oxylic acid derivatives (*2a, 2b, 2c*), (ii) nucleophilic addition-elimination giving α-hydroxy oxo acids (*3a, 3b*), and (iii) formation of α,β-unsaturated oxo acids (*4*) through dehydrochlorination by the 1,2-elimination mechanism. Besides the first two reactions the occurrence of reaction (iii) also is described in the older chemical literature (3). The reaction product analysis showed, however, that α,β-unsaturated acids (*4*) were not formed under the reaction conditions applied here. Therefore, the reaction (iii) was of no importance and could be ignored. Thus, the overall dechlorination of *1a, 1b* actually involved only two reactions, (i) and (ii), yielding dioic and hydroxy oxo acids in a weight ratio of 1 to 15.

The removal of chlorine from *1a, 1b* may occur either before or after addition of hydroxide ion to carbonyl group, i.e. through reactions (i) and (ii), respectively. Reaction (i) is so-called normal Favorskii rearrangement (6,7) involving the formation of a cyclopropanone, which further undergoes ring opening to give two dicarboxylic acid derivatives from both *1a* and *1b* (Scheme I). The dioic acids *2a* and *2c* are formed from *1a* and *1b*, respectively, while the same acid *2b* may be created from both chloro oxo acids applied.

The reaction product analysis showed that dechlorination of *1a, 1b* occurred predominantly through reaction (ii), giving α-hydroxy ketonic acids *3a* and *3b* as principal products (Scheme I). The initial reaction involves nucleophilic attack of hydroxyl ion to carbonyl group, followed by displacement of chloride ion by negatively charged oxygen to give hydroxyepoxy derivatives. The further reactions beyond this intermediate are not fully understood. The epoxy ring obviously is opened with a base producing trihydroxy intermediates, which lead to the final products *3a* and *3b* through dehydration and isomerization reactions. The opening of epoxy ring by an acid reagent can also occur during separation of dechlorination products from the acidified reaction product mixture.

The dechlorination reaction in Scheme I may also play a role in pulp bleaching processes. The treatment of pulp containing resin with chlorine dioxide leads to the formation of α-chlorooxo derivatives of alkenoic fatty compounds as shown by oleic and elaidic acids and their partly dehydrochlorinated derivatives (unpublished results from this laboratory). After treatment with chlorine, pulp is subjected to alkaline extraction, where the alkali-labile compounds formed, e.g. α-chlorooxo compounds may react with alkali and transform into more water soluble derivatives. The high reactivity of α-chloroketones toward alkali is also demonstrated by the unpublished observation that the resin of fully bleached softwood sulphite pulp was found to contain very little if any fatty compounds with α-chloroketonic structure.

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♣Determination of Ascorbyl Palmitate by High Performance Liquid Chromatography

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ABSTRACT

An HPLC method for the determination of ascorbyl palmitate in vegetable oil and lard has been developed. Chromatographic conditions consist of a diamine column, a mobile phase of 70:30 (v/v) methanol:0.02M monobasic potassium phosphate buffer, pH 3.5, and UV detection. Samples were extracted with methanol. An overall average recovery value of 96.7% was obtained for ascorbyl palmitate in five representative vegetable oils and lard.

INTRODUCTION

Ascorbyl palmitate (L-ascorbic acid, 6-hexadecanoate) has been shown to be very effective in the protection of vegetable oils (soybean, corn, peanut, safflower and sunflower) against oxidation (1). Although ascorbyl palmitate (AP) is not as efficient in the protection of animal fats per se, it effectively potentiates alpha- and gamma-tocopherol. AP also has been shown to synergize BHT, BHA, TBHQ and PG in safflower oil emulsions (2) and to synergize tocopherol in citrus oils and vitamin A (3). Recently (4) AP was shown to extend the stability and quality of frying fats.

Ascorbyl palmitate has been used widely in Europe for years. Klaui (5) demonstrated the activity of AP in butter fat, vegetable oils, vitamin A, beta-carotene, ethyl linoleate and ethyl arachidonate for both AP alone and in combination with alpha-tocopherol. Pongracz (6) has experimented extensively with AP in paste mixtures and demonstrated efficacy in butter and butter oil, salad dressings, biscuits, dried potatoes, ice cream mix and dried milk products.

