Bitter Taste of Virgin Olive Oil: Correlation of Sensory Evaluation and Instrumental HPLC Analysis

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ABSTRACT

A method for the extraction by C_{18} columns and HPLC analysis of bitter components of virgin olive oils was developed. Factorial design showed the suitability of using two columns and three HPLC analyses per sample. An equation obtained by multiple regression analysis, related bitterness with the area of peaks, which explained 92% or variance.

INTRODUCTION

VIRGIN OLIVE OIL, the only juice of the olive obtained by pressing, is one of the few oils that are consumed without any further refining process. Properly extracted from fresh mature fruits, the oil is characteristically fragrant and lightly bitter. The aroma of olive oil, like that of many foods, is composed of many volatile compounds, and separation and identification techniques place the number of volatile constituents close to 100 (Flath et al., 1973; Olías et al., 1980). Work has been reported concerning the actual aroma influence of some volatile compounds (Olías et al., 1978; Gutiérrez et al., 1981).

Nowadays, producers and consumers are concerned about the taste aspects of virgin olive oil but as yet these have not been studied. Even though sensory evaluation is the most important and common way to determine the taste quality of oil, it is time consuming. Therefore, there is a need for the development of a sensitive, simple and precise instrumental method which can measure the taste quality.

The objectives of this research were: (1) to develop a simple extraction method for nonvolatile bitter constituents from virgin olive oils; (2) to prepare an HPLC analysis of bitter compounds; and (3) to obtain an equation by multiple regression analysis between sensory and chromatographic analyses.

MATERIALS & METHODS

VIRGIN OLIVE OIL samples were obtained from a commercial source. Octadecyl (C_{18}) disposable extraction columns (6 mL) from J. T. Baker Chemical Company (Phillipsburg, NJ.). All solvents were HPLC grade from Romil Chemicals Ltd. (Shepshed, LE., England).

Procedure for extraction of bitter-tasting components

A sample of 1.0±0.1g virgin olive oil dissolved in 4 mL hexane was passed through an octadecyl (C_{18}) extraction column. After elution, 10 mL hexane were passed to eliminate the remaining fat, and finally the retained compounds were eluted with water:methanol (30:70) to 1 mL, measured in tared flask.

High performance liquid chromatography

Analyses were performed with an HP 1090 liquid chromatograph and peak areas were integrated by an HP-85 personal computer (Hewlett Packard, Palo Alto, CA). Two columns were used for reverse phase analysis; a 300 x7.8 mm stainless steel column packed with 10 μm, μBondapack C_{18} (Waters Associates, Milford, MA) was used for semi-preparative chromatographic separations and a 250 × 2 mm stainless steel column packed with 5 μm Ultrasphere ODS (C_{18}) (Beckman, Berkeley, CA) for analytical separations. The operational conditions in semi-preparative scale were: mobile phase water:methanol (70:30) and linear gradient of 1%/min of methanol, from the first minute; flow rate 1 mL/min, and 50 μL sample loop. In analytical separations the mobile phase was water:methanol (60:40), gradient of 0.5%/min of methanol for 20 min later increasing to 1%/min; the flow rate was 0.2 mL/min and injection volume 5 μL. In both HPLC separations the column temperature was kept at 40 ± 1°C and UV detector at 225 nm was used.

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Sensory evaluation

The oil samples were evaluated for bitterness by 10 panel members of the Institute de la Grasa. They were very familiar with oil flavor quality. A scale of 1 to 5 was used: 1 indicates imperceptible, 2 light, 3 moderate, 4 great, and 5 extreme. The panelists were seated in individual booths and the oil samples (15 mL) were served at 28°C in blue glass cups; duplicate determinations were made. To maintain the

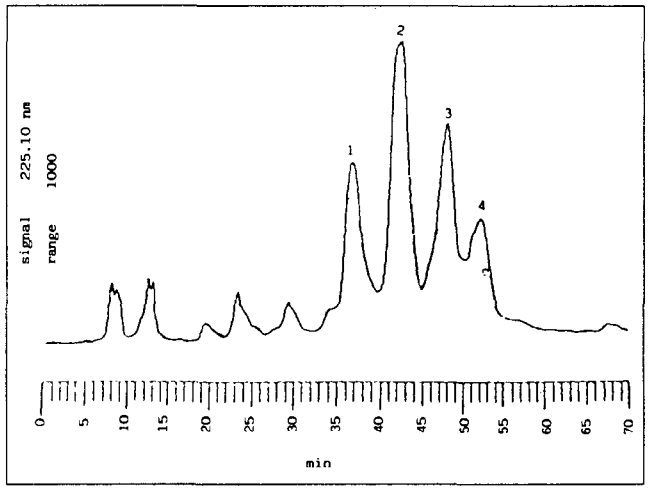

Fig. 1—Semi-preparative HPLC separation of bitter compounds of virgin olive oil; for conditions see Materials & Methods. Majority peaks of R, 36.5 (1) hot flavor, 42.4 (2) slightly bitter, 48.0 (3) strongly bitter, and 52.0 min (4) slightly bitter, respectively.

