The Storage of Cottonseed

II. The Effect of Ammonia Treatment On the Free Fatty Acids and Color of the Seed Oil, and On the Rate and Degree of Heating of the Seed

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Introduction

In a previous publication (2), it was shown that when the pH of cottonseed is raised to 8 by treatment with ammonia, the rate of formation of free fatty acids from oil in the seed during subsequent prolonged storage is effectively reduced. It was found, for example, that when prime cottonseed was moistened to raise its moisture content from 11 to 17 percent, treated with ammonia gas, and stored for 100 days along with untreated seed, the ammonia-treated seed developed far less free fatty acids than did the untreated seed. It was also found that the colors of the crude oils, obtained by solvent extraction, were significantly lighter in the case of the ammonia-treated seed compared with the untreated seed.

In actual practice, however, the conditions under which cottonseed becomes moist are not as well defined as in the above-mentioned experiments. There are many opportunities for the seed to become moist in the period between the opening of the bolls on the plant and the arrival of the seed at the oil mill. The moisture content of the seed of freshly opened cotton bolls is approximately 50 percent. It is only after the open bolls have dried in the sun for about a week that the moisture content of the seed decreases to the average normal value for prime cottonseed. Appreciable rainfall during this period may delay the drying process considerably. If the seed cotton is harvested before this drying process is completed, the moisture content of the seed will, of course, be high.

Another opportunity for the development of high moisture in the seed results from the fact that it is not always possible to harvest cotton at the most favorable stage of ripening. Frequently, cotton will remain in the field several days or weeks after it has dried and is ready for picking. Excessive rainfall during this period will cause the moisture content of relatively dry seed to rise again. An excellent example of the effect of rainfall on the moisture content of cottonseed after the opening of the boll is cited by Simpson (4). In this example, the cottonseed from freshly opened bolls had a moisture content of 52 percent. After six days of dry weather, the moisture content of the seed dropped to 11 percent. At this point there was a heavy rainfall which resulted in a rise of the moisture content of the seed to 34 percent within the next two days. It is apparent, therefore, that climatic factors, combined with delays resulting from labor shortage and transportation difficulties during the harvest season, can be responsible for the production of various types of cottonseed of high moisture content.

There is no *a priori* reason to believe that all seed with the same moisture content will behave similarly during storage. As a matter of fact, the evidence summarized in the previous publication (2), indicates that the history of the seed prior to storage plays an important role in determining its behavior during storage. For a complete understanding of the behavior of cottonseed in storage it is not sufficient, therefore, to determine the storage properties of artificially moistened seed. Seed must also be investigated which has become moist under natural field conditions and at various stages of growth and development.

Because of the bearing of the results of this investigation on oil-mill storage practice, the seed used in these experiments was obtained directly from oil mills and was representative of the type of moist cottonseed which may be received in appreciable volume at the oil mills during the course of an average crushing season. Two series of experiments were carried out with this type of seed. Small lots of seed were used in the first series, while in the second series much larger quantities of cottonseed were employed in order to determine the effect of ammonia treatment on the heating of the seed.

Analytical Methods

The methods used for the determination of the moisture content and pH of the seed, and percentage of free fatty acids in the seed oil, were described in the previous publication (2). The moisture content is expressed on the basis of the dry weight of the seed and the percentage of free fatty acids is given in terms of percentage of oleic acid. A Coleman double monochromator spectrophotometer with an absorption cell thickness of 13 mm. was used for all spectrophotometric measurements.

The spectrophotometric measurements were made on solutions of the oils in carbon tetrachloride which had been purified by washing successively with concentrated sulfuric acid, water, concentrated potassium hydroxide, and water, and then distilling (5). The dilution most frequently employed was one ml. of oil in a total volume of 50 ml. of solution. Whenever a dark oil was investigated, a much higher dilution was necessary in order to obtain spectrophotometric readings of maximum accuracy. It was occasionally necessary to use several different dilutions during the analysis of the same oil, as for example when wide variations occurred in light absorption in the different spectral regions. Repeated tests have shown that Beer's law is applicable to the pigments in cottonseed oil, thus making it possible to relate all dilutions used to one standard dilution without affecting the quantitative nature of the results. All absorption spectra data are reported in terms of the relative extinction coefficient which is related to the other spectrometric constants as shown below is the familiar form of the Beer-Lambert law:[1]

$$(1)\quad E = \frac{\log_{10} \frac{I_0}{I}}{c\,l}$$

where E is the relative extinction coefficient,
I_0 is the intensity of light transmitted through the solvent,
I is the intensity of light transmitted through the solution,
c is the concentration of the oil (one ml. of oil in 50 ml. of solution),
and l is the length of the cell (13 mm.).

