Chemometric Investigation on Italian Peppermint Oils

Franco Chialva,*,† Antonella Ariozzi,† Diego Decastri,† Paolo Manitto,‡ Sergio Clementi,§ and
Daniela Bonelli§

Centro Studi Maria Branca, Via Edoardo Porro 1, I-20158 Milano, Italy, Dipartimento di Chimica Organica e
Industriale, Università di Milano, Via Venezian 21, I-20133 Milano, Italy, and Dipartimento di Chimica,
Università di Perugia, Via Elce di Sotto 8, I-06100 Perugia, Italy

A set of 99 samples of Piedmontese peppermint oil was analyzed by means of HRGC. Principal component
analysis was applied to the GC data to find systematic patterns. The chemometric method used was
SIMCA. The results indicate that the main factors influencing the oil composition are geographic origin
of the plants, their harvest date, and interval of time since their latest transplantation. Linear PLS
models show which variables are involved in the maturation and aging process; it was not possible to
find a reliable classification model for oils of different geographic origin.

Since the eighteenth century, Italy has been a producer
of mint oil. The production has always been limited to a
small area (less than 250 km²) south of Turin, straddling
the rivers Po, Pellice, Variata, and Maira, with particular
soil and climatic conditions. The overall production of
this area (approximately 30 tons/year) is considered to be
of extremely high quality. The only variety of mint now
cultivated in Piedmont is *Mentha piperita* L. var. Italo-
Mitcham (black mint).

The 15 most abundant chemical components in pep-
permint oil account for about 80%, even though a normal
gas chromatogram reveals over 100 components. For many
of these, in addition to the name and chemical formula,
the biosynthetic pathway and various biochemical and
chemotaxonomic parameters are known.

Various studies (Murray et al., 1988; Clark and Menary,
1980; Lawrence and Chi-Keun Shu, 1986) have analyzed
possible factors influencing the quality or, in general, the
chemical composition of peppermint. The problem has
been analyzed from the chemical and biochemical stand-
points, including the study of actual in-field production;
however, the chemical composition has always been
evaluated on oils distilled from relatively small numbers
of plants, often sampled by unspecified criteria. Two
papers have recently been published (Chialva et al., 1992;
Marengo et al., 1991) that tackle the problem using samples
taken from actual production batches and which used
chemometric instruments to obtain a classification of the
oils based on their origin. These two studies clearly intended
primarily to evaluate the potential value of chemometrics
in the classification of essential oils, as can be seen from
the fact that samples of decidedly different origins and
compositions were classified. Furthermore, only 29 of the
most abundant components in the peppermint oils were
considered, and oils of very different geographic origins
were successfully distinguished on this basis.

However, nowadays, the classification by chemometric
techniques of products deriving from largely different
botanical varieties and geographic origin may be regarded
as a trivial result. It is much more challenging to
investigate whether the same tools could be used within
a much more homogeneous set of samples. Accordingly,
the present study included peppermint oils from a very

limited area and considered all of the compounds present
in amounts above 0.1%, to verify the significance of minor
constituents and to attempt a more in-depth chemometric
analysis. This research aimed not only to provide a more
precise characterization of Piedmontese peppermint oil
but also to determine elements useful to the producer in
controlling the composition of the oil, by means of methods
which are as valid as possible. A second phase, already
under way, will attempt to complete the picture by more
precisely correlating chemical composition and quality.

EXPERIMENTAL PROCEDURES

Description of Samples. All of the mint oil produced in
Piedmont comes from cultivations of peppermint which, being
a sterile hybrid, is only propagated agamically. This means that
any diversity found among oils of different production is due
exclusively to differences in cultivation techniques or climate.
Planting can be either in autumn or in sping; the plants are
harvested between July 15 and August 31 regardless of the year
they were planted, although there is a tendency to harvest later
those crops planted the previous spring. Every 2, or at most 3,
years, the plants lose vitality and are renewed by plating them
on new land; this means that there are no crops over 3 years old.

The sampling method used in this research, which covered
approximately 80% of the cultivated area, can be considered to
be amply representative of the overall Piedmontese production
for 1991.

Ninety-nine samples of crude peppermint essential oil were
collected directly from distilleries in production, where the oil
is produced by steam distillation of the entire aerial part of the
plant. Each sample was taken from a production batch origi-
nating from plant material grown by a single farmer. For all
samples the following information was available: name and
location of the distillery; type of distillation. For most samples
the following data were also known: date of distillation; the year
and season when the mint was planted; type of soil on which the
mint was grown.

