beginning with the addition of 9% sodium sulfate solution.

Discussion

Carbon tetrachloride has been used in all cases to extract the malathion from samples; however, hexane may be used in the extraction step (*2*) and probably in the decomposition step. Limited data indicate that methanol may be substituted for ethyl alcohol; however, isopropyl alcohol was unsatisfactory. The amount of sodium hydroxide to be added for decomposition of malathion should equal 1 ml. of 6*N*, plus that consumed by any free acidity or ester hydrolysis of the sample and should be determined experimentally for each type of product. The concentration may vary, but the volume should be held constant at 1 ml. in order to keep the water content at a minimum.

Usually, different techniques must be used to analyze each type of animal product—e.g., the whites and yolks of eggs were analyzed because the whites have a naturally occurring high alkalinity (pH 8.2 to 9.8 depending upon age) and must be acidified before extraction in order to prevent alkaline decomposition of the malathion. Because of this naturally occurring alkalinity, malathion would probably not be found in the whites of eggs from treated birds. In the development of a method for malathion in beef liver, data showed that malathion is probably converted by contact with raw beef liver macerate to a product which does not respond to the colorimetric procedure. This conversion does not take place if the liver is dried with anhydrous sodium sulfate, acidified to pH of about 3, or heated a few minutes at 80° C. before the malathion is added. Malathion will probably not persist without change for any length of time in livers of treated animals. The method reported, therefore, is satisfactory, provided the malathion is present at the time the sample is ground with anhydrous sodium sulfate. Use of sodium sulfate is a technique recommended by Jones and Riddick (*3*).

Emulsion difficulties with extracts of some animal products were minimized by a pre-extraction of concentrated carbon tetrachloride extracts with a strongly acidic sodium sulfate solution, and also after the alkali decomposition step by using an aqueous 9% sodium sulfate solution. Sodium sulfate was found preferable to sodium chloride because high chloride concentration caused decreased and somewhat erratic color development.

When determining malathion in the range of about 20 γ, interference due to trace amounts of metals, particularly copper, must be eliminated. Copper forms the carbon tetrachloride–soluble complex with the dimethyl dithiophosphoric acid which will be discarded before the color development step, thus producing low results. This interference may be eliminated by the addition of disodium ethylenediamine tetraacetate [(ethylenedinitrilo)tetraacetate, Versene, Sequestrene]. This technique was applicable to beef fat analysis but not for milk, so that a more reliable and generally applicable technique was developed. A small amount of carbon disulfide is added to the concentrated carbon tetrachloride extract taken for analysis. The carbon disulfide reacts with the ethyl alcohol and sodium hydroxide to produce sodium xanthate (CH_3CH_2—O—CSSNa) which forms a stable complex with copper. This complex is extracted and discarded, thus eliminating interference from copper. In addition, the xanthate probably serves to prevent oxidation of the dimethyldithiophosphoric acid.

Upon analysis of some products—eggs and liver, for example—a reducing material, probably a mercaptan, which interferes by reducing some of the copper to the cuprous ion, is extracted. Cuprous ion prevents formation of the colored cupric copper complex with the dithio acid. This interference may be eliminated by adding ferric chloride which prevents reduction of copper.

Control tests should be run whenever possible and suitable corrections made on samples known to contain no malathion. Controls on fat, meat, milk, eggs (whites or yolks), and liver have indicated approximate "apparent" malathion contents of 0.0, 0.0, 0.015, 0.05, and 0.2 p.p.m., respectively. Based upon results obtained by analysis of synthetic samples, the methods described appear satisfactory for determining malathion in fat, meat, milk, eggs (whites or yolks), and liver in concentrations down to 1.0, 0.5, 0.02, 0.5, and 1.0 p.p.m., respectively. The procedures described in detail are those which contain the latest improvements. Some of the results reported, however, were obtained before these modifications were made. Such cases have been indicated. The methods recommended for beef fat and milk have been applied by Claborn and associates (*1*) in connection with a series of experiments carried out by the U. S. Department of Agriculture on spraying cattle with malathion formulations.