All of the spectrophotometric analyses are presented in terms of E, Equation (1), and refer to a solution containing one ml. of oil in a total volume of 50 ml.

Formation of Free Fatty Acids and Heating of Seed During Storage

Experiment 1. A five-pound sample of moist cottonseed, obtained from a cotton oil mill, was divided into three equal parts. One portion was stored as received, in an air-tight glass jar at room temperature. Another portion was dried in vacuum desiccators over calcium chloride at room temperature until the moisture content had decreased to 15 percent and was then stored in a glass jar in the manner described for the first portion. A third portion was treated with ammonia gas in a closed chamber until the pH of the seed increased to 8.2. The excess ammonia was removed by aeration and the seed was then stored in a closed glass jar. The analytical data with reference to all three samples of the seed are given in Table 1.

TABLE 1
Effect of Ammonia Treatment on Formation of Free Fatty Acids in Stored Cottonseed

Treatment	Moisture content	pH	Free fatty acids content		
			Original	After 100 days storage	After 180 days storage
	percent		*percent*	*percent*	*percent*
Untreated	18.6	6.4	2.0	9.0	34.5
Dried at room temp.	15.3	6.5	2.0	4.0	5.2
Ammonia-treated	17.5	8.2	2.0	3.8	4.8

The untreated seed deteriorated rapidly and was quite moldy after storage for 100 days. Drying resulted in considerable improvement in the storage properties but the greatest inhibition of free fatty acid formation was obtained when the seed was treated with ammonia.

Experiment 2. One ton of moist cottonseed was obtained from a carload of seed as it was being unloaded at a cotton mill. This seed, which was very moist and had already heated to 50°C., was taken directly to the laboratory, spread on the floor, and cooled to room temperature by the use of a large fan. The seed was then divided into two equal parts; one portion was placed in a five-ton capacity storage tank; the other portion was treated with dry ammonia gas in a batch mixer so arranged that the gas could be introduced into the chamber containing the seed while the mixer was being rotated. Successive batches of cottonseed, of seventy-five pounds each, were treated with ammonia gas for three-quarters of an hour. All of the batches of ammonia-treated seed were combined and stored in a separate tank identical in all respects with that used for storing the untreated seed. The excess ammonia was removed from the air above the treated seed by ventilation. Both tanks were then closed and were kept closed during the entire storage period except as noted later for the ammonia-treated seed.

The free fatty acid content of the seed at the beginning of the storage period was 4.3 percent and the moisture content 17.5 percent. The pH values of the untreated and ammonia-treated seed were 6.4 and 7.8, respectively.

Thermocouples were placed in both tanks at distances of one and two feet above the floors and temperature readings were taken at regular intervals. The relationship between temperature and duration of storage for both treated and untreated seed is shown graphically in Figure 1. A record of the external atmospheric temperature for the same period is also given in the same figure for comparison.

