The Australian Wine Research Institute

Standard Method for the Measurement of Brix in Grapes and Must

Version Control

Version	Changes made in this version
1.0	First version of this method
1.1	Revised to reflect feedback from reference group

It should be noted that this procedure represents the current industry best practice for the measurement of brix in grapes. It is expected that this method will be open to modification and improvement as experience within industry dictates, as technology improves or the understanding of the science behind grape chemistry improves.

1. Introduction/Foreword

The purpose of this method is to define a standardised approach for the measurement of °Brix (or Baumé) in wine grapes or must. This method is specifically to address situations where Brix (or Baumé) measurement is used as part of the process for setting payment or rejecting grapes. While it is applicable to the general measure of Brix in grapes the level of quality assurance and rigor required for other purposes is at the discretion of the end user.

Validation of other automated equipment such as weighbridge sampling systems should be done in reference to this procedure and be able to demonstrate equivalent levels of accuracy and precision.

The Bé scale is generally used as an indication of the ripeness of grapes and is an indication of total dissolved solids (TDS) in the grape juice. The Bé of a must is also an approximate indication of the likely alcohol content of a dry wine produced from that must.

The Brix scale, commonly used by the brewing industry and to a lesser extent by the wine industry, relates directly to Bé via the following conversion formula:

1.00° Bé = 1.80° Brix

The °Brix is equivalent to the percentage sugar (sucrose) by weight, i.e. % w/w.

2. Scope

This method applies to the measurement of Brix (or Baume) in grapes and must.

3. Terminology

Must is defined as a mixture of grape juice, grape pulp, and grape skin that is fermented into wine.

A refractometer is a scientific instrument that measures the amount that light is bent (or refracted) when it moves from the air into a sample. Refractometers are typically used to determine the refractive index of a liquid sample.

The refractive index is a dimensionless number that describes how fast light travels through a material and provides a means of obtaining the sugar concentration in grams per liter for grape musts.

Refractive index meters come in both handheld and benchtop forms.

Handheld optical refractometers can also be known as Abbe refractometers.

4. Measurement Principle

A refractometer is an instrument that optically measures the density of a liquid. Light passes through the sample and is deflected in relation to the density of the sample. The instrument is calibrated in terms of refractive index and also usually contains a scale in terms of degrees Brix. Some instruments also can automatically convert between Brix and Baume units.

A common type of refractometer consists of two prisms between which a portion of the test sample is placed. A mirror will reflect light through the prisms and test sample. A telescopic tube with crosshairs is superimposed on the field of vision, correlating to a scale calibrated in terms of refractive index, degrees Brix, or both. There is also a compensator to correct for the chromatic dispersion of light, and a mechanism to correct for the temperature of the refractometer. This mechanism generally does not correct for the temperature of the sample except in the case of some benchtop units.

5. Units of Measurement

For use in the assessment of grapes measurements are generally in terms of Brix. A Brix value, expressed as degrees Brix (°Bx), is the number of grams of sucrose present per 100 grams of liquid.

The Australian wine industry also commonly uses the Baume when assessing the scale which can be reasonably be converted using the relationship;

1.00° Bé = 1.80° Brix

6. Metrological/Technical Requirements

The values adopted and published by the ICUMSA (International Commission for Uniform Methods of Sugar Analysis) are valid for the relation between the mass fraction of sucrose in sucrose-water solutions and the refractive index n for the wavelength l = 589.3 nm at a temperature of 20 °C. Refractometers should be marked indelibly with the following:

- the name or trademark of the manufacturer or representative
- the serial number
- the measuring ranges
- the year of production

For handheld or Abbe refractometers, the following shall be marked indelibly on the scales:

- scale for refractive index
- scale for the mass fraction
- for scales marked according to mass fraction, the type of liquid for which the refractometer has been adjusted (e.g.: aqueous solutions for which the relation between the refractive index and the mass

fraction is known and has been published by national bodies or by international commissions such as ICUMSA)

The maximum permissible errors on verification are the following:

- for analogue scales: ± 0.5 scale interval
- for digital scales: ± 1 scale interval

7. Health and Safety Considerations

Ensure safety glasses worn as mild discomfort if sucrose stock reagent comes into contact with eyes.