Acknowledgment

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PLANT ANALYSES

Measurement of Microgram Amounts of Chlorine in Plant Materials

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RECENT DEMONSTRATIONS of chlorine as an essential plant nutrient (*2*) have required the development of methods for the measurement of amounts of chlorine in the microgram range. Severely chlorine-deficient plants may contain as little as 35 γ of chlorine per gram of dry weight. This low minimal value for chlorine concentration in plant tissue requires that chlorine be assigned the status of a plant micronutrient along with molybdenum, zinc, copper, boron, manganese, and iron, thus requiring methods of analysis of the order of sensitivity and accuracy similar to those available for these latter elements.

The most successful attempts to pro-

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Procedures for the determination of microgram amounts of chlorine in plant materials have been developed. One procedure applies the microdiffusion technique of Conway to the determination of chloride in solutions of ash of plant materials and permits measurement of as little as 0.4 γ of chloride. Potentiometric titration of chloride to the apparent equivalent potential by the method of Kolthoff and Kuroda may be used for aqueous extracts of plant material as well as for solutions of ash of plant material. Pretreatment of plant samples with calcium hydroxide suspension or potassium carbonate solution before ashing is required to minimize losses of chlorine in the ashing procedure.

duce chlorine deficiency in plants result in very limited growth. In addition, analyses frequently have to be done on individual plant organs. Thus the available sample may be small and, also, low in chlorine content. Therefore, the analytical method should be as sensitive as possible—capable of measuring amounts of chlorine as low as a few tenths of a microgram with a reasonable order of accuracy and precision. The classical Mohr and Volhard titration methods, as well as the classical potentiometric methods, lacked the desired sensitivity, and attention was turned toward two alternative methods which showed promise.

In the Conway microdiffusion method (*3*), the chloride is oxidized to elemental chlorine by acid permanganate in a closed system. The chlorine then diffuses to a center well containing iodide which is oxidized to iodine. The iodine can then be measured spectrophotometrically as the starch-iodide complex. Preliminary trials with plant materials indicated that some modifications of Conway's procedure were necessary for the successful use of the method for the determination of chlorine in plant materials. A portion of this paper deals with these modifications.

Kolthoff and Kuroda (*5*) have described a potentiometric method in which precision and sensitivity for small amounts of chloride is obtained by titrating the sample in a supporting electrolyte to an apparent equivalence potential. The application of this method to the determination of chloride in plant ashes and extracts was studied.

Various methods of sample preparation, including ashing procedures and extraction techniques, were evaluated.

Microdiffusion Procedure

Conway (*3*) used the microdiffusion technique for the determination of as little as 0.1 γ of chloride in animal tissues and fluids without ashing. In the present work, however, the technique could not be applied directly to unashed plant materials and extracts. The reducing substances in plant tissues rapidly reduce the permanganate to lower oxides of manganese, which then are not capable of quantitatively oxidizing the chloride to chlorine, even though an excess of permanganate is present. For this procedure then, the plant samples had to be ashed.

