Method 3.5 – Juices: turbidity at 420 nm

1. Rationale

This method is applicable only to clear juice and is used to determine the turbidity in the juice. The result gives a useful measure of the effectiveness of the clarification process.

2. Principle

The clear juice is diluted to 5°Bx and divided in two portions. One portion is filtered through a membrane filter. The pH of both solutions is adjusted to 7.00 ± 0.02. The ICUMSA colour is determine from the respective Brix and absorbances at 420 nm. The turbidity is the difference between the ICUMSA colours of the filtered and unfiltered solutions.

3. Definitions

3.1 Transmittance of a solution

If I_1 represents the radiant energy incident upon the first surface of the solution, and I_2 represents the radiant energy leaving the second surface of the solution. Then:

$$T = \frac{I_2}{I_1} = \text{transmittance of the solution}$$

and $100 \times T$ = percentage transmittance

3.2 Transmittancy

Let T_{soln} represent the transmittance of a cell containing the solution and let T_{solv} represent the transmittance of the same cell containing the pure solvent. Then:

$$T_s = \frac{T_{soln}}{T_{solv}} = \text{transmittancy of the solution}$$

3.3 **Absorbancy** (extinction) measured in absorbance units (AU)

$$A_s = -\log_{10} T_s = \text{absorbancy of the solution}$$

3.4 **Absorbancy index** (extinction index)

Let b represent the length (mm) of the absorbing path between the boundary layers of the solution and let c represent the concentration (g/cm^3) of the sugar solution. Then:

$$A_i = \frac{A_s}{bc} = \text{absorbancy index of the solution.}$$

3.5 ICUMSA Colour

The value of the absorbancy index multiplied by 10 000 is reported as the ICUMSA Colour of the solution and the resulting value is expressed in ICUMSA Units (IU). Since the wavelength at which the determination of colour in solution is used is set at 420 nm the value is designated as being the ICUMSA 420 Colour.

4. Apparatus

4.1 **Spectrophotometer** capable of light transmission measurements at a wavelength of 420 nm with the narrowest practical bandwidth, *e.g.* ± 10 nm

4.2 **Optical glass cell**: 5 mm

4.3 **Membrane filters**: cellulose nitrate filters, 0.45 µm pore size, 50 or 47 mm ϕ

4.4 **Buchner funnel** or magnetic vacuum filtration funnel: 50-65 mm ϕ

4.5 **Buchner flask** and rubber bung: 500 cm^3

4.6 **pH meter** capable of measuring to 0.01 units

4.7 **Refractometer** operating at 20.0 ± 0.1°C

4.8 **Magnetic stirrer** with stirrer bar

4.9 **Laboratory balance** readable to 0.01 g

4.10 **Beakers**: 100, 250 and 1 000 cm^3

4.11 **Measuring cylinder**: 100 cm^3

4.12 **Volumetric flasks**: 2 × 200, 2 × 1 000 cm^3

4.13 **Pipettes**: 2 × 10 cm^3

5. Reagents

5.1 **Hydrochloric acid solution** (1 M)

Hydrochloric acid (HCl, 32%) is a corrosive acid and contact with the skin, eyes and through inhalation must be avoided. Work in a fume cupboard while wearing gloves and safety glasses.

Measure 98 cm^3 concentrated hydrochloric acid and add to approximately 700 cm^3 distilled water in a 1 000 cm^3 beaker. Always add the acid to the water and not the other way around. The acid dilution is exothermic and the solution will therefore heat. Allow the solution to cool down, transfer to a 1 000 cm^3 volumetric flask and make to the mark.

5.2 **Hydrochloric acid solution** (0.05 M)

Pipette 10 cm^3 of the 1 M hydrochloric acid solution into a 200 cm^3 volumetric flask and make to the mark with distilled water.

5.3 **Sodium hydroxide solution** (1 M)

Sodium hydroxide (NaOH) is a corrosive base and contact with the skin and eyes should be avoided. Wear gloves and safety glasses during use.

