Alternative Extraction Effects on the Free Fatty Acids and Phosphorus in Oil from Damaged and Undamaged Soybeans

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By varying the extracting conditions, it may be possible to produce high-quality, low-phosphorus and low-free fatty acid (FFA) oil extracted from water or mechanically damaged soybeans. The variability in phospholipids and FFA was studied in oil extracted by an alternative process from undamaged, damaged and aged soybeans subjected to various changes. Forrest and Hutcheson cultivars were used, and extractions were from finely ground flour rather than from flakes. Freezing caused the maximum increase in FFA and phosphorus levels compared to other levels in damaged or undamaged soybeans, but the levels were reasonable compared to flake extraction. Phosphorus and FFA increased when storage temperatures went from 25 to 45°C, extraction temperatures from 25 to 50°C and moisture of the flour from 6 to 10%. However, the storage time of soybeans with initially high moisture (20%) did not have a marked influence on FFA and phosphorus levels. Immediately after grinding moisture of the flour elevated or lowered the phosphorus level to a great extent, although it had little influence on the FFA level. Phosphatidic acid and phosphatidylcholine were identified as the main phospholipids present when total phosphorus was low in extracted oil. The time taken for the flour to dry to 6% moisture (after grinding and before it was extracted) was critical. The alternative extraction process moderated the expected increase in FFA and phospholipids as the result of soybean damage.

KEY WORDS: Alternative soybean extraction, free fatty acids, phospholipids, soybean damage.

The quality of fully refined soybean oil is influenced by the quality of crude oil and by the quality of the soybeans from which the oil is extracted. Soybeans can be damaged as a result of various preharvest and postharvest conditions. Poor preharvest conditions include adverse weather conditions such as frost, hail or drought (1). Adverse postharvest conditions that may cause damage include improper handling, high storage temperature, moisture (2) and shipment (3). Meloy (4) has shown that the atmospheric humidity during the period in which the seeds mature and dry in the field is the principal factor determining their subsequent storage properties. Seeds that mature at low humidity tend to be more stable than seeds maturing at high humidity.

Under certain conditions, stresses can develop in the soybeans that cause disruption of tissues and activation of enzymes within these tissues, resulting in a decrease in the quality of crude oil extracted. Activity of enzymes is more pronounced in disrupted tissues than in intact tissues (5).

Damaged soybeans result in an increased oil loss at the refinery and reduced stability of finished oil. Robertson *et al.* (6) reported that as a result of severe damage to the crop, oil losses rise from a normal level of 1–1.5% to over 4%, and that some processors blend damaged soybeans with sound beans to overcome high refinery losses. Increased refinery losses can be attributed to high free fatty acid (FFA) content of crude oil, and alteration and degradation of phospholipids.

Clark and Snyder (7) reported that extracting fine flour rather than flakes and keeping flour moisture below 6% yields crude oil with low phosphorus (15 ppm), but that phosphorus increases to 260 ppm at 9% moisture flour. The object of this study was to determine how an alternative extraction method could control FFA and phospholipids in crude oil, even though storage and handling conditions were manipulated to damage soybeans and to increase FFA and phospholipids. This information will be useful in determining conditions for extraction of high-quality oil from damaged soybeans.

MATERIALS AND METHODS

Soybeans used for this study were Forrest and Hutcheson cultivars obtained from Southern Farmers Association (Forrest City, AR).

Simulated water-damaged soybeans. These were prepared by soaking the soybeans in water for 24 h, then drying them at 60°C for another 24 h.

Simulated water- and mechanically-damaged soybeans. These were prepared by soaking the soybeans in water for 4 h. The beans were then broken with a blender and held at 25°C for 24 h. The beans were then dried at 60°C for 6 h.

Simulated freeze-damaged soybeans. These soybeans were prepared by soaking the soybeans in water for 4 h. The beans were then kept in a freezer at 0°C for 72 h. The frozen soybeans were thawed and dried at 60°C for 6 h.

Accelerated-aged soybeans. Separate batches of soybeans were stored over water in a desiccator at 25°C for 16 d, 35°C for 11 d and 45°C for 7 d until the moisture of the beans reached 21.4, 20 and 21%, respectively. Each of these separate batches of soybeans was then kept in storage for another 5 d to maintain the conditions of moisture and temperature.

