TABLE IV

Fatty Acid Composition and Iodine Numbers for Oil of Three Soybean Varieties in Uniform Test, Group VII, 1956[a] and 1957[b]

| Variety | Linolenic acid | | Linoleic acid | | Oleic acid | | Saturated acid[c] | | Iodine number | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 1956 | 1957 | 1956 | 1957 | 1956 | 1957 | 1956 | 1957 | 1956 | 1957 |
| | % | % | % | % | % | % | % | % | | |
| Jackson | 7.52 | 8.08 | 52.2 | 54.9 | 20.4 | 18.8 | 19.8 | 18.3 | 133.4 | 138.1 |
| Roanoke | 7.46 | 8.49 | 51.9 | 54.7 | 21.8 | 18.2 | 18.8 | 18.7 | 134.1 | 138.0 |
| Lee | 6.73 | 7.48 | 49.5 | 52.5 | 26.6 | 21.1 | 17.3 | 18.9 | 131.9 | 134.7 |
| Mean | 7.24 | 8.02 | 51.2 | 54.0 | 22.9 | 19.4 | 18.6 | 18.6 | 133.1 | 136.9 |
| Standard error for variety means | .08 | .10 | .29 | .63 | .98 | .93 | .77 | .43 | | |

[a] Mean of 14 locations.
[b] Mean of 5 locations.
[c] Contains some glycerol and other materials.

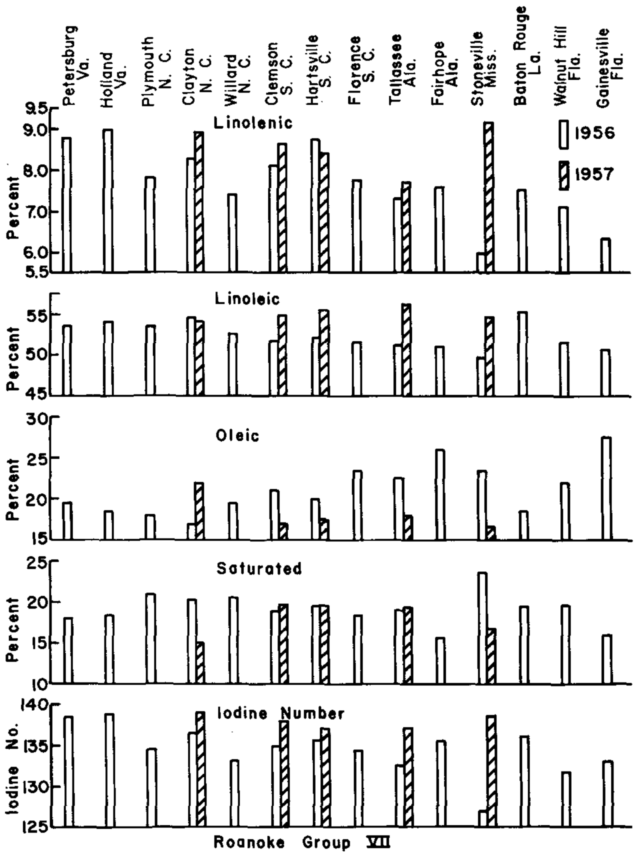

FIG. 5. Fatty acid composition and iodine number of oil from Roanoke soybeans grown at different locations, 1956–57.

prevailed at Ottumwa, Ia., during the critical period of fatty acid formation in seed filling.

These differences in temperature undoubtedly account for oil of Roanoke with 5.93% and 9.15% linolenic acid at Stoneville, and of Lincoln with 7.93% and 9.43% linolenic acid at Ottumwa in these two seasons.

## Summary

Fatty acid composition of soybean oil of 18 currently important varieties from 43 locations in 16 states of the United States ranged from about 5% to 11% in linolenic, 43% to 56% in linoleic, 15% to 33% in oleic, and 11% to 26% in saturated acids. Oil of all 18 varieties had wide ranges in composition at different locations in two crop years. Within each group varieties tended to maintain the same relative order of fatty acid compositions of oil at all locations in the two years of this study.

