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Multiple Discriminant Analysis in the Analytical Differentiation of Venetian Wines. 3. A Reelaboration with Addition of Data from Samples of 1979 Vintage Prosecco Wine

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Nineteen samples of Prosecco white wine, vintage of 1979, were analyzed for sodium, potassium, calcium, magnesium, chloride, pH, total acidity, phosphorus, ash content, and alkalinity of the ashes. Some parameters showed relevant differences with respect to the 1977 data. The new data were added to that already in our file, and the multiple discriminant analysis for the differentiation of the white wines Soave Classico, Prosecco di Conegliano-Valdobbiadene, and Verduzzo del Piave was applied. The apparent error rate obtained with all the parameters in the analysis was about 8.2%. Classification was improved (apparent error rate 6.6%) when the parameters calcium, alkalinity of the ashes, and ash content were excluded from the analysis. In this case the expected actual error rate, obtained by the jackknife procedure, was 9.8%. The number of parameters to be used can be reduced to five, without an error increment, or to four, with an apparent error rate of about 10%.

In previous papers, it was shown that the problem of differentiation of wines by means of analytical parameters can be successfully dealt with by statistical treatment of the experimental data according to the method of discriminant analysis (Moret et al., 1980; Scarponi et al., 1981). In such a method, the parameters are combined into one or more linear functions (the discriminant functions), the coefficients of which are computed to maximize the separation between the groups of different wine samples and to minimize the spread within the groups. Once the discriminant functions are obtained, it is possible to calculate values of them for each sample examined (discriminant scores) and, from these, to carry out the differentiation and the classification of the wines. The optimal combination of several parameters allows one to obtain a better exploitation of discriminant information contained in the data and to gain real means of differentiating the wines according to their origin.

Previous treatments of experimental data concerning the Venetian white wines Soave Classico, Prosecco di Conegliano-Valdobbiadene, and Verduzzo del Piave, vintage of 1977, showed that the three wines could be differentiated both in two by two comparisons, by binary discriminant analyses (Moret et al., 1980), and in a ternary comparison, by multiple discriminant analysis (Scarponi et al., 1981). The following analytical determinations were utilized as discriminating parameters: sodium, potassium, calcium, magnesium, chloride, pH, total acidity (TA), phosphorus, ash content (AC), and alkalinity of the ashes (AA).

The values of the selected parameters can, however, change significantly from year to year, especially as a consequence of climatic and environmental factors. Then, to obtain the discriminant functions that can be valid through the years, it is necessary to introduce into the analysis the data obtained from several years, so as to weigh the parameters according to their variability through the years.

In this paper we report the results of the analytical measurements mentioned above and performed on samples of Prosecco wine, vintage of 1979. Some significant differences from the previously obtained results (vintage of 1977) are evidenced. The new data were added to that already in our file, and the multiple discriminant analysis was repeated. Discriminant and classification function coefficients are reported, and the differentiation of the wines is evaluated.

EXPERIMENTAL SECTION

Collection and Analysis of Samples. Nineteen samples of Prosecco wine, vintage of 1979, were collected in the production zone from lots for which genuineness and typicality were guaranteed and certified by the Italian D.O.C. (Denominazione di Origine Controllata—Certified Brand of Origin) brand. This brand is obtained only by producers that follow the production disciplinary rules ("Gazzetta Ufficiale della Repubblica Italiana", 1969). These rules prescribe that, in order to be classified as D.O.C., the wine, in addition to being judged by its orga-

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Table I. Analytical Results Relative to 1979 Samples of Prosecco Wine: Comparison with 1977 Data

