World Journal of Agricultural Sciences 4 (1): 01-06, 2008
ISSN 1817-3047

Oil Quality of Winter Hardy Rapeseed Germplasm Relative to Biodiesel Production

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Abstract: There is increasing interest in using vegetable oils as feedstocks for biodiesel manufacturing. Most of the European biodiesel manufacturers use rapeseed oil whereas soybean oil is the feedstock of choice in the United States. However, no information regarding oil quality is available related to rapeseed grown in the mid-Atlantic region of the United States. We studied contents of oil and various fatty acids in 455 winter hardy accessions of *Brassica napus* L. and 44 winter hardy accessions of *Brassica rapa* L, grown in this region. These accessions were previously identified to be winter-hardy from field experiments conducted in Virginia. The oil in *napus* group contained following fatty acids in descending order C22:1, C18:1, C18:2, C18:3, C20:1, C16:0, C20:2, C16:1, C18:0, C20:0, C22:2, C14:0 and C22:0 fatty acids. The contents of fatty acids in *rapa* oil had similar distribution except that *rapa* group had higher content of C18:2 than C18:1 whereas *napus* group had higher C18:1 than C18:2. Based on desirability of low viscosity and higher cetane numbers, it was concluded that *napus* oil may be superior to *rapa* oil for biodiesel manufacturing. Selection index, a calculated value based on closeness of fatty acid profiles of *napus* and *rapa* accessions to an optimal fatty acid profile for soybean oil, also indicated that *napus* accessions may be more close to the target fatty acid profile than *rapa* accessions.

Key words: Biodiesel · *Brassica napus* · *Brassica rapa* · fatty acid profiles · biofuels

INTRODUCTION

There is increasing emphasis on using vegetable oils for manufactring of biofuels such as biodiesel. Industrial rapeseed (*Brassica napus* L. and *Brassica rapa* L.) is produced on a wideworld basis and is currently number three among oilseeds [1]. Until this increasing interest in biodiesel, erucic acid from rapeseed was a valuable and renewable raw material for manufacture of a wide array of industrial products. Even though U.S. industry uses the equivalent of 40 million pounds of high erucic acid oil annually, domestic production of rapeseed is meager and inconsequential relative to soybean, peanuts, sunflower, cottonseed and corn [1].

In North America, *Brassica napus* L. and *Brassica rapa* L. cultivars (oilseed rape or industrial rapeseed) refer to cultivars with high erucic acid (C22:1) content in the oil and high content of aliphatic glucosinolates in the meal. However, rapeseed in Europe refers to canola type cultivars. A genetic variant of rapeseed named canola has been gaining production acreage as a source of nutritious and edible oil. Canola is a coined name given to nutritionally superior seed, oil and meal produced by genetically modified rapeseed plants [2]. Canola refers to cultivars of oilseed rape that produce seed oils with less than 2% erucic acid (C22:1) and meals with less than 30 μmol of aliphatic glucosinolates per g. The canola oil is edible and the meal is suitable for livestock feeding whereas rapeseed oil and meal are not edible [3]. United States imported approximately 702,000 tonnes of canola oil and 1,456,000 tonnes of meal during 2005-06 from Canada for meeting the cooking oil and livestock feed needs [4]. Continuing interest in canola/rapeseed oil as a feedstock for biodiesel production indicates an increasing demand for this oil. Both rapeseed and canola are suitable feedstocks for biodiesel production. However, high contents of erucic acid in the oil are expected to result in biodiesel with cold flow problems as compared to canola oil. In order to meet the need for tremendous amounts of rapeseed oil to be used as a biodiesel feedstock, a need exists to produce more rapeseed in the United States.

Bringe [5] established a target fatty acid profile for ideal soybean oil for biodiesel. These target values consist of 2.1% C16:0, 1.0% C18:0, 71.3% C18:1; 21.4%

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Contribution of Virginia State University, Agricultural Research Station. Journal Article Series Number 260. The use of trade names or vendors does not imply approval to the exclusion of other products or vendors that may also be suitable

C18:2 and 2.2% C18:3. In contrast to these target values, the soybean typically contains 11.8, 4.6, 21.8, 53.1 and 8.0% of these fatty acids, respectively. Bringe [5] also presented fatty acid data on an experimental soybean line which had fatty acid contents closer to the target values.

