Quantity and Quality Evaluation of Girardinia Diversifolia and Girardinia Bullosa Seed Oil

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Abstract

Kenya imports more than 65% of its edible oil requirements. This is due to low oil production and limited sources that cannot adequately meet the high demand by the ever-increasing population. This study aimed to determine the fatty acid profile and assess the quantity and quality of Girardinia diversifolia and Girardinia bullosa seed oil. Its quality was assessed in terms of iodine value (IV), saponification value (SV), acid value(AV), peroxide value(PV), unsaponifiable matter, refractive index (RI), specific gravity(SG) and moisture content and volatile matter. The percentage oil content (18.4 to 21.2/100g dry) was low relative to most common edible oil plants. The highest moisture content recorded was 39.60 with the lowest being 31.24g/ 100g air-dried seeds. The RI ranged from 1.4662-1.4704. The IV ranged from 166-192. The PV was in the range of 8.68 to 13.6. The SV (164-179) was comparable to that of corn oil (187-147). The AV varied from 4.58-7.22. The percent unsaponifiable matter of the oil varied from 2.14-4.10. The fatty acids detected in the oil were decanoic acid, hexadecanoic acid, octadecanoic acid, 9-octadecaenoic acid, 9,12-octadecadienoic acid, eicosanoic acid and heneicosanoic acid. The results showed that Girardinia oil is stable in the first two hours of heating. The results indicated that the oil had a high content of long-chain unsaturated fatty acids, and this guarantees the possibility of using the oil in the food industry.

Keywords: Girardinia, Acid Value, Peroxide Value, Saponification Value

Background

Kenya produces only 34% of its edible oils and fat requirements[1]. As such, the country remains a net importer of vegetable oils as local production has not grown to meet the local demand and incurs huge expenditure on vegetable oil importation. In this study, Girardinia seeds were analyzed for their fatty acid composition with the aim of evaluating their potential as a source of oil/fat to boost oil production.

Girardinia Species

G. diversifolia (GD) and *G. bullosa* (GB) are members of the Urticaceae family commonly found in tropical Africa. The family consists of mostly herbs, and some trees with well-developed phloem, fibres and coarse. The leaves are usually simple. The flowers are usually in cones and mostly unisexual without petals; male flowers have as many stamens as sepals; female flowers with one to four sepals, sometime sheathing the ovary with a stigma and one basal ovule. GB has broader leaves, single stem med and is a shrub. There are two variants of GD, identified by virtue of their different leaf appearances; GD with lobed leaves (GDL), and GD with bladed leaves (GDS) or if lobed, the number of lobes cannot exceed three[2].

Phytochemical analyses of GD have revealed the presence of phytosterols, fatty acids, carotenoids, polyphenols and saponins. Fatty acids with an abundant portion of unsaturated derivatives (linoleic and linolenic acid at 22.0 and 9.7 mg/g respectively), vitamin C (2.9 mg/g) and vitamin B2 (0.12 mg/g) have are also present[3].

This plant has wide use; its leaves are cooked and eaten up together with ugali (a Kiswahili cake made from maize flour) by some tribes, mainly Kisii, Kalenjins, Luhyia and Kikuyus, in Kenya. The plant has been grown in demonstration projects in Kenya aiming at producing fibre, paper and insecticide from it.

Physical and Chemical Characteristics of Edible Oils

Physical Characteristics

The physical characteristics of edible fats and oils are majorly color, SG, RI, melting point, congeal point, smoke point, flash point, fire point, and viscosity[4]. The SG of edible fats ranges from of 0.90-0.92 at 25℃ except palm oil and the related oil that have slightly lower SG（0.89-0.90）at 25℃. The RI in the range of 1.44-1.47 at 25℃, and it depends on the fats and oils variety. The melting point of the vegetable oils also vary and is dependent on the fatty acid composition; Olive and rapeseed oils rich in oleic acid（18:1）have melting points ≥ 0℃, while corn and soybean oils rich in linoleic acid（18:2）have melting points ≤ 0℃. Palm oil and coconut oil, which are rich in saturated fatty acids, especially palmitic acid（16:0） have higher melting points[5].

Chemical Properties of Edible Oils

The main chemical parameters used for assessing the edibility of oils are AV, SV, PV, IV, unsaponifiable matter and fatty acid composition. Purification of oils is determined by it AV and a value ≤ 0.1 is desirable for refined edible fats and oils[6].

SV has been reported to be inversely related to the average molecular weight of the fatty acids in the oil fractions[7]. High saponification values of fats and oils are due to the predominantly high proportion of shorter carbon chain lengths of the fatty acids[8,9]. Most common vegetable oils such as corn, olive and soybean oils have an SV of 187-195, 187-196 and 188-196 respectively, whereas vegetable oils, such as the coconut and palm kernel oils containing large amounts of lauric (C12:0) and myristic (C14:0) acids have saponification values significantly higher (235–260 mg KOH/g oil) [5,10–12]

Determining the PV of edible oils is essential because it is one of the most typically used quality parameters to monitor lipid oxidation and control oil quality[13]. Several factors affect PV: presence of some minerals (copper, nickel and iron), moisture content, exposure to light and heat and degree of unsaturation. Generally, the greater the degree of unsaturation (i.e. higher IV) the greater the liability of the fat/oil to undergo autoxidation, though, extraction and storage conditions also have an effect on PV. The oxidation of edible oil leads to the production of off-flavor and toxic compounds and diminishes the oil quality and shelf life[14].

