani with an objective to assess the quality parameters of coconut oil and to detect adulteration by different techniques and to validate an easy and efficient method for the detection.

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

The present study entitled "Quality assessment of coconut oil and detection of adulteration" was undertaken at the Department of Plantation Crops and Spices, College of Agriculture Vellayani to assess the quality parameters of coconut oil and to detect adulteration by different techniques and to validate an easy and efficient method for the detection. The literatures related to composition of coconut oil, adulteration, standards of coconut oil, physical and chemical characteristics of pure coconut oil, microbial contamination, thin layer chromatography, fatty acid composition by GCMS and FTIR spectroscopy were reviewed and are presented in this chapter.

Coconut palm is generally known as "Kalpavriksha". Names prevailing in different regions are "Tree of life" or "Tree of heaven" or "Tree of abundance". These names indicate its uses and importance in lifestyle of individuals within the tropics. Each and every part of the palm is valuable and has multiple uses in agriculture, ayurvedic and religious fields. In South India and Southeast Asia, coconut oil is obtained mainly by processing of copra and is widely used for cooking. It occupies a major position as culinary fat in Kerala. Apart from its food value, it has medicinal and cosmetic value as it is rich in health factors (Ahuja *et al*., 2014).

According to ICAR (2016), in 2015-16 edible oil production in India was 25.3 million tonnes and it was obtained from an area of 26.13 million hectare. India imported 148.2 lakh tonnes of edible oils in 2015-16 while the net domestic accessibiliy was 86.37 lakh tonnes. For domestic consumption, India imports substantial amount of edible oils like groundnut oil, mustard oil, sunflower oil, soybean oil and palm oil. Among the imported edible oils, palm oil contributes a share around 60 per cent (Commodity Profile of Edible Oil for September – 2019).

Composition of Coconut Oil

Lipids comprise of fatty acids (FAs) and are categorized based on the presence or absence of double bonds. Saturated fatty acids (SFAs) are those without double

bonds and unsaturated fatty acids have one or more double bonds. Monounsaturated (MUFAs) ones are having one double bond and polyunsaturated fatty acids (PUFAs) have two or up to six double bonds (Orsavova *et al*., 2015). According to Eyres *et al.* (2016), coconut oil is composed of 92 per cent of saturated fatty acids from which 62 per cent corresponded to fatty acids with carbon number between 8 and 12, known as medium chain fatty acids.

Coconut oil belongs to a specific group of oils known as lauric oils. The major fatty acid present in the coconut oil is lauric acid (C12:0) and it accounts for 45 per cent of the total fatty acid composition. The health properties of coconut oil are contributed by the lauric acid. Moreover, palm kernel oil and babassu oil are also included in the category of lauric oil (Dayrit, 2014).

Kabara (1978) reported that bound fatty acids (FAs) (e.g., medium-chain saturates) and their derivatives (e.g., monoglycerides (MGs)) had adverse effects on varied microorganisms. Those microorganisms that were inactivated include bacteria, yeast, fungi, and enveloped viruses. Lauric acid and Monolaurin has antiviral, antibacterial, and antiprotozoal activity. Lipid-coated viruses, pathogenic bacteria like *Helicobacter pylori*, and protozoa such as *Giardia lamblia* can be destroyed with the help of Monolaurin monoglyceride.

Studies on virucidal effects of monolaurin on enveloped RNA and DNA viruses were conducted and they observed that the viruses had a lipid envelop and the presence of a lipid membrane on viruses made them especially vulnerable to lauric acid and its derivative monolaurin (Hierholzer and Kabara, 1982).

Strandberg *et al.* (2009) reported an inhibitory effect of monolaurin on *Staphylococcus aureus* in a human based study. Wang *et al*. (2014) reported the inhibition of monolaurin against *Helicobacter pylori* added in a mouthwash solution.

Berger and Andanar (1991) observed that palm kernel oil was comparable to coconut oil in composition and each exclusively supplied lauric oil within the world market. Coconut oil contained major proportion of saturated fatty acids and less amount of oleic acid which was an unsaturated fatty acid. However, palm kernel oil

was rich in oleic acid and babassu oil had a composition similar to that of palm kernel oil .

Adulteration in Oils

The Government of India has formed Prevention of Food Adulteration Act , 1954 according to which adulterant means any material which is or could be employed for the purpose of adulteration (GOI, 1954). Adulteration in coconut oil had become common and 45 brands of coconut oil were banned in Kerala on June 2018 (Times of India, 2018) followed by 74 brands in December 2018, by the State Food Safety Commissioner (Malayala Manorama, 2018).

Navya *et al*. (2017) reported that the poor who purchase loose edible oil across the country could run the risk of cancer, paralysis, liver problems and cardiopulmonary arrest as these oils are heavily impure. Apart from this, it was also found that, reputed brands were substituted with ordinary palm oil or other alternative low cost oils. In several cases it was observed that mineral oil, karanja oil, castor oil, and artificial colours were heavily utilized as adulterants in edible oil.

Damirchi and Torbati (2015) pointed out that adulteration of high priced oils with low priced oils and mixing of cold pressed oil with refined ones were the issues of adulteration. Highly expensive oils were substituted with low priced oils and were profitable for the producers. It was observed that high priced virgin olive oils were adulterated with oils of similar fatty acid profile. Limited availability of quality oils was another factor that leads to adulteration. Trans fatty acids and steradienes were formed during the refinement processes and were typically absent in cold pressed oil. Trans fatty acids were harmful to human body and its consumption would lead to coronary diseases.

A study was conducted to examine the consumer awareness towards the edible oil adulteration and to assess the quality through standard procedures. It was noticed that people with poor economic background preferred unpackaged oil samples over the packaged ones. Results obtained after the chemical analysis in mustard oil and soyabean oil showed the presence of adulterants in both packaged and unpackaged oil

samples. However, it was observed that coconut oil samples were free of adulterants (Pal and Jain, 2018).

Detection of adulterants like paraffin oil in coconut oil plays major role in assessing the quality of the oil. Paraffin oil is not an edible oil. Mineral oil derivatives like paraffin, paraffin oil, petroleum and propylene glycol will dissolve the natural oil of skin and thereby skin becomes highly dehydrated. It is tasteless and indigestible. Continuous use of such oils will lead to leukaemia. Due to their colourless and odourless nature, these oils become major candidates in adulteration and it is necessary to check the quality of coconut oil before using it (Sheeba *et al*., 2005).

Libish *et al*. (2011) conducted an experiment using fiber optic sensing system to detect the presence of paraffin oil in coconut oil. Liquid paraffin, palm oil and palm kernel oil were the most commonly used adulterants. They opined that liquid paraffin is extremely harmful to human body and it leads to severe health problems like liver disorders or cancer. In the experiment, pure coconut oil was mixed with paraffin oil in different proportions and the sensor was immersed in the mixture of coconut oil and paraffin oil. It was noticed that up to 3 per cent, the detection of adulteration was possible. Yadav (2018) reported that mineral oil belongs to group 1 carcinogens and are harmful to human life.

Heyst *et al*. (2018) conducted a survey to detect the presence of mineral oil in various food samples. In the survey, 217 packed samples were purchased from the market. It was found that food materials were comprised of mineral oil saturated hydrocarbons and mineral oil aromatic hydrocarbons. Liquid chromatography-gas chromatography coupled with flame ionisation detection (FID) was used for the quantification of mineral oil. Out of 217 samples, sampling strategy was not applicable to 19 samples. Among the remaining 198 samples, 23 samples exceeded the threshold limit of mineral oil aromatic hydrocarbons (MOAH) and only one sample exceeded the limit of mineral oil saturated hydrocarbon (MOSH). Threshold limits were proposed by Scientific Committee (SciCom) of the Belgian Food Safety Agency.

Standards of Coconut Oil

According to Dayrit *et al*. (2007) the Codex Alimentarius and the International Coconut Community (ICC) (formerly Asian and Pacific Coconut Community (APCC)) are the two international organizations responsible for implementing standards in coconut oil.

In India, Food Safety and Standards Authority of India (FSSAI) has been established under the Food Safety and Standards Act, 2006 which is a consolidating statute related to food safety and regulation in India. The FSSAI is responsible for protecting public health through regulation and supervision of food safety. The Codex Alimentarius Commission (CAC) is responsible for implementing Joint FAO/WHO Food Standards Programme. Their recommendations are used in international trade by more than 180 countries in their food legislation and regulations (Spink *et al*., 2019). The standards put forth for coconut oil by FSSAI and Codex are as follows

Table 1. Standards of coconut oil by FSSAI and Codex

Characters of coconut oil	FSSAI	Codex
Refractive index at 40°C	1.4481-1.4491	1.448-1.451
Moisture	Not more than 0.5%	0.2%
Insoluble impurities	Not more than 0.5%	0.05%
Saponification value	Not less than 250	248-265
Iodine value	7.5-10	6.3-10.6
Unsaponifiable matter	Not more than 1%	0.5
Acid value	Not more than 6	6
Polenske value	Not less than 13	13-18
Peroxide value	Not more than 15 meq	10meq

The ICC is an intergovernmental organization of coconut producing countries and it was established in 1969. All the activities under the coconut industry are coordinated to achieve maximum economic development. They are organized under the aegis of the United Nations Economic and Social Commission for Asia and the Pacific (UN-ESCAP). Over 90 per cent of world coconut production and exports of coconut products are contributed by 19 coconut producing member countries. ICC (former APCC) also sets standards for the coconut oil (APCC, 2003).

2.1. Chemical and Physical Characteristics of Coconut Oil

2.1.1. Refractive Index at 40°C

Adulteration and purity of oil can be checked by the refractive index. Pearson (1981) observed that refractometer could be used to determine the refractive index of oil and the value obtained for each oil would be unique. Rudan and Klofutar (1999) conducted a study in vegetable oils and reported a linear connection between refractive index and degree of unsaturation.

Atasie and Akinhanmi (2009) studied the physico chemical characteristics of palm kernel oil and the refractive index obtained was 1.453. Ariponnammal (2012) reported that percentage of adulteration in coconut oil was about thirty percent of palm oil and it could be detected using Abbe's refractometer of good accuracy.

According to FSSAI (2015), refractive index is defined as the ratio of velocity of light in vacuum to the velocity of light in the oil or fat or it is described as the ratio between the sine of angle of incidence to the sine of angle of refraction. Refractive index of the samples can be measured by using a suitable refractometer. FSSAI standard for refractive index of coconut oil at 40°C is 1.4481-1.4491.

In a study conducted by Srivastava *et al*. (2016), it was found that the refractive index of copra oil, hot extracted virgin coconut oil and cold extracted virgin coconut oil were 1.4480. Refractive index of homemade virgin coconut oil was 1.445. When it was deliberately adulterated with 5 to 25 per cent of palm oil an increase in the

refractive index was noticed (Premkumar and Joseph, 2018). Bahadi *et al*. (2019**)** studied the physico chemical properties of Malaysian crude palm kernel oil (CPKO) and reported that the refractive index of crude palm kernel oil at 28°C was 1.455.

2.1.2. Relative density

Rudan and Klofutar (1999) noticed an increase in relative density of oils as the molecular weight decreased and the saponification value was high. Kamariah *et al*. (2008) conducted a study in 10 virgin coconut oil samples to analyze the physico-chemical and quality characteristics. Virgin coconut oil samples were collected from Malaysian market. It was observed that the relative density of oil samples were within the range of 0.9185 to 0.9194.

2.1.3. Apparent density

Apparent density is described as the relationship between the mass and volume of the material, including pores and water (apparent volume) (Ramirez *et al*., 2012). Ali and Ali (2014) reported that density of the oils was determined by using a specific gravity bottle of 10ml capacity at 30 ± 0.1°C.

The density of palm kernel oil was between 0.9250 – 0.9350 g cc^{-1} (Thomas, 2000) and that of coconut oil was between 0.9190 – 0.9370 g cc^{-1} (Bailey and Shahidi, 2005).

According to Ramli *et al.* (2020), the analytical methods available are not suitable for routine application to discriminate palm oil from the sustainable and non sustainable sources. The classical physiochemical tests used are apparent density, refractive index, slip melting point, iodine value (IV), saponification value, peroxide value, and fatty acid composition. These classical methods, while relevant for quality analysis, are often time consuming, expensive, and are not suitable for routine application for traceability, especially those involving a large number of samples or batches, and require highly trained personnel.

2.1.4. Insoluble impurities

Amount of insoluble impurities should be very low and is a preferable characteristic in coconut oil (Keith *et al*., 1954). According to Cocks and Rede (1966), some substances remain insoluble in oil and are described as insoluble impurities. Petroleum ether or diethyl ether can be used to filter the dissolved impurities in fat or oil.

Gawad *et al*. (2015) evaluated the quality parameters of vegetable oils from the Egyptian market. It was noticed that the insoluble impurities of oil samples were high and it exceeded the maximum limit of Codex standards.

Agbaire (2012) reported that during the oil extraction, efficiency of clarification was determined by the amount of insoluble impurities. Hasan *et al.* (2018) investigated the physiochemical characteristics of virgin coconut oil and some marketed refined coconut oils. It was found that percentage of insoluble impurities in virgin coconut oil was less (0.16 per cent) when compared to other oils.

Insoluble impurities include dirt, debris and fibres. These substances dissolve in solvents like petroleum ether and are filtered off (ISO 663 (1992)). According to APCC and Codex standards maximum limit of insoluble impurities in oil should not be above 0.05 per cent.

2.1.5. Saponification value

Andrews (1933) observed that saponification value as an important quality parameter in the analysis of coconut oil. Marina *et al.* (2009) conducted a study on virgin coconut oil (VCO) which was collected from Malaysian and Indonesian market.

Odoom *et al.* (2015) evaluated the quality of coconut oil collected from different processing centres. It was found that the saponification value of oil samples did not meet the APCC standards. Thanuja (2015) reported that saponification values of virgin coconut oil obtained by different methods of extraction including

fermentation, induced fermentation, centrifugation and traditional boiling method were within the range 262.42 to 262.65 mg KOH g^{-1} of oil.

Saponification value of coconut oil according to FSSAI standard is above 250. The saponification value is the number of milligram of potassium hydroxide needed to saponify one gram of oil or fat. Fatty acids with larger molecular weight have low saponification (FSSAI, 2015). Codex standard for saponification value (SV) of coconut oil is between 248-265 mg KOH g^{-1} oil and the APCC Standards is 248-268 mg KOH g^{-1} of oil.

Pearson (1976) reported that large proportion of lower fatty acids have high saponification value. Saponification value is an important parameter that can be quantified and this quality is a desirable characteristic in soap production. Information regarding the mean weight of the acid, type of glycerides and the quantity can be obtained from saponification value. For industrial purposes, saponification value is used as an important parameter (Asiedu, 1989).

Kirk and Sawyer (1991) reported that fatty acid with shorter carbon chain length have high saponification value. Compounds present in the non saponifiable fraction affect the saponification value of vegetable oils which are unrefined. High saponification value of coconut oil is attributed by the presence of phenolic compounds and they react with KOH (Seneviratne and Dissanayake, 2005).

2.1.6. Iodine Value

Number of grams of iodine absorbed by 100 g of the oil or fat can be determined by iodine value. Wij's solution is used to determine the iodine value (FSSAI, 2015). According to FSSAI, iodine value of coconut oil is in the range 7.5-10.

According to Suzanne (1994), high iodine value occurs due to high amount of unsaturation and it leads to high absorption of iodine. Marina *et al*. (2009) reported that the iodine value of VCO samples ranged from 4.47 to 8.55. The low content of iodine value indicated that VCO has high degree of saturation. Amira *et al*. (2014)

conducted a study on physico chemical characteristics of palm kernel oil and found that the iodine value obtained for palm kernel oil was 15.86±4.02g and it depicted a higher level of unsaturation.

Odoom and Edusei (2015) evaluated the quality parameters in coconut oils collected from four centres of Jomoro district of western region of Ghana. It was found that the mean iodine value obtained from three centres met the APCC standard while coconut oil from only one centre met the Codex standard. APCC standard for iodine value is in the range 6.3-10.6 and according to Codex standard the iodine value is in the range between and 4.1-11.

Resistance to oxidative rancidity could be achieved by the low degree of unsaturation (Onyeike and Acheru, 2002). Highly unsaturated oils gave high iodine value and low iodine value was observed in saturated oils. High iodine value of oils led to the production of cosmetics, oil paints and varnish and they could also be used for nutritional purposes (Victor *et al*., 2012).

Atasie and Akinhanmi (2009) analysed the physico chemical characteristics of palm kernel oil and observed an iodine value of 41.24 g of iodine per 100g of oil. Ibrahim (2013) studied the physical and chemical characteristics of Malaysian palm kernel oil and observed an iodine value within the range 16.5 to 18.75.

2.1.7. Polenske value

The Polenske value is denoted as the number of milliliters of 0.1N aqueous alkali solution required to neutralise water insoluble and free fatty acids distilled from 5g of the oil or fat under the suggested conditions. It measures mainly caprylic, capric and lauric acids present in oil or fat which are steam volatile and are also water insoluble (FSSAI, 2015). According to FSSAI, Polenske value of coconut oil should not be less than 13.

Polenske value of coconut oil is within the range 15-20 and for palm kernel oil it is in the range 6-12 and for other oils and fats it is less than 1 (Singhal, 1980). According to Codex standards, the Polenske value for coconut oil is within the range

13-18, for palm kernel oil it is in the range 8-12 and babassu oils shows a range 8-10. According to Satheesh and Prasad (2012), virgin coconut oil extracted by natural fermentation method obtained a Polenske value of 13.9 ± 0.6 while induced fermentation method obtained a value of 13.9 ± 0.3. Thanuja (2015) observed that the Polenske value of virgin coconut oil recovered from traditional boiling method, fermentation, induced fermentation and centrifugation method were within the range 13-13.2.

2.1.8. Unsaponifiable matter

Oil consists of certain amount of unsaponifiable matter and is a mixture of hydrocarbons, aldehydes, ketones, alcohols, sterols, pigments and fat soluble vitamins. These substances are formed during processing or degradation and sometimes they occur naturally (Moura *et al*., 1975).

All naturally occurring fats and oils contain carboxylic acid glycerides and very small quantities of other substance besides the major constituent. The unsaponified constituent is mostly sterols. A small amount of tocopherols and phytosterols are present in coconut oil and are unsaponifiable (Krishna *et al*., 2010).

FAO (1986) defines unsaponifiable matter as substances which remain soluble in an oil after saponification. Sterols, higher open chain alcohols, pigments, vitamins and hydrocarbons, foreign organic matter including mineral oil are considered as unsaponifiable matter. According to FSSAI (2015) sterols, squalene, beta carotene, tocopherols and phenols are considered as unsaponifiable matter in the oil sample.

A study was carried out to evaluate the physico chemical properties of 10 virgin coconut oil samples and it was found that the average unsaponifiable matter was 0.116 per cent. Minimum value obtained was 0.085 per cent and maximum value was 0.135 per cent with a standard deviation of 0.0184 (Kamariah *et al*., 2008).

2.1.9. Acid value

Excess moisture and the action of lipase will lead to hydrolytic rancidity in coconut oil. Hydrolytic rancidity indicates the amount of free fatty acid content and

the aroma and flavour will change when the free fatty acid content increase (Hoover *et al*., 1973). According to Kirk and Sawyer (1991), acid value or free fatty acid (FFA) is often used to approximate the quantity of oil that would be vanished during refining steps in crude fats.

Kumar *et al.* (2018) conducted a study in refined and unrefined coconut oil samples collected from three survey regions of Godavari district. It was found that acid value of samples obtained were higher than the APCC standards. Oils with high acid value will have high fatty acid content and are undesirable for consumption.

Hydrolytic rancidity in coconut oil is due to the presence of fatty acids (Fernandez, 1988). A high acid value might depict a higher tendency to become rancid (Karim, 1997). Man *et al*. (1997) found that coconut oils with high moisture content had high amount of free fatty acids.

All vegetable oils contained naturally low amount of free fatty acids (FFAs). Residual water within the oil would react and additional amount of free fatty acids were formed during extraction and storage. Chemical or enzymatic mechanisms were responsible for hydrolysis. High levels of FFA led to the formation of unpleasant flavour (Dayrit *et al*., 2007).

FFA is one of the most important quality parameters in the palm kernel oil industry as it indicates the level of deterioration of the oil. A study was carried out to assess the physico chemical characters of crude palm kernel oil. The acid value noticed in the study was 10.4±0.1 mg NaOH g^{-1} for crude PKO (Bahadi *et al*., 2019).

The acid value denotes the amount of potassium hydroxide required to neutralize the free fatty acids which are present in one gram of fat. This is an indication of rancidity which represents the amount of free fatty acids developed in the decomposition of oil glycerides. The value is also expressed as per cent of free fatty acids calculated as oleic acid (FSSAI, 2015).

2.1.10. Peroxide value

The peroxide value of palm kernel oil was 14.3±0.8 meq kg^{-1}. This could be a sign of the degree of spoilage of palm kernel oil which was more liable to rancidity. Rancidity began to be noticeable once the peroxide value was well above 10 meq kg^{-1} (Pearson, 1976). Off flavour resulting from peroxidation of unsaturated fatty acids was the major cause of spoilage of stored oils (Semwal and Arya, 1992).

Oxidation in the initial stages could be determined by measuring the peroxide value of oils. Matthäus (2007) reported that the condition of cooking oils would not change after the refining process. Cooking oils might be refined or unrefined. Oxidation of oils depended on many factors like change in temperature, light, time, presence of moisture, metals etc.

Primary oxidation state of oil could be evaluated by the peroxide value. After oxidation one of the first products formed in the oil is a hydroperoxide. Peroxide value is the commonly used method for checking the oxidation in oils (FSSAI, 2015).

According to CODEX (2015), the specific limit for peroxide value is 15 meq kg^{-1} for virgin oils and APCC (2003) specifies 3 meq kg^{-1} oil for VCO. Oxidation state is generally classified as low, moderate and high. Peroxide value between 1 and 5 meq kg^{-1} represents low oxidation state and that between 5 and 10 meq kg^{-1} represents moderate oxidation and above 10 meq kg^{-1} indicates high oxidation state.

2.1.11. Matter volatile at 105°C

Kamariah *et al*. (2008) reported that matter volatile at 105°C for ten virgin coconut oil samples was within the range 0.08 – 0.15. The result was expressed in percentage by mass. According to Dayrit *et al*. (2007), an average of 0.04 per cent volatile matter was obtained for VCO samples and volatile matter was within the range of 0 to 0.08. It was also observed that refined bleached deodourised coconut oil (RBDCNO) contained water as volatile matter and no volatile organic compounds (VOC) were detected. In contrast, copra oil gave a high VOC level of 1.77 per cent.

2.2. Microbial Contamination

Yusuf *et al*. (2017) studied the microbial purity of locally extracted palm kernel oil and coconut oil. The presence of total aerobic mesophilic bacteria, fungal (mould), coliform counts and pathogenic bacteria (*E.coli*) were evaluated. Serial dilution, pour plate and Most Probable Number (MPN) techniques were applied along with biochemical tests. Eosine-Methylene Blue (EMB) test was used to detect the presence of *E.coli.* It was found that the bacterial and fungal colonies obtained were very few or even absent in the oil sample and no *E.coli* was detected. These microbial count was within the limited range of National Agency for Food and Drug Administration Control (NAFDAC) for oils.

Winter *et al*. (1971) developed a rapid method for the estimation of microbial contamination on food materials. Samples were swabbed with sterile diluents and they concentrated on the surface of membrane filters. They were incubated, heated, stained and finally the membranes were dried. Microscopic examination showed that microbial count using this technique provided reliable information on the data.

Kamariah *et al*. (2008) studied the microbial contamination of virgin coconut oil samples and the total plate count obtained was zero for almost all the samples.

Commercial samples of virgin coconut oil (VCO), refined, bleached and deodorized coconut oil (RBD CNO) and copra oil were analyzed using standard parameter for microbial contamination. Out of 33 samples taken, all 3 copra oil samples were <250 cfu ml^{-1}. The APCC standard for total plate count of coconut oil is < 10 cfu ml^{-1}. Failure to meet this standard indicates that the product, copra oil is of poor quality and is a potential health hazard (Dayrit *et al*., 2007).

2.3. Thin Layer Chromatography (TLC)

Thin layer chromatography (TLC) method is used for the detection of mineral oils and the spots were located with aqueous sodium fluorescein or 2',7'-dichlorofluorescein under UV light. Up to 3 per cent of adulterant mineral oil in different vegetable oils were detected using silica gel G layers sprayed and eluted

with benzene. The spots were detected by charring with 50 per cent ethanolic phosphoric acid (Mani and Lakshminarayana, 1968).

Gocan (2002) explained the properties of adsorbents in TLC. Silica gel is the most commonly used adsorbent in TLC. Along with silica gel, alumina, zirconium oxide, florisil, and ion-exchanger were used as stationary phase.

Bele and Khale (2011) explained the significance of thin layer chromatography (TLC). TLC technique is used to determine the impurities and it helps in the identification of compounds within the sample. It is widely used in many industries to assess the quality. This technique is simple, low cost and less time consuming when compared to the advanced techniques.

Kagan and Flythe (2014) applied TLC technique to check the antimicrobial compounds in plant extract. Silica coated TLC plates were used to separate the components and the separation was based on polarity of compounds. Bands obtained were visualized under UV light. Obtained zones were cut and they were incubated in plates with agar. Agar plates were stained with tetrazolium red in order to visualize the inhibitory bands.

Iodine vapour was used for the detection of FFAs and their glycerides on the Silica gel 60F254 TLC plates. Solvent systems A (hexane: ether: acetic acid 60:40:1 v/v/v) and B (hexane: ethyl acetate: acetic acid 60:40:0.5 v/v/v) were appropriate for separation of those compounds. TLC remains as a sensitive technique for the qualitative analysis of coconut oil (Pengon *et al*., 2012).

Kumar and Shree (2014) conducted a study to analyze the quality of different vegetable oils utilized in the ayurvedic oil preparations. Coconut oil, castor oil and sesame oil were collected and tested for mineral oil adulteration. Different concentrations (1, 5, 10, 50 and 80 per cent) of mineral oil were added to the vegetable oil. It was noticed that five formulations analysed were found to be free from the adulterant oil. A standard curve was plotted for the quantification.

Thin layer chromatography (TLC) or planar chromatography can be used for the detection of mineral oil. Silica gel coated plates can be used as stationary phase. Mineral oils are non polar and it exhibits faster moving spots on thin layer chromatographic plates, than the triglycerides (FSSAI, 2015).

2.4. Gas Chromatography Mass Spectrometry (GCMS) Analysis

2.4.1. Fatty acid composition

Structural elucidation of metabolites can be checked by the initiation of new methods. Along with library search, mass spectrometry with high resolution can be utilized for precise measurements, to interpret the mass spectrum, study of isotope ratios and for the study of the neutral losses (Lafferty *et al*., 1998).

GCMS is a robust and widely used technique. It combines high sensitivity and specificity for suitable analyte classes. Gas chromatography-mass spectrometry can be used for detailed profiling. This technique provides a detailed chromatographic profile of the sample and consequently relative or absolute amounts of the components can be measured. The number of components measured depends on the resolution of the chromatographic system and the specificity of the detection technique. A mass spectrometer will function as a highly specific chromatographic detector and if it is of high resolution, the analysis will get easier (Halket *et al*., 2005).

According to Colby (1992) samples could be characterized by their mass spectral patterns and GC retention indices. Chromatographic peaks or peaks obtained above a particular intensity could be used for the recognition of the sample. Non targeted analysis could be performed by GCMS. The number of measurement scan could be raised by deconvolution of the spectra using numerical methods. Metabolic profiling could be done with the help of several software programs.

The fatty acid composition put forth for coconut oil by Codex and ICC by gas liquid chromatography are as follows.

Table 2. Fatty acid composition of coconut oil based on gas liquid chromatography (expressed as percentage of total fatty acids)

Fatty acid	Codex Standard	ICC Standard
C 6:0	ND-0.7	0.10-0.95
C 8:0	4.6-10.0	4-10
C 10:0	5-8	4-8
C 12:0	45.1-53.2	45-56
C 14:0	16.8-21.0	16-21
C 16:0	7.5-10.2	7.5-10.2
C 18:0	2-4	2-4
C 18:1	5-10	4.5-10
C 18:2	1-2.5	0.7-2.5
C 18:3	ND-0.2	
C 20:0	ND-0.2	
C 22:0	ND	
C 24:0	ND	

(ND- Not Detected)

Mass spectra were scanned during the chromatographic peak elution in metabolic profiling. 1-6 spectra s^{-1} could be recorded with the help of advanced mass spectrometers. Peak 'skewing' could occur if the scanning speed was too slow. Skewing was corrected by using modern software. Required number of samples could be secured by undertaking sufficient number of scans and were recorded across the GC peak. The peak formed could be interpreted and quality measurements could be taken. Quantifying process depended on the areas or heights of selected ion chromatograms. Error would increase if the sampling rate was insufficient (Veriotti

and Sacks, 2003). Weckwerth *et al*. (2004) reported that instruments were designed with high scanning speeds or high mass resolution. Higher scanning speeds were used for metabolic profiling with mass spectrum. 'Ultrafast' GC, in which very high oven temperature was used for programming. They were used for fast chromatographic separations, could be used for fast scanning.

Fatty acids composition and the most vital physical-chemical parameters of tomato seed oil were distinguished by gas chromatography coupled with mass spectrometry. Each fatty acid had different retention times and it could be recognized by GC. Vaporous phase was achieved by converting the oil samples through esterification and the fatty acid from tomato seed oil were converted to fatty acids methyl esters. From the analysis it was observed that the most important element of tomato seed oil was linoleic acid (48.20 per cent), followed by palmitic acid (17.18 per cent) and oleic acid (9.20 per cent). This study concluded that tomato seed oil could act as a good supplier of essential fatty acids omega-6 (linoleic acid) and omega-9 (oleic acid) **(**Botineștean *et al*., 2012).

Moigradean *et al*. (2013) carried out an experiment in two completely different vegetables oils (walnut and coconut oils) to spot the composition of fatty acids. This was done with gas chromatography-mass spectrometry (GC-MS) method. The GC analysis of the fatty acid methyl esters (FAME) was performed by employing a Shimadzu QP 2010 GC-MS instrument. It was observed that content of saturated fatty acids in the walnut oil was 9.50 per cent, of which monounsaturated acids was 24.20 per cent, and that of polyunsaturated acids was 63.30 per cent. The oleic acid content of the walnut oil was 24.20 per cent of the total fatty acids, the linoleic acid content was 54.80 per cent and the linolenic acid was 8.50 per cent. The results showed that the principal fatty acids identified in coconut oil were lauric acid (44.60 per cent) and myristic acid (20.40 per cent).

A study was carried out to analyze the fatty acid composition in coconut oil and coconut oil was blended with different vegetable oils (palm, rice bran, sesame, mustard, sunflower, groundnut, safflower and soybean). Coconut oil contained C12:0 (lauric acid) as the major fatty acid. It was found that coconut oil contained 90 per

cent of saturated fatty acids and was deficient in unsaturated fatty acids. Mono unsaturated fatty acid accounted for 6 per cent and polyunsaturated fatty acid about 1 per cent. When coconut oil was blended with different vegetable oils there was an increase in percentage of unsaturation. Monounsaturated fatty acid increased to 8-36 per cent and polyunsaturated fatty acid showed an increase of 4-35 per cent (Bhatnagar *et al*., 2009).

Dorni *et al.* (2018) evaluated 320 edible oils and fats and their fatty acid profile was analysed. It was found that in coconut oil saturated fatty acids constituted the maximum proportion (90.84 per cent). Among the saturated fatty acids 49.57 percent was constituted by lauric acid. This was followed by myristic acid (21.12 per cent), palmitic, capric, stearic and caprylic acid. Among the unsaturated fatty acids, oleic acid accounted for 7.24 per cent followed by linoleic acid (1.9 per cent).

2.5. FTIR Spectroscopy

FTIR technique provide fast and accurate information about the components in a mixture. It is widely used in edible oils and fats for analysing the quality. Moreover, quantitative analysis can also be done. It is a non destructive analytical tool that requires minimum sample preparation. Oil samples obtained from different regions were distinguished with the help of non supervised grouping techniques (Dupuy *et al*., 1996).

Vlachos *et al.* (2006) investigated the adulteration in extra virgin olive oil with low priced oils which include (sunflower oil, soyabean oil, sesame oil, corn oil) using FTIR technique. Oxidation process of oil samples were also noticed. C-H stretching vibration of the *cis*-double bond was observed at 3009 cm^{-1}. Adulterant oils in the sample could be distinguished with the help of specific stretching or vibrations.

Alawi *et al*. (2004) developed a fast, practical and accurate FTIR methodology for the determination of FFA in edible oils. Analogous to the American oil chemists society (AOCS) volumetric analysis, the FTIR FFA determination was accomplished by an acid or base reaction however directly measures the product formed instead of utilizing an end point based on an electrode potential or colour change. Vibrations or

absorptions would vary with the nature of the bio chemical species. The infrared spectrum obtained would act as a fingerprint of the sample. Infrared profile obtained for each substance would be unique (Ellis *et al*., 2007).