Preparation of flour. The soybeans were dehulled and ground in a Udy Cyclone mill (UDY Corp., Fort Collins, Co.). Full-fat flour was sieved with an Alpine air-jet sieve (Alpine American Corp., Natick, MA) fitted with a 100-mesh sieve. The 100-mesh flour was used for this study. The moisture levels of the flour were 4–5% for simulated-damaged soybeans and 4–5, 7–8 and 9–10% for accelerated-aged soybeans. The flour was adjusted to a low moisture level by keeping it in a desiccator, with Drierite as the desiccant. High-moisture flour was obtained by keeping the flour over water in a desiccator until the desired moisture was reached.

Extraction. Oil was extracted from the flour by the rapid equilibrium method as developed by Snyder *et al.* (8). Flour with 4–5% moisture from simulated-damaged soybeans was extracted at 25°C for 35 min. Flours with 4–5, 7–8 and 9–10% moisture from soybeans stored at 25°C

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were extracted at 25°C and 50°C for 35 min each. High temperature of extraction was maintained by a thermostatically controlled water bath.

Hutcheson vs. *Forrest soybeans.* Moisture levels of the Hutcheson soybeans were 13 and 16%, whereas moisture levels of the Forrest soybeans were 12% and 18%. The soybeans from the two varieties were dehulled and ground in a Udy Cyclone mill, and the full-fat composite flour was used for analysis. Moisture of the flour was determined immediately after grinding. Moisture levels of the flour used were 5–6, 7–8 and 9–10% from each of the storage moisture levels of 13%, 16%, 12% and 18% soybeans. Low-moisture flours were obtained by oven drying the flours from 18- and 16%-moisture soybeans at 35°C for 4 h. Flours from 12% and 13% soybeans were dried with Drierite as the desiccant. Oil extracted from each flour was analyzed for FFA and phosphorus content.

Effect of retention time and flour moisture after grinding. Only Hutcheson soybeans were used in this study. The moisture contents of the soybeans were 9.1, 11.7 and 13.2%. These soybeans also were ground in a Udy Cyclone mill, and the composite flour obtained after grinding was immediately put in a hot air oven at 50°C for drying. Moisture content of the flour after grinding was determined. The moisture level of the flour used for analysis was between 5 and 6%. The oil extracted was analyzed for FFA and phosphorus content.

Determination of FFA and phosphorus content. Percent FFA was determined by a colorimetric method developed by Walde and Nastruzzi (9), which utilized phenol red solubilized in reverse micelles. The advantage of this method was the small quantity of oil (10 μL) needed for analysis. The phosphorus content was determined by Bartlett's (10) colorimetric method.

Determination of phospholipids by thin-layer chromatography. Thin-layer chromatography plates, silica gel H of 50 microns particle size (Analtech Newark, DE), were heated in an oven at 120°C for 1 h to activate. The plates were spotted with 15–90 mg of oil so that the total phosphorus in each lane would be approximately 6.5 μg. Phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol, phosphatidic acid and phosphatidylserine (Avantia Polar Lipids, Inc., Pelham, AL) standards were spotted between oil samples. The plates were developed with chloroform first to move the triglyceride fraction to the top of the plate. The plates were then air-dried for 10 min and developed in the same direction with chloroform/ethanol/water/triethylamine (30:34:8:35) (11) for 3 h. The plates were dried for 15 min and then sprayed with molybdenum blue spray reagent (Sigma Chemical Company, St. Louis, MO). Sample spots were identified by comparison with the standards.

Moisture analysis. Moisture of the ground soybean flour was determined by drying in an oven at 130°C (AOCS method AC 3-44; ref. 12); and for the whole soybeans by AOCS method AC 2-41 (12).