The iodine value or iodine number is the generally accepted parameter expressing the degree of unsaturation in fats or oils [7]. Olive oil, which is rich in monounsaturated fatty acids（18:1), has an IV ranging from 75 to 90, while soybean and corn oils rich in polyunsaturated fatty acids（18:2）have IV in the range of 120-140[5]. The determination of the IV is also important in classifying oils and fats as drying, semi drying and non-drying as follows: Drying oils: IV 200-130, Semi drying: IV 130-100 and Nondrying: IV lower than 100 [7,15].

Acid value is taken as an important indicator of oxidation of oil. In good oil, the AV should be very low (< 0.1) [6]. Increase in AV should be taken as an indicator of oxidation of the oil which may lead to gum and sludge formation besides corrosion.

Triacylglycerols (TAG) composition is a critical characteristic in the quality of oils because it can affect not only their physiological properties such as melting point and crystallization but also their nutritional properties such as susceptibility to lipase-hydrolysis[16].

Methods

Raw Material

Girardinia seeds were collected from different places in Kenya (Nyandarua, Mau Narok and Mount Kenya). A sample of the fresh seeds was taken and used for the determination of moisture content. The seeds were then sun-dried for three days and oven-dried at 60^0C for twelve hours to reduce the moisture content. The oven-dried seeds were ground using a grinding machine. The powdered seeds were parked in cleaned airtight plastic containers and stored in a deeper freezer. the flow chart diagram shows the processes for

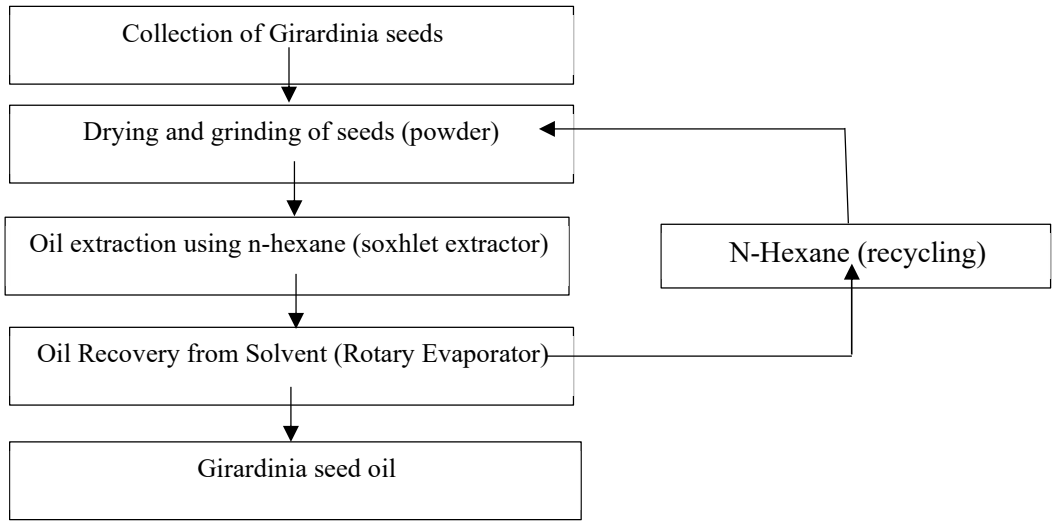

Figure 1 Flow chart diagram showing processes for obtaining oil from Girardinia seeds

Determination of Crude Lipid Content

The Crude Lipid Content of the seeds was determined using a standard procedure[17]. Quick fit flasks (500ml) were washed thoroughly with detergent, rinsed several times with distilled water and dried the flasks were dried in an oven maintained at 105^0C for two hours and left to cool to room temperature in a desiccator then weighed .About 10g of powdered dried Girardinia seeds were weighed in sextruplicate for each sample into clean dried extraction thimbles made of cellulose (Whatman size 29x80mm). The content was placed in a soxhlet extractor and connected to collection flasks. Petroleum spirit boiling point 40-60^0C (BDH, analar grade) was added (300ml) into each extractor high enough to siphon out. Immediately condensers were placed, tightened and water turned on for cooling. The solvent was heated using a heating mantle and refluxed for eight hours to extract the lipids from the powdered seeds.

On completion of fat extraction, the solvent was removed by evaporation at 37°c using a rotary evaporator. Acetone (20ml) was added to contents of each flask and evaporated to dryness using a rotary evaporator and a water bath at 60^0C. Crude lipid content (CLC) was determined using equation 1 below.

$$CLC = \frac{m_1}{m_2} x100 \quad \text{(1)}$$

Where m_1-mass of crude lipid; m_2- mass of dry powder Girardinia seed

Mean CLC (g/100g of fresh and dry matter), standard deviation (SD) and coefficient of variation (C.V) values for sextruplicate determinations of each sample were calculated.

Determination of Fatty Acid Composition

Transesterification[18]

Esterification of fatty acids was carried out using standard procedures[19]. Fifteen (15) grams of oil were added into 50ml of 0.5M sodium methoxide in a 250ml beaker. The contents were covered with a watch glass and mixed on a magnetic stirring/hot plate at low heat for approximately 3-5 minutes. The solution was clear indicating complete esterification. The reaction was quenched with 25ml of saturated salt/0.5% I-ICI solution. The esters were extracted with two portions of hexane and dried over anhydrous sodium sulphate before removing the solvent with a rotary evaporator.

