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Tartaric acid in frozen musts and wines. Optimization of Rebelein's method and validation by HPLC

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Rebelein's colorimetric method was adapted to determine tartaric acid in thawed samples of frozen musts and wines. The results were compared with those obtained by cation-exchange HPLC. This modification of Rebelein's method involved the elimination of the activated carbon step. Precipitated tartrates in the freezing stage were dissolved by acidification to pH 1·0 before colour reaction development or HPLC analysis. The data obtained agreed with those shown by HPLC, both for model solutions and for must and wine samples. The method was used to determine the tartaric acid content of thawed samples of frozen must and wines during vinification, making sure that the acidification of the samples achieved perfect redissolution of the tartrates.

INTRODUCTION

Tartaric acid is the most abundant and characteristic of the acids found in grapes and, consequently, in musts and wines. Depending on the pH of the solution, tartaric acid can be present in the anionic forms, tartrate or hydrogen tartrate. In non-stabilized wines these forms cause crystalline precipitations mainly with the calcium and potassium ions, the intensity of which depends upon such factors as alcoholic degree, temperature or ionic strength (Mazzoleni & Silva, 1985; Clark *et al.*, 1988). Numerous methods have been proposed to measure tartaric acid in musts and wines, including gravimetric (AOAC, 1970), potentiometric with selective ion electrode (Boulton, 1978), colorimetric (Hill & Caputi, 1970; Rebelein, 1973; Amerine, 1973), gas–liquid chromatography (Zanier & Tanner, 1977; Marcy & Carrol, 1982; Di Stefano & Bruno, 1983) isotachophoresis capillary (Karovicova *et al.*, 1990) and HPLC methods. Nowadays, HPLC is the most applied technique in the analysis of organic acids, and several systems have been developed including reversed phase (Mentasti *et al.*, 1985; Caccamo *et al.*, 1986; Koseki *et al.*, 1989; Marcé *et al.*, 1990), anion-exchange (Vrátny *et al.*, 1983) and cation-exchange (McCord *et al.*, 1984; Schneider *et al.*, 1987; Bissell *et al.*, 1989; Kupina *et al.*, 1991). Recently, the methods most commonly used employ a strongly cationic exchange resin and isocratic elution with diluted acid; with the use of an adequate detector, this method permits the simultaneous determination of organic acids and sugars, with high reproducibility.

For routine analysis, Rebelein's method (Rebelein, 1973), although not the officially adopted method, is recommended by the Office International de la Vigne et du Vin (O.I.V., 1978) and is widely used because it permits the simultaneous analysis of numerous samples. It employs activated carbon to decolorize the wine and it is this stage which is the most controversial; several authors have proposed modifications because of its poor reproducibility (Pilone, 1977; Vidal & Blouin, 1978; Mattick & Rice, 1981; Ubigli, 1981; Trossais & Asselin, 1985).

During fermentation it is necessary to process a large number of samples due to the rapid evolution of the must. This can be resolved by means of freezing the musts in order to stop the fermentation process. However, this freezing has a strong influence on the insolubilization of the tartrates, which are later difficult to redissolve even by heating or sonication.

In this work we compare Rebelein's and a HPLC cation-exchange method to carry out the analysis of thawed must and wine samples collected during vinification. The methods were used to determine the tartaric acid content, in a series of experiments during vinification of the Monastrell grape variety.

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MATERIALS AND METHODS

Samples

The musts and wines used came from red grapes of the Monastrell variety cultivated in the wine-producing area of Jumilla, Region of Murcia (Spain). Samples were frozen immediately and kept at -18 ± 1°C until analyzed. To determine the tartrate content, the must or wine samples were thawed in an ultrasonic bath to 40°C to help redissolution.

To chemically dissolve the tartrates precipitated in the freezing process, the thawed must or wine samples were acidified to pH 1 by the addition of 14 N nitric acid. The extract was stirred for 1 min and clarified by centrifugation. It was then ready for the tartrate determination.

Model solution

The recovery rate of the precipitated tartrates was studied by preparing a model solution containing: 4·80 g/litre potassium hydrogen tartrate, 7·00 g/litre citric acid, 3·00 g/litre sucrose, 10·00 g/litre glycerine, 0·27 g/litre monosodium phosphate, 0·14 g/litre calcium chloride, 0·21 g/litre magnesium chloride and 95·00 g/litre ethanol. The model solutions were submitted to the same treatment of freezing, thawing and acidification as the wine samples.

