A Comparison of Various Methods and Apparatus Available on the Market for Measuring Reducing Sugars in Wine

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Abstract: In this simple, non-comprehensive evaluation, various methods and apparatus for determining reducing (residual) sugars (RS) in wine were compared in their accuracy to measure standard solutions of known sugar concentrations. Since the initial amounts of RS in unadulterated wine samples were treated as unknowns, this evaluation looked at the correlation of measurements among methods and apparatus tested. This evaluation also looked at recovery of sugar in wine samples with 5 g/L of added glucose for methods and apparatus tested. The Hanna HI 902 unit measured RS levels and sugar recovery within 5% while the HI 83746 unit had significantly high errors, particularly with model solutions of low RS. The Vinmetrica RS Assay Kit measured RS levels and sugar recovery all within 8.5%, except for model solutions with 1 g/L and 2 g/L RS. The Fermentest kit, which uses color changes and RS evaluated against a color map, provided results sufficiently good to give a rough approximation of RS. Accuvin Residual Sugar test strips also make use of color changes but proved to be too difficult to make a satisfactory estimation of RS.

Keywords: Reducing sugars, residual sugars, Rebelein Method, Fehling, Hanna, Vinmetrica, Fermentest, Accuvin

Introduction. The objective of this simple, non-comprehensive study was to evaluate various methods and apparatus for determining reducing (residual) sugars (RS) in wine to provide home winemakers and small wineries guidance on choosing a method that best suits their needs.

Winemakers measure the amount of sugars remaining in wine to assess taste and microbial stability.

A wine with less than 2 g/L (0.2%) of residual sugars is considered *dry*, meaning, there is no perceptible taste of sweetness. As the amount of sugars increases, the taste of sweetness becomes more pronounced, particularly with low acidity. A wine with, for example, 5 g/L of residual sugar with relatively high acidity can still taste dry, but it can taste *off-dry* if there is much less acidity.

However, a wine with less than 2 g/L of residual sugars is considerably more stable, from a microbiological perspective, than a wine containing more; the latter will require special consideration and processing prior to bottling. If wine with more than 2 g/L of residual sugars is not processed to remove yeasts and bacteria, it is a great risk of refermenting or of microbial spoilage. Yeasts can convert any residual fermentable sugars into ethanol and carbon dioxide, and a greater problem if it occurs in bottles. Bacteria can also metabolize those sugars into other substances that can cause flaws or outright faults, possibly making the wine unappealing or undrinkable.

Wines with more than 2 g/L of residual sugars should be sterile filtered, as is done in commercial winemaking, to remove yeasts and bacteria down to a level at which they pose no danger. In home winemaking where sterile filtration may not always be possible, potassium sorbate (the potassium salt of sorbic acid) is used in conjunction with sulfite (e.g., potassium metabisulfite) to inhibit yeast cells and prevent refermentation, although this is only done in wines that have not undergone malolactic fermentation to avoid the risk of sorbic acid developing into unpleasant smells of rotting geraniums.

Although the term *residual sugars* is commonly used to refer to sugars remaining in wine, the technically correct terminology in terms of what is being measured is *reducing sugars*.

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Reducing sugars (RS), so-called for their ability to chemically "reduce" other substances, include fermentable glucose and fructose and unfermentable sugars like arabinose and ribose. RS measurements may not necessarily be accurate indicators of fermentation completion because of unfermentable sugars, but these are usually only present in very small amounts in wine. If a more accurate measurements is needed, for example, to make an accurate assessment of fermentation completion, an analysis of Glucose+Fructose concentration would be required.

Sucrose is a disaccharide comprising of glucose and fructose and is therefore not a reducing sugar. Sucrose only becomes fermentable once inverted and hydrolyzed into glucose and fructose. Therefore, sucrose can contribute to sweetness but is not measured in reducing sugars analysis. Since there is only trace amounts of sucrose in wine, it is only an issue when measuring reducing sugars when adding sugar to sweeten wine. Samples with added sucrose would require to be treated with an acid and heat to hydrolyze sucrose prior to measuring RS.

RS in wine can be measured analytically by copper reduction methods or by enzymatic assay.

