

Studies on Germinating Peanut Seeds

L. F. RABARI, R. D. PATEL, and J. G. CHOCHAN, Chemistry Department, Sardar Vallabhbhai Vidyapeeth, Vallabh Vidyanagar, India

A STUDY OF THE conversion of oil into carbohydrates during germination and of the conversion of carbohydrates into oil during the ripening period of seeds has been subject of many investigations since the time of Saussure (1).

Recently W. Mary *et al.* (2) have studied the composition of component fatty acids of oil during the germination of *Citrullus vulgaris*. They concluded from their experiment that major acids were metabolized at rates proportional to the quantities originally present with the exception of oleic acid, which is metabolized more rapidly while Kartha and Shethi (3) have reported nonselective metabolism of fat in a number of germinating seeds.

A review of previous literature indicates that no definite theory prevails to account for changes taking place during the germination of oleaginous seeds. The present investigation was undertaken therefore to throw some light on the problem of conversion of oil to carbohydrates.

Experimental

Dry peanut seeds were planted in soil in the first week of the month of June, 1957. The seedlings were removed at intervals of 3,5,7, and 8 days from the day of planting. Seedlings were immediately washed free of clay after removal from the soil. They were dried in sunshine after decanting off the water.

Dried seeds were crushed to a fine powder, then extracted with petroleum ether (40°-60°) in a glass Soxhlet apparatus. Most of the solvent was distilled off at atmospheric pressure, and traces were finally removed under reduced pressure. The cake of each sample was preserved in a stoppered bottle after the addition of a few drops of toluene.

The characteristics of oil samples extracted from seeds germinated for different intervals were determined according to the A.O.C.S. Methods (4).

Determination of Percentage Composition of Fatty Acids. The fatty acids required were prepared according to the method described by Hilditch (5). Saturated acids were obtained from mixed fatty acids by following Bertram's oxidation procedure (6) as modified by Palikan and von Mikusch (7). The methyl esters of these acids were fractionated in an E.H.P. column. The saponification equivalent of each fraction was determined, and the composition of component acid in mixed fatty acids was calculated.

The composition of unsaturated acids was determined by using the ultraviolet spectrophotometric technique (8). A Beckman spectrophotometer, DU Model, was used in the present investigation.

The composition of the cakes was determined according to methods recommended by the A.O.C.S. (9). The protein content was calculated from the nitrogen content, which was estimated by Kjeldahl's method.

Results of the investigation are given in Tables I-V.

TABLE II
Percentage Composition of Peanut Seeds Before and During Germination

	Before germination	3 days	5 days	7 days	8 days
Moisture.....	3.9	4.6	4.8	4.9	4.5
Ash.....	2.4	2.9	3.0	2.7	2.6
Oil content.....	46.8	41.5	26.0	16.5	9.3
Protein.....	26.7	27.2	26.6	26.2	26.6
Crude fiber.....	2.1	3.2	4.3	5.5	6.1
Reducing sugar.....	0.4	2.6	3.1	3.8
Sucrose.....	5.8	6.8	10.4	10.2	11.8
Starch.....	12.3	13.4	22.3	30.8	35.3

TABLE III
Percentage Composition of Mixed Fatty Acids of the Oil from Peanut Seeds Before and During the Germination Period

Acid	Before germination	3 days	5 days	7 days
Myristic.....	0.5	0.5	0.6
Palmitic.....	7.0	6.3	6.1	4.6
Stearic.....	2.8	2.8	3.5	4.7
Arachidic.....	3.8	3.4	2.5	2.2
Behenic.....	2.5	2.5	1.6	1.5
Lignoceric.....	1.3	1.4	2.3	3.1
Linoleic.....	21.7	24.0	26.9	27.5
Oleic (by difference).....	60.4	59.1	56.5	56.4

TABLE IV
Percentage Composition of the Component Fatty Acids of Oil from 100.0 g. of Peanut Seeds Before and During Germination

Acid	Before germination	3 days	5 days	7 days
Myristic.....	0.2	0.2	0.1
Palmitic.....	3.0	2.4	1.5	0.7
Stearic.....	1.2	1.1	0.8	0.7
Arachidic.....	1.6	1.3	0.6	0.3
Behenic.....	1.1	0.9	0.4	0.2
Lignoceric.....	0.5	0.5	0.5	0.5
Linoleic.....	9.4	9.1	6.4	4.2
Oleic.....	26.1	22.6	13.5	8.5

TABLE V
Percentage of Component Fatty Acids of Oil from Peanut Seeds During Germination, Taking 100.0 g. in Ungerminated Seeds

Acid	Before germination	3 days	5 days	7 days
Myristic.....	100	100.0	50.0
Palmitic.....	100	80.0	50.0	23.3
Stearic.....	100	91.6	66.6	58.3
Arachidic.....	100	81.2	37.5	18.7
Behenic.....	100	81.8	36.3	18.2
Lignoceric.....	100	100.0	100.0	100.0
Linoleic.....	100	96.8	68.0	44.7
Oleic.....	100	86.6	51.7	32.6

TABLE I
Characteristics of the Oil of Peanut Seeds Before and During the Germination Period

Characteristics	Before germination	3 days	5 days	7 days	8 days
Color.....	Yellow	Yellow	Dirty yellow	Green	Green
Refractive index.....	1.467 at 29.5°C.	1.468 at 31°C.	1.468 at 31°C.	1.4665 at 30.5°C.	1.466 at 31°C.
Density at 30°C.....	0.9106	0.9100	0.9108	0.9101	0.9098
% fatty acids.....	92.2	91.9	91.8	91.7
Iodine value.....	89.2	92.6	94.5	95.8	96.0
Saponification value.....	187.9	189.8	189.9	190.5	190.3
% unsaponification matter.....	0.3	0.4	0.6	0.6	0.65
Acid value (F.F.A. as oleic).....	0.7	3.5	7.4	10.5	12.1

Discussion

From the study of the characteristics of the oil presented in Table I it can be noted that iodine value, free fatty acids, and unsaponifiable increase during germination. The increase in I.V. may result from the synthesis of unsaturated acids. However this may arise from the disproportionate metabolism of saturated and unsaturated acids. Therefore a study of only I.V. does not help to form any conclusion. To draw a reliable conclusion it is necessary that the composition of component acids in 100 g. of seeds be considered. The increase in free fatty acids may be caused by the hydrolysis of glycerides by water, hence it will not be correct to say that free fatty acids are first accumulated before conversion of oil into glucides as concluded by Johnston and Sell (10).

