Variation in Lipid Composition of Niger Seed (*Guizotia abyssinica* Cass.) Samples Collected from Different Regions in Ethiopia

Paresh Chandra Dutta[1], Seved Helmersson, Eshetu Kebedu[2], Getinet Alemaw[3] and Lars-Åke Appelqvist[1,*]

Department of Food Hygiene, Swedish University of Agricultural Sciences, S-750 07 Uppsala, Sweden

Niger seed samples were collected from different regions in Ethiopia for determination of oil content, and of fatty acid, tocopherol and sterol composition in the seed oil by gas–liquid chromatography and high-performance liquid chromatography methods. There was a large variation in oil content, ranging from 29 to 39%. More than 70% of the fatty acids was linoleic acid (18:2) in all samples analyzed. The other predominant fatty acids were palmitic (16:0), stearic (18:0) and oleic (18:1) at a range of 6 to 11% each. Total polar lipids recovered after preparative thin-layer chromatography comprised a small fraction of the total lipids. They had higher 16:0 and lower 18:2 contents than the triacylglycerols. α-Tocopherol was the predominant tocopherol in all samples, 94–96% of the total amounting to 630–800 μg/g oil. More than 40% of the total sterols was β-sitosterol, *ca.* 2000 μg/g oil. The other major sterols were campesterol and stigmasterol, ranging from 11 to 14%. The Δ5- and Δ7-avenasterols were in the range of 4 to 7%. From the samples studied, no conclusion could be drawn regarding the influence of altitude or location on oil content, tocopherol and/or sterol contents. The results of the present study on niger seed oil are discussed in comparison with known data for common oils from Compositae, *viz*, safflower and sunflower.

KEY WORDS: Fatty acids, *Guizotia abyssinica* Cass., niger seed oil, sterols, tocopherols.

Niger (*Guizotia abyssinica* Cass.) is the most important oil crop in Ethiopia and a minor oil crop in India but is not involved in the world oilseed trade. Niger seed provides 50 to 60% of Ethiopia's indigenous edible oil but provides only 2% of India's total oil seed production. It is also a minor oilseed crop in some other African countries (1). Besides its use as an important edible oil in Ethiopia, niger seed oil is also used for the manufacture of soap, paint and illuminant in other countries (1).

Regarding the fatty acid composition, niger seed oil resembles that of safflower and sunflower with a high content of linoleic acid (18:2), up to 85% depending on the origin (1). In addition to the major importance of fatty acid composition in assessing oil quality, some minor components, e.g., tocopherols and sterols, are also of interest because of their antioxidant activity (2).

The present study is an extended investigation of the lipids of some uncertified niger seed varieties and of a few newly certified varieties collected from different regions in Ethiopia. The oil content and the fatty acid and sterol composition, determined by both packed-column and capillary-column gas chromatography, and content of tocopherols, determined by high-performance liquid chromatography (HPLC), are reported in this paper.

[1]Present address: Department of Food Science, Swedish University of Agricultural Sciences, Box 7051, S-750 07 Uppsala, Sweden.
[2]Present address: Food Research and Development Centre, Ethiopian Food Corporation, P.O. Box 5688, Addis Ababa, Ethiopia.
[3]Present address: Ethiopian Oilseeds Improvement Project, I.A.R., P.O. Box 2003, Addis Ababa, Ethiopia.
*To whom correspondence should be addressed at the Department of Food Science, Swedish University of Agricultural Sciences, Box 7051, S-750 07 Uppsala, Sweden.

MATERIALS AND METHODS

Seed samples. Most niger (*Guizotia abyssinica* Cass.) seed samples were collected from different agricultural regions (15 samples) in Ethiopia from noncertified seeds used by farmers, while three samples were certified varieties released by the Institute of Agricultural Research (IAR, Holeta, Ethiopia). The seed samples were provided by the Ethiopian Food Corporation (Addis Ababa, Ethiopia).

Solvents and reagents. All solvents and reagents were of analytical grade.

