Method for analysis of fatty acid distribution and oil content on a single Lesquerella fendleri seed

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A B S T R A C T

Lesquerella fendleri is a developing oilseed crop suitable for temperate growing regions in the US. The seed oil is rich in lesquerolic acid (57%) and could serve as a domestic source of hydroxy fatty acids. A method for the analysis of fatty acids and total oil content of a half or single lesquerella seed was developed. Lesquerella seeds are small with 1000 seed weights around 0.6 g (half seed mass of 200–500 μg). Conventional analytical balances provide mass accuracy to ±40 μg which fails to provide sufficient accuracy/precession (4–40% mass error) for the initial seed mass. A microbalance which measures to ±2 μg provided good reproducibility in initial seed weights but was not suitable for mass balance of the extracted oil. A normal phase HPLC coupled to an evaporative light scattering detector gave good response for oil in the mass range of 22 μg to 110 μg/mL. Therefore, micelle concentrations from single or half seed extractions could be determined with good reproducibility. This method was then evaluated on bulk seed that had been fractionated on a gravity table. Gravity table fractionation of L. fendleri seeds obtained from a large field plot provided seven fractions with increasing bulk density. These fractions were then analyzed in sets of 30 individual seeds and as an aggregate of 50 seeds. Oil content for individual seeds varied widely (15.6–44.2%) as did lesquerolic acid content (42.2–63.7%). The mean oil content increased (27–33%) with increasing bulk density (684–745 g/L). The mean lesquerolic acid content did not correlate with bulk density.

1. Introduction

Lesquerella fendleri (Gray) Wats. is a developing oilseed crop suitable for temperate growing regions in the US. The seed oil is rich in lesquerolic acid (57%) and could serve as a domestic source of hydroxy fatty acids. Lesquerella was first characterized almost 50 years ago in a massive screening of plant germplasm by the researchers at the USDA (Jones and Wolff, 1960). One of the main research efforts in the development of lesquerella has been directed toward improving the agronomics of the crop through plant breeding (Dierig et al., 1993).

Rapid development of any crop is dependent on improving the genetics of the crop through plant breeding. Consequently, selections to make genetic improvements often rely on wet chemical methods to characterize and quantitate the components within the plant. This characterization work needs to consume as little of the germplasm as possible yet provide an accurate and reproducible chemical profile of the genetic machinery in the plant. Crops, such as lesquerella as well as...
other oilseeds in the Brassicaceae family are cross pollinated and self-incompatible. A single flower produces multiple seeds that form inside a silique (pod). Lesquerella may contain up to 30 seeds inside a single silique and each has the potential to originate from a different pollen source causing each seed to be genetically different. Although more genetic variability for selection is generated, a desired trait is more difficult to select because the seeds from a single plant are half instead of full siblings, as self-pollination rarely occurs (Dierig et al., 2004).

Single and partial seed analysis methods for the determination of oil has been described in other crops (Knowles, 1989) and the literature has a number of reports for the development of near-infrared spectroscopy (NIRS) methods for specialty oilseeds such as rapeseed (Velasco et al., 1999; Hom et al., 2007; Reitzstein et al., 2007), sunflower (Perez-Vicha and Velasco, 1998), sesame (Sato et al., 2006) and cottonseed (Kohel, 1998). All of the NIRS methods rely on the development of primary wet chemical methods to develop calibration equations for the spectrometer. Variations in seed coats within diverse germplasms typically make NIRS difficult, and Perez-Vicha and Velasco (1998) reported that whole seed samples gave poor correlations for oil and fatty acids while husked seeds, meal and ground seed provided reliable results. Hom et al. (2007) noted that the NIRS analysis was suitable only for larger rapeseeds (250–450 mg). Seed size within lesquerella germplasm is in the range of 0.4–1.0 mg with significant variability in seed coats, therefore NIRS is not well suited for lesquerella seeds. A method for screening fatty acid profiles on a single or half seed was developed by us and results of its application in germplasm screening have already been published (Dierig et al., 2006). Because of the small seed size within lesquerella the development of a method for total oil on a single seed proved to be more difficult. Our objective here is to report on the first analytical methods developed for either a single or half seed for oil content along with the corresponding method for obtaining fatty acid profiles in a small seeded crop such as lesquerella.