Our purpose in conducting current studies was to characterize the variation among available rapeseed accessions for oil content and quality. We had previously reported that significant variation exists among rapeseed germplasm for oil, erucic acid and glucosinolate contents [6]. These observations were derived from our evaluation of 455 accessions of *Brassica napus* L. and 44 accessions of *Brassica rapa* L. These 499 accessions were observed to be winter-hardy under Virginia's climatic conditions. The generally prevalent climatic conditions in Virginia during rapeseed crop season (September-June) mandate the use of winter-hardy genotypes. These 499 accessions were selected from a germplasm collection of 938 accessions of *Brassica napus* L. and *Brassica rapa* L. based on their field performance at Petersburg, Virginia during 1993-94 crop season [6].

In the current studies, we evaluated these 499 winter-hardy lines for contents of various fatty acids and determined association, if any, between oil content and contents of various fatty acids. In addition, we compared suitability of *napus* and *rapa* accessions for biodiesel manufacturing.

MATERIALS AND METHODS

A germplasm collection consisting of 938 accessions of Brassica napus L. and Brassica rapa L., provided by the North Central Regional Plant Introduction Station of National Plant Germplasm System of US Department of Agriculture, was evaluated at the Randolph farm of Virginia State University (37°15' N and 77°30.8' W) during the 1993-94 crop season. Thirty seeds of each accession were planted on 14 October 1993 in a completely randomized design with one replication in rows 2-m long with 0.75-m spacing between the rows. The experiment was established on a uniform piece of land (abel sandy loam: Fine Loamy mixed, thermic Aquatic Hapridult). All plots received 100 kg/ha of nitrogen, phosphorus and potassium. The experimental area received a pre-plant-incorporated treatment of Treflan (trifluralin) herbicide. The number of emerged plants in each row were recorded two weeks after planting. All accessions that had a minimum of 12 plants per row were considered to have adequate plant stand. Among these accessions, those where at least 50% of the emerged plants survived the winter and matured were classified as winter hardy. This material consisted of 499 accessions (455 from *B. napus* and 44 from *B. rapa*). Each plot consisted of 12 or more plants that matured. These plants were harvested in bulk during first week of June, 1994 and the seeds of these accessions were used in studies to determine fatty acid contents. The seeds were stored in a freezer before analysis. These seeds were analyzed during 1995-1997.

The oil was extracted from 1 g of ground seed of 455 accessions of *Brassica napus* L. and 44 accessions of *Brassica rapa* L.) at room temperature by homogenization for 2 min in 10 ml hexane/isopropanol (3:2, v/v) with a Biospec Model 985-370 Tissue Homogenizer (Biospec Products, Inc. Racine, WI, USA) and centrifuged at 4000g for 5 min [7]. The oil extraction was repeated for each sample for three times to ensure full oil recovery. The hexane-lipid layer was washed and separated from the combined extract by shaking and centrifugation with 10 ml of 1% $CaCl_2$ and 1% NaCl in 50% methanol. The washing procedure was repeated and the purified lipid layer was removed by aspiration and dried over anhydrous Na_2SO_4. The oil percentage (g/100 g dry basis) was determined gravimetrically after drying in an under vacuum oven at 40°C and stored under nitrogen at -10°C until analysis.

The oil samples (5 mg) were vortexed with 2 ml sulfuric acid/methanol (1:99, v/v) in 10 ml glass vials containing a Teflon boiling chip. The open vials were placed in a heating block at 90°C until the sample volume was reduced to 0.5 ml [8]. After cooling to room temperature, 1 ml of hexane was added. The mixture was vortexed and dried over anhydrous Na_2SO_4. The hexane phase containing fatty acid methyl esters (FAME) was transferred to a suitable vial and kept under N_2 at 0°C for gas chromatographic analysis.

One µl aliquot of FAME in hexane was injected into a SupelcoWax 10 capillary column (25 m X 0.25 mm i.d. and 0.25 Fm film thickness, Supelco, Inc., Bellefonte, PA, USA) in a Varian model Vista 6000 Gas Chromatograph (GC) equipped with a Flame Ionization Detector (FID) (Varian, Sugar Land, TX, USA). Helium was used as a carrier gas at a flow rate of 1 ml/min with a split ratio of 1:100. The column temperature was isothermal at 220°C. The injector and detector temperatures were 250 and 260°C, respectively. A Spectra Physics Model 4290 Integrator (San Jose, CA, USA) was used to determine relative concentrations of the detected fatty acids. Peaks were identified by reference to the retention of FAME standards. The percentage of each peak was calculated as the percentage of the total area of all the peaks excluding the solvent.

