procedures agree fairly well. Both exhibited a tendency toward depression of the peroxide value as peroxygen equivalents were increased.

Summary

The Kokatnur-Jelling method as modified by Lingenfelter for the determination of peroxides in animal fat has been found to agree satisfactorily with other methods now in use. The modified method involves a microtitration in a homogeneous medium (isopropanol) and has the additional advantages of being both simple and rapid.

A statistical analysis of the peroxide values shows that the reaction time and temperature and the sample size must be standardized to obtain reproducible and reliable results. Close adherence to reaction specifications such as five minutes at 80°C. or seven minutes at 75°C. is necessary. Three minutes is too short a reaction time. The individual fat sample should contain no more than 50 microequivalents of peroxygen to avoid depression of the peroxide value.

REFERENCES

1. Kokatnur, V. R., and Jelling, M., Jour. Am. Chem. Soc. *63*, 1432-1433 (1941).
2. Lea, C. H., Proc. Royal Soc. London, *B108*, 175 (1931).
3. Lea, C. H., J. Soc. Chem. Ind. *65*, 286-290 (1946).
4. Liebhafsky, H. A., and Sharkey, W. H., Jour. Am. Chem. Soc. *62*, 190-192 (1940).
5. Lingenfelter, J. F., Thesis, Cornell University (1945).
6. Lips, A., Chapman, R. A., and McFarlane, W. D., Oil and Soap *20*, 240-243 (1943).
7. Lowen, L., Anderson, L., and Harrison, R. W., Ind. and Eng. Chem. *29*, 151-156 (1937).
8. Paschke, R. F., and Wheeler, D. H., Oil and Soap *21*, 52-57 (1944).
9. Snedecor, G. W., Statistical Methods Applied to Experiments in Agriculture and Biology, Iowa State College Press, Ames (1940).
10. Stansby, M. E., Ind. and Eng. Chem., Anal. Ed. *13*, 627-631 (1941).
11. Wheeler, D. H., Oil and Soap *9*, 89-97 (1932).
12. Yates, F., The Design and Analysis of Factorial Experiments, Imperial Bureau of Soil Science, Technical Communication No. 35, Harpenden (1937).

The Fatty Acids of Dormant Tung Buds

HAROLD M. SELL and SEYMOUR G. GILBERT [1]

United States Department of Agriculture
Gainesville, Fla.

Introduction

IN studies (1, 2) of the prolongation of dormancy of tung buds (*Aleurites fordii* Hemsl.), it was found that blossoming was effectively delayed, although always with attendant injury, by alpha-naphthalenacetic acid and certain of its derivatives or by indole-3-acetic acid, when applied in Crisco [2] or lanolin emulsions. However, when various materials containing alpha-naphthalenacetic acid were applied as sprays, or when an aqueous solution of its potassium salt was injected into the buds, dormancy was not prolonged. The rudimentary blossom buds of tung are embedded in a sticky wax. One would expect that if the radicals of the higher fatty acids in this wax are similar to those of Crisco or lanolin, penetration of growth-regulating substance emulsified in these materials should be facilitated since, according to the solubility rule (3), similarity in structure among organic compounds is conducive to mutual solubility. In view of these facts it appeared desirable to identify the higher fatty acid constituents of the wax, and an investigation was undertaken for this purpose. The techniques used and the results obtained are presented in this paper.

EXPERIMENTAL WORK

Extraction of the Wax from Tung Buds

In December, 1941, 14.5 kg. of buds were collected from the trees in the Alachua Tung Oil Orchards near Gainesville, Florida. The buds were extracted with ethyl ether in a two and one-half gallon Soxhlet extraction unit for 24 hours. They were then dried in vacuo at 70°C. and 10 mm. pressure, ground to pass a 2-mm. mesh sieve, and re-extracted with fresh ethyl ether for an additional 48 hours. The ether was removed from the combined extracts by distillation and the residue finally dried in vacuo at 70°C. and 6-mm. pressure. The yield was 1.8 kg. of crude wax, of which 45% was unsaponifiable and 55% was fatty acids.

The wax (1.67 kg.) was saponified by refluxing for 4 hours with 270 gm. of potassium hydroxide in 3 liters of 95% ethanol. After diluting the solution with 3 liters of water, it was extracted in a liquid-liquid extraction unit with petroleum ether for 24 hours to remove the unsaponifiable material. The aqueous-ethanol solution containing the potassium salts of the fatty acids was acidified with hydrochloric acid, and the liberated organic acids were extracted with ethyl ether. The fatty acids remaining after distillation of the ether and final drying in vacuo at 70°C. and 6-mm. pressure weighed 975.3 gm. No fatty acids of low molecular weight were detected in the distillate.

