TABLE III

Analysis of Oils and Fatty Acid Esters

	Per cent in sample							Per cent on chromatogram basis		
	Known ester[1] mixture		Pure soya oil[2]	Dehydrated castor oil[2]	Linseed-oiticica[3,5]	Soya alkyd No. 1[3]	Soya alkyd No. 2[3]	Soybean oil[6]	Soya alkyd No. 1	Soya alkyd No. 2
	Actual, %	Found, %								
Palmitate	15.4	15.3	9.9	0.8	3.9	7.1	8.0	13.5	11.5	13.3
Stearate	15.3	15.4	3.8	0.9	4.0	2.7	3.4	3.9	4.4	5.6
Oleate	15.5	15.5	23.5	4.0	9.7	17.6	19.2	22.2	28.4	30.7
Linoleate	22.6	22.6	49.7	24.4	5.2	29.2	26.6	51.4	47.1	42.8
Arachidate	15.7	15.9	.....	.....	.....	.....	.....	.....	.....	.....
Linolenate	15.5	15.5	9.3	.....	6.0	5.3	4.6	8.9	8.6	7.0
Other	.....	.....	4.4	20.0[4]	.....	.....	.4	.....	.....	.6
Polymer	0.0	0.0	0.0	49.9	71.2	38.1	37.7	N/A	N/A	N/A
Total	100.0	100.2	100.6	100.0	100.0	100.0	100.0	100.0	100.0	100.0

[1] Hormel Foundation. "Found, %" obtained using methyl margarate as internal standard.
[2] Oils.
[3] Extracted from paint.
[4] Includes conjugated linoleates.
[5] No licanate peak on the chromatogram.
[6] Reference (5).

by esterification of fatty acid and rosin acids alike with diazomethane followed by gas chromatography of the resultant ester mixtures (2), provided appropriate rosin ester detector response factors could be ascertained. However, the internal standard procedure reported herein should have a direct application to quantitative estimations of polymer content in heat-bodied oils.

REFERENCES

1. Gehrke, C. W., D. F. Goerlitz, C. O. Richardson, and H. D. Johnson, J. Dairy Sci. *43*, 839 (1960).
2. Hudy, J. A., Anal. Chem. *31*, 1754–1756 (1959).
3. Iden, R. B., and E. J. Kahler, JAOCS *39*, 171–173 (1962).
4. Lorette, N. B., and J. H. Brown, Jr., J. Org. Chem. *24*, 261–262 (1959).
5. Zielinski, W. L., Jr., W. V. Moseley, Jr., R. C. Bricker, Offic. Dig. *33*, 622–634 (1961).

[Received August 5, 1963—Accepted December 10, 1963]

Studies on the Changes in Fatty Acid Composition in Developing Seeds. I. *Recinus communis*

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Abstract

Oils from castor seeds at different stages of ripening have been studied. The fatty acid composition has been determined by paper chromatography. The ratio of the weight of the kernel to the weight of the seed coat changes from 1.0: 1.24 (14 days) to 1.0:0.48 (45 days) and the oil content of the seed coat is negligible. Amounts of the individual fatty acids in 1 g of kernel as well as in a single seed have been shown. The amounts of ricinoleic, linoleic and stearic acids gradually increase with the ripening of the seeds whereas the amounts of oleic and palmitic acid after an initial increase upto 28 days gradually decrease towards the later stages of growth when the amounts are calculated on the basis of a single seed.

Introduction

IN ORDER TO understand the mechanism of biosynthesis of fatty acids in plants, various investigations have been made of fats obtained from seeds at different stages of ripening, or grown at different climatic conditions. Recently Simmons and Quackenbush (1) published the results of their investigation with soybeans at different stages of ripening and Franzke (2) published similar investigations on sunflower seeds. In these reports the proportions of the individual unsaturated fatty acids and total saturated fatty acids, as well as their amounts in one g of the seed, were shown. But the proportions or amounts of the individual saturated fatty acids were not determined, probably because of the inadequacy of the method of analysis.

Burr and Miller (3) studied the formation of oil in castor beans and established its relation with the respiratory quotient. According to their observations, on the 7th day from the date of blossoming, about 3–6% oil in dry seeds was present. During the next 15 days, the respiratory quotient gradually increased with very little increase in the amount of the oil. It was only when the respiratory quotient was more than 1.0 that rapid oil formation took place. Appreciable increase in oil content was, however, noted after the respiratory quotient had again fallen below 1.0 or even 0.8. Fat metabolism in germinating castor beans also received the attention of Yamada (4).

