The Oven and Aeration Methods as Means of Accelerating Fat Oxidation[1] [2]

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In connection with other studies conducted by the authors pertaining to butterfat oxidation, considerable attention was directed toward two methods which have been utilized for accelerating oxidation in an effort to determine their reliability and the uniformity of results which may be obtained when they are used. The two methods studied were (a) the aeration method, involving the use of an apparatus usually referred to as "Swift's Fat Stability Apparatus," and (b) the hot air method.

The aeration method, developed in Swift's Laboratories by King, Roschen, and Irwin (8), has found acceptance in fat oxidation studies. Bull (3, 4, 5), Musher (10), and Stebnetz and Sommer (12, 13) utilized this method in research laboratories, and Freyer (7) reports on the use of this method in eleven commercial laboratories.

An analysis by Freyer (7) of a three-year committee study of the aeration method indicates that the cooperative testing of samples in 11 different laboratories gave inconsistent results. Freyer allowed an average tolerance in induction period time of 17 per cent, but, even so, about one-fourth of the laboratories which first adopted the method presented consistently uniform results. Two of the laboratories reported inconsistent results at first but after using the method for about a year, reported results that were in agreement with the other laboratories. Freyer (7) suggests that the lack of agreement between the different laboratories was apparently due to insufficient experience with the method, but feels that even though the method has many limitations, it still is the best accelerated procedure known for determining the relative stability of fats and oils toward oxidation.

Another method, the hot air oven method, has been rather widely used, and has been utilized recently by Barnicoat and Palmer (1), Bird (2), Dahle and Nelson (6), and Ritter and Nussbaumer (11). Barnicoat and Palmer decanted butter oil into straight-sided crystalizing dishes which were then heated for six hours in an air-stirred water-jacketed oven thermostatically controlled at 80°C. Bird (2) exposed small samples of fish oil to the atmosphere at 37°C. and Ritter and Nussbaumer (11) oxidized fat in 10 cm. petri dishes in an oven at 104°C. for eight hours.

Dahle and Nelson (6) placed equal volumes of butter fat in stoppered test tubes which were placed in a 60°C. oven in an upright position to insure uniform surface area exposure. In addition, these workers placed the fat in unstoppered 50 ml. centrifuge tubes which were permitted to lean at varying angles to obtain variations in the amount of surface exposed. They found that, although the temperature of different tubes did not vary by more than one degree, variation in the induction period between duplicate samples was as much as four days for the 15-day period, a 26.7 per cent deviation. No conclusions were drawn relative to the comparative uniformity of the test-tube and centrifuge-tube samples.

Information is not available of studies conducted in which a direct comparison is made, in the same laboratory, of the aeration and oven methods. Furthermore, literature records little information on the oven method per se and how the factors contributing to errors in the method may be controlled. This study was conducted, therefore, to secure additional data relative to these points.

Experimental Procedure

Butter oil used in the present studies was secured by churning sweet cream and by filtering the butter to remove the curd and water.

The aeration method was conducted using the standard apparatus similar to that shown by Stebnitz and Sommer (13). This consists of an oil bath maintained at 100°C. in which are placed the test tubes containing the fat sample. Air is permitted to flow through capillary glass tubing and into each tube of fat at a constant and uniform rate, after it first passes through a wet test meter which measures the volume, and also through a purifying solution of potassium permanganate. The rate of air flow was such as to maintain the butter oil in an air-saturated condition.

The oven method utilized a triple-walled, Cenco-DeKhotinsky, thermostatically controlled oven. In this oven, the heating elements are in the floor and ducts lead to the sides of the oven where the hot air enters at about six and twelve inches from the bottom. Two shelves were used in these studies, the bottom shelf being six inches and the top shelf ten inches from the floor. A thermometer was inserted through the top and extended to the top shelf of the oven. A Cenco air agitator was installed in the top of the oven to insure positive air agitation.

Twenty-five milliliters of butter oil were used both in the aeration and in the oven method. In the oven method, the butter oil was placed in 100 ml. beakers, whereas in the aeration method the fat was placed in 1″ x 8″ test tubes. Unless otherwise specified, both the oil bath used for the aeration procedure and the air oven were maintained at 100°C.

Peroxide determinations were conducted essentially by the Wheeler technique, and are reported as peroxide numbers (millimoles of peroxide per 1,000 gms. fat).

