

DETERMINATION OF FRUIT ACIDS BY TITRATION AND CALCULATION OF THE SUGAR/ACID RATIO

It is the sugar/acid ratio which contributes towards giving many fruits their characteristic flavour and so is an indicator of commercial and organoleptic ripeness. At the beginning of the ripening process the sugar/acid ratio is low, because of low sugar content and high fruit acid content, this makes the fruit taste sour. During the ripening process the fruit acids are degraded, the sugar content increases and the sugar/acid ratio achieves a higher value. Overripe fruits have very low levels of fruit acid and therefore lack characteristic flavour.

Titration is a chemical process used in ascertaining the amount of constituent substance in a sample, e.g. acids, by using a standard counter-active reagent, e.g. an alkali (NaOH).

Once the acid level in a sample has been determined it can be used to find the ratio of sugar to acid.

There are two methods specified for the determination of the titratable acidity of fruits:

- Method using a coloured indicator;
- Potentiometric method, using a pH meter, which should be used for very coloured juices.

Material:

- A laboratory burette of 25 or 50ml capacity or an automatic burette is used. A 10ml pipette, beaker (250ml), a filter (muslin cloth or fine filter) and an extractor or homogeniser.
- A bottle of distilled water.
- Sodium Hydroxide (NaOH): The Standard Laboratory solution of 0.1M which is used in the actual titration is considered to be dilute, and can readily be purchased in this form.
- Phenolphthalein: This is a 1% w/v solution of phenolphthalein in 95% v/v ethanol which is flammable and toxic if ingested. This is only required for the method using a coloured indicator.
- Indicator stripes To check the exact point of neutrality an indicator stripe should be used. Not necessary if pH Meter is used.

Sampling

To evaluate the lot selected for inspection, take a sample of at least 10 fruits of each size at random from the reduced sample. However, fruits should be free from defects such as sun scorch and pest or disease damage, which may have affected the normal ripening process.

Sample preparation

Depending upon the type of produce, either cut the fruit in half and squeeze out the juice with an extractor or a juice-press e.g. citrus fruits, or homogenise the flesh into a pulp. The juice of all squeezed fruits is mixed.

The skin and solids should not be included; the solids being filtered out through muslin cloth or fine filter extracting as much juice as possible.

Use a clean and dry safety 10ml pipette. Draw up 10ml of juice and discharge it into a 250ml beaker. Using another clean and dry pipette draw up 50ml of distilled water and add to the juice in the beaker.

Measurement

Method using a coloured indicator

Add 3 drops of phenolphthalein to the juice/water solution in each beaker from a dropping pipette which is specifically kept for that purpose.

Ensure the tap on the burette is shut and using a funnel pour the 0.1M solution of NaOH into the burette until it reaches the zero mark. Do not spill the solution onto the skin.

Slowly titrate the NaOH into the juice/water solution (with a 25ml burette or an automatic burette). Care must be taken that the NaOH is dropped directly into the solution and does not adhere to the glass, otherwise the reading may be false. While titrating care must be taken to continually swirl the solution in the beaker to keep it thoroughly mixed. This is essential, particularly when the solution nears neutrality. It is important to determine the point of neutrality or the end point of titration very exactly. The phenolphthalein indicator changes very rapidly from colourless to pink and the end point can easily be missed, which will give an inaccurate reading for the test. It is important therefore that towards the end of the titration the NaOH is added a drop at a time.

Using phenolphthalein as an indicator, the point of neutrality is reached when the indicator changes from colourless to pink. The indicator colour must remain stable (persisting for 30 seconds) and be light pink when viewed over a white background. However, the shade can vary depending on the type of juice being tested. If the point of neutrality is missed, i.e. the colour of the indicator is too dark, the test is not acceptable and must be repeated. An indicator stripe should be used to avoid the neutral point of pH 8.1.

- Read off the amount of the amount of NaOH used (titre) on the burette and record this figure.
- Re-fill the burette for each subsequent test.
- Clean the equipment thoroughly and rinse with distilled water. Detergents must not be used.

Note: When testing very acidic juices e.g. lemons and limes a larger amount of NaOH is required. Therefore, when the NaOH reaches the 25ml mark on the scale the burette tube should be recharged as described above. When the end point is reached the various readings are added together and recorded to produce a figure of NaOH used for each titration.

Method using a pH meter

The point of neutrality i.e. the end point of titration may also be determined using a pH meter. The precise method used will depend on the manufacturer instructions, but the following will provide a general guide.

Checking the pH meter

- Make sure the pH meter has warmed up before use - allow about 30 minutes.
- Remove the electrode from the distilled water in the storage beaker and dry.
- Place the electrode into the beaker containing a buffer solution of pH 7 and calibrate the meter to the same figure.
- Whenever readings are taken, ensure that the electrode is not in contact with the sides or base of the beaker.
- Remove the electrode and - after rinsing in distilled water - place in the solution to be tested; the electrode should not have any contact with the glass.