Lipid extraction. The lipids were extracted essentially as described elsewhere (3) except for the following modifications. Seeds (10 g) were extracted at room temperature in steel tubes, containing four steel balls to facilitate homogenization of the seeds, with 30 mL of heptane/isopropanol (3:2, vol/vol) under vigorous horizontal shaking for 1 h. The homogenates were filtered through defatted filter papers on a Buchner funnel, and the residues were washed three times with 5 mL of the same solvent. The total extracts were collected in volumetric flasks and filled up to volume. The content of total lipids was then estimated gravimetrically from a known volume of the total extracts. The extracted lipids were stored at −20°C until further analysis.

Preparation of fatty acid methyl esters. For total lipids (TL), *ca.* 20 mg of the lipids in hexane were treated consecutively with 0.01 M NaOH in dry methanol and 14% BF_3 in methanol at 60°C under shaking as described elsewhere (3). The fatty acid methyl esters were analyzed by packed-column gas–liquid chromatography (GLC) without further purification as described below. Purified triacylglycerols (TAG) and total polar lipids (PL), separated by thin-layer chromatography (TLC) as described below, were subjected to methanolysis with HCl in methanol (4%, vol/vol) without prior elution from the gels (4). In all cases, heptadecanoic acid (17:0) was used as internal standard to quantitate the amount of fatty acids.

TLC. Analytical TLC, to check the lipid patterns, was carried out on Silica Gel 60 precoated plates (0.25 mm; Merck, Darmstadt, Germany) in the solvent system hexane/diethyl ether/acetic acid (70:30:1, vol/vol/vol). Separation of lipids and purification of crude fatty acid methyl esters were accomplished by preparative TLC in the solvent system hexane/diethyl ether/acetic acid (85:15:1, vol/vol/vol). The separated fatty acid methyl esters were extracted by water-saturated diethyl ether.

Fatty acid methyl ester analysis by GLC. Packed-column GLC: Analyses of fatty acid methyl esters of TL were performed on a glass column (2.1 m × 0.2 cm) packed with 10% DEGS on Chrom W-AW 80-100 mesh (Scantec AB, Sollentuna, Sweden) and fitted in a Pye-Unicam (Cambridge, United Kingdom) GCD gas chromatograph with flame-ionization detector. GLC was conducted at a column

temperature of 170°C, and both the injector and detector temperatures were at 250°C. Nitrogen was used as carrier gas at a flow rate of 20 mL/min. A Hewlett-Packard 3390 A integrator (Avondale, PA) was used for recording peak areas. Fatty acid percentages were calculated after direct normalization of peak areas.

Capillary-column GLC: For analysis of fatty acid methyl esters of TAG and PL, two fused-silica WCOT capillary columns coated with CP Sil 88 (Chrompack International BV, Middelburg, The Netherlands) of 50 m and 20 m length were connected together and fitted in a Varian 3400 gas chromatograph (Walnut Creek, CA) equipped with flame-ionization detector and split/splitless injector. Both columns had 0.15 mm i.d. but 0.12 and 0.20 μm film thickness, respectively. Hydrogen was used as carrier gas at a velocity of 18 cm/s, and helium was used as make-up gas at a rate of 30 mL/min. A temperature program of 175°C for 20 min and an increase to 190°C at 1°C/min was used, and the split ratio was 66:1. The injector and the detector temperatures were 230 and 260°C, respectively. A Varian 4290 integrator was used for recording the peak areas. No response factors were used in calculating the fatty acid percentages either by packed- or capillary-column GLC because analysis of known reference fatty acids showed response factors rather close to unity by capillary-column GLC. Known amounts of heptadecanoic acid (17:0) were used as internal standard to calculate the absolute weight of the fatty acids of the PL.