The Rebelein Method, also known as the Titrametric–Rebelein Method and Gold Coast Method, is the most commonly used copper-reduction method. It consists of causing reducing sugars in a wine sample to react with copper (II) by using copper sulfate under alkaline conditions. Potassium iodide and sulfuric acid are then added to reduce excess copper to produce an equivalent amount of iodine, which is then titrated with sodium thiosulfate. Results are expressed in g/L reducing sugar (RS) or as % wt/vol. There are many variations of the Rebelein Method, including making a measurement by UV spectrometry instead of by titration.

For methods using copper reduction, red wines must first be decolorized using activated charcoal or polyvinylpolypyrrolidone (PVPP) to eliminate interference from anthocyanins as these are also reducing substances and would therefore skew measurements. Also, wines should not contain other reducing substances, such as ascorbic acid.

Enzymatic analysis of RS in wine involves a series of enzyme-enabled reactions that convert reducing sugars, but not pentoses, into substances that can be measured by observing a color change or by UV spectrometric measurements.

Materials and Methods

Model Solutions and Wine Samples. Model reference solutions of known concentrations and wine samples were used to evaluate RS methods and apparatus.

Model solutions with 10 g/L and 20 g/L RS were prepared by dissolving anhydrous, lab-grade *D*-glucose powder in demineralized water and adjusting the pH to 3.2–3.6 by adding lab-grade *L*-tartaric acid crystals. The 10-g/L solution was used to prepare model solutions with 1, 2 and 5 g/L RS, respectively.

Four wine samples of unknown RS were used: two whites and two reds. The base, unadulterated white wine was a Sauvignon Blanc vinified from grapes sourced from Lodi, California from the 2017 harvest. The second white-wine sample (adulterated) was prepared using the same base wine but with the equivalent of 5 g/L of *D*-glucose added. The base red wine was a Sangiovese vinified from frozen must (juice plus crushed grapes) sourced from Tuscany, Italy from the 2011 harvest but fermented in 2015, then decolorized using a PVPP treatment at 100 g/L followed by centrifugation at 1500 X g. A second sample was prepared using the decolorized base red wine with the equivalent of 5 g/L of *D*-glucose added. The purpose of sugar-adulterated samples was to verify recovery of the added *D*-glucose.

Methods and Apparatus. The following methods and apparatus were tested to measure RS in model solutions and wine samples. Knowing the range of expected RS will help decide the size of sample needed for analysis, particularly if sample dilution is required as methods have maximum ranges. Only a single test for each sample and each method or apparatus was performed.

A Hanna Potentiometric Titrator (HI 902) unit running Wine Pac V2.3 and measuring RS using the Titrametric–Rebelein method was used. The unit was first calibrated by analyzing a blank sample (distilled water) and 10- and 20-g/L non-acidified *D*-glucose standards. A simple titer determination cannot be performed, but rather a calibration factor must be determined by using glucose standards as the oxidation of sugars with Cu(II) takes place neither stoichiometrically nor linearly with time. 2.0-mL samples of DI water and appropriate volumes (Table 1) of standards, model solutions and wines were treated with 5 mL each of Fehling Solution A (copper sulfate) and Fehling Solution B (potassium sodium tartrate, Rochelle Salt) solutions, then heated to a gentle boil for 2 minutes. The cooled solutions were treated with 10 mL each of concentrated potassium iodide and 16% sulfuric acid, and brought to 100-mL volume with DI water. Solutions were titrated with a 0.1*M* sodium thiosulfate solution under the full control of the HI 902 to an inflection endpoint determined by a redox electrode. Results are automatically calculated and reported in "g/L reducing sugars" by the unit.

Expected RS (g/L)	Sample Volume (mL)	Dilution Factor
< 3	15.0	1
3 – 6	6.0	1
6 – 10	3.0	1
10 – 20	2.0	1

Table 1. Sample volume sizes and dilution factors for expected RS levels for analyzing samples with the HI 902

