Application of Pattern-Recognition Techniques to the Essential Oil of Hops[1]

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ABSTRACT

The essential oils from 148 hop samples grown in North America and Europe were collected by steam distillation. Capillary column gas chromatography was used to separate and measure the oil constituents. The results were analyzed by pattern-recognition and multivariable analysis techniques. The programs that were most successful in classifying the major hop varieties in the sample set were SIMCA and Stepwise Discriminant Analysis. These methods indicated which of the peaks in the chromatograms were characteristic of varieties and of growing regions. The results obtained enable estimation of the variety of unknown samples.

Key words: ARTHUR, BMDP, Gas chromatography, Multivariate analysis, SIMCA, Varietal identification

Researchers have attempted to identify varieties of hop samples by examining their essential oils. Luers (10) showed differences in the oils of hops grown in the Kent and Hallertau regions. Rabak (16) noted chemical differences in hop varieties. Howard and Slater (7) found that Japanese hops contained large amounts of hydrocarbons. Rigby and Bethune (17,18) noted differences in myrcene and humulene content and differentiated between European and North American varieties. Maier (11) and Roberts (19) divided hops into groups according to the profiles of their oils. Buttery and Ling (3) and Likens and Nickerson (9) developed methods of distinguishing between hop varieties by examining their gas chromatograph (GC) hop oil patterns. Hautke and Petricek (6) found varietal differences by examining the oil of a few hop cones. Kruger and Neumann (8) examined the oil of more than 100 hop varieties. Naya and Kotake (15) differentiated between five different varieties by introducing the hop lupulin directly into the mass spectrometer source.

These researchers examined chromatograms produced from hop oils and developed discrimination rules based mainly on the major peaks. With capillary gas chromatography, which is capable of separating a large number of components in these samples, efficiently choosing the peaks that contain the most information for classification can be very difficult: large peaks are not necessarily the most informative. In a preliminary study (20), pattern recognition and multivariate analysis showed promise in distinguishing between hop varieties and between the places where the samples were grown. For the work reported here, the sample set was expanded to include more samples and varieties.

EXPERIMENTAL

Distillation and Separation of Hop Oil Constituents

The 148 hop samples examined in this work included the following varieties: Northern Brewer, Tettnang, Hallertau, Saaz, Spalt, Lublin, Styrian Goldings, Super Styrians, Clusters, Galena, Eroica, Cascade, Fuggles, Comet, and Nugget (formerly USDA 21193). Most hop samples representing a variety were obtained from several suppliers. The sample set contained hops grown in 1980, 1981, and 1982. The hop oils were obtained by steam distillation according to Likens and Nickerson (9). Gas chromatography was performed on a Hewlett-Packard 5830 GC with a fused silica capillary column (60 meter × 0.32 mm i.d.) containing a nonpolar (SE-30) bonded liquid phase. The hop oil distillates were diluted in methylene chloride (50 μl/ml), and portions (5 μl) of the resulting solution were injected into the GC with a split ratio of 100:1. The flow rate of the carrier gas was 2 ml/min. After 7 min at 50°C, the column temperature was programmed to 180°C at 3°C/min. Peaks with less than 1,000 integrator counts were disregarded so that the very smallest peaks and baseline noise would be eliminated.

Identification of Hop Oil Constituents

The hop oil components were identified with a Hewlett-Packard 5985B Gas Chromatograph/Mass Spectrometer (MS)/Data System. The mass spectra obtained for unknown peaks were compared to the full spectra in the National Bureau of Standards mass spectral library and three other libraries (pollution, drugs, and biomedicine) supplied by Hewlett-Packard. Whenever possible, authentic compounds were injected into the GC/MS so that retention times and mass spectra could be compared under conditions identical to those used for the hop oil samples.

Data Handling

Integration was performed by the GC. A serial output port board (Hewlett-Packard) installed in the GC was used to transmit the results (retention times, integrated peak areas, and area percent calculation) to a microcomputer (C8P-DF Ohio Scientific, Aurora, OH). A BASIC program on the microcomputer acquired the data and stored it on an 8-in. flexible disk. A second BASIC computer program later concentrated the data by eliminating the area percent results and blank spaces between the retention times and integrated peak areas. Additional information, such as name and sample number, which described the sample, was entered at this stage. This program also had the ability to transfer the condensed data to a second disk for temporary storage.

With the aid of the Smart Terminal Operating System (STOS) program (Phil Lindquist, Union Lake, MI), the information was transferred to a large main-frame computer. A FORTRAN program was written to match the peaks with those expected (by comparing retention times) and to build files in the three different formats required by the various programs for pattern recognition.

Pattern Recognition and Multivariate Analysis

The following computer programs were used to analyze the data:

1. BMDP7M, stepwise discriminant analysis in the BMDP81 statistical software package (4) (Department of biomathematics, University of California, Los Angeles)
2. ARTHUR81 (2), a program specialized for chemical pattern recognition (available from Infometrix Inc., Seattle, WA) and containing routines for cluster analysis of samples, cluster analysis of measurements, nearest-neighbor analysis, nonlinear mapping, hyperplane separation, discriminant analysis, Bayesian classification, principal-components analysis, and two early versions of SIMCA
3. SIMCA-3B (statistical isolinear multicategory analysis), from the Department of Chemometrics of the University of Umeå, Sweden, and containing a series of BASIC programs (22).

