Phosphatides Isolated From Seeds of Commercial and Experimental Safflower Varieties

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Abstract

Studies on phosphatides from several safflower varieties show the following five major results. The total phosphatide contents of the various safflower seeds are quite similar (0.48% for a commercial and 0.58% for a brown-striped variety). The same three major and one minor phosphatides were present in all varieties: phosphatidyl choline (PC), phosphatidyl ethanolamine (PE), phosphatidyl inositol (PI) and phosphatidyl serine (PS). The amounts of these lipids present in the crude phosphatide mixture were quite similar in all varieties tested (~36% for PC, ~15% for PE, ~23% for PI, and less than 2% for PS). The fatty acid composition of the phosphatides of UC-1 high oleic safflower is very different from that of the other varieties, but it reflects the composition of the corresponding oil triglycerides as far as the major acid is concerned. All other safflower seed phosphatides investigated have linoleic acid as the major fatty acid constituent. A simple and very sensitive color test has been found which can differentiate phosphatides of the high linoleic from the high oleic type.

Introduction

In the course of work on a color problem which arose with oil of a new brown-striped safflower variety, we investigated phosphatides from several experimental varieties (1–3). We noticed a much higher degree of stability for phosphatides isolated from the UC-1 variety. UC-1 is known for the higher oxidative stability of its oil (4,5) caused by a newly introduced gene (6) which completely changes the composition of the oil. Commercial safflower oil has approximately 75% linoleic acid and 14% oleic acid. In UC-1 oil the ratios of these two fatty acids are essentially reversed. The replacement of diunsaturated linoleic acid by monosaturated oleic acid causes the substantial increase in oxidative stability which makes UC-1 oil preferable for many food and industrial uses (7,8).

Refined safflower oils are essentially free of phosphatides, but phosphatides are partially extracted with the crude oil from the seed and then removed by alkali-refining or degumming. Thus, large amounts of crude phosphatides are a valuable byproduct of oil processing and are used mostly as food additives for their emulsifying and nutritive properties. Since stability of phosphatides is as important as that of vegetable oil for industrial uses, we were interested in exploring the chemical causes for the observed higher stability of the UC-1 phosphatides in comparison to phosphatides obtained from commercial safflower seeds or oils.

Experimental Procedures

Materials

The following five safflower varieties were used in this study: Arizona Brown Stripe and a pigmentless, stiped variety, both bred by Rubis at the University of Arizona (9); a purple-striped variety, obtained from A.B. Hill at Cargill Co.; the high oleic UC-1; and a commercial variety, preponderantly Gila, obtained from Pacific Vegetable Oil Company.

Isolation and Analysis of Phosphatides

The extraction of seeds with hexane containing 3.5% methanol, the separation of crude phosphatide from the oil by precipitation with water, the thin layer chromatography (TLC) and paper chromatography, the column chromatographic separations on DEAE cellulose, the group analysis and qualitative and quantitative analysis of the fatty acids by gas liquid chromatography (GLC) were conducted by using the same methods used to obtain like data reported recently for a brown-striped variety (2).

Anisaldehyde Color Test

The phosphatides (1–25 μg) to be compared are applied in equinormal chloroform-methanol (19:1) solution to a TLC plate. They are separated into their individual components by developing with chloroform-methanol-water (60:50:4) before spraying with an anisaldehyde sulfuric acid spray (10). The plates are then heated in an oven at 100 C and checked for color differences. The colors developed depend on the length of heat exposure and are usually a dark purple for the high linoleic and a light grayish purple for the high oleic acid phosphatides.

Results and Discussion

It was assumed that the higher oxidative stability observed for the UC-1 phosphatide fraction was based upon either a different qualitative or quantitative composition of the crude phosphatide fraction or a different fatty acid composition of all or individual phosphatides.

To check these hypotheses we extracted the phosphatides from the seeds of five varieties and separated all the components present in the crude phosphatide extracts by TLC (Fig. 1). No qualitative differences

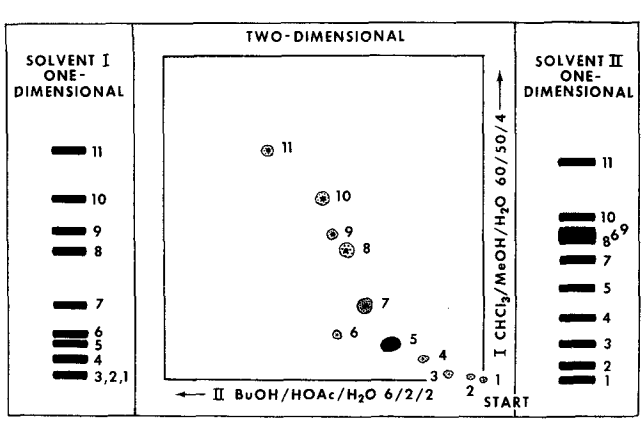

FIG. 1. Separation of crude phosphatide mixtures. Gila, UC-1 and Arizona Brown Stripe. Phosphatidyl choline, 5; phosphatidyl serine, 6; phosphatidyl inositol, 7; and phosphatidyl ethanolamine, 8.

