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# Effect of diverse enzyme preparations on the extraction and evolution of phenolic compounds in red wines

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**Abstract**

In this report, the effect of diverse enzyme preparations normally used in the elaboration of red wines, on certain colouring indices has been studied: the colouring intensity, tone, anthocyanins, index of total polyphenols, index of Folin-Cicalteu and anthocyanin broken up into monomers, red polymers and brown polymers. Wines elaborated with the Monastrell grape variety have been used and the evolution of these compounds during the vinification and the conservation process in bottles has been followed. It has been proved that all the enzyme preparations increase the extraction of polyphenols and the quality of colour obtained and the changes of these parameters in the conservation process, is the same in the control wine as in the treated wines. © 1999 Elsevier Science Ltd. All rights reserved.

*Keywords:* Enzyme preparations; Phenolic compounds; Colour; Red wine

## 1. Introduction

The pectic enzymes, or pectinases, belong to the group of carbohydrases which catalyse the breakdown of pectin substances. The grape and yeast both show this enzyme activity although its effect is limited by the sugars and the alcoholic content in the vinification process (Aryan, Wilson, Straus & Williams, 1987; Brillouet, Saulnier & Houtonnet, 1990). The use of these enzymes is a future technique within wine biotechnology as they are very specific and lead to an important series of benefits such as: a faster start to fermentation, an elevated efficiency of the must which flows from the drainage, easier pressing, quicker and more complete clarification of the wines obtained, a slight increase in the alcohol and methanol content, an important extraction of the phenolic compounds and the aromatic compound contents in the grape skin (Anocibar & Bertrand, 1994; Brown & Ough, 1981; Canal, 1990; Cordonnier, Blouin, & Guittard, 1988; Gnekow & Ough, 1976; Marteau, 1972; Nicolini & Mattivi, 1995).

The commercial preparations of pectic enzymes present diverse types of enzyme activity as they generally already contain a complex mixture of enzymes. For this reason it is of great interest to know the precise action of these enzymes as it differs according to the variety of grape and the type of vinification that is carried out. The effect which different commercial enzyme preparations produce in the phenolic wine compounds is the subject which has been studied. Wines have been elaborated from the Monastrell variety by means of the traditional red wine technique (from the maceration of the must with the solid parts of the bunch). The evolution of the phenolic compounds throughout the conservation of these wines during nine months in the bottle has also been studied.

## 2. Materials and methods

Grapes of the Monastrell variety, the most frequently used variety in Origin Denomination (D.O.) Jumilla

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(Murcia, SE Spain) wines, were picked during the 1995 harvest. They came from a smallholding totally representative of the area and were found to be in a perfectly healthy state and ripe.

They were distributed from the crushed grape harvest which came from an industrial process. A crushing-remove-stalk machine, with rollers and a capacity of 25000 kg/h, was used. The pressed grape harvest was placed in containers of 30 l capacity occupying only 25 l, to make allowances for the increase which the quantity undergoes, when it is in full fermentation. The tests were duplicated.

Each container was treated with a total of 80 mg/kg of $SO_2$. The enzyme preparations dissolved in water or must were added according to the indications of each wine company. Table 1 lists the characteristics of the enzyme preparations and their application doses.

The maceration for all the tests took place at 20–22°C and for a duration of 5 days. At the end of this time, the must was separated to be drained; the quantity that was left was pressed with a manual press and both fractions were remixed. After finishing fermentation, the wines obtained were moved and placed into bottles, which were kept laid out for nine months, which is the time the study lasted. In order to determine the evolution of the colouring material during vinification, samples were taken on the days that the enzyme preparations were added, day 0 and days 1,3,5,7,10,14 and 19 (the day which bottling took place). To determine the evolution during conservation, samples were taken every three months.

The analytical variables measured were: the colour density, the tone, the anthocyanins, the index of total polyphenols, and the pigment fractions in monomers, red polymers and brown polymers. The colour density and the tone were determined by absorbance of the samples at 420 and 520 nm (Sudraud, 1958). The index of total polyphenols was determined by means of the absorbance at 280 nm of diluted wine 1/100 (v/v). The content of anthocyanins was analyzed according to the method of discolouring with bisulphite (Ribereau-Gayon, Peynaud, Ribereau-Gayon & Sudrand, 1982). The fractionation of pigments was carried out by means of elutions of the sample on a column of PVPP-Gel of silica G60-Gel of silica 60, using methanol-HCl 0.1%, formic acid at 50%(v/v) and concentrated formic acid (Simard, Bourzeix & Heredia, 1980). Each analysis was repeated three times and the coefficients of variation in all of them were less than 9%.

