Peroxidizing and Carotene Bleaching Substances in Bacon Adipose Tissue[1]

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THE deterioration of bacon during storage causes considerable loss to meat packers, operators of frozen food locker plants and their patrons, and all those in general concerned with the production, processing, storage, sale, or consumption of this product. The problem is especially important to the armed forces under conditions where food must be stored for long periods and shipped to various parts of the world.

The development of rancidity in bacon may be attributed to both external and internal factors. Among the former are microorganisms, temperature, moisture, atmospheric oxygen, and light. Among the latter are the fat and proteins, enzymes, coenzymes, antioxidants, and minerals of the bacon tissue and chemicals which may be added during processing. The effects and control of microorganisms have been reviewed in detail (1) and the effects of curing, different curing mixtures, and impurities in the curing salts extensively studied (1, 2, 3, 4, 5, 6, 7).

A factor comparatively little investigated but probably of the first order of magnitude in importance is the effect of enzyme systems in the fatty tissue. Lea suggested (2, 3) that an enzyme system might be responsible for the rapid deterioration of bacon and later (8) presented evidence for its presence. Banks has reported (9) a similar enzyme in herring muscle. Watts and Peng have compared the catalytic effects of pork muscle extracts and hemoglobin solutions on peroxidation of lard (10). They concluded that the two are identical since the effects of both are destroyed by heat, decreased with dilution, and unaffected by cyanide at pH 5.3. Other oxidizing enzymes of fatty acids have been reported. Munoz and Leloir (11) prepared an enzyme from liver which catalyzes the oxidation of low molecular weight fatty acids, studies which have been considerably extended by Lehninger (12, 13) and Lang (14).

A "lipoxidase" present in legumes has been investigated and use made of it to study the mechanism of the oxidation of fatty acids (15, 16). The literature on this enzyme has recently been reviewed (17).

From the foregoing it is clear that effects of adipose tissue enzymes on rancidity development have been inadequately investigated. The studies of Lea (8) and Watts and Peng (10) were on muscle juice and the results inconclusive. The conclusion of the latter workers that hemoglobin is the active principle is predicated on the observation that muscle juice and hemoglobin are both heat labile, diminish with dilution, and are unaffected by cyanide. But these qualities might also be true of two different enzymes. They explained differences in activities of the two at increasing pH values by assuming a coacervation effect of hemoglobin and muscle proteins at the higher pH values.

The term "lipoxidase" has been very loosely used. It has been used to describe enzymes which cause rancidity, produce peroxides in unsaturated acids, bleach carotene, or oxidize saturated fatty acids to shorter chain products. In short, it has been used to name any enzyme which will produce any type of oxidation in any kind of lipid. Its use is therefore avoided in the present paper.

The original plan for the present study was to prepare extracts of bacon adipose tissue and to compare the peroxidation and carotene bleaching activities of the extracts with those of simple solutions of corresponding hemoglobin concentrations. This had to be abandoned when no practical method could be found to determine the hemoglobin concentration of either the adipose tissue or the cloudy extracts.

It was found that cured bacon, unsmoked, could be extracted with 0.1 M potassium acid phosphate and obtained free of hemoglobin. These extracts were then studied for their carotene bleaching effects at different pH values, reaction times, concentrations of the substrate, dilutions, and after heating or treatment with alcohol, cyanide, and fluoride. Similar studies were made with hemoglobin solutions. Observations were also made of the influence of hemoglobin solutions under different conditions on the formation of peroxides in lard and on peroxide formation in ground bacon adipose tissue.

Methods

Estimation of Peroxidizing Activity. To determine the influence of buffered hemoglobin solutions or tissue extracts on peroxide formation 100 mg. of gum acacia was dissolved in 0.5 ml. of the solution or extract and mixed with 1 gm. of lard. The mixture was stored as desired. For the estimation of peroxides 25 ml. of chloroform were added and stirred until the fat was dissolved. The fat content was determined on 8 ml. Eight ml. were pipetted to a 125-cc. Erlenmeyer flask, 12 ml. of glacial acetic acid added and the peroxides determined according to Lea (20) except that the potassium iodide was added after deaeration. The estimation of the peroxides in ground fatty tissue was made by the method of Rockwood *et al.* (21). Peroxide numbers are reported in milliequivalents per kilogram of fat.

