eral oils by the laboratory centrifuge method and the Official Method (Commercial Laboratory). In all cases the losses are considerably lower by the centrifugal method.

Summary

A centrifugal method is presented for the determination of refining loss of extracted soybean oils, entailing modifications of a procedure first introduced by the Swift and Company Research Laboratories and later improved by the Regional Soybean Laboratory. The method using glass cups in the International Centrifuge permits speed and accuracy in the laboratory refining of extracted soybean oils.

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Tangerine Seed Oil[1]

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A KNOWLEDGE of the chemical and physical properties of citrus seed oils is of importance for two practical reasons. One of these, of course, is concerned with the possible commercial uses of the oils when they are recovered in quantity as by-products of the canning industry. The other, and less obvious, reason is due to the possibility that the storage life of canned citrus juices is affected by the small amounts of the seed oils that get into them during the extraction process. Some evidence in support of this possibility was obtained by Nolte and von Loesecke (1) who showed that the lipids from orange juice that had deteriorated on storage gave positive tests for rancidity while those from fresh juice did not. Their work did not exclude the possibility that the lipids were oxidized preferentially while serving as antioxidants. Furthermore, it dealt with the whole lipid fraction of the juice, only a part of which was derived from seeds. Nevertheless, as part of an investigation of citrus juice lipids, a fairly detailed analysis of a seed oil seemed warranted and the results are reported below. Tangerine seed oil was chosen because no work on it had come to the author's attention and because, with the analysis of it in hand, any marked differences in its properties and composition from those obtained by other workers on other citrus seed oils would be apparent. The storage life of canned tangerine juice was known to be shorter than that of either canned orange or grapefruit juices, and this fact was thought to lend special significance to any differences found in the oils which might furnish an explanation of the different keeping qualities of the juices and the nature of the deterioration.

Experimental

Seeds of the Dancy tangerine (*Citrus nobilis* var. *deliciosa*) were obtained directly from the finisher at a cannery in Florida during the 1947-48 season. Most of the pulp was eliminated by stirring with large volumes of water and decanting. The seeds were then air-dried by spreading on a floor in front of a fan. After hand-sorting to remove broken seeds and fragments of pulp, the weight was 7.7 kg. The seeds were ground in a hammer mill and then in a food chopper. A small amount of oil (referred to in Table V as "cold-pressed" oil) was obtained as the seeds were passed through the food chopper and was treated separately in determining the chemical and physical properties. The bulk of the oil was obtained by extracting the ground seeds with acetone and then regrinding and re-extracting. A final extraction of the ground seeds was made with petroleum ether. The acetone extracts were evaporated and the petroleum ether extract was added and all of the solvent was evaporated by heating in vacuum. The entire yield on the basis of the air-dried seeds was 26.6%. The chemical and physical properties of the oils are given in Table V, standard methods of analysis being used throughout. In the determination of all thiocyanogen values the recommendations of Lambou and Dollear (2, 3) were followed closely.

To obtain the free fatty acids, 300 g. of the solvent-extracted oil were refluxed for six hours with 1,500 ml. of 95% ethanol and 180 g. of potassium hydroxide. After distilling off about half of the ethanol and diluting the remaining mixture with 1.5 volumes of water, the unsaponifiable matter was removed by extracting for 67 hours in a continuous liquid-liquid extractor with 30°-65° petroleum ether. The extract was washed thoroughly with potassium hydroxide solution and water, dried over anhydrous sodium sulfate, and set aside for possible later examination. The washings were added to the main solution of fatty acids soaps which was then acidified with dilute (1 + 3) sulfuric acid and again thoroughly extracted with petroleum ether using separatory funnels. The extract was washed with water, dried over anhydrous sodium sulfate, filtered into a flask, and the solvent distilled off under vacuum. A water bath was used as the source of heat throughout and nitrogen gas was bled into the system to prevent bumping. The yield of acids was 287 g., or 95.6%.

No attempt was made to separate the fatty acids into saturated and unsaturated fractions before esterification, the writer preferring to make saturated acid determinations on the individual ester fractions after distillation. The esterification was carried out by refluxing 255 g. of the mixed acids with 1 liter of absolute methanol and 9 ml. of concentrated sulfuric acid for five hours. The excess methanol was distilled off and the mixture of esters was dissolved by ethyl ether, washed with water, 10% sodium carbonate solution, and again thoroughly with water after which it was dried with sodium sulfate and the

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[1] Agricultural Chemical Research Division Contribution No. 241.

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ether distilled off, finishing under vacuum. From the washings 4.5 g. acids that had escaped esterification were recovered by acidifying, extracting with ether, drying, and evaporating. Since this was less than 2% of the original, it was discarded.

