EGER 1774

ESZTERHÁZY KÁROLY CATHOLIC UNIVERSITY

Institute for Viticulture and Oenology

FOOD CHEMISTRY AND ANALYSIS

Laboratory Practical Manual

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Contents

1. Laboratory rules and safe laboratory working practices

1. Eating, drinking and smoking are prohibited in the laboratory.

2. Order and cleanliness must be maintained at all times. Materials, vessels and equipment left lying around, spilled chemicals, accumulated waste, and neglected or damaged equipment and protective devices may cause accidents.

3. Passageways must never be narrowed, closed or obstructed, even temporarily. This is especially important for routes leading to exits.

4. Each laboratory, or its entrance area, must be equipped with the required fire extinguisher(s), a serviceable first-aid kit and an emergency safety shower.

5. Solutions of acetic acid, boric acid and sodium hydrogen carbonate at a maximum concentration of 2% should be available for emergency treatment when corrosive liquids contact the skin or eyes. Suitable eyewash vessels must also be provided.

6. If acid is splashed onto the skin, first wipe it off and then rinse with plenty of water. If necessary, neutralize with a weak alkali such as sodium hydrogen carbonate solution or borax. In the case of alkaline contamination, after wiping and rinsing with water, neutralize with 2% acetic acid or boric acid solution.

7. If a corrosive substance enters the eye, rinse immediately using an eyewash device. After rapid first aid, medical attention must always be sought.

8. Electrical fires must never be extinguished with water or foam. For larger laboratory fires, use suitable dry powder, carbon dioxide or sand. Fire-safety equipment must remain readily accessible and be used only in an emergency.

9. All staff and students must know the location and operation of the main electrical switch and the main water shut-off valves.

10. Before closing the laboratory, ensure that water, gas, vacuum and other service taps are closed, main shut-off valves are closed, windows are shut, and electrical equipment is switched off and disconnected where appropriate.

11. Utility lines and taps must be checked periodically for leakage. Soap solution may be used to locate gas leaks. Electrical equipment must have appropriate protection against electric shock.

12. Appropriate personal protective equipment must be used when required, including respiratory protection, rubber or protective gloves and safety goggles. PPE must be kept clean, serviceable and stored in its designated location.

13. Rubber gloves are mandatory when working with strong acids, alkalis and corrosive substances. They are also recommended when handling substances that may be absorbed through the skin. Gloves should be washed before removal and stored clean and dry.

14. Safety goggles or a non-flammable face shield must be worn whenever there is a risk of acids or alkalis entering the eyes, and during work under vacuum.

15. Alkalis, acids, toxic liquids and liquids releasing corrosive vapours must never be pipetted by mouth. Use an appropriate pipetting device.

16. Many laboratory accidents are caused by broken glassware. Cracked or damaged glassware must not be used and must be reported for replacement.

17. Do not place glass apparatus on the floor or where it can be kicked or knocked over.

18. When assembling apparatus on a laboratory stand, ensure that the stand is sufficiently stable for the combined mass of the apparatus and its contents. Check stability before adding materials.

19. Do not clamp glassware too rigidly. Glass expands on heating and dangerous stresses may develop. Metal clamps should be lined with cork, rubber or felt.

20. Never exceed the specified maximum temperature during boiling or heating.

21. Never add boiling chips to a liquid that is already close to boiling or superheated, and do not lean over or shake such a vessel. Add boiling chips before heating begins.

22. Smoking and the use of open flames are prohibited in the laboratory. Flammable materials must not be brought into the laboratory unless required and properly controlled.

23. Violation of laboratory rules may result in disciplinary action. Damage caused intentionally or through negligence must be compensated.