RESULTS AND DISCUSSION

Effect of soybean damage on FFA and phospholipids in extracted oil. FFA results in oil extracted from simulated-damaged soybeans and undamaged soybeans are shown in Figure 1. The mean FFA level from undamaged soybeans was 0.08%. The water-damaged and water- and

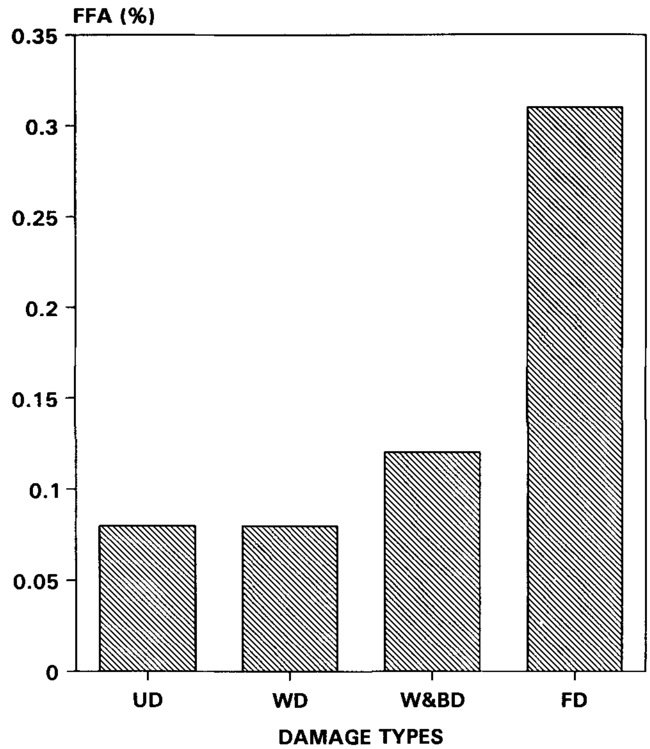

FIG. 1. Differences in free fatty acids (FFA) due to simulated damaged beans. Damage types: FD (freeze-damaged), W&BD (water- and blender-damaged), WD (water-damaged), and UD (undamaged). Experimental conditions: Moisture of beans, 6.33%; moisture of flour, 5%; extraction temperature, 25°C. Least significant difference = 0.0878 at 0.05 level.

blender-damaged soybeans had FFA levels of 0.08 and 0.12%, respectively, neither of which was significantly different from the control. Freeze-damaged soybeans had a significant increase in percent FFA as compared to the undamaged and to the other two types of damaged beans. This indicated that the degree to which the membranes were damaged was greater for the freeze-damaged soybeans, which probably released enzymes resulting in increased lipid hydrolysis.

The phosphorus levels in oil extracted from simulated-damaged soybeans are shown in Figure 2. The freeze-damaged soybeans showed maximum values in phosphorus levels up to 235 ppm, whereas water- and blender-damaged soybeans showed a phosphorus level of 115 ppm. On the other hand, water-damaged soybeans had a phosphorus level of 69 ppm in the extracted oil. All these levels were significantly greater than the level in undamaged soybeans (19 ppm phosphorus). The extraction of increased phospholipids may have been due to phospholipase activity, which increased with an increase in membrane damage.

Effect of accelerated-aging of soybeans on FFA and phospholipids in the extracted oil. During the accelerated storage studies a significant interaction was found between storage temperature and moisture on FFA content of extracted oil (Fig. 3). There was a significant interaction between storage temperature and extraction temperature on FFA, as well (Fig. 4).

In looking at flour moisture and storage temperature, the mean percent FFA ranged from 0.11 to 0.17% at 25°C

