generalization about the absence of thermal polymers in commercial potato chip frying oils.

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Alcoholic Extraction of Vegetable Oils. V. Pilot Plant Extraction of Cottonseed by Aqueous Ethanol[1]

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IT HAS BEEN POINTED OUT previously (10) by the authors that ethanol, because of its availability and low price relative to imported petroleum solvents, would be an attractive solvent for vegetable oils in Asiatic countries if it could be shown to have proper solvent properties. Data by the authors (8, 9, 10) and others (11, 12) have shown that ethanol is a good solvent at near boiling temperature for commonly used vegetable oils such as corn, cottonseed, peanut, sesame, and soybean.

The studies in this paper deal primarily with the pilot plant extraction of cottonseed meats by ethanol. Preliminary studies of the rate of extraction of cottonseed flakes of three different moisture contents by four concentrations of aqueous ethanol at three different temperatures over extraction periods from 10 to 100 minutes were made. The results of these studies were considered sufficiently favorable to justify pilot plant studies.

Extraction Rate Studies

Apparatus. The extraction rate apparatus (Figure 1) consisted of three major parts: a 3-liter round bottom flask serving as the solvent tank; a 16-in. straight tube condenser as a solvent preheater; and the extractor. The latter was a glass tube 1 in. in diameter, 6 in. in height, with a jacketed section through which water was circulated from a constant temperature bath. Water from the same supply was used to control accurately the temperature of the solvent.

Procedure. Prime cottonseeds were dehulled by passing the seed through a pair of corrugated rolls and separating the loosened hulls by screening. The resulting meats were adjusted to a moisture content of 10% and flaked on a set of laboratory flaking rolls.

A weighed sample of cottonseed flakes (about 15 g.), having a thickness of 0.013 in., was added to the extractor and heated to the desired temperature by circulating water from the heated water bath. At the same time heated solvent was allowed to flow into the extractor at a controlled rate. The starting time was taken as the first drop of miscella flowed from the extraction tube. The miscella was collected in 100-ml. graduated cylinders at the rate of 10 ml. per minute, transferred into tared flasks, and evaporated free of solvent on a water bath. At the end of the extraction experiment the remaining liquid was drained from the extractor, and the flake sample was transferred to a Soxhlet extractor where the residual

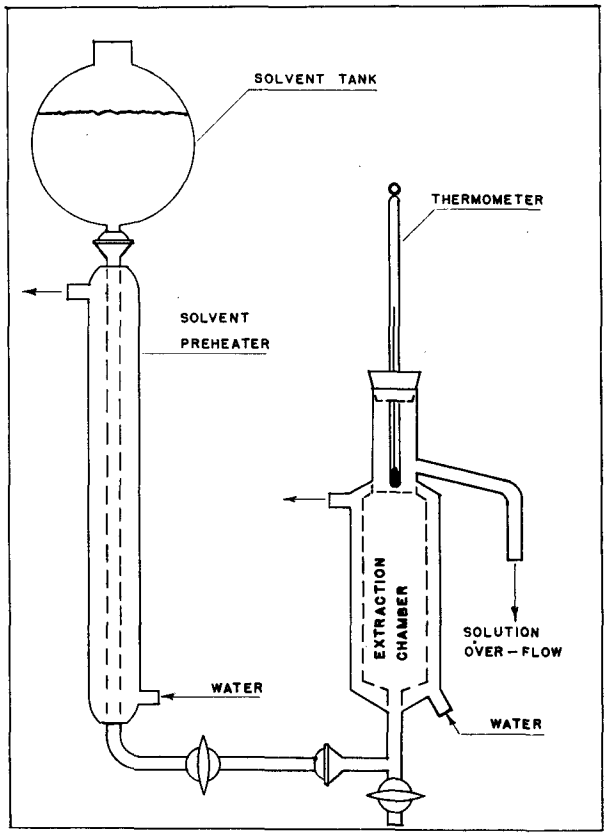

FIG. 1. Extraction rate apparatus.

