Seed oil content and fatty acid composition in a genebank collection of *Cucurbita moschata* Duchesne and *C. argyrosperma* C. Huber

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Abstract

Data on intra-specific variability for seed oil content, physical characteristics and fatty acid composition in *Cucurbita moschata* and *Cucurbita argyrosperma* are lacking in the scientific literature. We examined 528 genebank accessions of *C. moschata* and 166 accessions of *C. argyrosperma* – which included members of both subsp. *argyrosperma* and subsp. *sororia* – for seed oil content, oil physical characteristics and fatty acid composition. The oil of both species had near-identical viscosities, viscosity indices, colour and oxidative stabilities while the oil of *C. argyrosperma* had a slightly higher pour point, cloud point, percentage of free fatty acids and acid value when compared with *C. moschata*. Mean oil content values of the two species were similar at 28.7 ± /2.7 and 29.8 ± /2.6% for *C. moschata* and *C. argyrosperma*, respectively. The mean seed oil content of *C. argyrosperma* subsp. *argyrosperma* var. *palmeri* (32.1%) was significantly higher than that of the other taxa examined. The average (mean) percentage of total seed weight attributable to the kernel was 77.2% in *C. moschata* ($n = 34$) and 74.5% in *C. argyrosperma* ($n = 46$). The percentage of total seed weight attributable to the hull was correlated with seed oil content, in both species. Linoleic was the predominant fatty acid in all the samples analysed. Means for individual fatty acids in *C. moschata* were linoleic 48.5%, oleic 22.6%, palmitic 20.7% and stearic 7.5%. Means for individual fatty acids in *C. argyrosperma* were linoleic 47.3%, oleic 27.5%, palmitic 16.5% and stearic 8.0%.

Keywords: biodiversity; *Cucurbita mixta*; cushaw; distributions; physical properties; pumpkin; squash; var. *callicarpa*; subsp. *sororia*; var. *stenosperma*

Introduction

Pumpkins (*Cucurbita* spp.) are cultivated throughout the world for their food, seed oil and medicinal value. The term 'pumpkin' is commonly applied to various species

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within the genus *Cucurbita* (Ferriol and Pico, 2008). These include *C. pepo* L., *C. moschata* Duchesne, *C. maxima* Duchesne, *C. argyrosperma* C. Huber and *C. ficifolia* Bouche, although it has been applied to other species of *Cucurbita* and to members of other genera (i.e. Caili *et al.*, 2006; Stevenson *et al.*, 2007). The seed oil of these *Cucurbita* spp. is of local and regional importance. Pumpkin oil is highly digestible (Jacks *et al.*, 1972) and grown on a semi-industrial scale in Yugoslavia (Earle *et al.*, 1960; Markovic and Bastic, 1976), Austria, Hungary, the former USSR and India (Schormuller, 1969; Lal *et al.*, 1983). Pumpkin seed oil is credited with having anti-hypertension, anti-tumour, immune modulation, antibacterial, anti-hypercholesterolaemia, anti-inflammatory and analgesic properties (Caili *et al.*, 2006).

C. moschata is a tropical herbaceous annual (Saade and Hernandez, 1995) with fruits that vary considerably in size and shape. Current data suggest that *C. moschata* originated in the lowlands of northern South America from an ancestral species closely related to *C. argyrosperma* (Ferriol and Pico, 2008). The history of this species in cultivation can be traced to 3000 BC in coastal Peru and 2000 BC in Guatemala (Ferriol and Pico, 2008). *C. moschata* is the most frequently consumed vegetable in Korea (Kim *et al.*, 2012). Seeds of *C. moschata* contain high levels of various minerals and oil (Madaan and Lal, 1984; Fokou *et al.*, 2004, 2009) in addition to all nine essential amino acids (Kim *et al.*, 2012). Seeds of *C. moschata* cv. Butternut have been reported to have an oil content of approximately 33.5% (Bemis *et al.*, 1967). Linoleic acid is typically the predominant fatty acid (Fokou *et al.*, 2009). Numerous commercial varieties have been developed (Ferriol and Pico, 2008).

C. argyrosperma is a complex of domesticated, feral and wild forms closely related to *C. moschata* (Merrick, 1990). *C. argyrosperma and C. moschata* were originally considered a single species. However, two subspecies and four varieties of *C. argyrosperma* are now recognized (Merrick, 1990). Each of the subspecies and varieties has a distinctive seed morphology (Merrick, 1990). Seeds of both subspecies are consumed (Merrick, 1990). Oil content in the seed of three varieties of *C. argyrosperma* ranged from 30.6 to 43.0% (Applequist *et al*, 2006). The principal fatty acid is typically linoleic (Stevenson, 2007). Despite its long history of cultivation, relatively few varieties of *C. argyrosperma* have been developed (Ferriol and Pico, 2008).