All glassware coming in contact with the fat was thoroughly cleaned by washing in tri-sodium phosphate solution, followed by soaking overnight in a chromic acid cleaning solution. The glassware was rinsed free of the cleaning solution with hot water,

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and then leached in six or more changes of distilled water, each lot of water being allowed to remain over the glassware for 12 hours or more. This thorough rinsing is in line with the findings of Laug (9) which indicated that several successive leachings with distilled water were necessary to remove all of the chromic acid from glass.

Experimental Results

The oven method: Considerable attention was directed toward those factors which should be controlled if the oven method is to be used satisfactorily. Among the factors considered were temperature control, air agitation, position of the samples in the oven, and size and surface area of samples.

Temperature variations: To determine temperature variations within the oven preliminary trials were conducted in which a sample of fat was divided into nine portions in beakers. Three of these portions were placed on the floor of the oven, three on the bottom shelf (six inches from the floor), and three on the top shelf (10 inches from the floor). The air vent at the bottom of the oven was open, but no means of positive air circulation was used. At the end of 24 hours the temperatures of all nine samples were obtained and the peroxide determinations conducted.

The results secured clearly indicated the necessity for temperature control. The temperature of the oven varied widely and, consequently, influenced the rate of oxidation at different positions in the oven. The floor of the oven was considerable higher in temperature and varied to a greater extent than any other position in the oven. This is explained by the fact that the heating elements of the oven were located in the floor and only a portion of these elements were in operation. Samples with the higher temperature were close to or, perhaps, directly over one or more of the active heating elements. The respective variations in temperature and peroxide values for the three positions in the oven were as follows: top shelf, 7°C., 0.3 peroxide unit; bottom shelf, 13°C., 8.7 peroxide units; floor, 43°C., 48 peroxide units.

These preliminary trials indicated the undesirability of utilizing the bottom of the oven for oxidation studies. Consequently, all further studies pertaining to the oven method involved the use of the two shelves.

Arrangement of samples in oven: Trials were conducted to determine the influence of the arrangement of the samples on the shelves upon the control of temperature and the rate of oxidation. In these trials, the samples were distributed widely over the shelves (Diagram I, Figure 1), or grouped closely together (Diagram II, Figure 1).

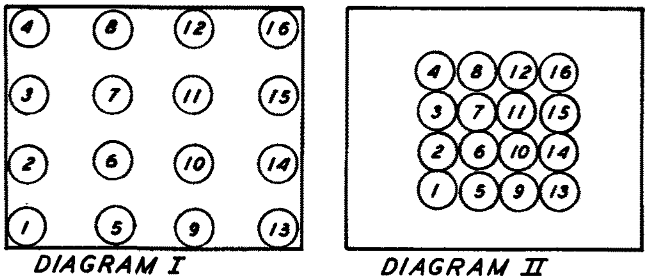

DIAGRAM I DIAGRAM II

FIG. 1. Diagrammatic sketch showing the arrangement of samples in the hot air oven. (Drawn approximately to scale.)

When the samples were widely distributed, and there was no positive air agitation, a maximum temperature difference of 6.4°C. was observed. The higher temperatures were in those samples located along the left wall near the heating vents and along the back wall, whereas the lower temperatures were in the samples placed along the front of the oven and along the right wall. The same general results were secured when the air agitator was running, although the maximum temperature difference in this case was only about 3°C. However, irrespective of the presence or absence of positive air circulation, wide variations in the rate of oxidation occurred, there being a general direct relationship between the temperature of the fat and the rate of oxidation. Only in these samples located near the center of the oven (samples 6, 7, 10, 11) was it possible to secure close agreement in peroxide values after uniform oxidation periods.

When the samples were grouped closely together as in Diagram II, Figure 1, and when no positive air agitation was utilized, a maximum temperature variation of 5.7°C. was obtained, a value of 0.7°C. less than was observed when the samples were widely distributed. When air agitation was used, the maximum variation was 1.9°C., 1.1°C. less than the variation secured when the samples were widely dispersed over the entire shelf. Although these results indicate only a comparatively slight difference in temperature control between those samples grouped closely together and those distributed widely over the shelves, there was a marked difference in the uniformity of oxidation. Those samples distributed widely over the shelves were found to vary greatly in the rate of oxidation, whereas those samples grouped closely together oxidized at a fairly uniform rate.

