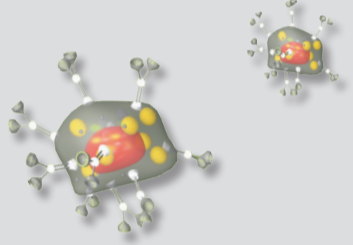


Evaluation of different colorimetric assays for routine analysis of tartaric acid in comparison to HPLC

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Abstract

The concentration of tartaric acid in must and wine is an important quality parameter since decades. Because there is no robust enzymatic method, researchers started early to develop the "Rebelein" method, which is based on the formation of a red complex out of tartaric acid and vanadate in acetic acidic solution. To transfer the principle to a commercial ready-to-use system, we used 28 wines of different origins and varieties which were analysed for tartaric acid by HPLC. The new assay and four other commercial colorimetric test kits were compared with these values. The new colorimetric assay of R-Biopharm shows very good correlation in comparison to HPLC and recovers around 100 % while the competitors failed in many cases.



Introduction

Besides sugars, alcohol and sulphite, the concentrations of organic acids and especially tartaric acid in must and wine are an important quality parameter since decades. In contrast to the other organic acids mentioned above, there is no robust commercial enzymatic method for the determination of tartaric acid and therefore researchers started early to develop an alternative which was established as the "Rebelein" method. The method is based on the formation of a red complex out of tartaric acid and vanadate in acetic acidic solution and the subsequent photometric determination of the extinction. Since the use of charcoal is mandatory, the determination gets quite cumbersome for the laboratory staff. The system seems simple but the transfer of the principle to a commercial ready-to-use system revealed problems. As a consequence, we started to evaluate a simple method for practitioners, which should be suitable both for white and red wines. For a precise examination, we used 28 wines of different origins and varieties which were analysed for tartaric acid by HPLC. Different commercial colorimetric test kits from Megazyme, BioSystems, Isitec, MTI diagnostics, and the new system of R-Biopharm were compared using the HPLC values as the reference.

Materials and methods

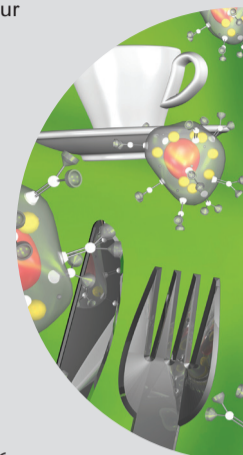
The pipetting scheme for the determination of tartaric acid in red wine using the Enzytec Colour Tartaric acid from R-Biopharm is shown in table 1.

Table 1. Scheme for pipetting the assay with the decolorization step in cuvettes (test volume 2.5 mL) and measurement at 520 nm.

	Sample	Calibrator	Blank
Wine	500 µL	-	-
Calibrator (5 g/L)	-	100 µL	-
Dist. water	-	400 µL	500 µL
Decolorant	200 µL	200 µL	200 µL
Dist. Water	1050 µL	1050 µL	1050 µL
Reagent 1	500 µL	500 µL	500 µL
Measure A1 at 520 nm			
Reagent 2	250 µL	250 µL	250 µL
Measure A2 at 520 nm			

A panel of 28 red, white and rose wines from all over the world were purchased from a local market in Germany. An overview on the panel of wines and results for the determination of pH-values, alcoholic strength, total acidity and different organic acids are given in the accompanying paper. The HPLC determinations for the tartaric acid were accomplished by combining a RP-18

column (Luna 5 µm C18; 250 x 4.6mm, Phenomenex) and an anion exchanger (Rezex Fast Fruit 8 % H, Phenomenex). The eluent was 1 % phosphoric acid in de-ionized water (HPLC quality). Separation was carried out isocratically at a flow rate of 0.7 ml min⁻¹, the detection is performed with a UV detector at 230 nm.



Results and discussion

The study of competitors revealed that they all chose different wavelengths to measure the OD. During the new development, this parameter was optimized and it could be shown that the wavelength has an influence on the sensitivity and the background values (fig. 1).

To analyze this observation more in detail, calibrators were measured at different wavelengths (figure 2). It is clear that 520 nm is a "compromise" wavelength where a low background value and a suitable sensitivity is observed. Linearity is given from 0.2 g/L up to 5 g/L (figure 3).

