Yield and Phytosterol Composition of Oil Extracted from Grain Sorghum and Its Wet-Milled Fractions

Vijay Singh,1,3 Robert A. Moreau,2 and Kevin B. Hicks2

ABSTRACT

Corn fiber contains an oil with high levels of three potential cholesterol-lowering phytosterol compounds. Little information is available about the levels and types of phytosterols in sorghum. In this study, phytosterols were evaluated in grain sorghum and its wet-milled fractions and were compared with the phytosterols in corn. The study showed that sorghum kernels can provide a significant source of two phytosterol classes, free phytosterols (St) and fatty acyl phytosterol esters (St:E).

Most of these phytosterols are concentrated in the wet-milled fiber fraction followed by the germ fraction. In addition to phytosterols, other lipid classes such as wax esters and an aldehyde (50% C28 and 50% C30) are also present in the sorghum oil. Comparison of sorghum and corn kernels show that corn has 72–93% more phytosterols than sorghum.

MATERIALS AND METHODS

Two sorghum hybrids, hybrid A (Cargill 737) and hybrid B (Cargill 888Y) with different genetics were obtained from a commercial seed company grown during the 2000 crop season. Sorghum samples were hand-cleaned to remove broken kernels and foreign material, packaged in plastic bags, and stored at 4°C until analysis. The whole kernel moisture content of the samples was measured using the 103°C convection oven method (Approved Method 44-15A, AACCC 2000). The fatty aldehyde standard was provided by the Department of Food Science and Technology, University of Nebraska-Lincoln, NE (Hwang et al 2002a,b).

The wet-milling process for sorghum is basically the same as the corn wet-milling process (Watson 1984). The wet-milling of sorghum samples was performed using the 1-kg laboratory corn wet-milling procedure as outlined by Eckhoff et al (1993) with slight modifications. The modifications included reducing the batch size to 500 g and using a Doxie A hydrocyclone (Singh and Eckhoff 1995) instead of starch tables to separate starch and protein fractions. The moisture content of all the fractions was determined using the two-stage convection oven method (Approved Method 44-18, AACCC 2000).

All the dried wet-milled fractions except the germ were ground (2–4 g) in a Wiley mill to 20-mesh size. The dried germ (1–2 g) was placed in a 55-mL screw-top vial, 40 mL of hexane was added, and the mixture was homogenized (Brinkman Polytron). The tube was shaken horizontally for 1 hr in a wrist-action shaker at room temperature. After extraction, the hexane extracts were filtered through a glass fiber filter (Whatman GF/A) fitted with a Buchner funnel under vacuum. A part of the sample was removed for HPLC analysis as previously outlined by Moreau et al (1996). Oil and phytosterols were extracted from the other dried wet-milled samples with hexane using an accelerated solvent extractor ( Dionex ASE200). Ground samples (1–2 g) were placed in 11-mL sample extraction cells. The extraction conditions in the cells were pressure of 1,000 psi, temperature of 100°C, heat time of 5 min, start time of 10 min, three static cycles, 100% flush volume, and a purge time of 60 sec.

For HPLC analysis, part of the sample was removed from the extracted solvent as outlined by Moreau et al (1996). The lipid classes, triacylglycerols (TAG), free fatty acids (FFA), free phytosterols (St), and fatty acyl phytosterol esters (St:E).

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RESULTS AND DISCUSSION

Initial analysis of oil extracted from sorghum kernels and their wet-milled fractions using the HPLC method (with a DioL column) indicated high concentration of phytosterol fatty acyl esters (St:E), however, previous reports had suggested that the high concentration of St:E may be due to other sorghum lipid classes coeluting. Bianchi et al (1979) reported that sorghum lipids are composed of fatty aldehydes, fatty alcohols, fatty acids, hydrocarbons, wax esters, and steryl esters. Hwang et al (2002a) reported that the sorghum fatty aldehyde is 50% C28 and 50% C30. The sorghum fatty aldehyde (C28 standard), sterol esters (cholesterol stearate as standard), fatty aldehyde (C28 and C30 standard), and hydrocarbons on a DioL column showed very similar retention times. Nordbäck and Lundberg (1999) reported a new method to successfully separate steryl ester and wax esters in animal lipid extracts using an HPLC with an alumina column. Use of an alumina column for analysis of oil from sorghum kernels and its wet-milled fractions resulted in separation of hydrocarbons, wax esters, sterol esters, and fatty aldehydes (Fig. 1). All further analysis of oil from sorghum and its wet-milled fractions for quantitative analysis of hydrocarbons, wax esters, fatty acyl phytosterol esters, and fatty aldehydes were done using the HPLC alumina method. For quantitative analysis of free phytosterols, ferulate phytosterol esters, free fatty acids and triacylglycerols, the HPLC DioL method was used.

