

Oil and Tocopherol Content and Composition of Pumpkin Seed Oil in 12 Cultivars

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Twelve pumpkin cultivars (*Cucurbita maxima* D.), cultivated in Iowa, were studied for their seed oil content, fatty acid composition, and tocopherol content. Oil content ranged from 10.9 to 30.9%. Total unsaturated fatty acid content ranged from 73.1 to 80.5%. The predominant fatty acids present were linoleic, oleic, palmitic, and stearic. Significant differences were observed among the cultivars for stearic, oleic, linoleic, and gadoleic acid content of oil. Low linolenic acid levels were observed (<1%). The tocopherol content of the oils ranged from 27.1 to 75.1 $\mu\text{g/g}$ of oil for α -tocopherol, from 74.9 to 492.8 $\mu\text{g/g}$ for γ -tocopherol, and from 35.3 to 1109.7 $\mu\text{g/g}$ for δ -tocopherol. The study showed potential for pumpkin seed oil from all 12 cultivars to have high oxidative stability that would be suitable for food and industrial applications, as well as high unsaturation and tocopherol content that could potentially improve the nutrition of human diets.

KEYWORDS: Pumpkin seed oil; pumpkin seed; oilseed; winter squash; Cucurbitaceae; fatty acid; tocopherol

INTRODUCTION

Vegetable oils are utilized globally for many food and other industrial purposes. Despite the vast range of sources for vegetable oils, world consumption is dominated by soybean, palm, rapeseed, and sunflower oils with 31.6, 30.5, 15.5, and 8.6 million tons consumed per year, respectively (1). The remaining vegetable oils possess different compositions, such as fatty acids and antioxidants, enabling improved performance in food and industrial applications, as well as nutritional benefits. Currently, pumpkin seed oil is not widely used commercially even though it has characteristics that are well suited for industrial applications and can contribute to healthy human diets. Pumpkin seed oil is, however, sold in most reputable health stores in the United States, typically formulated in capsules containing 1 gram of oil.

Pumpkin seed oil varies from dark green to brown in color. There has been a considerable amount of research conducted on the oil content and fatty acid composition of pumpkin seed oil, but a considerable proportion of this research has been published in journals that are difficult to access due to their low level of subscriptions. This difficulty has resulted in a lack of any review or summary of the fatty acid composition found

in various sources of pumpkin seed oil. Therefore, we have collated all references on pumpkin seed oil and summarized the content and fatty acid composition reported for the seed oil extracted from various pumpkin sources in **Table 1**. Pumpkin seed oil typically is a highly unsaturated oil, with predominantly oleic and linoleic acids present. Very low levels of linolenic acid or other highly unsaturated fatty acids are present, providing pumpkin seed oil with high oxidative stability for storage or industrial purposes and low free radical production in human diets. Studies of pumpkin seed oil triacylglycerol positional isomers found that oleic and linoleic acid distribution patterns are not random (65).

The highly unsaturated fatty acid composition of pumpkin seed oil makes it well-suited for improving nutritional benefits from foods. Pumpkin seed oil has been implicated in providing many health benefits (66). The most critical health benefit attributed to pumpkin seed oil is preventing the growth and reducing the size of the prostate (67, 68). There is also evidence that suggests pumpkin seed oil can retard the progression of hypertension (69) and mitigate hypercholesterolemia (70) and arthritis (71). Reduced bladder and urethral pressure and improved bladder compliance have been linked to pumpkin seed lipid components (72–75). Pumpkin seed oil has been found to alleviate diabetes by promoting hypoglycemic activity (66). Pumpkin seed oil has been found to provide a significant source of vitamin E (tocopherol) in Japanese diets (76). Diets high in pumpkin seeds have also been associated with lower levels of gastric, breast, lung, and colorectal cancer (77). There are also

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Table 1. Oil Content (Percent) and Fatty Acid Composition^a (Percent) of Previously Reported Studies on Pumpkin Seed Oil