The distilling column used for the fractionation was constructed by sealing male and female ball joints of appropriate size on the ends of a glass tube of which the internal diameter was about 22 mm. to give an over-all length of 135 cm. Indentations were made above the lower (male) joint to retain the packing. The column was wrapped with a double layer of asbestos paper upon which was wound about 4 meters of B and S gauge No. 20 Chromel wire. The turns were about 2.5 cm. apart at the bottom of the column and were wound with a gradually increasing interval between them until they were about 3.8 cm. apart at the top of the column. The whole assembly between the joints was then encased in asbestos steam pipe insulation about 2 cm. thick. Suitable leads were provided so that the resistance wire could be connected to a variable transformer. Some idea of the temperatures to be expected within the column was obtained by suspending a thermometer at different levels for each of a series of transformer settings, taking care that equilibrium was reached in each case before reading. The column was then filled with 6-mm. glass helices (mostly single-turn) and the head, patterned after one used by Baldwin and Longenecker (4), was attached. The still-pot used was a 250-ml. round-bottomed flask fitted with a thermometer well and heated by a glass-cloth mantle, the heat of which was regulated by a second variable transformer.

Into the flask was charged 91.15 g. of the mixed esters and the distillation was started. It was carried out at a pressure of 3.5-4.5 mm. Hg and was continued over 16 hours, an effort being made to maintain a reflux ratio of about 10 to 1 as nearly as could be estimated. The variable transformer settings for the pot heating mantle ranged from 60 to 68 volts as the distillation progressed and those for the column transformer from 65 to 90 volts. The fractions were received in weighed test tubes. The limited capacities of the receivers required the separate collection of fractions 3, 4, and 5 which distilled in about the same range of temperature. These fractions were subsequently handled separately, however; and some differences in composition were shown to be present by analytical means. Some of the distillation data are presented in Table I.

TABLE I

Fractionation of Esters

Fraction	Variable Transformer Settings		Distilling Range in °C.	Weight in g.
	Pot	Column		
1	60-68	65-69	155-165	15.59
2	68	69-71	165-175	8.44
3	68	71-72	175-178	22.18
4	68	72-76	176-178	21.68
5	68	76-90	176-178	20.84
Holdup	......	....	....	1.48
Flask residue	......	....	....	0.05
Recovery				90.71 g. or 99.5%

Analyses of the separate fractions (excluding the holdup and residue) and of the whole mixed esters were made to determine iodine value, thiocyanogen value, refractive index at 40°C., saponification equivalent, and saturated acids. These values are presented in Table II. The percentages of saturated esters in the whole mixed esters and in fractions 1, 2, and 5 were calculated from the percentages of saturated acids and the neutralization equivalents of these acids. However, the amounts of saturated acids in fractions 3 and 4 were too small to titrate accurately to determine the neutralization equivalents so these compositions were ascertained from the solidification points by the method of Schuette and Vogel (5), assuming, of course, that each was a binary mixture. From the compositions thus obtained, the neutralization equivalents were computed and the percentages of saturated esters were calculated from these and the percentages of saturated acids as was done with the other fractions. The results are given in Table III. The neutralization equivalent of the saturated acids from the whole mixed esters was 265.

TABLE II

Analyses of Mixed Esters and Distillation Fractions

Sample	Iodine value (Wijs, 30 min.)	Thiocyanogen value	Refractive index, 40°C.	Saponification equivalent	Saturated acids, % (Bertram)	Saturated esters, % (calc.)
Mixed esters	105.05	65.40	1.4464	291.27	25.91	27.30
Fraction 1	8.38	6.08	1.4342	269.92	79.00	83.30
Fraction 2	58.50	36.00	1.4403	272.84	50.10	52.80
Fraction 3	140.35	86.25	1.4504	291.06	4.58	4.83
Fraction 4	142.60	86.65	1.4507	295.70	3.74	3.92
Fraction 5	118.95	78.15	1.4492	296.66	16.51	17.30

The presence of linolenic and linoleic acids was proved by preparing the bromine derivatives according to the method of Eibner and Muggenthaler as given by Lewkowitsch (6), using acids obtained by the usual methods from remaining portions of ester fractions 3 and 4. The stearic hexabromide melted sharply at 181°C. without charring and the tetrabromide melted at 113°-114°C. The presence of oleic acid was shown by the preparation of the dihydroxy stearic acid from a similar source after first removing the saturated acids by the lead salt-ether procedure as given by Jamieson (7) and the subsequent removal of the linolenic and linoleic acids as the bromides. The remaining petroleum ether-soluble bromides were debrominated by the Riemenschneider, Swift, and Sando (8) modification of Kimura's procedure with zinc and absolute methanol and was then oxidized with dilute potassium permanganate according to the method of Sullivan and Bailey (9). The isolated oxidized acid melted at 131°C. after recrystallization from 95% ethanol. This agrees with values given in the literature for 9,10-dihydroxy stearic acid. No experimental evidence was found for the presence of palmitoleic acid.

