



# IDENTIFICATION OF FATTY ACIDS IN ESSENTIAL VEGETABLE OILS MANUFACTURED IN KENYA USING GC- FID

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## ABSTRACT

The aim of this research was to identify fatty acids using Gas Chromatography with a Flame Ionization Detector of edible vegetable oils manufactured in Kenya. Vegetable edible oils processed and refined in Kenya have not been characterized and hence it's impossible to distinguish between them. Sunflower, Corn and Soya bean vegetable oils by using physiochemical parameters dependently without an identification methods. Twenty two samples comprising of nine Sunflower of three different brand type, nine Corn oils of three different brand type and four Soya bean oils of two different brand type were collected from various supermarkets in Nairobi. The samples were transported, prepared and stored in the refrigerator at 4°C. GC-FID (Hewlett-Packard 6890 gas chromatograph - Agilent Technologies) was used for separation and quantification of FAMES. The GC-FID analysis was done under specific parameters and within the validation procedure linearity, precision and recovery, limit of detection (LOD) and limit of quantification (LOQ) were investigated.

**KEYWORDS:** sunflower oil, corn oil, soybean oil and Gas Chromatography with a Flame Ionization Detector (GC-FID)

## 1. INTRODUCTION

Vegetable oil is the key sub-sector of agriculture, currently there about 30 vegetable oil refiners in the country [16]. The larger companies include Bidco Oil Refineries, KAPA Oil Refineries, Pwani Oil Refineries, Palmac Oil Refineries and Unilever [18]. These companies engage in production of cooking oils, fats, edible oils, copra oil and corn oil among other oil products. [29] During extraction, purification and usage, oils undergo a variety of processing operations including heating, distillation and chemical modification which may alter their properties. To meet the quality and composition standards, oil and food industries use certain parameter to maintain the quality [11]. Free fatty acids content (acidity value) is one of the most frequently determined quality indices during the oil production, storage and marketing and it is often used to classify and /or evaluate oils. Other important parameter to be considered in oil analysis is the peroxide index. Peroxide is indicators of oxidative rancidity in foods. [5]

Around 14% of current oils production is used as starting material in manufacturing industry and around 6% are used as animal feed (indirectly)[18]. The remaining 80% is used for human food as spreads, frying oils, salads oils and cooking oils [23].

Sunflower is non- volatile oil expressed from sunflower (*Helianthus annuus*) seeds. The refined oil is clear and slightly amber – colour with a slightly fatty odour. Average oil content of the seed 40 – 50% of the entire fruit and 50 – 60% of the kernel only. The sunflower consist of phosphorus, wax, toropherols, carotenoids, chlorophlly, trace metals (Calcium, Magnesium and Iron). Sunflower oil has some unique characteristics that are popular with consumers, who use the oil in cooking and top dressing. [14]

Unlike many vegetable oils, corn oil (maize oil) is obtained from seeds (kernel). Production of corn kernel oil is costly due to low level of oils in the kernel because corn kernels contain high levels of starch 60 – 70%, a process of wet milling was developed to isolate pure starch efficiency from corn kernels. [19] Non starch portion of kernels are separated into four fractions: steep water soluble (about 70%), fiber (about 10%) corn gelatin meal (60%) and germ (7%). [22] Oil is usually obtained from dry milled corn germ by applying pressure. The level of total phytosterols in corn germ oil average a little more than 1%. [30] The corn oil consists of free fatty acids, tryacylglycerols, unsaponification and phytosteroids, tocophenola and tocotrienols and carotenoids. [22] Soya bean oil is a high- quality protein and valuable edible oil. It consist primarily of neutral lipids, which include tri-, di- and monoacylglycerols,



free fatty acids and polar lipids such as phospholipids[9]. It contains a minor amount of unsaponifiable matter that includes phytosterols, tocopherols and hydrocarbons such as squalene. Trace metals are found in soybean oil in ppm concentration. Soya bean oil has a high content of linoleic acids and a lower level of linoleic acid. [22] Triacylglycerols (TAG) are primarily neutral lipids in soybean oil. Due to high concentration of unsaturated fatty acid in soybean oil, nearly all the TAG molecules contain at least two unsaturated fatty acids, and di- and tri- saturated are essentially absent.

