Commercial soybean hulls (14.6% crude protein) were pin milled and then air classified into five fractions to determine whether the hull structure can be disrupted and the protein constituents concentrated. The number of pin millings had only a small effect on the weight distribution of the five fractions. After one grinding, the sum of fractions 1 and 2 (<15–18 µm) represented only 3% of total hulls, and on three grindings, they amounted to only 6% of the hulls. Fraction 3 (19–24 µm) shifted from 16 to 20% with three grindings, while fraction 4 (25–30 µm) remained unchanged at 5%. Fraction 5 (>30 µm) shifted from 75 to 69% on three pin millings to compensate for the shifts noted in fractions 1–3. Scanning electron microscopy revealed that fractions 1 and 2 consisted of the parenchymal cell layers (innermost portion) of the hulls; these fractions had three times the protein content and more than twice the amount of lipids found in the ground starting material. Fraction 3 contained many of the hourglass cells typically found in the middle layer of the hulls plus parenchyma cell material and exhibited about twice the amount of protein and lipid found in the starting hulls. Fraction 4 included large hourglass cells plus globular material and contained about twice the amount of protein and about one and one-half times the lipid of the starting material. Fraction 5 consisted primarily of clumps of palisade cells (outer cellular layer) adhering to each other and had a lower protein and lipid content than the starting hulls. Thus, pin milling causes some selective disruption of the hulls where parenchyma cell layers and hourglass cells are partially released. The palisade cells, however, are the most difficult to disrupt and constitute the fraction most resistant to pin milling. Our findings suggest that pin milling soybean hulls in combination with air classification can be used to concentrate the proteins and lipids in the fines fractions.

The soybean hull (seed coat) generally constitutes 6–8% of the seed (Bailey et al 1935; Calero et al 1981; Kawamura 1967). The hull structure consists of three distinct layers (Baker et al 1986; Wolf and Baker 1972). The outermost layer consists of palisade cells (macroclosoids) which are long thin (3–4 µm x 30–50 µm) cells oriented perpendicularly to the hull surface and serve as the primary protective tissue of the seed coat. The outer ends of the palisade cells that make up the external surface of the hull are covered with a thin cuticle. The next layer is made up of large thick hourglass cells (osteoclosoids) which are separated from each other by intercellular spaces. The hourglass cells lie below the palisade layer. They are 8–12 µm in diameter and vary in length from 15–70 µm (Baker et al 1986). The innermost layer of the hull is less distinctive than the other two layers and is made up of collapsed parenchyma cells below which occurs a single layer of cubical cells referred to as the aleurone layer.

Analysis of commercially produced hulls shows 9.9% protein, 1.0% fat, 42.0% crude fiber, 4.4% ash, 67.0% neutral detergent fiber, 15.7% hemicellulose, 51.2% acid detergent fiber, 50.0% cellulose, and 1.3% acid detergent lignin (Mitaru et al 1984). Polysaccharides are the major constituents of soybean hulls (Aspinall and Whyte 1964; Aspinall et al, 1966 1967b). Several proteins have been identified in the hulls including extensin (Cassab et al 1985), peroxidase (Sessa and Anderson 1981; Gillikin and Graham 1991; Gijzen et al 1993; Liu et al 1999), trypsin inhibitor (Sessa and Wolf 2001), and several allergens (Codina et al 1997).

In 1998, the United States produced 74.6 million metric tons of soybeans and processed 43.3 million metric tons into oil and meal (USDA 2000). The remainder was largely exported and processed overseas. Conventional soybean processing involves a dehulling step. Thus, based on a 7% yield of hulls, ~3.0 million metric tons of co-product hulls were produced in the United States in 1998 (Horan 1974).

At present, soybean hulls are utilized primarily as a fiber source for dairy and beef cattle, as an ingredient for reduced-fat diets for pets, and to add bulk to feeds (Central Soya 2000). Small amounts are used for commercial production of peroxidase, which has potential for use by the breadmaking industry. Workers in The Netherlands reported that addition of a combination of xylanase and soybean peroxidase to a cookie-type flour yielded bread doughs with good handling properties and good baking performance (Hillhorst et al 1999). Soybean peroxidase has also been evaluated as a biosensor for phenol (Bassi and McGrath 1999). Other potential commercial applications for hulls include carbonization to form adsorbents for bleaching soybean oil (Proctor and Harris 1996; Gnansansabandam and Proctor 1997) and chemical modification to convert the hulls into a cation exchange medium (Marshall et al 2001). Food-related studies on hulls include their use as an iron source for bread enrichment (Johnson et al 1985), metal binding as a model of insoluble dietary fiber (Laszlo 1987), plus water and lipid absorption properties in structured pork products (Muzilla et al 1989).