Since greater uniformity of results were secured when the samples were grouped closely together in the middle of the shelves, this arrangement was utilized in all subsequent trials. When this arrangement was used with positive air circulation, the temperatures on the bottom shelf varied from 99.2 to 99.9°C. (Avg. 99.6°C.), and those on the upper shelf varied from 97.6 to 99.5°C. (Avg. 98.9°C.).

Top and bottom shelves: The lower shelf consistently maintained a slightly higher temperature than the upper shelf even though they were only about four inches apart, and were both well removed from the heating elements. In addition, there was a marked tendency for the samples on the lower shelf to oxidize slightly more rapidly than those on the upper shelf. Data secured on 784 samples of fat in which one lot was oxidized on the top and the other lot on the bottom shelf, showed 66.5 per cent of the samples on the bottom shelf oxidized more rapidly than those on the top shelf. The average peroxide value of all samples taken from the bottom shelf was 8.10 whereas that for the top shelf was 7.16, an average difference of 0.94 peroxide units.

Further studies of the results secured with the top and bottom shelves of the hot air oven were conducted by utilizing the induction periods involved. A summary of these data is presented in Table I.

These data show that 50 per cent of the samples varied by not more than one hour, whereas 92.5 per cent varied by not more than two hours. Further analysis of the original data revealed that in 85 per cent of the trials, samples on the bottom shelf oxidized first and only in 10.5 per cent of the trials did those samples on the top shelf oxidize more rapidly. The average length of the induction period for the top and bottom shelves was 19.07 and 18.06 hours respectively, a difference of 1.01 hours. Thus, the normal difference between the two shelves was about 5.2 per cent.

TABLE I

Hours Difference in Induction Period of Fat Oxidized on the Top and Bottom Shelves of the Oven.*

Induction period (hours)	Number of samples	Number of samples varying in induction period when on top and bottom shelves.		
		Difference in hours		
		0-1.0	1.1-2.0	2.1-3.75
10.0-15.0	10	6	3	1
15.1-17.5	17	8	9	0
17.6-20.0	21	9	9	2
20.1-22.5	11	5	4	2
Above 22.5	7	4	3	0
Per cent		50.0	42.5	7.5

* Peroxide value of 5 indicated end of induction period.

Although the difference in the rate of oxidation between samples on the top and bottom shelves is not great, nevertheless, such a difference may materially influence the results in oxidative studies. In conducting subsequent studies, the difference in rate of oxidation in the two shelves was compensated for by placing one duplicate sample on each of the shelves and then removing the duplicates simultaneously for determinations.

Temperature of the oven: The temperature at which oxidation is accelerated is doubtless an important consideration, even though the temperatures used by workers for this purpose vary widely. One prevalent view is that lower temperatures of 60-80°C. are more desirable than higher temperatures since at these temperatures there is less destruction of the peroxides which result from oxidation. Possibly some temperature exists at which destruction of peroxides is at a minimum and formation at a maximum, thus increasing the sensitivity of rapid oxidation methods.

To determine the relative accuracy of accelerating oxidation at different temperatures, two identical Cenco-DeKhotinsky thermostatically controlled hot air ovens were utilized, each having a mechanical air agitator. Butter oil was oxidized in quadruplicate in these ovens, first at 70° and 100°C. then at 100° and 130°C. The results are presented in Table II.

TABLE II

Influence of Temperature of Hot Air Oven Upon the Rate of Oxidation and Upon the Uniformity of Results.*

Oven temperature (°C.)	Induction period			Deviation between minimum and maximum	
	Average (Hours)	Maximum (Hours)	Minimum (Hours)	(Hours)	(Per cent)
70	142.0	152.0	138.0	14	9.9
100	26.5	28.0	25.5	2.5	9.4
130	3.5	3.7	3.4	0.3	11.6

* Peroxide value of 5 indicated end of induction period.

As expected, the rate of oxidation varies directly with the temperature, with the 70°C. giving an extremely slow rate and the 130°C. an extremely rapid rate. From the standpoint of the percentage variation involved, there appears to be little difference between the 70°C. and the 100°C. temperature. However, the slow rate of oxidation induced by the 70°C. temperature is definitely a disadvantage unless it can be shown to appreciably contribute to the accuracy of measuring the induction period. In contrast, the extremely rapid rate of oxidation which occurred at 130°C. is also undesirable since it would not permit sharp differentiation between the induction periods of fats of different stabilities.

