

Fatty Acids Residues Composition in the De-Oiled Tea Seed Oil Cakes

Njuguna, D.G¹, *Wanyoko, J.K², Kinyanjui, T³ and Wachira, F.N².

¹Mount Kenya University, Pharmaceutical Chemistry Department, P.O Box 342-0100 Thika, Kenya

²Tea Research Foundation of Kenya, P.O Box 820-20200 Kericho, Kenya.

³Egerton University, Chemistry Department, P.O Box 536 Njoro, Kenya

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ABSTRACT

Studies were conducted on the fatty acids residues levels in the de-oiled tea seed oil cakes of six selected tea cultivars of Kenya. Fatty acids methyl esters were analysed by the use of gas liquid chromatography. De-oiled tea seed oil cake contained decanoic acid in the range 19-0.12mg/g, myristic acid in the range 18-0.05mg/g, palmitic acid in the range 10-0.21mg/g, stearic acid in the range 1-0.18mg/g, oleic acid in the range 2-0.24mg/g, linoleic acid in the range 0.59-0.006mg/g and α -linolenic acid in the range of 0.14-0.05mg/g. De-oiled tea was found to contain all these fatty acids and hence can be used as potential source to supplement some of the essential fatty acids in animal foods.

KEYWORDS: De-oiled tea seed cake, Fatty acids, Supplement

INTRODUCTION

The tea plant, depending on the variety can thrive in wide geological and climatic conditions (Owour et al., 2010). Tea plant in its wild state can grow up to 15 meters tall with a taproot to oblique root system (Bonheure, 1990). It can grow on a wide range of soils of different geological origin, but for economic production, certain specific requirements are necessary; deep well drained soils with a maximum depth of two meters and having an aggravated or crumb structure with about 50% pore spaces (Othieno, 1992). The flowers of the tea plant are white with a yellow center, 2.5-4cm in diameter, with 7 or 8 petals. The flowers are scented and occur singly or in clusters of two to four. Flowers are pollinated by insects and the wind. Tea is mostly self-sterile and almost entirely cross-pollinated. The fruits are 1-2cm in diameter, brownish-green in color when mature, and contain one to four spherical or flattened, brown seeds. The fruit ripens in 9-12 months, after which the seeds fall to the ground (Bonheure, 1990).

Tea seeds resulting from the cultivation of tea in Kenya and many parts of the world are often regarded as waste. Tea seeds contain oil, oil from tea seed could be thus harnessed to serve useful purpose such as oleochemical or chemical intermediate. It is well documented that most oil seeds are rich in nutrients such as digestible protein with a good array of nutrients and minerals (Fagbemi et al., 2004). In the World today, there is a geometrical increase for demand of vegetable oils for both domestic and industrial purposes (Ikhuoria et al., 2008).

Most industries in Kenya that depends on vegetable oils use raw materials such as olive oil, linseed oil, soybean oil etc sourced through importation and this is rather expensive and uneconomical. Generally, vegetable oils are well known for both their edible and technical applications (Fulmer, 1985). The need to reduce the competitive demand for these oils requires looking inward for other sources. Yahaya et al., (2011) in Nigeria reported that tea seeds have crude oil yield of 23%. This means about 87% of material is obtained as by-product. From the data available, a comprehensive fatty acids characterisation of Kenyan tea seed oil cake (by-products) of tea seed oil extraction has not been documented. It therefore becomes imperative that these would-be renewable resources that can be assayed to give insight about their potential use.

Fatty acids act as storage of energy and component of cell membranes (phospholipids), e.g. in adipose tissue and skeletal muscle, which also brings about the insulation of body from thermal, electrical and mechanical injury. Fatty acids are involved in transfer of signals like in the eicosanoids. Humans, as well as other species within the animal kingdom, lack the capacity for de novo synthesis of fatty acids that contain a double bond within the last 6 carbons from the methyl end. Consequently, they must rely on dietary sources for these fatty acids. Burr et al., (1929), discovered that certain fatty acids are essential components of the diet. He also determined that mammals were unable to synthesize linoleic or α -linolenic acids. These fatty acids are linoleic and α -linoleic acid and termed as essential fatty acids. The metabolites that linoleic and α -linolenic acid generates are the most important factors in the structure and function of every cell within the body.

Essential fatty acids (EFAs) are required for the survival of humans and other mammals and they cannot be synthesized in the body and hence, have to be obtained in diet and thus, are essential. They form an important constituent of all cell membranes, and confer on membranes properties of fluidity and thus, determine and influence the behavior of membrane-bound enzymes and receptors. There are two types of naturally occurring essential fatty acids in the body, the ω -6 series derived from linoleic acid and the ω -3 series derived from α -linolenic acid. Both ω -6 and ω -3 series of unsaturated fatty acids are metabolized by the same set of enzymes to their respective long-chain metabolites.

