

CHROM. 20 671

GAS CHROMATOGRAPHIC SEPARATION AND CHEMOMETRIC ANALYSIS OF MANDARIN ESSENTIAL OILS*

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(Received May 23rd, 1988)

SUMMARY

Capillary gas chromatography with flame ionization detection was applied to the separation of components of mandarin essential oils. Fifty-nine genuine samples were considered over the period October 1982–January 1983. Essential oils were obtained from unripened green fruits and ripened red fruits, as well as from fruits at intermediate ripening. Thirty-four pure components were systematically identified, but only 13 were used as variables for the further characterization by a chemometric procedure. Principal component analysis was applied to the differentiation of samples with different maturation and obtained with different technologies.

INTRODUCTION

Capillary gas chromatography is a frequently used method for the fractionation of components of essential oils, but few reports of the fractionation of Italian mandarin essential oils have appeared¹⁻³. In this study a systematic approach was adopted in order to characterize mandarin essential oils from Sicily. Two extreme types of fruits were considered for the extraction of the essential oils, green and red fruit, the former corresponding to the unripened and the latter to the completely ripened fruit. The first can be collected during October and a part of November, the second from December to January; in November, from 20 to 30 generally mixed samples can be collected. As essential oils extracted from green fruits seem to be different from those extracted from red fruits, their use is also different; therefore there is the problem of their identification, two traditional technological procedures (pressing or peeling) being commonly applied in Sicily¹. Chemometric analysis⁴ was applied as a tentative method to differentiate mandarin essential oils obtained from green and red fruits as well as the technologies involved in the extraction.

* 23rd paper of the series "On the Genuineness of Citrus Essential Oils".

EXPERIMENTAL

Sampling

Fifty-nine samples were first classified according to the technological procedure involved in the extraction of the essence, *i.e.*, pressing (marked in all figures by triangles) or peeling (circles). Each sample was representative of 50 kg of industrial essence and was further labelled according to the maturation stage of the fruit. Green fruits were therefore considered in the early period of maturation (October 21th to November 17th, 1982, in Sicily), red fruits later (December 1st, 1982 to January 31st, 1983). Green fruits were marked by open, red fruits by darkened circles or triangles. Stages of intermediate ripening (November 17th to 30th, 1982) were indicated by a horizontal dash.

Gas chromatographic analysis

The percentage composition based on the peak areas was determined by gas chromatography (GC) using a Carlo Erba gas chromatograph series Mega Model 5160 and a Shimadzu data processor C-R3A under the following experimental conditions: column, SE-52 glass or fused-silica capillary (25 m \times 0.32 and 0.45 mm I.D. respectively); column temperature, 60 (8 min) to 100°C at 3°C/min to 130°C at 2.5°C/min, to 180°C at 3°C/min; injector and detector temperatures, 280°C; carrier gas, hydrogen, 0.40 kg/cm²; injection mode, split; detection, flame ionization.

A certain number of essential oil samples were also fractionated by chromatography on neutral alumina columns, under the following conditions: column, glass, 1.6 cm I.D.; adsorbent, neutral alumina, grade II activity, 20 g; sample 100 μ l essential oil. Eluents: fraction 1 (hydrocarbons), light petroleum (b.p. 30–50°C; fraction 2 (esters) light petroleum–diethyl ether (97:3); fraction 3 (carbonyl compounds) light petroleum–diethyl ether (80:20, v:v) (20°C); fraction 4 (alcohols), diethyl ether.

Chemometric procedures

An $n \times q$ data matrix, consisting of $j = 1, 2, \dots, q$ variables and $i = 1, 2, \dots, n$ analyzed essential oils, was the starting point for further chemometric investigations. The selection of variables is discussed in detail in the next section. Standardization of the data vector for each variable produced an autoscaled Z matrix, from which the correlation matrix, R , was calculated and used as a starting matrix in principal component analysis (PCA)^{5,6}.

Principal components (PCs) were determined by considering eigenvalues, λ_j , and associated eigenvectors, v_j , calculated from the characteristic equation $(R - \lambda_j I)v_j = 0$ (I is the identity matrix). In order to choose the number, p of PCs ($p < q$) which is adequate to represent the q original variables, the criterion of explained variance was adopted. Correlations, s_{jk} , between the j -th variable and the k -th PC were calculated and represented vectorially. The Y matrix ($n \times p$) of PC scores was estimated from the eigenvector matrix, V . An orthogonal rotation (Normal Varimax) was also tentatively applied in order to ameliorate the separation among groups of scores. All calculations were performed by means of a SPSS-version 9 program⁷ run on a CDC Cyber 170-730 D computer.