## 2. MATERIAL AND METHODS

### Samples and sampling

Three types of Sunflower and Corn oils and two types of Soya bean oils were sampled. Out of each brand, 3 different batches were sampled at different supermarket. A total sample collected was 22 of 500ml size. Samples of vegetable edible oil: Sunflower oils (Table 2.1), Corn oils (Table 2:1) and Soya bean oils (Table 2:3) were collected from local supermarkets within Nairobi CBD during the period of October 2012 and February 2013.

**Table 2.1: Sunflower oils**

Lab code	Type of sample	Supermarket	Date of sampling	Batch No.	Expiry date
RS	Rinsun sunflower oil	Nakumatt	14/10/2012	6793913A	09/2014
		Naivas	07/11/2012	9612548C	12/2014
		Tuskys	13/01/2013	1253321F	01/2015
SS	Sungold sunflower oil	Tuskys	25/12/2012	02-34034	12/2014
		Naivas	07/11/2012	01-21650	08/2014
		Nakumatt	05/02/2013	05-25194	02/2014
ES	Equatorial naturals sunflower oil	Woolmatt	21/11/2012	1054A04T	10/2015
		Nakumatt	14/10/2012	1154G23L	06/2014
		Naivas	07/11/2012	851A24H	04/2015

**Table 2.2: Corn oils**

Lab code	Type of sample	Supermarket	Date of sampling	Batch No.	Expiry date
EC	Elianto corn oil	Woolmatt	21/11/2012	01-31018	06/2014
		Naivas	07/11/2012	01-26210	04/2014
		Tuskys	13/01/2013	03-15412	02/2013
CC	Captain corn oil	Nakumatt	14/10/2012	3515113A	12/2014
		Woolmatt	25/12/2012	4521627M	07/2014
		Tuskys	13/01/2013	54101225R	01/2015
DD	Chef corn oil	Tuskys	25/12/2012	A01/21524	07/2014
		Naivas	07/11/2012	A01/15133	09/2014
		Nakumatt	14/10/2012	A01/15467	02/2015

**Table 2.3: Soya bean oils**

Lab code	Type of sample	Supermarket	Date of sampling	Batch No.	Expiry date
SG	Soyagold oil	Woolmatt	21/11/2012	01-36638	06/2014
		Naivas	07/11/2012	01-26542	04/2014
HS	Hawaya soya bean oil	Tuskys	05/02/2013	B125045L	05/2015
		Woolmatt	21/11/2012	N16523J	03/2014

The sample was stored in plastic containers in the laboratory. To maintain the reactivity and the stability of the samples the following conditions was maintained:



### Preparation of FAMES

Each of the vegetable oil of the samples was transferred to a screw –cap test tube and a known concentration of internal standard was added. FAMES were prepared by using 1ml of 2M NaOCH<sub>3</sub> in water bath at 60 °C. Drops of concentrated glacial acetic were added to neutralize the NaOH. After the solvent was removed under nitrogen gas flow, the samples were re-dissolved in 1ml of methanol: toluene (2:1, vol.), and 100µl of TMS-DM (2M) in n-hexane was added at 60 °C for 5 minutes. Drops of glacial acetic acid were added until the yellow colour disappeared to remove unreacted TMS-DM and the reaction mixture was diluted with 1 ml of 0.5% NaCl solution. Methyl esters were extracted twice in hexane containing the FAMES were pooled and dried under nitrogen gas before stored at - 20° C until GC analysis.

