CROSS-CUTTING PROJECTS

**Title**: Oil analysis

**PI**: Ian Burke

**CoPI’s**: Pat Fuerst

**Graduate students**: none; Undergraduate: Jason Parsley (supported by the Biofuels project)

**Technical Support**: Scott Mattinson (partial support from Biofuels project), Dennis Pittmann, and John Fellman

**Background**: The purpose of this project is to provide oilseed quality analysis as a service in support of the field research component of this project. We are providing data on parameters such as oil yield from a seed crusher, total oil content, and fatty acid composition. Fatty acid composition is the key determinant of oil quality for biodiesel. This work will allow our research team to assess the suitability, locations and crop varieties, across growing seasons for oil content and quality.

Prior research indicates that environmental conditions can affect oil content and quality in oilseeds. Late-season insect infestations reduced both seed yield and seed oil content, with no effect on oil quality, in canola and rapeseed (Brown et al., 1999). Contamination of oilseeds with weed seeds can increase linolenic and erucic acids (Davis et al, 1999), both of which are highly undesirable in biodiesel. Production environment had a substantial effect on seed oil content of soybeans; the same six soybean lines averaged 27% more oil in seeds from plants grown in Mississippi, where seed-fill temperature was 27°C, than those from Indiana, where the seed-fill temperature averaged 21°C.

Gas chromatograph (GC) analysis is an efficient and precise method for assessing biodiesel quality, especially because it quantifies problematic fatty acids present in certain crops and cultivars. Certain fatty acids are very undesirable in biodiesel, including very long-chain fatty acids and highly unsaturated fatty acids. Canola breeding has dramatically decreased the undesirable fatty acids while increasing the desirable fatty acids like oleic acid. As it turns out, these are generally the best fatty acids for many purposes, including biodiesel, health, and food processing. In contrast to canola, there has been very little selection for oil quality in camelina, and the quality of this oil for biodiesel is substantially lower (Frohlich and Rice, 2005). Our own results confirm that camelina has relatively high levels of long-chain and highly unsaturated fatty acids which are undesirable in both biodiesel and food applications.

**Objectives**: Determine the cold press oil yield, total oil content, fatty acid composition, and potential biodiesel problems of canola, camelina, and other oilseed crops produced in field plots by other researchers in this project. Oil quantity and composition will be used along with yield data to make crop and variety recommendations to growers and processors.
Methods: In our part of this project, we determine seed crusher oil yield, total oil content, and fatty acid composition of canola, camelina, and other oilseed crops produced in field plots by other researchers. When analyses are complete, we provide a data summary to each investigator. Oil yield is determined using a Komet oil extractor (Fig. 1) to estimate maximum oil yield that could be obtained on an industrial scale. Total oil content is determined at the University of Idaho by nuclear magnetic resonance (NMR). Oil composition is determined in methyl-esterified extracts; methyl-esterification is also the process that produces biodiesel. Methyl-ester composition is determined by gas chromatography / flame ionization detection (GC-FID) and verified by gas chromatography / mass spectrometry (GC-MS). Developing efficient and highly repeatable methods for each procedure, especially GC, was the most time consuming part of our work to date.

Specific protocols:

A. Moisture: 10 grams of oilseed are placed in a weighed tin, dried overnight at 130C. Samples are immediately weighed after removing from the oven and % moisture is calculated (AOCS, Official Method 2-41).

B. Cold-press oil yield (seed crushing): The Komet oil crusher is pre-heated to 200F; after running blank oilseed sample to equilibrate crusher, 200-g samples are put through the crusher at low speed (5 minutes per sample) to maximize yield. Samples of oil and seed meal are collected over exactly a 2-minute period. Samples are weighed to calculate oil and seed meal yield (Fig. 2).

Figure 1. Komet oil crusher/extractor used to determine oil yield.

Figure 2. Canola oil and meal after seed crushing. After being weighed, the samples are analyzed for oil composition and quality.
C. Total Oil Content: Dried 12-gram samples are analyzed on a Newport MKIII NMR Analyzer, calibrating on an appropriate reference sample (Howard and Daun, 1991; Davis et al., 1999). The analysis is conducted by Lindy Seip in Dr. Jack Brown’s Oilseeds Laboratory at University of Idaho.