parameter	mean values ± 95% CI[a] (±95% TI[b])			F ratio
	1977 vintage[c]	1979 vintage[d]	1977 and 1979 vintages	
sodium, mg/L × 10^{-1}	1.3 ± 0.5 (±2.0)	1.8 ± 0.6 (±2.7)	1.6 ± 0.4 (±2.2)	1.6
potassium, mg/L × 10^{-2}	7.6 ± 0.7 (±3.2)	7.4 ± 0.6 (±3.0)	7.5 ± 0.4 (±2.6)	0.2
calcium, mg/L × 10^{-2}	1.2 ± 0.1 (±0.4)	1.2 ± 0.1 (±0.5)	1.2 ± 0.1 (±0.4)	0.02
magnesium, mg/L × 10^{-1}	12.0 ± 1.1 (±5.1)	7.4 ± 0.4 (±1.8)	9.4 ± 1.0 (±5.8)	87.6
chloride, mg/L × 10^{-1}	1.4 ± 0.3 (±1.5)	2.8 ± 0.6 (±2.8)	2.2 ± 0.4 (±2.6)	17.8
pH	3.39 ± 0.04 (±0.19)	3.26 ± 0.07 (±0.35)	3.31 ± 0.05 (±0.29)	8.5
total acidity, g/L	6.5 ± 0.3 (±1.4)	5.8 ± 0.6 (±2.6)	6.1 ± 0.4 (±2.1)	4.5
phosphorus, g of P_2O_5/L × 10^1	3.8 ± 0.6 (±2.4)	1.6 ± 0.2 (±0.9)	2.6 ± 0.5 (±2.8)	83.8
ash content, g/L	2.2 ± 0.2 (±0.8)	1.8 ± 0.1 (±0.6)	2.0 ± 0.1 (±0.7)	17.1
alkalinity of the ashes, mequiv/L × 10^{-1}	2.6 ± 0.2 (±0.7)	2.0 ± 0.2 (±0.7)	2.3 ± 0.1 (±0.8)	23.3

[a] CI = confidence interval. [b] TI = tolerance interval; coverage 90% (Kendall and Stuart, 1961; Mandel, 1978). [c] 14 samples. [d] 19 samples.

noleptic qualifications and a few chemical parameters, must originate from a vineyard registered in a special bulletin and be produced in a well-defined geographical area.

Analytical measurements were performed according to the methods previously reported (Moret et al., 1980); calcium was determined by atomic absorption spectrophotometry in an air–acetylene flame (Ough et al., 1979). An Orion Research pHmeter (Model 701) and a Perkin-Elmer atomic absorption spectrophotometer (Model 5000) were used.

Discriminant Analysis. A short introduction and literature survey on the multiple discriminant analysis and its application in the field of applied analytical chemistry (particularly in food and beverage analysis) were previously reported (Scarponi et al., 1981).

Calculations were performed by using the DISCRIMINANT subprogram of the SPSS statistical package for computer (Nie et al., 1975). The direct method (all variables enter into the analysis concurrently) and stepwise methods (variables are selected for entry on the basis of their discriminating power) were used. In stepwise methods, variable selection was performed according to all the criteria available in the program (i.e., Wilks, Mahal, Maxminf, Minresid, and Rao); the obtained selection order and the classification results were compared. So that the possibility of reducing the number of variables, compatibly with an acceptable correct classification, could be studied, several discriminant analyses were performed by excluding each time one, two, three, ..., and eight parameters, respectively, beginning from the less important one in the selection orders. The classification results were then compared.

The apparent classification error rate was obtained by performing the classification of the same samples used for analysis and determining the fraction erroneously classified (Lachenbuch, 1975). The expected actual error rate (a more correct estimate of the actual error of the procedure) was obtained by the jackknife procedure (Lachenbuch, 1975). In this method, one sample at a time is excluded from the analysis and classified on the basis of the discriminant functions obtained in this way. The procedure is applied to all the samples, and the obtained erroneous classifications are a good estimate of the expected actual error rate.

RESULTS AND DISCUSSION

Analytical Results. Table I gives the results of the analytical determinations performed on the 19 samples of Prosecco wine, vintage of 1979. Mean values, 95% confidence intervals, and 95% tolerance intervals, coverage 90% (Kendall and Stuart, 1961; Mandel, 1978), are reported. In addition (i) data relative to the samples of the 1977 year (Moret et al., 1980), (ii) the results obtained by grouping the two series of data, and (iii) the values of the F ratio for the evaluation of the difference of the two means are also reported for comparison purposes. It can be seen that the most significant changes are concerned, in decreasing order, with Mg, P, AA, Cl, and AC; conversely, a remarkable constancy is observed for Ca, K, and Na.

