Aroma Components of Olive Oil

Robert A. Flath,* Ralph R. Forrey, and Dante G. Guadagni

The polar volatile components of virgin olive oil were concentrated by codistillation with water, followed by solvent extraction and dry-column chromatography. Gas chromatographic–mass spectrometric examination of the polar concentrate yielded the identities of 77 components. Organoleptic assessment of some of these compounds indicated that several are significant contributors to olive oil aroma.

Olive oil is an important component in the daily diet of a large part of the world's population, as it has been throughout recorded history. Most of the world supply is produced in the countries surrounding the Mediterranean Sea; in recent years the annual production has approached 1.4×10^6 metric tons. Although the bulk of this production is consumed by the residents of the producing countries, a significant percentage is exported to Northern Europe and the Western Hemisphere. Approximately 2% of the world output is imported by the United States. Although olives are commercially grown in the U. S., the olive crop is devoted almost entirely to the production of canned ripe olives, which provide a better return to the processor than would pressing the crop for the oil. A small and variable amount of domestic oil is produced from culls, undersized fruit, and excess production.

Although olive oil is not an important domestic agricultural product, olive oil flavor is of interest to several segments of the food industry. Food processors use olive oil in certain specialty food preparations because it contributes to the resultant flavor of the product, even though olive oil costs considerably more than the more common bland vegetable oils. In addition, the ripe olive industry, centered in California, has recurring problems with off-flavors of various kinds. While the flavor of a processed ripe black olive appears to be rather subtle and complex, the oil contained within the olive is certainly a significant contributor to the olive's flavor, for approximately one-fifth of a ripe olive's weight is due to its oil content. Therefore, any study of ripe olive off-flavor must also consider the effect of oil off-flavor.

Relatively little information about olive oil flavor and volatiles composition appears in the literature. Two Italian researchers, Fedeli and Jacini (1968, 1970a,b; Fedeli, 1970) have recently reported preliminary results of their investigation of the flavoring constituents of olive oil. In the abstract of their 1970 report (Fedeli and Jacini, 1970a), they indicate that they have identified approximately 40 compounds, but their two publications (Fedeli, 1970; Fedeli and Jacini, 1970) list the identities of a somewhat smaller number. These include a series of saturated aldehydes ranging from C_7 to C_{12} (C_{13} ?), with C_{10} predominating. The authors do not indicate whether the aldehydes are all normal, or whether any branching is indicated by their data. In addition, C_{11} to C_{13} monounsaturated aldehydes were detected, with the double bond position unknown. Methyl palmitate, ethyl palmitate, methyl oleate, and methyl linoleate were identified as well. In one paper, Fedeli (1970) indicates the presence in low concentration of a series of terpenoid compounds. However, in another 1970 paper, Fedeli and Jacini instead suggest the presence of a series of aromatic compounds. Nawar (1969, 1970) has presented two reports on olive oil flavor and composition at national meetings, but has not yet published his findings in the literature. Only methyl palmitate, methyl oleate, ethyl palmitate, and ethyl oleate are specifically mentioned in the published abstracts of his talks, but apparently many more components of differing functionality were found. Both groups appear to be interested in correlating olive oil flavor with the constituents found, but they have not been entirely successful as yet, judging from the limited information available.

Western Regional Research Laboratory, Agricultural Research Service, U. S. Department of Agriculture, Berkeley, California 94710.

EXPERIMENTAL SECTION

Two problems became apparent when this study was started, selecting a representative sample of olive oil and finding an effective method for stripping aroma materials from the oil sample. Considerable aroma and flavor differences were obvious among the commercially available olive oil samples, not only among the many brands marketed, but in several instances between different lots from the same producer as well. A domestic virgin oil was used in most of the work reported below because of its ready availability and rather pronounced pleasant aroma and flavor. Several imported oils were also used, mostly for comparison purposes. Typically, these were lighter in both color and aroma than the domestic oil. Some samples of imported oil had hardly any aroma at all, but a light flavor could be detected when the sample was tasted.