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oil was removed by using the drained liquid as part of the extracting solvent.

The rate of extraction was determined from the total extractables, and the amounts were extracted during each time interval.

Results. The results are shown in Figures 2 to 6. The effect of the ethanol concentration on the extraction of cottonseed flakes containing 6.97% moisture for three different temperatures is shown in Figures 2, 3, and 4. Similar curves were obtained by plotting the data for flakes of other moisture contents. All the rate extraction curves follow the same general pattern. For the first 15 to 20 min. there is a rapid extraction rate period, followed by a slow extraction period in the last 80 min. Similar curves have been reported for cottonseed and other oils with other solvents. The best extraction was always obtained with the highest concentration of ethanol.

Increase in temperature on extraction with 99.9% ethanol (Figure 5) increases the extraction rate. The effect of moisture (Figure 6) is similar to that found by Arnold and Patel (3), using hexane as a solvent.

By using 99.9% ethanol at 78.3°C. with an extraction time of 90 min., it was possible to reduce the residual oil to less than 1.0%.

Pilot Plant Studies

Apparatus. The continuous countercurrent pilot plant, with minor changes, was that used in earlier studies (2, 4) with other solvents.

Procedure. The following variables were studied:

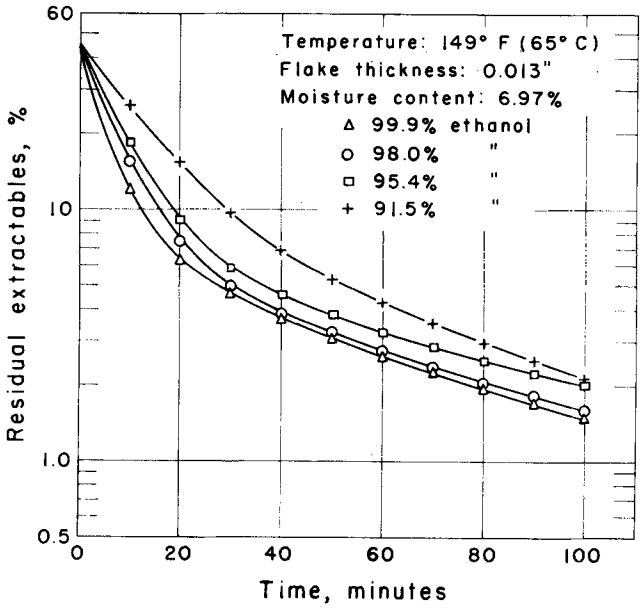

FIG. 2. Extraction rate curves for cottonseed flakes with 6.97% moisture at 65°C.

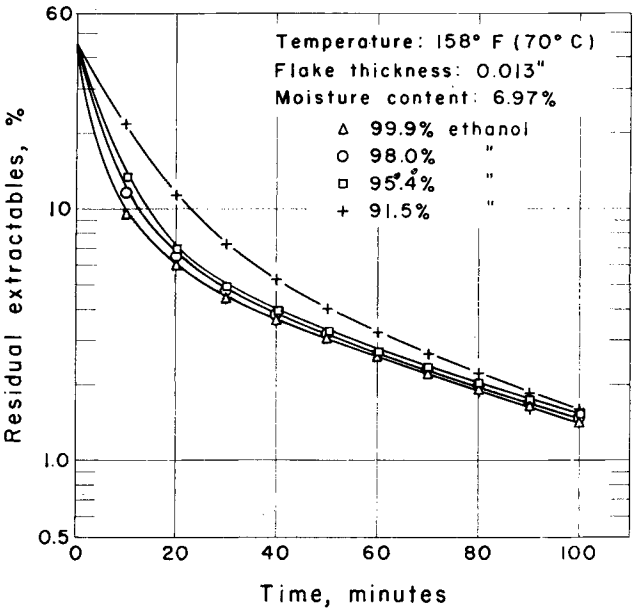

FIG. 3. Extraction rate curves for cottonseed flakes with 6.97% moisture at 70°C.