Measurement

Ensure the tap on the burette is shut and using a funnel pour the 0.1M solution of NaOH into the burette until it reaches the zero mark. Do not spill the solution onto the skin.

Slowly titrate the NaOH into the juice/water solution. Care must be taken that the NaOH is dropped directly into the solution and does not adhere to the glass, otherwise the reading may be false. While titrating care must be taken to continually swirl the solution in the beaker to keep it thoroughly mixed. This is essential, particularly when the solution nears neutrality. It is important to determine the point of neutrality or the end point of titration very exactly. The end point can easily be missed, which will give an inaccurate reading for the test. It is important therefore that towards the end of the titration the NaOH is added a drop at a time.

Using a pH meter, while titrating the digital readout will be seen to climb from around 4 or 5. When the reading reaches 7 proceed carefully. The point of neutrality or the end point of titration is reached at pH 8.1. If this figure is exceeded the test is not acceptable and must be repeated.

- When the pH meter reads 8.1 read off the amount of NaOH used on the burette and record.
- Remove the electrode and rinse it in distilled water ready for the next test. Do not allow it to become contaminated.
- Re-fill the burette for each subsequent test.
- Clean the equipment thoroughly and rinse with distilled water. Detergents must not be used.

Note: When testing very acidic juices e.g. lemons and limes a larger amount of NaOH is required. Therefore, when the NaOH reaches the 25ml mark on the scale the burette tube should be recharged as described above. When the end point is reached the various readings are added together and recorded to produce a figure of NaOH used for each titration.

Calculation of the Sugar/Acid Ratio

The °Brix value of the fruit concerned must also be obtained before calculation of the sugar/acid ratio is possible.

The calculations for determining the sugar/acid ratios of all produce are the same, but as some products contain different acids the appropriate multiplication factor must be applied to each calculation. Some products may contain more than one type of acid, it is the primary acid that is tested. A list of these acids and multiplication factors are found below.

Factor for:	- citric acid :	0.0064 (Citrus fruit)
	- malic acid :	0.0067 (Apples)
	- tartaric acid :	0.0075 (Grapes)

Using citric acid as an example, 1ml 0.1M NaOH is equivalent to 0.0064g citric acid.

Results expressed as percentage acid:

$$\text{Percentage acid} = \frac{\text{Titre} \times \text{acid factor} \times 100}{10 \text{ (ml juice)}}$$

$$\text{The sugar acid ratio} = \frac{\text{°Brix value}}{\text{Percentage acid}}$$

OR

Results expressed as acid in gram/litre:

$$\text{g/l acid} = \frac{\text{Titre} \times \text{acid factor} \times 100 \times 10}{10 \text{ (ml juice)}}$$

$$\text{The sugar acid ratio} = \frac{\text{°Brix value} \times 10}{\text{g/l acid}}$$

E.g.: In case of citric acid the result would be expressed as:

Percentage citric acid	Gram/ litre acid
Percentage Citric Acid = $\frac{\text{Titre} \times 0.0064 \times 100}{10\text{ml juice}}$	g/l Citric Acid = $\frac{\text{Titre} \times 0.0064 \times 100 \times 10}{10 \text{ (ml juice)}}$
This formula can be simplified to: Percentage Citric Acid = Titre x 0,064	This formula can be simplified to: g/l Citric Acid = Titre x 0,64
The sugar acid ratio = $\frac{\text{°Brix value}}{\text{Percentage acid}}$	The sugar Acid Ratio = $\frac{\text{°Brix value} \times 10}{\text{g/l Citric Acid}}$

Results

It is important to record the results, to one decimal place, as well as all the details concerning method, variety and stage of maturity and ripeness of the produce being tested.

If the result achieves the limit specified in the standard, the lot has reached the minimum maturity level.

If the result is at least 10 per cent below/above the limit specified in the standard, a second sample needs to be taken and analysed with other fruits of the reduced sample or from a new sample. If the average of the two samples is below/above the limit specified in the standard, the lot fails the minimum maturity level and needs to be rejected. No tolerance is applied.

Health and Safety Guidelines

Sodium Hydroxide in its undiluted form is extremely corrosive to body tissue. Skin contact causes irritation almost immediately and continued contact causes burns. The 0.1 Molar solution used in this test is much safer. However, it is recommended that protective coats are worn when using, and that it is used only in a well ventilated room.

Phenolphthalein is highly flammable and should be used with care. It should be stored and used away from naked flames or other sources of ignition. It is toxic if ingested.