In the United States ascorbyl palmitate is listed in the Code of Federal Regulations, Title 21, under section 182.3149 as a chemical preservative that is generally recognized as safe when used in accordance with good manufacturing practice. Although ascorbyl palmitate itself does not occur in nature, it is enzymatically broken down into ascorbic acid and palmitic acid, which are natural ingredients in food. Unlike many antioxidants, its use is not limited to 0.02% of the fat or oil, and AP may be used at higher levels if necessary.

A number of methods are reported in the literature for the analysis of ascorbyl palmitate. Budslawski and Pogorzelski (7) have reported a colorimetric procedure for the determination of AP. TLC procedures for AP have been reported by Alary et al. (8), Van Peteghem and Dekeyser (9), Pujol Forn (10) and De la Torre Boronat et al. (11). Woollard (12) and Melton et al. (13) have described HPLC

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procedures for the determination of AP in milk and flour, respectively. The purpose of this investigation was to develop an HPLC procedure that would permit the accurate determination of ascorbyl palmitate in vegetable oils and lard.

EXPERIMENTAL

High Performance Liquid Chromatography (HPLC)

The HPLC system used consisted of a model 510 HPLC pump (Waters Associates), a model LC 75 variable wavelength UV detector (Perkin-Elmer) operated at 255 nm, a model 7210 injector (Rheodyne) equipped with a 20-μl loop, and a Chromegabond diamine, 5 μm, column, 25 cm × 4.6 mm ID (E.S. Industries). Chromatograms were recorded and peak areas determined using both model 3380A and 3390A integrators (Hewlett-Packard). The mobile phase was 70:30 (v/v) methanol:0.02M monobasic potassium phosphate buffer, pH 3.5, with a flow rate of 1.0 ml/min. The injection volume was 20-μl. All solvents used were chromatographic grade (Burdick and Jackson) and were degassed and filtered prior to use. The ascorbyl palmitate used was NF-FCC. All other reagents used were reagent grade.

Sample Preparation

Approximately one gram of sample was accurately weighed into a 50-ml glass-stoppered centrifuge tube and a 20-ml portion of methanol added. The sample was extracted by mixing for one min in a vortex mixer and then centrifuged for five min at 2500 rpm. The resultant clear methanol extract was used for chromatography. If necessary, the samples were diluted with methanol to a concentration of approximately 10 μg/ml. Solidified animal fat was first liquified on a steam bath prior to extraction with methanol.

Standard Preparation

Accurately weighed 10 mg portions of ascorbyl palmitate were dissolved and diluted with methanol to a concentration of 10 μg/ml.

Recovery Procedures

Appropriate amounts (5 mg, 10 mg and 20 mg) of ascorbyl palmitate corresponding to 100 ppm, 200 ppm and 400 ppm were weighed into a 250-ml beaker containing 50 g of oil or lard. The mixture was heated gently with stirring until the ascorbyl palmitate was completely in solution, allowed to cool to room temperature, and assayed using the procedure described under Sample Preparation.

Results and Discussion

The procedure developed enabled the quantitative determination of ascorbyl palmitate in various representative vegetable oils. The unique selectivity of the polar bonded phase diamine column used also enabled the separation of ascorbyl palmitate and ascorbic acid. Equivalent results were not obtained from alkyl amine columns having a single amine group per active site. Possible explanations for the performance of the Chromegabond diamine column are the increases in resolution and retention gained from the presence of two active amine groups per active site and the high surface coverage, which tends to reduce adsorptive effects. The separation of ascorbyl palmitate and ascorbic acid is shown in Figure 1. A typical sample chromatogram showing ascorbyl palmitate in soybean oil is shown in Figure 2.

Both the reproducibility of the chromatographic system and the precision of the overall method were determined. System reproducibility was determined with five replicate injections of a 10 μg/ml ascorbyl palmitate standard solution. A relative standard deviation of 1.8% was obtained.