Although there is a substantial amount of data available on pumpkin seed oil, there are considerably less data available on seed oil content within and among individual species of *Cucurbita*. This study was undertaken in order to examine inter- and intraspecific variability for seed oil content and fatty acid composition within a genebanks' collections of *Cucurbita moschata* and *C. argyrosperma*.

Materials and methods

Plant material

All seeds used in this study were obtained from the USDA/ARS Plant Germplasm Collection in Griffin, GA (Jarret *et al.*, 1990). Individual taxa were identified by R.L.J. and L.C.M. based on field observations, examination of seed or digital images of seed, and/or herbarium records. Prior to analysis, all seeds (stored at $-20°C$ in foil pouches) were brought to room temperature for a minimum of 24 h. All analyses were conducted on intact seed, unless noted otherwise. Materials analysed in this study included: *C. moschata* Duchesne; *C. argyrosperma* C. Huber subsp. *argyrosperma* var. *argyrosperma*; *C. argyrosperma* subsp. *argyrosperma* var. *callicarpa* L. Merrick & D. M. Bates; *C. argyrosperma* subsp. *argyrosperma* var. *palmeri* (L. H. Bailey) L. Merrick & D. M. Bates; *C. argyrosperma* subsp. *argyrosperma* var. *stenosperma* (Pangalo) L. Merrick & D. M. Bates; *C. argyrosperma* subsp. *sororia* (L. H. Bailey) L. Merrick & D. M. Bates.

Preparation of oil standards

Oil standards were prepared from *C. moschata* cv. Waltham Butternut [Stokes Seed Company (Lot No. 200854), Buffalo, New York, USA] and *C. argyrosperma* cv. green-striped cushaw (Eden Brothers, Dahlonega, GA, USA) essentially as described by Jarret *et al.* (2011). For each of these, 200 g of dried seed were ground to a fine powder in a coffee mill (Black & Decker Model CBM205 – medium setting) and the powder transferred to a 1-litre round bottom flask. To the flask was added sufficient heptane (Acros Organics) to bring the volume of the mixture to ca. 500 ml. The flask was sealed, transferred to a rotary shaker (Thermolyne AROS 160) and the contents mixed for 24 h. The mixture was then allowed to settle for several hours and then twice vacuum filtered through Fisher Scientific P5 (Atlanta, GA, USA) filter paper. The filtrate was concentrated by rotary evaporation, yielding a yellow oil. Yields were typically 18–20% oil by seed weight.

TD-NMR analysis

Seed oil and moisture measurements were carried out by Time Domain Nuclear Magnetic Resonance (TD-NMR)

essentially as described by Krygsman and Barrett (2004) and Jarret *et al.* (2011) on a Bruker (Madison, WI, USA) mq10 Minispec NMR operating at a resonance frequency of 9.95 MHz and maintained at 40°C. For each signal acquisition, spin-echo parameters consisted of a 90° pulse of 10.44 μs and reading at 50 μs followed by a 180° pulse of 21.38 μs (pulse spacing = variable) and reading at 7 ms. A 2 s recycle delay between scans was used, and a total of 16 scans were collected for each sample. Bulk seed measurements were made in a 40 mm glass sample tube, and NMR signals were compared with oil and moisture calibration curves, generated by sample weight. All samples were measured in triplicate and the results were averaged. Moisture standards were prepared using seeds of known moisture content and calculating the mass of water present in different seed lots. Moisture content was predetermined by measuring the differences in masses of seeds before and after baking at 130°C for 3 h. All NMR oil analyses were conducted using separate seed samples drawn from the available inventory. Seeds were drawn from the 01 (first regeneration) inventory of each accession, unless noted otherwise. Seed oil in the samples of *C. moschata* was determined utilizing a standard curve prepared from oil extracted from cv. Waltham Butternut. Seed oil in the samples of *C. argyrosperma* was determined utilizing a standard curve prepared from oil extracted from cv. green-striped cushaw.