The temperature utilized for fat oxidation studies should naturally be that which is best adaptable to the laboratory program. Under the conditions of this experiment, the 100°C. temperature was considered best suited since it produced oxidation in the fat within 12 to 24 hours. Thus, it was possible to start the oxidation in the evening and complete the studies during the next day.

Sample size and surface area: Careful consideration should be given to the size of the sample and to the surface area of the sample if uniformity of results is to be secured by the oven method. An experiment was conducted to demonstrate the importance of these two factors. The results are illustrated in Figure 2.

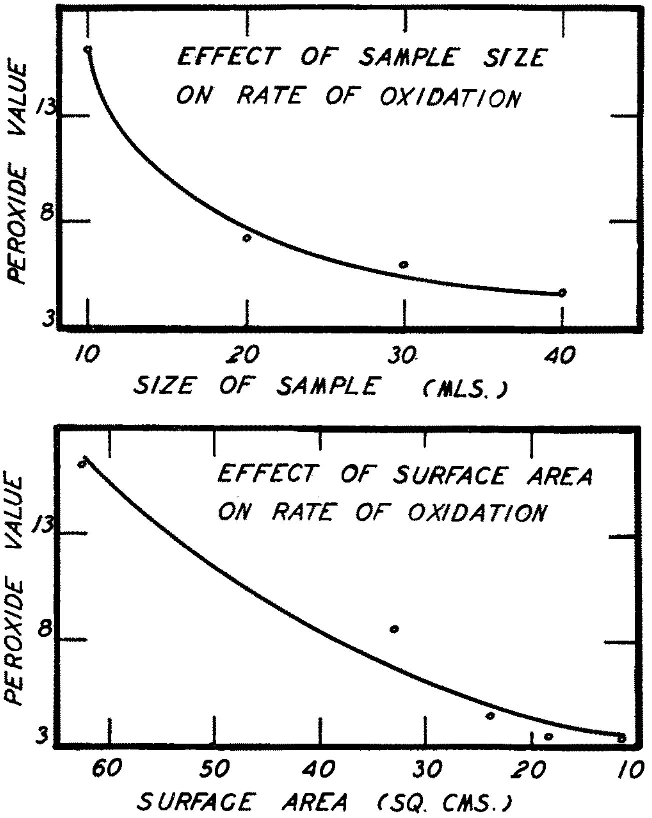

FIG. 2. Effect of sample size and surface area on rate of oxidation of butter oil.

This figure shows that either decreasing the size of the sample or increasing the surface area markedly affects the rate of oxidation. Thus, in oxidation studies, standardization of the size of sample and of the size of the container is essential if reliable results are to be secured. In this connection, the splashing of fat upon the side of the container should be avoided since obviously this would increase the surface area and thus hasten oxidation.

Comparison of oven and aeration method: Following the standardization of the oven method based on the factors studied, the reliability of the method was determined and a comparison made with the aeration procedure. In one series of 12 trials, quadruplicate samples of fat were oxidized in the oven, two samples in each set being on each of the two shelves. The results showed an average maximum difference in the induction period in each trial of 2.15 hours or a 7.8 per cent difference. The differences in induction periods ranged from 3.3 per cent to 11.1 per cent for the twelve trials. In other trials involving the use of the aeration method, the difference between the induction periods of triplicate samples of fat varied from 11.1 to 3.8 per cent, values similar to those secured by the oven method.

Following these general observations, more direct comparison of the reliability of the oven and aeration methods was made. The results of this comparative study are presented in Table III.

TABLE III

Induction Periods of Duplicate Samples of Fat Oxidized by Oven and Aeration Methods.*

Trial number	Oven method			Aeration method		
	Duplicates		Difference	Duplicates		Difference
	a	b		a	b	
	Hours	Hours	Hours	Hours	Hours	Hours
1	19.5	19.5	0.0	21.5	22.5	1.0
2	17.5	18.5	1.0	18.5	17.5	1.0
3	22.5	21.5	1.0	23.0	23.0	0.0
4	11.5	11.0	0.5	16.0	16.0	0.0
5	11.0	10.5	0.5	14.5	15.5	1.0
6	16.0	15.5	0.5	20.5	21.5	1.0
7	14.0	14.0	0.0	17.0	17.0	0.0
8	13.5	14.0	0.5	21.0	21.0	0.0
9	12.0	11.5	0.5	18.0	18.5	0.5
10	17.0	18.0	1.0	25.0	23.5	1.5
11	13.5	14.0	0.5	19.5	19.0	0.5
Average			0.55			0.59

* Peroxide value of 5 indicated end of the induction period.