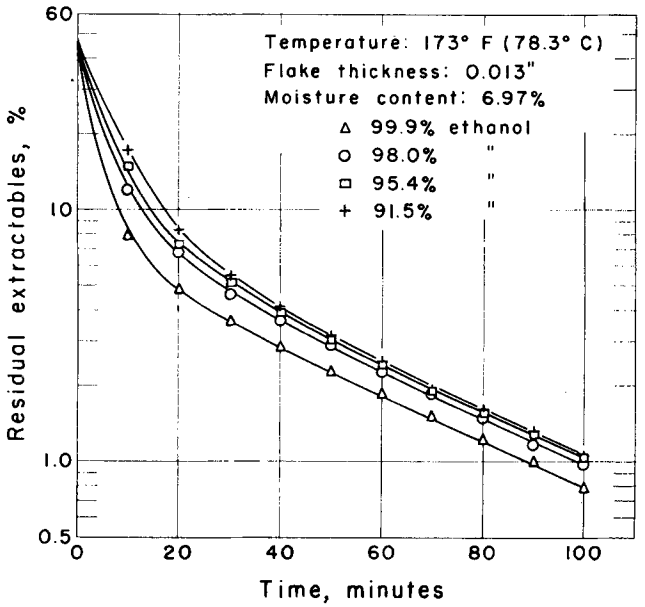

FIG. 4. Extraction rate curves for cottonseed flakes with 6.97% moisture at 78.3°C.

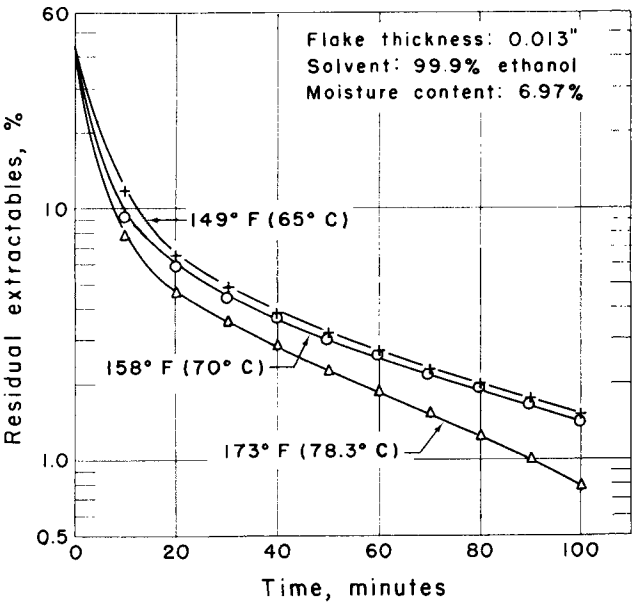

FIG. 5. Extraction rate curves for cottonseed flakes showing the effect of temperature on residual extractables.