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# A Comparison of Six Solvents for the Extraction of Jojoba Seed

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A PLANT, native to the desert areas of Southwestern United States, offers excellent possibilities as a potential crop since it would not compete with such oils as cottonseed, soybean, or linseed but would diversify American agriculture by giving this country a new industrial raw material in an area in which production is at present practically nonexistent.

*Simmondsia chinensis*, commonly known as jojoba, is unique in that its seed contain almost 50% of a liquid wax which appears to be a source of many chemical compounds of great interest in many applications (6). The residual meal, after the extraction of the wax, can apparently be utilized in animal feeds (12).

Research is at present being carried on by the Department of Agriculture along two major lines

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[1] One of the laboratories of the Southern Utilization Research and Development Division, Agricultural Research Service, U. S. Department of Agriculture.

TABLE I

Conditions of Extraction and Desolventization of Oil

| Solvent | Extraction | | Desolventization | | Remarks |
|---|---|---|---|---|---|
| | Maximum flaked meats temperature °C. during extraction | Number of solvent passes through flakes | Maximum temperature °C. | Minimum pressure (abs.) mm. Hg | |
| Carbon tetrachloride | 73 | 22 | 110 | 23 | Brownish gelatinous precipitate noted in desolventized oil |
| Benzene | 67 | 20 | 102 | 12 | Oil apppeared slightly cloudy after desolventization |
| Heptane | 86 | 21 | 105 | 6 | Oil apppeared slightly cloudy after desolventization |
| Tetrachloroethylene | 95 | 24 | 102 | 12 | Small quantity of flocculant precipitate noted after desolventizing |
| Hexane | 65 | 20 | 97 | 4 | Clear yellow oil |
| Isopropyl alcohol | 84 | 24 | 99 | 3 | White, sugarlike, precipitate removed as formed during desolventization and in concentrated miscella by standing over-night |

a) horticultural development of superior jojoba plants, methods of cultivation, and harvesting (6), and b) the utilization of the liquid wax and/or its components.

In connection with the latter phase of these investigations, practical economical methods of extracting the wax from the seeds must be developed if commercial production and utilization are to be accomplished.

Previous work (6) had shown that the solid wax obtained by hydrogenation of the liquid wax, which was produced by the cold-hydraulic pressing of cracked and flaked jojoba seeds had a melting point of 67–68°C. as compared to a melting point of 74–76°C. found in the literature (4). Because of this appreciable difference in melting points it was believed that product characteristics might vary, not only with the seed and the method of extraction (*viz.*, hydraulic pressing or solvent extraction), or the degree of extraction but also, in solvent extraction, with the particular solvent used for extraction.

Of the controllable variables influencing the yield and qualities of the products in the solvent extraction of oilseeds, the nature of the solvent is probably the most important (2, 7). Although the literature contains data on the composition and physical properties of jojoba oil (1, 4, 6, 8, 9, 11), no particular attention was paid to the method of obtaining the oil.

So far as is known to the authors, the effect of different solvents on the yield and properties of the oil (liquid wax), and subsequently the hydrogenated wax, from jojoba have not been previously explored on a comparative basis in which the solvent was the only significant variable.

It is the purpose of this paper to present data showing the effect of six different solvents on the yield and properties of the liquid wax from *Simmondsia*, and the subsequent effect of each solvent upon the characteristics of the hydrogenated waxes obtained from the liquid waxes. Soxhlet type of extractions were carried out under conditions in which the solvent was the only significant variable. For purposes of comparison, data on the characteristics of liquid wax and solid wax from *Simmondsia chinensis* which was obtained by cold-hydraulic pressing are included.

## Materials

*Seed.* This work was carried out on jojoba seed which were obtained from the Boyce Thompson Arboretum, Superior, Ariz., during December 1954. From receipt until used, they had been stored at 1–5°C. Analyses of the seed showed 4.2% $H_2O$, 2.5% nitrogen, and 44.0% liquid wax (using A.O.C.S. method Ba 3-38 for lipids). A portion of these seed were cold-hydraulic pressed (5). Samples of the oil and hydrogenated waxes were retained for comparison with the oils and hydrogenated waxes obtained in the work reported in this paper.