RESULTS AND DISCUSSION

Gas chromatographic analysis

In Fig. 1 is reported a typical chromatogram of a mandarin essential oil. Nearly all the thirty-four compounds identified can be baseline resolved on the phase and

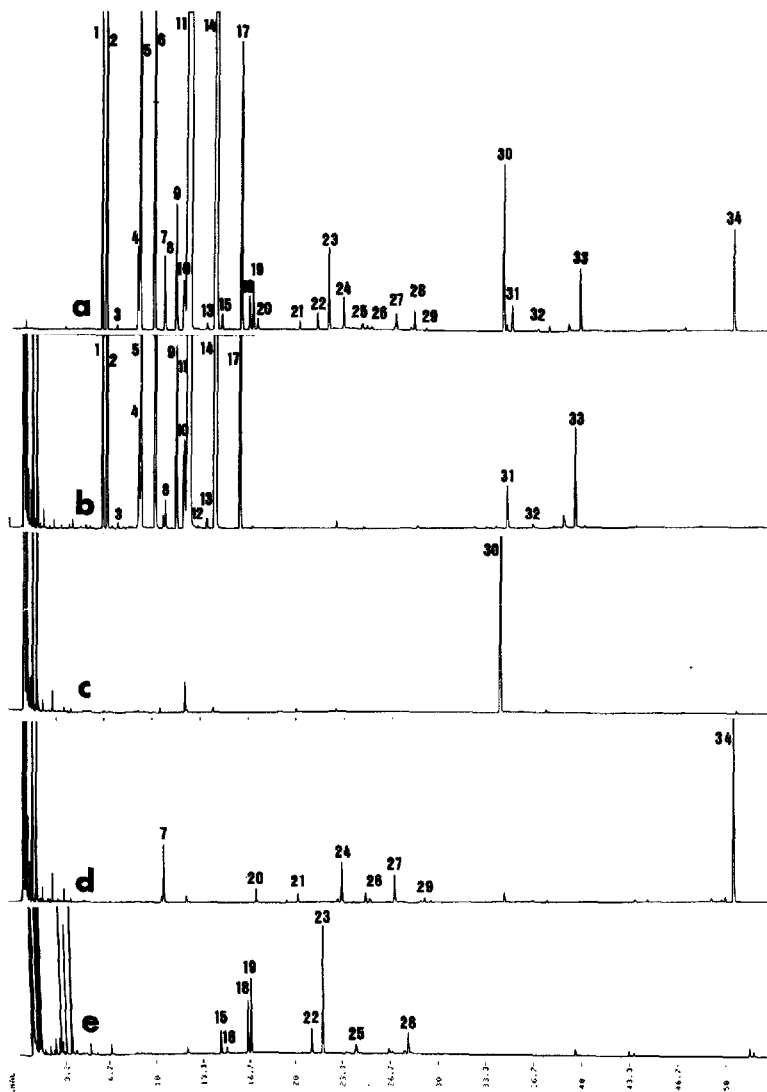


Fig. 1. Chromatogram of a mandarin essential oil and the fractions obtained from its separation on a neutral alumina column. (a) Mandarin essential oil; (b) terpene fraction; (c) esters; (d) carbonyl compounds; (e) alcohols. Peaks: 1 = α -thujene; 2 = α -pinene; 3 = camphene; 4 = sabinene; 5 = β -pinene; 6 = myrcene; 7 = octanal; 8 = α -phellandrene; 9 = α -terpinene; 10 = *p*-cymene; 11 = limonene; 12 = *cis*- β -ocimene; 13 = *trans*- β -ocimene; 14 = γ -terpinene; 15 = *cis*-thujenol; 16 = octanal; 17 = terpinolene; 18 = *trans*-thujenol; 19 = linalool; 20 = nonanal; 21 = citronellal; 22 = terpinen-4-ol; 23 = decanal; 24 = α -terpineol; 25 = nerol; 26 = neral; 27 = geranial; 28 = thymol; 29 = undecanal; 30 = methyl *N*-methylanthranilate; 31 = caryophyllene; 32 = humulene; 33 = farnesene; 34 = sinensal.

chromatographic conditions adopted. The values of the concentration (peak area) derived from this type of chromatogram were used for the determination of the relative amount of each compound. Other traces (Fig. 1b–e) corresponding to terpene fraction, esters, carbonyl compounds and alcohols were also used for the further identification of each component.