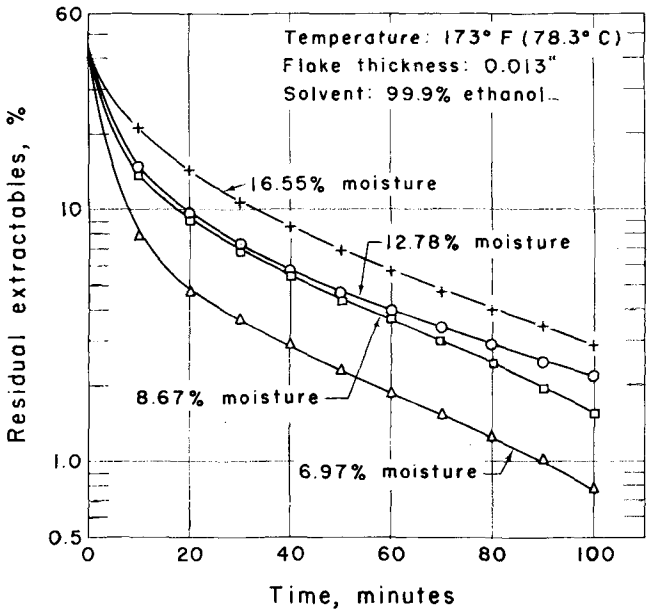

FIG. 6. Extraction rate curves for cottonseed flakes showing the effect of moisture content on the residual extractables.

extraction temperature, extraction time, flake moisture, aqueous ethanol concentration, and solvent-to-feed ratio. The general operational procedure for all runs consisted essentially of three steps: preparation of flakes, extraction, and analysis of products. The flakes were prepared by the same general procedure used for the rate extraction studies.

Before the start of actual extraction, a warm-up period was required during which the desolventizers and extractor loop were brought to the desired operating temperature. The extractor conveyor was then started, followed by the feed and solvent. The feed rate of the flakes was measured by recording the weight of flakes fed in each 20-min. interval. The solvent rate was determined from the rotameter reading. Approximately two hours after flake feeding started, the unit reached steady state conditions, and at this time two gallons of miscella sample and two samples of meal were collected. The length of each run varied with the extraction time used. After completion of the run the unit was emptied and made ready for the next run. The products obtained in each run were analyzed by official A.O.C.S. methods (1). The results of the pilot plant extractions are shown in Tables I to III.

Results. The data for Runs 1 to 6 in Table I as well as those from the laboratory extraction rate studies indicate, as would be expected, that an increase in extraction temperature increases the extraction rate. Since the best extraction was obtained at 172°F., subsequent extractions were run at this temperature. The increase in oil extracted with increasing extraction time is shown in Runs 10 to 13, Table II, and Runs 22 to 25, Table III.

TABLE I
Results of Extraction of Cottonseed with 91.5% Ethanol[a]

Run No.	Moisture content of flakes	Average extraction temperature	Solvent-to-feed ratio	Extraction time	Residual oil[b]
	%	°F.		min.	%
1	10.6	150	2	50	20.10
2	10.6	160	2	50	17.89
3	10.6	172	2	50	15.07
4	2.3	150	3	75	14.71
5	2.3	160	3	75	12.18
6	2.3	172	3	75	10.78
7	1.8	172	3	75	9.61
8	1.8	172	4	75	9.12
9	1.8	172	5	75	8.32

[a] Data common to all runs: average flake thickness = 0.013 in.
[b] Residual oil calculated on moisture-free basis.

TABLE II
Results of Extraction of Cottonseed with 95.4% Ethanol[a]

Run No.	Moisture content of flakes	Solvent-to-feed ratio	Extraction time	Residual oil[b]
	%		min.	%
10	6.8	4	50	5.95
11	6.8	4	75	4.50
12	3.7	4	50	4.85
13	3.7	4	75	3.65
14	3.7	5	75	3.46
15	2.3	4	75	2.53
16	2.3	5	75	1.90
17	2.3	4	75	2.71
18	1.8	2	75	1.98
19	1.8	3	75	1.54
20	1.8	4	75	1.48
21	1.8	5	75	1.42

[a] Data common to all runs: average flake thickness = 0.013 in; average extraction temperature = 172°F.
[b] Residual oil calculated on a moisture-free basis.

The effect of variation of residual oil content with varying moisture contents and solvent-to-feed ratios is shown in Table I for 91.5% ethanol, Table II for 95.4% ethanol, and Table III for 99.9% ethanol. The residual oil decreased with the decrease in moisture content of the flakes and with the increase in ethanol concentration. The decrease in the residual oil resulting from the increase in solvent-feed ratio is shown in Runs 18 to 21, Table II, and Runs 33 to 36, Table III.

