ing 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 semiquantitative, 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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hydroxy and keto acids, postulate that not only ω-hydroxylauric and ω-hydroxypalmitic acids, but also tetradecane dicarboxylic acid are formed by the omega oxidation of the corresponding *n*-saturated fatty acids. Therefore, it would be of interest on the one hand to detect the presence of arachidic and behenic acids—in the light of this hypothesis they should be present if the dicarboxylic acids contain an even number of carbon atoms—and, on the other, to prove or disprove the presence of heneicosanoic, the C_{21} acid. The occurrence in nature of a dicarboxylic acid containing an odd rather than an even number of carbon atoms would also be of interest since its biosynthesis cannot be readily rationalized by any of the prevailing hypotheses of fatty-acid formation.

With the foregoing as a point of departure, an investigation was begun of the saturated fatty acids of the monocarboxylic and the dicarboxylic series. With respect to the former it was of special interest to learn whether arachidic and behenic acids, which contain carbon chains corresponding in magnitude to the dicarboxylic acids reported as occurring in Japan wax, would be found. Added interest lay in an attempt at verifying the alleged presence of the intermediate acid, heneicosanoic.

The dicarboxylic acids of this fat present a much more difficult problem. Evidence for the formulas suggested to date has been based upon the highest melting point noted for small fractions, whereas a number of intermediate ones of indeterminate composition usually existed. The most probable of the formulas advanced, or $C_{22}H_{42}O_4$ and $C_{23}H_{46}O_4$, were based upon the results of an ultimate analysis, molecular weight determinations and, in the case of the C_{22} acid, a mixed melting point with a synthetic product. The same criteria were used in the case of the C_{21} product.

Therefore, in view of the unreliability of mixed melting points as a criterion of identity and the indeterminate character of the various fractions obtained by others, it was decided to investigate the problem by synthesizing the various dicarboxylic acids in question and by constructing either a solidification point or a melting point curve for binary mixtures of them. These curves would perhaps assist in the identification of binary mixtures in a manner analogous to the role which the solidification point curves of Schuette and Vogel (8) play in the identification of *n*-fatty acids. The synthesis of these acids, however, presents some difficulty and because certain of the starting materials have proved difficult to obtain to date this phase of the problem is still in progress.

Experimental

A commercial sample of Japan wax, deemed to be genuine because its simpler chemical constants (saponification and iodine numbers, 210 and 11.7[2], respectively) were in line with accepted standards, was used in this investigation.

Alcoholysis of a 1510-g. sample and subsequent removal by vacuum distillation through a modified Widmer column of *ca* 85 per cent of the esters yielded a residue of 310 g. containing dicarboxylic acid esters. The acids therein were regenerated and recrystallized from benzene with a yield of 77.7 g. of high-melting material. These acids were converted to ethyl esters after which they were fractionally distilled to yield six fractions, boiling from 170° to 204° at 0.25 mm., and a residue.

Regeneration of the acids from portions of each fraction and subsequent repeated crystallization indicated that fractions 2 to 5 inclusive were probably complex mixtures of mono- and dibasic acids. However, fraction 1 gave evidence of being composed of only monocarboxylic acids and fraction 6 yielded a crystalline acid (m.p. 125.5-126.3) of molecular weight 382.6 on the assumption that it is dibasic. These values correspond to those of a compound of formula $C_{23}H_{44}O_4$ (1, 4).

Identification of Arachidic and Behenic Acids. The first fraction obtained above had a molecular weight of 318.4. Assuming a binary mixture, this value corresponds to one consisting of arachidic acid, 79 per cent, and behenic acid, 21 per cent (9). Its solidification point (67.9° C.) was only 0.5° below that of a mixture of the above composition. Upon the quantitative addition of pure behenic acid and a redetermination of the solidification point, it was found that calculated and observed values agreed, thus confirming the existence of arachidic and behenic acids and precluding the occurrence of heneicosanoic acid. Quantitatively the above values correspond to 0.04 per cent and 0.01 per cent, respectively, of the total acids.

Ester-fractionation of the Mixed Saturated Esters. Unsaturated acids were removed by brominating (10) the mixed ethyl esters and rapidly distilling the esters of the saturated acids away from the brominated esters. The former were then fractionally distilled after the addition of a 50-gram mixture of the ethyl esters of arachidic and behenic acids. This addition was made to prevent the formation of a residue consisting of a mixture of all the higher components and part of the stearic acid, a procedure which, of course, sacrifices the possibility of making an analysis for arachidic and behenic acids, which in this case previously had been identified. The advantage of this procedure is a more exact analysis for palmitic and stearic acids.