From the chromatogram of Fig. 1a, limonene appears to be the predominant component of mandarin essential oil, followed by γ -terpinene, α -pinene, myrcene, β -pinene and other minor components.

The selection of the variables to be considered in the further chemometric data processing depends on (i) the mean abundance in the set of samples considered, (ii) the intra-laboratory repeatability and (iii) the classificatory power of each variable.

Only components having a mean concentration higher than 0.2% were first tentatively considered as variables. Under this condition, twelve variables were taken into consideration, but *p*-cymene was excluded, it is unstable and its concentration strongly depends on the time interval between preparation and analysis. However, two other variables with averages in the range 0.1–0.2% were considered. So the following variables were considered: (1) α -thujene; (2) α -pinene; (3) sabinene; (4) β -pinene; (5) myrcene; (6) α -terpinene; (7) limonene; (8) γ -terpinene; (9) terpinolene; (10) linalool; (11) α -terpineol; (12) methyl N-methylantranilate; (13) sinensal.

The intra-laboratory reproducibility was evaluated from six repeated analyses of a sample of essential oil. As far as the considered variables are concerned, the following mean values, \bar{x} (and coefficients of variation, C.V.) were obtained for each component: (1) 0.80 (0.87); (2) 2.18 (0.73); (3) 0.25 (1.21); (4) 1.50 (0.47); (5) 1.76 (0.63); (6) 0.40 (0.74); (7) 72.50 (0.13); (8) 17.53 (0.31); (9) 0.80 (0.62); (10) 0.10 (2.97); (11) 0.12 (1.74); (12) 0.36 (2.79); (13) 0.26 (5.68%). This list clearly shows that the predominant compounds (limonene, γ -terpinene) are characterized by a low C.V. ($<0.5\%$), and the C.V. is also low ($<1\%$) for some minor constituents (α -, β -pinene, myrcene). In the evaluation of the experimental variability, some compounds in the range $1.0 \geq \bar{x} \geq 0.1$ show a C.V. comparable with the previous ones (α -thujene, α -terpinene, terpinolene). Sabinene, linalool, α -terpineol, methyl N-methylantranilate and sinensal are characterized by a C.V. $\leq 6\%$ and this fact indicates the good analytical response obtained with the stationary phase SE-52, as well as with other experimental conditions, which allow an intra-laboratory repeatability adequate for further chemometric processing.

Differentiation of essential oils

In order to differentiate the essential oils from green and red fruits, a data matrix of $n = 38$ samples, $p = 13$ variables was first considered and analyzed by PCA. In Table I is reported as an example the corresponding *R* matrix from which the following sequence of positive eigenvalues are calculated: 8.71, 1.50, 1.20, 0.94, 0.35, 0.11, 0.09, 0.06, 0.02 and four values less than 0.01.

The number of PCs was estimated from the sequence of the eigenvalues. Only eigenvalues greater than 1, were considered, and so about 88% of the total variance is explained by this rule. In this way, thirteen variables were reduced to three PCs.

The PC scores were considered in projection (Fig. 2). The scores for green and red mandarin fruits appear to be completely separated in this projection. Moreover, the two technological extraction procedures are also differentiated.