Pumpkin common name	oil	12:0	14:0	16:0	16:1	17:0	18:0	18:1	18:2	18:3	20:0	22:1	TUFA
<i>Cucurbita argyrosperma</i>													
Cushaw green striped (2)	40.1			13.6			8.0	30.6	45.4				76.0
Japanese pie (2)	36.0			13.5			9.1	31.0	44.2				67.8
<i>Cucurbita ficifolia</i>													
not available (3)	43.5		0.16	15.4	0.3		4.2	14.7	61.0	1.9			77.9
not available (4) ^b			0.09	5.7	0.2		6.3	35.0	52.1				87.6
<i>Cucurbita foetidissima</i>													
buffalo gourd (5)	28												
buffalo gourd (6)	35.9	0.06	0.09	10.4			2.9	31.8	54.8				86.6
buffalo gourd (7)	36.0			11.8			3.5	21.9	60.6				82.5
buffalo gourd (8)				8.8			3.5	37.5	50.1				87.6
buffalo gourd (9)	32.9			9.7			5.3	26.7	59.2				
buffalo gourd (10)	28.5			9.3			2.1	25.0	63.6				
<i>Cucurbita maxima</i>													
not available (11) ^c			0.1	12.7	0.1	trace	6.0	38.1	42.1	0.2	0.3		80.6
Shintosa (12)	37.4		0.1	11.7	0.2		5.0	52.0	31.0		0.2		83.2
Kinuta (12)	32.1		0.3	12.0	0.1		5.6	45.9	36.0		0.2		82.0
Tokyo (12)	30.0		0.2	12.8			5.8	40.4	40.1	0.6			81.1
Chikanarihoko (12)	30.8		0.1	13.3			4.3	42.1	39.5	0.7			82.3
Hokkaido (2)	24.2			21.3			6.4	18.3	50.3				68.6
Indian (2)	42.3			18.9			7.4	22.8	48.2				71.2
butter (2)	33.1			15.0			9.7	29.4	43.1				72.5
not available (13)	52.1												
not available (14)	36.6			12.1			5.9	35.0	41.5		0.03		76.5
<i>Cucurbita mixta</i>													
not available (15)	50.6			13.8			5.9	21.4	58.9				80.3
<i>Cucurbita moschata</i>													
Egyptian (16)	43.0		0.16	13.1	0.3	0.28	6.0	26.2	53.2	0.12	0.17	0.14	80.3
not available (17)			0.09	18.3			6.0	22.3	52.6		0.2		75.4
Shirokikuza (12)	29.1			18.4			5.9	22.0	53.0	0.6			75.6
large sweet cheese (2)	39.3			12.4			5.4	25.8	53.5				79.3
North Carolina (2)	31.2			17.6			6.7	19.0	53.0				72.0
Zapallo (2)	37.6			15.5			8.2	18.7	54.5				73.2
Kentucky Field (2)	38.8			17.9			7.1	13.5	59.0				72.5
not available (4) ^d			0.04	10.8	0.3	0.27	8.1	30.0	48.5				78.8
Halva Kaddu (18)			1.2	21.6			6.5	38.7	31.5				70.2
Kaddu (19)	43.3												
not available (20)	40.2			20.8			6.7	14.5	57.0	0.9			72.4
<i>Cucurbita pepo</i>													
Iranian (16)	37.0		0.13	11.8	0.37	0.2	6.3	34.9	43.1	0.9	0.7	0.8	80.9
not available (21, 22)	51.0		0.17	13.4	0.44		10.0	20.4	55.6				76.5
not available (23)	45.4	trace	0.1	12.7	0.1	trace	5.4	37.8	43.1	0.3	0.3		81.3
not available (24)	47.0			9.9			6.2	41.0	41.2				82.2
not available (25)				13.6	0.3		4.8	30.2	47.9	0.4			78.8
not available (25)				49.2	0.9		11.2	31.6	4.9	0.9			38.3
not available (25)				25.4	0.5		8.5	31.7	24.5	0.4			57.1
not available (25)				16.7	0.2		7.0	33.9	34.3	0.5			68.9
Dubba (26)	35.0			11.2			8.2	28.3	50.3	0.2			79.3
Lady Godiva (2)	40.6			13.5			6.4	33.5	43.4				76.9
Mashidi (2)	34.9			15.6			4.8	23.1	53.8				76.9
Zapallo (2)	31.2			13.4			7.7	24.9	50.6				75.7
Oliva hull-less (2)	41.7			12.2			6.4	27.1	52.2				79.4
Bloomfield (2)	31.8			13.5			5.7	26.8	51.2				77.9
Japanese pie (27)	38.0			13.6			8.1	36.7	40.4				77.1
not available (28) ^e	34.8			14.6			8.2	47.0	19.8	0.8	1.0		
Ghia Kaddu (18)				18.2			8.4	43.5	30.0				73.5
not available (29)				13.0			9.4	37.4	40.7				
not available (30) ^f	34.2		0.1	20.1	0.1	0.1	8.2	17.0	52.5	0.4	0.6		70.7
not available (31)				11.4			6.2	29.2	52.8	0.3			
not available (32)	33.7			17.8			9.4	40.4	31.4				
<i>Lagenaria sicceraria</i>													
gourd (33, 34)	27.2			13.1			5.2	13.6	66.6				80.2
gourd (33, 34)	28.6			12.8			4.9	7.1	73.2				80.3
gourd (32)	9.8			13.2			5.2	13.6	73.2				
<i>Lagenaria vulgaris</i>													
not available (35)	50.6												
<i>Luffa acutangula</i>													
Luffa (36)	44.3		0.5	20.9			10.8	24.1	43.7				67.9
<i>Momordica charantia</i>													
Karela (18) ^g			1.8	2.8			21.7	30.0					73.7
<i>Momordica cochinchinensis</i>													
Gac (37) ^h				5.6	0.1		60.5	9.5	20.3	0.5	1.6		34.5
<i>Telfairia occidentalis</i>													
fluted (37)	64.4												
fluted (33)	26.4			16.3			13.5	29.8	39.6				69.3