RESULTS AND DISCUSSION

Gas Chromatography

Figure 1 is a typical hop oil chromatogram. Seventy to 117 peaks were quantitated for the various oil samples. The 148 hop oil samples were all chromatographed on the same column. The same

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conditions were used so that all of the results were obtained under nearly identical conditions.

Peak Identifications

Table I lists all 117 chromatographic peaks in order of elution and shows 25 peaks that have been identified; all of these are previously reported hop oil components and include monoterpenes, sesquiterpenes, methyl esters, and ketones. Some oxygenated derivatives of humulene, were also present, including humulene epoxide, humuladienone, and humulenol. A series of compounds with molecular weight of 204 and an empirical formula of $C_{15}H_{24}$ (the same as humulene) were observed, but these were not further characterized.

Application of Pattern Recognition

Cluster analysis is a multivariate technique that has fewer restrictions and makes fewer assumptions than most of the other methods of pattern recognition (12,13). Samples are not assigned to classes before the application of this technique. A representation of the closeness between samples according to distances between them in the multidimensional factor space is produced. This representation can be examined for evidence of a natural tendency for certain samples to group together. The dendrogram shown in Fig. 2 was obtained with unsupervised clustering from the ARTHUR package (5). A matrix was first created to express the distances between samples (ie, the vector lengths) in the multidimensional factor space. The "complete-link" method of clustering, which produced slightly better results than the "single-link" method, was used to depict the closeness of samples in two dimensions. The results shown are from eight different hop varieties, each of which was represented by a large number of samples. Samples included the North American variety Clusters and seven European varieties: Styrian Goldings, Hallertau, Northern Brewer, Saaz, Spalt, Super Styrian, and Tettnang. The samples clustered near the left side of the figure are the most similar. This method was somewhat successful because the program tended to associate some of the samples from major groups, such as Northern Brewer, Clusters, and Hallertau. Most groups, however, overlapped others.

Cluster analysis can also be used to search for relationships in the measurements; Fig. 3 shows such a dendrogram produced with the single-link method of clustering. In this presentation, two compounds whose concentration ratio is relatively constant in all or most samples have a high similarity value and will connect near the left side of the dendrogram. Some of the peaks that have been identified, such as α-pinene, methyl heptanoate, myrcene, limonene, and methyl octanoate appear to be closely related, as do unknowns 35 and 89. Most of the other compounds appear to have dissimilar behavior. Cluster analysis of measurements may provide information about compounds that are related by common biosynthetic pathways. This information may aid in the identification of unknown peaks, since peaks that cluster together may be of the same compound type or of similar structure.

Feature Selection

Through various selection techniques (13), the number of measurements or peaks used was reduced from the original 117. This reduction was intended to reduce the complexity of the data set but still retain the information necessary for the various classifications. Such simplification is mandatory for techniques that do not permit valid results with highly correlated variables (12) or with more measurements than samples. For most multivariate methods, there should be at least three times as many samples as measurements made on each sample (13). The selection technique

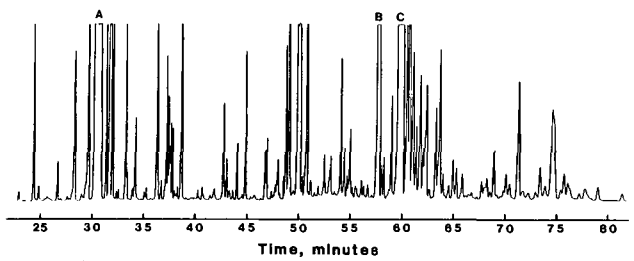

Fig. 1. Typical hop oil chromatogram. A = myrcene, B = caryophyllene, C = humulene.

TABLE I
Designation of the Peaks Observed in the Chromatograms of Hop Oils

Order of Elution	Chromatographic Peak[a]	Order of Elution	Chromatographic Peak[a]
1	U1	61	U42
2	U2	62	U43
3	Methyl hexanoate	63	U44
4	Pinene	64	U45
5	U3	65	U46
6	1-Butanol, 2-methyl-1-propanoate	66	U47
		67	U48
7	B-Pinene	68	Caryophyllene
8	U4	69	U49
9	Myrcene	70	U50
10	U5	71	Farnesene
11	U6	72	U51
12	U7	73	Humulene
13	Methyl heptanoate	74	U52
14	Limonene	75	U53
15	U8	76	U54
16	Ocimene	77	U55
17	U9	78	U56
18	Methyl octanoate	79	U57
19	2-Nonanone	80	U58
20	U10	81	U59
21	U11	82	U60
22	Linalool	83	U61
23	U12	84	U62
24	U13	85	U63
25	Methyl octanoate?	86	U64
26	U14	87	U65
27	U15	88	U66
28	U16	89	U67
29	U17	90	U68
30	U18	91	U69
31	U19	92	U70
32	U20	93	U71
33	Terpineol	94	U72
34	U21	95	U73
35	Methyl non-5-enoate	96	U74
36	Methyl nonanoate	97	U75
37	U22	98	U76
38	U23	99	Humulene epoxide
39	Geraniol	100	U77
40	2-Decanone	101	U78
41	U24	102	U79
42	U25	103	U80
43	U26	104	U81
44	U27	105	U82
45	U28	106	Humulenol
46	2-Undecanone	107	U83
47	U29	108	U84
48	U30	109	Humuladienone
49	U31	110	U85
50	U32	111	U86
51	U33	112	U87
52	U34	113	U88
53	U35	114	U89
54	U36	115	U90
55	U37	116	U91
56	U38	117	U92
57	U39		
58	U40		
59	2-Dodecanone		
60	U41		