2. Laboratory equipment, mass measurement of grape components, and linear interpolation

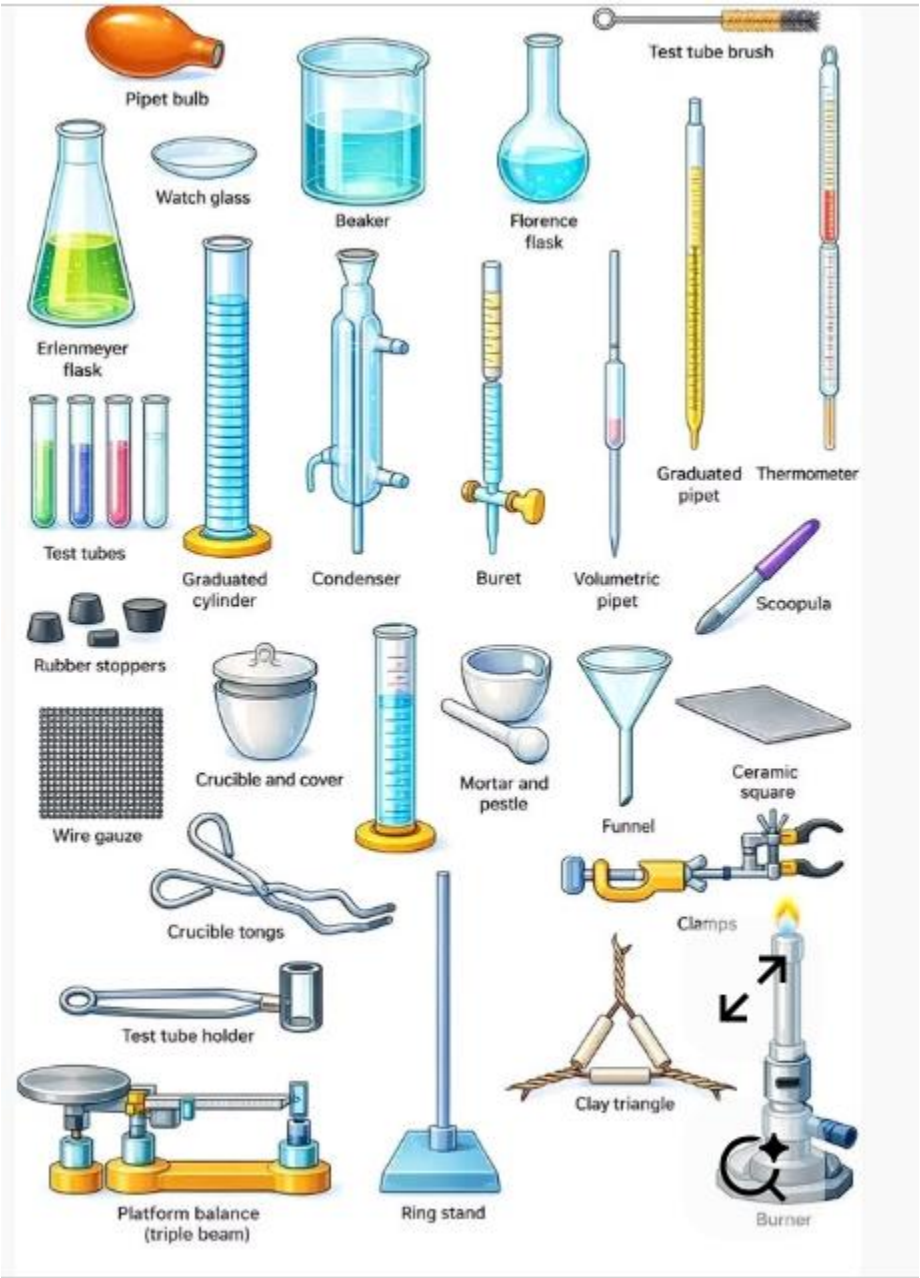

Figure 1. Laboratory equipments (Source: https://www.pinterest.com)

Figure 2. Laboratory equipments (Source: https://www.pinterest.com)

Mass measurement

Mass is a measure of the quantity of matter. Its SI unit is the kilogram (kg). The symbol for mass is m. Common laboratory balances include top-loading balances, tare balances and electronic analytical balances. Common prefixes include kilo-, hecto-, centi- and milli-.

In grape analysis, determine the total mass of the bunch and then the proportion of its components as percentages. In ripe grapes, the rachis/stem generally represents approximately 2-7% of bunch mass.

Stem (%) = stem mass (g) / bunch mass (g) × 100

Berry (%) = 100 − stem (%)

Skin (%) = skin mass (g) / berry mass (g) × 100

Seed (%) = seed mass (g) / berry mass (g) × 100

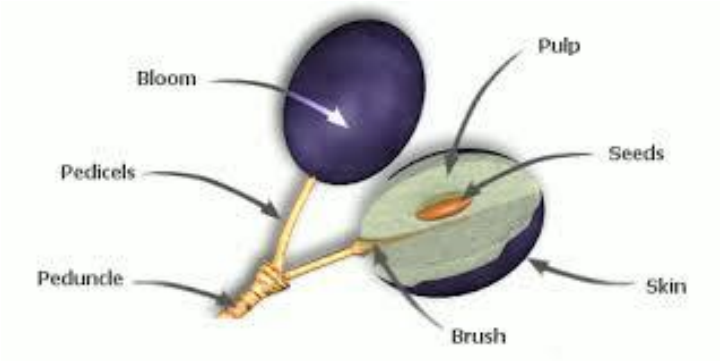

Figure 3. Structure of grape-berry (Source: https://www.youcellar.com)

3. Determination of carbohydrate content, potential alcohol content and pH of grape must

In Hungary, sugar content of grape must is commonly expressed as Hungarian must degree (MM°) and °Brix. Hungarian must degree indicates approximately how many decagrams of sugar are present in one kilogram of must and is related to density, sugar percentage and potential alcohol content.

°Brix expresses the mass percentage of sugar: grams of sucrose equivalent per 100 g of solution. A reading of 10 °Brix therefore corresponds to approximately 10 g sugar per 100 g of must.

°Brix = 1.176 × MM°

MM° = °Brix / 1.176

Potential alcohol (v/v%) = sugar content (g/L) / 17

Sugar content of must (g/L) = (must degree − 2.5) / 0.08

Density of must = 222 / (222 − must degree)

Measurement with a refractometer

A refractometer measures physical properties of liquids, particularly refractive index. Sugar solutions show a relationship between refractive index and sugar concentration: as sugar concentration increases, refractive index also increases.

Different refractometers require different procedures. Some instruments include automatic temperature compensation, whereas others require separate correction for sample temperature. Instruments that directly display sugar concentration calculate it from the measured refractive index using an internal calibration. If only refractive index is displayed, the result must be converted using a calibration curve or table.

Calibration: Calibrate the refractometer regularly according to the manufacturer's instructions, using suitable calibration standards.

Temperature correction: Temperature affects the result. Apply the required correction or use the instrument's automatic temperature-compensation function.

Sample preparation: Bring the sample to the appropriate temperature and place it on a clean, grease-free measuring surface without introducing air bubbles.

Replicate measurements: Perform repeated measurements and use the mean value to improve reliability and reduce random error.

A wine/grape refractometer may have a left-hand °Brix scale and a right-hand potential alcohol scale. Calibration is typically performed with 2-3 drops of distilled water, for which the reading should be zero.

