# Iron and Phosphorus Contents of Soybean Oil from Normal and Damaged Beans[1]

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## ABSTRACT

Analyses of commercial crude soybean oils showed a highly significant correlation of 0.74 between free fatty acid and iron content. Poor flavor characteristics exhibited by finished oils extracted from damaged beans may be caused in part by a higher free fatty acid and related higher iron content in crude oils. Source of the increased iron appears to be both damaged beans and steel processing equipment. Crude oil from damaged beans is 2-10 times higher in iron than crude oil extracted from sound beans. Iron appears loosely bound in soybeans, since autoclaving, spontaneous heating in storage, or treating with alcohol increased the level of iron in laboratory extracted crude oil from 0.2 to more than 1 ppm. Present data do not indicate that iron and phosphorus contents are associated statistically in extracted oils.

## INTRODUCTION

Toward the end of the 1971-1972 processing year for soybeans, private reports from the Southeastern U.S. indicated that the quality of crude oil, as measured by free fatty acid, and the quality of the finished oil, as indicated by flavor, were much lower than usual. Problems in oil refining brought inquiries to the staff of the Northern Laboratory. Earlier, North Carolina farmers had experienced one of the worst harvest seasons on record because of continued wet weather, including heavy rains early in November (1). Extensive field and storage damage to beans, estimated at 25-35%, resulted. Oil from damaged beans represents a challenge because of the effects on crude oil and the lower quality of the finished oils. Damage to soybeans is known to give increased free fatty acid (FFA) in the crude oil (2) and to lower flavor quality of refined oil (3,4). Consequently, we undertook a series of analyses to determine what chemical factors were related to poor flavor in oil from damaged beans (1971 crop year).

## MATERIALS AND METHODS

Soybeans were obtained from the Southeastern, Southwestern, and Midwestern areas of the U.S. Both field damaged and storage damaged beans came from the Southeastern area, along with sound beans grown in the Southeast and Midwest during the 1970, 1971, and 1972 crop years. Crude oils, partially processed oil samples and finished soybean salad oils, came from several sources in both the Southeast and Midwest. Abnormal oils characterized by high phosphorus and iron contents were provided by a Southeastern plant that was reportedly processing sound beans grown in Ohio and Indiana in 1972. These abnormal oils could not be degummed to meet the trading rule standards of less than 200 ppm phosphorus. The beans showed no visible damage, and the extracted oil had a normal FFA content.

To get noncontaminated oil for metal analysis, whole beans were crushed by hand in a porcelain mortar and extracted in glass. The beans (20-30 g) were dried overnight at 90 C so that they were sufficiently vitreous to be hand ground. Three separate extractions (by decantation) were made with petroleum ether (100 ml each), with a regrinding of the material after the first extraction. The first extraction was made overnight and the other two the next day. Solvent was removed on a steam plate under a stream of nitrogen until no further loss in wt occurred. The oil yield was calculated on these resultant wt.

FFA was determined on the oil by AOCS Method Ca 5a-40 (5). Iron and copper was measured on oil samples by atomic absorption, as described by List, et al. (6). Oils were refined chromatographically by AOCS Method Ca 9f-57 (5). To determine phosphorus, the AOCS Method Ca-12-55 (5) was modified slightly. An oil sample, 2 g for crude or 25 g for refined oil, was mixed with 1 ml 95% ethanol saturated with magnesium nitrate in a 100 ml Vycor evaporating dish. Evaporation and charring of the sample were conducted carefully on a rheostat controlled hot plate to prevent spattering. The samples were ashed for 16 hr in a muffle furnace at 600 C. The magnesium pyrophosphates were hydrolyzed by gentle boiling (watch glass reflux) for 1 hr with ca. 20 ml 5% sulfuric acid solution before proceeding with the phosphorus color determination. A freshly prepared stannous chloride solution was used for the reduction of the phosphomolybdate.