HPLC Analysis of Triglyceride Composition

High Performance Liquid Chromatography (HPLC) (Hewlet Packard 1100 series) instrument and an SGE 250SS4.6. CN 8/5 Packed Column at a temperature 20^0C was used. Run time, flow rate and sensitivity (attenuation) were set at 15 minutes, 0.8ml/min, 1000 and rnAv respectively. The method applied was reversed-phase HPLC. The mobile phase was a mixture (acetonitrile, isopropanol and tetrahydrofuran mixed in the ratio 5.'4:1) and hexane mixed at a ratio of 10:90. A Binary pump, detector (deuterium lamp) of peak width 0.4 min, set at 230 nm, and Ultra VGA 1280 Computer attached to LaserJet printer were also used. Auto sample injection (5»1) was done at room temperature.

Determination of fatty acid composition was done following a standard procedure [19]. The FAMES prepared above were dissolved in hexane and the samples put in the sample vials for auto-sample injection into the calibrated I•ffl4C (Calibration was done using known FANŒS). The HPLC was programmed to automatically inject 5pl of each sample in duplicate. A blank was prepared without the FAMES and put in a sample vial for

auto-injection. To identify the peaks on chromatograms for FAMES of the sample, comparisons of retention times was made with those of the FAMES of standard solutions.

Determination of Iodine Value

The IV of Girardinia seed oil(GSO) was determined using standard procedures[18]. The oil (0.2g) was weighed in the glass weighing scoops (4ml). The weighed fat/oil was dissolved in 2ml of carbon tetrachloride (BDH, analar grade) and poured in the ground necked bottle (250ml). The glass weighing scoop was rinsed three times with 2ml of carbon tetrachloride and the content poured into the ground necked bottle (250ml), followed by the addition of 7ml of carbon tetrachloride in order to make final volume of carbon tetrachloride to 15ml. This was followed by the addition of 2ml of Wijs reagent and the stopper inserted, the bottle covered by aluminum foil and kept in the dark for one hour.

After one hour, 20ml of potassium iodide solution (0. IN KI solution) was added followed by distilled-deionised water (150ml) and then the mixture shaken thoroughly. The resulting mixture was titrated with 0. IN standardized sodium thiosulphate using starch solution as the indicator. The titration was carried out until the blue colour just disappeared after a very vigorous shaking. A blank determination was carried out following the above steps except no oil was added. The IV was calculated using equation 2: -

$$IV = \frac{(bt- \quad)x\, n_{Na_2S_2O_3}\, x12.69}{w} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (\mathbf{2})$$

Where bt-blank titre; sv – sample volume; n-normality $Na_2S_20_3$; w- weight of sample used

Determination of Saponification Value (SV)[5,20]

Girardinia seed oil (2g) of each variant was weighed in the glass scoop (4ml). The oil was dissolved in 2ml of ethanolic potassium hydroxide solution (0.5N). The contents were poured into a 250ml Quick fit round-bottomed flask. The glass scoop was then rinsed three times with 2ml of ethanolic potassium hydroxide solution.

The solution mixture was boiled gently with occasional shaking. After one hour the heating was stopped and immediately 0.5ml of phenolphthalein indicator was added. The solution was titrated with 0.5N hydrochloric acid until the colour of the indicator changed and persisted for at least ten seconds. The determination of all the variant samples was carried out in triplicate. A blank determination was carried out following all the above steps except that no oil was added.

$$SV = \frac{(bt - sv)xn_{KOH}6.01}{w} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (\mathbf{3})$$

Where SV: saponification; *bt* – blank tire; *sv* – sample volume; *n*-normality of KOH; *w* – weight of sample

Determination of Acid Value (AC)

The oil (2.5g) was dissolved in 250ml of accurately neutralized solution mixture 95 per cent ethanol and diethyl ether (N.P, Normal analar grade). This resulting mixture was shaken thoroughly to dissolve the oil and titrated with 0.1N standardized potassium hydroxide solution and using phenolphthalein as indicator. The end point was reached when the pink colour of the phenolphthalein persisted for at least ten seconds after vigorous shaking. A blank determination was carried out following all the above steps except that no oil was added and titre. Similarly, the percent free fatty acids as 9-octadecaenoic (oleic), dodecanoic (lauric) and Hexadecanoic (palmitic) was calculated by multiplying the AV by the factors 0.282, 0.356 and 0.457 respectively. To

determine the effect of heat on acidity of GSO, the oil was heated for seven hours, and acidity was determined at every one-hour interval.

Determination of Peroxide Value (PV)[5]

Girardinia seed oil (2.5g) of each variant was weighed into the glass scoop (4ml). The fat/oil was dissolved into 2ml of chloroform (BDH, analar grade) and poured into ground necked flask with glass stoppers. The glass scoops were rinsed four times with 2ml chloroform, and the contents poured into the flask to make a total of 10ml chloroform and the contents were dissolved rapidly by shaking. Glacial acetic acid (15ml) (BDH, analar grade) was then added into the flask followed by Iml of potassium iodide (BDH, analar grade). The stopper was inserted immediately, and the contents shaken vigorously for one minute and left in the dark for exactly five minutes at a temperature of 22^0c.