TABLE III
Results of Extraction of Cottonseed with 99.9% Ethanol[a]

Run No.	Moisture content of flakes	Solvent-to-feed ratio	Extraction time	Residual oil[b]
	%		min.	%
22	10.4	2	25	14.10
23	10.4	2	50	7.75
24	6.8	2	50	6.64
25	6.8	2	75	5.57
26	6.8	4	75	3.95
27	3.7	3	50	5.10
28	3.7	4	75	3.02
29	3.7	5	75	2.84
30	2.3	3	75	2.64
31	2.3	4	75	2.35
32	2.3	5	75	2.16
33	1.8	2	75	1.76
34	1.8	3	75	1.36
35	1.8	4	75	1.28
36	1.8	5	75	1.25

[a] Data common to all runs: average flake thickness = 0.013 in.; average extraction temperature = 171 to 172°F.
[b] Residual oil calculated on a moisture-free basis.

When the miscellas from eight of the better extractions were cooled from the extraction temperature to room temperature, they each separated into two layers, one having from 2.7 to 3.3% oil and the other 91.1 to 92.9% oil. The free fatty acid content of the oils from these ran from 0.71 to 0.74%, refining loss from 7.3 to 7.4%, and refined oil color (photometric) from 5.2 to 5.4. The free gossypol content of these oils ranged from 0.064 to 0.118%. The gossypol in the corresponding meal samples was 0.0002%.

Discussion

Extraction rate studies are normally determined in this laboratory as a guide to the planning for pos-

sible pilot plant extraction studies. Experience has shown that there is no exact correlation between extraction rates and pilot plant results probably because the latter are obtained by countercurrent extraction in contrast to the fresh solvent extraction used in the former. Moreover in the current studies an added difference is introduced by the use of ethanol in the Soxhlet determination of the residual extractables in the extraction rate studies and hexane in the pilot plant studies. However the extraction rate studies show the general effect of flake moisture, extraction temperature, extraction time, and ethanol concentration. The general effects of temperature and extraction time in both the extraction rate and pilot plant studies are those shown in other extraction studies in this and other laboratories, using other solvents. This also was true of the effect of solvent-feed ratio in the pilot plant extraction.

The two variables having the most effect were moisture and ethanol concentration. For example, a comparison of data for Runs 3 and 23 at approximately 10.5% moisture shows a decrease in residual oil content from 15.1 to 7.8% as the ethanol concentration increased from 91.5 to 99.9%. When the flakes containing 1.8% moisture were extracted, the residual oil contents using 91.5, 95.4, and 99.9% ethanol were 9.6, 1.5, and 1.3%, respectively. When flakes containing higher moisture contents, such as 10%, are extracted with 95.4 or 99.9% ethanol, both oil and water would be extracted from the flakes, thus resulting in a decreasing water content in the flake mass. Thus water content of the ethanol increases so that extraction is being carried out by aqueous solutions of different concentrations across the extractor. While it is difficult to separate the direct moisture effects, it is obvious that the best extraction was obtained with flakes having a low moisture content.

One noticeable point is that after the flakes are dried to below 3%, 95.4% and 99.9% ethanol appear to be almost equally effective as solvents while 91.5% ethanol is definitely less effective. The results obtained for extraction runs with flakes of 1.8% moisture content are shown in Runs 18 to 21, Table II, and Runs 33 to 36, Table III. The maximum difference in the amount extracted in comparable runs is of the order of 0.20%. It is therefore possible to use 95.4% ethanol, which is more easily obtainable by distilling the ethanol layer of the miscella, with a sacrifice of 0.20% oil, than using absolute ethanol, which is expensive and not obtainable from the miscella without going to the complicated process of azeotropic distillation.

Commercial operation using the same type of equipment as the pilot plant would follow the flow sheet in Figure 7.

