High-Performance Liquid Chromatography Analysis of Peanut Phospholipids. II. Effect of Postharvest Stress on Phospholipid Composition

J.A. Singleton[a,*] and L.F. Stikeleather[b]

[a]USDA, ARS, Biological and Agricultural Engineering Department and [b]Department of Biological and Agricultural Engineering, North Carolina State University, Raleigh, North Carolina 27695-7625

ABSTRACT: Peanuts are harvested in late September, and sometimes the harvest season can extend through most of October. When weather patterns delay harvest, the result may cause an immature crop, curing problems, rain damage, and freeze damage. All of the above stress situations can affect oil quality and flavor of the peanuts by altering phospholipid composition. Such changes are related to refining problems as well as flavor problems. A new high-performance liquid chromatography (HPLC) method was used for the analysis of phospholipids from postharvest stressed peanuts. The concentrations of phosphatidic acid (PA), phosphatidylethanolamine (PE), and phosphatidylcholine (PC) were higher in immature seed when compared to mature seed. A slight increase in concentration was observed for phosphatidylglycerol (PG), and a decrease in phosphatidylinositol occurred in immature peanuts. All phospholipids increased in concentration except PG when peanuts were cured at a high temperature (40°C). When peanut seeds were frozen at −16°C (before curing), a significant increase in concentration was observed for PA and PG, whereas the concentrations of PC and PE decreased to very low levels when compared to the control. Where concentration permitted, molecular species were separated on a reverse-phase column by HPLC.
JAOCS 72, 485–488 (1995).

KEY WORDS: High-performance liquid chromatography, lipids, peanuts, phospholipids, stress.

Preharvest and postharvest conditions affect the quality of crude peanut oil and the overall quality of peanuts for edible purposes. Heavy rains during the harvest season, exposure to freezing temperatures, kernel immaturity at harvest, and drying at a higher-than-recommended temperature are the major factors contributing to a low-quality raw product (1–3). Enzymes, such as lipases and lipoxygenase, are activated during these stress events and result in the breakdown of phospholipids (4–7). Raw peanut oil usually has a high concentration of phosphatidylcholine (PC), which contributes to the efficiency of the degumming process during refining (4,5). A critical concentration of PC is needed to ensure that a gum is formed for the removal of phospholipids. Changes in phospholipid concentration may occur when peanuts are harvested prematurely, cured at a high temperature, and/or exposed to freezing temperatures. In many cases, the crude oil is unusable for edible purposes and becomes increasingly difficult to refine. The degree of damage due to treatment was determined by high-performance liquid chromatography (HPLC) analysis of the phospholipids after the peanuts were subjected to different postharvest treatments without prior removal of the triglyceride fraction. These data show that this new HPLC method may be used to determine peanut oil quality prior to refining.

*To whom correspondence should be addressed at USDA, ARS, 280 Weaver Bldg., NC State University, Raleigh, NC 27695-7625.

MATERIALS AND METHODS

Materials. Peanuts (VA NC7) were grown at the NC State Experiment Station (Lewiston, NC), and they were subjected to four different postharvest conditions. The control sample was dried to 6% moisture at ambient temperature. Immature peanuts were hand-harvested in early August, and the percentage of immature kernels was determined to be 40% by the hull scrape method (8). The immature sample also was dried to 6% moisture at ambient temperature. A sample was frozen at −20°C for 8 h. The internal peanut temperature was monitored with a thermocouple and an Easy Logger Recording System (Omnidata International, Logan, UT). The final peanut internal temperature was −16°C. After freezing, the peanuts were thawed and dried to 6% moisture at ambient temperature. Another sample was cured at 40°C and dried to 6% moisture at ambient temperature. All samples were stored in the shell at 45°C and 60% relative humidity. Peanuts were not sized prior to treatment. Solvents used in lipid extraction were reagent- and HPLC-grade (Fisher Scientific, Pittsburgh, PA). Phospholipid standards were obtained from Sigma Chemical Company (St. Louis, MO).