The multiple range test LSD and Tukey-HSD, with significance level 0.05, was applied to the results (Norusis, 1993).

Table 1
Enzyme preparations and application dose

| No | Enzyme preparations (EP) | Dose g/hL |
|---|---|---|
| 1 | Endozym contact pelliculatire (AEB, Brescia, Italy) | 3 |
| 2 | Biopectinasa (Sepsa, Barcelona, Spain) | 3 |
| 3 | Ultrazym 100 G (Agrovin, Ciudad Real, Spain) | 2 |
| 4 | Rapidase excolor (Laffort et Cia, Bourdeux, France) | 2 |

## 3. Results and discussion

The initial characteristics of the must and wines obtained can be seen in Table 2.

### 3.1. Colour density

The evolution of the colour density during vinification is shown in Fig. 1(a). All the enzyme treatments produce an increase of the colour density with respect to the control. The largest significant differences ($p < 0.05$) are obtained in the samples of day 7, in which the wine obtained with the enzyme preparation 2 is the one which gives lower values.

The evolution of the colour density during the nine months of wine conservation is presented in Fig. 1(b). There is a loss of colour density in all the wines, but the fall is less in those with enzyme treatments 1 and 2 and the highest fall takes place in the control wine.

### 3.2. Tone

The evolution during vinification is shown in Fig. 2(a). A similar conduct can be seen in all the tests, presenting a marked fall from the moment when the

Table 2
Analysis of the initial must and the wines obtained

| Sample | Must | Ep-1[a] | EP-2[a] | Ep-3[a] | EP-4[a] | Control |
|---|---|---|---|---|---|---|
| Density (g/l) | 110 | 993.2 | 992.8 | 992.9 | 992.8 | 992.1 |
| Volatile acidity (g/l acetic acid) | 0.15 | 0.37 | 0.37 | 0.33 | 0.33 | 0.33 |
| Titratable acidity (g/l tartaric acid) | 6.43 | 7.09 | 6.91 | 6.98 | 6.73 | 6.83 |
| pH | 3.36 | 3.42 | 3.38 | 3.35 | 3.36 | 3.38 |
| Alcohol content (% v/v) | – | 14.06 | 14.18 | 14.28 | 14.30 | 13.95 |

[a] The samples EP-1, EP-2, EP-3 and EP-4 correspond to the wines obtained with the enzyme preparations 1,2,3 and 4 respectively.