### GC analysis of FAMES

FAMES in all the test tube samples were re-dissolved in 100µl hexane and 1 µl volume of each sample was injected five times into GC-FID (Hewlett-Packard 6890 gas chromatograph -Agilent Technologies) for separation and quantification of FAMES. The analysis was carried out using a SP-2560 column (100 m × 0.25 mm ID, 0.20 µm film) from Supelco (Bellefonte, PA, USA). The run was under an optimized temperature programme as follows: initial column temperature 100 °C for 4 minutes, programmed to increase at the rate of 10 °C min<sup>-1</sup> up to 160 °C and then at 3 °C min<sup>-1</sup> up to 220 °C. This temperature was maintained for 5 minutes, then at 10 °C min<sup>-1</sup> up to final temperature of 260 °C and held for 5 minutes. Injector and detector temperature were at 260 °C and 280 °C, respectively. Helium was used as the carrier gas at a flow of 1 ml min<sup>-1</sup> with a split ration of 30:1

## 3. RESULTS AND DISCUSSION

Table 3.1 indicates the retention time and coefficient of correlation (r<sup>2</sup>) for fatty acids.

**Table 3.1: Linearity of the method**

Fatty Acids	Retention time (min)	Coefficient of correlation (r <sup>2</sup> )
Palmitic (C16:0)	22.759	0.99994
Palmitoleic (C16:1)	23.500	0.99990
Stearic (C18:0)	25.794	0.09987
Oleic (C18:1)	26.575	0.99994
Linoleic (C18:2)	27.320	0.99877
Linoleic (C18:3)	27.796	0.99886
Erucic ( C22:0)	29.330	0.99992

About the data obtained from the examination of the fatty acid profile, arithmetic mean (X), standard deviation (SD) and coefficient of variation (CV) were calculated.

**Table 3.2: Limit of detection and limit of quantification**

Fatty acids	LOD (µg/ml)	LOQ (µg/ml)
Palmitic (C16:0)	0.05	0.21
Palmitoleic (C16:1)	0.09	0.24
Stearic (C18:0)	0.07	0.24
Oleic (C18:1)	0.09	0.31
Linoleic (C18:2)	0.08	0.24
Linoleic (C18:3)	0.06	0.28
Erucic ( C22:0)	0.04	0.23

**Table 3.3: Repeatability, reproducibility and accuracy of the method**

Fatty acids	Repeatability RSD %			Reproducibility RSD %			Recovery %
	Oil sample			Oil sample			
	Sunflower n=9	Corn n=9	Soy bean n=6	Sunflower n=9	Corn n=9	Soy bean n=6	
Palmitic (C16:0)	1.26	1.28	1.74	2.19	3.55	4.23	98.21
Palmitoleic (C16:1)	1.57	1.49	1.59	2.47	3.91	3.92	98.95
Stearic (C18:0)	0.96	1.36	1.27	3.05	3.26	2.48	97.22
Oleic (C18:1)	1.98	1.89	1.84	3.14	2.09	2.91	96.83
Linoleic (C18:2)	1.79	1.25	1.28	2.91	2.74	2.65	102.26
Linoleic (C18:3)	1.26	1.06	1.57	2.15	2.75	3.49	100.13
Erucic (C22:0)	1.59	1.89	0.98	3.49	3.09	3.97	97.26