Isolation and analysis of fatty acids

For isolation of fatty acids, replicates of 50 seed samples were ground to a fine powder in a coffee bean mill. Approximately 50 mg of ground powder were transferred into a 16 × 100 mm test tube, and 5.0 ml of *n*-heptane (Fisher Scientific) were 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 (Fisher) were added to the test tube and mixed with the sample. The reaction was allowed to proceed for 2 h. Then, 7 ml of distilled water were 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 hexane containing 25 μg/ml of methyl nonadecanoate (C19:0) and analysed with a ThermoQuest Finnigan DSQII GC-MS system (ThermoFisher, San Jose, CA, USA). C19:0 was used as an internal standard. The mass spectrometer was operated in the electron impact mode and scanned at $m/z = 50$–400 during data acquisitions. Chromatographic separations were performed on a 30 m DB5®

column (0.25 mm inner diameter, 0.25 μm film thickness; Agilent, San Jose, CA, USA). Helium carrier gas flow was held constant at 1.5 ml/min and injection port temperature was 220°C. Injection was in the splitless mode. An oven initial temperature of 60°C was held for 1 min after injection and increased to 250°C at 8°C/min and held for 5 min. Peak assignments and quantification were based on the analysis of serial dilutions of a commercially available FAME mixture. The FAME mixture (GLC-10) and internal standard were purchased from Matreya LLC (Pleasant Gap, PA, USA). Data were collected on C16:0 (palmitic), C18:0 (stearic), C18:1 (oleic) and C18:2 (linoleic) acids, which in total accounted for more that 97% of the FAME present.

Determination of physical properties

Various physical properties of the oils used as standards (*C. moschata* cv. Waltham Butternut and *C. argyrosperma* cv. green-striped cushaw) were evaluated.

Pour point

Pour points were measured in accordance with Method D 97-96a (American Society for Testing Materials, 1996) to an accuracy of ± 3°C. The pour points were determined by placing a test jar with 50 ml of the sample into a cylinder submerged in a cooling medium. The sample temperature was reduced in 3°C increments at the top of the sample until the material stopped pouring. The sample no longer poured when the material in the test jar did not flow when held in a horizontal position for 5 s. The temperature of the cooling medium was chosen based on the expected pour point of the material. Samples with pour points that ranged from +9 to −6, −6 to −24 and −24 to −42°C were placed in baths of temperatures −18, −33 and −51°C, respectively. The pour point was defined as the coldest temperature at which the sample still poured. All pour points were determined in duplicate and the average values are reported.

Cloud point

Cloud points were determined in accordance with Method D-2500-99 (American Society for Testing Materials, 1999) to an accuracy of ± 1°C. The cloud points were determined by placing a test jar with 50 ml of the sample into a cylinder submerged into a cooling medium. The sample temperature was reduced in 1°C increments until any cloudiness was observed at the bottom of the test jar. The temperature of the cooling medium was chosen based on the expected cloud point of the material. Samples with cloud points that ranged from room temperature to 10, 9 to −6, and −6 to −24, −24 to −42°C were placed in baths of temperatures 0, −18, −33 and −51°C, respectively.

Table 1. Physical properties of the seed oil from *Cucurbita moschata* cv. Waltham Butternut and *Cucurbita argyrosperma* cv. green-striped cushaw

Traits	*C. moschata*	*C. argyrosperma*
Pour point (°C)	− 9	− 6
Cloud point (°C)	− 3	− 1
Viscosity		
0°C	13.8	13.9
40°C	4.7	4.5
Viscosity index	311	279
Gardner colour	6 +	7
Free fatty acids (%)	0.363	0.534
Acid value (mg/g)	0.722	1.064
Oxidative stability (°C)	150.45	150.56

All cloud points were determined in duplicate and the average values are reported.

Viscosity and viscosity index

Viscosity measurements were made using calibrated Cannon–Fenske viscometer tubes purchased from Cannon Instrument Co. (State College, PA, USA). Viscosity measurements were made in a Temp-Trol (Precision Scientific, Chicago, IL, USA) viscometer bath set at 40.0 and 100.0°C. Viscosity and *viscosity index* (VI) were calculated using American Society for Testing Materials Methods D 445-97 (1997) and D 2270-93 (1998), respectively. All viscosity measurements were run in duplicate and the average values are reported.

Oxidative stability

Pressurized-differential scanning calorimetry (P-DSC) analyses were conducted with a TA Instruments (New Castle, DE, USA) model DSC 2910 fitted with an HP 2910 model high-pressure DSC cell (maximum 7 MPa). A model 5000 personal computer-based controller was used for data acquisition and determination of oxidation onset temperature (OT). Purge gas outside the cell was low-pressure nitrogen. All scans were conducted with the cell pressurized with oxygen to 3000 ± 50 kPa

(440 ± 7 psig). A spring-action purge valve was fitted to the exhaust line to keep the cell at constant pressure during heating. P-DSC analyses were conducted using hermetically sealed aluminium pans with an ∼0.5-mm-diameter pinhole punched in the top cover to allow direct contact between the sample and pressurized oxygen. Samples were analysed simultaneously with an identical empty pan. OT data reported in this work are means determined from replicate scans on three fresh samples.