*Solvents.* Six solvents were selected for use as extractants on the basis of their ability to dissolve waxes, their boiling point range, and polarity or nonpolarity. They were carbon tetrachloride, benzene, isopropyl alcohol, heptane, hexane, and tetrachloroethylene. Of these the first three were reagent grade and the last three commercial grade.

The benzene contained 0.02% moisture, the isopropyl alcohol 0.23% moisture. All other solvents were moisture-free.

## Equipment

The equipment used for these experiments consisted of a stand of two-high (10) corrugated cracking rolls 12 in. wide by 12 in. in diameter, a two-high (10) stand of smooth flaking rolls 12 in. wide by 12 in. in diameter, a modified Soxhlet extractor and condenser, Whatman fat extraction thimbles, a glass boiler which could be heated either by a steam-heated oil bath or with an electric heating mantle, glass vacuum-stripping and desolventizing equipment, and small-scale glass laboratory hydrogenation equipment as described by Feuge *et al.* (3).

## Procedure

*Preparation of the Flakes for Extraction.* Upon removal from cold storage, the seed were equilibrated to room temperature in a sealed container. They were then cracked into 8–12 pieces by passing them through the corrugated rolls, which had been set at a clearance of 0.040 in. between the rolls. Cracking was carried out at the rate of approximately 250 lbs. per hour.

The cracked meats were passed through a pair of smooth rolls, which had been set at a clearance of 0.004 in. between rolls, at the rate of approximately 150 lbs. per hour. Well-formed flakes were produced that had an average thickness of 0.010 in. Sufficient flakes for 12 extractions were produced at one time and stored in sealed containers at 0–4°C. until used.

*Extraction.* For each extraction exactly 135 g. of the flakes were charged to a tared 6-in.-deep Whatman fat-extraction thimble. The thimble was placed in the Soxhlet extractor and 1,500 ml. of the solvent to be used was charged to the boiler. A total of 20 to 24 passes of solvent through the flakes were made. This resulted in a solvent-to-flake ratio of more than 75 to 1. All extractions were carried out at ambient pressure. Duplicate batches of flakes were extracted with each solvent. Table I shows the conditions of extraction used.

*Desolventization of Meals and Oils.* Immediately after the extraction was completed, the residual meals were spread on metal pans and desolventized in air at ambient temperature for 72 hrs. The meals from each pair of extractions were composited, sampled, and analyzed for moisture content.

The composite miscella from each pair of extractions was desolventized under vacuum at low temperature over a two-hour period by using water-pumped nitrogen as the sparging agent. The highest temperature recorded during desolventization (97–110°C.) was reached only for a short time at the end of the period. Table I gives the conditions of desolventization. The resulting stripped oils were dried in a vacuum oven at 105°C. for 2 hrs.

Material balances shown in Table II were based upon the weight of desolventized oil and meal recovered. Table III gives some of the physical characteristics of the oils.

*Hydrogenation.* It was noted that a fine crystalline precipitate developed in each of the desolventized solvent extracted oils. The quantity of precipitate varied from solvent to solvent (Tables II, III). To determine if the precipitate had a higher melting point or harder wax constituent the samples were each divided into two portions by decantation, the top (clear fraction) and the bottom (precipitate-containing fraction). Because of the excessive amount of precipitate, the bottom fraction from the isopropyl alcohol extraction was not hydrogenated. A 25-g. portion of each of these fractions was hydrogenated in glass equipment, using nickel catalyst (1% by weight). Hydrogen gas at 2.5 lbs. per sq. in. of gage pressure was passed through the sample, the temperature of which was maintained at 188–192°C. for 3 hrs. The hydrogenated waxes were filtered while hot, cooled, remelted, refiltered, and recooled. The clear fractions from the hexane, carbon tetrachloride, and heptane extractions had iodine numbers of 0.46, 0.39, and 0.26, respectively, while the precipitate fractions from these extractions had iodine numbers of 0.9, 0.9, and 0.8, respectively, after three hours of hydrogenation. Three samples of clear fractions and two precipitate fractions failed to hydrogenate to iodine numbers of less than 1 by this procedure. To ascertain the effect upon hardness and melting point and to determine the time required to reduce the iodine number to 1 or below, the hydrogenated waxes from the clear fractions of the benzene, tetrachloroethylene, and isopropyl alcohol extractions were rehydrogenated (using 1% fresh catalyst) for 1½ additional hours under the same conditions of temperature and pressure as described above. After a total of 4½ hrs. of hydrogenation these samples had iodine numbers of 0.36, 0.67, and 0.87, respectively. The small amount of the two precipitate samples (benzene and tetrachloroethylene) did not permit rehydrogenation.

