

**OECD SCHEME FOR THE APPLICATION OF INTERNATIONAL
STANDARDS FOR FRUIT AND VEGETABLES**

**GUIDANCE ON OBJECTIVE TESTS
FOR DETERMINING THE RIPENESS OF FRUIT**

FOREWORD

Within the framework of its activities aiming at promoting uniform quality control procedures, the Scheme for the Application of International Standards for Fruit and Vegetables has developed this Guidance on Objective Tests for Determining the Ripeness of Fruit.

The Scheme for the Application of International Standards for Fruit and Vegetables shall be open to States being Member countries of the United Nations Organization or its specialised agencies desiring to participate therein in accordance with the procedure for participation set out in Annex II to the Decision C(92)184/FINAL of the OECD Council dated 18 December 1992.

This report is published under the responsibility of the Secretary General of the OECD as recommended by the Plenary Meeting of the Scheme for the Application of International Standards for Fruit and Vegetables in April 1998.

OBJECTIVE TESTING TO DETERMINE THE RIPENESS OF FRUIT

In recent years there has become an increased awareness of the need for the consumer to have fruit available to eat which has reached a satisfactory state of ripeness and which exhibits the true organoleptic characteristics of the produce and of the variety concerned.

This document describes those methods of objective testing of fruits that have emerged as beneficial to both Inspection Services, and the fruit industry in general in determining acceptable levels of ripeness.

- 1) Determination of firmness of fruit by PENETROMETER.
- 2) Determination of the starch content of apples and pears using an IODINE Solution.
- 3) Determination of the Total Soluble Solids of sugar (TSS) by REFRACTOMETER.
- 4) Determination of fruit acids by TITRATION and calculation of the sugar/acid ratio.

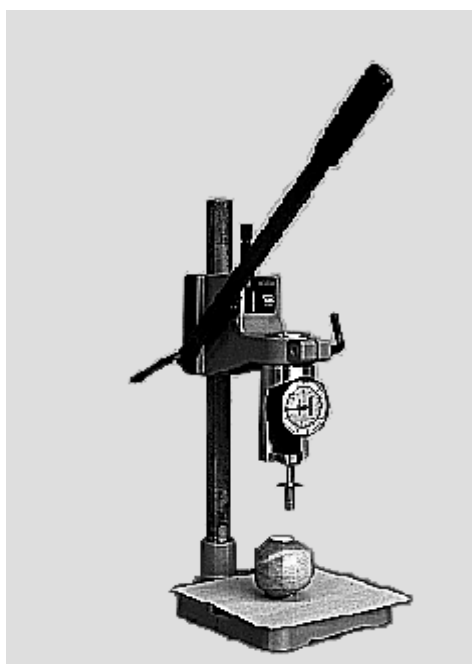
DETERMINATION OF FIRMNESS OF A FRUIT BY PENETROMETER

The firmness of a fruit is linked to the state of maturity and ripeness and may be influenced by the variety as well as the region of production and the growing conditions. This document describes an objective test to determine the firmness of fruit by means of a penetrometer.

The penetrometer is used by producers, packers and distributors to help to determine the stage of ripeness of a fruit and by the retail trade to determine palatability for the consumer and shelf life for their own records.

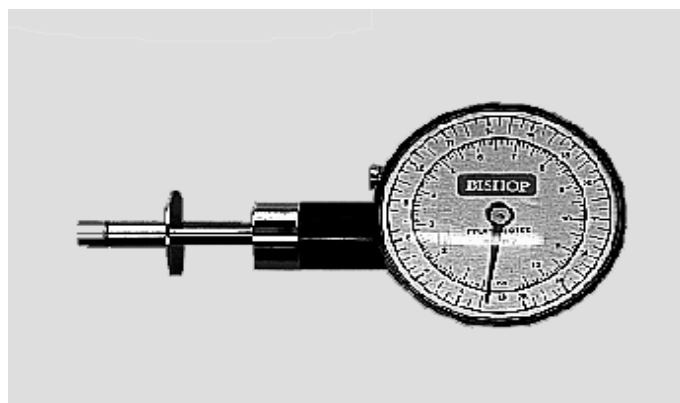
The determination of firmness of a fruit by means of the penetrometer is based on the pressure necessary to push a plunger of specified size into the pulp of the fruit up to a specific depth.