While some of the functions of essential fatty acids require their conversion to eicosanoids and other products, in majority of the instances the fatty acids themselves are active. The longer chain metabolites of linoleic and α -linolenic acid important in regulating membrane function, and are of major importance in the

brain, retina, liver, kidney, adrenal glands and gonads (Das et al., 1988). Essential fatty acids are also called polyunsaturated fatty acids (PUFAs) since they contain two or more double bonds. PUFAs are fatty acids some of which have at least two carbon-to-carbon double bonds in a hydrophobic hydrocarbon chain. There are at least four independent families of PUFAs, depending on the parent fatty acid from which they are synthesized. They include: the " ω -3" series derived from \pm -linolenic acid (α -linolenic acid, 18:3, ω -3), the " ω -6" series derived from cis-linoleic acid (linoleic acid, 18:2, ω -6), the " ω -9" series derived from oleic acid (oleic acid, 18:1, ω -9) and the " ω -7" series derived from palmitoleic acid (palmitoleic acid 16:1, ω -7). Linoleic is converted to γ -linolenic acid (18:3, n-6) by the action of the enzyme Δ^6 desaturase (d-6-d) and γ -linolenic acid is elongated to form dihomo- γ -linolenic acid (20:3, n-6), the precursor of the first series of prostaglandins. Dihomo- γ -linolenic acid can also be converted to arachidonic acid (20:4, n-6) by the action of the enzyme Δ^5 desaturase (d-5-d). Arachidonic acid forms the precursor of second series of prostaglandins, thromboxanes and the fourth series of leukotrienes. α -linolenic acid is converted to eicosapentaenoic acid (20:5, n-3) by d-6-d and d-5-d. Eicosapentaenoic acid forms the precursor of the third series of prostaglandins and the 5 series of leukotrienes. Linoleic, γ -linolenic, Dihomo- γ -linolenic, Arachidonic, α -linolenic, eicosapentaenoic, docosahexaenoic acids and docosahexaenoic acids are all PUFAs, but only linoleic and α -linolenic are essential fatty acids. Arachidonic and eicosapentaenoic acids also give rise to their respective hydroxy acids, which in turn are converted to their respective leukotrienes.

In addition, also give rise to certain anti-inflammatory compounds such as lipoxins and resolvins that have potent anti-inflammatory actions. These anti-inflammatory compounds are highly active in inflammation modulation, and are involved in various pathological processes such as atherosclerosis, bronchial asthma, inflammatory bowel disease, and other inflammatory conditions (Das, 2002).

MATERIALS AND METHODS

Sample collection and preparation

The experiment was established at the Tea Research Foundation of Kenya (TRFK) at Kericho station, Kenya. It comprised of six cultivars. For Assam variety cultivars were TRFK 91/1 and TRFK 12/28, for Cambod variety the cultivars were TRFK 301/2 and TRFK 301/3, and for China variety cultivars were GW Ejulu-L and TRFK K-purple. The six tea cultivars were randomly selected in the TRFK seed barie. For each clone dry mature and uninfected tea seeds were harvested from three tea bushes separately, each tea bush was taken as replicate. The tea seeds were sundried and then de-hulled and the kernels were subjected to oil extraction by soxhlet method.

Soxhlet oil extraction

A 100g of kernels were ground into fine powder using a high speed kitchen blender. Then 5g of powder was weighed and transferred into a clean extraction thimble and de-oiled cotton wool was used to stopper the thimble. The stoppered thimble was put onto a butt tube and the connected to a pre-weighed clean dry extraction flask containing 150ml of n-hexane. Soxhlet apparatus were assembled and water was allowed to run through the apparatus before electro mantle was switched on. Extraction was allowed to run for 6 hours at normal atmospheric pressure and at temperature of 40-60°C.

The thimble was removed from butt tube after extraction was completed the oil was distilled to recover hexane. The flask containing oil was dried in the oven at 120°C and desiccated to cool and then weighed. The tea seed oil cake was taken and dried in sun.

Analysis of Fatty Acids Composition in Tea Seed Oil Cakes by the GC

In this study, gas chromatography (GC) was used for detecting and identifying the components of a mixture of volatile fatty acids. Generally, the system generates a chromatogram which is plotted using information on the amount of the component as peak area with elution time as retention time. Contents and concentrations of various components of the sample are determined by comparing various chromatograms (McNair & Miller, 2009).

Extraction Procedure

To remove all potential lipid contaminants double distilled solvents were used. Ten grams (10g) of powdered oil cake was weighed out and extracted with 200mls of chloroform and methanol mixture (2:1 v/v) for four hours with continuous stirring at room temperature. The mixture was then filtered through sintered glass funnel with the aid of suction pump. A further 200mls of

chloroform methanol mixture (2:1 v/v) was added to the residue and extraction was done for four hours. The two filtrates which represented the “total lipids” were combined and the volume was determined. A 0.015g of heptadecanoic acid (the internal standard) was added and the contents stirred thoroughly.