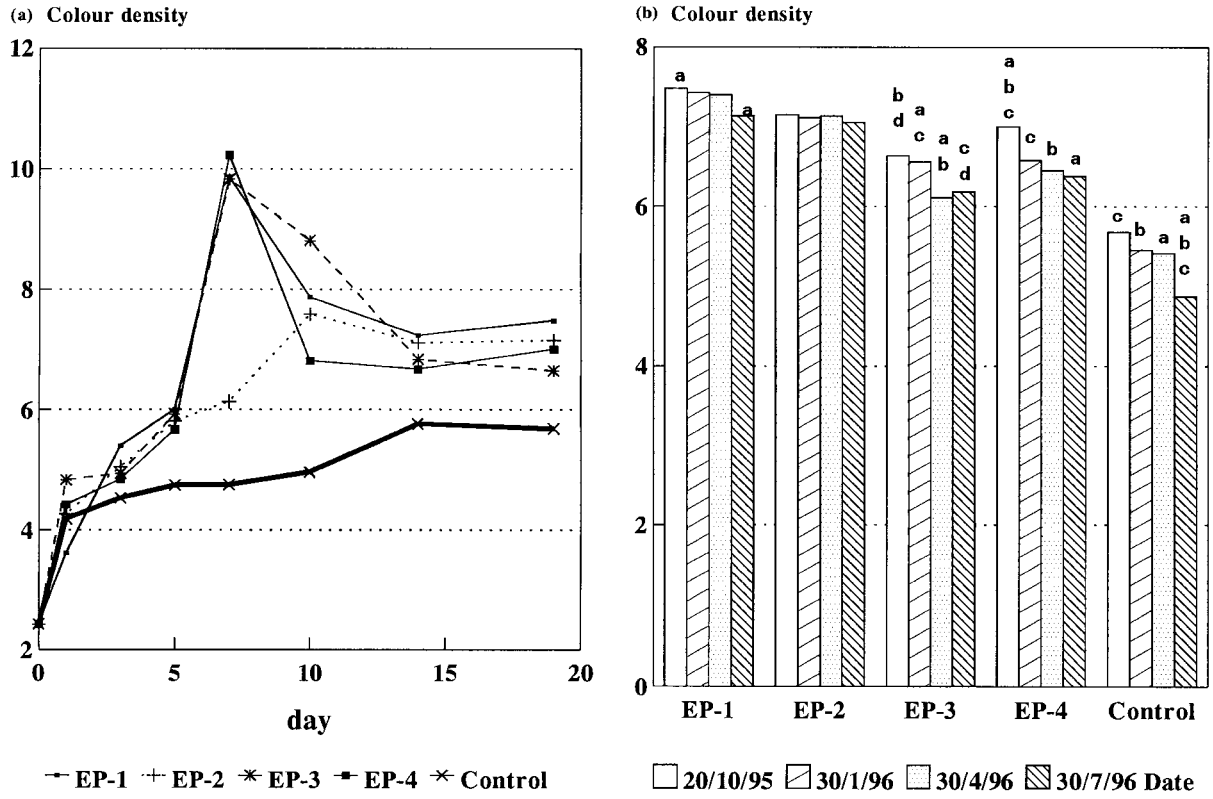


Fig. 1. Evolution of the colour density during vinification (a) and during conservation (b). EP-1, EP-2, EP-3, EP-4, correspond to the wines obtained with the enzyme preparations 1,2,3 and 4, respectively. a,b,c,d: identical letters indicate significant differences at level of probability of 5%.