Table 1. (Continued)

Pumpkin common name	oil	12:0	14:0	16:0	16:1	17:0	18:0	18:1	18:2	18:3	20:0	22:1	TUFA
not available (35)	51.4												
not available (39)	48.6												
fluted (40)	42.2			21.2			0.1	61.8	16.4	0.3			78.6
fluted (41) ⁱ	47.0		0.1	13.4		0.2	18.5	33.0	30.2		1.5		66.2
<i>Trichosanthes bracteata</i>													
snake gourd (42) ^j	31.6		0.3	13.1		0.2	5.3	8.6	30.0	0.1			80.5
unknown <i>Cucurbita</i> species													
Vitaminnaya (43)				13.5			6.3	25.0	55.2				
Stofuntovaya (43)				15.9			5.7	20.2	58.2				
Mindal'naya (43)				10.7			5.0	29.1	55.2				
Mozoleevskaya (43)				13.2			6.2	27.2	53.4				
Volzhskaya seraya (43)				11.0			5.5	26.1	56.4				
Ukrainskaya mnogoplodnaya (43)				11.8			5.4	27.6	55.2				
not available (44)								24.0	54.0	0.5			79.0
not available (45)				11.9			6.2	34.4	46.9	0.4	0.2		81.7
not available (46, 47)				11.2			5.1	28.5	55.2				83.7
not available (48)			0.1	12.9	0.1	trace	4.3	35.2	47.0	0.2	0.2		82.5
not available (49) ^k			0.2	14.7	0.4		6.3	12.8	64.7	0.2	0.3		78.2
not available (49, 50)			0.2	11.8	0.2	trace	5.6	28.9	51.9	0.3	0.2		81.6
not available (51) ^l			0.1	11.0	0.2		4.5	35.7	47.8	0.4	0.3		84.3
Kuriebisu (27, 52–54)				11.0			7.8	38.0	42.0				80.0
Rikyu (51)				12.2			8.9	53.0	22.0				75.0
Yatsuko (27, 52–54)				12.5			7.3	55.0	21.5				76.5
not available (55)				5.4			12.4	27.6	54.6				
not available (56)			0.1	11.7			5.0	52.0	31.0		0.2		83.0
not available (57) ^m		4.1	0.4	23.9	0.2		14.2	10.1	41.6	1.0	1.5	1.6	55.0
not available (58)	46.5			18.1			7.8	22.2	51.7				73.9
not available (50, 59)				7.1			6.7	24.4	48.6				
not available (50, 60)				7.1			6.7	24.4	46.6				
not available (61)				13.1			3.9	26.6	56.4				
not available (62)	36.0		trace	16.4			7.7	33.8	42.0				75.8
not available (63)				14.2			8.4	28.5	47.3				
not available (64)				16.4			7.7	33.8	42.0				

^a Fatty acid abbreviations are as follows: 12:0, lauric acid; 14:0, myristic acid; 16:0, palmitic acid; 16:1, palmitoleic acid; 17:0, margaric acid; 18:0, stearic acid; 18:1, oleic acid; 18:2, linoleic acid; 18:3, linolenic acid; 20:0, arachidic acid; 22:1, erucic acid; TUFA, total unsaturated fatty acids. ^b Also observed were 0.7% 9-dodecanoic acid (12:1), 0.06% pentadecenoic acid (15:1), 0.2% behenic acid (22:0), 0.22% lignoceric acid (24:0), 0.03% squalene (30:6), 0.02% 2,6,10-triene-1-dodecanal (15:3), and 0.03% 4,8,12-tetradecatrienal (17:3). ^c Also observed were trace levels of *trans*-18:2. ^d Also observed were 0.04% behenic acid (22:0), 0.02% squalene (30:6), 0.07% 2,6,10-triene-1-dodecanal (15:3), 0.07% 4,8,12-tetradecatrienal (17:3), 0.03% 7-hexadecenoic acid (16:1), 0.04% 11-octadecenoic acid (18:1), 0.03% 1,12-tridecadiene (13:2), and 0.27% 11,14-eicosadienoic acid (20:2). ^e Also observed were 0.4% gadoleic acid (20:1) and 0.7% eicosadienoic acid (20:2). ^f Also observed were 0.9% 18:1 Δ 7 and 0.1% gadoleic acid (20:1). ^g Also observed was 43.7% elaeosteric acid (18:3). ^h Also observed were 0.5% *cis*-vaccenic acid (18:1 Δ 11), 1.1% eicosa-11-enoic acid (20:1 Δ 11), and 3.0% eicosa-13-enoic acid (20:1 Δ 13). ⁱ Also observed were 3.0% of combined eicosapentaenoic acid (20:5) and behenic (maybe behenic?) acid (22:1). ^j Also observed was 41.8% punicic acid (18:3). ^k Also observed was 0.1% gadoleic acid (20:1). ^l Also observed was 0.1% gadoleic acid (20:1). ^m Also observed were 0.7% behenic acid (22:0) and 0.3% lignoceric acid (24:0).