Crude soybean oils were degummed in the laboratory, according to the method of Hayes and Wolff (7). Oils were protected by bubbling nitrogen through them before and during heating to 60 C after which the oils were transferred to a Waring blender. The required amounts of acetic anhydride were added as a pure material. Samples were agitated at high speed for 3 min, then water was added (2% by wt of oil), and agitation was continued for another 3 min. Gums were removed by centrifuging the oil in 250 ml bottles. Refining was conducted with various concentrations and excess of alkali, after which the oils were washed at room temperature in the blender with 20% water. After being centrifuged and dried under vacuum, oils were bleached with 0.5% earth. Deodorization was conducted for 3 hr at 210 C (8).

To orient the reader and to enable him to distinguish the important analytical differences between various processes and samples, Table I is introduced at this point. The approximate range of these minor constituents are indicated for the three types of commercial crude soybean oils and for the nonmetallic processed crude oils extracted in the laboratory.

## EXPERIMENTAL RESULTS

Phosphorus, iron, and copper contents were determined in several crude and processed oils identified according to the area of origin (Table II). Crude soybean oils varied in their phosphorus content from 312-910 ppm, while the damaged crudes as a group are the lowest in phosphorus. Abnormal crude oils and abnormal degummed oils are both high in phosphorus and iron. The iron content of commercially extracted crude oils varied from 0.9-6.1 ppm. Damaged crudes from the Southeast were nearly all above 3 ppm iron, a concentration level which marks badly damaged beans. Primary reduction in both iron and phosphorus occurs at the alkali refining stage, and oils with this degree

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TABLE I

Concentration Levels of Some Minor Constituents in Crude Soybean Oil

| Sample | Phosphorus, ppm | Iron, ppm | Copper, ppm | FFA[a] % |
|---|---|---|---|---|
| Commercial crudes | | | | |
| Normal | 500-700 | 1-3 | 0.03-0.05 | 0.3-0.7 |
| Abnormal | 700-900 | 3-7 | 0.03-0.05 | 0.4-0.7 |
| Damaged | <500 | 3-7 | 0.08-0.18 | 1.0-8.0 |
| Nonmetal processed, laboratory | | | | |
| Normal | --- | 0.1-0.3 | 0.02-0.06 | 0.1-0.3 |
| Modified | --- | 1.0-1.3 | 0.04-0.07 | 0.1-0.3 |
| Damaged | --- | 1.0-1.6 | 0.10-0.30 | 1.0-8.0 |

[a] Calculated as oleic acid. FFA = free fatty acid.

TABLE II

Phosphorus and Metal Contents of Commercial Soybean Oils

| Sample | Phosphorus, ppm | Iron, ppm | Copper, ppm |
|---|---|---|---|
| Crude | | | |
| Normal | | | |
| Midwest | 633 | 1.5 | 0.05 |
| Midwest | 673 | 1.2 | 0.03 |
| Midwest | 647 | 0.9 | --- |
| Southwest | 799 | 2.8 | 0.04 |
| Southwest | 700 | 2.4 | --- |
| Southwest | 570 | 2.9 | 0.05 |
| Abnormal | | | |
| Southeast | 820 | 6.1 | 0.05 |
| Southeast | 850 | 5.6 | 0.04 |
| Southeast | 910 | 3.3 | 0.03 |
| Southeast | 638 | 3.2 | 0.03 |
| Southeast | 857 | 3.8 | 0.03 |
| Southeast | 802 | 4.9 | 0.04 |
| Southeast | 708 | 3.2 | 0.04 |
| Damaged | | | |
| Southeast | 622 | 2.6 | 0.05 |
| Southeast | 616 | 3.9 | 0.04 |
| Southeast | 693 | 6.1 | 0.08 |
| Southeast | 312 | 4.5 | 0.06 |
| Degummed | | | |
| Normal | | | |
| Southwest | 222 | 1.3 | --- |
| Midwest[a] | 39 | 1.2 | 0.01 |
| Midwest[a] | 31 | 2.6 | --- |
| Abnormal | | | |
| Southeast | 378 | 2.7 | --- |
| Southeast | 386 | 2.6 | --- |
| Southeast | 398 | 2.9 | --- |
| Southeast | 373 | 3.3 | --- |
| Southeast | 426 | 2.9 | --- |
| Southeast | 458 | 3.5 | --- |
| Southeast | 461 | 3.5 | --- |
| Damaged | | | |
| Southeast | 295 | 1.3 | 0.03 |
| Refined | | | |
| Normal | | | |
| Midwest | 15 | 0.2 | --- |
| Midwest | 3.6 | 0.1 | --- |
| Southwest | 1.4 | 0.06 | --- |
| Southwest | 1.7 | 0.06 | --- |
| Damaged | | | |
| Southeast | 5 | 0.1 | --- |
| Deodorized | | | |
| Normal | | | |
| Midwest | 5.3 | 0.1 | 0.01 |
| Midwest | 1.4 | 0.08 | --- |
| Southwest | 3.7 | 0.2 | 0.01 |
| Southwest | 0.6 | 0.1 | --- |
| Damaged | | | |
| Southeast | 59 | 0.7 | 0.01 |
| Southeast | 4.6 | 0.1 | --- |