8. Materials/Apparatus

- Calibrated refractometer.
- Calibrated thermometer.
- 50mL plastic sample tubes for juice settling.
- Snap-lock sandwich bags for crushing grape samples.
- Lint free tissue (kimwipes or equivalent).
- Plastic Pasteur pipettes.
- Centrifuge, capable of approximately 3500 rpm and 50 ml centrifuge tubes (If required).

9. Reagents

- *10 º Baume = 18º Brix solution*
  Prepared by adding 18.0 g of AR grade sucrose to a 100 mL Schott bottle (or equivalent) then adding 82.0 g of Milli-Q water (total weight of 100 g). Sample to be stored in a fridge (4ºC) when not in use. Solution should be prepared fresh monthly.

- *14 º Baume = 25.2º Brix solution*
  Prepared by adding 25.2 g of AR grade sucrose to a 100 mL Schott bottle (or equivalent) then adding 74.8 g of Milli-Q water (total weight of 100 g). Sample to be stored in a fridge (4ºC) when not in use. Solution should be prepared fresh monthly.

- *RO water or similar (i.e. purified/distilled water)*

10. Verification/Calibration

Both the refractometer and the thermometers used within this protocol should be calibrated before use instead of set intervals if usage is infrequent.

Equipment without serial numbers or some other means of identification should be labeled with a property decal.

A. Thermometers

Thermometers used for checking the temperatures of food products should be tested biannually. The test is made by immersing the thermometer in an ice and water bath.

Fill an appropriate size beaker with ice and then water. Stir for 2 minutes and then immerse the thermometer for 2 minutes in the center of the mixture. Do not permit the thermometer bulb to rest against the side of the container. The thermometer may be held vertically by fitting it through a perforated piece of cardboard positioned across the top of the beaker. The thermometer should read within 1° of 0°C. Record results on the Thermometer Checks log.

B. Refractometer

Refractometers are generally factory calibrated.

The calibration checks outlined below are to be done in compliance with measurement procedure as outlined in section 12 of this document.

Daily Check
The refractometer must be checked daily for compliance by using distilled water demonstrating 0.0 ± 0.2 ºBrix. If the reading is outside of required ranges re-zero if possible and recheck. Results are to be recorded in refractometer checks log which includes the instrument identity, date and results as well as any corrective action taken if the reading was outside of the required range. Equipment which cannot meet the required results are to be taken out of use and marked as such until they can be repaired.

Weekly checks
The refractometer must be checked weekly for compliance by using 18º Brix and 25.2 º Brix standards demonstrating a reading ± 0.2 ºBrix within that stated for the standard. Results are to be recorded in refractometer checks log which includes the instrument identity, date and results as well as any corrective action taken if the reading was outside of the required ranges. Equipment which cannot meet the required results are to be taken out of use and marked as such until they can be repaired.

11. Environmental Conditions

Measurement temperatures should be maintained between 15 and 25°C and monitored correctly to minimize the levels of temperature correction.

Brix solutions prepared are to be stored in a fridge (4ºC) when not in use. Solution should be prepared fresh monthly. Brix solutions should be brought to room temperature prior to use.

12. Measurement Procedure

Sample Preparation

If whole grapes are to be analysed, the juice can be extracted from these berries by crushing by hand. The grape berries must be sourced and sub-sampled according to the ACCC approved sampling method. Samples must be as representative as possible. Squash approximately 500g of the berries in a plastic 'snap-lock" bag liberating as much juice as possible and ensuring all berries have been crushed. Decant 100ml of juice to an appropriate plastic tube and allow to settle. Measurement can be taken on the liquid portion of the settled sample. Alternatively, or if it the solids do not settle adequately the sample may be centrifuged at approximately 3500 rpm for 5 minutes to achieve a suitably solids free sample.

Tank samples should be treated in a similar manner with gross solids allowed to settle before sampling of the liquid fraction. All tanks should be suitably agitated before sampling to ensure no stratification.

