volume of whole wheat bread is affected most by the use of rancid fat. The volume of white bread is affected but not to the same degree as that of whole wheat bread. Cake volume is also affected but the effect on taste is much greater. There are many antioxidants that can be added to cereal products. Among those that can be considered for human consumption are lecithin and soya products. Table 2 shows this effect.

In the summary we will make the following points:

1. The fat content of the milled product is the most important factor to be considered in the evaluation of the keeping quality of milled products.

2. Bleaching and maturing treatments demand consideration when evaluating keeping quality. These agents in order of their effect on keeping quality are chlorine, nitrogen trichloride, and benzoyl peroxide.

TABLE II
Effect of Some Antioxidants

Mixture	Keeping time
Control	35 days
1% Lecithin	87 days
0.5 Lecithin	90 days
0.125 Lecithin	63 days
20% soy (extracted)	93 days
10% soy (extracted)	77 days
5% soy (extracted)	63 days

3. The metallic ingredients of the flour, such as copper, iron, etc., and of added ingredients, are instrumental in shortening the keeping time.

4. The addition of good hydrogenated shortenings do much to improve the taste and keeping quality of prepared mixes.

5. Certain antioxidants definitely improve the keeping quality.

Sampling Soybeans for Analysis[1]

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THE present situation in the soybean industry is similar to that described by Ainslie (1) for cottonseed when that commodity was first bought on the basis of chemical analysis. Much work is being done on the refinement of chemical methods in an effort to obtain reliable and consistent results, when in many cases, accuracy of sampling may be the limiting factor. It was early recognized by Barrow (2) and Munch and Bidwell (6) that accuracy of sampling is largely determined by sample size and uniformity of material sampled, and the more homogeneous the material, the smaller the sample may be.

Accurate sampling is imperative in soybean breeding work if reproducible and reliable chemical data are to be obtained. It is often desirable to composite seed of each strain from several uniform soybean nurseries in an area in order to obtain reliable varietal comparisons with a minimum number of analyses. A similar problem arises in the sampling of carloads of seed which may consist of a number of different lots varying widely in composition. According to Morse and Cartter (5), seed of different varieties may vary as much as 5% in oil content and 9% in protein content.

It seems to be quite generally assumed that any difference in results in the analysis of duplicate samplings is due to variations in the chemical analysis rather than to sampling differences. The purpose of this investigation is to determine the relative importance of sampling differences and how they may be reduced to a reasonable minimum.

In order to check the accuracy of generally accepted sampling methods, a mixture of soybeans was prepared consisting of equal weights of the Mandarin and Lincoln varieties. Aliquots were prepared from the mixture and the percentage of each variety in each aliquot was obtained. The aliquots were then analyzed for nitrogen by A.O.A.C. methods and for oil by A.O.C.S. methods. Variations in analytical results were then checked against variations in varietal composition to determine to what extent differences in chemical analyses were due to actual differences in varietal composition.

Methods and Materials

THE two varieties, Mandarin and Lincoln, were chosen because Mandarin is high in nitrogen and relatively low in oil content, and Lincoln is high in oil and relatively low in nitrogen content. The two varieties were readily separated to determine the varietal composition of an aliquot because Lincoln has a black hilum and Mandarin a colorless hilum. They have approximately the same seed size; Lincoln 14.2 grams and Mandarin 15.3 grams per hundred seed.

A bulk supply of each variety was screened to remove small or broken seed and foreign matter. A 480-gram lot of each variety was sampled from these bulk supplies and mixed thoroughly to make 960 grams of the mixture. This mixture was sampled by means of a Boerner Sampler (3) into 32 aliquots of approximately 30 grams each which were saved for chemical analysis. After each split with the sampler the two varieties were separated, and the percentage composition by weight of each aliquot was obtained. The two varieties were mixed thoroughly before the next sampling division was made. Figure 1 shows that there is a marked increase in range of percentage composition as the weight of the aliquots decrease. This is particularly true for samples smaller than 120 grams. Points on the curve represent the maximum differences obtained during the sampling from the two samples of approximately 480 grams each down

[1] The U. S. Regional Soybean Laboratory is a cooperative organization participated in by the Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U. S. Department of Agriculture and the Agricultural Experiment Stations of Alabama, Arkansas, Florida, Georgia, Illinois, Indiana, Iowa, Kansas, Louisiana, Michigan, Minnesota, Mississippi, Missouri, Nebraska, North Carolina, North Dakota, Ohio, Oklahoma, South Carolina, South Dakota, Tennessee, Texas, Virginia, and Wisconsin.

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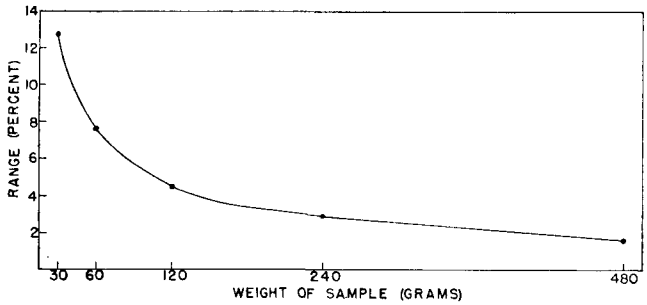

FIG. 1. The range for each sample size is expressed as the difference between the highest and lowest percentage of one soybean variety in an aliquot.

to the 32 samples of approximately 30 grams each. The sampling was then repeated from new lots of seed to obtain 32 samples of each weight. Plotting of these results gave a curve similar in general form to that shown in Figure 1.