Three-scale refractometers may display Mass Sacch% (°Brix), Oechsle and KMW. The Oechsle scale expresses how many grams per litre the density of must exceeds that of water; for example, 100 °Oe corresponds to a density of approximately 1100 g/L. KMW (Klosterneuburger Mostwaage) expresses sugar by mass. Measurements should be made on clean, filtered, unfermented must at the specified calibration temperature.

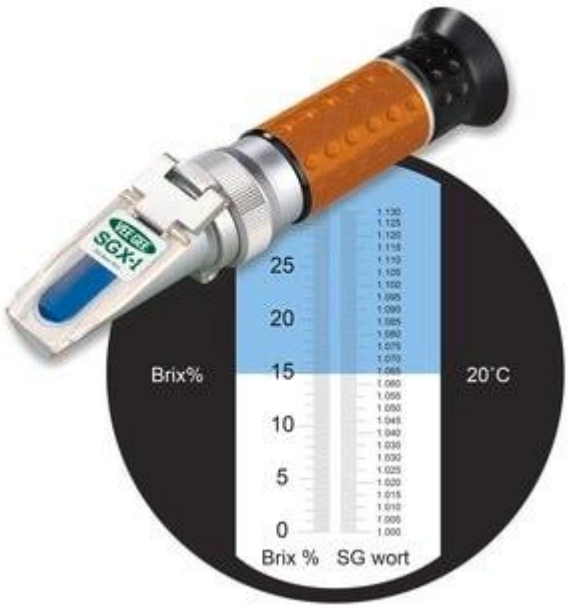

Figure 3. Refractometer (Source: https://www.sigmaaldrich.com)

Measurement with a must hydrometer

The certified Hungarian must hydrometer is a density meter, commonly equipped with a thermometer, with a scale approximately from 10 to 30 must degrees. If calibrated at 17.5 °C, a temperature correction is applied: for every 2 °C below the calibration temperature, subtract 0.1 must degree; for every 2 °C above it, add 0.1 must degree.

Figure 4. Density meter (Source: https://www.sigmaaldrich.com)

Measurement with a pH meter

The acidity of grape juice and wine, expressed by pH, strongly affects wine quality during production, development and ageing. pH influences the rate and equilibrium of chemical and biochemical reactions.

- Low pH inhibits bacterial growth and, to a lesser extent, yeast growth.
- pH strongly influences malolactic fermentation by lactic acid bacteria.
- pH affects the equilibrium among pigment forms and therefore wine colour; lower pH generally favours the red forms of anthocyanin pigments.
- Lower pH can slow several ageing reactions and influence the development of wine during maturation.

Figure 5. pH meter (Source:https://www.adwa.hu)

Practical task

- Destem and crush the grape sample, obtain the juice and filter it.
- Measure the extracted juice using a hand refractometer, a must hydrometer and a digital pH meter.
- Report sugar content in g/L, MM° and °Brix.
- Report potential alcohol in v/v% both as the instrument reading and as the calculated value.
- Report the pH of the must.

4. Methods for carbohydrate determination and the Rebelein method

Carbohydrates, particularly in foods of plant origin, often constitute the largest fraction of dry matter. Direct determination of total carbohydrate is rarely required. More commonly, mono- and oligosaccharides are determined as groups (reducing sugars), together with starch. Modern carbohydrate analysis increasingly relies on separation, purification and chromatographic determination of individual carbohydrate components.

Carbohydrate determination in selected food-industry sectors

Confectionery industry: Sugar is a major component of confectionery raw materials. Sugar beet contains approximately 20-25% dry matter and 15-18% sucrose. Sucrose may be determined after clarification, for example by polarimetry.

Bakery industry: Cereals contain substantial carbohydrate reserves as starch and structural carbohydrate as cellulose. Fibre and starch are particularly important analytical parameters. Fibre can be determined by Weende analysis; starch may be hydrolysed and the released glucose determined, for example, polarimetrically.

Dairy industry: Lactose and sucrose are important carbohydrates in milk and dairy products. Lactose is a reducing disaccharide, whereas sucrose is non-reducing. Methods include iodometric titration, polarimetry and near-infrared spectroscopy.

Alcoholic beverages / oenology: The sugar content of grape must consists mainly of glucose and fructose; sucrose, pentoses and pectin may also occur in smaller quantities. Physical methods such as density and refractive-index measurements are simple but less precise than chemical methods.

Meat industry: Only small quantities of glycogen and glucose occur in meat; their quantitative determination is usually of limited practical importance.

Preserving/canning industry: Carbohydrates are generally determined chemically after preparation and clarification of an aqueous suspension; total sugar may be measured after inversion.

Methods of carbohydrate determination

Calculation method: Carbohydrate content can be estimated by difference: 100% minus protein, fat, fibre, ash and other specified components.

Density measurement: Sugar-solution density changes monotonically with carbohydrate concentration; therefore density measurement, for example by pycnometer, can be used to estimate sugar content.

Refractometry: Refractive index is related to carbohydrate concentration. Refractometric sugar content is commonly reported in °Brix, approximately grams of sucrose per 100 g solution.

Polarimetry: The concentration of a single optically active sugar can be determined from optical rotation. Polarimetry is widely used for sucrose.

Near-infrared spectroscopy (NIR): Carbohydrates interact with infrared radiation at characteristic wavelengths; spectral position contributes qualitative information and absorbance can be used quantitatively.

Chemical methods: Examples include the Reischauer, Bertrand, Schoorl and Rebelein methods.

Enzymatic methods: Examples include glucose oxidase and glucose-6-phosphate dehydrogenase methods. NADPH formation can be measured photometrically at 340 nm.