potential health benefits to be gained from the various carotenoid pigments found in pumpkin seed oil (78), and carotenoids from all sources of pumpkin fruit have been linked to the prevention of prostate cancer (79, 80). Despite the aforementioned health benefits, pumpkin seed oil has been shown to exhibit no antimicrobial activity (81).

The antioxidant properties of tocopherols could play a significant role in the therapeutic effects of pumpkin seed oil. Roasted pumpkin seed oil was found to contain higher levels of α - and γ -tocopherol than roasted sunflower oil (82). Total tocopherol content was 20.1 mg/100 g, of which 87% was in the γ -form, and no β - or δ -tocopherol was detected. In a study on pumpkin seed oil from an unknown source, only γ -tocopherol (23.6 mg/100 g) was detected (48), whereas only α -tocopherol (12.6 mg/100 g) was found in oil extracted from a mixture of *Cucurbita pepo* and *Cucurbita maxima* pumpkins (11). Roasting time is also critical as both α - and γ -tocopherol contents were found to decrease during the first 40 min of roasting but subsequently to increase (55). Analysis of oil from fluted pumpkin found much higher levels of α -tocopherol (24 mg/100 g) (83), and even higher levels of α -tocopherol (0.9–9.0 mg/100 g) and γ -tocopherol (46.0–66.2 mg/100 g) were reported for Styrian pumpkins, with the absence of β - and δ -tocopherol (84). However, another study of oil extracted from

Styrian pumpkins found that α -tocopherol (2.0–4.9 mg/100 g), γ -tocopherol (1.5–5.4 mg/100 g), and δ -tocopherol (0.3–1.1 mg/100 g) were present (85). The most extensive study to date investigated the tocopherol contents of seed oil from 50 pumpkin breeding lines, with α -tocopherol ranging from 0.0 to 18.2 mg/100 g, γ -tocopherol ranging from 8.2 to 124 mg/100 g, and both β - and δ -tocopherol observed in just a few breeding lines (86).

In this study we investigate the oil content, fatty acid composition, and tocopherol content of 12 pumpkin cultivars, belonging to *C. maxima*, cultivated in Iowa. To date, nothing is known about the oil characteristics of any of the 12 cultivars selected in this study. Additionally, there are few studies (2, 11–14) on pumpkin seed oil from the many thousand cultivars of *C. maxima*. Knowledge gained from this study will help to determine the potential for seed oil from these pumpkin cultivars to be commercially exploited for industrial applications and incorporation into food formulations to improve human health.

MATERIALS AND METHODS

Pumpkin Cultivation. Twelve pumpkin (*C. maxima* D.) cultivars were cultivated at an Iowa State University farm site 1.7 miles south of Ames, IA (geographical location 41° 58' 57.5" N, 93° 38' 22.9"

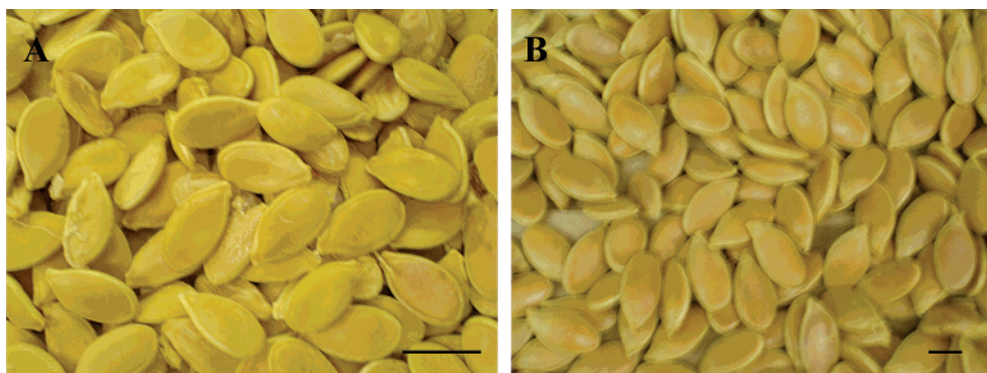


Figure 1. Images illustrating small pumpkin seeds for buttercup cultivars such as Cha Cha (A) and the large seeds of Zapallo Macre (B). Scale bar represents 1 cm.