A study was carried out to evaluate the effectiveness of Fourier transform infrared (FTIR) spectroscopy in the detection of palm kernel olein as an adulterant in virgin coconut oil. From pure and debased samples of virgin coconut oil, the reflectance measurements were analysed. Detection of adulteration up to 1 per cent was feasible. By analysing the structure of spectra, pure and adulterated samples were classified using the discriminant analysis with 10 principal components. A good linear regression of actual value was noticed in partial least square calibration method and a coefficient of determination (R^2) of 0.9875 was observed (Manaf *et al*., 2007).

Iodine value and saponification number of edible oils were determined by Fourier transform infrared (FTIR) spectroscopy with a support of disposable polyethylene (PE) films. Partial least squares calibration model was used for the quantification and it was done in the *cis* and *trans* double bond region (3206–2992 cm^{-1}) for obtaining the direct iodine value. This method can be used for edible oils with low to high iodine value. Vibration obtained in the region (781–650 cm^{-1}) was used for determining the saponification number (SN) by directly taking the area in carbon chain skeleton. It was found that less effort was required to prepare the sample and the results obtained were accurate when compared to the standard AOCS procedure (Xu *et al*., 2018).

The potential of the FTIR technique could be utilized for examining the freshness of edible oil. It has been verified that the sensorial and nutritional quality could be affected by the oxidation process (Sinelli *et al*., 2007). FTIR was most helpful for distinguishing chemicals that were either organic or inorganic. It was often utilized to quantify some components of an unknown mixture and for the analysis of solids, liquids and gases (IIT Kanpur, 2012).

Ramaiah *et al*. (2017) detailed the principle behind the FTIR. In this technique, infra-red region in the electromagnetic spectrum was used by the

molecules for absorbing light. Molecules will absorb light and as a result the spectra was obtained. IR spectroscopy provided fingerprint information about the composition of the sample. The frequency range was estimated as wave numbers. The infrared absorption bands recognized the molecular components and structure. So it was simple to spot the adulterants.

Coconut oil was mixed with different concentrations of paraffin oil (1, 2, 3, 4, 5, 10 and 100 per cent) and it was subjected to FTIR spectroscopic analysis. The FTIR analysis showed that the peaks corresponding to carbonyl groups at 1743 cm^{-1}, 1229 cm^{-1} and 1155 cm^{-1} and the peak at 1111 cm^{-1} corresponding to bending and deformation of –CH group were not observed in paraffin oil. These peaks could be taken as signature peaks for the detection of paraffin oil (Raj *et al*., 2018).

Hendl *et al.* (2001) determined the iodine value of vegetable oils using Fourier transform infrared spectroscopy. Oils with low iodine value to high iodine value were analysed in the experiment. Spectrum was obtained between the regions of 4000-400cm^{-1} and the heights of characteristic functional groups were analysed. Iodine value of oils obtained by spectroscopic technique was similar to that obtained through standard methods and a relative standard deviation of 5 per cent was noticed.

Among the commonly used fats and oils, virgin coconut oil had distinctive IR spectrum. In VCO spectrum, there was no peak at region close to 3008 cm–^{1} and 1654 cm^{-1}. Peaks at these regions correspond to unsaturated double bond (=CH; cis) and -C=C-(cis), respectively. These peaks were used to denote the unsaturation degree of triglyceride. VCO contained high level of lauric acid (about 50 per cent) and very low level of unsaturated FA of oleic and linoleic acids. Therefore, it is not pleasing if VCO has no peak at region near 3008 cm^{-1} and 1655 cm^{-1}. Additionally, at region of 1120–1090 cm^{-1}, due to C-O ester linkage vibration, VCO has one peak. At the same time, other edible fats and oils showed two peaks (Rohman and Man, 2011).

Man and Rohman (2013) investigated the chance to utilize Fourier transform infrared (FTIR) spectroscopy with multivariate chemometric analysis techniques. Principle component regression (PCR), partial least square (PLS) and discriminant

analysis (DA) were used as quantitative techniques to determine the canola oil (Ca-O) adulteration in virgin coconut oil (VCO). Normal and derivative FTIR spectra obtained were compared to derive the best technique for detection of adulteration. It was found that among the quantitative analytical techniques, DA was the best model to discriminate the pure VCO and adulterated VCO. Quantification of canola oil was done by selecting the frequency regions of 1200-900 cm^{-1} and 3027- 2985 cm^{-1} and a high correlation was found between the actual and predicted values of canola oil as adulterant in VCO. This study confirmed that FTIR spectroscopic technique could be used for authentication studies.

Virgin sesame oil adulteration with palm oil and groundnut oil were monitored using Fourier transform infrared spectroscopy. 5-15 per cent of adulterants were mixed with the virgin sesame oil and they were subjected to spectroscopic analysis. Chemometrics were applied in combination with FTIR. Principle component analysis, hierarchical cluster analysis and discriminant analysis models were used in the study. The principal component analysis, hierarchical cluster analysis and discriminant analysis using two principal components were able to classify virgin sesame oil and the same adulterated with palm oil and groundnut oil (Pandurangan *et al*., 2017).

Jiang *et al*. (2016) determined the acid value of edible oils by FTIR spectroscopic method. Acid value obtained was estimated based on the stretching of the O-H bond. Carbon tetrachloride was used to dilute the oil sample and the sample was placed on the crystal. In the spectra, peaks were obtained at 3535 cm^{-1} and 3508 cm^{-1} and the acid value was obtained by using the data range of 3340–3390 cm^{-1}.

MATERIALS AND METHODS

3. MATERIALS AND METHODS

The present study entitled "Quality assessment of coconut oil and detection of adulteration" was undertaken at the Department of Plantation Crops and Spices, College of Agriculture, Vellayani during the period 2018-2020. The study was conducted in order to assess the quality parameters of coconut oil and to detect adulteration by different techniques and to validate an easy and efficient method for the detection. The different techniques used to check the adulteration and the method used for analysis are presented in this chapter.

Pure coconut oil was obtained from the three different coconut expeller collected at three different periods and five different brands of coconut oil samples from three different shops at three different time were collected and analysed separately. Pure coconut oil obtained from the three expeller were mixed separately with 1, 5, 10, 15, 20 and 30 per cent of palm kernel oil and mineral oil. The treatments of the experiment were twenty and are shown in Plate 1. The details of the treatments is presented below.

Treatments

1. Pure coconut sample – 1 sample (T_1)

2. Branded coconut samples – 5 samples (T_2- T_6)

3. Pure coconut sample mixed with 1 per cent palm kernel oil (T_7)

4. Pure coconut sample mixed with 5 per cent palm kernel oil (T_8)

5. Pure coconut sample mixed with 10 per cent palm kernel oil (T_9)

6. Pure coconut sample mixed with 15 per cent palm kernel oil (T_{10})

7. Pure coconut sample mixed with 20 per cent palm kernel oil (T_{11})

8. Pure coconut sample mixed with 30 per cent palm kernel oil (T_{12})

9. Pure coconut sample mixed with 1 per cent mineral oil (T_{13})

10. Pure coconut sample mixed with 5 per cent mineral oil (T_{14})

11. Pure coconut sample mixed with 10 per cent mineral oil (T_{15})

12. Pure coconut sample mixed with 15 per cent mineral oil (T_{16})

13. Pure coconut sample mixed with 20 per cent mineral oil (T_{17})

14. Pure coconut sample mixed with 30 per cent mineral oil (T_{18})

15. Palm kernel oil sample-1 (T_{19})

16. Mineral oil sample-1 (T_{20})

Total number of samples –20

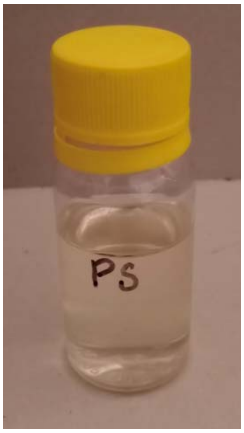
Replication - 3

These samples were tested for physical and chemical characteristics, microbial contamination, characterization by thin layer chromatography, fatty acid composition by GCMS and FTIR spectroscopy.

3.1. Chemical and Physical Characteristics of Coconut Oil

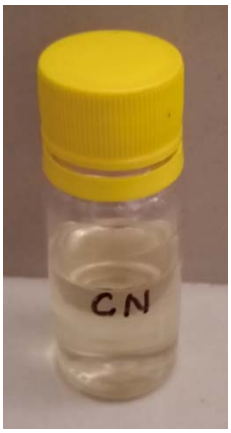
3.1.1. Refractive Index

Refractive Index of the oil at 40°C was determined by using a Butyro-refractometer (ATAGO RX – 50001) (Plate 2). Two drops of sample was placed on the lower prism. Prisms were closed and mirror was adjusted to get the sharpest reading. Refractive index is greatly affected by temperature, and hence care was taken to keep temperature constant. Temperature correction was undertaken automatically in the instrument itself. The reading of Butyro refractometer was converted to refractive index with the help of the table of FSSAI (FSSAI, 2015).



T_1

T_2

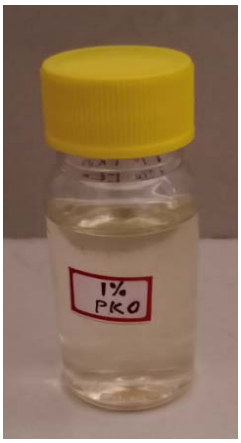


T_3

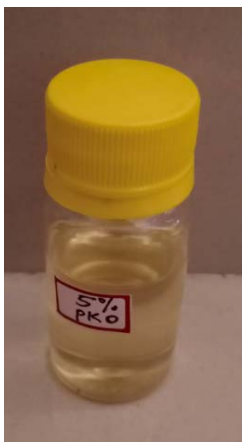
T_4

T_5

T_6



T_7



T_8

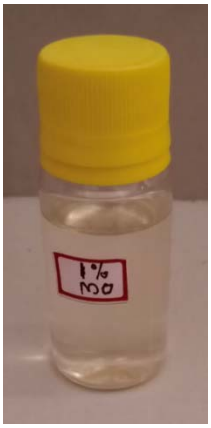
T_9

Plate 1. Treatments used in the analysis

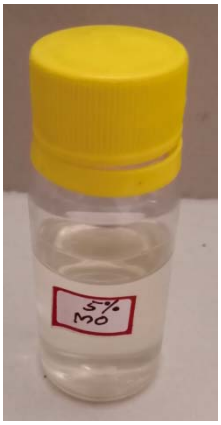
T_{10}

T_{11}

T_{12}

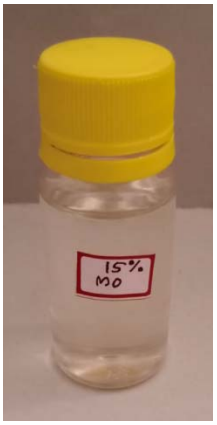


T_{13}

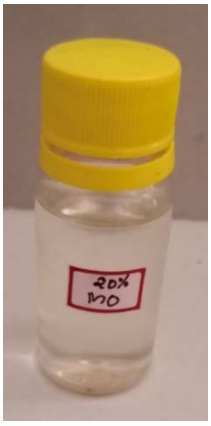


T_{14}

T_{15}



T_{16}



T_{17}

T_{18}

T_{19}

T_{20}

Plate 1. Treatments used in the analysis (Cont.)

Plate 2. Refractometer

3.1.2. Relative density

Relative density of the coconut oil samples were determined using a 50 ml pycnometer (CODEX, 2015). The pycnometer was wiped and weighed (M_1). Then the pycnometer was filled with samples which were maintained at (X°C) 40°C and weight was taken (M_2). The pycnometer was washed, dried and then filled with water maintained at 20°C and weighed (M_3). The relative density of samples were found out using the following formula

Relative Density XºC/water at 20ºC = $\frac{M_2-M_1}{M_3 - M_1}$

3.1.3. Apparent density

Apparent density was found out according to the CODEX standards (CODEX, 2015). Apparent density was calculated using the formula

Apparent density (ρ_0) = m/ V_0

ρ_0 is the apparent density expressed in g cm^{-3}

m is the mass in g of sample (W_2-W_1)

V_0 is the volume in natural state (including internal pores) expressed in cm^3 .

50 ml of samples were taken and the mass of the samples (g) were found out after subtracting the mass of the pycnometer (W_1) from the mass of pycnometer containing samples (W_2) and apparent density was calculated from the above formula.

3.1.4. Insoluble impurities

The insoluble impurities were determined according to CODEX (2015). Two g of the oil sample was taken in a 250ml conical flask. 20ml of 1:1 solvent mixture (petroleum ether + diethyl ether) was added to the flask and it was shaken vigorously. At 30°C, it was allowed to stand for 30 min. After 30 min, the liquid was filtered

through Whatman number 1 filter paper. 10 ml of the solvent mixture was used to wash the filter paper. The filter paper was dried in an oven at 103°C till it attained a constant weight. The increase in weight was denoted as the weight of impurities and it was expressed in percentage.

$$\text{Insoluble impurities (\%)} = \frac{a \times 100}{w}$$

Where,

a = increase in the weight of filter paper

w = weight of sample

3.1.5. Saponification value

Saponification value was determined according to the method described in FSSAI (2015). The samples were filtered through a filter paper to remove any impurities and moisture. After mixing the sample thoroughly, 2 g of dry sample was weighed into a 250 ml Erlenmeyer flask. Then 25 ml of the alcoholic potassium hydroxide solution was pipetted into the flask. A blank was run along with the sample. The sample flasks and the blank flask were connected with air condensers, kept on the water bath and boiled gently until saponification was completed. This was indicated by absence of any oily matter and appearance of clear solution. Clarity was obtained within one hour of boiling. When the flask and condenser have cooled, the inside of the condenser was washed down with about 10 ml of hot ethyl alcohol. The excess potassium hydroxide was titrated with 0.5N hydrochloric acid, using about 1.0 ml phenolphthalein indicator and saponification value was estimated using the formula,

$$\text{Saponification Value} = \frac{56.1\ (B-S)N}{W}$$

Where,

B = Volume in ml of standard hydrochloric acid required for the blank.

S = Volume in ml of standard hydrochloric acid required for the sample

N = Normality of the standard hydrochloric acid

W = Weight in g of the oil or fat taken for the test.

3.1.6. Iodine value

Iodine value was determined according to the procedure described in FSSAI (2015) using the Wij's iodine solution. Liberated iodine was titrated with standardized sodium thiosulphate solution, using starch as indicator. Iodine value is expressed as gram of iodine absorbed per 100 g of oil.

Six g of the sample was weighed into a 500 ml conical flask with glass stopper, to which 25 ml of carbon tetrachloride was added. The content was mixed well. Twenty five ml of Wij's solution was pipetted out and the glass stopper was replaced after wetting with potassium iodine solution. The flasks were kept in dark for half an hour. A blank was run simultaneously. After keeping for some time 15 ml of potassium iodide solution was added, followed by 100 ml of recently boiled and cooled water, rinsing the stopper also. Liberated iodine was titrated with standardized sodium thiosulphate solution, using starch as indicator at the end until the blue colour formed disappeared after thorough shaking with the stopper on. Slight variations in temperature appreciably affected titre of the iodine solution as chloroform has a high coefficient of expansion. Thus blanks and determinations were made at the same time. Iodine value was calculated as follows

$$\text{Iodine value} = \frac{12.69\,(B - S)\,N}{W}$$

Where,

B = Volume in ml of standard sodium thiosulphate solution required for the blank.

S = Volume in ml of standard sodium thiosulphate solution required for the sample

N = Normality of the standard sodium thiosulphate solution

W = Weight in g of the sample.

3.1.7. Polenske value

Polenske value was determined according to the method described by FSSAI (2015). The Polenske value is the number of ml of 0.1N aqueous alkali solution required to neutralize the water insoluble volatile fatty acids distilled from the oil sample.

Five g of the sample was weighed into a 300 ml distilling flask. Twenty ml of glycerine and 2 ml of concentrated sodium hydroxide solution was added and heated with swirling over a flame. The process was continued until the saponification completed, thereby the mixture become perfectly clear. The contents were cooled slightly and 90 ml of boiling distilled water was added. 0.7 g of pumice stone grains and 50 ml of dilute sulfuric acid solution were added and the flask was connected to the distillation apparatus. Heat was given very gently until the liberated fatty acids melted and separated. The flame was set so that 110 ml of distillate was collected within 19 to 21 min. The distillate was collected in a graduated flask. When the distillate exactly reached the 110 ml mark on the flask, the flame was removed and the flask was replaced by a 25 ml measuring cylinder. The graduated flask was kept in a water bath maintained at 15ºC for 10 min so that the 110 ml graduation mark was 1 cm below the water level in the bath. The graduated flask was then removed from the cold water bath, dried outside and the contents were mixed gently by inverting the flask 4 to 5 times without shaking. The liquid was filtered through Whatman No. 4 filter paper. The first 2-3 ml of the filtrate was rejected and the rest was collected in a dry flask. Hundred ml of the filtrate was pipetted out and titrated against standard 0.1N sodium hydroxide solution.

After titrating the soluble volatile acids, the still head was detached and the condenser was rinsed with three successive 15 ml portions of cold distilled water

passing each washing separately through the measuring cylinder, 110 ml graduated flask and was filtered using the filter paper. All the washings were discarded. The funnel was kept on a clean conical flask. The insoluble fatty acids were dissolved by three similar washings of the condenser, the measuring cylinder, the 110 ml flask with stopper, and the filter paper with 15 ml of ethyl alcohol. The alcoholic washings were combined in a clean flask and 5 drops of phenolphthalein indicator solution was added. Titration was done with standard (0.1N) sodium hydroxide solution.

Polenske value = 10 x V x N

where,

V = Volume in ml of standard sodium hydroxide solution required for the test and

N = Normality of the standard sodium hydroxide solution.

3.1.8. Unsaponifiable matter

Unsaponifiable matter was determined as per the procedure of FSSAI (2015). Saponification of the oil was first done with ethanolic potassium hydroxide solution. Extraction of the unsaponifiable matter was then carried out with petroleum ether for repeated times. After evaporating ether, residue was titrated against standard sodium hydroxide solution and was expressed in percentage.

Five g of the sample was taken and poured into 250 ml conical flask. To this 50 ml of alcoholic potassium hydroxide was added and boiled with reflux air condenser until saponification was completed. The condenser was washed with 10 ml ethyl alcohol. The saponified mixture was transfered immediately to a separating funnel. The flask was washed first with ethyl alcohol followed by cold water using 50 ml of water. The flask was cooled to 20-25°C and 50 ml of petroleum ether was added and shaked. The lower soap layer was transferred to a separating funnel and the ether extraction was repeated 3 times using 50 ml of petroleum ether. The ether extract was washed 3 times with 250 ml of aqueous alcohol followed by 25 ml of distilled water to ensure ether extract was free of alkali. Ether solution was then transferred to 25 ml beaker. The separating funnel was rinsed with ether and the rinsings were added to the

main solution. This was evaporated to about 5 ml and transferred quantitatively using ether to 50 ml of dried and weighed Erlenmeyer flask. The ether was separated. When all the ether was removed 2-3 ml of acetone was added and the solvent was completely removed by heating on steam or water bath. This was dried at 100°C for 30 minutes till constant weight was obtained thus removing the traces of ether. The residue was dissolved in 50 ml of warm ethanol and neutralised by phenolphthalein. This was titrated with 0.02 N NaOH.

Weight in g of the free fatty acids in the extract as oleic acid = 0.282VN

Where

V= Volume in ml of standard sodium hydroxide solution

N= Normality of standard NaOH solution

$$\text{Unsaponifiable matter} = \frac{100\,(A-B)}{W}$$

Where,

A = Weight in g of the residue

B = Weight in g of the free fatty acids in the extract

W = Weight in g of the sample

3.1.9. Acid Value

The acid value was determined by directly titrating the oil in an alcoholic medium against standard potassium hydroxide or sodium hydroxide solution using phenolphthalein as indicator (FSSAI, 2015). Acid value expresses the free fatty acids obtained. Two g of samples were weighed in a 250 ml conical flask and 50 ml of freshly neutralised hot ethyl alcohol was added. One ml of Phenolphthalein was also added to the solution. The mixture was boiled for about 5 min and titrated against standard alkali solution. Acid value was calculated as follows

Acid value = $\frac{56.1VN}{W}$

Where,

V = Volume in ml of standard potassium hydroxide or sodium hydroxide used

N = Normality of the potassium hydroxide solution or Sodium hydroxide solution; and

W = Weight in g of the sample

The acidity is frequently expressed as free fatty acid and was calculated as

Free fatty acids as oleic acid = $\frac{28.2VN}{W}$ percent by weight

Acid Value = Percent fatty acid (as oleic) x 1.99

3.1.10. Peroxide value

It is an indication of extent of oxidation suffered by oil. Peroxide value was determined according to the method described by CODEX (2015). Oil was treated with potassium iodide solution. The liberated iodine was titrated with 0.1N sodium thiosulphate solution. Peroxide value was expressed as milli equivalent of peroxide oxygen per kg of sample (meq/kg).

Five g of sample was weighed and taken in a 250 ml stoppered conical flask. Thirty ml of acetic acid chloroform solvent mixture was added and shaken to dissolve. 0.5 ml of saturated potassium iodide solution was added. It was kept for 1 min in dark with occasional shaking and then 30 ml of wáter was added. This was titrated with 0.1 N sodium thiosulphate solution until yellow color disappeared. Starch solution (0.5 ml) was used as an indicator. Vigorous shaking was given to release all iodine from $CHCl_3$ until blue color disappears. Blank was also run .The peroxide value was calculated as follows.

Peroxide value = $\frac{(S-B)\ N\ 100}{W}$

Where,

N = Normality of the sodium thiosulphate

S = Titre value of the sample

B = Titre value of the blank

W= Weight of the sample

3.1.11. Matter volatile at 105° C

The matter volatile at 105°C in oil sample was determined according to CODEX (2015). The oil sample was heated at 105°C until the volatile matter was completely removed. The loss in mass was recorded until it attained a constant mass. It was expressed as percentage by mass.

Aluminium dish with lid is taken and dried at 105°C for 2 h and cooled in a desiccator. It was weighed (W_1). Two g of well mixed sample was weighed and the weight was recorded (W_2). The sample was shaken to distribute the sample to maximum area. Lids were kept on each dish during the transfer to oven. In the oven lid was removed and the sample was kept. The dishes were kept for 3 h in the oven at 105°C. The dishes from the oven was removed and lids were replaced and transferred to a desiccators. This was cooled for 30 min before weighing. The weight (W_3) was recorded to 0.1 mg. The weight was taken when constant weight was attained when successive 1 h drying periods show additional loss of $\leq$ 0.5 per cent.

Matter volatile at 105 ^{o}C = $\{ (W_3 - W_1) / (W_2 - W_1) \}$ X 100%

Where: W_1 = Empty weight of container in grams

W_2 = Initial weight of sample in grams

W_3 = Dry weight of sample and container in grams

3.2. Microbial Contamination

Microbial contamination was determined by taking the total plate count. It included fungal, bacterial and actinomycete population in the oil sample. Serial dilution plate technique was used for estimating the microbial population. One ml of oil was taken and transferred to 9 ml of sterile water and shaken well for 5-10 minutes. From the stock suspension, different dilutions were prepared. 10^{-7} dilution was used for bacterial population while the fungal and actinomycete population was estimated at 10^{-3} dilution (Thanuja, 2015).

The number of colony forming units (cfu) per ml of the sample was calculated using the formula

$$\text{Number of colony forming units} = \frac{\text{Total number of colony formed} \times \text{Dilution factor}}{\text{Weight of oil taken}}$$

3.3. Thin Layer Chromatography (TLC)

Thin layer chromatography was used as a qualitative test for mineral oil detection. Faster moving spots were observed in the case of mineral oil on thin layer chromatographic plates. The spots were located with aqueous sodium fluorescein or 2',7'-dichlorofluorescein under UV light. Silica gel coated glass slides were taken for the experiment (Plate 3). Oil samples were spotted on the glass slide using a capillary tube. Slides were dried and placed in a developing tank containing petroleum ether (Plate 4). The tank was covered and the solvent was allowed to travel for 6 cm from the origin (about 4 min). The plate was removed from the tank and dried in air. Fluorescein solution was sprayed and then viewed under UV light. Presence of mineral oil was confirmed by the appearance of a yellow fluorescent spot on the solvent front. The vegetable oil forms a yellow streak about 2-3 cm long from the point of spotting (FSSAI, 2015).

Plate 3. Silica gel coated glass slide

Plate 4. Glass slides dipped in a bottle containing petroleum ether

3.4. Gas Chromatography Mass Spectrometry (GCMS) Analysis

3.4.1. Fatty Acid Composition

Gas chromatography coupled with mass spectrometry (GC-MS) was used to identify and measure the composition of fatty acids present in coconut oil sample. The samples were methyl esterified to enhance the volatility and separation of compounds. The oil samples were added with 1ml of hexane. Later, sodium methoxide (1 ml) prepared by adding 1.55 g of NaOH to 50 ml of methanol solution was also added. The mixture was shaken vigorously for 30 s. Centrifugation of the solution was done at 1200 rpm and incubation was done at room temperature for 10 min. The clear solution containing fatty acid methyl esters (FAME) was separated out from the cloudy layer. This hexane layer obtained can be used as a sample solution for GC (Kamatou and Viljoen, 2017).

The GC–MS analysis of the oil sample was performed on Agilent Technologies, 7890A gas chromatograph, coupled to a 5975C mass spectrometer with triple axis detector. The GC include a DB WAX capillary column (with stationary phase 5% Phenyl-95% dimethyl-polysiloxane; length 30 m; inner diameter 0.25 mm; film thickness 0.25 μm). Helium was used as carrier gas at flow rate of 1 ml min^{-1}. The injector temperature was set at 250°C and pressure at 6.8405 psi and the flow rate was 0.8ml min^{-1}. The oven program was started with an initial temperature of 80 °C and held for 5 min and then the oven temperature was raised at 4 °C min^{-1} to 230 °C and finally held isothermally for 5 min. 2μl of the samples were injected (split mode). For GC-MS detection, an electron ionization system, with ionization energy of 70 eV was used and the scanning covered a range of 40–450 amu. Chemstation software was used to obtain the relative percentage of compounds.. The mass spectrum obtained was submitted to National Institute of Standards and Technology software (NIST MS) for identification of the compound.

3.5. FTIR Spectroscopy

Attenuated total reflectance (ATR) sampling technique was used for infrared spectroscopy which requires little to no sample preparation. A windows based

operating system was used and the software implemented for FTIR data analysis was OMNIC^{TM}. This software is a full featured software which will collect the spectra and will analyze the data. A Nicolet iS50 FTIR spectrometer (Waltham, Massachusetts, USA) equipped with a SMART iTX Accessory with diamond crystal, deuterated triglycerine sulfate (DTGS) KBr detector and KBr beam splitter was used for the analysis (Plate 5). The crystal was cleaned by wiping with acetone or isopropyl alcohol and it was dried with a soft tissue to avoid cross contamination. The background spectrum obtained was collected and it was optimized before the analysis of each sample. A dropper or syringe was used to place the thin film of sample over the crystal. The sample was placed in the centre of the crystal directly under the pressure point and it was covered for obtaining better result. The collection of FTIR spectra was carried out with a resolution of 4 cm^{-1} and 32 scans. All spectra were recorded within the mid infrared region from 4,000 to 525 cm^{-1} and the percentage of transmittance was measured (Rohman, 2017).

3.6. Economics of Adulteration

The economics of adulteration by palm kernel oil and mineral oil per one quintal of coconut oil was worked out. Cost per 100 kg of coconut oil and adulterants like palm kernel oil and mineral oil were taken into account. Profit obtained during the mixing of adulterant oils in coconut oil was also calculated. Benefit cost ratio was calculated by the following formula

$$\text{Benefit Cost ratio} = \frac{\text{Profit obtained during adulteration}}{\text{Cost of one quintal of oil}}$$

Statistical Analysis

Chemical and physical characteristics of coconut oil were analysed by analysis of variance technique. Multivariate analysis consisting of principal component analysis was also performed (Dayrit *et al*., 2007).

Plate 5. Fourier Transform Infrared Spectrometer (FTIR)

RESULTS

4. RESULTS

The study entitled, "Quality assessment of coconut oil and detection of adulteration" was carried out at the Department of Plantation Crops and Spices, College of Agriculture, Vellayani, Thiruvananthapuram during the period 2018-2020. The data generated on physical and chemical characters on pure coconut oil, five branded coconut oil and coconut oil mixed 1, 5, 10, 15, 20 and 30 per cent of palm kernel oil and mineral oil as well as characterization of these oils by thin layer chromatography, fatty acid composition by GCMS and FTIR spectroscopy in the laboratory were analysed and the results are presented in this chapter.

Pure coconut oil was obtained from the three different coconut expeller collected at three different periods and five different brands of coconut oil samples from three different shops at three different time were collected and analysed separately. Pure coconut oil obtained from the three expeller were mixed separately with 1, 5, 10, 15, 20 and 30 per cent of palm kernel oil and mineral oil and all the samples were analysed for physical and chemical characteristics, microbial contamination, thin layer chromatography, fatty acid composition by GCMS and FTIR spectroscopy.

4.1. Chemical and Physical Characteristics of Coconut Oil

The chemical and physical characteristics of coconut oil samples were analysed and they were subjected to analysis of variance. Significant variations were observed in all the characters studied. Principal component analysis was also performed to analyze the variation contributed by these parameters.

4.1.1. Refractive Index at 40° C

The effect of refractive index on the quality assessment of oil samples is presented in Table 3. Significant variation was noticed with respect to refractive index at 40°C among different oil samples. The refractive index of samples T_1 to T_{13} which included pure coconut samples collected from expeller, branded coconut oil, coconut oil mixed with 1, 5, 10, 15, 20 as well as 30 per cent palm kernel oil and 1 per cent mineral oil was 1.449. The highest refractive index was noticed for treatment T_{20}

(mineral oil) and the value obtained was 1.467. This was followed by T_{18} (coconut oil mixed with 20 per cent mineral oil) which showed a refractive index of 1.453. The refractive index started changing from coconut oil mixed with 5 per cent mineral oil onwards.

4.1.2. Relative density

Relative density of the samples obtained are given in Table 3. Significantly different relative density was observed among oil samples. The relative density of pure coconut oil collected from expeller (T_1) and branded coconut oils (T_2, T_3, T_4, T_5 and T_6) ranged from 0.910 in T_3 (Branded sample 2) to 0.921 in T_5 (Branded sample 4), T_2 (Branded sample 1) and T_6 (Branded sample 5). Relative density of pure sample (T_1) was on par with T_5, T_2 and T_6. The relative density of coconut oil mixed with palm kernel oil varied from 0.907 to 0.911 while that for pure palm kernel oil (T_{19}) was 0.903. Lower relative density was observed for mineral oil (T_{20}) and the value obtained was 0.852 followed by T_{18} (coconut oil mixed with 30 percent mineral oil) with a value of 0.892. This was on par with T_{17} (coconut oil mixed with 30 percent mineral oil) with a value of 0.895.

4.1.3. Apparent density

Apparent density of the oil samples analysed are presented in Table 3. The highest apparent density was recorded for branded coconut oils T_6 (Branded sample 5), T_5 (Branded sample 4), T_2 (Branded sample 1) with 0.907g ml^{-1} which was on par with T_1 (Pure sample) with apparent density of 0.906 g ml^{-1} and T_4 (Branded sample 3) with an apparent density of 0.903 g ml^{-1}. The apparent density of pure palm kernel oil (T_{19}) was 0.889 g ml^{-1} while the coconut oil mixed with 1, 5, 10, 15, 20 and 30 per cent palm kernel oil ranged from 0.890 to 0.897. The lowest apparent density was noticed for mineral oil (T_{20}) with a value of 0.844 g ml^{-1} which was followed by T_{18} (coconut oil mixed with 30 per cent mineral oil) with 0.879 g ml^{-1} and they were found to be on par.