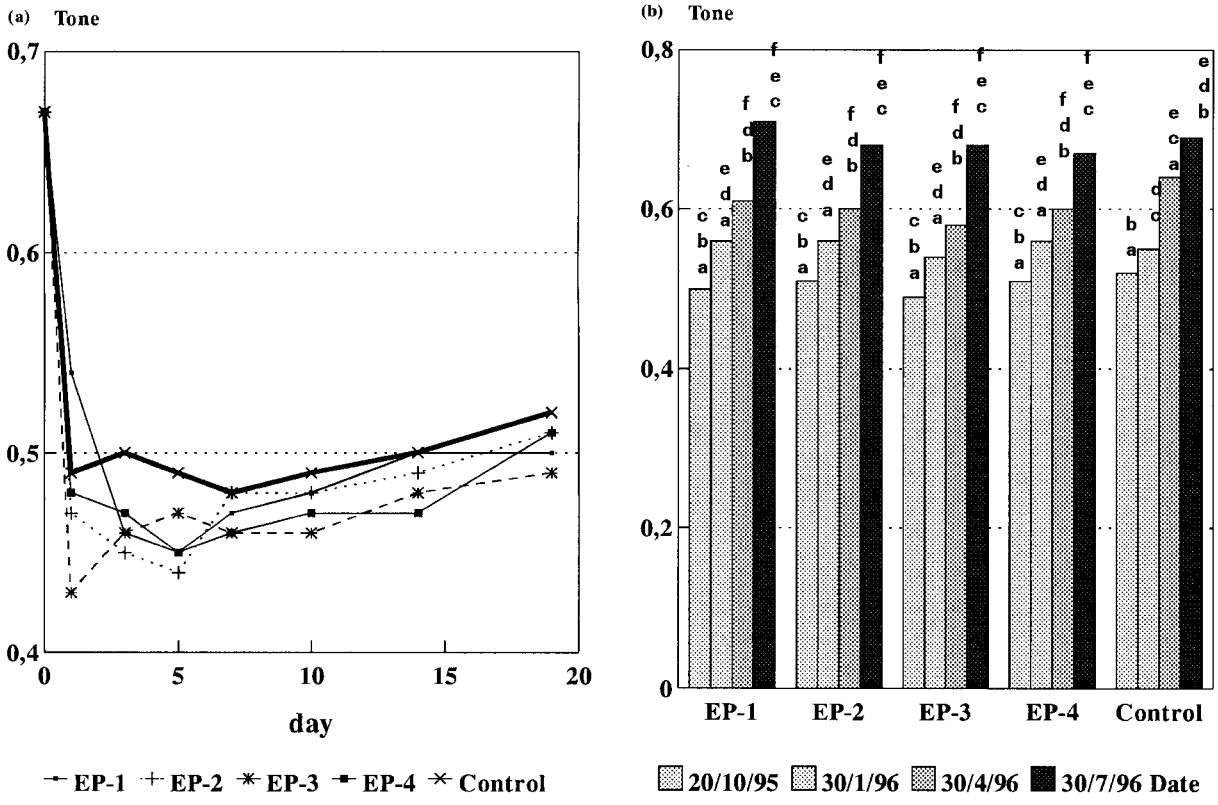


Fig. 2. Evolution of the tone during vinification (a) and during conservation. EP-1, EP-2, EP- 3, EP-4, correspond to the wines obtained with the enzyme preparations 1,2,3 and 4, respectively. a,b,c,d,e,f: identical letters indicate significant differences at level of probability of 5%.