Sterol analysis. The separation and subsequent trimethylsilyl (TMS) derivatization of the sterols were performed after saponification of the TL. Cholestane (10 μg) as internal standard was added to a weighed amount of lipids (*ca.* 10 mg) in glass tubes with a ground-glass stopper. After evaporating the solvent under a stream of nitrogen, 2 mL of 1 M KOH in methanol was added, and the stoppers were secured with clips. The tubes were placed in a glycerol bath at 60–70°C for 30 min with continuous shaking. After cooling in running water, 2 mL water and 2 mL hexane were added, and the tubes were shaken vigorously. The hexane layer was transferred with a disposable pipette to small tubes. The solvent was evaporated under a stream of nitrogen, and 100 μL Tri-Sil reagent (Pierce, IL) was added to the tubes and incubated in an oven at 60°C for 45 min. Thereafter, the solvent was evaporated under a stream of nitrogen, and the TMS-ether derivatives of the sterols were dissolved in 1 mL hexane. About 1 μL of sample was analyzed by GC (Varian 3700) equipped with a flame-ionization detector and a falling needle injector. An OV-1 fused-silica capillary column (Hewlett-Packard, length 25 m, i.d. 0.32 mm and film thickness 0.52 μm) was used for the separation of the unsaponifiables. Helium was used as carrier gas at an inlet pressure of 8 PSIG and as make-up gas at a rate of 30 mL/min. The column temperature was maintained at 246°C, and the detector temperature was at 300°C. The peaks were computed by an HP 3390 A integrator (Hewlett-Packard), and the absolute weight and weight percentage composition were determined from the known amount of cholestane as internal standard.

HPLC. The tocopherol content was determined by direct injection of the oil samples into an HPLC column (detailed method to be published elsewhere). In brief, a Spectra Physics SP 8700 solvent delivery system with a Perkin-Elmer LS-2 filter fluorimeter detector were used at wavelengths of 295 nm and 320 nm for excitation and emission, respectively. Two HPLC-glass columns (100 mm, 3 mm i.d.) connected in series, packed with Hypersil APS (NH_2, particle size 5 μm; Chrompack International BV) were fitted with a 10-μL loop manual valve injector. Isocratic elution was carried out with a mixture of hexane/*tert*-butylmethylether/tetrahydrofuran/methanol (79:20:0.98:0.02, by vol) at a flow rate of 0.7 mL/min. A Hewlett-Packard HP 3390 A Integrator was used for calculating the peak areas. Standard tocopherol (Merck) samples were run regularly to identify the peaks. Duplicate samples were analyzed in all the methods described in this paper.

RESULTS AND DISCUSSION

The oil contents and the fatty acid compositions, determined by packed-column gas chromatography (GC), are presented in Table 1. There was a fairly large variation in oil content (28.5 to 38.8%). To find out whether this variation is predominantly due to variety or location (including soil condition, elevation, rainfall and temperature), further investigation is needed. However, samples from the same region displayed largely different oil contents (samples no. 001–004 had from 28.5 to 35.8% oil, Table 1). This is the first systematic study of total variability in lipids of niger seed, and studies on a single variety planted at different experimental stations have to be undertaken to reveal the major contributors to the overall variation. Reports in the literature showed the oil content in niger seed to be in the range of 30 to 50% (5–9). Thus the results of the present study lie in the lower range of data reported earlier.

The major fatty acids, as determined by packed-column GC, are palmitic, stearic, oleic and linoleic acids (Table 1). The linoleic acid (18:2) content was in the range of 71.4 to 79.2%, irrespective of variety and/or location. This range is within the data given in a report on Ethiopian varieties (9). However, Indian varieties have been shown to contain linoleic acid in a wide range from 45 to 74% (1,8). The contents of other major fatty acids reported here also concur with published results, except for those Indian varieties, where lower linoleic acid content was compensated for by increased oleic acid (8–11).

Because a few "minor" fatty acids are impossible or difficult to quantitate by packed-column GC, a capillary-column GC system was used on some samples. In addition, this second fatty acid study on six selected samples was preceded by a separation of the TAG and total PL from the crude lipids. The significant difference in the fatty acid compositions between TAG and PL was that the TAG had lower 16:0 percentages, *ca.* 10% compared with *ca.* 16% in PL. The higher percentages of 16:0 in PL were mainly compensated for by lower percentages of 18:2 (Table 2). Visual observation of analytical TLC plates showed that TAG was the most predominant fraction in TL. However, it was also of interest to quantitate the PL fraction in TL and its fatty acid composition. The PL comprised only a small fraction of the total lipids, *ca.* 0.7 to 0.8% fatty acids of the TL, corresponding to *ca.* 1% of PL (Table 2).

