How do you like your sugar: reducing, residual or total?

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Introduction

There are a number of different ways to measure the sugar content of juice, must and wine. They range from the simple and inexpensive to the complex and expensive, although all have their applications. As usual, it depends upon how accurate you want to be and how much time and money you have. In this article we outline the main ways that this important parameter in wine is measured, try to reduce some confusion about the terminology used, and point out which technique may be best for your application.

Sugars in grape juice and wine

There are many different types of sugars but the ones that are of most interest to oenologists and winemakers are fructose and glucose.

Fructose and glucose are the main sugars in the juice from *Vitis Vinifera* grapes, occurring in fairly similar levels. The amount of these sugars increase as the grape ripens and at full ripeness is of the order of 200 gram per litre (g/L). Traces of other sugars may also be present; these are usually non-fermentable sugars and can include small amounts of sucrose.

Sugars are the main component of the juice of ripe grapes by weight (apart from water), the next most common are acids in the range of about 5 to 10 g/L. That is, acids only account for about 5% of the total carbohydrate load of juice, the bulk being sugars. The major component of juice is water, typically around 75 - 80% by weight.

Units of measurement

Unlike other parameters of juice and wine, sugar content can be stated in a number of different units. This does make a simple situation more complex than it needs to be and can probably be explained by historical factors. For example hydrometers have been in use since the 1700s, HPLC only since the 1970s. The ideal situation would of course to have only one unit used; an oenologist would probably choose g/L, a viticulturalist degrees Brix.

The units typically used are:

Baumé scale

This was originally devised in France in 1768 as a measure of salt in water, 0^{o} Baumé being pure water, 15^{o} Baumé a 15% salt solution. It was found that ^{o} Baumé roughly (we stress roughly!) equates to the Potential Alcohol content of grape juice and has been in use ever since.

Brix scale

At least this scale was developed for sugar solutions, albeit sucrose. For grape juice it represents the percentage of soluble solids on a weight per weight basis, including sugars. Again it is an approximation, not an accurate measure of grape sugars.

Gram/litre
This is a unit of concentration in on a weight per volume basis, and when used for grape sugars, does refer to the actual number of grams of sugar per litre of juice, must or wine.

A discussion of how these different units relate to each other is an interesting one but beyond the scope of this article.

Physical methods for measuring grape sugars
If we consider that grape juice is made up of only water and sugars and ignore the effect of other dissolved materials, we can use this as a way to measure the approximate level of sugar in juice using fairly simple physical measurements.

There are two main physical techniques for measuring sugar content; both rely upon measuring the density of the juice. As grape juice is not a pure solution of sugar and water it must be remembered that both these techniques are approximations only. These techniques are widely used, as they are simple to perform, portable and quite inexpensive.

1. Hydrometry
One way to measure the density of a solution is by hydrometry. An hydrometer is placed in a measuring cylinder full of juice and a density reading is taken from the hydrometer scale at the surface of the liquid. It is important to measure the density at 20^{o}C or to correct readings if the temperature is far from 20^{o}C. The advantages of using an hydrometer is that they are quite cheap (typically around $30) and easy to use, however about 200mL of juice is needed per measurement. Hydrometers typically use the Brix or Baume scales as a measure of dissolved materials, although they can be made to read g/L directly. As the juice ferments however, the alcohol formed interferes with the measurement and affects the accuracy of the reading.

2. Refractometry
When a beam of light passes from one medium to another it undergoes a change of direction; this is known as refraction (Glasstone & Lewis). The degree to which the beam changes is proportional to the density of the material is goes through. This property of light is used in a refractometer to give a measure of the density of the material placed in the refractometer. When a drop of grape juice is placed in a refractometer the density of the juice is measured, usually in Brix units. The advantages of using a refractometer is that there are portable models, they cost only several hundred dollars and only a drop of liquid is needed. Temperature correction is used for more accurate measurements with bench top laboratory models.

Chemical methods for measuring grape sugars
There are 3 main chemical ways to measure sugars in grape juice, must or wine.

