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# Classification of Variety Musts by Statistical Analysis of their Electrophoretic Protein Pattern

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*ABSTRACT*

*Unsupervised classification techniques (Principal Components Analysis and Cluster Analysis) have been applied to electrophoretic analytical data from seventeen grape must samples of six different cultivars. A good grouping of samples according to their cultivar has been obtained.*

## INTRODUCTION

The characterization of wines and musts according to their geographical origin, the variety of grape from which they proceed, the types of wine, and so on, is very interesting and is the subject of many papers published during recent years (Martin-Alvarez *et al.*, 1987; Larrechi *et al.*, 1987).

A review of papers up to 1986, in which statistical methods for data processing had been applied to variety characterization, has recently been published (Cabezudo *et al.*, 1986). Cáceres (1987) and Martin-Alvarez *et al.* (1987) also applied statistical methods to data variables of variety musts. The variables had been obtained by chemical analysis and gas and liquid chromatographic analysis. Protein fraction data have not been used in any of these works or subsequent ones although it is known that protein fractions differ with variety (Koch & Sajak, 1959; Bayly & Berg, 1967; Radola *et al.*, 1967; Drawert & Gorg, 1974; Wolfe, 1976; Yokotsuka *et al.*, 1977; Correa *et al.*, 1988).

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The protein fraction is not influenced by the nature of the soil or by the climate, but it is genetically defined. Part of the protein fraction of the grape remains in the must and in the wine in spite of the processes used in order to eliminate proteins (Somers & Ziemelis, 1973; Heatherbell *et al.*, 1985).

In this work, two multivariate statistical methods will be applied to the data obtained by electrophoretic analysis of variety grape musts in order to test the fitness of this information for their characterization.

## MATERIALS AND METHODS

### Must samples

Seventeen grape musts of six different varieties and from various areas of Spain, have been studied. These musts were obtained in the laboratory by slight pressing. They were centrifuged at 4000 *g*, for 15 min and were kept at a temperature of −20°C until analyzed.

### Protein purification and concentration

The musts were dialyzed against tap water during 18 h in Spectra POR 3 membranes which retain molecules of a molecular weight of more than 3500 daltons. They were then concentrated 40 times by introducing dialysis bags in a 20 M polyethylene glycol solution, at 20 percent.

### Polyacrylamide gel electrophoresis

According to Hillier (1976) this was carried out in polyacrylamide (180 × 140 × 0·7 mm) of $T = 9{\cdot}4\%$ and $C = 4{\cdot}25$. To stain the plates, tinctures with Blue Coomassie G-250 (Blakesley & Boezi, 1977) and silver nitrate (Merril *et al.*, 1981) were used.

The densitometric measures were carried out at 600 nm with Shimadzu equipment composed of a spectrophotometer (Chromato Scanner CS-930) as well as an integration and graphic impression system (Data Recorder DR-2).

### Data processing

The statistical method used for the data processing was Principal Component and Cluster Analysis.

The BMDP4M program (Dixon, 1983) has been used for Principal Component Analysis. Cluster Analysis was carried out by using the

**TABLE 1**

Percentual Distribution of the Bands Separated by PAGE and Stained with Coomassie Blue G-250 for Grape Musts

| *Grape varieties* | | *Mobility* | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | *0·09* *I* | *0·13* *II* | *0·18* *III* | *0·30* *IV* | *0·35* *V* | *0·39* *VI* | *0·43* *VII* | *0·46* *VIII* | *0·51* *IX* | *0·55* *X* | *0·60* *XI* | *0·63* *XII* | *0·65* *XIII* | *0·70* *XIV* |
| White grapes | | | | | | | | | | | | | | | |
| Airen ($n = 4$) | $\bar{X}$ | — | — | — | — | 26·6 | 34·3 | — | 12·3 | 9·8 | 2·7 | — | — | — | 12·5 |
| | $s$ | — | — | — | — | 4·7 | 5·0 | — | 2·0 | 4·0 | 3·4 | — | — | — | 1·7 |
| Moscatel ($n = 2$) | $\bar{X}$ | 0·5 | 1·5 | — | — | 19·7 | — | 21·6 | 5·1 | 9·7 | 3·7 | — | 35·1 | 1·0 | 3·1 |
| | $s$ | 0·7 | 0·7 | — | — | 15·5 | — | 7·3 | 7·1 | 1·5 | 2·6 | — | 14·8 | 0 | 0 |
| Red grapes | | | | | | | | | | | | | | | |
| C. Sauvignon ($n = 2$) | $\bar{X}$ | 1·0 | 1·0 | 1·0 | 7·3 | 10·8 | 4·8 | 27·5 | 35·2 | 10·3 | — | — | — | — | — |
| | $s$ | 0 | 0 | 0 | 0·7 | 1·3 | 0·8 | 0·7 | 1·1 | 0·5 | — | — | — | — | — |
| Garnacha ($n = 3$) | $\bar{X}$ | — | — | 6·4 | — | 19·1 | 16·5 | — | 16·9 | 6·0 | 11·2 | 22·9 | — | — | — |
| | $s$ | — | — | 8·0 | — | 10·0 | 7·6 | — | 3·1 | 5·1 | 5·5 | 5·9 | — | — | — |
| Tempranillo ($n = 4$) | $\bar{X}$ | — | — | — | 1·5 | 2·5 | 2·0 | 11·1 | 8·4 | 8·2 | 13·9 | 42·1 | 7·4 | — | — |
| | $s$ | — | — | — | 1·1 | 1·2 | 1·1 | 2·3 | 2·4 | 2·0 | 1·0 | 8·1 | 3·1 | — | — |
| Cencibel ($n = 2$) | $\bar{X}$ | — | — | — | — | — | 1·0 | 10·8 | 11·3 | — | 20·5 | 47·4 | 8·6 | — | — |
| | $s$ | — | — | — | — | — | 0 | 0·1 | 4·5 | — | 0·7 | 3·3 | 0·7 | — | — |