Weigh 40.0 g sodium hydroxide pellets and dissolve in some distilled water. This dissolution is exothermic and the solution will therefore heat. Allow the solution to cool and dilute to 1 000 cm^3 in a volumetric flask.

5.4 **Sodium hydroxide solution** (0.05 M)

Pipette 10 cm^3 of the 1 M sodium hydroxide solution into a 200 cm^3 volumetric flask and make to the mark with distilled water.

6. Procedure

6.1 Calibration of pH meter

Following the manufacturer's directions, calibrate the pH meter using the 4.00 and 7.00 pH buffer solutions (compensated for a temperature different from 20°C) while stirring at a constant rate. Calibrations should be done at the beginning of each day or shift using fresh buffer solutions only. The buffer solutions should be at room temperature.

6.2 Sample analysis

Allow the clear juice to cool to room temperature. Mix the sample thoroughly. Determine the approximate Brix of the unfiltered solution and calculate the mass of juice needed to prepare a 5°Bx solution using the formula below.

$$\text{Mass juice (g)} = \frac{\text{total mass (g)}}{\text{original Brix (°Bx)}} \times \text{final Brix (°Bx)}$$

Dilute this mass of well-mixed sample to 100.00 g using distilled water.

Divide the solution into two parts and filter the one through a 0.45 µm cellulose nitrate membrane under vacuum into a clean, dry Buchner flask.

Transfer both solutions into 50 or 100 cm^3 beakers. Stir the solutions on the magnetic stirrer and adjust the pH to 7.00 ± 0.02 using either hydrochloric acid (0.05 M) to bring the pH down or sodium hydroxide (0.05 M) to bring the pH up. Allow ample time for the pH reading to stabilize (1 minute).

Measure the absorbance of the solutions in a 5 mm cell using the spectrophotometer at 420 nm against distilled water as a reference. Also measure the Brix of the solutions.

7. Calculations

Use the Brix readings at 20.0°C to obtain the concentration of total solids in g/cm^3 using the formula indicated below. Determine the ICUMSA colour of the filtered and unfiltered solutions.

$$\text{Total solids (g/cm}^3\text{)} = \frac{\text{Brix} \times (a + b \times \text{Brix} + c \times \text{Brix}^2 + d \times \text{Brix}^3 + e \times \text{Brix}^4)}{100}$$

where $a = 0.9971843$

$b = 3.85738 \times 10^{-3}$

$c = 1.254916 \times 10^{-5}$

$d = 8.125659 \times 10^{-8}$

$e = 5.611455 \times 10^{-10}$

$$\text{ICUMSA 420 Colour} = \frac{A_s \times 10\,000}{bc}$$

where A_s ≡ absorbance at 420 nm (AU)
b ≡ cell length (mm)
c ≡ concentration of total solids (g/cm^3)

Report results in ICUMSA Units (IU) to the nearest 10 units.

8. Example

Obtain the concentration of total solids in g/cm^3 from the Brix reading at 20.0°C using the equation in 7.

Unfiltered solution:

Brix at 20.0°C	=	5.0°Bx
Concentration of total solids	=	0.051 g/cm^3
Absorbance at 420 nm	=	0.529 AU
ICUMSA 420 colour	=	$\frac{0.529\ \text{AU} \times 10\,000}{5\ \text{mm} \times 0.051\ \text{g/cm}^3}$
	=	20 745 IU

Filtered solution:

Brix at 20.0°C	=	5.0°Bx
Concentration of total solids	=	0.051 g/cm^3
Absorbance at 420 nm	=	0.742 AU
ICUMSA 420 colour	=	$\frac{0.742\ \text{AU} \times 10\,000}{5\ \text{mm} \times 0.051\ \text{g/cm}^3}$
	=	29 098 IU
Turbidity	=	Unfiltered Colour - Filtered Colour
	=	(29098 - 20745) IU
	=	8353 IU

Report as 8 350 IU

9. References

SASTA (1985). *Laboratory Manual for South African Sugar Factories.* 3rd Edition: 262 - 263.

SMRI (2004). Determination of the colour and turbidity of clear juice. *SMRI Test Methods,* TM020.