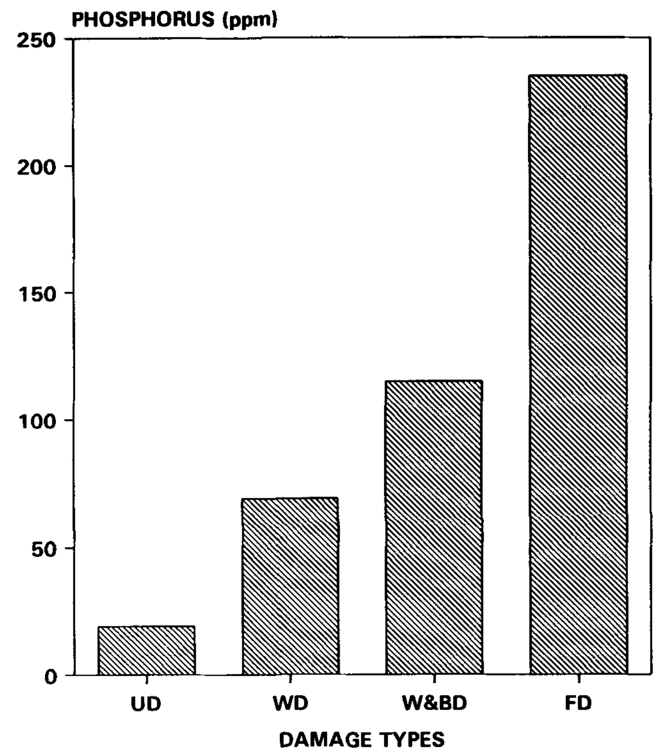

FIG. 2. Changes in phosphorus among types of simulated damaged beans. Abbreviations as in Figure 1. Extraction conditions: Moisture of beans, 6.33%; moisture of flour, 5%; extraction temperature, 25°C. Least significant difference = 18.4 at 0.05 level.

storage temperature, 0.28 to 0.49% at 35°C storage at 35°C storage temperature, and 0.84 to 1.10% at 45°C storage temperature (Fig. 3). Thus, an increase in storage temperature produced a significant increase in FFA. At 25°C storage temperature, the increase in moisture of flour from 5 to 8% or 8 to 10% did not produce a significant increase in percent FFA. At 35°C storage temperature, an increase in the moisture content of the flour from 5 to 8% gave a significant increase in FFA. At 45°C storage temperature, each increase in flour moisture gave a significant increase in FFA. Control of moisture at the time of flour extraction could provide control of FFA in crude oil.

Results from the interaction of storage temperature and extraction temperature showed that the percent FFA ranged from 0.12 to 0.94% at 25°C extraction temperature, and from 0.16 to 0.98% at 50°C, as shown in Figure 4. At each extraction temperature the FFA increased as storage temperature was elevated. The increase in FFA due to extraction temperature was minimal.

The increase in FFA can be explained by the breakdown of triglycerides by lipase. The enzyme activity was probably raised as a result of the increase in storage temperature and the high moisture of the flour at the time of extraction. Lipase could have originated from the damaged soybean tissue. Under commercial conditions, microbial growth could have taken place on the soybeans at the high moisture content. However, it is unlikely that the increase in FFA was due to mold growth because the soybeans used for the study were intact and mold mycelium did not penetrate the soybeans.

Storage temperature studies indicated that the temperatures at which soybeans were stored influenced the phosphorus level to a significant extent (Fig. 5). Soybeans stored at 25, 35 and 45°C had 21, 73 and 98 ppm phosphorus, respectively, in the extracted oil.

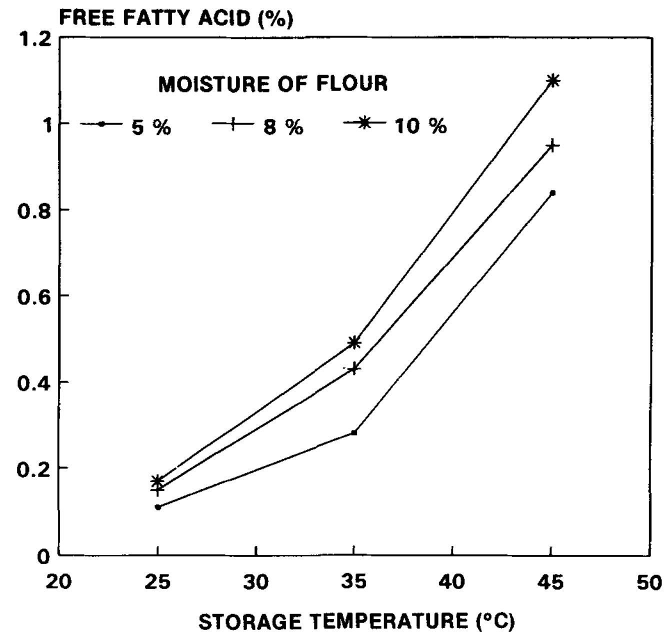

FIG. 3. Effect of storage temperature and moisture interaction on free fatty acid analysis in oil from accelerated-aged soybeans. Experimental conditions: moisture of soybeans, 20%; storage time for 25, 35 and 45°C soybeans were 21, 16 and 12 d, respectively; moisture of flour, 5, 8 and 10%; extraction temperature, 25°C. Least significant difference 0.045 at 0.05 level.