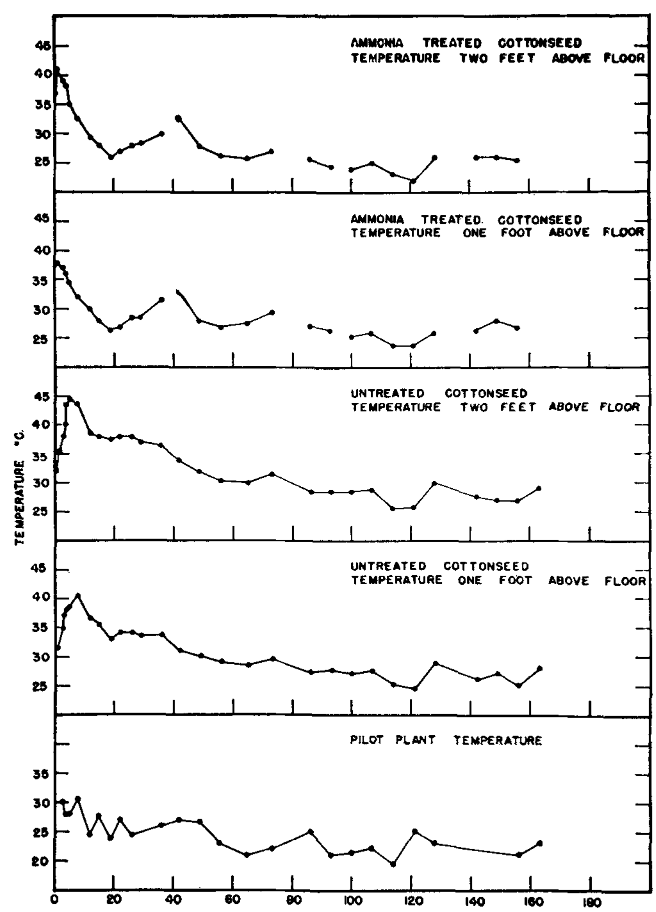

FIG. 1. The effect of treatment with ammonia on the heating of cottonseed during storage.

The untreated seed began to heat almost as soon as the tanks were closed and the temperature rose to a maximum of 45°C. in about a week, after which

[1] See Hogness and Potter (1) for a general discussion of the Beer-Lambert law.

the seed cooled slowly. The ammonia-treated seed heated immediately upon exposure to the gas and therefore was at an elevated temperature when it was first put into the storage tank. It is probable that the rise in temperature resulted from the heat of the reaction of the seed constituents with ammonia and that, as soon as this reaction was completed, the seed cooled rapidly to approximately room temperature.

After storage for 20 days the temperature of the ammonia-treated seed began to rise slowly. During the same period the pH of this seed dropped from 7.8 to 7.3. Accordingly, more ammonia was introduced into the seed while still in the storage tank. Upon completion of the second ammonia treatment the heating ceased and the temperature again decreased rapidly to the minimum level. Three more ammonia treatments were made as indicated by the breaks in temperature records in Figure 1. The excess ammonia was not removed after these treatments. These latter treatments were employed more as a precaution to keep the pH of the seed at a high level than to counteract any additional rise in temperature.

The effect of the ammonia treatment upon the rate of formation of free fatty acids in the seed is shown graphically in Figure 2. It is obvious that treatment with ammonia results in a marked reduction in the rate of formation of free fatty acids.

After storage for 170 days, approximately 500 pounds each of the treated and untreated seed were processed for oil by pressing in an Export-model type of French hydraulic box press. At the time of processing, the untreated seed was moldy and had decreased in moisture content to 13.5 percent. There

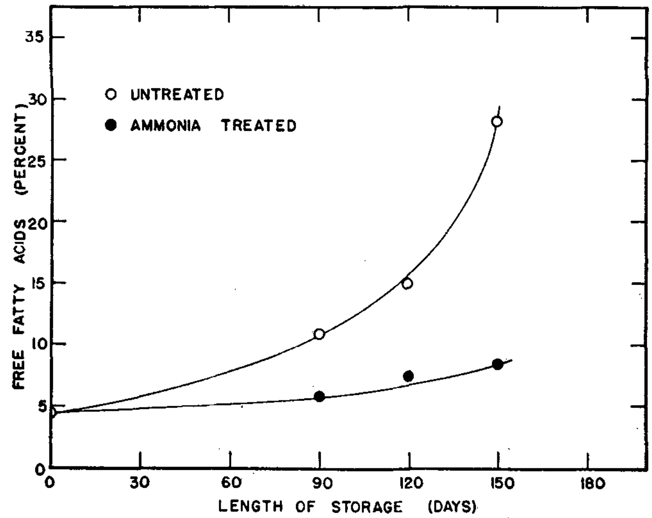

FIG. 2. The effect of treatment with ammonia on the rate of formation of free fatty acids in cottonseed during storage.

was very little evidence of mold in the ammonia-treated cottonseed and its final moisture content was 15.5 percent.