[a] U + number = unknown compound.

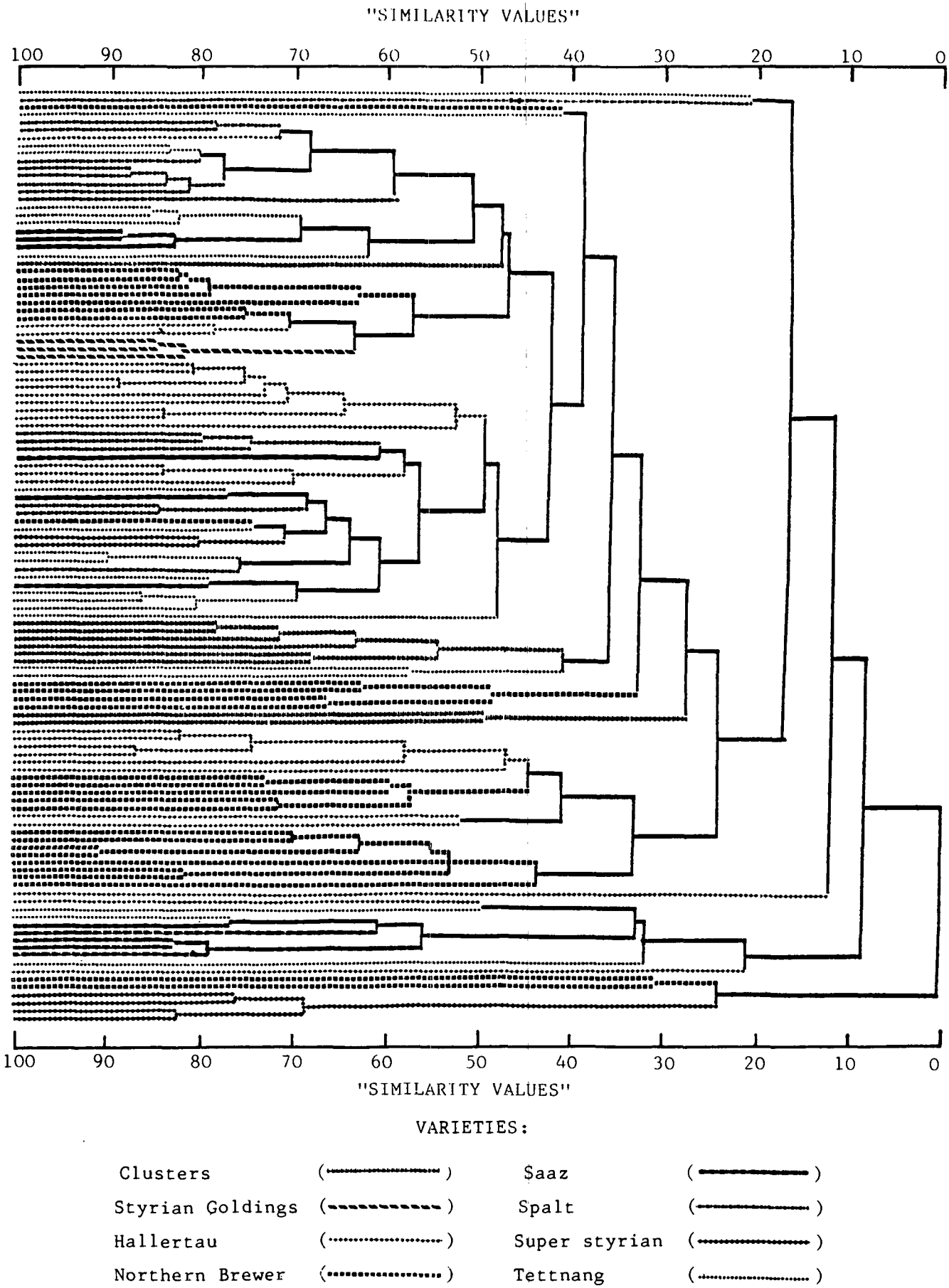

Fig. 2. Cluster analysis of samples (ARTHUR).

in ARTHUR was used to choose unrelated (orthogonal) peaks, which had the highest tendency to separate the samples into the specified variety groups (2,5). In the ARTHUR manual, this tendency is called "correlation to property weighting"; peaks were selected until the normal cut-off tolerance (0.001) was reached. This resulted in a reduction to 54 peaks.