Based on the target contents of C16:0, C18:0, C18:1, C18:2 and C18:3 fatty acids in a suitable soybean oil for

biodiesel manufactring (Bring, 2005), we developed an equation to characterize rapeseed lines for their suitability for biodiesel manufacturing. This equation is described below:

$$(\text{Selection Index=SI}) = \text{SQRT}((C16{:}0\text{-}2.1)^2) + \text{SQRT}((C18{:}0\text{-}1.0)^2) + \text{SQRT}((C18{:}1\text{-}73.1)^2) + \text{SQRT}((C18{:}2\text{-}21.4)^2) + \text{SQRT}((C18{:}3\text{-}2.2)^2) \quad (1)$$

Essentially, we recorded the difference between actual content of a given fatty acid and the target content [5], squared the differences to eliminate minus or plus signs and calculated the square root. These deviations for all five fatty acids (C16:0, C18:0, C18:1, C18:2 and C18:3) were summed. The genotype with lowest magnitude of the summed deviations was considered closest to the optimal fatty acid profile and the genotype with the highest magnitude was considered farthest from the optimal fatty acid profile.

The data were analyzed using the procedures of SAS Institute, Inc. [9]. The two *Brassica* species were compared for the 455 accessions of *B. napus* as a group with the 44 accessions of *B. rapa* as a group using the completely randomized design. The mean separation was based on Least Significant Difference at 5% level of significance.

RESULTS AND DISCUSSION

Significant differences existed between *napus* and *rapa* groups for contents of oil and contents of C14:0, C16:0, C20:0, C22:0, C16:1, C18:1, C18:3, C20:2, C22;1 fatty acids in the oil (Table 1). Both species had similar contents of saturated and unsaturated fatty acids. Both groups had statistically similar mean values for MUFA (mono-unsaturated fatty acids) and PUFA (poly-unsaturated fatty acids) but an evaluation of ranges among each species indicated that accessions form *napus* group contained accessions with higher MUFA and lower contents of PUFA. Based on mean values, *napus* oil contained C22:1, C18:1, C18:2, C18:3, C20:1, C16:0, C20:2, C16:1, C18:0, C20:0, C22:2, C14:0 and C22:0 fatty acids in descending order. Corresponding order for *rapa* oil was C22:1, C18:2, C18:1, C18:3, C20:1, C16:0, C20:2, C16:1, C18:0, C20:0, C22:2, C14:0 and C22:0 fatty acids in descending order. The *napus* and *rapa* oils differed only in the contents of C18:1 (oleic) and C18:2 (linoleic) with *napus* having higher content of oleic over linoleic whereas *rapa* had higher mean content of linoleic over oleic.

Correlation analysis (Table 2) indicated that most relationships between contents of oil and fatty acids were

Table 1: Contents of oil and fatty acids in *Brassica napus* L. and *B. rapa* L. seeds, grown in Virginia during 1993-94

	Brassica napus L.		*Brassica rapa* L.		
Trait	Mean[z]	Range	Mean[z]	Range	*napus* vs. *rapa*
Oil	37.441	29.563-49.220	36.577	29.498-40.837	*[y]
14:0	0.207	0.125-0.305	0.197	0.140-0.250	*
16:0	4.707	2.840-6.950	4.770	3.130-5.620	*
18:0	0.683	0.130-1.526	0.719	0.315-1.470	ns
20:0	0.497	0.000-1.355	0.659	0.115-1.400	**
22:0	0.112	0.000-0.675	0.179	0.010-0.400	**
16:1	0.713	0.120-0.196	0.855	0.260-1.460	**
18:1	23.922	10.215-53.905	15.910	9.875-29.230	**
18:2	19.775	5.800-31.620	19.372	14.775-25.385	ns
18:3	12.176	2.075-18.610	13.478	9.150-18.735	**
20:1	9.998	1.520-16.985	9.761	6.200-13.800	ns
20:2	0.868	0.000-2.985	1.441	0.290-2.750	**
22:1	26.064	0.275-56.155	32.598	12.905-50.305	**
22:2	0.278	0.000-2.005	0.361	0.125-1.425	ns
Long chain	37.817	2.205-75.020	44.997	23.185-58.810	**
Short chain	62.183	24.970-97.785	55.002	41.190-76.815	**
SFA	6.205	4.220-9.060	6.224	4.290-7.850	ns
UFA	93.795	90.935-95.775	93.776	92.145-95.700	ns
MUFA	60.697	44.980-83.895	59.124	50.180-66.890	ns
PUFA	33.098	9.270-47.880	34.652	26.910-43.160	ns
Si[x]	65.999	31.682-81.173	73.894	63.653-81.299	**

z: Based on 455 accessions of *Brassica napus* and 44 accessions of *Brassica rapa*., y: Significant differences between mean values of *Brassica napus* and *Brassica rapa*, at 5% (*) or 1% (**), level of significance. NS: differences between mean values of *Brassica napus* and *Brassica rapa* were not significant