Table II. Univariate F Ratios (One-Way Analysis of Variance Test for Equality of Group Means on a Single Discriminating Variable)[a]

parameter	F ratio
pH	36.4
AC	34.4
AA	23.4
Na	14.1
P	12.9
Cl	12.4
K	10.8
Ca	9.6
Mg	3.3
TA	1.9

[a] From data of 14 samples of wines Soave, Prosecco, and Verduzzo, vintage of 1977, and 19 samples of Prosecco wine, vintage of 1979 (degrees of freedom 2 and 58).

Analysis of Variance and Correlation. Table II gives the results of the analysis of variance performed for a simultaneous evaluation of the difference between the means of the three sample groups. Data relative to samples of Prosecco of 1979 have been grouped with those relative to samples of 1977. Comparing this table with that previously reported (Scarponi et al., 1981), one can note that the order of importance of the variables is only slightly changed. In particular, the parameter P has gained and Mg and Ca have lost, respectively, some discriminating power.

Table III gives the correlation matrix obtained from all the available data. The reported values confirm the correlations previously found, except those of calcium–magnesium and magnesium–phosphorus that result less and more significant, respectively.

Discriminant Analysis. *Direct Method.* Table IV reports the necessary information for judging how many discriminant functions obtained by the direct method should be derived. For this purpose two measures were computed. The first one is the eigenvalue (and the eigenvalue relative percentage) associated with each function. This is a measure of the relative importance of the function. The second measure is the Wilks' Λ, computed as each function is derived. This is an inverse measure of

Table III. Pooled Within-Group Correlation Matrix

	Na	K	Ca	Mg	Cl	pH	TA	P	AC	AA
Na	1.000									
K	0.036	1.000								
Ca	0.028	0.312	1.000							
Mg	0.092	0.157	0.465	1.000						
Cl	0.207	0.168	−0.039	−0.330	1.000					
pH	−0.048	0.478	0.261	0.306	−0.101	1.000				
TA	−0.046	−0.080	0.025	0.201	−0.194	−0.419	1.000			
P	−0.039	0.142	0.270	0.680	−0.177	0.387	0.185	1.000		
AC	0.069	0.619	0.351	0.457	−0.120	0.707	0.003	0.462	1.000	
AA	0.019	0.647	0.394	0.516	−0.162	0.699	−0.047	0.424	0.894	1.000

Table IV. Discriminating Power of the Discriminant Functions Obtained Using the Direct Method

Eigenvalues Associated with Functions			
discriminant function	eigenvalue	rel %	canonical correlation
1	3.60	75.4	0.885
2	1.17	24.6	0.735

Change in Wilks' Λ				
functions derived	Λ	χ^2	df[a]	significance
0	0.100	123.2	20	0.000
1	0.460	41.6	9	0.000

[a] df = degrees of freedom.

the discriminating information not already accounted for by the earlier functions.

Values reported in Table IV indicate that it is useful to derive the second (and last) discriminant function, since it contributes significantly to the ability of discriminating between the groups. In fact, (i) the eigenvalue associated to the second function contributes about 25% to the total sum of eigenvalues and (ii) the Λ computed after the first function is derived (i.e., 0.460) and its associated χ^2 test indicate that a statistically significant amount of discriminating information still exists. Consequently, the remaining computations are based on the two functions.

Results of the multiple discriminant analysis performed by the direct method are reported in Tables V and VI and in Figure 1. In the last one, points corresponding to the 1977 and 1979 Prosecco wine samples are marked by two different symbols. According to the values of the reported standardized discriminant function coefficients, one can deduce that the most significant parameters for the differentiation are pH, P, Cl, and K, in the first function, and P, AC, AA, and Na, in the second one, respectively. Conversely, parameters such as Ca, Mg, and TA give negligible contributions to both functions. Comparing the present results with those previously reported (Scarponi