Significant amounts of free phytosterols (St) and phytosterol fatty acyl esters (St:E) were observed in sorghum kernels and their wet-milled fractions for both the hybrids (Tables I and II). Both these phytosterol classes were mainly recovered from the fiber, germ, and protein fractions of the sorghum kernel. High concentrations of these two phytosterols were observed in the oil recovered from the fiber and the protein fraction, compared with the other wet-milled fractions. For hybrid A, sorghum fiber had ≥2.5% of oil and ≥1.3% of that oil was St fraction and ≥1.4% was the St:E fraction (Table I). Compared with St and St:E, significantly low amounts of ferulate phytosterol esters (FPE) were also present in the sorghum fiber oil. The sorghum germ fraction (for hybrid A) had the maximum amount of oil (13.6%) compared with the other fractions, however, the concentration of the St (≥0.5%) and St:E (≥0.3%) are low compared with the oil recovered from fiber and protein. The higher concentration of phytosterols in the fiber oil compared with the germ oil was also observed by Moreau et al (1996, 1999) for corn kernels. Analysis of other lipid classes showed that TAG were the most abundant component. TAG constituted ≥91.0% of the oil recovered from sorghum kernels or their wet-milled fractions. A low concentration of FFA (<0.05%) was observed in all the oils. Significant amounts of wax esters (0.83–2.86%) and fatty aldehydes (2.93–3.17%) were observed in the oil from the fiber and protein fractions. However, negligible or very low (0.08%) concentrations of wax esters and fatty aldehydes were observed in the oil recovered from the germ fraction. A negligible amount of oil was recovered in the starch and steep-water fraction of the grain sorghum.

The trends in results as observed for hybrid A (Table I) were similar to those observed for hybrid B (Table II). Sorghum hybrid B had significantly higher amount of oil in fiber, germ, and protein fractions compared with hybrid A. The amount of oil recovered from the fiber was ≥3.5%, and ≥1.1% of that oil was St and ≥1.08% was St:E. The amount of oil in the germ was ≥18.9% and ≥0.6% of that oil was St and ≥0.27% was St:E. Although the amounts of oil in the germ were significantly lower than in corn germ, wax esters and fatty aldehydes were essentially absent in the germ.

Total Phytosterol Recovery

Previously, we reported the levels of phytosterols in different wet-milled fractions of a yellow dent corn (Moreau et al 1999).
Comparison of the sorghum and corn wet-milling fractions show comparable yields for germ and protein fractions (Table III). For sorghum kernels, depending on the hybrid, the amount of fiber (≈1.8–3.9%) and steepwater solids (≈2.1–2.4%) were high and the amount of starch was low (≈3.8–4.9%) compared with the corn kernels. Although the germ yields for the sorghum and corn kernels were comparable, the amount of oil in the germ was ≈1.8 to 2.5 times higher in the corn kernels. The germ oil content in sorghum kernels is lower than the oil content in the corn kernels (Watson 1984). The protein fraction yield for the sorghum and corn kernels was also comparable, however, the amount of oil in the sorghum protein fraction was ≈4 to 7 times higher compared with the oil in the corn protein fraction (Table III). This lipid fraction in sorghum consists of some phytosterols but mainly wax esters and an aldehyde. Clean separation between starch and protein is a problem during the sorghum wet-milling process because small pieces of pericarp interfere in the separation (Watson 1984) or there is a highly cross-linked protein matrix in sorghum endosperm (Hamaker et al. 1992). It is possible that these lipid compounds (wax esters and fatty aldehydes) found mainly in protein fraction and small amounts in the starch fraction could also have an effect on the starch-protein separation during sorghum wet-milling process.

