yield (determined by GLC area percent values), was shown to be nootkatone by comparison of its infrared spectrum and GLC retention time with those for an authentic sample.

Acetylation of Nootkatol (5) to Acetate 3. To 100 mg of compound 5 was added 300 μL of a 2:1 mixture of acetic anhydride and pyridine. The mixture was allowed to stand overnight and was then separated by preparative TLC in solvent 1. Compound 3 was afforded in about 80% yield.

Conversion of Ester Mixture 2 and 3 to Nootkatone (6). To 12 g of crude ester mixture in 100 mL of diethyl ether was added 50 mL of sodium hydroxide solution (58 g of NaOH in 500 mL of 1:1 methanol–water), and the solution was stirred overnight. The aqueous layer was separated and extracted two times with 25-mL portions of diethyl ether, and the ether extracts were added to the original ether layer.

The combined ether solution was dried over magnesium sulfate and concentrated to afford 9.0 g of crude alcohol mixture. Where the crude mixture was separated by preparative TLC (solvent 2), alcohol 4 was recovered in about 65% yield.

The crude alcohol mixture in 100 mL of diethyl ether was treated with 35 mL of aqueous chromic acid and the mixture stirred overnight. The layers were then separated, the aqueous layer was extracted two times with diethyl ether, and the extracts were added to the original ether solution. The combined ether solution was then dried over magnesium sulfate and concentrated to afford 6.6 g of crude product. Qualitative and quantitative GLC analysis of the crude mixture showed it contained 85.5% nootkatone (6) assuming the sample was 100% GLC volatile (47% overall yield from valencene).

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Quantitative Composition of Cold-Pressed Grapefruit Oil

Charles W. Wilson, III* and Philip E. Shaw

The major volatile components of Florida cold-pressed white grapefruit oil were quantitatively analyzed by gas chromatography. Corrected weight percentages were determined from response factors and the precentages of nonvolatiles in the whole oil. Of the 24 identified constituents, 19 were quantitated. Data for only 9 of the 19 could be compared with literature values, but the agreement was generally good. The octanal to decanal ratio was slightly greater than 1. Quantities of the two major esters, octyl and neryl acetates, are reported for the first time.

Reliable quantitative information on individual components of cold-pressed grapefruit oil is scarce relative to comparable data on orange, mandarin, and lemon oils (Shaw, 1977). With such data on grapefruit, the contribution of individual compounds to the total flavor profile of grapefruit oil could be assessed, the chemotaxonomy of grapefruit hybrids could be studied, and entomologists may be better able to study reasons for survival of insect larvae in grapefruit peel.

Reports to date on quantitative analysis of grapefruit oil constituents have emphasized the aldehydes because they are major contributors to citrus oil flavors. Yokoyama et al. (1961) compared colorimetric methods for quantitating citral in various citrus oils. Stanley et al. (1961) and Braddock and Kesterson (1976) quantitated the major aldehydes in citrus oils, but the aldehyde values of Stanley et al. (1961) could only be related to total oil by use of an estimated value for total aldehydes present (Shaw, 1978).

U.S. Citrus and Subtropical Products Laboratory, Science and Education Administration, Federal Research, U.S. Department of Agriculture, Winter Haven, Florida 33880.

Other studies on grapefruit oil quantitation involved injection of the whole oil onto a gas chromatographic (GLC) column (Ashoor and Bernhard, 1967; Attaway et al., 1967); but in one of these (Attaway et al., 1967) the workers used relative peak heights to determine the relative percentage of each component rather than the more accurate quantitation based on peak areas. Ikeda et al. (1962) quantitated the major hydrocarbons in grapefruit oil by GLC after a preliminary separation by thin-layer chromatography (TLC). However, in none of those studies were the GLC response factors or the considerable number of nonvolatiles present in grapefruit oil taken into account.

In the current study, whole cold-pressed grapefruit oil was directly injected onto a GLC column, and the weight percentage of each major oil component was determined from response factors and the precentage of nonvolatiles retained by the column.

