Full Length Paper

Extraction and analysis of tea (*Camellia sinensis*) seed oil from different clones in Kenya

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Kenyan tea (*Camellia sinensis*) is widely grown for its leaves and is commercialized as black tea. Product diversification and value addition is currently an area of great interest. This study provides data on the physico-chemical properties of Kenyan tea seed oil from selected clones of tea seeds to ascertain its potential applications. Soxhlet extraction using hexane was employed to obtain tea seed oil followed by chemical analysis to assess its properties. Oil yield, iodine value, saponification value, peroxide value, free fatty acids, total polyphenols and antioxidant activity were determined. The oil yields ranged between 16 to 25% w/w. Iodine value was in the range of 86 to 91 g I_2/100 g, peroxide value < 3.5 meq O_2/kg, saponification value between 182 to 187 mg KOH/g, free fatty acid < 1.5% oleic acid, total polyphenols 0.036 to 0.043 mg/L gallic acid and antioxidant activity of between 14 to 21% 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging activity. Tea seed oil is stable and can be a potential source of natural antioxidants.

Key words: Tea seed oil, iodine value, saponification value, peroxide value, free fatty acids, total polyphenols, antioxidant activity.

INTRODUCTION

Many varieties of seeds and nuts are good sources of edible vegetable oils which are also known as important raw materials in the chemical industries. Importation of vegetable oils as raw materials annually in some tea growing countries including Kenya has been an expensive exercise and yet there are possibilities of harnessing oil from tea seeds to complement this situation. In Kenya, the marginal areas are associated with poor quality and low yields of tea, not competitive hence less beneficial to farmers. Oil from *Camellia oleifera* has an extensive use in China as cooking oil (Yu et al., 1999). *Camellia* species cultivated in western countries as ornamentals (*C. japonica, C. reticulate* and

C. sasaqua) have oil-rich seeds (Chen et al., 2008), but the study of these oils have attracted minimal attention as tea seed oil (TSO) has been underutilized and unexploited in a number of tea growing countries, especially in Kenya.

The method employed in the extraction of oil from seeds and the type of solvent used to some extent have notable effects on the percentage oil yield and the quality of the extracted oil (Devesh et al., 2011). Supercritical fluid extraction method using carbon dioxide has been shown to obtain similar or higher yields of tea seed oil compared to solvent extraction and other methods. Tea seed oil extracted by this method is clearer than the one extracted by solvents (Rajaei et al., 2005). Further, highest tea (*C. sinensis*) seed oil yield has been obtained under optimal SC-CO_2 extraction conditions compared to oil yield obtained by soxhlet extraction (Wang et al., 2011). Tea seed oil (Iranian) is stable and suitable in nutri-

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tional properties, has a shelf life value identical to that of the olive oil at a temperature of 63°C, has higher shelf life than that of sunflower oil, and is capable of increasing the shelf life of sunflower oil when mixed (Ataii et al., 2003). Crude tea (*C. sinensis*) seed oil (South Indian) is highly stable to peroxide formation, but after refining or bleaching it has been suggested that the addition of antioxidants may be required (Ravichandran, 1993).

Tea (*C. sinensis)* seed oil is highly edible and equally important as a health-promoting food resource in human diet due to its good antioxidant activity (Wang et al., 2011). Tea (*C. sinensis*) seed oil comprises essentially monoene and can be a suitable feedstock for inedible applications which may include surfactant production, biodiesel and lubricants, biopolymers, etc, hence can serve as alternative to petrochemicals which are limited in supply (Yahaya et al., 2011). The antioxidant capacity and stability of tea seed oil has been previously reported (Sahari et al., 2004; Rajaei et al., 2008) and is similar to that of sesame oil thus tea seed oil has been suggested to be an potential alternative source of natural antioxidants (Rajaei et al., 2008). Therefore, this study aims at reporting some physico-chemical properties of Kenyan tea seed oil and to provide a comprehensive documentation of these properties with the aim of having an insight on its possible potential applications.

MATERIALS AND METHODS

Tea seeds of different clones were obtained from the seed barie at the tea research foundation of Kenya, Kericho, Kenya and identified as TRFK301/3, TRFK301/4, TRFK301/5, GW-Ejulu, K-Purple, TRFK306, TRFK91/1 and SFS150. The control samples (maize germ, sunflower and soybean seeds) were obtained from Bidco Oil Refineries Limited, Nakuru, Kenya. All chemicals used in this study were of analytical grade and the reagents used were standardized.

