Varietal Differentiation of Must and Wines by Means of Protein Fraction

ENCARNACIÓN PUEYO[1], MARTA DIZY[2], and M. CARMEN POLO*[3]

Native electrophoresis, SDS electrophoresis, and isoelectric focusing were used to determine electrophoretic patterns of 10 grape musts and 14 varietal wines made from white grapes. Nine of the musts from the Macabeo, Xarel-lo, and Parellada grape varieties were fermented on an industrial scale under similar conditions. Five wines were produced in a pilot plant (500 L), with different inoculation of yeasts and with or without added SO_2 from the Malvar grape variety. The electrophoretic patterns obtained with the three techniques were similar for musts from the same variety, but different when musts from different varieties were compared. The electrophoretic patterns of the resulting wines were different from original musts. The electrophoretic patterns of the wines from the same variety were very similar in spite of the different conditions under which they were produced.

KEY WORDS: protein, varietal musts, varietal wine, electrophoresis, isoelectric focusing

Isozyme electrophoretic patterns can be produced from many grapevine organs, including shoots, leaves, and berries, as indicators of the variety from which they originate (2,14,16). During grape must production a large part of the enzymatic activity is lost due to interaction between polyphenols and proteins (9), but electrophoretic patterns of musts proteins from the same variety are similar and in turn different from those of musts from other varieties (5,13).

Important changes occur in proteins during vinification. Some of the proteins become insoluble and are later eliminated in wine clarification treatment; they may also be hydrolyzed through the proteolytic action of exocellular protease enzymes in the yeasts (4); or there may even be a transfer of proteins to the wine in the processes of autolysis in the yeasts.

The objective of this study was to investigate whether the protein transformations occurring during vinification from the same grape variety influences the electrophoretic patterns of the original musts. For this purpose, two types of samples were used; one type was produced on an industrial scale and the other on a pilot plant with different inoculations of yeasts and with or without added SO_2.

Materials and Methods

Production of musts and wines: Table 1 summarizes the conditions under which each of the musts and wines used in this study were prepared. The white Macabeo, Xarel-lo, and Parellada musts (3 from each variety) came from the Penedés area in the North-East of Spain. The Malvar must came from the area of Madrid. Each of the wines was racked for clarification and was cold stabilized to eliminate potassium bitartrate. The musts were frozen and the wines were refrigerated until analyzed.

Determination of soluble protein: The Bradford method (1) was used directly on the sample.

Preparation of the sample for electrophoretic study: One hundred milliliters of must or wine was centrifuged at 10 000 × *g* for 20 minutes. The supernatants were collected and dialyzed against running wa-

Table 1. Conditions under which musts were obtained and wines produced.

Grape variety	Must obtained	Wine produced
Macabeo Xarel-lo Parellada	Pressing: Belt press Clarification: Filtration in rotary filter, through Perlite	Industrial (125 000 L) Temperature 18°C 30-40 mg SO_2/L Inoculation of yeasts *
Malvar	Pressing: Horizontal press Clarification: Static separation	Pilot plant (500 L) Temperature 15°±1°C **I:** Spontaneous fermentation **II:** *Kloeckera apiculata* ** *Torulaspora rosei* ** *Saccharomyces ellipsoideus* ** **III:** 70 mg SO_2/L *Kloeckera apiculata* ** *Torulaspora rosei* ** *Saccharomyces ellipsoideus* ** **IV:** 70 mg SO_2/L L.S.A. *Saccharomyces cerevisiae* 71B *** **V:** 70 mg SO_2/L Spontaneous fermentation

Sources of yeast cultures: * Collection of company supplying samples. ** Collection of Instituto de Fermentaciones Industriales. *** Agrovin. Isolated by INRA Narbonne, France.

[1,2,3]Instituto de Fermentaciones Industriales. CSIC. Juan de la Cierva, 3. 28006 Madrid. Spain.

