| | |
|---|---|
| Fat No. 5 (blended) | 1.762 |
| Fat No. 4 (all hydrogenated) | 1.777 |
| Fat No. 1 (blended) | 1.792 |

The spread between the highest and lowest being 0.146.

When the question of cost is considered, the blended shortenings were the cheapest to use due to their lower initial cost.

Since the free fatty acid content, color, and smoke point of fats and oils, have been shown to change rapidly when used for deep fat frying, perhaps this paper should have been entitled "Unstability of Fats Used for Deep Fat Frying."

# The Occurrence Of Phosphorus In Soybeans

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**Abstract**

The phosphorous compounds present in soybeans have been tentatively divided into four groups. Methods for determining these groups have been studied and applied to the analysis of a sample of soybeans.

WHENEVER phosphorus is mentioned in reference to soybeans it is usually associated solely with the phosphatides known to be present in this legume. The phosphatides, however, contain only a small part of the phosphorus present in soybeans. It is the purpose of this paper to point out the other compounds of phosphorus occurring in soybeans and to indicate their relative abundance. Compounds of phosphorus found in seeds may be classified in four groups, namely, phytins, phosphatides, nucleic compounds, and inorganic phosphorus compounds (5).

The phytins are salts of inositol phosphoric acid and on the basis of the work of Anderson (1) are recognized as hexaphosphates. In the isolation or separation of these substances from natural sources one or more phosphate groups may be removed. Consequently phytins have often been reported as containing only four or five phosphate groups. Phytins usually occur as calcium, magnesium, or potassium salts.

Any lipid ester of phosphoric acid may be defined as a phosphatide. Soybean phosphatides have been discussed in several recent papers appearing in this journal (12) (7) and no further discussion is needed here.

The nucleic-phosphorus compounds include phosphoproteins and nucleic acid derivatives. They occur in various amounts in living material, but only in relatively small amounts in soybeans. Comparatively little is known about them. The inorganic phosphorus compounds may occur either as potassium acid phosphates, as suggested by Osborne (9), or as other similar substances.

In connection with certain experimental work of the U. S. Regional Soybean Industrial Products Laboratory, it was considered essential to be able to determine the relative amounts of the different types of phosphorus compounds present in soybeans. When considering the analysis of the soybeans in connection with the agronomic data available for each sample, the change in total phosphorus is of value, but it is more important to know in which of the several possible compounds the variation occurs. Information of this type is essential in attempting to reach an understanding of the metabolism of the bean.

Some of these phosphorus compounds are relatively unstable when removed from the soybean. For example, the phosphatides are easily oxidized and the soybean proteins in general are altered by almost any kind of treatment. Separation by solvents at temperatures not much above that of the room, while perhaps not the ideal way, is the best method available. It is exceedingly difficult to ensure complete removal of any one type of phosphorus compound from the bean by the use of solvents.

Nottbohm and Mayer (8) analyzed several samples of commercial lecithin from soybeans as well as from other sources. They determined phosphorus, nitrogen, and choline and showed that the value for lecithin varied greatly according to whether it was calculated from the phosphorus, nitrogen, or choline content. Other phosphorus compounds such as the salts of phosphatidic acid (6) can lead to erroneous values for lecithin if calculated solely on the basis of phosphorus content. The recently proposed color reaction for choline (2) is not specific since similar colors are developed with many other amino compounds. In this laboratory the precipitation of choline with iodine and subsequent titration of the choline-iodine complex (10) has proved most satisfactory. The precipitation is not affected by ethanolamine and can therefore be carried out in the presence of cephalin.

Experimental work carried out so far in this laboratory on the distribution of phosphorus in soybeans has been of a preliminary nature. Typical values for the amounts of phosphorus extracted from soybeans by various solvents are shown in Table 1.