Recently, Ozawa et al (2000) described pin milling of hulls and fractionation by air classification. They obtained a fine fraction (36–37%) and a coarse fraction (63–64%). The fine fraction was enriched in protein and lipid and may be useful for general food applications, while the coarse fraction was rich in fiber and appropriate as a dietary fiber source.

Our objectives were to further investigate the effects of pin milling and air classification of the hulls as a means for greater enrichment of the protein fractions which may have potential industrial and food uses. We also wanted to more fully define the structural characteristics of the various fractions through the use of the scanning electron microscope.

MATERIALS AND METHODS

Soybean hulls prepared without heat treatment were obtained from Central Soya Company (Fort Wayne, IN). Stem fragments and other obvious foreign materials were removed by hand before pin milling. In handling the bulk hull sample, we noted that it contained a fine fraction that adhered to our hands. Mechanical screening of a 100-g sample of hulls on an orbital shaker (model 2001, VWR Scientific Products, Chicago IL) through a 20-mesh (850 µm)
Screening electron microscopy (SEM) was conducted by mounting the samples on carbon tape, sputtering coating with 20 nm of gold-palladium in a Sputter SPI coater and then examining in a JEOL JSM-6400 instrument. Moisture, Kjeldahl nitrogen, ash, and lipid analyses were done according to official methods (AOAC 1998). Protein contents are expressed as Kjeldahl nitrogen x 6.25.

RESULTS AND DISCUSSION

Characterization of Starting Material

The starting material consisted of hull fragments approximately one-half of the original seed size or smaller; the composition is shown in Table I. The low protein and fat contents reflect the high content of polysaccharides found in hulls. Defatted hulls have been estimated to contain 40-50% cellulose by weight, plus other polysaccharides (Aspinall et al 1967a; Mitaru et al 1984). The major structural features of soybean hulls were readily observed in the commercial product using SEM. Figure 1A shows a fracture surface of a hull fragment in which the three major cellular layers are identifiable. Damage resulting from the mechanical cracking during the dehulling operation is apparent in the ragged appearances of the palisade and parenchyma layers. An interesting feature we noted in some of the hull fragments was the presence of only palisade and hourglass layers; the parenchyma layer was cleanly stripped off, exposing the flared bases of the hourglass cells (Fig. 1B).

The fines fraction obtained by sieving the hulls through a 20-mesh screen was enriched in crude protein compared with the coarse fraction (Table I), whereas the coarse fraction was not changed appreciably because it represented 92% of the starting hulls. The fines fraction contained a variety of particle sizes and shapes (Fig. 1C). Hull fragments of ≤1 mm in length are apparent; the long thin fibrous materials may be contaminants. The large fragments were palisade cell layers with hourglass cells attached. Higher magnification revealed parenchyma cell material plus spherical particles =10 μm in diameter (not shown). These latter particles may be protein bodies that are prevalent in the cotyledon cells (Wolf and Baker 1972) and probably were dislodged from the broken cotyledon particles during the commercial cracking operation. They may account for some of the higher protein content of the fines as compared with original hulls (Table I). Fragments of the hourglass cell layer free of palisade layer but with residual parenchyma cell layer still adhering to them were also observed. Isolated hourglass cells were observed infrequently.

Effects of Pin Milling

One passage through the pin mill at 18,000 rpm reduced the particle size of the unscreened hulls, but particles of 600-700 μm still remained (Fig. 2A). These large particles were fragments of palisade cell layer and palisade layer with hourglass cells still attached; parenchyma cell layers were largely removed and mechanical damage from the milling was evident. In some instances, milling removed parenchyma layers very cleanly leaving a surface network of the bases of the hourglass cell layer. These particles may have originated by disruption of larger fragments similar to those shown in Fig. 1B. Isolated hourglass cells occurred and fragments of palisade cell layer with evidence of shredding were also seen.

After three passes through the pin mill, the particle size was decreased (Fig. 2B) compared with that in one pass (Fig. 2A). Fragments of 100-200 μm were still prevalent along with occasional particles of 300-500 μm. The large fragments were mainly palisade cells still attached to each other. Although the hourglass cells had largely been removed from the palisade layer, isolated hourglass cells or obvious fragments of them were not as prevalent as anticipated. As expected, grinding did not change the chemical composition of the hulls (Table I).