W), in a randomized complete block (8.23 m × 3.05 m blocks) with two replicates (36 plants/replicate). Mean maximum and minimum temperature, rainfall, and solar radiation for each 2 week period of the entire growing season from mid-May to the end of September have been previously reported (87). Normal crop husbandry was followed as required. Pumpkin cultivars studied were four buttercups (Cha Cha, Delica, Kurijiman, and Sweet Mama), one cross between a buttercup, Green Delicious, and a non-buttercup, Table Queen (Hyvita), two Halloween-type (Big Max and Rouge Vif D'Etampes), one Hubbard-type (Warren Scarlet, also known as Red Warren), one Crown-type (Whangaparoa Crown), one Native American Indian pumpkin (Lakota), and two noncommercially developed pumpkins, one from Burkina Faso (Yogorou) and one from the Bolivian/Peruvian border (Zapallo Macre). Seeds were purchased for Kurijiman, Warren Scarlet, and Whangaparoa Crown from Webling and Stewart Ltd., Petone, New Zealand, for Delica from Yates New Zealand Ltd., Onehunga, New Zealand, for Sweet Mama from Henry Field Seed and Nursery Co., Shenandoah, IA, for Lakota and Big Max from W. Atlee Burpee and Co., Warminster, PA, for Rouge Vif D'Etampes from J. W. Jung Seed Co., Randolph, WI, and for Cha Cha from Johnny's Select Seeds Co., Winslow, ME. Hyvita was received as a gift from Dr. Henry Munger, Department of Plant Breeding, Cornell University, Ithaca, NY. Yogorou and Zapallo Macre were both obtained from the USDA, ARS Plant Genetic Resources Unit, Cornell University, Geneva, NY, with the accession numbers being PI 490352 and PI 298818, respectively. Pumpkin fruit maturity was adjudged when stalks became woody (88).

Oil Extraction. For each replicate of each pumpkin cultivar, four randomly selected fruits were cut open at harvest, and the seeds were collected with fibrous material removed and stored at $-20\text{ }^{\circ}\text{C}$ until oil was extracted. Pumpkin seeds from each replicate for each of the 12 cultivars were ground, with seed coat intact, in a coffee grinder, and oil was extracted using both supercritical fluid extraction (SFE) and accelerated solvent extraction (ASE) (oil samples kept separate for each replicate). Oil content was determined gravimetrically. SFE was performed with neat carbon dioxide (CO_2) at $100\text{ }^{\circ}\text{C}$ and 8500 psi using an Isco SFX 3560 extractor (Teledyne Isco Inc., Lincoln, NE), and ASE was conducted with hexane at $100\text{ }^{\circ}\text{C}$ and 1500 psi using an ASE 200 accelerated solvent extractor (Dionex Corp., Sunnyvale, CA). Because ASE and SFE methods did not yield sufficient oil for both fatty acid and tocopherol analysis, additional oil was extracted from seeds ground in a Waring blender (Waring Corp., New Hartford, CT; high mode used) using a methanol/chloroform (1:2) mixture for 36 h at $25\text{ }^{\circ}\text{C}$. Methanol/chloroform extracted oil was used for the determination of fatty acid composition and tocopherol content.

Fatty Acid Composition. The fatty acid composition of oil extracted from pumpkin seeds was analyzed by injecting fatty acid methyl esters (89) into a Hewlett-Packard 5890 series II gas chromatogram (Hewlett-Packard, Santa Clarita, CA) equipped with a flame ionization detector and an SP-2380 column (60 m × 0.25 mm i.d., 0.20 μm film thickness, Supelco, Bellefonte, PA) using helium as the carrier gas at a linear flow velocity of 18 cm/s (90).

Tocopherol Analysis. An aliquot of solvent-extracted pumpkin seed oil was transferred to a preweighed 1.5-mL HPLC autosampler vial, and any residual solvent or moisture was evaporated in a vacuum oven (National Appliance Co., Portland, OR) for 6 h at an ambient temperature of $22\text{ }^{\circ}\text{C}$. After evaporation, the weight of the oil was determined by weighing the vial again, and then the oil was redissolved in 1 mL of HPLC grade hexane. Duplicate HPLC samples were prepared in this manner.

An AOCS standard method (Ce 8-89) (91) for tocopherol analysis was followed with minor modification. External standards of α -, γ -, and δ -tocopherols (Sigma-Aldrich, Inc., St. Louis, MO) were used to calculate the individual amounts of each tocopherol in oil sample. A 15 μL aliquot of sample was injected using a commercial automatic injector for quantification. Sample was eluted with 1% (v/v) isopropanol in HPLC grade hexane at a 0.650 mL/min flow rate with a Beckman Coulter HPLC system, which has System Gold 126 solvent delivery system, a model 508 autosampler, and a model 168 UV detector (Beckman Coulter, Inc., Beckman, CA) at a wavelength of 292 nm. The column was a 250 mm × 3.1 mm Lichrosorb Si60 (Merck, Darmstadt, Germany) with a 7- μm silica particle size. The tocopherol content was expressed as parts per million concentration relative to oil.