Table 3. Refractive Index at 40°C, relative density and apparent density of oil samples

Treatments	Refractive Index at 40°C	Relative density	Apparent density(g/ml)
T_1 (Pure sample)	1.449	0.920	0.906
T_2 (Branded sample 1)	1.449	0.921	0.907
T_3 (Branded sample 2)	1.449	0.910	0.896
T_4 (Branded sample 3)	1.449	0.917	0.903
T_5 (Branded sample 4)	1.449	0.921	0.907
T_6 (Branded sample 5)	1.449	0.921	0.907
T_7 1% PKO+ 99 % Coconut oil	1.449	0.911	0.897
T_8 5% PKO + 95 % Coconut oil	1.449	0.907	0.893
T_9 10% PKO+ 90% Coconut oil	1.449	0.910	0.896
T_{10} 15% PKO+ 85 % Coconut oil	1.449	0.907	0.893
T_{11} 20% PKO + 80% Coconut oil	1.449	0.908	0.890
T_{12} 30% PKO+ 70% Coconut oil	1.449	0.910	0.896
T_{13} 1% Mineral oil + 99% Coconut oil	1.449	0.906	0.892
T_{14} 5% Mineral oil + 95 % Coconut oil	1.450	0.904	0.890
T_{15} 10% Mineral oil+ 90% Coconut oil	1.451	0.903	0.890
T_{16} 15% Mineral oil+85% Coconut oil	1.451	0.901	0.887
T_{17} 20% Mineral oil+ 80% Coconut oil	1.452	0.895	0.881
T_{18} 30% Mineral oil+ 70% Coconut oil	1.453	0.892	0.879
T_{19} PKO	1.450	0.903	0.889
T_{20} Mineral Oil	1.467	0.852	0.844
SE(m) ±	0.00000068	0.002	0.002
CD (0.05)	0.0001	0.005	0.005

4.1.4 Insoluble impurities

Insoluble impurities of the oil samples analysed are represented in Table 4. Insoluble impurities of pure coconut oil and branded coconut oils T_2, T_3, T_4 and T_5 were 0.024 per cent while that for T_6 (Branded sample 5) was 0.040. The highest value was observed in coconut oil mixed with 30 per cent mineral oil (T_{18}) and the value obtained was 0.047 per cent. This was followed by coconut oil mixed with 10 percent mineral oil (T_{15}) and the value observed was 0.043 per cent. The insoluble impurities in coconut oil mixed with varying concentrations of mineral oil ranged from 0.024 to 0.047 per cent. The insoluble impurities in coconut oil mixed with varying concentrations of palm kernel oil ranged from 0.024 to 0.043 per cent while that for pure palm kernel (T_{19}) was 0.033 per cent. Lowest percentage of insoluble impurities was observed in coconut oil mixed with 20 per cent palm kernel oil (T_{11}) with a value of 0.024 per cent.

4.1.5. Saponification value

The saponification value of the oil samples analysed showed significant variation among treatments and the values are given in Table 4. The highest saponification value was noticed for treatment T_1 (Pure sample) with a saponification value of 266.29 mg KOH g of oil^{-1}. This was followed by T_2 (Branded sample 1) with a value of 259.18 mg KOH g of oil^{-1}. The saponification value of pure coconut oil as well as branded coconut oil ranged from 250.31 to 266.29 mg KOH g of oil^{-1}. The saponification value of pure palm kernel oil (T_{19}) was 250.67 mg KOH g of oil^{-1} and that of coconut oil mixed with varying concentration of palm kernel oil ranged from 236.79 to 247.09 mg KOH g of oil^{-1}. Saponification value was the lowest in T_{20} (mineral oil) with 8.48 mg KOH g of oil^{-1} followed by T_{18} (coconut oil mixed with 30 per cent mineral oil) with 23.80 mg KOH g of oil^{-1}.

4.1.6. Iodine value

Iodine value obtained for all the treatments in the analysis are presented in Table 4. Significant differences in iodine value were observed among the treatments. The iodine value of pure coconut oil (T_1) was 9.27 g of iodine per 100 g of oil and

was on par with T_5 (9.23 g of iodine 100 g of oil^{-1}). Similarly, treatments T_6 (8.37 g of iodine 100 g of oil^{-1} and T_4 (8.233 g of iodine 100 g of oil^{-1}) were on par. The iodine value of pure palm kernel oil was 20.26 g of iodine 100 g of oil^{-1} while that of coconut oil mixed with different concentration of palm kernel oil varied from 10.03 to 17.61 g of iodine 100 g of oil^{-1}. The highest iodine value was observed for pure palm kernel oil, T_{19} (20.26 g of iodine 100 g of oil^{-1}) followed by coconut oil mixed with 30 per cent palm kernel oil, T_{12} (17.61 g of iodine 100 g of oil^{-1}). The lowest iodine value was noticed for mineral oil, T_{20} (2.43 g of iodine 100 g of oil^{-1}) which was followed by coconut oil mixed with 30 per cent mineral oil, T_{18} (3.20 g of iodine 100 g of oil^{-1}). Thus lower iodine values were noticed in mineral oil as well as coconut oil mixed with different concentration of mineral oil (2.43 to 8.67 g of iodine 100g of oil^{-1}).

4.1.7. Polenske value

Polenske value obtained in the experiment are shown in Table 5. Treatment T_1 (Pure sample) showed high Polenske value (14.17) in the analysis. This was followed by T_3 (Branded sample 2) with a value of 13.81, T_5 (Branded sample 4) with 13.75, T_6 (Branded sample 5) with 13.65, T_2 (Branded sample 1) with 13.50 and they were found to be on par. The Polenske value of coconut oil mixed with 1, 5, 10, 15, 20 and 30 per cent of palm kernel oil were 13.03, 12.91, 12.50, 12.22, 11.04 and 7.80 respectively. Pure palm kernel oil (T_{19}) showed a Polenske value of 7.73. The lowest Polenske value (0.26) was obtained for mineral oil (T_{20}) followed by T_{18} (coconut oil mixed with 30 per cent mineral oil) with a value of 5.63.

4.1.8. Unsaponifiable matter

Unsaponifiable matter observed in different oil samples varied significantly among treatments and are presented in Table 5. The unsaponifiable matter in pure coconut oil and branded coconut oil ranged from 0.13 to 0.33 per cent. The lowest value was observed for treatment T_2 (Branded sample 1) with a value of 0.13 per cent. This was followed by T_8 (coconut oil mixed with 5 per cent palm kernel oil) with a value of 0.15 per cent and they were found to be on par. The unsaponifiable matter for pure palm kernel oil (T_{19}) was 0.21 per cent. The unsaponifiable matter for

coconut oil mixed with varying concentration of palm kernel oil ranged from 0.15 to 0.33 per cent. The highest unsaponifiable value was noticed for treatment T_{20} (mineral oil) with a value of 89.12 per cent followed by T_{18} (coconut oil mixed with 30 per cent mineral oil) with 37.94 per cent.

4.1.9. Acid value

Acid value obtained for all the tested coconut samples are presented in Table 5. Significant difference in acid value was noticed among the oil samples. The acid value of the pure coconut sample (T_1) was 3.13 mg KOH g of oil^{-1} and it varied from 1.29 to 3.53 mg KOH g of oil^{-1} in five branded coconut oil samples tested. The highest acid value was noticed in pure palm kernel oil (T_{19}) with 9.83 mg KOH g of oil^{-1}. This was followed by coconut oil sample with 30 per cent of palm kernel oil (T_{12}) with an acid value of 8.06 mg KOH g of oil^{-1}. The acid value of coconut oil mixed with different concentration of palm kernel oil ranged from 3.56 to 8.06 mg KOH g of oil^{-1}. The lowest acid value was observed for mineral oil (T_{20}) with 0.57 mg KOH g of oil^{-1}. This was followed by T_2 (Branded sample 1) with a value of 1.29 mg KOH g of oil^{-1} which was on par with T_6 (Branded sample 5) with an acid value of 1.30 mg KOH g of oil^{-1}.

4.1.10. Peroxide value

The peroxide value of oil samples obtained tested are presented in Table 6. Peroxide value was significantly different among the oil samples analysed. The peroxide value for the pure coconut oil (T_1) was 5.33 meq kg of oil^{-1}. The branded coconut oils T_2 (Branded sample 1), T_3 (Branded sample 2) T_4 (Branded sample 3), T_5 (Branded sample 4) and T_6 (Branded sample 5) recorded peroxide values of 2.15, 4.35, 6.13, 6.06 and 3.58 meq kg of oil^{-1} respectively. Peroxide value of coconut oil mixed with 1, 5, 10, 15, 20 and 30 per cent of palm kernel oil were 9.32, 11.03, 10.86, 12.13, 12.86 and 13.06 meq kg of oil^{-1} respectively. The lowest peroxide value was observed for branded sample 1 (T_2) with 2.15 meq kg of oil^{-1}. This was followed by branded sample 5 (T_6) with a peroxide value of 3.58 meq kg of oil^{-1}. The highest

peroxide value was noticed for palm kernel oil, T_{19} (14.56 meq kg of oil^{-1}) followed by coconut oil mixed with 30 per cent palm kernel oil (T_{12}) with 13.06 meq kg of oil^{-1} and coconut oil mixed with 20 per cent palm kernel oil (T_{11}) with 12.86 meq kg of oil.

4.1.11. Matter volatile at 105°C

Matter volatile at 105°C obtained for all the oil samples tested are presented in Table 6. The matter volatile at 105° C for pure coconut oil was 0.077 per cent which was the lowest. Branded coconut oils had matter volatile at 105°C varying from 0.123 to 0.147 per cent. Highest matter volatile at 105°C was noticed for treatment T_{12} (coconut oil mixed with 30 per cent palm kernel oil) with a value of 0.157 per cent followed by T_{20} (mineral oil) with 0.150 per cent and T_{18} (coconut oil mixed with 30 per cent mineral oil) and T_3 (Branded sample 2) with 0.147 per cent which were on par. Matter volatile at 105 °C of coconut oil mixed with different concentration of mineral oil were 0.080, 0.097, 0.117, 0.127, 0.137 and 0.147 per cent respectively.

Table 4. Insoluble impurities, saponification value and iodine value of oil samples

Treatments	Insoluble impurities (%)	Saponification value (mg KOH/g of oil)	Iodine value (g/100g of oil)
T_1 (Pure sample)	0.024	266.29	9.27
T_2 (Branded sample 1)	0.024	259.18	7.63
T_3 (Branded sample 2)	0.024	250.31	7.97
T_4 (Branded sample 3)	0.024	251.54	8.23
T_5 (Branded sample 4)	0.024	254.92	9.23
T_6 (Branded sample 5)	0.040	257.12	8.37
T_7 1% PKO+ 99 % Coconut oil	0.024	236.79	10.03
T_8 5% PKO + 95 % Coconut oil	0.043	238.91	11.17
T_9 10% PKO+ 90% Coconut oil	0.024	242.42	12.50
T_{10} 15% PKO+ 85 % Coconut oil	0.043	244.37	14.70
T_{11} 20% PKO + 80% Coconut oil	0.024	244.88	15.61
T_{12} 30% PKO+70% Coconut oil	0.024	247.09	17.61
T_{13} 1% Mineral oil + 99% Coconut oil	0.024	106.16	8.67
T_{14} 5% Mineral oil + 95 % Coconut oil	0.024	69.91	7.40
T_{15} 10% Mineral oil+ 90% Coconut oil	0.043	64.11	6.80
T_{16} 15% Mineral oil+85% Coconut oil	0.037	41.83	5.43
T_{17} 20% Mineral oil+ 80% Coconut oil	0.040	36.13	4.70
T_{18} 30% Mineral oil+ 70% Coconut oil	0.047	23.80	3.20
T_{19} PKO	0.033	250.67	20.26
T_{20} Mineral Oil	0.043	8.48	2.43
SE (m) ±	0.004	0.421	0.081
CD (0.05)	0.013	1.209	0.231

Table 5. Polenske value, unsaponifiable matter and acid value of oil samples

Treatments	Polenske value	Unsaponifiable matter (%)	Acid value (mg KOH/g of oil)
T_1 (Pure sample)	14.17	0.28	3.13
T_2 (Branded sample 1)	13.50	0.13	1.29
T_3 (Branded sample 2)	13.81	0.17	1.78
T_4 (Branded sample 3)	13.13	0.15	3.53
T_5 (Branded sample 4)	13.75	0.33	1.63
T_6 (Branded sample 5)	13.65	0.25	1.30
T_7 1% PKO+ 99 % Coconut oil	13.03	0.23	3.56
T_8 5% PKO + 95 % Coconut oil	12.91	0.15	4.76
T_9 10% PKO+ 90% Coconut oil	12.50	0.17	5.39
T_{10} 15% PKO+ 85 % Coconut oil	12.22	0.21	6.26
T_{11} 20% PKO + 80% Coconut oil	11.04	0.24	7.67
T_{12} 30% PKO+70% Coconut oil	7.80	0.33	8.06
T_{13} 1% Mineral oil + 99% Coconut oil	10.84	1.18	3.64
T_{14} 5% Mineral oil + 95 % Coconut oil	10.56	3.38	3.35
T_{15} 10% Mineral oil+ 90% Coconut oil	10.35	8.01	3.17
T_{16} 15% Mineral oil+85% Coconut oil	7.63	13.84	2.95
T_{17} 20% Mineral oil+ 80% Coconut oil	6.20	24.95	2.80
T_{18} 30% Mineral oil+ 70% Coconut oil	5.63	37.94	2.15
T_{19} PKO	7.73	0.21	9.83
T_{20} Mineral Oil	0.26	89.12	0.57
SE (m) ±	0.748	0.065	0.058
CD (0.05)	2.147	0.187	0.167

Table 6. Peroxide value and matter volatile at 105° C

Treatments	Peroxide value (meq/Kg of oil)	Matter volatile at 105°C (%)
T_1 (Pure sample)	5.33	0.077
T_2 (Branded sample 1)	2.15	0.123
T_3 (Branded sample 2)	4.35	0.147
T_4 (Branded sample 3)	6.13	0.133
T_5 (Branded sample 4)	6.06	0.140
T_6 (Branded sample 5)	3.58	0.140
T_7 1% PKO+ 99 % Coconut oil	9.32	0.083
T_8 5% PKO + 95 % Coconut oil	11.03	0.103
T_9 10% PKO+ 90% Coconut oil	10.86	0.117
T_{10} 15% PKO+ 85 % Coconut oil	12.13	0.137
T_{11} 20% PKO + 80% Coconut oil	12.86	0.143
T_{12} 30% PKO+70% Coconut oil	13.06	0.157
T_{13} 1% Mineral oil + 99% Coconut oil	6.23	0.080
T_{14} 5% Mineral oil + 95 % Coconut oil	6.55	0.097
T_{15} 10% Mineral oil+ 90% Coconut oil	8.35	0.117
T_{16} 15% Mineral oil+85% Coconut oil	10.32	0.127
T_{17} 20% Mineral oil+ 80% Coconut oil	11.42	0.137
T_{18} 30% Mineral oil+ 70% Coconut oil	11.63	0.147
T_{19} PKO	14.56	0.110
T_{20} Mineral Oil	3.64	0.150
SE (m) ±	0.01	0.004
CD (0.05)	0.03	0.012

Principal Component Analysis

Principal component analysis (PCA) was done on physical and chemical characteristics of oil samples. Total variation of 74.16 per cent was observed in the data and is shown in Table 7. In the analysis, two principal components were obtained, PC 1 and PC 2. PC 1 accounted for 55.36 per cent of variation followed by PC2 with 18.80 per cent variation. Loadings of principal components 1 and 2 from PCA analysis is shown in Table 8. In PC1, high coefficient was obtained for the parameter unsaponifiable matter (-0.968) followed by relative density (0.943), apparent density (0.942), Polenske value (0.938), refractive index (-0.935) and saponification value (0.826) and the lowest coefficient was obtained for peroxide value (0.009). In PC 2, high coefficient was noticed for acid value (0.891) followed by peroxide value (0.713) and iodine value (0.701). The lowest coefficient was noticed refractive index (-0.030) in PC 2. The score plot obtained is shown in fig 12. A variable plot was also constructed based on the data and is shown in fig 13.

Table 7. Total Variance Explained

Component	Initial Eigen Values			Extraction Sums of Squared Loadings		
	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %
1	6.089	55.357	55.357	6.089	55.36	55.36
2	2.068	18.797	74.154	2.068	18.80	74.16
3	.987	8.976	83.130			
4	.917	8.340	91.470			
5	.633	5.753	97.222			
6	.233	2.122	99.344			
7	.044	.402	99.746			
8	.014	.129	99.874			
9	.012	.105	99.979			
10	.002	.020	99.999			
11	.000	.001	100.000			

Table 8. Loadings of principal components 1 and 2 from PCA analysis of physical and chemical properties of oil samples

Sl No	Physical and chemical properties	Principal component 1	Principal component 2
1.	Refractive Index	- 0.935	-0.030
2.	Relative Density	0.943	-0.244
3.	Apparent Density	0.942	-0.262
4.	Insoluble Impurities	-0.562	0.252
5.	Saponification Value	0.826	0.061
6.	Iodine Value	0.602	0.701
7.	Polenske Value	0.938	-0.243
8.	Unsaponifiable Matter	-0.968	-0.059
9.	Acid Value	0.363	0.891
10.	Peroxide Value	0.009	0.713
11.	Matter Volatile at 105°C	-0.362	0.115

4.2. Microbial Contamination

4.2.1. Total plate count

Total plate count of the oil samples were analysed and is represented in Table 9. This include bacterial, fungal and actinomycete count. It is expressed in cfu ml^{-1}. Fungal and actinomycete count was zero for all the treatments (Plate 7). The highest value of bacterial count observed was 7×10^{-7} cfu ml^{-1} in T_{12} (coconut oil mixed with 30 per cent palm kernel oil) (Plate 6b). This was followed by T_{10} (coconut oil mixed with 15 per cent palm kernel oil) and T_{17} (coconut oil mixed with 20 per cent mineral oil) with 6×10^{-7} cfu ml^{-1}. The lowest bacterial count or no colony was observed for pure sample (T_1) (Plate 6a.) followed by T_2 (Branded sample 1) with 1×10^{-7} cfu ml^{-1}.

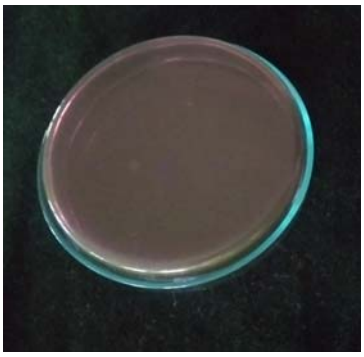
4.3. Thin Layer Chromatography (TLC)

Thin layer chromatography was carried out for all the samples. Results obtained during the analysis is presented in Table 10. TLC profiles obtained during the analysis of oil samples are also depicted (Plate 8).

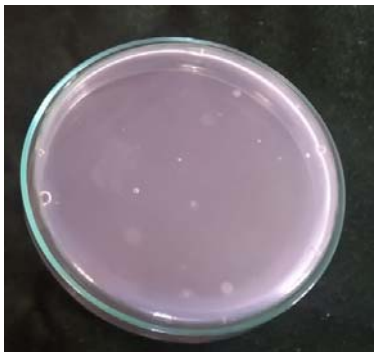
Among the samples, presence of mineral oil was detected for treatments T_{13} (coconut oil mixed with 1 percent mineral oil), T_{14} (coconut oil mixed with 5 per cent mineral oil) T_{15} (coconut oil mixed with 10 per cent mineral oil), T_{16} (coconut oil mixed with 15 per cent of mineral oil), T_{17} (coconut oil mixed with 20 per cent of mineral oil) T_{18} (coconut oil mixed with 30 per cent of mineral oil and T_{20} (mineral oil).Yellow fluorescent spots were observed on these treatments. Yellow streaks were appeared on other treatments which showed the absence of mineral oil.

Table 9. Microbial population of oil samples

Treatments	Microbial count (cfu/ml)		
	Mean Bacterial population ($x10^7$)	**Mean Fungal population ($x10^3$)**	**Mean Actinomycete population ($x10^3$)**
T_1 (Pure sample)	0	0	0
T_2 (Branded sample 1)	1	0	0
T_3 (Branded sample 2)	2	0	0
T_4 (Branded sample 3)	3	0	0
T_5 (Branded sample 4)	2	0	0
T_6 (Branded sample 5)	3	0	0
T_7 1% PKO+ 99 % Coconut oil	5	0	0
T_8 5% PKO + 95 % Coconut oil	3	0	0
T_9 10% PKO+ 90% Coconut oil	4	0	0
T_{10} 15% PKO+ 85 % Coconut oil	6	0	0
T_{11} 20% PKO + 80% Coconut oil	4	0	0
T_{12} 30% PKO+70% Coconut oil	7	0	0
T_{13} 1% Mineral oil + 99% Coconut oil	4	0	0
T_{14} 5% Mineral oil + 95 % Coconut oil	5	0	0
T_{15} 10% Mineral oil+ 90% Coconut oil	5	0	0
T_{16} 15% Mineral oil+85% Coconut oil	5	0	0
T_{17} 20% Mineral oil+ 80% Coconut oil	6	0	0
T_{18} 30% Mineral oil+ 70% Coconut oil	4	0	0
T_{19} PKO	4	0	0
T_{20} Mineral Oil	3	0	0
SE(m)±	0.835		
CD	1.508		



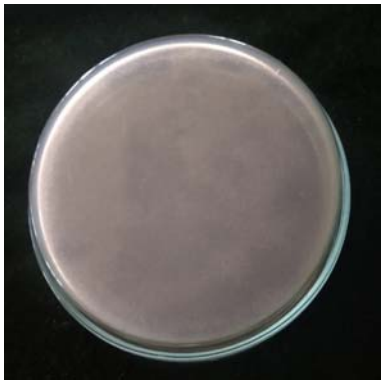
(a) T_1



(b) T_{12}

Plate 6. Treatments showing(a) lowest and highest (b)bacterial count

(a)

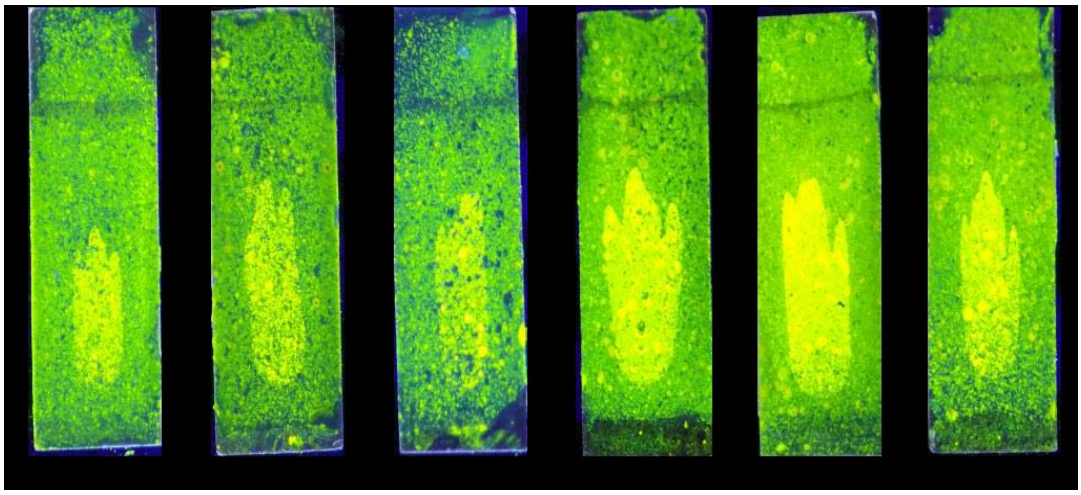


(b)

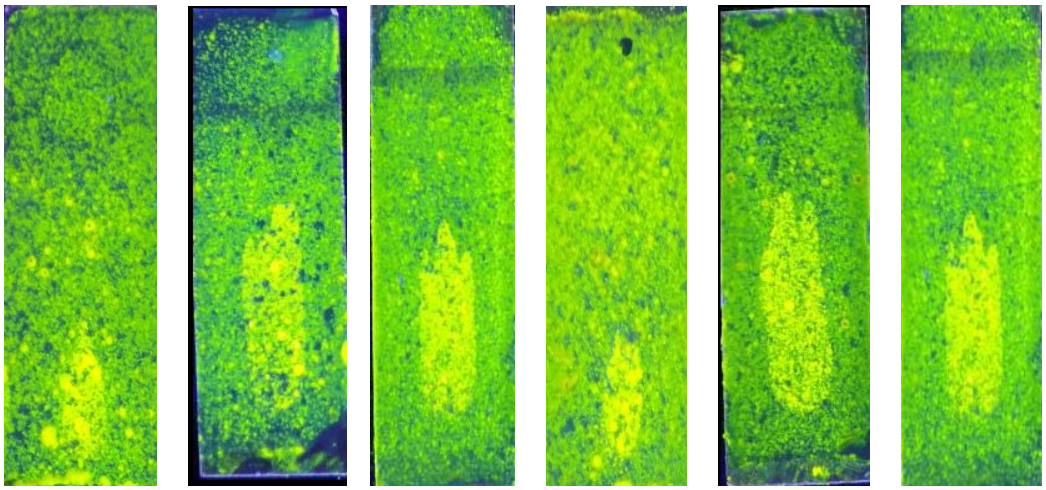
Plate 7. Treatment showing (a) absence of fungal count and (b) absence of actinomycete count

Table 10. Thin layer chromatography of oil samples

Treatments	Presence of mineral oil
T_1 (Pure sample)	Not detected
T_2 (Branded sample 1)	Not detected
T_3 (Branded sample 2)	Not detected
T_4 (Branded sample 3)	Not detected
T_5 (Branded sample 4)	Not detected
T_6 (Branded sample 5)	Not detected
T_7 1% PKO+ 99 % Coconut oil	Not detected
T_8 5% PKO + 95 % Coconut oil	Not detected
T_9 10% PKO+ 90% Coconut oil	Not detected
T_{10} 15% PKO+ 85 % Coconut oil	Not detected
T_{11} 20% PKO + 80% Coconut oil	Not detected
T_{12} 30% PKO+70% Coconut oil	Not detected
T_{13} 1% Mineral oil + 99% Coconut oil	Detected
T_{14} 5% Mineral oil + 95 % Coconut oil	Detected
T_{15} 10% Mineral oil+ 90% Coconut oil	Detected
T_{16} 15% Mineral oil+85% Coconut oil	Detected
T_{17} 20% Mineral oil+ 80% Coconut oil	Detected
T_{18} 30% Mineral oil+ 70% Coconut oil	Detected
T_{19} PKO	Not detected
T_{20} Mineral Oil	Detected

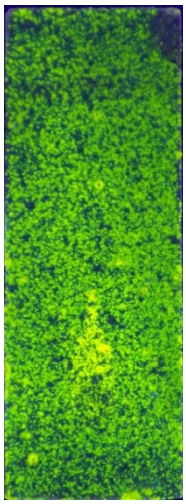


T_1 T_2 T_3 T_4 T_5 T_6



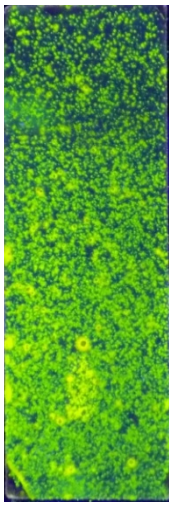
T_7 T_8 T_9 T_{10} T_{11} T_{12}

Plate 8. TLC profile of oil samples

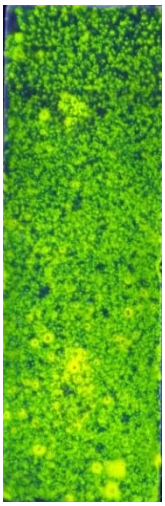


T_{13}

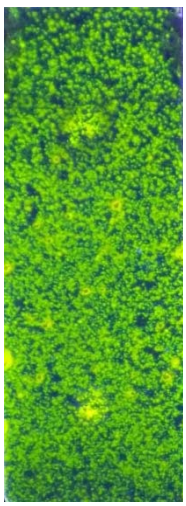
T_{14}



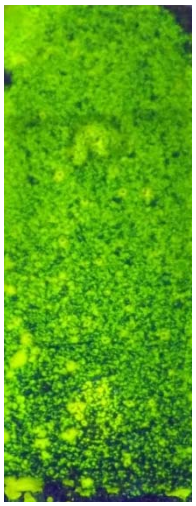
T_{15}



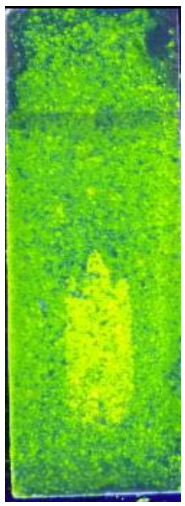
T_{16}



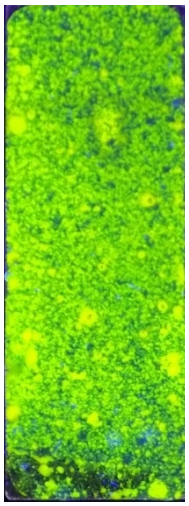
T_{17}



T_{18}



T_{19}



T_{20}

Plate 8. TLC profile of oil samples (Cont.)

4.4. Gas Chromatography Mass Spectrometry (GCMS) Analysis

4.4.1. Fatty acid composition (%)

Gas chromatography coupled with mass spectrometry was used to analyze the fatty acid composition in the oil samples. In the analysis, fatty acids were not detected in mineral oil (T_{20}). Caprylic acid (C8:0), capric acid (C10:0), lauric acid (C12:0), myristic acid (C14:0), palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2), arachidic acid (C20:0), behenic acid (C22:0) and lignoceric acid (C24:0) were the fatty acids detected from the oil samples and the composition of each fatty acid is shown in Table 11. It is evident from the table that the fatty acids like caproic acid (C6:0) and linolenic acid (C18:3) were not detected in any of the treatments. Among the fatty acids, C24:0, showed maximum variability (CV-44.02 per cent) between the treatments with an average of 0.03 per cent. The fatty acids C22:0, C20:0, C18:2, C18:1, C18:0, C16:0 and C14:0 showed a variability of 42.30, 14.41, 31.35, 21.92, 19.28, 9.50 and 15.40 respectively. The coefficient of variation obtained was minimum (CV-7.19 per cent) for C12:0 and the average obtained was 37.19 percent. The fatty acids C10:0 and C8:0 showed a variability of 15.48 and 20.69 per cent respectively.

Principal component analysis (PCA) was performed to get an idea about the contribution or importance of different fatty acids. PCA extracted two PCs which accounted for 79.76 per cent variation in the entire data and is presented in Table 12. The first PC explained a variation of 61.21 per cent and PC 2 explained a variation of 18.55 per cent. The loadings of PC 1 and PC 2 obtained are presented in Table 13. It is evident from the table that the fatty acid C16:0 (0.978), C12:0 (0.954), C14:0 (0.936), C20:0 (0.895) and C18:2 (0.853) had high loadings on PC 1. However, a low coefficient was noticed for C22:0 (0.216) which was followed by C24:0 (0.485). In PC 2, high coefficient was obtained for C8:0 (-0.707) followed by C10:0 (-0.595) and the least coefficient was observed in C16:0 (-0.055). The score plot based on two PCs is depicted in fig 13 and the variable plot is depicted in fig 14.

Table 11. Fatty acid composition

Treatment	Caproic acid C6:0 (%)	Caprylic acid C8:0 (%)	Capric acid C10:0 (%)	Lauric acid C12:0 (%)	Myristic acid C14:0 (%)	Palmitic acid C16:0 (%)	Stearic acid C18:0 (%)	Oleic acid C18:1 (%)	Linoleic acid C 18:2 (%)	Linolenic acid C 18:3 (%)	Arachidic acid C 20:0 (%)	Behenic acid C 22:0 (%)	Lignoceric acid C 24:0 (%)
T_1 (Pure sample)	ND	1.31	2.33	42.97	19.28	11.46	9.55	8.14	2.64	ND	0.19	0.04	0.04
T_2 (Branded sample 1)	ND	1.54	2.56	38.67	19.49	12.36	9.35	8.23	2.83	ND	0.17	0.03	0.05
T_3 (Branded sample 2)	ND	1.11	1.94	38.30	19.84	10.82	8.03	10.22	2.27	ND	0.19	0.02	0.03
T_4 (Branded sample 3)	ND	1.50	2.35	40.78	18.23	11.72	7.61	10.86	2.16	ND	0.18	0.06	0.08
T_5 (Branded sample 4)	ND	1.53	2.28	38.97	16.81	11.95	7.28	10.53	2.05	ND	0.20	ND	ND
T_6 (Branded sample 5)	ND	1.21	2.15	40.63	16.98	12.69	7.54	10.24	2.09	ND	0.18	0.03	0.02
T_7 1% PKO+ 99% Coconut oil	ND	1.54	2.55	38.52	19.14	11.24	9.16	9.22	2.81	ND	0.17	0.02	0.02
T_8 5% PKO + 95 % Coconut oil	ND	1.51	2.54	37.95	18.66	11.55	9.62	9.51	3.07	ND	0.18	0.04	0.03
T_9 10% PKO+ 90% Coconut oil	ND	1.32	2.50	37.82	18.55	12.19	10.53	9.65	3.16	ND	0.17	0.03	0.02
T_{10} 15% PKO+ 85 % Coconut oil	ND	1.31	2.33	35.18	18.23	12.92	10.65	10.13	3.64	ND	0.19	0.04	0.03

Table 11. Fatty acid composition (Cont.)