The results of the present study concur with those of earlier investigations, that showed detailed fatty acid composition (9,10). However, the earlier reports did not show

TABLE 1

Oil Content, Tocopherol Content and Fatty Acid Composition of Total Lipids of Niger Seed Samples from Ethiopia

Sample number	Variety and/or location	Altitude (m)	Oil content (%), as is	Tocopherol[a] μg/g	Major fatty acids (% of total)			
					16:0	18:0	18:1	18:2
	Western Shewa region							
001	GENCHI	2220	28.5	761	9.0	5.9	8.0	77.1
002	AMBO	2350	35.8	770	8.5	5.6	6.7	79.2
003	GEDO	2400	31.6	790	8.7	7.0	5.9	78.3
004	GEDO	2460	32.1	700	8.6	6.7	6.8	77.8
	Wellega region							
005	GEDO-FINDHA Road	2300	34.5	769	8.6	6.7	8.3	76.4
006	FINCHA-SHAMBU Road	2400	36.4	699	8.0	6.1	7.6	78.2
007	SHAMBU-ALIBO Road	2500	29.6	813	8.9	6.7	7.0	77.2
	Near Hareto							
008	SHAMBU-BAKO Road	2250	29.5	723	8.6	6.4	6.6	78.1
	Between Sire and Gute							
009	BAKU-NEKEMIT Road	1850	33.9	683	8.4	6.9	6.7	77.8
010	NEKEMIT-GIMBI Road	2250	38.8	714	8.6	6.1	6.7	78.5
	Gojam							
013	DENBECHA	1900	33.9	695	9.0	8.1	11.0	71.4
014	FNOTESELAM	1650	34.4	657	8.9	5.9	10.2	74.5
015	ADDIS KIDAME	2300	35.1	718	9.2	6.2	7.4	77.1
016	DANGLA	1940	34.0	727	9.2	6.6	10.3	73.8
	IAR[b] station at Gojam							
017	ADET LOCAL	2240	32.1	703	9.0	6.3	7.8	76.9
	New variety released by IAR, Holeta							
018	ESETE-1	2480	36.6	728	8.6	6.3	6.6	78.4
019	SENDAFA	2480	34.3	765	8.5	6.2	6.4	77.7
020	FOGERA-1	2480	37.0	853	8.6	6.4	6.3	78.4

[a]α-Tocopherol, 94–96%; β-tocopherol *ca.* 1.0%; γ-tocopherol, 3–5%.
[b]Institute of Agricultural Research.

TABLE 2

Fatty Acid Composition of the Triacylglycerols (TAG) and of Total Polar Lipids (PL) Analyzed by Capillary-Column Gas Chromatography of Niger Seed Samples from Ethiopia

Sample[a] number	TAG (fatty acids, % of total)									
	16:0	16:1	18:0	$18:0^{\Delta 9}$	$18:1^{\Delta 11}$	18:2	18:3	20:0	22:0	24:0
004[b]	9.7	0.1	6.6	5.4	0.1	77.0	0.1	0.3	0.3	0.2
007	9.6	0.1	7.1	6.0	0.1	75.7	0.2	0.3	0.4	0.3
016	9.6	0.1	8.2	9.3	0.1	71.1	0.1	0.5	0.6	0.2
018	9.6	0.1	6.7	5.0	0.1	77.4	0.1	0.3	0.4	0.2
019	10.6	0.1	6.6	5.1	0.1	76.5	0.1	0.3	0.3	0.2
020	9.4	0.1	6.7	5.2	0.2	77.5	0.1	0.2	0.3	0.2
	PL[c]									
004	16.3	0.2	8.0	4.2	0.2	70.0	0.5	0.2	0.2	0.2
007	16.6	0.1	7.7	3.7	0.1	71.0	0.6	0.2	0.1	0.1
016	16.5	0.1	7.6	4.9	0.3	69.6	0.4	0.2	0.1	0.1
018	15.6	0.1	7.7	3.7	0.2	71.5	0.6	0.1	0.3	0.1
019	15.4	0.1	7.0	3.4	0.2	72.6	0.7	0.1	0.2	0.2
020	15.4	0.1	7.5	3.4	0.2	72.2	0.6	0.2	0.2	0.2