The ethanol-rich miscella fraction plus the ethanol recovered from the desolventization of the oil and meal and any necessary make-up would be used as the solvent. The use of the ethanol-rich miscella should give satisfactory extraction in view of the results obtained by Beckel (5) with soybean oil. It has been shown by Othmer and Agarwal (7), using hexane for the extraction of soybean oil, that dilute miscellas can be used effectively for extraction. It is probable that there would be a build up of alcohol-soluble nonglyceride materials in the dilute miscella. While it is not expected that this would cause difficulty, this possibility needs experimental checking. The ethanol

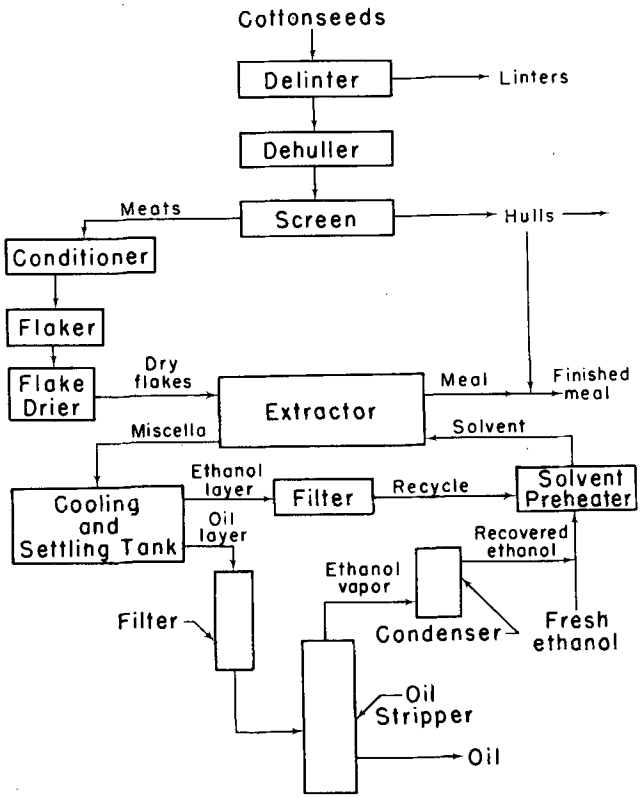

FIG. 7. Flow diagram for the ethanol extraction of cottonseed.

recovered from the oil and meal could be run into the extractor at a point above the point of entrance of the dilute miscella so as to wash the extracted flakes free from surface miscella and reduce to a minimum the oil deposited on their surface as the solvent is evaporated.

The optimum conditions for extraction must be determined as in any extraction process from the costs as affected by such factors as extraction time, flake drying, and solvent recovery in relation to the value of the oil and meal produced. It is believed that conditions in Run 19 are probably optimum for profitable operation. While the residual oil in the meal could be reduced somewhat by the use of 99.9% ethanol, this would involve higher solvent recovery costs than justified by the small increase in oil yield. The use of flakes containing only 1.8% moisture not only will assure good oil extraction but will prevent any dilution of the alcohol. In commercial operation it should be possible to produce thinner flakes than those produced experimentally, and this should result in somewhat better extraction.

The free fatty acid content, refining loss, and color of the oil are within the limits for a prime oil (6). The low gossypol content, 0.0002%, of the meal should make a preferred meal for feeding. Since it is not necessary to heat the meal to destroy the gossypol, heat damage to the protein could be limited to that used in the extraction. The possible coagulating effect of the alcohol on the protein needs study.

Summary

Cottonseed flakes were extracted by aqueous ethanol in a countercurrent pilot plant unit to determine the effect of operating variables and the optimum operating conditions.

This investigation has shown that direct extraction of cottonseed, using aqueous ethanol as a solvent, is a feasible process in the type of equipment developed previously in this laboratory. The optimum operating conditions for the ethanol extraction of cottonseed have been established.

The pilot plant extractions have shown that in this process a prime quality of crude oil and light-colored meal of good quality, with negligible free gossypol content, are obtained.