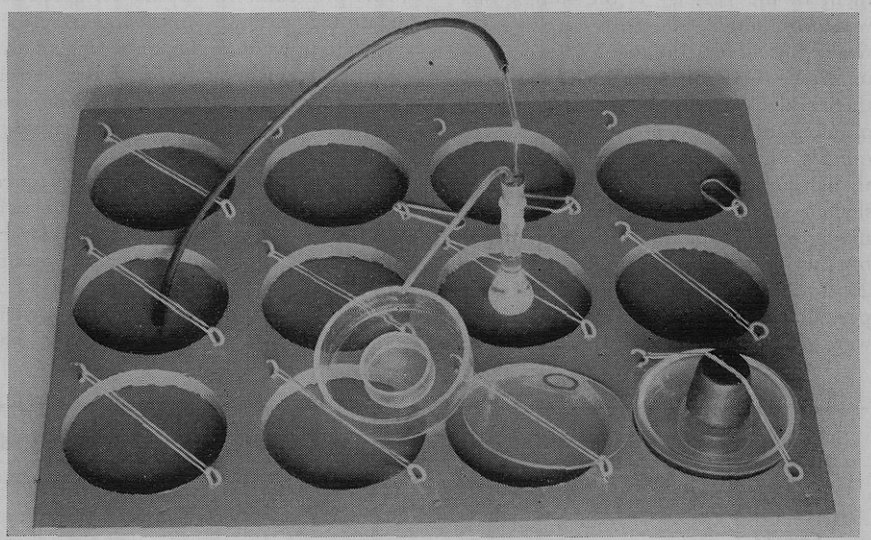

Figure 1. Tray, Conway vessels, lid supports, and aspiration device for transfer of solution from inner chamber of Conway vessel to volumetric flask

Apparatus

Standard Conway microdiffusion units —70 mm. in diameter and 18 mm. deep —provided with glass lids were used. The lids were sealed in place with a fixative prepared by melting together 30 grams of heavy liquid petrolatum and 12.5 grams of paraffin (melting point, 50–5° C.). The proportions of wax and petrolatum may be varied to provide a fixative of suitable consistency for prevailing operating temperatures. The fixative was conveniently applied to the vessel rims by spreading a thin film on a glass plate and inverting the vessel on this surface. Some grades of wax and petrolatum may absorb small amounts of chlorine; in this event, chlorination of the fixative, as recommended by Gordon (*4*), may be desirable.

The vessels were supported in insets in wooden trays, for convenience in handling and manipulation on the shaker. The lids of the vessels were secured by rubber bands stretched over the top of a 1-inch rubber stopper, placed in the center of the lid as shown in Figure 1. The vessels were shaken on a reciprocating shaker, which had a stroke of 1.25 inches, at the rate of 60 complete cycles per minute.

Spectrophotometric measurements were made in a Beckman Model B spectrophotometer.

Reagents

Sulfuric acid, 60% v./v., prepared by dilution of ACS reagent grade concentrated sulfuric acid.

Potassium permanganate solution, 6% in distilled water, prepared weekly.

Oxidant solution, prepared immediately before use by adding slowly, and with chilling in an icebath, 15 ml. of the 60% sulfuric acid to an equal volume of the 6% permanganate solution. The temperature of the oxidant mixture was kept as low as possible during the mixing to prevent decomposition of permanganic acid with the evolution of oxygen bubbles. To aid in keeping the temperature low, measured volumes of the 60% sulfuric acid were stored in a deep freeze at −20° C. Evolution of oxygen, as the result of improper mixing conditions, always resulted in a reagent that was useless in the quantitative recovery of chloride in the procedure. Therefore, such solutions were always discarded and fresh solutions prepared. The oxidizing solution usually contained small amounts of chloride, which could be removed easily by holding the solution under low pressure for about 5 minutes. This precaution resulted in low and uniform blanks.

Starch solution, prepared by grinding 4 grams of soluble starch and 10 mg. of

mercuric iodide with water to a thin paste and pouring, with stirring, into 1 liter of boiling distilled water. The solution was stored in a dark bottle.

Starch-iodide solution, prepared fresh daily by dissolving 3 grams of potassium iodide in 100 ml. of the starch solution.

Procedure

Starch-iodide solution, 3 ml., was added to the center well of the Conway vessel. Standard or sample solution, 2 ml., was placed in the outer compartment of the vessel. If the sample size was less than 2 ml., sufficient distilled water was added to make a total volume of 2 ml. Oxidant solution, 2 ml., was then added rapidly from a wide-tipped pipet, and the cover glass was placed quickly on the vessel. The vessels were then secured in the tray (Figure 1) and shaken for 1.5 hours. No effort was made to maintain constant temperature, but usually temperatures were about 20° to 25° C. At the end of the shaking period, the contents of the center well were transferred by aspiration into a 5-ml. volumetric flask. The center well was washed repeatedly with small amounts of distilled water—the washings were also aspirated into the volumetric flask. The solution was made to volume, mixed, and transferred to spectrophotometer cuvettes, and the absorbance was determined at 540 mμ, the absorption maximum.