Treatment	Caproic acid C6:0 (%)	Caprylic acid C8:0 (%)	Capric acid C10:0 (%)	Lauric acid C12:0 (%)	Myristic acid C14:0 (%)	Palmitic acid C16:0 (%)	Stearic acid C18:0 (%)	Oleic acid C18:1 (%)	Linoleic acid C 18:2 (%)	Linolenic acid C 18:3 (%)	Arachidic acid C 20:0 (%)	Behenic acid C 22:0 (%)	Lignoceric acid C 24:0 (%)
T_{11} 20% PKO + 80% Coconut oil	ND	1.24	2.26	35.04	17.81	13.16	10.86	11.82	3.83	ND	0.17	0.03	0.02
T_{12} 30% PKO+70% Coconut oil	ND	1.11	1.94	34.33	17.13	13.65	11.33	13.22	3.97	ND	0.19	0.02	0.01
T_{13} 1% Mineral oil + 99% Coconut oil	ND	1.55	2.35	38.64	24.21	11.72	7.60	10.86	2.86	ND	0.16	0.02	0.01
T_{14} 5%Mineral oil + 95 % Coconut oil	ND	1.53	2.28	36.43	24.92	11.27	6.28	10.50	2.55	ND	0.16	0.01	0.01
T_{15} 10%Mineral oil+ 90% Coconut oil	ND	1.22	2.15	35.83	25.16	11.12	7.54	10.24	2.09	ND	0.15	0.03	0.01
T_{16} 1 5% Mineral oil+ 85% Coconut oil	ND	1.15	2.05	34.91	25.14	10.83	9.16	9.72	1.82	ND	0.15	0.02	0.01
T_{17} 20% Mineral oil+ 80% Coconut oil	ND	1.13	2.04	33.76	24.66	10.55	9.62	9.51	1.47	ND	0.14	0.04	0.03
T_{18} 30% Mineral oil+70% Coconut oil	ND	1.10	1.83	33.15	23.75	10.19	10.82	8.65	1.16	ND	0.14	0.03	0.02
T_{19} PKO	ND	0.45	1.11	34.66	18.13	14.64	13.57	18.68	4.14	ND	0.25	0.06	0.03
T_{20} Mineral Oil	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Average	ND	1.28	2.19	37.19	20.32	11.90	9.27	10.52	2.66	ND	0.18	0.03	0.03
CV (Coefficient of variation)	ND	20.69	15.48	7.19	15.40	9.50	19.28	21.92	31.35	ND	14.41	42.30	44.02

Table 12. Total Variance Explained

Component	Initial Eigen Values			Extraction Sums of Squared Loadings		
	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %
1	6.733	61.213	61.213	6.733	61.21	61.21
2	2.040	18.546	79.759	2.040	18.55	79.76
3	.977	8.885	88.644			
4	.662	6.017	94.660			
5	.357	3.250	97.910			
6	.127	1.151	99.062			
7	.054	.492	99.553			
8	.041	.374	99.927			
9	.007	.062	99.989			
10	.001	.009	99.998			
11	.000	.002	100.000			

Table 13. Loadings of principal components 1 and 2 from PCA analysis of fatty acid composition of oil samples

Sl. No.	Fatty acid composition	Principal component 1	Principal component 2
1.	C 8:0 (Caprylic acid)	.677	-.707
2.	C 10:0 (Capric acid)	.787	-.595
3.	C 12:0 (Lauric acid)	.954	-.279
4.	C 14:0 (Myristic acid)	.936	-.298
5.	C 16:0 (Palmitic acid)	.978	-.055
6.	C 18:0 (Stearic acid)	.771	.383
7.	C 18:1 (Oleic acid)	.644	.492
8.	C 18:2 (Linoleic acid)	.853	.319
9.	C 20:0 (Arachidic acid)	.895	.296
10.	C 22:0 (Behenic acid)	.216	.356
11.	C 24:0 (Lignoceric acid)	.575	.556

4.5 FTIR Spectroscopy

FTIR spectra was obtained using FTIR spectrometer. Spectra of all the treatments were analysed. In pure sample (T_1) major peaks were formed at regions of 2800-2900 cm^{-1}, 1743 cm^{-1}, 1465 cm^{-1}, 1417 cm^{-1}, 1377 cm^{-1}, 1229 cm^{-1}, 1155 cm^{-1}, 1111 cm^{-1}, 962 cm^{-1} and 722 cm^{-1} (Fig 16). All the branded coconut oil samples showed peaks at 2800-2900 cm^{-1}, 1743 cm^{-1}, 1465 cm^{-1}, 1417 cm^{1}, 1377 cm^{-1}, 1229 cm^{-1}, 1155 cm^{-1}, 1111 cm^{-1}, 962 cm^{-1} and 722 cm^{-1} (Fig 17, 18, 19, 20, 21). Adulteration was not detected in any of these branded coconut oil samples. The peaks noticed in the regions of 2921 cm^{-1} and 2853 cm^{-1} were common for all treatments used in the analysis. In palm kernel oil (T_{19}) peaks were observed at 3006 cm^{-1}, 2921 cm^{-1}, 2853 cm^{-1}, 1735 cm^{-1}, 1465 cm^{-1}, 1417 cm^{-1}, 1377 cm^{-1}, 1238−1165 cm^{-1} and 722 cm^{-1} (Fig 34). When palm kernel oil was used as an adulterant in different concentrations, peaks appeared at 3006 cm^{-1}, 2921 cm^{-1}, 2853 cm^{-1}, 1735 cm^{-1}, 1465 cm^{-1}, 1417 cm^{-1}, 1377 cm^{-1}, 1238−1165 cm^{-1} and 722 cm^{-1} (Fig 22, 23, 24, 25, 26, 27). Mineral oil was also used as an adulterant in different concentrations in coconut oil and the peaks were observed at 2800-2900 cm^{-1}, 1743 cm^{-1}, 1465 cm^{-1}, 1417 cm^{-1}, 1377 cm^{-1}, 1229 cm^{-1}, 1155 cm^{-1}, 1111 cm^{-1}, 962 cm^{-1} and 722 cm^{-1} (Fig 28, 29, 30, 31, 32, 33). The peaks for mineral oil (T_{20}) were obtained at 2954 cm^{-1}, 2923 cm^{-1}, 2854 cm^{1}, 1466 cm^{-1}, 1378 cm^{-1} and 721 cm^{-1} (Fig 35).

4.6. Economics of Adulteration

Economics of adulteration of coconut oil by palm kernel oil and mineral oil is depicted in Table 14. Cost of 1 quintal pure coconut oil sample (T_1) was Rs 20500. Cost of branded coconut sample 1 (T_2) and branded sample 3 (T_4) obtained per quintal was Rs.21000. While, cost of one quintal branded sample 2 (T_3) was Rs.22000. Cost of branded sample 4 (T_5) was Rs.18700 and branded sample 5 (T_6) was Rs.18500. Cost of one quintal palm kernel oil (T_{19}) was Rs.10000 and for one quintal mineral oil (T_{20}), it was Rs.4500. Cost per quintal was less (Rs.15700) when coconut oil was mixed with 30 percent of mineral oil (T_{18}) and the profit obtained was Rs.4800. In this case, BC ratio obtained was 0.30 and was found to be the highest among other treatments. When coconut oil was mixed with 1 percent palm kernel oil (T_7) cost

Table 14. Economics of adulteration of coconut oil by palm kernel oil and mineral oil

Treatments	Cost of one quintal of oil (Rs.)	Profit (Rupees)	BC Ratio
T_1 (Pure sample)	20500		
T_2 (Branded sample 1)	21000		
T_3 (Branded sample 2)	22000		
T_4 (Branded sample 3)	21000		
T_5 (Branded sample 4)	18700		
T_6 (Branded sample 5)	18500		
T_7 (1% PKO+ 99 % Coconut oil)	20395	105	0.005
T_8 (5% PKO + 95 % Coconut oil)	19975	525	0.026
T_9 (10% PKO+ 90% Coconut oil)	19450	1050	0.05
T_{10} (15% PKO+ 85 % Coconut oil)	18925	1575	0.08
T_{11} (20% PKO + 80% Coconut oil)	18400	2100	0.11
T_{12} (30% PKO+70% Coconut oil)	17350	3150	0.18
T_{13} (1% Mineral oil + 99% Coconut oil)	20340	160	0.007
T_{14} (5% Mineral oil + 95 % Coconut oil)	19700	800	0.04
T_{15} (10% Mineral oil+ 90% Coconut oil)	18900	1600	0.08
T_{16} (15% Mineral oil+85% Coconut oil)	18100	2400	0.13
T_{17} (20% Mineral oil+ 80% Coconut oil)	17300	3200	0.18
T_{18} (30% Mineral oil+ 70% Coconut oil)	15700	4800	0.30
T_{19} PKO	10000		
T_{20} Mineral Oil	4500		

(Price of coconut oil, branded coconut oil samples, palm kernel oil and mineral oil are listed in Appendix II.)

obtained was Rs. 20395. Profit attained in this case was Rs 105 and a low BC ratio (0.005) was obtained.

DISCUSSION

5. DISCUSSION

The results of the experiment entitled "Quality assessment of coconut oil and detection of adulteration" conducted during the year 2018-2020 are discussed in this chapter.

5.1. Chemical and Physical Characteristics of Coconut Oil

Chemical and physical characteristics of coconut oil samples were analysed and it include refractive index at 40°C, relative density, apparent density, insoluble impurities, saponification value, iodine value, Polenske value, unsaponifiable matter, acid value, peroxide value and matter volatile at 105°C. Significant variations were observed in these parameters among the various oil samples. Principal component analysis was also performed and the results were analyzed.

5.1.1. Refractive Index at 40°C

Effect of refractive index at 40°C on the quality assessment of oil samples is depicted in Fig. 1. The refractive index of pure coconut sample (T_1), branded coconut oil samples (T_2, T_3, T_4, T_5 and T_6), coconut oil mixed with 1, 5, 10, 15, 20 as well as 30 per cent palm kernel oil and 1 per cent mineral oil was 1.449. The refractive index was the highest for treatment T_{20} (mineral oil) and the value obtained was 1.467. This was followed by T_{18} (coconut oil mixed with 30 per cent mineral oil) which showed a refractive index of 1.453. This indicates that pure coconut oil, branded coconut oil samples and coconut oil mixed with different concentrations of palm kernel oil and 1 per cent mineral oil showed similar refractive index. Refractive index started increasing when more quantity of mineral oil substituted the coconut oil. In the analysis, treatment T_{19} (palm kernel oil) obtained a refractive index (1.450) which was above the value of pure coconut oil.

According to FSSAI (2015), refractive index at 40°C for pure coconut oil is 1.4481-1.4491. In the experiment all the branded coconut oil samples showed a refractive index within the standard value. When palm kernel oil was used as an adulterant in different concentrations, the values obtained were within the FSSAI

standard for coconut oil and it was difficult to detect the adulterant. When mineral oil was used as an adulterant, adulteration could be detected from the addition of 5 per cent of mineral oil. Ariponnammal (2012) reported that coconut oil was found to be adulterated with thirty per cent of palm oil and it was detected by Abbe's refractometer of good accuracy. In a study conducted by Srivastava *et al*. (2016), it was found that the refractive index of copra oil, hot extracted virgin coconut oil and cold extracted virgin coconut oil were 1.448. Refractive index of homemade virgin coconut oil was 1.445. When it was deliberately adulterated with 5 to 25 per cent of palm oil an increase in the refractive index was noticed (Premkumar and Joseph, 2018). However in the present experiment there was no difference in the refractive index of coconut oil mixed with various concentration of palm kernel oil.

Atasie and Akinhanmi (2009) reported that the refractive index of palm kernel oil is 1.453. Refractive index of crude palm kernel oil at 28°C was 1.4559 (Bahadi *et al*., 2019). According to FSSAI (2015), refractive index for palm kernel oil is 1.4490-1.4520.

5.1.2. Relative density

Relative density of the samples obtained are given in Fig. 2. Significantly high relative density was observed in pure and branded coconut oils which were on par. T_5 (Branded sample 4), T_2 (Branded sample1) and T_6 (Branded sample 5) recorded a relative density of 0.921, followed by T_1 (Pure sample) with a value of 0.920 and T_4 (Branded sample 3) with a value of 0.917. The relative density started decreasing in coconut oil mixed with palm kernel oil. In coconut oil mixed with varying concentration of palm kernel oil, the relative density ranged from 0.907 to 0.911. The relative density of the pure palm kernel oil (T_{19}) was 0.903. A lower relative density was observed for mineral oil (T_{20}) and the value obtained was 0.852 followed by T_{18} (coconut oil mixed with 30 per cent mineral oil) with a value of 0.892.The relative density of coconut oil mixed with varying concentration of mineral oil ranged from 0.892 to 0.906.

According to CODEX standards, the relative density of coconut oil should be within the range 0.908 to 0.921 (CODEX, 2015).

The relative density of palm kernel oil ranges from 0.899- 0.914 according to codex standard (CODEX, 2015). An increase in relative density of pure coconut oil and branded coconut oil might be due to high saponification value of coconut oil samples. Rudan and Klofutar (1999) noticed an increase in the relative density of oils as the molecular weight decreased and the saponification value was high. Kamariah *et al*. (2008) studied the physio-chemical and quality characteristics of coconut oil. Ten virgin coconut oil samples from Malaysian market were taken and analysed and observed that the relative density of the virgin coconut oil ranged from 0.9185 to 0.9194.

5.1.3. Apparent density

Apparent density of the treatments observed are presented in Fig. 3. The apparent density of pure coconut oil (T_1) was 0.906 g ml^{-1} and that for branded coconut oils T_2 (Branded sample 1) was 0.907g ml^{-1}, T_3 (Branded sample 2) was 0.896 g ml^{-1}, T_4 (Branded sample 3) was 0.903 g ml^{-1}, T_5 (Branded sample 4) and T_6 (Branded sample 5) was 0.907 g ml^{-1}. High apparent density was recorded for treatments T_6 (Branded sample 5), T_5 (Branded sample 4) and T_2 (Branded sample 1) and were on par with T_1 (Pure sample) and T_4 (Branded sample 3). The apparent density of coconut oil mixed with 1, 5, 10, 15, 20 and 30 per cent of palm kernel oil were 0.897, 0.893, 0.896, 0.893, 0.890 and 0.896 g ml^{-1} respectively. The apparent density of pure palm kernel oil (T_{19}) was 0.889 g ml^{-1}. The lowest apparent density was noticed for mineral oil (T_{20}) with a value of 0.844 g ml^{-1} which was followed by T_{18} (coconut oil mixed with 30 per cent mineral oil) with a value of 0.879 g ml^{-1} and they were found to be on par.

Apparent density is defined as the relationship between the mass and volume of the material, including pores and water (apparent volume) (Ramirez *et al*., 2012). The density of coconut oil was between 0.9190 – 0.9370 g cc^{-1} (Bailey and Shahidi, 2005) and that of palm kernel oil was between 0.9250 – 0.9350 g cc^{-1} (Thomas, 2000) According to CODEX (2015), the apparent density of palm kernel olein was 0.904-0.907 g ml^{-1} and that of palm stearin was 0.904-0.906 g ml^{-1}. In the experiment the samples mixed with 15 per cent, 20 per cent and 30 per cent mineral oil showed less apparent density compared to pure and branded coconut oils. So a mixing of less than

15 per cent mineral oil would be unable to identify. Since no standards are available with respect to apparent density in coconut oil and palm kernel oil, it was difficult to compare the apparent density due to adulteration of coconut oil with palm kernel oil.

5.1.4. Insoluble impurities

Insoluble impurities include substances like dirt, debris and fibres. Insoluble impurities of all the given treatments are represented in Fig 4. Insoluble impurities of pure coconut oil sample and branded coconut oil was 0.024 per cent except that of T_6 (Branded sample 5) which recorded a value of 0.040 per cent. The highest value was observed in coconut oil mixed with 30 per cent mineral oil (T_{18}) and the value obtained was 0.047 per cent. This was followed by coconut oil mixed with 5 per cent palm kernel oil (T_8), 15 per cent palm kernel oil (T_{10}), 10 percent mineral oil (T_{15}) and mineral oil (T_{20}) and the value observed was 0.043 per cent. The lowest value observed was 0.024 per cent.

Amount of insoluble impurities should be very low and is a preferable characteristic in coconut oil (Keith *et al*., 1954). Cocks and Van (1966) observed that some substances remain insoluble in oil and are described as insoluble impurities. Petroleum ether or diethyl ether can be used to filter the dissolved impurities in fat or oil. Gawad *et al*. (2015) evaluated the quality parameters of vegetable oils from the Egyptian market and noticed that the insoluble impurities of oil samples were high and it exceeded the maximum limit of Codex standards. Hasan *et al.*(2018) investigated the physiochemical characteristics of virgin coconut oil and some marketed refined coconut oils. It was found that percentage of insoluble impurities in virgin coconut oil was less (0.16 per cent) when compared to other oils. The maximum percentage by mass of insoluble impurities in oils according to both the ICC and Codex standards should not exceed 0.05 per cent. The insoluble impurities of all the samples used in the experiment were within the ICC and Codex limits. Though different types of mixing was done with palm kernel oil and mineral oil the insoluble impurities remained within the limits prescribed by ICC and Codex

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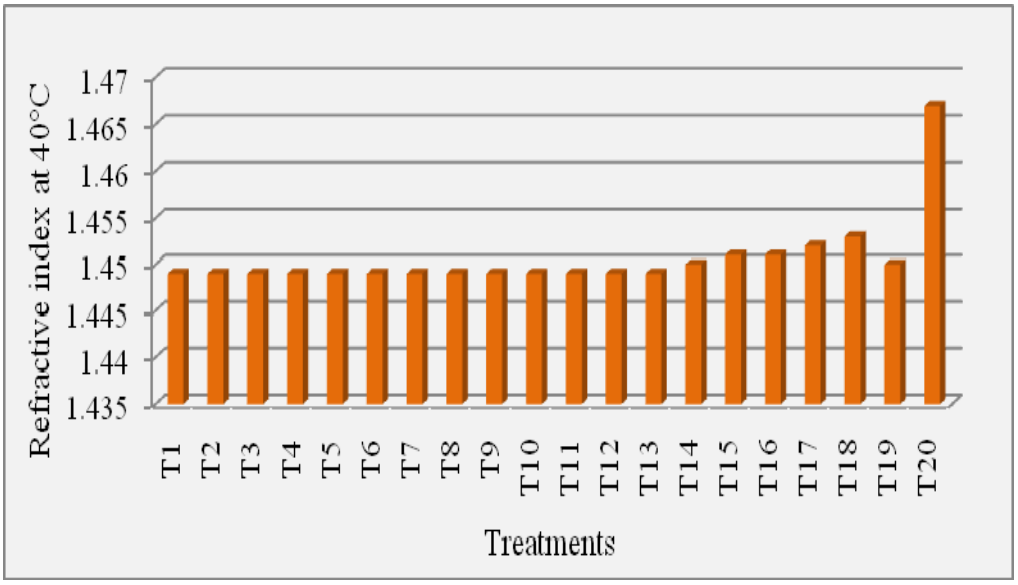

Fig 1. Refractive Index at 40°C of the treatments obtained from the analysis

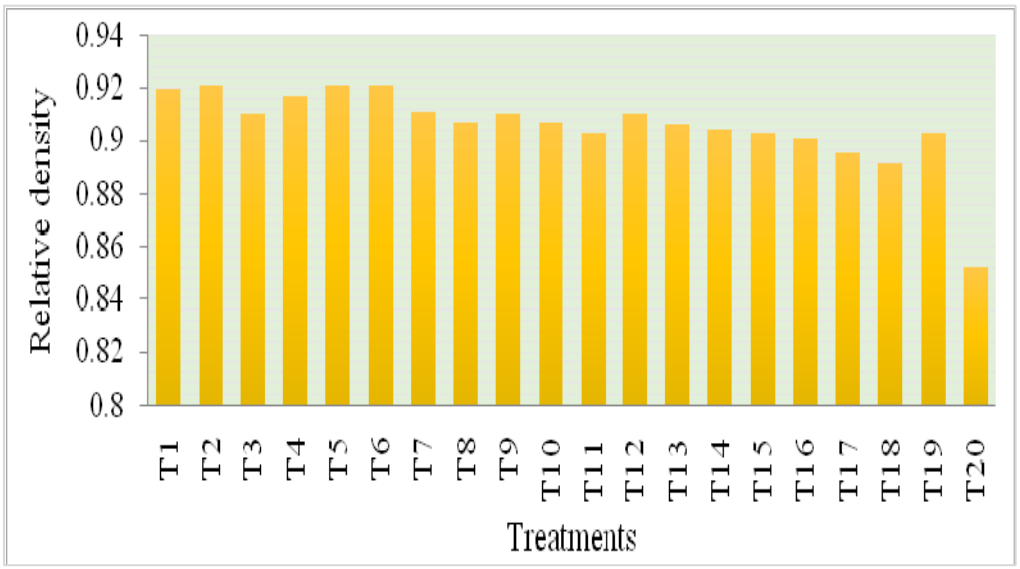

Fig 2. Relative density of the treatments obtained from the analysis

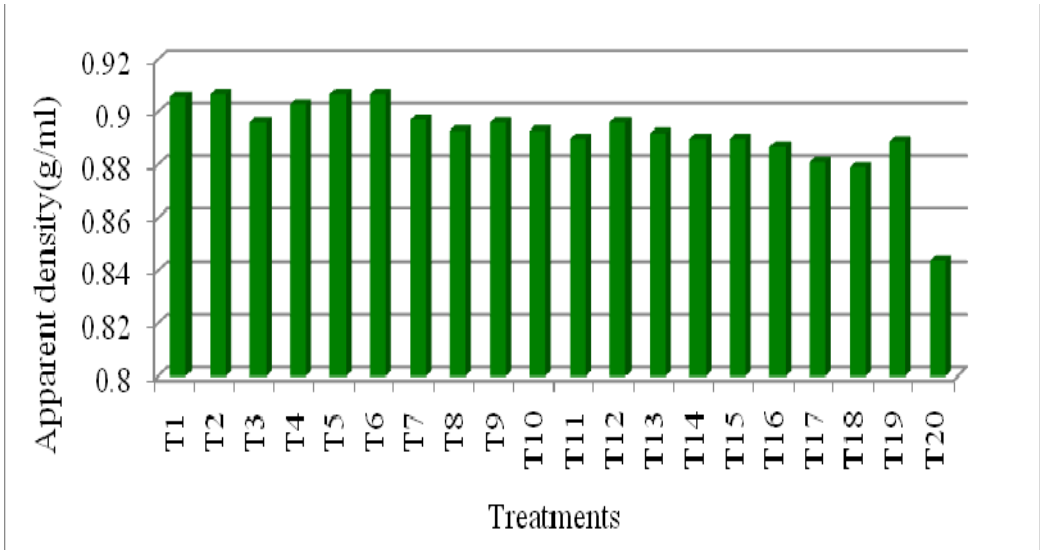

Fig 3. Apparent density of the treatments obtained from the analysis

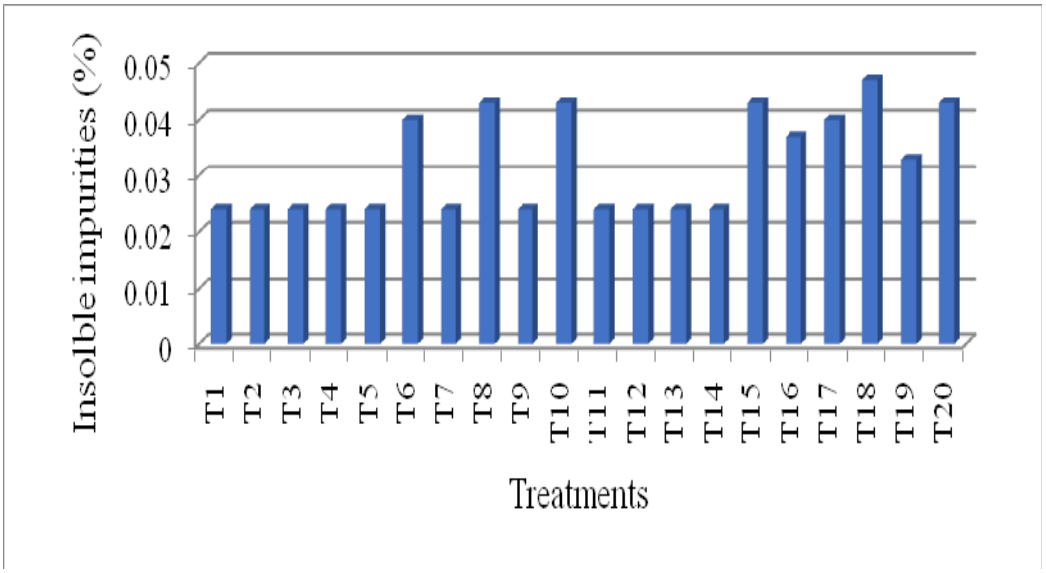

Fig 4. Insoluble impurities in the treatments obtained from the analysis

5.1.5. Saponification value

Saponification value (SV) obtained for all the treatments are given in Fig 5. From the figure it can be understood that all the samples mixed with mineral oil showed less saponification value and it was the least for the mineral oil, T_{20} (8.48 mg KOH g of oil^{-1}). This means mineral oils are composed of long chain fatty acids. The highest saponification value was noticed for treatment T_1 (pure sample) and the value obtained was 266.29 mg KOH g of oil^{-1}. This was followed by T_2 (Branded sample 1) with a value of 259.18 mg KOH g of oil^{-1}. According to FSSAI (2015), saponification value for pure coconut oil is above 250. In the experiment, pure sample and all the branded coconut oil samples showed a value above 250.

When palm kernel oil was used as an adulterant in different concentration from 1 per cent to 30 per cent, the value obtained was below 250 mg KOH g of oil^{-1}. This indicates that the coconut oil is adulterated. In the experiment, saponification value for palm kernel oil (T_{19}) was 250.67 mg KOH g of oil^{-1}. FSSAI standard of saponification value for coconut oil is not less than 250 mg KOH g of oil^{-1} while palm kernel oil is in the range 237-255 mg KOH g of oil^{-1}. According to Codex Alimentarius, the saponification values (SV) of coconut oil range between 250-260 mg KOH g of oil^{-1}.

When palm kernel oil or mineral oil was used as an adulterant in different concentration, the saponification value showed a variation from the FSSAI standard. Andrews (1933) observed that saponification value is an important quality parameter in the analysis of coconut oil. Kirk and Sawyer (1991) reported that fatty acid with shorter carbon chain length will have high saponification value. Compounds present in the non saponifiable fraction will affect the saponification value of vegetable oils which are unrefined. Abayeh *et al*. (1998) also reported that saponification values are inversely related to the average molecular weight of the fatty acids in the oil fractions. High saponification value of coconut oil is attributed to the presence of phenolic compounds and they will react with KOH (Seneviratne and Dissanayake, 2005). Marina *et al.* (2009) conducted a study on virgin coconut oil (VCO) which was

collected from Malaysian and Indonesian market. Saponification value obtained was within the range 250.07 to 260.67 mg KOH g^{-1} .

The saponification value refers to all fatty acids present in the sample (free and esterified). For the determination of saponification value, the sample was completely saponified with an excess of alkali, which excess was then determined by titration (in mg KOH g^{-1}). The saponification number depends on the molecular weight and the percentage concentration of fatty acid components present in fatty acid methyl esters (FAMEs) of oil. The SV is effectively used to determine the average relative molecular mass of oils and fats. Lauric oils, with a higher percentage of ester bonds than longer chain oils, have a higher SV (240–250 mg KOH g^{-1} for coconut oil (Bart *et al*., 2010).

The saponification value of the coconut oil mixed with varying concentrations of the palm kernel oil was less compared to pure and branded coconut oil. This shows that mean molecular mass of pure coconut oil was less than the palm kernel oil and coconut oil mixed with palm kernel oil. Since variation was shown in the saponification value when mixed with palm kernel oil and mineral oil, it could be an effective character to identify adulteration in coconut oil.

5.1.6. Iodine value

Iodine value obtained for all the treatments in the analysis are presented in Fig 6. Significant differences were observed among the treatments. The highest iodine value was observed for treatment palm kernel oil (T_{19}) with a value of 20.26 g of iodine 100g of oil^{-1}. This was followed by coconut oil with 30 per cent of palm kernel oil (T_{12}) which showed a value of 17.61 g of iodine 100g of oil^{-1}, coconut oil with 20 per cent palm kernel oil (T_{11}) showed 15.6 g of iodine 100 g of oil^{-1}, coconut oil with 15 per cent palm kernel oil (T_{10}) showed 14.70 g of iodine 100g of oil^{-1}, coconut oil with 10 per cent palm kernel oil showed 12.50 g of iodine 100g of oil^{-1}, coconut oil with 5 per cent palm kernel oil showed 11.17 g of iodine 100 g of oil^{-1} and that with 1 per cent palm kernel oil showed 10.03 g of iodine 100 g of oil^{-1}. These values were higher than iodine value of pure coconut oil, T_1 (9.267g iodine 100g of oil^{-1}) and the

branded coconut oils T_2 (7.633 g of iodine 100g of oil^{-1}), T_3 (7.967g of iodine 100g of oil^{-1}), T_4 (8.23 g of iodine 100 g of oil^{-1}), T_5 (9.233g of iodine 100 g of oil^{-1}) and T_6 (8.367g of iodine 100 g of oil^{-1}). Treatments T_1 (Pure sample) and T_5 (Branded sample 4) were found to be on par. Similarly, T_6 (Branded sample 5) and T_4 (Branded sample 3) were also found to be on par. The lowest iodine value was noticed for treatment T_{20} (mineral oil) and the value obtained was 2.43 g which was followed by T_{18} (coconut oil with 30 per cent mineral oil) with an iodine value of 3.20 g, T_{17} (coconut oil with 20 per cent mineral oil) with an iodine value of 4.70 g, T_{16} (coconut oil with 15 per cent mineral oil) with an iodine value of 5.43 g, T_{15} (coconut oil with 10 per cent mineral oil) with an iodine value of 6.80 g, T_{14} (coconut oil with 5 per cent mineral oil) with an iodine value of 7.40 g and T_{13} (coconut oil with 1per cent mineral oil with an iodine value of 8.67g of iodine 100g of oil^{-1}.

Iodine value is used as an important parameter to check adulteration of oil. The iodine value (IV) of oil is a measure of its total unsaturation. It is the percentage by weight of which an oil or fatty acid absorb halogens such as iodine under the test conditions. According to FSSAI (2015), iodine value of coconut oil is in the range 7.5-10. In the experiment, pure sample and all the branded coconut oil samples showed iodine value within the range 7.5-10. When palm kernel oil was used as an adulterant, iodine value exceeded the standard value. Palm kernel oil showed an iodine value of 20.26 g. When mineral oil was used as an adulterant, iodine value showed a decreasing trend. Since iodine value changed with even minor adulteration, iodine value could be used as a source for detecting adulteration. With 1 per cent palm kernel oil the iodine value exceeded the limit. However treatment with 1 per cent mineral oil had an iodine value within the range specified by FSSAI.