With iodine numbers of less than one, all samples had melting points between 65 and 68°C., and hardness values of 90 on the trionic gauge.

## Discussion of Results

The data reported in Table II demonstrate that in so far as oil yield is concerned, carbon tetrachloride, benzene, heptane, and hexane all extract substantially the same amounts. Isopropyl alcohol extracted more material; however a considerable amount of precipitate formed upon cooling. Possible explanation for the formation of the precipitate at this point is that the solubility of the wax in isopropyl alcohol varies with the temperature. While the greater portion of this precipitate redissolved upon heating, it reformed when the concentration of the wax-alcohol miscella reached approximately 50%.

It is postulated that the isopropyl alcohol extracted considerable matter other than wax, such as sugars, which are insoluble in the miscella at 50% concentration. This seemed probable in view of the fact that when desolventization of the wax was continued after the precipitate formed, it gradually assumed a brown color and became amorphorous. This strongly suggests caramelization. As a consequence of this phenomenon, additional extractions were made with isopropyl alcohol, and the precipitate was removed as soon as it formed during concentration and stripping operations. Samples of the precipitate were tested and found to be sugars.

Small amounts of a precipitate were noted in all wax samples after standing for 7–10 days except in the wax obtained by cold pressing (Tables II and III). When fractions of these waxes containing the precipitate were hydrogenated, no significant differences were noted in the characteristics of the resulting hard waxes. Melting points were between 64 and 67°C., hardness between 80 and 90 as measured by the trionic gage, and iodine values all below 1.0. Hydrogenation of a sample from which the precipitate was removed by acid washing resulted in a hard wax with a melting point of 65–66°C., hardness of 90, and an iodine number of 0.8. As a control a sample of cold-hydraulic pressed wax which contained no precipitate was acid-washed and hydrogenated. This product had a melting point of 66–67°C.,

TABLE II

Materials Balances for Different Solvents

| Solvent | Feed flakes–$H_2O$ free g.[a] | Extracted flakes | | | Precipitate recovered g. | Oil recovered g. |
|---|---|---|---|---|---|---|
| | | Recovered g. | $H_2O$ % | Free $H_2O$ g. | | |
| Carbon tetrachloride | 258.9 | 142.7 | 6.6 | 133.3 | Trace | 122.6 |
| Benzene | 259.2 | 143.4 | 6.5 | 134.0 | Trace | 122.2 |
| Hexane | 259.2 | 140.5 | 7.2 | 130.4 | Trace | 126.4 |
| Isopropyl alcohol | 259.2 | 109.8 | 6.5 | 102.7 | 52.8 | 93.5 |
| Tetrachloroethylene | 259.2 | 152.5 | 5.8 | 143.7 | Trace | 113.4 |
| Heptane | 259.2 | 142.4 | 6.5 | 133.1 | Trace | 124.7 |

[a] Initial moisture of feed flakes — 4%.

TABLE III

Oil (liquid wax) Characteristics

| Solvent | Index of refraction $N^{25/D}$ | Density (20°C.) | Iodine value wijs | Phosphorous % | Unsaponifiables % | Oil color | Remarks |
|---|---|---|---|---|---|---|---|
| Carbon tetrachloride | 1.46656 | 0.8644 | 83.6 | 0.004 | 49.6 | Dark yellow | White crystalline precipitate in evidence after standing |
| Benzene | 1.46657 | 0.8645 | 83.6 | 0.001 | 50.1 | Dark yellow | White crystalline precipitate in evidence after standing |
| Heptane | 1.46649 | 0.8641 | 83.2 | 0.006 | 50.2 | Dull yellow | White crystalline precipitate in evidence after standing |
| Isopropyl alcohol | 1.46676 | 0.8649 | 84.3 | <0.001 | 49.9 | Dull yellow | White crystalline precipitate in evidence after standing |
| Tetrachloroethylene | 1.46686 | 0.8631 | 83.3 | 0.006 | 50.1 | Dark brownish yellow | Dark brown precipitate in evidence upon standing |
| Hexane | 1.46642 | 0.8631 | 83.3 | <0.001 | 49.8 | Bright yellow | Slight white crystalline precipitate on standing |
| Cold-hydraulic pressed | 1.46440 | 0.8646 | 83.2 | ...... | 50.3 | Bright yellow | No precipitate on standing |

a hardness of 92, and an iodine value of 0.6, which demonstrates that acid washing had no significant effect on these characteristics of the hard waxes.