2. Materials and methods

2.1. Germplasm

*L. fendleri* seed was grown at the University of Arizona, Maricopa Agricultural Center in Maricopa, AZ and harvested in June of 2006. Seeds were prepared by cutting each seed with a scalpel to include the distal portion of the cotyledon for analysis under a dissecting microscope for analysis. The remaining half seed with the proximal portion of the cotyledon and the entire hypocotyl was planted. Lesquerella oil was obtained from cold pressed seed and then subsequently alkali refined, bleached and deodorized. Acetone and hexanes for extractions and high-pressure liquid chromatography (HPLC) were obtained from Sigma–Aldrich (St. Louis, MO).

2.2. Instrumentation

2.2.1. Gas chromatography

Gas chromatography (GC) was performed with a Hewlett-Packard 6890N gas chromatograph (Palo Alto, CA), equipped with a flame-ionization detector and an autosampler/injector. Analyses were conducted on a SP 2380 30 m × 0.25 mm i.d. (Supelco, Bellefonte, PA) column. Saturated C₈–C₄₀ FAMEs provided standards to make fatty acid methyl ester assignments. SP 2380 analysis was conducted as follows: total flow 15 mL/min with helium head pressure of 172 kPa (25 psi) with a split flow of 10.7 mL/min to give a split ratio of 6.9:1; programmed ramp 180–210 °C at 7 °C/min, 210–265 °C at 30 °C/min; hold 5 min at 265 °C; injector and detector temperatures set at 265 °C. Injection volume of 5 μL was used.

2.2.2. High-pressure liquid chromatography (HPLC)

HPLC separations were performed on a Thermo Separations (Fremont, CA) AS3000 autosampler with 100 μL loop using a PS 2000 binary gradient pump coupled to a Varex (Alltech Associates, Deerfield, IL) evaporative light scattering detector (ELSD). A 4.6 mm × 25 cm Rainin (Varian, Walnut Creek, CA) column was used for the analysis of triglycerides. An isocratic hexane/acetone 50:50 mixture at a flow rate of 1 mL/min eluted the oil from the column with a retention time of 3.1 min. The eluent from the column was connected to the ELSD which used a drift tube temperature of 50 °C and a nitrogen flow rate of 21 L/min at 103 kPa (15 psi) back pressure. A five-point standard curve was developed to determine the response of the ELSD with concentrations of 22.4, 44.8, 67.2, 89.6 and 112.0 μg/mL for single seed analysis. For 50 seed analysis a five-point curve was developed using oil concentrations of 99.8, 199.7, 299.5, 399.4 and 499.2 μg/mL. This response curve was then used to calculate oil concentrations from seed extracts. Check standards for both bulk seed analysis (220 and 380 μg/mL) and single seed analysis (30 and 47 μg/mL) where injected with each sample set to verify detector response.

2.3. Gravity table fractionation of bulk lesquerella seed

Dry lesquerella seeds from the field were screened and aspirated to remove crop residue and other dockage. The clean bulk seeds were then density-graded using a vacuum gravity separator (Model TKV-25, Forsberg, Inc., Thief River Falls, MN). The fractionation diagram is shown in Fig. 1. The seeds were fed into the gravity table using a flexible screw conveyor at the rate of 136 kg/h. The deck inclination was fixed at 1° (side to side). The air flow through the deck and the deck speed were adjusted accordingly to main-

![Fig. 1 – Schematic diagram of gravity table fractionation.](image-url)
tain full coverage of the deck. The first pass on the gravity table separated the bulk seeds into a light, intermediate and heavy fractions noted 1-3, 1-2 and 1-1, respectively. The heavy 1-1 fraction was screened using 11 mesh (1.803 mm opening) and 16 mesh (1.181 mm opening) screens. This provided three additional fractions. The first fraction +11 for seeds that did not pass through the 11 mesh screen. The second fraction is −11 +16 for those seeds passing through the 11 mesh screen but remained above the 16 mesh screen. The last fraction was obtained from the seeds of the 1-1 fraction which passed through both 11 and 16 mesh screens. The remaining two gravity table fractions 1-2 and 1-3 were placed back onto the gravity table independently to further classify a light and heavy fraction, respectively. The bulk density was obtained by recording the mass of 1L of seed using a 1L graduated cylinder as the volume measurement.

