Phospholipids of Three Xerophytic Cucurbit Seed Oils[1]

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The composition of seed phospholipids was determined in three species of xerophytic cucurbits, *Cucurbita digitata* Gray, *C. foetidissima* HBK and *Apodanthera undulata* Gray. The phospholipid fractions were isolated using silicic acid chromatography and quantitated by colorimetric analysis. The component phospholipids were separated using thin layer chromatography. All three species contained phosphatidylcholine, phosphatidylethanolamine and phosphatidylinositol as their major component phospholipids. Analysis by gas liquid chromatography of fatty acids in total phospholipid samples revealed linoleic acid as the major component and myristic acid in significant amounts in each species. Small amounts of conjugated unsaturated fatty acids in the phospholipids of each species were determined by ultraviolet spectrometry. Close similarities in the composition of specific phospholipids were found in all species.

Xerophytic cucurbits that produce root starch, seed oil and protein may have promise as crops in semi-arid environments. The purpose of this study was to examine the composition of phospholipids in the seed of three species of the family Cucurbitaceae: *Cucurbita digitata* Gray (CD), *C. foetidissima* HBK (CF) and *Apodanthera undulata* Gray (AU). Seed from these plants has been reported to contain about 30% oil (1). Studies of the properties and laboratory processing of CF oil (2-4) indicate that it could be processed commercially as an edible or industrial oil. The presence of a substantial quantity of conjugated unsaturated fatty acids in CD and AU oils (5,6) implies that they would be useful primarily in industrial products rather than in food. Furthermore, the utilization of these plants might not be limited to oil production; seeds and roots of these plants could also be processed to yield commercially valuable protein (1,7) and starch (8,9).

EXPERIMENTAL

Chemical, chromatographic and spectrophotometric analyses are reported as means of triplicate determinations. All solvents were of nanograde quality with 0.005% butylated hydroxytoluene (BHT) added as a preservative.

Seed was obtained from plants grown at the University of Arizona-Marana Agricultural Center. Seed which appeared to be fully developed and undamaged was cleaned and stored under nitrogen in sealed containers at room temperature in the dark.

Seed (10 g) was ground at high speed in a Waring blender for approximately two min. The blended material was placed in a mortar containing 15 ml of chloroform/methanol (2:1, v/v) and ground further to assure complete oil extraction. This slurry was transferred to a 250-ml actinic flask into which an additional 85 ml of chloroform/methanol was added. The flask was stoppered and agitated on a shaker for one hr. This mixture was filtered through a sintered glass funnel, and the residue was reground and reextracted for an additional hour. The filtrates from both extractions were combined and washed according to the method of Folch et al. (10).

The sample was then concentrated with a rotary film evaporator, dried with nitrogen and resuspended in chloroform for storage. All lipid extracts were stored at 0 C in the dark in desiccators that were flushed periodically with nitrogen.

Phospholipids were separated from neutral lipids by modifying the method of Rouser et al. (11), which called for elution with the following sequence of solvents: chloroform (10 column volumes), acetone (40 column volumes) and methanol (10 column volumes). The least polar lipids were eluted in chloroform, glycolipids and sulfolipids in acetone and phospholipids in methanol. Elution with acetone proved ineffective with the oils in this study, and separation was therefore effected with chloroform and methanol as indicated. Glycolipids could not be detected in the methanol fraction with napthoresorcinol-sulfuric acid spray reagent during subsequent thin layer chromatography (TLC) procedures.

One- and two-dimensional TLC was used to identify specific phospholipids. One-dimensional TLC was performed on Pre-Adsorbent Silica Gel G plates (Analabs, Inc., North Haven, Connecticut) using the solvent system chloroform-methanol-acetic acid-water (65:25:8:4, v/v/v/v) developed by Skipski et al. (12). After development, the spots were visualized by spraying the plates with a phosphorus spray reagent (13). Identification was made by comparison to reference standards (Sigma Chemical Co., St. Louis, Missouri).

Two-dimensional TLC was performed on either Redi-Coat 2-D plates (Supelco, Inc., Bellefonte, Pennsylvania) or on Pre-Adsorbent Silica Gel G plates. The plates were developed in the first dimension with chloroform-methanol-water-28% aqueous ammonia (130:70:8:0.5, v/v/v/v) and in the second dimension with chloroform-acetone-methanol-acetic acid-water (100:40:20:20:10, v/v/v/v/v) as suggested by Parsons and Patton (14). Detection and identification were accomplished by application of one or more of the following reagents and by comparison with standards:

(i) Rhodamine 6G spray—detection of lipid-containing compounds (15).

(ii) Iodine vapor—detection of unsaturated lipids (15).

(iii) Phospholipid spray—for the detection of all phospholipids (13).