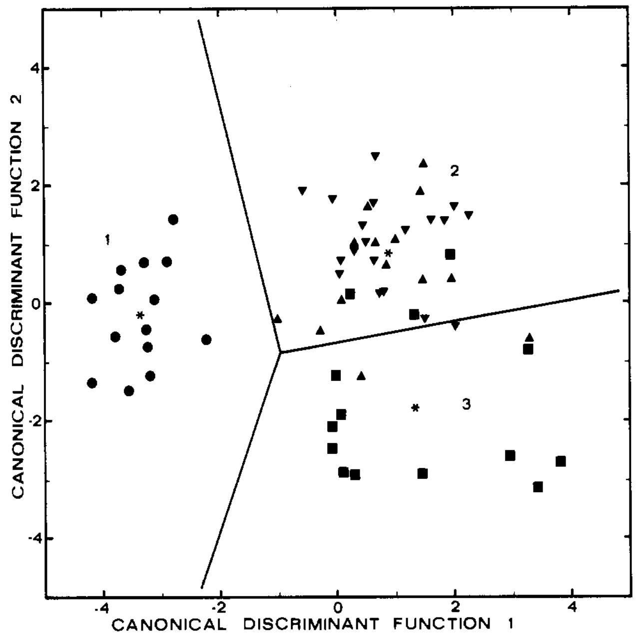

Figure 1. Graphical representation of wine groups in the discriminant plane. Area 1, Soave 1977 (●); area 2, Prosecco 1977 (▲) and 1979 (▼); area 3, Verduzzo 1977 (■). (*) Group centroids.

et al., 1981), and relative to 1977 vintage, it is possible to note that a remarkable decrease of importance is obtained for the parameters Ca and Mg, while an increased contribution to differentiation is given by Na, Cl, and, particularly, P.

As far as the classification obtained with the new functions is concerned, it is to be noted that the 1977 and 1979 samples of Prosecco are well grouped together in the discriminant space (it is not possible to recognize any subgroup in Figure 1) and that the apparent classification error (about 8.2%) can be accepted, especially if one considers that in the direct discriminant method all the analytical parameters are used and that some of these are remarkably different in the 2 years. In fact, exclusion of

Table V. Discriminant and Classification Function Coefficients Obtained with the Direct Method

	discriminant function coefficients				classification function coefficients		
	standardized		unstandardized				
variable	funct 1	funct 2	funct 1	funct 2	wine Soave	wine Prosecco	wine Verduzzo
Na	0.096	−0.517	7.19×10^{-3}	-3.87×10^{-2}	5.19×10^{-1}	5.10×10^{-1}	6.15×10^{-1}
K	−0.558	−0.410	-3.34×10^{-3}	-2.45×10^{-3}	-1.58×10^{-2}	-3.25×10^{-2}	-2.76×10^{-2}
Ca	0.153	0.115	7.12×10^{-3}	5.37×10^{-3}	1.15×10^{-1}	1.50×10^{-1}	1.39×10^{-1}
Mg	0.399	0.443	1.56×10^{-2}	1.73×10^{-2}	1.90×10^{-1}	2.74×10^{-1}	2.35×10^{-1}
Cl	0.620	0.083	5.67×10^{-2}	7.60×10^{-3}	9.00×10^{-1}	1.15	1.15
pH	0.990	0.163	7.70	1.27	6.84×10^{2}	7.18×10^{2}	7.18×10^{2}
TA	0.453	0.231	5.34×10^{-1}	2.72×10^{-1}	5.70×10^{1}	5.95×10^{1}	5.90×10^{1}
P	−0.883	−0.872	−7.20	−7.11	-1.46×10^{2}	-1.84×10^{2}	-1.68×10^{2}
AC	0.357	−0.728	1.07	−2.19	-1.21×10^{2}	-1.18×10^{2}	-1.12×10^{2}
AA	0.011	0.590	2.82×10^{-3}	1.54×10^{-1}	−3.03	−2.86	−3.26
(constant)			-2.94×10^{1}	−2.43	-1.11×10^{3}	-1.23×10^{3}	-1.24×10^{3}

Table VI. Classification Results: Direct Method

actual group	no. of samples	predicted group membership			apparent correct classification, %[a]
		Soave	Prosecco	Verduzzo	
Soave	14	14	0	0	100.0
Prosecco	33	0	31	2	93.9
Verduzzo	14	0	3	11	78.6

[a] Total apparent correct classification 91.8%.