Purification of Lipid Extracts

One hundred milliliters (100mls) of 0.88% of potassium chloride solution in water was added to the total lipid extracts and the mixture was thoroughly shaken before being allowed to settle. The solvents portioned into lower and an upper layer. The lower layer contained the purified lipids and upper phase contained the non-lipid contaminants. The purified lipid layer was filtered before the solvent was removed at 35°C on a rotary evaporator.

Preparation of Fatty Acid Methyl Esters (FAMES)

Base-catalyzed transesterification was used in conjunction with acid-catalyzed trans esterification. Ten milliliters (10ml) of 0.5M methanolic NaOH was added to the lipid mixture in 100ml round bottomed flask fitted with a condenser. The mixture was refluxed for 10 minutes until the lipids were dissolved. Twelve milliliters (12ml) of boron trifluoride-methanol complex (14% w/w BF₃) was added through the top of the condenser and refluxing done for a further 2 minutes.

The solution was allowed to cool, and then 5ml of hexane added and the mixture boiled once more for 2 minutes and then allowed to cool. Five milliliters of saturated sodium chloride was then added, the organic layer was separated and dried with anhydrous sodium sulphate.

Under the above conditions no isomerization of double bonds in polyunsaturated fatty acid occurs.

Analysis of FAMES by GC

The column (DB-wax column) was first conditioned by passing nitrogen gas through at 180°C for 5 hours. The column was fitted on gas chromatograph (Varian 3300) equipped with flame ionization detector (FID) and integrator (Varian, 4290) as the read out. The column temperature was isothermal throughout the column length at 180 ±1°C. Injector-detector temperature was 220 ±1°C, nitrogen gas flow rate was 40ml/min. 3µl sample of the FAMES in hexane solution was injected into GC using microlitre syringe.

Identification and Quantification of the FAMES

Identification of the individual fatty acids was done by direct comparison of the retention times of their methyl esters with those of known standard of methyl esters. For the purpose of quantification of the fames the entire sample together with the internal standard was injected and area response recorded for the internal standard represented its weight. The weights of the other peaks were then directly calculated from the weight of the internal standard (IS). The following relationship was used to calculate response factors on equal weight basis;

$$\text{Response factor} = \frac{\text{Peak area of internal standard}}{\text{Weight of Intrnal standard}} \div \frac{\text{Peak area of component}}{\text{Weight of component}}$$

And then the quantity of fatty acids methyl esters was calculated as follows;

$$\text{Weight of component} = \frac{\text{Response factor} \times \text{Weight (IS)} \times \text{Peak area of component}}{\text{Peak area (IS)}}$$

Table 1: Response Factor Used in the Gas Liquid Chromatograph

Fatty acid methyl ester	Response factor calculated
Decanoic	0.9715
Myristic	0.9558
Palmitic	0.9997
Stearic	0.9994
Oleic	1.0000
Linoleic	1.0000
α-Linolenic	0.9883

Data Analysis

All statistical analysis was carried out using (MSTAT-C, 1993) statistical software using one factor randomized complete block design model. ANOVA was used to determine the means, coefficient of variation and any differences in the parameters determined. The probability limit was set at p≤0.05 significant level. The data was presented in tables. Results of the parameters determined were expressed as a mean of the triplicate determination.

RESULTS AND DISCUSSION

RESULTS

Decanoic acid levels were highest in the clone GW-Ejulu-L with a value of 19mg/g and lowest in the clone TRFK 301/2 which had 0.12mg/g. Decanoic acid levels were not detected in the clones TRFK 91/1, AHPSC 12/28 and TRFK K-Purple and in the control sample of soybean assayed. Clone GW-Ejulu-L had significantly higher levels of decanoic acid at p≤0.05 than the rest of clones and the maize meal and sunflower meal control samples.

Myristic acid levels were highest in the clone TRFK K-purple with a value of 18mg/g and lowest in the clone TRFK 91/1 which had 0.05mg/g. Clone TRFK K-purple had significantly high levels of myristic acid content at p≤0.05 than the rest of the clones. Clone TRFK K-Purple had a significantly high amount of myristic acid at p≤0.05 than maize meal which had 15mg/g. Myristic acid levels were not detected in the control samples of soybean and sunflower meals. Palmitic acid levels were highest in the clone TRFK K-purple with a value of 10mg/g and lowest in the clone TRFK 91/1 which had 0.21mg/g. Clones TRFK 91/1 and GW Ejulu-L had significant low levels of palmitic acid at p≤0.05 than the

rest of the clones. Clone TRFK K-purple had significantly higher levels of palmitic acid content at p≤0.05 than the control samples sunflower and maize meals, but low amount than soybean meal.

