

Screening of the Entire USDA Castor Germplasm Collection for Oil Content and Fatty Acid Composition for Optimum Biodiesel Production

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ABSTRACT: Castor has tremendous potential as a feedstock for biodiesel production. The oil content and fatty acid composition in castor seed are important factors determining the price for production and affecting the key fuel properties of biodiesel. There are 1033 available castor accessions collected or donated from 48 countries worldwide in the USDA germplasm collection. The entire castor collection was screened for oil content and fatty acid composition by nuclear magnetic resonance (NMR) and gas chromatography (GC), respectively. Castor seeds on the average contain 48.2% oil with significant variability ranging from 37.2 to 60.6%. Methyl esters were prepared from castor seed by alkaline transmethylation. GC analysis of methyl esters confirmed that castor oil was composed primarily of eight fatty acids: 1.48% palmitic (C16:0), 1.58% stearic (C18:0), 4.41% oleic (C18:1), 6.42% linoleic (C18:2), 0.68% linolenic (C18:3), 0.45% gadoleic (C20:1), 84.51% ricinoleic (C18:1–1OH), and 0.47% dihydroxystearic (C18:0–2OH) acids. Significant variability in fatty acid composition was detected among castor accessions. Ricinoleic acid (RA) was positively correlated with dihydroxystearic acid (DHSA) but highly negatively correlated with the five other fatty acids except linolenic acid. The results for oil content and fatty acid composition obtained from this study will be useful for end-users to explore castor germplasm for biodiesel production.

KEYWORDS: *Ricinus communis*, castor germplasm, oil content, nuclear magnetic resonance (NMR), alkaline transmethylation, fatty acid composition, gas chromatography (GC), biodiesel production

INTRODUCTION

Castor belongs to the family Euphorbiaceae (consisting of 280 genera), and the genus *Ricinus* (containing only one species, *Ricinus communis* L.) and has been cultivated for over 6000 years.^{1,2} Castor oil is not edible but has multiple uses ranging from medicine to cosmetics to industrial applications.³ From ancient times, castor oil had been used as a popular stimulant laxative. Currently, castor oil still has many uses in modern medicine such as eye drops for lipid-deficiency dry eyes,^{4,5} an ingredient with balsam and trypsin for reducing edema and scabbing of patient wounds and skin disorders,⁶ and oil inducer for women to start labor.³ Castor oil is also used as a cosmetic ingredient in lipsticks, shampoos, hand lotions, and other coating materials.⁷ Castor oil comprises a high percentage of ricinoleic acid (C18:1–1OH). The hydrogen-bonding property from the hydroxyl group of ricinoleic acid leads to castor oil having high viscosity. Due to its high viscosity, castor oil has been used as an efficient lubricant for high-speed engines such as racing cars and airplanes. Castor seed contains ricin, a heterodimeric glycoprotein toxin, which can inhibit protein synthesis and lead to cell death.⁸ However, it was found that there are no toxic protein (including ricin and allergenic proteins) residuals in castor oil.³

Because castor seed contains a high percentage of oil, castor has great potential to become one of the feedstocks for biodiesel production.^{9,10} Obviously, oil content in castor seed can significantly affect the cost of biodiesel production. However, castor

oil cannot be directly used as fuel due to its extremely high viscosity and high water content, which are not suitable for combustion engines. High viscosity leads to poor fuel atomization during the spray and incomplete combustion.¹¹ As the temperature decreases, the viscosity increases. Therefore, high viscosity causes excessive fuel injection pressure during engine warm-up and more problems for the engine operation at low temperatures.^{12,13} Castor oil has to be converted to methyl or ethyl esters (i.e., biodiesel) by transesterification. The viscosity can be reduced 11.8-fold from castor oil to castor biodiesel by transesterification.¹⁴ Other fuel properties of biodiesel (such as cloud point, oxidative stability, and NO_x exhaust emission) can also be improved by altering the fatty acid composition (length of hydrocarbon chain and number of double bonds) of the original oil.¹⁵

Variability for oil content and fatty acid composition exists in the castor germplasm collection. A natural mutant (OLE-1) with an altered fatty acid composition (a high level of oleic acid and a low level of ricinoleic acid) has been identified by screening 191 castor germplasm accessions.^{16,17} The USDA castor germplasm collection contains 1033 accessions and is maintained by the

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USDA-ARS, Plant Genetic Resources Conservation Unit (PGRCU), in Griffin, GA. The variability for oil content and fatty acid composition within the entire USDA germplasm collection needs to be assessed. In a previous study,¹⁸ the variability for oil content was determined and reported in a short paper by our laboratory but without fatty acid composition. Therefore, the objectives of this study were (i) to determine the variability of fatty acid composition within the entire USDA castor germplasm collection, (ii) to detect correlations among fatty acids and between the oil content and fatty acids, and (iii) to identify castor accessions with a high percentage of oil and/or optimum fatty acid composition as parents to develop castor cultivars for biodiesel production.