Soxhlet extraction

Dried tea seeds were de-husked and the husks were carefully separated from the kernels. The desired tea seed kernels were finely ground using a kitchen blender. Solvent extraction was carried out in a laboratory set up of a standard soxhlet apparatus using hexane. Employed solid-solvent ratio was 1:20 with an extraction time of 8 h. After extraction, the hexane extract was concentrated by rotary evaporation at 60°C using a rotor evaporator (Model R-3000, Buchi, Switzerland) and then oven dried for an hour to evaporate any residual solvent and to obtain the crude TSO. Further, a stream of nitrogen gas was blown into the head space of the sample bottles, tightly closed and stored in a refrigerator below -4°C for subsequent chemical analysis. Color and weight of the oil yield in grams were noted.

Oil content (OC)

This was determined by expressing the mass of the oil extracted as a percentage by mass of the oil bearing material (milled seeds) used in the extraction (Paquot, 1979).

Determination of iodine value (IV)

This was determined according to Wijs as indicated by AOCS

Official Methods of Analysis (1988), Cd 1-25 using the formula $12.69(T)(V_2-V_1)/W$ and the results were expressed as $gI_2/100$ g of oil, where; W is the weight (g) of oil, V_1 the volume (ml) of thiosulphate solution (test solution), V_2 the volume (ml) of thiosulphate solution (blank), T is the titre (conc.) of thiosulphate solution [C $(Na_2S_2O_3)$] and 126.9 the atomic mass of iodine.

Determination of saponification value (SV)

This was determined according to AOCS method Cd 3-25 (AOCS, 1978) with slight modifications. About 5 g of oil was weighed into a 250 ml round bottom flask. Using a volumetric flask, 50 ml of 0.5 M ethanolic KOH was added to the sample. The mixture was boiled under reflux for 1 h. The hot soap solution was then titrated with 0.5 M HCl using phenolphthalein indicator. A blank was also run in the same manner. The saponification values were calculated using the formula $56.1T (V_2-V_1)/W$ and reported in mgKOH/g oil. Where, 56.1 is the molecular weight of KOH, W is the weight (g) of fat, V_1 is the volume (ml) of HCl used in the sample, V_2 is the volume (ml) of HCl used in the blank and T is the concentration (mol/litre) of HCl.

Determination of peroxide value (PV)

This was determined according to the IUPAC method 2.501 (Paquot, 1979) with slight modifications. Approximately 2 g of oil was weighed into a flask. Twenty milliliter (20 ml) of solvent (2:1 v/v, glacial acetic acid: chloroform) was added to the sample followed by 1 ml of freshly prepared saturated KI solution. A homogenous solution resulted. After a few minutes, 30 ml of water was added and the mixture titrated with sodium thiosulphate solution (0.01 M) with starch solution as the indicator. A blank solution was carried out in the same manner at the same time.

This parameter was monitored periodically (0, 7, 14 and 21days). The peroxide values were calculated using the equation $1000T (V_1-V_2)/W$ and reported in meqO$_2$/kg oil, where: W is the weight (g) of oil, V_1 is the volume (ml) of $Na_2S_2O_3$ used in the test, V_2 is the volume (ml) of $Na_2S_2O_3$ used in the blank and T is the concentration of $Na_2S_2O_3$ (mol/l).

Determination of free fatty acids (FFA)

Free fatty acid was determined according to AOCS Ca. 5a-40 (AOCS, 1978) with slight modifications. Approximately 2 g of oil was weighed and dissolved gently in 25 ml of solvent system (96% ethanol and ethoxyethane: 1:1 v/v) neutralized just before use by titration with NaOH solution. The mixture was heated in a water bath at 60°C for 10 min. The hot fat solution was then titrated while stirring with 0.1 MNaOH using phenolphthalein indicator. This parameter was also monitored periodically (0, 7, 14 and 21 days). Free fatty acids was calculated and expressed as percentage oleic acid using the formula $MVT/10W$, where M is the mean molecular weight of fatty acids, W is the weight of oil (g), V is the volume of NaOH (ml) used in titration and T is the concentration of NaOH.

Samples for total polyphenols (TP) and antioxidant capacity (AC) assays

Sample extraction for antioxidant assay was achieved by dissolving approximately 2 g of the oil formulations in 2 ml of *n*-hexane. The mixture was vigorously stirred using a vortex until dissolution followed by liquid-liquid extraction using 5 ml methanol/water (80:20 v/v) mixture in order to assay the polar fraction. The final mixture was further vortexed for a few seconds and then centrifuged at 5000 rpm for 10 min. The supernatant (methanolic extract) was col-

Table 1. Some properties of Kenyan tea seed oil from different clones.