The percentages of the individual unsaturated acids were calculated by the use of formulas derived from those recommended by the American Chemical Society Committee on the Analysis of Commercial

TABLE III

Analyses of Saturated Acids of Various Fractions

Fraction No.	Neutral equiv.	Solid. pt., °C.	% Esters in Saturated Fractions			% Esters in Whole Mixed Esters		
			Palmitate	Stearate	Arachidate	Palmitate	Stearate	Arachidate
1	256.93	......	100.0	0	0	14.65	0	0
2	260.88	......	80.5	19.5	0	4.04	0.98	0
3	263 calc.	53.8	72.1	27.9	0	0.87	0.34	0
4	281 calc.	66.8	8.4	91.6	0	0.08	0.88	0
5	291.75	......	0	71.6	28.4	0	2.91	1.15

Fats and Oils (10). The modifications were necessary to permit use with mixtures of esters instead of acids and were based on the assumption that equivalent quantities of unsaturated acids and their esters absorb the same amounts of iodine and thiocyanogen. These modified formulas are as follows:

% Methyl linolenate=1.305 S—130.54—0.134 I.V.—1.67 T.V.
% Methyl linoleate =1.424 I.V.—3.365 T.V.—1.643 S—164.3
% Methyl oleate =66.24 —0.6624 S—1.29 I.V.—1.695 T.V.

where S = per cent saturated esters; I. V. = iodine value; and T. V. = thiocyanogen value. Difficulty was encountered when these formulas were applied to certain of the ester fractions, notably number 1, some of the calculated ester percentages being negative in sign. The saturated esters in these cases appeared to be too low in amount. An acetyl value was determined on the solvent-extracted oil and on the mixed esters which gave results of 8.19 and 6.30, respectively. From this latter value, assuming a monohydroxy acid ester of molecular weight 258, it appeared that 2.90% of the whole ester mixture was of this type Such a compound would account for the discrepancy because it might well escape detection as a saturated acid by the Bertram method by reason of its being somewhat water soluble or susceptible to further oxidation and, on the other hand, it would absorb no iodine or thiocyanogen. The distribution of this ester among the various fractions was not determined because of lack of sample so the modified formulas were applied to the whole ester mixture, the percentage of hydroxy acid ester being added to the sum of the determined percentages of saturated esters to make S equal 28.80 for purposes of calculating the individual unsaturated acids. The greatest concentration of the hydroxy acid ester appeared to be in fractions 1 and 2; hence the molecular weight assigned to it for purposes of calculation was of an appropriate order for the boiling ranges of these fractions. It is likely that the saponification equivalent of fractions 1 and 2 are somewhat low because of the concentration in them of this ester. An attempt was made to isolate the acid from a small sample of fraction 1 after saponification. The acids were freed from the soaps in the usual way and extracted with ethyl ether. After evaporation of this solvent, petroleum ether was added. A portion of the acids was found to be much less soluble in this solvent than most fatty acids, but was soluble in ethanol and ether. It was slightly soluble in water and was crystallized from a large volume of this solvent. After filtering and drying it was found to melt at about 58°C., but the melting point was not very sharp. There was too little of it to attempt a purification. It cannot be stated with certainty that this material was an hydroxy acid, but it had the solubility characteristics ordinarily ascribed to these compounds. This fact, when taken with the other evidence presented above, leaves little doubt that such an acid is present. Work on the identity of the acid as the subject of a later paper is under consideration.

TABLE IV

Summary of Analyses of Mixed Methyl Esters

Methyl linolenate	2.1
Methyl linoleate	46.6
Methyl oleate	22.5
Methyl hydroxy acid ester	2.9
Methyl palmitate	19.6
Methyl stearate	5.2
Methyl arachidate	1.1
	100.0

The final results of the analyses of the esters are presented in Table IV. The percentages of the fatty acid glycerides in the oil would, of course, differ but slightly from the percentages of methyl esters in the ester mixture.

Discussion

For the sake of comparison, the characteristics of several citrus seed oils as determined by different investigators are given in Table V. From these data there appears to be little difference in the physical and chemical properties; certainly not enough to justify any conclusions as to effect on storage life of the juices, especially in view of the facts that so few samples of the oils have been analyzed and the histories of these are not known to be similar. Somewhat more linolenic acid was found in the present investigation than had been reported for the other oils, but this is probably not significant.