There is no reference in the literature about the changes in fatty acid composition of ripening castor seeds. Hence, an investigation was undertaken as it was considered to be of interest because of the presence of hydroxy acids in the oil.

Experimental

Preparation of Samples

Flowers of castor plants grow in bunches and all the buds in a bunch do not blossom on the same day. Also, a proportion of flowers which bloom may not be fertilized. Hence a large number of flowers were tagged on the day of blossoming and the rest of the

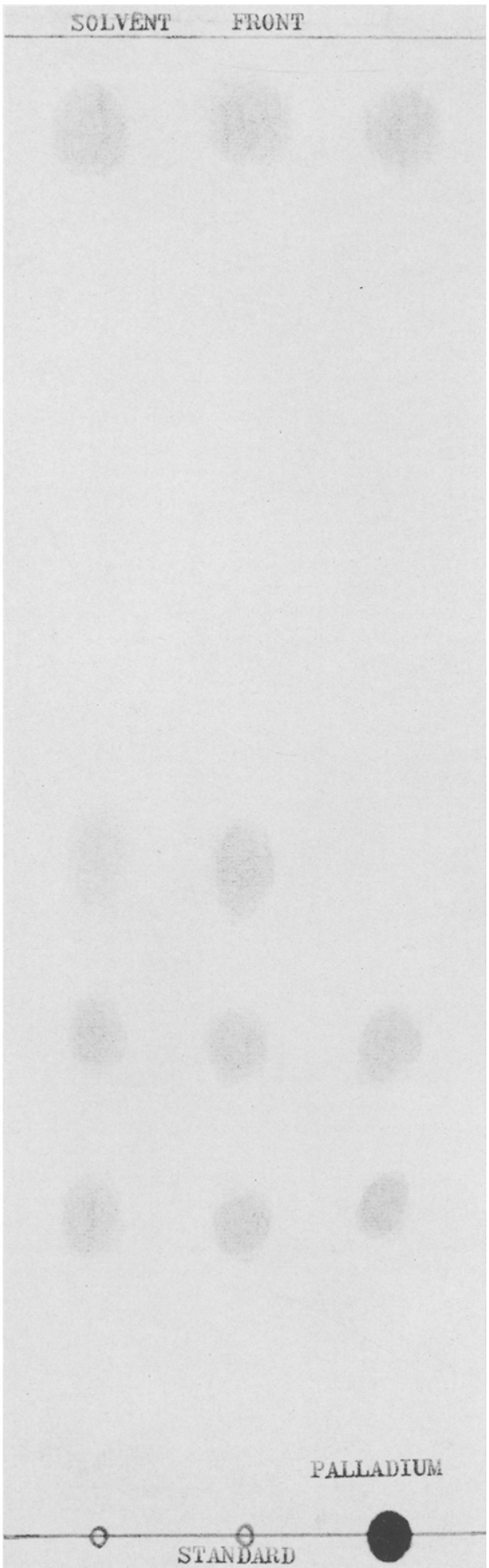

FIG. 1.

buds were carefully removed. The fruits were gathered after intervals of 14,21,28,35, and 45 days from the day of blossoming. These were fully ripe on the last day of collection.

The black, small seeded variety of the plant was chosen for the experiment. The plants were grown in a garden at Calcutta and the seeds were collected during Sept. and Oct., 1960 when average temperatures varied between 20–25C. After collection, the fruit-coat was quickly removed in a cold room and the seeds weighed. Then the seed-coat was removed and the kernel weighed. The kernel was then dried under reduced pressure at 40C in an atmosphere of N_2. The dried seeds were crushed and extracted with petroleum ether bp 60–80C in an atmosphere of CO_2. Oil was recovered after removal of solvent and moisture at reduced pressure at 40C in an atmosphere of CO_2.

A suitable amount of oil was saponified with 0.5 *N* alcoholic KOH and, after removal of the unsaponifiables, the soap was broken with very diute sulphuric acid in the cold in an atmosphere of CO_2. The mixed fatty acids were then extracted with ether, washed free from mineral acid, and dried over anhydrous sodium sulphate. The mixed fatty acids were then obtained after removal of ether at 10C under reduced pressure in an atmosphere of CO_2 to avoid oxidation of the fatty acids or formation of estolides.

Analysis of the Mixed Fatty Acids

The composition of the mixed fatty acids was determined by reversed phase paper chromatographic method as described by Chowdhury, Chandra, and Mukherjee (5a), a modification of the method of Kaufmann and Nitsch (5b).