Air Classification

Both the 1x and 3x pin-milled hulls were air classified into five fractions. The yield distributions of the resulting fractions (1-5) are shown in Table I. Fraction 1 was very small (0.7%) but increased threefold (2.0%) on three grindings. Fraction 2 nearly doubled from 2.0 to 3.8% on regrinding. The increase in fraction 3 (from 16.3 to 20.0%) on regrinding was smaller than in fractions 1 and 2. Fraction 4 was the same (5.1%) irrespective of the number of

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Particle Size (μm)</th>
<th>Yield (%)</th>
<th>Protein (%)</th>
<th>Fat (%)</th>
<th>Ash (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Starting hulls</td>
<td>Un-sized</td>
<td>100</td>
<td>14.56</td>
<td>3.29</td>
<td>4.76</td>
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<td>Coarse</td>
<td>&gt;850</td>
<td>92.2</td>
<td>14.38</td>
<td>1.32</td>
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<tr>
<td>Fines</td>
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<td>7.8</td>
<td>21.88</td>
<td>6.22</td>
<td>6.58</td>
</tr>
<tr>
<td>Pin milled 1x</td>
<td>Un-sized</td>
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<td>14.25</td>
<td>4.80</td>
<td>5.08</td>
</tr>
<tr>
<td>Pin milled 3x</td>
<td>Un-sized</td>
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<td>14.57</td>
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<td>Fraction 1 (1x)</td>
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<tr>
<td>Fraction 1 (3x)</td>
<td>&lt;15</td>
<td>2.0</td>
<td>43.31</td>
<td>11.33</td>
<td>4.96</td>
</tr>
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<td>Fraction 2 (1x)</td>
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<td>42.88</td>
<td>12.16</td>
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<tr>
<td>Fraction 2 (3x)</td>
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<td>42.75</td>
<td>11.44</td>
<td>5.94</td>
</tr>
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<td>Fraction 3 (1x)</td>
<td>19-24</td>
<td>16.3</td>
<td>28.44</td>
<td>8.41</td>
<td>6.60</td>
</tr>
<tr>
<td>Fraction 3 (3x)</td>
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<td>20.0</td>
<td>27.57</td>
<td>7.06</td>
<td>6.11</td>
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<tr>
<td>Fraction 4 (1x)</td>
<td>25-30</td>
<td>5.1</td>
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<td>6.84</td>
<td>6.37</td>
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<tr>
<td>Fraction 4 (3x)</td>
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<td>5.1</td>
<td>26.31</td>
<td>6.61</td>
<td>5.37</td>
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<td>Fraction 5 (1x)</td>
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<td>2.10</td>
<td>4.44</td>
</tr>
<tr>
<td>Fraction 5 (3x)</td>
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<td>69.1</td>
<td>11.63</td>
<td>2.52</td>
<td>4.26</td>
</tr>
</tbody>
</table>

* Dry weight basis.
* N x 6.25.
* Obtained by sieving on a 20-mesh screen.
* 1x, 3x = pin milled once and three times, respectively.

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grindings, and the yield was smaller than that of the preceding and following fractions. Reregrinding resulted in a decrease (from 75.9 to 69.1%) in fraction 5, as expected, because of the increases observed in fractions 1-3. A plot of the yields revealed a bimodal distribution with fractions 3 and 5 predominating (Fig. 3). The air-classified fractions were then examined by SEM.

**Fraction 1.** The particle size distribution agreed with the set point on the air classifier (<15 μm); many particles were <10 μm in diameter/length, and a few palisade cells appeared to be present (Fig. 2C). The particles appeared to be primarily parenchyma cells and possibly their contents that were dislodged from the bases of the hourglass cells during pin milling. Ovoid and spherical particles were also present and were generally 5–10 μm in diameter, although some exceeded this range. The spherical particles fall into the size range typical of cotyledon protein bodies (Wolf and Baker 1972). Occasional long, thin particles were present which may be individual palisade cells or fragments of them. No hourglass cells were observed. A large shift in protein content was noted in fraction 1 as compared with the ground hulls (Table I); the protein content was almost three times that of the starting material. The lipid content was also increased.

**Fraction 2.** The particles were mainly globular (Fig. 4A) but larger than in fraction 1 (Fig. 2C). The bulk of the sample was parenchyma cells although a small number of individual and clumps of palisade cells were present. At high magnification, the spherical particles appeared to be aggregates of <1 μm particles (not shown). Small hourglass cells (=30 μm long) were observed occasionally (not shown). As noted for fraction 1, fraction 2 also contained about three times the protein content of the original hulls (Table I).