(iv) Ninhydrin spray—detection of aminophosphatides such as phosphatidylethanolamine and phosphatidylserine (15).

(v) Dragendorff spray—for the detection of phosphatidylcholine and lysophosphatidylcholine (15).

Quantitative analysis of phospholipids. A quantitative phosphorus analysis was performed for two purposes, to

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determine the percentage of each component phospholipid within a sample, and to determine the total amount of phospholipid extracted from the original oil. Individual phospholipids were separated using the two-dimensional TLC procedure previously described (14), and the plates were visualized with the phosphorus spray (13). Each spot was identified and then scraped into a 15-ml Kjeldahl flask for digestion.

Samples used for determining the total phospholipid content were prepared by pipetting 25 ml of the washed lipid extracts into 15-ml Kjeldahl flasks. They were then heated in a boiling water bath until all traces of solvent evaporated. Samples were allowed to stand overnight after addition of one ml concentrated nitric acid.

Both silica gel scrapings and lipid extracts were then digested and analyzed for phosphorus as described by Dittmer and Wells (16). Samples containing silica gel were centrifuged for 10 min at 5,000 rpm to remove adsorbent.

Conversion factors used to calculate phospholipid content from % phosphorus were determined according to Chapman (17) and Singleton and Pattee (18).

Gas liquid chromatography of nonconjugated unsaturated fatty acids. Methyl esters of the total phospholipid fraction were prepared by placing a drop of each extract into 10-ml screw cap test tubes. After drying under a nitrogen stream, two ml of toluene and 4.5 ml of 0.5% (v/v) sulfuric acid in methanol were added to each sample together with one boiling stone. Tubes were capped and heated at 100 C for three hr; when cool, two ml of hexane and four ml of water were added, followed by shaking to extract the esters into the hexane-toluene phase. Aliquots were removed from the upper layer and placed into one-ml Micro Reaction vessels (Supelco, Inc., Bellefonte, Pennsylvania). The solvent was evaporated with nitrogen, and the samples were then redissolved in hexane.

Methyl esters of the component phospholipids were prepared after first separating the phospholipids by two dimensional TLC (14). Spots were visualized with Rhodamine 6G and scraped into 10-ml screw cap test tubes. Samples were then treated according to the method of Feldman and Rouser (19) except that four ml of 0.5% (v/v) sulfuric acid in methanol was used for esterification, and the samples were heated five hr rather than overnight.

Gas liquid chromatographic analysis of all samples was performed with a Perkin-Elmer Model 880 gas chromatograph, equipped with a Speedomax W recorder and a flame ionization detector, under the following conditions: 1.8m × 3.2mm stainless steel column packed with 10% Silar 7 CP on acid-washed Chromosorb W (100-120 mesh); isothermal operation at 195 C; injector 205 C; detector 195 C; nitrogen carrier gas 40 ml/min.

Ultraviolet spectrometry of conjugated fatty acids. Aliquots (100 μl) of individual phospholipid extracts were pipetted into tared five-ml flasks. The solvent was evaporated under nitrogen and phospholipid concentrations determined. They were then brought to volume with cyclohexane. Samples were scanned from 350-220 nm in quartz cells of one cm pathlength, and the percentage of conjugated unsaturated fatty acids was calculated (20).

RESULTS AND DISCUSSION

The total phospholipid content of the respective oils was determined as 3.3% for CD, 2.9% for CF and 4.0% for AU (Table 1). Phosphatidylcholine was the major component in each case, ranging from about 56 to 75%; phosphatidylinositol and phosphatidylethanolamine were second or third, with concentrations ranging from 10 to 19% of total phospholipid content, and lysophosphatidylcholine was present in a concentration of less than 5%. Three unidentified spots were found on TLC plates, but none contained phosphorus. Levels of phosphatidylcholine were much higher than those in soybean and cottonseed phospholipid fractions. Comparison of profiles from xerophytic cucurbits with those from common sources (22,24) revealed that each possesses a unique spectrum of component phospholipids. The squash-melon, *Citrullis vulgaris* var. *fistulosis,* was found to have much lower phosphatidylcholine content (34.1%) and higher phosphatidylinositol content (25.4%) than CD, CF or AU oils (25). Variations in component composition should not, however, affect the possible usefulness of xerophytic cucurbit phospholipids as commercial emulsifiers.