Rebelein's method

Thawed sample (25 ml) was added to 0·1 g of activated carbon and stirred for 15 min. The mixture was then filtered using a no. 2 Whatman filter paper. Five ml of the resulting colourless liquid was added to 10 ml of solution I (100 mM silver nitrate in an aqueous solution of acetic acid 30% (v/v)) and to 10 ml of solution II (85 mM ammonium metavanadate, 150 mM sodium hydroxide and 656 mM sodium acetate). The acetic–acetate buffer used adjusted the pH to an adequate value for the development of the reaction with metavanadate without depending upon the acidity of the sample. This mixture was stirred and the colour allowed to develop for 15 min at room temperature. Then, the coloured solution obtained was filtered through a no. 2 Whatman filter paper. Colour absorbance was measured at 530 nm, using solution II as reference with a Perkin-Elmer (Norwalk, Connecticut, USA) spectrophotometer, model 554.

In a parallel experiment with the activated carbon omitted, the spectrophotometric absorbance, measured at 530 nm after the colour reaction, was corrected by subtracting the absorbance reading of the wine diluted with equal proportions of metavanadate solution.

The stability of the reagents used was checked to see if they had changed with time, temperature and light.

HPLC method

The HPLC equipment consisted of a modular Shimadzu (Kyoto, Japan) liquid chromatography system equipped with two LC-6A pumps operated from a Shimadzu SCL-6A controller. A Shimadzu SPD-M6A photodiode UV/Vis detector and a Rheodyne (Cotati, California, USA) injector (model 7125) were used. Analysis was performed in a polymeric column ION 300 (Interaction Chemicals Inc., Mountain View, California, USA) (300 mm × 7·8 mm) at 70°C, with isocratic elution using 0·008 N sulphuric acid as mobile phase with a flow rate of 0·5 ml/min. Prior to the injection, samples were filtered through a 0·45 μm pore size nylon filter (Lida Manufact. Corp., Kenosha, Wisconsin, USA).

Reagents

The charcoal used was A.R. quality from Merck (Darmstadt, Germany), and was checked to prove it did not release acids into the medium. The rest of the reagents used were of A.R. quality. In the chromatographic separation they were HPLC quality, and water was MilliQ quality.

Calculations

The tartaric acid content of all samples was determined from the regression equations of standard curves.

RESULTS AND DISCUSSION

Table 1 shows the results obtained when Rebelein's method (Rebelein, 1973) was applied to previously frozen must samples, thawed in an ultrasonic bath.

Owing to the poor results, an HPLC analysis was carried out in order to obtain more accurate data. However, these new data were no better than those obtained by Rebelein's method. This provoked us, on the one hand, to optimize the conditions of the Rebelein assay, and on the other to redissolve the tartrates precipitated in the freezing stage. The modifications introduced consisted of the omission of the activated carbon, the study of the reagents' stability and the redissolution in acidic medium of the precipitated tartrates.

Table 1. Tartaric acid concentration of must samples frozen during fermentation. Determination by Rebelein's method

Days*	g/litre
2	1·53
3	1·62
4	1·10
5	1·05
6	0·98
7	1·19
8	1·21
9	1·13
10	1·14
12	1·26

* Days elapsed since the beginning of fermentation.

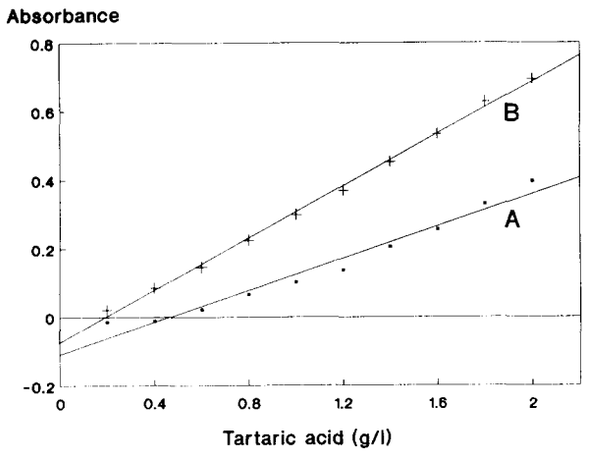

Fig. 1. Graphical representation of the absorbance values obtained in model solutions of tartaric acid following (A) Rebelein's method $r = 0.9587$ and (B) the optimized method $r = 0.9987$.