Chemical Analytical Methods. Quantitative analysis on
the 99 samples was carried out by HRGC with an internal standard
method (ethyl octanoate, Fluka, purity 98%, standard solution
1% volume). For these analyses a Hewlett-Packard 5890 Series
II gas chromatograph with autosampler was used, equipped with
flame ionization detector and HP ALICE data collection and
processing system (release 2.01). Chromatographic separation
was achieved on a fused silica DB-Wax (J&W Scientific) capillary
column (i.d. 0.32 mm, film thickness 0.25 μm, column length 60
m). The temperature program was as follows: 4 min at 75 °C;
gradient of 4 °C/min to 100 °C; gradient of 5 °C/min to 180 °C;
gradient of 8 °C/min to 210 °C; isotherm at 210 °C for 15 min.
Sample preparation was performed as follows: 0.5 mL of standard
solution was added to 100 μL of essential oil, and the mixture was

† Centro Studi Maria Branca.
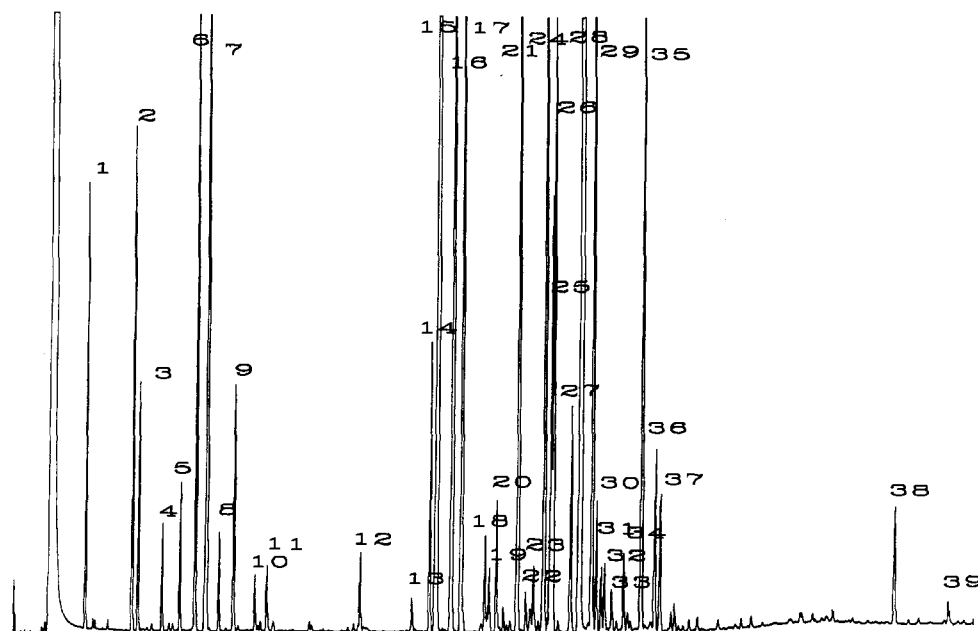‡ Università di Milano.
§ Università di Perugia.

Chemometric Investigation on Italian Peppermint Oils

J. Agric. Food Chem., Vol. 41, No. 11, 1993 **2029**

Figure 1. Typical chromatogram of a Piedmonteses peppermint oil. Peaks: 1, α-pinene; 2, β-pinene; 3, sabinene; 4, myrcene; 5, α-terpinene; 6, limonene; 7, 1,8-cineole; 8, cis-ocimene; 9, γ-terpinene; 10, p-cimene; 11, terpinolene; 12, 3-octanol; 13, 1-octen-3-ol; 14, trans-sabinene hydrate; 15, menthone; 16, menthofuran; 17, isomenthone; 18, β-bourbonene; 19, B; 20, linalool; 21, menthyl acetate; 22, C; 23, D; 24, neomenthol; 25, terpinen-4-ol; 26, β-caryophyllene; 27, isomenthol; 28, menthol; 29, pulegone; 30, E; 31, F; 32, G; 33, H; 34, α-terpineol; 35, germacrene D; 36, piperitone; 37, piperitenone; 38, viridiflorol; 39, tymol.