TABLE 1

Phospholipid Content of Cucurbit and Other Seed Oils

Phospholipid	Composition (%)[a]				
	CD[b]	CF[b]	AU[b]	SOY[b,c]	COT[b,d]
Phosphatidylcholine	74.9	55.8	68.5	45.0	38.2
Phosphatidylethanolamine	10.5	18.7	13.3	26.3	10.9
Phosphatidylinositol[e]	13.7	17.2	13.7	14.1	13.9
Lysophosphatidylcholine	Trace	0.8	4.5	—	12.6
Other[f]	0.8	5.5	Trace	14.6	24.4
Total phospholipid in oil	3.3	2.9	4.0	1.8-3.5[g]	2.4

[a]Values shown are means of triplicate determinations.
[b]CD, *C. digitata;* CF, *C. foetidissima;* AU, *A. undulata;* SOY, soybean; COT, cottonseed.
[c]O.S. Privett et al. (21).
[d]J.P. Cherry et al. (22).
[e]May contain lysophosphatidylethanolamine.
[f]Probably phosphatidic acid.
[g]H. Witcoff (23).

Fatty acid composition of the phospholipids and triglycerides of each species is shown in Table 2. Fatty acid compositions of all phospholipids were similar, and the most abundant fatty acid in both fractions was linoleic acid. Fatty acids of the polar plant lipid fraction generally are considered to parallel those of the neutral lipid fraction, except that a higher portion of saturated acids is expected in the polar fraction (23). Mean values of approximately 36% for saturated fatty acids in phosphatides and 16% in triglycerides are reported in Table 2. Myristic acid was found in phospholipids at levels of 7.4 to 9.2% but was not present in neutral triglycerides. A study of heritability of fatty acid composition in CF seed oil revealed wide variability; ranges for palmitic, stearic, oleic and linoleic acids were found to be 7.3-12.1%, 3.8-6.8%, 12.9-61.7% and 27.4-73.0%, respectively (26).

Conjugated unsaturated fatty acids have been reported in certain xerophytic cucurbit oils (3,5,6). Consequently, their levels were determined in the total phospholipid fractions, and values are shown in Table 2. With the exception of CF, these unusual fatty acids appear to accumulate

TABLE 2

Fatty Acid Composition of Cucurbit Triglycerides and Total Phospholipids

	Composition (%)					
	Triglycerides			Phospholipids[a]		
Fatty acid	CD[b,c]	CF[b,d]	AU[b,e]	CD	CF	AU
14:0				7.5	7.4	9.2
16:0	10.0	11.8	10.4	22.5	21.4	22.4
18:0	5.0	3.5	7.4	5.2	5.6	6.5
18:1	24.0	21.9	22.9	16.5	13.0	18.8
18:2	43.0	60.6	30.8	45.6	50.0	37.4
20:0 and greater	—	—	11.2	—	—	—
Conjugated[f]	18.3	2.3	17.3	2.7	2.6	5.7

[a]Values shown are means of triplicate determinations.
[b]CD, *C. digitata;* CF, *C. foetidissima;* AU, *A. undulata.*
[c]W.P. Bemis et al. (5).
[d]J.A. Vasconcellos et al. (3).
[e]J.A. Vasconcellos et al. (6).
[f]Total of di-, tri-, tetra and pentaenoic fatty acids.

TABLE 3

Fatty Acid Composition of the Component Phospholipids of Cucurbit Oils

	Fatty acids (%)[a]				
Phospholipid	14:0	16:0	18:0	18:1	18:2
Phosphatidylcholine					
CD[b]	6.9	22.1	5.8	19.8	45.4
CF[b]	9.7	22.4	5.5	13.8	48.6
AU[b]	9.9	18.5	8.6	29.5	33.5
Phosphatidylethanolamine					
CD	20.8	23.7	11.2	13.4	30.9
CF	13.4	18.2	5.5	13.9	49.0
AU	14.7	26.1	5.7	15.2	38.3
Phosphatidylinositol					
CD	18.0	37.1	11.9	8.8	24.2
CF	27.3	24.1	15.8	9.5	23.3
AU	19.3	29.3	11.8	11.1	28.6
Lysophosphatidylcholine					
CD	35.1	31.8	11.0	12.2	9.9
CF	21.2	30.6	46.3	—	1.9
AU	(not determined)				
Other[c]					
CF	41.5	19.9	9.9	12.1	16.6

[a]Values shown are means of triplicate determinations.
[b]CD, *C. digitata;* CF, *C. foetidissima;* AU, *A. undulata.*
[c]Probably phosphatidic acid.

in the triglyceride fraction rather than in the polar lipids (27).

Fatty acid compositions of the component phospholipids are presented in Table 3. Individual fatty acids are reported as a percentage of the total of saturated and nonconjugated unsaturated acids; the contribution of conjugated unsaturated acids was not measured. Specific phosphatides had similar composition from species to species with the exception of lysophosphatidylcholine. The predominant phosphatidylcholine contained about 64% unsaturated fatty acids, compared to 62% for the total phospholipid fraction. Phosphatidylethanolamine and phosphatidylinositol, the next most abundant components, contained about 54% and 35% unsaturated fatty acids, respectively.

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