AGRICULTURAL AND FOOD CHEMISTRY

Oil and Fatty Acid Contents in Seed of Citrullus lanatus Schrad.

Robert L. Jarret*,[†] and Irvin J. Levy[§]

[†]Plant Genetic Resources, Agricultural Research Service, U.S. Department of Agriculture, 1109 Experiment Street, Griffin, Georgia 30223, United States

[§]Department of Chemistry, Gordon College, 255 Grapevine Road, Wenham, Massachusetts 01984, United States

ABSTRACT: Intact seed of 475 genebank accessions of *Citrullus* (*C. lanatus* var. *lanatus* and *C. lanatus* var. *citroides*) were analyzed for percent oil content using TD-NMR. Extracts from whole seed of 96 accessions of *C. lanatus* (30 var. *citroides*, 33 var. *lanatus*, and 33 egusi), *C. colocynthis* (n = 3), *C. ecirrhosus* (n = 1), *C. rehmii* (n = 1), and *Benincasa fistulosa* (n = 3) were also analyzed for their fatty acids content. Among the materials analyzed, seed oil content varied from 14.8 to 43.5%. Mean seed oil content in egusi types of *C. lanatus* was significantly higher (mean = 35.6%) than that of either var. *lanatus* (mean = 23.2%) or var. *citroides* (mean = 22.6%). Egusi types of *C. lanatus* had a significantly lower hull/kernel ratio when compared to other *C. lanatus* var. *lanatus* or *C. lanatus* var. *citroides*. The principal fatty acid in all *C. lanatus* materials examined was linoleic acid (43.6–73%). High levels of linoleic acid were also present in the materials of *C. colocynthis* (71%), *C. ecirrhosus* (62.7%), *C. rehmii* (75.8%), and *B. fistulosa* (73.2%), which were included for comparative purposes. Most all samples contained traces (<0.5%) of arachidonic acid. The data presented provide novel information on the range in oil content and variability in the concentrations of individual fatty acids present in a diverse array of *C. lanatus*, and its related species, germplasm.

KEYWORDS: watermelon, citron, egusi, roundmelon, seed oil, TD-NMR, Citrullus rehmii, Citrullus ecirrhosus

INTRODUCTION

The plant family Cucurbitaceae is a large one, with approximately 130 genera and 900 species.¹ It contains numerous taxa that produce edible seeds or seeds used for the production of oil at the subsistence level in various African countries and in the Middle East, although none of these appear to currently be cultivated on an industrial scale.^{2,3} Within the Cucurbitaceae, two species of the genus Citrullus are of commercial interest for their oilseed-producing capacity. The first of these is Citrullus lanatus (Thunb.) Matsum. & Nakai, which includes the common watermelon (C. lanatus var. lanatus), the citron or preserving melon (C. lanatus var. citroides (L. H. Bailey) Mansf.), and the "egusi" melon, a unique form of C. lanatus, the seed of which has a fleshy pericarp. A second species, Citrullus colocynthis L. (Schrad.), is a wild perennial that thrives in desert regions⁴ and is commonly mistaken for C. lanatus.⁵ Two additional species include Citrullus ecirrhosus Cogn. and Citrullus rehmii De Winter, although these are little studied.

In addition to being a form of *C. lanatus* var. *lanatus*, the term "egusi" appears in the literature as a generic term used to denote the fat- and protein-rich seeds of certain cucurbitaceous plants that are used as a source of food and/or oil. "Egusi", in a generic sense, plays various and important roles in the food and farming systems in West Africa,⁶ India,⁷ the Mediterranean area,⁸ and elsewhere.⁹ A rather detailed discussion of the culture and socioeconomic impact of egusi seed production in West Africa (specifically Benin) was given by Achigan-Dako et al.⁶ In Benin, as in other West African countries, "egusi" may refer to seed of *C. lanatus*, *C. colocynthis*, *Cucumeropsis mannii*, certain varieties of *Lagenaria siceraria*, and other genera/species as well. In that region, egusi is often a cash crop and one associated with a variety of sociocultural roles including food production, income generation, and soil fertility management.

C. lanatus egusi (or subsp. *mucosospermus*) is the egusi of choice in Benin and Nigeria and is preferentially cultivated there both on larger holdings and in home gardens.