[a]Variety and/or location/altitude are given in Table 1.
[b]Sample 004 also had 0.1% of 20:1, and other samples had trace amounts of 20:1 in TAG.
[c]In polar lipids, the fatty acids comprised from 0.66 to 0.84% of total lipids.

the presence of *cis*-vaccenic acid ($18:1^{\Delta 11}$), which was detected in trace amounts (Table 2) in both TAG and PL fractions of all the samples analyzed by capillary GC (tentatively identified from the retention time of standard *cis*-vaccenic acid in our laboratory). The extent of other minor fatty acids present in niger seed oil in the present study also concurs with those published results, except that the amount of lignoceric acid, reported by Seegler (9), was 2%

TABLE 3

The Qualitative and Quantitative Composition of Sterols in Some Samples of Niger Seed from Ethiopia

Sample number	μg/g lipids (% composition)						
	Cholesterol	Campesterol	Stigmasterol	Sitosterol	Δ5-Avenasterol	Δ7-Avenasterol	Others[a]
004	24 (0.4)	690 (13.1)	750 (14.2)	2280 (43.2)	350 (6.6)	240 (4.5)	950 (18.0)
007	19 (0.4)	620 (12.8)	690 (14.2)	2090 (43.1)	310 (6.4)	200 (4.1)	920 (19.0)
016	31 (0.8)	490 (11.8)	560 (13.5)	1590 (38.4)	210 (5.1)	190 (4.6)	1070 (25.8)
018	10 (0.2)	620 (13.0)	680 (14.2)	2010 (42.0)	300 (6.3)	210 (4.4)	950 (19.9)
019	14 (0.3)	600 (12.8)	630 (13.4)	1980 (42.2)	290 (6.2)	200 (4.2)	980 (20.9)
020	11 (0.2)	550 (12.6)	600 (13.8)	1860 (42.7)	260 (6.0)	180 (4.1)	900 (20.6)

[a]Representing peak areas in chromatograms with retention time (RT) > cholesterol and < Δ7-avenasterol. The major component in this group is Δ7-stigmastenol, but a compound with RT = 24-methylenecycloartanol was also present in minor amounts.

while our samples contained a maximum of 0.5% (Table 2). The differences in fatty acid composition between TAGs and PLs are of interest because the fatty acid pattern of the TAG fraction will predominate in a refined, bleached and deodorized oil, whereas that of the PL fraction may be of interest if a "lecithin" fraction is recovered in a refining process.

α-Tocopherol was the major component of the total tocopherols in the samples investigated. The levels varied from 680 to 850 μg/g oil, comprising more than 90% of the total tocopherol content (Table 1). γ-Tocopherol was present at a level of 3–5% of the total tocopherols, and β-tocopherol was present as a minor component at a rather constant level of *ca.* 1% (Table 1). The only previous report on one sample, to our knowledge, on tocopherol content in niger seed oil (12) concurs well with the present investigation.

Because tocopherol levels are of interest as antioxidants (2), and the high content of linoleic acid would make the oil prone to oxidation, it was of interest to investigate if there was any correlation between these two components. When the content of total tocopherols (y) was calculated in relation to linoleic acid content (x) we got the following equation:

$$y = 14.31 + 9.33x, R = 0.37 \quad [1]$$

Apparently there seems to be a weak positive correlation between these two compounds. According to a previous publication (13), sunflower and safflower oil, belonging to the Compositae family, had 510 and 400 μg tocopherol/g oil, respectively. Niger seed oil in the present study, on the other hand, contained much higher amounts of tocopherol, 660 to 850 μg tocopherol /g oil (Table 1), which may contribute to its great stability toward oxidation in spite of higher linoleic acid content than sunflower and safflower oils.