MATERIALS AND METHODS

Plant Material. The entire USDA castor germplasm collection containing 1033 accessions was obtained from two locations: 364 accessions from the USDA-ARS, Plant Genetic Resources Conservation Unit, Griffin, GA, and 669 accessions from the USDA-ARS, National Center for Genetic Resources Preservation, Fort Collins, CO. These castor seeds were used in this study.

Oil Content by NMR Analysis. Because our shorter paper did not elaborate on the method for measuring oil content, we wish to present more detailed information here. In a magnetic field, certain atomic nuclei resonate at specific radio frequencies. This resonance can be converted to a signal and measured to determine the amount and nature of the particular nuclei in a sample. As the nuclei return to equilibrium, their collective NMR signal decays at different rates depending on their chemical state and surroundings. Solids and liquids are easily distinguished, and although their nuclear relaxation properties are similar, oils and water can be differentiated by employing specifically timed radio pulses, provided the moisture content is relatively low (<15% of the total mass). NMR signals are calibrated against standards of known amounts to establish curves for measuring oil and water contents in castor seed samples. The oil and water contents were measured using a Mini-spec mq10 nuclear magnetic resonance (NMR) analyzer (Bruker Optics Inc., Houston, TX). The NMR was maintained at 40 °C and operated at a resonance frequency of 9.95 MHz. 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. The temperature of the castor sample can affect the results of oil measurement using NMR.¹⁸ Before measurement, the samples were tempered to 40 °C for 90 min to avoid temperature fluctuation effects. No other preparation of the seeds is necessary. To establish an oil standard curve, 12 standards were prepared by weight. To prepare each standard, shredded paper was added into a test tube along with a carefully measured mass of oil. Moisture standards were prepared using castor seeds of known moisture content and calculating the mass of water present in different amounts of seeds. Seed moisture content was predetermined by measuring the difference in the mass of seeds before and after baking at 130 °C for 3 h. Seed oil and water content were measured, and the mass of each was converted to a percentage of the total weight of each sample. All samples were measured in triplicate (25 seeds for each replication) and the results averaged.

Fatty Acid Composition by GC Analysis. Fatty acid methyl esters (FAMEs) were prepared from castor seed by alkaline transmethylation.¹⁹ Because castor seed contains toxic ricin and allergen proteins, the isolation of castor fatty acids was carefully conducted in the laboratory fume hood (Two River, WI). Two seeds were used, and the seed coat was removed. The endosperm and cotyledons were ground

with a glass rod to fine particles. Approximately 150 mg of these fine particles was transferred into a screw-cap glass tube, and 3.0 mL of *n*-heptane (Fisher Scientific) was added to the extracted castor oil. For conversion of fatty acids to methyl esters, 500 μL of 0.5 M sodium methoxide (NaOCH₃) in methanol solution (Sigma-Aldrich, St. Louis, MO) was added to each tube and mixed with the sample. The reaction was allowed to proceed for 2 h. Five milliliters of distilled water was then added to separate the organic layer from the aqueous layer and residue. An aliquot of the organic layer (1.5 mL) containing the methyl esters was transferred to a 2.0 mL autosampler vial for gas chromatography (GC) analysis.

Fatty acid composition was determined using an Agilent 7890A gas chromatograph (GC) equipped with a flame ionization detector (FID) and an autosampler. Peak separation was performed on a DB-225 capillary column (15 m × 0.25 mm i.d. with a 0.25 μm film) from Agilent Technologies. The carrier gas was helium set to a constant inlet pressure of 15 psi (~1.5 mL/min at 190 °C initial temperature). A FAME standard mix RM-3 (Sigma-Aldrich) was used to establish peak retention times for palmitic (C16:0), stearic (C18:0), oleic (C18:1), linoleic (C18:2), linolenic (C18:3), gadoleic (C20:1), ricinoleic (C18:1–1OH), and dihydroxystearic (C18:0–2OH) acids. One microliter of sample was injected at a 60:1 split ratio into the column with the following thermal profile: 190 °C for 2 min; raised from 190 to 200 °C at 2.5 °C/min and from 200 to 220 °C at 4 °C/min; 220 °C for 2 min. The inlet and detector were set to 280 and 300 °C, respectively. Total run time for each sample was 13 min. Fatty acid composition was determined by identifying and calculating relative peak areas. All samples were prepared and injected twice.