Several techniques were tried in attempts to isolate volatile constituents from the olive oil's mixed triglyceride base. Short-path molecular distillation in a wiped film still was first tried, but the recovery of lower-boiling material was quite poor. By bubbling purified nitrogen gas through large batches of olive oil and then passing the gas through a trapping train, small quantities of material, mostly water, could be scrubbed from the gas stream by the cold traps. This approach was tried at both atmospheric and reduced pressure, with the oil either at room temperature or heated. Material yields were again quite low, even after several days of sweeping. Most of this investigation was conducted on material obtained by codistillation with water under nitrogen at atmospheric pressure.

Codistillation with Water. Domestic virgin olive oil (7 l.) was combined with distilled water (3.7 l.) in a nitrogen-flushed 12-l. round-bottomed three-necked flask fitted with a large glass–Teflon stirrer, a thermometer, a modified Likens and Nickerson extraction head (1964), and a heating mantle. Purified *n*-pentane (75 ml) was the extracting solvent. The thoroughly stirred oil–water mixture was heated to boiling (108°) under a slight positive pressure of nitrogen. The *n*-pentane was heated to boiling with a water bath at approximately 40°. Reflux extraction was continued for 3 hr, and then the *n*-pentane extract was concentrated by distillation of the solvent through a 30 × 2.5 cm Raschig ring-packed column (maximum head T = 37°; maximum water bath T = 50°). Solvent was further removed by slow distillation through a small Claisen head (maximum head T = 37°; maximum bath T = 45°). A