Table VII. Selection Order of Variables in the Stepwise Methods

selection order	selection methods		
	Wilks and Minresid	Mahal and Maxminf	Rao
1	pH	AC	pH
2	P	Cl	P
3	Na	Na	Na
4	Mg	P	Mg
5	Cl	Mg	Cl
6	TA	K	TA
7	K	AA	K
8	AC	pH	AC
9	AA	TA	Ca
10	Ca	Ca	AA

a few parameters will actually lead to an improvement in the classification (see below).

Stepwise Methods and Optimization. The order by which parameters are selected for inclusion in the stepwise discriminant analyses is given in Table VII. The same order of entry is obtained with methods Wilks and Minresid and methods Mahal and Maxminf, respectively. Method Rao gives a selection order slightly different from the first one; i.e., only the order of the last two variables is reversed.

It can be seen that, as previously observed (Scarponi et al., 1981), after pH is entered, the parameters AC and AA (that, as reported in Table II, have a high discriminating power when considered singly) reach the last positions. The opposite is verified when AC is the first variable selected for entry. The existing correlations between parameters pH, AC, and AA can explain this fact.

Classification results obtained in the optimization procedures are given in Table VIII. Figure 2 shows the change in the percentage of the samples correctly classified against the number of parameters used in the discriminant analysis (selection methods Wilks and Minresid). It can be seen that deletion of some of the less important parameters (i.e., Ca, AA, and AC) leads to improved classification (93.4%). This verifies one of the major potentialities of the stepwise methods in discriminant analysis, i.e., to recognize and to delete redundant variables (Coomans et al., 1979). Moreover, one can note that it is possible to delete up to five variables (either Ca, AA, AC, K, TA, and methods Wilks, Minresid, and Rao or Ca, TA, pH, AA, K, and methods Mahal and Maxminf) without an increment in the apparent error rate. The error can be kept within 10% by using only four parameters (pH, P, Na, and Mg), while it increases up to 13.1% and 16.4%, respectively, if one use only the first three or two parameters of the previous series (methods Wilks, Minresid, and Rao).

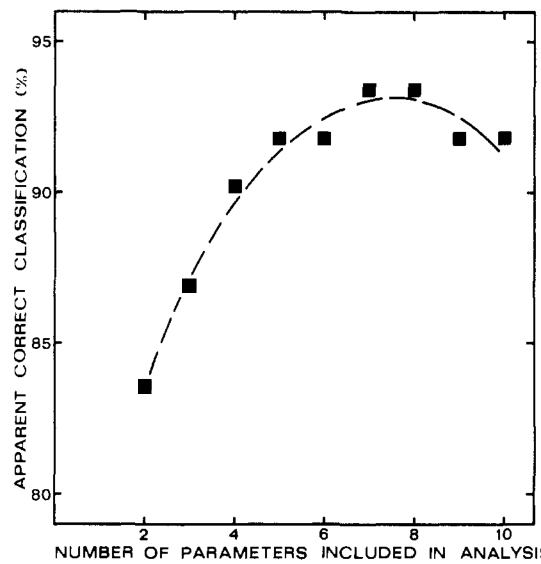

Figure 2. Percentage of samples correctly classified against the number of parameters used in the discriminant analysis (selection methods were Wilks and Minresid).

Tables IX–XI give the weight coefficients of the discriminant and classification functions obtained for the better series of seven, five, four, three, and two parameters, respectively; methods used for selection of variables and the apparent classification errors are also reported.