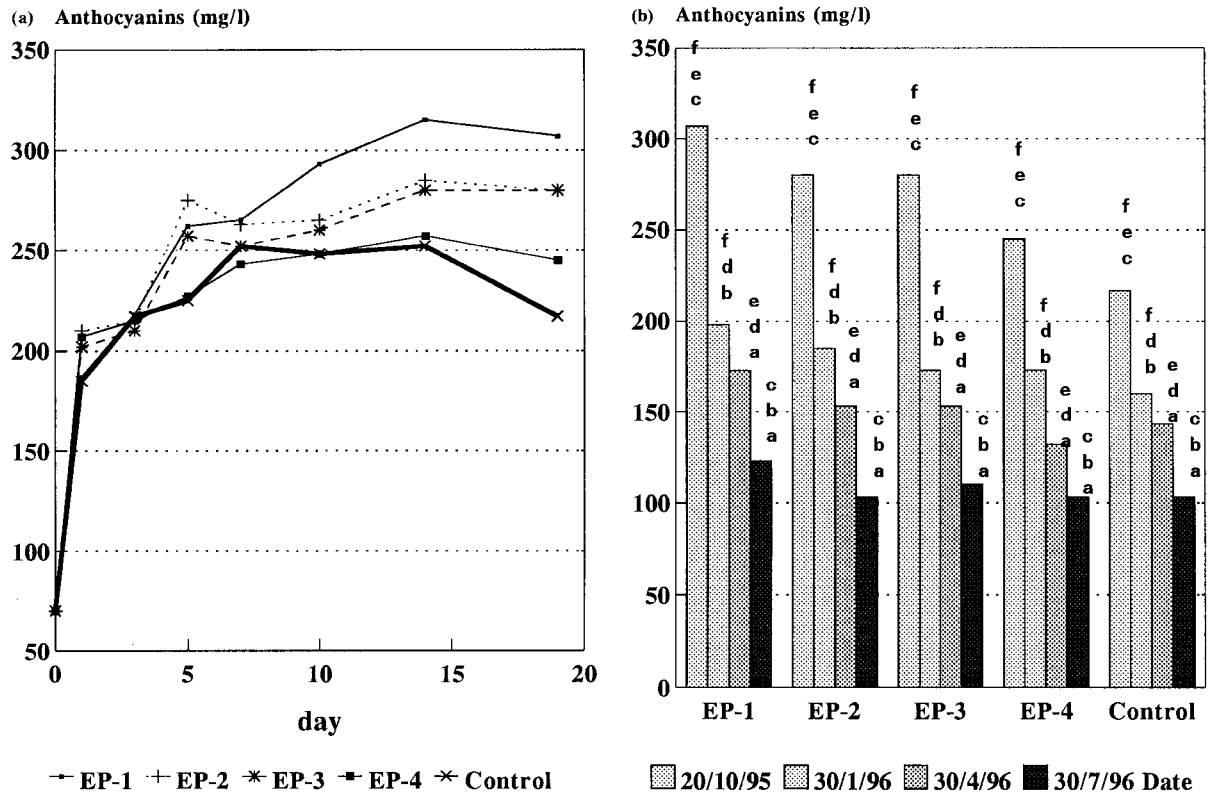


Fig. 3. Evolution of the anthocyanins during vinification (a) and during conservation (b). EP-1, EP-2, EP-3, EP-4, correspond to the wines obtained with the enzyme preparations 1,2,3 and 4, respectively. a,b,c,d,e,f: identical letters indicate significant differences at level of probability of 5%.

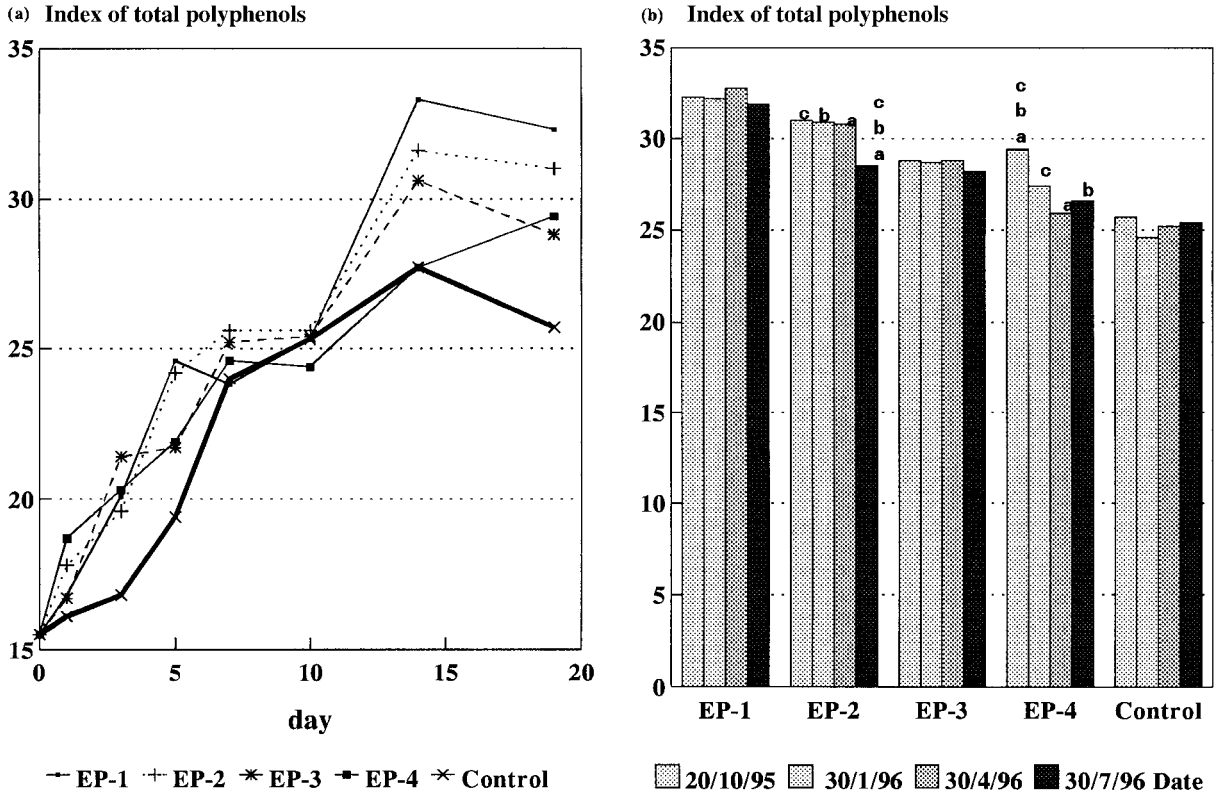


Fig. 4. Evolution of the index of total polyphenols during vinification (a) and during conservation (b). EP-1, EP-2, EP-3, EP-4, correspond to the wines obtained with the enzyme preparations 1,2,3 and 4, respectively. a,b,c: identical letters indicate significant differences at level of probability of 5%.