Samples should be relatively clear at the time of measurement and as close to 20°C as possible. The temperature correction function of most refractometers corrects for the temperature of the instrument rather than that of the sample. Room temperature is advised to be as close to 20°C as possible.

Benchtop or Digital refractometers

The degrees Brix or Baume can typically be read directly from a digital refractometer. Follow the instrument manual for detailed instruction on use. A typical procedure is outlined below.

1. Turn on the instrument and allow the startup procedure to complete.
2. Ensure the correct method (either Brix% or Baume) is selected.
3. Clean the sample cell by rinsing with distilled or Milli-Q water and drying with a lint free tissue or clean cloth.
4. Perform a zero check by covering the sample cell (~0.3 mL) with distilled or Milli-Q water and running a test.
5. This result must be 0.0 ± 0.2 units.
6. If a value of 0.0 ± 0.2 units is not achieved, wipe the water off the cell and repeat. If not achieved after the 2nd attempt, re-zero the instrument.
7. Once achieved, ensure the cell is clean and dry.
8. Measure the temperature of your sample with the calibrated thermometer and take note. (Note: all samples and solutions should be at room temperature, that is the same temperature as the instrument) prior to analysis. Generally speaking the temperature correction applied refers to the temperature of the instrument and not that of the sample.
9. Place approximately 0.3 mL of sample on the cell and run a test.
10. The result will be displayed.
11. Once all analysis is complete, clean the sample cell with distilled or Milli-Q water and dry with a lint free tissue or clean cloth.
12. If no further samples are to be analysed, turn off the Refractometer.

Note: Temperature greatly influences the Brix reading, and it is essential that the reading be corrected to the instrument's standard temperature if necessary (usually 20 degrees C). This correction is made using the chart detailed in **section 13**. It is suggested that all samples (including solutions and reagents) be brought to room temperature prior to analysis.

When sample temperatures are above or below the temperature at which the instrument is calibrated, corrections are based on the standard temperature of the instrument. The Temperature correction chart included in Section 13 is only for instruments standardized at 20 degrees C.

Handheld or Abbe refractometers

1. Look into the eyepiece to ensure the scale appears clearly.
2. Open the prism.
3. Carefully clean the outer surface with a lint free tissue or clean cloth.
4. Perform a zero check by placing a few drops of distilled water onto the prism.
5. Close the prism and look into the eyepiece against a natural light source.

6. Take reading at the point where the contrast line (difference between light and dark) crosses the scale.
7. This indicates the concentration of sugar in the liquid.
8. This result must be 0.0 ± 0.2 units.
9. If a value of 0.0 ± 0.2 units is not achieved, wipe the water off the cell and repeat. If not achieved after the 2nd attempt, re-zero the instrument.
10. Once achieved, ensure the cell is clean and dry.
11. Repeat the steps above with your test sample.
12. Measure the temperature of your sample with the calibrated thermometer and take note.
13. Take reading at the point where the contrast line (difference between light and dark) crosses the scale.
14. Once all analysis is complete, clean the prism with distilled or Milli-Q water and dry with a lint free tissue or clean cloth.

Note: Temperature greatly influences the Brix reading, and it is essential that the reading be corrected to the instrument's standard temperature if necessary (usually 20 degrees C). This correction is made using the chart detailed in **section 13.** It is suggested that all samples (including solutions and reagents) be brought to room temperature prior to analysis.

When sample temperatures are above or below the temperature at which the instrument is calibrated, corrections are based on the standard temperature of the instrument. The Temperature correction chart included in Section 13 is only for instruments standardized at 20 degrees C.

13. Calculations/Corrections

Table 1: Temperature correction chart for obtaining Brix from refractometer readings.