Finally, it is to be noted that, owing to the observed changes in some important parameters, any attempt to classify the samples analyzed here (Prosecco 1979) according to the discriminant functions previously obtained,

Table VIII. Optimization Procedure and Comparison of the Various Selection Methods[a]

selection method	included variables	apparent correct classification, %	excluded variables
Wilks and Minresid	pH P Na Mg Cl TA K AC AA	91.8	Ca
	pH P Na Mg Cl TA K AC	93.4	Ca AA
	pH P Na Mg Cl TA K	93.4	Ca AA AC
	pH P Na Mg Cl TA	91.8	Ca AA AC K
	pH P Na Mg Cl	91.8	Ca AA AC K TA
	pH P Na Mg	90.2	Ca AA AC K TA Cl
	pH P Na	86.9	Ca AA AC K TA Cl Mg
	pH P	83.6	Ca AA AC K TA Cl Mg Na
Mahal and Maxminf	AC Cl Na P Mg K AA pH TA	91.8	Ca
	AC Cl Na P Mg K AA pH	91.8	Ca TA
	AC Cl Na P Mg K AA	91.8	Ca TA pH
	AC Cl Na P Mg K	91.8	Ca TA pH AA
	AC Cl Na P Mg	91.8	Ca TA pH AA K
	AC Cl Na P	86.9	Ca TA pH AA K Mg
	AC Cl Na	82.0	Ca TA pH AA K Mg P
	AC Cl	82.0	Ca TA pH AA K Mg P Na

[a] Results for method Rao are equal to that of methods Wilks and Minresid with the exception of the first step where parameter AA rather than Ca is deleted and an apparent correct classification of 93.4% is obtained.

Table IX. Discriminant and Classification Function Coefficients Obtained Using the First Seven Parameters Selected by Methods Wilks, Minresid, and Rao (Apparent Error Rate 6.6%)

	discriminant function coefficients				classification function coefficients		
	standardized		unstandardized				
variable	funct 1	funct 2	funct 1	funct 2	wine Soave	wine Prosecco	wine Verduzzo
Na	−0.120	−0.616	-9.00×10^{-3}	-4.62×10^{-2}	1.99×10^{-1}	1.93×10^{-1}	3.11×10^{-1}
K	0.420	−0.400	2.51×10^{-3}	-2.39×10^{-2}	-1.20×10^{-1}	-1.33×10^{-1}	-1.28×10^{-1}
Mg	−0.546	0.609	-2.13×10^{-2}	2.38×10^{-2}	-5.63×10^{-2}	5.44×10^{-2}	2.75×10^{-3}
Cl	−0.638	0.064	-5.83×10^{-2}	5.89×10^{-3}	1.16	1.41	1.41
pH	−1.250	−0.015	−9.72	-1.14×10^{-1}	4.56×10^{2}	4.97×10^{2}	5.01×10^{2}
TA	−0.556	0.119	-6.56×10^{-1}	1.40×10^{-1}	4.39×10^{1}	4.58×10^{1}	4.66×10^{1}
P	0.917	−0.984	7.48	−8.02	-1.65×10^{2}	-2.03×10^{2}	-1.86×10^{2}
(constant)			3.50×10^{1}	2.53	-7.73×10^{2}	-9.10×10^{2}	-9.32×10^{2}

Table X. Discriminant and Classification Function Coefficients Obtained Using the First Five Parameters Selected with All the Methods (Apparent Error Rate 8.2%)

	discriminant function coefficients				classification function coefficients		
	standardized		unstandardized				
variable	funct 1	funct 2	funct 1	funct 2	wine Soave	wine Prosecco	wine Verduzzo
			Methods Wilks, Minresid, and Rao				
Na	−0.167	−0.631	-1.25×10^{-2}	-4.72×10^{-2}	9.26×10^{-2}	8.09×10^{-2}	1.99×10^{-1}
Mg	−0.577	0.733	-2.25×10^{-2}	2.86×10^{-2}	1.02×10^{-2}	1.20×10^{-1}	7.32×10^{-2}
Cl	−0.514	0.056	-4.70×10^{-2}	5.09×10^{-3}	1.89×10^{-1}	3.55×10^{-1}	3.83×10^{-1}
pH	−0.832	−0.120	−6.47	-9.35×10^{-1}	2.08×10^{2}	2.28×10^{2}	2.36×10^{2}
P	0.722	−1.138	5.88	−9.28	-5.61×10^{1}	-8.67×10^{1}	-7.05×10^{1}
(constant)			2.27×10^{1}	4.21	-5.10×10^{2}	-3.78×10^{2}	-4.09×10^{2}
			Methods Mahal and Maxminf				
Na	0.142	−0.573	1.06×10^{-2}	-4.29×10^{-2}	-5.43×10^{-3}	-3.51×10^{-2}	7.41×10^{-2}
Mg	0.427	0.870	1.66×10^{-2}	3.39×10^{-2}	6.67×10^{-2}	1.63×10^{-1}	1.07×10^{-1}
Cl	0.538	0.155	4.92×10^{-2}	1.42×10^{-2}	1.74×10^{-1}	3.37×10^{-1}	3.64×10^{-1}
P	−0.641	−1.186	−5.22	−9.66	6.22	-2.27×10^{1}	−7.12
AC	0.845	−0.160	2.54	-4.80×10^{-1}	1.05×10^{1}	1.72×10^{1}	2.13×10^{1}
(constant)			−6.05	1.43	-1.38×10^{1}	-2.57×10^{1}	-3.87×10^{1}