Preparation of the Methyl Esters of the Fatty Acids

The fatty acids (963.0 gm.) were converted to the methyl esters by refluxing for 3 hours in 2.5 liters of absolute methanol containing 5% sulfuric acid (by weight). The solution was neutralized with anhydrous sodium carbonate and then diluted with 2.5 liters of water. The methyl esters of the fatty acids were extracted with ether and dried over anhydrous sodium sulfate for about 3 days. The ether was removed by distillation, the last traces being removed in vacuo at 70°C. and 6-mm. pressure. A yield of 975 gm. of crude methyl esters was obtained.

Fractionation of the Methyl Esters of the Fatty Acids

a) *Description of Distilling Column.* A column packed with a spiral screen as described by Lecky and

[1] Associate chemist (resigned) and associate plant physiologist, respectively, Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, United States Department of Agriculture.

[2] The trade name for a partially hydrogenated vegetable fat.

TABLE 1.

Fractionation of the Methyl Esters of the Fatty Acids in Tung Buds.

Fraction number	Boiling point in degrees C. at 1-2 mm. pressure	Weight of fraction in grams	Total heating time in minutes	Iodine number	Refractive index at 25°C.	Saponification equivalent		Melting point of derivative degrees C.		Acid identified
						Fraction	Known acid	Unknown	Known	
Original methyl ester	.....	385.4	.....	.....	.....	.....	.....	.....	.....	.....
1[1]	75-115	18.3	95	.....	.....[2]	.....	.....	.....	.....	.....
2	115-120	7.0	145	72.7	1.4508	256.3	.....	.....	....	.....
3	120-125	11.3	170	72.0	1.4555	249.1	242	80.0[3]	81.0	Myristic
4	125-130	13.5	190	73.9	1.4558	263.6	.....	.....	.....	.....
5	130-135	7.7	215	66.0	1.4541	265.7	.....	.....	.....	.....
6	135-140	19.7	270	61.0	1.4520	266.8	270	85.5[3]	86.0	Palmitic
7	140-145	49.3	345	57.9	1.4537	268.5	270	86.0[3]	86.0	Palmitic
8	145-150	23.2	415	63.3	1.4566	266.8	.....	.....	.....	.....
9	150-155	25.5	490	76.8	1.4579	269.9	270	85.0[3]	86.0	Palmitic
10	155-160	52.4	575	71.5	1.4578	273.1	{296 294	131.5[4] 173.0[4]	132.0 174.0	Oleic Linoleic
11	160-165	50.7	695	54.9	1.4568	287.2	296	133.0[4]	132.0	Oleic
12	165-170	23.9	735	38.7	1.4588	288.4	.....	.....	.....	.....
13	170-175	10.4	765	51.0	1.4600	290.8	.....	.....	.....	.....
14	175-180	16.6	800	48.7	1.4610	295.0	298	90.0[3]	90.0	Stearic
15	180-185	9.6	830	53.6	1.4618	297.4	.....	.....	.....	.....
16	185-190	4.9	845	53.5	1.4625	298.0	298	91.0[3]	90.0	Stearic
17	190-195	5.8	870	52.2	1.4640	311.0	298	89.5[3]	90.0	Stearic
Residue	.....	33.9	.....	.....	.....	.....	.....	.....	.....	.....

[1] Non-fatty acid material containing a trace of methyl ester. [2] Only a trace of methyl ester present. [3] P-bromo-phenacyl ester. [4] Hydroxy acids.

Ewell (4) was constructed by F. H. Hayes of the Drake Laboratory of the University of Florida for use in this study. The spiral, having a length of 122 cm. with 1.5 turns per centimeter, was constructed of stainless steel washers, having an outside diameter of 35 mm. and an inside diameter of 17 mm. To provide support a glass tube, sealed at both ends, was inserted in the spiral which was then forced into a pyrex tube of 34 mm. inside bore. The pyrex tube was wound with a nichrome wire of the desired resistance to permit the column to be heated electrically. The heating jacket was insulated and protected by another pyrex tube of 75-mm. diameter. The one-liter flask was heated by a hemispherical unit with internal nichrome heating elements. The temperature of both the flask and column was controlled by means of an A.C. variable transformer. A modified Penn State still head with an enclosed thermometer was used at the top of the column. The reflux ratio was regulated by a 2-mm. stopcock located just below the condenser of the still head. By using a mixture of carbon tetrachloride according to the method of Morton (5) and the data of Rosanoff and Easley (6), the column was found to have 15 theoretical plates with an H.E.T.P. equivalent to 3.2 inches.

