ed up to about 7 μg of any acid injected. The retention times and relative retention times of the standard organic acids are listed in Table I.

A comparison of the two silylating reagents used in this study, Regisil + 1% TMCS and BSA, is shown in Table II. The response of aconitic acid diminished only 17% after 30 min reaction time with Regisil + 1% TMCS, whereas the response of aconitic acid using BSA decreased 89% in the same period of time. The silylation of both shikimic and quinic acid was essentially complete after 3 min reaction time with Regisil + 1% TMCS. In fact, there was a slight loss of response of shikimic acid with time, as was found with aconitic acid, indicating the need to use a constant reaction time for all standards and samples. The incomplete silylation of shikimic and quinic acid with BSA is very evident. Response of shikimic acid was still increasing after 30 min, while the response of quinic acid was very inconsistent due to its incomplete silylation, plus interference from the partially silylated shikimic acid peak. Quantification of these two acids, and in particular quinic acid, was nearly impossible.

The separation of silylated derivatives of shikimic and citric acid is difficult, especially when the concentration of shikimic acid is very low compared to citric acid. This is often the case, since many plant species are known to contain large amounts of citric acid. Nonseparation of these acids results in shikimic acid eluting as a shoulder on the citric acid peak, thus complicating quantification of both acids. A series of liquid phases, OV-1, OV-3, and OV-7, were tested with regard to overall separation and speed of analysis. A 9.5% coating of OV-3 was found to give the best separation of the standard organic acids when used with the programming conditions stated previously. Injection at 150° allowed separation of the smaller 2, 3, and 4 carbon acids, while the rapid rate of temperature programming increased the resolution and consequently the separation of shikimic and citric acid, and also reduced analysis time. While these operating parameters resulted in good separation with this chromatograph, other operators using different instruments may have to vary these conditions somewhat in order to obtain optimum separation.

A representative chromatogram illustrating the separation of organic acids in orchard grass is presented in Figure 1. Identification of the acid was made using the relative retention times of the standard acids listed in Table I. The results of multiple gc analysis on each of three orchard grass subsamples are presented in Table III. Malonic and oxalic acid are not included in Table III, since they were only present in trace quantities (<0.01%) in this sample. Error attributable to the gc analysis is very low. Repeatability of this procedure as indicated by the standard error values among subsamples is also quite good. This precision coupled with the speed of analysis represents an improvement in glc analysis of plant organic acids and should be beneficial to many laboratories involved in analytical determination of organic acids.

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Enzymatic Hydrolysis of Fatty Acids in Orange Juice Phospholipids

Palmitic acid was found to be located primarily at the 1 position and linoleic acid was at the 2 position of phosphatidyl choline and phosphatidyl ethanolamine in orange juice. Lipase or phospholipase hydrolysis of lipids during storage of commercial orange juice is questioned.

A recent publication by Nagy and Nordby (1970) has described changes during storage in the fatty acid content of chilled orange juice. These authors postulated that action by phospholipases on phospholipids in the juice caused significant changes in the proportions of palmitate (16:0) and oleate (18:1) remaining after storage. Also, Vandercook *et al.* (1970) have listed the quantities of the major phospholipids in some citrus juices.

Studies in our laboratory (Braddock, 1972) have described the distribution of the major fatty acids of phosphatidyl ethanolamine (PE) and phosphatidyl choline (PC) from orange juice. The purpose of this communication is to present evidence which indicates that lipolytic enzymes were not primarily responsible for phospholipid hydrolysis in stored orange juice, as suggested by Nagy and Nordby (1970).

EXPERIMENTAL SECTION

Experimental procedures have been described in detail in a previous publication (Braddock, 1972). Essentially, these methods entail purification of the phospholipids from orange juice and gas chromatographic analyses of fatty acid methyl esters from PC, PE, and their respective lyso derivatives.

Table I. Fatty Acid Composition of Valencia Orange Juice Phospholipids and of Fatty Acids in the 2 Position

	% glc peak area[a]					
	16:0	16:1	18:0	18:1	18:2	18:3[b]
PE						
Phospholipid	24	4	2	19	42	9
2 position	3	0	0	23	62	12
PC						
Phospholipid	22	5	2	26	34	9
2 position	2	0	0	31	57	10

[a] Average of nine different juice samples. [b] Notation signifying carbon chain length:number of double bonds.