Comparison of total phytosterol recovery shows that more phytosterols can be recovered from corn than from sorghum. From the wet-milled fractions most of the phytosterols (for both corn and sorghum) were recovered from the fiber fraction followed by the germ fraction. Depending on the sorghum hybrid, the total recovery of phytosterols from the wet-milled fractions is considerably lower (27–47%) compared with the total amount of phytosterols present in the whole sorghum kernels. The total recovery of phytosterols from wet-milled corn fractions is also lower (≈53%) compared with the amount of phytosterols present in the whole corn kernels. The reasons for lower recovery in the wet-milled fractions compared with the whole kernels are difficult to ascertain. It is likely that there are some losses during the milling of grain into their individual components.

The results from this study indicate that sorghum kernels are a relatively significant source for two phytosterol classes: St and St:E. Most of these phytosterols were concentrated in the fiber fraction. In addition to phytosterols, other lipid classes such as wax esters and fatty aldehyde are also present in the sorghum oil. The presence of wax esters and fatty aldehydes in sorghum could make recovery of phytosterols difficult. Comparison of sorghum and corn kernels showed that corn has higher recovery of phytosterols from the wet-milled fractions compared with the whole kernels are difficult to ascertain. It is likely that there are some losses during the milling of grain into their individual components.

### CONCLUSIONS

Sorghum kernels can provide a significant source of two phytosterol classes: St and St:E. Removal of fiber in a dry grind ethanol plant using sorghum (in addition to corn) can provide a source for these phytosterol compounds. New value-added dry grind ethanol technologies such as the Quick Fiber (Singh et al. 1999; Wahjudi et al. 2000) would be applicable to the dry grind ethanol plants that use corn as well as sorghum as their feedstock.

### TABLE I

**Oil Yield and Its Phytosterol and Other Lipid Classes** in Sorghum Kernels: Hybrid A

<table>
<thead>
<tr>
<th>Sample</th>
<th>Oil %</th>
<th>FPE</th>
<th>St</th>
<th>St:E</th>
<th>FFA</th>
<th>TAG</th>
<th>Hydrocarbons</th>
<th>Wax Esters</th>
<th>Fatty Aldehyde</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kernels</td>
<td>3.22 ± 0.08</td>
<td>0.03 ± 0.00</td>
<td>0.96 ± 0.07</td>
<td>0.61 ± 0.05</td>
<td>0.03 ± 0.00</td>
<td>96.64 ± 0.19</td>
<td>0.16 ± 0.01</td>
<td>0.12 ± 0.01</td>
<td>1.47 ± 0.07</td>
</tr>
<tr>
<td>Fiber</td>
<td>2.53 ± 0.18</td>
<td>0.15 ± 0.00</td>
<td>1.32 ± 0.10</td>
<td>1.36 ± 0.37</td>
<td>0.04 ± 0.01</td>
<td>92.77 ± 0.83</td>
<td>0.60 ± 0.03</td>
<td>0.83 ± 0.44</td>
<td>2.93 ± 0.81</td>
</tr>
<tr>
<td>Germ</td>
<td>13.67 ± 1.39</td>
<td>0.01 ± 0.00</td>
<td>0.52 ± 0.09</td>
<td>0.32 ± 0.03</td>
<td>0.04 ± 0.01</td>
<td>91.39 ± 0.40</td>
<td>0.72 ± 0.20</td>
<td>0.00 ± 0.00</td>
<td>0.08 ± 0.01</td>
</tr>
<tr>
<td>Protein</td>
<td>3.53 ± 0.55</td>
<td>0.01 ± 0.00</td>
<td>1.99 ± 0.66</td>
<td>0.83 ± 0.42</td>
<td>0.04 ± 0.00</td>
<td>91.28 ± 0.05</td>
<td>0.68 ± 0.09</td>
<td>2.86 ± 0.35</td>
<td>3.17 ± 0.58</td>
</tr>
<tr>
<td>Starch</td>
<td>0.10 ± 0.00</td>
<td>0.00 ± 0.00</td>
<td>2.63 ± 0.52</td>
<td>0.00 ± 0.00</td>
<td>0.03 ± 0.00</td>
<td>99.03 ± 0.14</td>
<td>0.03 ± 0.09</td>
<td>2.01 ± 0.96</td>
<td>3.35 ± 0.77</td>
</tr>
</tbody>
</table>

*Average ± standard deviation.