The Determination of Carotene Bleaching Activity. The method used in the present paper was essentially that of Reiser and Fraps (18). Five ml. of the extract were pipetted into a 125-ml. Erlenmeyer flask. One ml. of a carotene solution (50 mg. of crystalline carotene + 300 mg. of Wesson oil to 500 ml. in acetone) was added directly into the extract, mixed by one gentle whirl, and allowed to stand the desired time. For routine work the time was 90 minutes. Ten ml. of methanol, 100 ml. of U.S.P. ether, and 10 ml. of water were added and well shaken in turn. The density of the carotene in the resulting ether solution was compared to controls without bacon extracts in a Beckman spectrophotometer. The percentage of carotene bleached, above that of the control, was the criterion of the activity of the extract.

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Occult Hemoglobin. The presence of hemoglobin in the extract was determined by the phenolphthalin test (19) except that a drop of ammonia solution was added before the extract was boiled. It was found that this test gives a strong positive test for hemoglobin in dilutions of 1:100,000,000.

Hemoglobin Solutions. Commercial hemoglobin was found to have lost much of its activity. Fresh hemoglobin solutions were prepared as follows: Fresh swine blood cells were washed several times with physiological saline and diluted 1:100 in 0.1 M KH_2PO_4 or whatever other extraction solution or medium was being studied. Concentration of hemoglobin was determined by the o-phenanthraline determination of the constituent iron and later by the alkaline pyridine hemochromogen as follows: To 5 ml. of a 1:1,000 solution of the cells were added in turn 0.5 ml. of 50% potassium hydroxide, 0.25 ml. of pyridine and a few grains of sodium hydrosulfite. The extinction coefficient at 1%, 1 cm. was found to be 16.7 at 560 mμ.

Preparation of Extracts. The bacon used in this work was prepared from swine slaughtered in the Meats Laboratory of the Department of Animal Husbandry. After the carcasses were chilled, the sides were removed and each 100 pounds salted with 5 pounds of a mixture of 8 pounds of sodium chloride, 2 pounds of sugar, and 3 ounces of 9:1 sodium nitrate-sodium nitrite. Curing was continued for 11-12 days at 38°F. Hemoglobin-free extracts of cured (but not uncured) bacon adipose tissue were prepared as follows: After washing away superficial salt the fatty tissue was carefully cut away from the lean, diced, and placed in a Waring blendor with an equal volume of 0.1 M potassium acid phosphate. About 100 gm. of fatty tissue is optimum. The mixture was homogenized until a thick emulsion formed. This occurred quite suddenly after about 2 or 3 minutes in the blendor. The emulsion was chilled in an ice salt bath, preferably after transfer to a beaker, and the extract worked out with a heavy glass rod. The extract can be filtered through qualitative filter paper. Centrifugation at low speed for a few minutes may sometimes aid the filtration.

If the filtrate gave a test for occult hemoglobin, it was adjusted to pH 4.5, allowed to stand in the refrigerator for several hours or over night and again filtered. The pH was adjusted to 5 for carotene bleaching effect.

It was found that fresh, uncured bacon fat extracts could not be freed of hemoglobin by this procedure. If 1 gm. of salt was added to each 100 ml. of the potassium acid phosphate solution, the extract could be obtained free of hemoglobin.

Observations

Peroxidation Activity of Hemoglobin Solutions. In Table I are shown typical results of the effect of hemoglobin solutions on lard. In no case was any activity ever noticed for hemoglobin solutions of less than 0.2 mg. per ml. even though incubated at 37°C. for 8 days. As also evidenced from this table, heating hemoglobin prepared as the buffered solutions of red blood cells always reduced to some extent its peroxidizing activity, indicating the presence in the cells of a heat labile lipoxidase.