Table I. Explanation of the Objects' Codes

descriptor	character (meaning)
zone of origin	C (Cavallermaggiore); P (Pancalieri); R (Racconigi); T (Moretta)
distillery	A–E (Pancalieri); F, G (Cavallermaggiore); H, L (Moretta); N (Racconigi)
degree of maturation (distillation period)	1 (July 15–31); 2 (Aug 1–8); 3 (Aug 9–15); 4 (Aug 16–23); 5 (Aug 24–Sept 15)
years since latest transplantation (age)	P (1); S (2); T (3)
type of soil	S (sandy); C (partly argillaceous)
tye of still	I (internal steam production); E (external boiler)

brought to 100 mL with absolute ethanol; 5 μL of prepared sample was injected in split mode, split ratio 1:50. Helium was used as carrier gas, with 104 kPa of pressure at the head of the column. For some of the chemical components detected by gas chromatography, identification was provided by the mass spectra recorded by GC/EI-MS analysis (GC/MS: HP 5988A; analytical conditions as described above) and Kovats indices. Chemometric analysis was applied to the areas (corrected by the internal standard method) of the 39 chromatographic peaks of compounds that were present in all of the samples examined in quantities above 0.1%. The 32 most abundant components were all identified; the remaining 7 were assigned the letters B–H (Figure 1).

Data were analyzed statistically by the SIMCA method, which entails autoscaling of the data, analysis of the principal components (NIPALS algorithm), and the separate modeling of each class (software package SIMCA 3B, from Umeå University, Sweden) (Wold and Sjöstrom, 1977).

RESULTS AND DISCUSSION

Principal Component Analysis. Each sample was assigned a code comprised of five letters and one number (Table I), which indicate, respectively, the zone of origin, the distillery, the period of distillation (degree of maturation), the years since the latest transplantation (age), the type of soil where grown, and the type of still. The whole data matrix, the 39 parameters used (variables) for each of the 99 oil samples analyzed (object), was subjected to principal component analysis (the data table is available as supplementary material).

The preliminary PC model allowed us to detect six samples that had to be excluded from the subsequent analysis. Two of them could easily be recognized as anomalous on the basis of the known conditions of production. The other four showed an unacceptably high leverage in driving the first components: since statistics should model the systematic pattern and not the specific points, it is appropriate that the model does not rely on peculiar samples.

The final PC model was then carried out on 93 samples and required 7 principal components. The projections of the samples on the plane defined by the first two principal components are illustrated in Figures 2–4. The relative position of the samples appears to show that oils from different distilleries (i.e., originating from different areas) tend to be grouped separately (Figure 2), especially for groups H and N which belong to Moretta (T) and Racconigi (R), respectively, whereas this is not as clear for the larger groups of Pancalieri (P) and Cavallermaggiore (C). Nevertheless, the same figure redrawn with the codes describing the plants' degree of maturation (Figure 3) and age (Figure 4) also shows a regular trend: samples of older age distilled earlier tend to occupy the upper right quadrant of the score plot. It is noteworthy that this statistical result implies the existence of a relationship between the composition of the samples and the maturity and/or age of the plants.

Since one of the aims of this research was to derive rules for the classification of the essential oils, the first result of this study was to realize that the GC data cannot be used as such for an unambiguous classification of unknown samples upon their geographic origin. This also implies that the other discrete factors taken into account as possibly influencing the compositions of samples, such as the type of soil and still, do not show up as relevant to the classification found. However, the result showed that the plant's biochemistry must be taken into account.