2.4. Oil extraction by AOCS method Ae 3-52

Butt extraction of lesquerella seed was conducted using AOCS method Ae 3-52 (Firestone, 1994). Lesquerella seed (30 g) was finely ground using a coffee grinder prior to Butt extractions and three 5 g portions were extracted. These replicated sets were independently evaluated at extraction times of 24 and 27 h. A second set of lesquerella seed (30 g) was ground in a coffee grinder, extracted for 24 h then reground and re-extracted for an additional 3 h. A third set of lesquerella seed (20 g) was ground using a ball mill. The ground seed was extracted 3 × 24 h with grinding between the second and third butt extraction. The combined dry oil masses were used to determine total oil extracted.

2.5. Oil extraction of bulk lesquerella seeds

Bulk lesquerella seeds (50 seeds) were massed into a 11 dram scintillation vial and 5 mL of hexane was placed over the seed. The seeds were thoroughly ground in the hexane solution by a tissue homogenizer (Cole Parmer, Vernon Hills, IL) with a 10 mm coarse generator for approximately 60 s until no whole seeds were visible. The shaft of the mixer was then rinsed with 25 mL of hexane to bring the total volume in the vial to 30 mL. An aluminum lined cap was placed on the vial and the vial was placed in a heating block maintained at 60 °C. After 30 min the heating block the vial was removed and allowed to cool to room temperature then 2 mL of solution was drawn from the top portion of the solution and transferred to a target vial. A screw cap was placed on the target vial and the solution was injected onto the HPLC to determine oil concentration as described above using the response curve developed from the lower oil concentration standards. All samples were run on 30 individual seeds from each gravity table fraction.

2.7. Synthesis of methyl esters for FAMES analysis

The target vials containing the extracted oil in 2 mL of hexane described in Section 2.6 had the screw caps removed and the hexane was evaporated in a heating block at 60 °C under a stream of nitrogen. Once all the hexane had been removed, 0.25 mL of 0.5 M potassium hydroxide in methanol was added to the vial. A new screw cap was placed on the vial and the vial was placed into a heating block at 60 °C. After 60 min the vial was removed, allowed to cool to room temperature, the cap removed and 0.25 mL of 1 M sulfuric acid in methanol was added to the vial. The vial was capped and placed back into the heating block at a temperature of 60 °C. After 15 min the vial was removed from the heating block, allowed to cool to room temperature and the crimp cap removed. To the vial 0.25 mL of saturated sodium chloride solution and 1 mL of hexanes were added. The contents were mixed thoroughly and the two layers were allowed to separate. The top hexane layer was transferred to a clean target vial and injected onto the GC for FAMES analysis as described above. All bulk 50 seed samples were run in triplicate and individual seeds were replicated 30 times for each gravity table fraction.

2.8. Oil content by pulsed NMR

A Bruker Minispec NMR (The Woodlands, TX) was used to determine oil content of the whole seed. Approximately 3.5 g of the whole seed from each gravity table fraction was placed into test tubes and sealed with a stopper. The test tubes were then placed in a 40 °C heating block and heated for 2 h. Each test tube was then placed in the NMR and the oil content in grams was recorded based on a calibration curve made from a linear regression of aliquots of whole seeds with a mass range between 0.08 and 0.80 g of oil.
3. Results and discussion

3.1. AOCS standard method for oil from bulk seeds

A suitable method for the determination of oil concentration in bulk lesquerella samples was evaluated and these results are reported in Table 1. The oil content of the bulk seed will serve as a reference standard for the development of a method for determining the oil content of individual seeds which may have significant variability. AOCS method Ae 3-52 failed to provide a suitable extraction of oil from lesquerella seed even when the extraction time was extended from 4 to 27 h (only 23.7% oil was recovered). Pulsed NMR of the bulk sample calculated the seed oil content to be 29.9% and pulsed NMR evaluation of the extracted seed meal indicated 5.2% residual oil. In an effort to reduce particle size for improved extractability, seed was ground in a ball mill prior to butt extraction. The bulk seed was ground in a ball mill and extracted three times for 24 h with grinding of the mark between the second and third extractions. This modified method increased the extracted oil content to 24.4% but failed to duplicate the NMR values.