In dynamic (positive gas flow) mode, the cell was pressurized and then sealed off. After the cell was equilibrated at 25°C, the inlet valve was opened wide and the outlet valve cracked slightly open to allow a steady flow of oxygen through the cell. Oxygen flow rate was set manually to 100 ± 10 ml/min and monitored by a calibrated gas flow meter connected downstream from the cell outlet valve. Once oxygen flow was established and pressure restabilized, the cell was equilibrated at 30°C and then heated with a ramp rate of 10°/min to a terminal temperature of 300°C. Sample mass for dynamic mode scans was 1.50 mg.

Acid value (AV) and free fatty acids (FFA)

A 751 GPD Titrino from Metrohm Ltd (Herisau, Switzerland) was used for measurements. AVs and percentage of FFA were determined by the official AOCS Method Te 2a-64 with ethanol substituted for methanol to increase the solubility of the estolide ester during the titration (Firestone, 1994). All AVs were run in duplicate and the average values are reported.

Gardner colour

Gardner colour was measured on a Lovibond 3-Field Comparator from Tintometer Ltd (Salisbury, England) using AOCS Method Td 1a-64. The Gardner colour scale is from 1 to 18 with 1 containing the least amount of colour and 18 with the maximum amount of colour. In many cases, the Gardner colour of materials can be

Table 2. Percentage of oil content and related general statistics on the seed of *Cucurbita moschata* and *Cucurbita argyrosperma*

Genus/species	N	% Oil	Minimum	Maximum	Range	Std. Dev.
C. moschata	528	28.7[B]	15.8	34.2	18.4	2.7
C. argyrosperma						
subsp. *argyrosperma*						
var. *argyrosperma*	63	29.4[B]	24.4	32.9	8.5	1.8
var. *callicarpa*	41	29.1[B]	19.9	34.7	14.8	2.9
var. *palmeri*	23	32.1[A]	27.5	36.3	8.8	2.3
var. *stenosperma*	14	29.5[B]	26.1	34.7	8.6	2.4
subsp. *sororia*	25	29.0[B]	23.3	32.3	9.0	2.6

[A,B]Mean values within columns followed by the same capital letters are not significantly different ($\alpha = 0.05$) based on Tukey's studentized range (HSD) test.
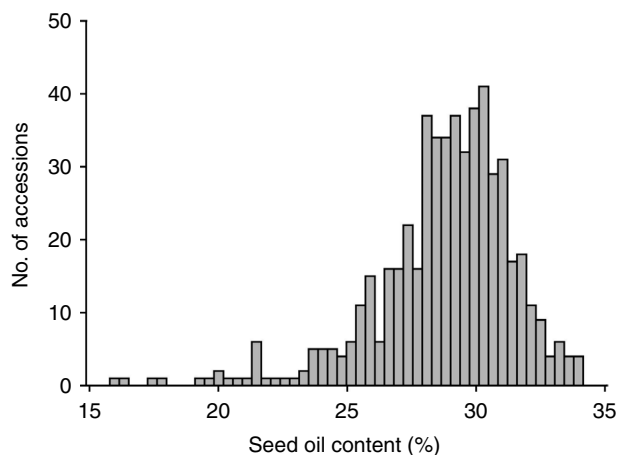
Fig. 1. Frequency histogram depicting the distribution of seed oil content in the 528 genebank accessions of *Cucurbita moschata*.

susceptible to the interpretation of the recorder, thus the + and − notation was employed to designate samples that did not match one particular Gardner colour.

Determination of the hull/kernel ratio

Representative examples of each species having high, low and intermediate seed oil content values were utilized. For each accession, five intact seeds were divided into hull and kernel, and each portion weighed. This process was replicated four times (total of 20 seeds/accession) and the hull/kernel ratios calculated. Thirty-four accessions of *C. moschata* and 46 accessions of *C. argyrosperma* were thus analysed.

Statistical analysis

Pearson's coefficient analysis was performed to determine significant correlations. An analysis of variance was performed on the data, and means were separated using Tukey's studentized range Honestly Significant Difference (HSD) test. General statistical data were generated and analysed using SigmaPlot 11.2 and SAS.