Table 1—Reproducibility of the isolation and separation of bitter compounds

Trials	Peak areas (counts)			
	2	2a	3	4
A	4230	3600	5927	3271
	4318	3675	5774	3230
	4384	3675	5883	3206
B	4040	3411	5518	2873
	4113	3298	5451	2855
	4279	3622	5603	2873
C	4035	3384	5366	3353
	4196	3735	5390	3043
	4455	3810	5177	3043
D	4606	3525	5780	3271
	4694	3374	5841	3353
	4318	3277	5689	3043

temperature during the evaluation period, the cups were set in holes drilled into heavy aluminium blocks preheated to the desired temperature. The bitterness value assigned to each sample corresponded to the average of the intensities given by the panelist.

To determine the basic taste of the extracts from virgin olive oil, samples of 1g of oil were extracted, as described above, solvents were removed under vacuum at 30–35°C and the mixture of components taken up in 250 μL of water. Freshly prepared extracts were used in evaluation session. The samples were taken for panelists using Pasteur capillary pipettes. The fractions obtained by semi-preparative HPLC from the bitter extracts were prepared for evaluation of sensory characteristics as stated above.

Table 2—Precision of testing schemes

Trials (columns)	HPLC analyses per column	Total HPLC analyses	$S_{\bar{x}}^2$	$S_{\bar{x}}$	Confidence limits (95%)
1	1	1	37.382	193	394
	2	2	28.280	168	343
	3	3	25.245	159	324
	4	4	23.728	154	314
2	1	2	18.692	137	279
	2	4	14.140	119	243
	3	6	12.622	112	229
	4	8	11.864	109	222
3	1	3	12.460	112	228
	2	6	9.426	97	198
	3	9	8.415	92	187
	4	12	7.909	89	181
4	1	4	9.346	97	197
	2	8	7.070	84	172
	3	12	6.311	79	162
	4	16	5.932	77	157

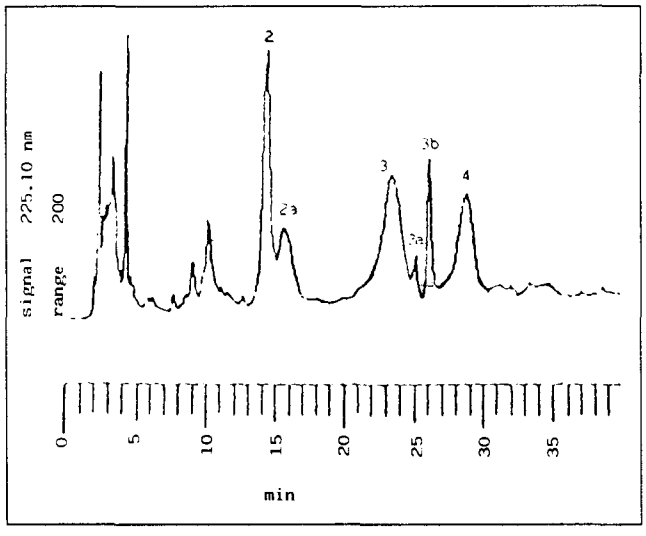

Fig. 2—Analytical HPLC separation of bitter compounds of virgin olive oil; for conditions see Materials & Methods. Numbered peaks correspond to those peaks obtained in semi-preparative HPLC separation. Peak 1 (R_t 10.1 min) in analytical was isolated from peak 1 (R_t 36.5 min) in semi-preparative; 2 (R_t 14.3 min) and 2a (R_t 15.6 min) from 2 (R_t 42.4 min); 3, (R_t 23.4 min), 3a (R_t 25.1 min), and 3b (R_t 26.1 min) from 3 (R_t 48.0 min); 4 (R_t 28.8 min) from 4 (R_t 52.0 min).

Statistical analysis

The study of method error and the search for the best operational conditions were carried out by means of variance analysis in a factorial design. The equation of correlation between the objective and subjective trials was carried out assuming a linear multiple regression between the intensity of bitterness and the peak chromatographic areas of the bitter extract.

RESULTS & DISCUSSION

Evaluation of bitter-tasting components

The method of isolation set up achieved complete recovery of the bitter components of oil, as shown by the fact that the oil recovered after passing through the column had no bitter flavor, while the extract had an intense bitter-taste.

