From the adsorption data no suitable theories could be postulated as to why one builder acts better than another. The beneficial effect of builders may be chiefly due to the nature of builder-detergent solution effects, *e.g.*, ability to prevent redeposition of soil. In this connection it is known that, at moderate concentrations, polyphosphates do not promote the deposition of soil onto cotton but that more alkaline builders do promote soil deposition.

Acknowledgment

Acknowledgment is made to Michael Galan and Leonard Markowitz, who performed most of the test work. The test was conducted under the supervision of Leonard Zoole and was sponsored by the Bureau of Ships, Department of the Navy, under the direction of Vincent L. Saitta.

Thee views expressed in this article are the author's and should not be construed as representing those of the Department of the Navy.

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The Phosphorus Content of Refined Soybean Oil as a Criterion of Quality

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DURING THE PAST TWO DECADES or more, ways for improving the flavor stability of soybean oil used for edible purposes have received much attention. However present procedures for refining crude soybean oil generally place major emphasis on the attainment of a minimum loss of oil and on a specified color for the finished product. The refiner varies the quantity and concentration of alkali solution used in the neutralization step in accordance with the characteristics of each lot of oil and thus determines to a large extent the resultant color reduction and oil loss. Although flavor stability of the refined oil is influenced by conditions employed in the neutralization step, a search of the literature revealed little information on this aspect. The Soybean Oil Committee of the Soybean Research Council for the National Soybean Processors' Association published a number of factors that affect flavor stability (9). One of the factors listed as favorable was the use of a slight excess of caustic. Mattikow stated that the phosphorus content of a refined oil should not exceed 0.5 part per million (6). Neither Mattikow nor the Soybean Oil Committee gave any data to support their conclusions. Sanders found a correlation over a restricted range between bleached color and stability where reduction in color favored stability (8).

Because of the lack of specific information on the subject, a study on a pilot-plant scale was undertaken to investigate the relationship between conditions employed in refining a crude oil and the stability of the finished oil. Both degummed and non-degummed crude oils were used in the tests. The data obtained in this study and the conclusions reached are presented here.

Small individual batches of oil taken from a large blended batch were refined with lyes ranging from 8° to 30° Baumé and with several amounts of sodium hydroxide varying from 0.05 to 0.30% in excess of the amount required to neutralize the free fatty acids, based on weight of the oil. Samples of the refined, bleached, and deodorized soybean oil produced by the refining treatments were analyzed for iron, tocopherol, phosphorus, and ash. The effect of refining conditions on the removal of these substances, and on the color and stability of the deodorized oil as measured by the Active Oxygen Method could then be determined. All of the oils were refined by the batch method.

Experimental Methods

Each refining test was conducted with 120 lbs. of oil. The oil was agitated vigorously at room temperature in a kettle while the alkali solution was slowly added, and mixing was continued for 15 min. After the speed of the agitator had been reduced to a low rate, the material was heated to 150°F.(65°C.) and pumped to a centrifuge. About 25 min. were required to complete the separation of the oil and soapstock. The refined oil was next washed with 15% of softened water, heated to 205° to 210°F.(95° to 98°C.), and centrifuged. The warm oil was dried by slowly drawing it back into the kettle under a vacuum of 28 to 29.5 in. of mercury, heating it to 210° to 215°F.(98° to 102° C.), and filtering it.

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[1] One of the Branches of the Agricultural Research Service, U. S. Department of Agriculture.

The refined oil was bleached with 1% of a 15:1 mixture of natural bleaching earth and activated carbon for 15 min. at 215° to 220°F.(102° to 107°C.). The bleached oil was deodorized for 4 hrs. at 425°F. (218°C.) and 6 mm. of mercury absolute pressure, using about 2% blowing steam per hour. The batch was cooled to 120°F.(49°C.) in the deodorizer before samples were taken. Except for a few minor items, all equipment in which the oil was treated was constructed of stainless steel. The deodorizer was cleaned with refluxing acetone after each deodorization (2). Operating conditions were as uniform as possible for each test.