### TABLE II

**Oil Yield and Its Phytosterol and Other Lipid Classes** in Sorghum Kernels: Hybrid B

<table>
<thead>
<tr>
<th>Sample</th>
<th>Oil %</th>
<th>FPE</th>
<th>St</th>
<th>St:E</th>
<th>FFA</th>
<th>TAG</th>
<th>Hydrocarbons</th>
<th>Wax Esters</th>
<th>Fatty Aldehyde</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kernels</td>
<td>3.66 ± 0.30</td>
<td>0.03 ± 0.00</td>
<td>0.59 ± 0.07</td>
<td>0.63 ± 0.08</td>
<td>0.05 ± 0.00</td>
<td>96.92 ± 0.41</td>
<td>0.17 ± 0.03</td>
<td>0.28 ± 0.03</td>
<td>1.34 ± 0.20</td>
</tr>
<tr>
<td>Fiber</td>
<td>3.46 ± 0.56</td>
<td>0.08 ± 0.00</td>
<td>1.11 ± 0.29</td>
<td>1.08 ± 0.18</td>
<td>0.05 ± 0.00</td>
<td>93.15 ± 0.15</td>
<td>0.44 ± 0.02</td>
<td>1.55 ± 0.94</td>
<td>2.54 ± 0.63</td>
</tr>
<tr>
<td>Germ</td>
<td>18.93 ± 1.67</td>
<td>0.01 ± 0.00</td>
<td>0.56 ± 0.00</td>
<td>0.27 ± 0.02</td>
<td>0.04 ± 0.00</td>
<td>91.47 ± 0.05</td>
<td>0.46 ± 0.17</td>
<td>0.06 ± 0.01</td>
<td>0.13 ± 0.02</td>
</tr>
<tr>
<td>Protein</td>
<td>6.17 ± 0.01</td>
<td>0.00 ± 0.00</td>
<td>1.50 ± 0.03</td>
<td>0.62 ± 0.11</td>
<td>0.03 ± 0.01</td>
<td>92.38 ± 0.05</td>
<td>0.44 ± 0.08</td>
<td>3.94 ± 0.41</td>
<td>1.98 ± 0.31</td>
</tr>
<tr>
<td>Starch</td>
<td>0.15 ± 0.02</td>
<td>0.00 ± 0.00</td>
<td>1.67 ± 0.33</td>
<td>0.00 ± 0.00</td>
<td>0.03 ± 0.01</td>
<td>98.93 ± 0.05</td>
<td>0.03 ± 0.00</td>
<td>4.63 ± 0.30</td>
<td>0.84±0.11</td>
</tr>
</tbody>
</table>

*Average ± standard deviation.

### TABLE III

**Comparison Between Sorghum (Hybrids A and B) and Corn for Yield,** Oil Content, and Total Phytosterols in Different Wet-Milling Fractions

<table>
<thead>
<tr>
<th>Sample</th>
<th>Hybrid A (%)</th>
<th>Hybrid B (%)</th>
<th>Corn (%)</th>
<th>Total Phytosterols (mg/100 g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kernels</td>
<td>100.0</td>
<td>3.22 ± 0.08</td>
<td>100.0</td>
<td>3.66±0.30</td>
</tr>
<tr>
<td>Fiber</td>
<td>15.9</td>
<td>2.53 ± 0.18</td>
<td>18.05</td>
<td>3.46 ± 0.56</td>
</tr>
<tr>
<td>Germ</td>
<td>4.9</td>
<td>13.67 ± 1.39</td>
<td>4.73</td>
<td>18.93 ± 1.67</td>
</tr>
<tr>
<td>Protein</td>
<td>8.91</td>
<td>3.53 ± 0.55</td>
<td>7.69</td>
<td>6.17 ± 0.01</td>
</tr>
<tr>
<td>Starch</td>
<td>64.77</td>
<td>0.10 ± 0.00</td>
<td>63.62</td>
<td>0.15 ± 0.02</td>
</tr>
<tr>
<td>Steepwater</td>
<td>5.47</td>
<td>0.00 ± 0.00</td>
<td>5.76</td>
<td>0.00 ± 0.00</td>
</tr>
</tbody>
</table>

*All yields are averages of two values.

Data previously reported by Moreau et al. (1999).

Average ± standard deviation.
concentration of phytosterol compounds (St and St:E) and an additional class of phytosterol compounds, ferulate phytosterol esters (FPE) can also be recovered from corn kernels.

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LITERATURE CITED


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