PLS Analysis. Chemometric modeling is further complicated by the nature of the samples available. The
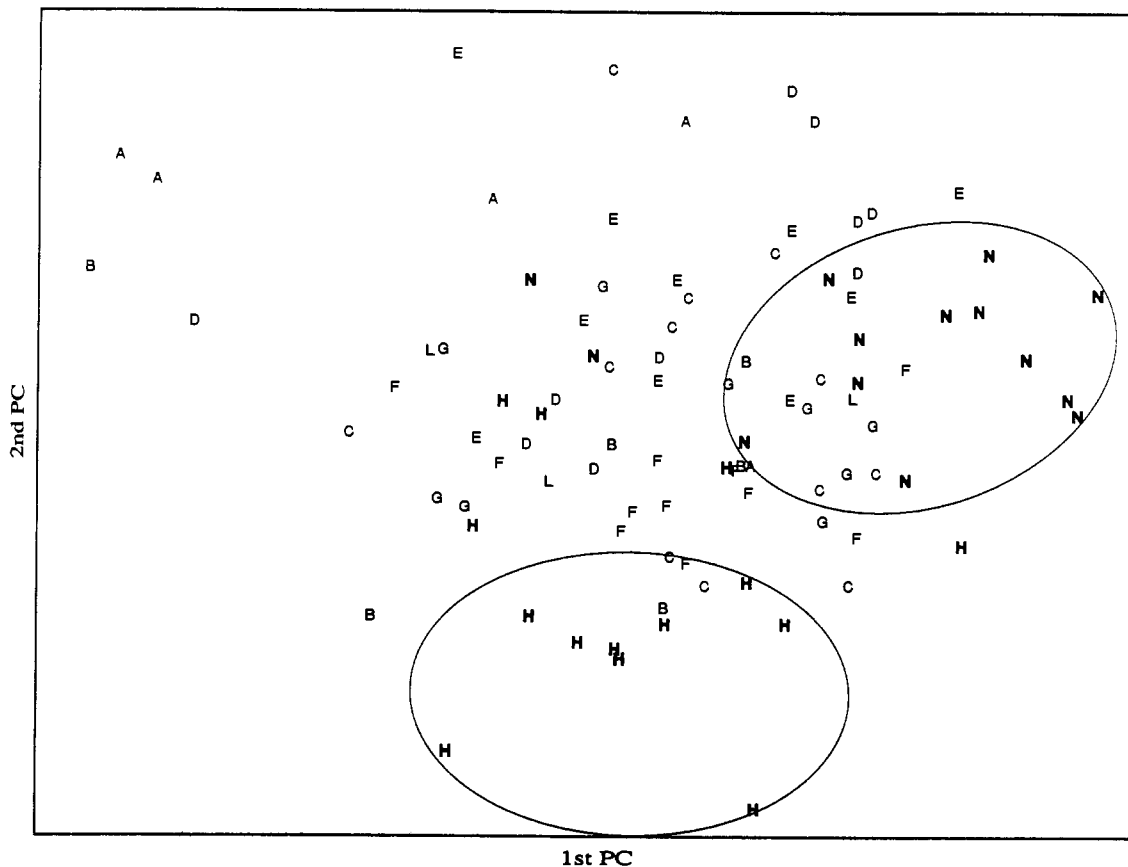
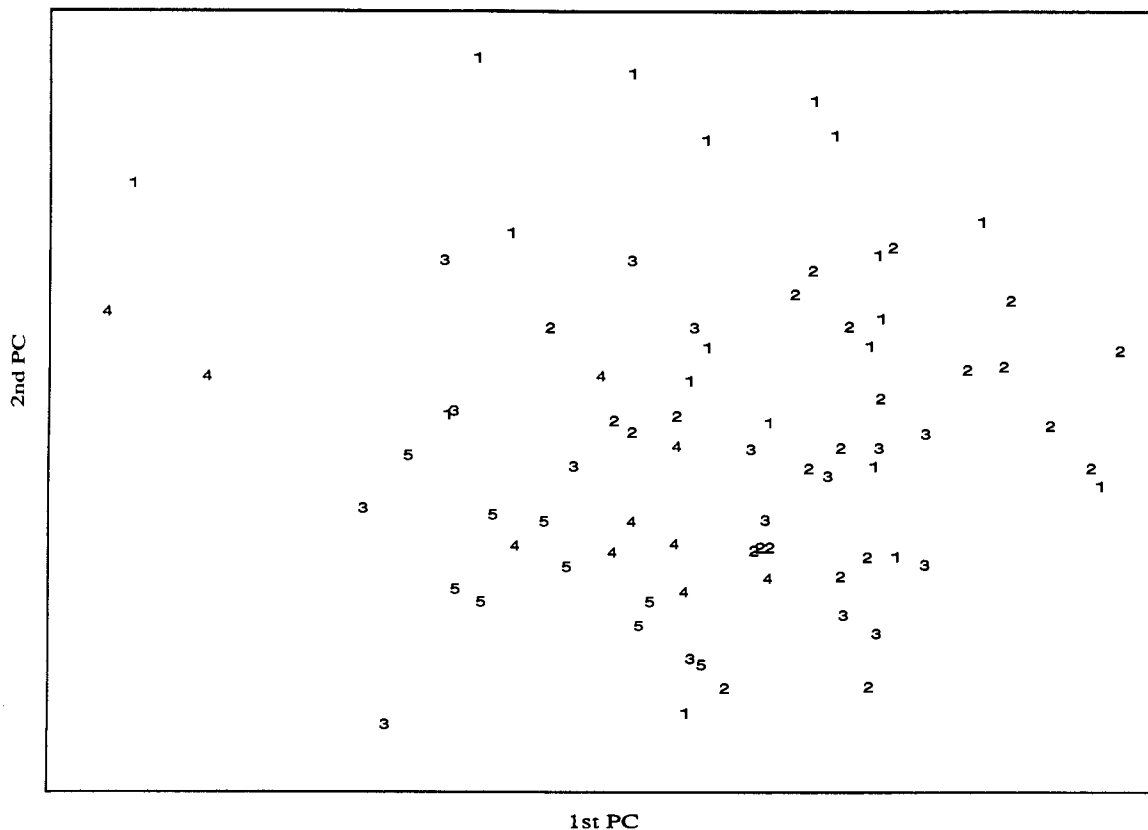**Figure 2.** Plot of the scores of PC1 and PC2 (distilleries).