Samples of the deodorized oil were aerated for 6 hrs. at 100°C. in an Active Oxygen Method Apparatus, and the peroxide values were determined. Stability evaluation of samples by organoleptic methods was not undertaken because of the number of samples involved, the limited facilities available, and the difficulty of translating evaluations by a taste panel to a broad numerical scale. However, reliability of the correlation between A.O.M. peroxide values (P.V.) and flavor stability for liquid soybean oil has been demonstrated repeatedly at this laboratory by taste panel evaluation of laboratory, pilot-plant, and commercial-plant samples (3, 4).

Oil colors were determined by the A.O.C.S. spectrophotometric method, using the Coleman Model 6B[2] spectrophotometer. This instrument was used also in all the colorimetric methods described later.

The ash content was determined by charring a 20-g. sample of oil contained in a 100-ml. beaker, on a hot plate, and ashing the residue in a muffle furnace at 450°C. until free of carbon. After the ash was weighed, it was dissolved in 2 ml. of hydrochloric acid for a determination of the iron content colorimetrically by the dipyridyl method (1).

Tocopherol was determined in the deodorized oil by the colorimetric method of Rawlings, Kuhrt, and Baxter (7). Phosphorus was determined by the reduced phosphomolybdate colorimetric method (10). Ten grams of oil and 1 ml. of a saturated solution of magnesium nitrate in ethanol were slowly heated in a 100-ml. beaker until the alcohol was evaporated. The oil was then charred on a hot plate and ashed in a muffle furnace for 8 hrs. at 500°C. or until all carbon disappeared. The ash was dissolved with 15 ml. of distilled water and 1 ml. of concentrated sulfuric acid, and the solution was diluted to 20 ml. with distilled water. An aliquot of this solution was mixed with the molybdate and stannous chloride reagents and was made up to a standard volume; the light transmission was read at 700 mμ. From results obtained with standard phosphate solutions the phosphorus content of the oil sample was calculated.

Results

Quality vs. Constituents. Several interesting relationships found between quality of the deodorized oil and concentration of minor constituents are expressed in Figures 1, 2, and 3. Two measurements of quality, oxidative stability, and color, have been plotted *versus* phosphorus content in Figures 1 and 3. The two minor constituents, iron and phosphorus, are plotted against each other in Figure 2. The scale for phosphorus has been made logarithmic in order to emphasize the relationship in the lower ranges. The points represent data obtained with two shipments of non-degummed soybean oil from a single commercial source, and from tests carried out on a batch of degummed oil prepared in the pilot plant from one of the non-degummed oils. Considering that varia-

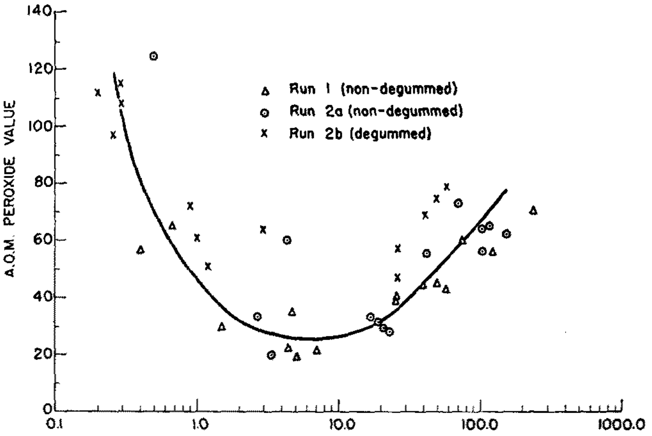

FIG. 1. Relationship between residual phosphorus content and oxidative stability of deodorized soybean oil.