Oil sample	OC (%)	TP (mg/LGA)	IV(gI$_2$/100 g oil)	SV (mg KOH/g)	AC(% DPPH)
TRFK91/1	22.0	0.038	90.8	187	16.6
TRFK301/3	16.9	0.036	86.6	186	15.6
TRFK301/4	17.5	0.038	89.8	182	17.9
TRFK301/5	17.9	0.036	91.4	181	14.3
GW-Ejulu	23.4	0.043	89.8	186	20.6
K-Purple	24.3	0.038	91.3	181	15.7
TRFK306	25.2	0.040	90.5	181	21.1
SFS150	21.5	0.038	91.9	186	19.5
*Corn	17.4	0.025	120.4	190	34.8
*Sunflower	37.8	0.029	127.2	190	12.8
*Soybean	18.6	0.028	127.7	189	14.1
Mean	22.08	0.035	99.82	185.9	18.5
CV (%)	4.18	4.29	2.16	1.00	22.02
LSD($p \leq 0.05$)	1.573	0.002	3.666	3.153	6.94

*Samples used as controls; CV, coefficient of variation; LSD, least significant difference.

lected in a clean test tube. The extraction was repeated twice only with the addition of 5 ml methanol/water (80:20 v/v), and each time, the supernatant was collected in the same tube (with the same sample).

Total polyphenols analysis

This was determined according to the Folin-Ciocalteau spectrophotometric method at 725 nm, using gallic acid as a standard (Samaniego et al., 2007). After sample extraction, a 1 ml aliquot of the methanolic extract was mixed with 5 ml Folin-Ciocalteau reagent in 100 ml volumetric flask, followed by the addition of 10 ml of 20% w/v sodium carbonate solution and then completed to 100 ml with distilled water. The mixture was left to stand and after 1 h, absorbance was read at 725 nm on a UV-Vis spectrophotometer (Model JENWAY6705, multi-cell changer) against a blank (5 ml of Folin-Ciocalteau reagent, 10 ml of 20% w/v sodium carbonate and completed to 100 ml with distilled water). Each sample was measured in triplicates and quantification was finally carried out on the basis of the standard curve of gallic acid:

$y = 0.066x + 0.024$

Where, x is the absorbance; y is the mg/L gallic acid and $(r^2) = 0.9631$

DPPH free radical scavenging activity

This was estimated according to the method of Morales and Jime´nez-Pe´rez (2001) procedure with slight modifications. A 400 µl aliquot of the methanolic extract of the different oil samples was mixed with 3 ml of DPPH (74 mg/l in 80% methanol). The mixture was shaken and left to stand in the dark for 1 h; absorbance was then read at 520 nm using a UV-Vis spectrophotometer (Model JENWAY 6705 multi-cell changer). A blank solution of DPPH (74 mg/l in 80% methanol) was also read and noted. The antioxidant activities of the oils were finally estimated using the formula 100*(AB-AA/AB)* and expressed as percentage DPPH scavenging effect, where *AB* is the absorbance of the blank sample, *AA* the absorbance of the test sample.

RESULTS

Oil appearance and yield

All the tea seed oil extracts were golden yellow in color and liquid at room temperature. Clone TRFK306 had the highest oil yield which was not significantly different from that of clone K-purple. TRFK301/3 had the lowest oil yield amongst the tea seed oil samples. All these values were reported at $p \leq 0.05$ and the data is presented in Table 1.

Iodine value

Tea seed oil from clone SFS150 had the highest iodine value though this value was not significantly different from tea seed oils of the other clones except for clone TRFK301/3 which statistically had the lowest iodine value at $p \leq 0.05$. All the tea seed oil samples had the lowest iodine values compared to the control samples. This data is also presented in Table 1.

Saponification value

Tea seed oil from clones TRFK91/1, GW-Ejulu, TRFK301/3 and SFS150 had the highest saponification values (all these values were not statistically different at $p \leq 0.05$). There was no significant difference in the SV for the rest of the tea seed oils. This data is also presented in Table 1.

Peroxide value

There were generally no significant differences in the peroxide value of all the tea seed oils at $p \leq 0.05$. On day

Table 2. Peroxide value and free fatty acid levels in tea seed oil.