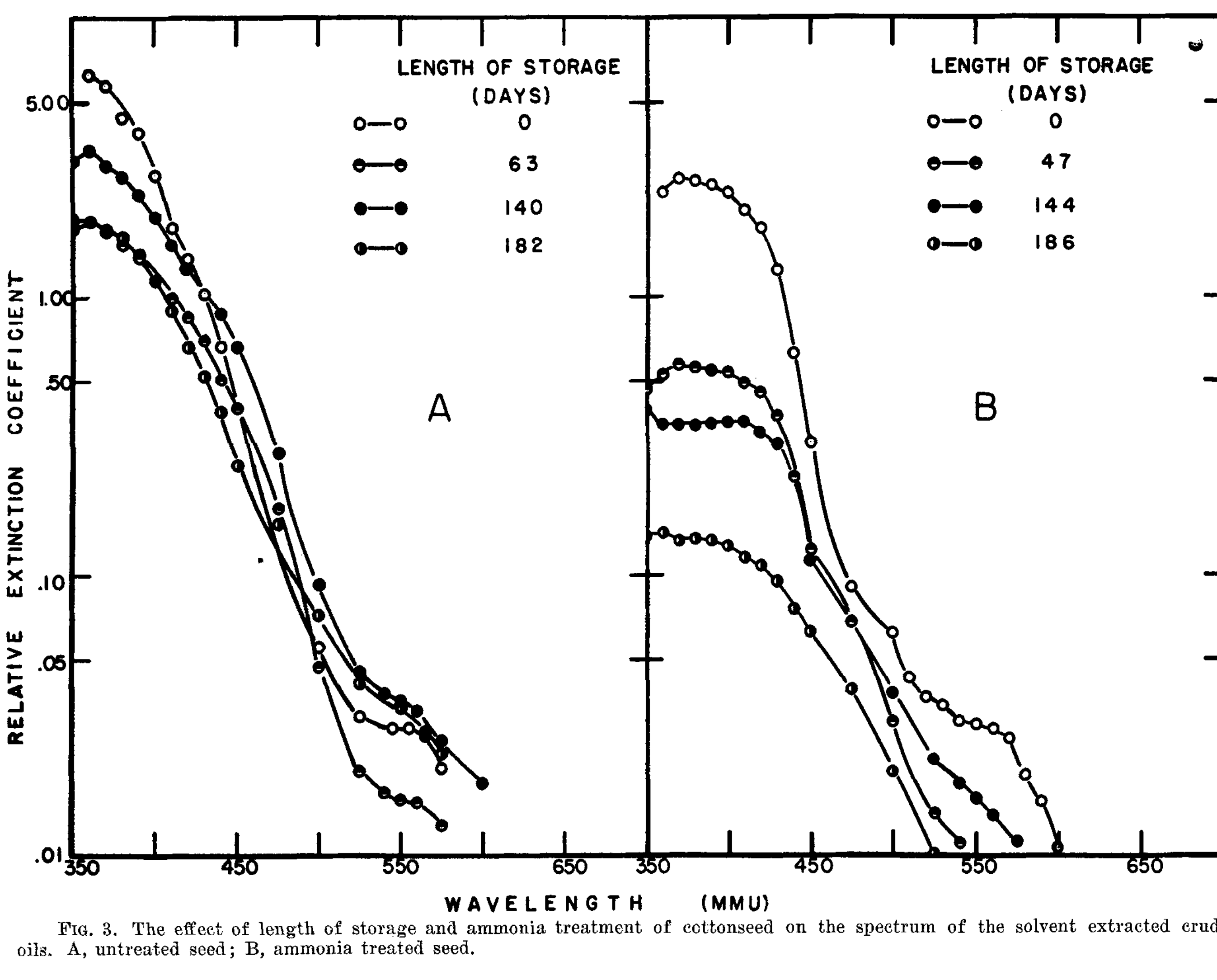

FIG. 3. The effect of length of storage and ammonia treatment of cottonseed on the spectrum of the solvent extracted crude oils. A, untreated seed; B, ammonia treated seed.

After storage for 185 days another 50 pounds each of the treated and untreated seed were processed. This time, however, the oil was removed by solvent extraction using solvent naphtha having a boiling range of 30° to 60° C.