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TABLE I

Quantitative Phosphatide Analyses

Seed type	Oil yield, %	Crude phosphatide, %	Individual phosphatides[a]		
			PC[b]	PE	PI
G[b]	40.4	0.48	35.9	15.7	26.7
AB St	45.3	0.58	32.0	16.2	23.0
Pu St	44.8	0.56	.....	.....	.....
Pig St	42.9	0.50	.....	.....	.....
UC-1	37.9	0.49	39.5	13.5	20.5

[a] Per cent of crude phosphatide.
[b] Abbreviations: G, Gila; AB St, Arizona Brown Stripe; Pu St, purple-striped variety; Pig St, pigmentless striped variety; PC, phosphatidyl choline; PE, phosphatidyl ethanolamine; PI, phosphatidyl inositol.

were detectable; all varieties showed 11 separated compounds, four of which were phosphatides.

The same three major phosphatidyl choline (PC), phosphatidyl ethanolamine (PE) and phosphatidyl inositol (PI) and one minor phosphatide, phosphatidyl serine (PS), were present in all varieties. To determine the relative quantities and exact identities of each individual phosphatide, the crude mixture of the commercial and UC-1 phosphatides were separated by column chromatography on DEAE cellulose and compared to each other and to values obtained earlier in exactly the same manner for a brown-striped variety (2). The results (Table I) show clearly that qualitative and quantitative compositions are very similar for all varieties and do not seem to be the cause for the observed differences in stability. Each individual phosphatide was then positively identified by comparison of R_f values of deacylated and intact phosphatides with those of known reference compounds and by determination of molar ratios of ester, glycerol, choline, inositol, nitrogen and phosphorus. The results presented in Table II show that all three major phospholipids of each of the three investigated varieties were correctly identified as PE, PC and PI.

To analyze for fatty acid composition each phosphatide was deacylated in methanolic KOH and the methyl esters qualitatively and quantitatively determined by GLC. A striking difference was found as far as the major acid is concerned. The commercial and brown-striped varieties have linoleic acid and UC-1 oleic acid as their major constituent (Table III). Hence it can be assumed that the reversal of the linoleic-oleic acid ratio in UC-1 is the major cause for the observed higher stability of the UC-1 phosphatides.

Crude or individual pure phosphatides from UC-1 can be quickly differentiated from phosphatides of the other safflower varieties by a color test on TLC plates after spraying with an anisaldehyde sulfuric acid spray. When color formation is observed after spraying and during or after heating, an easily recognizable color difference can be observed when high linoleic and high oleic phosphatides are compared. Similar color differences were observed when triolein and trilonolein are compared.

Recently published data on the fatty acid composition of safflower phosphatides by Osman et al. (11) agree essentially with the data presented here if their values for total phosphatide are compared with data computed for total phosphatide (PC + PE + PI) from Tables I and II. However, there is no agreement in the composition of individual phosphatides PC and PE. While Osman et al. report arachidic acid as the major constituent (11), we found less than 0.5%. On the other hand, they report no linoleic and oleic acid for PC and we found 72.5% and 8.3% for the commercial variety.

We were called upon to explore the reasons for these discrepancies. Pure PC, isolated from commercial safflower seeds, as well as pure methyl linoleate, when *trans* esterified under the strenuous conditions described by Osman, both gave rise to a reaction product which had a retention time under conditions of our GLC analysis close to that of arachidic acid. However, analysis by mass spectroscopy, NMR and IR revealed that the compound had a molecular weight of 330, contained one chlorine atom and one double bond. We suggest, therefore,

TABLE II

Characterization of Phosphatides[a]

Seed type	Assigned structure	Analyses					
		Phosphorus	Glycerol	Ester	Choline	Inositol	Nitrogen
G[b]	PC[b]	1.00	0.99	2.03	0.95		1.01
	PI	1.00	1.04	2.06		0.98	
	PE	1.00	0.90	1.92			0.97
AB St	PC	1.00	1.07	2.02	1.02		1.01
	PI	1.00	1.06	1.98		0.95	
	PE	1.00	1.11	1.89			0.99
UC-1	PC	1.00	1.03	2.01	1.01		1.00
	PI	1.00	1.04	1.97		0.99	
	PE	1.00	0.96	1.85			0.99

[a] Values are given as molar ratio relative to phosphorus.
[b] Abbreviations: see Table I.

TABLE III

Fatty Acid Composition of Safflower Seed Phosphatides[a]

Seed type	Phospholipid	Fatty acids[b]					Other	Total unsaturated
		14:0	16:0	18:0	18:1	18:2		
G[c]	PC[c]	1.2	14.0	3.7	8.3	72.5	0.3	80.8
	PE	0.7	15.6	2.9	5.9	74.6	0.3	80.5
	PI	0.9	26.1	5.6	3.5	61.5	2.4	65.2
AB St	PC	0.2	13.8	4.1	12.1	68.1	1.7	80.7
	PE	0.2	19.5	2.9	8.3	69.1	0.0	77.4
	PI	0.1	29.0	4.8	4.4	59.7	2.0	64.1
UC-1	PC	1.4	7.5	0.9	75.9	14.1	0.2	90.0
	PE	1.0	9.7	0.8	71.2	16.8	0.5	88.1
	PI	1.4	19.4	4.9	53.7	20.0	0.6	73.7

[a] Composition expressed as corrected area percentage.
[b] Ratio of carbon length to degree of unsaturation.
[c] Abbreviations: see Table I.

that the compound represents a chloro octadecenoic acid methyl ester obtained by addition of one mole of HCl to methyl linoleate.

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