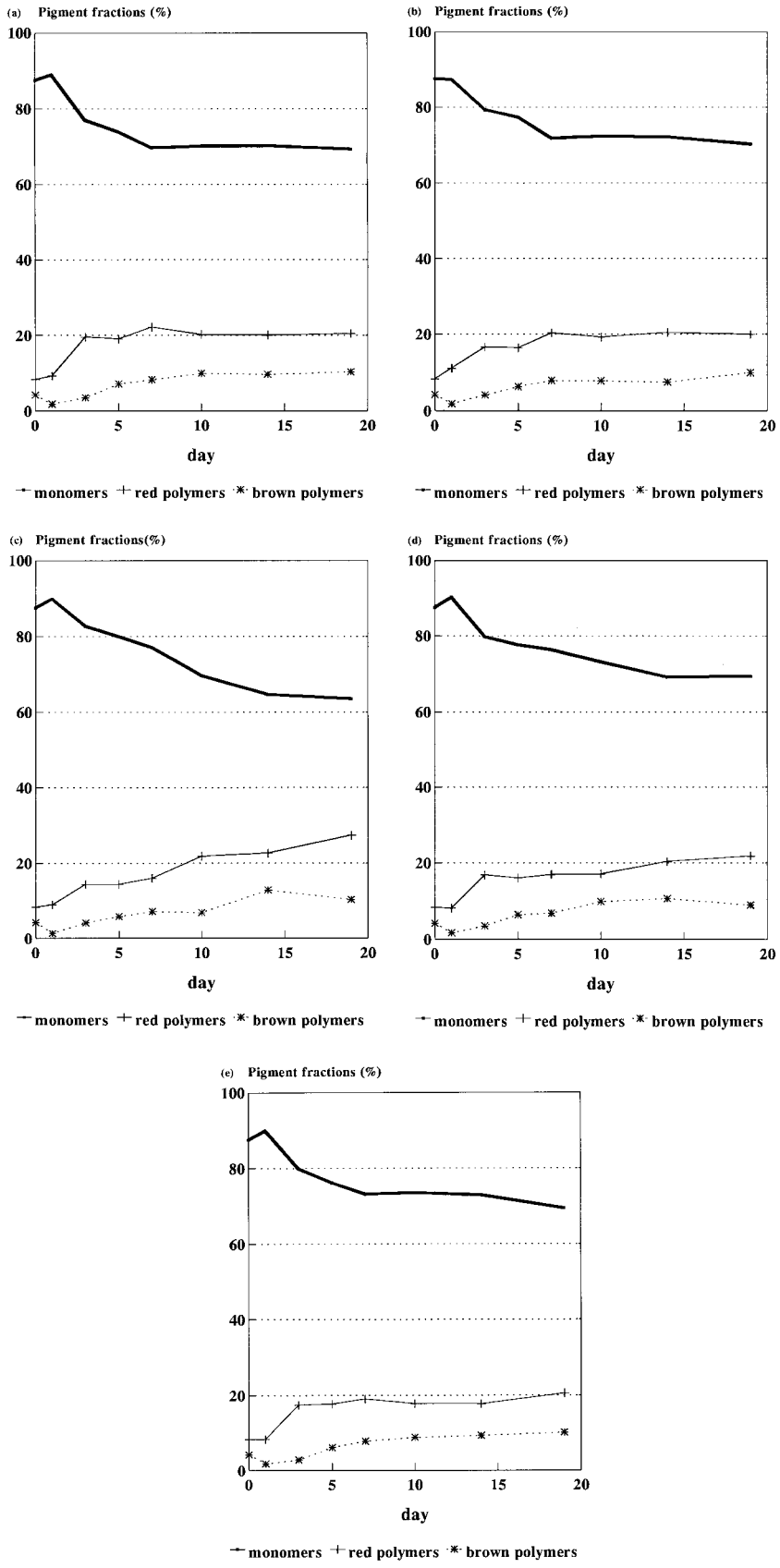


Fig. 5. Evolution of pigment fractions during vinification: (a) wine treated with enzyme preparation 1, EP-1, (b) with enzyme preparation 2, EP-2, (c) with enzyme preparation 3, EP-3, (d) with enzyme preparation 4, EP-4, and (e) control wine.