b). *Distillation.* The 793 gm. of crude mixed methyl esters of the fatty acids (which consisted of 68% unsaturated acids and 32% saturated acids estimated by the lead salt-ether method) were distilled in the previously described column at pressures between 3 and 5 mm. and at temperatures ranging from 75°C. to 300°C. A yield of 386 gm. of mixed methyl esters having a faint straw color was obtained. This mixture of methyl esters was then carefully fractionated in the column into 17 fractions of different boiling points. Data giving the weight of each fraction, heating time, refractive index, iodine number, and saponification equivalent are given in Table 1.

c) *Identification of Fractions.* The saponification equivalent (7) of each fraction was determined by refluxing 2 gm. of methyl ester in 25 ml. of .5 N alcoholic potassium hydroxide for 30 minutes and then titrating the unused potassium hydroxide with .5 N sulfuric acid to the phenolphthalein end point. The observed saponification equivalents correspond approximately with the theoretical values (Table 1) expected for the methyl esters of the C_{14}, C_{16}, and C_{18} saturated fatty acids but not for the unsaturated fatty acids. The identification of the saturated fatty acids was accomplished by the preparation of their p-bromo-phenacyl derivatives according to the method given by Shriner and Fuson (8). After further fractionation of the distillate by the lead salt-ether procedure (9) the unsaturated acids were identified by the preparation of the hydroxy acid derivatives (10). The p-bromo-phenacyl esters were recrystallized to constant melting point from 95% ethanol and the hydroxy-acid derivatives from absolute ethanol or from 1:1 benzene-ethanol mixture. The melting points of the known (8, 9) and the unknown derivatives are given in Table 1. Myristic acid was identified in fraction 3; palmitic acid in fractions 6, 7, and 9; 9:10-oleic acid in fractions 10 and 11; 9:10-, 12:13-linoleic acid in fraction 10; and stearic acid in fractions 14, 16, and 17. Oleic and linoleic acids were also identified in the original distillate before fractional distillation, by separation of the unsaturated acids by means of lead salt-ether procedure with subsequent preparation of the hydroxy acids. After separation by the lead salt-ether procedure, fraction 4 gave an unsaturated fatty acid having an iodine value of 85, a thiocyanogen value of 65, and an equivalent weight

of 268. Upon oxidation with alkaline permanganate this fatty acid failed to yield a crystalline derivative. The residue left from the distillation, upon further purification, yielded no other fatty acid having a higher equivalent weight than fraction 17.

Conclusions and Summary

The methyl esters of the fatty acids in the wax of dormant tung buds were prepared and fractionated in a column packed with a spiral screen. Myristic, palmitic, linoleic, oleic, and stearic acids were identified in some of the fractions by the saponification equivalents and by the melting points of the p-bromophenacyl derivatives of the saturated acids and the hydroxy derivatives of the unsaturated acids. The identification of these acids proved the presence of some of the higher fatty acid radicals similar to those found in Crisco and lanolin. It is believed that the mutual solubility of these fatty acids may have facilitated penetration of the alpha-naphthalenacetic or indole-3-acetic acid in Crisco and lanolin emulsions, into the bud tissue and in this way increased their effectiveness in prolonging dormancy.

Acknowledgment

The authors are indebted to Albert H. Best for suggestions in building the distilling column and to F. H. Hayes for the construction of the column.

REFERENCES

1. Sell, H. M. Reuther, W., Fisher, E. G., and Lagasse, F. S., Bot. Gaz. *103*: 788-793 (1942).
2. Sell, H. M., Taylor, H. A., and Potter, G. F., Bot. Gaz. *106*: 215-223 (1944).
3. Kamm, O., Qualitative Organic Analysis, p. 10, New York (1932).
4. Lecky, H. S., and Ewell, R. H., Ind. Eng. Chem., Anal. Ed. *12*, 544 (1940).
5. Morton, A. A., Laboratory Technique in Organic Chemistry, p. 86, New York (1938).
6. Rosanoff, M. A., and Easley, C. W., J. Am. Chem. Soc. *31*, 970 (1909).
7. Dean, H. K., Utilization of Fats, p. 56, London (1938).
8. Shriner, R. L., and Fuson, R. C., The Systematic Identification of Organic Compounds, Laboratory Manual, pp. 132, 182, New York (1940).
9. Jamieson, G. S., Vegetable Fats and Oils, pp. 345, 404, New York (1943).
10. Lapworth, A., and Mottram, E. N., J. Chem. Soc. *127*, 1628 (1925).