Lipid extraction. Lipids were extracted from peanuts with chloroform/methanol ($CHCl_3$/MeOH, 2:1, vol/vol). A 50-g sample was blended with 300 mL of $CHCl_3$/MeOH (2:1, vol/vol) in a Sorvall blender (Ivan Sorvall, Inc., Norwalk, CT) for 1 min. The slurry was suction-filtered through filter paper

in a Buchner funnel. A saturated solution of NaCl was added to the filtered solution in a separatory funnel, shaken, and allowed to stand until phase separation occurred. The $CHCl_3$ layer was saved, and the water layer was discarded. The solvent layer was washed twice with saturated NaCl, and the $CHCl_3$ was removed by flash evaporation. Extracted lipid material was stored in a freezer at –20°C until analyzed.

HPLC of phospholipids. Peanut phospholipids were separated on a silica column (100 mm × 8 mm) with a combination gradient and isocratic program of mixed solvents and detected at 205 nm with an ultraviolet (UV) detector. Solvent A was a mixed solvent of isopropanol/hexane (4:3, vol/vol), and solvent B was a mixed solvent of isopropanol/hexane/water (8:6:1.5, vol/vol/vol). Phospholipids were separated with a gradient starting at 100% solvent A to 100% solvent B in 20 min, isocratic with 100% solvent B for 15 min, and regeneration of the column for the next analysis with 100% solvent A for 10 min. Phospholipids were identified from retention times by running authentic standards under the same conditions. Individual phospholipids were collected manually and stored at –20°C for further analysis. Triplicate analyses were run on each sample for comparative purposes, and multiple analyses were run for collection of individual phospholipids. The injection volume for samples from all postharvest treatments was 1 mL.

HPLC of molecular species. Collected phospholipid fractions were further purified by adding hexane and water to the collected fraction to create a two-phase system for the control, immature, and high-temperature cured samples. The water phase was saved and reextracted with $CHCl_3$/MeOH (2:1, vol/vol). Each sample was further concentrated by flash evaporation prior to analysis. Molecular species of PC were separated by HPLC reverse-phase chromatography on a column (150 mm × 4.6 mm, 3 μ; Supelco, Inc., Bellefonte, PA) with an isocratic elution of MeOH/acetonitrile/water (91:3:6, vol/vol/vol). Sample size of PC from each treatment for molecular species analysis was 1 mL injected *via* a 1-mL sample loop. A flow rate of 1.5 mL/min was used, and molecular species were detected with a UV detector at 205 nm.

RESULTS AND DISCUSSION

HPLC of peanut phospholipids. The major phospholipids of peanut oil are phosphatidic acid (PA), phosphatidylglycerol (PG), phosphatidylethanolamine (PE), phosphatidylinositol (PI), and PC. These phospholipids were concentrated and separated under the conditions outlined in the Materials and Methods section. Figure 1 shows a typical HPLC chromatogram of the four different postharvest treatments identified in the figure legend. Samples were injected automatically onto the HPLC column with a 1-mL sample loop. Individual phospholipids were identified by retention time of known standards that had been previously characterized by mass spectrometry. Other unidentified components are probably hydroperoxides of the phospholipids and are also present in the freeze-damaged sample.

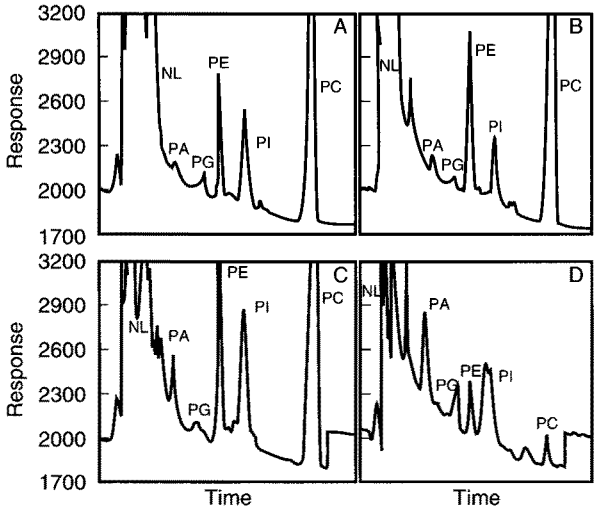

FIG. 1. Effect of different postharvest treatments on the phospholipid profile of peanuts: (A) undamaged, (B) immature, (C) high-temperature cured, and (D) freeze-damaged; NL, neutral lipids; PA, phosphatidic acid; PG, phosphatidylglycerol; PE, phosphatidylethanolamine; PI, phosphatidylinositol.

