Distribution of Fatty Acids during Germination of Soybean Seeds

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ABSTRACT

Gas chromatographic determination of the fatty acids in the seeds of soybean (*Glycine max*) showed mainly linoleic, oleic and palmitic acids with linoleic acid being the major component. Changes in the distribution of fatty acids were measured during germination in the cotyledons and roots. A decrease in palmitic and oleic acids was observed in the cotyledons from 6 to 12 days, while linoleic acid increased during the same period. In roots also, the major fatty acid was linoleic acid, while palmitic and linolenic acids were higher in roots in comparison with the cotyledons. During the 3-12 days of germination period, no major changes in the distribution pattern of fatty acids were observed in the roots. The possible significance of these changes is discussed.

INTRODUCTION

Increased production and utilization of the soybean (*Glycine max*) seeds in the U.S. have created renewed interest in studies regarding its chemical composition and genotypes by chemists and geneticists, respectively. Soybean seeds contain large amounts of protein and oil. Chronology of fatty acid formation in the germinating or maturing soybean seeds has been reported by several investigators (1-3). Brown et al. (1) determined the fatty acid composition of soybean seeds (Chippewa variety) during the germination, and reported a significant decrease in oleic acid during the early phase. The sequence of formation of fatty acids in the developing soybean seeds was reported by Simmons and Quackenbush (2). Excised soybean (Lincoln) stems bearing pods and leaves were fed C^{14}-labeled sucrose, and the radioactivities were found to appear in the fatty acids in the following order: oleic, saturated, linoleic and linolenic. The presence of the highest specific activity in oleic acid was interpreted by these workers to suggest that oleic acid was the precursor for the other fatty acids. The mechanism of desaturation of fatty acids has also been investigated by Dutton and Mounts (3) in photosynthesizing flax, soybean and safflower plants exposed to $^{14}CO_2$ for 1 hr at seed-setting stage. Of the C_{18}-unsaturated fatty acids, oleic was the first to acquire radioactivity, which subsequently and successively appeared in linoleic and linolenic acids. Brown et al. (1) and Simmons and Quackenbush (2) determined the fatty acids by the 11% glycerol-KOH isomerization methods of Brice et al. (4), while Dutton and Mounts (3) used the gas chromatographic method.

The present report concerns the distribution of the saturated and unsaturated fatty acids in the cotyledons and the roots of the soybean seeds during 0-12 days of germination. The fatty methyl esters were analyzed by the gas liquid chromatographic procedure.

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EXPERIMENTAL PROCEDURES

Seed

Lee variety soybean seeds were obtained from Beaumont, Tex., through J.I. Kirkwood (director, Experiment Station, Prairie View A&M College).

Germination and Extraction of Oil

Germination of the seeds was conducted in the dark at 25 C. At 3, 6, 9 and 12 days following germination, the seedlings were separated into cotyledons and roots and placed in an oven for 10-12 hr. The dried samples were ground to a fine powder in a Waring blender and stored in a freezer.

Oil from the ground material was extracted with petroleum ether in a soxhlet apparatus. The solvent was removed by evaporation under low heat, and the oil was stored at refrigerator temperature.

Preparation of Derivatives

The fatty acids were obtained from the glycerides by alkaline hydrolysis (5), and the methyl esters were prepared for gas liquid chromatographic analysis by the method of Huston and Albra (6).

Gas Chromatography

Methyl ester fractions obtained by the above procedure were injected into a Beckman GC 2A gas chromatograph. A 0.6 cm x 7.0 ft stainless steel column, treated with 20% polydiethylene glycol succinate on acid-washed Chromosorb W, was operated at a temperature of 220 C with

TABLE I

Fatty Acids of Soybean Cotyledons during Germination

	Content, %				
	Days of germination				
Fatty acid[a]	0	3	6	9	12
Palmitic	15.1	16.6	17.0	12.3	11.8
Stearic	5.3	5.3	5.5	3.9	3.0
Oleic	28.8	27.1	27.5	19.5	18.3
Linoleic	42.3	42.0	43.3	60.2	63.4
Linolenic	8.5	8.6	6.3	3.8	3.2

[a]Fatty acids were identified as methyl esters by comparing their retention time with that of authentic samples. Per cent composition of fatty acids was calculated on the basis of their gas chromatographic areas, which were obtained by multiplying peak heights by widths at half peak heights. Oil contents of cotyledons at 0, 3, 6, 9 and 12 days were 18.3, 18.0, 17.4, 17.2, 16.9%, respectively.

helium as the carrier gas and a current of 200 ma. Elution time was ca. 10 min. A standard solution containing a mixture of authentic samples of fatty methyl esters was run under the same experimental conditions prior to running the samples. The retention times of the unknown samples of methyl esters were compared with the standards for identification purposes.

