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Received for review June 13, 1983. Accepted October 24, 1983.

Quantitation of Individual and Total Aldehydes in Citrus Cold-Pressed Oils by Fused Silica Capillary Gas Chromatography

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Total aldehydes in cold-pressed oils from California and Florida citrus cultivars were quantified by fused silica capillary gas chromatography using a nonpolar bonded-phase fused silica column. Total aldehydes by GC were from 93 to 100% of the U.S.P. total aldehyde value and were within the limits of precision for the U.S.P. method. The major aldehydes quantified in oils were octanal and decanal, and these values were higher in oils with the higher total aldehydes with one exception. Most values for individual aldehydes were within or below aldehyde values reported earlier.

Volatile aldehydes are important to the flavor and aroma of citrus cold-pressed oils and total aldehydes are part of the standards of identity for determining oil quality (Kesterson et al., 1971). The current U.S.P. ("United States Pharmacopeia", 1965) method for total aldehydes is nonspecific, since the reagent used for color formation (hydroxylamine hydrochloride) reacts with any aldehyde or ketone carbonyl present in the oils. Thus, some of the compounds detected may not contribute in the same manner as the main aldehydes to the flavor or aroma of citrus oils.

A reliable gas chromatographic (GC) procedure would provide quantitative values for individual volatile aldehydes and ketones, total aldehydes, and quantitative values for many other desirable volatile flavor components in citrus oils. Early GC methods using packed analytical columns for quantifying volatile components of citrus oils met with limited success because of incomplete separation of individual components (Shaw, 1979). Development of glass capillary GC columns and improved instrumentation gave a method for analyzing complex mixtures where improved resolution and sensitivity were achieved (Jennings, 1979). Jennings (1980) discussed the advantages of high-resolution glass capillary chromatography for analyzing complex mixtures of volatiles in foods and essential oils. Wilson and Shaw (1980) reported the quantification of 32 constituents of Florida cold-pressed grapefruit oil using a Carbowax 20M glass capillary column. Vora et al. (1983) reported the concentration of 24 volatile flavor components from Florida Valencia and midseason cold-pressed oils using a nonpolar OV-101 fused silica column. They quantified most of the major aldehydes present but were unable to separate and quantify the major aldehyde, octanal. Staroscik and Wilson (1982a) quantified 37 components in a mixture of California and Arizona cold-pressed lemon oil with a nonpolar SE 54 glass capillary column and reported quantitative differences in lemon oil mixtures due to seasonal and regional variations (Staroscik and Wilson, 1982b). They found that SE 54 gave good separation of aldehydes and that it was superior to the widely used Carbowax 20M for analyzing citrus oils. Their value for citral determined by capillary GC was about 0.4% lower than the citral value determined by the hydroxylamine test. However, total aldehydes determined by capillary GC afforded up to 96% of the total aldehydes determined by the nonspecific U.S.P. method.

The current report describes the determination of total aldehydes in cold-pressed citrus oils from several California and Florida citrus cultivars using a bonded-phase fused silica capillary column and compares the results to those obtained by the U.S.P. method.

EXPERIMENTAL SECTION

Two commercial samples each of California navel orange, Florida Valencia orange, midseason orange, tangerine, and white graphefruit cold-pressed oils were obtained for this study. Each oil had a different U.S.P. total aldehyde value.

Oil samples were analyzed in triplicate on a Hewlett-Packard 5840A GC equipped with a fused silica capillary column with a bonded phase equivalent to SE 54 (DB-5, 30 m × 0.32 mm i.d., 1.0-μm film thickness, J & W Scientific, Inc., Rancho Cordova, CA). The oven temperature was held at 40 °C for 0.5 min, raised to 60 °C at 20 °/min, and then programmed to 250 °C at 4 °C/min. Injection port and detector temperatures were 250 and 350 °C, respectively. The carrier gas (H_2) flow (U) was 38 cm/s at 250 °C, and makeup gas (N_2) was 30 mL/min. the sample size for both oils and calibration mixtures was 0.1 μL with an injection port split ratio of 100:1. Compounds used as calibration standards were obtained from commercial sources (Aldrich Chemical Co., Milwaukee, WI; C. A. Aromatics, Floral Park, NY). Calibration mixtures for determining response factors were prepared as previously described (Wilson and Shaw, 1978). Ethyl cinnamate was