Stearic acid levels were highest in the clone TRFK K-purple with a value of 1mg/g and lowest in the clone AHPSC 12/28 which had 0.18mg/g. Clone AHPSC 12/28 had significantly low levels of stearic acid content at p≤0.05 than the rest of the clones. In all the oil cakes of the clones the levels of stearic acid were significantly lower at p≤0.05 than in the controls samples.

Oleic acid levels were highest in the clones TRFK 301/2 and AHPSC 12/28 with a value of 2mg/g and lowest in the clone TRFK K-purple which had 0.24mg/g. Clone TRFK 91/1 was exceptional since no levels of oleic acid were detected. Clone TRFK K-purple had significantly lower levels of oleic acid content at p≤0.05 than the rest of the clones. All the control samples used in this study had significantly high levels of oleic acid content at p≤0.05 than all the tea seed oil cakes of all the clones studied.

Linoleic acid levels were highest in the clone AHPSC 12/28 with a value of 0.59mg/g and lowest in the clone TRFK 301/2 which had 0.06mg/g. In the clone TRFK 91/1 the levels of linoleic acid were not detected. There were no much significant differences in the levels of linoleic acid content at p≤0.05 between the clones studied. In all clones, the levels of linoleic acid were significantly lower at p≤0.05 than in the control samples.

α-linolenic acid was only detected in two clones i.e. TRFK 301/3 and TRFK 12/28 which had 0.05mg/g and 0.14mg/g respectively. The levels of α-linolenic acid content in the tea seed oil cakes of the two clones were significantly different at p≤0.05. No levels of α-linolenic acid were detected in the control samples.

Table 2: Residues of Fatty Acids in the De-oiled Oil Meals

Oil cake	Fatty acid content (mg/g)						
	Decanoic	Myristic	Palmitic	Stearic	Oleic	Linoleic	α-Linolenic
TRFK 301/2	0.12	0.51	0.35	0.67	2	0.06	ND
TRFK 301/3	0.22	4	2	0.35	1	0.18	0.14
TRFK 91/1	ND	0.05	0.21	0.18	ND	ND	ND
AHPSC 12/28	ND	0.97	0.39	0.50	2	0.59	0.05
TRFK K-Purple	ND	18	10	1	0.24	0.51	ND
GW Ejulu-L	19	0.23	0.28	0.65	0.91	0.17	ND
*Soybean	ND	ND	12	10	34	24	ND
*Sunflower	0.16	ND	3	2	6	6	ND
*Maize meal	6	15	5	4	12	10	ND
Mean	5	6	4	2	8	5	0.10
CV	9.1	5.1	6.1	10	2.7	5.9	18
LSD (p≤0.05)	0.44	0.37	0.38	0.39	0.30	0.46	0.01

ND = Not Detectable

*Samples used as positive control

Mean values of three replicate determination expressed as mg/g of dry weight

DISCUSSION

From this work de-oiled tea seed cakes clones with higher levels of unsaturated fatty acids, were identified and could be used in manufacturing of animal feed supplement with higher levels unsaturated fatty acid levels. The clones are; TRFK 301/2, AHPSC 12/28, GW-Ejulu-L, TRFK K-Purple and TRFK 301/3 having most of the unsaturated fatty acids assayed. In general the levels of accumulation of fatty acid in the tea seed oil cakes from most abundant was lauric > oleic > myristic > linoleic > decanoic > Palmitic > stearic > α -linolenic. The order of unsaturated fatty acid from the highest to least is oleic > linoleic > α -linolenic. The order of abundance of essential fatty acids in tea seed cakes was linoleic > α -linolenic. Oleic acid tends to increase HDL (good) cholesterol in human blood stream; linoleic acid is known to have no effect on it. Both oleic and linoleic acid are known to lower LDL (bad) cholesterol (Erickson et al., 1964), hence decreasing the risks of heart stroke and coronary heart disease. The results of fatty acid residues composition present in the tea seed oil cakes of the clones assayed were lower than those reported by Wanna & Wipada, (2007) in local plant seeds of *Sesamum indicum*, *Perilla frutescens*, *Hibiscus sabdariffa*, and *Hibiscus cannabinus*. It was also observed that accumulation of fatty acid in various samples was clonally dependent.

CONCLUSIONS

De-oiled tea seed oil cakes contained saturated, unsaturated and essential fatty acids are of paramount importance in health of animals. It is, therefore, the recommendation of this work these by-products should be tried as animal feed supplement for livestock keeping.

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CONFLICT OF INTEREST

The authors of this article declare no conflict of interest.

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