*Author to whom correspondence should be addressed.

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ter in Spectra POR 3 membranes (Spectrum Medical Industries, Los Angeles, CA, USA) for 48 hours. The dialyzed liquid was lyophilized, and the resulting residue was dissolved in 2 mL of pH 8.3 buffer (0.6 g *tris* (hydroxymethyl) aminomethane + 2.9 g glycerine per liter of water).

Polyacrylamide gel electrophoresis (PAGE): Polyacrylamide gel electrophoresis was performed as described by Hillier (8). The sample was applied to a polyacrylamide gel (80 × 80 × 0.75 mm), contained 9.0 g acrylamide and 400 mg N,N'-methylenebisacrylamide in 100 mL buffer of pH 8.9. Electrophoresis was performed for approximately one hour at a constant current setting of 12 mA per gel.

The gel was stained with Coomassie Brilliant Blue R-250 (15).

SDS-polyacrylamide gel electrophoresis (SDS-PAGE): Laemmli's method (11) for discontinuous electrophoresis was followed using a concentration gel of 3.89 g acrylamide and 108 mg N,N'-methylenebisacrylamide in 100 mL buffer of pH 6.8, and a resolution gel of 12.16 g acrylamide and 340 mg N,N'-methylenebisacrylamide in 100 mL buffer of pH 8.8, on plates of 140 × 130 × 0.75 mm. Electrophoresis was performed at a constant current setting of 15 mA per gel for approximately four hours.

A Pharmacia Fine Chemicals (Pharmacia LKB, Uppsala, Sweden) low molecular weight electrophoresis calibration kit was used as a marker to determine the molecular weight of the SDS proteins. Standard proteins were: α-lactalbumin (MW 14 400), tripsin inhibitor (MW 20 100), carbonic anhydrase (MW 30 000), ovalbumin (MW 43 000), albumin (MW 67 000), phosphorylase b (MW 94 000).

The gels were stained with Coomassie Brilliant Blue R-250 (7).

Isoelectric focusing: A Multiphor M-2117 apparatus and a Multitemp II LKB M-2219 thermostatic circulator (Pharmacia LKB, Uppsala, Sweden) were used. LKB Ampholine PAGplates with dimensions of 245 × 110 × 1 mm and pH range 3.5 to 9.5, were used for electrophoresis performance. Electrophoresis was performed at 12°C for three hours at 1500 V, 50 mA, and 10 W. pH gradient was measured using a Multiphore Electrode M-2117-111 LKB surface electrode (Pharmacia LKB, Uppsala, Sweden) before staining with Coomassie Brilliant Blue R-250.

Densitometrics: Densitometric measurements of electrophoretic bands were performed at 600 nm with Shimadzu (Shimadzu, Tokyo, Japan) equipment made up of a spectrophotometer (Chromato Scanner CS-930) and an integration and graphic impression system (Data Recorder DR-2).

Results and Discussion

Protein content: Protein concentration in the musts analyzed ranged between 20.2 and 50.3 mg BSA/L (Table 2). These values were similar to those we have found in white grape musts of other varieties (6). The mean value of protein content in the Xarel-lo grape musts (46.1 mg BSA/L), and protein content in the Malvar must (49.9 mg BSA/L) was higher than the mean values of protein content in the Macabeo and Parellada grape musts, although there were no significant differences between them. There was a reduction in protein content in the wines which were industrially produced, so that mean protein value in the wines was 29% of mean protein value in the musts. By contrast, the five wines produced in a pilot plant from Malvar grape must contained, on average, 78% of the must protein content.

Table 2. Protein concentration (mg BSA/L) in musts and wines.

