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SCHLIESSMANN
SCHWÄBISCH HALL
Tel. 07 91 - 9 71 91-0 • Fax 9 71 91-25
C. Schliessmann Kellerei-Chemie GmbH & Co.KG
Auwiesenstr. 5 • D-74523 Schwäbisch Hall

Getränkeanalytik

Determination of sugar according to Dr. Rebelein

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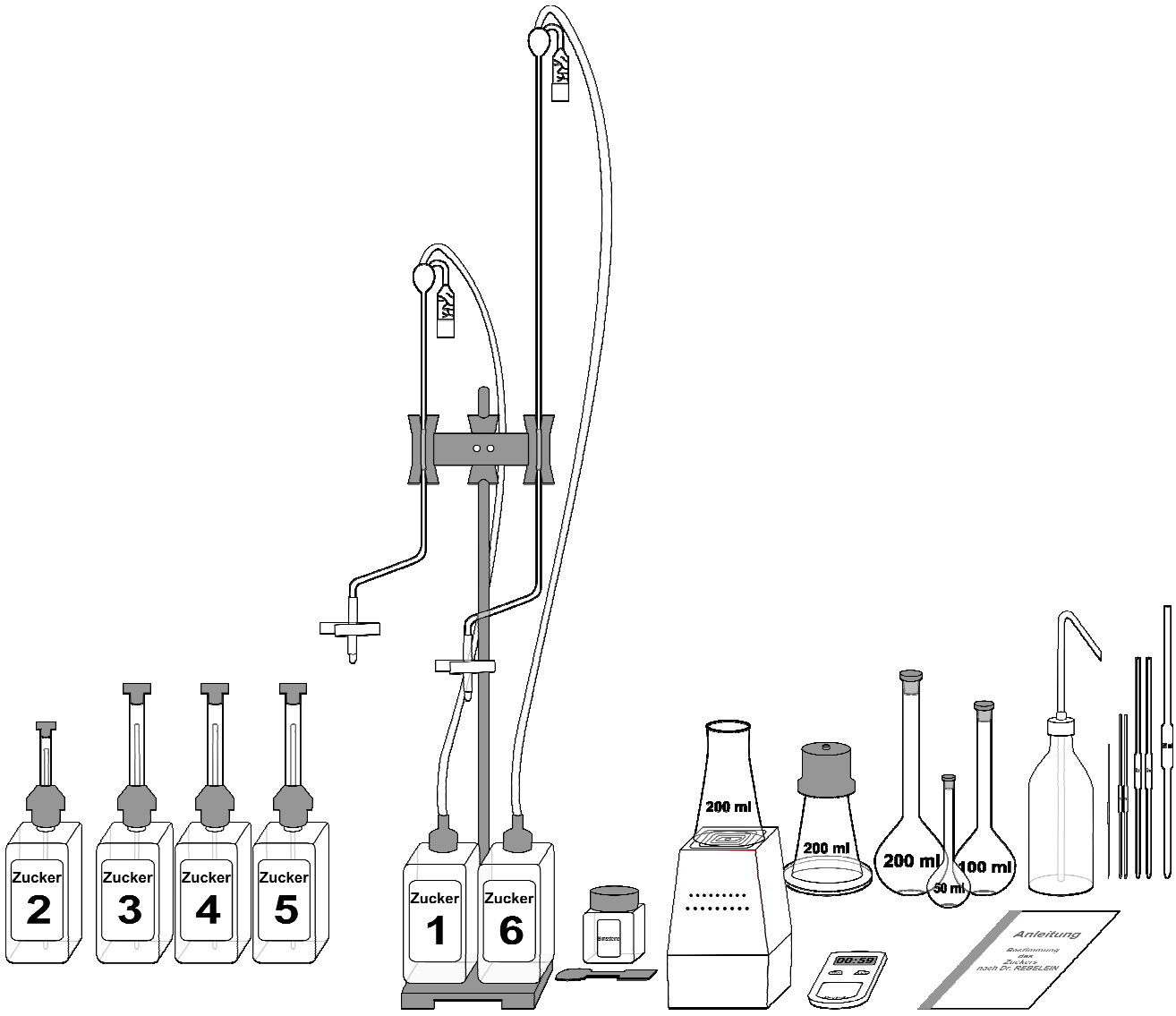

Principle of the method:
The determination of reducing sugars according to Dr. Rebelein has been in use for more than four decades as a proven method for measuring the fermentable sugars glucose, fructose and - after inversion - also sucrose, which are important in fruit juices and wines.
The method is based on the quantitative oxidation of reducing sugars using an alkaline copper sulfate solution. The bivalent copper is reduced to copper oxide, the unused copper is reacted with potassium iodide and the iodine formed is then titrated back with sodium thiosulfate.
The exact observance of the analytical procedure, in particular the heating conditions, guarantees the quantitative sequence of the chemical reactions and significantly ensures the accuracy of the result found.