Oil sample	Peroxide value (meq O_2/kg oil)				Free fatty acids (% oleic acid)			
	Day 0	Day 7	Day 14	Day 21	Day 0	Day 7	Day 14	Day 21
TRFK91/1	3.44	3.54	3.65	3.80	1.30	1.36	1.44	1.50
TRFK301/3	3.26	3.38	3.43	3.48	1.08	1.29	1.44	1.58
TRFK301/4	3.27	3.36	3.50	3.58	1.04	1.20	1.21	1.37
TRFK301/5	3.32	3.43	3.57	3.65	1.12	1.22	1.28	1.37
GW-Ejulu	3.29	3.36	3.45	3.56	1.13	1.25	1.31	1.39
K-Purple	3.39	3.52	3.66	3.77	0.89	1.12	1.23	1.34
TRFK306	3.35	3.44	3.54	3.70	1.19	1.33	1.34	1.50
SFS150	3.36	3.63	3.79	3.91	0.99	1.15	1.27	1.40
*Corn	2.58	3.08	3.70	3.89	2.03	2.25	2.33	2.48
*Sunflower	1.96	2.22	2.43	2.55	0.98	1.26	1.42	1.52
*Soybean	2.13	2.43	2.61	2.79	1.09	1.29	1.41	1.55
Mean	3.04	3.22	3.39	3.52	1.17	1.34	1.43	1.54
CV (%)	7.14	5.60	4.33	4.30	12.08	8.48	8.07	5.90
LSD ($p \leq 0.05$)	0.369	0.309	0.253	0.258	0.241	0.194	0.194	0.152

All means are values of triplicate determinations.

7, no significant difference was noted in TSO of clones SFS150, TRFK91/1, TRFK306, TRFK301/5 and K-purple. On day 14, TSO of clone SFS150 had the highest peroxide value and was significantly different from the peroxide values of sunflower and soybean oils which had lower values. The other tea seed oils were not statistically different from corn oil with an exception of TSO of clones TRFK301/3 and GW-Ejulu. On day 21, TSO of clone SFS150 had the highest peroxide value. These results are presented in Table 2.

Free fatty acid (FFA)

On day 0, free fatty levels were found to be high in corn oil, low in TSO of clone K-Purple. FFA level in Soybean oil was not significantly different from the value in all the tea seed oil samples. There was also no significant difference between the FFA level in sunflower oil and TSO of clone K-purple. On day 7, FFA levels were higher in corn oil and lower in TSO of clone K-Purple. There was no significant difference however in TSO of clone K-purple, sunflower and soybean oils. On day 14, a high FFA level was noted in corn oil. No significant difference was noted in soybean oil, sunflower oil and the rest of the tea seed oils with an exception of TSO of clones TRFK301/4 and K-purple. On day 21, FFA levels were higher in corn oil. The levels in soybean and sunflower oil did not differ significantly with those of TSO of clones TRFK301/3, TRFK91/1 and TRFK306. TSO of clone K-purple had the lowest levels of FFA. This data is also presented in Table 2.

Total polyphenols and antioxidant activity

Kenyan tea seed oil had some levels of polyphenols as

reported in this study which could be attributed to the antioxidant activity exhibited by it. Tea seed oil from clone GW-Ejulu had the highest amount of total polyphenols compared to the rest of the oil samples at $p \leq 0.05$ followed by TRFK306. There was no significant difference in total polyphenols levels in the rest of the tea seed oil samples. There was no significant difference in the antioxidant capacity in the tea seed oil samples (all values at $p \leq 0.05$). These results are also presented in the Table 1.

DISCUSSION

Oil content of 23% has been reported for *C. sinensis* seeds (Yahaya et al., 2011) though is a lower value compared to between 30 to 32% (Ravichandran and Dhandapani, 1992). Geographical and climatic differences could be attributed to this apart from the method of extraction which has been previously shown to have an effect on the oil yields. The use of supercritical carbon dioxide in the extraction of tea (*C. sinensis*) seed oil yields 29.2 ± 0.6% oil compared to 25.3 ± 0.1% by Soxhlet method (Wang et al., 2011). Other factors include the method used in drying the seeds before extraction as in the extraction of oil from sea buckthorn seeds and pulp (Gutierrez et al., 2008).

A recent study reports an iodine value of 74.23 gI_2/100 g of oil for *C. sinensis* seed oil (Yahaya et al., 2011) which is lower than the one obtained from this work. Several studies show higher levels of oleic acid than linoleic and linolenic in tea seed oil (Jinlin et al., 2011; Yahaya et al., 2011; Wang et al., 2011). Tea seed oil is more monounsaturated than polyunsaturated hence, pro-

bably a reason for its lower iodine value compared to corn oil which is more polyunsaturated (Eqbal et al., 2011) hence it's higher iodine value. The fatty acid composition of the triacylglycerol greatly influences this parameter. The saponification value of tea seed oil from this study is in agreement with the reported value of 186.5 mgKOH/g of oil (Yahaya et al., 2011). As most of the mass of a fat/trimester is found in the three fatty acids, saponification value allows for comparison of the average fatty acids chain length. This work also concurs with a study that shows no significant differences in SV of tea seed oil (Indian), sunflower and olive oils (Sahari et al., 2004).