Purified kerosene oil, bp 180–200C, was shaken with 95% acetic acid and allowed to stand overnight. The hydrocarbon layer was used as stationary phase, and the acid layer was used as the mobile phase. Strips of Schliecher and Schuell filter paper No. 2043 b, 8 x 30 cm, were used. About 3–4 µl of a 2% solution of the mixed fatty acids in benzene was dropped on the start line for the preparation of chromatograms. Chromatograms were also prepared with mixed fatty acids after hydrogenation with finely divided Pd-catalyst deposited on paper (6). The chromatograms (Fig. 1) of the mixed fatty acids both before and after hydrogenation along with standard fatty acids were developed with copper acetate and rubianic acid (7) and evaluated photometrically with Photovolt Densitometer Model 525, by the method of Seher (8).

The Rf values for authentic samples of different fatty acids and their derivatives as obtained during

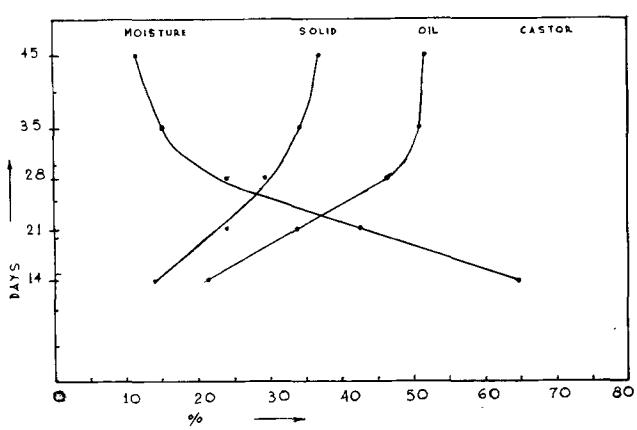

FIG. 2.

TABLE I
Characteristics of the seeds and oils

	14 days	21 days	28 days	35 days	45 days
A. Seeds					
No. of seeds taken	60	60	100	88	270
Wt in g of above seeds	4.36	4.56	6.9	7.5	43
Wt in g of kernels	1.946	2.24	3.48	4.78	29
Wt in g of seed-coat	2.414	2.32	3.42	2.72	14
Hence, kernel:seedcoat	1:1.24	1:1.04	1:1	1:0.57	1:0.48
Wt in g of oil in kernel	0.4128	0.7493	1.6189	2.4144	14.95
Wt in g of oil in seedcoat	0.0063	0.0058	0.0068	0.0049	0.0196
Wt in g of kernel solid matter	0.2787		1.0238	1.64	10.67
Hence:		0.5398			
% oil in kernel	21.21	33.45	46.52	50.71	51.56
% kernel solid matter	14.3	24.1	29.42	34.29	36.81
% H_2O in kernel	64.49	42.45	24.06	15.00	11.63
% oil in seedcoat	0.26	0.25	0.20	0.18	0.14
B. Oils from kernel					
Sap. value	192.8	192.4	189.6	183.6	183.4
Iodine value (Wijs' 30 min)	93.7	92.5	89.3	87.3	84.2
Acid value					2.67
Acetyl value					305.5
Refractive index (40C)	1.4802	1.4793	1.4770	1.4760	1.4758
% Unsap.	0.32	0.28	0.22	0.21	0.22

this investigation are:

Acids	Avg Rf values
Ricinoleic acid	0.95
9,10-dihydroxy stearic acid	0.95
Acetyl ricinoleic acid	0.94
12-hydroxy stearic acid	0.94
Stearic acid	0.22
Linoleic acid	0.45
Oleic acid	0.33
Palmitic acid	0.32

The proportions of the "critical partners" oleic acid and palmitic acid were determined by the hydrogenation difference technique of Kaufmann, as oleic acid after hydrogenation was converted to stearic acid and intensified the spot due to the stearic acid originally present; palmitic acid which was left alone produced a separate spot. But the critical partners, ricinoleic acid and 9,10-dihydroxy stearic acid, do not form separate spots either after hydrogenation or on acetylation, and hence they could not be separately estimated. So during this investigation no separate estimation of 9,10-dihydroxy stearic acid was made.

The proportion of linoleic acid was also determined by spectrophotometric method after alkali isomerization according to the method of Hilditch et al. (9). The acetyl value was determined by standard AOCS methods (10).