EXPERIMENTAL SECTION

Cold-pressed white grapefruit oil from Duncan and Marsh seedless grapefruit that had been stored several days at 0 °C or less (winterized) was obtained from a local processor in November 1977 and stored at 4 °C until used. Authentic samples of individual compounds for response

Table I. Quantitative Analytical Data for Cold-Pressed Grapefruit Oil

	digital integrator area %				
	grapefruit oil	synth mixt	calcd wt % in synth mixt	corrected wt % in oil[a]	previously reported range[b]
α-pinene	0.44	0.37	0.45	0.49	(0.2-1.6)
myrcene + sabinene	1.84	1.48	1.85	2.12	(1.4-1.9)
d-limonene	93.47	95.29	94.35	85.60	(86-95)
octanal	0.97	1.17	0.93	0.71	(0.3-0.6)
nonanal	0.10	0.09	0.09	0.04	(0.04-0.1)
octyl acetate	0.10	0.19	0.11	0.09	
citronellal	0.16	0.18	0.17	0.14	
decanal	0.61	0.57	0.60	0.60	(0.3)
linalool	0.27	0.22	0.26	0.30	(0.4)
α-copaene	0.07	0.09	0.09	0 06	
β-copaene	0.10	0.09	0.12	0.01	
citronellyl acetate[c]					
β-elemene	0.09			0.06[d]	
caryophyllene	0.29	0.31	0.29	0.25	
Δ-cadinene	0.09	0.07	0.09	0.11	
neral[c]					
neryl acetate	0.18	0.12	0.16	0.22	
geranial	0.12	0.05[e]	0.10	0.11	(0.1-0.2)
geranyl acetate[c]					
decyl acetate	0.22	0.32	0.24	0.15	
carvone[c]					
perillaldehyde	0.04	0.03	0.04	0.2	(0.003-0.1)
elemol	0.07	0.15	0.09	0.04	
nootkatone[f]					(0.3-0.8)
nonvolatiles				7.5[g]	

[a] Corrected for 7.5% nonvolatiles. [b] Shaw, 1978. [c] Identified by IR and RT, but not quantitated because compound is part of a mixture. [d] Calculated using copaene response factor. [e] Area % determined with citral (51% geranial) and corrected wt % adjusted accordingly. [f] Peak too broad due to long retention time to integrate properly. [g] Determined by distillation (see Experimental Section).

factors, spectral comparisons, and determinations of retention times were obtained commercially or were isolated from citrus essential oils by previously described techniques (Shaw, 1977).

Infrared (IR) spectra of the samples as films were determined with a Perkin-Elmer Model 137-A infrared spectrometer. Identifications were made by comparison of IR spectra with those for authentic compounds and by comparison of retention times with those of authentic compounds that were added individually to the cold-pressed oil.

Response factors for the identified components in the cold-pressed peel oil were determined by a previously described internal normalization method (Shaw and Coleman, 1971). The synthetic mixture was prepared in *d*-limonene with standards of compounds identified in the peel oil. The compounds were mixed in the proportions indicated by the GLC area percentage analysis. Thus, corrected wt% (column 5 in Table I) = area % whole oil (column 2)/area % synthetic mixture (column 3) x calcd wt % (column 4).

The percentage of nonvolatiles (Shaw and Coleman, 1974) was determined from 1.24 kg of grapefruit cold-pressed oil which was first distilled in a rotary evaporator at 40 °C and 1 mmHg. The pot residue (122 g) was then further distilled in a 2-in. molecular still at 170 °C and 0.01 mmHg. The residue was 88 g, 7.5% of nonvolatiles.

For qualitative analyses a Perkin-Elmer Model 900 gas chromatograph was used. It was equipped with a Hewlett Packard Model 3380 A computing integrator, and the 20 ft × 0.20 in i.d. stainless steel columns were packed with either 20% UCW-98 or Carbowax 20M on 60–80 mesh Gas-Chrom P. The injection port and thermal conductivity detector temperatures were 250 and 265 °C, respectively. The oven temperature was programmed from 100–220 °C at 2 °C/min and He flow was 100 mL/min. The cold-pressed grapefruit oil was concentrated on a rotary evaporator at 40 °C (1 mmHg) and then separated by GLC for product isolation and identification. Individual compounds were trapped in glass capillary tubes with liquid nitrogen as they eluted from the GLC column.

For quantitative GLC analysis of whole cold-pressed oil, 10-μL samples were injected onto a 20 ft × 0.125 in. i.d. stainless steel column packed with 20% Carbowax 20M on 60–80 mesh Gas-Chrom P. The oven was programmed from 80–210 °C at 4 °C/min, and He flow rate was 30 mL/min. Area percentages were averaged from three consecutive runs.

RESULTS AND DISCUSSION

Nineteen major components of a Florida white grapefruit oil were quantitated (Table I). Distillation showed the whole oil contained 7.5% nonvolatiles; thus, 92.5% of the sample was believed volatile enough to be eluted from the GLC column. Using the values in column 2 to 4 of Table I and the percentage of nonvolatiles, we calculated the corrected weight percentages shown in column 5 (Shaw and Coleman, 1971).