The bitter extract, separated by semi-preparative liquid chromatography was fractioned in four majority peaks of R_t 36.5 (1), 42.4 (2), 48.0 (3), and 52.0 min (4), respectively (Fig. 1). In ten successive 50 μL injections, a sufficient quantity was collected from each peak for its sensory evaluation by the panelists. Peak 1 had a hot flavor, peaks 2 and 4 were slightly bitter and 3 was strongly bitter.

Using an analytical column, it was possible to resolve peak 2 into two peaks with R_t 14.3 (2) and 15.6 (2a) min, respectively. Peak 3 was separated into three with R_t 23.4 (3), 25.1 (3a), and 26.1 (3b) min, respectively. A chromatogram of the total extract carried out with the analytical column is shown in Fig. 2.

Determination of analytical errors and choice of optimum scheme

For this study a virgin olive oil of bitterness 3.9 was used. Four samples (1.0 ± 0.1g) of the oil were extracted in the same number of columns; four HPLC analyses (5 μL injection) were performed for each extract. The experiment was considered to be a factorial design, with one fixed factor, peak areas with four levels and one random factor, columns also with four levels. The values of the areas of peaks 2, 2a, 3, and 4 obtained from the four samples, which are denominated A, B. C, and D are shown in Table 1.

The analysis of variance showed the existence of real variations between the columns and an interaction between the peaks and columns, indicating that column behavior was not independent of the component nature. From quantities estimated by the mean squares, the following variance values were determined.

$$\text{Columns } S_c^2 = 14{,}538$$

$$\text{Interaction peak, column } S_{pc}^2 = 18{,}554$$

$$\text{Replicates } S_0^2 = 18{,}206$$

Table 3—Samples of virgin olive oil, flavor scores (bitterness)[a] and means of peak areas of bitter compounds

Samples	Bitter score	HPLC peak area (means) 1	2	2a	3	3a	3b	4
1	0.1 ± 0.2[b]	170	1282	350	0	0	0	0
2	0.6 ± 0.3	781	1640	880	500	229	306	712
3	1.0 ± 0.4	239	2679	919	1010	178	413	1729
4	1.0 ± 0.4	388	3419	1501	1139	150	822	1745
5	1.4 ± 0.5	401	2721	1610	1300	178	629	1684
6	2.0 ± 0.6	900	2903	3043	2699	100	336	2051
7	2.5 ± 0.6	2300	3169	2311	3930	519	210	2700
8	2.6 ± 0.6	317	2541	3168	1900	366	477	2089
9	3.3 ± 0.7	3059	4624	2893	2538	100	1888	2090
10	3.5 ± 0.7	1560	3268	1611	3879	301	888	2375

[a] A scale of 1 to 5 was used for evaluation of bitterness: 1 indicates imperceptible, 2 light, 3 moderate, 4 great, and 5 extreme.
[b] Confidence limits (95%)

The total variance was 51,298; the variability due to the columns means 28.3%, to the interaction 36.2%, and to the replicates 35.5% of whole variance, respectively.

To have a general view of the method error, Table 2 was prepared showing several of the possible analytical schemes, from 1 to 4 extraction columns and from 1 to 4 chromatographic analyses for extract; this includes the confidence limits for levels of probability p = 0.05, for the mean obtained for whichever of the analytical schemes. From these values, it was estimated that an adequate analytical performance would be to use two columns and three chromatographic analyses per sample.

To determine the method error of the proposed scheme (two samples, three injections), the errors presented by individual peaks were studied. Thus, it was seen that the typical errors of the means obtained were a function of the areas, in agreement with the equation.

$$S_x = 0.027 \text{ peak area} + 18$$

According to this equation, for example, an area of 500 presented confidence limits of ±65 and an area of 3,000±202, which indicated the suitability of the analytical procedure.

Correlation between chromatographic peak areas and intensity of bitterness

Ten virgin olive oils, whose intensities of bitterness, shown in Table 3, go from imperceptible (samples 1 and 2) to high (samples 9 and 10) were studied. They are representative of the range of virgin olive oils in the market each oil-producing season. In each sample, two extractions of bitter compounds and three chromatographic analyses per extract were carried out. The mean areas of the peaks studied are shown in Table 3.

From an initial study by multiple linear regression, including all the peaks, a positive correlation only with peaks 2a, 3, 3a and 3b was indicated. The functional equation which correlated these with the intensity of bitterness (B.I.) was:

$$B.I. = (2.18\ A_{2a} + 5.04\ A_3 + 8.19\ A_{3a} + 8.15\ A_{3b})10^{-4} - 0.214$$

The correlation coefficient between instrumental liquid chromatographic and sensory analyses for virgin olive oil was 0.963, which permitted us to conclude that approximately 92% of the total variance of bitterness for the four peaks included in the regression could be justified. Using this equation, the bitter score calculated had a standard error of 19.5%.

