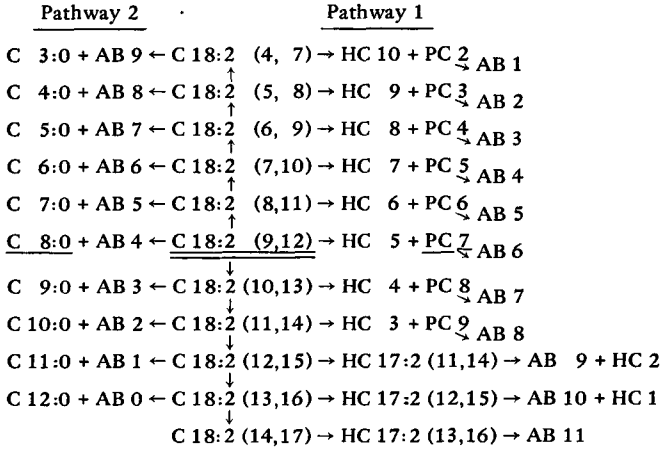

AB = *n*-alkylbenzenes; PC = ω-phenylcarboxylic acids; C = fatty acids; HC = hydrocarbons.

SCHEME II. Summary of possible reactions taking shift of double bonds into consideration.

radicals can cyclize in a manner entirely analogous to the one just discussed. The products formed this way then represent the complete spectrum of all AB, PC and fatty acids found.

Branched or more unsaturated fatty acids with at least 3 double bonds must be considered as precursors for the formation of 1-butyltetralin and 1-pentylindane (ca. 0.2% each) in experiments 1 and 4, respectively; for a summary of possible reactions see Scheme II. In this reaction scheme the main products of the pyrolysis of 9,12-octadecadienoic acid without shift of double bonds are underlined.

With the double bond shifted into the direction of the carboxylic group, the isomerized acid reacts to C7:0 and AB5. A further shift of the double bonds apparently takes place only to a very small extent. If the double bonds are shifted in the opposite direction, decarboxylation can take place before or after cyclization.

ACKNOWLEDGMENTS

We express our thanks to "Österreichische Nationalbank" for the financial support as well as to Westvaco Corp., Charleston, SC, and G. Billek for providing a large number of samples. A. Nikiforov helped in operating the mass spectroscopical equipment.

REFERENCES

1. Totani, N., and N. Matsuo, Yukagaku 25:467 (1976).
2. Pines, H., and Ch. T. Goetschel, J. Org. Chem. 30:3530 (1965).
3. Traitler, H., and K. Kratzl, JAOCS (in press).
4. Grob, K., G. Grob and K. Grob, Jr., Chromatographia 10:181 (1977).
5. Ackman, R., J. Gas Chromatogr. 2:173 (1964).
6. Liardon, R., and U. Kühn, J. High Resol. Chromatogr. Chromatogr. Commun. 1:47 (1978).
7. Bruce, B.A., M.L. Sivain, S.F. Herb, P.L. Nichols and R.W. Riemenschneider, JAOCS 29:279 (1952).

[Received December 26, 1979]

The Effect of Soybean Moisture during Storage on the Lipid Composition of Extracted Crude Oil

G.W. CHAPMAN, Jr., and **J.A. ROBERTSON,** Field Crops Research Unit, Richard B. Russell Agricultural Research Center, USDA, SEA, PO Box 5677, Athens, GA 30613

ABSTRACT

The quality of soybean oil extracted from seed stored under constant temperature and relative humidity for 42 days was evaluated over a wide range of moisture levels. Storage of soybeans at 9, 13 and 18% moisture had little affect on the major lipid components (neutral lipids), even though seed stored at 18% moisture became infected with mold. The level of phospholipid in the extracted crude oil decreased during the last 3 weeks of storage in seeds stored at 13 and 18% moisture from 4 to 2.5% of the total oil. During the same period, the level of free fatty acids, (FFA) (primarily 16:0 and 18:2) in these samples increased. This study indicated that the increase in FFA during seed storage at high moisture levels was the result of soybean lipase and possibly phospholipase activity. These findings suggested that soybeans should be kept at less than 13% moisture for long-term on-farm storage to preserve oil quality.

INTRODUCTION

The recommended soybean moisture for harvest is 13% (1); however, in actual practice, soybeans are harvested between 13-18% (2). Soybeans harvested at the higher moisture levels should be dried to 13% or less for storage (1,3). When soybeans are stored for very long periods (2 yr), a moisture of 12.0% is recommended to maintain a good seed grade (4). At present, no scientific reason has been given for the selection of this particular moisture level. However, high levels of phospholipids have been found in extracted crude oil from soybeans stored for very short storage periods under high moisture and temperature conditions (5).