Seed oil yields of *Citrullus* were reported as early as 1945,¹⁰ again in 1952,¹¹ and more recently by Kamel et al.¹² Das et al.¹³ noted that yields of oil from *C. lanatus* were significantly lower than published yields of sunflower and groundnut, but higher than soybean, whereas the densities of *C. lanatus* seed oil were comparable with those of soybean, sunflower, and groundnut. They also noted very small amounts of secondary oxidation products, indicative of the high stability of that oil. Seeds of four varieties of *C. lanatus* from Ghana and Botswana yielded oil contents of from 24.8 to 28.0%. Oil of egusi (*C. lanatus*) seed was reported as being less viscous than that of most other drying oils and had a specific gravity of 0.93.¹⁴

Nolte and Loesecke¹⁵ reported the fatty acid composition of seed oil of *C. lanatus* cv. Cuban Queen as 68.3% linoleic, 13% oleic, 8.8% palmitic, and 6.6% stearic acids. Whereas earlier reports may exist, the accuracy of the taxonomic identification of the materials utilized can be difficult to ascertain.¹⁰ Dhingra and Biswas¹⁰ reported a fatty acid composition of 48.7% linoleic, 35.3% oleic, 7.6% palmitic, and 6.1% stearic and suggested *Citrullus* oil as a substitute for almond oil. T-Sao and Potts¹¹ described the predominant fatty acids in seeds of *C. lanatus* as 62% linoleic, 19% oleic, 10.5% palmitic, and 6.1% stearic. Numerous subsequent studies have examined the fatty content of *C. lanatus* seed.^{3,12–14,16–18} The ranges of individual fatty acids reported by those authors were 55.2–71.3% linoleic, 10.3–15.9% oleic, 9.9–20.8% palmitic, and 6.7–13.5% stearic.

Received:	January 4, 2012
Revised:	April 21, 2012
Accepted:	April 28, 2012
Published:	April 28, 2012

ACS Publications © 2012 American Chemical Society

Citrullus seed oil is also known to contain traces of linolenic, myristic, and lauric acids.¹⁷ Seeds of *C. lanatus* store well, and both the oil and the fatty acid contents are stable after 6 months in storage.¹⁹

The eating of watermelon seeds is a cultural trait and is common today in North African and Eastern Mediterranean countries.²⁰ In many West African countries, egusi (C. lanatus) is a major soup ingredient and a common component in daily meals.¹⁴ Ground seeds are used to thicken soups and stews. Seeds are sometimes soaked, fermented, boiled, and wrapped in leaves to form a favored food seasoning.²¹ The seeds are roasted with those of peanut and pepper and ground into an oily paste spread on kolanuts or eggplant. The seed meal can be compacted into patties that serve as a meat substitute.¹⁴ Seeds are roasted and used in beverages and the seed cake as cattle feed,¹⁹ or seeds are fried and eaten as snacks.²² Curtis⁴ noted the potential of seed oil of various cucurbits for use in soapmaking. Oil of *Citrullus* seed may have potential in the production of biodiesel,²³ whereas immature fruits are consumed as a vegetable.¹³ The egusi crop also serves as an effective groundcover, reducing erosion and conserving soil moisture.²⁴ Even the hulls of watermelon seed are useful as a raw material for the preparation of furfural.⁷

The world's production of vegetable oil is approximately 100 million metric tons, and demand is increasing due to novel nonfood uses including biofuel, oleochemicals, lubricants, pharmaceuticals, and cosmetics.¹⁷ No exact figures exist for the current production of egusi (*C. lanatus*) seed inasmuch as confusion regarding its taxonomic identification persists and because much of the crop is wild or semicultivated in remote rural areas. It is often regarded as a backyard garden crop. In 1963, it was estimated that 73000 metric tons of egusi seed was produced annually in Nigeria.²⁵ Kamel et al.¹² reported the production (in Nigeria) of >66000 metric tons of melon seeds. More recently, Giwa et al.²⁶ noted that in Nigeria, egusi (melon seed) was cultivated over an area of 361 000 ha, resulting in the production of 347 000 metric tons of seed. However, it is not clear which species were included in that production data. *C. lanatus* is also cultivated for seed in the arid and semiarid areas of the northern and western parts of India in both summer and rainy seasons, where fruit production is about 8000–12000 kg/ ha/year with fruit containing 10–12% seed by weight.²⁷

In recognition of the social and economic importance of *C. lanatus* seed oil, we examined a broad range of genebank accessions of *Citrullus* including var. *lanatus*, var. *citroides*, and egusi forms to determine the range in their seed oil contents and fatty acid profiles as a basis for future utilization and/or improvement.