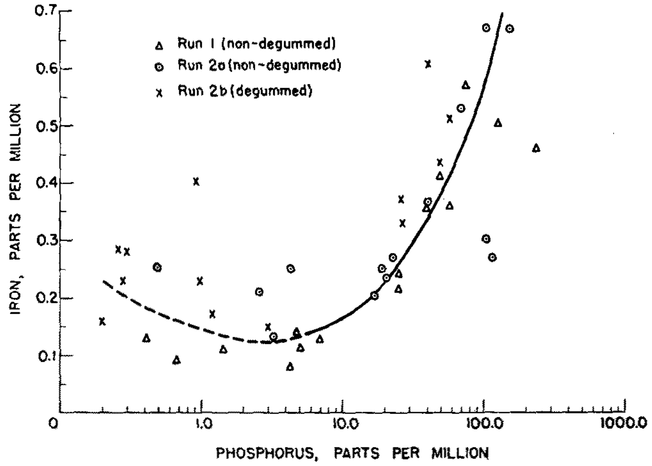

FIG. 2. Relation between residual phosphorus and residual iron contents of deodorized soybean oil.

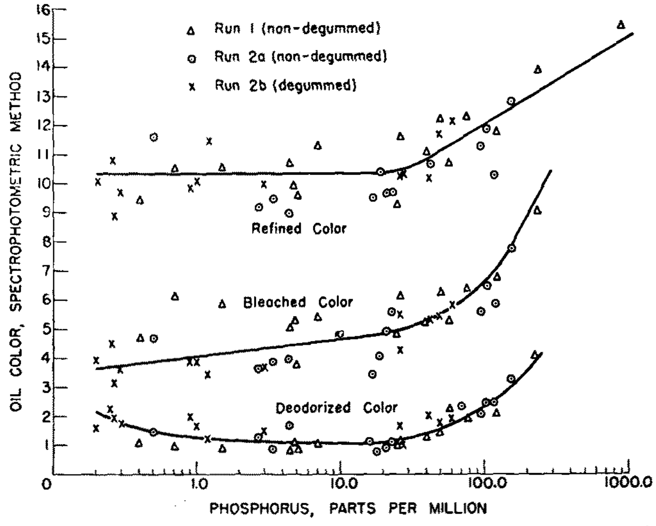

FIG. 3. Relation between residual phosphorus content of deodorized soybean oil and the spectrophotometric color of the refined, bleached, and deodorized oils.

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[2] The mention in this article of commercial equipment under names of their manufacturers does not constitute endorsement by the U. S. Department of Agriculture of such firms or products.

tions in processing were unavoidable, the data are believed to be quite consistent.

The similarity in shape of the curves representing the relation between phosphorus content of the deodorized oils and their stability, color, and iron content is obvious. However the implications to be derived from these relations may not be so manifest. At residual phosphorus contents greater than about 20 p.p.m. the stability decreased (P.V. increased), and the deodorized color increased more or less in proportion to the increase in phosphorus. While the correlation of color to phosphorus content in this range is perhaps to be expected, the drop in stability is less easily explained. In fact, as will be discussed later, the phosphorus should either contribute toward better stability or else have no effect, depending upon its chemical identity. When it is noted that the iron content rises in this range as the phosphorus content becomes greater, the decrease in stability can be rationalized. The fact is well known that small amounts of iron reduce the stability of an oil although the effect of the iron may be modified or reduced by the concomitant occurrence of phosphorus. Other undetermined factors may also be involved.

It has been demonstrated that pro-oxidant metals other than iron may be present in crude soybean oils (4). It is also possible for metallic pro-oxidants to exist in oils in both active and inactive forms (2). These facts should be borne in mind when the data are closely examined since failure of some points to fall near the curves may be due to such factors.

The interrelations of iron, phosphorus, color, and stability at phosphorus levels below 10 p.p.m. are somewhat more difficult to interpret. Here too it is likely that iron is the predominating factor. The anomaly in this case is the rise in iron content at extremely low phosphorus levels. There is some indication from data, which have not been included, that these oils increased in iron content after the refining stage. If this is true, then iron present in the deodorized oil, not being associated with adequate amounts of phosphorus and not being in the chemical state in which it occurred in the crude oil, probably is an extremely active catalyst for oxidation. No reason was found to explain why oils which have been refined to low phosphorus and iron levels should increase in iron content during the bleaching or deodorization steps. In consideration of the foregoing discussion, the rapid decrease in stability of oils with phosphorus contents of less than about 2 p.p.m. appears more reasonable.