Acknowledgments

Thanks are due to the Southern Utilization Research and Development Division, U. S. Department of Agriculture, for the standard gossypol, to Swift and Company for the cottonseed used in the extraction rate studies, and to Southern Cottonseed Oil Company for the cottonseed used in the pilot plant study.

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A Modified Indophenol-Xylene Extraction Method for the Determination of Ascorbic Acid in Soybeans[1]

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RAPID DECOLORIZATION of the dye, 2,6-dichlorophenolindophenol, to the leuco form is the basis of several methods used to measure ascorbic acid in extracts of food and other materials (6, 15), including germinating soybeans (3, 23, 24). Decolorization of the dye by sulfhydryl groups is generally presumed to occur at a much slower rate than by ascorbic acid (6, 15). However interference of certain sulfhydryl compounds in the indophenol estimation of ascorbic acid appears to depend to a great extent upon the relative concentration of the sulfhydryl components (20) and may possibly be affected by the hydrogen ion concentration (1).

When the indophenol-xylene extraction method, using the formaldehyde treatment (15), was adapted by the authors to measure ascorbic acid in untoasted solvent-extracted soybean oil meal, possible interference resulting from sulfhydryl groups in the meal (11, 13, 20) was suspected. Blocking of these groups was achieved with *p*-chloromercuribenzoic acid (*p*- CMB), previously utilized in estimating the sulfhydryl content of soybean meal (13). Analysis for ascorbic acid in the absence of sulfhydryl groups verified their interference and the absence of ascorbic acid in the meal (13).

Results of studies in which *p*-CMB suppressed completely the ability of hydrogen sulfide, cysteine, and glutathione to decolorize the dye were reported by Owen (18) during the time this reagent was being investigated by the authors to eliminate sulfhydryl interference in ascorbic acid analysis of soybean meals. Owen recommended that *p*-CMB be used in indophenol-ascorbic acid analysis of biological materials containing sulfhydryl compounds and subsequently used *p*-CMB in the ascorbic acid analysis of such materials as human plasma, erythrocytes, and urine (19).

Since sulfhydryl groups of the soybean meal interfered in the indophenol method for ascorbic acid, these might be suspected of affecting ascorbic acid determination in the germinating beans. Instead of reported increases in ascorbic acid during germination, as determined by indophenol methods (3, 23), these values may stem from interference of an increasing sulfhydryl content (21).

Data reported here were obtained to emphasize the need for, and the applicability of, a modified indophenol-xylene extraction method to eliminate sulfhydryl interference in the ascorbic acid analysis of soybeans.

Experimental

Hawkeye soybeans, harvested in 1955 and stored at 4°C., were culled, and the choice beans were soaked in a solution of filtered calcium hypochlorite (5 g. in 150 ml. of water) to inhibit mold growth (3, 24). The beans were washed thoroughly with water and placed between multiple layers of moistened cheesecloth in a glass tray. Germination proceeded in the dark at 23°–25°C. Sprinkling the contents of the tray with water several times a day helped to maintain adequate moisture contact with the beans.

After sprouting, the beans were transferred to a Waring Blendor [2] bowl and covered with 2% metaphosphoric acid. Nitrogen was introduced prior to and during the grinding operation by means of a glass tube passing through the bowl cover and extending below the surface of the acid. The slurry was diluted to volume with additional metaphosphoric acid and centrifuged under nitrogen in capped bottles.

The Waring Blendor was not satisfactory for processing whole ungerminated soybeans. Whole beans in 20-g. lots were ground under metaphosphoric acid, using a Vir-Tis "45" homogenizer and a heavy-walled specially constructed flask adapted with a side-arm nitrogen inlet. The beans were first soaked in filtered

[2] Reference to commercial equipment in this publication is not intended to be a recommendation of this equipment by the U. S. Department of Agriculture over others not mentioned.