Table XI. Discriminant and Classification Function Coefficients Obtained Using the First Four, Three, and Two Parameters Selected by Methods Wilks, Minresid, and Rao (Apparent Error Rate 9.8, 13.1, and 16.4, Respectively)

	discriminant function coefficients				classification function coefficients		
	standardized		unstandardized				
variable	funct 1	funct 2	funct 1	funct 2	wine Soave	wine Prosecco	wine Verduzzo
			First Four Parameters				
Na	−0.348	−0.601	-2.61×10^{-2}	-4.50×10^{-2}	1.32×10^{-1}	1.54×10^{-1}	2.78×10^{-1}
Mg	−0.378	0.728	-1.48×10^{-2}	2.84×10^{-2}	-2.57×10^{-2}	5.23×10^{-2}	6.81×10^{-4}
pH	−0.939	−0.075	−7.30	-5.84×10^{-1}	2.07×10^{2}	2.28×10^{2}	2.36×10^{2}
P	0.710	−1.168	5.79	−9.52	-5.37×10^{1}	-8.24×10^{1}	-6.58×10^{1}
(constant)			2.40×10^{1}	3.21	-3.09×10^{2}	-3.72×10^{2}	-4.02×10^{2}
			First Three Parameters				
Na	−0.456	−0.501	-3.42×10^{-2}	-3.75×10^{-2}	1.26×10^{-1}	1.66×10^{-1}	2.78×10^{-1}
pH	−0.982	0.226	−7.63	1.76	2.07×10^{2}	2.29×10^{2}	2.36×10^{2}
P	0.394	−0.943	3.21	−7.68	-5.73×10^{1}	-7.51×10^{1}	-6.57×10^{1}
(constant)			2.47×10^{1}	−2.57	-3.08×10^{2}	-3.72×10^{2}	-4.02×10^{2}
			First Two Parameters				
pH	1.072	0.166	8.33	1.29	2.07×10^{2}	2.28×10^{2}	2.35×10^{2}
P	−0.568	0.924	−4.63	7.53	-5.76×10^{1}	-7.56×10^{1}	-6.64×10^{1}
(constant)			-2.59×10^{1}	−6.72	-3.07×10^{2}	-3.69×10^{2}	-3.95×10^{2}

and from data relative to samples of 1977 only (Scarponi et al., 1981), would lead to misclassification in most cases. Then, practical applications of the reported functions to unknown samples are to be performed, for the present, with caution. So that conclusive results can be obtained, further study is necessary, in order to acquire analytical data from several vintage years, possibly by extending the determinations to organic and aroma compounds of wines.

Estimate of Expected Actual Error Rate. As mentioned previously (Scarponi et al., 1981), the apparent error rate (used until now) is an optimistic estimate of the expected actual error rate [see also Lachenbuch (1975)]. For determination of the present difference between the two errors, the last one was estimated according to the jackknife procedure for the classification rule obtained by using the first eight parameters selected by the Wilks method (apparent error 6.6%). The new calculation has led to misclassification of 6 cases in 61, with an error of 9.8%. This result confirms the existence of a difference from the apparent and the actual errors, but is also shows that this difference is relatively small.