In summary, this preliminary study showed that instrumental HPLC analyses could be used to measure the bitter tasting quality of virgin olive oil. Since only ten samples of oil were analyzed, more work is still needed to develop a standard method to evaluate the flavor quality of virgin olive oil.

REFERENCES

Flath, R.A., Forrey, R.R., and Guadagni, D. G. 1973. Aroma components of olive oil. J. Agric. Food Chem. 21: 948.
Gutiérrez, R., Dobarganes, M.C., Gutiérrez, F., and Olías, J.M. 1981. Componentes volátiles en el aroma del aceite de oliva virgen. V. Aceites obtenidos de frutos atrojados. Grasas y Aceites 32: 299.
Olías, J.M., Dobarganes, M.C., Gutiérrez, F., and Gutiérrez, R. 1978. Componentes volátiles en el aroma del aceite de oliva virgen. II Identificación y análisis sensorial de los eluyentes cromatográficos. Grasas y Aceites 29: 211.
Olías, J. M., Gutiérrez, F., Dobarganes, M. C., and Gutiérrez, R. 1980. Componentes volátiles en el aroma del aceite de oliva. IV. Su evolución e influencia en el aroma durante el proceso de maduración de los frutos en la variedades Picual y Hojiblanca. Grasas y Aceites 31: 391.
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column for primary sorption of amino acids from the supernatant.

Present studies demonstrated that crawfish chitosan was an excellent polymeric substrate as a ligand-exchange chromatographic support for recovery of amino acids from crawfish processing wastewater. Work is needed to establish whether differences between commercial chitosan and that derived from crawfish may be due to dissimilarities in the respective amino group content of the two substrates. Nevertheless, the concentration of potentially recoverable compounds obtained was quite significant in view of the magnitude of the total amount of water discharged from the pigment extraction process. Further significance may be seen when possible byproduct recovery from other seafood processing plants in this country is considered.

In order to effectively exploit the aforementioned findings, the quality of the raw material certainly is a primary consideration. Critical aspects in recovery of flavor-based compounds from seafood processing discharge streams certainly will depend on the freshness of the waste and the water effluent, both of which are related to the logistics of "waste" product collection and processing time involved.

REFERENCES

Chen, H. M. and Meyers, S. P. 1982. Extraction of astaxanthin pigment from crawfish waste using a soy oil process. J. Food Sci. 47: 892.
Hemmasi, B. 1975. Ligand-exchange chromatography of amino acids on nickel-Chelex 100. J. Chromatogr. 104: 367.
Hemmasi, B. and Bayer, E. 1975. Ligand-exchange chromatography of amino acids on copper-, cobalt-, and zinc-Chelex 100. J. Chromatogr. 109: 43.
Meyers, S. P. and Rutledge, J. E. 1973. Utilization of economically valuable byproducts from the shrimp processing industry. In "Food and Drugs from the Sea," L. R. Worthen (Ed.), p. 75. Proc. Marine Technol. Soc., Washington, DC.
Meyers, S. P. and Sonu, S. C. 1974. Nucleotides and amino acids in shrimp blanching water. Feedstuffs 46: 23.
Muzzarelli, R. A. A. 1977. "Chitin." Pergamon Press, Oxford.
Muzzarelli, R. A. A. 1978. Modified chitosans and their chromatographic performances. In "Proceedings of the First International Conference on Chitin/Chitosan," R. A. A. Muzzarelli and E. R. Pariser (Ed.), p. 335. MIT Sea Grant Program, Cambridge, MA.
Muzzarelli, R. A. A. Rocchetti, R., and Muzzarelli, M. G. 1978a. The isolation of cobalt, nickel, and copper from manganese nodules by chelation chromatography on chitosan. Sep. Sci. Technol. 13: 153.
Muzzarelli, R. A. A., Tanfani, F., Muzzarelli, M. G., Scarpini, G., and Rocchetti, R. 1978b. Ligand-exchange chromatography of amino acids on copper-loaded chitosan. Sep. Sci. Technol. 13: 869.
No, H. K. 1987. Application of crawfish chitosan as a coagulant in recovery of organic compounds from seafood processing wastes. Ph.D. dissertation, Louisiana State University, Baton Rouge, LA.
Perkins, B. E. and Meyers, S. P. 1977. Recovery and application of organic wastes from the Louisiana shrimp canning industry. In "Proc. 8th Natl. Symp. on Food Processing Wastes" (EPA-600/2-77-184), p. 292. Seattle, WA.
Siegel, A. and Degens, E. 1966. Concentration of dissolved amino acids from saline waters by ligand- exchange chromatography. Science 151: 1098.
Ms received 5/5/88; revised 9/2/88; accepted 9/2/88.

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