MATERIALS AND METHODS

Plant Material. All seeds used in this study were obtained from the USDA/ARS Plant Germplasm Collection in Griffin, GA. 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. For the purposes of this study, we define egusi as *C. lanatus* var. *lanatus* having seeds with a thin seed coat, varying in shape and size, but generally tan/whitish in color, essentially as described and pictured by Oyolu.⁵ In addition to seed of *C. lanatus*, representative examples of *C. colocynthis* Schrad., *C. ecirrhosus, C. rehmii*, and *Benincasa fistulosa* (Stocks) H. Schaef. & S.S. Renner) (formerly *Praecitrullus fistulosus* (Stocks) Pangalo) were included in the analysis of fatty acids for comparative purposes.

Preparation of Oil Standards. Oil standards were prepared from *C. lanatus* var. *lanatus* cv. Charleston Gray and *C. lanatus* var. *citroides*

(genebank accession PI 295843) essentially as described by Jarret et al.²⁸ For each of these, 200 g of dried seed was ground to a fine powder in a coffee mill (Black & Decker model CBM205, medium setting) and the powder transferred to a 1 L 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 and transferred to a rotary shaker (Thermolyne AROS 160), and the contents were 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) 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 TD-NMR as described previously²⁸ 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 μ s and reading at 50 μ s followed by a 180° pulse of 21.38 μ s (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 to oil and moisture calibration curves, generated by sample weight. All samples were measured in triplicate, and the results were averaged. Oil standards were generated using oils extracted from C. lanatus var. lanatus cv. Charleston Gray and C. lanatus var. citroides (genebank accession PI 295843). Twelve oil standards were prepared by oil weight. 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 in triplicate using separate seed samples drawn from the available inventory. Seeds were drawn from the 01 (first regeneration) inventory of each accession, unless noted otherwise.

Determination of Hull/Kernel Ratio. Eight accessions were selected from each of three groups (*C. lanatus* var. *lanatus, C. lanatus* var. *citroides,* and egusi) on the basis of their previously determined seed oil content. Representative examples having high, low, and intermediate seed oil content values were utilized. For each accession, three intact seeds were divided into hull and kernel, and each portion was weighed. This process was replicated four times (total of 12 seeds/ accession), the hull/kernel ratios were determined, and the averages were calculated.

Isolation and Analysis of Fatty Acids. For isolation of fatty acids, replicate 20 seed samples were ground to a fine powder in a coffee bean mill. Approximately 150 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, 500 μ L of 0.5 M sodium methoxide in methanol solution was added to the test tube and mixed with the sample. The reaction was allowed to proceed for 2 h. Seven milliliters 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. The fatty acid composition was determined using a Hewlett-Packard (HP) 5890 series II gas chromatograph (GC) equipped with a flame ionization detector (FID) and an HP-7673 autosampler. A fatty acid methyl ester (FAME) standard mix RM-3 (Sigma Chemical Co., St. Louis, MO, USA) was used to establish peak retention times for palmitic (16:0), stearic (18:0), oleic (18:1), and linoleic (18:2) acids. The peak separations were performed on a DB-225 capillary column (15 m \times 0.25 mm i.d. with a 0.25 μ m film, Agilent Technologies, Santa Clara, CA, USA). The carrier gas was helium set to a flow rate of ~1.0 mL/min. One microliter of sample was injected onto the column maintained isothermally at 200 °C, with an injection temperature of 280 °C and a detection temperature of 300 °C. The total run time for each sample was 12 min. Fatty acid composition was determined by identifying and calculating relative peak areas.

Journal of Agricultural and Food Chemistry

Statistical Analysis. A 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 (HSD) test. General statistical data were generated and analyzed using SigmaPlot 11.2 and SAS.