Grape variety	Musts	Wines
Macabeo	23.5	7.3
Macabeo	37.9	12.9
Macabeo	27.2	7.6
Xarel-lo	40.9	15.3
Xarel-lo	50.3	14.3
Xarel-lo	47.2	15.8
Parellada	20.2	6.6
Parellada	42.1	5.7
Parellada	31.3	6.8
Malvar	49.9	I 30.9
		II 45.2
		III 42.1
		IV 34.4
		V 41.3

The different quantitative data to be found in the literature cannot be compared among themselves due to the diversity of methods with which they were obtained, but the values shown in Table 2 are similar with those obtained by Hsu and Heatherbell (9), who also used the Bradford method, and by Feuillat *et al.* (3), who estimated protein content as nitrogen of the Sephadex G-25 exclusion volume, multiplied by 6.25.

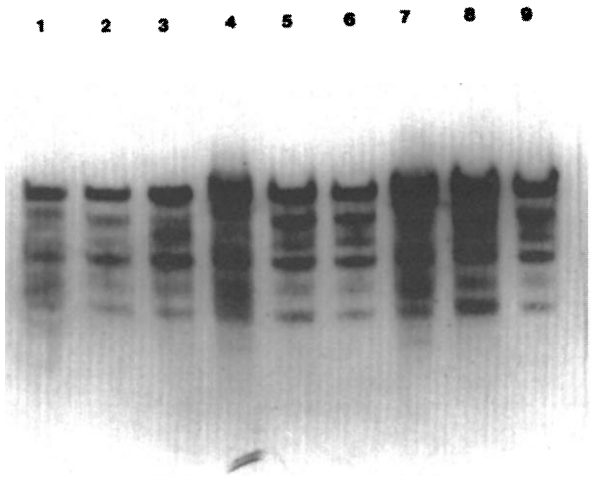

Fig. 1. Polyacrylamide gel electrophoresis of must and wine proteins: (lanes 1, 4, 7) Xarel-lo wines, (lanes 2, 3, 5, 6, 8, 9) Xarel-lo musts.

Polyacrylamide gel electrophoresis (PAGE): From five to eight bands with mobilities from 0.31 to 0.91 were separated in each of the musts and wines. Table 3 shows the values of band intensity expressed as a percentage of the total. Figures 1, 2, and 3 show the electrophoretograms of some of the musts and wines analyzed.

Electrophoretic patterns obtained by PAGE of proteins in musts from the same grape variety were similar, while there were differences between electrophoretograms of musts from different varieties, as was postulated by Kock and Sajak in 1959 (10) and confirmed by us in earlier works (5,13).

The electrophoretic patterns of the wines produced industrially differ slightly from those of the musts from which they originate. In the Xarel-lo and Parellada wines, the 0.40 mobility band has disappeared; a 0.39 mobility band appeared in the Xarel-lo wines, and a 0.41 mobility band in the Parellada wines. High mobility bands (0.86, 0.89, 0.91), which were not present in the musts, also appeared in the nine industrially produced wines (Table 3 and Fig. 2). There were only small qualitative differences between the Malvar must and the wine elaborated from it.

In 13 of the 14 wines studied, the electropherogram of the wines originating from the same grape variety were identical in spite of the fact that in some cases they had been produced with different inoculations of yeasts and without SO_2 (Malvar wines I and II) or with SO_2 (Malvar wines III, IV, and V). One of the Parellada wines differed from the other two by the presence of the 0.86 mobility band and the absence of the 0.91 mobility band (Table 3).

SDS-polyacrylamide gel electrophoresis (SDS-PAGE): Protein bands were detected with molecular weights from 14 000 to 94 000; the most intense bands had molecular weights of between 25 000 and 35 000. Similar electrophoretic patterns were also obtained with this electrophoretic technique for musts of the same variety. The electrophoretograms of wines originating from the same variety musts were also similar, although different from those of the musts themselves. During vinification, some bands disappeared or their intensity diminished, *i.e.*, bands of MW 36 000 in Malvar wines and bands of MW 34 600 in Xarel-lo wine (Fig. 4).