Although the addition of 0.01% citric acid to the oil prior to deodorization increased the stability of all samples, limited tests indicated that the phosphorus level for maximum stability was the same when citric acid was employed as it was when this stabilizer was not used.

On close examination of the data it was found that the light absorption of deodorized oils, particularly at 460 mμ (not shown), correlates well with stability of the oil. This becomes evident if it is postulated that iron salts can be responsible for much of the color of the deodorized oils, especially when other pigments have been removed adequately. This postulate has been partially confirmed in the laboratory.

Phosphorous Removal

Since phosphorus-containing compounds constitute the bulk of impurities removed in the refining process and since some of the important minor constituents are removed at the same time, it is of interest to examine in detail the removal of phosphorus during refining.

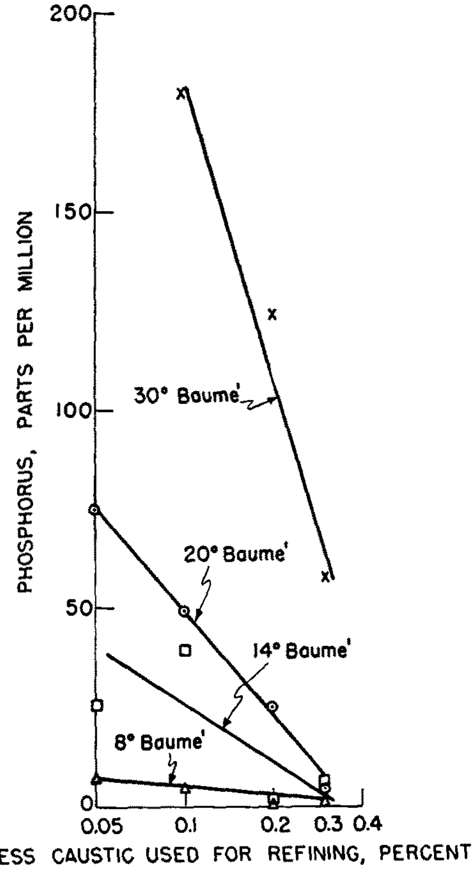

FIG. 4. Relation between residual phosphorus content of deodorized soybean oil and the strength and the amount of lye used for refining the oil.

In Figure 4 the phosphorus content of a deodorized oil is presented as a function of the amount of caustic used in the neutralization step. These experiments were made with one batch of non-degummed oil containing 880 p.p.m. of phosphorus. The resulting plot is a family of curves; weaker lyes and greater excesses of caustic removed more phosphorus. It will be noted that for a constant and comparatively small

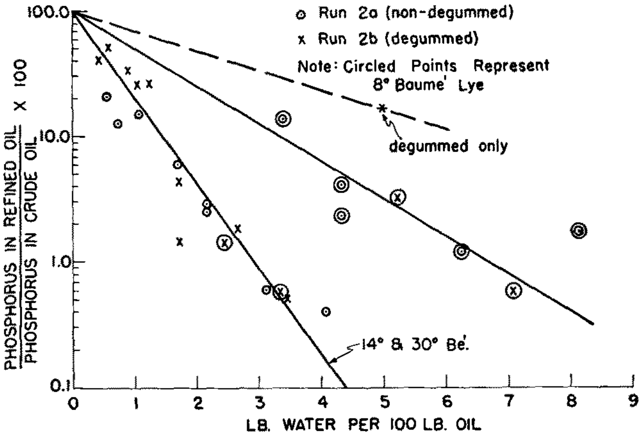

FIG. 5. Relation between the amount of water (from the lye) used for refining soybean oil and the percentage of original phosphorus remaining in the refined oil.

excess of alkali, such as 0.1%, there is less phosphorus remaining in the oil as more dilute lye is used. Higher excesses remove more phosphorus, but the differences in the degree of removal become smaller. This is true because, for lyes of constant strength, more water as well as more caustic are provided by the greater excess, and this additional water removes more phosphorus.

