TABLE I

Summary of Replicate Detergent Analyses

Sample	"As is" detergent, ppm of P found	Detergent samples, each fortified with 15 ppm of P ppm of P found
1	12	31
2	11	28
3	13	25
4	13	25
5	13	26
Average ± average deviation	12.4 ± 0.7	27.0 ± 2.0

TABLE II

Recovery of Phosphate From Fortified Detergent Samples

Sample no.	Amount of phosphate added, ppm as P	Total P found, ppm	Added P recovered, ppm[a]	Per cent recovery
1	15	31	18.6	124
2	15	28	15.6	104
3	15	25	12.6	84
4	15	25	12.6	84
5	15	26	13.6	91
Average	15	27.0	14.6	97.5

[a]Determined by subtracting initial phosphorus content of 12.4 ppm from total P found.

phosphomolybdic acid into the upper organic phase. A 25 ml aliquot of the separated organic phase is transferred to a 50 ml volumetric flask. A 20 ml portion of 2% H_2SO_4 in methanol is added, followed by 1 ml of stannous chloride, prepared by dissolving 10 g of stannous chloride in 25 ml of concentrated HCl and diluting 0.5 ml of this solution to 100 ml with water. This diluted solution must be prepared fresh each day. After mixing, the solution is diluted to the mark with methanol. Color development is allowed to proceed for a 10 min period and then absorbance is measured at 650 nm, using 1 cm cells. Samples are not allowed to stand more than 30 min. The concentration of phosphorous is determined directly from a standard curve, prepared by taking known amounts of an ortho-phosphate solution, transferring them to extraction cylinders, diluting to 48 ml, continuing as described above, and plotting phosphorous concentration against absorbance. The standard curve will obey Beer's law between concentrations of 10-200 μg of phosphorous (Fig. 1).

Using the above procedure, a group of five 1 g samples taken from the same carton of a silicate-containing, "phosphate free" detergent were analyzed. Additionally a second group of five 1 g samples from the same carton were taken, and each fortified with phosphate equivalent to 15 ppm as phosphorous, prior to the ashing step. The reproducibility of the method, in terms of the amount of phosphorous found in each of the samples, is shown in Table I. Table II shows the amount of phosphate recovered from fortified samples of detergent. A statistical analysis of the data indicates reliability of low level assays to be ±13%, with the average percentage recovery of replicate samples of phosphate being 98.5%. The reliability of an assay for a sample containing 25 ppm of phosphorous would be ±3 ppm.

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Grapefruit Seed Oil Sterols

ABSTRACT

Grapefruit seed oil sterols separated from other lipids by Florisil column chromatography were characterized by gas liquid chromatography. The presence of stigmasterol, campesterol and β-sitosterol is indicated. Expressed in terms of peak area, the three sterols are present in proportions of 2.5%, 7.4% and 90.1% of the total, respectively.

Seeds of citrus fruits contain about 30% oil (1). While the proportion of seed to fruit is relatively small, there is considerable potential of citrus seed oil as a by-product of fruit processing. Processing and refining methods have been developed for the commercial production of citrus seed oils (1,2). Certain characteristics of grapefruit seed oils have been published (3-5). While the identification of sterols in other portions of citrus fruit has been reported (5-7), there is a lack of information regarding seed oil sterols. This investigation reports the nature of sterols in grapefruit seed oil as characterized by gas liquid chromatography (GLC).

Seeds were obtained from grapefruit, *Citrus paradisi* (Macf.), representing two varieties: Yuma pink and Yuma yellow. The seeds were removed from the fruit, washed with distilled water, air-dried and extracted in a high speed mixing blender with chloroform-methanol 1:1 v/v (8). The