Unusually wet weather during soybean harvest is sometimes a problem in southeastern United States. High-moisture soybeans may become damaged in the field or during storage. Because of the high temperatures (35-45 C) of silos and storage bins in this geographical area, soybeans not dried to proper moisture levels can be severely damaged. Soybeans damaged in such a manner are characterized by high levels of free fatty acids, (FFA) high peroxide values and Lovibond color in extracted crude oil, which greatly reduces the commercial grade of an oil (6-8). A penalty is imposed on crude oil for export markets with FFA levels exceeding 0.75% of the total oil (8). These undesirable oil qualities are attributed to mold growth on oilseeds harvested at or stored under high moisture (9,10).

The purpose of this study was to determine the effects of several constant soybean moisture levels on the lipid composition of extracted crude oil during short-term storage and to establish the involvement of soybean enzymatic reactions on extracted oil quality.

MATERIALS AND METHODS

Locally produced soybeans (mixed var.) that had been

stored on-farm for 1 year were used in this study. The soybeans were of good quality with very few splits and a moisture content of 9.8%.

Two 500-g samples of soybeans were adjusted to 12 and 14% moisture by mixing calculated amounts of distilled water and soybeans in inflated air-tight plastic bags and allowing them to equilibrate for 18-20 hr. These soybeans were spread evenly in aluminum wire-screen containers and uniformly exposed to constant temperature (30 C) and predetermined relative humidity in Climate-labs. Relative humidities of 61, 69 and 78% were required to equilibrate and maintain constant moistures of 9, 13 and 18% in these soybean samples during 42-day storage periods. Chamber relative humidities were periodically monitored with a Humidity Indicator (Bacharach Instrument Co., Pittsburgh, PA). The sample equilibrated at 18% moisture was divided equally, and one part treated with 50% Captan (*N*-[trichloromethyl)thio]-4-cyclohexene-1, 2-dicarboximide) to inhibit mold growth during storage. Samples at the other 2 moistures were not treated, since a moisture content higher than 14% usually is required to support mold growth for short storage periods (10).

Samples were taken weekly for moisture determination (11) and lipid analysis. Phospholipid and FFA contents were analyzed every 14 days in captan-treated samples. Three replicate soybean samples (7 g each) were ground in liquid nitrogen and their crude oil extracted with chloroform/methanol (2:1, v/v) as previously described (5). The crude oil was pooled and stored in glass vials at -15 C, and lipid analysis was conducted as soon as possible after extraction. The extracted soybean meal was air-dried and stored in glass jars at -5 C.

Total phospholipid content of crude oil was determined after separation of polar and neutral lipids by thin layer chromatography (TLC), digestion of polar lipids with perchloric acid and spectrophotometric assay of lipid phosphorous (12,13).

FFA content and FFA composition of crude oil was determined by gas liquid chromatography (GLC) (14).

Three major lipid classes of crude oil were separated by silicic acid (BioSil A, 100/200 mesh, BioRad Labs, Richmond, CA) column chromatography with elution rates of 0.5 ml/min with all solvents (15). The chloroform fraction contained neutral lipid oil (NLO), the acetone fraction contained glycolipids (GL) and FFA, and the 1.0 N ammonium hydroxide/methanol fraction contained phospholipids and other polar lipids. The weight percentage of each lipid class was based on total weight of lipid recovered, which averaged 98.2% of the crude oil applied. The 3 lipid classes were monitored by TLC on 20 x 20 x 0.025 cm precoated Silica-Gel 60 plates to insure uniformity of separation of each lipid sample during silicic acid column chromatography. The plates were developed first with chloroform for 14 cm, followed by chloroform/methanol/7 N ammonium hydroxide (65:30:4) for 10 cm in the same direction.

NLO (60 mg) was saponified with 2.0 ml 0.5 N NaOH/CH_3OH for 6 min at 83 C, then esterified with 3.0 ml 10% BCl_3/CH_3OH for 5.5 min at the same temperature. The fatty acid composition of NLO was determined as area percentage by GLC analysis of fatty acid methyl esters using GLC conditions previously described (14).

GL were determined by subtracting the weight of FFA from the weight of the acetone fraction. Other polar lipids were obtained by subtracting the weight of phospholipids from that of the NH_4OH/methanol fraction.