RESULTS AND DISCUSSION

Seed oil content varied widely within and among the groups of materials analyzed. As indicated in Table 1, seed of the egusi

 Table 1. Descriptive Statistics for Percent Oil Content in

 Seed of Citrullus lanatus

variety/form	n	mean ^a	SD	range	max	min	median
var. lanatus	293	23.2b	2.3	14.8	29.8	15.0	23.5b
var. citroides	108	22.6b	3.0	17.0	32.6	15.5	22.8b
egusi	74	35.6a	3.8	17.7	43.5	25.8	35.8a
^a Values with	n colu	imns foll	lowed	by the	same	letter	are not
significantly d	ifferent	$(\alpha = 0)$).05) I	per Tuk	ey´s Sti	adentize	ed range
(HSD) test.							

types of *C. lanatus* had significantly higher oil content than did seeds of either var. *lanatus* or var. *citroides* accessions. The range of oil content values across all analyzed accessions was 28.7%. Accessions within groups having the highest percentages of oil included var. *lanatus* PI 385964 (29.2%) and PI 197416 (29.8%), var. *citroides* PI 482302 (30.5%) and PI 482342 (32.9%), and egusi types PI 490379 (42.8%) and PI 186975 (43.5%). Accessions with the lowest percent seed oil content/ group were *C. lanatus* var. *lanatus* PI 274034 (15.0%) and PI 542116 (15.6%), *C. lanatus* var. *citroides* PI 596692 (15.5%) and PI 532669 (15.6%), and egusi type PI 560011 (25.8%) and PI 184800 (25.9%). Only minimal overlap of the distributions of the oil content values of the *C. lanatus* var. *lanatus* and *C. lanatus* var. *citroides* accessions and the egusi types was observed (Figure 1).



Figure 1. Frequency histograms of seed oil content in combined accessions of *Citrullus lanatus* var. *lanatus* and var. *citroides* (A) and egusi forms of *C. lanatus* (B) as determined by TD-NMR.

Various authors have reported seed oil yields from decorticated seed of *Citrullus* spp. For example, Dhingra and Biswas¹⁰ reported a seed oil yield of 45.5% from an Indian variety of *C. lanatus*, whereas four commercial varieties of *C. vulgaris* (syn. *C. lanatus*) from Egypt contained an average of ~52% oil.¹² More recently, Ziyada and Elhussien¹⁸ reported the oil content of *C. colocynthoide* Pangalo (syn. *C. lanatus* var. *citroides*) 'Garum' seed from Sudan as 35%. We chose to analyze the entire seed due to the large number of accessions analyzed. T-Sao and Potts¹¹ reported that entire seeds of a large green citron (*C. lanatus* var. *citroides*) yielded ~19% oil of a light yellow color, whereas Lakshminarayana et al.⁷ recovered 21.3% oil from watermelon seed. The values presented in the current study compare favorably with these previously published values while extending the documented range.

The fruit mesocarp of the egusi form of *C. lanatus* is extremely bitter. However, the seeds are an important regional source of vitamin E, protein, and oil. Five seed types of egusi (*Colocynthis citrullus* L., syn. *C. lanatus*) have been reported. They vary considerably in size, shape, and thickness of the seed coat, but not in chemical composition.²⁵ The type I seed (small, elongated) described by Oyolu⁵ was similar to PI 326516 (Figure 2). The seed (entire) oil content of PI 326516 was



Figure 2. Seed of egusi type Citrullus lanatus PI 326516.

35.4%. The resemblance of this seed to that reported (and pictured) by Loukou et al.²⁹ is noteworthy as those authors identified their material as *C. lantus* var. *citroides*. Thus, some confusion exists within the scientific literature regarding the proper taxonomic classification of egusi melons.⁵ We regard this as a result of not only changes to the taxonomy of this genus over time but also the recognition that "egusi" is commonly used as a generic term that reflects the various cultural uses of the seed of a number of indigenous cucurbits (Table 2).

We observed average hull/kernel ratios of 1.29, 1.37, and 0.49 for seed of *C. lanatus* var. *citroides*, *C. lanatus* var. *lanatus*, and the egusi form of *C. lanatus*, respectively (Table 3). Within the 24 accessions examined, the highest hull/kernel ratios were seed of PI 596692 (2.59–*C. lanatus*. var. *citroides*) and PI 274035 (2.0–*C. lanatus* var. *lanatus*). The lowest hull/kernel ratios were found for PI 505604 (0.73–*C. lanatus* var. *citroides*) and PI 197416 (0.79–*C. lanatus*. var. *citroides*). A hull/kernel ratio of 0.92 for *C. lanatus* seed was reported by Lakshminarayana et al.⁷ Abd-El-Akhar et al.³⁰ reported an