[a] Acetic anhydride degumming.

of processing should have less than 5 ppm of phosphorus and 0.2 ppm of iron. The acetic anhydride degumming process markedly lowers the phosphorus content of undamaged crude oils by ca. 95%, but the process does not remove iron.

Decrease of phosphorus in commercial refining of a Southeastern crude (damaged bean oil, FFA 1.6%) was as follows: crude, 622; degummed, 295; bleached, 5.8; and deodorized, 4.7 ppm, respectively. These changes in phosphorus content are normal and typical of results obtained in water degumming and alkali refining of undamaged soybean oil. Iron may or may not be removed with the phosphorus, but, in the previous example, degumming removed 47% of the phosphorus and 50% of the iron (from 2.6 ppm). Commercial degumming in a different plant but by a similar process reduced phosphorus 32% (from 700 ppm) but reduced iron 54% from a crude oil level of 2.4 ppm. A commercial sample of finished salad oil of poor stability processed from Southeastern damaged crude had both high iron (0.65 ppm) and high phosphorus (59 ppm) contents. Good stable soybean salad oils, regardless of the various processing techniques employed by the oil industry, should only have a few ppm phosphorus and an iron content of less than 0.1 ppm.

The FFA analysis and metal contents of oils from normal and damaged beans extracted by hand in nonmetallic equipment appear in Table III. Oil from sound beans was low in both its iron content and in the amount of FFA present, regardless of the geographic area in which the beans were grown. Low iron contents of less than 0.4 ppm with FFA of less than 0.3% appear normal for all sound beans. Damaged beans, processed in procelain and glass, show a marked increase in both their iron and FFA contents. The maximum value for iron in oil from damaged beans of 1.6 ppm for hand processed oil is ca. half the value found for commercially processed crudes. This difference would indicate that the origin of iron in commercial crudes is twofold: from natural iron in the bean but, mainly, from iron being picked up from processing equipment. Iron taken up by high fatty acid oils would be expected to be in the form of iron soaps. Natural iron in the bean itself may be chelated in some form and tied to proteins, phosphatides, or other lipid or nonlipid carriers. Ohlson (9) discussed the organic complexes of metals effecting oil stability. He also reported considerably higher values than we found for iron and copper in four native soybean oils which had not been in contact with metals.

Copper content varied little with quality or source of crude soybean oils. Copper values did not vary with the iron content and decreased with processing to the limits of detection in the finished oil. The degumming step also removed ca. 50% of the copper from the crude oil. Because of its low levels, copper contamination is not believed to be a serious factor in the stability or quality of oil from damaged beans. All hand prepared oil samples extracted