Equipment:



BENCH MOUNTED PENETROMETER ¹

1. These instruments are presented for information only. The OECD does not recommend the usage of any particular make.



HAND HELD PENETROMETER ¹

Penetrometers are available with dial gauges calibrated in both metric (kg) and imperial (lbs) measurements and can be obtained to cover different ranges of pressure suitable for measuring either soft or harder types of fruit. One range covers 1.5 - 12 kg or 3 - 27 lbs and is generally suitable for use in testing harder fruit, e.g. apples, pears, etc ..., and the other range 0 - 5 kg or 0 - 11 lbs for softer produce, e.g. peaches, plums, etc.

Each instrument is supplied with two detachable plungers of 8 mm ($\frac{1}{2}$ sq. cm) and 11 mm (1 sq. cm) diameter. These plungers are interchangeable to enable practical tests to be made on harder or softer fruit. There is also a pointed plunger available to measure avocados.

The small plunger is generally used to measure harder produce and the larger plunger for softer produce, depending on the variety and the stage of ripeness of the produce to be tested.

Ideally the penetrometer should be bench mounted on a fixed, rigid drill stand to ensure that pressure is applied at a steady controlled rate and at a constant angle to the fruit i.e. vertically downwards. This is more difficult to achieve when using a hand-held penetrometer.

If it is not practical to use a stand mounted penetrometer and it is necessary to use a hand-held one as in the field or market place - then particular care must be taken to ensure a smooth and uniform application of pressure when taking readings. The method is the same for both the hand-held and the mounted penetrometer and must be identical for each item of produce tested in order to obtain consistent results.

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Sampling:

- i) Take a sample of 10 fruits of each size at random from different places in the lot selected for inspection, and assumed to be representative of that lot. 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:

- ii) Remove up to 2 cm² (¾ sq. ins) disc of peel (only skin depth) from opposite sides of the equatorial area of the fruit.

Where fruit is of mixed colour, e.g. apples, the tests should be carried out where possible between the highest and the lowest coloured portion of the surface.

Measurement:

- iii) Hold the fruit firmly with one hand, rest it on a rigid surface, such as a table top or the plate at the base of the stand.

The choice of plunger size and scale range used will depend on the type and the variety of the produce being tested and its stage of maturity and ripeness. It is recommended that the size of the plunger chosen and the particular scale used should be such as to give readings in the middle range of the scale.

- iv) Zero the penetrometer and place the plunger head against the flesh in the peeled area of the fruit. Apply steady downward pressure until the plunger has penetrated the flesh of the fruit up to the depth mark (half way up) on the plunger. Slow steady pressure is essential as sharp uneven movements may give unreliable results. Remove the plunger and note the reading on the penetrometer dial, to one decimal place.
- v) Repeat the process on the opposite side of the same fruit after first zeroing the penetrometer.

It is very important to conduct all tests as uniformly and carefully as possible in order to allow an accurate comparison of results.

Results:

The sum total of the 20 readings should be averaged to give a mean figure.

It is important to record the results as well as all the details concerning plunger size, scale range used, the variety and stage of maturity and ripeness of the produce being tested.

There are no limits set out in the UN/ECE quality standards. For limits set by national regulations or recommendations, please refer to document AGR/CA/FVS(91)4/Last revision.

DETERMINATION OF THE STARCH CONTENT OF APPLES AND PEARS USING AN IODINE SOLUTION

During the development of the flesh of a fruit, nutrients are deposited as starch which during the ripening process are transformed into sugars. The progression of the ripening process leads to decreasing starch levels.