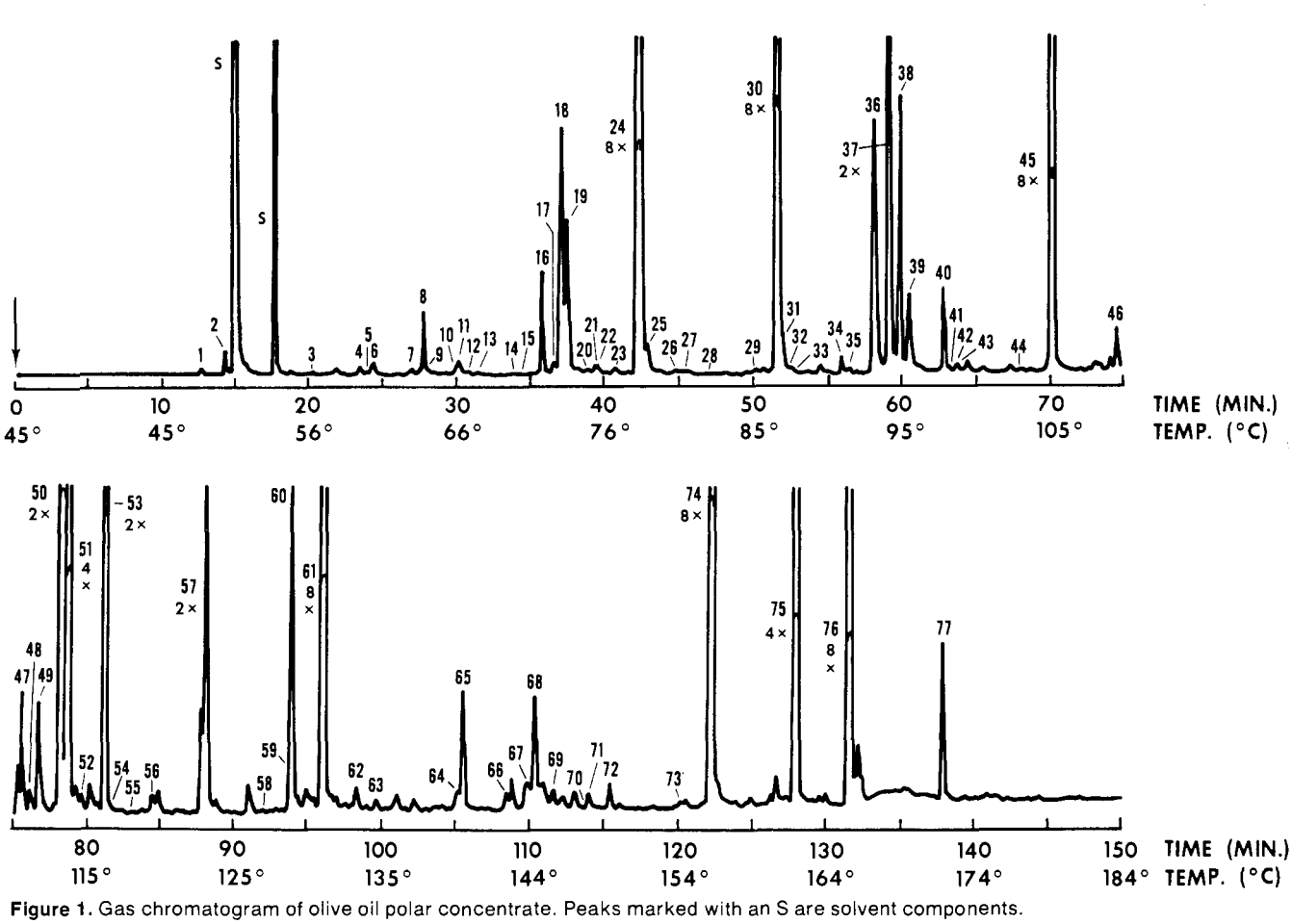

Figure 1. Gas chromatogram of olive oil polar concentrate. Peaks marked with an S are solvent components.

small quantity of Antioxidant 330 (Ethyl Corp.; 1,3,5-trimethyl-2,4,6-tri(3,5-di(1,1-dimethylethyl)-4-hydroxybenzyl)benzene) was added to the residual solution. The procedure above was repeated twice with 7.6-l. batches of oil, and with extraction times of 4 and 5 hr. The total residues were combined, yielding 1.6 g of concentrated solution in pentane (approximately 50–60 ppm in the starting oil).

Dry-Column Fractionation of Extract. A portion of the extract solution (1 g) was separated into two fractions, a polar and nonpolar, by the dry-column method of Loev and Goodman (1967). A 40 × 1.5 cm column of silica gel (Mallinckrodt SilicAr CC-7, 100–120 mesh, containing 15% added water by weight, with 0.5% Woelm fluorescent green indicator) was prepared in nylon tubing. The extract solution was then placed at the top in a thin layer of sand, and the column was developed with *n*-pentane. The two diffuse but well separated bands were cut from the column and the organic material was washed from each portion of adsorbent with pentane–ether. A small quantity of Antioxidant 330 was added to each solution, and the solvent was removed from each by distillation as described above. Approximately 0.53 g of polar material and 0.23 g of nonpolar material were obtained, both still containing residual solvent.

Component Separation and Identification. All gas chromatographic separations were carried out with 0.03 in. i.d. × 500 ft stainless steel open-tubular columns prepared and coated in our laboratory. These were coated with methyl silicone oil, either OV-101 (Ohio Valley) or SF-96(50) (General Electric) containing 5% Igepal CO-880 (General Aniline and Film). After sample injection, the columns were held at 35–40° for 10 min; then the oven temperature was programmed at 1°/min to 175–185°. Analytical runs were monitored with a flame ionization detector (Varian Aerograph). A dual thermistor detector (Carle Instruments) was used when the eluted bands were sniffed for organoleptic assessment (see Figure 1).

Identifications are based upon mass spectral data obtained by coupling the effluent end of the gas chromatographic column to a quadrupole-type mass spectrometer (Quad 300, Electronic Associates, Inc.) using a single-stage methyl silicone membrane-type interface held at 140–150°. The membrane area exposed to the column effluent measured ¼ × 2 in. Tentative identifications were checked by comparing the olive oil component's spectrum with that of an authentic sample run on the same mass spectrometer. Authentic samples were either purchased or synthesized by standard methods. The sample's gc retention time behavior was also checked by coinjection with a small quantity of the olive oil volatile concentrate.