Restrictions / Disturbances of the method:
The also reducing but not fermentable sugars rhamnose and arabinose (in grape juice and wine approx. 0.5 - 1.5g/L) and plant dyes (flavonoids) especially in dark red and fruit wines may give somewhat higher results than those obtained with the official reference methods (enzymatic photometric or HPLC) can be found in the same sample.
For this reason rhamnose and arabinose in grape wine are taken into account by a calculated reduction of 1g/L in the vine yield. Flavonoids, which can fake up to 4g sugar per litre, especially in deep dark red wines, can be removed with the following simple sample preparation:

Sample preparation (only necessary or recommended for deep dark red and fruit wines):
- Stir approx. 5 g PVPP (polyvinylpolypyrrolidone) into approx. 50 mL wine in the 100mL Erlenmeyer flask,
- Wait 5 minutes while stirring occasionally,
- Filter the mixture using the MN 615 ¼ or SS 595 ½ pleated filter into clean 100 mL conical flasks,
- Carrying out the sugar determination in the bright filtrate, which is weakened in colour.

Sugar determination with or without inversion?
The natural invert sugar (glucose, fructose) of saccharose free samples is determined according to method 1. If the sample contains sucrose, it must first be inverted, i.e. split into glucose and fructose. The inverting of the sucrose and subsequent determination of the total invert sugar is carried out according to method 2.

Detection limits of the method / dilution of the sample:
Beverages with a maximum sugar content of 28 g/L are analysed undiluted (sample quantity 2.0 mL). The result is read off directly at the burette "Zucker nach Dr. Rebelein", expressed as invert sugar in g/l.
Beverages with a sugar content higher than 28 g/L are diluted as follows before the test and then 2,0 mL of this dilution is subjected to the analysis. Multiplying the burette value read by the dilution factor gives the calculated sugar content of the undiluted beverage sample.

Preparation of dilutions:
Beverage with 28 - 56 g/l sugar (**dilution factor 2**)
Pipette 25,0 ml of beverage into 50 ml measuring flask. Fill up to mark with distilled water, adjust exactly.
Beverage with 56 - 112 g/l sugar (**dilution factor 4**)
Pipette 25,0 ml of beverage into 100 ml measuring flask. Fill up to mark with distilled water, adjust exactly.
Beverage with 112 - 224 g/l sugar (**dilution factor 8**)
pipette 25,0 ml of beverage into 200 ml measuring flask. Fill up to mark with distilled water, adjust exactly.
Beverage with 224 - 560 g/l sugar (**dilution factor 20**)
pipette 10,0 ml of beverage into 200 ml measuring flask. Fill up to mark with distilled water, adjust exactly.

Alternative Variant for Examination Range 0 - 56 g of Sugar per Litre:
If instead of 2,0 ml only 1,0 ml of undiluted beverage is taken for analysis, the same result is obtained as with a dilution of the beverage with distilled water in the relation 1:1. The burette figure must be multiplied by 2. Through this variant a dilution free examination range of 0 - 56 g of sugar per litre is obtained. The working method is of interest when the sugar figures often are around 28 g of sugar per litre.

Pipetting technique:
The exact dimension of the sample is decisive for the accuracy of the test result:
- Always rinse the pipette with the liquid to be measured;
- Place the liquid approx. 2 cm high above the ring mark, dry the pipette with a cellulose cloth;
- Set the sample to the mark by placing the tip of the pipette against the wall of a special beaker to drain off excess liquid and then wiping it off;
- Transfer sample into the prepared Erlenmeyer flask without loss (place the pipette tip against the flask wall to allow it to drain, blow out the pipette after approx. 15 seconds and remove the pipette tip).

Our information sheet "Notes on working equipment for the analytical methods according to Dr. Rebelein" provides information on the correct handling of burettes and dosing cylinders as well as the disposal of analytical waste.

Method 1: Determination of Natural Inverted Sugar (without Inversion):

- Preheat infrared laboratory heater for approx. 5 minutes.
- Check burettes. The meniscus must be on a level with the top mark of the burettes. Remove drops from the burette jets, fill up dose cylinders.
- Fill 200 ml conical flask slowly with 10,0 ml of "Sugar 1", wipe remaining drop off the jet of the burette on the inside wall of the flask.
- Add 5 ml of "Sugar 2" (dose cylinder) and 5-8 pumice stones.
- Pipette 2,0 ml of the liquid to be analysed (Blowing-out Pipette). Refer to "Correct pipetting technology" on page 2!
- Place the conical flask on the heated infrared laboratory heater and set the laboratory alarm clock to **2 ½ minutes**.
- ☒ After this time put rubber cap on conical flask, place the flask into the Petri dish and cool it with running water.
- Once the liquid has cooled down to room temperature (after about 2-3 minutes) add 10 ml each of "Sugar 3", "Sugar 4", and "Sugar 5" (dose cylinder) in the listed order while shaking the flask gently.
- Titrate the now deep blue liquid with "Sugar 6" to "creamy yellow". (See "Comments on Titration".)
- Read content of sugar off titration burette. If necessary multiply this figure with the dilution factor (see calculation example below!).
- Fill up burettes to the starting mark for the next determination. Do not leave burettes completely or partly empty.

Calculation example for a dilution:
Dilute 25 ml beverage sample in measuring flask with water to 100 ml (dilution factor 4). The sugar determination in this dilution results in a burette reading of 24.8g/L. The beverage sample contains 24.8 x 4 = 99.2 g/L sugar.