The content of most of the common 4-desmethylsterols in the six selected niger seed oils are presented in Table 3. The most predominant component was β-sitosterol, comprising 38 to 43% of the total sterols. The next major components were campesterol and stigmasterol, both comprising *ca.* 14% each. Other components, e.g., Δ5-avenasterol, were present at a level of *ca.* 5–7%, and Δ7-avenasterol was present at *ca.* 4%. A small amount (0.2 to 0.4%) of cholesterol, identified by retention time only, was present in all the samples analyzed. There were no remarkable differences between the six samples in terms of sterol composition. The only published results on niger seed oil sterols (14), analyzed by packed-column GLC, showed a rather similar pattern, i.e., the sitosterol was the most predominant, and the next two major components were campesterol and stigmasterol. There are numerous published results on sterols of some other oils of the Compositae family, e.g., sunflower and safflower (15). These oils had higher sitosterol content, at a range of 50–75% of total sterols, compared to niger seed oils (38 to 43%). Because the present analytical conditions could not separate Δ7-stigmastenol from 24-methylenecycloartanol, we presented the former sterol under "Others" in Table 3. The peak tentatively assigned to Δ7-stigmastenol was the most prominent in the group "Others," and the other unidentified peaks eluting between cholesterol and Δ7-avenasterol were minor. However, it is known that the oils from other Compositae, sunflower and safflower, contain a high percentage of this sterol (15). Hence, it is highly likely that this is the case with niger seed as well.

From the results of the present and previous studies, it is clear that niger seed is a good source of high linoleic acid-containing oil, even higher than sunflower and safflower oils. In addition, it is a good source of vitamin E because almost all of the tocopherols are α-tocopherol.

ACKNOWLEDGMENTS

This work was financially supported by SAREC, Sweden (SAREC project no. S/5. 11 SWE-87-034). Gunilla Eriksson performed lipid extraction and the fatty acid composition analysis by packed-column gas–liquid chromatography.

REFERENCES

1. Riley, K.W., and H. Belayneh, in *Oil Crops of the World*, edited by G. Röbbelen, R.K. Downey and A. Ashri, McGraw-Hill Publishing Company, New York, 1989, p. 394.

2. Appelqvist, L.Å., *Ibid.*, 1989, p. 22.
3. Appelqvist, L.Å., *Arkiv För Kemi, Kungl. Svenska Vetenskapsakademien 28*:551 (1968).
4. Stobart, A.K., and S. Stymne, in *Methods in Plant Biochemistry*, edited by J.L. Harwood, and J.R. Bower, Academic Press, London, 1990, p. 20.
5. Nema, N.P., and L. Singh, *Indian Oilseeds J. 9*:192 (1965).
6. Khidir, M.O., and A.K. Ahmed, *Sudan J. Food Sci. Technol. 7*:86 (1975).
7. Ghiglione, C., and D. Lemordant, *Bull. Trav. Soc. Pharm. Lyon 21*:3 (1977).
8. Nasirullal, T., Mallika, S. Rajalakshmi, K.S. Pashupathi, K.N. Ankaiah, S. Vibhakar, M.N. Krishnamurthy, K.V. Nagaraja and O.P. Kapur, *J. Food Sci. Technol. 19*:147 (1982).
9. Seegler, C.J.P., in *Oil Plants in Ethiopia, Their Taxonomy and Agricultural Significance*, Centre for Agricultural Publishing and Documentation, Wageningen, 1983, p. 122.
10. Grieco, D., and G. Piepoli, *Oleagineux 22*:611 (1976).
11. Seher, Von A., and U. Gundlach, *Fette. Seifen. Anstrichm. 84*:342 (1982).
12. Piorr, W., L. Toth and N. Novakovic, *Z. Lebensmitt.-Untersuch. 138*:11 (1967).
13. Slover, H.T., R.H. Thompson Jr. and G.V. Merola, *J. Am. Oil. Chem. Soc. 60*:1524 (1983).
14. Sil, S., J. Chakrabarti and R.S. Gandhi, *J. Food. Sci. Technol. 27*:234 (1990).
15. Kochhar, S.P., *Prog. Lipid Res. 22*:161 (1983).

[Received September 15, 1993; accepted April 30, 1994]