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Thermal Stability of Trypsin Inhibitor Activity in Winged Bean (*Psophocarpus tetragonolobus*)

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The thermal stability of trypsin inhibitor activity (TIA) in six varieties of winged beans was investigated. The TIA in winged bean meals was extremely resistant to dry heat treatment. Prolonged cooking (45–60 min) of the whole bean was required to reduce the TIA substantially. Autoclave treatment at 120 °C, 1.05 kg/cm^2, is, however, very effective in destroying TIA in the beans or bean meals. There are significant varietal variations in the effectiveness of autoclave inactivation of winged bean TIA. The TIA in the winged bean meal extracts is, however, heat labile.

The winged bean (*Psophocarpus tetragonolobus*) is indigenous to the humid tropics. Its exceptional agronomic and nutritional peculiarities have been well documented (National Academy of Sciences, 1975; Masfield, 1973; Claydon, 1975). All parts of the plant can be consumed. The bean protein content ranges from 28.8% to 42.5%, with 13.6% to 21.4% oil (Masfield, 1973; Khor et al., 1980). Analysis of the bean protein indicated a more desirable amino acid composition than for most legumes. Raw winged beans are toxic to rats and have a low digestibility (Jaffe and Korte, 1976). Cerny et al. (1971) reported that the nutritive quality of winged bean protein, as assessed by the PER and NPU values, compared well with that of soybean. Other trial experiments have also confirmed the promising properties of the winged bean as livestock feeds (Wong, 1975).

Early studies on the winged bean have indicated the presence of protease inhibitor activity (Sohonie and Bhandarkar, 1954). Several trypsin inhibitors and a chymotrypsin inhibitor have been isolated and purified from the winged bean (Kortt, 1979, 1980; Tan el al., 1979; Chan and de Lumen, 1982a). Trypsin inhibitor activity of the winged beans ranges from 18 500 to 41 000 IU/g of bean (Khor et al., 1980).

The nutritional significance of plant trypsin inhibitors has been extensively investigated (Liener and Kakade, 1980). High levels of trypsin inhibitor activity (TIA) stimulate pancreatic juice secretion and cause pancreatic hypertrophy and growth inhibition. The evidence of the antinutritional role of winged bean TIA came from the studies of Chan and de Lumen (1982b). They demonstrated that feeding rats with casein plus isolated winged bean trypsin inhibitors caused pancreatic hypertrophy and growth inhibition.

Cerny et al. (1971) reported that winged bean TIA could be destroyed easily by moist heat treatment. Jaffe and Korte (1976), however, demonstrated that autoclaved winged beans could still induce pancreatic hypertrophy. Presumably, the TIA in the winged bean variety used by the latter authors is particularly heat resistant and that autoclave treatment could not abolish its deleterious effects.

A cosmopolitant collection of winged bean varieties at the Agricultural University of Malaysia afforded the opportunity for evaluation of the varietal differences of winged bean TIA. This paper reports the results of our investigation on the thermal stability of TIA in six varieties of winged beans grown on the experimental farm at the Agricultural University of Malaysia.

MATERIALS AND METHODS

Materials. Winged beans of varieties 207, 046, 185, 100, 141, and 095 were grown locally at the experimental farm of Agricultural University of Malaysia. Soybeans were obtained from commercial outlets in Kuala Lumpur. Winged bean meals were prepared by grounding the mature beans manually with a pestle and mortar, and the fine powder was stored below 0 °C in a glass container before use. Bovine trypsin and *N*-benzoyl-DL-arginine-*p*-nitroanilide (BAPNA) were purchased from Sigma Chemicals. *p*-Nitrophenyl *p*-guanidinobenzoate hydrochloride was from Merck. All other chemicals are of analytical reagent grade and were purchased from Sigma Chemicls and Merck.

Determination of the Purity of Trypsin. The purity of the commercial trypsin was determined by the active-site titration method of Chase and Shaw (1967), using *p*-nitrophenyl *p*-guanidinobenzoate as the titrant. Gen-

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