This document describes an objective test to determine the amount of starch in the flesh of a fruit using an iodine solution. Iodine turns a blue-black colour when it comes into contact with starch. This test is particularly suitable for fruit such as apples, and to a lesser extent pears. As a fruit ripens more starch is converted to sugar, and the blue-black area becomes less prominent. Ripening usually takes place from the core of the fruit towards the skin. Ripening fruit will generally show an increasing white ring around the core, if treated with iodine.

Requirements:

- Iodine solution

The iodine solution is prepared by dissolving 10g of potassium iodide in 30ml of distilled water, and then adding 3g. of iodine. Once the iodine has dissolved the mixture is made up to 1 litre by adding distilled water at 10° - 30° C. This solution can be stored for up to 6 months in a cool (4 to 7° C) dark place.

Note: The chemicals and the prepared solution will stain, so should be kept away from the skin and from fabrics.

Sampling:

Take a sample of 10 fruits of each size at random from different places in the lot selected for inspection, and assumed to be representative of that lot. 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:

Using a sharp knife each fruit is sliced in half. It is very important that the surfaces are cleanly cut, without any additional damage occurring to the flesh of the fruit or the skin. Additional damage of this type may cause further starches to be released, from the damaged cells, leading to an inaccurate result.

Method:

The two freshly cut surfaces of the fruit are evenly coated with iodine solution. This can be applied using a dropper bottle, pipette or spray bottle.

Measurement:

The two cut sections are left for one minute before the results are recorded.

The percentage surface of the fruit changing to a blue-black colour must be recorded.

The amount of blue-black colour present on a tested sample may be directly related to the ripeness of the fruit.

Note: Care should be taken when interpreting the results of this test as many varieties of apple and pear ripen in different ways, and produce differing starch patterns. Varieties are suitable for eating at different stages of maturity and ripeness, dependent on individual consumer preference.

Results:

The sum total percentages of the 20 readings should be averaged to give a mean figure.

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

There are no limits set out in the UN/ECE quality standards. For limits set by national regulations or recommendations, please refer to document AGR/CA/FVS(91)4/Last revision.

Health and Safety Guidelines:

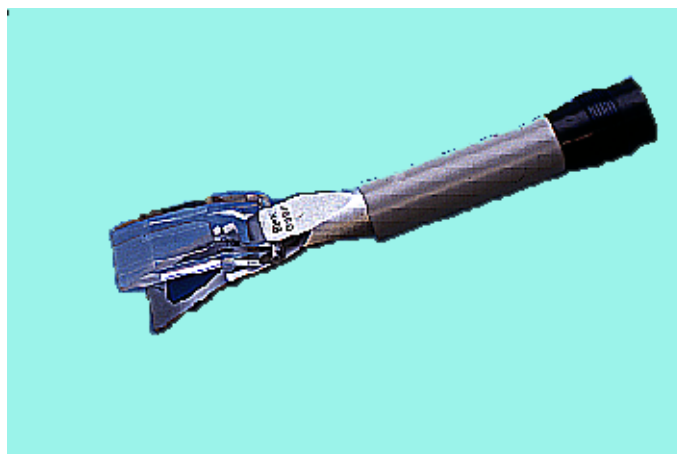
Pure **Iodine** can cause severe irritation to the respiratory system, skin and eyes, and the solid will burn the skin. It is recommended that gloves, goggles and protective clothing be worn when handling.

DETERMINATION OF TOTAL SOLUBLE SOLIDS OR SUGAR (TSS) BY REFRACTOMETER

During the development of the flesh of a fruit, nutrients are deposited as starch which during the ripening process are transformed into sugars. The progression of the ripening process leads to increasing sugar levels.

This document describes an objective test to determine the total content of soluble solids (TSS) of sugar in a fruit by means of the refractometer. The method is especially suitable for ripe and juicy fruit, with significant sugar content, as the determination of TSS is based on the capacity of sugars in a juice to deviate light.

Equipment:



HAND-HELD MODEL ¹

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LCD DIGITAL BENCH MODEL^{1 2}



TEMPERATURE CORRECTED MODEL¹

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 2. The text describes the calibration and method of operation for the more traditional hand-held refractometer. When using digital battery/mains operated models similar principles apply, however, the manufacturer's instructions must always be followed.