Discriminant Analysis

Stepwise discriminant analysis employs a different type of selection than that used for ARTHUR (4,13). In this procedure, the groups to which samples are thought to belong are specified, and the program selects the measurement that best separates the samples into those groups. The measurement that best improves the classification is then added. (This measurement is added to the first selected.) Measurements are added singly in this manner either until satisfactory separation has been obtained or until the error term becomes large (12,13). The canonical variable plot in Fig. 4 was obtained with the BMDP7M stepwise discriminant analysis program (4), using the normal assumptions (F-ratio to enter a new variable = 4.0; F-ratio to remove a variable = 3.996. Included here are results for seven hop varieties for which we had significant

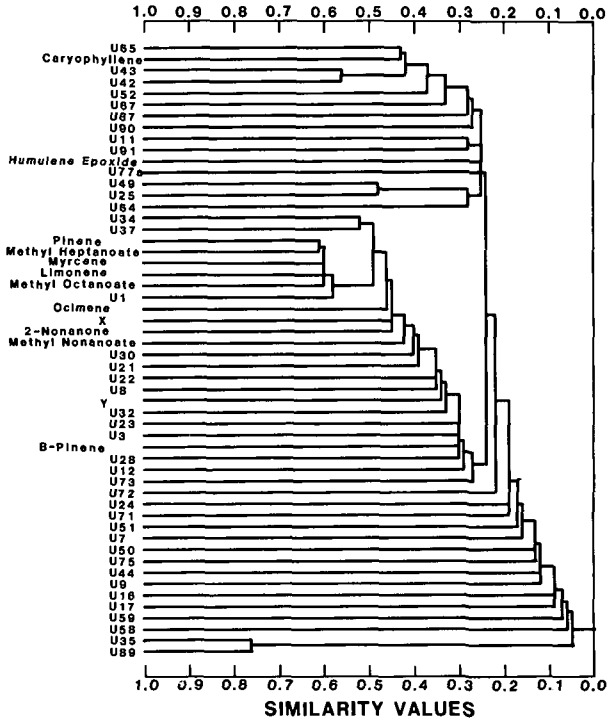

Fig. 3. Cluster analysis of measurements (ARTHUR). X = methyl non-5-enoate, Y = 1-butanol, 2-methyl-1-propanoate.

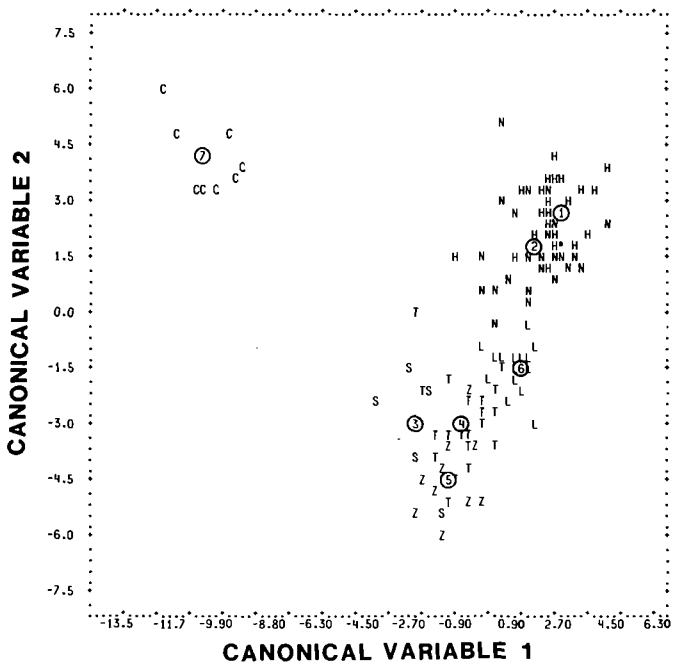

Fig. 4. Stepwise discriminant analysis with Cluster samples included (BMDP7M). C = Clusters, Z = Saaz, S = Styrian, H = Hallertau, L = Spalt, T = Tettnang, N = Northern Brewer.

TABLE II
Peaks Selected by Stepwise Discriminant Analysis (BMDP7M) for Classification into Varietal Groups

With Clusters	Without Clusters
U39	1-Butanol, 2-Methyl-1-Propanoate
U58	Linalool
U29	U21
U40	Methyl Nonanoate
U26	U36
U9	U37
U91	Caryophyllene
Humulenol II	Farnesene
U76	U51
U46	U69
U65	U79
U68	U80
	U82
	U83