The association of variable vectors is indicated in the first quadrant of the

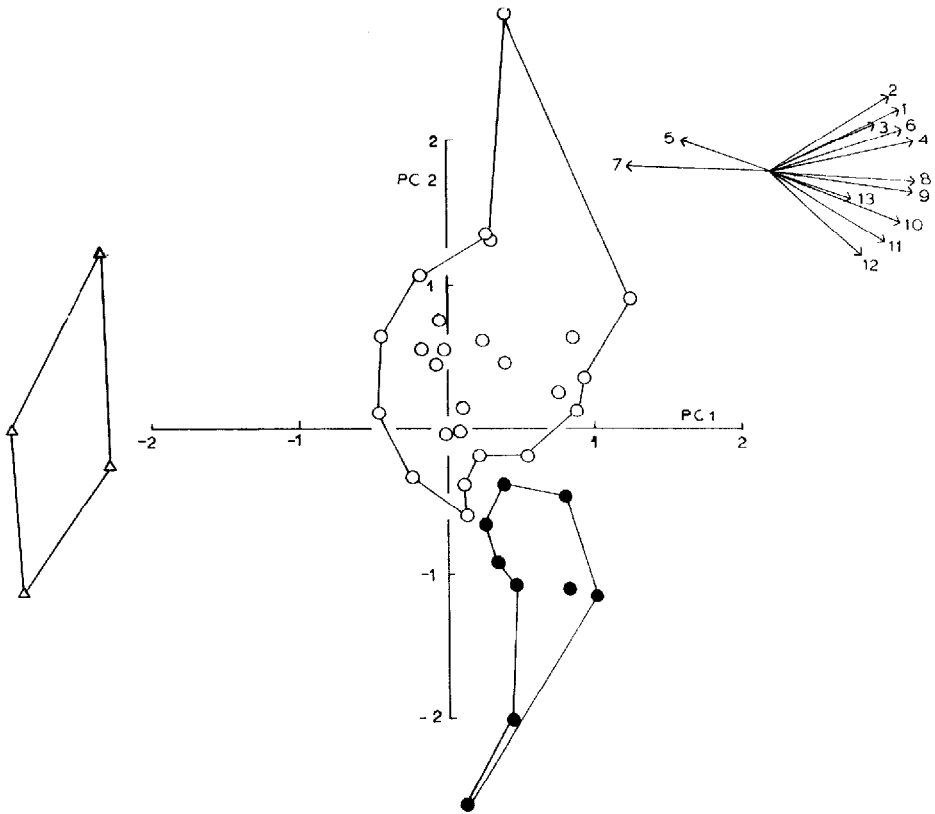


Fig. 2. Scores of the principal components (PC 1, PC 2) of 38 mandarin essential oils, obtained from green fruits by pressing (○) or peeling (●), and from red fruits by pressing (△). The association between the PCs and variable vectors is indicated.

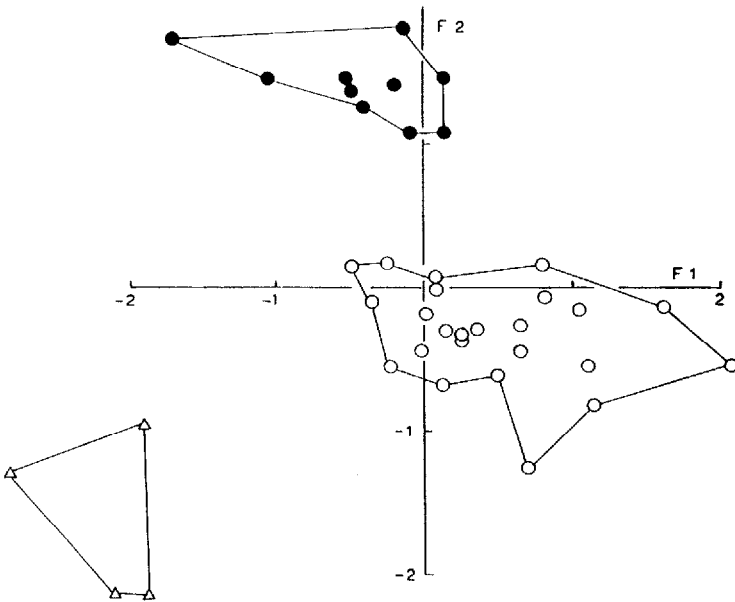


Fig. 3. Projection of scores on the two principal factors (F1, F2) of 38 mandarin essential oils, obtained after an orthogonal rotation. Symbols as in Fig. 2.