RESULTS AND DISCUSSION

Research has been published which disclosed that the proportions of palmitate decreased and oleate increased considerably in the PE and PC remaining after storage of orange juice at 85°F (Nagy and Nordby, 1970). They reported a loss of approximately 90% of the PE and PC during 85°F storage of orange juice. This means that the changes in palmitate and oleate were measured from fatty acid determinations on only the remaining 10% of PE and PC. Also, Nagy and Nordby showed in their Table III that the four major fatty acids in the free fatty acid fraction increased by 85–90% during storage at 85°F. These data do not indicate any selective hydrolysis of the 90% of PE and PC reported as destroyed, but only that the 10% of unhydrolyzed PE and PC had less palmitate and more oleate than in the initial concentrations of these two phospholipids. These authors postulated that the changes in these two fatty acids were due to a specific action of phospholipases in the juice, resulting in complete hydrolysis of certain phospholipids, as no lyso-phospholipids were detected.

The findings presented in Table I show that practically all of the palmitate is at the 1 position of both PE and PC and that the predominant acid at the 2 position is linoleate. It could be reasoned that since no lyso compounds were present, the complete enzymatic hydrolysis of both the 1 and 2 positions of PE and PC should also result in significant decreases of linoleic acid. The number of phospholipids containing two palmitate molecules would also be small, because only about 2–3% of this acid is esterified at the 2 position.

Since the data of Nagy and Nordby (1970) do not indicate any significant decrease during storage of any fatty acid other than palmitate in the remaining PE and PC, it appears that the changes reported were not entirely due to lipase or phospholipase hydrolysis. Knowing that orange juice is an acidic substrate, is subjected to heat treatment during processing, and contains many degradable compounds which may interact with phospholipids causing losses, it seems that phospholipid changes during storage are primarily due to random nonenzymatic degradation, not specific hydrolysis by phospholipases. A careful study of the activity of lipolytic enzymes in citrus juices is needed in order to more fully characterize changes in the lipids which may lead to quality changes in the juice.

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Occurrence of Chlorophyll in Dried Bract of Cotton Plant

Dried bracts from field-grown frost-killed cotton plants but not from hothouse grown plants were shown by visible spectroscopy and thin-layer chromatography to contain chlorophyll a and chlorophyll b. The ratio of chlorophyll a to b was 54:46 in dried bract but 65:35 in green living bract. The total chlorophyll content of dried bract was about 0.3%. An investigation as to whether chlorophyll and pheophytin can be released from bract by lung tissue and the effect of chlorophyll and pheophytin on lung tissue to determine if these compounds can contribute to the acute response of byssinosis may be worthwhile.

Bract, a leaf-like part under the boll of the cotton plant and a reportedly prominent trash component of cotton dust that arises during textile processing, is thought to contain the chemical causative of byssinosis (Bouhuys, 1966; Bouhuys and Nicholls, 1966; Stoll, 1971; Taylor *et al.*, 1971). Plant pigments, in addition to many other compounds, have been suggested (Taylor *et al.*, 1971) as a cause of this industrial pulmonary disease. We, therefore, have undertaken a study of the chemical composition of the dried bract of the mature cotton plant, with special emphasis on the pigments present in this friable material. In this study, we report the identification of chlorophyll (Chl) in dried bract.

EXPERIMENTAL SECTION

Bracts from frost-killed field-grown cotton (High Plains, Lubbock, Texas area; variety, Paymaster 111) were collected (in November of 1971) by hand with special care being taken that the sample contained only dried bracts from under mature bolls (FDB). Samples of dried bract (HHDB), green leaf, and green bract (1 day prior to anthesis) were collected from glanded varieties of cotton grown in a hothouse. The dried bract samples were ground in a Wiley mill (room temperature; 20 mesh) and the green materials were cut into small pieces with scissors. A portion (2–3 g) of each was separately dispersed in methanol (50 ml) or acetone, each containing $MgCO_3$ (100 mg). After stirring at 0° for 1 hr, the dispersion was filtered (Whatman No. 41 paper), and the methanolic solution was partitioned with CCl_4 and the acetone was partitioned with ether. To obtain the green (red fluorescing) pigments, water saturated with $MgCO_3$ was added to the solvent mixture. The CCl_4 or ether phase, containing the green pigments, was evaporated to dryness in a stream of N_2 gas. For thin-layer chromatographic analysis, the samples of the extracts and of authentic Chl a and b were chromatographed on silica gel G (Merck) thin layers at room temperature in a lined tank using the following sol-