Chromatographic methods: Thin-layer chromatography, high-performance liquid chromatography (HPLC), gas chromatography (GC) and gel chromatography can be used for carbohydrate analysis.

Rebelein method

Standard referenced in the original manual: MSZ 9479-80.

Principle: reducing sugars are oxidized with alkaline copper sulfate solution. In acidic medium, after addition of potassium iodide, an amount of iodine equivalent to the copper(II) ions is liberated. The iodine is titrated with sodium thiosulfate in the presence of starch indicator. The consumption of titrant is proportional to the sugar content of the wine.

The method is applicable up to approximately 28 g/L sugar; samples above this concentration must be diluted.

Procedure

- **Place 10 mL copper sulfate solution, 5 mL potassium sodium tartrate (Seignette salt) solution, 2 mL wine or must, and a few boiling chips in a 200 mL Erlenmeyer flask.**
- **Boil for 1.5 minutes, then cool under running tap water.**
- **While swirling the flask, add 10 mL potassium iodide solution, 10 mL 16% sulfuric acid and 10 mL starch solution.**
- **Titrate with sodium thiosulfate solution to a bone-white endpoint.**
- **Blank: perform the procedure using 2 mL distilled water instead of wine. Correctly prepared solutions require 30.0 ± 0.25 mL sodium thiosulfate solution for the blank.**

For a normal burette: sugar content = 30 − n (g/L), where n is the volume (mL) of sodium thiosulfate solution consumed for titration of the wine. Apply the appropriate dilution factor when the sample has been diluted.

5. Determination of fat content in foods and iodine value of fats and oils

The iodine value is a dimensionless analytical value related to the degree of unsaturation of fats and oils, i.e. to the number of carbon-carbon double bonds. Iodine adds across double bonds; therefore a higher iodine value generally indicates a higher degree of unsaturation. It also relates to physical state: fats with lower iodine values tend to be harder, whereas highly unsaturated lipids with higher iodine values and lower melting points are commonly oils.

Heating may substantially decrease the iodine value. Polymerisation can cause liquid oils spread in thin layers to solidify; this phenomenon is referred to as drying of oils.

Required equipment and reagents

- iodine-value flask
- burette
- chloroform
- 0.1 N potassium bromate titrant
- potassium bromide
- 20% hydrochloric acid
- potassium iodide
- 0.1 N sodium thiosulfate titrant
- starch indicator solution

Calculation: subtract the titrant volume consumed by the sample from that consumed by the blank. The molar mass of molecular iodine (I_2) is 254 g/mol. Practical task: determine the iodine value of different grape-seed oils.

6. Determination of titratable acidity of wines by potentiometric titration

Titratable acidity is the amount of titratable acidic groups in wine or must and is expressed as tartaric-acid equivalent.

Procedure

- **Measure 15 mL wine into a 200 mL Erlenmeyer flask.**
- **Heat the wine to boiling to remove dissolved carbon dioxide, then cool to room temperature.**
- **Add 5 drops of bromothymol blue indicator.**
- **Titrate with 0.2 mol/L sodium hydroxide to the colour-change endpoint corresponding to pH 7.0.**

Titratable acidity and pH are different concepts. Titration measures the total amount of replaceable acidic hydrogen under the defined conditions, whereas pH is a physicochemical parameter reflecting hydrogen-ion activity/concentration in the equilibrium state. Wines with the same titratable acidity can therefore have different pH values.

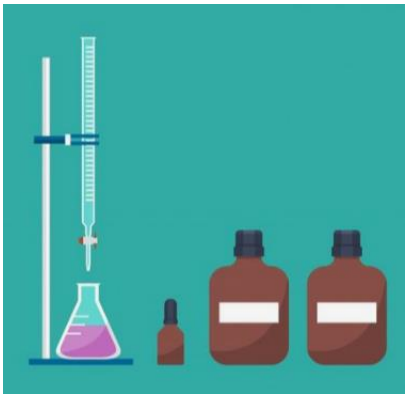

Figure 6. Titration method (Source: https://www.pinterest.com)

7. Determination of free and total sulfur dioxide in wines

Sulfur dioxide in must and wine occurs in several forms, including molecular sulfur dioxide and bisulfite. Their equilibrium depends on pH and temperature. Total sulfur dioxide is the sum of all free and bound forms.

Principle: the reducing properties of sulfurous acid are used in an iodometric/oxidimetric determination. Sulfurous acid reduces iodine to iodide. Excess iodine forms a blue complex with starch. Only free sulfur dioxide reacts directly; bound sulfur dioxide must first be released under alkaline conditions for determination of total sulfur dioxide. Results are expressed as mg/L SO_2.

Free sulfur dioxide

- **Pipette 50 mL wine into a 200 mL Erlenmeyer flask.**
- **Add 1-2 crystals of potassium iodide.**
- **Add 5 mL starch solution.**
- **Add 10 mL of 16-17% sulfuric acid.**
- **Titrate with 1/768 mol/L $KH(IO_3)_2$ solution until a blue colour persists for at least 30 seconds.**
- **Multiply the consumed volume of $KH(IO_3)_2$ solution in mL by 10 to obtain free sulfur dioxide content according to the procedure used in the manual.**

Total sulfur dioxide

- **Place 25 mL of 1 mol/L NaOH in an airtight 200 mL flask.**
- **Pipette 50 mL wine into the alkali with the pipette tip immersed in the solution.**
- **Close the flask tightly and allow it to stand for approximately 20 minutes, shaking several times.**
- **In the specified order add potassium iodide, 2-3 mL starch solution and 15 mL of 16-17% sulfuric acid.**
- **Titrate with 1/768 mol/L $KH(IO_3)_2$ solution to a blue iodine-starch endpoint persisting for at least 30 seconds.**
- **Multiply the consumed titrant volume in mL by 10 to obtain total sulfur dioxide content according to the procedure used in the manual.**