Figure 3. Plot of the scores of PC1 and PC2 (degree of maturation).

producers tend to harvest the older plants first, and only at the end of the season do they harvest the younger ones, so that it is extremely difficult to distinguish the effects due to seasonal biochemical alternations (which we will indicate as maturation) from those depending on the

interval of time since transplanting (age). Furthermore, since the distilleries supplied samples throughout the entire harvesting period, it is reasonable to suppose that the differences due to the maturation of the plant, present in the group of samples from each distillery, might mask
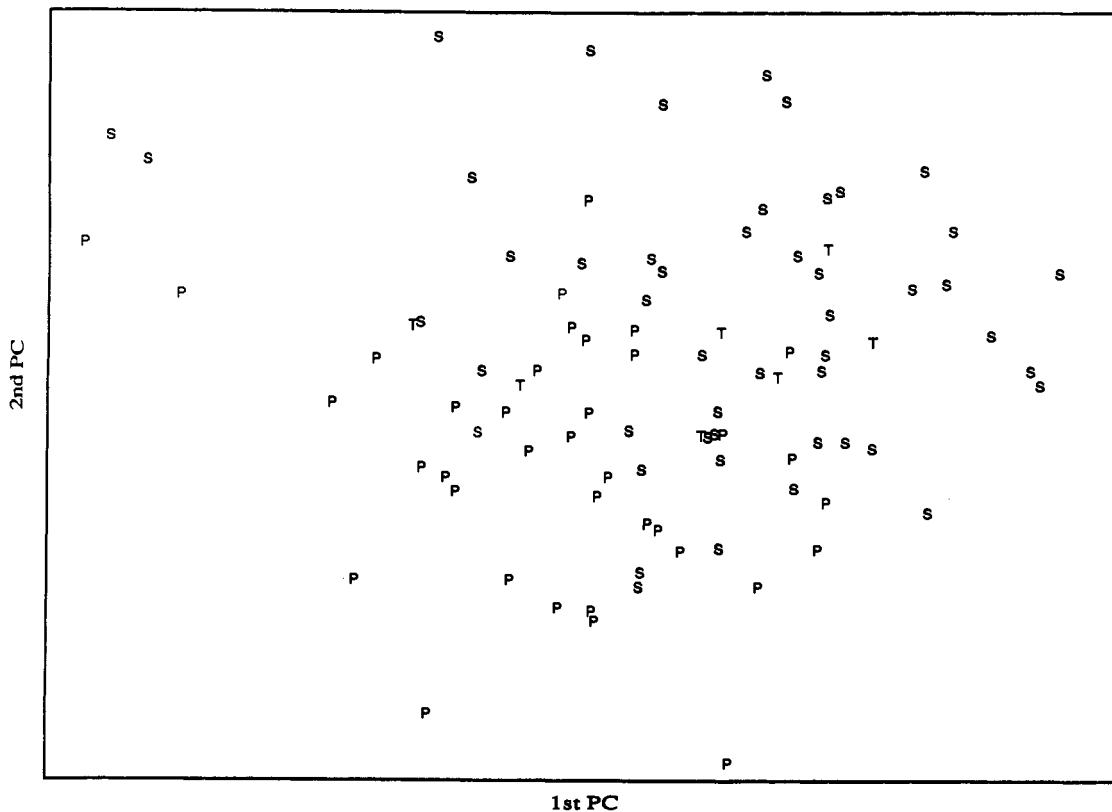
Chemometric Investigation on Italian Peppermint Oils