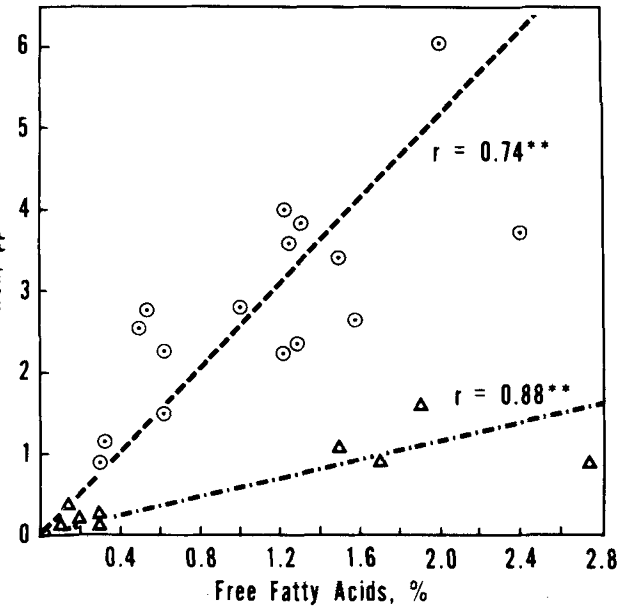

FIG. 1. Correlations of iron contents with the amount of free fatty acid in commercial crude soybean oils and for oils extracted in the laboratory under metal-free conditions. ⊙ = Commercially extracted and △ = extracted in glass.

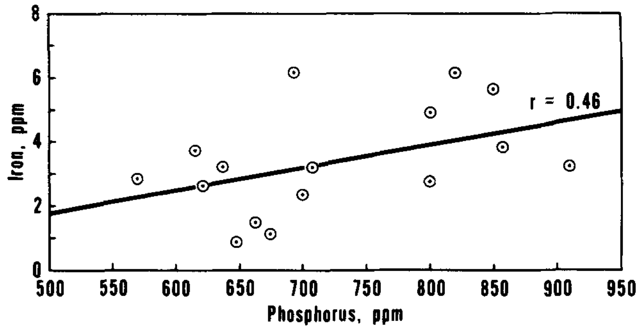

FIG. 2. Relationship between iron and phosphorus contents of commercial crude soybean oils. ⊙ = Commercially extracted.

from damaged beans increased appreciably in their copper contents, but this increase was somewhat smaller than that observed for iron.

After correlation analysis for 16 commercial crude oils, iron values were plotted against the FFA content (Fig. 1). The correlation coefficient for this relationship was 0.74. The correlation is significant at the 1% confidence level for this amount of data. Iron and FFA contents of hand ground and laboratory extracted oil are also plotted in Figure 1. Data plotted for oil from sound beans are well separated from the area of damaged oil and demonstrate the 10-fold increase in iron content of damaged bean oil. A highly significant correlation of r = 0.88 was obtained for the nonmetallic processed oils.

A nonsignificant association between the iron and phosphorus contents of commercial crude soybean oils is depicted in Figure 2, where a correlation of only 0.46 was obtained. Solvent extraction studies on phosphatides, were all the iron remains in the oil (unpublished data), and acetic anhydride degumming studies also indicated that iron and phosphorus are not associated in crude soybean oil.

Laboratory degumming of oils from damaged beans with acetic anhydride has shown substantial differences in amounts of iron and phosphorus removed by this system (Table IV). Low levels of acetic anhydride removed greater amounts of phosphorus and lesser amounts of iron. As concentration of the reagent was increased, the phosphorus removed remained substantially the same, but the amount of iron removed was increased to 96%. A difference in concentration of 0.1-0.25% acetic anhydride increased the iron removal 6.3-fold. Higher levels of acetic anhydride did not increase the removal of either phosphorus or iron substantially. Alkali refining of damaged oils in the laboratory removed 99% of both iron and phosphorus.

TABLE III

Analysis of Crude Soybean Oils
Hand Processed in Glass Equipment

| Sample | FFA$^a$ % | Iron, ppm | Copper, ppm |
|---|---|---|---|
| Sound beans | | | |
| Certified seed | 0.10 | 0.06 | 0.02 |
| Certified seed | 0.14 | 0.4 | 0.05 |
| Southeast | 0.19 | 0.2 | 0.04 |
| Southeast | 0.30 | 0.2 | 0.02 |
| Southeast | 0.30 | 0.2 | 0.02 |
| Midwest | 0.31 | 0.3 | 0.06 |
| Damaged beans | | | |
| Southeast | 1.49 | 1.1 | 0.34 |
| Southeast | 1.70 | 0.9 | 0.18 |
| Southeast | 1.90 | 1.6 | 0.09 |
| Southeast | 2.76 | 0.9 | 0.11 |
| Southeast | 7.20 | 0.1 | 0.05 |

[a] Calculated as oleic acid. FFA = free fatty acid.