The characteristics of the seeds and oils at different stages of growth are shown in Table I, and the composition of the mixed fatty acids obtained from these oils is shown in Table II. The amounts of individual fatty acids present in one g of kernel are shown in Table III and those in a single seed are shown in Table IV. Figure 2 represents the changes in oil, moisture, and solid matter contents of the seeds calculated on the basis of kernel at successive stages of growth.

The seed coats obtained at different stages of growth were dried at 100C in an air oven, crushed, and extracted with petroleum ether for 18 hr. After removal of the solvent and moisture, the amount of the oil in the seed coat was determined.

Discussion

During the growth of castor seeds, weight of the kernel increases at a greater rate than that of the seed coat (Table I) and the ratio of the weight of the kernel to that of the seed coat changes from 1.0:1.24 (14 days) to 1.0:0.48 (45 days). The seed coats at different stages are found to contain very little oil and hence the percentage of oil calculated on total seed will be different from that calculated on kernel only. In the present report percentages of oil, solid matter and moisture have been shown on the basis of fresh kernel.

The compositions of the mixed fatty acids show that the percentages of ricinoleic and stearic acids gradually increase till the seeds are fully ripe, whereas those of linoleic, oleic and palmitic acids gradually decrease (Table II). When the amounts of individual fatty acids in 1 g of kernel are considered, it is found that the amounts of ricinoleic and stearic acids gradually increase but the amounts of linoleic, oleic, and palmitic acids, after initial increase up to 28 days from the date of blossoming, show rapid decrease in the later stages of ripening (Table III). But when the amounts of the individual fatty acids in a single seed are considered, it is found that the amounts not only of ricinoleic and stearic acids, but linoleic acid also, gradually increase till the seeds are fully ripe, whereas those of palmitic and oleic acids, after initial increase up to 28 days, decrease in the later stages (Table IV).

It is now generally agreed that the percentage compositions of the fats obtained at different stages of

TABLE II
Fatty acid composition

	14 days	21 days	28 days	35 days	45 days
% Ricinoleic acid	63.82	67.84	73.61	82.25	88.86
% Linoleic acid	16.77	14.6	11.1	9.3	6.5
	18.1[a]	15.6[a]	12.2[a]	8.8[a]	6.3[a]
% Oleic acid	13.92	12.23	10.6	4.13	1.54
% Palmitic acid	4.09	3.8	2.91	1.2	0.45
% Stearic acid	1.4	1.53	1.78	3.1	3.65

[a] Indicates results of spectrophotometric analysis.

TABLE III
Amounts of individual fatty acids, mg in 1 g of kernel

	14 days	21 days	28 days	35 days	45 days
Ricinoleic acid	161.8	206.5	312.0	378.7	416.8
Linoleic acid	32.38	44.46	47.04	42.82	30.48
Oleic acid	26.87	37.24	44.92	19.01	7.22
Palmitic acid	7.89	11.57	12.33	5.52	2.11
Stearic acid	2.70	4.65	7.54	14.28	27.13
Total Amount	231.64	304.42	423.83	460.53	483.74

TABLE IV
Amounts of individual fatty acids, mg per seed

	14 days	21 days	28 days	35 days	45 days
Ricinoleic acid	5.278	7.71	10.86	20.36	44.81
Linoleic acid	1.062	1.66	1.64	2.3	3.28
Oleic acid	0.88	1.39	1.565	1.02	0.78
Palmitic acid	0.259	0.43	0.43	0.3	0.23
Stearic acid	0.089	0.174	0.26	0.77	2.9
Total Amount	7.568	11.364	14.755	24.75	52.00

growth of the seed do not represent a correct picture of the formation of fatty acids in the seeds. The rapid variation of the ratio of the weight of the kernel to the seed coat, as well as the fact that the seed coat does not contain appreciable amount of oil, will indicate that calculation of the amounts of individual fatty acids in 1 g of the seed does not give us a correct picture of the formation of fatty acids. The number of seeds that would produce one g of kernel differ at different stages of growth of the seeds. As each seed is a unit center of synthesis and storage of fatty acids, it is considered that calculations of the amounts of individual fatty acids in one seed, or a fixed number of seeds at each stage of growth, would give a better picture of biosynthesis of the fatty acids.

ACKNOWLEDGMENTS

Laboratory facilities supplied by R. M. Roy. Financial assistance by the Director of Public Instructions, Government of West Bengal.