Effect of postharvest treatment on phospholipid composition. Table 1 shows the concentration of the five individual phospholipids and how they are affected by postharvest stress. In immature peanuts, all phospholipids except PI increased in concentration when compared to the control sample. The increase in concentration of PA and PC in immature peanuts might be explained on the basis that these phospholipids are the precursors to the other phospholipids (9), and the phospholipid fraction content of the total lipid fraction in immature peanuts is somewhat higher than the phospholipid fraction in mature peanuts. Total phospholipids in the immature sample were 700 mg/100 g dry weight (DWT), whereas total phospholipids in the control sample were 500 mg/100 g DWT (Table 1).

Effect of high-temperature cure. When peanuts are subjected to heat stress during curing, cells become leaky, and the metabolic changes activate lipase enzymes and lipoxygenase. Increased temperature results in increased fluidity of the lipids, which leads to disruption of the phospholipid bilayer, resulting in "holes" in the membrane (10). Increased temper-

TABLE 1
Effect of Postharvest Treatment on Total Phospholipids[a]

Treatment	Phospholipid (% area)					Total phospholipid (mg/100 g DWT)
	PA	PG	PE	PI	PC	
Control	2.2	2.5	13.3	15.7	66.4	500
Immature	4.5	2.3	14.0	7.6	71.7	700
Heat cured	9.5	1.1	16.0	15.4	58.1	900
Freeze-damaged	28.3	14.1	15.2	33.5	8.8	250

[a]PA, phosphatidic acid; PG, phosphatidylglycerol; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PC, phosphatidylcholine; DWT, dry weight.

ature may also stimulate lipid biosynthesis (11). In peanuts, an increase in the concentration of the phospholipid fraction would probably be at the expense of the large triglyceride fraction that is present in peanuts (approximately 98%). In the heat-damaged sample, PA increased significantly when compared to the control (Table 1). A 3% increase in PE content was observed in the heat-damaged sample when compared to the control sample, whereas PG showed a slight decrease. PI and PC content (mg basis) also increased due to this postharvest treatment. Total phospholipids in the heat-damaged sample increased to a level of 900 mg/100 g DWT as compared to 500 mg/100 g DWT for the control sample. The increased concentration of total phospholipids in the heat-damaged sample reflects the increases in PA, PE, PI, and PC and may be due to the fact that PA and PC are known to be precursors to the other phospholipids. A slight decrease was noted in the concentration of PG, which may have become more saturated due to the increased curing temperature. However, if heat stress is prolonged or the temperature is excessive, the phospholipid bilayer becomes disorientated, and the proteins (enzymes) become immobile, leading to the oxidation of unsaturated lipids (12). An increase in the total phospholipid fraction, coupled with potential oxidation of unsaturated fatty acids, would render the oil more difficult to refine and affect the quality and the stability of the finished product.

Freeze damage. In the freeze-damaged sample, the concentration of PA and PG increased significantly, whereas the concentration of PC decreased greatly when compared to the control sample (Table 1). The increase in PA and the large decrease in PC may be related to the fact that freezing induces phospholipase-D activity. The susceptibility of phospholipids to attack by enzymatic activity are in the order PC > PI > PE, resulting in greater amounts of PA being formed (3). Therefore, some increase in PA concentration resulted in the removal of the choline group from PC. It is well established that freeze injury leads to an increase in the total phospholipid fraction, unsaturation (13), and an increase in free phosphorus (14). However, in this case, the total phospholipid content of the sample decreased due to the almost complete destruction of PC. The actual concentration of PE also decreased in the freeze-damaged sample due to the much lower content of total phospholipids in this sample. PE is know to have a synergistic antioxidant role with the tocopherols (15). The unsaturation of the fatty acids in PG increases as well as their concentration in chilling sensitive plants. The concentration of PI remained essentially the same (mg basis) as that of the control sample. During freeze damage, respiration goes anaerobic, and the phospholipid bilayer becomes more solidified and restricts protein movement. At this stage, cellular constituents leach out and change the metabolic activity of the cells. Unsaturated lipids are highly susceptible to oxidation during this process. Freeze damage to peanuts results in an oil that is difficult to refine, and the production of hydroperoxides would certainly affect the stability of the oil.