Soybean lipase activity was determined in samples of defatted meal from soybeans stored for 28 days for each moisture level. Soymeal (200 mg) and NLO (100 mg) from column chromatography were incubated with 0.5 ml 0.1 M Tris buffer (pH 8.0) at 40 C for 2 hr. The reaction mixture was stirred periodically with a glass rod and was stopped by adding 3.0 ml of chloroform/methanol (2:1) and stirring with a Vortex mixer. The chloroform/methanol layer was removed with a Pasteur pipet and filtered into a small round-bottomed flask. The soymeal was reextracted and stirred twice more with solvent and filtered into the flask.

TABLE I

Soybean Lipid Composition and Moisture during Storage[a]

	Percent (w/w) of total lipid recovered				
Days stored	NLO	GL	FFA	PL	Other polar lipids
9.1% Ave. moisture					
0	95.5 ± 0.3	0.9 ± 0.1	0.2 ± 0.03	2.1 ± 0.07	1.1 ± 0.4
14	94.0 ± 0.8	1.7 ± 0.8	0.3 ± 0.01	2.5 ± 0.01	1.8 ± 0.4
21	93.6 ± 1.8	1.7 ± 1.8	0.3 ± 0.01	2.6 ± 0.65	1.7 ± 0.1
28	93.2 ± 3.2	2.6 ± 1.2	0.3 ± 0.06	2.1 ± 0.57	1.5 ± 0.1
35	94.3 ± 0.4	1.1 ± 0.1	0.3 ± 0.02	2.5 ± 0.21	1.5 ± 0.1
42	93.7 ± 1.9	2.2 ± 1.7	0.3 ± 0.01	2.4 ± 0.14	1.3 ± 0.4
13% ave. moisture					
0	92.1 ± 3.9	0.9 ± 0.1	0.2 ± 0.01	1.7 ± 0.14	2.1 ± 0
7	90.0 ± 4.8	0.5 ± 0	0.3 ± 0.01	2.8 ± 0.31	2.7 ± 0
14	88.3 ± 3.4	1.3 ± 0.1	0.3 ± 0.01	4.0 ± 0.03	3.4 ± 0.3
21	92.1 ± 2.7	1.1 ± 0	0.3 ± 0.06	2.9 ± 0.13	1.3 ± 0.1
28	88.1 ± 3.7	2.8 ± 0.5	0.3 ± 0.01	3.7 ± 0.20	2.3 ± 0.4
35	92.8 ± 2.7	0.05 ± 0.1	0.4 ± 0.03	3.1 ± 0.11	1.5 ± 0.1
42	91.8 ± 1.5	1.1 ± 0	0.5 ± 0.08	2.7 ± 0.05	2.6 ± 0.1
18.3% ave. moisture					
0	92.1 ± 3.9	0.9 ± 0	0.2 ± 0.01	1.7 ± 0.14	2.1 ± 0
7	86.9 ± 7.3	1.9 ± 0.2	0.4 ± 0.03	3.5 ± 0.25	1.5 ± 0.1
14	87.2 ± 6.9	1.4 ± 0.1	0.3 (0.3)	3.0(2.5)	2.8 ± 0.3
21	89.3 ± 2.5	2.1 ± 0.1	0.4 ± 0.05	3.7 ± 0.06	2.5 ± 0.1
28	93.0 ± 0.8	0.5 ± 0	0.6(0.6)	3.0(3.3)	2.1 ± 0.1
35	94.0 ± 0.8	0.8 ± 0	0.7 ± 0.01	2.5 ± 0.01	1.2 ± 0
42	94.6 ± 1.3	1.7 ± 0	0.8(0.8)	2.1(3.0)	1.8 ± 0.1

[a]Values are averages of two determinations with ± SD; values for captan-treated soybeans are given in parentheses. NLO=neutral lipid oils, GL=glycolipids, FFA=free fatty acids, PL=phospholipids.

The solvent was removed from the combined extracts by reduced pressure, and the remaining lipid was redissolved with hexane and shaken with saturated sodium chloride in a small separatory funnel. The hexane layer was collected and removed under reduced pressure at 35 C. Five μl of the oil and oleic acid standards were subjected to TLC with the procedure just described. The TLC plate was stained with iodine and a visible increase in FFA (identical R_F with standards) from zero time was considered an indication of lipase activity. The validity of the enzyme assay procedure was checked by incubating *Candida cylindracea* lipase (Sigma Chemical Co., St. Louis, MO) under identical conditions.

RESULTS AND DISCUSSION

Moisture equilibration was achieved within the first 24 hr after the soybeans were placed in Climate-labs. During storage, soybean moistures averaged 9.1 ± 0.2, 13.0 ± 0.1 and 18.3 ± 1.2% (Table I). Untreated soybeans held at 18% moisture for 14 days of storage were moldy; no other samples became moldy throughout the storage period.