J. Agric. Food Chem., Vol. 41, No. 11, 1993 **2031**

Figure 4. Plot of the scores of PC1 and PC2 (age).
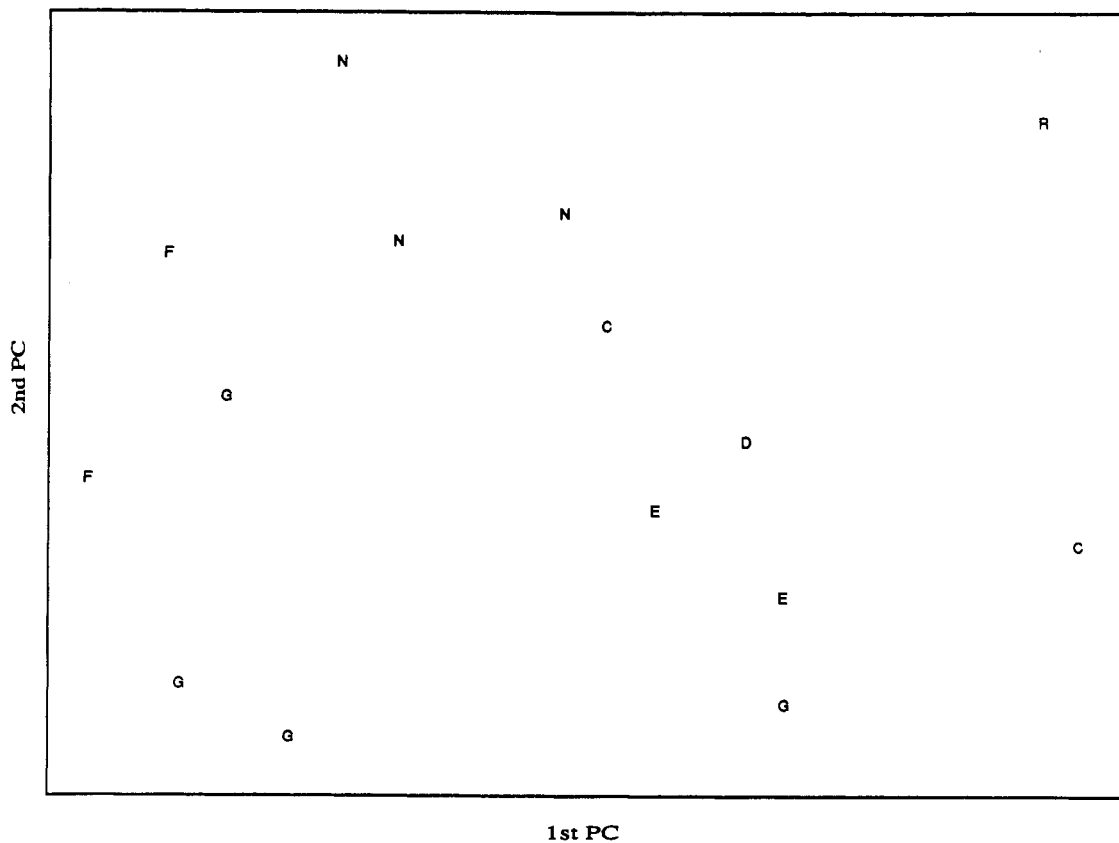

Figure 5. Plot of the scores of PC1 and PC2 [distilleries (only samples distilled during the third period)].

the differences in chemical composition deriving from geographic position.

Thus, it was decided to verify whether a chemometric analysis could be used to confirm the existence of a systematic relation between the chemical composition of the essential oil and the maturation (already demonstrated in other ways) or age of the plant. To this end, two variables were added to the data matrix, and their dependence on the chemical composition of the samples was investigated. The date of distillation was expressed as the number of days elapsing from July 1, 1991; the age was expressed as the estimated number of months elapsing between the

Table II. Loadings (p) and Modeling Powers (MP) Higher than 0.1 Obtained from PLS Analyses of Chemical Composition and Degree of Maturation