Carotene Bleaching Activity of Hemoglobin. The relation of concentration of hemoglobin solutions in 0.1 M potassium acid phosphate to their carotene bleaching power is shown in Figure 1. The curve was prepared from 3 different preparations of hemoglobin as described above, the reaction time being 90 minutes. The curve is smooth, the increases in the amount of carotene bleached rising very rapidly up to a hemoglobin concentration of about 0.03 mg. per ml. The increase in bleaching effect with increase in concentration then slowly tapers off until between 0.2 mg. and 0.3 mg. of hemoglobin per ml., it is comparatively slight.

TABLE I

Peroxidation Activity of Hemoglobin

Approximate Erethracyte Dilution	Concentration Hemoglobin mg. per ml.	Milliequivalents of Peroxides per Kilogram of Lard After Storage at 10°C. for 9 days	
		Boiled Hemoglobin	Unheated Hemoglobin
1:100	3.00	127	147
1:200	1.50	83	122
1:400	0.75	55	85
1:800	0.38	32	42
1:1600	0.19	23	31
1:3200	0.094	20	21
1:6400	0.047	20	21
	Control, Lard-Buffer	16	16

Acetate buffer pH 5 was used as dilutant.

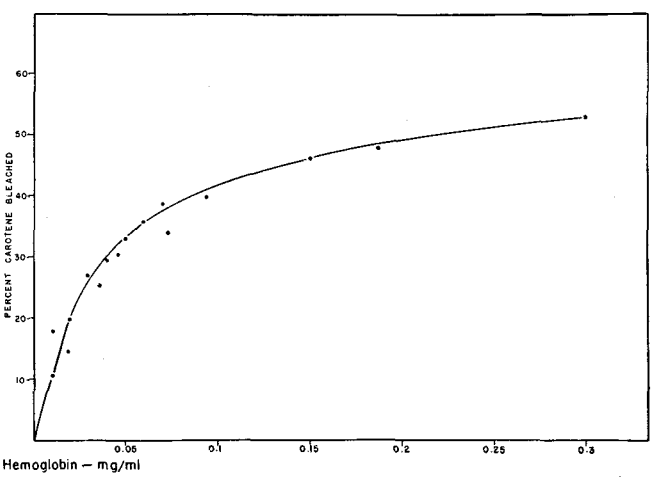

FIG. 1. Carotene bleaching activity of hemoglobin. Effect of concentration. Reaction time 90 minutes.

Similar to their peroxidation activities, heated blood cell solutions bleached somewhat smaller percentages of carotene than the unheated, the differences being noticeable in hemoglobin concentrations above 0.4 mg. per ml. and amounting to about 10%.

In Figure 2 is shown the effect of reaction time on the carotene bleaching activity of a solution containing 0.05 mg. of hemoglobin per ml. After about the first 10 minutes there is a straight line relationship of about 12% bleached each hour. During the first minute, however, 8% was bleached and 17% during the first 5 minutes.

It can be seen from Table II that for a reaction

TABLE II

Carotene Bleaching Activity of Hemoglobin Effect of Concentration of Substrate

90 Minutes		20 Minutes	
μg. Carotene	% Bleached	μg. Carotene	% Bleached
49	25	26	0
97	23	47	5.1
146	21	82	16
186	24	159	15
233	22	.....	.....

Hemoglobin concentration = 0.05 mg./ml.

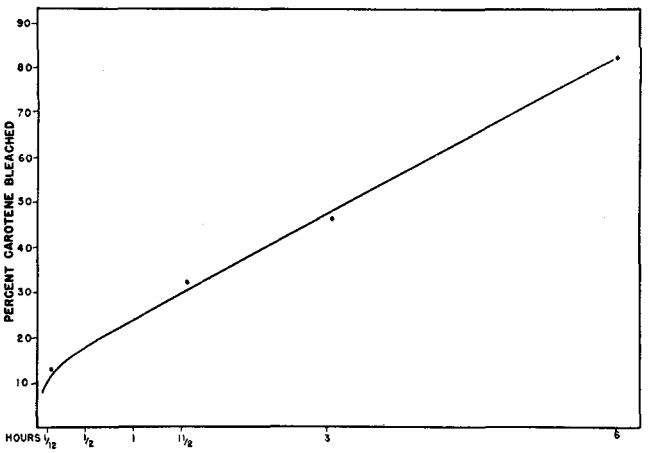

FIG. 2. Carotene bleaching activity of hemoglobin. Effect of time. 0.05 mg. hemoglobin per ml.

time of 90 minutes the per cent of carotene bleached is independent of the concentration of the substrate. At 20 minutes, however, the percentage bleached does vary with the concentration of carotene at lower concentrations.