The cold-pressed liquid wax was used as the control since it was believed that this method of extraction would have the least undesirable effects on the liquid wax product. It was recognized that cold-hydraulic pressing of the jojoba flakes was inefficient in that residual lipids were about 11%. However it was assumed that the cold-pressed wax would be representative of the liquid waxes as they exist in the seed. The assumption made is deemed valid since no significant differences were found in the iodine value, hardness, and melting points of the solvent-extracted waxes from the values obtained for the cold-hydraulic pressed waxes.

Tetrachloroethylene extracted significantly less material than any of the other solvents. In addition, the wax fraction after concentration and stripping was considerably darker (Table III) than the waxes obtained from the other solvents, suggesting that some of the pigments occurring in the seed skins were extracted.

From Table III it will be noted that the density values reported for the waxes obtained from the extractions made with carbon tetrachloride, benzene, heptane, and isopropyl alcohol, as well as the wax obtained by cold hydraulic pressing, all had values which fell between 0.8641 and 0.8648 g. while the waxes obtained by extraction with hexane and tetrachloroethylene were significantly less dense at 0.8631.

From Table III it will be seen that a variation exists in the values reported for the index of refraction for the various liquid waxes, the lowest value (1.46440) being reported for the cold-hydraulic pressed wax and the highest value (1.46686) for the tetrachloroethylene-extracted wax.

No significant variation was found in the Wijs iodine value (83.2–84.3), phosphorous content (0.001–0.006%), and unsaponifiable matter of the waxes extracted by cold-hydraulic pressing and those extracted by the various solvents (49.6–50.3%).

Some of the liquid waxes did not hydrogenate to iodine values of less than 1.0 in a 3-hr. period. However when samples were hydrogenated for an additional 1½ hr. with fresh catalyst, iodine values of less than 1.0 were obtained. The fact that some samples of wax hydrogenated more readily than others is not considered of great importance. Very minute quantities of impurities may have been responsible for poisoning the catalyst, particularly in view of the fact that crude liquid waxes were hydrogenated. It is also known that some solvents contain small amounts of degradation products that are catalyst poisons.

On the basis of these data it appears that the difference in melting point between these samples (66–68°C.) and those reported in the literature (74–76°C.) does not result from the solvent used but may be attributable to the variety of seed and/or the treatment of liquid wax before hydrogenation.

## Summary

Data are presented which show the effects of different solvents on the yield and properties of liquid wax from *Simondsia chinensis* (jojoba) and on the characteristics of the hydrogenated waxes obtained from the liquid waxes.

Three reagent grade solvents, carbon tetrachloride, benzene, and isopropyl alcohol, and three commercial grade solvents, heptane, hexane, and tetrachloroethylene, were evaluated as extractants for the liquid wax from jojoba. Soxhlet-type of extractions were carried out under conditions in which the solvent was the only significant variable.

Four of the solvents extracted essentially the same amount of material from the seed while isopropyl alcohol extracted significantly more material and tetrachloroethylene significantly less. Obviously the difficulties involved in separating the solids recovered from the isopropyl alcohol extraction preclude its use as the extracting solvent for jojoba wax.

The density of the liquid waxes varies from 0.8631 to 0.8648; the waxes from the tetrachloroethylene and hexane extractions had the lowest value and the wax from isopropyl alcohol the highest.

In each case, regardless of the solvent used, a precipitate developed in the liquid wax after it had been desolventized and stored for 7–10 days. Hydrogenation of clear fractions and precipitate containing fractions of these liquid waxes showed that the precipitate had no apparent effect upon the melting point or hardness of the resulting solid wax.