Organoleptic Assessment. A preliminary judgment of each component's odor character was made by having various individuals smell the gc column effluent, and then describe their impressions as the component bands were eluted. Selected components were then added to an odorless oil base for comparison with authentic olive oil samples. Purified corn oil was used at first, but in most of the sample preparation work food grade high oleic safflower oil (Oleinate 181, Pacific Vegetable Oil Corp.) was used. This material, which is odorless when freshly refined, has a fatty acid distribution (6% palmitic, 2% stearic, 79% oleic, and 13% linoleic; Kirschner, 1971) very similar to that of a typical olive oil (Gracian Tous, 1968). Most of the test mixtures contained three to ten olive oil components, and the total additive concentration in the base oil ranged from 0.1 to 10 ppm, depending upon which aroma constituents were added. Only compounds identified in this study were used as additives (except for acetic acid; see Table I); their relative concentrations in the safflower

Table I. Composition of Safflower-Olive Oil Component Mixtures

Component	Concentration in sample no., ppm						
	1	2	4	5	6	7	11
Hexanal			0.5	0.3	blank	0.3	
Octanal			0.5	0.5		0.5	
Nonanal	0.4	0.2				0.4	
trans-2-Hexenal			0.2				
trans-2-Heptenal	0.2	0.2					
trans-2-Octenal	0.1	0.1					
trans-2-Decenal	0.2		0.6	0.2		0.5	
2,4-Heptadienal[a]	0.2	0.3	0.2	0.2			0.6
2,4-Decadienal[a]	0.4	0.2	1.1	1.0		0.5	1.2
2-Octanone			0.06				
3-Methylbutan-1-ol	0.1	0.2					
cis-3-Hexen-1-ol			0.17	0.3		0.3	
2-Phenylethanol	0.2	0.2					
Acetic acid			1.5	1.0		1.0	

[a] Mixture of geometric isomers.

Table II. Identified Olive Oil Components

1. Acetaldehyde
2. Ethanol
3. Ethyl acetate
4. 3-Methylbutanal
5. Methylpropan-1-ol
6. 2-Methylbutanal
7. 3-Methylbutan-2-one
8. Pentanal
9. 3-Pentanone
10. 1-Penten-3-ol
11. Ethyl propionate
12. 3-Pentanol
13. Methyl butyrate
14. 2-Methyl-2-butenal
15. Pentenal (*cis*-2-?)
16. *trans*-2-Pentenal
17. Ethyl 2-methylpropionate
18. 3-Methylbutan-1-ol
19. 2-Methylbutan-1-ol
20. 2-Methyl-1-propyl acetate
21. Methyl 3-methylbutyrate
22. Methyl 2-methylbutyrate
23. 2-Hexanone
24. Hexanal
25. Ethyl butyrate
26. Propyl propionate
27. *n*-Octane
28. Methyl pentanoate
29. *cis*-2-Hexenal
30. *trans*-2-Hexenal
31. Ethyl 2-methylbutyrate
32. Ethyl 3-methylbutyrate
33. 1-Propyl 2-methylpropionate
34. 3-Methyl-1-butyl acetate
35. 2-Methyl-1-butyl acetate
36. *cis*-3-Hexen-1-ol
37. 1-Hexanol
38. Heptanal
39. *trans*-2-Hexen-1-ol
40. 2-Methyl-1-propyl 2-methylpropionate
41. 2,4-Hexadienal
42. Methoxybenzene (anisole)
43. Methyl hexanoate
44. Heptenal (*cis*-2-?)
45. *trans*-2-Heptenal
46. Benzaldehyde
47. 2-Methyl-2-hepten-6-one
48. 2-Octanone
49. 1-Heptanol
50. Octanal
51. 2,4-Heptadienal (isomer A)
52. *cis*-3-Hexenyl acetate
53. 2,4-Heptadienal (isomer B)
54. 2-Methyl-1-butyl 2-methylpropionate
55. Methyl heptanoate
56. 1,8-Cineole
57. *trans*-2-Octenal
58. Acetophenone
59. 2-Nonanone
60. 1-Octanol
61. Nonanal
62. Linaloöl
63. Methyl octanoate
64. 1,2-Dimethoxybenzene (veratrole)
65. *trans*-2-Nonenal
66. Ethyl benzoate
67. 2-Phenylethanol
68. 1-Nonanol
69. Ethyl octanoate
70. α-Terpineol
71. 1-Octyl acetate
72. 2,4-Nonadienal
73. Ethyl phenylacetate
74. *trans*-2-Decenal
75. 2,4-Decadienal (isomer A)
76. 2,4-Decadienal (isomer B)
77. *trans*-2-Undecenal