Seed Weight and Seed-Coat Color. Two samples of available seeds were counted and weighed. The average weight for each accession was expressed as grams per 100 seeds. Seed-coat color was also recorded and classified into four categories and expressed numerically: 1, gray with brown stripes; 2, light brown to dark brown stripes; 3, reddish brown with brown stripes; and 4, largely dark brown, black, or a solid color.

Statistical Analysis. An analysis of variance was performed on the data, and means were separated using Tukey's multiple-comparison procedure (SAS, 2008, Online Doc 9.2; SAS Institute Inc., Cary, NC). Significant correlations between response variables were determined using Pearson correlation coefficients.

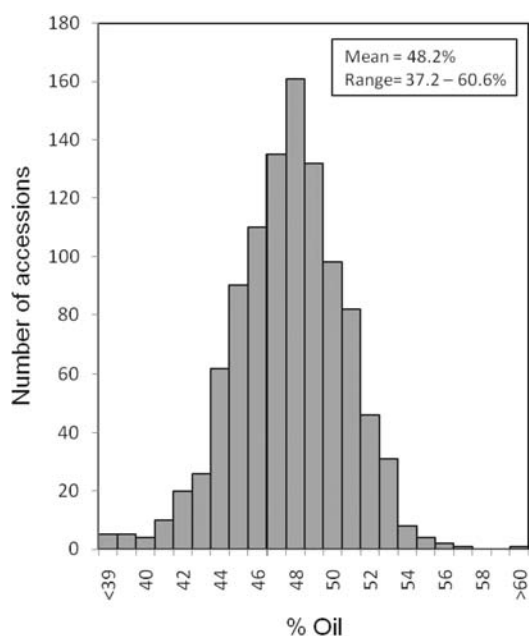
RESULTS AND DISCUSSION

Variability in Oil Content. The results for oil content, fatty acid composition, 100-seed weight, seed-coat color, and country of origin for these castor accessions have been entered into the germplasm resources information network database (<http://www.ars-grin.gov/npgs>). Castor seed from the 1033 accessions on average contained 48.2% oil and ranged significantly from 37.2 to 60.6% among the U.S. germplasm accessions (Table 1). Tukey's studentized range (honestly significant differences, HSD) test for oil was 0.5927%. The variation range and sample distribution of oil content are graphed in Figure 1 and generally formed a normal distribution. In a previously published paper,²⁰ 36 castor varieties were surveyed and the oil content ranged from 39.6 to 59.5% (narrower than 37.2–60.6%) with an average of 51.6% (higher than the 48.2% in our study). The sample size (1033 accessions versus 36 varieties) from these two studies may be a good explanation for the differences. Among 1033 accessions, there were only 4 accessions that contained oil at <38%: NSL 86416, 37.24%; PI 167238, 37.62%; PI 250573, 37.81%; and PI 384013, 37.88%. There were only 8 accessions that contained oil at >55%: PI 240673, 60.62%; NSL 15621, 57.13%; NSL

Table 1. Variability of Seed Oil Content, Weight, and Fatty Acid Composition within the USDA Castor Germplasm Collection^a

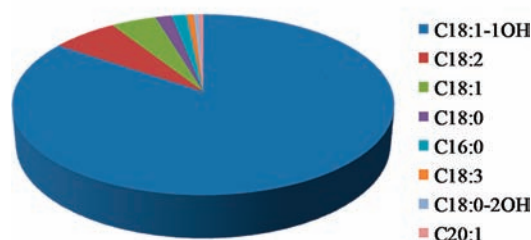
variable	no.	mean	SD	min	max
oil (%)	1033	48.22	2.94	37.23	60.62
seed weight (g)	1033	28.30	5.82	10.12	73.32
fatty acids					
C16:0	1031	1.48	0.19	1.04	2.44
C18:0	1031	1.58	0.34	0.87	4.60
C18:1	1031	4.41	1.09	1.81	9.77
C18:2	1031	6.42	0.65	4.76	9.27
C18:3	1031	0.68	0.16	0.45	1.45
C20:1	1031	0.45	0.12	0.11	1.16
C18:1–1OH	1031	84.51	1.44	78.29	87.98
C18:0–2OH	1031	0.47	0.09	0.19	0.92

^a Fatty acid composition is expressed as % (ME) = % of total methyl esters found in oil samples. SD, standard deviation.

