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Oil and Fatty Acids in Seed of Eggplant (*Solanum melongena* L.) and Some Related and Unrelated *Solanum* Species

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Abstract: The seed oil content of 305 genebank accessions of eggplant (*Solanum melongena*), five related species (*S. aethiopicum* L., *S. incanum* L., *S. anguivi* Lam., *S. linnaeanum* Hepper and P.M.L. Jaeger and *S. macrocarpon* L.) and 27 additional *Solanum*s pecies, was determined by NMR. Eggplant (*S. melongena*) seed oil content varied from 17.2% (PI 63911317471) to 28.0% (GRIF 13962) with a mean of 23.7% (std. dev = 2.1) across the 305 samples. Seed oil content in other *Solanum* species varied from 11.8% (*S. capsicoides*-PI 370043) to 44.9% (*S. aviculare*-PI 420414). Fatty acids were also determined by HPLC in genebank accessions of *S. melongena* (55), *S. aethiopicum* (10), *S. anguivi* (4), *S. incanum* (4) and *S. macrocarpon* (2). In all samples examined, the predominant fatty acid was linoleic acid (18:2) followed by oleic acid (18:1) and palmitic acid (16:0). Levels of linoleic acid ranged from 57% (*S. aethiopicum*-PI 194166) to 74.5% (*S. anguivi*-PI 183357). Oleic and palmitic acid levels ranged from 11.3% (*S. anguivi*-PI 183357) to 25.2% (*S. aethiopicum*-PI 194166) and 8.4% (*S. melongena*-PI 115507) to 11.2% (*S. melongena*-PI 600912), respectively. Oil extracted from seed of *S. melongena* cv. Black Beauty had a pour point of -12°C, viscosities of 28.8 (40°C) and 7.3 (100°C), a viscosity index of 240, an oxidation onset temperature of 160°C and a Gardner Color of 6+.

Keywords: Brinjal, Aubergine, Physicochemical Properties, Genebank, TD-NMR

Introduction

Eggplant arose in Africa and was subsequently dispersed throughout the Middle East and thence to Asia (Weese and Bohs, 2010). The cultivated eggplant (*S. melongena*) and several of its related taxa form what is sometimes referred to as the *S. melongena* complex. This complex is composed of the Asian *S. melongena* eggplant (groups E, F, G and H), the African *S. incanum* eggplant (groups A, B, C and D) and (more recently) *S. linnaeanum*. Numerous studies have characterized the genetic, morphological and breeding characteristics of the members of this complex and space here does not permit a summarization of these; however, readers are referred to the publications of (Daunay *et al*., 1991; 1999; 2001; Doganlar *et al*., 2002; Kahn, 1979; Lester and Hasan, 1991; Lester and Niakan, 1986) and the references cited therein. Two additional species of interest include*Solanum aethiopicum* (scarlet eggplant) and *Solanum macrocarpon* (Gboma eggplant). These species are cultivated in Africa and elsewhere, but are not generally believed to be closely related to *S. melongena* (Whalen, 1984).

Eggplant is an important vegetable in Asia, Africa, the Middle East and, to a lesser extent, the Americas. Approximately 2×10^3 Ha of eggplant were planted in 2012 in the USA as compared to 8.0×10^5 Ha in China and 1.85×10^6 Ha globally (FAO, 2015). World production of eggplant in 2013 yielded an estimated 48.4×10^6 metric tons of fruit, most of which was produced in China (58%) and India (25%). Other leading producers included Iran, Egypt and Turkey, while the

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largest exporters of the crop in 2013 were Spain, Jordon and Mexico. On the basis of tonnage produced, eggplant production ranks sixth after tomatoes, watermelons, onions, cabbages and cucumbers (FAO, 2015).