Results

Physical properties of the seed oil standards

The physical characteristics of the oils from *C. moschata* (cv. Waltham Butternut) and *C. argyrosperma* (cv. green-striped cushaw), used as standards for the TD-NMR oil analysis, were generally similar (Table 1) to near-identical viscosities, VIs and colour. The pour point values of *C. moschata* and *C. argyrosperma* oils

were − 9 and − 6°C, respectively. The oil of the *C. moschata* sample had a slightly lower pour point, cloud point, FFA value and AV when compared with *C. argyrosperma*. The P-DSC OT (as a measure of oxidative stability) for both samples was near 150°C. VIs for both samples were also similar at 311 and 279 for *C. moschata* and *C. argyrosperma* seed oils, respectively. Seed oil estimates (via TD-NMR) were 31.6 and 28.2% for cvs. green-striped cushaw and Waltham Butternut, respectively. The fatty acid profiles of both cultivars were likewise similar [Waltham Butternut: palmitic (17.7%), stearic (6.4%), oleic (29.2%) and linoleic (45.5%) vs. green-striped cushaw: palmitic (11.3%), stearic (7.9%), oleic (34.4%) and linoleic (45.4%)] but differed primarily in their concentration of palmitic acid.

Seed oil content and hull/kernel ratio

Seed oil content in the (entire) seed of 528 accessions of *C. moschata* and 166 accessions of *C. argyrosperma* is presented in Table 2. The mean oil content values for both species were similar at 28.7±/-2.7 and 29.8 ± /-2.6% for *C. moschata* and *C. argyrosperma* (all subspecies and varieties combined), respectively ($P = 0.337$). However, the seed oil content of *C. argyrosperma* subsp. *argyrosperma* var. *palmeri* (32.1%) was significantly higher than that of other *C. argyrosperma* subsp. *argyrosperma* varieties, *C. argyrosperma* subsp. *sororia* and *C. moschata*. Ranges in oil content within species were also similar [*C. moschata* − 15.8% (PI 442269) to 34.2% (PI 494850); *C. argyrosperma* − 19.9% (PI 511899) to 36.3% (PI 511987)]. The distributions of seed oil content were likewise similar for both species (Figs 1 and 2).
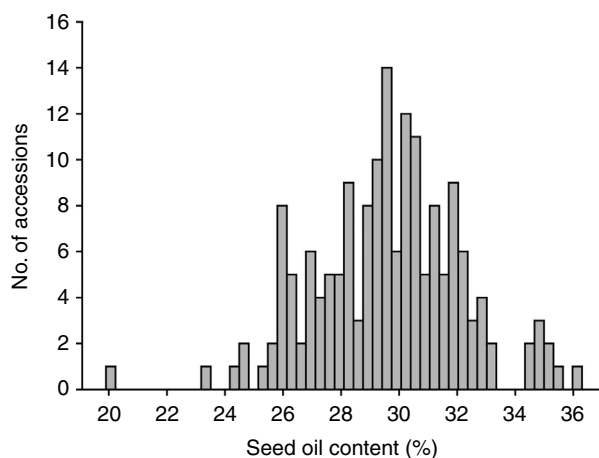

Fig. 2. Frequency histogram depicting the distribution of seed oil content in the 166 genebank accessions of *Cucurbita argyrosperma*.

Table 3. One hundred-seed weight (mean) and related general statistical data on the seed of *Cucurbita moschata* and *Cucurbita argyrosperma* genebank accessions

Genus/species	N	100-Seed weight (g)	Minimum	Maximum	Range	Std. Dev.
C. moschata	528	12.61[C]	3.86	34.18	32.55	4.02
C. argyrosperma						
subsp. *argyrosperma*						
var. *argyrosperma*	63	23.86[A]	14.05	40.68	26.63	6.12
var. *callicarpa*	41	18.93[B]	9.82	30.23	20.41	0.80
var. *palmeri*	23	7.86[D]	3.98	12.57	8.58	2.51
var. *stenosperma*	14	20.78[B]	17.41	24.73	7.31	0.59
subsp. *sororia*	25	4.00[E]	1.90	7.84	5.93	0.26

[A,B,C,D,E]Mean values within columns followed by the same capital letters are not significantly different ($\alpha = 0.05$) based on Tukey's studentized range (HSD) test.