component	distillery B (6 samples; A = 1; 74% expl var)		distillery C (12 samples; A = 1; 78% expl var)		distillery D (10 samples; A = 1; 87% expl var)		distillery E (10 samples; A = 1; 72% expl var)		distillery F (11 samples; A = 1; 92% expl var)	
	p	MP	p	MP	p	MP	p	MP	p	MP
1 α-pinene	-0.08		0.05		0.12		0.1		0.14	
2 β-pinene	-0.1		0.06		0.07		0.08		0.12	
3 sabinene	-0.1		0.04		-0.1		0.13		0.12	
4 myrcene	-0.1		0.19	0.16	0		0.04		0.04	
5 α-terpinene	-0.18	0.3	0.09		-0.09		-0.11		-0.14	
6 limonene	-0.5		0.13		0.17	0.33	-0.03		-0.06	
7 1,8-cineole	-0.16	0.21	0.03		0.01		0.07		-0.07	
8 cis-ocimene	-0.17	0.22	0.11		-0.18	0.36	0.09		0.02	
9 γ-terpinene	-0.21	0.52	0.08		-0.15	0.23	-0.15	0.1	-0.15	0.11
10 p-cymene	-0.21	0.5	-0.23	0.29	-0.2	0.56	-0.13		-0.21	0.27
11 terpinolene	-0.18	0.26	0.06		-0.13	0.12	-0.13		-0.17	0.14
12 3-octanol	-0.09		0.13		0.05		0		-0.09	
13 1-octen-3-ol	-0.05		0.01		-0.13	0.14	-0.2	0.25	-0.07	
14 trans-sabinene hydrate	-0.11		-0.17	0.11	-0.22	0.72	0.11		0.05	
15 menthone	-0.17	0.26	-0.13		-0.22	0.75	-0.25	0.5	-0.23	0.35
16 menthofuran	0.16	0.21	0.27	0.46	0.2	0.54	0.26	0.63	0.27	0.59
17 isomenthone	-0.17	0.24	-0.14		-0.2	0.5	-0.19	0.23	-0.25	0.48
18 β-bourbonene	-0.15	0.16	-0.29	0.55	-0.22	0.7	-0.26	0.58	-0.21	0.28
19 B	-0.21	0.49	-0.28	0.49	-0.1		-0.12		-0.03	
20 linalool	-0.21	0.49	0.14		-0.02		0.09		-0.08	
21 menthyl acetate	-0.21	0.49	-0.22	0.25	-0.13	0.13	0.13		0.15	0.1
22 C	-0.21	0.51	0.21	0.22	0.13	0.13	0.17	0.15	-0.19	0.22
23 D	-0.21	0.53	-0.26	0.39	-0.14	0.17	-0.04		0.09	
24 neomenthol	-0.19	0.34	-0.24	0.33	-0.17	0.33	-0.12		-0.14	
25 terpinen-4-ol	-0.22	0.61	-0.09		-0.2	0.5	-0.22	0.36	-0.28	0.72
26 β-caryophyllene	-0.14	0.1	-0.13		-0.22	0.66	-0.24	0.46	-0.23	0.33
27 isomenthol	-0.22	0.61	-0.12		-0.06		0.09		-0.06	
28 menthol	-0.19	0.33	-0.01		-0.08		0.19	0.22	-0.03	
29 pulegone	0.16	0.21	0.26	0.41	0.21	0.63	0.26	0.6	0.07	
30 E	-0.08		-0.06		-0.2	0.5	-0.19	0.22	-0.13	
31 F	-0.2	0.46	-0.25	0.37	-0.12		-0.11		-0.09	
32 G	-0.15	0.15	0.09		-0.1		-0.06		-0.15	
33 H	-0.15	0.15	-0.2	0.18	-0.22	0.71	-0.24	0.45	-0.25	0.46
34 α-terpineol	-0.13		0.11		-0.14	0.16	-0.19	0.23	-0.19	0.21
35 germacrene D	-0.12		0.05		-0.21	0.63	-0.11		-0.06	
36 piperitone	-0.19	0.33	-0.2	0.18	-0.22	0.71	-0.22	0.32	-0.28	0.68
37 piperitenone	-0.14		0		-0.2	0.53	-0.1		-0.09	
38 viridiflorol	-0.12		-0.14		-0.22	0.79	-0.22	0.35	-0.18	0.17
39 thymol	-0.04		0.06		-0.2	0.48	-0.13		-0.26	0.55

date of planting and the date of harvest. To eliminate any error stemming from differences in the oils due to the area of origin, this analysis was carried out on subgroups of samples from the same distillery and was limited to those distilleries that had provided a sufficient number of samples.

The PLS models obtained from the first variable were much better than those from the second, mainly because of the large number of levels available to define "maturation", whereas only five could be used to define "age". Loadings and modeling powers resulting from the PLS models, which relate the chemical compositions to the date of distillation of the mint plants, are reported in Table II. The analysis of the results showed that the maturation of the plant is linked to a reduction of γ-terpinene, p-cymene, methone, isomenthone, β-bourbonene, terpinen-4-ol, β-caryophyllene, constituent H, and piperitone and a corresponding increase in menthofuran and pulegone. This would seem to agree, at least partially, with the results from previous studies conducted with techniques traditionally applied to the study of phytochemistry (Murray et al., 1988; Clark and Menary, 1980; Lawrence and Chi-Keun Shu, 1989); in particular, the increase of pulegone and menthofuran and the decrease of isomenthone, menthone, and piperitone on maturation are confirmed, whereas the variations in the remaining cited compounds were never reported previously.