Historically, eggplant and *Solanum* spp. in general, have not been cultivated for their seed or seed oil. Neither has breeding or selection been practiced with an aim towards increasing their seed yield or characterizing or altering their seed oil yield or composition. The genus *Solanum* contains a large number of species, many of which produce edible fruit or plant parts (D'Arcy, 1986). However, the literature contains little or no information on the seed oil characteristics of most of these. New uses for vegetable seed oil have prompted studies on the yield, fatty acid composition and physicochemical characteristics of oil derived from seed of tomato (*Solanum lycopersicon*) and pepper (Giuffre *et al*., 2015; Jarret *et al*., 2013). Yields of tomato seed oil averaged 20-23% (Giuffre *et al*., 2015) whereas seed oil yields among *Capsicum* spp. ranged from 11 to 36% (Jarret *et al*., 2013). Dhellot *et al*. (2006; Nzikou *et al*., 2007) proposed the use of seed oil of *S. nigrum* as a means of diversifying sources of lipids eaten by the peoples of Africa. Thus, this study was undertaken to examine the seed oil content in a genebank's holdings of eggplant (*S. melongena*), several of its related species and also in a variety of miscellaneous *Solanum* spp. for which seed were available. Also examined was the fatty acid composition of a subset of these materials.

Materials and Methods

Plant Material

All seed used in this study were obtained from the USDA/ARS Plant Germplasm Collection in Griffin, GA (Jarret *et al*., 1990). A total of 386 genebank accessions were examined. Prior to analysis, all seed (stored at -20°C in foil pouches) were brought to room temperature for a minimum of 24 h. Species examined are presented in Table 1.

Preparation of Oil Standards

Oil standards were prepared from *S. melongena* cv. Black Beauty (Eden Bros., Asheville, NC) essentially as described by Jarret *et al*. (2013).

Table 1. Seed oil content (mean +/- standard deviation) in eggplant (Solanum melongena) and other Solanum species in the S-9 genebank

			Oil content	
No. accessions	Mean	Low	High	Taxon
Solanum aculeatissimum Jacq.	2	23.9	23.8	24.1
Solanum aethiopicum L.	16	25.3	22.6	31.0
Solanum americanum Mill.	6	33.7	29.5	36.2
S. anguivi Lam.	1	32.7	32.7	32.7
Solanum atropurpureum Schrank	1	24.6	24.6	24.6
Solanum aviculare G. Forst.	2	42.8	40.7	44.9
Solanum capsicoides All.	4	15.3	11.8	20.8
Solanum chippendalei Symon	1	20.1	20.1	20.1
Solanum dulcamara L.	2	32.0	31.8	32.2
Solanum incanum L.	2	22.3	22.3	22.4
Solanum laciniatum Aiton	3	35.8	33.6	38.5
Solanum lasiocarpum Dunal	1	21.6	21.6	21.6
Solanum lichtensteinii Willd.	1	20.4	20.4	20.4
Solanum linnaeanum Hepper and P.M.L. Jaeger	3	25.6	23.7	27.0
Solanum macrocarpon L.	2	23.4	22.4	24.4
Solanum mammosum L.	4	18.8	16.8	22.3
Solanum melongena L.	305	23.7	17.2	28.0
Solanum ochranthum Dunal	1	17.0	17.0	17.0
Solanum pseudocapsicum L.	4	23.6	21.7	24.6
Solanum quitoense Lam.	1	18.8	18.8	18.8
Solanum retroflexum Dunal	3	33.9	32.8	34.8
Solamum richardii Dunal	1	28.7	28.7	28.7
Solanum rostratum Dunal	1	34.5	34.5	34.5
Solanum sarrachoides Sendtn.	1	32.4	32.4	32.4
Solanum scabrum Mill.	1	33.7	33.7	33.7
Solanum sessiliifolium Dunal	1	20.0	20.0	20.0
Solanum sisymbriifolium Lam.	4	29.3	28.5	30.3
Solanum sitiens I.M. Johnst.	2	25.1	24.3	26.0
Solanum stramoniifolium Jacq.	1	25.7	25.7	25.7
Solanum suaveolens Hunth and C.D. Bouche	4	32.7	31.4	34.3
Solanum viarum Dunal	3	20.6	20.0	21.3
Solanum villosum Mill.	1	35.0	35.0	35.0
Solanum virginianum L.	2	25.9	25.1	26.8

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TD-NMR Analysis

Seed oil and moisture measurements were carried out on intact seed by TD-NMR as described by Krygsman and Barrett (2004) and Jarret *et al*. (2013) on a Bruker (Madison, WI) mq10 Minispec. NMR analyses were conducted on seed drawn from single inventories (01) without replacement. All samples were measured in triplicate and the results were averaged.