After five minutes, 75ml of distilled-deionised water was added and shaken vigorously. The liberated iodine was titrated using 0.002N standardized sodium thiosulphate solution using starch as indicator and shaking vigorously. The end point was reached when the blue colour disappeared and grey colour or almost colourless appeared. The GSO samples were analyzed in triplicate. A blank determination was also carried out. PVwas calculated using equation 4.

$$\mathrm{PV}({}^{\mathrm{Meq}}/_{100\mathrm{g\ of\ oil}}) = \frac{(\mathrm{st}-\mathrm{bt})\mathrm{x}n_{\mathrm{Na_2S_2O_3}}\ \mathrm{x}100}{\mathrm{w}} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots . \mathbf{(4)}$$

Where *st*-sample titre; *n*-normality of $Na_2S_2O_3$; *w*-weight of oil used

Determination of Unsaponifiable Matter[5]

The GSO samples were weighed in triplicate into 250ml Quickfit flasks with ground joints. This was followed by addition of 25ml of potassium hydroxide solution and some pumice grains. The reflux condenser was attached and the contents boiled gently for one hour. After that, heating was stopped followed by addition of 50ml of distilled deionised water through the top of the condenser and swirled. After cooling, the solution was transferred to a separation funnel (500ml). The flask was rinsed several times with diethyl ether (50ml in total) and contents poured into the separating funnel. The separating funnel was then shaken vigorously for one minute, periodically releasing pressure.

The separating funnel was allowed to settle until the two phases separated and the soap solution was drawn off as completely as possible into a second separating funnel. The aqueous ethanoic soap extract was extracted twice more with diethyl ether. The three extracts were combined in one separating funnel containing 20ml of distilled-deionised water. The separating funnel was rotated gently. After the layers separated completely, the lower aqueous layer was drawn off. The ethereal solution was washed twice with 20ml portions of distilled-deionised water, while shaking vigorously each time and discarding the lower aqueous layers after separation. The ethereal solution was washed successively with 20ml aqueous potassium hydroxide solution distilled-deionised water and again with 2ml potassium hydroxide solution followed by five times more 20ml of distilled-deionised water.

The washing was stopped when wash-water gave no pink colour on addition of a drop of phenolphthalein solution. The ethereal solution was transferred quantitatively a little at a time through the top of the separating funnel into a 250ml Quick flask and the solvent evaporated on a boiling water bath. Acetone (5ml) was added and the volatile solvent removed completely in a gentle current of air, holding the flask obliquely while turning it in a boiling water bath. The residue was dried in the oven maintained at 105^0Cfor fifteen minutes while placing

the flask in horizontal position, followed by cooling in desiccator and weighing. The process was repeated until constant weight is achieved.

The residue was then dissolved in diethyl ether (2ml) followed by addition of 10ml of ethanol. The solution mixture was titrated with 0.1M ethanolic potassium hydroxide solution using phenolphthalein as indicator. A blank determination was carried out following all the steps except no oil was added.

$$\text{FA content ether extract} = \text{ ml of NaOH x molarity of NaOH x 0282 as oleic acid (g)} \ldots\textbf{(5)}$$

$$\text{\% unsaponifiable matter} = \frac{\text{Wt og residue} - \text{wt of FA in the residue)x100}}{\text{wt of sample taken}} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\textbf{(6)}$$

Determination of Moisture Content and Volatile Matter[21]

Fresh weighed Girardinia seeds were transferred into each beaker from the polythene bags then dried in an oven maintained at 105^{0}C for twenty hours. The beakers and the contents were transferred into a desiccator to cool to room temperature then weighed accurately. The loss in mass in fresh seeds after oven drying would be determined. The latter loss in mass represented moisture content (g/100g) in each of the seed variants [21]. The same procedure for the determination of moisture content was repeated for the oven-dried (60^{0}C) Girardinia seeds.

Determination of Specific Gravity[21]

The pyknometer was thoroughly cleaned with detergent, rinsed with chromic acid and finally with distilled-deionised water. The pyknometer was dried in an oven maintained at 105^{0}C for two hours. It was then weighed in a water bath maintained at 20^{0}C until it reached the temperature, after fixing the volume of liquid to mark. After thirty minutes the pyknometer was removed from the water bath and allowed to stand for short time and weighed. The pyknometer was emptied and dried at 105^{0}C for one hour. It was filled with a sample of the GSO and kept in water bath maintained at 40^{0}C for one hour. The volume of oil in the pyknometer was adjusted to the fixed mark. The pyknometer was removed from the water bath and allowed to stand for a short time and weighed. The weighing was made in air with all weights adjusted to balance brass weights in air. The SG was adjusted using equation 7.

$$\text{SG} = {}^{w_1}/_{w_2} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \textbf{(7)}$$

Where w_1-weight of oil; w_2-weight of water

Determination of Refractive Index(RI)[22]

The oil was kept in water maintained at 50^{0}C for one hour, until the temperature of the sample stabilized. The Abbe refractometer was calibrated by measuring the RI of the glass plate ensuring optical contact with the prism by means of a drop of the alpha-bromophthalein.