It is, moreover, very difficult to be certain that the biochemical variations revealed by the chemometric model are really due to the maturation of the plants during their annual cycle and do not depend, rather, on the age of the plant in years. It has already been said that the earlier samples have generally been distilled from the older plants and vice versa. Only a few of the samples included in this study are to the contrary (first-year plants distilled relatively early and older plants distilled relatively late). A classification criterion based on the mean values of these different groups of samples would seem to imply that the variation due to maturation is more significant than that due to the age of the plant. Nevertheless, the database on which this speculation could be attempted is so small that it is probably better only to speak of maturation without specifying whether this depends on the annual cycle or on the age of the plant.

Final PC Model. Lastly, it is interesting to verify whether, if the variations induced by maturation are eliminated, the series of data permits a more precise classification of the samples by area of origin. To this end, principal component analysis was conducted on the subgroup of samples distilled during the same period and thus homogeneous in terms of vegetative stage. The most interesting results were obtained from samples produced during the third distillation period, which is also the period in which the largest number of batches of mint are distilled

Chemometric Investigation on Italian Peppermint Oils

J. Agric. Food Chem., Vol. 41, No. 11, 1993 **2033**

in all of the areas considered. The diagram of the principal components, reported in Figure 5, shows the separation of the various distilleries/areas of origin is much improved compared to the original plot, as indicated in Figure 2. Nevertheless, it is equally clear that at least two samples are in an inappropriate place on the diagram, if the classification model is to be used a posteriori and to give unambiguous results.

Conclusions. Thus, it must be concluded that, at the present state of knowledge and on the basis of the specific characteristics considered in this study, it is not possible to find a fully certain classification model for Piedmontese mint in the various areas of origin/producing distilleries. The fact that in no case during all of the phases of the analysis did any indications emerge of a possible influence of the other factors considered (type of still, type of soil) would appear to indicate that any effects due to these factors are of a decidedly lower order than those detected. Nevertheless, the study has provided better knowledge of the problem and made it possible to characterize the prevalent composition for at least some of the production zones and to show that the variations in chemical composition attendant on maturation are of the same order of magnitude as those deriving from the different geographic origin, because of the limited area considered here. Should further work be undertaken to further clarify the problem, the sampling should be in line with the aims it is intended to address; i.e., samples should be of the same maturity if one wishes to look for geographic classification.

ACKNOWLEDGMENT

LITERATURE CITED

Chialva, F.; Ariozzi, A.; Decastri, D.; Manitto, P. *Riv. Ital. EPPOS* **1992**, 533–550.

Clark, R. J.; Menary, R. C. Environmental and Cultural Factors Affecting the Yield and Composition of Peppermint Oil (*Mentha piperita* L.). *Int. Congr. Essent. Oils, [Papers], 8th* **1980**.

Lawrence, B. M. Essential Oils Production. A Discussion of Influencing Factors. In *Biogeneration of Aromas*; Parliment, T. H., Croteau, R., Eds.; American Chemical Society: Washington, DC, 1986; pp 363–369.

Lawrence, B. M.; Chi-Keun Shu. Peppermint Oil Differentiation. *Perfum. Flavor.* **1989**, *14*, 21–30.

Marengo, L.; Baiocchi, C.; Gennaro, M. C.; Bertolo, P. L.; Lanteri, S.; Garrone, W. Classification of Essential Mint Oils of different origin by applying pattern recognition methods to gas chromatographic data. *Chemom. Intell. Lab. Syst.* **1991**, *11*, 75–88.

Murray, M. J.; Marble, P.; Lincoln, D.; Hefendehl, F. W. Peppermint Oil Quality Differences and the Reason of them. In *Flavor and Fragrances: A World Perspective*; Lawrence, B. M., Mookherjee, B. D., Willis, B. J., Eds.; Elsevier Press: Amsterdam, 1988; pp 189–210.

Wold, S.; Sjöstrom, M. In *Chemometrics theory and application*; Kowalski, B. R., Eds.; ACS Symposium Series 52, American Chemical Society: Washington, DC, 1977; p 243.