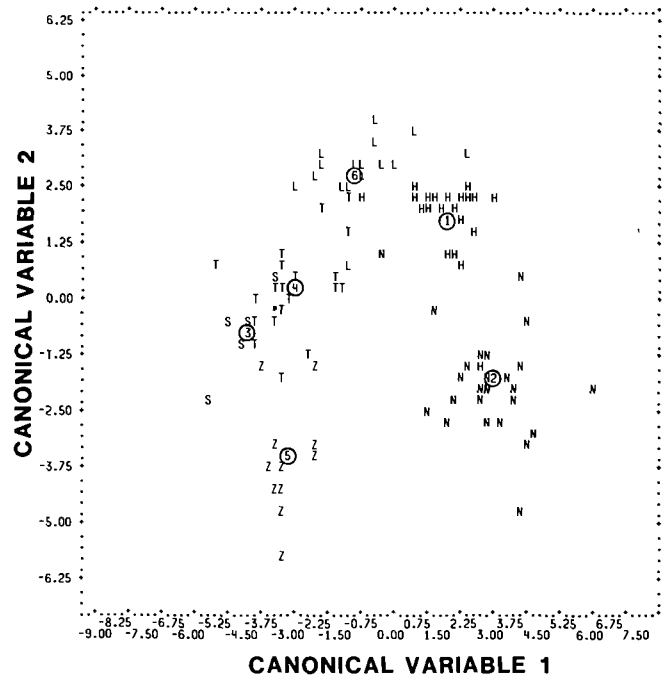

Fig. 5. Stepwise discriminant analysis without Clusters (BMDP7M). T = Tettnang, H = Hallertau, N = Northern Brewer, Z = Saaz, L = Spalt, S = Styrian.

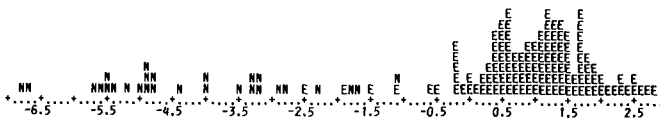

Fig. 6. Stepwise discriminant analysis, varieties assigned to place of growth (BMDP7M). E = hops grown in Europe, N = hops grown in North America.

numbers of samples. The program was able to establish a set of classification rules that easily separated the North American variety Clusters from the European varieties. These varieties might be better separated than they appear to be, because a 12-dimensional factor space projected into only two dimensions is represented. In Fig. 5, the samples of Clusters were removed from the data set. The program was then able to establish a set of rules showing improved separation of the European varieties. Table II gives the two lists of peaks selected by stepwise discriminant analysis as important for differentiating between the varieties. The two lists do not contain a single common peak, which means that an entirely different set of rules was chosen to discriminate between the varieties when the Clusters samples were and were not included. For discriminant analysis and for the other pattern-recognition techniques, knowing the identity of the peaks is not a requirement and offers no advantage.

The histogram in Fig. 6 shows the result of stepwise discriminant analysis of all 148 samples. Included are some samples of typical European varieties (Hallertau, Tettnang, and Styrian) that were grown in North America. The varieties were first assigned to groups according to their actual place of growth, and the program was instructed to separate the hops grown in North America from the hops grown in Europe. A separation with a small degree of overlap was obtained, indicating that the program had some difficulty establishing a set of rules that would separate the two groups. The histogram in Fig. 7 shows the result of stepwise discriminant analysis when the European-type samples grown in North America were assigned to the European class. The program had no difficulty in separating the two classes. The two separate lists of peaks chosen are shown in Table III, and peaks common to both lists are marked with superscripts. The unmarked peaks in the top list are presumably determined genetically and characterize the differences between the variety groups normally grown in Europe and in North America. The unmarked compounds in the bottom list are highly influenced by the place of growth.

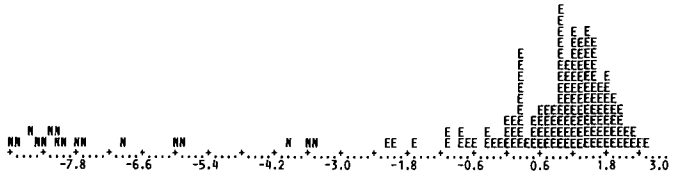

Fig. 7. Stepwise discriminant analysis, North American-grown European varieties assigned to European class (BMDP7M). E = European varieties, N = hops grown in North America.

Bayesian Classification

With the ARTHUR program, the results of many classification methods can be displayed in the form of a misclassification matrix like the one in Fig. 8. If all of the samples had been correctly classified, the squares running diagonally would have had 100% in them. Bayesian classification compares frequency distributions for each measurement in each class (13,21). Conceptually, this is equivalent to preparing histograms in which the number of samples in each of a number of subdivisions of the concentration range covered are depicted for each class. Two of these histograms are overlayed at a time to determine whether the distributions overlap or are mutually exclusive. The ARTHUR Bayesian-classification routine, using unsmoothed histograms (2,5), achieved an average percentage of correct classification of only 31.6, indicating that this procedure was unsuccessful in developing classification rules for these samples.