The in-house performance criteria for the assay are summarized in table 2. It is obvious that the determination in cuvettes is highly robust and that the performance data at all are suitable for routine analysis.

The influence of other organic acids on the tartaric acid colour reaction was investigated and it could be shown that increasing the amounts up to 10 g/L clearly shows decreasing values of tartaric acid of about 20 %. From a practical point of view, these high values will never be observed in wines but maybe in musts.

We decided to blind the competing manufacturers of other commercial test kits (table 3). Competitor 1 gave slightly better results for white wines but too high values for all red wines. In contrast, competitor 2 worked quite well with red wines but revealed too low values for white wines. When using the test kit of competitor 3, it was clear that there were problems with both kinds of wine. One of these systems (competitor 4) resulted in instable colour developments of the calibration solution (not shown) and therefore high variations in the tartaric acid concentration of a sample. In some cases

(competitor 1 and 2), the mandatory inclusion of malic acid concentrations to correct the tartaric acid results did not help to improve the assay results. In contrast, the new test kit Enzytec Colour Tartaric acid from R-Biopharm shows a very good accordance with HPLC results. To our opinion the reduction of some assay systems to two assay components (vanadate solution and acidic solution) is the main problem of most of these assays. This is the result of limited pipetting capacities of biochemistry analysers.

Figure 1. Wavelength scan (from 650 nm to 450 nm) for a tartaric acid calibrator (solid circles) and a reagent blank (open circles).

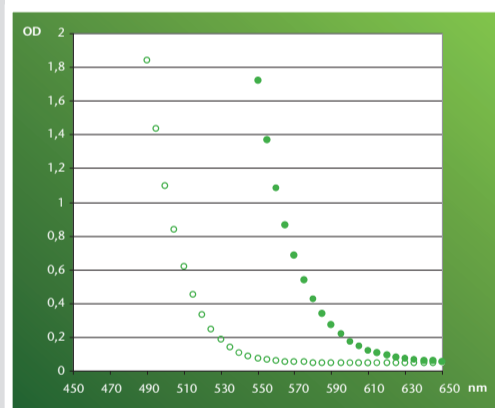


Figure 3. Calibration graph ranging from 0.2 up to 5 g/L measured as ΔOD at 520 nm (difference between A2 and A1). Insert: Calibration graph from 0.2 up to 1 g/L. Double determinations with CVs less than 1.7%.

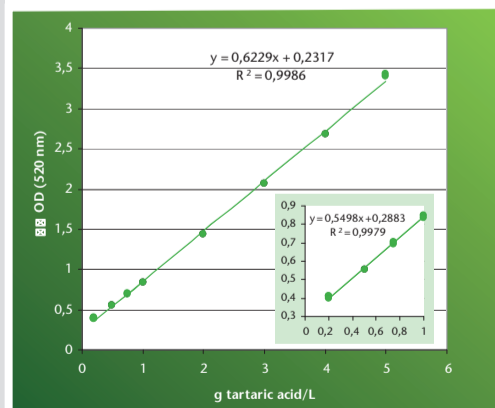


Figure 2. Calibration curves ranging from 0 g/L up to 2 g/L (n=1) measured at different wavelengths (500 nm, 510 nm, 520 nm, 540 nm). Linearity and the coefficient of variation are given.

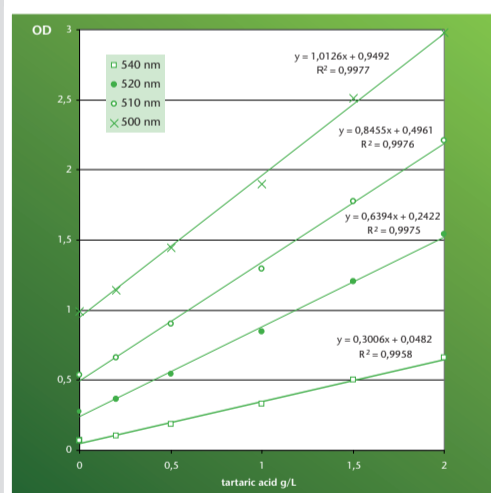


Table 2. In-house performance data for the assay Enzytec Color tartaric acid.