The effectiveness of water as an agent for the removal of phosphorus is shown in Figure 5. Here the ratio of phosphorus remaining in the refined oil to the phosphorus content of the crude oil, expressed as a percentage, is compared with the amount of water in the lye used in the neutralization step. The weight of sodium hydroxide has been disregarded. It is interesting to note that a family of three convergent lines was obtained. In one experiment a batch of non-degummed crude oil was treated with water alone, and the result is represented by the point "*" through which the upper, dashed line was drawn. The results obtained by use of 8° Bé lye are given by the middle line, with the exception of two points, and those for both 14° and 30° Bé by the lower line. It is apparent from a study of Figures 4 and 5 that the reduction in phosphorus content is determined largely by the amount of water present in the alkali solution used in the neutralization step and that the addition of sodium hydroxide to the water aids in removal of the phosphorus. It should be pointed out that equally good results might have been obtained by use of the 8° Bé lye as with 14° and 30° Bé lyes, but a highly emulsified oil-lye mixture apparently was produced, and a satisfactory separation of oil from the soapstock could not be made in the centrifuge. This naturally would be a disadvantage in operations on a commercial scale.

It will be noted that in Figure 5 two of the points for 8° lye fell on the lowest line, which represents the 14° and 30° concentrations. This is considered a significant observation since work with crude oils different from those reported here strongly indicates that 12° lyes almost invariably and 8° lyes often behave in a manner equal to the stronger lyes. Indeed it is suspected that, depending upon the nature of the phosphorus, water alone may sometimes prove to be sufficient for effective phosphorus removal.

A rather striking implication of these results is that the conventional basis for calculating the amount of caustic to be used in the neutralization step gives no indication of the conditions leading to optimum removal of phosphorus. In the case of domestic, crude soybean oil, where the amount of material containing phosphorus usually is several times the free fatty acid content, the distinction seems to be especially important.

It was observed that phosphatides may be removed from crude oils by treatment with hot water or by warm or even cold caustic solutions; however washing the refined oil with hot water has no further effect on phosphorus removal unless an incomplete separation of oil and soapstock was made in the primary centrifuge. This fact, which is borne out by laboratory centrifugation of samples from the pilot-plant, indicates that the chemical nature of the residual phosphorus in the refined oil may have been changed by the caustic treatment.

Figure 6 demonstrates the degree of removal of phosphorus during the bleaching of oils 2a and 2b. The data follow the Freundlich equation for adsorption. By use of Figure 6 the phosphorus values given in Figure 4 for oil 1 after it was refined, bleached, and deodorized can be translated to the phosphorus content of refined oil 1, assuming no change in phosphorus content during deodorization. The results will show that the same general relations given in Figure 5 for oils 2a and 2b will hold for oil 1. Analyses for phosphorus contents of the refined oils were not performed for oil 1.

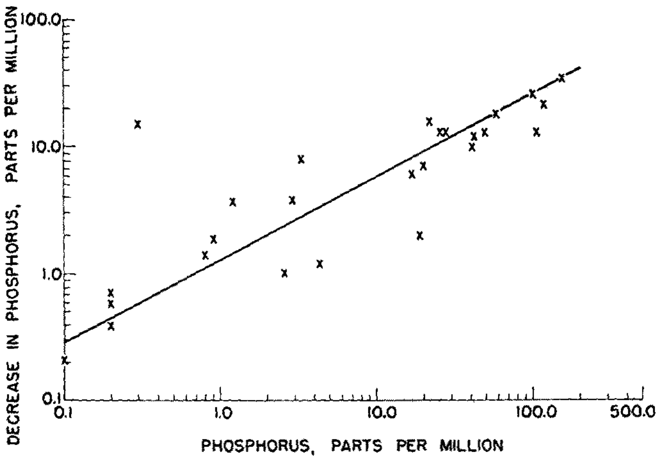

Fig. 6. Removal of phosphorus during bleaching. Phosphorus in bleached oil *versus* amount removed by bleaching.