In our sampling of the 34 accessions of *C. moschata* and the 46 accessions of *C. argyrosperma*, the means for the percentage of hull were 22.7 ± 5.2 and $25.5 \pm 4.2\%$ for these species, respectively. A significant difference was detected between these means ($P = 0.013$). Seed oil content was positively correlated with the percentage of hull (as the percentage of total seed weight) in both *C. moschata* ($R^2 = 0.585$) and *C. argyrosperma* ($R^2 = 0.386$), respectively. The percentage of total seed weight attributable to the kernel varied within and between ($P = 0.011$) species. The average percentage of total seed weight attributable to the kernel ranged from 62.0 to 85.3% (mean 77.3%) in *C. moschata* and from 65.1 to 80.4% (mean 74.5%) in *C. argyrosperma*. No correlation was found between 100-seed weight and oil content for either *C. moschata* ($R^2 = 0.065$) or *C. argyrosperma* ($R^2 = 0.051$). Significant differences in the 100-seed weight values of the various taxa examined were also detected (Table 3).

Fatty acids

The mean concentrations of individual fatty acids in the seed of *C. moschata* are indicated in Table 4. The mean values of individual fatty acids in the seed of *C. moschata* ($n = 38$) were as follows: palmitic 20.7%; stearic 7.5%; oleic 22.7%; linoleic 48.5%. Among these same materials, low and high values (and ranges) for each were as follows: linoleic 24.7–61.7% (37.0%); oleic 10.0–53.8% (43.8%); palmitic 13.6–27.8% (14.2%); stearic 3.7–9.5% (5.8%); arachidonic 0.2–0.6% (0.4%). Average concentrations of palmitic acid were significantly higher in the seed of *C. moschata* when compared with *C. argyrosperma* (Table 4). Published values for individual fatty acids present in *C. moschata* seed, as previously cited, fall within the ranges reported here, though the ranges presented in this study are larger than those in earlier reports.

The mean concentrations of individual fatty acids in the seed of *C. argyrosperma* are also indicated in Table 4. Linoleic acid was the predominant fatty acid present in the seed of *C. argyrosperma*, reaching both a maximum (62.3%) and a minimum (25.4%) in subsp. *argyrosperma* var. *callicarpa* (range 36.8%). Concentrations of palmitic acid were highest (19.0%) in var. *argyrosperma* (range 4.9) and lowest (12.1%) in var. *callicarpa* (range 6.8). Concentrations of stearic acid were similar among all taxa (including *C. moschata*),

Table 4. Principal fatty acids (mean %($\pm$ SD)) in the seed of genebank accessions of *Cucurbita moschata* and *Cucurbita argyrosperma*

Genus/species	N	Palmitic	Stearic	Oleic	Linoleic
C. moschata	38	20.74 (2.52)[A]	7.47 (1.43)[A]	22.66 (8.94)[B]	48.52 (8.37)[A,B,C]
C. argyrosperma					
subsp. *argyrosperma*					
var. *argyrosperma*	15	17.41 (1.17)[B]	8.17 (0.92)[A]	24.31 (8.94)[A,B]	49.56 (5.13)[A,B,C]
var. *callicarpa*	9	14.96 (2.23)[C]	7.55 (1.19)[A]	32.21 (5.25)[A]	44.77 (10.71)[B,C]
var. *palmeri*	7	15.43 (2.14)[B,C]	8.04 (0.92)[A]	32.07 (11.07)[A]	44.00 (9.04)[C]
var. *stenosperma*	3	16.56 (0.71)[B,C]	8.27 (0.98)[A]	20.66 (1.23)[B]	54.23 (1.10)[A]
subsp. *sororia*	10	17.57 (1.79)[B]	7.96 (1.72)[A]	21.34 (5.52)[B]	52.61 (6.82)[A,B]

[A,B,C]Mean values within columns followed by the same capital letters are not significantly different ($\alpha = 0.05$) based on Tukey's studentized range (HSD) test.

and concentrations of oleic acid reached a maximum of 49.0% and a minimum of 12.7% in the seed of var. *callicarpa*. Concentrations of oleic acid and linoleic acid were significantly higher in the seed of *C. argyrosperma* subsp. *argyrosperma* var. *palmeri* and *C. argyrosperma* subsp. *argyrosperma* var. *stenosperma*, respectively. However, we acknowledge that the number of accessions examined for each taxon varied widely (i.e. var. *argyrosperma*, $n = 15$; var. *stenosperma*, $n = 3$).