**Figure 1.** Range and sample distribution of oil content in the entire USDA castor germplasm collection.

85845, 56.62%; NSL 85838, 56.04%; NSL 15620, 55.57%; NSL 4687, 55.54%; NSL 85832, 55.40%; and NSL 15626, 55.07%. The oil content of these 8 accessions was much higher than that of 3 released cultivars in Texas:^{21,22} Lynn, 53%; Dawn, 50%; and Hale, 48.3% (measured from this study). Among these 8 high-oil accessions, only PI 240673 was collected from Uruguay, whereas the other seven accessions were donated from the United States. These 8 accessions represent potential breeding material to use for enhancing oil content in castor breeding programs. In our study, we measured the oil from stored seeds, which were not necessarily collected from the same location and year. Our results may differ from those oil extraction results obtained from fresh seeds that are collected from the same location and year.

Variability in Fatty Acid Composition. Castor oil is mainly composed of eight fatty acids. The average proportion of each

Fatty acid composition**Figure 2.** Average fatty acid composition in the castor oil from the entire USDA castor germplasm collection.

fatty acid in the oil from high to low obtained from our analysis is shown (clockwise) in Figure 2: 84.51% ricinoleic (C18:1–1OH), 6.42% linoleic (C18:2), 4.41% oleic (C18:1), 1.58% stearic (C18:0), 1.48% palmitic (C16:0), 0.68% linolenic (C18:3), 0.47% dihydroxystearic (C18:0–2OH), and 0.45% gadoleic (C20:1) acids. Four C18 unsaturated fatty acids (C18:1–1OH, C18:1, C18:2, and C18:3) account for about 96% of the total fatty acid composition in castor seed oil. The variation range and sample distribution for each fatty acid are shown in Table 1 and Figure 3. Significant variability was detected in ricinoleic acid (RA), linolenic acid, oleic acid, and stearic acid, ranging from 78.29 to 87.98%, from 4.76 to 9.27%, from 1.81 to 9.77%, and from 0.87 to 4.60%, respectively. This implies that there is potential to increase or decrease the amounts of these four fatty acids in castor oil depending on their attributes to key fuel properties of castor biodiesel. Because the variation range for the other four fatty acids is small (<1.5%), there is not much potential to decrease or increase these four fatty acids.

The results for fatty acid composition analysis can be affected by extraction and esterification methods of lipids and the sensitivity of the equipment employed. The fatty acid composition of castor oil had been analyzed previously by conventional methods,²³ but this method had limitations and could not distinguish each of the major saturated fatty acids (such as palmitic and stearic acids). Using gas chromatography for fatty acid analysis is more sensitive and accurate than conventional methods. Lipid extraction and esterification have been combined into a one-step reaction using either acidic or alkaline catalysts. Free fatty acids can be transesterified by acidic catalysts but not by alkaline catalysts. However, alkaline transmethylation (using sodium methoxide) is a popular method for esterification and can produce accurate fatty acid analysis with high efficiency and minimum cost.¹⁹ In this study, the alkaline transmethylation method was employed and methyl esters were prepared from the castor meal derived from seeds that had been stored for several years. It is not clear whether the duration of seed storage could lead to high levels of free fatty acid accumulation. If this is the case, the fatty acid composition from castor seeds in this study may be slightly different from that of freshly harvested seeds.

Variability in Seed-Coat Color and Seed Weight. Among 1033 accessions, 641 (62.05%), 299 (28.95%), 60 (5.81%), and 33 (3.19%) were classified into categories 2, 1, 4, and 3, respectively. Light brown to dark brown stripes was the most common seed-coat color, whereas reddish brown with brown stripes was the rarest seed-coat color. A significant variation for 100-seed weight was detected in the collection. The average of 100-seed weight was 28.3 g, ranging from 10.1 to 73.3 g.