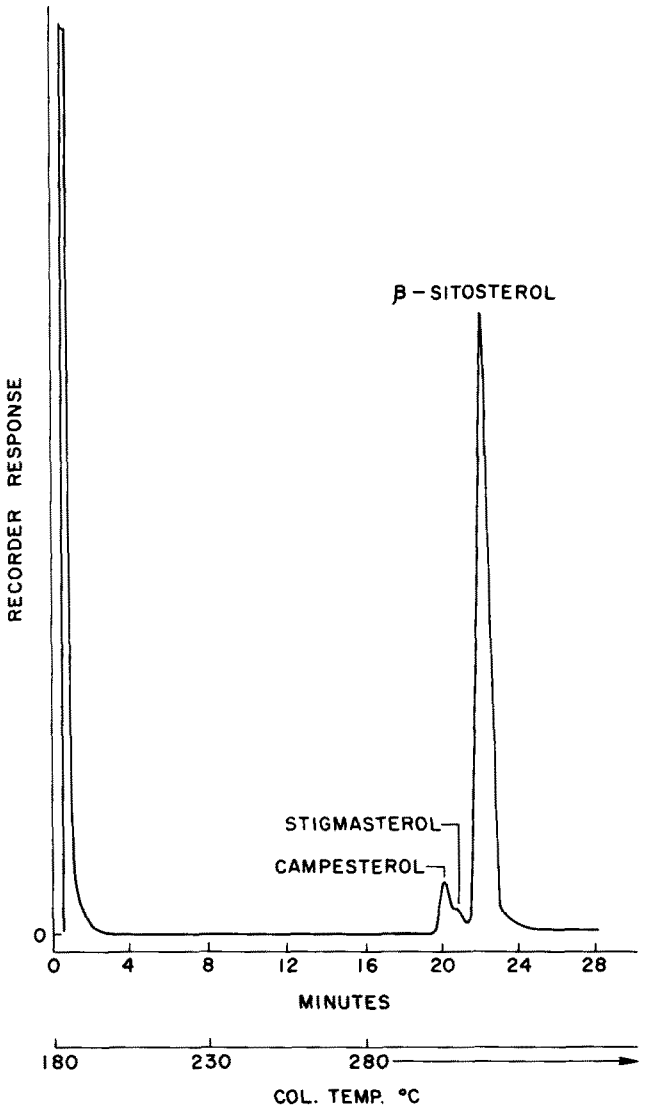

FIG. 1. Gas chromatographic separation of grapefruit seed oil sterol trimethylsilyl ethers on 3% SE-52.

extract was filtered and the solvent removed under reduced pressure. The extracted oil was saponified and the unsaponifiables fractionated by Florisil column chromatography (9,10). The seed oil from the two varieties of grapefruit had an average total sterol content of 0.53% based on the amount of the Florisil column fraction eluted from the unsaponifiable matter. The sterols obtained from this separation were converted to trimethylsilyl ethers by dissolving in chloroform (30 μg/μl) and mixing 0.5 ml of this solution with 0.5 ml of N, O-Bis-(trimethylsilyl)-acetamide (BSA) and 20 μl of trimethylchlorosilane (TMCS) in a 2 ml vial. The vial was tightly stoppered and allowed to stand at room temperature for 30 min. A portion of this mixture was injected directly into the gas chromatograph. GLC was on a 4 mm x 180 cm glass column of 3% silicone gum (methyl-phenyl type, SE-52) on AW-dimethyldichlorosilane treated Chromosorb W, 60-80 mesh at 180 to 280 C (6.25 C/min), at 40 psi and 45 ml/min, utilizing on-column injection. The sterols were identified by comparison with retention times of pure compounds. A typical chromatogram indicates the presence of stigmasterol, campesterol and β-sitosterol (Fig. 1).

Expressed in terms of peak area, the three sterols are present in proportions of 2.5%, 7.4% and 90.1% of the total, respectively. Similar analyses obtained from other seed oils indicate average values for the amount of β-sitosterol in soybean, safflower and cottonseed to be 72.9%, 81.9% and 96.2%, respectively (10). Values for stigmasterol in this report indicate that cottonseed has none of this sterol while safflower and soybean oils contain an average of 5.6% and 12.5%, respectively. Average γ-sitosterol values for cottonseed, safflower and soybean oils are reported by these workers to be 3.8%, 12.5% and 14.6%, respectively. Because of the similarity of retention times, it is probable that the component identified as campesterol in the present work is the same as γ-sitosterol in the earlier report (10).

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