8. Spectrophotometric determination of polyphenol content by the Folin-Ciocalteu method

Calibration and sample preparation

- Prepare gallic acid standard solutions in the range 200-1000 mg/L by accurate weighing and measure their absorbance using the same analytical procedure as for the samples.
- Transfer 1 mL of wine sample into a 100 mL volumetric flask; add 60 mL distilled water and 5 mL Folin-Ciocalteu reagent.
- Allow the sample to stand for 20 minutes.
- Add 3.0 g solid Na_2CO_3, make up to 100 mL and allow to stand for 2 hours.
- Measure absorbance at 765 nm.
- Determine the polyphenol content of the wine from the calibration line and express it as gallic acid equivalents.

The original manual also provides an alternative working procedure using a 1:5 diluted wine sample, 50 mL distilled water, 5 mL Folin-Ciocalteu reagent and 20 mL sodium carbonate solution, followed by dilution to 100 mL, 30 minutes reaction time and absorbance measurement at 750 nm against a distilled-water blank.

Figure 7. Folin-Ciocalteu reagent (Source: https://www.libios.fr/en/analytical-solutions/oxydative-stress-antioxidant-capacity/oxydative-stress-antixodidant-capacity-assay-kits/polyphenols-folin-ciocalteu)

9. Measurement of wine turbidity by nephelometry

Method referenced in the original manual: OIV-MA-AS2-08: R2009. Turbidity is measured instrumentally using a turbidimeter/nephelometer.

Measurements depend strongly on instrument design. Important sources of variation include stray light, sample colour, electronic drift, light-source characteristics, photodetector design and measurement-cell geometry.

The principle is nephelometric measurement of light scattered at 90° to the incident beam. Turbidity is caused by very fine dispersed particles that reduce transparency and scatter light. Results are expressed in NTU (Nephelometric Turbidity Units).

The laboratory manual specifies a HI83749 turbidimeter (Hanna Instruments) with calibration standards below 0.1, 15, 100 and 500 NTU. The listed measuring ranges are 0.00-9.99 NTU, 10.0-99.9 NTU and 100-1200 NTU, with resolution depending on range.

Figure 8. Turbidity standards (Source: JRC Publications)

10. Determination of alcohol content in wines

Wine alcohol content may be determined by physical or chemical methods. Physical approaches include boiling-point, density, refractive-index and surface-tension measurements. In the practical exercise, ethanol is distilled from the wine and the distillate is adjusted to the original sample volume. Its relative density is then determined with a pycnometer and alcohol content is obtained by interpolation from the appropriate alcohol-density table.

Method referenced in the original manual: MSZ 9458-72 (withdrawn standard), determination of distillate density by pycnometer, classical analysis.

Definition and principle

Alcohol by volume is the volume of ethyl alcohol, in litres, contained in 100 litres of wine when both volumes are measured at 20 °C. It is expressed as % (v/v). Ethanol, its homologues and their esters that pass into the distillate are included in the analytical alcohol fraction under this procedure.

Principle: distil wine made alkaline with calcium hydroxide, then determine the alcohol content of the distillate. The reference approach determines distillate density with a pycnometer and converts density to alcohol by volume using the relevant table.

Equipment and procedure

- 1 L round-bottom flask
- distillation head
- Liebig condenser
- 100 mL volumetric flask
- pycnometer
- analytical balance
- glass rod
- 50 mL pipette

Measure 100 mL wine at 20 °C into a volumetric flask, transfer it to the distillation flask, add 2-3 boiling chips and begin distillation after starting the cooling water. Collect approximately 95 mL distillate in the volumetric flask and make up to the mark with distilled water.

Meanwhile, determine the mass of the dry pycnometer and the mass of the pycnometer filled with distilled water (water value). Determine the relative density of the distillate from equal-volume mass measurements, then obtain alcohol by volume from the corresponding density/alcohol table by interpolation.

Relative density = (mass of pycnometer + sample − mass of dry pycnometer) / (mass of pycnometer + water − mass of dry pycnometer)