As has been shown by Watts and Peng, Table III indicates that the "lipoxidase" activity of hemoglobin increases as the pH approaches five. One hundredth molar cyanide and fluoride had no effect on the carotene bleaching activity of hemoglobin solutions.

TABLE III

Carotene Bleaching Activity of Hemoglobin
Effect of pH

pH	Per Cent Bleached
5.5	60
6.0	46
6.5	44
7.0	41
7.5	38
8.0	37

Approximately 3 mg. of hemoglobin per cc. for 20 minutes.

Peroxidation Activity of Bacon Adipose Tissue. Similar to blood cell solutions, heated ground bacon adipose tissue showed evidence of containing both heat stable and heat labile peroxide forming factors. Thus, in a typical experiment, five samples of ground tissue heated and unheated were incubated at 37°C. when no peroxides were produced after one week at 8°C. After 11 days heated samples number 3, 4, and 5 had peroxide numbers of 77, 202, and 150 respectively, while unheated samples number 1, 3, 4, and 5 had peroxide numbers of 88, 197, 349, and 307. Heated samples number 1 and 2 and unheated sample number 2 developed no peroxides.

Carotene Bleaching Activity of Adipose Tissue Extracts. The carotene bleaching activity of the adipose tissue extracts varied from 8% to 65% of the carotene in 90 minutes. The most active extracts were obtained by extracting a fresh sample with a previous extract. The effect of dilution on the carotene bleaching activity of adipose tissue extracts is shown in Table IV. The results would indicate the presence of an inhibiting substance which is more affected by dilution than the lipoxidase. This is

TABLE IV

Carotene Bleaching Activity of Bacon Fat Extracts
Effect of Dilution

Dilution	Per Cent of Carotene Bleached		
	Extract 1	Extract 2	Extract 3
0	39	24	38
1/2	33	19	24
1/4	23	15	22
1/8	18	19	26
1/16	....	27	24
1/32	....	....	23
1/64	....	....	19
1/128	....	....	14

Reaction time = 90 minutes.

especially pronounced in extract 2 in which case the lipoxidase activity actually increased in dilutions greater than 1:4. The effect of reaction time on the carotene bleaching activity of representative adipose tissue extracts is shown in Figure 3.

A large percentage of the carotene is bleached almost immediately after contact. The rate of bleaching rapidly falls off during the first hour at 37° and somewhat more gradually at 8°C., is negligible for the following 7 hours, and then is constant for the remainder of the 24-hour period tested, at which time about 90% is bleached.

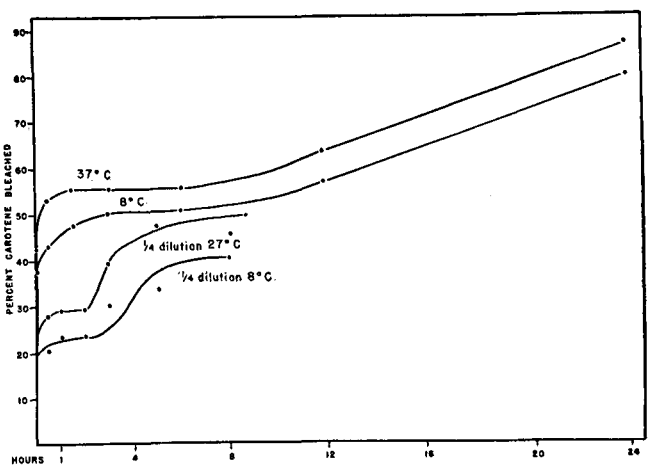

FIG. 3. Carotene bleaching activity of bacon fat extract. Effect of time.