Table 2. Various Taxonomic Classifications Utilized in the Scientific Literature To Denote Egusi Melon

classification	ref
Colocynthis citrullus (= C. lanatus)	5, 37
C. lanatus var. lanatus	14
C. lanatus var. colocynthoide (= C. lanatus var. citroides)	18
C. colocynthis	26
Cucumeropsis mannii	35
C. lanatus subsp. mucosospermus, Cucumeropsis mannii, Lagenaria siceraria	6
Cucumeropsis mannii, Cucurbita maxima, Cucurbita moschata, Cucumis sativus, Lagenaria siceraria	36
C. lanatus var. citroides, Cucumeropsis mannii, Cucumis melo var. agrestis	29
Cucumeropsis mannii, Cucurbita maxima, Cucurbita moschata, Lagenaria siceraria. Cucumis sativus	24

 Table 3. Descriptive Statistics for Seed Hull/Kernel Ratios in

 Citrullus lanatus

variety/form	n	mean ^a	SD	range	max	min	median
var. lanatus	8	1.37a	0.67	1.81	2.60	0.80	1.09
var. citroides	8	1.28a	0.65	1.93	2.58	0.66	1.10
egusi	8	0.49b	0.15	0.48	0.75	0.26	0.48

^{*a*}Values within the column followed by the same letter are not significantly different ($\alpha = 0.05$) per Tukey's Studentized range (HSD) test.

average hull/kernel ratio of 1.05 for three varieties of watermelon cultivated in Egypt. Within the egusi forms examined here, the highest value observed was PI 184800 (0.62) and the lowest for PI 254737 (0.26). The hull/kernel ratios of egusi were significantly lower than those of either *C. lanatus* var. *lanatus* or *C. lanatus* var. *citroides* and likely reflect the effect of the unusual seed coat characteristic of egusi (*eg*) on this trait. Oyolu²⁵ reported hull/kernel ratios for seed of five seed types of the egusi form of *C. lanatus* as 0.71–0.85, as compared to the range of 0.48–0.75 observed in the present study. A negative correlation ($R^2 = 0.83$) was detected between seed oil content (Figure 3) and the hull/kernel ratio on a



Figure 3. Linear regression of percent seed oil content against the hull/kernel ratio in seed of *Citrullus lanatus*.

weight basis. Decreased hull weight relative to the kernel weight results in increased oil content. This relationship accounts for the higher percent oil content of the egusi type seed as seed of egusi types have a seed coat that is noticeably thinner than that of a typical *C. lanatus* seed.

A summary of the fatty acid data for 96 genebank accessions of *C. lanatus* is presented in Table 4. In all instances, linoleic acid was the predominant form present, with values ranging from a low of 45.37% (var. citroides PI 244017) to a high of 73.0% (var. citroides PI 542116). No differences were detected among the means of the three categories (var. lanatus vs var. citroides vs egusi) for percent linoleic acid. However, the relative concentrations of stearic, palmitic, and oleic acids varied. In \sim 64% of the samples analyzed, concentrations of fatty acids increase from stearic to palmitic to oleic. In other samples, for example, PI 485583 and PI 271132, concentrations increased from stearic to oleic to palmitic. In PI 254740 and PI 306367 concentrations increased from oleic to stearic to palmitic, whereas in PI 560015 and PI 596669 concentrations increased from palmitic to stearic to oleic. In agreement with the report of Crombe and Comber,³¹ small amounts of arachidonic acid (20:4) were detected in most samples, although at a concentration never exceeding 0.5%, with one exception (PI 500310 = 0.51%). Oyolu and Mcfarlane³² noted that egusi seed oil contained approximately 70% unsaturated fatty acids primarily linoleic (54.3-64.0%) and oleic (13.4-18.7%), in agreement with the study of Oyenuga and Fetuga.³³ These authors noted a positive correlation between concentrations of palmitic and oleic acids and a negative correlation between concentrations of palmitic and linoleic acids. We did not find either a positive concentration between levels of palmitic and linoleic acids $(R^2 = 0.105)$ or a negative correlation between concentrations of palmitic and oleic acids ($R^2 = 0.023$). However, significant differences in the levels of individual fatty acids among the three groups examined were evident (Table 4).

Various papers have noted the high degree of unsaturation in *Citrullus* seed oil.^{17,32} We observed an average of 78.91% unsaturated fatty acids (linoleic plus oleic) across all accessions with a high of 83.35% (var. *lanatus* PI 306364) and a low of 74.27% (var. *lanatus* PI 260773), with no significant differences between the means of each group. However, a wider range of values was observed within var. *lanatus* (9.0) when compared to var. *citroides* (6.4) or egusi (5.7). This may be a reflection of the more extensive collection of var. *lanatus* from which samples were chosen for analysis, or perhaps it is a reflection of existing genetic diversity for this characteristic within each group.