A Vinmetrica Residual Reducing Sugar (RRS) Assay Kit (Figure 1) using the Rebelein method with Version 1.0 of the User Manual was used. The method requires no dilution of samples for expected RS levels less than 28 g/L. 2.0-mL samples of DI water (blank), model solutions and wines were treated with 10 mL each of Copper Sulfate (Fehling Solution A) and RRS Binding Solution (Fehling Solution B), then heated in a boiling-water bath for 5 minutes. The cooled solutions were treated with 10 mL of RRS (1+5) Sulfuric Acid Solution, 2 mL of RRS Developer Solution (potassium iodide) and 2 mL of Starch Indicator Solution, and immediately titrated to a light cream-colored endpoint using the RRS Titrant (0.20*N* sodium thiosulfate). The volume V_a and V_b of RRS Titrant used to titrate samples and the blank, respectively, were then used to calculate the RS in g/L reducing sugars according to the following formula (this formula has been updated in Version 1.21of the User Manual):

$$RS = 28 - \left[28 \times \left({}^{V_a}/_{V_b}\right)\right]$$

Figure 1. Vinmetrica Residual Reducing Sugar Assay Kit reagents and test apparatus

A Hanna Photometer for the Determination of Reducing Sugars in Wine (HI 83746) unit (Figure 2) measuring RS using the UV Spectrometric–Rebelein method at a wavelength of 610 nm was used. The method requires no dilution of samples for expected RS levels less than 50 g/L. Vials (HI 83746A-0) containing Fehling Solution A were dosed with 1.0 mL of Fehling Solution B (HI 83746B-0) and 200 µL of sample (blank, model solutions, wines) to be analyzed. The vials were incubated in a Hanna Reactor (HI 839800) at 105°C (221°F) for exactly 7 minutes and then allowed to cool to room temperature for 40 minutes, mixing once after the first 10 minutes. The photometer was zeroed using the blank, then each sample was measured.

Figure 2. Hanna Photometer for the Determination of Reducing Sugars in Wine, Reactor (heater), and reagents

A Laboratoires Dujardin-Salleron Fermentest kit (Figure 3) that uses tablets for the copper-reduction reaction was used. A tablet containing copper sulfate and alkaline salts is added to diluted volumes of samples of each model solution and wine. For samples with an expected RS less than 5 g/L, 25 drops of wine were diluted with 15 drops of DI water in a small test tube, and for samples with an expected RS between 5 and 20 g/L, 6 drops of wine were diluted with 34 drops of DI water, as per instructions. The mixture generates a temperature rise that causes the characteristic reaction of reducing sugars. The result is obtained by observing the color change with the excess of blue copper salts and/or red copper oxide formed. Each test tube was held for several seconds under hot water to accelerate the reaction. The color at the end of the reaction, which took up to two minutes, was then compared against the supplied color chart for the appropriate range, i.e. 0–5 g/L or 0–20 g/L.

Figure 3. Laboratoires Dujardin-Salleron Fermentest kit

Accuvin AV – Residual Sugar test strips (Figure 4) were used. These are based on the change in color exhibited by a Trinder color indicator during a reaction involving hydrogen peroxide in the presence of the enzymes peroxidase and glucose oxidase. The method requires no dilution of samples for expected RS levels less than 2 g/L. A small sample of model solution or wine was applied to the pad on a test strip and the color at the end of the reaction, approximately two minutes, was compared to a supplied color chart.

Figure 4. Accuvin Residual Sugar test strips

Results and Discussion

Table 2 tabulates test results obtained using the HI 902, Vinmetrica method, and HI 83746, showing expected RS values for samples, measured values along with absolute and percent differences between those values. Given the challenges of all methods in accurately measuring RS in wine, differences under 10% were considered "good."