As was the case with hemoglobin solutions after 90 minutes reaction time, adipose tissue extracts bleach the same percentage of carotene in concentrations of from 50 to 250 μg. of the substrate per 6 ml. of reaction mixture during a 15-minute reaction time (Table V). As also shown on Table V the effect of increasing pH is much less pronounced in the present work than in muscle extracts as described by Watts and Peng. Nevertheless, the activity increases as the pH approaches 5, in conformity with the previous report.

TABLE V

Carotene Bleaching Activity of Bacon Fat Extracts

Effect of Concentration of Substrate		Effect of pH	
μg. Carotene	% Bleached	pH	% Bleached
46	35	5	29
95	38	6	27
150	39	7	25
254	38	8	16
....	....	9	17

Reaction time = 15 minutes.

When heated on the boiling water bath for 5 minutes and filtered, the filtrate possessed no carotene bleaching activity. When the unfiltered, heated extract was assayed, the results were inconsistent. Some extracts lost almost all their activity upon heating while some retained a large portion of their activity. Cyanide and fluoride were without effect. One to one dilution with 95% alcohol resulted in somewhat less activity than 1:1 dilution with 0.1 M potassium acid phosphate.

Discussion

It appears from the above evidence that there are at least two factors in bacon adipose tissue and its extracts capable of producing fatty acid peroxides and bleaching carotene. That one of these factors is hemoglobin is probable. Both its peroxide catalyzing and carotene bleaching activities have previously been demonstrated. Although the concentration of hemoglobin in adipose tissue has not been determined, it is obviously present.

According to Watts and Peng hemoglobin is inactivated by heat. The present study indicates that it is not. From consideration of the known stability of hemoglobin as well as the evidence that its peroxidase activity is due to the heme fraction rather than the entire molecule (22, 23, 24, 25) it is difficult to interpret experiments indicating that it is labile at 70°-90°C.

Haurowitz *et al.* (22) have shown that heating hemoglobin or heme with fat at 38°C. destroys them, and in the present work, during attempts to determine hemoglobin in adipose tissue, it was found that heating hemoglobin with lard at 50°C. completely destroyed its ability to form alkaline pyridine hemochromogen. Watts and Peng, however, neither filtered their heated hemoglobin solutions nor heated them with fat.

It is academic to discuss whether the action of hemoglobin is catalytic or enzymatic, the answer depending purely on definition. The low sensitivity of the peroxidation as compared to the carotene bleaching activity of hemoglobin is probably due to the nature of the substrate. Thus, under the conditions used, no peroxides were observed when 0.5 ml. of hemoglobin of concentrations less than 0.2 mg. per ml. were mixed with 1 gm. of lard while carotene bleaching effects were pronounced at a concentration of 0.02 mg. per ml. Had linoleic acid been used as substrate, it is logical to assume that peroxides would have been evident at lower concentrations.

At low concentrations of carotene and short reaction time the percentage bleached varies with the concentration. At higher concentrations and longer reaction time this variation disappears (Table II).

The lessened peroxidation activity of adipose tissue and diluted blood cells upon heating and the carotene bleaching activity of extracts free of hemoglobin show the presence of one or more non-hemoglobin factors in bacon which may be responsible for the rapid development of rancidity in that meat product. The complete loss of activity of heated and filtered extracts is almost conclusive evidence that these factors are enzymes. The irregular behavior of unfiltered heated extracts might be due to the presence of some catalytic factor which is adsorbed by the coagulum. This might be a decomposition production of hemoglobin not detected by the phenolphthalin text.