Isoelectric focusing: Isoelectric focusing separated from 5 to 14 bands with isoelectric points in the range of 3.0 to 5.6 were separated in each of the samples. Table 4 shows the percentage distribution of the bands grouped in intervals of 0.5 units and Figure 5 the

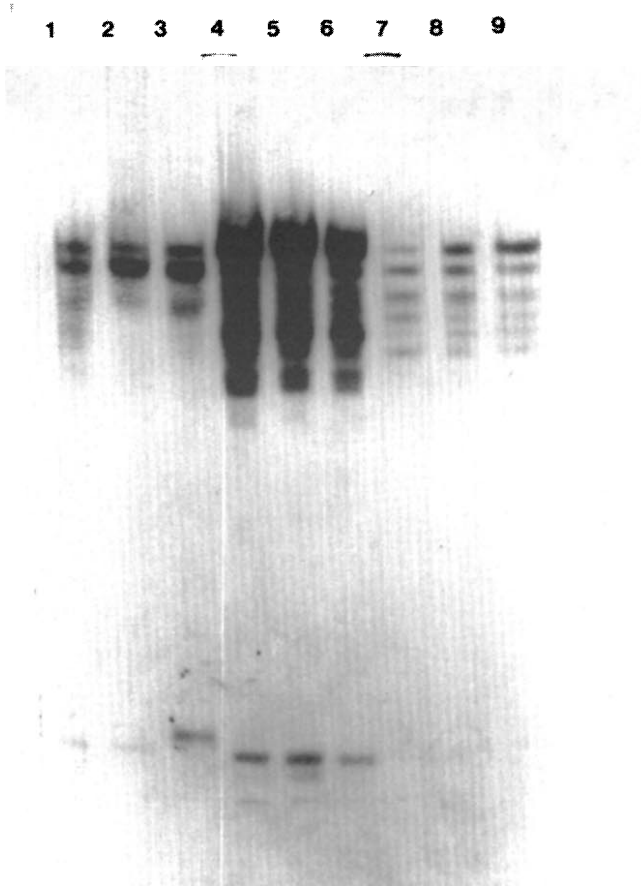

Fig. 2. Polyacrylamide gel electrophoresis of wine proteins: (lanes 1 - 3) Parellada, (lanes 4 - 6) Xarel-lo, (lanes 7 - 9) Macabeo.

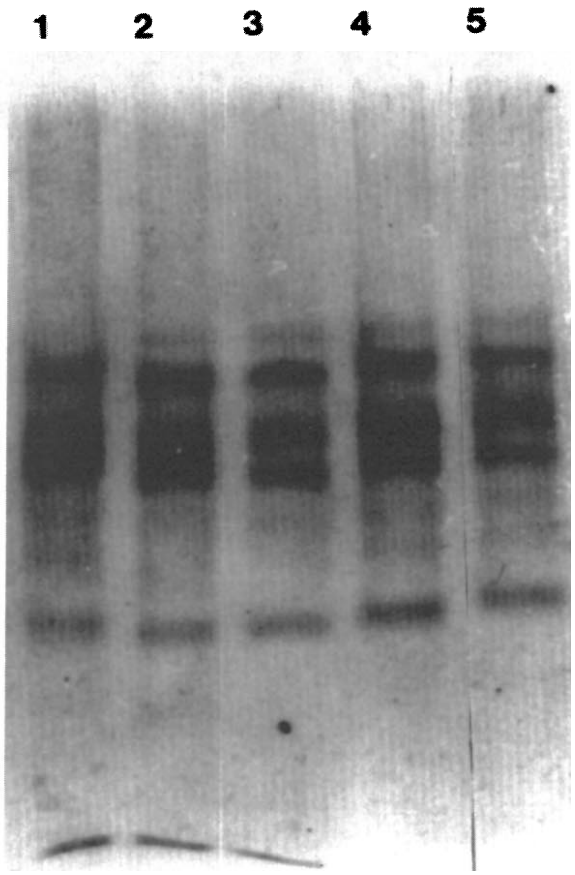

Fig. 3. Polyacrylamide gel electrophoresis of the proteins of Malvar wines: (lanes 1 - 5) wines I-V.