TABLE III
Peaks Selected by Stepwise Discriminant Analysis (BMDP7M) for Classification into North American and European Groups

Hop Varieties Assigned to	Selected Peaks
North American or European type[a]	Myrcene, U8, U11, U14, U21, U26,[b] U31, U38,[b] U40, U46,[b] U55,[b] U56, U64, U65, U67, U74, U76,[b] Humulenol II, U91
Actual place of growth	U9, U13, Methyl octanoate, U22, U23, 2-decanone, U24, U26,[b] 2-undecanone, U37, U38,[b] U46,[b] U47, U55,[b] U73, U76,[b] U77

[a] Includes European varieties grown in North America.
[b] Peaks in common.

COMPUTED CLASS

TRUE CLASS	1	2	3	4	5	6	7	8
1	26.9	0.	15.4	0.	0.	3.8	19.2	34.6
2	0.	11.1	11.1	0.	0.	0.	33.3	44.4
3	10.7	3.6	46.4	0.	3.6	7.1	14.3	14.3
4	0.	0.	33.3	66.7	0	0	0	0
5	0.	0.	0.	0.	0.	16.7	83.3	0.
6	13.6	4.5	9.1	0.	0.	13.6	45.5	13.6
7	0.	0.	9.1	0.	0.	18.2	54.4	18.2
8	6.7	0.	13.3	0.	6.7	6.7	33.3	33.3

Fig. 8. Misclassification matrix from Bayesian classification (ARTHUR).

COMPUTED CLASS

TRUE CLASS	1	2	3	4	5	6	7	8
1	80.8	0.	3.8	0.	0.	11.5	0.	3.8
2	0.	77.8	0.	0.	11.1	11.1	0.	0.
3	14.4	0.	60.7	3.6	0.	3.6	3.6	14.3
4	0.	0.	0.	100	.0	0.	0.	0.
5	0.	0.	0.	0.	66.7	16.7	0.	16.7
6	0.	0.	0.	0.	0.	81.8	4.5	13.6
7	0.	0.	0.	0.	0.	18.2	72.7	9.1
8	0.	0.	0.	0.	0.	26.7	0.	73.3

Fig. 9. Misclassification matrix from K-nearest neighbor analysis (K = 1) (ARTHUR).

Nearest-Neighbor Analysis

K-nearest-neighbor analysis is another technique based on the distance between samples in multidimensional space (13,21). The class of the K-nearest samples is determined for each sample. The classification of the test sample is then assigned according to the class of most of its nearest neighbors. In the ARTHUR procedure used (1,5), results were calculated for the 1-, 3-, 4-, 5-, 6-, 7-, 8-, 9-, and 10-nearest neighbors. Of these, results for the 1-nearest neighbor gave the highest average percentage of correct classification, 76.7, (Fig. 9).

SIMCA

Most of the multivariate analysis methods described above operate at what has been called pattern-recognition level 1 (1). At this level, results simply show which sample class a given sample most resembles. No information indicates that a particular sample is intermediate between two defined groups (a hybrid, for instance) or that it is an outlier (an erroneous result or a member of a previously unrepresented class). Programs that operate at pattern-recognition level 2 can detect such occurrences.

COMPUTED CLASS

TRUE CLASS	1	2	3	4	5	6	7	8
1	96.2	0.	3.8	0.	0.	0.	0.	0.
2	0.	100	0.	0.	0.	0.	0.	0.
3	3.6	0.	96.4	0.	0.	0.	0.	0.
4	0.	0.	0.	100	.0	0.	0.	0.
5	0.	0.	0.	0.	100	0.	0.	0.
6	0.	0.	4.5	0.	0.	81.8	0.	13.6
7	0.	0.	0.	0.	0.	0.	90.9	9.1
8	0.	0.	0.	0.	0.	0.	0.	100

Fig. 10 Misclassification matrix from SIMCA principal components method (ARTHUR).

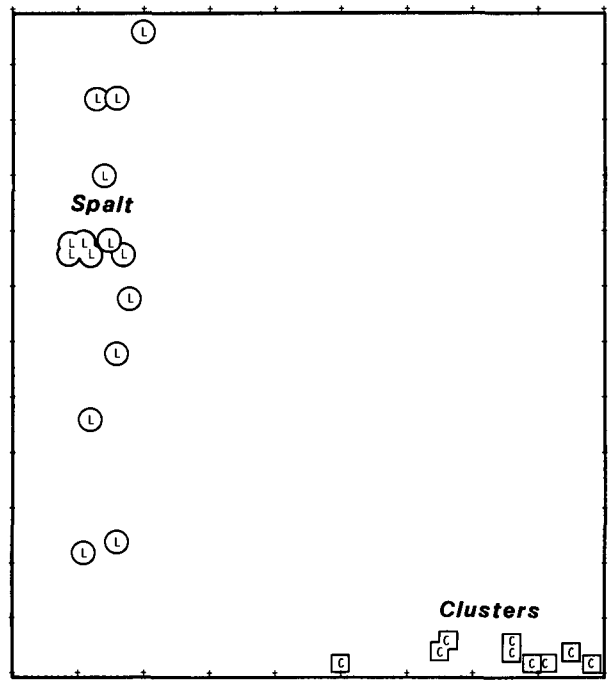

Fig. 12. Principal components extracted from sample classes shown (SIMCA-3B). *x*-axis = eigenvector 1, *y*-axis = eigenvector 2.