**Fraction 3.** A characteristic of this fraction was the presence of numerous hourglass cells (Fig. 4B) despite the fact that they greatly exceeded the nominal size setting of the air classifier for this fraction (19–24 μm). Separations by air classification depend on density and shape as well as size; their unusual shape may play a role in their behavior in the air classifier. The hourglass cells varied from 70–140 μm in length. Surprisingly, many of the hourglass cells appeared intact and obvious fragments were seen infrequently, suggesting that these cells are relatively tough and resistant to breakage during milling. Some of the isolated hourglass cells were flattened and others were twisted, possibly as a result of the commercial cracking and dehulling operation or the pin milling. Figure 4C shows an example of an intact hourglass cell and also reveals a structural detail of the hourglass cells, namely their ribbed surface. The ribbed surface was first reported by Baker et al (1986) and is a useful characteristic in identification of the hourglass cells. Another distinctive structural feature of the hourglass cells is the flaring out at both ends of the cells. This is seen clearly in Fig. 4C, where an hourglass cell is resting on what may be a fragment of a second hourglass cell (arrow) or an intact hourglass cell that is wrapped around the larger cell. The remainder of fraction 3 consisted of oval to spherical particles that appeared to be agglomerates of smaller particles (not shown) and probably consist of larger clumps of parenchyma layer material than in fraction 2. The composition of fraction 3 was considerably lower in protein (≈28%) and lipid (≈8%) than fraction 2, but still about twice as high in these constituents as the starting hulls. The lower protein and lipid contents suggest that the hourglass cells may be largely structural polysaccharides.

**Fraction 4.** This fraction also contained hourglass cells (Fig. 5A), but they were very long (=100 μm). Globular material similar to that seen in fraction 3 (Fig. 3A) was also present. The protein and lipid contents of fraction 4 were lower than for fraction 3 but still almost twice the values for the original hulls.

**Fraction 5.** As expected, fragments of the palisade cell layer were very numerous in this fraction (Fig. 5B) because we found little evidence of palisade cells in fractions 1–4. Surprisingly, many of the particles had rounded edges, suggesting abrasion during processing. Because we did not see evidence for such smoothing off of sharp edges before air classification (Figs. 2A and 2B), it is likely that this abrasion occurred during air classification rather than pin milling. Fraction 5 was air classified 10–15x because the coarse fractions were each reclassified two to three times at each step to ensure removal of the smaller particles. Higher magnification of fraction 5 clearly showed the presence of the palisade

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**Fig. 4.** Scanning electron micrographs of (A) commercial hull fracture surface showing palisade cells (p), hourglass cells (hg), and parenchyma layer (pa); (B) hull fracture surface where parenchyma cell layer has been cleanly removed leaving the bases of hourglass cells exposed (arrow); (C) fines obtained by sieving (arrows indicate fibers that may be contaminants).
cells (not shown). Also present were occasional palisade layer particles with hourglass cells still attached but fairly free of parenchyma cells.

The toughness of the palisade cell layer to grinding apparently is also reflected in their resistance to microbial degradation. Dintzis et al. (1979) incorporated milled soybean hulls into bread and fed it to humans. Recovery of undigested material from feces showed that only the palisade cells survived passage through the gastrointestinal tract. Grenet and Barry (1990) conducted in vivo digestibility studies of soybean hulls in fistulated sheep using the nylon bag technique. They found that the aleurone and parenchyma layers disappeared first (8 hr), followed by the hourglass cells (24 hr), and after 72 hr, only a few palisade cells survived along with the cuticle layer.

An abrupt change in protein and lipid content was noted in fraction 5 as compared with fractions 1–4 (Table I). In fact, the protein and lipid values were below those observed for the starting hulls. This suggests the presence of greater amounts of polysaccharides in the palisade cells than in the hourglass and parenchyma cells, which, in turn, may be responsible for the resistance of the hulls to grinding and microbial degradation noted earlier.

As shown in Table I, the hulls and their air-classified fractions contained variable amounts of lipids. Examination of the fractions after defatting did not reveal changes in structure as compared with the undefatted samples.

The only previous work on pin milling and air classification of soybean hulls appears to be that of Ozawa et al. (2000). Our results are in general agreement with those of Ozawa and coworkers. They separated pin-milled hulls into a fine and a coarse fraction. The fine fraction range was 2–100 μm with a median particle size of 12 μm and corresponds approximately to our fractions 1–4. They reported a protein content of 26% (db) for their fine fraction, which compares with 30% on a weighted basis for our fractions 1–4.