Isolation and Analysis of Fatty Acids

For isolation of fatty acids, replicate 100 mg seed samples were ground to a fine powder with a mortar and pestle in liquid nitrogen. Approximately 50 mg of ground powder was transferred into a 16×100 mm test tube and 5.0 mL of n-heptane (Fisher Scientific) was added to extract the oil. For conversion of Fatty Acids to Methyl Esters (FAME), 500 µL of 0.5 M sodium methoxide ($NaOCH_3$) in methanol solution was added to the test tube and mixed with the sample. The reaction was allowed to proceed for 2 h. Seven mL of distilled water was then added to separate the organic layer from the aqueous layer and residue (45 min). An aliquot of the organic layer (1.5 mL) containing the methyl esters was transferred to a 2.0 mL autosampler vial for GC analysis.

FAME extracts were diluted 100-fold in heptane containing 25 µg mL^{-1} methyl nonadecanoate (C19:0) and analyzed with a Thermo Quest Finnigan DSQII GC-MS system (Thermo Fisher, San Jose, CA, USA) using C19:0 as an internal standard (ISTD). The mass spectrometer was operated in the electron impact mode and scanned at m/z = 50 to 400 during data acquisitions. Chromatographic separations were on a 30 m DB5® column, 0.25 mm i.d., 0.25 µ film (Agilent, San Jose, CA, USA). Helium carrier gas flow was held constant at 1.5 mL min^{-1} and injection port temperature was 220°C. Injection was in the splitless mode. Oven initial temperature of 60°C was held 1 min after injection and increased to 250°C at 8°C/min and held for 5 min. Peak assignments and quantitation were based on analysis of serial dilutions of a commercially available FAME mixture. The FAME mixture (GLC-10) and ISTD were purchased from Matreya LLC (Pleasant Gap, PA, USA).

Determination of Physicochemical Properties

Physicochemical properties including viscosity, viscosity index, oxidative stability and Gardner color, were determined as described previously (Jarret *et al*., 2013).

Statistical Analysis

A Pearson's co-efficient analysis and an analysis of variance (ANOVA) were performed on the data. Means were separated using Tukey's Studentized Range (HSD) Test. General statistical data were generated and analyzed using Sigma Plot 13 and SAS.

Though not cultivated, data from *Solanum* species other than *S. melongena* and *S. aetheopicum* are presented for comparative purposes. Seed oil content and fatty acid composition data are available at www.ars-grin.gov.

Results and Discussion

Seed Oil Content in S. melongena and Eggplant-Related Spp.

Seed oil content in 305 genebank accessions of *S. melongena* ranged from 17.7 to 28% (mean = 23.7%, standard deviation = 2.1). Oil contents in seed of its related species *S. incanum* and *S. linnaeanum* were 22.3, 25.6 and 23.4%, respectively. Oil content in seed of the *S. aethiopicum* (scarlet eggplant), *S. anguivi* and *S. macrocarpon* were 25.3, 23.2 and 23.4%, respectively. We were unable to locate published values for seed oil content in these species and so a direct comparison cannot be made of values obtained in the present study, with previous studies. However, the average values for these species are quite close to those reported for tomato (20-23%) and pepper (ave. = 23.5%) (Giuffre *et al*., 2015; Jarret *et al*., 2013). In addition to being consumed as a vegetable (with the exception of *S. linnaeanum*), these species are reported to possess phytochemical and/or medicinal properties (Oyeyemi *et al*., 2015).

Seed Oil Content in Miscellaneous Solanum Spp.