Statistical Analysis. All statistical significance tests were calculated using SAS (92), utilizing ANOVA followed by a multiple comparison, the Tukey difference test (93). Correlations were calculated using Microsoft Excel.

RESULTS AND DISCUSSION

Seed Characteristics and Oil Content. The 12 pumpkin cultivars studied had considerable variation in seed size, weight, and color. Because few people are familiar with the 12 pumpkin cultivars studied, an example of small seeds from the buttercup pumpkin, Cha Cha, and large seeds from Zapallo Macre are shown in **Figure 1**, panels A and B, respectively. The four buttercup pumpkin cultivars had similar seed size and color, and all had brown/gold seed coats; Whangaparoa Crown also had some resemblance in shape and color. Hyvita, which is a buttercup crossed with non-buttercup, had much larger seeds than the buttercup pumpkin cultivars, and seeds were white instead of brown/gold. The largest seeds were observed for Big Max, Rouge Vif D'Etampes, and Zapallo Macre (**Figure 1B**). Seed size was not correlated to seed weight (**Table 2**). Analyzing the data by a paired *t* test, oil contents, overall for all cultivars, determined by ASE results were significantly lower than those determined by SFE (**Table 2**). Oil content overall mean for the accelerated solvent extractions was 19.9% compared to 20.6% for the SFE results, and oil contents from the two methods were highly correlated ($r = 0.98$). The oil content of pumpkin cultivars ranged from 10.9 to 30.9% of the seed weight, and

Table 2. Seed Average Weight and Oil Content (Percent of Seed Weight) of the 12 Pumpkin Cultivars Extracted by Accelerated Solvent Extraction (ASE) or Supercritical Fluid Extraction (SFE)

pumpkin cultivar	av seed wt (mg)	oil content (%), ASE	oil content (%), SFE
Big Max	269	19.3	20.1
Cha Cha	226	13.3	13.4
Delica	340	15.1	16.1
Hyvita	271	24.4	26.8
Kurijiman	280	15.3	16.7
Lakota	245	24.3	21.8
Rouge Vif D'Etampes	346	19.7	20.3
Sweet Mama	285	17.4	18.8
Warren Scarlet	291	24.2	25.0
Whangaparoa Crown	198	10.9	11.5
Yogorou	298	30.1	30.9
Zapallo Macre	313	25.1	26.1
<i>P</i> ^a	0.08	0.16	0.21

^a *P* represents the probability of the *F* statistic exceeding the value expected for each comparison among pumpkin cultivars in the respective column.

there were no significant differences among cultivars (**Table 2**). However, when the two cultivars with the highest variation in oil content, Zapallo Macre and Cha Cha, were omitted from the statistical analysis, there was a significant difference ($P = 0.04$) among the remaining 10 cultivars. Furthermore, when Rouge Vif D'Etampes was eliminated from the analysis, there was a strong significant difference ($P = 0.004$) in oil content among the remaining 9 cultivars. Hyvita, a genetic cross, and the two uncommercialized cultivars (Yogorou and Zapallo Macre) appeared to have the highest oil contents, suggesting that obtaining *C. maxima* pumpkins with higher oil content may be possibly best achieved by plant breeding or exploring underutilized pumpkins. Relative to other studies, the majority of pumpkin cultivars we studied had lower oil content than reported for other *C. maxima* cultivars (2, 11–14), and oil content was lower than for the majority of pumpkins in Cucurbitaceae (**Table 1**).

Fatty Acid Composition. The fatty acid composition of the 12 pumpkin cultivars is shown in **Table 3**. Pumpkin seed oil from all 12 cultivars was high in unsaturated fatty acids with total unsaturated fatty acid (TUFA) content ranging from 73.1% (Zapallo Macre) to 80.5% (Big Max). TUFA of the 12 pumpkin cultivars we studied was similar to other studies of *C. maxima* pumpkin seed oil (2, 11–14) and for all pumpkin species in Cucurbitaceae (**Table 1**). Vegetable oils high in TUFA have been well documented to provide numerous health benefits (94–100); therefore, incorporation of pumpkin seed oil, extracted from all cultivars in this study, into the diet would be very salubrious. The predominant fatty acids in pumpkin seed oil from all 12 cultivars studied were linoleic, oleic, palmitic, and stearic. Despite high TUFA, all pumpkin seed oils were low in linolenic acid (<1%), which is in agreement with all other studies of pumpkin seed oil (**Table 1**). Pumpkin seed oil low in linoleic acid content provides high oxidative stability, making it suitable for industrial applications and long shelf life. Three of the most predominant fatty acids in pumpkin seed oil, linoleic, oleic, and stearic, were significantly different among the 12 cultivars. Big Max seed oil had significantly higher linoleic acid than the four buttercup cultivars (Cha Cha, Delica, Kurijiman, and Sweet Mama) and the closely related buttercup cultivar, Hyvita, and Warren Scarlet. Whereas Big Max seed oil did not have significantly higher linoleic acid