These data show a maximum variation of 1.0 hour for the oven method as compared to 1.5 hours maximum variation for the aeration method. On the basis of percentage variation, the hot air oven varied 5.6 per cent whereas the aeration method varied 6.4 per cent. However, the average percentage difference between the duplicates is 3.6 per cent in the case of the hot air oven as compared to 3.0 per cent for the aeration method, an insignificant difference.

Another means of determining the reliability of the acceleration methods is to ascertain the uniformity of peroxide values of fat samples oxidized for equal periods of time. In this connection, data were secured of 491 duplicate samples of butter oil, 163 samples oxidized by the aeration method, 378 by the oven method. The results are shown in Figure 3.

Approximately 37 per cent of those duplicates oxidized in the aeration apparatus varied between themselves by less than 0.6 peroxide units as contrasted to about 29 per cent for the oven method. However, 65.6 per cent of the samples from the aeration method and 74.8 per cent of the samples from the oven method varied by not more than 2.0 peroxide units between duplicates. Only 7 per cent of the 328 duplicates oxidized in the oven varied by more than 5 peroxide values as contrasted to 13.5 per cent of the 163 samples oxidized by the aeration procedure. On the basis of these results, there appears to be no appreciable difference between the reliability and uniformity of the results secured by these two acceleration methods under carefully controlled conditions.

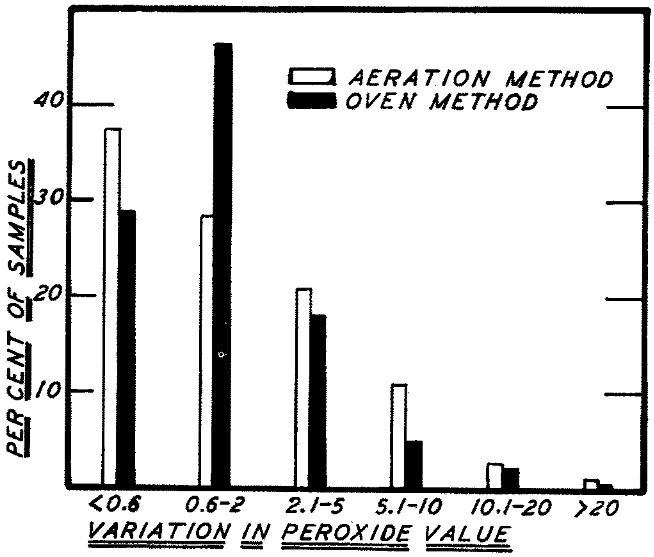

FIG. 3. Variation between duplicate samples in the aeration and oven methods.

Speed and extent of oxidation: When comparisons were made between the aeration and oven methods, it was observed that the oven method tended to produce oxidation more quickly. This may be observed in the data in Table III, which reveal that the oven method on the average gave a shorter induction period by 4.25 hours, a reduction of approximately 22 per cent. However, even though the fat in the oven method oxidized sooner the oxidation failed to proceed as rapidly or to the extent as in the case of the fat in the aeration apparatus. This relationship is illustrated by Figure 4. In this connection, it should be emphasized that the fat in the aeration procedure was in an air saturated condition and that the same volume of fat was utilized by both procedures.

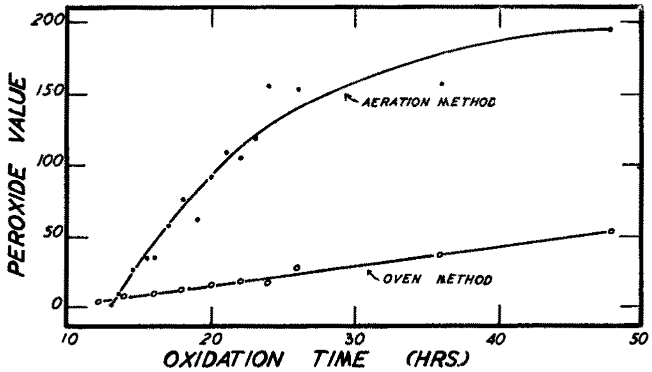

FIG. 4. Rate and extent of oxidation of butter oil by the aeration and oven methods.