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Table I. Aldehyde Values of Florida and California Citrus Cold-Pressed Oils

	cultivar, wt %									
	Valencia		midseason[a]		CA navel		tangerine		grapefruit	
	1	2	1	2	1	2	1	2	1	2
octanal	0.505	0.393	0.374	0.342	0.172	0.150	0.285	0.456	0.520	0.466
nonanal	0.075	0.069	0.060	0.057	0.022	0.022	0.085	0.056	0.081	0.072
citronellal	0.090	0.094	0.060	0.055	0.081	0.064	0.100	0.080	0.091	0.077
decanal	0.415	0.375	0.338	0.277	0.142	0.131	0.207	0.177	0.421	0.395
neral	0.073	0.072	0.064	0.065	0.062	0.045	0.018	0.013	0.040	0.046
geranial	0.180	0.120	0.114	0.113	0.104	0.080	0.024	0.015	0.066	0.087
perillaldehyde	0.033	0.040	0.029	0.031	0.017	0.013	0.062	0.047	0.018	0.024
dodecanal	0.083	0.080	0.067	0.055	0.034	0.030	0.046	0.043	0.050	0.048
β-sinensal	0.056	0.081	0.048	0.047	0.026	0.019			0.018	0.027
α-sinensal	0.037	0.060	0.034	0.034	0.024	0.016	0.256	0.142		
nootkatone	0.051	0.024	0.035	0.015	0.045	0.043			0.237	0.147
total	1.60	1.41	1.22	1.09	0.729	0.613	1.08	1.04	1.54	1.39
U.S.P. total aldehydes	1.66	1.41	1.22	1.11	0.75	0.66	1.11	1.05	1.55	1.46

[a] Mixture of pineapple and other citrus cultivars.

used as an internal standard. Identification of aldehydes and ketones known to be present in citrus oils was based on results from coinjection with known standards and peak enrichment as well as disappearance of these peaks after sodium borohydride reduction of a sample of each oil (Wilson and Shaw, 1977). The coefficient of variation for individual aldehydes ranged from about 6 to less than 1%, and compounds present in smaller concentrations had the higher coefficients of variation.

RESULTS AND DISCUSSION

The major aldehydes in cold-pressed citrus oils from one California and four Florida citrus cultivars were quantified by using a DB-5 fused silica capillary GC column, and the results were compared to total aldehydes obtained by the U.S.P. method (Table I). Much information can be obtained by this GC method that is not available by the U.S.P. total aldehyde method; the total analysis time is about the same for the two methods. Table I lists the compounds quantified in order of their increasing retention times on the GC column. Two oils with different U.S.P. total aldehyde values were analyzed for each cultivar. The higher total aldehyde sample is oil no. 1 in each case.

An important consideration in a new method of oil analysis is how the values obtained compare with those obtained by earlier methods, especially those recognized as standard methods. The values obtained by totaling the weight percent values for all aldehydes quantified by GC were compared with those obtained by the standard U.S.P. total aldehyde method (Table I). Total aldehydes by GC were from 93 to 100% of the U.S.P. total aldehyde values for all oils, which is within the limit of precision for the U.S.P. method (Dennis, 1983). None of the values obtained by the GC method were higher than those for the U.S.P. method. Since minor aldehydes and ketones present in the oils would contribute to the U.S.P. total aldehyde method, but would not be included in the GC total aldehyde method, this result was to be expected.

The two major aldehydes quantified in all oils, except the high-aldehyde tangerine oil, were octanal and decanal (Table I). The octanal and decanal values were higher in the oil with higher total aldehydes with one exception. The α-sinensal value for the tangerine oil with the higher total aldehydes was greater than the decanal value and the octanal value was substantially higher in the tangerine oil sample, which had the lower total aldehyde value.

In the orange oil samples, all values except those for perillaldehyde and nootkatone were either within or below the range of values reported earlier (Shaw, 1979). Since fused silica capillary GC affords better separation of components than packed column GC used for much of the earlier work, some lower values might be expected when fused silica capillary columns are used for quantitation. The perillaldehyde values were close to those reported earlier (0.02–0.03%) from our laboratory using a packed column (Shaw and Coleman, 1974). The nootkatone values were higher in this study in all orange oil samples than the value (<0.01%) determined by an earlier preparative GC method where losses due to collection of the sample were possible (MacLeod and Buigues, 1964).

In the tangerine and grapefruit oil samples most values found in this study were equal to or slightly lower than those reported earlier (Shaw, 1979). In tangerine oil, only the nonanal value was higher than found earlier (Calvarano et al., 1974). However, the oil used in the earlier study was from the Clementine cultivar, while the Florida tangerine oil in this study was mostly from Dancy fruit. None of the values for grapefruit oil were higher than the range of values reported earlier (Shaw, 1979; Wilson and Shaw, 1980).

Use of the nonpolar bonded-phase DB-5 fused silica capillary column has several advantages for quantitative analysis of aldehydes in citrus oils over columns used in earlier studies. The major aldehyde, octanal, was resolved from myrcene on this column but was unresolved on other nonpolar columns. Although octanal was resolved from other components on a polar Carbowax 20M column (Wilson and Shaw, 1980), the nonpolar DB-5 column was more stable and resulted in shorter total analysis time.