All four samples of oil were analyzed for free fatty acids and refining loss by the official methods of the American Oil Chemists' Society. The results are shown in Table 2. It was found that the content of

TABLE 2
Analysis of Cottonseed Oil from Untreated and Ammonia-treated Seed After 6 Months' Storage

Treatment	Method of oil extraction	Free fatty acids	Refining loss
		percent	*percent*
Untreated	Hydraulic press	21.0	58.8
Untreated	Solvent extraction	40.2	95.8
Ammonia-treated	Hydraulic press	9.6	27.8
Ammonia-treated	Solvent extraction	10.7	30.0

free fatty acids in the oil from the untreated seed rose from 21 percent to 40 percent during the 15 days which elapsed between the processing of the two lots of seed whereas the content of free fatty acids in the oil from the ammonia-treated seed rose only one percent in the same period. The possibility that hydraulic pressing left a considerable quantity of free fatty acids in the cake was disproved by an examination of the residual oil in the press cake. It was found that the free fatty acids content of the oil remaining in the cake was 27 percent. It would appear, therefore, that the untreated seed was deteriorating so rapidly that the free fatty acids content actually doubled in the 15 days of additional storage.

Effect on the Color of the Oil

The intensity of the color in cottonseed oil is of considerable concern to the oil miller and in some respects is of greater interest to him than the free fatty acids content of the oil. While the free fatty acids are readily removed by treatment with alkali not all of the color is readily removed by bleaching and it may persist in abnormal degree throughout all of the refining operations.

In Figure 3 are given the results of spectrophotometric analyses of the crude oil obtained during the periodic examinations of the seed described in Experiment 1. It is of interest to note the absence of chlorophyll absorption at 620 and 660 millimicrons in all of the oils examined. This fact is more remarkable when it is realized that chlorophyll is found in many crude cottonseed oils, as is evident from the work of McNicholas (3). It is very likely that chlorophyll is found only in immature or green seed and is not a substantial color factor in fully ripened cottonseed.

There are two regions of interest in the spectrum of cottonseed oil, one of which is in the near ultraviolet and violet region from 350 to 400 millimicrons, and the other in the green region from 500 to 600 millimicrons. In both of these regions there is a specific absorption which is due to the presence of pigments in the oil. In the case of the untreated seed (Figure 3, A), there is evident very little change in the overall absorption in either of these regions during the 182 days of storage. The treatment of the seed with ammonia, however, caused a marked progressive drop in absorption in both regions (Figure 3, B). It was observed that the oil obtained from ammonia-treated seed which had been stored for six months no longer exhibited any specific absorption but did exhibit a low diffuse absorption in the green region of the spectrum. This oil had practically no red color.

The spectra of all the oils obtained in Experiment 2 are shown in Figures 4 and 5, and the Lovibond color values of the same oils are listed in Table 3. All

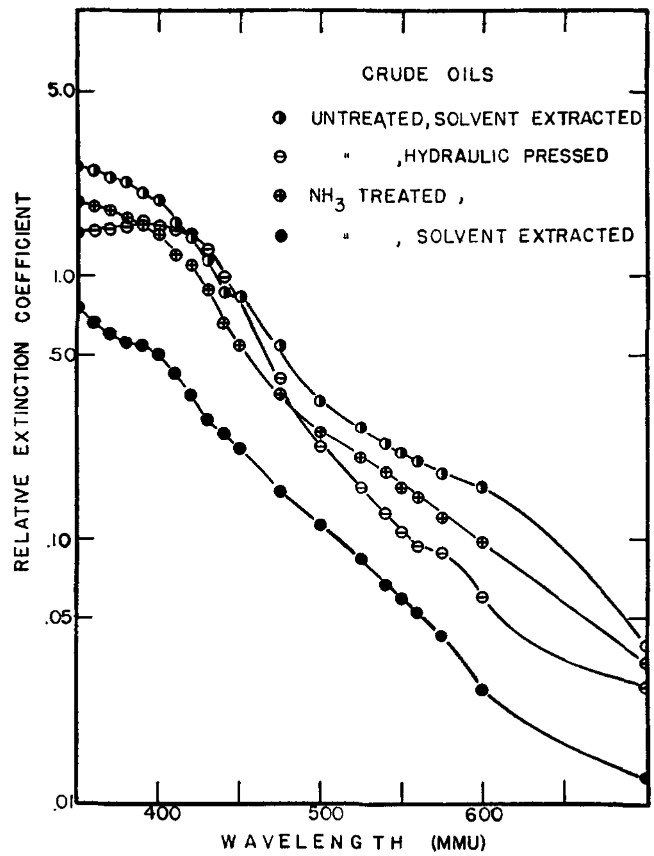

FIG. 4. The spectra of the crude oils produced by pilot plant processing.