Table 1. Density - Alcohol transfer count table

d	Alkohol		d	Alkohol		d	Alkohol	
20°/20°	g/l.	%(v/v)	20°/20°	g/l.	%(v/v)	20°/20°	g/l.	%(v/v)
0,9889	63,7	8,07	0,9829	103,6	13,13	0,9769	147,1	18,63
8	64,4	8,16	8	104,3	13,21	8	147,9	18,73
7	65,0	8,24	7	105,0	13,30	7	148,6	18,82
6	65,5	8,32	6	105,7	13,39	6	149,3	18,91
5	66,3	8,40	5	106,4	13,48	5	150,1	19,01
4	66,9	8,48	4	107,1	13,56	4	150,8	19,10
3	67,5	8,55	3	107,8	13,65	3	151,5	19,19
2	68,2	8,64	2	108,5	13,74	2	152,2	19,28
1	68,8	8,71	1	109,2	13,83	1	153,0	19,38
0	69,4	8,79	0	109,9	13,92	0	153,7	19,47
0,9879	70,1	8,88	0,9819	110,7	14,02	0,9759	154,4	19,56
8	70,7	8,96	8	111,4	14,11	8	155,2	19,66
7	71,4	9,04	7	112,1	14,20	7	155,9	19,75
6	72,0	9,12	6	112,8	14,29	6	156,6	19,84
5	72,7	9,21	5	113,5	14,38	5	157,4	19,93
4	73,3	9,29	4	114,2	14,7	4	158,1	20,02
3	74,0	9,37	3	114,9	14,56	3	158,8	20,12
2	74,6	9,45	2	115,7	14,65	2	159,6	20,21
1	75,3	9,54	1	116,4	14,74	1	160,3	20,30
0	75,9	9,62	0	117,1	14,83	0	161,0	20,39
0,9869	76,6	9,70	0,9809	117,8	14,92	0,9749	161,7	20,48
8	77,2	9,78	8	118,5	15,01	8	162,5	20,57
7	77,9	9,86	7	119,3	15,11	7	163,2	20,67
6	78,5	9,94	6	120,0	15,20	6	163,9	20,76
5	79,1	10,02	5	120,7	15,29	5	164,7	20,85
4	79,8	10,10	4	121,5	15,39	4	165,4	20,94
3	80,4	10,18	3	122,2	15,48	3	166,1	21,03
2	81,1	10,27	2	122,9	15,57	2	166,9	21,13
1	81,8	10,36	1	123,6	15,66	1	167,6	21,22
0	82,5	10,44	0	124,4	15,75	0	168,3	21,31
0,9859	83,1	10,52	0,9799	125,1	15,84	0,9739	169,1	21,41
8	83,8	10,61	8	125,8	15,93	8	169,8	21,51
7	84,5	10,70	7	126,6	16,03	7	170,5	21,59
6	85,1	10,78	6	127,3	16,12	6	171,2	21,68
5	85,8	10,87	5	128,0	16,22	5	172,0	21,78
4	86,6	10,96	4	128,8	16,31	4	172,7	21,87
3	87,2	11,05	3	129,5	16,40	3	173,4	21,96
2	87,8	11,13	2	130,2	16,49	2	174,2	33,06
1	88,5	11,21	1	130,9	16,58	1	174,9	22,16
0	89,2	11,30	0	131,6	16,67	0	175,6	22,25
0,9849	89,9	11,39	0,9789	132,4	16,77	0,9729	176,3	22,34
8	90,6	11,48	8	133,1	16,86	8	177,0	22,42
7	91,2	11,56	7	133,8	16,95	7	177,8	22,52
6	91,9	11,64	6	134,5	17,04	6	178,5	22,61
5	92,6	11,73	5	135,3	17,14	5	179,2	22,70
4	93,3	11,82	4	136,0	17,23	4	179,9	22,79
3	94,0	11,91	3	136,7	17,32	3	180,6	22,88
2	94,7	12,00	2	137,4	17,41	2	181,3	22,97
1	95,5	12,08	1	138,2	17,50	1	182,1	23,07
0	96,0	12,16	0	138,9	17,60	0	182,8	23,16
0,9839	96,7	12,25	0,9779	139,7	17,70	0,9719	183,5	23,24
8	97,4	12,34	8	140,4	17,79	8	184,2	23,33
7	98,1	12,42	7	141,2	17,89	7	184,9	23,42
6	98,8	12,51	6	141,9	17,98	6	185,6	23,51
5	99,5	12,60	5	142,7	18,08	5	186,3	23,60
4	100,2	12,69	4	143,4	18,17	4	187,0	23,69
3	100,9	12,78	3	144,2	18,26	3	187,7	23,78
2	101,6	12,87	2	144,9	18,35	2	188,4	23,87
1	102,3	12,96	1	145,7	18,45	1	189,1	23,96
0	103,0	13,05	0	146,4	18,54	0	189,8	24,04

Figure 9a. Alcohol distillation equipments (without wine)