A refractometer measures TSS as % Brix in 0.1% graduations. There are hand-held refractometers as well as digital battery/mains operated models available. All models apply similar principles. However, the manufacturers instructions must always be followed.

Some refractometers automatically compensate for changes in temperature, whereas others may be calibrated to read accurately at a fixed temperature (usually 20°C). To obtain accurate readings at temperatures other than 20°C you should refer to the International Temperature Correction Table (1974) which is usually supplied with the instrument or ISO standard 2173 - (First edition 15 November 1978).

Refractometers should not normally require re-calibration, however, the following calibration instructions may prove useful. If there is any doubt as to the accuracy of any reading it is important to consult the manufacturers instructions.

Checking the refractometer:

Requirements:

- A bottle of distilled water.
- A small bottle of 6% sucrose solution. This should be stored in a bottle, kept away from daylight and used within 48hrs of preparation.

Checking and re-calibration to zero:

- Place several drops of distilled water on the prism surface.
- Hand-held model: Close the prism lid and look through the instrument, towards the light. If necessary focus the eye piece until a clear image appears.
- The position at which the demarcation line between the light and dark regions crosses the vertical scale gives the percentage soluble solids reading.
- LCD Digital model: Push the button to get a reading.
- Distilled water should give a reading of zero. If not adjust the zero adjustment screw on the refractometer until the demarcation line crosses the vertical scale at zero.

- Wipe the prism plate dry with a soft tissue.
- Place several drops of 6% sucrose solution onto the clean and dry prism plate.
- The refractometer should give a reading of 6%. If the reading is not accurate:
 - a) new fresh solution of accurate 6% sucrose may be required.
 - b) the refractometer may need to be repaired or replaced

Taking care of the refractometer:

Optical glass is relatively soft and damage can easily occur to prism surfaces. Care should be taken therefore to keep metal and glass objects away from the surface.

Samples should be washed off the instrument as soon as practicable with distilled water. A prism is susceptible to alkalis and acids if left in contact for any length of time. They should be washed clean with a suitable solvent before being rinsed with distilled water and dried off with a soft tissue.

Periodically it is an advantage to wipe the prism plate with alcohol to remove any oils which may adhere. Alcohol must not be used on battery/mains operated models.

It is always advisable to keep any liquids confined to the prism end of the refractometer.

Sampling:

Take a sample of 10 fruits of each size at random from different places in the lot selected for inspection, and assumed to be representative of that lot. 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:

It is important that the juice sample used for measuring soluble solids is extracted in a uniform way. In some fruits like the orange the whole fruit can be squeezed. In others like melons and kiwifruit a specific method needs to be followed in order to take into account natural differences in the distribution of soluble solids within the fruit.

Although it is not possible to lay down precise guidelines for all produce which could be tested. Where guidance is given, for example in the OECD Kiwifruit brochure it should be followed. The overriding criteria is that the juice sample must be as far as possible representative of the whole fruit. Dry fruit should be used as any external moisture mixing with the juice will lower the reading.

- Oranges - Cut each fruit in half crosswise and squeeze to extract all the juice.
- Apples - Cut two thin slices across the equatorial section, from about a third of the length of the apple measured from both the stalk and eye cavities.
- Kiwifruit - Cut the stem and blossom ends at a distance of 15 mm from each end of the fruit to be sampled.
- Melons - Using a small diameter metal borer (1 - 4 mm) core of melon should be extracted from the equatorial axis area. Each end of the core should be discarded i.e. the skin and the flesh area immediately beneath it and also the soft pulpy seed area. The remaining flesh should be used to extract the juice for testing.

Measurement:

Place an equal number of drops (1 or 2) from the prepared fruit juice or the prepared fruit onto the refractometer prism plate. Note the reading on the prism scale to one decimal place. A second reading should be obtained from each single fruit. After each test the prism plate must be cleaned with several drops of distilled water and wiped dry with a soft tissue.