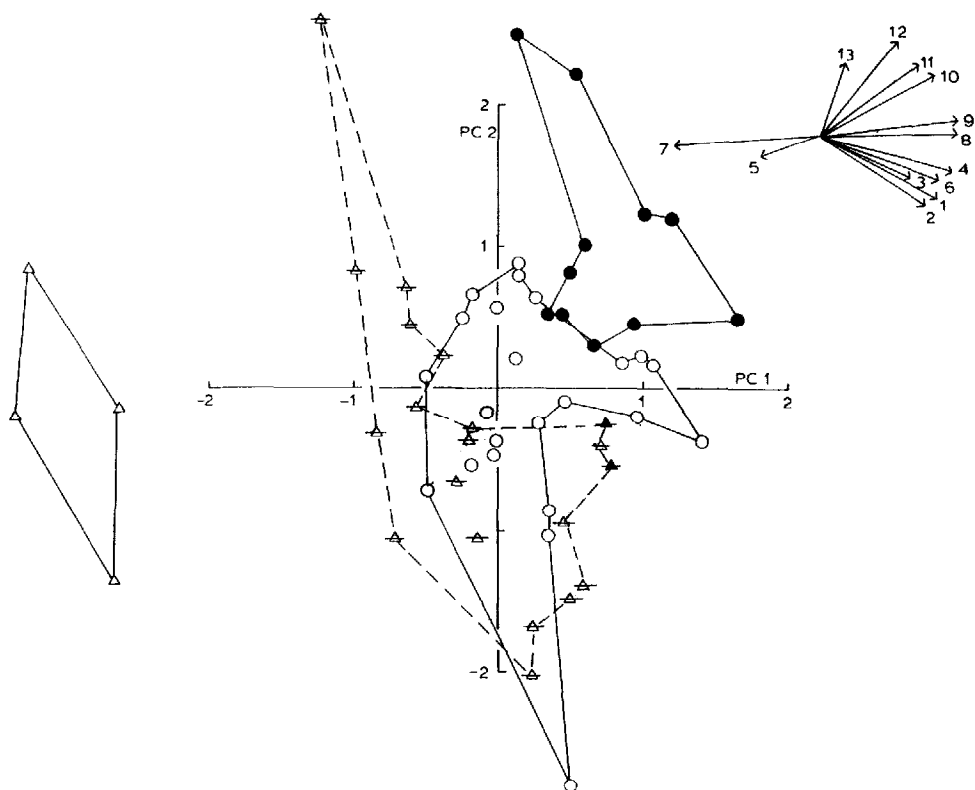


Fig. 4. Scores of the two principal components (PC 1, PC 2) of 59 mandarin essential oils, obtained from green fruits by pressing (\circ) or peeling (\bullet), and from red fruits by pressing (\triangle). Twenty-one oils having an intermediate degree of ripening are marked by an horizontal dash (\triangle , essential oils obtained by pressing; \blacktriangle , essential oils obtained by peeling). Associations between PCs and variable vectors are indicated.

projection. Nearly all variables appear to be positively or negatively correlated to the first PC, except for variable 13, which is strongly associated to the third PC. An orthogonal rotation further improves the separation between the factor scores, as is seen in Fig. 3.

In order to investigate the effect of the presence of essential oils of mandarin fruits having an intermediate maturation, a 13×59 data matrix was considered. From the corresponding \mathbf{R} matrix (not reported), four eigenvalues greater than 1 were extracted, explaining about 93% of the total variance.

The PC scores are displayed in Fig. 4. In this projection a clear separation is again observed between the few points pertaining to pressed red fruits and other points. Moreover a tendency to separation is seen between points for pressed and peeled green fruits, but the points for the samples at intermediate ripening appear to be superposed upon those for green pressed fruits. In the same plot the variable association is also reported. The vector fan is essentially the same as in that in Fig. 2, with some minor variations. An orthogonal rotation improves the separation among

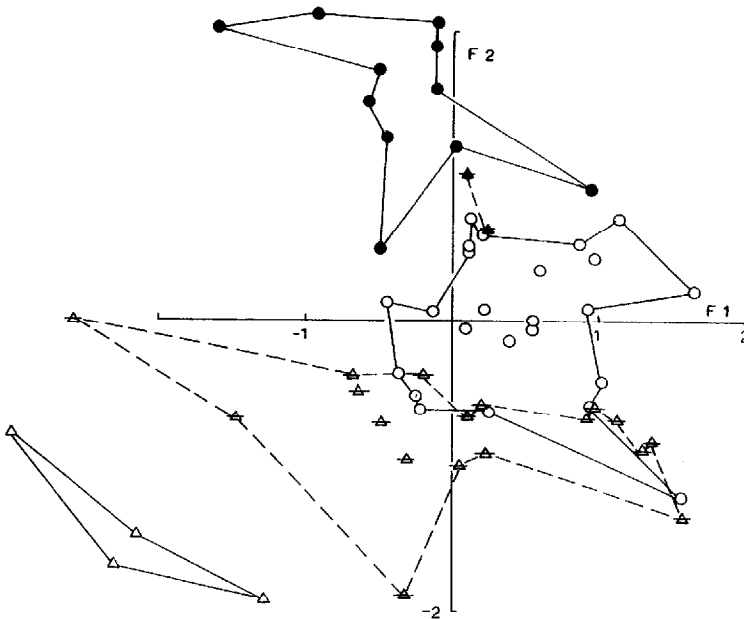


Fig. 5. Projection of scores on the two principal factors (F1, F2) of 59 mandarin essential oils, obtained after an orthogonal rotation. Symbols as in fig. 4.

different clusters of points (Fig. 5), although a small degree of overlapping is observed for the pressed green fruits and those of intermediate ripening.

From an analytical point of view, gas chromatographic procedures, in combination with chemometric methods of data handling, seem to be a fruitful approach to resolving problems of product differentiation in the essential oils industry.

ACKNOWLEDGEMENT

We gratefully acknowledge the assistance of Mrs. Rossana Levi.

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