Table 2: Correlations between oil and various fatty acids in *Brassica napus* L. and *B. rapa* L. seeds, grown in Virginia during 1993-94

Traits	*Brassica napus* L.	*Brassica rapa* L.
Oil and C14:0	0.08	0.02
Oil and C16:0	0.08	0.03
Oil and C18:0	-0.20**	0.12
Oil and C20:0	+0.10*	0.05
Oil and C22:0	-0.09*	0.23
Oil and C16:1	-0.13**	0.26
Oil and C18:1	0.00	0.14
Oil and C18:2	+ 0.10*	0.24
Oil and C18:3	+ 0.18**	0.29
Oil and C20:1	0.02	0.20
Oil and C20:2	0.06	0.09
Oil and C22:1	0.06	0.05
Oil and C22:2	0.01	0.17
Oil and Long chain	-0.04	0.13
Oil and Short chain	0.04	-0.13
Oil and SFA	-0.10*	0.10
Oil and UFA	+ 0.10*	0.10
Oil and MUFA	-0.14**	0.31*
Oil and PUFA	+ 0.16**	-0.31*

*, **: Correlation coefficient significantly different from zero at 5 and 1% level of significance, respectively

significant for *napus* and non-significant for *rapa*. In *napus*, positive relationships existed between oil on one hand and contents of C20:0, C18:3 (linolenic), total unsaturated fatty acids and total poly unsaturated fatty acids whereas negative relationships existed between oil on one hand and contents of C18:0, C22:0, C16:1, total saturated fatty acids and total mono-unsaturated fatty acids. Most of these correlations were of low magnitude. However, a positive correlation between contents of oil and C18:3 (r=0.18 significant at 1% level) and that between oil and PUFA (r=0.16, significant at 1% level), indicating that both these traits are expected to increase or decrease simultaneously with content of oil, may prove to be a limitation for breeding programs where objective may be to increase oil content and decrease the content of C18:3 and PUFA due to their contributions towards un-stability of oil.

A part of our study dealt with suitability of *napus* and *rapa* oil for manufacture of biodiesel. In light of the observation [10] that viscosity of biodiesel increases with increasing chain length, a comparison of contents of long chain (greater than C20:0) vs. short chain (less than C20:0)

Table 3: Ten rapeseed accessions with lowest and ten with highest SI[x] values in two *Brassica* species

	Brassica napus L.			*Brassica rapa* L.		
No.	Accession	SI[x]	Country of origin	Accession	SI	Country of origin
1.	PI-458945	31.68	Germany	PI-392024	57.77	Canada
2.	PI-531281	33.89	Germany	PI-537003	63.65	Yugoslavia
3.	PI-535861	35.66	Germany	PI-263055	64.61	USSR
4.	PI-531283	36.39	Germany	PI-458618	66.76	New Zealand
5.	PI-535869	36.73	Sweden	PI-458972	67.35	USA
6.	PI-535872	37.17	Poland	PI-470064	67.46	Korea
7.	PI-531276	37.44	Germany	PI-458615	68.78	New Zealand
8.	PI-432392	37.59	Bangladesh	PI-458932	69.54	Sweden
9.	PI-469936	38.36	Korea	PI-458977	69.99	USA
10.	PI-548954	38.62	Germany	PI-406312	70.30	USSR
11.	PI-269449	80.19	Pakistan	PI-162778	78.39	Afghanistan
12.	PI-469988	80.33	Japan	PI-135821	78.63	Afghanistan
13.	PI-469967	80.44	Japan	PI-458973	78.11	USA
14.	PI-469868	80.49	Korea	PI-207463	79.22	Afghanistan
15.	PI-469757	80.73	Korea	PI-269452	79.24	Pakistan
16.	PI-469726	80.81	Korea	PI-254360	79.75	India
17.	PI-469884	80.91	Korea	PI-254543	79.88	Afghanistan
18.	PI-469752	80.92	Korea	PI-251326	80.91	Iran
19.	PI-469862	81.08	Korea	PI-271041	81.21	India
20.	PI-469781	81.17	Korea	PI-391550	81.30	China