3.2. Development of an HPLC method

Poor oil recoveries using the AOCS standard method of analysis and the need to develop a method that can screen numerous samples with very low oil prompted us to develop a method suitable for all the oil analysis (bulk, 50 seeds and single seed). Normal phase silica HPLC provided a solution when using a 50:50 hexane/acetone solvent mixture a single oil peak was observed. Standard response curves for an evaporative light scattering detector (ELSD) connected to this HPLC were developed. For bulk 50 seed analysis, an oil concentration of 99–499 g/mL was used and resulted in a linear detector response ($R^2 = 0.9999$, $P < 0.01$) over this range. For single seed analysis an oil concentration of 22–112 µg/mL was used and resulted in a linear detector response ($R^2 = 0.9993$, $P < 0.01$) over this range. As can be seen in Table 2. The lightest mass fraction was found in the 1-3 light fraction of the grav-
ity table. Consequently, the lowest oil mass found occurred within this fraction where 24.2 μg oil measured. This value was within the linear response of the ELSD and demonstrated the robustness of the HPLC/ELSD to measure this low oil level.

3.3. Hexane soak method for oil determination

The HPLC method just described was then employed to determine the oil content of crude extracts of bulk gravity table fractionated lesquerella seed. Seeds were placed in 11 dram vials in 5 mL of hexane and either hand crushed or ground by tissue homogenizer. The ground seed in hexane solution was then extracted at 60 °C for 30 min and the resulting crude hexane extract supernatant analyzed directly by HPLC (pulsed NMR of this mark found no residual oil within the meal).

Table 1 indicates that 50 seeds crushed by hand with a glass stirring rod or ground finely in the tissue homogenizer provided similar oil concentrations and agreed well with the value for this seed obtained by pulsed NMR. The close agreement of hand crushing to fine grinding with the mixer indicates that single seed analysis not capable of grinding in the mixer will still give good oil recoveries when crushed by hand.

3.4. Gravity table fractionation of bulk lesquerella seed

Gravity table fractionation of bulk lesquerella seed yielded seven fractions. A separation diagram is shown in Fig. 1. Table 2 lists the bulk densities of each fraction and its relative percent to the whole lot. The bulk seed had a density of 691 g/L. The largest fraction (60.7%) was the 1-3 light fraction which had a bulk density of 684 g/L. The oil content of this fraction was 26.3% with a percent oil range of 15.6–35.5%. There does not appear to be a direct correlation of bulk density to oil content as shown in Table 2. However, the combined average oil percentage and bulk density of the three gravity table fractions 1-1, (bulk density 717, oil 33.0%), 1-2 (bulk density 691, oil 29.7%) and 1-3 (bulk density 692, oil 27.0%) indicate a general relationship where higher bulk densities yield higher oil containing seed. There is a good correlation between bulk density and average seed mass with higher bulk densities indicating higher seed mass. As expected from the correlation of bulk density to average mass, the higher bulk density fractions contained seeds with heavier maximum masses than those with lower bulk densities. Unfortunately, the fractions with the highest oil and bulk densities represent the smallest fraction of the seed from a field plot.

Table 3 – Main fatty acid distribution means of 30 individual seeds from gravity table fractionation