TEMPERATURE CORRECTIONS FOR OBTAINING BRIX FROM REFRACTOMETER READINGS

Temp. Degrees C.	Degrees Brix										
	0	5	10	15	20	25	30	40	50	60	70
	Subtract from Brix Reading										
10	.50	.54	.58	.61	.64	.66	.68	.72	.74	.76	.79
11	.46	.49	.53	.55	.58	.60	.62	.65	.67	.69	.71
12	.42	.45	.48	.50	.52	.54	.56	.58	.60	.61	.63
13	.37	.40	.42	.44	.46	.48	.49	.51	.53	.54	.55
14	.33	.35	.37	.39	.40	.41	.42	.44	.45	.46	.48
15	.27	.29	.31	.33	.34	.34	.35	.37	.38	.39	.40
16	.22	.24	.25	.26	.27	.28	.28	.30	.30	.31	.32
17	.17	.18	.19	.20	.21	.21	.21	.22	.23	.23	.24
18	.12	.13	.13	.14	.14	.14	.14	.15	.15	.16	.16
19	.06	.06	.06	.07	.07	.07	.07	.08	.08	.08	.08
	Add to Degrees Brix Reading										
21	.06	.07	.07	.07	.07	.08	.08	.08	.08	.08	.08
22	.13	.13	.14	.14	.15	.15	.15	.15	.16	.16	.16
23	.19	.20	.21	.22	.22	.23	.23	.23	.24	.24	.24
24	.26	.27	.28	.29	.30	.30	.31	.31	.31	.32	.32
25	.33	.35	.36	.37	.38	.38	.39	.40	.40	.40	.40
26	.40	.42	.43	.44	.45	.46	.47	.48	.48	.48	.48
27	.48	.50	.52	.53	.54	.55	.55	.56	.56	.56	.56
28	.56	.57	.60	.61	.62	.63	.63	.64	.64	.64	.64
29	.64	.66	.68	.69	.71	.72	.72	.73	.73	.73	.73
30	.72	.74	.77	.78	.79	.80	.80	.81	.81	.81	.81

14. Uncertainty of Measurement

The uncertainty of measurement should be estimated using the top-down approach described in ISO/IEC Guide 98-3:2008 Uncertainty of measurement -- Part 3: Guide to the expression of uncertainty in measurement (GUM:1995) or Assessment of uncertainty in measurement (Cook 2002).

The estimation of the UOM also needs to include an assessment of bias and the uncertainty in the bias. It should then be determined whether the bias is significant in regard to the combined uncertainty. If so, the bias should be accounted for the combined uncertainty for the result or stated separately with the result. The guideline for this estimation can be seen in the NATA 'General Accreditation Guidance' – Estimating and reporting measurement uncertainty of chemical test results. Robust QA data across time and analysts is useful for this estimation.

Sound professional judgement may be used by an appropriate designated representative to amend the UOM if the data appears unrealistic based on previous experience.

Estimates of the UOM should be reviewed every 18 to 24 months or when trending data indicates a history of non-conformances.

15. Limits of Detection

Check the product manual as each supplier will have subtle variations for the range, resolution and accuracy of the results, however typically refractometers will have capabilities as per below:

Digital refractometers

Measurement Range:

Brix % or °Brix: [0.0 to 45.0% or 0.0 to 45.0°]
Baume: [0.0 to 21.0°]

Resolution:

- Brix % or °Brix: [0.1% or 0.1°]
- Baume: 0.1°

Accuracy:

- Brix % or °Brix: [± 0.1%]
- Baume: ± 0.2°

Handheld or Abbe Refractometers

Measurement Range:

Brix % or °Brix: [0 to 32% or 0 to 32°]

Resolution:

- Brix % or °Brix: [0.2% or 0.2°]

Accuracy:

- Brix % or °Brix: [± 0.2%]

16. Reporting Results

After adjusting the reading for temperature variation (if required), report results of Brix readings to the closest 0.1 degree, or to the appropriate degree per the grading instructions. Corrections for the presence of fruit acids, minerals, and similar ingredients are not made unless specified in the standard or specification.

17. Validation Requirements

The minimum validation requirement required to use this method within a testing or processing facility is for 10 replicate samples to be sent to an independent laboratory for comparative testing. This can be achieved using the following process.