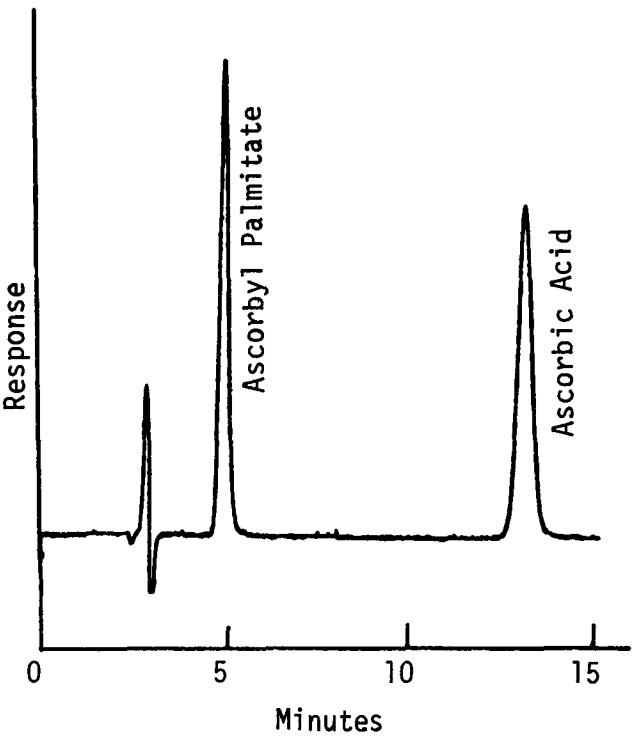

FIG. 1. HPLC separation of ascorbyl palmitate (10 μg/ml) and ascorbic acid (5 μg/ml).

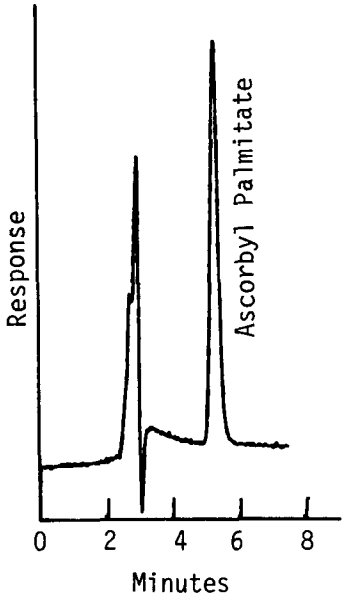

FIG. 2. HPLC chromatogram of ascorbyl palmitate in soybean oil.

Response was linear within the range of 2.5 to 25 μg/ml with a correlation coefficient of 0.99995 obtained from linear regression analysis. The accuracy of the method was determined and verified through a series of recovery experiments in which ascorbyl palmitate was added directly to representative vegetable oils at three levels (100, 200 and 400 ppm) and the oils assayed for ascorbyl palmitate using the described procedure. Peanut oil, fractionated coconut oil, animal/vegetable oil, corn oil, soybean oil and pork lard were chosen as representative vehicles. Recovery results are shown in Table I. Average recovery values ranged from 95.7% to 97.4%, with an overall average value of 96.7%. All levels tested showed acceptable recoveries: 96.4% at the 100 ppm level, 96.4% at the 200 ppm level, and 97.3% at the 400 ppm level.

The versatility of the method has been determined on

TABLE I

Recovery of Ascorbyl Palmitate

	Level (ppm)	Percent recovery: Corn oil	Peanut oil	Fractionated coconut oil	Soybean oil	Animal/ vegetable oil[a]	Pork lard
	100	96.6	95.0	97.9	96.3	97.1	95.8
	100	95.7	95.3	95.3	95.1	98.6	98.2
	200	95.5	96.5	92.4	97.5	95.5	96.5
	200	102.0	95.2	94.8	96.0	96.5	98.7
	400	95.8	96.5	95.8	96.5	96.7	95.3
	400	97.3	99.3	97.9	103.0	98.2	95.4
Average		97.2	96.3	95.7	97.4	97.1	96.7
Standard deviation		2.5	1.6	2.1	2.9	1.1	1.5
Relative standard deviation		2.6%	1.7%	2.2%	3.0%	1.1%	1.6%

[a]75% animal:25% vegetable.

TABLE II

Analysis of Ascorbyl Palmitate in Various Samples

Sample	Claim (%)	Assay (%)
Yogurt bars	0.1	0.10
Soybean oil	0.02	0.016
Soybean oil (4 days at 350 F)	0.02	0.012
Soybean oil (6 days at 350 F)	0.02	0.010
Carotene emulsion	1.30	1.32
Cosmetic sample A	10.0	9.9
Cosmetic sample B	10.0	10.5
Cosmetic sample C	5.0	4.8
Flour sample no. 1	0.02	0.019
Flour sample no. 2	0.044	0.042

a wide range of samples. In addition to the determination of ascorbyl palmitate in vegetable oils, the method has been used to assay ascorbyl palmitate in flour, yogurt bars and various creams. Experimental results obtained for samples are presented in Table II. As the use of ascorbyl palmitate becomes more widespread, the procedure described in this paper can provide a means for its accurate determination.

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