The temperature of the prism of the Abbe refractometer was maintained at 50^{0}C by circulating through it water from the water bath controlled to the nearest 0. I ^{O}C. The temperature of the water coming out of the Refractometer was monitored. The surface of the prism was wiped with a soft cloth moistened with a few drops of petroleum spirit, 4060^{0}C.The RI of the oil was carried out in triplicate in accordance with the working of the Refractometer. The RI as read to nearest 0.0002 as an absolute value and the temperature of prism recorded. Immediately after the measurement, the surface of the prism was wiped with a soft piece of cotton wool moistened with a few drops of the solvent. The RI was then calculated using the equation below:

$$n_D{}^{40} = n_D{}^{40} + 0.0036 \ldots\ldots\ldots\ldots\ldots \ldots (8)$$

Where D is the Sodium line.

Determination of Heat of Combustion (D_c)

The heat of combustion of the samples was calculated using equation 9 below.

$$\Delta_C = IV - 9.15(SV) \ldots\ldots\ldots\ldots\ldots \ldots (9)$$

Where IV and SV are the iodine and saponification values of the oil respectively.

Statistical Data Analysis

Comparison of oil contents in seeds of various variants collected from different places was made. Similar statistical analysis was used to determine whether there were significant differences between oil content from the two varieties. The contents were quoted as comparable when there is no significant difference between the values at 99% confidence limit (A statistical test was done using the T-test, at 99% confidence level). Some data was subjected to Duncan Multiple Range Test (DMRT) to separate the means and determine the difference between them. Finally, the values obtained from various experimental tests were compared with published and/or available data collections of vegetable oils already in the market for the purpose of identifying the economic use of the oil/fat under study.

Results

Chemical Characteristics of Girardinia Seed Oil (GSO)

The chemical characteristics GSO, as defined by its IV, SV, PV, AV and unsaponifiable matter are recorded in Table 1. The IV ranged from 168 $\pm$1.83 to 188 $\pm$ 3.91. Oil from variant GDS (188 $\pm$ 3.91) registered the highest IV, followed by GDL (183 $\pm$ 3.01). The GB oil had the lowest IV (168 $\pm$ 1.83). it should also be noted that GB oil was significantly different (P>O.OI) from GD oil as far IV parameter was concerned.

The SV values ranged from 167.6 $\pm$ 3.14 to 178.2 $\pm$ 1.14. The GDS oil had the highest value with GDL registering the lowest. The values of GB and GDS were comparable and almost similar (P>0.01). The PV for the oils ranged from 8.86 $\pm$ 0.18 to 13.5 $\pm$ 0.07. Oil from variant GDS registered the highest amount of peroxides (*see table 1*).

The percent unsaponifiable matter of GSO varied from 2.67 $\pm$ 0.53 to 3.49 $\pm$ 0.61. Oil from variant GDL had the highest amount of unsaponifiable matter. However, the percentage unsaponifiable matter of oil from the other variant, which registered the smallest amount, was similar and comparable (P>0.0I) to it. Oil from GB did not show any pronounced difference with that from either GDL or GDS (P< 0.0I) in terms of unsaponifiable matter (P>0.0I). The AV, determined at room temperature, ranged from 4.81 $\pm$ 0.23 to 7.46 $\pm$ 0.24. GDS had the highest amount of AV which was higher (P>0.01) than that of GDL (6.56 $\pm$ 0.24). GB oil had the lowest AV (4.81 $\pm$0.23) (see *table 1*).

Table 1: Chemical Properties of Girardinia Seed Oil

Parameter		GB	GDL	GDS
Iodine value		168± 1.83	183 ±3.01	188 ± 3.91
Saponification value		176 ± 2.02	168 ± 2.56	175 ± 0.93
Peroxide Value (Meq/Kg)		9.50 ± 0.03	8.86 ± 0. 15	13.55 ± 0.06
% Unsaponifiable matter		2.98 ± 0.38	2.67 ± 0.53	3 49 ± 0.61
Acid value		4.81 ±0.23	6.56 ± 0.24	7.46± 0.24
% FFA as	i) Oleic	2.43 ±0.13	3.31 ±0.11	3.77 ± 0.11
	ii)Lauric	1.71 ± 0.09	2.31 ±0.09	2.66 ± 0.09
	iii) Palmitic	2.20 ±0.03	3.00 ± 0.12	3.41 ±0.10

Physical Characteristics of *Girardinia* Seed Oil

The moisture content, specific gravity, refractive index, heat of combustion and lipid content of GSO are recorded in table 2. The GD seed oil, after extraction, was light yellow while that of GB was golden yellow. The percent oil contents ranged from 18.4 to 21.2/100g dry matter. GB seeds, with the lowest oil content (18.4 ± 0.35%), were significantly different from those of the two variants, GDL and GDS, of GD species (P>0.01) at 99% confidence limit. This is Probably due to their different generic properties. A significant difference in oil content was also observed between the variants, GDS and GDL. The varietal classification of the two variants of GD could explain the difference in oil contents.

The mean moisture contents and standard deviations of the samples were determined at 60^0C and 105^0C. GB seeds registered the highest mean moisture content (37.2 ± 2.40g/ 100g air-dried seeds) while seeds of GDL had the lowest 31.5 ±0.26g/ 100g air-dried seeds). However, the moisture contents of seeds from GDL (50.25 ±3.15) and GDS (51.0 ± 0.30) variants of *diversifolia* species were similar and comparable (P>0.01).

The specific gravity (SG) of GB was the highest (0.9109 ± 0.0071) while that of variant GDL was the lowest (0.8979 ±0.0040). The values of GDS and GDL were found to be similar (P<0.01). However, a slight difference was observed between GD and GB.