Limit of detection	0.1 g/L
Linearity	0.2 – 5 g/L
Intraassay CV (calibrator)	< 2 %
(sample)	< 1 %
Intraassay CV (sample)	< 5 %
Recovery (HPLC)	88 – 113 %
(reference samples)	90 – 100 %
(certified reference)	99 – 101 %
Specificity	D-tartaric acid, L-tartaric acid but not meso-tartaric acid
Interferences	D-malic-, L-malic-, L-lactic acid above 5 g/L (table 4)
Robustness (wavelength)	515 nm – 525 nm
(person)	Interassay CV is below 5 %
(day)	Interassay CV is below 5 %
(time)	measure OD of A2 within 2 h

Table 3. Panel of different wines; Comparison with of 4 commercial test kits, the new R-Biopharm system (measured in cuvettes) and HPLC (reference values). **Pale green:** recovery outside the 90 and 110 % range; **dark green:** recovery outside the 80 and 120 % range compared to HPLC.

No.	Origin	Variety	HPLC g/L	Rebelein g/L	R-Biopharm g/L	Comp. 1	Comp. 2	Comp. 3	Comp. 4
1	Germany	Spätburgunder	1.60	1.5	1.51	1.96	1.39	-0.97	1.33
3	Italy	Valpolicella	1.99	2.1	2.16	2.76	1.98	2.01	1.92
4	Argentina	Tempranillo	1.55	1.6	1.61	2.76	1.67	1.80	1.52
5	South Africa	Cabernet Sauvignon	2.41	2.3	2.32	3.70	2.06	-0.31	2.19
6	Italy	Chianti	2.17	2.3	2.39	3.32	2.22	2.54	2.40
9	Italy	Cuvee	2.31	2.6	2.58	2.95	2.20	1.28	2.12
10	Argentina	Cabernet Sauvignon	1.93	1.9	1.74	3.06	1.77	1.46	1.54
11	Bosnia-Herzegovina	Blatina	1.69	1.9	1.76	2.43	1.69	2.59	1.69
12	Spain	Tempranillo	2.33	2.4	2.18	3.47	2.03	0.82	2.12
13	South Australia	Shiraz Cabernet	2.42	2.4	2.38	3.90	2.07	1.09	2.17
14	France	Cuvee	1.91	1.9	1.69	2.61	1.61	1.97	1.68
15	Italy	Bardolino	2.02	2.1	2.29	2.67	1.92	0.88	2.07
2	Spain	Cuvee	1.48	1.5	1.52	1.48	1.18	1.32	0.77
17	Chile	Cabernet Sauvignon	3.52	3.9	3.88	3.45	3.18	3.32	3.12
7	Italy	Cuvee	2.86	2.9	2.93	2.44	2.44	3.06	2.38
8	Italy	Pinot grigio	2.14	2.1	1.96	1.65	1.65	2.22	1.53
16	Italy	Pinot bianco	2.10	2.2	2.14	1.92	1.64	1.71	1.93
18	Italy	Malvasia	1.86	1.9	1.81	1.47	1.44	1.95	1.29
19	Turkey	cuvee	3.28	3.5	3.45	2.95	2.84	3.20	2.87
20	Spain	cuvee	1.75	1.8	1.65	1.41	1.17	2.10	1.13
21	Spain	cuvee	2.70	2.6	2.64	2.24	2.11	2.62	1.83
22	South Africa	Sauvignon blanc	1.88	2.0	1.79	1.67	1.32	2.02	0.89
23	California	Chardonnay	1.39	1.4	1.40	1.02	0.99	1.93	1.08
24	South Australia	Chardonnay	1.99	2.0	1.80	1.56	1.38	1.95	1.19
25	France	Chablis	2.02	2.0	2.04	1.56	1.43	2.28	1.78
26	Greece	Muskat Cavino	1.39	1.4	1.46	1.19	1.05	2.01	1.15
27	Germany	Müller Thurgau	2.07	2.2	2.01	1.89	1.64	1.10	1.69
28	Germany	Riesling	3.36	3.5	3.47	2.87	2.59	3.38	2.21

Conclusion

The new colorimetric assay of R-Biopharm shows a very good correlation in comparison to HPLC and recovers around 100 %. The assay is calibrated to a certified reference material and therefore traceable according to ISO guides. A validated assay system for the use in microtitreplates will be launched soon and will give analysts the opportunity to run it on automated systems.

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