RESULTS

The results of the distribution patterns of fatty methyl esters of the cotyledons during germination (Table I) show that the seeds prior to germination contain largely linoleic (42.3%), with decreasing concentrations of oleic (28.8%) and palmitic (15.1%) acids. During 6-12 days of germination, the concentrations of saturated and oleic acids decreased gradually, while the concentration of linoleic acid increased.

The changes in the fatty acid composition of the roots during the 3-12 days of germination are presented in Table II. At three days following germination, linoleic acid was the major component of the roots as well as the seed; however concentrations of palmitic and linolenic acids were higher in the oil from the roots than in the oil from the cotyledons. There were no major changes in the pattern of fatty acids of the roots between 3-12 days of the germination period.

DISCUSSION

Of the leguminous crops, soybean seeds are reported to have a relatively higher concentration of oil. The predominant unsaturated fatty acids are linoleic and oleic, which comprise over 70% of the total fatty acids. Furthermore soybean seed oil is classified as "linoleic-rich" because linoleic acid constitutes ca. 50% of the total fatty acids.

Holman (7) reported a decrease in both linoleic and linolenic acid content of the whole soybean seedling during 6 days of germination in the dark. On the other hand, Brown et al. (1) reported no major change in the linoleic acid content during germination. These workers used a combination of iodine value and spectrophotometric method (3) and analyzed the whole seedlings. The results of the present study show the changes in the concentrations of the individual fatty acids in the cotyledons and in the roots during the 12 days of germination period, as measured by gas liquid chromatographic procedure. The linoleic acid content of oil in the cotyledons increases from 43.3 to 63.2% durng the 6-12 day period, while the oleic acid in the oil decreases from 27.5 to 18.3%. The results may be explained on the basis of possible conversion of oleic acid to linoleic acid, as suggested by Simmons and Quackenbush (2) and by Dutton and Mounts (3) for soybean plants.

TABLE II

Fatty Acids of Soybean Roots during Germination

	Content, %			
	Days of germination			
Fatty acid[a]	3	6	9	12
Palmitic	21.3	22.8	22.5	18.3
Stearic	7.5	6.3	4.8	4.7
Oleic	10.4	10.8	11.8	10.8
Linoleic	43.0	41.4	40.5	45.8
Linolenic	17.6	21.5	21.1	20.1

[a]Procedure for determination of fatty acids was same as described under Table I. Oil content of root was 2% and did not change significantly during germination.

The roots of the soybean seeds during the 3-12 days of germination contain a lower level of oleic acid and higher levels of linolenic and palmitic acids in the oil, in comparison with the oil from the cotyledons.

Singh et al. (8) studied the distribution of individual fatty acids in various parts of the soybean seeds before germination and reported a consistently higher value for linolenic acid in the oil from the section of the seed containing the rootshoot axis. The differences in the pattern of fatty acids of the cotyledons and the roots show the importance of analyzing the individual components of the seedling during germination.

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REFERENCES

1. Brown, B.E., E.M. Meade and J.R. Butterfield, JAOCS 39:327 (1962).
2. Simmons, R.O., and F.W. Quackenbush, Ibid. 31:441 (1954).
3. Dutton, H.J., and T.L. Mounts, J. Lipid Res. 7:221 (1966).
4. Brice, B.A., M.L. Swain, S.F. Herb, P.L. Herb, P.L. Nichols and R.W. Reimenschneider, JAOCS 29:279 (1952).
5. Nesbitt, J.A., and W.J. Lennarz, J. Bacteriol 89:425 (1964).
6. Huston, C.K., and P.W. Albra. Ibid. 88:425 (1964).
7. Holman, R.T., Archiv. Biochem. 17:459 (1948).
8. Singh, B.B., H.H. Hadley and R.I. Collins, Crop Sci. 8:171 (1968).

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