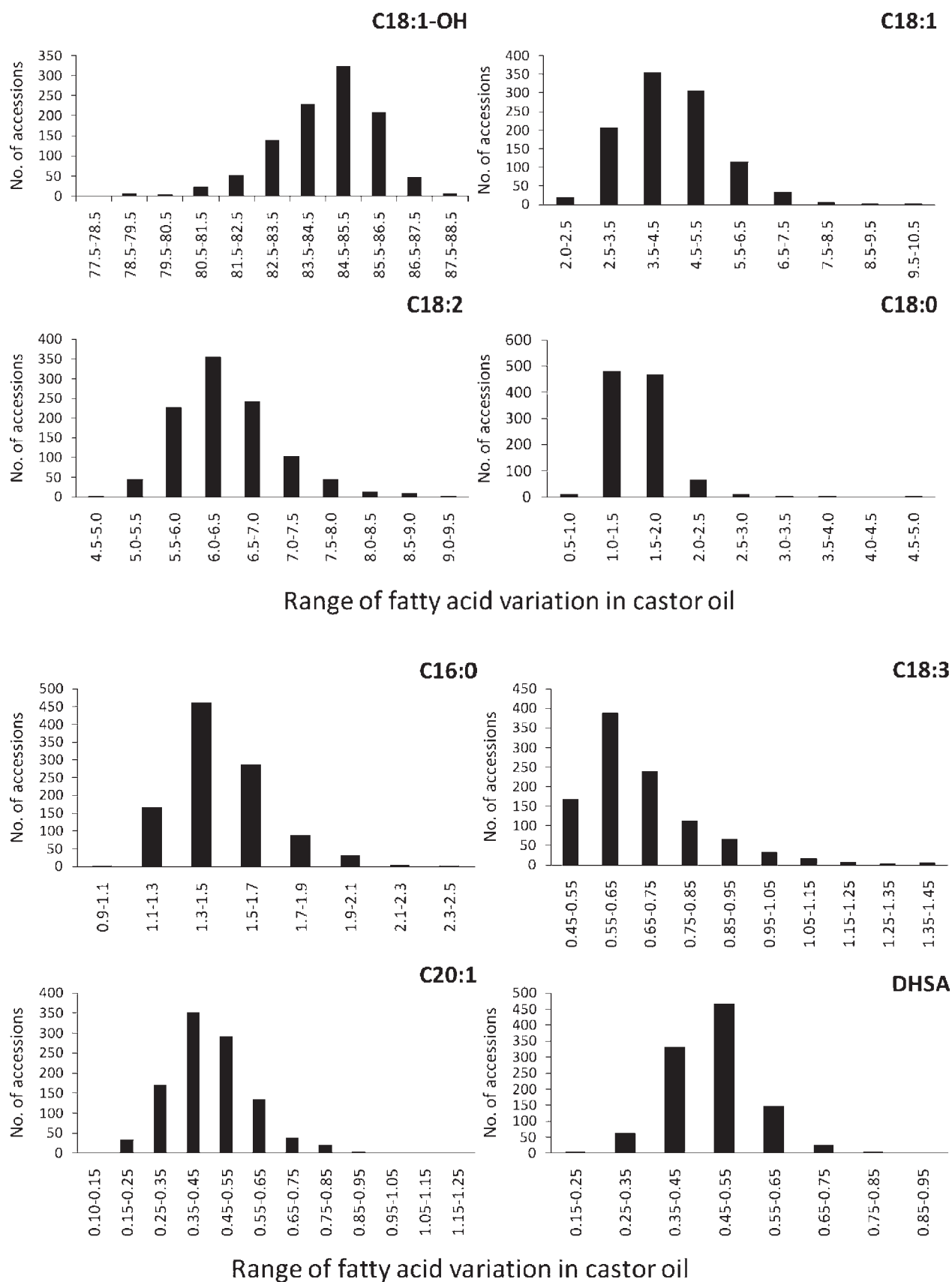


Figure 3. Range and sample distribution of each fatty acid in the entire USDA castor germplasm collection.

Table 2. Pearson Correlation Coefficients, Probability, and Number of Observations for Oil Content, Seed-Coat Color, Seed Weight, and Fatty Acid Composition of the USDA-ARS Germplasm Collection^a