Some of the liquid waxes required a longer hydrogenation time to attain an iodine value of about 1. At this iodine value all of the solid waxes had melting points between 66 and 68°C.

Hardness values of all the solid waxes as measured by the Trionic hardness gauge were 90.

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# Isolation and Identification of Carbonyl Compounds Formed by Autoxidation of Ammonium Linoleate

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VARIOUS STUDIES, involving the volatile decomposition products formed by autoxidation of edible fats and oils, have been carried out during the past few years. Among these products many carbonyl compounds have been identified (4, 7, 8, 9, 12, 14, 15, 16, 19, 20, 21, 22).

It is commonly accepted that the autoxidation of nonconjugated unsaturated fatty acids starts with the formation of $\alpha$-methylene radicals and that the hydroperoxides produced in the so-called propagation stage decompose to form carbonyl compounds, keto acids, etc. As will be discussed later, these theories imply that specific carbonyl compounds are to be expected as the (primary) products of the decomposition of the hydroperoxides derived from a specific unsaturated fatty acid.

However too complicated a mixture of unsaturated fatty acids has usually been studied. A mixture involves the production of a whole series of carbonyl compounds, each of which may have been formed in several ways. Swift, O'Connor, Brown, and Dollear (22) and Lea (17) have shown that specific carbonyl compounds are to be expected and have, in fact, been found in autoxidizing systems containing linoleate.

Using a method published by van Duin (11), we have studied the main carbonyl compounds produced after a limited autoxidation of a system containing linoleic acid (in the form of ammonium linoleate) as the only fatty acid, with the object of discovering whether these carbonyl compounds in fact were those to be expected on theoretical grounds.

## Methods

*Autoxidation of Ammonium Linoleate.* Linoleic acid of a high degree of purity (prepared from the urea addition compound, giving 99.3% purity) has been used in the work described. A limited autoxidation was carried out according to a method described by Tappel (23). A solution of 0.02 M. ammonium linoleate in 0.1 M. of phosphate buffer of pH 6.9, containing $10^{-4}$ M. $CuSO_4$ as a catalyst, was subjected to autoxidation in air at 37.2° C., using a Warburg apparatus. After an uptake of ½ mol. $O_2$ per mol. of linoleate, the reaction was stopped.

*Concentration of Carbonyl Compounds.* The carbonyl compounds were collected by extracting the reaction mixture three times with 25 ml. of carbonyl-free benzene. This extract was chromatographed, using a column of aluminum oxide containing 5% water; the oxidation products, such as acids and peroxides, were retained. In this way a benzene solution containing the carbonyl compounds free from acids, peroxides, etc., was obtained.

*Conversion into D.N.P.-Ones.* The conversion of the carbonyl compounds into their D.N.P.-ones (abbr. for 2,4-dinitrophenyl hydrazones) was carried out by adding 0.5 g. of 2,4-dinitrophenyl hydrazine and 2.5 g. of trichloroacetic acid to the benzene solution and leaving the mixture in the dark at 20°C. for 12 hours. The reaction mixture was shaken twice with 5% sodium bicarbonate solution, then three times with water, and dried over anhydrous magnesium sulphate. The benzene was removed by distillation under reduced pressure and a temperature not to exceed 30°C. Finally the D.N.P.-ones were extracted from the residue with four 10-ml portions of carbonyl-free petroleum ether (b.p. 60–80°C.).

*Separation of the D.N.P.-Ones.* Separation and identification were carried out, using the method of van Duin (10, 11).[2] By partition chromatography on a column with nitromethane on silica gel as the immobile phase and petroleum ether (b.p. 60–80°C.) as the mobile phase, a separation based on the chain length of the carbonyl compounds was carried out. The existence of a rectilinear relation between the logarithms of the retention volmes of the D.N.P.-ones of the single members of various homologous series of carbonyl compounds and the sequence of these members in these series was used for the determination of their chain length.

A further separation of each fraction from the partition column was necessary because D.N.P.-ones of different types of carbonyl compounds may possess the same retention volume in spite of a different chain-length. This was effected by van Duin *(loc. cit.)* by

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[2] This method will be described in more detail in a thesis which will be published in the near future.