D. Fatty Acid Composition of Oil:  
* Methyl-esterification: Methods were adapted from several closely related procedures (Davis et al., 1999; O’Fallon et al., 2007; Hammond, 1991; Barthet and Daun, 2005; Barthet et al., 2002). A 15 µL aliquot of oil is hydrolyzed and methylated with sodium methoxide. Fatty acid methyl esters are separated from other reaction products by partitioning into hexane. The fatty acid composition of the hexane phase is analyzed by GC-FID or GC-MS.

* GC Analysis: We have two HP 5890 gas chromatographs in Dr. Burke’s laboratory. Initial analyses were conducted with “DB-1MS” columns we have used extensively in our laboratory and all fatty acids ≥ 1% of the total fatty acids present were identified by mass spectrometry (Fig. 4). However, separation of linoleic and linolenic acid was inadequate and we purchased an “HP-88” column, specifically made for separating fatty acids. With this column, we obtained remarkable separations of all relevant fatty acids, and revealed numerous (as yet unidentified) minor fatty acids, many of them representing < 0.1% of the total fatty acids present. Although such fatty acids would not likely affect biodiesel quality, it is indicative of the quality of our procedure. At this point it is not possible for us to study the effects of field treatments or crop varieties on these minor fatty acids due to the sheer number of constituents and the number of samples we analyze.

* Preliminary Analysis of GC data: Fatty acid composition is the most complicated aspect to report and we have not settled on a final method of reporting. We quantify and analyze results on all fatty acids representing ≥ 1% of the total fatty acids present. In canola we have 6 peaks to analyze whereas camelina has 13. Compiling all this data across hundreds of oilseed samples represents a big challenge and as yet unfinished business. Most likely we will summarize results as pertinent to biodiesel quality. This would include the % composition of the following fatty acids, grouped according to general biodiesel properties:

1. Fatty acids ≤ 16 carbons (≤ C16)
2. Palmitic acid and other C18 fatty acids not including those below
3. Oleic + linoleic acids (C18 with 1 and 2 unsaturated bonds, respectively); these are very desirable constituents of biodiesel.
4. Linolenic acid (C18 with 3 unsaturated bonds; undesirable biodiesel constituent)
5. Fatty acids ≥ 20 carbons (most are undesirable in biodiesel)

E. Final report to Researchers:
Field researchers provide us with a spreadsheet showing the experimental design, treatments, and replication. Based on this data sheet, we add data columns from the above tests, including moisture, cold-press oil yield, total oil content, and fatty acid composition, described above.

Duration: 7/1/07 – 6/30/09, and renewed for the current biennium (2009-2011)

Results and Discussion:
Following literature review and method development, all laboratory analyses of oilseed samples have been completed (Table 1). We are compiling the results for each investigator and will send
their final report in early January. We are receiving 2009 crop year oilseed samples now and have begun processing them.

Table 1. Progress in Analyzing 2008 Oilseed Samples.

<table>
<thead>
<tr>
<th>Investigator</th>
<th>Study</th>
<th># Samples</th>
<th>moisture</th>
<th>crush</th>
<th>NMR</th>
<th>GC</th>
<th>analyze and summarize data</th>
<th>Send final report to scientists</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stevens</td>
<td>Canola Variety</td>
<td>24</td>
<td>√</td>
<td>√</td>
<td>√</td>
<td>√</td>
<td>√</td>
<td>√</td>
</tr>
<tr>
<td>Stevens</td>
<td>Canola Line source</td>
<td>160</td>
<td>√</td>
<td>√</td>
<td>√</td>
<td>√</td>
<td>√</td>
<td>√</td>
</tr>
<tr>
<td>Stevens</td>
<td>Canola Fertility</td>
<td>54</td>
<td>√</td>
<td>√</td>
<td>√</td>
<td>√</td>
<td>√</td>
<td>√</td>
</tr>
<tr>
<td>Hulbert</td>
<td>Camelina Variety</td>
<td>75</td>
<td>√</td>
<td>√</td>
<td>√</td>
<td>√</td>
<td>√</td>
<td>√</td>
</tr>
<tr>
<td>Young</td>
<td>Young - Canola seeding</td>
<td>6</td>
<td>√</td>
<td>√</td>
<td>√</td>
<td>√</td>
<td>√</td>
<td>√</td>
</tr>
<tr>
<td>Hammac</td>
<td>Canola Fert, 2 sites</td>
<td>80</td>
<td>√</td>
<td>√</td>
<td>√</td>
<td>√</td>
<td>√</td>
<td>√</td>
</tr>
</tbody>
</table>