Color Removal

In considering color reduction during refining, it is convenient to think of the many pigments which occur in crude soybean oil as being of three types: those which are largely removed during refining and which may be associated with iron or phosphorus or both, those remaining in the oil after refining which are adsorbable by bleaching clays, and those which change to a colorless form or are destroyed upon heating the oil to a high temperature during deodorization.

From the data plotted in Figure 3 it is seen that the amount of color at all stages of processing is related somewhat to phosphorus but that the influence of "phosphorus-associated" pigments on color of refined oil practically disappears below a phosphorus content of about 25 to 30 p.p.m., leaving only the influence of the other two types. It is possible that the conditions which were responsible for greatest phosphorus removal during bleaching were not those which produced optimum bleachable-color removal. Examination of the relationship of refined color to the amount of water and of caustic used in refining indicates that, at a constant percentage of water, the total amount of caustic used influences color removal; the more caustic used, the lower the color of the refined oil. The issue is somewhat obscured by the fact that part of the color removal can be connected with pigments of the first kind, which are removed by water, not by caustic.

During deodorization two color-change reactions can occur. Certain plant pigments are destroyed or removed as far as their effect on color is concerned, and phosphorus, in certain forms at least, can cause darkening of the oil. Since in a phosphorus range greater than 10 p.p.m. the curves for bleached and deodorized oils are parallel, the latter effect does not seem to be in evidence. As for the convergence of the curves at lower phosphorus levels, color seems to increase in opposition to heat bleaching, and, as has been previously mentioned, this seems to be due to or coincidental with the presence of iron.

Addition of Phosphatides

To bring the foregoing into stronger focus, results of a series of experiments with refined oil from a different crude oil are summarized in Figure 7. Here crude gums were added in varying amounts to several portions of a batch of refined oil. The resulting mixtures were then bleached and deodorized. A close relationship between phosphorus content of the deodorized oil and its color and stability is noted. The refined oil had a phosphorus content of about 0.35 p.p.m. so its original stability was of the order shown in Figure 1 for that level of phosphorus. As gums were added to the oil, its stability increased until, at 5 p.p.m. phosphorus, it was equal to those in Figure 1 at 5 p.p.m. Over this range the added phosphorus had little, if any, effect on deodorized color. At levels above 5 p.p.m. phosphorus the stability continued to increase while the color rose very sharply. At high phosphorus levels color of the deodorized oils exceeded that of the bleached oil and at very high levels, even that of the refined oil. From a comparison of Figures 3 and 7 it is evident that the behavior of the oil at phosphorus levels above 5 p.p.m., when the added phosphorus-containing compounds have not undergone treatment with lye, thus differs markedly from that of oils containing phosphorus-associated compounds which remain in the oil after refining. Phosphorus compounds in the form obtained by degumming are good stabilizers, but they cause extreme darkening of the deodorized oil when added at levels which give excellent stability. Phosphorus remaining in the refined oil may be in a form which behaves similarly to the sodium phosphates although these have been shown to have a more or less neutral effect on stability compared to phosphoric acid (5).

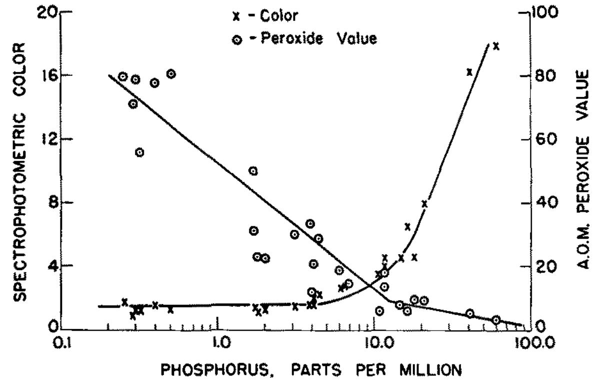

Fig. 7. Effect of phosphatide addition to refined soybean oil in color and stability of the deodorized oil.