Discussion

Pour point values observed for *C. moschata* and *C. argyrosperma* were similar to each other and similar to or identical with pour point values observed for canola ($-9°C$), soyabean ($-9°C$), safflower ($-6°C$) and meadowfoam ($+3°C$) seed oils (Asadauskas and Erhan, 1999). Cloud point (phase separation) values for *C. moschata* ($-3°C$) and *C. argyrosperma* ($-1°C$) were similar to those of canola ($-2°C$) and sunflower ($-1.0°C$) oils (Knothe *et al.*, 2005). Though the lower temperature properties of these and other vegetable oils make them less attractive for use as industrial lubricants, they have qualities such as high VI that make them ideal as industrial fluids. Petroleum oil typically has a VI of 90–100, significantly lower than that of soyabean oil ($VI = 222$). Since the viscosity of an oil with a higher VI changes less than that of an oil with a lower VI for a given change in temperature, for the purposes of use as an industrial fluid, a higher VI is desirable. The high VI values observed in the present study suggest that these oils have potential as industrial fluids.

The seed oils from *C. moschata* and *C. argyrosperma* were similar in colour, being somewhat darker than peanut oil which typically has a Gardner colour value of ∼4 (Dean *et al.*, 2011). AV is a measure of the FFA present in the oil. It is used as a measure of oil quality, lower values being preferable to higher. Fokou *et al.* (2009) reported a range in the acid index values from 1.77 to 7.81 mg KOH/g oil (mean 4.31 ± 2.69) for four varieties of *C. moschata* grown in Cameroon. Chemical refining could be used to improve the AV, FFA and colour values (Evangelista, 2005). In general, P-DSC OTs range from 143 to 178°C for most vegetable oils (Adhvaryu *et al.*, 2000). The cucurbits examined in this study had a P-DSC OT (∼150°C) similar to that of cotton seed oil (149.9°C). Oxidative stability is affected by various factors such as the percentage of FFA present and the process used to remove the oil. Many seeds have natural antioxidants that provide resistance to oxidation. The relatively high oxidative stability of oils of *C. moschata* and *C. argyrosperma* is indicative of their potential for use in cooking and/or industrial applications. Inter- and intra-specific variability for these and other physical characteristics is to be expected, and it would be premature to suggest that the values presented are fully representative of the taxa examined.

The mean seed oil content values reported here are similar to those reported by Bemis *et al.* (1967) who noted an average of 33.5% oil in the (apparently entire) seed of *C. moschata* (cv. Butternut), 31.2% in the seed of cv. Seminole Pumpkin (*C. argyrosperma*) and 37.4% in the seed of *Cucurbita mixta* (syn. *C. argyrosperma*) cv. Tucson 2. The literature contains a variety of other reports on oil content in *C. moschata* and, to a lesser extent, *C. argyrosperma*. However, data on the seed oil content of individual subspecies and varieties of *C. argyrosperma* do not appear to be available. Many previous studies of seed oil content have utilized only kernels (decorticated seed) in their analyses, while others have examined the entire seed. Decorticated seed of *C. moschata* yielded 46.1% oil as determined by Lal *et al.* (1983). Teotia *et al.* (1989) reported an oil yield of 46.2% in the kernels of *C. moschata*, whereas Al-Khalifa (1996) reported an oil content of 43% in the seeds of this species. Fokou *et al.* (2009) reported an average oil content in the decorticated seed of four varieties of *C. moschata* as 50.8 (41.9–54.4)%, while kernals of Egyptian varieties of *C. moschata* yielded 44.5% oil (El-Aziz and El-Kalek, 2011).

In addition to the values reported by Bemis *et al.* (1967) for decorticated seed, the entire seed of *C. moschata* (four varieties) and *C. argyrosperma* (three varieties) examined by Applequist et al. (2006) yielded a range from 31.2 to 39.2% oil and from 30.6 to 43.0% in each of these species, respectively. In that study, seeds of cv. green-striped cushaw (*C. argyrosperma* subsp. *argyrosperma* var. *callicarpa*), one of the few cultivated varieties of *C. argyrosperma*, were found to contain 41.1% oil. We found the entire seed of this cv. to contain 31.2% oil. Stevenson *et al.* (2007) reported on the oil content in the seed of both *C. moschata* and *C. argyrosperma*. The values ranged from 29.1 to 43.3% and from 36.0% to 40.1% for *C. moschata* and *C. argyrosperma*, respectively. In general, published values for seed oil content in *C. moschata* were much higher than the 22.1% reported by Sae-lim *et al.* (2008) but somewhat similar to the 35.7% oil present in the entire seed of a Korean variety of *C. moschata* (Kim *et al.*, 2012). The range of oil content values reported in the present study is similar to the values reported by Madaan *et al.* (1982) in a study of 91 accessions of *Cucumis melo* L. Madaan and Lal (1984) also reported that approximately 75% of the total seed weight of *C. moschata* was attributable to the kernel and ∼25% to the hull. The range of values observed in the present study was considerably smaller than the range of values reported by Madaan *et al.* (1982) for a large number of *C. melo* accessions in which the percentage of

the total seed weight attributable to the kernel ranged from 25.0 to 74.0%. In a subsequent study of various *Cucurbita* spp. (Madaan and Lal, 1984), the percentage of total seed weight attributable to the kernel ranged from 54.5 to 75.5%.