Canola samples were analyzed for oil yield, oil content, and seed meal composition, summarized in Table 2. Seed meal analysis indicated that seed crushing extracted 85% of the oil in the seed, with 15% remaining in the seed meal; this is a very high efficiency. Based on this data we estimated total oil content in these samples to be 47%. Total oil content estimated at the UI by NMR was 43%. The discrepancy was small but we will attempt to determine the reason for this. Seed meal analysis (Table 2) indicated 30% protein content and 74% total digestible nutrients; this is a very high quality feed but protein is lower than usual for canola, which typically is more like 40%.

Table 2. Canola oil yield from crusher, total oil content, and seed meal composition, average of 6 samples of ‘Rapier’. Samples from Frank Young’s field plots in Okanogan.

<table>
<thead>
<tr>
<th>WSU Analysis</th>
<th>UI Analysis</th>
<th>Contract Lab: Seed meal composition</th>
</tr>
</thead>
<tbody>
<tr>
<td>% seed meal from crusher</td>
<td>% oil from crusher</td>
<td>% oil from seed meal</td>
</tr>
<tr>
<td>60%</td>
<td>40%</td>
<td>7%</td>
</tr>
</tbody>
</table>

Camelina oil yields from our seed crusher varied among varieties (Figure 3). The cultivar ‘Calena’ is consistently one of the highest yielding in field plots, and also has one of the highest oil contents; combined, these make it a very desirable variety. Total oil contents among these varieties showed a similar pattern among cultivars, similar to Figure 1, as determined by NMR at UI.
Figure 3. Oil yield from 20 camelina cultivars (field research by Scot Hulbert). Ranking of cultivars was similar for total oil content.
Fatty acid composition of each oil sample was analyzed by GC (Figure 4). Fatty acids are separated by retention time on a capillary column and either quantified by flame ionization (FID) or quantified and identified by mass spectral (MS) analysis. Figure 4 is from a GC-MS. Using MS, the “fingerprint” of each peak is compared with a library of known fatty acid fingerprints and allows us to positively identify each major component.

Figure 4. Fatty acid-methyl ester profile from rapeseed (low erucic acid type). Separations occurred over 90 minutes on the GC-MS. Fatty acids were identified by mass spectral matches with known fatty acids and confirmed by samples with known fatty acid composition. Nomenclature: C18 indicates fatty acid with 18 carbons; C18:1 indicates one unsaturated bond (this is oleic acid, the most abundant). The C17 standard is artificial and allows us to estimate sample-to-sample variations.
We present below (Figure 5) a comparison of fatty acid composition of canola, rapeseed, and camelina. Canola varieties have been intensively developed as food oil, and this is why it has the highest levels of oleic and linoleic acids, both of which are highly desirable for biodiesel and food oils. High content of linolenic acid (camelina) and long-chain fatty acids (≥C20) is undesirable in biodiesel.

**Figure 5.** WSU GC analysis of fatty acid composition of canola, rapeseed (high erucic acid type), and camelina.

**Impact/Potential Outcomes:**
Given the differences in agronomic zones, differences in the seed oil content and quality can be expected among the experimental locations. The knowledge generated by this cross-cutting project, together with crop yields determined by cooperating researchers, will allow us to make recommendations as to which crop types and varieties are best across a diversity of locations in eastern Washington.

**Publications:** None as yet

**Future directions in the upcoming year:** We will send final reports on crop year 2008 oilseed samples to cooperating field researchers by early January 2010. Using protocols established in our laboratory, we will continue to service multiple projects, analyzing samples from the 2009 crop year.
**Literature Citations:**