Two other items should be mentioned briefly. Measurements of the ash content showed it to be very closely correlated with the phosphorus content (Figure 8). This observation has two implications. It suggests that phosphorus in a refined oil is present as a sodium salt and thus is not volatilized during the ashing step. Secondly, the association suggests that ash content can be used as a control method for estimating phosphorus, at least at the higher levels.

The remaining measurement carried out on these samples was the tocopherol content. As reported by Kuhrt *et al.*, little tocopherol was removed in the neutralization step (7). Affecting the amount removed was the quantity of caustic used. Weak lyes tended to remove less tocopherol. It is interesting to note that this is in direct contrast to the removal of phosphorus.

Discussion

The greatest difference in the response of three batches of soybean oil to a given refining treatment with lye was in the reduction of phosphorus, and even here there was no significant difference if the response is considered on the basis of the original phosphorus content. And while crude oils 1 and 2a differed in absorption at each of the wavelengths measured, the numerical value for color as calculated from these absorptions differed very little. The subtractive effect of 670 μ counterbalanced the additive effect at other wavelengths.

Regardless of the uncertainty which exists in accounting for the rise in iron content at low phosphorus levels and its apparent relation to increased color and decreased stability of the deodorized oils, these data make the concept of "over-refining" inescapable. The general phosphorus level for optimum results has a lower as well as an upper boundary and seems to be between 2 and 20 p.p.m. phosphorus. Exceeding the maximum value has more effect on color than on stability while falling below the minimum brings about serious impairment of stability and, to a lesser degree, an increase in color of the deodorized oil.

If the reduction in phosphorus content is determined largely by the amount of water used in the alkali solution, which it apparently is, and the relationship between phosphorus, iron, and color holds true, the refining treatment necessary to achieve a given quality level should be predictable for a given system. For example, if a residual phosphorus content of 5 p.p.m. is desired, the data shown in Figure 5 indicate that with a non-degummed oil having an original phosphorus content of 1,000 about 3 lbs. of water per 100 lbs. of oil should be used whereas with a degummed oil containing 100 p.p.m. of phosphorus only about 1.7 lbs. are needed. As long as the lye used is 14° Bé or stronger, the deodorized oils would be equal in quality. With weaker lyes, larger amounts

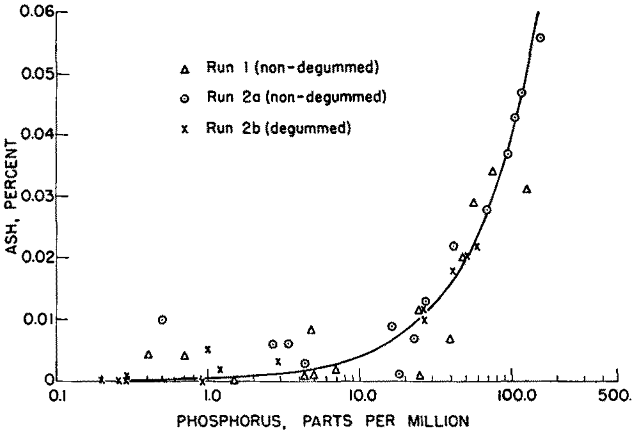

Fig. 8. Relation between phosphorus and ash contents of deodorized soybean oil.

of water but smaller amounts of sodium hydroxide would be required to obtain the same results. If the two oils had the same initial free fatty acid content and were as a consequence refined in the same manner, say, using 3 lbs. of water per 100 lbs. of oil, the degummed oil would be expected to be lower in quality. The resulting "over-refining" would have reduced its residual phosphorus to 0.5 p.p.m.