These values were obtained with a four-hour extraction in a Butt type extractor. The samples contained 6 to 7 per cent water, and the phosphorus removed by petroleum ether was low. This has been confirmed by other experiments showing that within limits higher moisture content increases manifold the phosphorus removed by petroleum ether. The phosphorus extracted by these solvents is probably derived chiefly from phosphatides and perhaps also from some inorganic compounds. Phytin probably is unaffected by the treatment. Although repeated extraction with some of the more efficient solvents failed to remove additional phosphorus, it is questionable whether all the phosphatides were removed.

Phytin, which contains most of the phosphorus present in soybeans, is difficult to determine. The

[1]A cooperative organization participated in by the Bureaus of Chemistry and Soils and Plant Industry of the U. S. Department of Agriculture, and the Experiment Stations of the North Central States of Illinois, Indiana, Iowa, Kansas, Michigan, Minnesota, Missouri, Nebraska, North Dakota, Ohio, South Dakota, and Wisconsin.

TABLE 1.—PHOSPHORUS EXTRACTED FROM SOYBEANS BY VARIOUS SOLVENTS, EXPRESSED AS MILLIGRAMS OF PHOSPHORUS PER GRAM OF WHOLE BEAN

| Sample Number | First Extraction Solvent | First Extraction Phosphorus Extracted mg | Second Extraction Solvent | Second Extraction Phosphorus Extracted mg | Third Extraction Solvent | Third Extraction Phosphorus Extracted mg | Total Phosphorus Extracted mg |
|---|---|---|---|---|---|---|---|
| 65 | Petroleum Ether | 0.014 | Alcohol 99% | 0.70 | | .... | 0.71 |
| 65 | Petroleum Ether | 0.014 | Benzene-Alcohol (70%—30%) | 0.70 | | .... | 0.71 |
| 67 | Petroleum Ether | 0.018 | Alcohol 99% | 0.64 | | .... | 0.66 |
| 73 | Petroleum Ether | 0.003 | Alcohol 99% | 0.72 | | .... | 0.72 |
| 73 | Petroleum Ether | 0.003 | Benzene-Alcohol (70%—30%) | 0.71 | | .... | 0.71 |
| 77′ | Benzene-Alcohol (70%—30%) | * | 1-4 Dioxane | 0.008 | | .... | ... |
| 77′ | Benzene-Alcohol (70%—30%) | * | n-Butyl Alcohol | 0.013 | | .... | ... |
| 77′ | Benzene-Alcohol (70%—30%) | * | Pyridine | 0.026 | Alcohol 95% | 0.067 | ... |
| 77′ | Petroleum Ether | * | Benzene-Alcohol (70%—30%) | * | Ethyl Acetate | 0.005 | ... |
| 77′ | Petroleum Ether | * | Benzene-Alcohol (70%—30%) | * | Trichlorethylene | 0.005 | ... |
| 77′ | Ethylether | 0.14 | Alcohol 95% | 0.63 | | .... | 0.77 |
| 77′ | Alcohol 95% | 0.79 | Alcohol-Benzene (80%—20%) | 0.013 | | .... | 0.80 |
| 77′ | Benzene | 0.35 | Alcohol 95% | 0.41 | | .... | 0.76 |
| 77′ | Alcohol-Benzene (80%—20%) | 0.71 | Alcohol 95% | 0.053 | | .... | 0.76 |

*No determinations of phosphorus made.

method of Heubner and Stadtler (4) has proved more satisfactory in this laboratory than several modifications of it which have been proposed. By this method phytin is extracted with 1.8 per cent hydrochloric acid and titrated with ferric chloride, using ammonium thiocyanate as an indicator. The solution becomes very turbid and the endpoint is difficult to determine, but with continued practice reproducible results can be obtained.

Inorganic phosphorus is extracted by the method of Collison (3) using alcohol acidified with a little hydrochloric acid. Since the acid alcohol would undoubtedly remove phosphatides as well as inorganic compounds, the order of removal must be phosphatides, inorganic compounds, and phytin.

The series of extractions outlined above was tried on a sample of Dunfield soybeans with the results shown in Tables 2 and 3.