Concentrations of linoleic acid in the seed of *C. colocynthis, B. fistulosa, C. rehmii,* and *C. ecirrhosus* were 71.5, 73.2, 75.8, and 62.7%, respectively (Table 5). Due to the extremely limited number of seeds available, these materials were not examined for oil content. The predominance of individual fatty acids within these plant materials varied with the taxa under consideration. Concentrations of palmitic acid were highest in *C. ecirrhosus* (12.3%) and lowest in *C. rehmii* (9.4%). Levels of oleic acid were highest in *C. ecirrhosus* (17.8%) and lowest in *C. rehmii* (6.7%). The limited number of accessions of these taxa available for examination suggests that the values presented here are unlikely to represent the average values for the species or the extent of the variability present in the gene pool.

Previous papers in the literature on the fatty acid composition of *C. rehmii, C. ecirrhosus,* or *B. fistulosa* were not found. In contrast, various studies have examined the seed oil yield and fatty acid content of *C. colocynthis.* Used in ancient times as a source of oil for candlelight and for its various medicinal properties, the seed of *C. colocynthis* has recently been used as a source of cooking oil^{8,34} and biofuel.^{8,23,26} Sawaya et al.³⁴ reported a fatty acid profile of this perennial vine as 50.6% linoleic, 25% oleic, 13.5% palmitic, and 10.5% stearic. Yaniv et al.⁸ reported a range of values for 28 accessions of *C. colocynthis* as linoleic (67–73%), oleic (10.1–16.0%), palmitic (8.6–12.0%), and stearic (5.2–8.2%). Values reported by Giwa

Table 4. Principal Fatty Acids in Seed Extracts of Citrullus lanatus var. citroides (Citron), C. lanatus Egusi-Type (Egusi), and C. lanatus var. lanatus (Lanatus)

	16:0 (palmitic)		18:0 (stearic)		18:1 (oleic)			18:2 (linoleic)				
	ctron	egusi	lanatus	citron	egusi	lanatus	citron	egusi	lanatus	citron	egusi	lanatus
Ν	30	33	33	30	33	33	30	33	33	30	33	33
mean ^a	10.6b	10.31b	11.15a	8.05b	10.16a	8.32b	16.42a	12.88b	13.81b	63.37a	65.17a	65.15a
SD	0.73	1.02	0.95	1.13	0.96	1.64	5.53	2.61	4.80	5.55	2.72	5.34
range	3.10	3.14	4.70	4.55	5.21	6.49	25.70	9.12	17.77	24.78	12.15	24.30
max	12.82	12.82	14.38	11.01	13.84	11.52	33.95	17.26	25.67	70.15	71.10	73.00
min	9.72	9.68	9.68	6.46	8.63	5.03	8.24	8.14	7.89	45.37	58.95	48.70
median	10.34	10.27	11.04	7.88	10.12	8.68	15.42	13.50	12.47	64.66	65.03	65.44
^{<i>a</i>} Within each fatty acid type, values followed by the same letter are not significantly different ($\alpha = 0.05$) per Tukey's Studentized range (HSD) test.												

Table 5. Principal Fatty Acids (Percent of Total) in Seed Extracts of Three *Citrullus* spp. and *Benincasa fistulosa*

genus/species	Ν	palmitic	stearic	oleic	linoleic
C. colocynthis	3	9.87	6.86	11.35	71.51
C. ecirrhosus	1	12.27	6.59	17.79	62.76
C. rehmii	1	9.40	8.09	6.72	75.81
B. fistulosa	3	10.38	6.78	8.66	73.23

et al.²⁶ were slightly outside those ranges (linoleic, 61.4%; oleic, 17.9%; stearic, 9.7%). The average values of individual fatty acids of the three accessions of *C. colocynthis* reported in the present study fell within the ranges reported by Yaniv et al.⁸

AUTHOR INFORMATION

Corresponding Author

*Phone: +1 (770) 228-7303. Fax: +1 (770) 229-3323. E-mail: bob.jarret@ars.usda.gov.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

We acknowledge Sarah Moon for expert technical assistance in the TD-NMR analysis of oil content and the extraction of fatty acids, Brandon Tonnis for the GC analysis of fatty acids, and Dr. M. L. Wang for the use of laboratory equipment.