Sample	Expected RS (g/L)	HI 902				Vinmetrica				HI 83746			
		Sample Size (mL)	RS (g/L)	Diff	%Diff	Sample Size (mL)	RS (g/L)	Diff	%Diff	Sample Size (mL)	RS (g/L)	Diff	%Diff
Model Solutions													
1 g/L RS	1.00	15.0	1.0391	0.04	3.91	2.0	1.80	0.80	80.12	0.2	1.75	0.75	75.00
2 g/L RS	2.00	15.0	2.0522	0.05	2.61	2.0	2.78	0.78	39.18	0.2	4.25	2.25	112.50
5 g/L RS	5.00	6.0	5.1977	0.20	3.95	2.0	5.24	0.24	4.80	0.2	3.25	-1.75	-35.00
10 g/L RS	10.00	2.0	10.504	0.50	5.04	2.0	10.15	0.15	1.52	0.2	8.50	-1.50	-15.00
20 g/L RS	20.00	2.0	19.404	-0.60	-2.98	2.0	18.34	-1.66	-8.30	0.2	19.75	-0.25	-1.25
2017 Sauvignon Blanc													
Unadulterated wine	Unknown	6.0	5.3254			2.0	5.40			0.2	6.25		
Unadulterated wine + 5 g/L	10.34		10.323	-0.02	-0.16		10.32	-0.02	-0.23		10.25	-0.09	-0.86
Incremental amount of *D* -glucose (g/L)	5.01		4.998	-0.02	-0.33		4.91	-0.10	-2.03		4.00	-1.01	-20.22
2011 Sangiovese													
Unadulterated wine	Unknown	6.0	3.2824			2.0	3.60			0.2	3.75		
Unadulterated wine + 5 g/L	8.29		8.1106	-0.18	-2.18		8.19	-0.10	-1.26		8.75	0.46	5.53
Incremental amount of *D* -glucose (g/L)	5.01		4.8282	-0.18	-3.61		4.58	-0.42	-8.47		5.00	-0.01	-0.18

Table 2. Test results

The HI 902 provided excellent results with almost all having a difference from expected values of 5% or less. Recovery of added sugar (5 g/L) were very good with differences of approximately 0.3% and 3.6% for the white- and red-wine samples, respectively.

The Vinmetrica method provided very good results when the expected RS is greater than 5 g/L. The difference for the 1- and 2-g/L model solutions were approximately 80% and 39%, respectively. Vinmetrica has updated its procedure and now recommends using 4.0-mL samples instead of 2.0 mL for greater accuracy in samples containing less than 4 g/L RS; the updated procedure (Version 1.21 of the User Manual) also has a new calculations to compute RS from the volumes of titrant used. Recovery of added sugar (5 g/L) was also very good with differences of approximately 2.0% and 8.5% for the white- and red-wine samples, respectively.

The HI 83746 provided significantly less accurate results with differences ranging from about 15% to 112% for model solutions with 10 g/L RS and less. There was however very good correlation with results for the adulterated wine samples obtained using the HI 902 and Vinmetrica methods. Recovery of added sugar (5 g/L) in the red wine sample is almost exactly as expected (difference of about 0.2%), but the difference in the recovery of added sugar in the white wine sample is more than 20%.

The RS values of both the base (unadulterated) white and red wines were unknown. Results demonstrated very good correlation between the HI 902 and Vinmetrica methods for both wines. The HI 83746 measured a somewhat higher RS, 6.25 g/L versus 5.33 g/L and 5.40 g/L for the white wine using the HI 902 and Vinmetrica methods, respectively. Correlation was better for all three methods and apparatus for the red wine with RS values of 3.28 g/L, 3.60 g/L and 3.75 g/L for the HI 902, Vinmetrica method, and HI 83746, respectively.

Given the difficulty in assessing a color change in samples against a chart, the Fermentest kit provided only a rough estimate of RS within, typically, between two colors. For example, for expected RS less than 5 g/L, the reaction color of the 2-g/L model solution sample was between 1.5 g/L and 2 g/L while the 5-g/L model solution sample was between 3 g/L and 5 g/L; for expected RS greater than 5 g/L, the reaction color of the 20-g/L model solution sample was between 12 g/L and 20 g/L. Both unadulterated white and red wine samples were in the 3- to 5-g/L color range while the adulterated samples were in the 8- to 10-g/L color range.

Assessing color changes with Accuvin test strips proved to be a much bigger challenge with, for example, no apparent color difference between the 5- and 10-g/L model solution samples. Diluting samples 20-fold, as per instructions, yielded colors so light that it was very difficult to make a reading, which would be erroneously much greater once multiplied by 20.

Conclusions

For home winemakers and small wineries on a budget, the Vinmetrica Residual Reducing Sugar (RRS) Assay Kit provides very good results with a per-test cost of approximately US$3.00. The method does require some time to perform, particularly if red-wine samples need to be decolorized, and handling of several reagents, but is relatively simple. Although the method requires detection of a color change for determining the titration endpoint, the color change happens quickly and crisply with only an incremental amount of 0.05 mL of titrant.

The Fermentest method is quick and cheap at a per-test cost of approximately US$1.85, but only provides an estimate of RS. Matching the color of reacted samples to a color can prove difficult.