The level of NLO averaged ca. 92% of the total lipid at all moisture levels and fluctuated very little during storage (Table I). The fatty acid composition of this lipid fraction did not change during storage at all moisture levels and was; 16:0, 11.3%; 18:0, 4.1%; 18:1, 19.8%; 18:2, 56.8%; 18:3, 7.8%. The fluctuation and range of GL levels were rather large, but were not associated with soybean moisture (Table I).

The very polar lipids (PL and other polar) from soybeans stored at 13.0 and 18.3% moisture fluctuated more and attained slightly higher levels than did those from soybeans at 9% moisture (Table I). The average PL values (excluding day 0) during 42 days of storage were 2.4, 3.2 and 3.0% for 9, 13 and 18% moisture soybeans, respectively.

The level of extractable phospholipids from 13 and 18% moisture soybeans increased during the first half of the storage period (21 days) and reached maximal levels of ca. 4.0% (Table I). The increase in PL also agrees with an earlier study in which PL levels in extracted crude oil increased significantly within 6 days of storage from soybeans stored under high relative humidity (85%) and temperature (35 C) (5). After reaching maximal levels, the PL from soybeans stored at 13 and 18% moisture declined (Table I). Declines in total PL levels in extracted crude oil also have been reported in soybeans with severe field damage and in soybeans stored for 6 weeks during overseas shipment (7,16).

The FFA levels in crude oil from 9% moisture soybeans remained low (0.3%) during storage, but increased 2- and 4-fold in crude oil from soybeans at 13 and 18%, respectively (Table I). The FFA levels obtained by chloroform/methanol (2:1) extraction of 9% moisture soybeans (Table I) was similar to FFA levels (0.4%) from 24 soybean varieties using AOCS procedures (17), and would suggest that chloroform/methanol (2:1) and commercial hexane extractions would yield essentially identical FFA levels from whole soybeans. The highest FFA level (0.8%), attained in crude oil from soybeans at 18% moisture, slightly exceeds the allowable levels in crude oil for export without penalties (8). It could be estimated that the FFA levels from soybeans at 13% moisture could approach this level by an additional 21 days of storage.

The weight percentage of each individual FFA (Table II) in extracted crude oil was calculated from FFA composition data and total FFA levels (Table I), during storage. Free linolenic acid increased 4-fold during storage, whereas the other FFA increased 2-fold in crude oil extracted from 13% moisture soybeans. Linolenic acid increased 6-fold during storage whereas the other FFA increased 4-fold in 18% moisture soybeans. There was little change in individual FFA levels in crude oil from 9% moisture soybeans after 14 days of storage. Although free linolenic acid increased at a much faster rate, free palmitic and linoleic

TABLE II

Weight Percentage of Free Fatty Acids in Extracted Soybean Crude Oil from Soybeans at Different Moisture Levels

Fatty acid	Days stored						
	0	7	14	21	28	35	42
9% ave. moisture							
16:0	.04	–	.06	.06	.06	.07	.06
18:0	.01	–	.01	.01	.01	.01	.01
18:1	.02	–	.03	.03	.03	.02	.03
18:2	.12	–	.16	.17	.18	.19	.17
18:3	.01	–	.02	.02	.02	.01	.02
13% ave. moisture							
16:0	.04	.04	.06	.06	.06	.07	.09
18:0	.01	.01	.01	.01	.01	.01	.02
18:1	.02	.02	.02	.02	.02	.03	.04
18:2	.12	.17	.18	.18	.18	.24	.30
18:3	.01	.02	.02	.02	.02	.04	.04
18% ave. moisture							
16:0	.04	.08	.06	.09	.11	.12	.14
18:0	.01	.01	.01	.01	.02	.03	.03
18:1	.02	.03	.02	.03	.07	.08	.09
18:2	.12	.24	.18	.24	.36	.41	.47
18:3	.01	.03	.02	.02	.04	.06	.06
18% ave. moisture[a]							
16:0	.04	–	.06	–	.12	–	.15
18:0	.01	–	.01	–	.02	–	.03
18:1	.02	–	.03	–	.06	–	.08
18:2	.12	–	.18	–	.36	–	.47
18:3	.01	–	.02	–	.04	–	.06

[a]Soybeans treated with 50% captan during storage.

acids remained the major FFA throughout the storage period at all moisture levels (Table II).

In association with increased FFA levels, we found that lipase became active in defatted meal from 13 and 18% moisture soybeans stored for 28 days, but was inactive in meal from 9% moisture soybeans stored for 28 days under identical assay conditions.