Omission of the activated carbon

Figure 1 shows the results obtained when model solutions of tartaric acid were measured by Rebelein's method (A) and when the activated carbon was omitted in a modification of the same method (B). It can be observed that the correlation coefficient of line A is clearly lower, and even shows negative absorbancies for low concentrations. This proves that tartaric acid is adsorbed by the charcoal. To confirm this adsorption we carried out an experiment where several solutions of tartaric acid in aqueous ethanol of concentrations between 1 g/litre and 7 g/litre were treated with the charcoal and, after this, the acidity of the filtered solutions was determined by standard titration and by HPLC. The results showed that the charcoal adsorbed between 10 and 25% of the acid, according to the level of tartaric acid in the solution. The higher percentage of absorption corresponded to the more diluted solution. These results are in accordance with the observations of Vidal and Blouin (1978) and Mattick and Rice (1981) and could be interpreted as a non-selective adsorption by the activated carbon of the components which take part in the reaction, in agreement with Pilone's conclusions (Pilone, 1977).

Study of the reagents' stability

Tables 2 and 3 summarize the results obtained when the following influences are considered: time elapsed since the colour reaction process, and storage conditions of the reagents employed.

The time elapsed (up to 90 min) after the end of the colour reaction process is not critical. Neither do the storage conditions have any significant influence, as long as the silver salt is adequately protected against the light (in a topaz flask, for example). The greatest differences in adsorbance were noted when analyses realized with different reagents and standards were compared. A new calibration straight line was necessary every time new standard solutions of reagents were prepared.

Table 2. Influence on the spectrophotometric absorbance of time elapsed since the colour reaction

Tartaric acid (g/litre)	Time elapsed (min)		
	15	45	90
0·40	0·060 ± 0·007[a]	0·059 ± 0·007	0·057 ± 0·007
0·60	0·100 ± 0·007	0·095 ± 0·007	0·093 ± 0·007
1·00	0·238 ± 0·010	0·235 ± 0·008	0·230 ± 0·008
1·40	0·389 ± 0·011	0·387 ± 0·012	0·380 ± 0·011
1·80	0·546 ± 0·014	0·545 ± 0·013	0·537 ± 0·014
2·00	0·652 ± 0·014	0·652 ± 0·014	0·645 ± 0·012

[a] Mean ± standard deviation of three determinations.

Redissolution in acidic medium of the precipitated tartrates and quantification by Rebelein's and HPLC method

To study the redissolution of the tartrates precipitated by freezing, standard solutions prepared in the manner previously described were kept for 10 days at −18 ± 1°C, 4 ± 1°C and 20 ± 2°C. After this time, a slight precipitation was observed in the sample maintained at 4 ± 1°C and an intense precipitation in the sample at 18 ± 1°C. In all samples, the tartrates were quantified by means of acid redissolution. The results obtained, expressed as tartaric acid, are shown in Table 4. The recovery percentages of all samples showed similar values for both methods of between 95% and 105%.

To check the recovery level in red wines, several samples were prepared by addition of known quantities of potassium hydrogen tartrate to 5 ml of previously analyzed wine (1·93 g/litre of tartaric acid). These samples were submitted to the freezing and thawing processes already described. Table 5 shows the quantification of tartaric acid by the optimized Rebelein's and HPLC methods in these samples.

Application of the optimized Rebelein method

This method was used to study the evolution of the tartrates in the vinification process of red Monastrell grapes. Figure 2 shows the dependence between the tartaric acid content and the alcoholic degree parameters which have the greatest influence on tartrate precipitation. Samples were frozen immediately after being taken

Table 3. Influence on the spectrophotometric absorbance of the storage conditions (light and temperature) of the reagents used

Tartaric acid (g/litre)	Storage conditions		
	Topaz flask, room temperature	Darkness, room temperature	Darkness 4°C
0·40	0·057 ± 0·007[a]	0·056 ± 0·008	0·057 ± 0·007
0·60	0·090 ± 0·007	0·088 ± 0·007	0·091 ± 0·006
1·00	0·226 ± 0·011	0·235 ± 0·008	0·234 ± 0·006
1·40	0·393 ± 0·012	0·379 ± 0·012	0·390 ± 0·007
1·80	0·573 ± 0·014	0·541 ± 0·014	0·557 ± 0·010
2·00	0·634 ± 0·011	0·630 ± 0·010	0·629 ± 0·009

[a] Mean ± standard deviation of three determinations.