REFERENCES

1. Simmons, R. O., and F. W. Quackenbush, JAOCS, *31*, 601 (1954).
2. Franzke, Cl., Fette, Seifen, Anstrichmittel, *58*, 340 (1956).
3. Burr, G. O., and E. S. Miller, Bot. Gaz., *99*, 773 (1938).
4. Mitsuhiro Yamada, Sci. Papers Coll. Gen. Educ., University of Tokyo *5*, 149 (1955), Chem. Abs., *51*, 5914 (1957).
5a. Chowdhury, D. K., K. S. Chandra, and S. C. Mukherji, Die Nahrung, *3*, 252 (1959).
5b. Kaufmann, H. P., and W. H. Nitsch, Fette, Seifen, Anstrichmittel, *56*, 154 (1954).
6. Kaufmann, H. P., and D. K. Chowdhury, Chem. Ber. *91*, 2117 (1958).
7. Ballance, P. E., and W. Mary Crombie, Biochem. J., *69*, 632 (1958).
8. Seher, A., Fette, Seifen, Anstrichmittel, *58*, 498 (1956).
9. Hilditch, T. P., C. B. Patel, and J. P. Riley, Analyst, *76*, 81 (1951).
10. *American Oil Chemists' Society, Official and Tentative Methods*, 2nd ed., rev. to 1962, Chicago, Ill., Cd 4–40.

[Received May 28, 1962—Accepted April 16, 1963]

Complex Formation Between Oxidized Lipids and Egg Albumin[1]

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Abstract

The nature of the reactions leading to the formation of complexes between oxidized lipids and proteins is little understood. Complexes were formed between thermally oxidized and thermally polymerized corn oils and egg albumin. The results indicated that the thermally oxidized oil was much more reactive than the thermally polymerized oil. The nature and extent of oxidative groups in the lipid were varied and reacted with egg albumin. The keto and epoxy groups seemed to have a pronounced influence on complex formation while the hydroxy and hydroperoxide groups were observed to be less reactive. Acetylation of egg albumin did not significantly decrease its complexing ability with thermally oxidized corn oil. Further confirmation that the reactive groups in the protein were not covalently tied up to the lipid was obtained by estimating these groups in the original protein and in the complexed protein. IR analyses of oxidized lipid-alumina, oxidized lipid-egg albumin and fatty acid-urea complexes indicated that the characteristic absorption was due to bonded O—H stretching vibration in the first two complexes and the bonded N—H stretching vibration in the two latter complexes.

Introduction

THE BROWNING of food materials is a familiar example of complex formation between proteins and reducing sugars (1,2). Complex formations between carbohydrates and lipids are generally not well known (3,4). The complex reactions that occur during the oxidation of fats in the presence of proteins have been considered to be responsible for the yellowing of bacon and rusting of fish, and oxidized fats have been implicated in the formation of brown pigment in the adipose tissue of vitamin E deficient rats and ceroid pigment in choline deficient rats (5,6,7). However very little work has been devoted to studying the chemical and physical properties of the oxidized lipid-protein complexes which may be formed *in vitro*.

The linkage between the lipid and the protein in the case of the complex conjugated proteins, known as lipoproteins, probably involves Vander Waals forces and weak electrostatic attractions (8,9,10). In most cases, these lipoprotein complexes are labile and the lipid can be separated by suitable extraction methods. On the other hand, oxidized lipid-protein complexes are not amenable to similar easy separations (11).

It has been recently observed that oxidized methyl linoleate interacts *in vitro* with serum low density lipoproteins while unoxidized methyl linoleate does not (12). Hartroft has observed the presence of ceroid pigments in the aortas of man exhibiting atheromatous changes, and he also observed the formation of ceroid-like pigments *in vitro* by reacting heparinized red cells with cod liver oil in the presence of air (13,14). The system used in these *in vitro* experiments has been complex and therefore very little information could be obtained regarding the type of interaction between the oxidized lipid and the protein.

In a previous study (11), a relatively simple system such as oxidized linoleic acid and egg albumin was studied and the results indicated absence of covalent bonding between the oxidized lipid and the protein. It was further suggested that hydrogen bonds were apparently responsible for complex formation. However no definite information could be obtained from that study as to the extent to which each of the oxidative groups in the lipid was involved in complex formation with the protein. Therefore, in the present investigation, an atempt was made to understand the nature of the linkage between the oxidized lipid and the protein by a) varying the nature and extent of the reactive groups in the lipid, b) blocking the reactive groups in the protein and estimating the total reactive groups in the original pro-

[1] Supported by research grant No. C-1932 and A-1671 from the National Institutes of Health; U. S. Public Health Ser.; Dept. of Health, Education, and Welfare; and The American Dairy Assoc.
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