REFERENCES

(1) Jeffrey, C. A review of the Cucurbitaceae. J. Linn. Soc. Bot. 1980, 81, 233–247.

(2) Lazos, E. S. Nutritional, fatty acid, and oil characteristics of pumpkin and melon seeds. *J. Food Sci.* **1986**, *51*, 1382–1383.

(3) Akoh, C. C.; Nwosu, C. V. Fatty acid composition of melon seed oil lipids and phospholipids. J. Am. Oil Chem. Soc. **1992**, 69, 314–316.

(4) Curtis, G. C. The possibilities of using species of perennial cucurbits as source of vegetable fats and protein. *Chemurg. Dig.* **1946**, 13 (5), 223–224.

(5) Oyolu, C. A quantitative and qualitative study of seed types in egusi (*Colycynthis citrullus* L.). *Trop. Sci.* **1977**, *19*, 55–62.

(6) Achigan-Dako, E. G.; Fagbemissi, R.; Avohou, H. T.; Vodouhe, R. S.; Coulibaly, O.; Ahanchede, A. Importance and practices of Egusi crops (*Citrullus lanatus* (Thunb.) Matsum. & Nakai, *Cucumeropsis mannii* Naudin and *Lagenaria siceraria* (Molina) Standl. cv. 'Aklamkpa' in sociolinguistic areas in Benin. *Biotechnol. Agron. Soc. Environ.* **2008**, *12*, 393–403.

(7) Lakshminarayana, T.; Surendranath, M. R.; Kristappa, G.; Viswanadham, R. K.; Thirumala Rao, S. D. Processing of Indian watermelonseed. *Ind. Oil Soap J.* **1968**, 33, 323–330.

(8) Yaniv, Z.; Shabelsky, E.; Schafferman, D. Colocynth: potential arid land oilseeds from an ancient cucurbit. In *Perspectives on New*

Crops and New Uses; Janick, J., Ed.; ASHS Press: Alexandria, VA, 1999; pp 251-261.

(9) Munisse, P.; Andersen, S. V.; Jensen, B. D.; Christiansen, J. L. Diversity of landraces, agricultural practices and traditional uses of watermelon (*Citrullus lanatus*) in Mozambique. *Afr. J. Plant Sci.* 2011, *5*, 75–86.

(10) Dhingra, D. R.; Biswas, A. K. Component fatty acids of oil of *Citrullus vulgaris*, Schrad. (water melon) seed. *J. Ind. Chem. Soc.* **1945**, 22, 119–122.

(11) T-Sao, C. M.; Potts, W. M. The analysis and characterization of the oil from the seed of *Citrullus vulgaris*. J. Am. Oil Chem. Soc. **1952**, 29, 444–445.

(12) Kamel, B. S.; Dawson, H.; Kakuda, Y. Characteristics and composition of melon and grape seed oil and cakes. *J. Am. Oil Chem. Soc.* **1985**, *62*, 881–883.

(13) Das, M.; Das, S. K.; Suthar, S. H. Composition of seed and characteristics of oil from karingda (*Citrullus lanatus* (Thumb) Mansf.). *Int. J. Food Sci. Technol.* **2002**, *37*, 893–896.

(14) Oluba, O. M.; Ogunlowo, Y. R.; Ojieh, G. C.; Adebisi, K. E.; Eidangbe, G. O.; Isiosio, I. O. Physicochemical properties and fatty acid composition of *Citrullus lanatus* (egusi melon) seed oil. *J. Biol. Sci.* **2008**, *8*, 814–817.

(15) Nolte, A. J.; von Loesecke, H. W. Characteristics and composition of watermelon seed oil (Cuban Queen variety). J. Am. Chem. Soc. **1939**, *61*, 889–891.

(16) Sodeke, V. A. Extraction of oil from water melon seed and analysis. *Q. Res. Serv.* **2005**, 25–30.

(17) Mabaleha, M. B.; Mitei, Y. C.; Yeboah, S. O. A comparative study of the properties of selected melon seed oils as potential candidates for development into commercial edible vegetable oil. *J. Am. Oil Chem. Soc.* **2007**, *84*, 31–36.

(18) Ziyada, A. K.; Elhussien, S. A. Physical and chemical characteristics of *Citrullus lanatus* var. *colocynthoide* seed oil. *J. Phys. Sci.* **2008**, *19*, 69–75.