Table 3. Relative proportions (% of total) and mobility of the bands separated by polyacrylamide gel electrophoresis after staining with Coomassie Brilliant Blue R-250.

Sample	Mobility														
	0.31	0.36	0.39	0.40	0.41	0.43	0.44	0.46	0.50	0.53	0.54	0.60	0.86	0.89	0.91
Macabeo															
must	9.3	22.4	—	30.9	—	27.3	—	—	10.1	—	—	—	—	—	—
wine	23.4	25.6	—	16.0	—	16.4	—	—	12.0	—	—	—	—	6.1	0.5
must	8.6	23.9	—	33.1	—	25.7	—	—	8.7	—	—	—	—	—	—
wine	21.9	22.9	—	21.0	—	17.1	—	—	10.0	—	—	—	—	6.7	0.4
must	6.3	22.4	—	36.1	—	26.4	—	—	8.8	—	—	—	—	—	—
wine	16.8	30.4	—	21.1	—	13.3	—	—	12.2	—	—	—	—	5.8	0.4
Xarel-lo															
must	38.9	7.9	—	17.8	—	—	—	21.5	7.9	6.0	—	—	—	—	—
wine	30.7	12.9	8.4	—	—	—	—	23.7	8.4	9.3	—	—	—	6.2	0.4
must	40.1	24.8	—	11.0	—	—	—	13.5	5.9	4.7	—	—	—	—	—
wine	45.5	9.2	6.4	—	—	—	—	20.4	4.4	5.7	—	—	—	7.2	1.2
must	35.4	26.2	—	12.2	—	—	—	15.7	5.4	5.1	—	—	—	—	
wine	45.6	11.3	8.0	—	—	—	—	11.0	8.6	8.5	—	—	—	6.7	0.3
Parellada															
must	5.2	24.3	—	56.0	—	—	10.0	4.5	—	—	—	—	—	—	—
wine	20.6	41.0	—	—	21.6	—	5.2	3.8	—	—	—	—	7.3	0.5	—
must	8.3	20.3	—	53.7	—	—	13.2	4.5	—	—	—	—	—	—	—
wine	13.3	58.4	—	—	13.0	—	4.4	1.5	—	—	—	—	—	5.4	0.4
must	6.4	22.7	—	55.6	—	—	10.3	5.0	—	—	—	—	—	—	—
wine	21.1	28.2	—	—	18.9	—	13.6	11.2	—	—	—	—	—	5.9	1.1
Malvar															
must	10.2	12.6	—	—	—	23.5	—	24.5	—	10.7	6.3	12.2	—	—	—
wine I	10.3	20.0	—	—	—	21.1	—	22.1	—	10.3	7.1	9.0	—	—	—
wine II	12.0	24.6	—	—	—	20.8	—	18.3	—	7.4	5.7	11.2	—	—	—
wine III	12.1	26.5	—	—	—	21.3	—	19.0	—	6.0	3.7	11.4	—	—	—
wine IV	10.6	24.6	—	—	—	22.0	—	18.9	—	7.5	5.8	10.5	—	—	—
wine V	9.4	21.0	—	—	—	23.7	—	20.6	—	8.5	6.2	10.5	—	—	—