Chromatographic refining completely removed all nontriglyceride components from the crude oil. It gave a high correlation with FFA content, and the intercept at 0% FFA was ca. 2.2%, which is attributed to phosphatide content. The removal of iron by chromatographic refining was not so complete as expected. Iron removal was high for oils having both high iron and high FFA contents. At normal levels of iron and FFA, the removal rate of iron ranged from 50-80%. Iron content of chromatographically refined oil was from 5-10 times higher than alkali refined oil (0.05-0.10 ppm). Table V presents data on the removal of iron by chromatographic refining of oil from normal and highly damaged beans.

How beans are treated before extraction of the oil also may affect the amount of iron removed during extraction. In Table VI, it is shown that factors other than FFA may affect the extraction of iron. Sound beans processed by hand under nonmetallic conditions had iron levels of ca. 0.2-0.4 ppm, whereas damaged beans had levels in the range of 1-2 ppm. However, if sound beans are soaked 24 hr with 50% alcohol and if the alcohol is allowed to evaporate (no extraction), iron content in the crude oil rose from 0.38-1.15 ppm, a factor of threefold. Oil extracted from steam treated sound beans (10 min, 100 C) showed a fivefold increase in the amount of iron extracted with the oil and a twofold increase in the amount of copper. The release of oil-soluble metals may be ascribed to protein degradation, denaturation, or such biological modifications as enzyme inactivation. Although the magnitude of these increases in oil-soluble metals is significant, the amounts involved are probably a small percentage of the total amount of iron or copper present in the soybean.

## DISCUSSION

The phosphatide and phosphorus content of crude soybean oil always has been an important factor in oil refining (10-12). Robertson, et al., (13) reported a decrease in phospholipid content of the oil in direct relationship to the severity of bean damage. Refining problems may result, not only from a higher FFA content, but also from increased content and difficulty of removing nonhydratable phospholipids. Hvolby (12) reports that normal oil contains 135 ppm phosphorus (4.36 mmole/kg) in a nonhydratable form and that this could not be removed even after 16 water washing steps. Iron could well be associated with this

TABLE IV

Reduction in Iron and Phosphorus Content by Laboratory Acetic Anhydride Degumming of Oil from Damaged Beans

| | | | Removal of | |
|---|---|---|---|---|
| Sample | Phosphorus, ppm | Iron, ppm | Phosphorus, % | Iron, % |
| Crude | 312 | 5.5 | --- | --- |
| Degummed | | | | |
| 0.1% Acetic anhydride | 57 | 1.9 | 82 | 65 |
| 0.25% Acetic anhydride | 76 | 0.3 | 76 | 94 |
| 0.50% Acetic anhydride | 80 | 0.2 | 74 | 96 |
| Refined and deodorized | | | | |
| 0.1% Excess alkali | 0.9 | 0.09 | 99 | 98 |
| 0.3% Excess alkali | 1.2 | 0.09 | 99 | 98 |