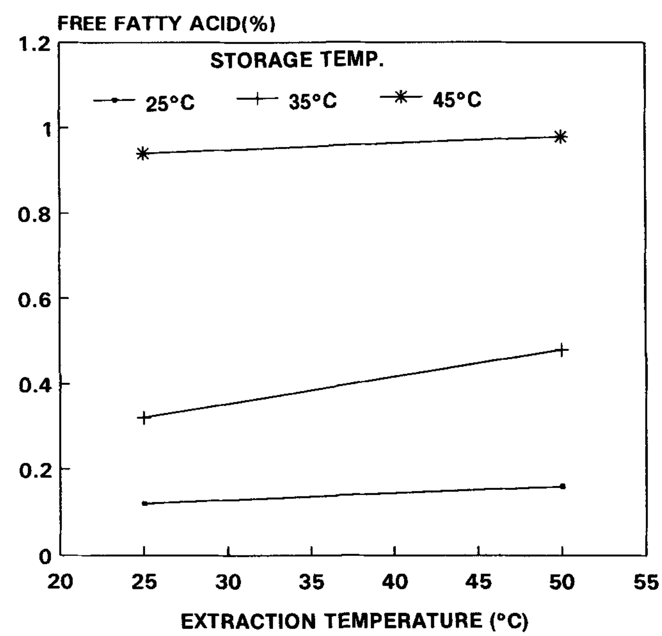

FIG. 4. Effect of storage and extraction temperature interaction on free fatty acid analyzed in oil from accelerated-aged soybeans. Experimental conditions: moisture of soybeans, 20%; storage time for 25, 35 and 45°C soybeans were 21, 16 and 12 d, respectively; moisture of flour, 5%; extraction temperatures, 25 and 50°C. Least significant difference, 0.037 at 0.05 level.

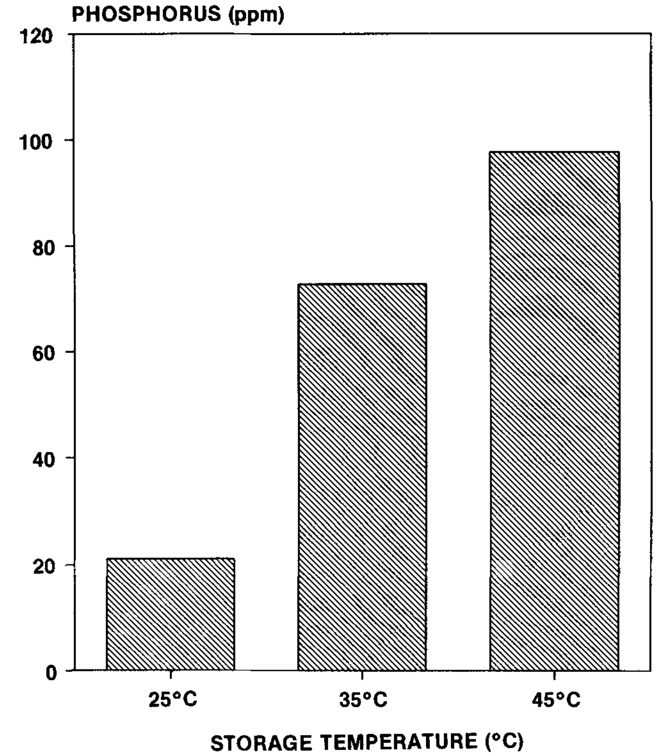

FIG. 5. Comparison of accelerated-aged beans stored at different temperatures with respect to phosphorus analysis. Experimental conditions: moisture of soybeans, 20%; storage time of 25, 35 and 45°C soybeans were 21, 16 and 12 d, respectively; moisture of flour 5%; extraction temperature, 25°C. Least significant difference = 17.2 at 0.05 level.