Peroxide value may vary in different seed oils depending on the extraction methods, storage conditions and sample varieties. An earlier study comparing tea (*C. sinensis*) seed oil to olive and sunflower oils points out that both TSO and olive oil have identical shelf life values at 63°C and are stable oils (Ataii et al., 2003). A higher peroxide value can be caused by amongst others, the storage method of the oil (Kamau and Nanua, 2008) as psychotropic organisms secreting oxidative enzymes can grow at temperatures as low as 5°C or even below (Marfil et al., 2011). It could also be a reflection of high levels of oxidative rancidity of the oils, the absence or low levels of antioxidants (Kyari, 2008). It has been shown in corn oil that the exact peroxide value at which organoleptic rancidity sets in depends upon conditions such as the amount of oxygen available, the temperature and the amount of surface exposed (Lowell, 2006). So, it is possible that some of these factors may have led to higher initial peroxide values for the tea seed oil as there existed some air spaces in the storage bottles even and the blowing of nitrogen in the air spaces before storage might not have been that efficient to expel all the oxygen available.

Free fatty acid levels is an index of the quality of fats and oils and among the parameters that have been studied and monitored periodically (in terms of days or months) under certain conditions alongside peroxide value by several researchers on their respective works for example (Kyari 2008). In all these different studies, both the free fatty acid level and peroxide value rises with an increase in the number of storage period of the oils. This study also reports a similar trend. Such changes have been interpreted before to be due to some structural changes in the triglyceride leading to the formation of new chemical properties and products. Auto-oxidation or photo-oxidation of the oils due to the presence of double bonds could be a reason. Infra Red spectra gives an identification of rancidity of the condiment oil due to bands observed at 3400 to 2700 and 1705 cm^{-1} suggesting a possible formation or absence of acid and aldehyde respectively, the two being products of oxidative rancidity (Chindo et al., 2010).

The initial values were significantly different in the oils. It suggests that the degree of breakdown of the triacylglycerols due to lipolysis or hydrolysis in the oils were different. Low levels of free fatty acids in oils suggest low

levels of hydrolytic and lipolytic activities in the oils (Kyari, 2008). Several factors can lead to this for example; oils extracted carelessly, and/or from poor quality seeds suffer from a very significant breakdown of the triacylglycerol into free fatty acids. The triglyceride can break down, one, two or all of them. In general, high initial level of free fatty acids can be due to seed/fruit fly infestation, delays between harvesting and extraction (if seeds/fruits are bruised during harvesting), fungal diseases in seeds/fruits, prolonged contact between oil and vegetation water (after extraction) and careless extraction methods (Gunstone et al., 1986).

The antioxidant activity in tea seed oil from this study (16 to 21% scavenging of DPPH) is in agreement with a recent study on the antioxidant activity in tea (*C. sinensis*) seed oil which suggests TSO extracted by soxhlet and SC-CO$_2$ shows concentration-dependent scavenging of the DPPH free radical. The study reports antioxidant activity of 12.1 ± 2.7 to 67.8 ± 6.4% for TSO extracted by Soxhlet method and 17.2 ± 0.1 to 93.4 ± 1.8% by SC-CO$_2$ method (Wang et al., 2011). Tea seed oil has been previously reported to have polyphenols (Ravichandran, 1993) and antioxidant capacity responsible for its stability and can be used as a natural antioxidant (Sahari et al., 2004; Rajaei et al., 2008). Further, some bioactive compounds have been isolated from tea (*C. oleifera*) seed oil such as sesamin, which exhibits remarkable antioxidant activity. The antioxidant capacity of oils can also be due to a high percentage of main constituents, but also to the presence of other constituents in small quantities or to synergy among them. Kaempferol glycosides take a role in antioxidant activity of tea seed oil as these compounds have been found in tea seeds. However, it has been reported that these minor antioxidants may be destroyed during the long term required for soxhlet extraction (Li et al., 2010).

Conclusion

Some clones of Kenyan tea seeds have high oil content compared to maize germ and soybean seeds which are known commercial vegetable oils. Apart from the known edible properties of tea seed oil, Kenyan tea seed oil, having low free fatty acid level and low peroxide value is stable oil. The presence of polyphenols and antioxidant properties adds it more stability and thus can also be a potential source of natural antioxidants. It is not a good drying oil due to its low iodine value and can possibly find use together with the amino resins as finishes for certain appliances or even act as plasticizers, used as a lubricant and for protective coatings. Its relatively high saponification value also makes it a good raw material in the soap making industry.

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