The final aliquots from the first run consisting of 32 samples of about 30 grams each were then analyzed for oil and nitrogen. The results are shown in Figure 2.

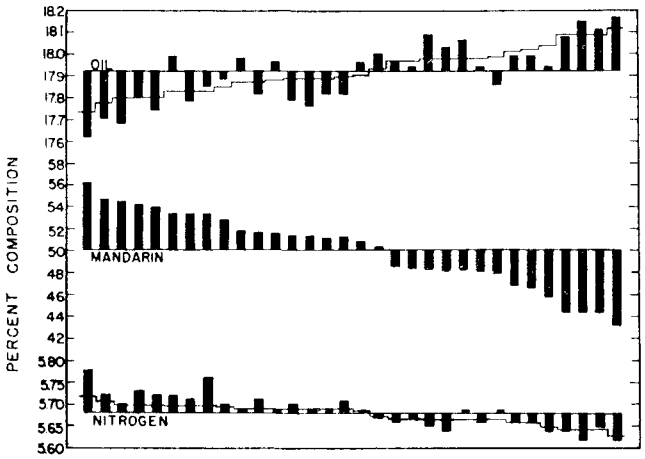

FIG. 2. Deviations from average composition of 32 samples in terms of per cent oil, per cent nitrogen and varietal composition arranged in order of decreasing percentage of the Mandarin variety in the mixture. Broken line crossing bars of graph indicates theoretical percentage of oil and of nitrogen as calculated from the varietal composition.

The bar graphs show the deviation of each sample from the mean in varietal composition, percentage of oil, and percentage of nitrogen. A high percentage of Mandarin soybeans in the sample should cause the percentage of nitrogen to be higher than the mean and the percentage of oil to be lower than the mean. Statistical analysis of the results shows highly significant correlations between percentage nitrogen and percentage Mandarin (.899); between percentage oil and percentage Mandarin (—.837) and between percentages oil and nitrogen (—.823). These correlations indicate that more than two-thirds of the deviations in analyses may be attributed to known differences in sampling.

WHEN curves showing frequency distribution of percentage composition are plotted, a normal distribution curve would be expected with a peak at a composition of 50% of each variety since that is the composition of the original mixture sampled. This

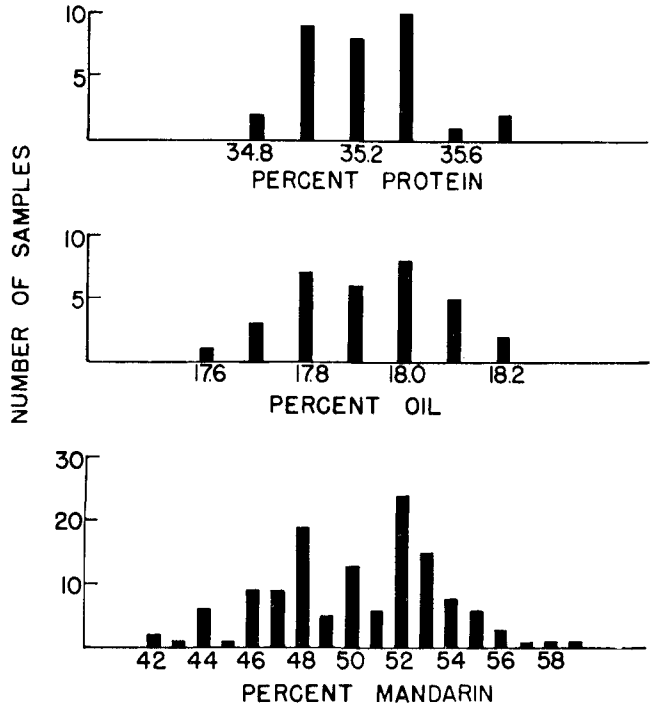

FIG. 3. Bar graphs showing frequency distribution of percentage composition of samples in a two-variety mixture of soybean seed.

was found to be true for samples of 120 grams or definitely bimodal as shown in Figure 3. The two larger, but in the case of smaller samples the curve is peaks are at one or two per cent on each side of the expected peak. When per cent oil and per cent protein as determined by chemical analysis are plotted in a similar way, bimodal curves are also obtained in agreement with the varietal percentage composition curves. The number of samples involved in the case of the chemical analyses is too small to make the form of these curves very significant. Samplings were repeated with two different Boerner samplers by three different operators with similar results. The cause of the bimodal form of the curves may be the basis of interesting speculation but is not within the scope of this investigation. A similar bimodal curve was found by Fisher and Halton (4) in a wheat sampling study. The fact that a sample of the same composition as the original mixture is obtained in only 13 cases out of 128 is important, particularly since percentages differing from it by about two per cent are more common.