	oil	SeedCl	SeedWt	C16:0	C18:0	C18:1	C18:2	C18:3	C20:1	RA	DHSA	T_HFA
oil	1	0.09	0.16	-0.11	0.08	0.2	-0.16	-0.39	-0.04	-0.04	0.02	-0.04
		0.0033	<0.0001	0.0007	0.013	<0.0001	<0.0001	<0.0001	0.2494	0.1868	0.4856	0.1779
	1033	1033	1033	1031	1031	1031	1031	1031	1031	1031	1031	1031
SeedCl	0.09	1	0.03	-0.02	0.06	0.07	-0.06	-0.02	0.04	-0.04	-0.03	-0.02
		0.0033	0.345	0.6254	0.0689	0.0284	0.0643	0.5863	0.2044	0.2412	0.3583	0.4294
	1033	1033	1033	1031	1031	1031	1031	1031	1031	1031	1031	1031
SeedWt	0.16	0.03	1	-0.21	-0.17	-0.03	-0.1	-0.14	0.07	0.14	0.15	0.14
	<.0001	0.345		<0.0001	<0.0001	0.2709	0.0008	<.00001	0.0168	<0.0001	<0.0001	<0.0001
	1033	1033	1033	1031	1031	1031	1031	1031	1031	1031	1031	1031
C16:0	-0.11	-0.02	-0.21	1	0.23	-0.08	0.74	0.46	0.09	-0.48	-0.46	-0.5
	0.0007	0.6254	<.0001		<.0001	0.0109	<.0001	<.0001	0.0063	<.0001	<.0001	<.0001
	1031	1031	1031	1031	1031	1031	1031	1031	1031	1031	1031	1031
C18:0	0.08	0.06	-0.17	0.23	1	0.41	0.01	-0.07	0.09	-0.56	-0.22	-0.54
	0.013	0.0689	<.0001	<.0001		<.0001	0.863	0.027	0.0062	<.0001	<.0001	<.0001
	1031	1031	1031	1031	1031	1031	1031	1031	1031	1031	1031	1031
C18:1	0.2	0.07	-0.03	-0.08	0.41	1	-0.19	-0.42	0.49	-0.74	-0.18	-0.71
	<0.0001	0.0284	0.2709	0.0109	<0.0001		<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
	1031	1031	1031	1031	1031	1031	1031	1031	1031	1031	1031	1031
C18:2	-0.16	-0.06	-0.1	0.74	0.01	-0.19	1	0.44	0.25	-0.46	-0.15	-0.45
	<0.0001	0.0643	0.0008	<0.0001	0.863	<0.0001		<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
	1031	1031	1031	1031	1031	1031	1031	1031	1031	1031	1031	1031
C18:3	-0.39	-0.02	-0.14	0.46	-0.07	-0.42	0.44	1	0.02	-0.03	-0.07	-0.04
	<0.0001	0.5863	<0.0001	<0.0001	0.027	<0.0001	<0.0001		0.5267	0.2881	0.0184	0.267
	1031	1031	1031	1031	1031	1031	1031	1031	1031	1031	1031	1031
C20:1	-0.04	0.04	0.07	0.09	0.09	0.49	0.25	0.02	1	-0.61	0.09	-0.57
	0.2494	0.2044	0.0168	0.0063	0.0062	<0.0001	<0.0001	0.5267		<0.0001	0.0027	<0.0001
	1031	1031	1031	1031	1031	1031	1031	1031	1031	1031	1031	1031
RA	-0.04	-0.04	0.14	-0.48	-0.56	-0.74	-0.46	-0.03	-0.61	1	0.25	0.96
	0.1868	0.2412	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.2881	<0.0001		<0.0001	<0.0001
	1031	1031	1031	1031	1031	1031	1031	1031	1031	1031	1031	1031
DHSA	0.02	-0.03	0.15	-0.46	-0.22	-0.18	-0.15	-0.07	0.09	0.25	1	0.31
	0.4856	0.3583	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.00184	0.0027	<0.0001		<0.0001
	1031	1031	1031	1031	1031	1031	1031	1031	1031	1031	1031	1031
T_HFA	-0.04	-0.03	0.14	-0.5	-0.54	-0.71	-0.45	-0.04	-0.57	0.96	0.31	1
	0.1779	0.4294	<.0001	<.0001	<.0001	<.0001	<.0001	0.267	<.0001	<.0001	<.0001	
	1031	1031	1031	1031	1031	1031	1031	1031	1031	1031	1031	1031

^a SeedCl, seed-coat color; SeedWt, 100 seed weight; C16:0, palmitic acid; C18:0, stearic acid; C18:1, oleic acid; C18:2, linoleic acid; C18:3, linolenic acid; C20:1, gadoleic acid; RA, ricinoleic acid; DHSA, dihydroxystearic acid; T_HFA, total hydroxyl fatty acid (RA + DHSA).