The study of the composition of the component fatty acids in 100 g. of seeds has revealed that none of the acid has been synthesized during germination. All acids are metabolized at different rates during germination. Myristic acid has been metabolized completely. Stearic acid is metabolized slowly while lignoceric acid is not metabolized in the initial stages of germination. The metabolism of lignoceric acid during later stages of germination was found to be very slow. Other saturated acids are metabolized more rapidly. Among unsaturated acids, oleic acid is metabolized more rapidly than linoleic acid. The rate of the metabolism of unsaturated acids is slower than that of saturated acids. Thus a disproportionate metabolism of saturated and unsaturated acids is observed in the present investigation. Hence increase in I.V. is due to this disproportionate metabolism of component acids and cannot be attributed to the synthesis of unsaturated acids.

The study of the composition of seeds has revealed the significant change in crude fiber, oil, and glucide

contents of seeds. Reducing sugar, which was not originally present, has been synthesized in the first stage and grows during subsequent stages. Sucrose and starch contents also increase during germination. It is therefore concluded that the oil is metabolized during germination and that reducing sugar, sucrose, and starch are synthesized at the expense of oil. This observation is in close agreement with those reported by previous workers (10,11,12). The nitrogen content of seeds was found to be constant. Ash content and moisture content have been increased to a small extent.

From the present investigation it has been concluded that saturated acids are metabolized at a greater rate than that of unsaturated acids during germination; some acids are not metabolized during the initial stages of germination; and fatty acids are converted into soluble and insoluble glucide during the course of germination.

REFERENCES

1. Saussure T., de Froriepe's Notizen, *24*, 29 (1842).
2. Mary, W., Crombie, L., and Comber, R., *J. Exptl. Botany*, *7*, 166-180 (1956).
3. Kartha, A. R. S., and Shethi, A. S., *J. Sci. Industr. Res.*, *17B*, 34 (1958); *ibid.*, *17C*, 104 (1958).
4. Official and Tentative Methods of the American Oil Chemists' Society, Cd 1-25, Cd 3-25, Ca 6a-40, Ca 5a-40, 2nd ed., including additions and revisions, 1947-1951, Am. Oil Chem. Soc., Chicago, Ill.
5. Hilditch, T. P., "The Chemical Constitution of Natural Fats," 3rd ed., Chapman and Hall Ltd., London, 1956, p. 571.
6. Bertram, S. H., *Z. dtshch. Öl-u. Fettindustr.*, *45*, 733-36 (1925).
7. Palikan and von Mikusch, *Oil and Soap*, *15*, 149 (1938).
8. Hilditch, T. P., Patel, C. B., and Riley, J. P., *Analyst*, *76*, 81 (1951).
9. Official and Tentative Methods of Analysis of the A.O.A.C., 27.28, 27.32, 34.39, 34.40, 6th ed., 1945, Association of Official Agricultural Chemists, Washington, D. C.
10. Johnston, F. A. Jr., and Sell, H. M., *Plant Physiol.*, *19*, 694-698 (1944).
11. Miller, E. C., *Ann. Bot.*, *24*, 693 (1910); *ibid.*, *26*, 889 (1912).
12. Houget, Jacques, *Compt. rend.*, *215*, 387-388 (1942); *ibid.*, *216*, 821-822 (1943).

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Unsaturated Lipid Peroxidation Catalyzed by Hematin Compounds and Its Inhibition by Vitamin E¹

A. L. TAPPEL, W. DUANE BROWN,² H. ZALKIN, and V. P. MAIER,³ Department of Food Science and Technology, University of California, Davis, California

Evidence favoring hematin catalysis over autoxidation as the dominant mechanism of lipid peroxidation in animal tissues is presented. Lipid peroxidation in Erlich ascites tumor cells and isolated electron transport particles has been studied. Random destruction of the cytochromes and a loss of catalytic activity correlate with peroxidation of the electron transport particle.

Mixtures of α -, β -, and γ -tocopherols show no synergistic effect. Synergism with ascorbate and citrate greatly enhance the antioxidant activity of α -tocopherol. A tocopherol-ascorbate-glutathione-triphosphopyridine nucleotide couple could act synergistically to inhibit lipid peroxidation in animal tissues.

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² Present address: Institute of Marine Resources, University of California, Berkeley.

³ Present address: U.S.D.A. Fruit and Vegetable Laboratory, Pasadena, Calif.

LIPID PEROXIDATION catalyzed by hematin compounds is a basic pathological reaction *in vivo* and a deteriorative reaction *in vitro*. In vitamin E-deficient animals hematin-catalyzed lipid peroxidation appears to be widespread but is particularly damaging in the mitochondria and microsomes, where the free radical intermediates react with enzymes and lead to metabolic derangements (28-30,33). Lipid peroxidation products have been found in human atherosclerosis (6,11); this pathological reaction may involve catalysis by hemoglobin (9). Stored whole blood appears to deteriorate by hemolysis involving hemoglobin-catalyzed oxidation of the unsaturated