COMPUTED CLASS

TRUE CLASS	1	2	3	4	5	6	7	8
1	100	0.	0.	0.	0.	0.	0.	0.
2	0.	100	0.	0.	0.	0.	0.	0.
3	0.	0.	100	0.	0.	0.	0.	0.
4	0.	0.	0.	100	0.	0.	0.	0.
5	0.	0.	0.	0.	100	0.	0.	0.
6	0.	0.	0.	0.	0.	100	0.	0.
7	0.	0.	0.	0.	0.	0.	100	0.
8	0.	0.	0.	0.	0.	0.	0.	100

Fig. 11. Misclassification matrix from SIMCA-Jacobi method (ARTHUR).

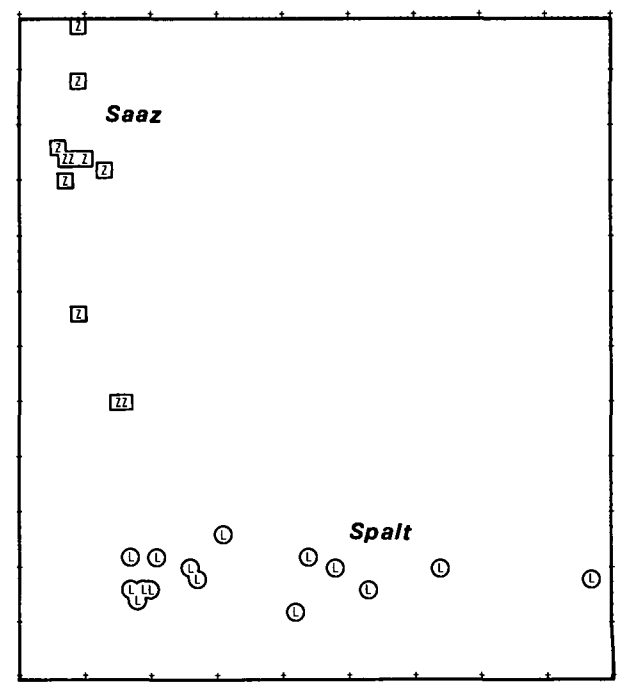

Fig. 13. Principal components extracted from sample classes shown (SIMCA-3B). *x*-axis = eigenvector 1, *y*-axis = eigenvector 2.

SIMCA operates at pattern-recognition level 2 (1,23,24). With SIMCA, each class is separately examined. The procedure excludes each sample in the class one at a time and computes principal components and the degree of scatter for the class. From this information, it is possible to determine whether a sample falls within the space defined by that class.

ARTHUR contains two earlier versions of SIMCA, SIMCA Principal Components and SIMCA-Jacobi method (2,5). The earlier versions were highly successful in classifying the hop oil samples, achieving 96% (Fig. 10) and 100% (Fig. 11) correct classifications, respectively. Unfortunately, the versions of SIMCA in ARTHUR cannot be displayed graphically.

Graphs can be prepared with the newer SIMCA-3B program (22). Fig. 12 shows an example of principal components extracted from sample classes with SIMCA-3B. This program can project results for any samples into two dimensions defined by the principal components for two of the sample classes. SIMCA-3B easily separated the Spalt samples from the Clusters, and the Saaz samples from the Spalt samples (Fig. 13). Two of the Tettnang samples appear to be outliers (Fig. 14). These samples may have been incorrectly identified originally, or the data-reduction program may have mismatched some of the peaks in the chromatogram. The Hallertau group was not well-separated from the Northern Brewer group (Fig. 15). This is not surprising because these two varieties are grown in the same region. Confusion or mixing in the fields, as well as at several stages in the handling process, can thus occur. In Fig. 16, the Hallertau samples are shown to be reasonably well separated from the Tettnangs.

A summary of the success rates obtained with the different methods of ARTHUR classification is given in Table IV. The most successful were the SIMCA-Jacobi technique (100%) and the SIMCA principal-components method (96%). Discriminant analysis was successful 80% of the time, nearest-neighbor analysis with 1-nearest neighbor 77%, and Bayesian classification only 32%. The success rate for the BMDP stepwise discriminant analysis program was higher than that for the ARTHUR routine just mentioned.

In our experience with these data and with beer aroma volatiles results (14), SIMCA usually has been more successful than any