**TABLE 2**
Percentual Distribution of the Bands Separated by PAGE and Stained with Silver Nitrate for Grape Must

| *Grape varieties* | | *Mobility* | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | *0·09* *I* | *0·13* *II* | *0·18* *III* | *0·30* *IV* | *0·35* *V* | *0·39* *VI* | *0·43* *VII* | *0·46* *VIII* | *0·51* *IX* | *0·55* *X* | *0·60* *XI* | *0·63* *XII* | *0·65* *XIII* | *0·70* *XIV* | *0·79* *XV* | *0·85* *XVI* |
| White grapes | | | | | | | | | | | | | | | | | |
| Airen ($n = 4$) | $\bar{X}$ | — | — | — | — | 23·7 | — | — | 9·3 | 19·1 | 11·6 | — | — | — | 25·6 | 1·6 | — |
| | $s$ | — | — | — | — | 7·8 | — | — | 4·1 | 2·2 | 3·9 | — | — | — | 3·6 | 0·6 | — |
| Moscatel | | — | — | — | — | 30·9 | — | 10·8 | 9·5 | 8·6 | 3·5 | — | 28·1 | 1·0 | 7·0 | — | — |
| Red grapes | | | | | | | | | | | | | | | | | |
| C. Sauvignon ($n = 2$) | $\bar{X}$ | — | — | 12·0 | 13·5 | 15·2 | 1·0 | 12·2 | 11·8 | 12·3 | 13·5 | — | — | — | 3·0 | — | 2·4 |
| | $s$ | — | — | 2·4 | 2·8 | 2·0 | 0 | 0·9 | 1·7 | 0·5 | 2·2 | — | — | — | 1·0 | — | — |
| Garnacha ($n = 2$) | $\bar{X}$ | — | — | 1·0 | — | 15·3 | 12·0 | — | 12·5 | 10·1 | 14·0 | 25·9 | — | — | — | — | — |
| | $s$ | — | — | 0 | — | 4·6 | 2·8 | — | 0·7 | 3·8 | 0·7 | 1·4 | — | — | — | — | — |
| Tempranillo ($n = 4$) | $\bar{X}$ | — | — | — | 3·5 | 4·5 | 1·0 | 9·8 | 5·2 | 13·5 | 21·3 | 40·5 | — | — | — | — | — |
| | $s$ | — | — | — | 0·5 | 0·4 | 0 | 1·6 | 0·8 | 1·6 | 0·9 | 2·3 | — | — | — | — | — |
| Cencibel ($n = 2$) | $\bar{X}$ | — | — | — | 1·6 | 1·6 | 2·7 | 15·2 | 9·3 | — | 16·3 | 37·9 | — | — | — | — | — |
| | $s$ | — | — | — | 2·2 | 2·2 | 2·4 | 5·1 | 1·1 | — | 0·8 | 5·7 | — | — | — | — | — |

CLUSTAN program (Wishart, 1978). These programs were run in a CDC Cyber 180/855 computer.

## RESULTS AND DISCUSSION

The mean values and the standard deviations of the percentages of the bands separated by electrophoresis in polyacrylamide gel and stained with Coomassie Blue G-250 and silver nitrate are listed in Tables 1 and 2. There is a total of 16 bands with mobilities from 0·09 to 0·85, and most of the must proteins (from 60% to 93%, according to the variety) were grouped in the intermediate mobility zone (0·30–0·60). Some of the bands may be observed only with one of the two colorants which have been used. For example, the bands I and II have only been detected with Coomassie Blue and the bands XV and XVI only with silver nitrate. This does not very much affect quantitative data if we consider that the bands in which the behaviour is different in relation to the tincture are small. It may be considered that both systems of tincture are complementary.