oil base were determined in part by their relative abundances in the concentrate from a high quality virgin imported oil. The intensity and odor impression of each compound during the gc sniffing runs were also considered in selecting additives likely to contribute to an olive oil-like aroma. The compositions of mixtures submitted for panel evaluation are shown in Table I.

The mixtures were prepared and placed in 30-ml screw-cap vials. Aromas were compared by sniffing the coded vials, which were approximately three-quarters full of oil. Several different brands of imported olive oil were also included for comparison.

The panel used in these studies was selected from a group of people who indicated that they were frequent users of olive oil. After extensive training on the best imported olive oils available, a panel of 23 persons was selected to evaluate the aromas of the various samples.

The evaluations were conducted in a room supplied with odor-free air at a slight positive pressure and maintained at 74 ± 2°F and 50% relative humidity. The vials were jacketed with tissue paper and the individual booths were illuminated with 7.5-W green bulbs to eliminate any possible influence from color differences.

The samples were evaluated by ranking four samples per session according to the most desirable olive oil aroma. The same samples were ranked at least twice in random order by 20 judges, and the results are expressed as the average of two replications. Some of the samples were also compared in pairs. In this case the panelists were asked to indicate which sample had the more desirable olive oil aroma. In these tests some of the pairs were replicated as many as four times.

RESULTS AND DISCUSSION

Components identified in the present study are listed in Table II. As indicated above, identifications are based upon low-resolution mass spectral data and relative retention time comparisons. As a result, there remains a degree of uncertainty in some of the identifications, particularly those of the compounds containing one or two olefinic bonds. Those compounds whose double bond positions and geometries are designated in Table II display retention behaviors and mass spectral cracking patterns which agree in all respects with those of fully characterized reference compounds. The double bond geometries of the various 2,4-dienal reference samples have not been rigorously determined, so the olive oil dienals are not fully characterized. In addition, several minor components, each having the same mass spectrum as the corresponding major aldehyde, are found approximately 1–2 min before *trans*-2-pentenal, *trans*-hexenal, and *trans*-2-heptenal (15, 29, 44). It was suspected that these were the corresponding cis isomers, but they could also have been positional isomers such as the 3-enals. *cis*-3-Hexenal, prepared from *cis*-3-hexen-1-ol, was found to have a retention time several minutes shorter than that of the peak of interest. Obvious mass spectral differences were noted as well. Attempts to synthesize *cis*-2-hexenal and purify it were unsuccessful, but photolysis of a sample of commercial *trans*-2-hexenal in a hydrocarbon solvent yielded a mixture in which a minor component, having the same mass spectrum and gc retention time as the olive oil component, was increased in concentration to approximately 15–20% of the *trans*-2-hexenal isomer. A sample of this minor component was isolated by preparative open-tubular gas-liquid chromatography and examined by infrared spectrometry. The ir spectrum is consistent with a *cis*-2-enal structure, showing a conjugated carbonyl but no evidence for the prominent trans double bond absorption found in the spectrum of the trans isomer. Sufficient material could not be isolated by this method for nmr examination in a reasonable length of time, so a ¼ in. × 20 ft packed column (1% SF-96(50) on Chromosorb G) was used to purify a sample containing both the *trans*-2-hexenal and the minor component. This mixture was exam-