According to Suzanne (1994), high iodine value occurred due to high amount of unsaturation and it led to high absorption of iodine. Marina *et al*. (2009) reported that the iodine value of VCO samples ranged from 4.47 to 8.55. The low content of iodine value indicated that VCO had high degree of saturation. Amira *et al*. (2014) conducted a study on physico chemical characteristics of palm kernel oil and found

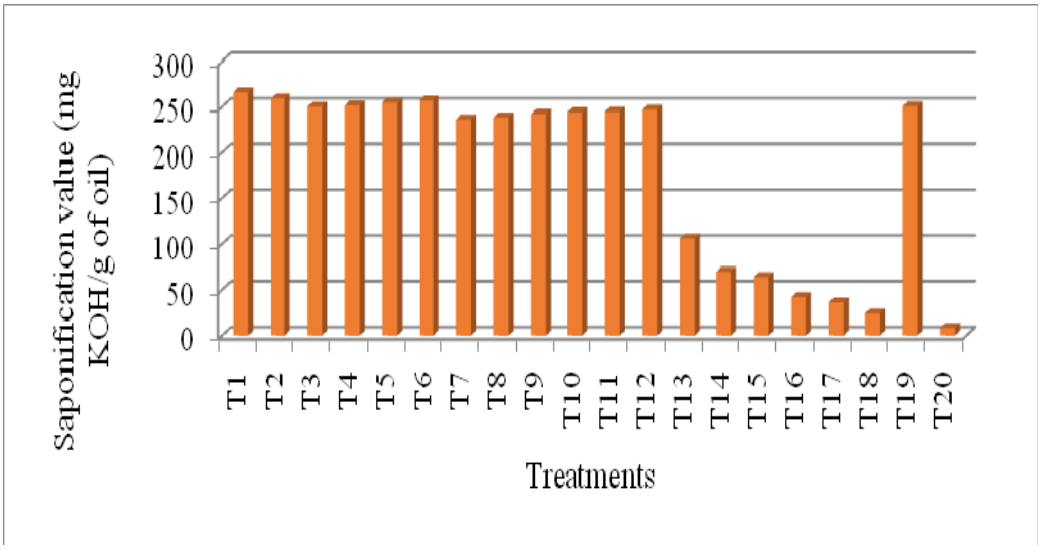

Fig 5. Saponification value of the treatments obtained from the analysis

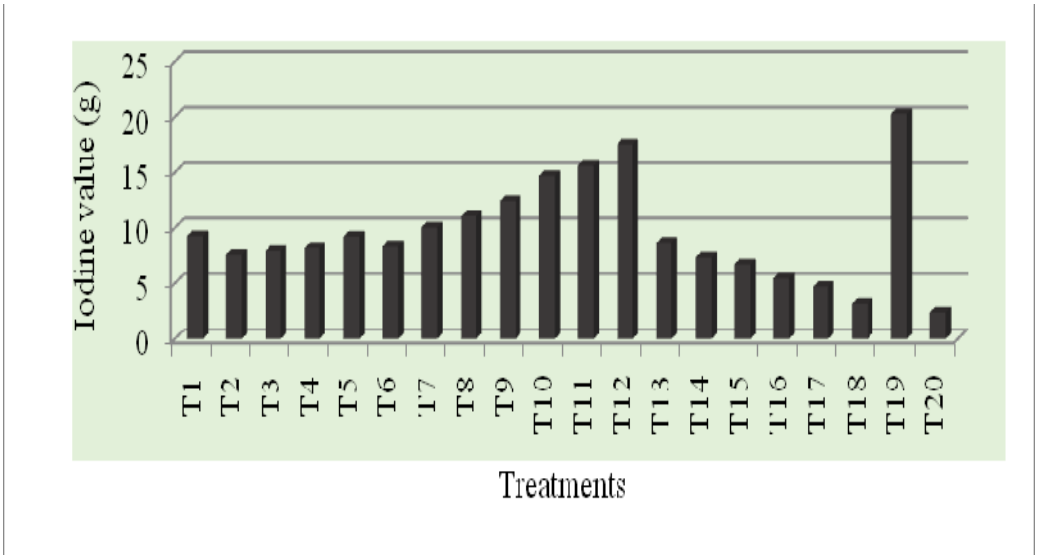

Fig 6. Iodine value of the treatments obtained from the analysis

that the iodine value obtained for palm kernel oil was 15.86±4.02 g and it depicted a higher level of unsaturation. Odoom and Edusei (2015) evaluated the quality parameters in coconut oils collected from four centres of Jomoro district of western region of Ghana. It was found that the mean iodine value obtained from three centres met the APCC standard while coconut oil from only one centre met the Codex standard. APCC standard for iodine value of coconut oil is in the range 4.1-11 (APCC, 2003) and according to CODEX (2015), standard the iodine value is in the range between 6.3-10.6.

5.1.7. Polenske value

Significant variation in Polenske value of oil samples is presented in Fig.7. Treatment T_1 (Pure sample) showed high value (14.17) in the analysis which was on par with branded coconut oils and coconut oil mixed with 1, 5, 10 and 15 per cent palm kernel oil. The Polenske value of T_2 (Branded sample 1) was 13.50, T_3 (Branded sample 2) was 13.81, T_4 (Branded sample 3) was 13.13, T_5 (Branded sample 4) was 13.75 and T_6 (Branded sample 5) was 13.65. The Polenske value of coconut oil mixed with 1, 5, 10, 15 and 20 per cent palm kernel oil (T_7, T_8, T_9, T_{10}, T_{11},) were 13.03, 12.91, 12.50, 12.22 and 11.04 respectively which were on par. However the Polenske value of coconut oil mixed with 30 per cent palm kernel oil showed significantly lower Polenske value (7.80). The Polenske value of pure palm kernel (T_{19}) was 7.73. The lowest Polenske value (0.26) was obtained for mineral oil (T_{20}) followed by T_{18} (coconut oil mixed with 30 per cent mineral oil) with a value of 5.63. The Polenske value of coconut oil mixed with varying percentage of mineral oil ranged from 5.63 in T_{18} (coconut oil mixed with 30 per cent mineral oil) to 10.84 in T_{13} (coconut oil mixed with 1 per cent mineral oil). In the experiment, pure sample and branded coconut oil samples showed a Polenske value according to the standard prescribed by FSSAI. When palm kernel oil was used as an adulterant, Polenske value obtained was below 13. In this case detection of adulterant was possible from coconut oil samples adulterated with 5 per cent palm kernel oil. When mineral oil was used as an adulterant, even 1 per cent adulteration could be detected since it varied from the FSSAI standard. According to FSSAI (2015), Polenske value of coconut oil should not be less than 13.

According to Singhal (1980), Polenske value of coconut oil is within the range 15-20 and for palm kernel oil it is in the range 6-12 and for other oils and fats it is less than 1. Polenske value measures caprylic, capric and lauric acids present in the oil and are steam volatile and water insoluble fatty acids (FSSAI, 2015). The Polenske value for coconut oil is in the range 13-18, for palm kernel oil it is in the range 8-12 and for babassu oil it is in the range 8-10 (CODEX, 2015). According to Satheesh and Prasad (2012), virgin coconut oil extracted by natural fermentation method obtained a Polenske value of 13.9 ± 0.6 while induced fermentation method obtained a value of 13.9 ± 0.3. Thanuja (2015) observed that the Polenske value of virgin coconut oil recovered from traditional boiling method, fermentation, induced fermentation, and centrifugation method was within the range of 13-13.2.

Thus in the present experiment it could be inferred that adulteration of coconut oil with palm kernel oil above 5 per cent and mineral oil with even 1 per cent adulteration could be detected by recording the Polenske value. Thus Polenske value is a good indicator of adulteration of coconut oil with palm kernel oil and mineral oil.

5.1.8. Unsaponifiable matter

Unsaponifiable matter obtained for all the oil samples tested are presented in Fig. 8. The highest percentage of unsaponifiable matter was noticed for the treatment T_{20} (mineral oil) with a value of 89.12 per cent followed by T_{18} (coconut oil mixed with 30 per cent mineral oil) with 37.94 percent. The unsaponifiable matter for coconut oil mixed with varying percentage of mineral oil varied from 1.18 to 37.94 per cent. The unsaponifiable matter for pure palm kernel oil (T_{19}) was 0.21 per cent and that of coconut oil mixed with varying concentration of palm kernel oil ranged from 0.15 to 0.33 per cent. However not much variation in unsaponifiable matter was obtained between pure coconut sample and coconut oil samples adulterated with varying percentage of palm kernel oil. The lowest value was observed for treatment T_2 (Branded sample 1) with a value of 0.13 per cent. This was followed by T_8 (coconut oil mixed with 5 per cent palm kernel oil) with a value of 0.15 per cent and they were found to be on par. According to FSSAI (2015), unsaponifiable matter of coconut oil should not be more than 1 per cent. Pure sample and branded coconut oil

samples obtained values within the standard. When palm kernel oil was used as an adulterant in different concentrations, the observed values met the FSSAI standard. Hence it was difficult to detect the presence of palm kernel oil with this parameter. In the case of mineral oil adulteration, even 1 per cent adulteration could be detected by unsaponifiable matter. So by estimating unsaponifiable matter it would be possible to detect the adulteration with mineral oil since even 1 per cent addition of mineral oil exceeded the standard limit.

FAO (1986) defines unsaponifiable matter as substances which remain soluble in an oil after saponification. Sterols, higher open chain alcohols, pigments, vitamins and hydrocarbons, foreign organic matter including mineral oil are considered as unsaponifiable matter. A study was carried out to evaluate the physico chemical properties of 10 virgin coconut oil samples and it was found that the average unsaponifiable matter was 0.116 per cent. Minimum value obtained was 0.085 per cent and maximum value was 0.135 per cent with a standard deviation of 0.0184 (Kamariah *et al*., 2008). Krishna *et al*. (2010) reported that the small proportion of tocopherols and phytosterols are present in coconut oil which serves as unsaponifiable matter. Unsaponifiable matter of oil serves as a check for contamination by foreign materials like mineral oil. According to FSSAI (2015), sterols, squalene, beta carotene, tocopherols and phenols are considered as unsaponifiable matter in the oil sample.

According to IARC (1984), mineral oils refined from petroleum crude oils are complex and variable mixtures of straight and branched chain paraffinic, naphthenic (cyclo paraffinic) and aromatic hydrocarbons with carbon numbers of 15 or more and boiling points in the range of 300–600°C (IARC, 1984).

The presence of different types of hydrocarbons in mineral oil as well as the coconut oils mixed with varying percentage of mineral oil might have resulted in the higher percentage of unsaponifiable matter as revealed from the experiment.

5.1.9. Acid value

Acid value of pure coconut oil, palm kernel oil, mineral oil as well as the adulteration of coconut oil with varying percentage of palm kernel oil and mineral oil is given in Fig 9. The highest acid value was noticed for palm kernel oil (T_{19}) with 9.83 mg KOH g of oil^{-1}. This was followed by coconut oil sample with 30 per cent of palm kernel oil (T_{12}) with an acid value of 8.06 mg KOH g of oil^{-1}. The acid value of coconut oil mixed with 20, 15, 10, 5 and 1 per cent palm kernel oil ranged from 3.56 to 7.67 mg KOH g of oil^{-1}. Acid value of pure coconut sample was 3.13 mg KOH g of oil^{-1} and that of branded coconut oils were T_2 (1.29 mg KOH g of oil^{-1}), T_3 (1.78 mg KOH g of oil^{-1}), T_4 (3.53 mg KOH g of oil^{-1}), T_5 (1.63 mg KOH g of oil^{-1}) and T_6 (1.30 mg KOH g of oil^{-1}). The lowest acid value was observed for mineral oil (T_{20}) with 0.57 mg KOH g^{-1}. This was followed by T_2 (Branded sample 1) with a value of 1.29 mg KOH g^{-1} which was on par with T_6 (Branded sample 5). Pure sample and branded coconut oil samples obtained an acid value within the standard. FSSAI standard for acid value is not more than 6. In samples where palm kernel oil was used as an adulterant in different concentrations, acid value showed an increasing trend with increasing percentage of palm kernel oil. In the experiment, acid value of palm kernel oil was 9.83. When mineral oil was used as an adulterant in different concentrations, acid values obtained were within the FSSAI standard.

Acid value is one of the most important quality parameters in the oil industry which indicates the level of deterioration of the oil. Hydrolytic rancidity indicates the amount of free fatty acid content and the aroma and flavour change when the free fatty acid content increases (Hoover *et al*., 1973). According to Kirk *et al*. (1991), acid value or free fatty acid (FFA) was often used to approximate the quantity of oil that vanished during refining steps in crude fats. Man *et al*. (1997) found that coconut oils with high moisture content had high amount of free fatty acids. All vegetable oils contain naturally low amount of free fatty acids (FFAs). Residual water within the oil react and additional amount of free fatty acids are formed during extraction and storage. Chemical or enzymatic mechanisms are responsible for hydrolysis. High levels of FFA lead to the formation of unpleasant flavour (Dayrit *et al*., 2007).

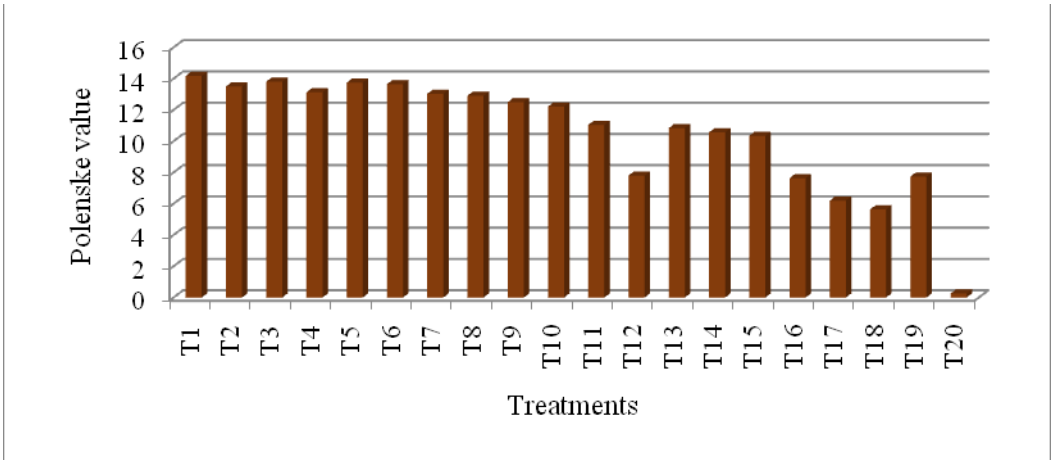

Fig 7. Polenske value of the treatments obtained from the analysis

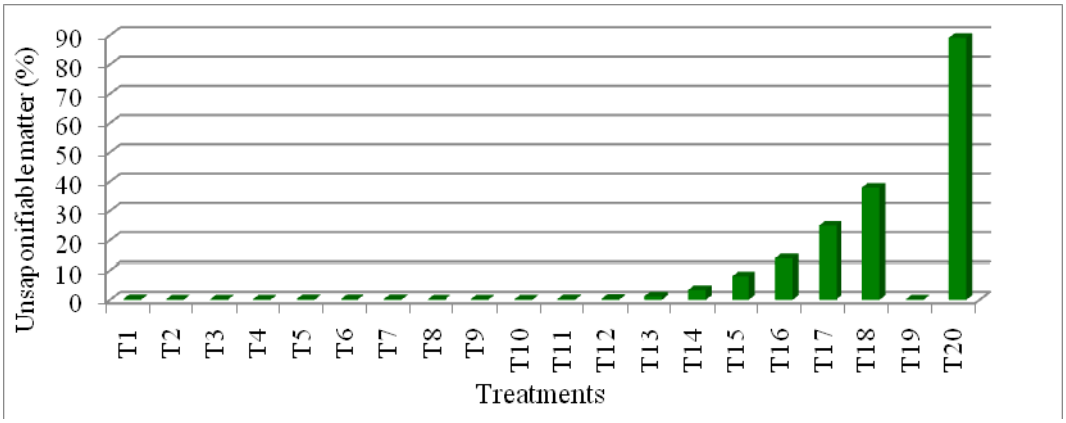

Fig 8. Unsaponifiable matter in the treatments obtained from the analysis

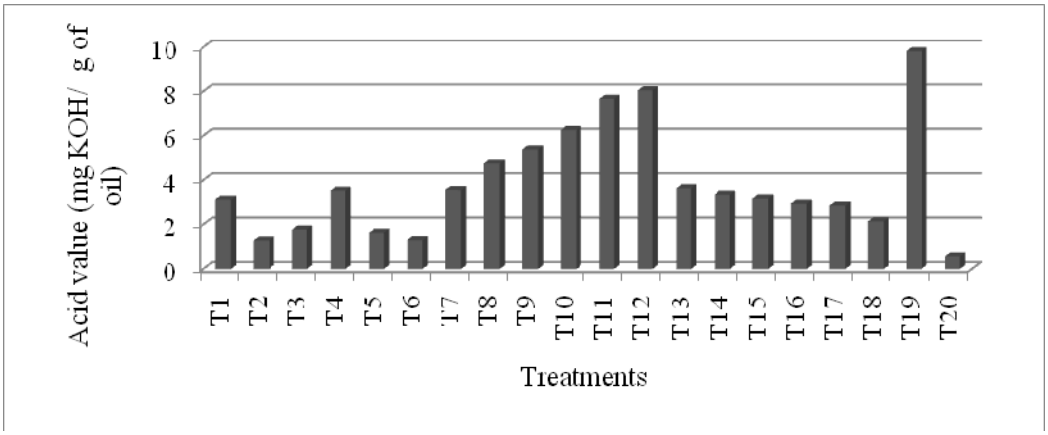

Fig 9. Acid value of the treatments obtained from the analysis

5.1.10. Peroxide value

The results of peroxide value of oil samples analysed are presented in Fig 10. The peroxide value for the pure coconut oil (T_1) was 5.33 meq kg of oil^{-1}. The peroxide values of branded coconut oils T_2, T_3, T_4, T_5 and T_6 were 2.15, 4.35, 6.13, 6.06 and 3.58 meq kg^{-1} respectively. Peroxide value of coconut oil mixed with 1, 5, 10, 15, 20 and 30 per cent of palm kernel oil were 9.32, 11.03, 10.86, 12.13, 12.86 and 13.06 meq kg^{-1} respectively. The highest peroxide value was noticed for coconut palm kernel oil (T_{19}) with 14.56 meq kg^{-1} followed by coconut oil mixed with 20 per cent palm kernel oil (T_{11}) with12.86 meq kg^{-1}. The lowest peroxide value was observed for T_2 (Branded sample 1) with 2.15 meq kg^{-1}. This was followed by T_6 (Branded sample 5) with a peroxide value of 3.58 meq kg^{-1}. Quality of coconut oil can be assessed by the peroxide value. In the experiment conducted, pure sample and branded samples obtained a peroxide value which was less than 10 meq kg^{-1}. When palm kernel oil was used an adulterant in different concentrations, the oxidation values were above 10 meq kg^{-1}. The peroxide values observed when 1, 5, 10, 15, 20 and 30 per cent mineral oil were used as an adulterant were 6.23, 6.55, 8.35, 10.32, 11.42 and 11.63 meq kg^{-1} of oil.

According to FSSAI (2015), fresh coconut oils have a peroxide value below 10 meq kg^{-1}. Rancid taste will begin when the peroxide value is above 20 meq kg^{-1}. Coconut oil with a value above 40 meq kg^{-1} is not good for health. According to CODEX (2015), peroxide value between 1 and 5 meq kg^{-1} represents low oxidation state and that between 5 and 10 meq kg^{-1} represents moderate oxidation and above 10 meq kg^{-1} indicates high oxidation state. Generally, Codex gives a peroxide value limit of 15 meq kg^{-1}.

In a study conducted by Pearson (1976), it was found that the peroxide value of palm kernel oil was 14.3±0.8 meq kg^{-1}. It indicated the degree of spoilage of palm kernel oil which is more liable to rancidity. Off flavour resulting from peroxidation of unsaturated fatty acids was the major cause of spoilage of stored oils (Semwal and Arya, 1992). Oxidation in the initial stages can be determined by measuring the peroxide value of oils. Matthäus (2007) reported that the

condition of cooking oils would not change after the refining process. Cooking oils might be refined or unrefined. Oxidation of oils depended on many factors like change in temperature, light, time, presence of moisture, metals etc.

The moderate increase in peroxide value of coconut oil mixed with palm oil might be due to the increase in peroxidation of the unsaturated fatty acid making the oil more liable to rancidity. Similarly an increase in the peroxide value of coconut oil adulterated with increasing percentage of mineral oil also might be due to more peroxidation of unsaturated fatty acid of mineral oil.

5.1.11. Matter volatile at 105°C

Matter volatile at 105°C obtained for all oil samples are presented in Fig 11. The matter volatile at 105° C for pure coconut oil was 0.077 per cent which was the lowest. Branded coconut oils had matter volatile at 105°C varying from 0.123 to 0.147 per cent. The highest matter volatile at 105°C was noticed for treatment T_{12} (coconut oil mixed with 30 per cent palm kernel oil) with a value of 0.157 per cent followed by T_{20} (mineral oil) with 0.150 per cent and T_{18} (30 per cent mineral oil + 70 per cent coconut oil) and T_3 (Branded sample 2) with 0.147 per cent which were on par. The lowest value was observed for pure sample (T_1) with a value 0.077 per cent. This was followed by coconut oil mixed with 1 per cent mineral oil (T_{13}) with 0.080 per cent. CODEX standard for matter volatile at 105°C is 0.2 per cent. In the experiment, all the treatments showed values within the standard. Kamariah *et al*. (2008) reported that matter volatile at 105°C for ten virgin coconut oil samples were within the range 0.080 – 0.150 per cent. The result was expressed in percentage by mass. According to Dayrit *et al*. (2007), an average of 0.040 per cent volatile matter was obtained for VCO samples and volatile matter was within the range of 0 to 0.080 per cent. It was also observed that RBDCNO contains water as volatile matter and no volatile organic carbon was detected. In contrast, copra oil gave a high VOC level of 1.770 per cent.

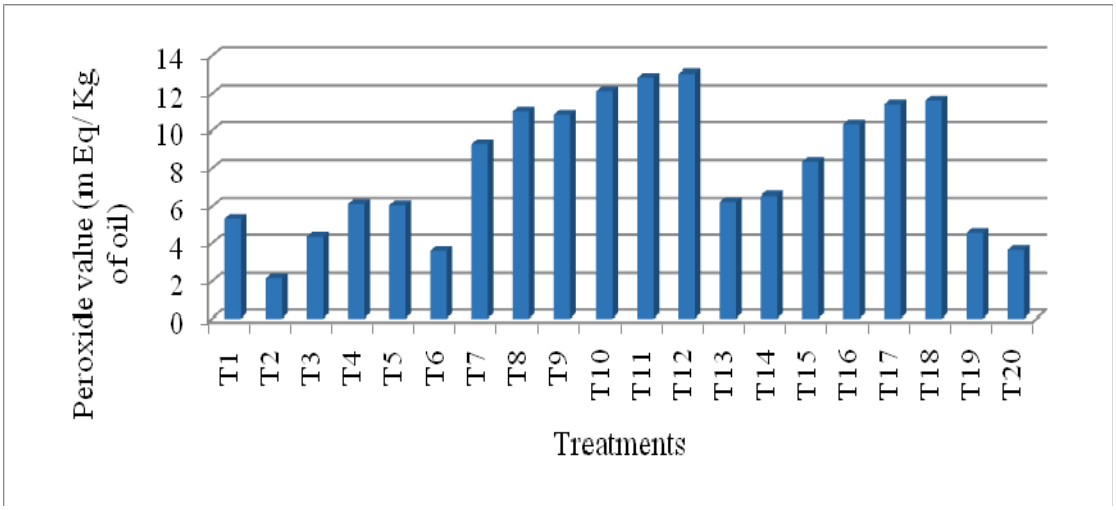

Fig 10. Peroxide value of treatments obtained from the analysis

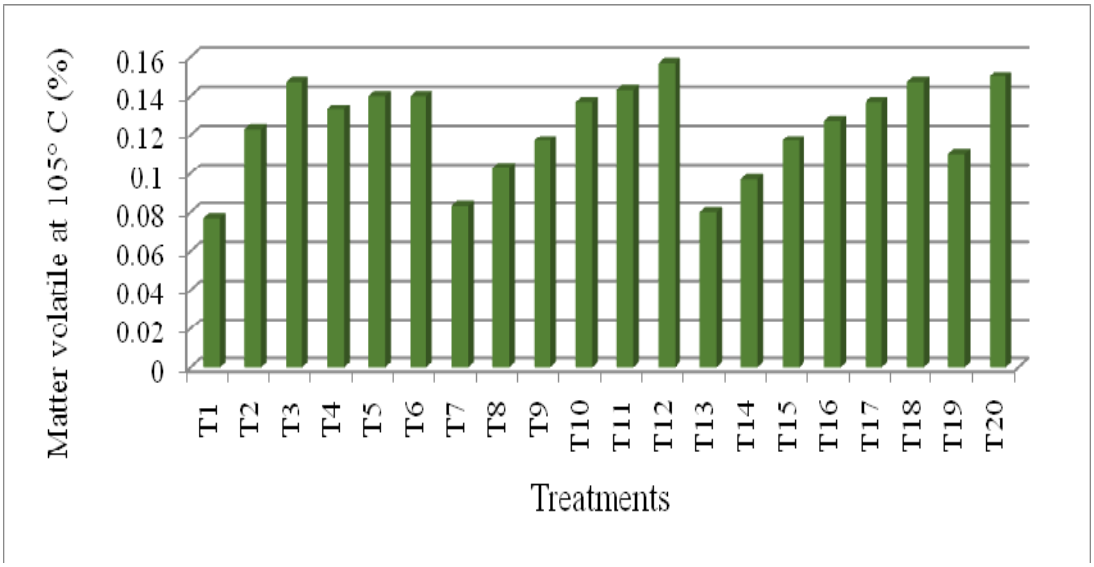

Fig 11. Matter volatile at 105°C for treatments in the analysis

Principal Component Analysis

Principal component analysis (PCA) was performed on physical and chemical characteristics of oil samples. PCA emphasizes variation and transforms large sets of variable to smaller one. Total variation of 74.16 per cent was observed in the data and is shown in Table 6. In the analysis, it was clear that there were two eigen values were greater than unity. So two principal components were obtained principal component 1 (PC 1) and principal component 2 (PC 2). Principal component 1 accounted for 55.36 percent of variation followed by principal component 2 with 18.80 percent variation. Loadings of principal components 1 and 2 from PCA analysis is shown in Table 7. In PC1, high coefficient was obtained for the parameter unsaponifiable matter (-0.968) followed by relative density (0.943), apparent density (0.942), Polenske value (0.938) refractive index (-0.935) and saponification value (0.826). The negative values of loadings of variable in the components of the PCA means the existence of an inverse correlation between the factor PCA and the variables. Low coefficient was noticed for peroxide value (0.009) followed by matter volatile at 105°C (-0.362). This means peroxide has small role, whereas unsaponifiable matter, relative density, apparent density, Polenske value, refractive index and saponification value have sizable roles in explaining the variation due to adulteration. In PC 2, high coefficient was noticed for acid value (0.891) followed by peroxide value (0.713) and iodine value (0.701). Low coefficient was observed for refractive index (0.030) followed by unsaponifiable matter (0.059) in PC 2.

A score plot was constructed based on the physical and chemical parameters and is depicted in Fig 12. Examination of score plot indicates the nature and type of oil sample. Pure coconut oil and branded coconut oil samples clustered in the same quadrant when compared to other treatments. Coconut oil mixed with different concentrations of palm kernel were found to cluster in another quadrant. Mineral oil adulterated samples were clustered in the quadrant which is entirely opposite to coconut oil. This indicates that maximum variation occurs due to mineral oil adulteration. A variable plot was also constructed based on the data and is shown in fig 13.

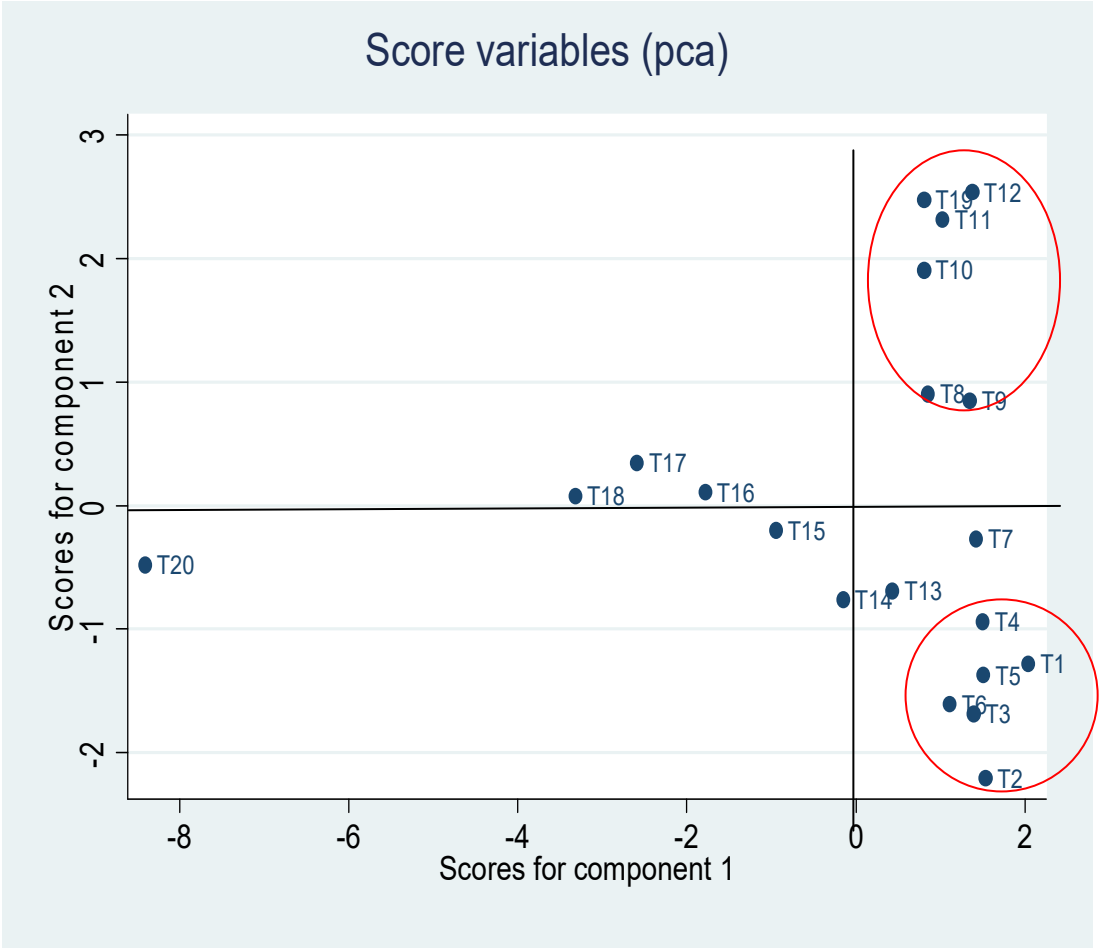

Fig 12. Score plot of PCA constructed based on the physical and chemical characteristics of coconut oil.

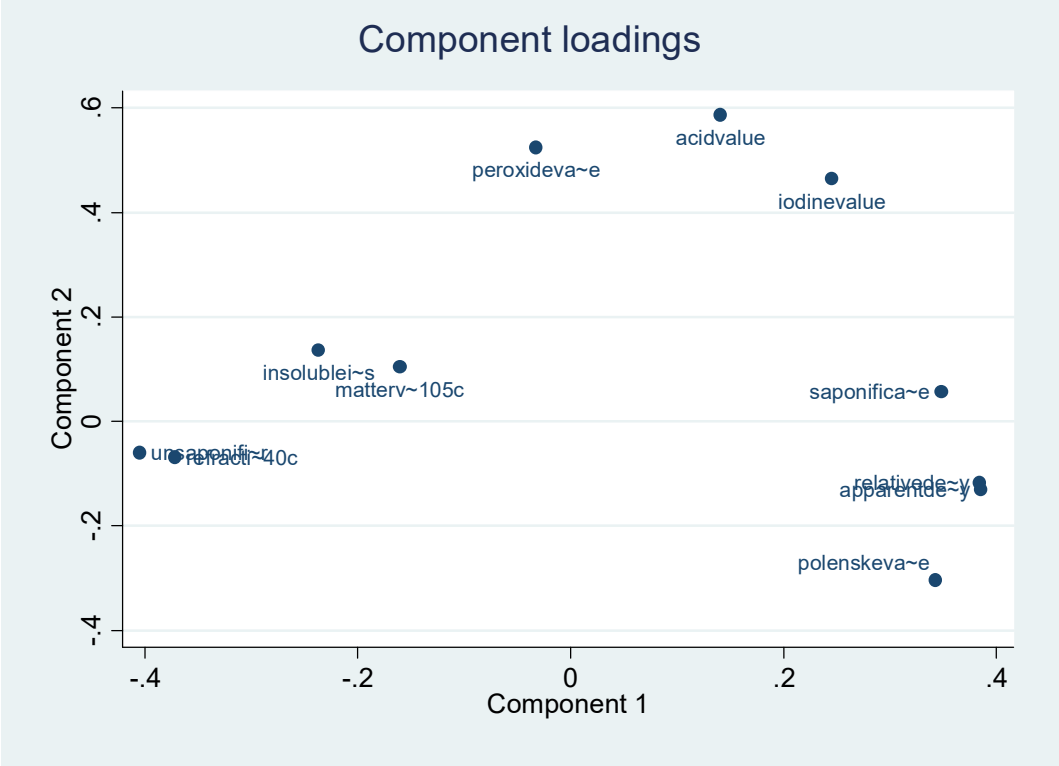

Fig 13.Variable plot constructed based on the chemical and physical characteristics of coconut oil.

By comparing score plot with variable plot we could identify the parameters leading to variation. Thus variation in mineral oil was contributed by the parameters unsaponifiable matter, refractive index at 40°C, relative density, apparent density ad Polenske value. But for palm kernel oil, the variation was highly influenced by the parameters acid value, iodine value and peroxide value. So these parameters can be effectively used to check adulteration in the oil samples.

In an experiment conducted by Dayrit *et al*. (2007) chemical parameters were selected and PCA was performed to analyze the combination of characters which leads to maximum variation. It was concluded that percentage of moisture, volatile organic carbon (VOC) and FFA were capable of differentiating virgin coconut oil from refined bleached deodourized coconut oil (RBD CNO) and copra oil.

5.2. Microbial contamination

5.2.1. Total plate count

Total plate count was taken for all the treatments and is represented in Table 10. This include bacterial, fungal and actinomycete count. It is expressed in cfu ml^{-1}. Fungal and actinomycete count was zero for all the treatments which indicated the absence of fungal and actinomycete population. However bacterial population was noticed in all the oil samples except the oil sample collected from expeller (T_1 - pure coconut oil). The highest value of bacterial count observed was 7×10^{-7} cfu ml^{-1} in T_{12} (coconut oil mixed with 30 per cent palm kernel oil) (Plate 6b). This was followed by T_{10} (coconut oil mixed with 15 per cent palm kernel oil) and T_{17} (coconut oil mixed with 20 per cent mineral oil) with 6×10^{-7} cfu ml^{-1}. No bacterial colony was observed for pure sample (T_1) (Plate 6a.). But the branded coconut oil T_2 (Branded sample 1) had a bacterial population of 1×10^{-7} cfu ml^{-1}. Oils are usually extracted by traditional methods and many workers are employed in the production process. As a result, sometimes oils are prone to contamination by micro-organisms. This include bacteria, fungi and actinomycete. Microbes are usually found from the materials used for production or from the environment or due to improper storage and

distribution. In the experiment, it was found that there were no fungal colonies and actinomycete. But a few colonies of bacteria were detected..