Evidence indicated that the FFA and phosphorus levels were not influenced by the initial moisture of the soybeans. Soybeans that were at 6.33% moisture and flour at 5% moisture showed 19 ppm phosphorus and 0.08% FFA, whereas soybeans at 20% moisture and flour at 5% showed 21 ppm phosphorus and 0.11% FFA in the extracted oil. These results indicated that although 20% moisture soybeans were stored for 21 d, there was a negligible effect on FFA and phosphorus levels.

It can be concluded that the levels of phosphorus and FFA were more dependent on the moisture of the soybeans at the time of grinding than on the moisture during storage. The high-moisture soybeans were oven-dried at 40°C for 24 h to less than 5% before grinding.

Influence of moisture and retention time of flour immediately after grinding. During the course of this study, it was realized that the moisture of the flour, determined immediately after grinding, had a great influence on the quality of extracted oil. To investigate this further, Forrest soybeans were studied at two different moisture levels, *i.e.,* 12% and 18%, and Hutcheson soybeans were studied at 13% and 16% moisture. Oil was extracted from the flours at three different moisture levels, *i.e.,* 5–6, 7–8 and 9–10%. From moisture determination of the flour obtained immediately after grinding, Forrest soybeans (18 and 12% moisture) dropped to 12.5 and 10%, respectively. Hutcheson soybeans (16 and 13% moisture) dropped to 12% and 10.5%, respectively, after grinding. Although the moisture of the flour after grinding from Hutcheson soybeans (13% moisture) and Forrest soybeans (12% moisture) were approximately the same, there was a difference in the FFA (Fig. 6) and phosphorus (Fig. 7) levels between the two. Phosphorus levels from Forrest soybeans (12% moisture) ranged from 36 to 299 ppm, and phosphorus levels from Hutcheson soybeans (13% moisture) ranged from 164 to 515 ppm. Similarly, FFA ranged from 0.13 to 0.18% for Forrest soybeans (12% moisture) and from 0.25 to 0.31% for Hutcheson soybeans (13% moisture). Results from Forrest soybeans at 18% moisture showed FFA ranging from 0.13 to 0.19% and phosphorus from 120 to 288 ppm. On comparing the results with those from Forrest soybeans at 12% moisture, there is not much difference in FFA levels. However, there is a great increase in phosphorus levels.

A thin-layer analysis of the kinds of phospholipids being extracted showed that the predominant phospholipid for low levels of phosphorus (20–50 ppm) was phosphatidic acid. Along with phosphatidic acid, phosphatidylcholine was identified. For phosphorus levels of 50 to 500 ppm, the usual phospholipids were identified (phosphatidylcholine, phosphatidylinositol and phosphatidylethanolamine, with minor amounts of phosphatidic acid). The significance of this pattern of phospholipid extraction is not known, but perhaps at low levels of moisture only

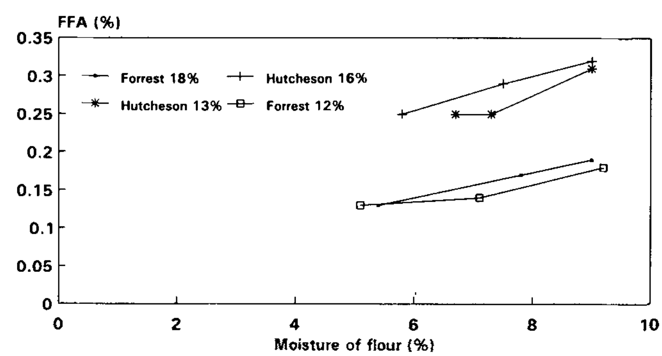

FIG. 6. Effect of moisture of soybeans from Forrest and Hutcheson cultivars on free fatty acid (FFA) levels in extracted oil. Experimental conditions: moistures of Forrest soybeans were 12 and 18%. Moistures of Hutcheson soybeans were 13 and 16%. Moistures of flour used were 5–6, 7–8 and 9–10% for each cultivar.