ined by nmr, and the spectrum obtained was compared with that of pure *trans*-2-hexenal. The aldehydic proton of the minor component appears as a doublet (J = 7.8 Hz) at δ 10.06, while that of the *trans*-2-hexenal appears at δ 9.49, also as a doublet (J = 7.8 Hz). Both samples were run at 100 MHz in $CDCl_3$ with TMS reference. Such a downfield shift, relative to the position for the aldehydic proton of a trans compound, is typical for the corresponding cis isomer (Chan *et al.*, 1968). The minor component in commercial *trans*-2-hexenal is therefore very likely the cis isomer. Since it has the same mass spectrum and retention time as the minor companion peak of *trans*-2-hexenal in olive oil, this latter compound is probably *cis*-2-hexenal as well. A similar sequence was not followed with the minor components appearing before *trans*-2-pentenal and *trans*-2-heptenal, but by analogy they are tentatively assigned the cis geometry as well.

Most of the identifications listed were made by gc-ms examination of polar material obtained from a domestic virgin oil. Several runs were also made with concentrates from various imported oils. The qualitative results from these latter runs agree well with those obtained with the domestic oil, although there are considerable quantitative differences among the various olive oil aroma concentrates.

The major volatile olive oil components are typical products of triglyceride oxidation, but a considerable number of minor oxygenated compounds were also found. Unfortunately, only half of the detected components are identified, even tentatively. Most of the unknown constituents are present in very small amounts, and/or their mass spectra are confused due to multiple peak overlap in the gc separation. As with any identification scheme relying upon low-resolution mass spectra, numerous compounds remain unknown because their spectra could not be interpreted.

All results reported in this paper were obtained using polar material from the olive oil aroma concentrate. The nonpolar fraction was not examined extensively, partly because its contribution to olive oil aroma was considered minimal. However, preliminary examination shows that it includes numerous isomeric alkylbenzenes, several *n*-alkanes, and at least six terpenoids, as well as two major hydrocarbons, *n*-octane and an unidentified compound with a molecular weight of 182.

Selection of organoleptically important compounds was found to be rather difficult. Gc effluent sniffing was of some value, but was complicated at higher temperatures and longer retention times by a continuous oily background odor which developed in the column effluent stream. This was attributed to the presence of very polar components which were not resolved in the methyl silicone column or to higher molecular weight material which collected in the column with use and then was gradually eluted at higher column temperatures. As indicated above, one of the major problems was establishing a standard for olive oil aroma against which to compare synthetic mixtures. In general, the aroma of commercial olive oil samples varied from delicate and fruity to very pronounced and heavy, with some samples suggesting 2,3-butanedione, although this compound was not found in the concentrate examined. Finally, seven different olive oils were selected and submitted to the evaluation panel for ranking. Four of these were selected by the panel as having the best aromas. The best four samples of imported oils could not be distinguished significantly from one another, but some of the other three could be identified readily by the panel as inferior.

When ranked together, some of the more promising mixtures of olive oil components in safflower oil were not significantly distinguishable. However, when one of the best four brands of olive oil was included in a series of four samples, the authentic olive oil always ranked significantly better than the other synthetic samples. When one or more of the poorest authentic oil samples were included in the four-sample group, one of the safflower-olive oil component mixtures was ranked significantly better than the safflower oil alone or than the worst of the authentic olive oils. In most of these ranking trials it appeared that mixture 2 (Table I) was ranked ahead of the other mixtures tried. Therefore this mixture, along with the olive oils and several other promising mixtures, was evaluated further by direct pairing of samples (Table III). Different samples of the same brand of high-quality olive oil C were not distinguishable from one another. However, samples of olive oil C were significantly better in olive oil aroma than any of the safflower oil-component mixtures tried. Mixture 2 was significantly better than olive oil G and not distinguishable from olive oils E and F. In summary, it appears that mixture 2 in the safflower oil base has an aroma approaching that of olive oil, but still lacks certain components needed to provide the fruitiness characteristic of a quality olive oil.