Dayrit *et al*. (2007) reported that commercial samples of virgin coconut oil (VCO), refined, bleached and deodorized coconut oil (RBD CNO) and copra oil were analyzed using standard parameter for microbial contamination. Out of 33 samples taken, all 3 copra oil samples had < 250 cfu ml^{-1}. The APCC standard for total plate count of coconut oil is < 10 cfu ml^{-1}. Failure to meet this standard indicates that the product, copra oil is of poor quality and is a potential health hazard. Kamariah *et al*. (2008) conducted a study on virgin coconut oil and reported that total plate count is very important in determining the quality VCO. Result from the analysis showed that most of the samples had zero or less than 10 colony forming unit (cfu). Since the bacterial count observed was less than 10 colonies for all the oil samples tested it was safe with respect to bacterial population.

Yusuf *et al*. (2017) studied the microbial purity of locally extracted palm kernel oil and coconut oil. The presence of total aerobic mesophilic bacteria, fungal (mould), coliform counts and pathogenic bacteria (*E.coli*) were evaluated. Serial dilution, pour plate and Most Probable Number (MPN) techniques were applied along with biochemical tests. Eosine-Methylene Blue (EMB) test was used to detect the presence of *E.coli.* It was found that the bacterial and fungal colonies obtained were very few or even absent in the oil sample and no *E.coli* was detected. These microbial count was within the limited range of National Agency for Food and Drug Administration Control (NAFDAC) for oils.

5.3. Thin Layer Chromatography (TLC)

Thin layer chromatography was carried out for all the samples and results obtained during the analysis is presented in Table 11. TLC profile obtained during the analysis is represented. Yellow streaks were appeared in the profile of all coconut oil samples except in those mixed with mineral oil and pure mineral oil. Pure coconut oil sample, branded coconut oil samples, and coconut oil mixed with different concentrations of palm kernel oil showed yellow steaks. Yellow fluorescent spots

were observed in the profile among the coconut oil samples mixed with mineral oil as well as in pure mineral oil sample. Among the samples, presence of mineral oil was detected for treatments T_{13} (coconut oil mixed with 1 per cent mineral oil), T_{14} (coconut oil mixed with 5 per cent mineral oil) T_{15} (coconut oil mixed with 10 per cent mineral oil), T_{16} (coconut oil mixed with 15 per cent of mineral oil), T_{17} (coconut oil mixed with 20 per cent of mineral oil) T_{18} (coconut oil mixed with 30 per cent of mineral oil and T_{20} (mineral oil).

Mani and Lakshminarayana (1968) used TLC method for the detection of mineral oils and the spots were located with aqueous sodium fluorescein or 2',7'-dichlorofluorescein under UV light. Up to 3 per cent of adulterant mineral oils in different vegetable oils were detected using silica gel G layers sprayed with silver nitrate solution and eluted with benzene. The spots were detected by charring with 50 per cent ethanolic phosphoric acid. Gocan (2002) observed that silica gel was the most commonly used adsorbent in TLC. According to Pengon *et al.* (2012) TLC was a sensitive technique used for qualitative analysis of coconut oil. Kumar and Shree (2014) conducted a study to analyze the quality of different vegetable oils utilized in the ayurvedic oil preparations. Coconut oil, castor oil and sesame oil were collected and tested for mineral oil adulteration. Different concentrations (1, 5, 10, 50 and 80 per cent) of mineral oil were added to the vegetable oil. It was noticed that five formulations analysed were found to be free from the adulterant oil. A standard curve was plotted for the quantification.

In the present experiment it was observed that yellow streaks were absent from T_{13} where coconut oil was mixed with 1 per cent mineral oil. Thus thin layer chromatography could be used as a technique to detect mineral oil as adulterant in coconut oil.

5.4. Gas Chromatography Mass Spectrometry (GCMS) Analysis

5.4.1. Fatty acid composition (%)

Gas chromatography coupled with mass spectrometry was used to analyze the fatty acid composition in the oil samples. In the analysis, fatty acids were not detected

in mineral oil (T_{20}). Caprylic acid (C8:0), capric acid (C10:0), lauric acid (C12:0), myristic acid (C14:0), palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2), arachidic acid (C20:0), behenic acid (C22:0) and lignoceric acid (C24:0) were the fatty acids detected from the oil samples. Chromatographic profiles obtained for the coconut oil and branded samples are illustrated (Fig. 14, 15, 16, 17, 18, 19). Similarly, chromatographic profile of coconut oil mixed with 1, 5, 10, 15, 20 and 30 per cent of palm kernel oil and mineral oil are also shown (Fig. 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31). Chromatographic profile obtained for palm kernel oil, T_{19} is depicted (Fig. 32) and no peaks were observed for mineral oil, T_{20} (Fig. 33). Fatty acids like caproic acid (C6:0) and linolenic acid (C18:3) were not detected in any of the treatments. Among the fatty acids, C24:0, showed maximum variability (CV-44.02 per cent) between the treatments with an average of 0.03 per cent. The fatty acids C22:0, C20:0, C18:2, C18:1, C18:0, C16:0 and C14:0 showed a variability of 42.30, 14.41, 31.35, 21.92, 19.28, 9.50 and 15.40 respectively. The coefficient of variation obtained was minimum (CV-7.19 per cent) for C12:0 and the average obtained was 37.19 percent. The fatty acids C10:0 and C8:0 showed a variability of 15.48 and 20.69 per cent respectively.

Principal component analysis (PCA) was performed to get an idea about the contribution or importance of different fatty acids. PCA extracted two PCs which accounted for 79.76 per cent variation in the entire data. The first PC explained a variation of 61.21 per cent and PC 2 explained a variation of 18.55 per cent. The fatty acids C16:0 (0.978), C12:0 (0.954), C14:0 (0.936), C20:0 (0.895) and C18:2 (0.853) had high loadings on PC 1. However, a low coefficient was noticed for C22:0 (0.216) which was followed by C24:0 (0.485). In PC 2, high coefficient was obtained for C8:0 (-0.707) followed by C10:0 (-0.595) and the least coefficient was observed in C16:0 (-0.055).

Among the fatty acids, C16:0, C12:0, C14:0, C20:0 and C18:2 contributed the maximum variation whereas C22:0 showed only less variation. Saturated fatty acids were observed in large proportion for all the treatments. Among the saturated fatty acids, lauric acid (C12:0) constituted the highest proportion. This was followed by myristic acid (C14:0) and palmitic acid (C16:0).Thus observing C16, C12 andC14 the

adulteration in coconut oil composition can be understood. Oleic acid and linoleic acids were the major unsaturated fatty acids observed in the oil samples. Percentage of oleic (C18:1) and linoleic (C18:2) acids were found to increase with increase in concentration of palm kernel oil. On the other hand, both saturated and unsaturated fatty acids were found to decrease with increase in concentration of mineral oil increased. A score plot was constructed based on the data and is shown in fig 13. The score plot revealed that that the treatments with similar fatty acid composition were in a cluster. Palm kernel oil adulterated samples and branded coconut oil samples were tightly clustered around the pure coconut oil.

Location of treatment T_{19} (palm kernel oil) indicated the difference in its fatty acid composition when compared to the pure coconut oil sample. Coconut oil adulterated with different concentrations of mineral oil were also found within the cluster but T_{18} which was mixed with 30 per cent of mineral oil was away from the other coconut oil mixtures. Fatty acids were not detected in treatment T_{20} (mineral oil) and its location was far away from the cluster. Similarly a variable plot was also obtained and is shown in fig 34. The variable plot explained the influence of fatty acids in the treatments (Fig 35). The fatty acid composition of oils were highly influenced by lauric acid (C12:0) and palmitic acid (C16:0). In palm kernel oil, variation was contributed by stearic acid (C18:0), oleic acid (C18:1) and linoleic acid (C18:2).

Ten types of commercially available vegetable oils including extra virgin olive oil, olive oil, canola oil, palm oil, soybean oil, corn oil, sunflower oil, rice bran oil, peanut oil and coconut oil were collected from the market and they were subjected to GCMS analysis. Principal component analysis (PCA) was performed to analyse the extent of variation and a score plot was obtained. The score plot obtained from the GCMS data showed that coconut oil clustered far away from other oils and fats due to the presence of methyl laureate. Olive oil and extra virgin olive oils were clustered in the middle of upper score plot. In contrast, soya bean oil and corn oil clustered in the negative quadrant of score plot (Fang *et al*., 2013).

GCMS is a robust and widely used technique. It combines high sensitivity and specificity for suitable analyte classes. Gas chromatography-mass spectrometry can be used for detailed profiling (Halket *et al*., 2005). A study was carried out to analyze the fatty acid composition in coconut oil and coconut oil was blended with different vegetable oils (palm, rice bran, sesame, mustard, sunflower, groundnut, safflower, and soybean). Coconut oil contains C12:0 (Lauric acid) as the major fatty acid. It was found that coconut oil contains 90 percent of saturated fatty acids and is deficient in unsaturated fatty acids. Mono unsaturates accounts for 6 per cent and polyunsaturates about 1 per cent. When coconut oil was blended with different vegetable oils there was an increase in percentage of unsaturation. Monounsaturates were found to be 8-36 per cent and polyunsaturates showed an increase of 4-35 per cent (Bhatnagar *et al*., 2009). Dorni *et al.* (2017) evaluated 320 edible oils and fats and their fatty acid profile was analysed. It was found that in coconut oil saturated fatty acids constituted the maximum proportion (90.84 per cent). Among the saturated fatty acids 49.57 per cent was constituted by lauric acid. This was followed by myristic acid (21.12 per cent), palmitic, capric, stearic and caprylic acid. Among the unsaturated fatty acids, oleic acid accounted for 7.24 per cent followed by linoleic acid (1.9 per cent).

Moigradean *et al*.(2013) carried out an experiment in two completely different vegetables oils (walnut and coconut oils) to spot the composition of fatty acids. This was done with gas chromatography-mass spectrometry (GC-MS) method. Coconut oil contained 87.20 per cent of saturated fatty acid and 8.40 per cent unsaturated fatty acid. The results showed that the principal fatty acids identified in coconut oil were lauric acid (44.60 per cent) and myristic acid (20.40 per cent). Among the unsaturated fatty acids, the presence of oleic acid was about 5.50 per cent. It was observed that content of saturated fatty acids in the walnut oil was 9.50 per cent. Monounsaturated acids were 24.20 per cent and polyunsaturated acids were 63.30 per cent. The oleic acid content of the walnut oil was 24.20 per cent of the total fatty acids. The linoleic acid content was 54.80 per cent and the linolenic acid was 8.50 per cent.