content than some cultivars, the 62.8% content was considerably higher than that of any other cultivar we studied and all other previous studies on *C. maxima* (2, 11–14) (**Table 1**). Pumpkin seed oil with greater than 60% linoleic acid has been previously reported for a few cultivars (3, 7, 33, 49). Conversely, due to high linoleic acid content, Big Max seed oil had significantly lower oleic acid than Hyvita and was 43–76% lower than all other cultivars studied. The oleic acid content observed for all cultivars we studied was similar to other results of pumpkins from *C. maxima* (**Table 1**). The Halloween-type pumpkin Rouge Vif D'Etampes had seed oil with significantly higher stearic acid than the other Halloween-type pumpkin, Big Max. Previous reports on the stearic acid of *C. maxima* seed oils (2, 11–14) were within the range we observed for the 12 cultivars in this study. Gadoleic acid was also significantly different among the pumpkin cultivar seed oils, but it was a minor component.

Tocopherol Content. The tocopherol content of the 12 pumpkin cultivar seed oils is shown in **Table 4**. The α -, γ -, and δ -tocopherol isomers were observed in seed oil from all pumpkin cultivars, and no β -tocopherol was present. In general, the total tocopherol levels we observed in pumpkin seed oil in this study were substantially higher than that reported for most pumpkin seed oils (82–86). The Halloween-type cultivar Big Max had significantly higher γ -tocopherol than all other cultivars except the other Halloween-type cultivar (Rouge Vif D'Etampes), indicating that seed oils from Halloween-type pumpkins may have higher levels of beneficial tocopherols. Levels of α -tocopherol were low relative to the very high levels of δ -tocopherol, but the latter has only 1% bioavailability of the former isomer (103). High variability for the δ -tocopherol content of Sweet Mama resulted in no significant differences observed. However, when Sweet Mama was omitted from the analysis, a highly significant difference ($P = 0.01$) in δ -tocopherol content was observed among the remaining 11 cultivars. γ -Tocopherol content of the pumpkin seed oils was typically 2–8 times higher than the isomer currently regarded as having the greatest bioavailability, α -tocopherol. However, recent research has found that γ -tocopherol may have greater antioxidant activities than α -tocopherol (104–106) and that the latter may suppress the bioavailability of the former isomer (107). In which case, the considerably higher γ -tocopherol content of pumpkin seed oils could make the oil even more attractive from a nutritional perspective.

Correlations. Significant correlations were observed for the 12 pumpkin cultivar seed oil tocopherol contents and fatty acid composition. γ -Tocopherol content of the 12 pumpkin cultivar seed oils was negatively correlated to content of δ -tocopherol ($r = -0.95$, $P < 0.0001$), total tocopherol ($r = -0.84$, $P = 0.0005$), and oleic acid ($r = -0.77$, $P = 0.005$) and positively correlated to linoleic acid content ($r = 0.85$, $P = 0.0004$). Because of its high content relative to the other isoforms in the pumpkin seed oils, δ -tocopherol was positively correlated to total tocopherol content ($r = 0.97$, $P < 0.0001$) and, in contrast to the γ -tocopherol, was also positively correlated to oleic acid content ($r = 0.79$, $P = 0.004$) and negatively correlated to linoleic acid content ($r = -0.88$, $P = 0.0001$). No correlations were observed between α -tocopherol and the other isoforms, which has been previously reported for canola oil (108). The highly significant correlation we observed between γ -tocopherol and total tocopherol content and the lack of correlation between α - and γ -tocopherol contents both agree with that reported for corn (109) and pecan (110) oils, but the latter correlation

Table 3. Fatty Acid Composition (Percent) of Pumpkin Seed Oil from the 12 Cultivars