Results:

The sum total of the 20 readings should be averaged to give a mean figure.

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

There are limits set out in the UN/ECE quality standards for kiwifruit (FFV-46) and for melons (FFV-23). However for other fruit there are no limits set out. For limits set by national regulations or recommendations, please refer to document AGR/CA/FVS(91)4/Last revision.

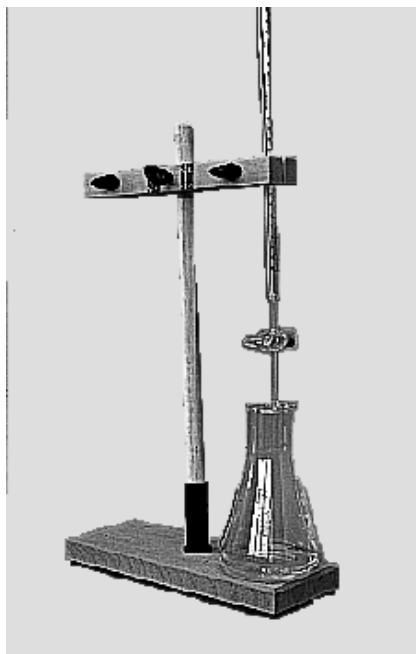
DETERMINATION OF FRUIT ACIDS BY TITRATION AND CALCULATION OF THE SUGAR/ACID RATIO

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).

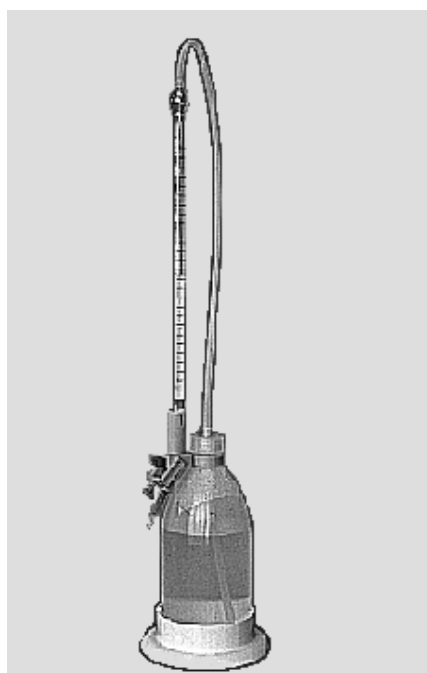
Once the acid level in a sample has been determined it can be used to find the ratio of sugar to acid.

Equipment:



STANDARD LABORATORY EQUIPMENT ¹

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COMMERCIAL TITRATOR ¹



EXAMPLE OF pH METER ¹

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EXAMPLE OF pH METER ¹

A laboratory burette of 25 or 50ml capacity is used. 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.

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Requirements:

- 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.

Sampling:

Take a sample of 10 fruits of each size at random from different places in the lot selected for inspection, and assumed to be representative of that lot. 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 it in half and squeeze out the juice with an extractor e.g. citrus fruits, or homogenise the flesh into a pulp e.g. apples.

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.

Using a clean and dry safety pipette for each fruit. Draw up 5ml 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. Two further beakers are similarly filled.

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. 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.

- 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.
- Repeat the titration process with the second and third beakers and record the results.
- 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 25 ml 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.

Measurement 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 manufacturers 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.

Testing:

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.
- Repeat the titration process with the second and third beakers and record the results.
- 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 25 ml 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)

Average the results of the three titration tests.

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

$$\text{Percentage Citric Acid} = \frac{\text{Average Titre} \times 0.0064 \times 100}{5\text{ml juice}}$$

This formula can be simplified to: $\frac{\text{Titre} \times 0.64}{5}$

$$\text{The Sugar Acid Ratio} = \frac{\% \text{Brix value}}{\text{Percentage Citric Acid}}$$

Results:

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

There are no limits set out in the UN/ECE quality standards. For limits set by national regulations or recommendations, please refer to document AGR/CA/FVS(91)4/Last revision.

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.