of these crude oils had a more intense color than the crude oils described in Experiment 1 and had a more general type of absorption in all the regions of the spectrum. It would appear that oxidation of the pigments of the seed took place upon exposure to air during prolonged storage with the result that more intense color development and less specific absorption occurred. The oil from untreated cottonseed obtained by solvent extraction had the highest color, whereas the oil from ammonia-treated seed obtained by the same method had the lowest color. These results follow the same trend observed in the first experiment. There was, however, a reversal of color intensity relationships when the oils were removed from the seed by hydraulic pressing. The hydraulic pressed oil ob-

TABLE 3
Lovibond Color Readings for Alkali-refined and for Refined and Bleached Oil. (Figures in parenthesis are for 5¼-inch cell; all others for 1-inch cell.)

Treatment of seed	Treatment of oil	Hydraulic pressed		Solvent extracted	
		Yellow	Red	Yellow	Red
Untreated	Alkali-refined	35	5.4	35	31.9
Untreated	Refined and bleached	35 (35)	2.3 (15)	35	17.4
Ammonia-treated	Alkali-refined	35	9.4	35 (35)	4.5 (29.9)
Ammonia-treated	Refined and bleached	35	6.0	(35)	(10.6)

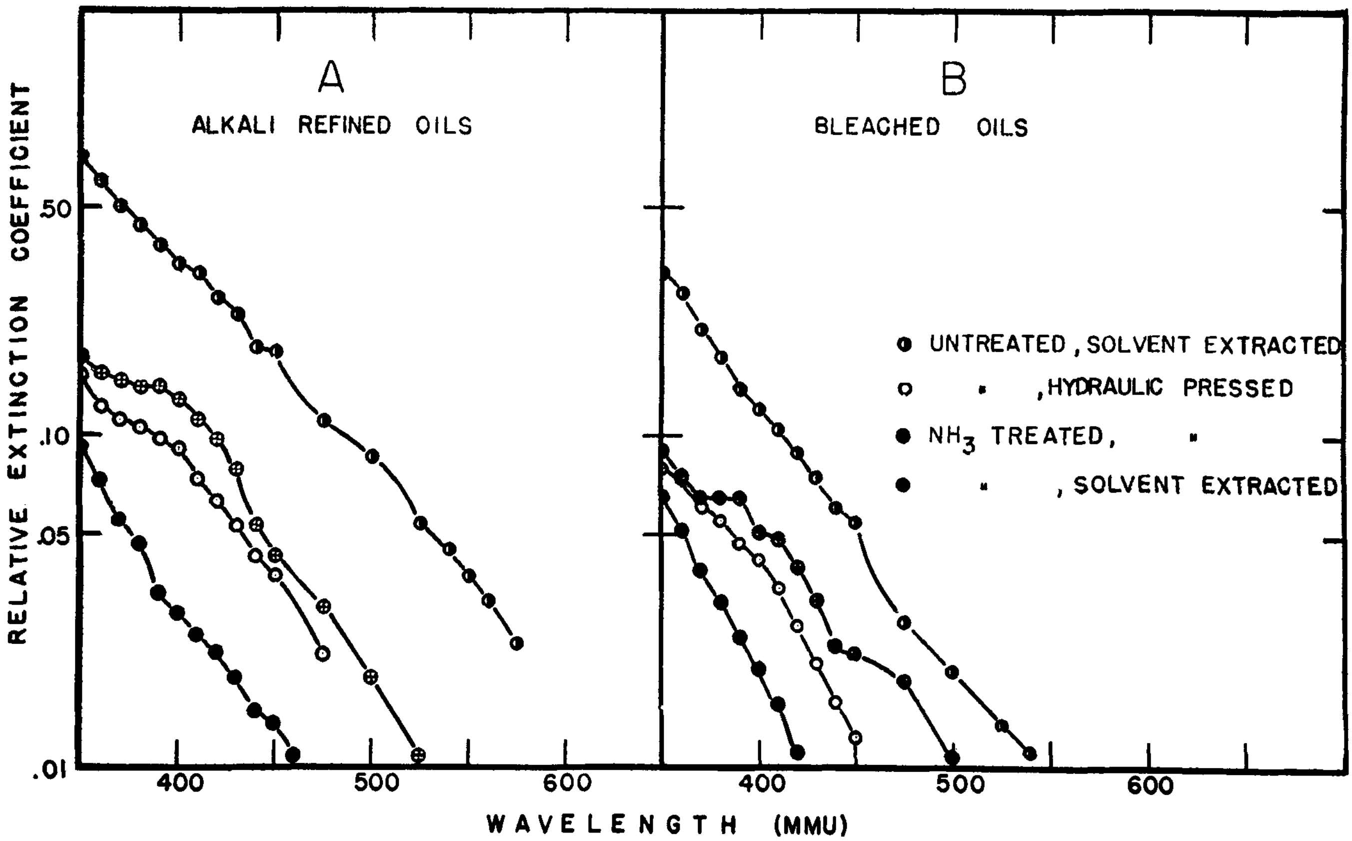

FIG. 5. The effect of alkali refining and of bleaching on the spectra of oils obtained in Experiment 2.

tained from ammonia-treated seed had more color than the pressed oil obtained from the untreated seed. It should be noted, however, that the ammonia treated seed had a higher moisture content at the time of processing than the untreated seed. Inasmuch as no attempt was made to bring the moisture contents of the two lots of seed to the same level prior to the cooking of the meats, it is possible that this difference in color may be due to the conditions of cooking rather than to intrinsic differences in the two lots of seed.

Discussion

Treatment of cottonseed with ammonia has a profound effect on the biological systems of the treated seed. Such treatment reduces the heating of the cottonseed, inhibits the formation of free fatty acids in the seed oil, and reduces the color of the extracted oil. In the previous publication (2) it was shown that treatment of cottonseed with ammonia destroys its