That the remaining factors are multiple must be concluded from the behavior of the hemoglobin free extracts. In Figure 3 are shown the effects of reaction time on the carotene bleaching activity of a representative hemoglobin free extract. A remarkable effect is the bleaching of a large percentage of the carotene almost immediately after contact. Equally peculiar is the lack of any activity between one and eight hours at 37°. The change is more gradual at 8°C. If the equilibrium is caused by an anti-enzyme or inhibitor one would expect the effect to be in the beginning and show an induction period. The curves shown might result from a mixture of two enzymes and an inhibitor, the rapidly acting enzyme being independent of the inhibitor. Another possibility is that the rapid acting principle is a reactant. Although nothing in these experiments indicates the identity of the inhibitor, the manner in which the extracts were prepared and the heavy cloudiness of the filtered extract makes it likely that lecithin is present.

There are other evidences of an inhibitor. As shown in Table IV, the carotene bleaching activity of diluted extracts may first slowly decrease and increase again before finally decreasing. This phenomenon can also be explained by the presence of an inhibitor that is diluted away more rapidly than the enzyme. It was also found that heating diluted whole blood increased its carotene bleaching activity. A similar increase has been reported in bacon fat catalase after heating (26) and was attributed to the destruction of an anticatalase.

The relation between concentration of carotene and percentage bleached by hemoglobin is not true of percentage bleached by hemoglobin free extracts. Typical enzymes should show such relations if the systems are uncomplicated. However, it has been indicated that these adipose tissue extracts may contain two active principles acting at different rates and an inhibitor.

Summary and Conclusion

The peroxidation activity of hemoglobin solutions was studied for the effects of concentration of hemoglobin and heat. The carotene bleaching activity of hemoglobin was studied for the effects of pH, concentration of hemoglobin, concentration of carotene, time, heat, and poisons. These observations were compared to similar studies of hemoglobin free extracts of salt cured bacon fatty tissue. It was found that hemoglobin activity is not influenced by boiling but that there is a heat labile factor in hemoglobin free extracts. Evidence is presented that the hemoglobin free extracts contain at least two principles which may be responsible for the rapid development of rancidity in bacon and one inhibitor.

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REFERENCES

1. Lea, C. H., Rancidity in Edible Fats, 1939, Chemical Publishing Co., Inc., New York, N. Y.
2. Lea, C. H., J. Soc. Chem. Ind., *50*, 215T, 343T (1931).
3. Lea, C. H., *Ibid.*, *52*, 57T (1933).
4. Hills, C. L., Conochie, J., J. Council Sci. Ind. Research, *18*, 35T (1945).
5. Hills, C. L., and Conochie, J., *Ibid.*, *19*, 1 (1946).

6. Greenwood, D. A., Striter, J. E., and Kraybill, H. R., Proc. Inst Food Tech., 1945, p. 58.
7. Oser, B. L., Food Ind., *18*, 1683 (1946).
8. Lea, C. H., J. Soc. Chem. Ind., *56*, 376T (1937).
9. Banks, A., Dept. Sci. Ind. Research (British) Rept. Food Invest. Board, *1937*, 73 (1938).
10. Watts, B. M., and Peng, D., J. Biol. Chem., *170*, 441 (1947).
11. Munoz, J. M., and Leloir, L. F., J. Biol. Chem., *147*, 35T (1943).
12. Lehninger, A. L., J. Biol. Chem., *161*, 413, 437 (1945).
13. Lehninger, A. L., Kennedy, E. P., J. Biol. Chem., *173*, 753 (1948).
14. Lang, K., J. Physiol. Chem., *261*, 240 (1939).
15. Bergström, S., Arkiv. Kemi. Minerol. Geol., *21A*, 14, 15 (1945).
16. Holman, R. T., Arch. Biochem., *15*, 403 (1947).
17. Holman, R. T., Advances in Emzymologya, *8*, 425 (1948).
18. Reiser, R., and Fraps, G. S., J. Assoc. Off. Agn. Chem., *26*, 186 (1943).
19. Gettler, A. O., and Kaye, S., Am. J. Clin. Path., Tech. Sec., *7*, 77 (1943).
20. Lea, C. H., J.S.C.I., *65*, 286 (1946).
21. Rockwood, B. N., Ramsbottom, J. M., and Mehlenbacher, V. C., Anal. Chem., *19*, 853 (1947).
22. Haurowitz, F., Schwerin, P., and Yenson, M. M., J. Biol. Chem., *140*, 353 (1941).
23. Kiese, M., Naturwessenschraften, *33*, 123 (1946).
24. Haurowitz, F., and Schwerin, D., Enzymologya, *9*, 193 (1941).
25. Robinon, M. E., Biochem. J., *18*, 250 (1924).
26. Bodnar, J., and Bartfoi, J., J. Physiol. Chem., *244*, 225 (1936).