The outer chambers of the vessels were cleaned by removing the contents by aspiration, followed by thorough rinsing with water. This procedure proved to be preferable to tipping the spent reagent and washings out of the vessel because it prevented the spreading of the fixative over the interior of the vessel. Saturated oxalic acid was used to remove manganese dioxide deposits as necessary. Only occasionally was it necessary to wash the vessels with detergent to remove fixative. After such washing, one or two conditioning runs with chloride samples were necessary before reliable results could again be obtained.

Calibration Curve. The starch-triiodide complex was measured at 540 mμ—the region of maximum absorbance. Absorbance data for amounts of starch-triiodide complex developed from amounts of chloride from 0.44 to 21.27 γ are presented in Table I. In the range of 3.55 to 21.27 γ of chloride, Beer's law was obeyed, but in the lower ranges marked deviations were observed. For this reason, calculations for samples containing less than 3.55 γ of chloride were based on standard data obtained at the same time in the appropriate range. Agreement among values obtained by different analysts was good.

Comments on Procedure. Conway (*3*) presented data on the effects of variations in permanganate and sulfuric acid concentrations on the recovery of chloride. Data obtained in the present study are in substantial agreement with those of Conway, with optimal concentrations of permanganate and sulfuric acid being established at 1.5% w./v. and 15% v./v., respectively.

Conway observed a slight back diffusion of iodine from the center well to the outer chamber, amounting to about

Table I. Spectrophotometric Data for Known Amounts of Chloride by the Microdiffusion Method

(Absorbance values, A_s, obtained at 540 mμ, 1-cm. path, at 25° C.)

Chloride Taken, γ	A_s Individual Values						A_s Mean	C.V.[a]	Molar Absorbance Index[b]
0.44	0.012	0.013	0.019	0.019	0.011	0.013	0.015	23.7	5,600
0.89	0.033	0.038	0.028	0.043	0.038	0.041	0.037	14.9	7,400
1.77	0.083	0.093	0.087	0.093	0.083	0.087	0.088	5.11	8,800
3.55 (analyst A)	0.190	0.200	0.193	0.191	...	...	0.194	2.31	9,700
3.55 (analyst B)	0.182	0.210	0.201	0.203	0.208	0.203	0.201	4.97	10,550
10.64 (analyst A)	0.591	0.610	0.638	0.603	0.615	0.621	0.613	2.62	10,216
10.64 (analyst B)	0.600	0.605	0.590	0.632	0.618	0.604	0.608	2.43	10,133
21.27 (analyst A)	1.251	1.251	1.261	1.260	1.252	...	1.255	0.40	10,458
21.27 (analyst B)	1.231	1.253	1.244	1.231	1.218	1.231	1.235	0.99	10,292

[a] C.V. equals $\frac{\text{standard deviation of individuals}}{\text{mean of individuals}} \times 100$.

[b] Molar absorbance index equals $\frac{A_s}{bc}$, where b is length of absorbing path in centimeters, and c is concentration of chloride expressed in moles per liter.

Table II. Chloride Concentrations in Plant Samples

(Comparison of values obtained by various preparative and determination procedures)

Sample	Preparative Procedure[a]	Determination Procedure	Cl, P.P.M. Mean	Cl, P.P.M. C.V.
T.C.1	Ashed 2 hr. at 550° C., no added base	Potentiometric titration	747	2.44
T.C.1	Ashed 2 hr. at 550° C., CaO added	Potentiometric titration	900	2.90
T.C.1	Aliquots of above ash solution	Microdiffusion method	902	2.31
T.C.1	Ashed 2 hr. at 550° C., K_2CO_3 added	Potentiometric titration	909	1.28
T.C.1	Ashed 1 hr. at 550° C., K_2CO_3 added	Potentiometric titration	927	0.62
T.C.1	Aliquots of above ash solution	Microdiffusion method	919	1.52
T.C.1	Filtrate of plant sample extracted with hot H_2O without ashing	Potentiometric titration	915	0.98
T.C.1	Filtrate of plant sample extracted with hot 0.1N HNO_3 without ashing	Potentiometric titration	918	2.51
T.C.1	Supernatant after centrifuging equilibrium hot H_2O extract	Potentiometric titration	922	0.60
T.C.1	Plant sample extracted with hot H_2O, residue not filtered off	Potentiometric titration	935	0.76
T.C.2	Ashed 1 hr. at 550° C., K_2CO_3 added	Potentiometric titration	40.8	3.00
T.C.2	Aliquots of above ash solution	Microdiffusion method	40.4	6.40
T.C.2	Filtrate of plant sample extracted with hot H_2O	Potentiometric titration	34.0	4.78
T.C.2	Plant sample extracted with hot H_2O, residue not filtered off	Potentiometric titration	84.0	12.4