It has been suggested that the genus *Solanum* contains approximately 1500 to 2000 species (Vander Burgt and van Medenbach de Rooy, 1996)-though the actual number is unknown. Limited research has been conducted on many of the non-cultivated species; yet, some data are available. Nolasco *et al*. (2001) reported a yield of 20.6% from seed of *S. sisymbriifolium*. The four genebank accessions of *S. sisymbriifolium* included in the present study averaged 29.3%. Dhellot *et al*. (2006; Nzikou *et al*., 2007) reported oil yields of between 34.5 to 37.5% and 37.1 to 38.8%, respectively, from seed of *S. nigrum*. (synonym-*S. americanum* Mill.). The six samples of *S. amercanum* available for analysis in the current study averaged 34% oil. Other reports in the literature include *S. elaeagnifolium* Cav (2.95% -Feki *et al*., 2013), *S. ferox* L. (2.7%- Garg and Gupta, 1966), *S. platanifolium* Hook. (6.5% -Puri and Bhatnagar, 1975) and *S. argentinum* Bitter and Lillo (21.1% -Lucini *et al*., 1994). Unfortunately, seed of these later species were not available for analysis. Zygadlo (1994) analyzed sterols in seed of 13 *Solanum* species-but oil yields were not determined. Our analysis of the oil content of the 25 remaining misc. *Solanum* spp. revealed maximum and minimum values of 27.5 and 42.8%, respectively and with a mean of 26.5% ±7.2. The highest oil content was found in seeds of *S. aviculare* (42.8%) and *S. laciniatum* (35.8%). *Solanum aviculare* is a soft-wooded Australian species sometimes referred to as poropora.

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Table 2. Fatty acid composition (% of total +/- standard deviation) in seed of eggplant (*Solanum melogena*), five related species, pepper (*Capsicum annuum*) and tomato (*Solanumlycopersicum*)

Taxa	No. accessions	16:0	18:0	18:1n-9	18:2n-6	18:3n-6
Solanum aethiopicum	10	9.7 (0.8)	4.1 (0.4)	16.5 (3.6)	67.8 (4.1)	1.0 (0.03)
Solanum anguivi	4	9.8 (0.6)	3.8 (0.3)	14.4 (3.1)	70.5 (3.1)	0.90 (0.06)
Solanum incanum	4	9.6 (0.07)	3.8 (0.2)	15.2 (0.9)	69.8 (1.7)	0.85 (0.05)
Solanum linnaeanum	3	9.4 (0.6)	3.7 (0.4)	15.6 (1.8)	70.1 (2.3)	0.98 (0.7)
Solanum macrocarpon	2	8.6 (0.3)	4.0 (0.4)	16.2 (2.3)	69.5 (3.1)	1.00 (0.03)
Solanum melogena	55	9.68 (0.6)	3.9 (0.4)	16.7 (2.8)	68.3 (2.8)	0.98 (0.1)
Capsicum annuum[Z]	80	13.4 (1.34)	3.6 (0.6)	6.6 (1.1)	76.1 (1.5)	0.30 (0.1)
Solanum lycopersicum L.[y]	8	14.8 (0.5)	3.1 (0.1)	20.48 (1.7)	56.7 (14)	1.99 (0.3)

[Z]Jarret *et al*. (2013)
[Y]Giuffre *et al*. (2015)

It is a coastal lowland shrub bearing (toxic) green immature fruit and yellow to orange mature fruit. *Solanum laciniatum* (Tasmanian kangaroo apple) is a closely related species, also native to Australia and New Zealand. The lowest average oil content was found in *S. capsicoides*, a weed species native to eastern Brazil.

Fatty Acids in Seed of S. melongena and its Related Spp.