(19) Teotia, M. S.; Ramakrishna, P. Chemistry and technology of melon seeds. J. Food Sci. Technol. 1984, 21, 332–340.

(20) Cox, A.; van der Veen, M. Changing foodways: watermelon (*Citrullus lanatus*) consumption in Roman and Islamic Quseir al-Qadim, Egypt. Veget. Hist. Archaeobot. 2008, 17 (Suppl.), S181–S189. (21) Achi, O. K. Traditional fermented protein condiments in Nigeria. Afr. J. Biotechnol. 2005, 4, 1612–1621.

(22) Badifu, G. I. O.; Ogunsua, A. O. Chemical composition of kernels from some different species of Cucurbitaceae grown in Nigeria. *Plant Foods Hum. Nutr.* **1991**, *41*, 35–44.

(23) Pal, A.; Kachhwaha, S. S.; Maji, S.; Babu, M. K. G. Thumba (*Citrullus colocynthis*) seed oil: a sustainable source of renewable energy for biodiesel production. *J. Sci. Ind. Res.* **2010**, *69*, 384–389.

(24) Achu, M. B.; Fokou, E.; Tchiegang, C.; Fotso, M.; Tchouanguep, F. M. Nutritive value of some Cucurbitaceae oilseeds from different regions in Cameroon. *Afr. J. Biotechnol.* **2005**, *4*, 1329–1334.

(25) Oyolu, C. Extraction rates and chemical composition of seed types in egusi (*Colocynthis citrullus* L.). *Acta Hortic.* **1977**, *53*, 287–290.

(26) Giwa, S.; Abdullah, L. C.; Adam, N. M. Investigating "Egusi" (*Citrullus colocynthis* L.) seed oil as a potential biodiesel feedstock. *Energies* **2010**, *3*, 607–618.

(27) Joshi, A. Thesis, *Chemical Composition and Nutritional Evaluation of Pumpkin, Watermelon and Karingda Seed Oil;* Department of Food Science, SP University, Anand, India, 1990.

(28) Jarret, R. L.; Wang, M. L.; Levy, I. J. Seed oil and fatty acid content in okra (*Abelmoschus esculentus*) and related species. J. Agric. Food Chem. **2011**, 59, 4019–4024.

(29) Loukou, A. L.; Gnakri, D; Dje, Y.; Kippre, A. V.; Malice, M.; Baudoin, J.-P.; Bi, I. A. Z. Macronutrient composition of three cucurbit species cultivated for seed consumption in Cote d'Ivoire. *Afr. J. Biotechnol.* **2007**, *6*, 529–533.

(30) Abd-el-Akhar, M.; Tamini, A. H. K.; Khairy, M.; Morsi, S.; El-Sharkawy, A. Water melon seeds. I. Chemical composition and oil content of seeds. *Agric. Res. Rev.* **1975**, *53*, 79–87.

(31) Crombe, W. M.; Comber, R. Fat metabolism in germinating *Citrullus vulgaris. J. Exp. Bot.* **1956**, *7*, 166–180.

(32) Oyolu, C.; Macfarlane, N. A study of the oil and soluble protein components of five Egusi (*Colocythis citrullus* L.) cultivars. *Trop. Sci.* **1982**, *24*, 93–98.

(33) Oyenuga, V. A.; Fetuga, B. L. Some aspects of the biochemistry and nutritive value of the water melon seed *Citrullus vulgaris* Schrad. J. Sci. Food Agric. **1975**, *26*, 643–654.

(34) Sawaya, W. N.; Daghir, N. J.; Khalil, J. K. *Citrullus colocynthis* seeds as a potential source of protein for food and feed. *J. Agric. Food Chem.* **1986**, *34*, 285–288.

(35) Kapseu, C.; Kamga, R.; Tchatchueng, J. B. Triacylglycerols and fatty acids composition of egusi seed oil (*Cucumeropsis mannii* Naudin). *Grasas Aceites* **1993**, *44*, 354–356.

(36) Fokou, E.; Achu, M. B.; Tchouanguep, F. M. Preliminary nutritional evaluation of five species of egusi seeds in Cameroon. *Afr. J. Food Agric. Nutr. Dev.* **2004**, *4*, 1–11.

(37) Girgis, P.; Said, F. Lesser known Nigerian edible oils and fats. I. Characteristics of melon seed oils. *J. Sci. Food Agric.* **1968**, *19*, 615–616.