Table III. Paired Comparisons: Selected Safflower-Olive Oil Component Mixtures and Olive Oil Samples[a]

Comparison	N	Best olive oil aroma in: First sample	Best olive oil aroma in: Second sample	Exact probability
Olive oil C *vs.* olive oil C′	37	22	15	
Olive oil C *vs.* olive oil C″	37	20	17	
Olive oil C′ *vs.* olive oil C″	37	17	20	
Olive oil C *vs.* mixture 2	43	28	15	0.0660
Olive oil C *vs.* mixture 4	43	31	12	0.0054
Mixture 2 *vs.* mixture 3	43	31	12	0.0054
Olive oil C″ *vs.* mixture 2	44	28	16	0.0961
Olive oil C″ *vs.* mixture 5	44	34	10	0.0004
Olive oil C″ *vs.* mixture 7	44	36	8	0.000025
Olive oil C′ *vs.* mixture 2	86	56	30	0.0066
Mixture 2 *vs.* mixture 7	86	62	24	0.000051
Mixture 2 *vs.* mixture 5	86	64	22	0.000007
Olive oil C′ *vs.* mixture 2	44	31	13	0.0095
Olive oil C″ *vs.* mixture 2	44	30	14	0.0226
Olive oil C *vs.* mixture 2	44	29	15	0.0487
Mixture 2 *vs.* olive oil G	46	35	11	0.0005
Mixture 2 *vs.* olive oil E	46	24	22	
Mixture 4 *vs.* olive oil G	46	35	11	0.0005
Mixture 4 *vs.* olive oil E	46	21	25	
Mixture 2 *vs.* mixture 4	46	29	17	0.1038
Mixture 2 *vs.* olive oil E	46	27	19	0.3020
Mixture 2 *vs.* olive oil F	46	28	18	0.1839
Mixture 2 *vs.* safflower oil	46	40	6	0.000001

[a] C, C′, and C″ are different samples of the same olive oil.

ACKNOWLEDGMENT

The authors wish to thank R. G. Buttery for supplying some of the unsaturated aldehyde reference samples and T. R. Mon for preparing the open-tubular gc columns used in this study.

LITERATURE CITED

Chan, K. C., Jewell, R. A., Nutting, W. H., Rapoport, H., *J. Org. Chem.* **33**, 3382 (1968).
Fedeli, E., *Riv. Ital. Sostanze Grasse* **47**, 14 (1970).
Fedeli, E., Jacini, G., presented at the 42nd National Meeting, American Oil Chemists Society, New York, N. Y., October 1968.
Fedeli, E., Jacini, G., presented at the 44th National Meeting, American Oil Chemists Society, Chicago, Ill., September 1970a.
Fedeli, E., Jacini, G., *Chim Ind. (Milan)* **52**, 161 (1970b).

Gracian Tous, J., "Analysis and Characterization of Oils, Fats, and Fat Products," Vol. 2, Boekenoogen, H. A., Ed., Interscience, New York, N. Y., 1968, p 315.
Kirschner, E., Pacific Vegetable Oil Corporation, private communication, 1971.
Likens, S. T., Nickerson, G. B., *Proc. Amer. Soc. Brew. Chem.* 5 (1964).
Loev, B., Goodman, M. M., *Chem. Ind.* 2026 (1967).
Nawar, W. W., presented at the 29th National Meeting, Institute of Food Technologists, Chicago, Ill., May 1969.
Nawar, W. W., presented at the 44th National Meeting, American Oil Chemists Society, Chicago, Ill., September 1970.

Received for review July 5, 1973. Accepted September 7, 1973. Reference to a company or product name does not imply approval or recommendation of the product by the U. S. Department of Agriculture to the exclusion of others that may be suitable.

Thin-Layer Chromatographic Investigation of Color Developer Involved in Pinking of White Onion Purees

Chiranjib Bandyopadhyay* and Gyanendra M. Tewari

The compounds responsible for pinking of white onion purees were isolated and studied by thin-layer chromatography. At least three compounds were found to have color-developing properties. The major one among these was tentatively characterized by infrared spectrophotometry and gas-liquid chromatography as thiopropanal *S*-oxide, the lachrymatory factor of onion. Preliminary analysis of the other two components indicated that they were also thioalkanal-*S*-oxide types of compounds having lachrymatory properties hitherto unreported.