Principal fatty acids in seed of all *S. melongena* accessions and in its five related species (Table 2) were C18:2n-6 > C18:1n-9 > C16:0 > C18:0 > C18:3n-6. Also detected were traces (< 0.25%) of C14:0, C16:1n-7, C20:0, C22:0; C24:0 and C26:0. Ranges for each of the fatty acids for the 55 *S. melongena* accessions examined were 8.4 to 11.2% (C16:0), 2.8 to 4.7% (C18:0), 11.8 to 23.7% (C18:1n-9) and 61.3 to 74.0% (C18:2n-6). The average values presented in Table 1 are similar to those reported by Kaymak (2014) who noted concentrations of C18:2n-6, C18:1n-9, C16:0 and C;18:0 in seed of eggplant cv. Pala-49, as 74.2, 12.6 and 7.9%, respectively. Kaymak (2014) did not report the presence of C18:3n-6, but did detect traces of C15:1 and C17:0. These latter two were not detected in the current study. Grosso *et al*. (1991) had earlier reported on the fatty acid composition of a variety of *Solanum* species, including *S. melongena*. Concentrations of C18:2n-6, C18:1n-9, C16:0 and C18:0 were determined to be 69.2, 17.3, 9.6 and 2.9%, respectively. Also detected were trace amounts of C14:0, C16:1, C18:3, C20:0, C22:0 and C24:0, in agreement with the results of the current analyses.

Data on the seed fatty acid composition of eggplant-related species is lacking in the scientific literature. As noted previously, Grosso *et al*. (1991) examined the fatty acid composition of various *Solanum* species including the common cultivated eggplant. However, no eggplant-related species were included in that study. They did however, note the uncommon occurrence of high (> 1.0%) levels of C14:0 in *S. diflorum* Vell. and *S. elaeagnifolium* Cav.Data on fatty acid composition for more distantly related species such as *S. elaeagnifolium*, *S. argentinum*, *S. platanifolium*, *S. lycopersicon* and others have been published (Puri and Bhatnagar, 1975; Grosso *et al*., 1991; Lucini *et al*., 1994; Lazos *et al*., 1998; Ceron *et al*., 2012; Feki *et al*., 2013). As Table 2 indicates, the fatty acid composition of the five eggplant-related species examined does not differ significantly from that of *S. melongena*. In all instances, C18:2n-6 was the predominant fatty acid followed by C18:1n-9, C16:0 and C18:0. The high concentration of linoleic acid makes these oils susceptible to oxidation (Feki *et al*., 2013). Inadequate seed supplies prevented the analysis of the full range of species listed in Table 1.

We examined several physicochemical parameters of the seed oil of eggplant cv. Black Beauty-a widely planted and commercially significant cultivar. 'Black Beauty' seed oil had a pour point of -12°C, viscosities of 28.8 (40°C) and 7.3 (100°C), a viscosity index of 240, an oxidation onset temperature of 160°C and a Gardner Color of 6+. These may be compared to oil extracted from seed of pepper cv. California Wonder which had a pour point of -9°C, viscosities of 25.1 and 6.6 at 40 and 100°C, respectively, a viscosity index of 239 and a Gardner color of 6+ (Jarret *et al*., 2013).

Conclusion

Seed oil of eggplant varied from 17.2 to 28.0% among 305 genebank accessions, with a mean of 23.7%. Seed oil content in other *Solanum* species varied from 11.8% in *S. capsicoides* to 44.9% in *S. aviculare*. The predominant fatty acid in seed of eggplant and several of its related species was in the order of: Linoleic acid >oleic acid > palmitic acid. Levels of linoleic acid ranged from 57% in *S. aethiopicum* to 74.5% in *S. anguivi*. Oleic and palmitic acid levels ranged from 11.3% in *S. anguivi* to 25.2% in *S. aethiopicum* and from 8.4% in 11.2 in *S. melongena* to 11.2%, respectively. Oil extracted from seed of *S. melongena* cv. Black Beauty had a pour point of -12°C, viscosities of 28.8 (40°C) and 7.3 (100°C), a viscosity index of 240, an oxidation onset temperature of 160°C and a Gardner Color of 6+, similar to oil from tomato and pepper.

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Author's Contributions

Robert Jarret: Planned the experiment, conducted the NMR analysis and coordinated the preparation of the manuscript.

Irvin Levy: Prepared the oil standards and contributed to the preparation of the manuscript.

Thomas Potter: Conducted the FAME analysis and contributed to the preparation of the manuscript.

Steven Cermak: Conducted the physicochemical properties analyses and contributed to the preparation of the manuscript.

Ethics

This article presents original and unpublished material. The corresponding author confirms that all of the authors have read and approved the manuscript and that no ethical issues are involved.

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