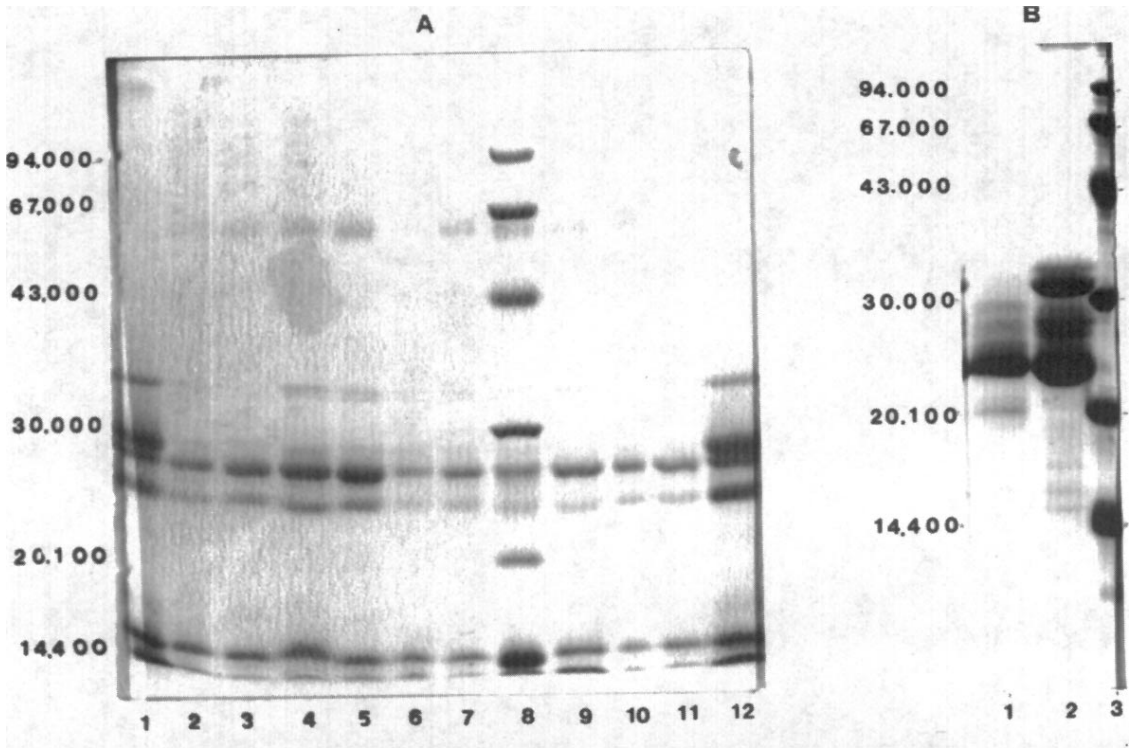

Fig. 4. SDS-Polyacrylamide gel electrophoresis of must proteins, wine proteins and standard proteins used as molecular weight markers. (**A**) Malvar variety (lanes 1 and 12) must, (lanes 2 and 3) wine I, (lanes 4 and 5) wine II, (lanes 6 and 7) wine III, (lane 8) standards plus wine IV, (lane 9) wine IV, (lanes 10 and 11) wine V. (**B**) Xarel-lo variety (lane 1) must, (lane 2) wine, (lane 3) standards. Molecular weights of standards are given on the left side of each gel.

Table 4. Percentage distribution of bands obtained using isoelectric focusing after staining with Coomassie Brilliant Blue R-250.

Sample	Isoelectric point				
	3.0 - 3.5	3.6 - 4.0	4.1 - 4.5	4.6 - 5.0	5.1 - 5.6
Macabeo					
must	—	45.0	40.0	15.0	—
wine	—	47.5	12.0	40.5	—
must	—	43.3	41.4	15.3	—
wine	—	37.1	11.4	51.5	—
must	—	41.0	44.1	14.9	—
wine	—	38.4	11.8	49.8	—
Xarel-lo					
must	1.9	50.9	28.3	18.9	—
wine	—	31.0	10.0	59.0	—
must	3.3	52.8	19.3	24.6	—
wine	—	33.0	11.5	55.5	—
must	2.8	52.1	26.6	18.5	—
wine	—	33.7	15.0	85.2	—
Parellada					
must	—	10.6	41.5	7.6	—
wine	—	22.8	7.4	69.8	—
must	—	25.5	58.9	15.6	—
wine	—	21.9	7.2	70.7	—
must	—	17.7	56.5	25.8	—
wine	—	28.2	8.4	63.8	—
Malvar					
must	9.3	9.3	48.1	22.8	10.5
wine I	14.8	3.2	51.3	16.4	14.3
wine II	13.0	3.0	45.2	20.6	18.2
wine III	8.9	1.8	39.3	22.9	27.1
wine IV	6.1	2.5	45.2	19.8	26.4
wine V	12.5	—	48.3	16.2	23.0