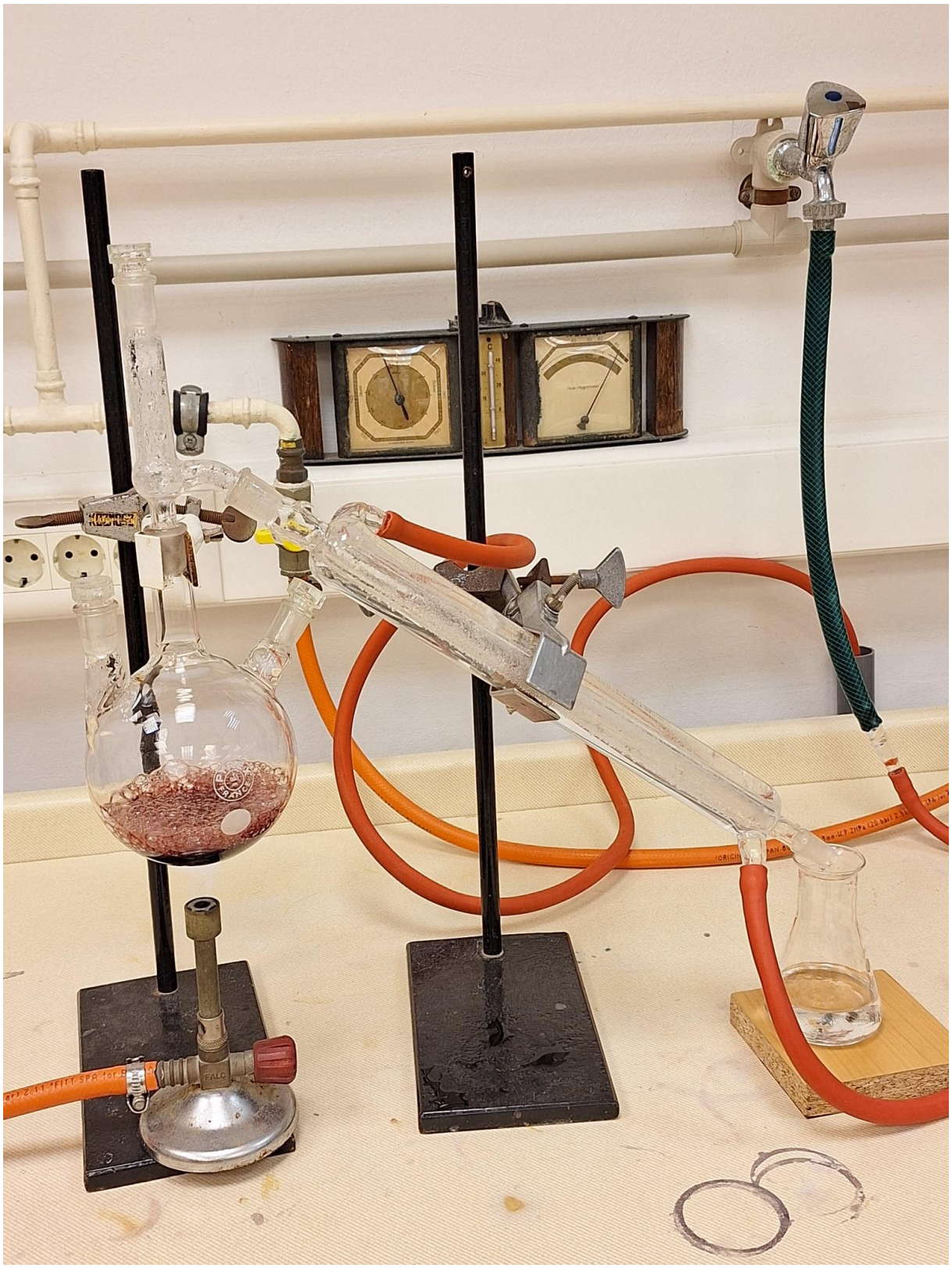

Figure 9b. Alcohol distillation equipments (with wine and distillatum)

11. Determination of energy value and calculation exercises

Conversion factors used in the original manual:

1 kJ = 0.2388458966275 kcal; 1 kcal = 4.1868 kJ

Nutritional values are generally expressed per 100 g of product. To calculate the nutritional value of a product, the formulation, amount of each ingredient, nutritional composition of each ingredient and final product mass must be known. Ingredient information may be obtained from packaging or supplier specifications.

Energy value is a calculated nutritional parameter expressed in both kJ and kcal. Each nutrient is multiplied by the appropriate conversion factor.

1. Determine the amount of each ingredient in the formulation.

2. Obtain the nutritional composition of the ingredient, normally per 100 g.

3. Calculate how much of each nutrient is present in the amount of ingredient actually used.

4. Multiply the amount of each energy-yielding nutrient by its applicable energy conversion factor.

5. Repeat for every nutrient and every ingredient.

6. Add the calculated energy contributions.

7. Express the final nutritional information on the required product basis, typically per 100 g.

Component	Conversion factor
Carbohydrate (except polyols)	17 kJ/g - 4 kcal/g
Polyols	10 kJ/g - 2.4 kcal/g
Protein	17 kJ/g - 4 kcal/g
Fat	37 kJ/g - 9 kcal/g
Salatrims	25 kJ/g - 6 kcal/g
Alcohol (ethanol)	29 kJ/g - 7 kcal/g
Organic acid	13 kJ/g - 3 kcal/g
Fibre	8 kJ/g - 2 kcal/g
Erythritol	0 kJ/g - 0 kcal/g

Tolerances for foods – excluding food supplements – including measurement uncertainty:

Nutrient	Tolerance
Vitamins	+50% -35%
Minerals	+45% -35%
Carbohydrate, sugars, protein, fibre	<10 g / 100 g: ±2 g 10-40 g / 100 g: ±20% >40 g / 100 g: ±8 g
Fat	<10 g / 100 g: ±1.5 g 10-40 g / 100 g: ±20% >40 g / 100 g: ±8 g
Saturated fatty acids, Mono-unsaturated fatty acids, Polyunsaturated fatty acids	<4 g / 100 g: ±0.8 g ≥4 g / 100 g: ±20%
Sodium	<0.5 g / 100 g: ±0.15 g ≥0.5 g / 100 g: ±20%
Salt	<1.25 g / 100 g: ±0.375 g ≥1.25 g / 100 g: ±20%

Example from the manual: if an ingredient contains 42 g protein per 100 g and 50 g of that ingredient is used, the protein contribution is 42 × (50/100) = 21 g. Using the stated factors, this corresponds to 21 × 17 = 357 kJ and 21 × 4 = 84 kcal.

Foods exempt from mandatory nutrition declaration

1. Unprocessed products consisting of a single ingredient or category of ingredients.

2. Processed products for which the only processing has been maturation and which consist of a single ingredient or category of ingredients.

3. Water intended for human consumption, including water whose only added ingredients are carbon dioxide and/or flavourings.

4. Herbs, spices or mixtures thereof.

5. Salt and salt substitutes.

6. Table-top sweeteners.

7. Specified coffee and chicory extract products, whole or ground coffee beans and decaffeinated coffee beans.

8. Herbal and fruit infusions, tea, decaffeinated tea, instant/soluble tea or tea extract without added ingredients other than flavourings that do not modify nutritional value.