Comments on Titration:
Before the titration the liquid has a deep blue colour. As soon as the liquid becomes lighter the titration should be slowed down. Aim of titration is a mixed colour of white, grey, and beige. The technical term for this colour is "creamy yellow". It is hard to characterise but good and clear to recognise. When the titration comes near to the described mixed colour the burette reading is remembered and one lets fall another drop into the centre of the liquid surface and observes whether the area where the drop has fallen in becomes lighter. If this is not the case, the previous burette reading is the-final one. If, on the contrary, the colour is still brightening up the liquid is mixed by shaking, and the process is repeated until the colour remains constant.

Method 2: Determination of Natural Inverted Sugar and Saccharose (with Inversion):

- Put a few pumice stones, 10,0 ml of "Sugar 1", 2 ml of inversion solution, and 2,0 ml of the sample to be determined in 200 ml conical flask.
- Place flask on heated infrared laboratory heater and set laboratory alarm clock to **2 minutes**.
- After the 2 minutes let 5 ml of "Sugar 2" (dose cylinder) run into the centre of the boiling liquid without removing flask from the infrared laboratory heater.
- Set alarm clock to **2½ minute**, during which time the liquid continues to boil.
- Proceed to method 1 at ☒.
- Burette value read off = sugar content after inversion in g/l; **consider dilution if necessary**!

Calculation of Saccharose:
("Sugar with inversion" – "sugar without inversion" x 0,95)

Example:

Sugar with inversion	26,0 g/l
Sugar without inversion	- 22,0 g/l
Saccharose	4,0 g/l x 0,95 **= 3,8 g/l**

Determination of the Total Sugar:
("Sugar without inversion" + saccharose)

Example:

Sugar without inversion	22,0 g/l
Saccarose	+ 3,8 g/l
Content of total sugar	**25,8 g/l**

Determination of the Sugar Free Extract:
(Total extract - saccharose – "sugar without inversion" +1g/L)

Example:

Total extract	45,0 g/l
Saccharose	- 3,8 g/l
Sugar without inversion	- 22,0 g/l
	19,2 g/l
Factor	+ 1,0 g/l
sugar free extract	**20,2 g/l**

Blank Titration for the Control of the Solutions:
Proceeds according to Method 1, but use 2,0 ml of distilled water as sample. A sugar content of 0 g/l should be obtained after titration with a tolerance not significantly exceeding ± 0,2g/l.

Check the heating capacity of the heater:
The heating capacity of a laboratory heater is sufficient for Dr. Rebelein analyses if it preheats 13mL of water in the open 200 mL Erlenmeyer flask to a maximum of 3 mL within four minutes.

Determination of sugar according to Dr. Rebelein - parts list -

- ☐ 1 Infrared laboratory heater (instructions)
- ☐ 1 laboratory alarm clock –digital-
- ☐ 1 Stand with staff 600 x 12 mm for burettes
- ☐ 1 holder for burettes 10 – 10 / 12
- ☐ 1 burette 10 ml TTS for „sugar 1“
- ☐ 1 burette „sugar according to Dr. Rebelein“ TTS for „sugar 6“
- ☐ 1 blowing out-pipette 1 ml
- ☐ 2 blowing out-pipettes 2 ml
- ☐ 2 blowing out-pipettes 10 ml
- ☐ 1 blowing out-pipette 25 ml
- ☐ 1 dose cylinder 5 ml for „sugar 2“
- ☐ 3 dose cylinder 10 ml for „sugar 3“, „sugar 4“ and „sugar 5“
- ☐ 2 conical-flasks 200 ml (Reaction flask)
- ☐ 1 rubber cap Size 4a as Hood
- ☐ 1 petri dish approx 95 mm Ø
  for putting into the reaction flask during the cooling with tap water
- ☐ 1 polyamide bottle 500 ml for distilled water
- ☐ 1 x pumice stones for analysis (Riedel-de-Haen)
- ☐ 1 spatula for pumice stones
- ☐ 1 measuring flask 50 ml for dilution
- ☐ 1 measuring flask 100 ml for dilution
- ☐ 1 measuring flask 200 ml for dilution
- ☐ 1 map with information-material

original allocation of equipment of reagents:	**Need per analysis:**
☐ sugar 1 500 ml in polyamide bottle	(10,0 ml / burette)
☐ sugar 2 500 ml in polyamide bottle	(5 ml / dose cylinder)
☐ sugar 3 500 ml in polyamide bottle	(10 ml / dose cylinder)
☐ sugar 4 500 ml in polyamide bottle	(10 ml / dose cylinder)
☐ sugar 5 500 ml in polyamide bottle	(10 ml / dose cylinder)
☐ sugar 6 500 ml in polyamide bottle	(variable / burette)

only on express order for determination of sugar with inversion:

☐ 500 ml inversion solution (2 n sulfuric acid) in polyamide bottle	(2 ml / dose cylinder)
☐ dose cylinder 2 ml	

recommendable accessories:

- ☐ draining rack for laboratory glass
- ☐ pipette stand from polypropylene