Tukey's studentized range (HSD) test for 100-seed weight was 7.4 g. There were only 3 accessions that had 100-seed weight of <11 g: PI 370042, 10.1 g; PI 232858, 10.3 g; and PI 267814, 10.5 g. There were only 4 accessions that had 100-seed weight of >50 g: PI 486318, 73.3 g; PI 243214, 67.32 g; PI 243200, 54.0 g; and NSL 15620, 51.0 g. Seed weight is an important component that can contribute to castor seed yield. The accessions with 100-seed weight of >50 g may need to be considered as parents for improving seed yield in castor breeding programs.

Correlations among Different Traits. There were 12 traits investigated in this study, and their correlations are shown in Table 2. Oil content was significantly correlated (at $p < 0.0001$ level) with seed weight ($r = 0.16$), oleic acid ($r = 0.20$), linoleic acid ($r = -0.16$), and linolenic acid ($r = -0.39$), but none of the correlation (r) values were >0.4. This implies that if the oil

content increases, this increase may not significantly affect the fatty acid composition. For example, NSL 15621 had significantly higher oil content (57.1%) than PI 250573 (37.8%), whereas both had a similar level of ricinoleic acid (85.3 and 85.0% in Figure 4). Conversely, if the fatty acid composition of castor oil is altered for the purpose of biodiesel production, it may be possible to maintain the castor oil content at a similar level. For example, NSL 86150 had a significantly higher level of ricinoleic acid (88.1%) than NSL 86268 (78.0%), but their oil contents were very similar (49.0 and 47.4% in Figure 4). Seed-coat color was not significantly correlated with any traits investigated at the $p < 0.0001$ level. Seed weight was significantly correlated with palmitic acid ($r = -0.21$), stearic acid ($r = -0.17$), linolenic acid ($r = -0.14$), ricinoleic acid ($r = 0.14$), dihydroxystearic acid ($r = 0.15$), and total hydroxyl fatty acid ($r = 0.14$), but the

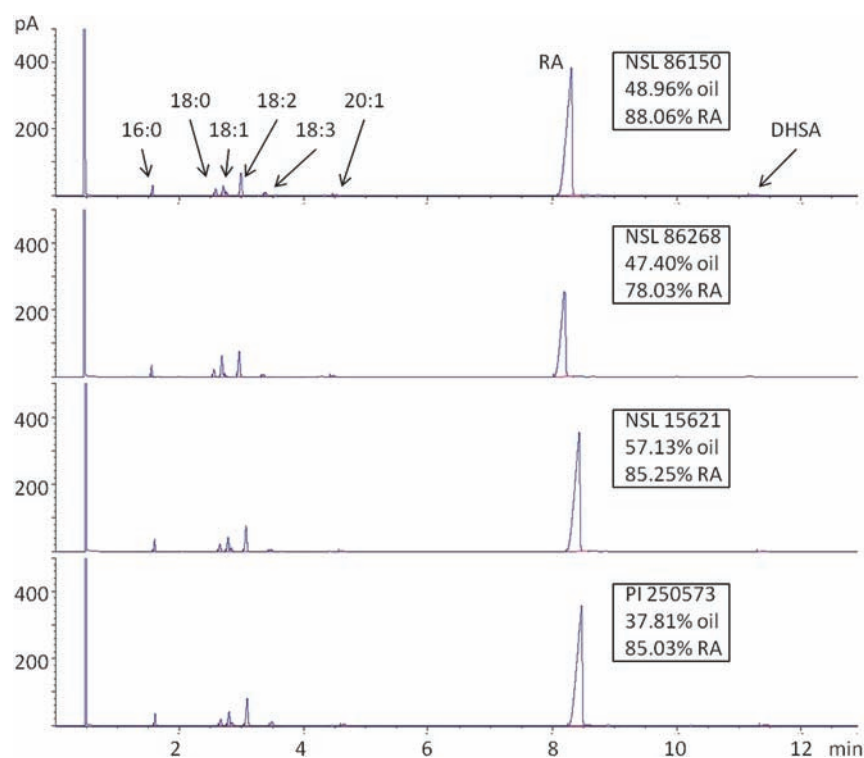


Figure 4. Chromatogram examples showing there was no highly significant relationship between oil content and fatty acid composition.