The Refractive index (RI) of GSO was in the range of 1.4662 ± 0.00012 to 1.4704 ± 0.00032. Oil from GB species had the lowest RI while that from GDS seeds highest. The RI of GDS oil was similar (P>0.01) to that of oil from GDL seeds. The two types of seeds were obtained from plants of the same species. However, a significant difference (p<0.01) was observed between oil from GB and the two variants, GDS and GDL.

The heat of combustion ranged from 9580 ± 32.3 to 9663 ± 32.32 cal/g. The heat of combustion of GDL oil seeds was the highest, while oil from GB seeds had the lowest. However, all values compared favorably (P>0.01) with each other.

Table 2: Physical Properties of Girardinia seed Oil

Parameter	GB	GDL	GDS
Mw of FFA	305.3 ± 7.73	321.5 ±6.33	308.4 ± 4.56
% moisture Content of Seeds at: (i)60^{0}c	61.4±2.87	50.25±3.15	51.0±0.30
(ii)105^{0}c	37.2±2.40	31.5±0.26	33.2±0.21
Specific gravity (20^{0}c)	0.91067± 0.00709	0.8979±0.000397	0.8984 ± 0.00225
Refractive Index (400c)	1 4662 ± 0.00012	1.4705 ± 0.00032	1.4707 ± 0.00012
Heat of Combustion(Cal/g)	9580.22 ±8.43	9663.41 ±32.32	9602.48 ± 19.76
Oil content (% yield). Mean ±SD	18.4 ± 0.35	20.3 ± 0.42	21.2 ± 0.40
C.V	5.10	1.92	1.42

***Key**: Values are means and standard deviations of triplicate experiments except % yield*

The effect of heat on AV and FFA was analyzed using the least significant difference test (LSD) and the results are recorded in Table 3. The oil sample from GB and GDS, one of the two variants of *GD*, remained stable in the first two hours of heating unlike GD. All samples were affected during the subsequent heating period. Figure 1 illustrates the effect of heat on AV.

Table 3: Effect of Heat on Acidity of Girardinia Seed Oil

Heating Period (hrs)	**Sample**	**AV (±SD)**	**^{0}/0 FFA as**		
			Oleic (±SD)	**Lauric (±SD)**	**Palmitic(±SD)**
0	GB	4.810 ± 0.23	2.247 ± 0.13	1.71 ±0.09	2.20 ± 0.12
	GDL	6.560 ± 0.24	3.310±0.11	2.34 ± 0.09	3.00 ± o. 12
	GDS	7.227 ± 0.24	3.767 ±0.11	2.66 ± 0.09	3.41 ±0.10
1	GB	5.793 ± 0.95	2.590 ± 0.48	1.825 ± 0.34	2.343 ± 0.43
	GDL	8.340 ± 0.61	4.213 ± 0.31	2.970 ±0.21	3.813 ± 0.28
	GDS	7.780 ± 0.64	3.927 ± 0.33	2.770 ± 0.23	3.557 ± 0.29
2	GB	7.593 ± 0.37	3.833 ±0.19	2.707 ± 0.13	3.470 ±0.17
	GDL	10.693 ± 0.53	5.403 ± 0.27	3.803 ± 0.19	4.893 ± 0.27
	GDS	9.353 ± 0.86	4.723 ± 0.44	3.333 ±0.15	4.273 ± 0.40
3	GB	10.287 ± 0.1	5.190±0.51	3.660 ± 0.36	4.700 ± 0.46
	GDL	12.713 ± 0.14	6.420 ± 0.07	4.523 ± 0.05	5.810 ± 0.07
	GDS	I l. 143 ± 0.44	5.630 ± 0.21	3.967 ± 0.15	5.093 ± 0.19
4	GB	12.083 ± 0.41	5.770 ±0.21	4.300 ± 0.14	5.520 ± 0.19
	GDL	14.063 ± 0.34	7.103 ± 0.17	5.003 ± 0.12	6.427 ± 0.16
	GDS	12.377 ± 0.46	6.250 ± 0.23	4.403 ± O. 16	5.657 ± 0.21
5	GB	14.474 ±0.43	7.313 ± 0.22	5.150±0.14	6.613 ±0.19
	GDL	15.330 ± 0.35	7.743 ± 0.12	4.457 ± 0.12	7.200 ± 0.26
	GDS	13.570±0 18	6.857 ± 0.09	4.830 ± 0.07	6.207 ± 0.08
6	GB	15.623 ± 0.62	7.890±0.13	5.560 ± 0.10	7.140 ± 0.13
	GDL	15.330 ± 0.35	8.373 ± 0.07	5.923 ± 0.05	7.570 ± 0.07
	GDS	15.670 ± 0.43	7.917 ± 0.23	5.577 ± 0.15	7.150 ± 0.19
7	GB	16.530 ± 0.25	8.350 ± 0.12	5.883 ± 0.08	7.493 ± 0.14
	GDL	18.070 ±0.12	9.127 ± 0.25	6.433 ±0.18	8.257 ± 0.23
	GDS	17.053 ± 0.51	8.610 ± 0.26	6.070 ± 0.18	7.790 ± 0.24