[a] Data for ashed samples obtained from six individual ashings and six individual potentiometric or microdiffusion determinations on each ash solution. Data for extracts and suspensions obtained from six extractions with a single potentiometric titration on each extract.

1.2% per hour, and applied appropriate correction in his calculations. The use of starch-iodide solution in the center chamber in the proposed modification has prevented measurable diffusion of iodine from the center chamber.

Shaking the reaction vessels reduced the time required for attainment of equilibrium—reaction times as long as 16 hours, without shaking, failed to give as complete recovery as did 1.5 hours with shaking. This finding appears to be at variance with Conway's. He stated that the rate of oxidation of chloride was so much slower than the diffusion rate of chlorine gas that the time of analysis is largely determined by the rate of oxidation. Agitation of the reaction vessels would hardly be expected to affect the reaction rate, but would have an effect on the diffusion rate.

The absorption spectrum of the starch-triiodide complex was different from that reported by Lambert (*6*) for linear starch and lower iodide concentrations. Conway also noted that, in the presence of a large excess of iodide, the complex had a purplish color rather than the usual blue. Therefore, the absorption spectrum must be ascertained for the particular starch reagent and concentration of iodide employed in the procedure. Likewise, each worker must prepare his own calibration curves.

Potentiometric Procedure

Potentiometric titration of the chloride to the apparent equivalence potential, as described by Kolthoff and Kuroda (*5*), gave excellent results with amounts of chloride in plant ash solutions over the entire range studied. Where sample size is not limiting, the method is faster and less laborious than the microdiffusion method. Even with amounts of chloride in the order of 35.5 p.p.m., the precision was about 5%, and the results agreed closely with those obtained by the microdiffusion technique.

This method has an additional advantage over the microdiffusion method in that chloride may be determined directly in aqueous extracts of the plant material, thus eliminating time-consuming ashing procedures. Results obtained with aqueous extracts of plant material were slightly higher than those obtained on ash solutions. This is in agreement with the results of Best (*7*), who attributed the differences to losses incurred in ashing of the plant sample. Samson (*8*) likewise reported good results when titrating aqueous extracts directly. Both of these investigators were dealing with samples containing larger amounts of chloride than are under consideration here. Nevertheless, even when small amounts of chloride were present, there was remarkably good agreement between ashed samples and aqueous extracts, as is shown by current results in Table II. Considering the ease with which chloride may be lost from samples on ashing, even with due care being taken to maintain the ashing temperature in the range of 500° to 550° C., the higher results obtained on the aqueous extracts probably more nearly represent the amounts present initially.

Comparison of Results Obtained by Potentiometric Procedure and Microdiffusion Procedure. For purposes of comparison of results obtained by the two determination procedures, samples of plant material were ashed at 550° C. for 2 hours. Previous experiments showed that longer ashing times gave lower results, and that shorter ashing times occasionally gave residues containing large amounts of carbon and often water-soluble charred material that interfered with the microdiffusion procedure. Comparison of the relative efficiency of calcium oxide and potassium carbonate in preventing loss of chloride in the ashing was also made.