Previous quantitative results and methods for analyzing grapefruit oil were reviewed recently by Shaw (1978), but quantitative comparisons could be made for only 9 of the 19 compounds we quantitated (see Table I). The data for most of the nine compounds agreed closely with those reviewed by Shaw (1978). Values for limonene and linalool were slightly less and values for octanal, decanal, and perillaldehyde were greater in this study than have been reported. Quantitative information on linalool in grapefruit oil is variable. Kesterson and Hendrickson (1964) using terpeneless oil reported linalool to be absent in white grapefruit oil. These same investigators, however, later reported that a measurable amount of linalool was present initially, but that it gradually disappeared during 1 year of storage (Kesterson and Hendrickson, 1967). Attaway et al. (1967) found 0.4% of linalool in a mature grapefruit

sample, using relative peak heights from a GLC curve to follow changes in oil composition with maturity.

The ratio of octanal to decanal in grapefruit oils has been suggested as a quality index. Kesterson et al. (1971) reported ratios of 1:1.1 to 1:1.4 for white grapefruit oil and ratios of 1.2:1 to 1.3:1 for red grapefruit oil. A later study (Braddock and Kesterson, 1976) indicated that the ratio was greater than 1 in a white grapefruit oil sample. The ratio we found was about 1.2:1.

Several of the identified compounds were part of mixtures and/or present in quantities too small to be accurately quantitated. Nootkatone eluted as small, broad peak with a very long retention time (95 min) and could not be integrated accurately. Only the peak for one mixture (myrcene and sabinene) was sufficiently large and sufficiently well resolved from surrounding peaks to make integration possible. The response factor for the sabinene–myrcene peak was determined from that of myrcene only in the synthetic mixture.

In other cases, adjustments had to be made for accurate quantitation. Sufficient quantities of chromatographically pure β-elemene were not available to allow us to determine its response factor; so its corrected weight percent is based on the response of β-copaene. The response factor for citral (containing 51% geranial) was used as that for geranial, and the corrected weight percentage of geranial is based on the percentage of geranial present in the citral sample.

Thus, we quantitated 19 major components of a typical Florida white grapefruit oil by a procedure involving GLC without preliminary separation steps that uses response factors and corrects for the presence of nonvolatiles. Only few comparisons could be made, but our results are similar to previously reported values. We quantitated ten components not previously quantitated, including octyl and neryl acetates. These esters were reported by Moshonas (1971) to be two of the major carbonyl flavor components present in grapefruit oil.

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Isolation and Characterization of Arabinogalactan from Black Gram (*Phaseolus mungo*)

N. S. Susheelamma* and M. V. L. Rao

An arabinogalactan type of polysaccharide has been isolated from black gram by extracting the meal with aqueous 10% (w/v) trichloroacetic acid and precipitation with acetone. Reprecipitation and dialysis gave an ultracentrifugally homogeneous preparation with a high molecular weight (ca. 144 000) as determined by gel filtration through Bio-Gel P-200. Aqueous dispersions possessed high viscosity around pH 5–7 which decreased with increase in temperature.

Black gram (*Phaseolus mungo*) has been the traditional choice among the common grain legumes as an essential ingredient of some of the most popular and typical Indian breakfast foods which possess a characteristic soft, spongy texture and are made out of leavened mixtures of the legume and cereals (usually rice). A noteworthy feature of batters containing this legume is their high viscosity ascribed to the presence in it of a mucilagenous principle that is also held to be responsible for the gas-holding and dough-raising qualities. Other legumes lack this principle and hence are considered unsuitable for such food preparations.

Kadkol et al. (1961) attempted to isolate the mucilagenous principle from black gram by extracting with an acetate buffer, deproteinizing by repeated treatment with Sevag's solvent, and finally precipitating with acetone. The resultant crude preparation still contained 20% protein and was designated as a mucopolysaccharide. In our preliminary studies on the texture principles of this legume (Susheelamma and Rao, 1974), it was demonstrated that the highly surface-active proteins in the grain function producing the spongy texture and an arabinogalactan type polysaccharide closely associated with the proteins (and generally coprecipitated with them during isolation) protect this spongy framework against thermal disruption

Discipline of Biochemistry and Applied Nutrition, Central Food Technological Research Institute, Mysore 570013, India.

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