The increased levels of FFA in extracted oils have been attributed to mold growth on safflower seed and soybeans stored at high moisture (9,10). However, in this study, FFA may have resulted from the action of soybean lipases which probably were activated in soybeans at 13 and 18% moisture during storage. Total FFA levels increased significantly (2-fold) in soybeans with 13% moisture during the latter part of storage (Table I). No mold was visible on these samples as well as those treated with 50% captan at 18% moisture. Yet, increased FFA levels were identical in both soybean samples held at 18% moisture. These findings would indicate soybean lipase activity (Table I and II). Soybean lipases apparently were not activated in beans maintained at 9% moisture.

The results of this study indicate that changes in PL and FFA levels in extracted crude oil are directly related to soybean moisture, and under the storage conditions used, mold growth on 18% moisture soybeans did not significantly affect any of the lipid components measured. The fluctuations in the other lipid components apparently are not associated with soybean moisture. A portion of the increased levels of free palmitic and linoleic acids (Table II) could possibly have been derived from phospholipase action, since these are the 2 major fatty acids of soybean PL (13).

This study shows that significant changes can occur in relatively short storage times (42 days) in soybean crude oil extracted from soybeans maintained at moderate to high moisture levels. It is unknown what effect long-term storage (i.e., 6-12 months) would have on the lipid components of soybean crude oil under these constant moisture conditions, but the effect probably depends on the ability of soybeans to take up moisture and on the activation-deactivation of soybean lipases and possible phospholipases during storage. Prior treatment of soybeans with fungicide would not prevent the occurrence of high levels of FFA in extracted oil, unless the fungicide has the ability to inactivate soybean acylhydrolases. Therefore, soybeans should be dried to less than 13% moisture for long-term on-farm storage if low levels of FFA are desirable in extracted crude oil. Therefore, the recommended moisture of 12% (4) for long-term storage of soybeans seems to be valid based on the results of this study. A constant relative humidity of 67% would be needed to maintain soybeans at this moisture level at 30 C. Relative humidity was calculated from the linear relationship, % RH = 1.88(% moisture) + 44.19, which we derived (r = 0.999) from experimental data.

Further investigation is needed to determine at what precise moisture level soybean lipases are activated and what mechanism is involved. The probable involvement of phospholipases in the production of FFA during storage also needs further study. A better understanding of the mechanisms of these enzymatic reactions could lead to the development of controls for moisture damage to soybeans during storage that do not involve costly drying techniques.

REFERENCES

1. Siemens, J.C., and H.J. Himing, Harvesting and Drying Soybeans, Circular 1094, College of Agriculture, Cooperative Extension Service, University of Illinois, Urbana, IL, 1974.
2. Soybean Digest, National Soybean Processors Association, Mar. 1978: p. 15, Sept. 1978: p. 10, Oct. 1978: p. 10.
3. Botts, R., Mold in Feeds: problems and prevention, Feed Management, Watt publication, Mount Morris, IL, 1979. pp. 24-26.
4. Wolf, W.J., and J.C. Cowan, in "Soybeans as a Food Source," CRC Press, Cleveland, OH, 1971, pp. 16-17.
5. Chapman, G.W., Jr., and J.A. Robertson, JAOCS 54:195 (1977).
6. Evans, C.D., G.R. List, R.E. Beal and L.T. Black, Ibid. 51:444 (1974).
7. Robertson, J.A., W.H. Morrison, III, and D. Burdick, Ibid. 50:443 (1973).
8. Year Book and Trading Rules 1976-1977, National Soybean Processors Association, pp. 46-52, 78.
9. Heaton, T.C., P.F. Knowles, D.S. Mikkelsen and J.E. Ruckman, JAOCS 55:465 (1978).
10. Milner, M., and W.F. Geddes, Cereal Chem. 23:225 (1946).
11. "Official and Tentative Methods of the American Oil Chemists' Society," Vol. I and II, Third Edition, AOCS, Champaign, IL, 1964 (revised to 1972), Method Ac 2-41.
12. Rouser, G., A.N. Siakotos and S. Fleischer, Lipids 1:85 (1966).
13. Chapman, G.W., Jr., JAOCS (in press).
14. Chapman, G.W., Jr., Ibid. 56:77 (1979).
15. Robertson, J.A., G.W. Chapman, Jr., and R.L. Wilson, Jr., Ibid. 55:266 (1978).
16. Mounts, T.L., G.R. List and A.J. Heakin, Ibid. 56:883 (1979).
17. Chapman, G.W., Jr., J.A. Robertson, D. Burdick and M.B. Parker, Ibid. 53:54 (1976).

[Received March 27, 1980]