correlation values were low (<0.3). Seed weight is an important yield component and may directly affect seed yield. This implies that if the fatty acid composition of castor oil is altered for the purpose of biodiesel production, the castor seed yield may not be affected significantly. Palmitic acid was significantly correlated with oleic acid ($r = 0.23$), highly positively correlated with linoleic acid ($r = 0.74$) and linolenic acid ($r = 0.46$), and highly negatively correlated with ricinoleic acid ($r = -0.48$), dihydroxystearic acid ($r = -0.46$), and total hydroxyl fatty acids ($r = -0.50$). This implies that decreasing palmitic acid can significantly decrease polyunsaturated fatty acids (C18:2 and C18:3) and increase ricinoleic acid and total hydroxyl fatty acids. Stearic acid was significantly correlated with dihydroxystearic acid ($r = -0.22$), highly positively correlated with oleic acid ($r = 0.41$), and highly negatively correlated with ricinoleic acid ($r = -0.56$) and total hydroxylated fatty acids ($r = -0.54$). Oleic acid was significantly correlated with linoleic acid ($r = -0.19$) and dihydroxystearic acid ($r = -0.18$), highly positively correlated with gadoleic acid ($r = 0.49$), and highly negatively correlated with ricinoleic acid ($r = -0.74$) and total hydroxyl fatty acids ($r = 0.71$). Linoleic acid was significantly correlated with gadoleic acid ($r = 0.25$) and dihydroxystearic acid ($r = -0.15$) and highly negatively correlated with ricinoleic acid ($r = -0.46$) and total hydroxyl fatty acids ($r = -0.45$). Gadoleic acid was highly negatively correlated with ricinoleic acid ($r = -0.61$) and total hydroxyl fatty acids ($r = -0.57$). Ricinoleic acid was significantly correlated with dihydroxystearic acid ($r = 0.25$) but highly positively with total hydroxyl fatty acids ($r = 0.96$), whereas dihydroxystearic acid was significantly but not highly correlated with total hydroxyl fatty acids ($r = 0.31$). These correlation coefficients are useful in developing strategies in breeding programs for altering the fatty acid composition of castor oil for biodiesel production.

Fatty Acid Composition for the Key Fuel Properties of Castor Biodiesel. Fatty acids in the castor oil can be converted to methyl esters by transesterification. The levels of different methyl esters in biodiesel directly affect the key fuel properties. The key fuel properties affected by methyl esters include ignition quality, heat of combustion, fuel oxidative stability, lubricity, viscosity, cold flow, and nitrogen oxide (NO_x) exhaust emission.^{24–26} Some methyl esters may contribute positively to some fuel properties, but other methyl esters may contribute negatively to other fuel properties. Balancing these fuel properties is important in developing castor oil with a desirable fatty acid composition for biodiesel production. In general, castor oil displays better lubricity than other vegetable oil esters.²⁷ In comparison with petrodiesel, biodiesel with high levels of methyl esters from linoleate (C18:2) and linolenate (C18:3) will have poor fuel stability and high NO_x emission. Therefore, the levels of linoleic and linolenic acids should be reduced as low as possible in the castor oil. Cold flow is an important fuel property. Biodiesel with high levels of methyl esters from palmitate (C16:0) and stearate (C18:0) will have poor cold flow properties compared with petrodiesel.²⁸ The levels of palmitic and stearic acids should be reduced as low as possible. From various studies,^{23–26,29,30} biodiesel with high levels of methyl oleate will have excellent characteristics (such as ignition quality, NO_x exhaust emission, and oxidative stability). From our study, castor oil contains nearly 89% oleate (84.5% ricinoleic acid and 4.4% oleic acid). Castor seed should be a good feedstock for biodiesel production, although a serious concern is the high viscosity. Viscosity is one of the most important fuel properties (or bottlenecks) to determine whether biodiesel can be used as fuel. In general, biodiesel has higher viscosity than petrodiesel.^{9,31} To reduce the viscosity, castor biodiesel should be mixed or blended with petrodiesel, other additives,³² or biodiesel from soybean or brassica.

The entire USDA castor germplasm collection (1033 accessions) has been screened for oil content and fatty acid composition. The ideal germplasm accessions for biodiesel production should have not only a high oil content but also an appropriate fatty acid composition (high content of oleic and ricinoleic acids). Among the 1033 accessions screened, 8 accessions contained oil content above 55%. Among these 8 accessions, PI 240673 contained the highest oil content (60.6%) and also a higher oleic acid (6.2%) than the average (4.4%). This accession was identified as potential breeding material to use for enhancing oil content and improving fatty acid composition in castor breeding programs. The content of ricin and allergic proteins in castor seed is also an important limiting factor preventing castor from being used as feedstock for biodiesel production. Variability in ricin and allergic proteins within the USDA castor germplasm collection is not clear. Screening the germplasm collection for identification of accessions with low content of ricin and allergenic proteins in seed is warranted.

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