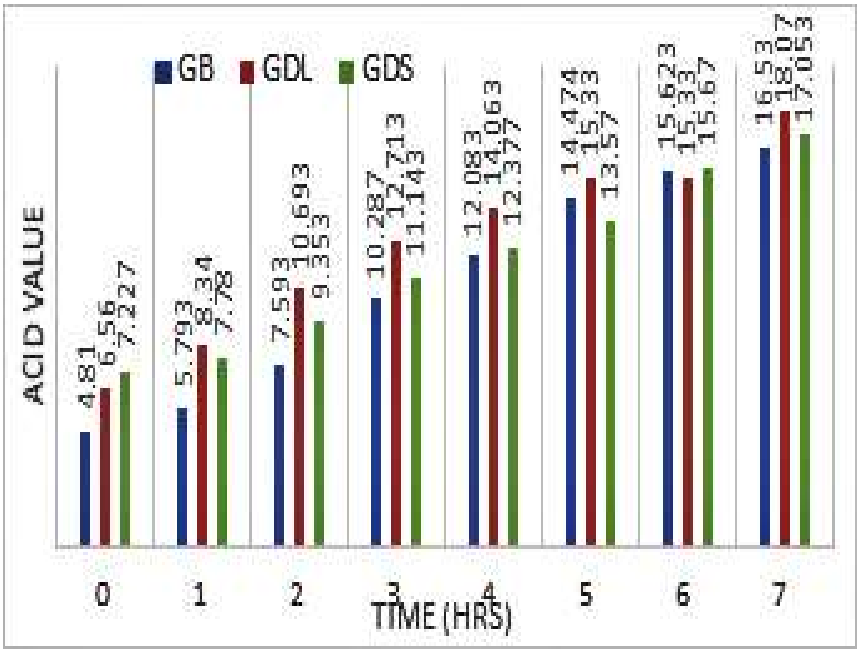

Figure 2: Effect of heat on acidity of Girardinia seed oil

Fatty Acid Composition of Girardinia Seed Oil

The fatty acid profile was determined by HPLC[29] and the chromagrams of both the standards and samples are shown as figures 2 to 6. The detected the fatty acids in of GSO are given in Table 4.

Table 4: Fatty acid composition of Girardinia seed oil.

Chain Length	Name	Structural Formula	Amount %		
			GDL	GDS	GB
C_7:0	Heptanoic acid	$CH_3(CH_2)_5COOH$	-	1.57	--
C_8:0	Octanoic acid	$CH_3(CH_2)_6COOH$	1.1	1	1.0
C_{IO}:0	Decanoic acid	$CH_3(CH_2)_8COOH$	1.4	3.17	1.7
C_{16}:0	Hexadecanoic(palmitic) acid	$CH_3(CH_2)_7COOH$	-	1	6.9
C_{18}:0	Octadecanoic (stearic) acid	$CH_3(CH_2)_{16}COOH$	5.8	34.97	-
C_{18}:1	9-Octadecaenoic(oleic) acid	$CH_3(CH_2)_7CHCH(CH_2)_7COOH$			
C_{18}:2	9, 12-Octadecadienoic (linoleic) acid	$CH_3(CH_2)_7(CH)_2CH_2(CH)_2(CH_2)_7COOH$	-	5.7	-
C_{20}:0	Eicosanoic acid	$CH_3(CH_2)_{18}COOH$	1.16	4.47	1.3
C_{21}:0	Heneicosanoic acid	$CH_3(CH_2)_{19}COOH$	-	-	13.4

Discussion

Chemical Properties Girardinia Seed Oil

The findings on IV suggest that the oil from GD species contains higher amount of unsaturated fatty acids than oil extracted from GB. However, the fatty acid composition and degree of unsaturation of the acids in oil extracted from the variants of GD, could be similar; there was no statistical difference (at the level of 99%) between the IV of GDL oil and GDS oil samples. The relatively high IV puts GSO under drying oil category.

The values also indicate that the oil is highly unsaturated[23]. The IV value of GSO compared very well with linseed oil (170-190) that is commonly used for paints [23]. The unsaponifiable matter of Girardinia seed oil was also found closer to that of almond oil (3.0) except oil from GDS.

The GSO had low SV relative to most vegetable oils [23]. With low SV and being liquid at room temperature, the oil is expected to have high proportion of long chain unsaturated fatty acids. The fatty acids in the oil from GB, with highest SV, had the lowest mean molecular weight (305.3 $\pm$ 7.73). GEL oil, with the lowest SV, registered the largest fatty acid mean molecular weight (321.5 $\pm$ 6.33). However, there was no statistical difference (at the level of 99%) between the weights ($p>0.01$). The results suggest that the oil samples would have fatty acids with almost the same carbon chain length.

From the findings, GDS, which registered the highest amount of peroxides Its value was remarkably different from that of oil obtained from other variant, GDL. GB had the lowest PV, implying that it is more stable oil than that from GD in undergoing autoxidation. GB oil, being less susceptible to autoxidation, could be more preferred to GD oil, if GSO is to be used for edible purposes.

The acid values obtained indicated that GB oil had lowest AV implying that it is likely to be less susceptible to rancidity (Ajiwe et. al., 1996). However, rancidity also depends on other factors such as temperature conditions during storage and extraction. The AV of GSO was comparable to that of some vegetable oils such as physic nut and some varieties of mango kernels, Dnjekir (4.90*)* and *Gedong gilletti* (4.90).