The individual fatty acid methyl ester standards (FAMES Palmitic (C16:0), Palmitoleic (C16:1), Stearic (C18:0), Oleic (C18:1), Linoleic (C18:2), Linoleic (C18:3) and Erucic (C22:0) acid were dissolved in n-hexane and added to the vegetable oil to the concentration of 1,000 ppm (w/v) and. Fatty acids were derivatized to fatty acid methyl esters (FAME) using BF<sub>3</sub>/MeOH (14% boron trifluoride) and analyzed by GC according to AOAC 969.33 (16) with some modification. FAME was analyzed by Hewlett-Packard 6890 gas chromatograph (Agilent Technologies) with a FID, and a SP-2560 column (100 m × 0.25 mm ID, 0.20 μm film) from Supelco (Bellefonte, PA, USA). The oven temperature started at 100oC for 4 min, increased to 225oC at 3oC/min, and held at 225oC for 20 min. The temperatures of injector and detector were 225 and 285oC, respectively. The flow rate of helium carrier gas was 0.75 mL/min, the injection volume was 1 μL, and the split ratio was 1:200. Peaks of GC chromatograms were identified comparing the retention times of a mixture of standard fatty acid methyl esters. Each peak of fatty acid was quantified using an equivalent of the concentration of the internal standard. Samples were separately analyzed in triplicate.

The precision of the method was evaluated through repeatability and reproducibility and the results are expressed as the relative standard deviation (RSD, %) (Table 4.9). Repeatability of the method was established by six fold analyses of three different samples, while the reproducibility was established by three fold analyses of three different samples in three consecutive days.

The recovery (%) of the method was established by spiking a sample with a standard working solution at one concentration level (10.0 mg/ml), and assaying it in triplicate (Table 4.7). Accuracy of the method was verified through the recovery. The fatty acid composition of the vegetable oil samples are presented in Table 4.9.

**Table 4.9: Fatty acid profiles for sunflower, corn and soya bean oils**

Fatty acids	Samples											
	Sunflower				Corn				Soya bean			
		X	SD	CV		X	SD	CV		X	SD	CV
Palmitic (C16:0)	7.1	4.7	1.0	21.27	11.7	10	2.0	20.0	10.2	9.0	2.0	22.22
Palmitoleic (C16:1)	6.08	8.8	0.8	9.09	11.67	25.1	1.8	7.17	11.75	13.5	0.93	6.89
Stearic (C18:0)	3.26	2.0	0.4	20.0	1.85	3.5	1.5	42.86	3.7	4.0	0.9	22.5
Oleic (C18:1)	16.93	31.5	4.5	14.29	25.16	26.8	1.2	4.48	20.8	31.5	1.2	3.81
Linoleic (C18:2)	73.73	59.5	7.5	12.61	60.60	48	4.5	9.38	55.53	49.5	6.5	13.13
Linoleic (C18:3)	1.0	32.5	4.5	13.85	0.48	26.8	1.2	4.48	9.3	28.5	1.2	4.21
Erucic (C22:0)	0.9	59.5	7.5	12.61	0.3	48	4.5	9.38	0.2	57.5	2.2	3.83

#### 4. CONCLUSION

In spectroscopic methods, the oils were analyzed by means of UV/Vis and IR spectroscopy. UV/Vis analysis was able to discriminate oils by differentiating in their λ<sub>max</sub> absorbance. IR analysis revealed that each vegetable oil could be discriminated by looking at the pattern of each individual spectra.

UV/Vis spectroscopy was used to discriminate oils by the intensity of their λ<sub>max</sub>. Sunflower, Corn and Soya bean oil had maximum wavelength as per Table 4.18. Sunflower oil, Corn oil and Soya bean oil samples showed similar values of maximum wavelength hence this disqualify out ultraviolet/visible spectroscopy for identification of different oil samples.



The results indicate that specific IR spectra regions prove to be very useful for identification of Sunflower, Corn and Soya bean oils. Vegetable oils contain the same type of fatty acids especially those C16 and C18 and triglycerides content is similar (C50, C52, C54), however, subtle spectral differences exist in Sunflower, Corn and Soya bean oils, so it is possible to identify the addition of foreign. The IR spectra for sunflower, corn and soya bean oil sample were distinguished by specific fingerprint. The use of infrared for analysis of edible vegetable oils is rapid because no sample preparation. Infrared spectroscopy can be used at the differentiation and classification stages of vegetable oils. This technique also allow for classification as well as determination of purity and authenticity of vegetable oils, such as corn, soya bean and sunflower oils

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