viability. Evidence is available that treatment of cottonseed with ammonia also affects respiration and diminishes the rate of autolysis or self-disintegration of the tissue proteins. Ammonia is capable of effecting those changes in both artificially moistened and in naturally moist samples of cottonseed. A categorical statement that treatment with ammonia is equally effective on all types of cottonseed would be premature since the number of samples so far investigated has been relatively small, but some evidence has been obtained that the effect of ammonia on the rate of formation of free fatty acids is much greater for freshly moistened seed than for seed which has been in a moist condition for some time.

A number of the changes induced by treatment of cottonseed with ammonia appear to make possible a greater and more efficient utilization of cottonseed. Experimental work thus far has been confined to a laboratory and small pilot plant scale and it may be expected that there will be marked differences in the effectiveness of the ammonia treatment on a large scale where the heating of the seed and greater exposure to air create difficulties which are not encountered in laboratory experiments.

Summary

Treatment of moist cottonseed with ammonia prior to storage has been shown to reduce self-heating of the seed and the rate of formation of free fatty acids during storage.

The color of oils obtained by solvent extraction from ammonia-treated cottonseed is considerably lighter than that of oils obtained from untreated cottonseed.

Acknowledgment

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LITERATURE CITED

1. Hogness, T. R., and Potter, V. R., Spectrometric Studies in Relation to Biology, Ann. Rev. Biochem. *10*, 509-530 (1941).
2. Karon, M. L., and Altschul, A. M., The Storage of Cottonseed. I. Effect of Moisture and of Treatment with Acid and Alkali on Rate of Formation of Free Fatty Acids. Plant Physiology. (In press.)
3. McNicholas, H. J., Color and Spectral Transmittance of Vegetable Oils, J. Res. Natl. Bur. Stds. *15*, 99-121 (1935).
4. Simpson, D. M., and Stone, B. M., Viability of Cottonseed as Affected by Field Conditions, J. Agr. Research *50*, 435-447 (1935).
5. Weissberger, Arnold, and Proskauer, Erich, Organic Solvents, 210 pp., Oxford (1935).