One-gram samples of ground plant material, in porcelain dishes, were treated with either 0.25 gram of calcium oxide, as recommended by Piper (*7*), or 6 ml. of a 2% solution of potassium carbonate prior to ashing. The calcium oxide was thoroughly mixed with the dry sample; then water was added to make a thin slurry. The slurry was dried on a steam bath before being placed in the cold muffle furnace. The samples treated with the potassium carbonate solution were likewise dried on a steam bath prior to ashing. The cooled ash was treated with 10 to 20 ml. of distilled water and sufficient nitric acid to give an approximate final pH of 1. The solution was transferred to a volumetric flask without filtering off the carbon residue, made to volume, and mixed. Appropriate aliquots were taken for the potentiometric titrations and for the microdiffusion procedure. Care was taken to avoid transfer of carbon particles to the reaction vessels. The use of an ultramicroburet facilitated titration of the samples with 0.01N silver nitrate. Blank measurements were made, and appropriate corrections were applied for chloride in the calcium oxide and potassium carbonate.

The data of Table II provide a comparison, not only of the results obtained by the two determination procedures on a given ash solution, but also of the effectiveness of calcium oxide and potassium carbonate in preventing loss of chloride during the ashing. The mean value for six samples ashed without any alkali added was 747 p.p.m. compared with values of 900 and 909 p.p.m., when calcium oxide or potassium carbonate, respectively, were added prior to ashing. Ashing for 1 hour only with potassium carbonate added, gave a slightly higher result of 927 p.p.m. than did a 2-hour ashing. Ashing times of 1 hour or less at 400° to 450° C., frequently gave poor results in the microdiffusion procedure. Therefore, ashing at 500° to 550° C. for at least 1 hour, but not in excess of 2 hours, was preferable. Potassium carbonate solution was more effective than calcium oxide in preventing loss of chloride—possibly because it more thoroughly wetted the sample than did the calcium oxide slurry. In all cases, the agreement between the values obtained on a given ash solution by the two procedures was good.

Titration of Aqueous Extracts and Suspensions. Aqueous extracts of plant materials, dried and ground to pass the 40-mesh screen of the Wiley mill, were prepared by gently boiling 1-gram samples with 10 ml. of distilled water for 5 minutes, filtering on a sintered-glass filter, and washing with small portions of hot water to a total volume of 30 ml. The extracts were titrated potentiometrically with 0.01N silver nitrate, after acidification to about pH 1 with nitric acid. Longer extraction times were tried, but yielded similar titration results. Extraction with 0.1N nitric acid gave similar results. Some plant extracts tend to filter poorly, clogging the pores of the sintered funnel. Centrifuging such suspensions yielded satisfactory supernatants for titration. Titration of these supernatants yielded results similar to those obtained with filtrates. Direct titration of suspensions gave high and erratic results, especially in the case of the low chloride sample (TC 2). The end point in the titrations of suspensions was indefinite and drifting, even with the TC 1 sample.

As shown in Table II, titration values for aqueous extracts of the high chloride sample, TC 1, were in good agreement with both the titration and microdiffusion results on solutions of ash of the sample. The results for the potentiometric titration of the low chloride sample filtrate were lower than those obtained by either potentiometric titration or microdiffusion determinations on solutions of ash of the same material. As noted earlier, chloride in plant extracts cannot be determined by the microdiffusion method.

Potentiometric titration of filtrates or supernatants of samples containing moderate to large amounts of chloride (about 200 p.p.m. of chlorine) is more rapid and convenient than either titration or microdiffusion analysis of ashed samples. In addition, the possible loss of chloride on ashing is eliminated. However, with small amounts of chloride in the sample, titration or microdiffusion analysis of ash solutions is to be preferred because of the low results obtained on titration of aqueous extracts of dry plant material.

None of the methods described here distinguish between chloride, bromide, and iodide when present together in plant materials. For most purposes, this

is not a serious handicap because bromide and iodide usually occur in relatively small amounts in plant materials and do not contribute a large error in chloride analyses.

