The Australian Wine Research Institute

Standard Method for the Measurement of °Brix (or °Baume) in Grapes Juice

Version Control

Version	Changes made in this version
1.0	First version of this method
1.1	Revised to reflect feedback from reference group
1.2	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 grape juice. 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 grape juice. 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 and ° Baume in grape juice, the level of quality assurance and rigor required for other purposes is at the discretion of the end user.

This does not necessarily replace in house methods, but any such method needs to be suitably validated against this method. The approaches for cross validation of in house methods which use the same principle as this method are outlined in section 17 of this procedure.

Where a different analytical principal is used to determine °Brix or °Baume in grape juice (i.e. a secondary method such as MIR spectroscopy) a more extensive form of validation against this reference method is required. This is outlined in a separate procedure (ref. IESP4.0, Development and validation of secondary analytical methods for the determination of grape parameters).

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

The the Brix and Baume scales are generally used as an indicatior of the ripeness of grapes and are an indication of total dissolved solids (TDS) in the grape juice.

The Baume scale of a grape juice is also an approximate indication of the likely alcohol content of a dry wine produced from that juice.

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 °Baume = 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, seeds 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 through correlation with standards provides a means of estimating the sugar concentration in grams per liter for grape juice derived from grapes or must.

Refractive index meters come in both handheld and benchtop forms.

Handheld optical refractometers can also be known as Abbe refractometers after a common manufacturer, however they are currently produced by a range of manufacturers.

4. Measurement Principle

A refractometer is an instrument that optically measures the density of a liquid. Light passes through the sample and is deflected to varying degrees 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 ºBrix. Some instruments also can automatically convert between ºBrix and º 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, ºBrix, 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 ºBrix. A ºBrix (°Bx), is the number of grams of sucrose present per 100 grams of liquid.

The Australian wine industry also commonly uses the Baume (°*Bé)* 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 manual 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 when preparing and using sucrose stock reagent as mild discomfort can occur 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

- *18 ^{o}Brix solution = 10 ^{o}Baume*

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) and mixing thoroughly until dissolved. Sample to be stored in a fridge (4ºC) when not in use. Solution should be prepared fresh monthly.

- *25.2 ºBrix solution = 14 ºBaume*
  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) and mixing thoroughly until dissolved. 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 in a 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 18 ºBrix and 25.2 ºBrix 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

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

ºBrix 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 (between 15 and 25°C) prior to use and preferably as close to 20 ºC as possible.

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 ag greed industry standard 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 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.

When sampling from winery tanks, they should be suitably agitated before sampling to ensure no stratification. Gross solids allowed to settle before sampling of the liquid fraction.

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.

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.

Benchtop or Digital refractometers

The ºBrix or ºBaume 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 ºBrix or ºBaume) 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) and as close to 20 °C as possible 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. If necessary correct for sample temperature using the table in section 13 of this procedure (check the instrument manual).
12. 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.
13. Repeat from step 8 for the next sample.
14. If no further samples are to be analysed, turn off the Refractometer.

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. Take reading at the point where the contrast line (difference between light and dark) crosses the scale.
13. Measure the temperature of your sample with the calibrated thermometer and take note.
14. If necessary correct for sample temperature using the table in section 13 of this procedure.
15. Once all analysis is complete, clean the prism with distilled or Milli-Q water and dry with a lint free tissue or clean cloth.

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

Uncertainty of measurement (UoM) is calculated by determining the average, standard deviation and the coefficient of variation of reproducibility data of at least 7 replicates. From this it can be estimated that the MU at the 95% confidence interval is equal to 2 x SD and 2 x CV (%). The maximum UoM for ºBrix or ºBaume for this method as described should be in the region of ± 5 % for an individual laboratory on replicate homogenates. Each laboratory must demonstrate its typical UoM as part of its validation procedure.

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.

It should be noted that these results are those expected for standard solutions and do not reflect the variations introduced by sampling of materials such as grapes.

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. 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 (or a substantially similar method as already used in the facility) 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 refrigerated.
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. For each sample calculate the difference between the local laboratory and the independent laboratories result.
7. Take the average of these differences.
8. Average difference should not vary by more than 10% of the mean value of all of the results for the procedure to be considered valid.
9. No individual result should vary by more than 15% from the independent laboratory result for the procedure to be considered valid.
10. At the beginning of each season this procedure should be repeated to ensure no changes have been introduced that may degrade the results.
11. 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 which is outside the scope of this document, but can be found in various references including "NATA - General Accreditation Guidance – Validation and verification of quantitative and qualitative test methods". This is not necessary if the testing site is using this procedure as is (or a substantially similar procedure) and does a cross validation as highlighted above with an independent accredited laboratory.

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 at the start of the batch and at a suitable frequency within each batch. The result is to be 0 ± 0.2 ^{o}Brix.

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

Association of Official Analytical Chemists. Official Methods of Analysis. 14th ed. Virginia; AOAC; 1984: 1050-1055.

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Zoecklein, B.W., Fugelsang, K.C., Gump, B.H., Nury, F.S. Production Wine Analysis. AVI Van Nostrand Reinhold. New York, 1989; 26-29.

USDA, Technical Procedures Manual
https://www.ams.usda.gov/sites/default/files/media/TechnicalProceduresManual%5B1%5D.pdf

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