A study of the data for the light absorption at 465, 550, 620, and 670 mμ indicates that the higher strengths should remove more of some pigments than of others, but it is possible, as in this case, that the net effect on the official spectrophotometric color could not be observed. This is expected to be true especially with "green" oils which show high initial absorption at 670 mμ. The higher strengths would also remove more tocopherol, slightly reducing the stability.

Summary

Restricted tests in pilot-plant equipment have indicated that, starting with good quality crude soybean oil, the phosphorus level of the deodorized oil is closely associated with its color and oxidative stability but that the refined or bleached color is not a good criterion for predicting the quality of the finished oil. Phosphorus is removed by water in the presence of adequate concentrations of alkali, and optimum oil quality is achieved at phosphorus levels in the deodorized oil of no less than about 2 and no greater than about 20 p.p.m. Over-refining to a lower phosphorus content, by the use of too great an excess of caustic (and therefore of water) was harmful to oxidative stability in every instance and generally increased the color of the deodorized oil. The ash analysis of the deodorized oil is a fair indication of its residual phosphorus content at levels above 20 p.p.m.

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Fat, Cholesterol, and Atherosclerosis[1]

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THE TRUTH ABOUT FATS in the diet is of tremendous importance to the edible fat and oil industry of the United States. Considerable publicity has been directed to a potential danger from fats in human food which relates to cholesterol content, to meat fats *versus* vegetable oils, and to various and sundry other allegations. It is our purpose in this paper to review and discuss known and published facts about fats in metabolism and nutrition. We believe that present research in the field of fat metabolism, particularly as it relates to such abnormalities as atherosclerosis, is not only quite inadequate but wholly contradictory and confusing. Much more research is vitally needed before the truth can be definitely established. A large part of this research is now being sponsored and financed by the food industry. What are some of the facts concerning fat and cholesterol in relation to atherosclerosis?

Cholesterol

Cholesterol is a solid, fat-like monoatomic alcohol with a cyclic structure containing the cyclopentanophenanthrene ring. The word cholesterol is derived from the Greek and means "solid bile," referring to its physical character and its source. Cholesterol is found in all animal cells and is particularly abundant in fat, brain, spinal cord, bile, milk, egg yolk, liver, kidney, and adrenal. Since it is a universal constituent of all animal cells, cholesterol occurs in all foods of animal origin.

The cholesterol present in the blood is distributed between the plasma and the cells in about equal amounts. Normally the serum cholesterol level in man is between 130 and 220 mg. per cent. This level is not altered appreciably by feeding 2 to 10 g. of cholesterol, either in crystalline form or as cholesterol-rich food (4, 65, 67, 68, 69, 70). The blood cholesterol level is fairly constant for continued cholesterol intakes up to 700 mg. per day but can be increased with diets very rich in fats and in such conditions as pregnancy, alcoholism, icterus, nephritis, and diabetes. In 1950 London and Rittenberg (1) studied the regeneration rate of cholesterol in man with the aid of deuterium-labeled cholesterol and found it to be 50% in eight days. It has been estimated therefore that cholesterol synthesis in man is at the rate of 1.5 to 2 g. per day (2).

Cholesterol and phospholipid circulate in blood plasma as combination products with protein. These lipoproteins account for 90% of the total plasma lipid and consist of free cholesterol, cholesterol esters, neutral fat, phospholipids, and protein. There are two types of lipoproteins, α and β, depending on whether the conjugated protein is α- or β-globulin.

The recent work by Gofman and others (3) has shown that these plasma lipoproteins differ in size and density. They have different sedimentation and flotation rates and may therefore be separated into classes according to the speed of the analytical ultracentrifuge that is required for their migration. By using solvents of high density, the less dense β-lipoproteins can be made to float in the centrifugal field. Thus, at a specified density of solvent (usually 1.063

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[1] Presented at the 47th Annual Meeting, American Oil Chemists' Society, Houston, Tex., April 23–25, 1956.