While an extensive amount of information has been accumulated regarding the long-chain fatty acids in the seeds of pumpkins (*Cucurbita* spp.; Hopkins, 1990), information specific to *C. moschata* is somewhat less abundant. Bemis *et al.* (1967) noted the occurrence of four fatty acids in the seed of *C. moschata* cv. Butternut (palmitic 19.0%, stearic 7.0%, oleic 40.0% and linoleic 34.0%) and cv. Seminole Pumpkin (palmitic 17.0%, stearic 7.0%, oleic 50.0% and linoleic 26.0%). Al-Khalifa (1996) noted that linoleic acid was the predominant fatty acid in *C. moschata* (53.2%) followed by oleic (26.2%), palmitic (13.1%) and stearic (6.0%). Sae-lim *et al.* (2008) found the fatty acids in *C. moschata* seed to be 56.0% linoleic, 20.4% oleic, 13.4% palmitic and 9.9% stearic – similar to soyabean (46.9% linoleic, 26.0% oleic, 10.5% palmitic and 3.4% stearic). Fokou *et al.* (2009) reported an average fatty acid composition (four varieties) of *C. moschata* as 49.5% linoleic, 19.6% oleic, 19.2% palmitic and 9.2% stearic with traces (1.7%) of arachidonic (C20:0) acid. In more recent reports, Applequist et al. (2006) reported average fatty content values (four varieties) in *C. moschata* of 55.0% linoleic, 19.2% oleic, 15.8% palmitic and 6.9% stearic. Raharjo *et al.* (2011) reported that the predominant fatty acids associated with seed phospholipids were oleic and palmitic in phosphatidylcholine and phosphatidylethanolamine, and oleic and linoleic in phosphatidylserine. Kim *et al.* (2012) identified four fatty acids in the seed of *C. moschata*, these being linoleic (35.7%), oleic (31.3%), palmitic (12.8%) and stearic (7.3%). Fatty acid composition may be subject to certain environmental effects, as alluded to by Applequist et al. (2006). Thus, the ranges established for individual subspecies and varieties may be subject to modification.

Fewer published reports are available on the fatty acid content in the seed of *C. argyrosperma*, compared with *C. moschata*. Bemis *et al.* (1967) reported a fatty acid profile in the seed of *C. mixta* cv. Tucson 2 as 34.0% linoleic, 46.0% oleic, 12.0% palmitic and 8.0% stearic. Applequist et al. (2006) reported average values (three varieties) of *C. argyrosperma* as 55.0% linoleic, 19.2% oleic, 15.8% palmitic and 6.9% stearic. Previously published values for individual fatty acids present in *C. argyrosperma* seed fall within the ranges reported here. However, the literature does not contain previous reports of fatty acid composition in the seed of *C. argyrosperma* at the level of individual subspecies or varieties. Hence no comparison with previously published data is possible.

Reports in the literature regarding seed oil and fatty acid composition (and other characteristics such as medicinal properties) associated with 'pumpkins' and pumpkin seed oil do not always clearly identify the taxonomic classification of the plant materials utilized. Several of the reports on the medicinal properties associated with the use of pumpkin seed oil, as cited by Stevenson *et al.* (2007), were conducted utilizing a commercially available product marketed as 'Pepon' (Mepaco-Medifood, Egypt). The literature on this product notes that 'Pepon' contains 'pumpkin seed oil, which is composed mainly from many oils'. As noted previously, the term 'pumpkin' is commonly used to refer to *C. pepo*, *C. moschata*, *C. argyrosperma*, *C. maxima* and possibly fruit of other taxa (Stevenson *et al.*, 2007; Ferriol and Pico, 2008). Reports on seed oil content that do not identify the taxa from which the oils were obtained, or indicate whether entire or decorticated were utilized, can be difficult to interpret in relation to the published literature and can confound efforts to establish ranges.

Acknowledgements

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