1. Reduction of copper
Although it may not be obvious at first glance, there are several techniques that use the chemical principle of reduction of copper salts to determine the level of fructose and glucose. Both these sugars have the ability to undergo reduction reactions, although sucrose does not. They are often called reducing sugars because of this ability. Other reducing substances in wine also react (eg pentoses), so these techniques do overstate the actual level of fructose and glucose. In our experience, these techniques exceed the true amount by as much as 2 g/L.

a. Lane and Eynon titration
This method relies upon the reaction between reducing sugars and an alkaline solution of copper sulfate; followed by a titration of the boiling mixture with a standard glucose solution:

- the wine sample has to be decolourised and boiled to remove alcohol
- the wine is then added to a fresh mixture of Fehlings A (aqueous copper sulfate) and Fehlings B (alkaline sodium potassium tartrate, Rochelle salt) and placed on a hotplate
- whilst boiling the solution is titrated with a standard glucose solution
- the Fehlings mixture is titrated separately with the glucose solution to the red brown colour of cuprous oxide
- the difference between the titres is used to calculate the reducing sugar content of the wine

c. Clinitest tablets
These tablets are used for wine at the end of fermentation and use the same principle of reducing copper salts to form a red brown colour in the presence of reducing sugars, the colour formed being related to the level of sugars in the wine.

- a small sample of wine is added to a test tube
- a Clinitest tablet is added
- after the reaction is complete the colour formed is noted and compared to a colour chart

b. Rebelein titration
This is also based upon the reduction of copper by reducing sugars but uses a back titration of iodine with sodium thiosulfate

- The decolourised wine sample is added to Fehlings A and B and boiled
- Portions of iodide solution and acid are added
- Starch indicator is added to form a inky blue/black colour
- The excess iodine is titrated with thiosulfate to a cream endpoint
- The difference between the blank and sample titrations is the reducing sugar content in g/L

Because these 3 methods tend to overstate the true glucose and fructose content, they are not very reliable for determining the accurate level of sugar at low levels.

2. Spectrophotometric
There are 2 main spectrophotometric techniques, both use very specific enzymes that react with both fructose and glucose to give accurate results for these sugars only.

a. Reflectometry
A fairly recent development has been the introduction of a very simple reflection instrument. This can be used for several measurements including combined glucose and fructose in wine. The technique is very easy:

- a sample tube is filled to the mark with decolourised wine
- water is added to the mark
- a strip is inserted for a fixed time
- the strip is inserted into the instrument and a result read from the digital display after a fixed time

This technique has been investigated in our lab and found to be more accurate than the copper reduction methods for wine containing less than 10 g/L of sugars. The initial cost of the Reflectometer is around $1100. Whilst operators need some rudimentary skills the technique is very easy compared with the other chemical methods.

b. Visible absorption spectrophotometry
A very familiar method to larger laboratories uses enzyme test kits that are specific to particular analytes. In this case a kit is used to measure glucose and fructose either separately or as a total. The technique does require good laboratory skills, a Visible spectrophotometer and other ancillary equipment. The set-up costs start at around $5000, but give the most accurate results. Multiple samples can be analysed during the same test run, depending upon the type of spectrophotometer used. All enzyme test kits contain instructions on their use, some are made specifically for wine analysis.

3. High Performance Liquid Chromatography (HPLC)
Another very accurate technique and with the right equipment up to 100 samples can be analysed in one run. The major disadvantages are the initial $50000+ for the instrument, the length of time needed per sample and the need for very skilled operators. For labs that have large sample throughputs they are certainly a great way to go.

Summary
The main methods of measuring the sugars fructose and glucose in juice, must and wine have been discussed. The particular technique used will depend upon whether the sample is from the vineyard, the fermentation

vessel or the finished wine. It will also depend upon the expertise of the tester and the budget of the winery. One final point; if we could all talk about the sugar content in one measurement unit only, communication between all interested parties would be much simpler!

References.

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