Figure 4 shows the rapid and great extent of oxidation which occurs with the aeration method and also illustrates the fact that over a relatively long period of time the oven method gives a fairly straight line relationship between oxidation time and peroxide increases.

The longer induction period which results when the aeration method is used is doubtless due to the presence of water which is carried into the fat by the water-saturated air. When this air was dried by passing it through sulfuric acid, the induction period was shortened by some 15-20 per cent.

Summary and Conclusions

Investigations were made to compare the aeration and hot air oven methods of accelerating fat oxidation at 100°C. Results show these two methods to be equally reliable when careful technique is exercised and when consideration is given the influence of certain variable factors. On the basis of the findings in this study, ten per cent variation in induction periods between samples of the same fat constitutes a generous allowance. In these studies, the average variations for the oven method in two different series of trials were 7.8 and 3.6 per cent, respectively. Similar results were secured by the aeration procedure. The results with the oven method are in marked contrast to the four day or 26.7 per cent variation reported in the work of Dahle and Nelson (6).

Approximately two-thirds of the aeration samples and three fourths of the oven samples varied between duplicates by less than 2.0 peroxide units.

Especial attention should be given to temperature control, air agitation, and arrangement of the samples if the oven method is to give uniform results. Furthermore, factors of surface area and size of samples must also be controlled.

The temperature at which the oven is operated should be such as to permit the oven method to be adapted to the laboratory routine. Excessively high temperatures should be avoided, but in this experiment 100°C. gave as satisfactory results as did 70°C. The temperature used, however, may be varied depending upon the type of fat or oil being studied, the size of the sample, and the surface area involved.

Under the conditions of this experiment the oven method gave a shorter induction period for the fat than the aeration procedure but the extent of oxidation over a relatively long period of time was less.

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The Saturated Fatty Acids of Japan Wax[1]

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Japan wax is the fruit-coat fat—the designation "wax" is a misnomer—of a sumac *(Rhus succedanea L.)* abundant in southwestern Japan where it is produced in commercial quantities. It has some unique physical features, such as plasticity, and a comparatively high density. Its chief chemical characteristics are an extremely high content of the glycerides of palmitic acid and a relatively small percentage of high molecular weight dicarboxylic acids, the latter providing much of the research interest in this fat. They are, in fact, held to be responsible for its plasticity (1).

Despite the very considerable amount of work which has been done on this fat, the constitution of its dicarboxylic acids is still in doubt. Eberhardt (2), who first isolated them, assigned to its chief acid of this type the formula $C_{20}H_{38}O_4$. Since then formulas involving 19 to 23 carbon atoms have been proposed. More recent studies in this field have been by Ruzicka *et al.* (3) who suggested the formula $C_{21}H_{40}O_4$ on the basis of molecular weight, ultimate composition, and a mixed melting point with a synthetic product. Later Flaschenträger and Halle (4) claimed the isolation of an acid of formula $C_{23}H_{44}O_4$. They established the straight-chain character of the molecule and the position of the carboxyl groups on the terminals of the chain. Tsujimoto (1) then verified this formula. More recently Siina (5), on the grounds of mixed melting points with synthetic products, reported the formula $C_{22}H_{42}O_4$.

The constitution of the bulk of the *n*-monocarboxylic acids has not received such extensive attention. In fact, not until 1935 was any quantitative, or even semi-quantitative, attempt at the analysis of the total acids made other than the detection of major quantities of palmitic acid. It was then that Tsujimoto (6) reported that he had found an unbleached sample of Japan wax to consist of palmitic acid (77 per cent), stearic acid (5 per cent), arachidic acid in traces, dibasic acids (6 per cent), oleic acid (12 per cent) and linoleic acid (less than 1 per cent). Crude ester fractionation and recrystallization techniques had been used in arriving at these results. It is also a matter of interest that Flaschenträger and Halle (4) have claimed the isolation of a heneicosanic ($C_{21}H_{42}O_2$) acid in the course of their studies on the dicarboxylic acids, a unique discovery, indeed, if factual!

The fatty-acid composition of Japan wax is also of interest from a biochemical viewpoint. Chibnall and Piper (7), in a discussion of the biosynthesis of

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