The mixed, saturated esters were subjected to fractional distillation at 0.25 mm. through a modified Widmer column with the following results.

TABLE 1

Fractionation of Saturated Fatty Esters

Fraction	Boiling range °C.	Weight g.	Properties of acids	
			Mol. wt.	Sol. pt. °C.
1	-139	12.3	250.9	57.35
2	139-140	89.9	256.2	60.55
3	140-162	61.1	263.7	54.15
4	162-179	47.1	305.9	65.95

The percentage composition of the fatty-acid mixture was calculated both from the molecular weight and the solidification-point diagrams of Schuette and Vogel (8). The resulting values for total composition did not vary by more than 1.5 per cent in any instance for the two methods employed. The calculated percentages of total mixed acids found are: lower molecular weight acids, as myristic, 1.9 per cent, palmitic 67.6 per cent, and stearic 11.6 per cent. The percent-

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[2] Commercial samples of this fat are usually subjected to a bleaching procedure. They are usually contaminated with some of the seed oil and for this reason have iodine numbers ranging from 4.2 to 15; hence they are unsuitable for an investigation as to the nature of the unsaturated acids.

age of total saturated acids (86.4 per cent) was calculated from the iodine number by virtue of the fact that the unsaturated acids are known to be almost entirely oleic.

The dicarboxylic acid content (5.3 per cent) was found by the method of Tsujimoto (1) which is based, essentially, upon the petroleum-ether insolubility of these acids.

Identification of Myristic Acid. In view of the unsatisfactory nature of the identification of myristic acid (see foregoing section), said not to exist in Japan wax glycerides by Tsujimoto (6), further studies were made of the low-boiling constituents of the 1600 g. of esters resulting from the original alcoholysis. On fractionally distilling them at 0.25 mm. through a modified Widmer column, the following results (Table 2) were obtained:

TABLE 2

Identification of Myristic Acid

Fraction	Boiling range °C.	Weight g.	Properties of acids	
			Mol. wt.	Sol. pt. °C.
A	111-124	19.1	235.6	44.9
B	124-135	25.1	253.0	58.9

Fraction A was found to consist of myristic acid 72.8 per cent and palmitic acid 27.2 per cent; fraction B of myristic 11.4 per cent and palmitic 88.6 per cent. Methods analogous to those used in the identification of arachidic and behenic acids above were used. The presence of myristic acid is, therefore, assured.

Summary

Myristic and behenic acids, hitherto not reported therein, have been shown to be components of the glycerides of Japan "wax" in contrast to the nonexistence of an alleged heneicosanoic acid. Ester-fractionation analysis revealed the following composition: myristic acid 1.9 per cent, palmitic acid 67.5 per cent, stearic acid 11.6 per cent, arachidic acid 0.04 per cent, behenic acid 0.01 per cent, unsaturated acids 13.6 per cent, and dicarboxylic acids 5.3 per cent. A preliminary investigation of the latter resulted in the isolation of an acid which by virtue of its melting point and molecular weight has been tentatively characterized as heneicosane dicarboxylic acid (C_{23}). However, final proof of identity must rest upon subsequent synthesis of this acid.

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Report of the Soap Analysis Committee—1942

The report of the Soap Analysis Committee for the current year must necessarily be brief and represents only a report of progress on unfinished subjects which have been studied previously by the Committee. Due to the fact that most of the members have been exceedingly busy under present existing conditions, it has been practically impossible to carry on any appreciable amount of cooperative studies. Such work as has been done has not yet been completed and, consequently, we are not in position to make an official report or recommendations.

Last year (1941) the Committee conducted some extensive studies in the determination of caustic potash and potassium carbonate in paste type soaps. The complete data of these studies were contained in our 1941 report and at the Committee meeting in October, 1941, some further studies were outlined to be undertaken by a sub-committee on the same problem. These studies are now under way but unfortunately no results are available at this time. It is hoped that these studies can be continued by the sub-committee and that a further report will be available at our next meeting.

One other item should be carried forward as a matter of record. The volumetric method for determination of tetra sodium pyrophosphate in soap was recommended for tentative adoption by the Committee at the 1941 Fall meeting. It is now recommended that this method be considered for official adoption at this time.

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