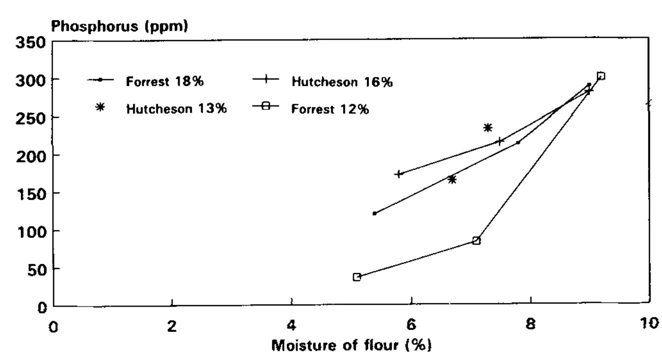

FIG. 7. Effect of moisture of soybeans from Forrest and Hutcheson cultivars on phosphorus levels in extracted oil. Experimental conditions: moistures of Forrest soybeans were 12 and 18%. Moistures of Hutcheson soybeans were 13 and 16%. Moistures of flour used were 5–6, 7–8 and 9–10% for each cultivar.

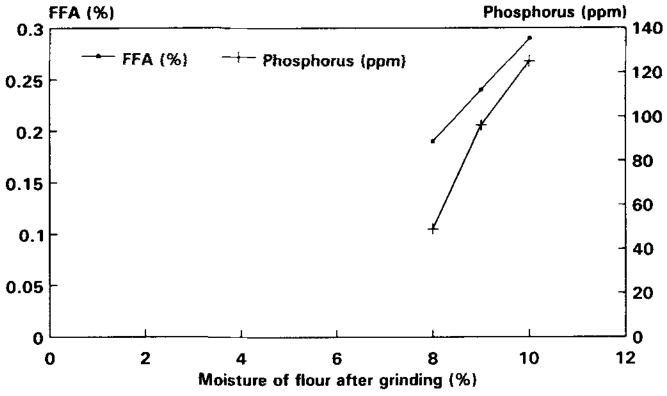

FIG. 8. Influence of retention time and moisture of flour immediately after grinding on free fatty acids (FFA) and phosphorus levels in extracted oil. Experimental conditions: moistures of flour immediately after grinding were 8, 9 and 10% from 9.4, 11.7 and 13.2%-moisture Hutcheson soybeans, respectively. Moisture of the flour used for extraction was between 5–6%.

phospholipids not yet incorporated in membranes are being extracted. Phosphatidic acid and phosphatidylcholine are precursors of other phospholipids (13).

Results from Hutcheson soybeans (16% moisture) showed FFA ranging from 0.25 to 0.32%, and phosphorus from 172 to 280 ppm. On comparing the results with those of Hutcheson soybeans at 13% moisture, as mentioned earlier, there was not much difference in FFA or phosphorus level. The results clearly indicate that the moisture of the flour immediately after grinding influenced the phosphorus level to a great extent, although it had little influence on the FFA level.

During this study it was determined that the time taken for the flour to dry after grinding, before it was ready for extraction, was critical. The time required for drying was influenced to some extent by the relative humidity in the grinding room.

To investigate this further, Hutcheson soybeans were briefly studied at three different moisture levels—9.4, 11.7 and 13.2%. Moisture levels of the flours after grinding were about 8, 9 and 10%, respectively. As can be seen from the results in Figure 8, the FFA values in the crude oil (flour extracted at 5–6% moisture) for 8, 9 and 10% moisture flour were 0.19, 0.24 and 0.29%, whereas the phosphorus levels were 49, 96 and 125 ppm, respectively. The times for the flours to dry to 5–6% after grinding were 10 min, 20 min and 45 min for 9.4, 11.7 and 13.2%-moisture soybeans, respectively. As mentioned earlier, flour from 13%-moisture Hutcheson soybeans dried at 35°C for 4 h showed phosphorus of 164 ppm in oil extracted from 5.1%-moisture flour. On the other hand, flour from 13.2%-moisture Hutcheson soybeans dried at 50°C for 45 min showed 125 ppm phosphorus in oil extracted from 5.3%-moisture flour. This shows that the duration of drying the flour also influences the phosphorus level in extracted oil. If the moisture of the flour after grinding can be reduced to 6% or lower in a short time (5–10 min), we can minimize phosphorus and FFA in the extracted oil. Thus it may be possible to get high-quality, low-phosphorus and low-FFA oil extracted even from inferior quality soybeans.

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