1. Source 10 separate samples of grapes.
2. From each set randomise 400 grams of berries and then randomly split the sample into 2 200g sample sets.
3. Each sample should be then rapidly frozen.
4. One of each sample duplication should be submitted to an accredited independent laboratory using the reference method as outlined in this procedure to determine the brix content of each sample.
5. Within a one-week timeframe the samples should also be analysed in the source laboratory using the inhouse procedure.
6. Average differences between results should not vary by more than 10% and no individual result should vary by more than 20% for the procedure to be considered valid.
7. At the beginning of each season this procedure should be repeated to ensure no changes have been introduced that may degrade the results.
8. Document all results from the process in an appendix to the procedure validation.

If the facility wishes to independently validate their method or use a technology significantly different to that outlined in this standard procedure a much more rigorous validation is required as outlined below. This is not necessary if the testing site is using this procedure as is and does a cross validation as highlighted above with an independent accredited laboratory.

In order to make a complete assessment of fitness-for-purpose of a method there are several characteristics of its performance that must be established. NATA's 'General Accreditation Guidance' – Validation and verification of quantitative and qualitative test methods provides valuable insights into the validation requirements for laboratory instruments. However, it is not always necessary to meet all of these criteria. Selecting the minimum requirements to satisfy fitness-for purpose from the complete list requires a clear understanding of the purpose of the method and a judgement of the important characteristics of the method.

Full compliance includes assessment of method in respect of:

- selectivity or specificity
- range

- linearity
- sensitivity
- limit of detection
- limit of quantitation
- accuracy
- precision
- measurement uncertainty

Once established these parameters should be clearly stated in the documented method to enable users to assess the suitability of the method for their needs. Method validation may also include establishing procedures for sampling, handling (including storage of samples) and transportation.

Characteristics to be evaluated	**Procedures which may be followed**
Limit of detection and quantitation	Replicate analysis at multiple concentrations including a concentration close to zero (graphical method), or replicate analysis at a concentration estimated to be equal to twice the LOQ (statistical method). Use blanks and a range of standards or samples containing low concentrations of (naturally occurring or artificially contaminated) analytes. Separate determinations may be required for different matrices.
Sensitivity	Analysis of spiked or artificially contaminated samples or standards prepared in sample extract solutions. Initial check for satisfactory gradient for plot of response vs concentration. (More appropriately a QC issue following initial check).
Selectivity	Analysis of reagent and matrix blanks, standards and matrix samples spiked with standards (in working range) to which known concentrations of suspected interferents have been added.
Range in which the calibration equation applies (linearity of calibration)	Duplicate measurements of standards evenly spaced over expected concentration range of samples.
Measuring interval	Evaluation of bias and possibly LOQ determinations
Matrix effects	Analysis of matrix blanks or matrix spiked with standards (at least once or in duplicate at each of 3 concentrations in each sample matrix type).
Trueness; bias	Analysis of replicates. Reference samples should be matrix and concentration matched with samples.
Precision (repeatability and reproducibility) / Accuracy	Replicate analysis for each sample matrix type (if possible selected to contain analytes at concentrations most relevant to users of test results) under stipulated conditions. For comparing precision of two methods, the F-test is recommended. For accuracy, compare each mixture's true value vs. the measured result.
Ruggedness	Introduce appropriate limits to method parameters likely to impact results if not carefully controlled. Investigate if necessary: i) single variable tests

	(test and re-test with small change to one method parameter); ii) multi variable tests (Plackett-Burman designed experiment).
Measurement Uncertainty (MU)	Calculate a reasonable, fit-for-purpose estimate of MU. Ensure estimates are aligned with the concentration(s) most relevant to the users of results.

18. Quality Assurance

The reliability of the method is monitored using the following procedure:

1. At least one sample is to be performed in duplicate per batch. Duplicates specifications should agree with outcomes of the validation.

2. The water solution and 25.2 ° Brix Solution should be analysed at the start of the batch and at a suitable frequency within each batch. The result is to be 0 ± 0.2 °Brix and 25.2 ± 0.2 °Brix, respectively.

If the duplicate analysis criteria is not met, the analysis should be repeated in another batch.

If the Brix solution standards criteria is not met, the data from that batch is rejected and analysis stopped. The cause of the problem should be ascertained in conjunction with a senior staff member before proceeding.

19. References

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NATA - General Accreditation Guidance – Validation and verification of quantitative and qualitative test methods