Factors Influencing Vegetable Oil Bleaching by Adsorption

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ABSTRACT

Vegetable oil bleaching by adsorption involves the removal of color particles which are either dissolved or colloidally dispersed in the oil. This type of adsorption follows an exponential relationship and the Freundlich equation was utilized in correlating the data. In utilizing this equation, the color of the oil was assumed proportional to the concentration of the color particles, and a color measurement system based on the Beer-Lambert Law was devised. This system involves a measurement of the transmittance of light of 475 millimicrons wave length through the oil. A Coleman, Model 11, Spectrophotometer was used for the transmittance measurements. Spectral transmittance curves for cottonseed and soybean oil, bleached and unbleached, are given.

The effect of the variables of temperature, time of contact, and particle size on the activity of several adsorbents was determined. It was found that the activity of each adsorbent is at a maximum at some particular temperature. The adsorbents were grouped into three general classes: acid-activated clays, natural earths, and materials high in silica content, and it was found that adsorbents in the same class have a temperature of maximum activity within the same range.

The effectiveness of 16 different adsorbents in bleaching a refined cottonseed oil was determined by utilizing the Freundlich equation and plotting adsorption isotherms for each adsorbent. Similar data are given for 10 different adsorbents in bleaching a refined soybean oil. On the basis of adsorbent activity, acid-activated clays were 1½ to 2 times as effective as natural earths.

THIS paper is the second in a series of studies made by the Chemical Engineering Department of Washington University on the application of adsorbents to industrial processes. The first paper (10) showed the applicability of reclaiming dry cleaning solvents by adsorption. Although adsorption is practically the only method used for bleaching oils which are to be used in edible products, there has been little work reported in the literature on the determination of optimum operating conditions for specific adsorbents and the relative activities of various adsorbent materials. It is recognized that adsorbent activity is only one of several factors that affect the choice of an adsorbent to a specific bleach operation. Other factors involved are cost, oil retention value, filtration characteristics, taste imparted to the oil, and effect of the adsorbent on the free fatty acid concentration in the oil. However, with most of the commercial materials studied in this report, the last three factors were of little consequence: all materials studied had approximately the same filtration characteristics; all oils were deodorized after bleaching, and very low free fatty acid concentrations were not demanded for the product under consideration.

While it is known that the free fatty acid content of an oil will vary with the nature of the adsorbent clay used in its treatment and that a large part of the free fatty acid will be lost during the deodorization, it was felt that economically this factor was small compared to the others listed and hence could be safely ignored. The selection of an adsorbent then resolved itself into an economic balance of: cost of adsorbent, adsorbent activity, and oil retention value (1). Knowing these three factors, nomographs (5) are available for evaluating a series of adsorbents.

Actually adsorbent activity should be determined by tests made on plant size equipment. With the number of adsorbents now available this was considered impractical. It was believed that at least an indication of the relative activities of a number of adsorbents can be obtained from laboratory data. However, the authors would like to state here, that as this work was carried out in small scale laboratory apparatus, the results cannot be properly extrapolated to plant size equipment directly without further work in large scale equipment under plant operating conditions. It should be further emphasized that the ultimate selection of an adsorbent rests on economic balance of three main factors, one of which, oil retention value, was not considered in this work. The object of this work was to determine the effect of the variables of time of contact, temperature, and particle size on adsorbent activity and to evaluate a series of adsorbents as to their activity in bleaching a typical refined soybean and a cottonseed oil. Obviously laboratory data on oil retention values would correlate poorly with actual plant data, and no attempt was made in this work to determine oil retention values.

At the present time much of the bleaching is car-