Pinking of white onion bulb purees was first reported by Joslyn and Peterson (1958). Lukes (1959) investigated the cause of the development of such pink pigment in onion purees and showed that at least two steps were involved in color formation. The first one was enzymic, where an ether-soluble colorless compound named as color developer was rapidly produced, and the second one was nonenzymic, where the color developer reacted with naturally occurring amino acids and carbonyls, resulting in the final pigment. Pigment-forming reactions and precursors, involved in the formation of pink color in onion purees, were elaborated by Shannon *et al.* (1967a,b). They demonstrated that the color developer was produced rapidly by the enzymic action of allinase on *S*-1-propenyl cysteine sulfoxide (PECSO), the primary pigment precursor of onion. However, the nature of color developer was still illusive and to our knowledge no further studies in this regard have been reported. In the present investigation an attempt has been made to elucidate further the nature of color developer compound present in white onion purees.

EXPERIMENTAL SECTION

Fresh white globe onions, stored for about a month after harvest, were purchased from local market and kept at 0° for several hours before extraction.

All solvents and reagents were analytical grade. The solvents were redistilled before use.

Onion Extract. Extraction was carried out at 0° in four batches, each having 500 g of peeled onion. The ether-soluble color developer was obtained by extracting chilled onion juice several times with cold peroxide-free diethyl ether, as described by Lukes (1959). The residual pulp was also repeatedly extracted with the same solvent in a Waring blender. The ether layer from both the pulp and juice was pooled together after cold centrifugation. The combined ether extract was dried over anhydrous sodium sulfate, filtered, and finally concentrated in a rotary evaporator at room temperature. The residue (combined extract) was transferred into a tared flask with a minimum volume of diethyl ether. Ether was removed by blowing a stream of nitrogen and the final residue, a greenish pasty mass, was kept under nitrogen at −40° until use. In a similar way ether extracts from onion juice and the residual pulp were separately prepared from two batches of onions.

Thin-Layer Chromatography (tlc). Glass plates (20 × 20 cm), spread with silica gel G (E. Merck), layers of thickness 400 μ and 0.5 mm (preparative), respectively, were used. Silica gel slurry was prepared in distilled water (1:2 w/v). The plates were dried at room temperature and activated at 120° for 1.5 hr.

The plate was divided into two halves and on both the halves 500 μg of each extract in ether solution was spotted with the help of a micropipette. The plate was developed in a chromatographic tank containing petroleum ether (40°–60°)–diethyl ether–acetic acid (60:40:1 v/v). After development and subsequent removal of the solvent at room temperature, one half of the plate was sprayed with 50% sulfuric acid and the other half with glycine–formaldehyde reagent (4.5 ml of 0.1 *M* glycine and 0.5 ml of 3×10^{-4} *M* formaldehyde) prepared according to the method of Shannon *et al.* (1967a), and this half of the plate was covered with a cleaned glass plate. The chromatograms were visualized within 1 hr by heating the plate at 100°. With glycine–formaldehyde reagent, pink-colored spots were noted only after heating, while with sulfuric acid (Bandyopadhyay *et al.*, 1970) several colored spots were visible even before heating.

Isolation of Color Developer. Color developer compound was isolated from combined onion extract by high vacuum distillation based on the principle of closed system high vacuum transfer (Merritt *et al.*, 1959). The distillation assembly was comprised of a high vacuum pump coupled with a two-stage silicon oil diffusion pump, which was connected to a vacuum manifold. The distillation was accomplished within 6 hr under a pressure of 10^{-3} Torr at 40° in a closed unit consisting of two gas bottles of suitable size fitted with stopcocks and attached to the vacuum manifold by means of glass tubings, according to the procedure described by Merritt *et al.* (1959). The distil-

Biochemistry and Food Technology Division, Bhabha Atomic Research Centre, Trombay, Bombay 400085, India.