TABLE V

Removal of Iron by Chromatographic Refining of Crude Oil

| Sample | FFA[a] % | Iron, ppm | Chromatographic refining loss, % | Iron removed, % |
|---|---|---|---|---|
| Normal | | | | |
| Midwest | 0.36 | 1.2 | 1.7 | 58 |
| Southwest | 0.47 | 2.8 | 2.1 | 79 |
| Midwest | 0.61 | 2.3 | 2.6 | 52 |
| Midwest | 0.62 | 1.5 | 3.0 | 60 |
| Southwest | 1.0 | 2.8 | 2.7 | 86 |
| Abnormal | | | | |
| Southeast | 0.43 | 3.2 | 2.3 | 66 |
| Southeast | 0.57 | 3.3 | 2.4 | 63 |
| Southeast | 0.63 | 3.8 | 2.1 | 58 |
| Southeast | 0.65 | 4.9 | 2.5 | 69 |
| Southeast | 0.68 | 3.2 | 2.5 | 46 |
| Southeast | 0.78 | 5.6 | 2.5 | 77 |
| Southeast | 0.80 | 6.1 | 3.4 | 64 |
| Damaged | | | | |
| Southeast | 1.21 | 3.8 | 3.2 | 84 |
| Southeast | 1.31 | 4.0 | 3.7 | 87 |
| Southeast | 1.56 | 2.6 | 4.2 | 68 |
| Southeast | 1.99 | 6.1 | 4.6 | 69 |
| Southeast | 3.32 | 2.0 | 5.2 | 80 |
| Southeast | 5.69 | 4.5 | 7.8 | 93 |
| Abnormal, degummed | | | | |
| Southeast | 0.49 | 3.5 | 1.3 | 16 |
| Southeast | 0.50 | 2.9 | 0.8 | 7 |
| Southeast | 0.53 | 2.9 | 1.0 | 19 |
| Southeast | 0.55 | 2.6 | 1.1 | 15 |
| Southeast | 0.55 | 3.3 | 1.0 | 18 |
| Southeast | 0.59 | 3.5 | 1.7 | 30 |
| Southeast | 0.62 | 2.7 | 1.0 | 16 |

[a] FFA = free fatty acid.

increased phosphatidic acid content of damaged bean oil and could remain in the oil with deleterious results.

Refining damaged bean oil to remove both iron and phosphorus becomes a difficult processing problem because of stable emulsion formation and high refining losses. If phospholipids are essentially destroyed in damaged beans, then high levels of nonhydratable phosphatidic acids must be present. Removal of nonhydratable phospholipids has been discussed by Hvolby (12) who indicated that an acidulation treatment is beneficial. He believes that dilute acids dissociate the Ca/Mg phosphatidates and that the phosphatidic acid disappears from the oil phase into the water phase in the form of micells. A similar explanation could apply to the removal of iron salts. Braae (11) and Sullivan (14) use acidulation to increase yield of neutral oil in refining.

The low quality of refined oil from damaged beans can arise from many factors. Some of the evidence for poor quality, however, can be associated with higher than normal

TABLE VI

Metal Content of Oil as Affected by Treatment of Beans

| Beans[a] | Treatment | FFA[b], % | Iron, ppm | Copper, ppm |
|---|---|---|---|---|
| Midwest, sound | None | 0.3 | 0.3 | 0.06 |
| Certified seed | None | 0.1 | 0.4 | 0.05 |
| Same plus treatment | Alcohol[c] | 0.3 | 1.2 | 0.04 |
| Southeast, sound | None | --- | 0.2 | 0.04 |
| Same plus treatment | Steamed[d] | --- | 1.0 | 0.07 |
| Field damaged | None | 1.7 | 0.9 | 0.18 |
| Field damaged | None | 1.9 | 1.6 | 0.09 |

[a] Hand ground and extracted in glass.

[b] FFA = free fatty acid.

[c] Beans soaked 24 hr in 50% ethyl alcohol and then dried under vacuum.

[d] Beans autoclaved 10 min at 212 F and oil extracted in the usual fashion.

levels of iron. The presence of iron in soybean salad oils at levels above 0.1 ppm is known to be detrimental (15,16). Our interpretation of the data indicates that poor refining will give both high phosphorus and high iron contents. If the iron is not removed completely, quality oil cannot be produced regardless of its phosphorus content. Processing steps that remove phosphorus also remove iron, becasue the two elements are associated closely. In oil from damaged beans, the iron and FFA both increase with the degree of damage. In crude oils, we found no relationship between the iron content and the phosphorus content. Iron is readily available from the bean itself, as has been shown for both damaged beans and treated beans, and also from processing equipment.

The analysis of phosphorus in partially processed or refined oils is not an easy or routine determination. Iron can be determined easily and quickly by atomic absorption analysis, and this technique allows the processor to follow the refining of damaged bean oil much better than does a phosphorus analysis. We have no evidence that phosphorus contributes to the poor quality of damaged bean oil, and the phosphorus level, as reported by Beal, et al. (10), are deemed satisfactory. Although high phosphorus indicates poor refining, it is the iron not the phosphorus that gives poor flavor.

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