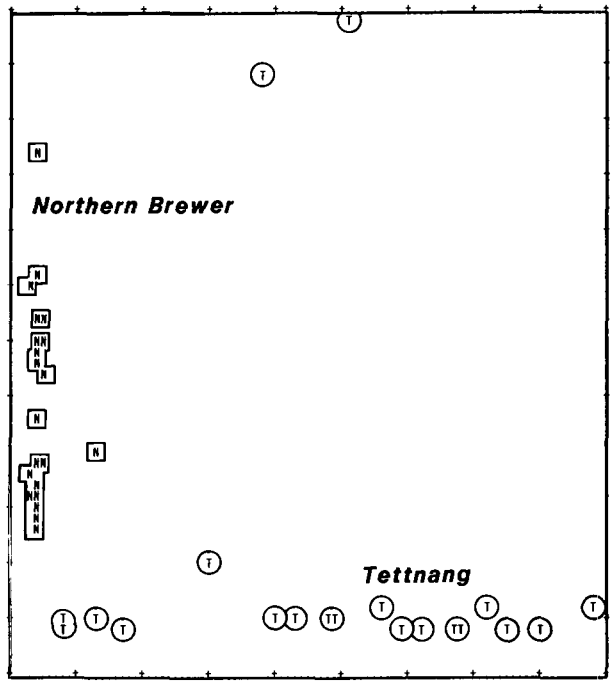

Fig. 14. Principal components extracted from sample classes shown (SIMCA-3B). *x*-axis = eigenvector 1, *y*-axis = eigenvector 2.

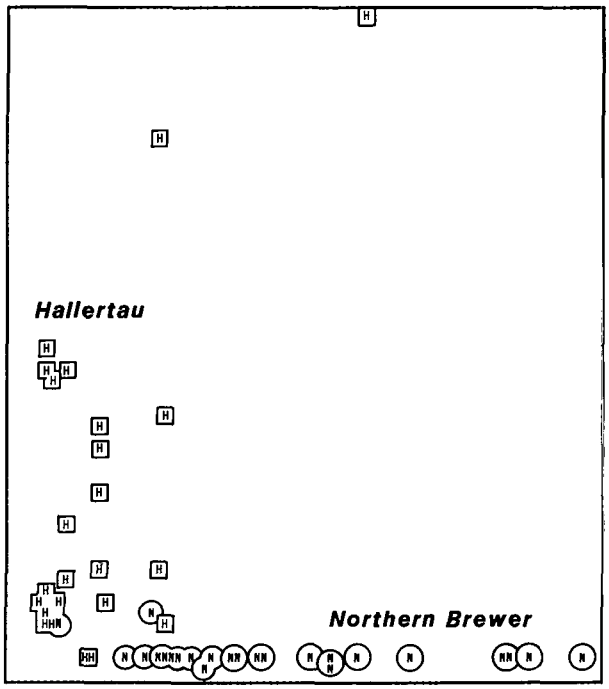

Fig. 15. Principal components extracted from sample classes shown (SIMCA-3B). *x*-axis = eigenvector 1, *y*-axis = eigenvector 2.

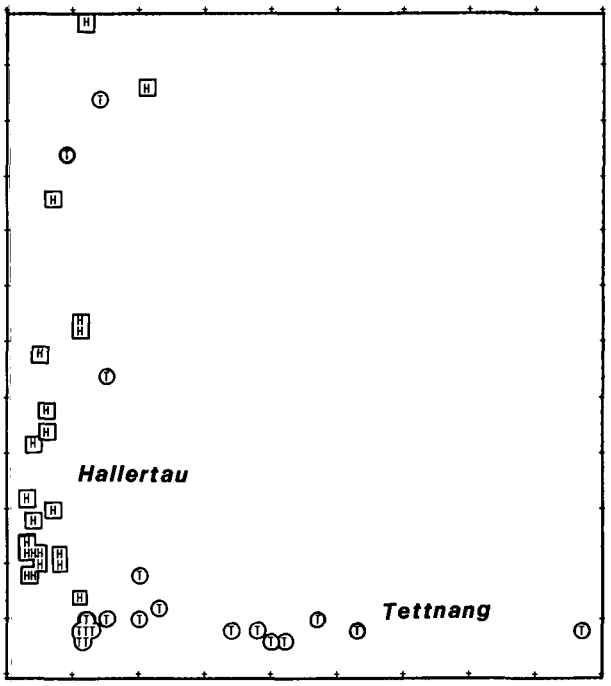

Fig. 16. Principal components extracted from sample classes shown (SIMCA-3B). *x*-axis = eigenvector 1, *y*-axis = eigenvector 2.

TABLE IV
Summary of Results Obtained with Multivariate Methods Tested

Classification Method	Percent Success
Discriminant analysis	80
Nearest-neighbor analysis	77
Bayesian classification	32
SIMCA principal component	96
SIMCA-Jacobi method	100

other procedure for establishing classification rules that separate sample groups. The mathematical sophistication of the method probably contributes to its success.

CONCLUSION

This paper was written to demonstrate the capabilities of available multivariate analysis techniques. The results given here may not be universally applicable. For universal solutions to be found, the chromatographic techniques used must be screened for ruggedness of peak separation, and the peaks chosen should contain a single predominant compound. Despite these limitations, classification rules obtained either with SIMCA or with discriminant analysis should aid in assigning hop samples to the correct variety based on their oil patterns. The original 117 peaks could be reduced to as few as 12–13 peaks that still retained the necessary discriminating information. This selection of the peaks was independent of peak size; in fact, some of the smaller peaks had high discriminating power. Such information can guide priorities for further peak identification and can simplify handling of data. The programs also gave insight into the compounds that characterize similarities and differences of North American and European varieties as well as the compounds that indicate where a sample was grown.

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