In order to measure the variation in sampling within a variety a new 480-gram sample of soybeans was obtained with the sampler from the original bulk supply of each variety. Each of these varieties was divided with the Boerner sampler into 16 samples of

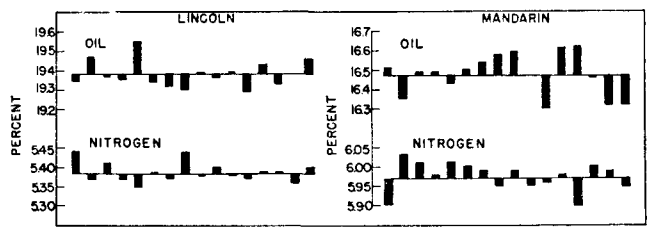

FIG. 4. Deviations from average composition of 16 small (30 gm.) aliquots from a single large sample—for two varieties of soybeans.

approximately 30 grams each, and each aliquot was analyzed for oil and nitrogen content. Figure 4 shows the random deviations from the mean in percentages of oil and nitrogen of the individual samplings.

Variations in these groups of analyses are much smaller than in the two-variety mixture samples, and deviations may be largely accounted for by variations in chemical analyses. There seems to be no accurate means of separating chemical errors from sampling differences in this case. Generally, the inverse correlation is quite high between oil and nitrogen content of a group of samples which differ appreciably in nitrogen and oil. If we assume that this is true for small variations in aliquots from a single lot of beans, we may use this correlation coefficient as a measure of agreement between oil and nitrogen analyses. The coefficient of correlation between oil and nitrogen analyses was —.377 for the Lincoln variety and —.244 for the Mandarin variety. This low correlation seems to indicate no appreciable error due to sampling difference when small lots of carefully selected soybeans of a single variety are sampled.

Conclusion

Under the conditions of this study, 30-gram samplings of mixtures of soybeans were found to differ significantly in oil and nitrogen content indicating the desirability of larger samples. The use of 120 to 240-gram aliquots from mixtures of soybeans which vary widely in chemical composition should tend to reduce differences due to sampling to a reasonable minimum. Differences among 30-gram samplings of highly uniform soybean seed of a single variety seem to be of slight significance. The limitations of present sampling methods should be recognized in any comparison or interpretation of chemical analyses of soybean seed.

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Changes Occurring in Fat Autoxidation[1, 2]

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Introduction

THE FACT that a rancid fat has a lower mean unsaturation and mean molecular weight, and lesser amounts of unsaturated fatty acids than a freshly refined fat has been known for some time. The impression seems to prevail that these characteristics cannot be used as criteria of the extent of oxidative rancidity. The basis for this impression is not readily apparent in the absence of rate studies on the decrease in iodine value, saponification equivalent, and the disappearance of specific unsaturated fatty acids. Therefore, it seemed desirable to study the rates of change of peroxide and iodine values, saponification equivalents, and the amounts of "linoleic and linolenic acids" during accelerated rancidification of a fat. An attempt has been made in the present investigation to correlate and interpret the experimental data in terms of existing theories concerning autoxidative deterioration.

Experimental

The substrates examined were commercial edible fats and oils purchased on the local market. Two of the samples were hydrogenated vegetable oil shortenings and the third was a refined cottonseed oil. The fatty acid compositions were such that the percentage of octadecadienoic acids ranged from 10 to 55% of the total fatty acids.

[1] The work described in this paper was carried out under a contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and the University of Pittsburgh. Contribution No. 563 from the Department of Chemistry, University of Pittsburgh, Pittsburgh, Pennsylvania.

The suggestions of Dr. B. F. Daubert in the preparation of this manuscript are gratefully acknowledged.

[2] Presented at the 35th Annual Meeting of the American Oil Chemists' Society, May 10 to 12, 1944, New Orleans, La.

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Method of Oxidation

The oxidation was carried out under the conditions of the modified Swift stability test (1). A large number of samples was oxidized in order to follow the chemical changes occurring over a relatively long period of time.

If an antioxidant or synergist was incorporated into the substrate, precautions were taken to secure a uniform dispersion. Samples were removed from the heated oil bath at definite time intervals and refrigerated until the entire series of samples from one experiment was available for analyses. The cooled samples were warmed until fluid and well agitated before weighed aliquots were removed for chemical analysis.

Analytical Constants

(a) Peroxide values (P.V.) were determined by a modified Wheeler method (2) and were expressed as milliequivalents of sodium thiosulfate per kilogram of fat.

(b) Iodine values (I.V.) were determined by the approved A.O.A.C. method, using Wijs reagent for a 30-minute reaction time.

(c) Saponification equivalents (S.E.) were determined by a modification of the approved A.O.A.C. method.

Spectrophotometric Determination of Unsaturated Fatty Acids

The amounts of diene and triene conjugated material originally present in the fat samples or induced in them during the course of the oxidation were determined spectrophotometrically in purified isooctane. The amount of diene and triene material present was calculated, using the $E^{1\%}_{1\,cm.}$ values (corrected to methyl esters) previously accepted and used