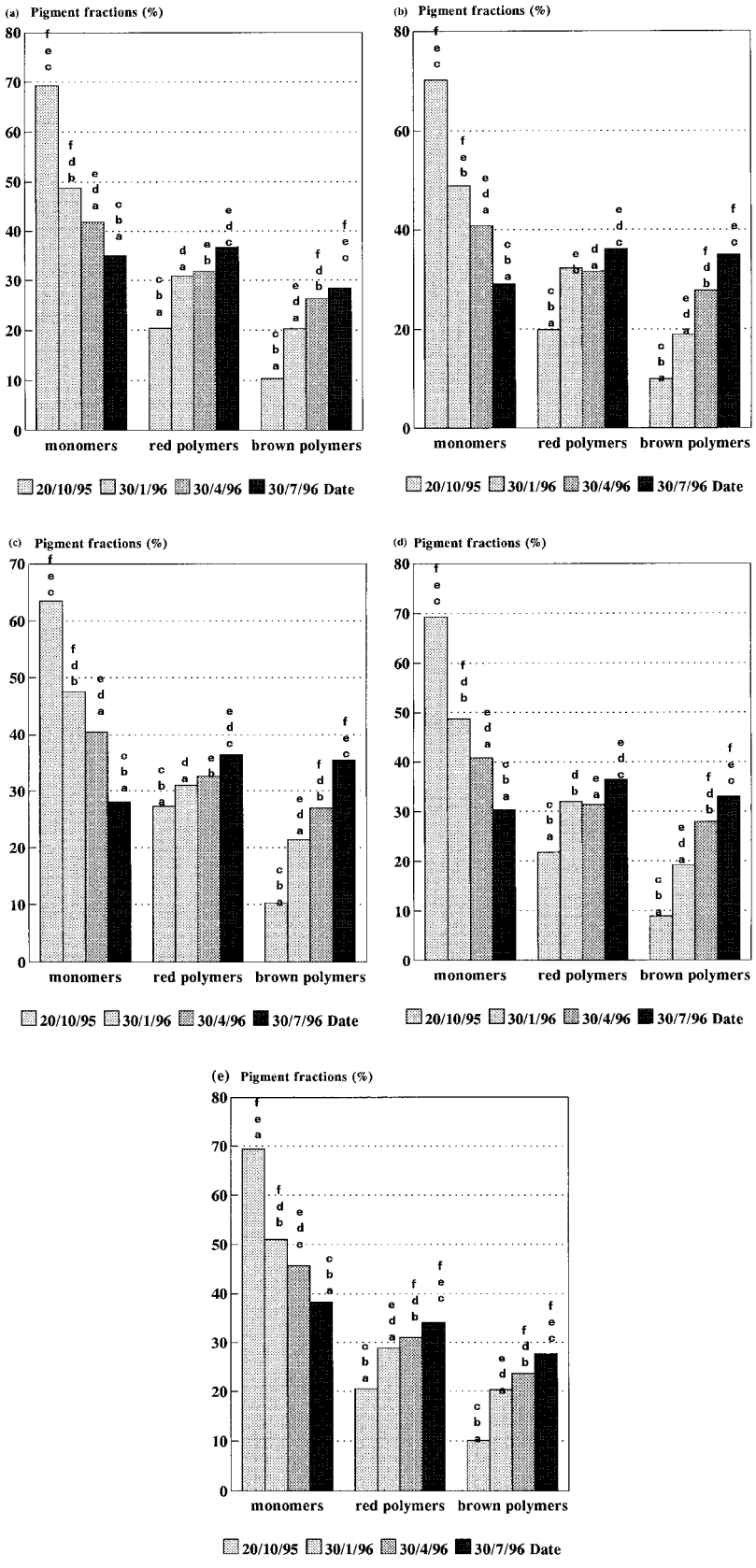


Fig. 6. Evolution of pigment fractions during conservation: (a) wine treated with enzyme preparation 1, EP-1, (b) with enzyme preparation 2, EP-2, (c) with enzyme preparation 3, EP-3, (d) with enzyme preparation 4, EP-4, and (e) control wine. a,b,c,d,e,f: identical letters indicate significant differences at level of probability of 5%.

maceration was started, as there is an important dissolution of the compounds which contribute to the red colour of the must. After the initial fall, stabilization occured and there was a slight increase towards the end of the process. Significant differences only occurred during days 3 and 5 (the tumultuous phase of fermentation). The final values show, in all the tests treated with enzymes, a favourable situation with respect to the control (inferior values).