electrophoretograms of Malvar wines. The most intense bands have isoelectric points in the range of 3.6 to 5.0. Similarities can again be observed between the electrophoretograms of musts and wines produced from the same grape varieties.

The electrophoretograms of the industrially produced wines differed from those of their musts, especially in the isoelectric point range of the most intense bands: from 3.6 to 4.5 in the musts and from 4.6 to 5.0 in the wines. The electrophoretograms obtained by isoelectric focusing of the Malvar wines were similar to the electrophoretograms of the musts from which they originated. Moio and Addeo (12) also obtained very similar isoelectrophoretic patterns in musts and the wines produced from them.

Conclusions

Using different electrophoretic techniques, polyacrylamide gel electrophoresis, SDS-polyacrylamide gel electrophoresis, and isoelectric focusing, similar electrophoretic patterns were obtained from musts of the same grape variety, while those from musts of different varieties differed. On the other hand, the electrophoretic patterns of the wines were different from those of the musts from which they originated. The electrophoretic patterns of the wines studied which originated from the same grape variety were similar among themselves, even though they were produced with different inoculations of yeasts and with or without added SO_2.

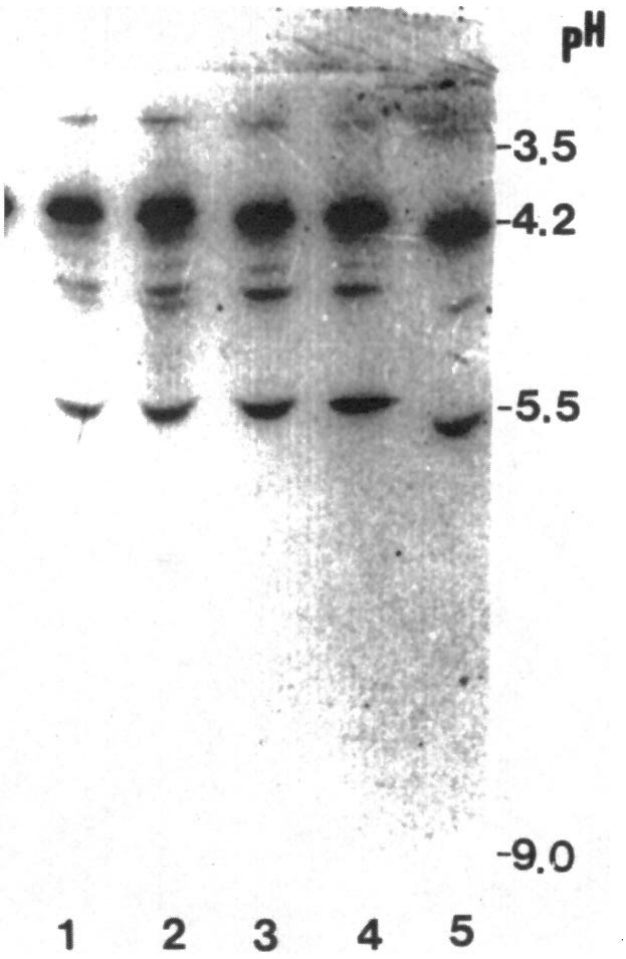

Fig. 5. Isoelectric focusing of proteins of Malvar wines.

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