Table 4. Tartaric acid recovery of optimized Rebelein's and HPLC methods after acidification with nitric acid of the standard solutions kept at different temperatures

Tartaric acid (g/litre)	Conservation temperature											
	20°C				4°C				−18°C			
	HPLC		Opt. Reb		HPLC		Opt. Reb		HPLC		Opt. Reb.	
	g/litre[a]	%[b]	g/litre	%	g/litre	%	g/litre	%	g/litre	%	g/litre	%
3·84	3·69	96	3·65	95	3·73	97	3·70	96	3·75	98	3·73	97
1·92	1·85	96	1·82	95	1·88	98	1·86	97	1·90	99	1·88	98
0·77	0·80	104	0·81	105	0·79	103	0·79	103	0·77	100	0·78	101
0·38	0·40	105	0·39	103	0·40	105	0·40	105	0·38	100	0·39	103
0·19	0·18	95	0·20	105	0·20	105	0·20	105	0·20	105	0·20	105

[a] Recuperated tartaric acid.
[b] Recovery.
Average of three replications.
Opt. Reb. = Optimized Rebelein.

Table 5. Quantification of tartaric acid by Rebelein's and HPLC methods in fortified wine samples submitted to the freezing and thawing processes

Sample	Tartaric added[a] (mg)	Total tartaric (g/litre)	Rebelin		HPLC	
			g/litre	%	g/litre	%
1	7·98	3·22	3·38	105	3·25	101
2	15·96	3·66	3·77	103	3·70	101
3	23·94	4·20	4·16	99	4·33	103
4	31·92	4·62	4·67	101	4·63	100
5	39·90	4·96	4·81	97	5·06	102
6	47·88	5·93	5·63	95	6·17	104

[a] From a standard solution of 10·0 g/litre potassium hydrogen tartrate.
Average of three replications.

and kept at −18 ± 1°C. The same acid redissolution procedure already described was used in the analysis.

The decrease in tartaric acid is significantly important during the first 12–14 days following the start of fermentation. The tartrate content showed no significant dependence upon pH, which varied slightly in our experiment. A greater dependence was established between the degree of alcohol and the precipitation of tartaric acid; indeed, the ethanol present modified the dissociation constant of the tartaric acid (Usseglio-Tommasset & Bosia, 1978; Postel, 1983; Clark *et al.*, 1988), increasing the level of insoluble salts. In our experiments the greatest precipitations occurred when the degree of alcohol was higher than 12 degrees.

CONCLUSIONS

Quantification of tartaric acid by Rebelein's method showed the same inconveniences mentioned by other authors. Omission of the activated carbon used as decolorizer improved the determination. The acidification of frozen samples achieved perfect redissolution of the tartrates, rendering feasible the freezing of samples to stop the evolution processes of musts and wines, so that later analysis could be perfectly carried out. Cation-exchange HPLC verified the accuracy of the optimized Rebelein method. Although this latter method is slower than HPLC, it costs less and enables a large number of samples to be analyzed at the same time without affecting accuracy, thus offsetting the shorter time needed per individual sample with the HPLC technique.

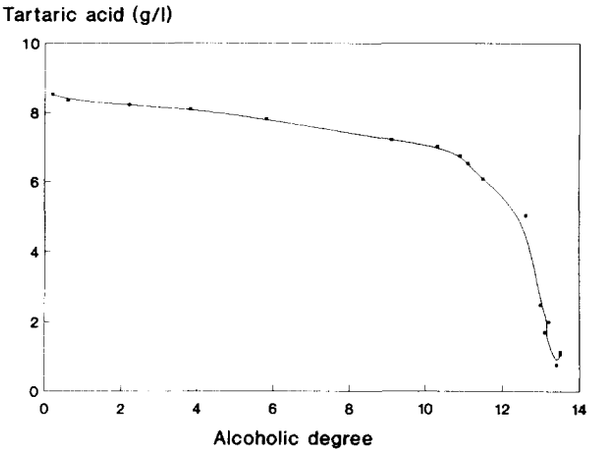

Fig. 2. Influence of the alcohol degree on the tartaric acid content during vinification of Monastrell grape.

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