Effect of postharvest treatment on the molecular species of PC. The major molecular species found in PC are C18:2/C18:2 and C18:2/C18:1, and these species were separated on the basis of unsaturation on reverse-phase columns. The most unsaturated species eluted first (16). Table 2 shows the relative content of the molecular species of PC as affected by postharvest treatment. The control sample had a distribution of 40% C18:2/C18:2, whereas the distribution in the immature sample was equal for both major species. However, the total concentration of molecular species was much greater in the immature sample than the control. Part of the difference in the total concentration of molecular species in the immature sample can be attributed to the increased amount of the C18:2 fatty acid moiety in this sample, and increased unsaturated increases the amount detected by a UV detector. Molecular species found in the high-temperature cured sample had a higher degree of saturation due to the presence of C18:1/C16:0 molecular species. This was probably due to the oxidation of some of the more unsaturated molecular species, particularly the C18:2 fatty acid moiety, by heat stress. All of the molecular species shown in Table 1 have been identified in PC isolated from peanuts by HPLC and mass spectrometry. Phospholipids act in a synergistic manner with tocopherols in lengthening the onset of the induction period of lipid oxidation. The degree of unsaturation of the acyl fatty acid chains has an added effect on the length of the induction period (17). The above factors can definitely affect the quality of the oil and, therefore, the refining process. Due to the destruction of PC in the freeze-damaged sample, the concentration was too low to permit detection of the molecular species in this sample.

TABLE 2
Effect of Postharvest Treatment on the Major Molecular Species of Phosphatidylcholine

Sample	Molecular species	%	Amount (mg/100 g DWT)[a]
Control	C18:2/C18:2	40.7	135.1
	C18:2/C18:1	59.3	196.9
Immature	C18:2/C18:2	50.0	254.6
	C18:2/C18:1	50.0	254.6
Heat-cured	C18:2/C18:1	27.2	142.2
	C18:1/C16:0	72.8	380.7

[a]DWT, dry weight.

Results of this study have illustrated the use of a new HPLC method for the analysis of peanut phospholipids and the collection of phospholipids for molecular species analysis. The results presented here also show that HPLC of phospholipids and molecular species of phospholipids in crude peanut oil can be useful for evaluating peanut oil quality as affected by postharvest stress.

ACKNOWLEDGMENT

Paper number BAE 94-12 of the Journal series of the Department of Biological and Agricultural Engineering, NC State University, Raleigh, NC 27695-7625.

REFERENCES

1. Dickens, J.W., and H.E. Pattee, in *Peanuts: Cultivars and Uses*, American Peanut Research and Education Association Inc., Stillwater, 1978, p. 509.
2. *Soybean Digest 32*:7 (1971).
3. Mounts, T.L., and A.M. Nash, *J. Am. Oil Chem. Soc. 67*:757 (1990).
4. Robertson, J.A., W.H. Morrison and D. Burdick, *Ibid. 50*:443 (1973).
5. Evans, C.D., G.R. List, R.E. Beal and L.T. Black, *Ibid. 51*:444 (1974).
6. Pattee, H.E., E.O. Beasley and J.A. Singleton, *Proceedings Third National Peanut Research Conference*, Peanut Improvement Working Group, Auburn University, Auburn, 1964, p. 66.
7. Singleton, J.A., H.E. Pattee and E.B. Johns, *J. Agric. Food Chem. 19*:130 (1971).
8. Drexler, J.S., and E.J. Williams, *Proc. Am. Peanut Research and Education Soc. 11*:57 (1979).
9. Jaworski, J.C., in *Lipids: Structure and Function*, edited by P.K. Stumpf, Academic Press Inc., Orlando, 1980.
10. Kuiper, P.J.C., *Plant Physiol. 45*:684 (1970).
11. Byfield, J.E., and O.H. Scherbaum, *Science 156*:1504 (1967).
12. Levitt, J., in *Responses of Plants to Environmental Stresses*, edited by T.T. Koylowski, Academic Press, New York, 1972, p. 265.
13. Levitt, J., *Ibid.*, 1972, p. 125.
14. Adhikari, C., H.E. Snyder, T.W. Kwon and P.K. Clark, *J. Am. Oil Chem. Soc. 69*:1141 (1992).
15. Dziedzic, S.Z., and B.J.F. Hudson, *Ibid. 61*:1042 (1984).
16. Demandre, C., A. Tremolieres, A.-M. Justin and P. Mazliak, *Phytochemistry 24*:481 (1985).
17. Ohsima, T., Y. Fujita and C. Koizumi, *J. Am. Oil Chem. Soc. 70*:269 (1993).

[Received April 1, 1994; accepted January 3, 1995]