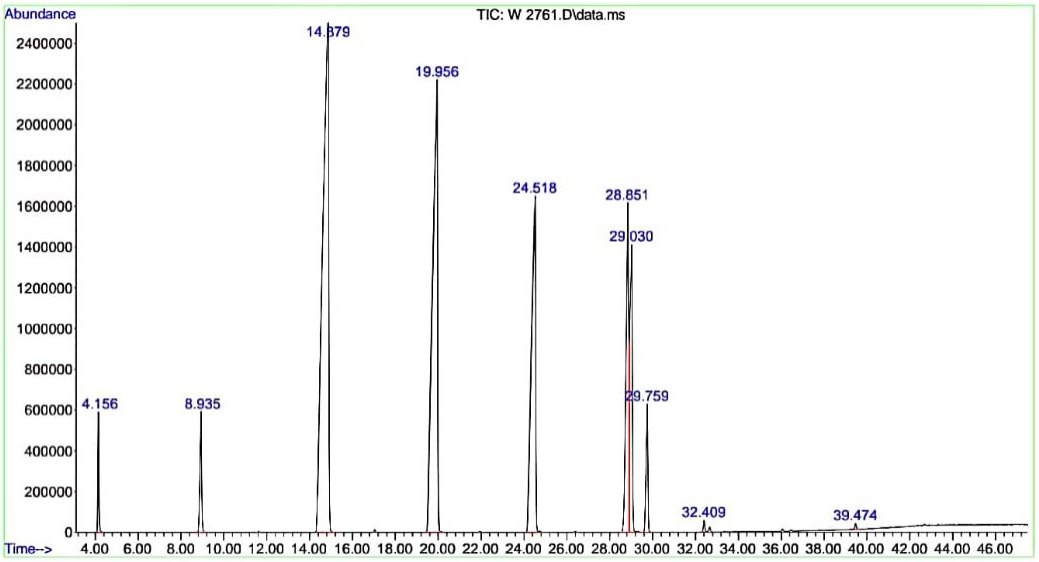

Fig 14. Chromatographic profile obtained for the treatment T_1

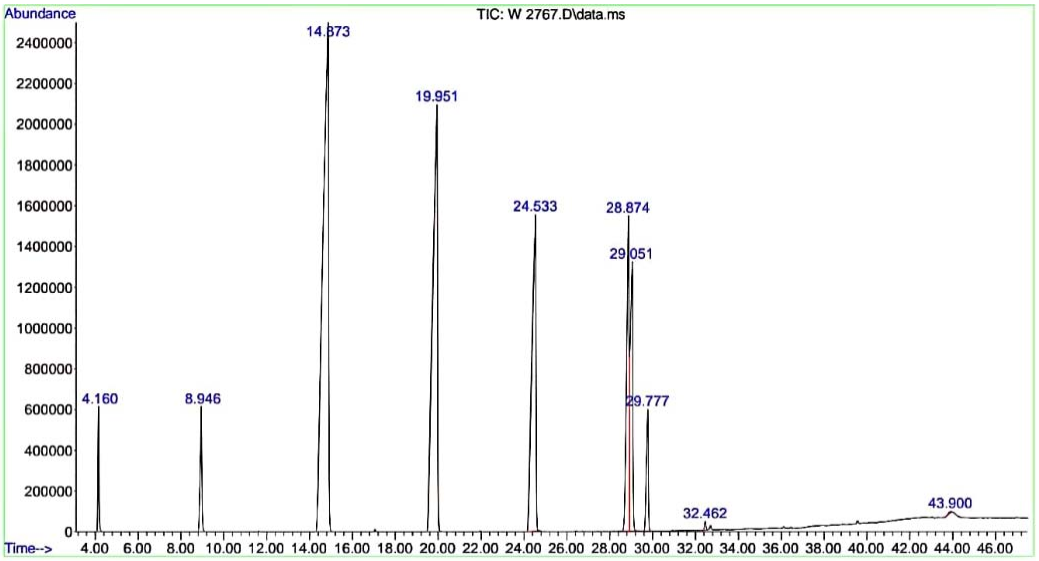

Fig 15. Chromatographic profile obtained for the treatment T_2

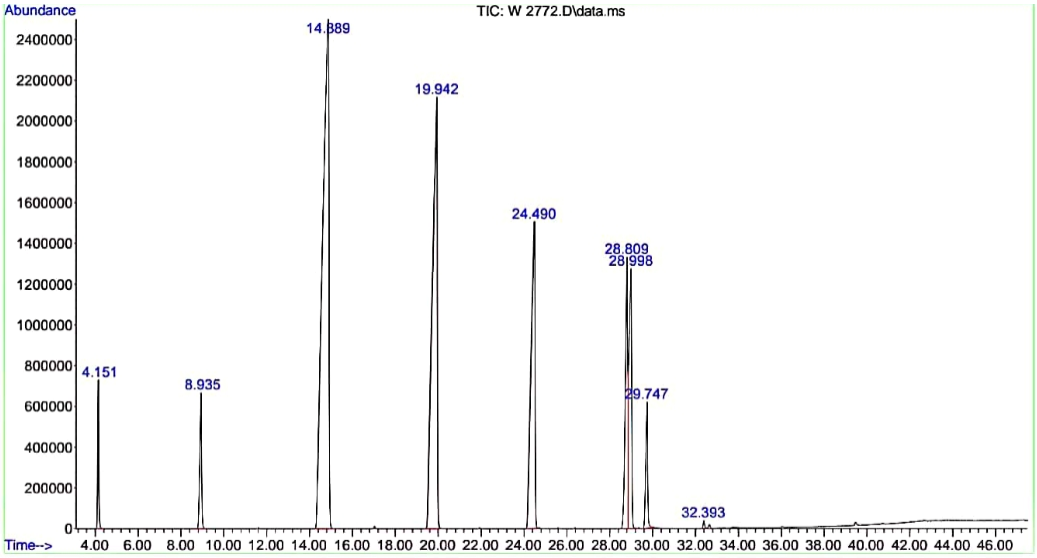

Fig 16. Chromatographic profile obtained for the treatment T_3

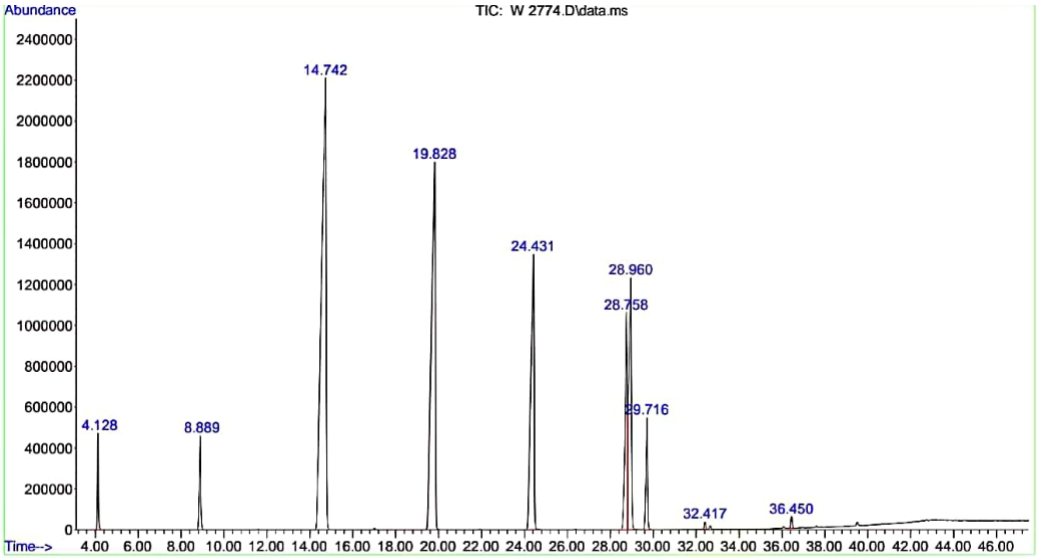

Fig 17. Chromatographic profile obtained for the treatment T_4

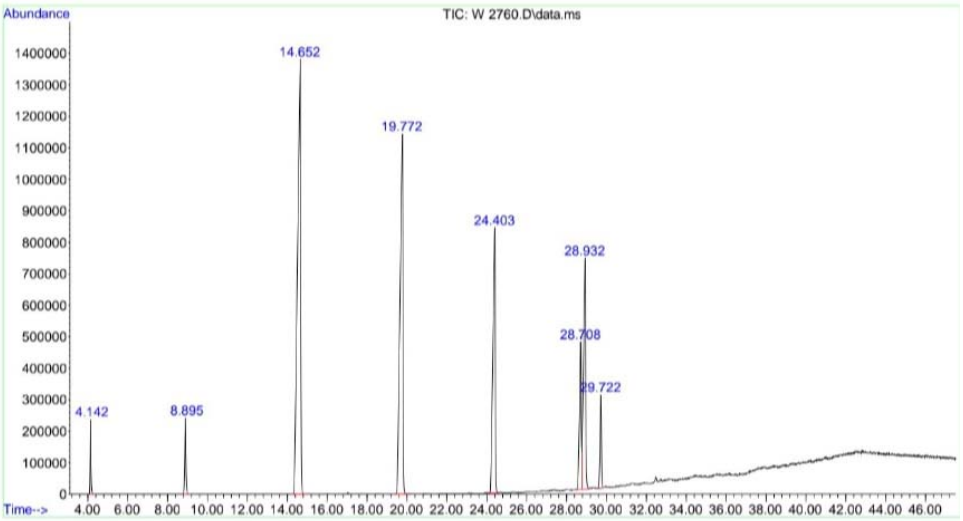

Fig 18. Chromatographic profile obtained for the treatment T_5

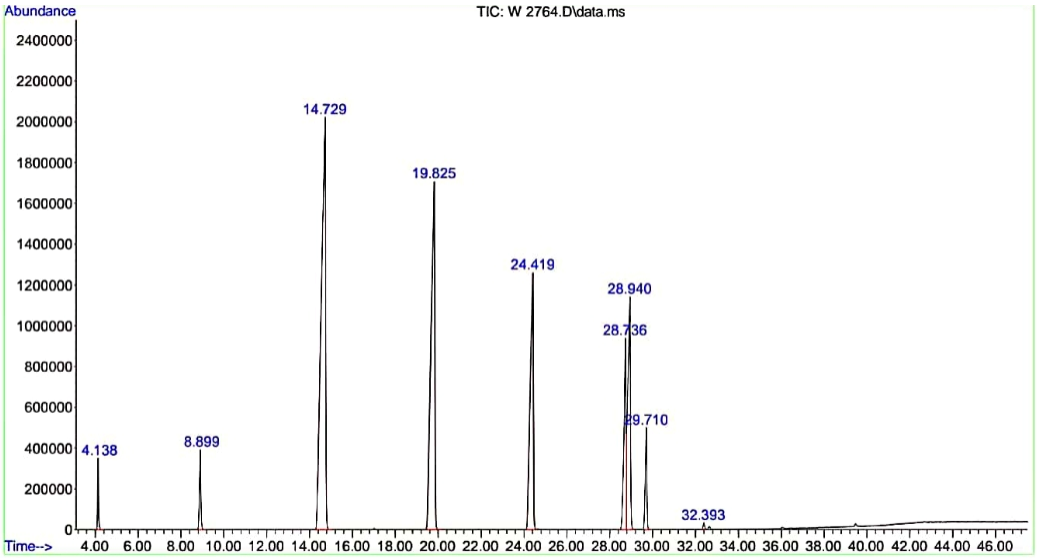

Fig 19. Chromatographic profile obtained for the treatment T_6

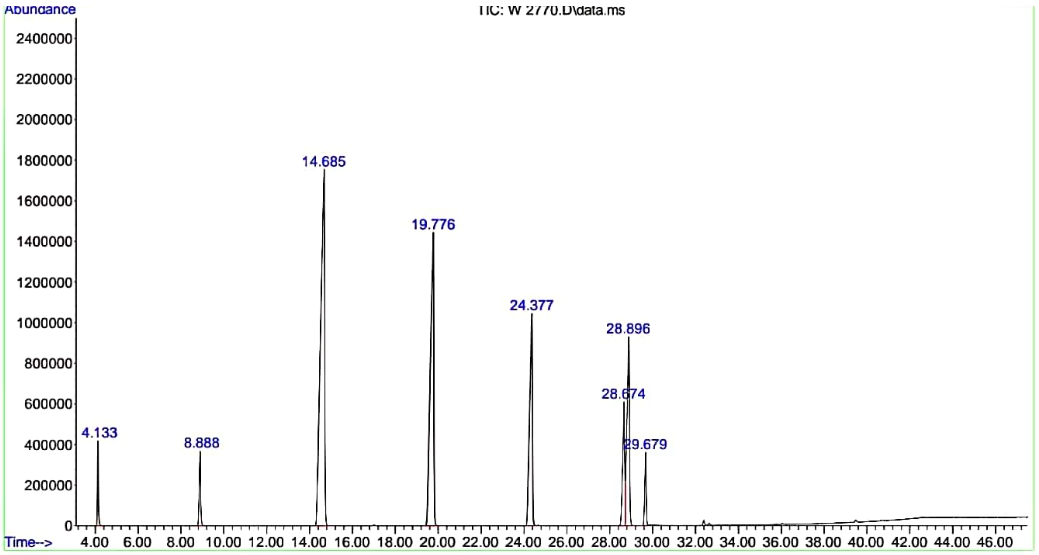

Fig 20. Chromatographic profile obtained for the treatment T_7

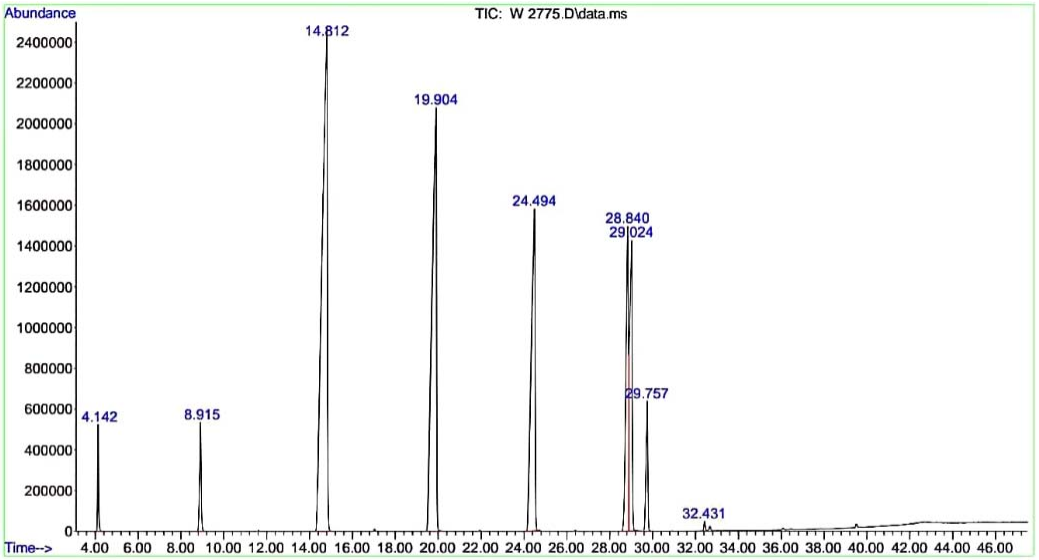

Fig 21. Chromatographic profile obtained for the treatment T_8

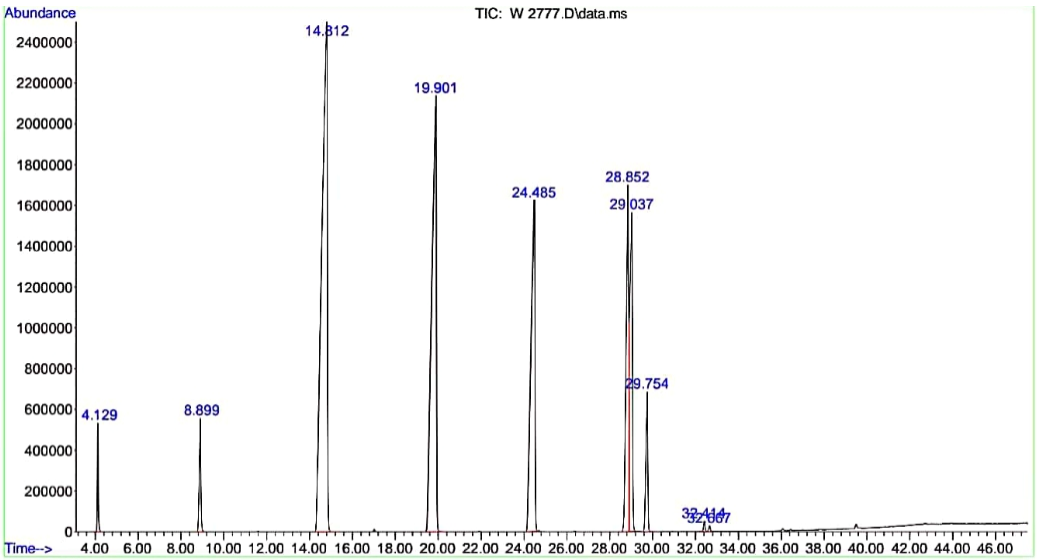

Fig 22. Chromatographic profile obtained for the treatment T_9

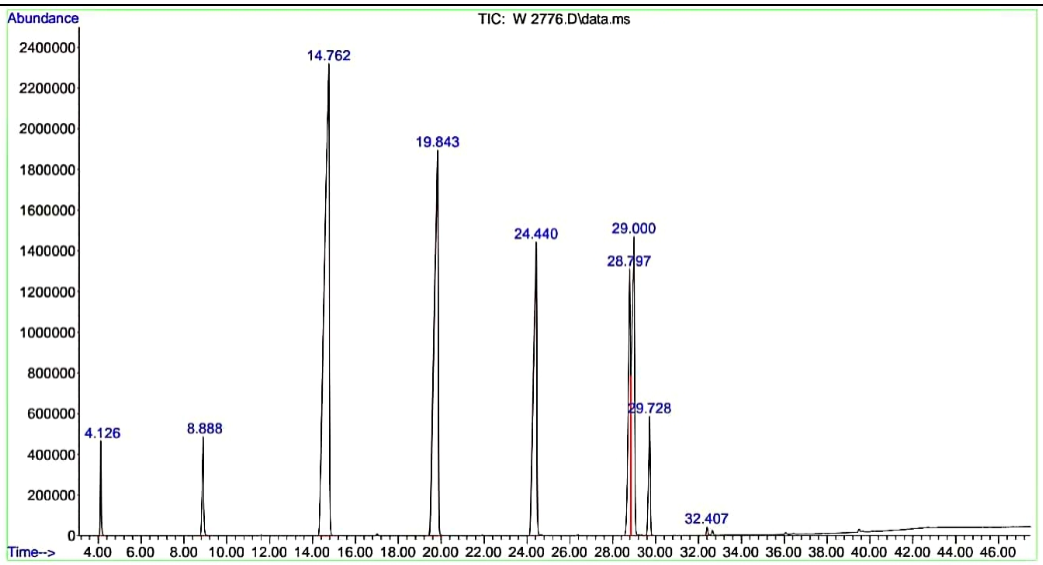

Fig 23. Chromatographic profile obtained for the treatment T_{10}

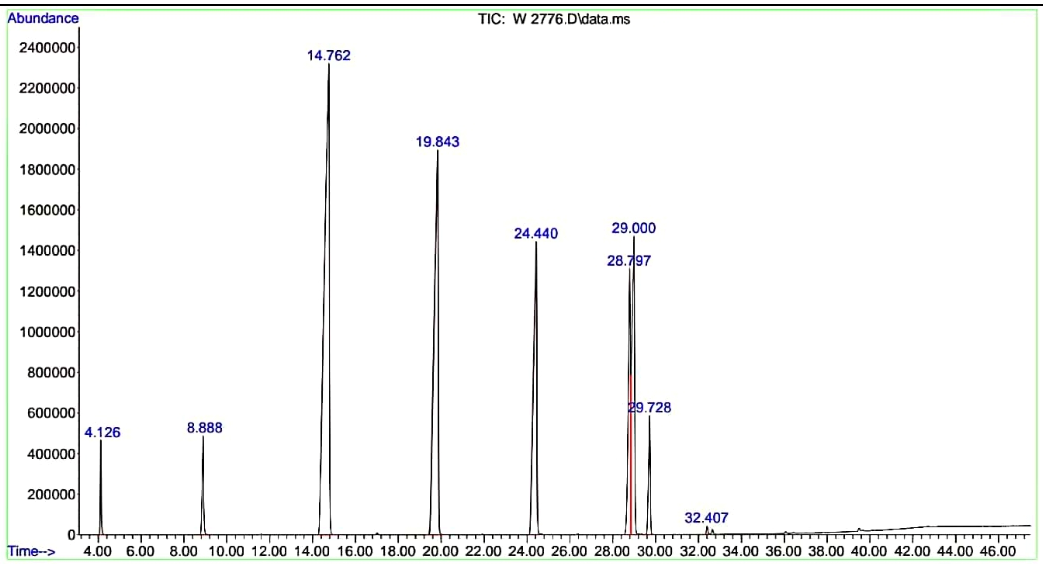

Fig 24. Chromatographic profile obtained for the treatment T_{11}

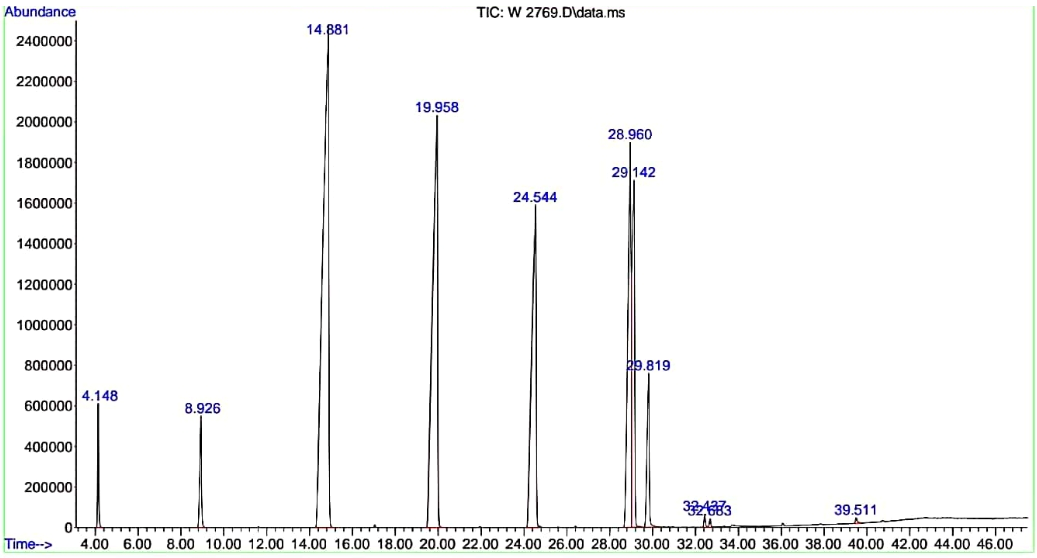

Fig 25. Chromatographic profile obtained for the treatment T_{12}

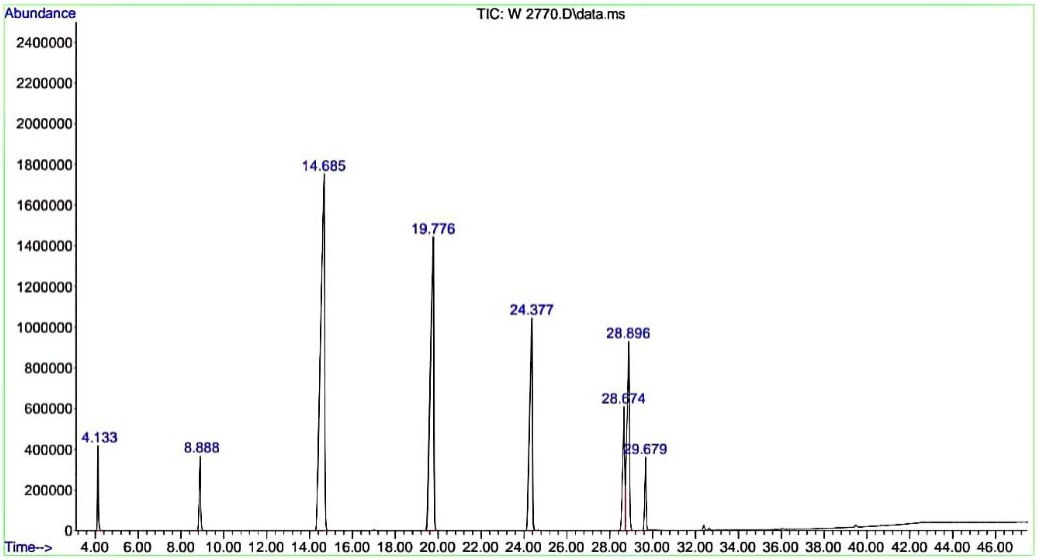

Fig 26. Chromatographic profile obtained for the treatment T_{13}

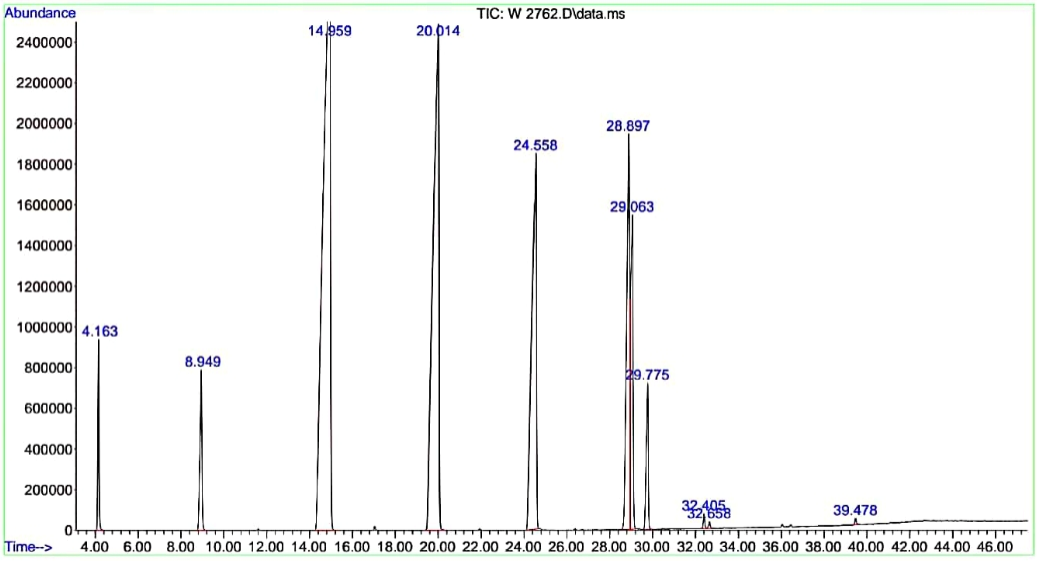

Fig 27. Chromatographic profile obtained for the treatment T_{14}

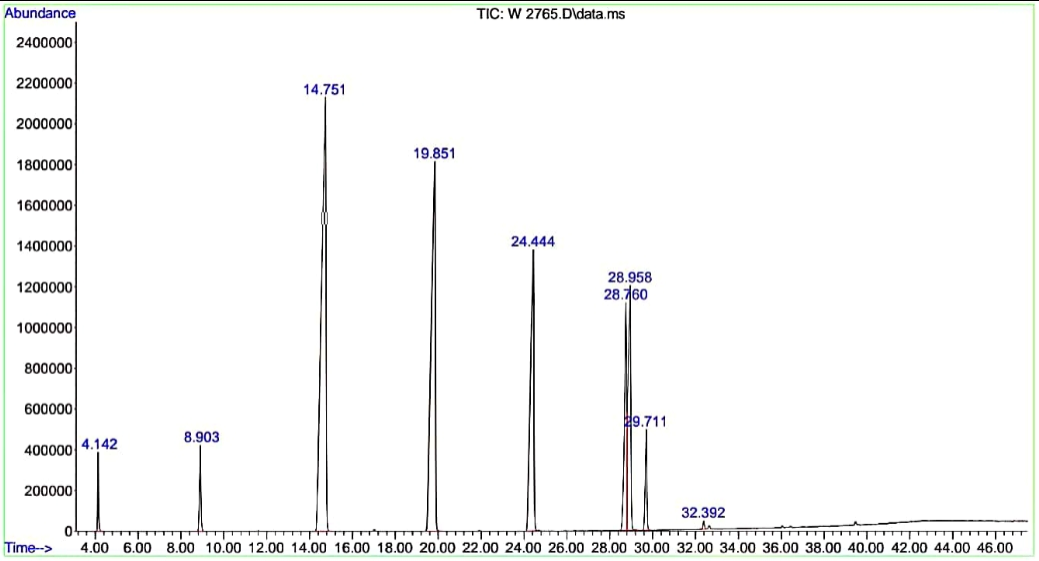

Fig 28. Chromatographic profile obtained for the treatment T_{15}

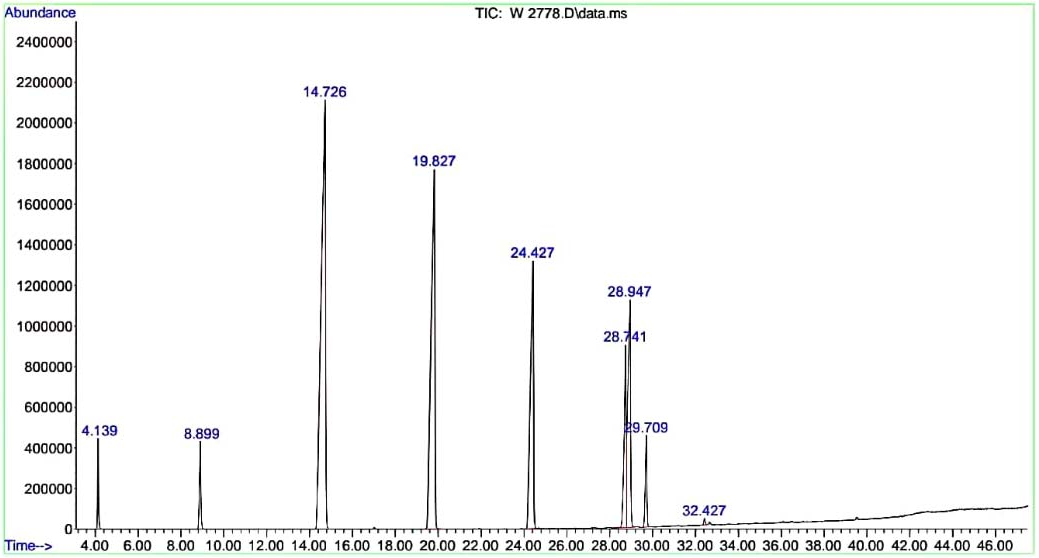

Fig 29. Chromatographic profile obtained for the treatment T_{16}

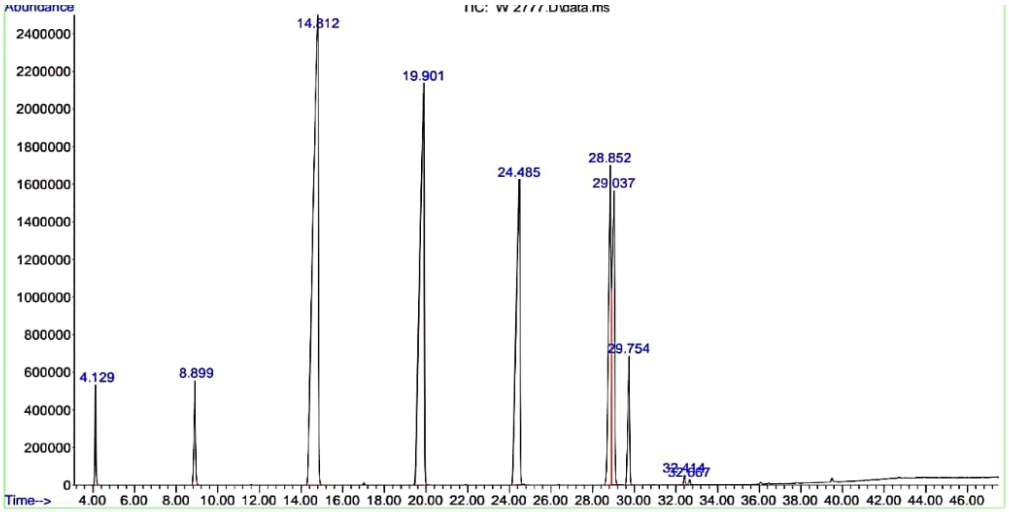

Fig 30. Chromatographic profile obtained for the treatment T_{17}

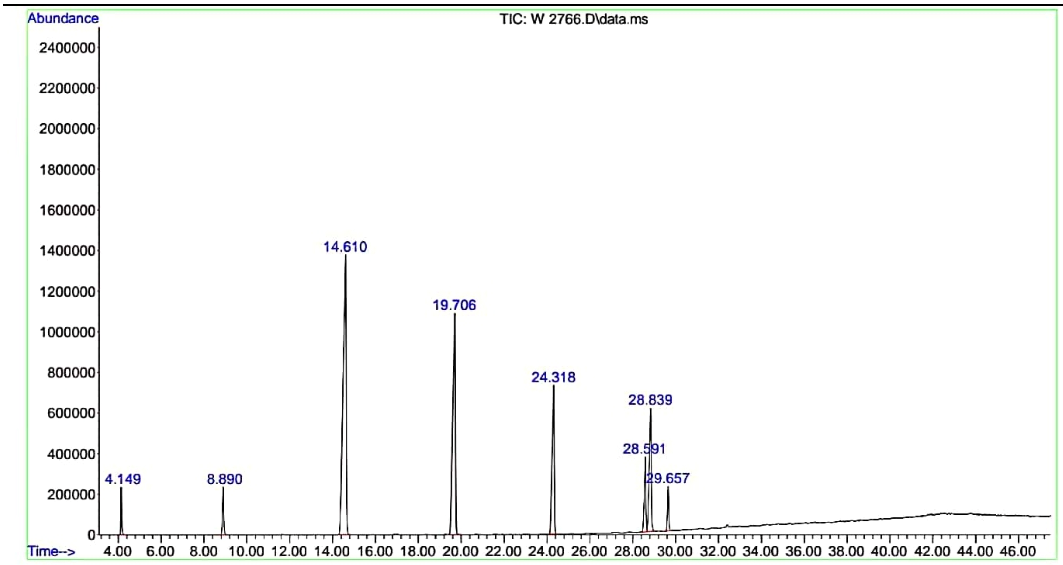

Fig 31. Chromatographic profile obtained for the treatment T_{18}

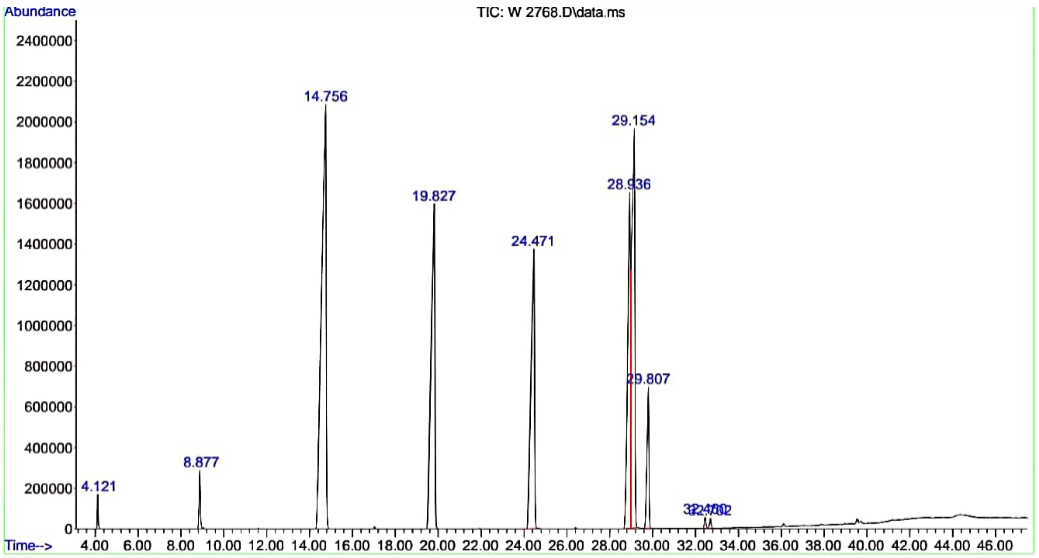

Fig 32. Chromatographic profile obtained for the treatment T_{19}

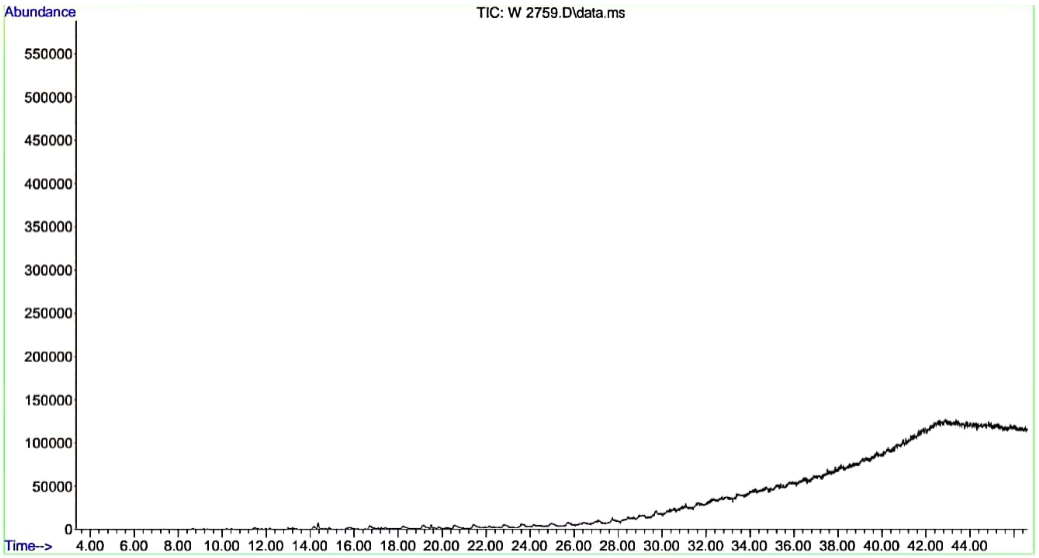

Fig 33. Chromatographic profile obtained for the treatment T_{20}

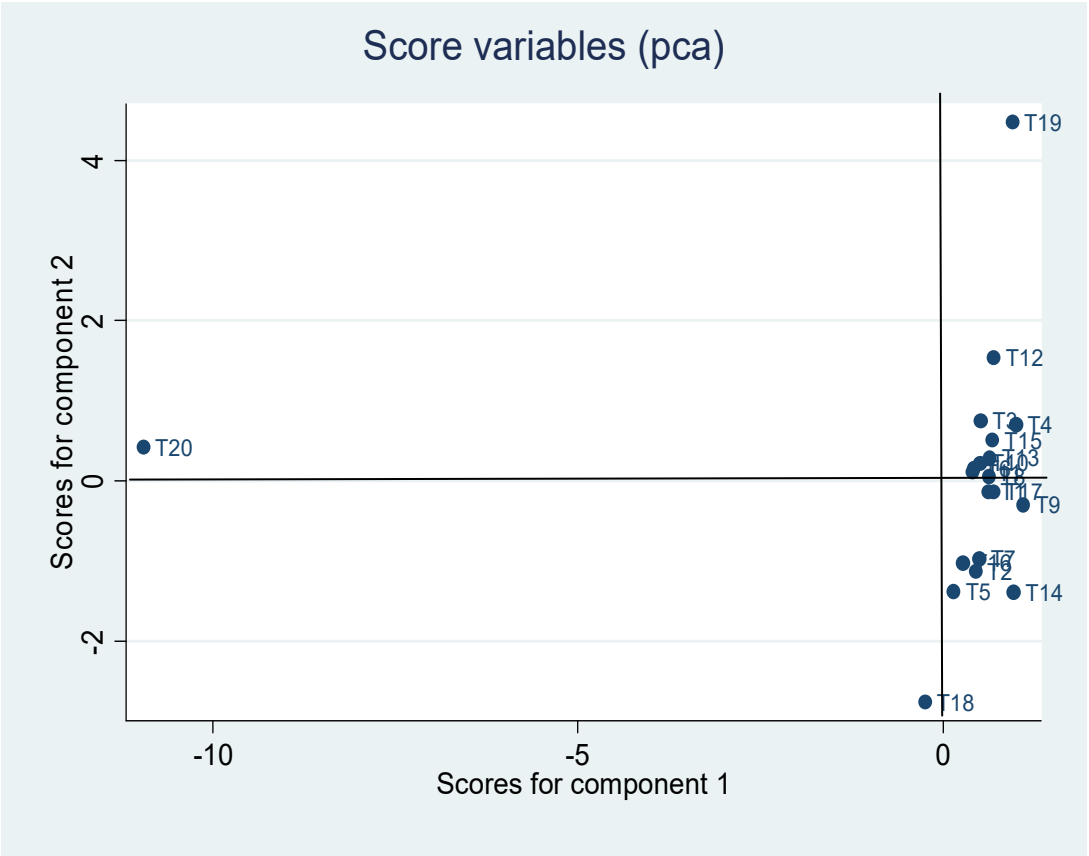

Fig 34. Score plot of PCA constructed based on the fatty acid composition of oil samples

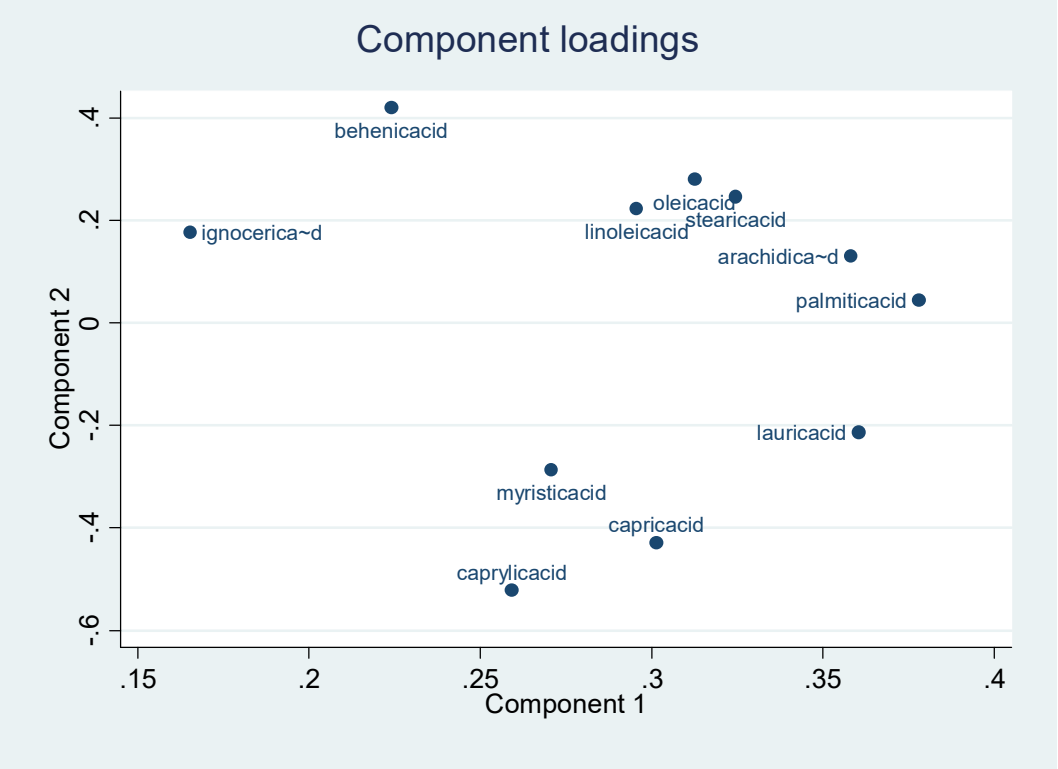

Fig 35. Variable plot of PCA constructed based on the fatty acid composition of oil samples

5.5 FTIR Spectroscopy

FTIR spectra was obtained using FTIR spectrometer (Plate 5). This is a rapid and non destructive analysis. Spectra of all the treatments were analysed. In pure sample (T_1) major peaks were formed at regions of 2800-2900 cm^{-1}, 1743 cm^{-1}, 1465 cm^{-1}, 1417 cm^{-1}, 1377 cm^{-1}, 1229 cm^{-1}, 1155 cm^{-1}, 1111 cm^{-1}, 962 cm^{-1} and 722 cm^{-1} (Fig 36). All the branded coconut oil samples showed peaks at 2800-2900 cm^{-1}, 1743 cm^{-1}, 1465 cm^{-1}, 1417 cm^{1}, 1377 cm^{-1}, 1229 cm^{-1}, 1155 cm^{-1}, 1111 cm^{-1}, 962 cm^{-1} and 722 cm^{-1} (Fig. 37, 38, 39, 40, 41). Adulteration was not detected in any of these branded coconut oil samples. The peak noticed in the region of 2921 cm^{-1} and 2853 cm^{-1} was common for all treatments used in the analysis. In palm kernel oil (T_{19}) peaks were observed at 3006 cm^{-1}, 2921 cm^{-1}, 2853 cm^{-1}, 1735 cm^{-1}, 1465 cm^{-1}, 1417 cm^{-1}, 1377 cm^{-1}, 1238−1165 cm^{-1}, 722 cm^{-1} (Fig 54). When palm kernel oil was used as an adulterant in different concentrations, peaks appeared at 3006 cm^{-1}, 2921 cm^{-1}, 2853 cm^{-1}, 1735 cm^{-1}, 1465cm^{-1}, 1417cm^{-1}, 1377cm^{-1}, 1238cm^{-1}, 1165cm^{-1} and 722cm^{-1} (Fig 42, 43, 44, 45, 46, 47). Mineral oil was also used as an adulterant in different concentrations in coconut oil and the peaks were observed at 2800-2900 cm^{-1}, 1743 cm^{-1}, 1465 cm^{-1}, 1417 cm^{-1}, 1377 cm^{-1}, 1229 cm^{-1}, 1155 cm^{-1}, 1111 cm^{-1}, 962 cm^{-1} and 722 cm^{-1} (Fig 48, 49, 50, 51, 52, 53). The peaks for mineral oil (T_{20}) were obtained at 2954 cm^{-1}, 2923 cm^{-1}, 2854cm^{1},1466 cm^{-1}, 1378 cm^{-1} and 721 cm^{-1} (Fig 55).

In the FTIR spectra, peak formed in the region between 2800-2950 represents the stretchings of C-H group. The peak formed at 1743 cm^{-1} indicated the presence of C=O group which is an exclusive characteristic peak for coconut oil. Next peak was formed at 1465 cm^{-1} which indicated the C-H scissoring and bending for methylene. At 1417 cm^{-1} rocking of C-H bond was observed. Another peak was observed at 1377 cm^{-1} which indicated the presence of CH_3 deformation. Stretching of -C-O group was observed at 1229 cm^{-1} . Similar stretching of C-O group was observed at 1155 cm^{-1}. The peak at 1111 cm^{-1} showed the C-H bending and C-H deformation. At 962 cm^{-1}, bending of C-H groups in Trans-olefin was observed. C-H group vibration was also

obtained at 722 cm^{-1}. In palm kernel oil adulterated samples, at 3006 cm^{-1}, C=C bending vibration was observed. Intensity of this bending increased with increasing concentration of palm kernel oil and even 1 per cent adulteration can be detected. C-H stretching vibration was observed at 2921 cm^{-1} and 2853 cm^{-1}. One major peak was observed at 1739.4 cm^{-1} which indicated C=O stretching vibration. At 1465 cm^{-1}, C-H scissoring and bending was observed. =C-H bond was observed at 1417 cm^{-1}. CH_3 deformation was found at 1377 cm^{-1}. In the region of 1238-1165 cm^{-1}, stretching of C-O group was observed. At 722 cm^{-1}, C-H group vibration was noticed. Stretching or bending of methyl group was observed at these points. Characteristic peak at 1743 cm^{-1}, 1229 cm^{-1} and 1155 cm^{-1} which indicated the carbonyl group (C=O) was absent in the case of mineral oil.

FTIR is most helpful for distinguishing chemicals that are either organic or inorganic. It is often utilized to quantify some components of an unknown mixture and for the analysis of solids, liquids, and gases (IIT Kanpur, 2012). Rohman (2017) observed that the spectrum obtained for virgin coconut oil was unique when compared to other edible oils and fats. In the analysis, spectrum of VCO was compared with olive oil and palm oil. VCO contains high amount of saturated fatty acids when compared to unsaturated fatty acids. No peaks were observed in the region near 3008 cm^{-1} and 1654 cm^{-1} for VCO. These peaks are used to indicate the degree of unsaturation. Moreover, in the region of 1120–1090 cm^{-1}, VCO has only one peak due to C-O ester linkage vibration while olive oil and palm oil showed two peaks.

A study was carried out to evaluate the effectiveness of Fourier transform infrared (FTIR) spectroscopy in the detection of palm kernel olein as an adulterant in virgin coconut oil. From pure and debased samples of virgin coconut oil, the reflectance measurements were analysed. Detection of adulteration up to 1 per cent was feasible. By analysing the structure of spectra, pure and adulterated samples were classified using the discriminant analysis with 10 principal components. A good linear regression of actual value was noticed in partial least square calibration method and a coefficient of determination (R^2) of 0.9875 was observed (Manaf *et al*., 2007). Coconut oil was mixed with different concentrations of paraffin oil (1, 2, 3, 4, 5, 10, and 100 per cent) and it was subjected to FTIR spectroscopic analysis. The FTIR

analysis showed that the peaks corresponding to carbonyl groups at 1743 cm^{-1}, 1229 cm^{-1} and 1155 cm^{-1} and the peak at 1111 cm^{-1} corresponding to bending and deformation of C-H group were not observed in paraffin oil. These peaks can be taken as signature peaks for the detection of paraffin oil (Raj *et al*., 2018).

Among the commonly used fats and oils, virgin coconut oil has distinctive IR spectrum. In VCO spectrum, there was no peak at region close to 3008 cm^{-1} and 1654 cm^{-1}. Peaks at these regions corresponded to unsaturated double bond =CH (cis) and C=C (cis), respectively. These peaks are used to denote the unsaturation degree of triglyceride. VCO contained high level of lauric acid (about 50 per cent) and very low level of unsaturated FA of oleic and linoleic acids, therefore, it is not pleasing if VCO has no peak at region near 3008 cm^{-1} and 1655 cm^{-1}. Additionally, at region of 1120–1090 cm^{-1}, due to C-O ester linkage vibration, VCO had one peak. At the same time, other edible fats and oils showed two peaks (Rohman and Man, 2011). Man and Rohman (2013) investigated the chance to utilize Fourier transform infrared (FTIR) spectroscopy with multivariate chemometric analysis techniques. Principle component regression (PCR), partial least square (PLS) and discriminant analysis (DA) were used to determine the canola oil (Ca-O) adulteration in virgin coconut oil (VCO). It was found that among the quantitative analytical techniques, discriminate analysis was the best model to discriminate the pure VCO and adulterated VCO. Quantification of Ca-O was done by selecting the frequency regions of 1200-900 cm^{-1} and 3027-2985 cm^{-1} and a high correlation was found between the actual and predicted values of Ca-O as adulterant in VCO. This study confirmed that FTIR spectroscopic technique can be used for authentication studies.

In the experiment, it was found that palm kernel oil adulterated samples showed an extra peak at 3006 cm^{-1} which indicates the C=C bending vibration. Intensity of this bending increased with increasing concentration of palm kernel oil and even 1 per cent palm kernel oil adulteration can be detected. For mineral oil adulterated samples, intensity of characteristic peaks at 1743 cm^{-1}, 1229 cm^{-1} and 1155 cm^{-1} which indicated the carbonyl group (C=O) were found to decrease . These peaks were absent in mineral oil.