<table>
<thead>
<tr>
<th>Fraction</th>
<th>16:0</th>
<th>16:1</th>
<th>18:0</th>
<th>18:1</th>
<th>18:2</th>
<th>18:3</th>
<th>20:1</th>
<th>18:1OH</th>
<th>20:1OH</th>
<th>20:2OH</th>
<th>Maximum 20:1 OH</th>
<th>Minimum 20:1 OH</th>
<th>Median 20:1 OH</th>
</tr>
</thead>
<tbody>
<tr>
<td>+11</td>
<td>1.1</td>
<td>0.4</td>
<td>2.1</td>
<td>15.7</td>
<td>7.2</td>
<td>14.1</td>
<td>1.1</td>
<td>0.5</td>
<td>54.3</td>
<td>3.5</td>
<td>58.5</td>
<td>44.6</td>
<td>55.2</td>
</tr>
<tr>
<td>-16</td>
<td>1.1</td>
<td>0.4</td>
<td>1.9</td>
<td>15.0</td>
<td>7.1</td>
<td>12.9</td>
<td>1.0</td>
<td>0.5</td>
<td>57.0</td>
<td>3.2</td>
<td>59.4</td>
<td>53.8</td>
<td>57.3</td>
</tr>
<tr>
<td>1-2 Heavy</td>
<td>1.0</td>
<td>0.4</td>
<td>1.6</td>
<td>14.4</td>
<td>6.9</td>
<td>13.0</td>
<td>0.8</td>
<td>0.4</td>
<td>58.2</td>
<td>3.3</td>
<td>59.9</td>
<td>50.9</td>
<td>57.5</td>
</tr>
<tr>
<td>1-2 Light</td>
<td>1.1</td>
<td>0.2</td>
<td>1.7</td>
<td>14.3</td>
<td>7.4</td>
<td>13.8</td>
<td>0.7</td>
<td>0.1</td>
<td>57.5</td>
<td>3.0</td>
<td>61.7</td>
<td>55.5</td>
<td>57.7</td>
</tr>
<tr>
<td>1-3 Heavy</td>
<td>1.4</td>
<td>0.8</td>
<td>1.5</td>
<td>14.2</td>
<td>7.3</td>
<td>14.1</td>
<td>0.6</td>
<td>0.3</td>
<td>56.5</td>
<td>3.4</td>
<td>62.4</td>
<td>42.2</td>
<td>57.5</td>
</tr>
<tr>
<td>1-3 Light</td>
<td>1.1</td>
<td>0.2</td>
<td>1.8</td>
<td>15.3</td>
<td>7.5</td>
<td>13.9</td>
<td>0.6</td>
<td>0.1</td>
<td>56.9</td>
<td>2.6</td>
<td>63.3</td>
<td>44.7</td>
<td>57.7</td>
</tr>
<tr>
<td>Bulk Seed</td>
<td>1.8</td>
<td>1.1</td>
<td>1.4</td>
<td>13.9</td>
<td>8.5</td>
<td>15.8</td>
<td>0.4</td>
<td>1.2</td>
<td>52.0</td>
<td>3.0</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

Fatty Acids are average of 30 individual seeds. Fatty acid legand: 16:0 is palmitic; 16:1 is palmitoleic; 18:0 is stearic; 18:1 is oleic; 18:2 is linoleic; 18:3 is linolenic; 20:1 is eicosenoic; 18:1 OH is ricinoleic; 20:1 OH is lesquerolic; 20:2 OH is auricolic.
3.5. **Fatty acid profiles of gravity table fractions**

Individual seed FAMEs analysis did not prove to be difficult with good signal to noise ratios, 3200:1 for lesquerolic (57% of total FAs) and 120:1 for palmitic (1% of total FAs) as can be seen by Fig. 2. Total oil mass at the lowest concentration observed was 24 μg/mL with an injection volume of 5 μL and a split ratio of 6.9:1 to give an on column mass of 17 ng. Table 3 presents the fatty acid profiles of all the gravity table fractions. Average lesquerolic acid content was the highest (58.2%) in the 1-2 gravity table heavy fraction. However, individual seed maximum tended toward the lightest seeds with a lesquerolic acid content of 63.3% in the 1-3 light fraction. The 1-3 fraction represents the majority (60.7%) of the seed harvest from a field plot. The median lesquerolic acid content centered around 58% in all fractions except the +11 fraction.

4. **Conclusions**

The HPLC method for total oil from hexane extracted single lesquerella seeds provided good reproducibility (standard deviation 0.4–1.8%) even when the mass of the seed was low. This method has now been successfully applied to half seed oil analysis for lesquerella (data not shown). Subsequent conversion of extracted oil into FAMEs and analysis by GC gave good signal to noise ratios for all of the major fatty acids in lesquerella. Lastly, gravity table fractionation of bulk seed may provide a means to screen for seeds with higher oil content, unfortunately the largest mass fraction from the gravity table had the lowest oil content.

**Acknowledgments**

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**REFERENCES**