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VEGETABLE OILS

Review of Chemistry and Research Potential of *Simmondsia Chinensis* (Jojoba) Oil

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The known chemistry and research potential of *Simmondsia chinensis* (Jojoba), a unique agricultural material that occurs only in the wild, is summarized. Its nuts contain 50% of an oil which is a liquid wax, similar in some respects to sperm whale oil. Current interest in this seed stems from the fact that the United States is dependent on foreign sources of plant wax. The cultivation of jojoba offers promise in reducing this dependence. Preliminary industrial evaluations of jojoba oil and hydrogenated wax are discussed.

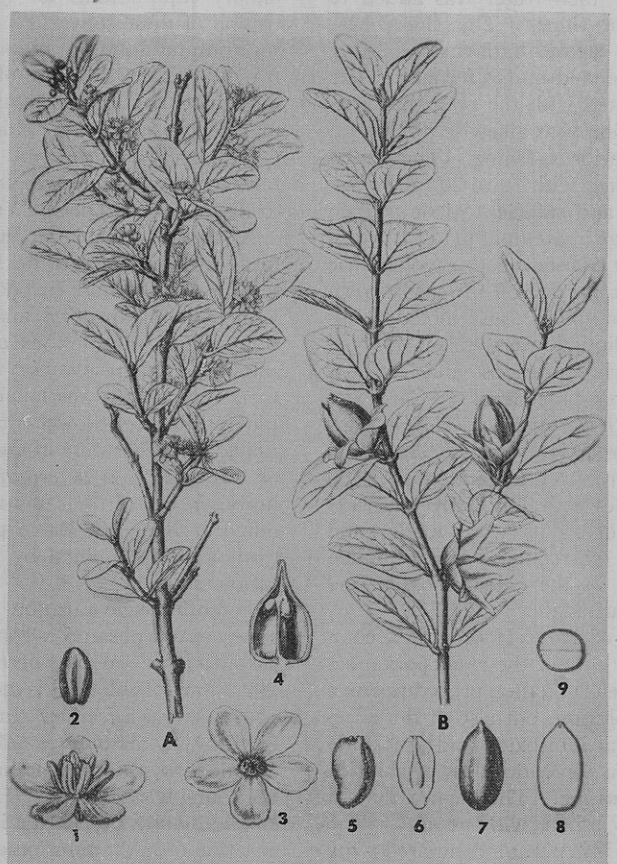

Figure 1. *Simmondsia chinensis*

A. Flowering branch of male plant
B. Branch of female plant
1. A male flower, enlarged
2. Anther, more enlarged
3. Male flower, with stamens removed
4. Ovary, magnified and longitudinally divided
5. Ovule, more highly magnified
6. Longitudinal section of ovule
7. Seed, somewhat enlarged
8. Longitudinal section of seed
9. Transverse section of seed (*18*)

THE POTENTIAL VALUE of *Simmondsia chinensis* (jojoba), a wild desert shrub, native to southwestern United States and northern Mexico, is centered in the unusual oil that occurs in its seed. The seeds, or nuts, are reddish brown in color and measure approximately $^1/_2$ by $^3/_8$ inch (Figure 1). They contain up to 50% by weight of an oil which is a liquid wax (esters of long-chain acids and alcohols) rather than a glyceride fat (*5, 7–9, 11, 13, 20*). In this respect jojoba oil is most similar to sperm whale oil, which is an oddity in animal fats.

The current interest in jojoba stems from the fact that the United States is dependent on foreign sources for almost all of the plant waxes of commerce. Because of chemical similarity to sperm whale oil, the cultivation of jojoba offers promise of reducing the dependence of the United States on foreign sources of sperm waxes, large quantities of which are imported yearly (*5, 17*) (Table I).

Hydrogenation of jojoba oil produces a white, crystalline wax with a melting point of 70–4° C., of excellent hardness (*5, 6, 14, 16*). Melting point of carnauba wax is 85° C. and various grades and types of candelilla wax melt at 64° to 77° C. Jojoba appears to be a possible replacement for some applications of other imported vegetable waxes.

There have been several recorded domestic plantings of jojoba (*5, 17*). Wild jojoba plants produce approximately 2 pounds of seed per plant; this could, conceivably, be increased to 4 or