Relatively, all samples registered had high refractive indices. This suggested that GSO has unsaturated fatty acids with large molecular weights. the refractive indices set by FAO/WHO as standard for four edible vegetable oils, canola oil, sunflower oil, corn oil and olive pomace oil are 1.465 – 1.467, 1.467 – 1.474, 1.472-1.474 and 1.4680-1.4707 respectively [27,28]. The RI of oil from GB seeds was comparable to values of some vegetable oils: corn oil (1.465 -1.469), sesame seed oil (1.465 -1.4609) and Soya bean (1.4677 - 1.470). The index value of GD seeds was comparable to olive oil (1.4677 - 1.4705). The heat of combustion was also determined and the value obtained was close to 9500 cal/g, the approximate value for edible oils [26]. The results suggested that this oil could be used for edible purposes.

Physical Properties Girardinia Seed Oil

The oils were liquid at room temperature, suggesting the presence of unsaturated fatty acids. The percentage oil content of Girardinia seeds is comparable to commercially useful oil seeds such as Soya bean (16-25%), cottonseed (18-25%) and rubber-seed (21-25%)[23]. It also compares with Sunflower (*Helianthus annuus* L.) which considered to be one of the most important oil plants having 22–55% oil content[24]. Thus, oil processing from the seeds is a viable commercial enterprise.

The moisture content of Girardinia seeds is considered to be low relatively to reported values of other seeds such as acha, groundnut and mango (42.1 - 67.6). The low moisture content of Girardinia seeds remains an asset in storage and preservation of its nutrients[30,31]. The specific gravities of GDL and GDS were comparable to those of wild mango (0.85 $\pm$ 0.3) and esculentus tuber oil (0.90) [23,26]. The SG of GB was comparable to those of other vegetable oils that have been analyzed: groundnut oil (0.914 - 0.917), mustard seed oil (0.910-0.921), sesame seed oil (0.915-0.920).

The difference in SG between GD and GB observed in the results could be attributed to the difference in the component fatty acids, which may differ considerably in terms of the level of unsaturation as indicated by their differences in IV, where, low IV indicates the presence of high amounts of saturated fatty acids[25].

Nevertheless, the relative densities of the oil samples of two species are high implying that GSO contains long-chain fatty acids that are unsaturated. Oils of this nature are recommended for edible purposes [23]. The values also conform to the FAO/WHO standards for edible oils[27,28].

- **Fatty Acid Composition**

Stearic acid (34.97%), oleic acid (4.47%), decanoic acid (3.17%) and heptanoic acid (1.57%) were detected in GDS oil sample. GDL contained heneicosanoic acid (63%), octadecanoic (stearic) acid (5.8%), 9,13-octadecadienoic (linoleic) acid (1.16%), decanoic acid (1.4%) and octanoic acid (l. 1%). The results indicate that both long- and short-chain fatty acids are present in GB seed oil, though the former are, relatively, in higher concentration. The long-chain unsaturated fatty acids detected in Girardinia are 9-octadecaenoic (oleic) and 9, 12-octadecadienoic (linoleic) acids; octadecanoic (stearic) and heneicosanoic acids were the long-chain saturated fatty acids detected.

G. Bullosa oil was also found to contain long chain fatty acids: eicosanoic acid (13.39%) and 9,12-octadecadienoic acid (1.34%). Other fatty acids detected were hexadecanoic (palmitic) acid (6.89%), decanoic acid (1.69%) and octanoic acid (0.98%). Linoleic acid was present in both GD and GB oil samples an essential fatty acid when in □-form. Thus, *Girardinia* seed oil could be used in food industry.

Conclusion

The percentage oil content of Girardinia seeds (18-21%) is comparable to those of oil seeds such as cotton seed (18-25%), soyabean (16-25%) and rubber-seed (21-25%). Thus, the processing of oil from Girardinia seeds can be a viable commercial enterprise.

As a result of its low SV, Girardinia seed oil is expected to have high proportion of long chain unsaturated fatty acids. The IV of Girardinia seed oil is quite high indicating that the oil is highly unsaturated. This also puts the oil under drying oil category. The oil is likely to be less susceptible to rancidity because of the low concentration of free fatty acids in it as indicated by its low AV. . The AV is relatively much lower than the reported values of some vegetable oils recommended for commercial use [6]. The values ranged from 4.57 to 7.70. Most of the physical and chemical characteristics (RI, SG, AV, PV and SV) of the Girardinia oil conformed with FAO/WHO Standards for edible oils [32]. The PV was also well below the recommended acceptability standards (100meq or 75-120 meq/Kg value) for edible oils and fats[33–35]. Its Heat of Combustion was close to 9500 cal/g, the approximate value for edible oils [26]. Hence, Girardinia seed oil is most suitable for use in food industry

Abbreviations

AV	-	Acid Value
CLC	-	Crude lipid content
FFA	-	Free fatty acid
DMRT	-	Duncan Multiple Range Test
GB	-	*G. bullosa*
GDS	-	*G. diversifolia* with bladed leaves
GDL	-	*G. diversifolia* with lobed leaves
GSO	-	Girardinia seed oil
HPLC	-	High Performance Liquid Chromatography
IV	-	Iodine Value
PV	-	Peroxide Value

RI	-	Refractive index
SV	-	Saponification Value
SG	-	Specific gravity
UFA	-	Unsaturated Fatty Acids

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Declaration

The author declares that he has no competing interests.

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