During conservation, an increase of tone ($p < 0.05$) is produced in all the wines. The results are shown in Fig. 2(b). The best conservation of tone, and therefore quality of colour, corresponds to the control wine.

### 3.3. Anthocyanins

The evolution of the anthocyanins during vinification is shown in Fig. 3(a). During the maceration an initial rapid increase is presented ($p < 0.05$ in all the samples), and continues until the end of the maceration, in order to maintain itself constant later or undergo small increases. The evolution is similar in all the tests. At the end of vinification, the results show that all the wines treated with enzyme preparations have a higher concentration of anthocyanins ($p < 0.05$) with absolute values which give the largest difference for preparation 1 (190 mg/l), the smallest difference for preparation 4 (30 mg/l) and similar differences for preparations 2 and 3 (63 mg/l).

The evolution of the anthocyanins during conservation is represented in Fig. 3(b). A marked fall can be seen in the contents of anthocyanins in each one of the wines ($p < 0.05$), the highest fall occurring in wine treated with preparation 1 and the lowest in the control wine, therefore, this fall is higher in the wines whose initial concentration is higher. The percentage of loss of anthocyanins due to the effect of conservation is higher in the treated wines than in the control wine.

### 3.4. Index of total polyphenols

The evolution which follows the index of the total polyphenols content can be seen in Fig. 4(a). A net increase can be observed during all vinification, which is faster during the maceration phase and more moderated when it is finished.

The results obtained at the end of vinification indicate that, with respect to the control, a higher extraction of polyphenols occurs in all the treated wines in which the enzyme action ($p < 0.05$) has taken place. The highest index is obtained with preparation 1, followed by preparation 2; the lowest index is for preparations 3 and 4.

In Fig. 4(b) the evolution of this index during the conservation of the wines is shown. Significant differences were not found in the treated wines with preparations 1 and 3 and the control, and only a small decrease of the treated wines with preparations 2 and 4 can be observed.

### 3.5. Pigment fractions

The results of the evolution of monomers, red polymers and brown polymers during vinification in each one of the wines are shown in Fig. 5. The evolution of different fractions in which the pigments are separated show, in all cases, a decrease in the percentage of monomers and an increase in the polymers, the red ones as much as the brown ones.

With respect to the control, significant differences in the monomers were not found, although the higher percentages for the treated wines with preparations 1 and 4 and those slightly superior to those in the wine treated with preparation 2 can be valued. With respect to only the red polymers, the treated wine with preparation 3 showed values significantly higher than the control at the end of the process. The treated wine with preparation 2 is the one which shows the least proportion of red polymers at the end of vinification. Significant differences for the brown polymers between the control wine and the treated wines were not found; nevertheless the lower percentages can be valued in the treated wines with preparations 2 and 4. According to the results, the enzyme preparation 2 shows the best characteristics; although it does not give rise to the highest proportion of monomers, it does give rise to the lowest proportions of both types of polymers.

The evolution of these variables throughout the conservation of the wines can be seen in Fig. 6. In all the wines, we evaluate the important fall in monomers and so consequently an increase of the red polymers and the brown ones.

At the end of the conservation experiment, the control wine and the wine treated with preparation 1 presented the highest percentages of monomer compounds and the lowest percentages of these compounds were obtained with the enzyme preparation 3.

## 4. Conclusions

During the maceration carried out during vinification, all the enzyme preparations produced an increase in the quantity of polyphenols extracted from the solid parts, with respect to the elaborated control test without the addition of enzyme preparation. Highest values obtained are in colour density, anthocyanins and index of total polyphenols. The quality of the colour obtained is better in the tests treated with enzyme preparations, as the values of tone are lower and larger in proportion of monomers. The best results are obtained with the enzyme preparations 1 and 2, specific for the extraction of colour in the maceration. The use of diverse enzyme

preparations does not have a significant effect during conservation of all wines, given that the behaviour of all the treated wines is very similar to the control.

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