![Fig. 2. Scanning electron micrographs of (A) 1x pin-milled hulls; (B) 3x pin-milled hulls; and (C) air-classified fraction 1 (<15 μm) showing mainly parenchyma cell material with long thin structures (arrows) that are probably individual palisade cells.](image)

![Fig. 3. Distribution of yields of fractions obtained by air classification of hulls after one and three passes through the pin mill.](image)

### TABLE II

Summary of Major Structural Components in Hull Fractions Obtained by Sieving, Pin Milling, and Air Classification

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Structural Components</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fines by sieving</td>
<td>Palisade hourglass cell clumps, palisade layer fragments, hourglass-parenchyma layer, protein bodies (Fig. 1C)</td>
</tr>
<tr>
<td>Pin milled 1x&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Palisade cell layer, palisade hourglass fragments, clumps of hourglass cells, protein bodies (Fig. 2A)</td>
</tr>
<tr>
<td>Pin milled 3x&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Palisade cell layer, protein bodies, hourglass cells (Fig. 2B)</td>
</tr>
<tr>
<td>Fraction</td>
<td>Structural Components</td>
</tr>
<tr>
<td>----------</td>
<td>-----------------------</td>
</tr>
<tr>
<td>1</td>
<td>Parenchyma cells and protein bodies (Fig. 2B)</td>
</tr>
<tr>
<td>2</td>
<td>Parenchyma cells (Fig. 4A)</td>
</tr>
<tr>
<td>3</td>
<td>Hourglass and parenchyma cells (Figs. 4B–C)</td>
</tr>
<tr>
<td>4</td>
<td>Palisade cell layer, protein bodies, hourglass cells (Fig. 2B)</td>
</tr>
<tr>
<td>5</td>
<td>Clumps of palisade cells (Fig. 3B)</td>
</tr>
</tbody>
</table>

<sup>a</sup> 1x, 3x = pin milled once and three times, respectively.

![Distribution of yields of fractions obtained by air classification of hulls after one and three passes through the pin mill.](image)
(3× pin-milled series). Their fines fraction contained hourglass cell fragments, which is in agreement with our fractions 3 and 4. The coarse fraction of Ozawa et al (2000) was 20–800 μm with a median particle size of 163 μm, and resembles our fraction 5 in composition and structural components. The coarse fraction of the Japanese workers had a protein content of 8.6% (db) compared with 11.6% for our fraction 5. Their coarse fraction contained palisade cell layer fragments as also noted in our fraction 5.

Table II summarizes the distribution of the structural components of soybean hulls among the fractions that we obtained by sieving, pin milling, and air classification.

Fig. 4. Scanning electron micrographs of (A) air-classified fraction 2 (15–18 μm) revealing parenchyma cells with occasional palisade cells (arrows); (B) air-classified fraction 3 (19–24 μm) showing numerous hourglass cells (arrows); (C) isolated hourglass cell in fraction 3 showing ribbed surface and flared ends with another hourglass cell (arrow) below it.

CONCLUSIONS

Although commercial hulls contained 7% of fines (20 mesh), the fraction was very heterogeneous in particle size and would require grinding before air classification. Its low yield makes it relatively unattractive as a starting material, hence we did not attempt to pin mill and air classify it. Soybean hulls proved to be quite resistant to grinding in a pin mill. Even after three grindings, fragments of 100–200 μm were numerous, and a few particles were 300–500 μm in length. The most resistant to disruption was the palisade cell layer, although this characteristic favored its isolation in fraction 5. Pin milling released the hourglass cells from their respective layer in the hulls and they appeared relatively undamaged by the grinding. Studies on alternative methods of grinding are desirable. It would be advantageous to be able to grind the parenchyma cell layer to a more uniform size and to be able to concentrate the parenchyma cells in one or two fractions such as fractions 1 and 2 instead of having them distributed through four fractions as we observed. Greater disintegration of the parenchyma cell layers may facilitate their removal before fractions 3 and 4, thus yielding a cleaner preparation of hourglass cells, particularly in fraction 3.

Pin milling and air classification was successful in concentrating the hull cellular layers into parenchyma cells (fractions 1–2), hourglass cells (fraction 3) and palisade cells (fraction 5), which suggests that this fractionation may be a useful step in separating protein fractions if the proteins are located in specific cellular layers. Thus, peroxidase is of interest, although evidence for its cellular location is conflicting. Gillikin and Graham (1991) reported peroxidase in both the hourglass cells and palisade cells, whereas Gijzen et al (1993) presented evidence that it is present primarily in the hourglass cells.

Fig. 5. Scanning electron micrographs of (A) air-classified fraction 4 (25–30 μm) containing large hourglass cells and (B) air-classified fraction 5 (>30 μm) showing abraded fragments of palisade cell layer.
ACKNOWLEDGMENTS

We thank M. L. Schaer and A. Taves for technical assistance, A. M. Kelly-Webb for analytical analyses, and B. D. Deadmond for performing the air classifications.

LITERATURE CITED


[Received June 20, 2001. Accepted January 22, 2002.]