x: Equation-1: (Selection Index=SI) = $SQRT((C16:0-2.1)^2) + SQRT((C18:0-1.0)^2) + SQRT((C18:1-73.1)^2) + SQRT((C18:2-21.4)^2) + SQRT((C18:3-2.2)^2)$. Essentially, we recorded the difference between actual content of a given fatty acid and the optimal content, squared the differences to get rid of the minus or plus signs and calculated the square root. These deviations for all five fatty acids (C16:0, C18:0, C18:1, C18:2 and C18:3) were summed

fatty acids in *napus* (a mean of 37.8%) and *rapa* (a mean of 45.0%) oils indicated that *napus* oil may be more suitable for manufacturing of biodiesel with lower viscosity which is considered desirable in biodiesel. Desirability of *napus* oil as a feedstock for biodiesel is also supported based on cetane numbers given that long chain fatty acids in the feedstock oil are also expected to enhance cetane numbers of biodiesel [11].

The viscosity of biodiesel is also affected by contents of MUFA and PUFA with higher content of PUFA and lower contents of MUFA resulting in lower viscosity. The oils from *napus* and *rapa* did not differ in mean contents of MUFA and PUFA (Table 1), however, MUFA ranged from 45 to 84% in *napus* and 50 to 67% in *rapa* oils indicating that rapeseed accessions with lower MUFA can be more easily selected from *napus* as compared to *rapa* germplasm. Similarly, based on PUFA, *napus* oil was considered better for biodiesel manufacture since PUFA in *napus* ranged from 9 to 48% and 27 to 43% in *rapa* since lower contents of PUFA in feedstock oil result in lower viscosity of biodiesel.

Desirability of *napus* oil is also indicated by contents of C18:1, C18:2, C18:3, C16:0 and C18:0 fatty acids in the oil. Bringe [5] established target values for these fatty acids to be 71.3, 21.4, 2.2, 2.1 and 1.0 percent, respectively for an ideal oil for use as a feedstock for biodiesel. Even though *napus* oil was considerably different from these target traits, it was considerable closer to the target values as compared to *rapa* oil. Bringe also indicated that lower contents of C16:0 and C18:0 fatty acids are also desirable for cold flow quality of biodiesel. In our study, *napus* oil had significantly lower C16:0 as compared to *rapa* oil.

The mean selection index (a calculated number denoting similarity or dis-similarity with optimal ratio of fatty acids in an oil for biodiesel manufacturing, lower values indicate desirability for use as feedstock for biodiesel manufacturing), for 455 *napus* accessions was 66.0 and that for 44 *rapa* accessions was 73.89 with significant difference between two species (Table 1). Selection index (varied from 31.68 to 81.17 in *napus* whereas that in *rapa* varied from 57.77 to 81.30 (Table 3). Based on the criterion used by Bringe [5], *napus* accessions are expected to be a better feedstock than *rapa* accessions for biodiesel manufacturing. The selection index values (Table 3) also indicated that most *napus* accessions desirable for biodiesel manufacturing originated in Germany whereas less desirable accessions originated from Korea. In the case of *rapa*, most accessions desirable for biodiesel manufactring originated mostly in Europe whereas less desirable accessions originated in the general geographic area of Afghanistan, Pakistan, Iran and India. Based on the selection index, we identified ten most desirable (low selection index values) and least desirable (high selection index values) accessions in each species (Table 3).

Our results are based on 455 *napus* and 44 *rapa* accessions that were previously categorized as winter-hardy. These results may not reflect true differences between the two species relative to fatty acid profiles rather are representations from a select group of accessions i.e. accessions considered winter-hardy under Virginia' conditions. Additionally, the results from only one environment. The authors realize that results from replications over time and/or space are desirable but given the size of material, replication of such studies will be un-economical and cumbersome. The authors believe that with all the limitations, the result still add to the scientific knowledge.

Europe uses rapeseed oil predominantly for biodiesel manufacturing. However, soybean oil is the feedstock of choice in the United States, mainly due to extensive production of soybean resulting in extensive availability of oil [1]. The rapeseed research in USA has indicated higher rapeseed seed yields and higher oil content over that from soybean. Our studies indicate that *napus* may be the choice of breeders attempting to develop rapeseed germplasm for biodiesel production.

ACKNOWLEDGMENTS

This material is based upon work supported by Cooperative State Research, Education and Extension Service, U.S. Department of Agriculture, under Cooperative Agreement No. 93-COOP-1-9523. Any opinions, findings, conclusions, or recommendations expressed in this publication are those of the authors and do not necessarily reflect the views of the U.S. Department of Agriculture.

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