Phosphorus was determined on the various extracts by the colorimetric method described by Truog and Meyer (11), except in the case of the phytin extract, phosphorus was calculated from the ferric chloride titration. An alcoholic solution of magnesium nitrate was used to assist in ashing the samples. In making successive extractions it was necessary to correct for the amount of solvent left in the residue of the first extraction. Likewise, the phosphorus retained on the filter papers at earlier stages of the extractions was distributed proportionately among the determinations which followed, as shown in Table 3.

The results for total phytin are in agreement within the precision of the method. A total phosphorus determination made on the first phytin extract agreed within 1 per cent with the phytin phosphorus measured by ferric chloride titration. If the phosphatides are removed first, the results for inorganic phosphorus are in agreement. It is uncertain exactly how much inorganic phosphorus was extracted with the phosphatide fraction and how much phosphatide remained to appear as inorganic phosphorus. This phase of the problem is still being studied. The nature of the residual unextracted phosphorus is also under investigation. It is expected that this residue contains the nucleic phosphorus compounds. Only about 6 per cent of the total phosphorus of the soybeans is unaccounted for in the present investigation.

TABLE 2.—PHOSPHORUS REMOVED FROM DUNFIELD SOYBEANS, SAMPLE NO. 77, BY SUCCESSIVE EXTRACTIONS, USING VARIOUS SOLVENTS[1,2]

| Solvent | Type phosphorus removed | Phosphorus removed from samples, numbered— 1 mg | 2 mg | 3 mg | 4 mg | 5 mg | 6 mg |
|---|---|---|---|---|---|---|---|
| Petroleum ether | Phosphatide | ... | ... | .02 | .02 | .03 | .03 |
| Alcohol, 95%, 1st extract | Phosphatide | .80 | .77 | ... | ... | .71 | .71 |
| Filter papers from above | | .11 | .11 | .13 | .13 | .14 | .15 |
| Alcohol-HCl, 1st extract | Inorganic | .20 | .19 | .76 | .75 | .17 | .19 |
| Alcohol-HCl, 2nd and 3rd extract | Inorganic | .06 | .07 | .11 | .12 | .08 | .08 |
| Filter papers from above | | .12 | .12 | .16 | .13 | .09 | .10 |
| HCl, 1.8% in water, 1st extract | Phytin | 4.09 | 3.86 | 3.86 | 3.92 | 3.90 | 3.86 |
| HCl, 1.8% in water, 2nd and 3rd extract | Phytin | .15 | .18 | .33 | .15 | .23 | .19 |
| Phosphorus in residue | | .23 | .31 | .34 | .35 | .31 | .30 |

[1]Results expressed as milligrams of phosphorus per gram of whole bean.
[2]Total phosphorus in bean, 6.02 milligrams per gram.

TABLE 3.—DISTRIBUTION OF PHOSPHORUS IN DUNFIELD SOYBEANS CONTAINING 6.02 mg PHOSPHORUS PER GRAM OF WHOLE BEAN

| Sample Number | Phosphatide Phosphorus mg | Inorganic Phosphorus mg | Phytin Phosphorus mg | Phosphorus in Residue mg | Total Accounted for mg |
|---|---|---|---|---|---|
| 1 | .80 | .27 | 4.45 | .24 | 5.76 |
| 2 | .77 | .26 | 4.29 | .33 | 5.61 |
| 3 | .91* | .. | 4.44 | .36 | 5.71 |
| 4 | .91* | .. | 5.29 | .37 | 5.57 |
| 5 | .74 | .26 | 4.33 | .33 | 5.66 |
| 6 | .74 | .28 | 4.26 | .32 | 5.60 |

*Also includes inorganic phosphorus.

## SUMMARY

1. The types of phosphorus compounds probable in soybeans are discussed.

2. A tentative method of separating these compounds of phosphorus by various solvents is tested on soybeans and the results tabulated.

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