TABLE V

Comparison of Properties of Some Citrus Seed Oils

	Grapefruit Seed Oil		Orange Seed Oil		Tangerine Seed Oil (Present work)	
	(11)	(12)	(11)	(13)	Cold-pressed	Solvent-extracted
Specific gravity 25/25°C	0.9170	0.9197	0.921 -0.925$^{15.5°}$	0.9153	0.9168	0.9165
Refractive index, 25°C	1.4700	1.4698	1.4638-1.4647$^{40°}$	1.4686	1.4698	1.4702
Acid value	2.5	0.95	....	....	4.83	4.31
Saponification value	194.1	193.0	194-197	197.5	193.78	193.55
Unsaponifiable matter, %	0.7	0.48	....	0.95	0.34	0.54
Iodine value	106.3 (H)	100.9 (H)	98-104	101.7 (W)	107.4 (W)	107.3 (W)
Thiocyanogen value	....	....	....	....	66.22	66.37
Acetyl value	7.7	2.4	....	....	....	8.2

Numbers in parentheses are literature references.
(W) and (H) refers to Wijs and Hanus methods, respectively.

Conclusions

Some physical and chemical properties of tangerine seed oil were determined. Evidence for the presence of linolenic, linoleic, oleic, palmitic, stearic, arachidic, and an unidentified hydroxy acid was obtained and the percentages of these acids were determined.

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The Flavor Problem of Soybean Oil. V. Some Considerations in the Use of Metal Scavengers in Commercial Operations[1]

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CITRIC acid appears to have been used in Denmark as early as 1928 in the refining of soybean oil (1). While its use became widespread in Europe before the second World War, it seems not to have been adopted in this country although numerous studies on its synergistic function with antioxidants have been reported (2).

On receipt of reports by Goss on the water-washing citric acid process used in Germany (3), work was undertaken at the Northern Regional Research Laboratory to evaluate the process. This work, carried out on a laboratory scale, showed that the addition of citric acid to soybean oil did improve its flavor stability (4). Still further studies revealed that certain polyhydric alcohols and certain polybasic acids also increased the oxidative and flavor stability of soybean oil and supported previous suggestions (7, 8) that these compounds might function as metal scavengers by complexing pro-oxidant metals, thus decreasing the rate of oxidation of the oil (9).

After these studies practical considerations in the use of metal scavengers remained to be clarified. These included the concentration of scavenger required, the effect of temperature on the activity of the citric acid, the point during deodorization at which citric acid should be added for best results, the value of the water-washing step, the role of the phosphatides, and the efficacy of metal scavengers in commercial operations. These considerations are the subject of the present communication.

Concentration of Metal Scavenger

To determine the optimal concentration for addition of citric acid and sorbitol to oils, 0.001, 0.01, and 0.1% of each compound as a 20% aqueous solution was flashed into three aliquots of alkali-refined and bleached soybean oil at the beginning of deodorization. These aliquots and a control aliquot were deodorized simultaneously for three hours in the four-sample, glass laboratory deodorizer at 210°C. (10). Oxidative stability was assayed by determining the peroxides of the oil after holding it eight hours under Active Oxygen Method conditions and after holding it three days at 60°C. The samples held at 60°C. were also evaluated organoleptically according to this Laboratory's procedure (11). Results on citric acid addition are given in Table I. The peroxide data show that citric acid imparts increasing oxidative stability at concentrations of 0.001, 0.01, and 0.1%. In contrast to the oxidative stability data, flavor stability data indicate that 0.001% affords insufficient protection, but 0.01% is highly effective, and 0.1% appears to hold no advantage over 0.01%.[3] Sorbitol behaves similarly to citric acid as the concentration is varied. Thus the addition of citric acid or sorbitol in concentrations higher than 0.01% does not appear necessary or economically desirable.

TABLE I

Effect of the Citric Acid Concentration Upon the Oxidative and Flavor Stability of Soybean Oil

0.1% Citric	0.01% Citric	0.001% Citric	Control	Significant difference[1]
Flavor Score at 0 Time				
8.1 (0.47)[2]	8.5 (0.36)	8.7 (0.39)	8.7 (0.56)	+
After 3 days' storage at 60°C.				
6.9 (1.40)	7.1 (1.50)			+
7.0 (1.30)		5.7 (5.23)		*
6.8 (1.19)			4.9 (4.89)	**
	7.4 (1.65)	6.2 (5.50)		*
	7.8 (1.56)		6.0 (5.66)	**
		5.7 (5.13)	5.8 (5.11)	+
Peroxide values (A.O.M. conditions—8 hours)				
6.5	13.1	39.8	56.9	

[1] + No significant difference.
* Significant difference (5% level).
** Highly significant difference (1% level).
[2] Peroxide value at time of organoleptic evaluation.

Effect of Temperature on the Activity of Citric Acid

Since normal deodorization temperatures are above the decomposition temperature of citric acid, the question is raised thereby whether the application of heat is essential to the activity of citric acid. An answer was sought by the following experiment. To one

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[2] One of the laboratories of the Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture.

[3] In further experiments in which samples were stored at 60°C. and tasted at intervals up to 10 days, no significant difference in flavor scores were found between the 0.1 and 0.01% levels.