Abstracts

Oils and Fats

Edited by
M. M. PISKUR and MARIANNE KEATING

DECOLORIZATION AND DEODORIZATION OF MUSTARD OIL. S. C. Ukel and M. N. Goswami. *Indian Soap J. 12*, 258-65 (1947). S compounds are removed by deodorization at 100°. Optimum decolorization was with 9 parts of Fuller's earth and 1½ parts carbonit in the oil for 45 minutes at 75°.

CONTINUOUS DEACIDIFICATION OF OLIVE OIL IN A PACKED COLUMN. J. M. Martinez Moreno. *Anales fis. y. quim.* (Madrid) *43*, 261-78 (1947). Oleic acid is extracted from olive oil by 96° alcohol in a tower packed with Raschig rings or pieces of solid glass. The latter was the more effective. On passing through the tower, the mixture separates into an oil phase and an alcohol phase, the latter containing 75-90.5% oleic acid. (*Chem. Abs. 41*, 6420).

VACCENIC (ISOÖLEIC) ACID AS A MEANS FOR DETERMINING BEEF FAT IN PORK FAT. A SEMIMICRO METHOD. Oskar Bauer (Staatlichen Anstalt Lebensmitteluntersuch. Innsbruck). *Z. Lebensmittel-Untersuch. u. -Forsch. 86*, 223-8 (1943). The isoöleic acid detn. of Grossfeld was modified to a semimicro procedure. Results of analyses for isoöleic acid gave: various lards 0.21-0.41 (average 0.28), beef fats from various parts of the carcass 1.15-1.55 (1.30), and butter oils 1.10-1.29 (1.19)%. Using 0.3 and 1.3% as the average isoöleic content of lard and beef fat, resp., the error in approximating beef fat content in mixt. with lard was about 10%.

THE EFFECT OF THE FAT CONTENT OF CREAM ON THE HARDNESS, MOISTURE CONTENT, AND OTHER PROPERTIES OF THE BUTTER. H. Mulder (Rijkslandbouwproefstation, Hoorn, Netherlands). *Verslag Landbouw.* Onderzoek. No. 52C, 269-75 (1946). In view of the widespread belief that it is more difficult to prepare hard butter from cream low in fat than from rich cream, comparative experiments were made with cream containing 8% fat (I), and with 25-40% fat (II). It was found that I takes a longer time to churn and yields round, smooth butter particles, whereas II churns rapidly and gives flocs composed of small particles. The hardness of the butter is not affected by the fat content of the cream. The butter from I contained more moisture, more buttermilk, and was more difficult to dry than that from II. Centrifuging removed more fat from the buttermilk obtained from I, and the loss in fat upon churning was greater. But the phospholipid content of the buttermilk was about the same with or without centrifuging, and the difference in the fat loss could not be explained on this basis. Churning at a temperature of 9° gave harder butter than churning at 15°. (*Chem. Abs. 41*, 6349.)

SOME MODIFICATIONS OF THE SCHIBSTED FAT ALDEHYDE TEST. W. R. Mummery (Dairy Res. Inst., Palmerston North, New Zealand). *J. Dairy Research 15*, 55-6 (1947). The application of the Schibsted fat aldehyde test for the routine examination of butter and butterfat is facilitated by the following modifications: storage of the reagent at cool temperatures, use of Na metabisulphite instead of SO_2, preparation of the standard color at a lower pH.

EFFECT OF STORAGE AND ACIDITY ON THE PROTECTION OF VITAMIN A IN SHARK-LIVER OIL BY ANTIOXIDANTS. S. M. Bose (Indian Inst. Sci., Bangalore). *Current Sci.* (India) *16*, 119-120 (1947). Vitamin A in shark-liver oil was protected by the use of antioxidants. Highest protection was achieved with 0.04% isobutyl gallate and 0.02% citric acid. Stored at room temperature in dark bottles the controls lost 10% activity in one month and about 60% within 10 months, whereas the activity in protected samples was retained up to this time. However, once deterioration had started the rate of destruction was comparable to those of the controls. (*Chem. Abs. 41*, 6021.)

VITAMIN A AND OIL CONTENT OF FISH LIVERS AND VISCERA. FISHES OF THE OREGON COAST. R. O. Sinnhuber and D. K. Law (Oregon Agr. Exper. Sta.,