From the direct observation of the electrophoretic pattern and from the data in Tables 1 and 2, it may be observed that there is a similitude in the

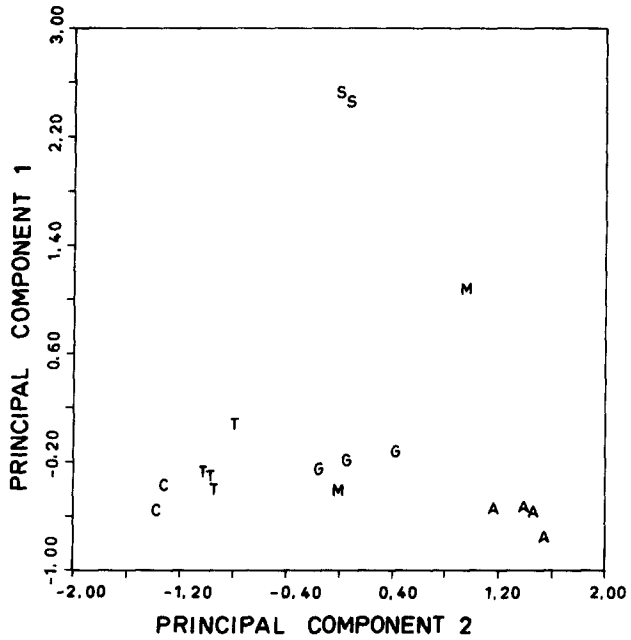


**Fig. 1.** Representation of six groups of variety musts in the plane defined by the two first principal components. Samples: A = Airén, C = Cencibel, G = Garnacha, M = Moscatel, S = Cabernet Sauvignon, T = Tempranillo.

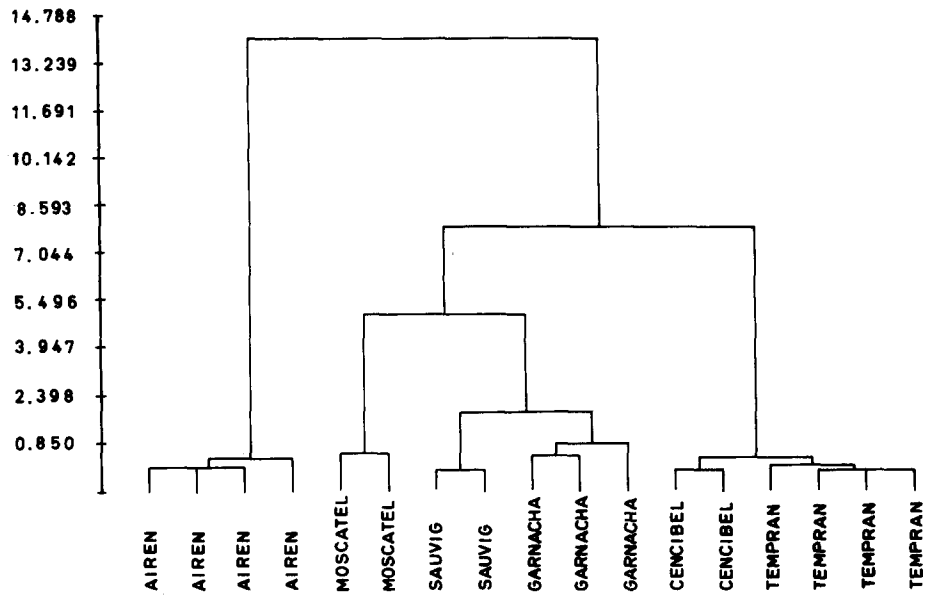


**Fig. 2.** Dendogram revealing associations between varieties following Cluster Analysis.

distribution of the same-variety must bands and thus differences between different varieties of musts.

The data of the electrophoretic pattern of the 17 analyzed samples (stained with Coomassie Blue G-250) have been subjected to Principal Component Analysis. The scores of the two first principal components are plotted in Fig. 1; these explain 60·4% of the total variance. The first component is highly correlated with bands I, IV, VIII, II and VII (loadings > 0·70), while V, XI, XIV, X and VI contribute more strongly to the second component (loadings > 0·80). In the figure there is a satisfactory grouping of must samples according to the different types of grapes except for the two samples of the Moscatel variety. The third component that explains 18·6% of the total variance is correlated with bands XIII and XII (loadings > 0·90).

Figure 2 shows the results of the application of Cluster Analysis (Ward's method) to the 17 samples. The Euclidean distance was taken as a measure of the proximity between two samples. It can be seen that samples are perfectly grouped according to their cultivar.

It is interesting to show the proximity of the must samples proceeding from Cencibel and Tempranillo varieties with both statistical methods used. It is considered that these two varieties, of which one (Cencibel) has been cultivated in La Mancha and the other (Tempranillo) in Rioja proceed from the same variety. Similar results have been obtained (Cáceres, 1987) from data on volatile components, free amino acids, and on the determinations

which are conventionally carried out for the grape musts (°Bé, pH, acidity, and so on) for these varieties.

Multivariate statistical methods applied to electrophoretic data show that protein patterns give valuable information on the variety origin of grape musts.

Supervised classification techniques have also been applied (Martín-Alvarez *et al.*, 1987) to the data of volatile compounds, the amino acids and global composition of grape musts of the same varieties with satisfactory results. Nevertheless, electrophoretic analysis is less time-consuming and involves less laboratory work than chemical and chromatographic analysis.

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