CONCLUSION

Major aldehydes in cold-pressed citrus oils can be separated and quantified on a DB-5 fused silica capillary column to afford results in accord with those by the U.S.P. total aldehyde method and in about the same length of analysis time. However, information about individual aldehydes is obtained by the GC method that is not available by the present U.S.P. standard method. This could be of potential value in correlating oil composition with quality.

Registry No. octanal, 124-13-0; nonanal, 124-19-6; citronellal, 106-23-0; decanal, 112-31-2; neral, 106-26-3; geranial, 141-27-5; perillaldehyde, 2111-75-3; dodecanal, 112-54-9; β-sinensal, 60066-88-8; α-sinensal, 17909-77-2; nootkatone, 4674-50-4.

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Received for review September 30, 1983. Accepted November 21, 1983. Mention of a trademark or proprietary product is for identification only and does not infer a warranty or guarantee of the product by the U.S. Department of Agriculture over others that may also be suitable.

Formation and Chemical Characterization of Some Nitroso Dipeptides N Terminal in Proline

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The reaction products from sodium nitrite in dilute acid and six dipeptides that were N terminal in proline were investigated. Chemical identity of the reaction products was based on spectral characteristics using ultraviolet, infrared, and mass spectrometry correlated with spot tests obtained from thin-layer chromatographic plates. The nitrosation products from the dipeptides were *N*-nitroso dipeptides with the nitroso group on the imino nitrogen.

Dipeptides are biologically important compounds that occur in foods and can be formed upon digestion of proteins in the upper gastrointestinal tract. The potential for formation of *N*-nitroso derivatives of peptides in foods would be of considerable concern since *N*-nitrosamines and *N*-nitrosamides represent two classes of potent carcinogens (Magee et al., 1976).

Several investigators have considered the possibility of nitrosation of amide bonds. White (1955) and Bonnett and Nicolaidou (1977) demonstrated that the amide linkage is relatively unreactive toward nitrosation by nitrite in aqueous solution. Kakuda and Gray (1980) reported that N-nitrosation of amides decreased rapidly as the pH increased, and little reaction occurred above pH 3. Pollock (1982) reported nitrosation of the peptide bond in a series of dipeptides. These same investigators, however, have more recently claimed that the reaction products were (*N*-nitrosoimino)alkanoic acids rather than *N*-nitroso peptides (Outram and Pollock, 1983).

Bonnett and Nicolaidou (1977) suggested that peptides with the amino acids proline, hydroxyproline, or sarcosine in the N-terminal position could form stable *N*-nitroso derivatives. For this investigation we chose to study the formation of *N*-nitroso derivatives of a number of peptides that are N terminal in proline. Such dipeptides would be expected to undergo nitrosation at the imino nitrogen to produce a stable *N*-nitrosamine. These compounds have neither been synthesized nor been characterized. The existence in nature of *N*-nitroso derivatives of dipeptides N terminal in proline has not been reported, and the carcinogenic properties of these compounds are not known. The purpose of this study was to synthesize and characterize products from the nitrosation of dipeptides N terminal in proline.

EXPERIMENTAL SECTION

Chemicals. L-Proline (Pro), L-prolylglycine (Pro-Gly), L-prolylhydroxyproline (Pro-Hyp), L-prolylisoleucine (Pro-Ile), L-prolylphenylalanine (Pro-Phe), L-glycylglycine (Gly-Gly), and L-glycylproline (Gly-Pro) were obtained from Aldrich Chemical Co. L-Prolylglutamic acid (Pro-Glu) and L-prolylserine (Pro-Ser) were purchased from Bachem, Inc.

Purity of the above dipeptides was checked by thin-layer chromatography on silica gel 60, F-254, using propanol–water (7:3) as a developing system. Griess and ninhydrin reagents were used separately to visualize the plates. Commercial dipeptides were free of *N*-nitroso compounds and free amino acids. The lower limit for detection of *N*-nitroso compounds and free amino acids in the dipeptides was 1%. All solvents were analytical grade.

Synthesis of *N*-Nitroso Derivatives of Dipeptides. The method of Hansen et al. (1974) previously developed for the synthesis of *N*-nitrosoamino acids was modified for the synthesis of *N*-nitroso derivatives of dipeptides. One gram of dipeptide was dissolved in 50 mL of water. Ten grams of sodium nitrite was added, and the solution was acidified to pH 3 with hydrochloric acid. Since Pro-Phe and Pro-Ile were not soluble in water, they were added directly to the acidified sodium nitrite solution. The reaction was allowed to proceed in the dark while being stirred at room temperature for 3 h. The mixture was then acidified to pH 1, water was evaporated under reduced

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0021-8561/84/1432-0401$01.50/0