pumpkin cultivar	14:0 ^a	16:0	16:1	18:0 ^b	18:1	18:2	18:3	20:0	20:1	22:0
Big Max	0.20	13.6	0.16	5.1 b	17.0 b	62.8 a	0.53	0.44	0.00 b	0.23
Cha Cha	0.25	15.6	0.23	7.7 ab	36.3 ab	38.1 b	0.81	0.62	0.17 a	0.30
Delica	0.22	14.0	0.15	7.9 ab	37.9 ab	38.4 b	0.75	0.46	0.00 b	0.30
Hyvita	0.19	12.6	0.13	6.8 ab	39.5 a	39.4 b	0.71	0.31	0.13 ab	0.23
Kurijiman	0.24	14.8	0.18	8.5 a	38.5 ab	36.2 b	0.82	0.45	0.06 ab	0.28
Lakota	0.22	14.5	0.17	5.9 ab	30.6 ab	47.6 ab	0.56	0.32	0.00 b	0.22
Rouge Vif D'Etampes	0.22	15.6	0.19	9.0 a	25.5 ab	47.4 ab	0.81	0.72	0.07 ab	0.54
Sweet Mama	0.09	17.4	0.52	6.3 ab	34.4 ab	39.7 b	0.34	1.12	0.06 ab	0.12
Warren Scarlet	0.25	13.0	0.18	7.0 ab	34.6 ab	43.7 b	0.69	0.28	0.06 ab	0.23
Whangaparoa Crown	0.23	14.0	0.17	7.0 ab	29.2 ab	47.8 ab	0.68	0.56	0.14 ab	0.33
Yogorou	0.18	13.7	0.12	7.7 ab	26.0 ab	51.3 ab	0.58	0.26	0.00 b	0.18
Zapallo Macre	0.27	18.4	0.27	7.3 ab	22.3 ab	49.8 ab	0.76	0.56	0.00 b	0.45
<i>P</i> ^c	0.41	0.53	0.75	0.02	0.02	0.0008	0.35	0.66	0.03	0.24

^a Fatty acid abbreviations: 14:0, myristic acid; 16:0, palmitic acid; 16:1, palmitoleic acid; 18:0, stearic acid; 18:1, oleic acid; 18:2, linoleic acid; 18:3, linolenic acid; 20:0, arachidic acid; 20:1, gadoleic acid; 22:0, behenic acid. ^b Values with different letters denote differences at the 5% level of significance for each comparison among pumpkin cultivars in the respective column. ^c *P* represents the probability of the *F* statistic exceeding the value expected for each comparison among pumpkin cultivars in the respective column.

Table 4. α -, γ -, and δ -Tocopherol Contents (Micrograms per Gram) of Seed Oil from 12 Pumpkin Cultivars

pumpkin cultivar	α -tocopherol	γ -tocopherol ^a	δ -tocopherol	total
Big Max	61.3	492.8 a	35.3	589.4
Cha Cha	29.9	74.9 b	967.1	1071.9
Delica	27.1	85.1 b	933.3	1045.5
Hyvita	36.2	77.1 b	820.5	933.8
Kurijiman	37.4	87.0 b	1109.7	1234.2
Lakota	53.8	148.6 b	800.4	1002.8
Rouge Vif D'Etampes	35.3	285.7 ab	431.0	752.0
Sweet Mama	35.6	75.8 b	850.6	962.0
Warren Scarlet	75.1	172.1 b	659.9	907.1
Whangaparoa Crown	36.7	114.6 b	771.3	922.6
Yogorou	29.6	227.0 b	433.3	689.9
Zapallo Macre	57.6	99.3 b	850.2	1007.2
<i>P</i> ^b	0.26	0.0008	0.15	0.80

^a Values with different letters denote differences at the 5% level of significance for each comparison among pumpkin cultivars in the respective column. ^b *P* represents the probability of the *F* statistic exceeding the value expected for each comparison among pumpkin cultivars in the respective column.

disagrees with oil from Brassicaceae (111). The Brassicaceae oils had significant correlations between the four tocopherol isoforms and stearic, palmitic, linolenic, or gadoleic acid, but not for oleic or linoleic acid, which we observed for pumpkin seed oil (111). In contrast to our findings, a positive correlation between γ - and δ -tocopherol was observed in soybean seeds (112). Although unable to confirm our findings of correlations between contents of γ - or δ -tocopherol and oleic or linoleic acid, studies of sunflower seeds also found no correlation between α -tocopherol and linoleic acid content (113). Correlation analysis indicated that higher levels of TUFA are obtained when palmitic acid levels are low ($r = -0.87$, $P = 0.0003$). Although both present in low amounts, there was a strong correlation between myristic acid and linolenic acid content ($r = 0.82$, $P = 0.0009$). We did not observe any correlations among oil content, percentage unsaturated fatty acids, and total tocopherol or individual isomers.

This study showed that the 12 pumpkin cultivars studied all have seed oils with high levels of unsaturated fatty acids, allowing the potential for pumpkin seed oil to replace oils with higher saturated fatty acid contents. Pumpkin seed oils were low in oxidatively unstable linolenic acid, thereby making the oil suitable for food and other industrial applications and potentially having extended shelf life. Seed oil tocopherol

contents of the 12 cultivars were considerably higher than most other studies, and therefore these 12 cultivars and other genetically similar cultivars should be explored further for their potential to improve the nutrition of human diets. Studies on other *C. maxima* cultivars and cultivars of other pumpkin species could discover other beneficial oils. Further study is also needed to understand how stage of maturity, harvesting conditions, storage period, storage environment, and processing procedures influence pumpkin seed oil characteristics.

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