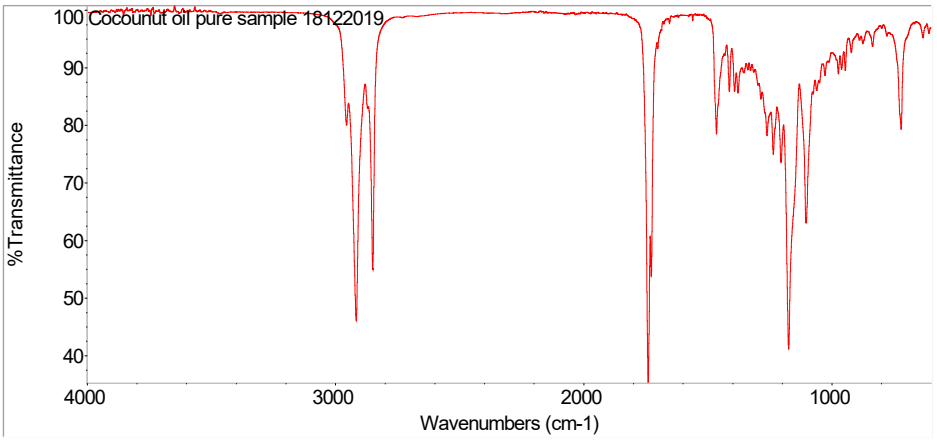

Fig 36. FTIR spectra of pure sample (T_1)

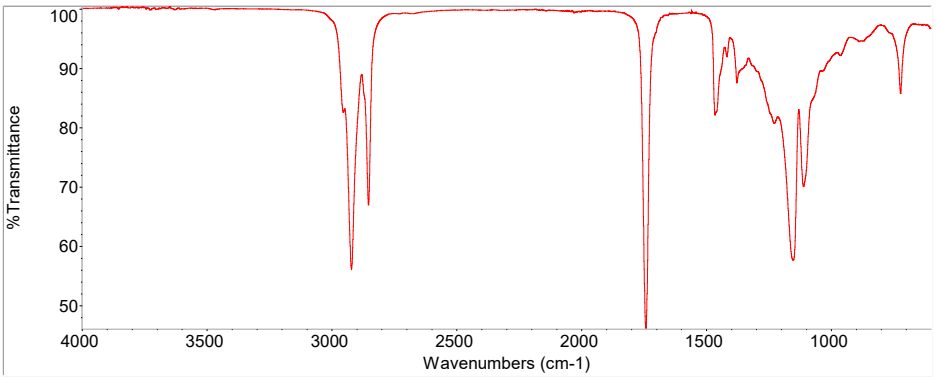

Fig 37. FTIR spectra of branded sample 1 (T_2)

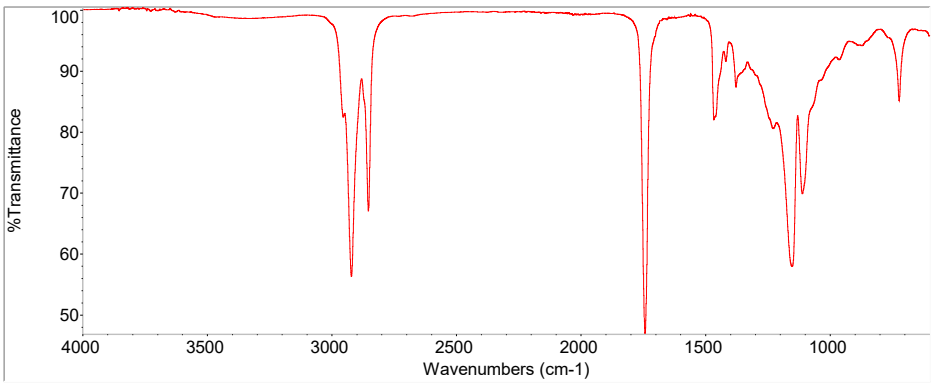

Fig 38. FTIR spectra of branded sample 2 (T_3)

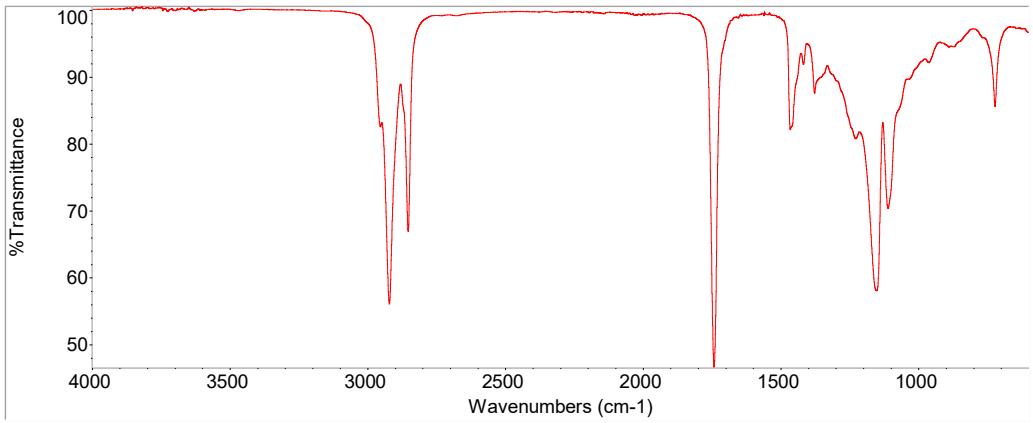

Fig 39. FTIR spectra of branded sample 3 (T_4)

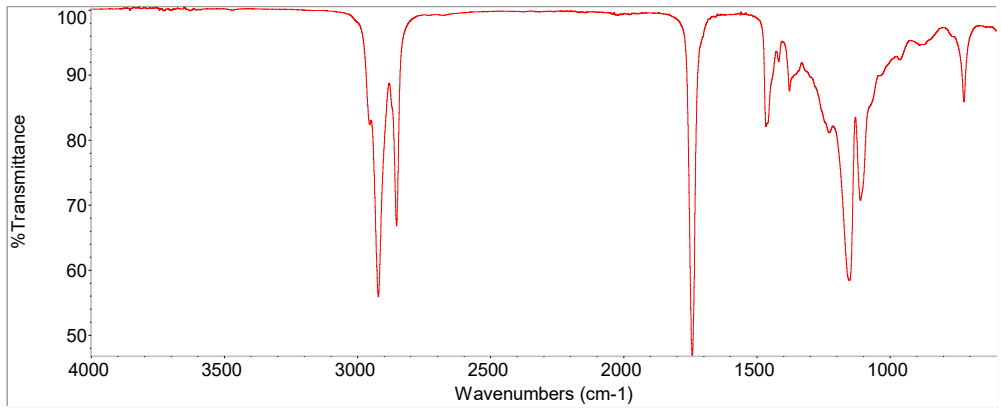

Fig 40. FTIR spectra of branded sample 4 (T_5)

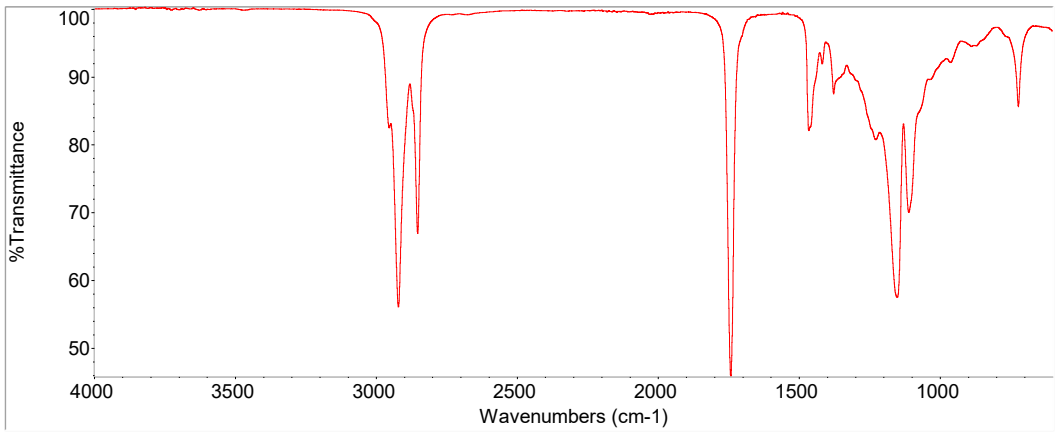

Fig 41. FTIR spectra of branded sample 5 (T_6)

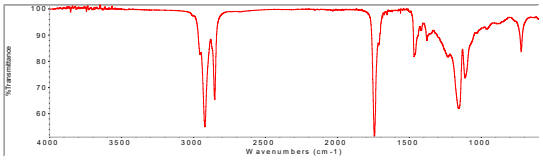

Fig 42. FTIR spectra of coconut oil adulterated with 1 per cent palm kernel oil (T_7)

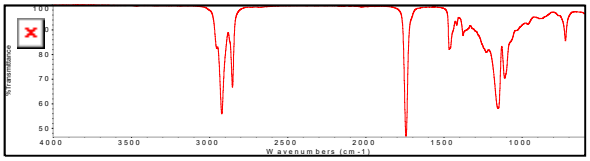

Fig 43. FTIR spectra of coconut oil adulterated with 5 per cent palm kernel oil (T_8)

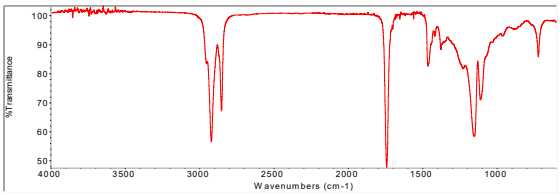

Fig 44. FTIR spectra of coconut oil adulterated with 10 per cent palm kernel oil (T_9)

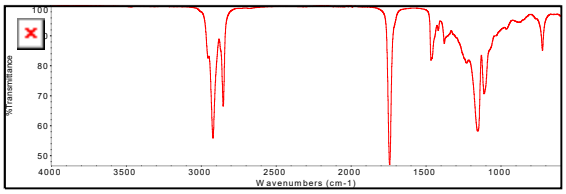

Fig 45. FTIR spectra of coconut oil adulterated with 15 per cent palm kernel oil (T_{10})

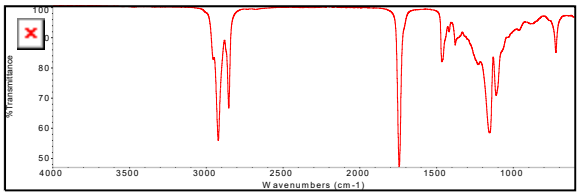

Fig 46. FTIR spectra of coconut oil adulterated with 20 per cent palm kernel oil (T_{11})

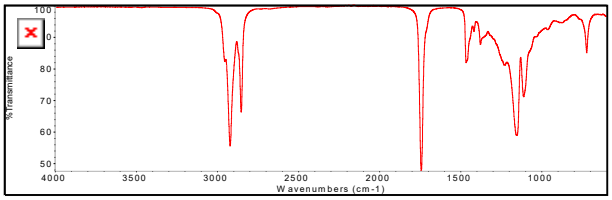

Fig 47. FTIR spectra of coconut oil adulterated with 30 per cent palm kernel oil (T_{12})

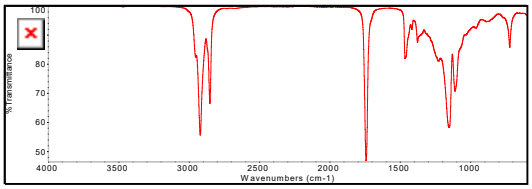

Fig 48. FTIR spectra of coconut oil adulterated with 1 per cent mineral oil (T_{13})

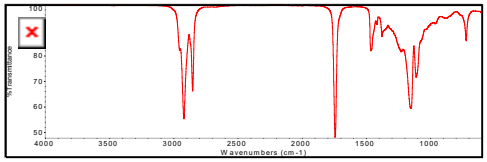

Fig 49. FTIR spectra of coconut oil adulterated with 5 per cent mineral oil (T_{14})

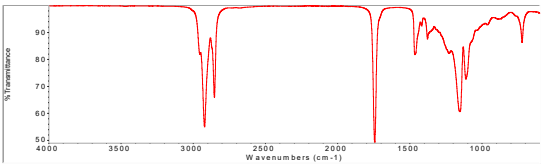

Fig 50. FTIR spectra of coconut oil adulterated with 10 per cent mineral oil (T_{15})

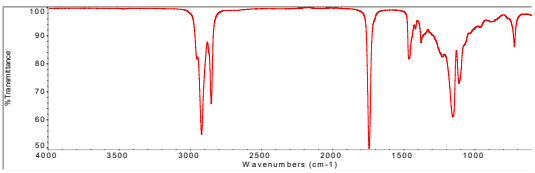

Fig 51. FTIR spectra of coconut oil adulterated with 15 per cent mineral oil (T_{16})

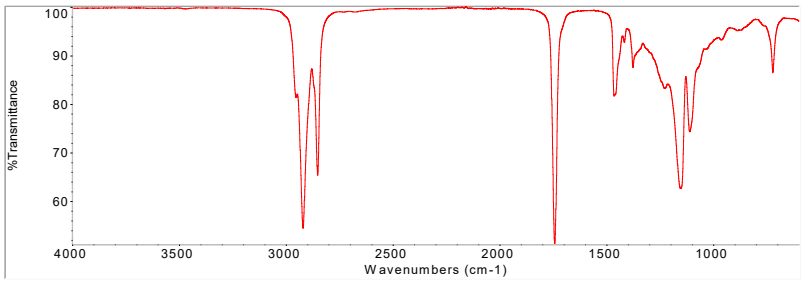

Fig 52. FTIR spectra of coconut oil adulterated with 20 per cent mineral oil (T_{17})

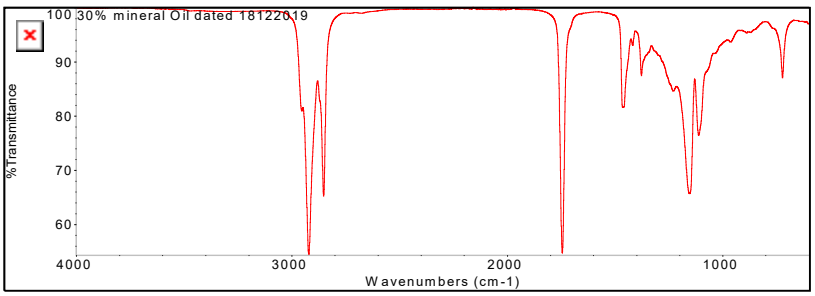

Fig 53. FTIR spectra of coconut oil adulterated with 30 per cent mineral oil (T_{18})

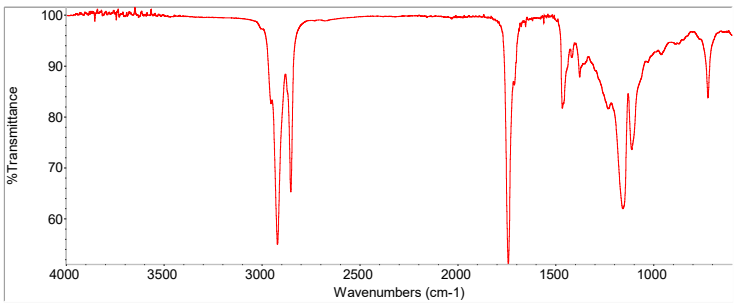

Fig 54. FTIR spectra of palm kernel oil (T_{19})

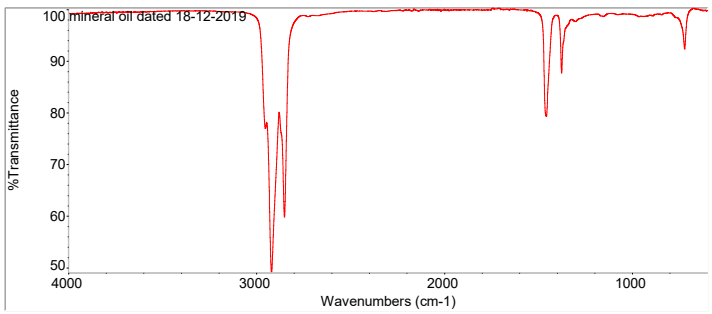

Fig 55. FTIR spectra of mineral oil (T_{20})

5.6. Economics of Adulteration

Economics of adulteration of coconut oil by palm kernel oil and mineral oil is depicted in Table 15. Cost of 1 quintal pure coconut oil sample (T_1) was Rs 20500. Cost of 1 quintal pure coconut oil sample (T_1) was Rs 20500. Cost of branded coconut sample 1 (T_2) and branded sample 3 (T_4) obtained per quintal was Rs.21000. While, cost of one quintal branded sample 2 (T_3) was Rs.22000. Cost of branded sample 4 (T_5) was Rs.18700 and branded sample 5 (T_6) was Rs.18500. Cost of one quintal palm kernel oil (T_{19}) was Rs.10000 and for one quintal mineral oil (T_{20}), it was Rs.4500. Cost per quintal was less (Rs.15700) when coconut oil was mixed with 30 per cent of mineral oil (T_{18}) and the profit obtained was Rs.4800. Hence, BC ratio comes to 0.30 and is found to be the highest among other treatments. This was followed by coconut oil mixed with 20 per cent mineral oil (T_{17}) with a BC ratio of 0.18. Similar BC ratio was obtained when coconut oil was mixed with 30 per cent palm kernel oil (T_{12}). When coconut oil was mixed with 1 per cent palm kernel oil (T_7) cost of production was Rs. 20395. Profit obtained was Rs 105 and a low BC ratio of 0.005 was obtained.

Adulterants like palm kernel oil and mineral oil are available at a cheaper rate when compared to the pure coconut oil sample. Hence adulteration becomes a common process in the edible oil industry. In this study, it was observed that a considerable amount of profit was obtained by adding 30 per cent of palm kernel oil and mineral oil.

SUMMARY

6. SUMMARY

The present study entitled "Quality assessment of coconut oil and detection of adulteration" was undertaken at the Department of Plantation Crops and Spices, College of Agriculture Vellayani during the period 2018-2020. The study was conducted in order to assess the quality parameters of coconut oil and to detect adulteration by different techniques and to validate an easy and efficient method for the detection.

Twenty samples were taken for experiment. A sample of pure coconut oil was obtained from the coconut expeller and five different brands of coconut oil samples were collected. Pure coconut oil of 1, 5, 10, 15, 20 and 30 per cent were substituted with palm kernel oil and mineral oil. These samples were tested for physical and chemical characteristics, microbial contamination, thin layer chromatography, fatty acid composition by GCMS and FTIR spectroscopy.

Physical and chemical and characteristics of coconut oil samples were analysed and it include refractive index at 40°C, relative density, apparent density, insoluble impurities, saponification value, iodine value, Polenske value, unsaponifiable matter, acid value, peroxide value and matter volatile at 105°C. FSSAI standard for refractive index in coconut oil is 1.4481-1.4491. All branded coconut oil samples and coconut oil mixed with different concentrations of palm kernel oil obtained a value within the FSSAI standard while mineral oil adulterated samples exceeded the FSSAI limit. High refractive index (1.4674) was noticed for mineral oil. Codex standard for relative density is within the range 0.908-0.921. Branded coconut oil samples and palm kernel oil adulterated samples obtained a value within the standard. Coconut oil samples adulterated with different percentage of mineral oil obtained a value less than the Codex standard. Relative density was higher for branded samples 1, 4 and 5 (0.921). Similarly apparent density was also higher for branded samples 1, 4 and 5 (0.907 g ml^{-1}). According to Codex standards, insoluble impurities should be below 0.05 per cent. Insoluble impurities of all the samples were

within the range 0.024-0.047 per cent. Higher insoluble impurities (0.047 per cent) were obtained for coconut oil mixed with 30 per cent mineral oil.

According to FSSAI, saponification value for pure coconut oil is above 250. Saponification value was significantly higher (266.29 mg KOH g of oil^{-1}) for pure coconut oil. All the branded coconut oil samples showed a value above 250. Coconut oil mixed with different concentrations of palm kernel oil and mineral oil obtained a value below 250. High iodine value was noticed for palm kernel oil (20.26 g of iodine 100g of oil^{-1}). FSSAI standard for iodine value of coconut oil is in the range 7.5-10. When palm kernel oil was used as an adulterant, iodine value exceeded the standard value. Mineral oil adulterated samples obtained iodine values less than the standard limit. In the analysis, pure coconut oil sample showed a high Polenske value (14.17). FSSAI standard for Polenske value is above 13. Pure coconut sample and branded coconut oil samples obtained Polenske values as per the standard. Coconut oil samples mixed with different concentrations of palm kernel oil and mineral oil attained values below 13. Unsaponifiable matter is another parameter which helps in the detection of adulteration. High value was noticed for mineral oil (89.12 per cent). According to FSSAI, unsaponifiable matter in coconut oil should not be more than 1 per cent. Branded coconut oil samples and palm kernel oil adulterated samples obtained values within the standard. On the other hand, mineral oil adulterated samples exceeded the FSSAI limit.

Acid value indicates the level of deterioration of oil. The highest acid value was noticed for palm kernel oil with 9.83 mg KOH g of oil^{-1}. Branded coconut oil samples and coconut oil adulterated with mineral oil showed values within the limit. FSSAI standard for acid value is not more than 6. However, acid value showed an increasing trend, when 15 per cent palm kernel oil was used. Oxidation of oil can be determined by peroxide value. Generally, Codex gives a peroxide value limit of 15 meq kg^{-1}. In the analysis highest peroxide value was noticed for palm kernel oil (14.56 meq kg of oil^{-1}). Pure sample and branded samples obtained a peroxide value which was less than 10 meq kg^{-1}. When palm kernel oil was used an adulterant in different concentrations, the peroxide values were above 10 meq kg^{-1}. Different peroxide values were observed when mineral oil was used as an adulterant. Matter

volatile at 105°C was highest for coconut oil mixed with 30 per cent palm kernel oil and the value obtained was 0.157 per cent. Codex standard for matter volatile at 105°C should not be more than 0.2 per cent. In the experiment, all samples obtained values within the standard.

Principal component analysis (PCA) was performed on physical and chemical characteristics of oil samples. PCA emphasizes variation and transforms large sets of variable to smaller one. Total variation of 74.16 percent was observed in the data and two principal components obtained PC1 and PC2. PC1 accounted for 55.36 percent of variation and PC2 with 18.80 per cent variation. In PC1, high coefficient was obtained for unsaponifiable matter (-0.968) and it contributed maximum variation followed by relative density (0.943), apparent density (0.942), Polenske value (0.938), refractive index (-0.935) and saponification value (0.826). In PC2, high coefficient was noticed for acid value (0.891) followed by peroxide value (0.713) and iodine value (0.701). Score plot was constructed based on the data and it indicated the nature and type of oil sample. Pure coconut oil and branded coconut oil samples clustered in the same quadrant. On the other hand, palm kernel oil adulterated samples were found to cluster in another quadrant. Mineral oil adulterated samples clustered in a quadrant which was entirely opposite to coconut oil and this indicated that maximum variation occurred due to mineral oil adulteration. By comparing score plot with variable plot we could identify the parameters leading to variation. Thus variation in mineral oil was contributed by the parameters unsaponifiable matter, refractive index at 40°C, relative density, apparent density ad Polenske value. But for palm kernel oil, the variation was highly influenced by the parameters acid value, iodine value and peroxide value. So these parameters can be effectively used to check adulteration in the oil samples.

Microbial contamination was another parameter used to assess the quality of oil sample. Total plate count was taken to evaluate the bacterial, fungal and actinomycete count and was expressed in cfu ml^{-1}. Fungal and actinomycete count was zero for all the oil samples. The highest bacterial count (7×10^{-7} cfu ml^{-1}) was observed in coconut oil mixed with 30 per cent palm kernel oil. This was followed by T_{10} (coconut oil mixed with 15 per cent palm kernel oil) and T_{17} (coconut oil mixed

with 20 per cent mineral oil) with 6×10^{-7} cfu ml^{-1}. No bacterial colony was observed for pure sample. But the branded coconut oil sample 1 had a bacterial population of 1×10^{-7} cfu ml^{-1}. Microbes are usually found from the materials used for production or from the environment or due to improper storage and distribution. In the experiment, it was found that there were no fungal colonies and actinomycete. But a few colonies of bacteria were detected. The bacterial count observed was less than 10 colonies for all the samples.

Thin layer chromatography was used as a qualitative test for adulteration detection. Among the samples, yellow streaks were appeared in the profile of pure coconut oil sample, branded coconut oil samples and coconut oil mixed with different concentrations of palm kernel oil. On the other hand, presence of mineral oil was detected for coconut oil mixed with 1 per cent mineral oil onwards as revealed from yellow fluorescent spots on the chromatographic profile of mineral oil adulterated samples.

Gas chromatography coupled with mass spectrometry was used to analyze the adulteration and fatty acid composition in the oil samples. Principal component analysis was carried out to analyze the variation contributed by the fatty acids. C8:0, C10:0, C12:0, C14:0, C16:0, C18:0, C18:1, C18:2, C20:0, C22:0 and C24:0 are the fatty acids detected from the oil samples. Total variation observed was 79.76 per cent and the variation was contributed by two principal component PC 1 and PC 2. PC 1 showed a variation of 61.21 per cent and PC 2 showed a variation of 18.55 per cent. In PC 1, high coefficient was observed for the fatty acid C16:0 (0.978), followed by C 12:0 (0.954) and C 14:0 (0.936). In PC 2, high coefficient was obtained for C 8:0 (-0.707) followed by C 10:0 (-0.595).

Among the fatty acids, C16:0, C 12:0 and C 14:0 contributed the maximum variation. Saturated fatty acids were observed in large proportion for all the oil samples. Among the saturated fatty acids, lauric acid (C12:0) constituted the highest proportion. This was followed by myristic acid (C14:0) and palmitic acid (C16:0). Oleic acid and linoleic acids were the major unsaturated fatty acids observed in the oil samples. Percentage of oleic (C18:1) and linoleic (C18:2) acids were found to

increase with increase in concentration of palm kernel oil. Score plot constructed based on the GCMS data revealed that oil samples with similar composition clustered together. Palm kernel oil adulterated samples and branded coconut oil samples were tightly clustered around the pure coconut oil. Coconut oil adulterated with different concentrations of mineral oil were also found within the cluster. Fatty acids were not detected in mineral oil and its location was far away from the cluster. The variable plot explained the influence of fatty acids in the treatment. The fatty acid composition of oils were highly influenced by lauric acid (C12:0) and palmitic acid (C16:0). In palm kernel oil, variation was contributed by stearic acid (C18:0), oleic acid (C18:1) and linoleic acid (C18:2).

FTIR spectra was obtained using FTIR spectrometer. Analysis of FTIR spectra showed that pure coconut oil sample formed peaks at regions of 2800-2900 cm^{-1}, 1743 cm^{-1}, 1465 cm^{-1}, 1417 cm^{-1}, 1377 cm^{-1}, 1229 cm^{-1}, 1155 cm^{-1}, 1111 cm^{-1}, 962 cm^{-1} and 722 cm^{-1}. All the branded coconut oil samples obtained peaks at similar regions and adulteration was not detected in any of these branded coconut oil samples. Palm kernel oil adulterated samples obtained peaks at regions similar to those in coconut oil. At 3006 cm^{-1}, C=C bending vibration was observed. Intensity of this bending increased with increasing concentration of palm kernel oil and based on this, even 1 per cent adulteration can be detected. Mineral oil was also used as an adulterant in different concentrations in coconut oil and similar peaks were observed. Intensity of the peaks at 1743 cm^{-1}, 1229 cm^{-1} and 1155 cm^{-1} and 1111 cm^{-1} were found to decrease with increase in percentage of mineral oil. The peaks for mineral oil were obtained at 2954 cm^{-1}, 2923 cm^{-1}, 2854cm^{1}, 1466 cm^{-1}, 1378 cm^{-1} and 721 cm^{-1}. Characteristic peaks at 1743 cm^{-1}, 1229 cm^{-1} and 1155 cm^{-1} which indicated the carbonyl group (C=O) was absent in the case of mineral oil.

Economics of adulteration of coconut oil by palm kernel oil and mineral oil was calculated. In this study, it was observed that a considerable amount of profit was obtained by adding 30 and 20 per cent of mineral oil and 30 per cent palm kernel oil. Coconut oil mixed with 30 per cent of mineral oil obtained a high BC ratio (0.30) when compared with others. Adulterants like palm kernel oil and mineral oil are available at a cheaper rate when compared to the pure coconut oil sample.

Physical and chemical parameters such as unsaponifiable matter, relative density, apparent density, Polenske value and refractive index could be used to identify the adulteration in coconut oil by mineral oil while saponification value, iodine value and Polenske value could detect the adulteration due to palm kernel oil. Adulteration of coconut oil with palm kernel oil and mineral oil could be easily detected by FTIR spectroscopic technique. Besides, GCMS analysis provided information regarding the fatty acid composition. Addition of even 1 per cent palm kernel oil in coconut oil changed the percentage of unsaturated fatty acid in the oil sample.

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7. REFERENCES

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QUALITY ASSESSMENT OF COCONUT OIL AND DETECTION OF ADULTERATION

by

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ABSTRACT

The research programme entitled “Quality assessment of coconut oil and detection of adulteration” was undertaken at the Department of Plantation Crops and Spices, College of Agriculture Vellayani during the period 2018-2020. The study was conducted in order to assess the quality parameters of coconut oil and to detect adulteration by different techniques and to validate an easy and efficient method for the detection.

Coconut oil was adulterated with 1, 5, 10, 15, 20 and 30 per cent of palm kernel oil and mineral oil. Apart from this pure coconut oil, pure palm kernel oil, pure mineral oil and five branded coconut oils were also collected altogether contributing twenty samples. These samples were analysed for physical and chemical characteristics, microbial contamination, thin layer chromatography, fatty acid composition by GCMS and FTIR spectroscopy to detect adulteration and to find an easy and efficient method for detection of adulteration.

Physical and chemical characteristics analysed revealed refractive index and relative density of pure coconut oil, branded coconut oil and coconut oil mixed up to 30 per cent palm kernel oil were within the codex standard range. The apparent density of pure coconut oil differed significantly from adulterated samples. Insoluble impurities of all the samples were in the range 0.024-0.047 per cent which was within the standard prescribed by Codex (< 0.05 per cent). Saponification value of pure sample and all the branded coconut oil samples showed a value above 250 mg KOH g of oil^{-1} which was in tune with standard specified by FSSAI. Palm kernel oil and mineral oil as adulterant in different percentage showed less than 250 mg KOH g of oil^{-1}. FSSAI standard for iodine value of coconut oil is in the range 7.5-10g and the iodine value of pure sample and all the branded coconut oil samples tested were within the range 7.5-10g. Iodine value of coconut oil adulterated with 5 per cent palm kernel oil and above exceeded the standard value (10) while adulteration with mineral oil above 1 per cent showed less than 7.5g. Standard Polenske value as prescribed by FSSAI and Codex (not less than13) was noticed in all samples except coconut oil with

palm kernel oil 5 per cent and above and all mineral oil combinations. Pure sample, branded coconut oil and coconut oil mixed with varying percentage of palm kernel oil had unsaponifiable value within the limit of standards (not more than 1 per cent) while that of coconut oil mixed with even 1 per cent mineral oil and above exceeded the limit (1.18 per cent). Acid value of not more than 6 was the standard put forward by FSSAI and Codex and all oil samples except palm kernel oil and coconut oil mixed with 15 per cent palm kernel oil and above recorded values above 6. FSSAI standard for peroxide content in fresh coconut oil is below 10 meq/Kg and higher peroxide value were obtained from coconut samples mixed with palm kernel oil at 5 per cent and above and mineral oil at 15 per cent and above. Codex standard for matter volatile at 105°C is < 0.2% and all samples were within the limit.

Principal component analysis (PCA) performed on physical and chemical characteristics of oil revealed high coefficient in PC1 for unsaponifiable matter and it contributed to maximum variation followed by relative density, apparent density, Polenske value, refractive index, saponification value and iodine value. In PC2, high coefficient was noticed for acid value followed by peroxide value and iodine value .

Microbial contamination assessed by total plate count was within the APCC standard of less than 10 (< 10) colony forming units/ml. Fungal and actinomycete population was however not detected in the oil samples.

Thin layer chromatography revealed yellow streaks in the profile of pure coconut oil sample, branded coconut oil samples and coconut oil mixed with different concentrations of palm kernel oil while yellow streaks were absent in coconut oil samples mixed with mineral oil.

Gas chromatography coupled with mass spectrometry revealed the fatty acid composition in the oil samples. Principal component analysis carried out to analyze the variation contributed by the fatty acids revealed C16:0, C 12:0 and C 14:0 contributed the maximum variation in PC1. In PC2 high coefficient was noticed for C8:0 and C10:0. Percentage of oleic (C18:1) and linoleic (C18:2) acids were found to increase with increase in concentration of palm kernel oil and fatty acids were not detected in mineral oil.

FTIR spectra formed peaks at regions of 2800-2900 cm^{-1}, 1743 cm^{-1}, 1465 cm^{-1}, 1417 cm^{-1}, 1377 cm^{-1}, 1229 cm^{-1}, 1155 cm^{-1}, 1111 cm^{-1}, 962 cm^{-1}, 722 cm^{-1} in pure coconut oil sample. Branded coconut oil samples and palm kernel oil adulterated samples obtained peaks at similar regions. Intensity of the peak at 3006 cm^{-1} increased with increasing concentration of palm kernel oil. The peaks for mineral oil were obtained at 2954 cm^{-1}, 2923 cm^{-1}, 2854cm^{1}, 1466 cm^{-1}, 1378 cm^{-1} and 721 cm^{-1}. Intensity of the peaks at 1743 cm^{-1}, 1229 cm^{-1}, 1155 cm^{-1} and 1111 cm^{-1} were found to decrease with increase in percentage of mineral oil which indicated the absence of carbonyl group in mineral oil.

Economics of adulteration of coconut oil by palm kernel oil and mineral oil revealed that maximum of Rs 4800/- per quintal was obtained by adulteration when 30 per cent of coconut oil was substituted by mineral oil followed by 20 per cent mineral oil (Rs 3200/- per quintal) and 30 per cent palm kernel oil (Rs 3150/- per quintal).

The present study implies that among the physical and chemical parameters, unsaponifiable matter, relative density, apparent density, Polenske value and refractive index could be used to identify the adulteration in coconut oil by mineral oil. Similarly thin layer chromatography could also detect the presence of mineral oil. Saponification value, iodine value and Polenske value could detect the adulteration due to palm kernel oil. The use of FTIR spectroscopic technique is an easy method to identify adulteration in coconut oil through identification of specific peaks. GCMS analysis could provide information about the fatty acid composition.

APPENDICES

APPENDIX I

Media compositions

1. Nutrient Agar

Peptone	-	5g
Sodium chloride	-	5g
Beef extract	-	3g
Agar	-	20g
Distilled water	-	1000ml
pH	-	7

2. Martin's Rose Bengal Agar

Glucose	-	10g
Peptone	-	5g
KH_2PO_4	-	1g
$MgSO_4.7H_2O$	-	0.5g
Rose Bengal	-	35mg
Agar	-	15g
Distilled water	-	1000ml

3. Ken knight's Agar

Dextrose	-	1g
KH_2PO_4	-	0.1g
$NaNO_3$	-	0.1g
KCl	-	0.1g
$MgSO_4.7H_2O$	-	0.5g
Agar	-	15g
Distilled water	-	1000ml

APPENDIX II

Market price of oil samples

Sl. No.	Name of Input	Market price for 1 litre (Rupees)
1	Coconut oil	205
2	Branded sample 1	210
3	Branded sample 2	220
4	Branded sample 3	210
5	Branded sample 4	187
6	Branded sample 5	185
7	Palm kernel oil	100
8	Mineral oil	45