9. Fermented vinegars and vinegar substitutes, including those whose only added ingredients are flavourings.

10. Flavourings.

11. Food additives.

12. Processing aids.

13. Food enzymes.

14. Gelatine.

15. Jam-setting compounds.

16. Yeast.

17. Chewing gum.

18. Food in packaging or containers whose largest surface has an area of less than 25 cm^2.

19. Food, including handcrafted food, supplied directly by the manufacturer in small quantities to the final consumer or to local retail establishments directly supplying the final consumer.

Nutrition declaration according to Regulation (EU) No 1169/2011:

Component	Unit
energy	kJ/kcal
fat	g
of which	
- saturates	g
- mono-unsaturates	g
- polyunsaturates	g
carbohydrate	g
of which	
- sugars	g
- polyols	g
- starch	g
fibre	g
protein	g
salt	g
vitamins and minerals	the units specified in point 1 of Part A of Annex XIII

For wines, the manual notes two approaches for energy-value calculation: calculation from alcohol, sugar and acid content, or use of category-specific values. It cites Regulation (EU) 2021/2117 of the European Parliament and of the Council (2 December 2021).

Figure 10. Wine-label instruction (Source: https://www. https://www.scantrust.com/eu-wine-label-requirements-2021-2117/)

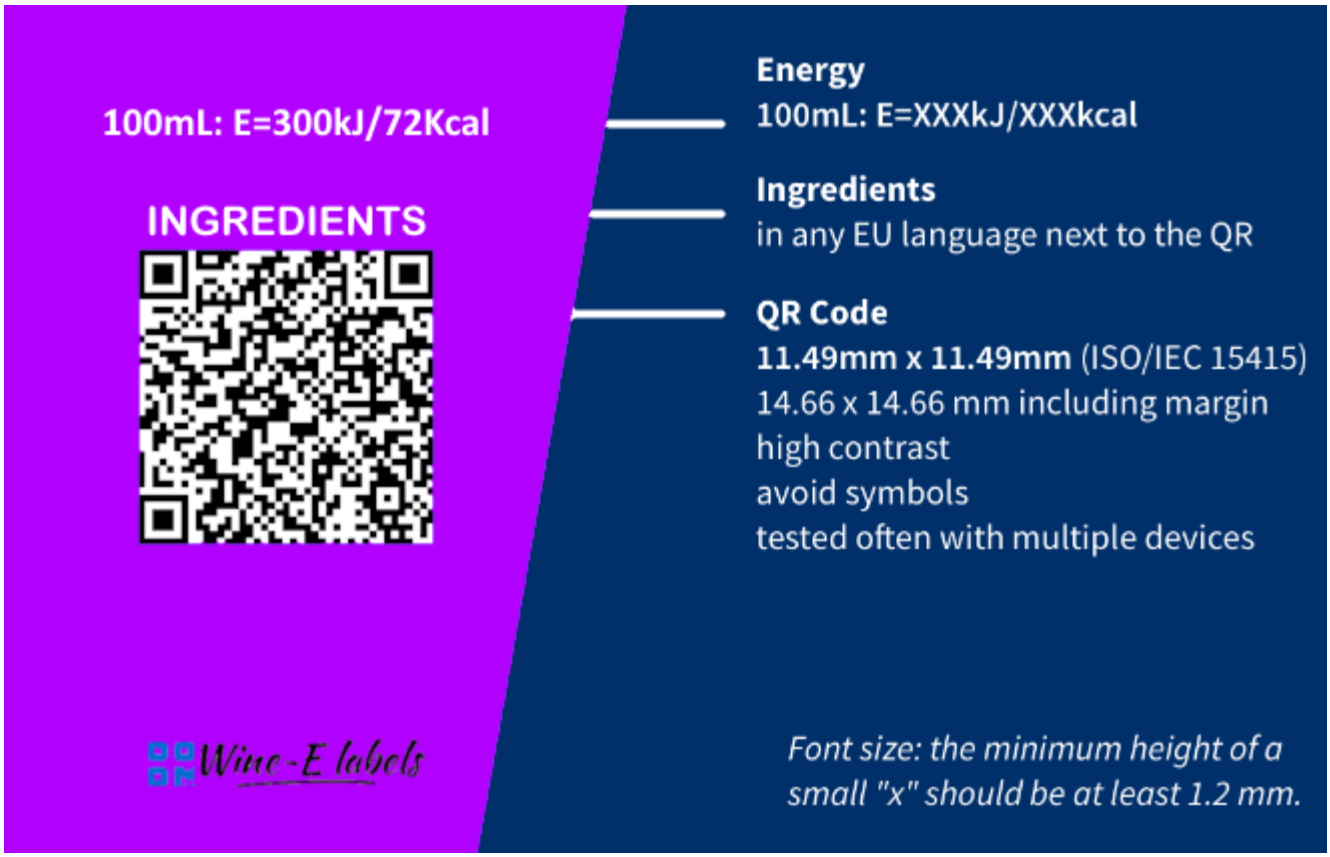

Figure 11. Wine label instruction (Source: https://www.wine-elabels.eu)

12. Review questions and topics for independent study

1. When is a carbohydrate reducing?

2. What is meant by an essential amino acid, and which amino acids are essential?

3. What are the most important biological properties of lipids? Describe the function of biological membranes.

4. What is water activity and what is its significance in foods?

5. Describe the main constituents of foods. Select one group and discuss its physiological role, classification and characteristic chemical reactions.

Topics to be developed

- Microplastics in foods
- Microplastics in drinking water
- Toxins
- Comparison of consumption habits
- Foods for special dietary needs
- Dietary antioxidants and their characteristic sources
- Probiotics and prebiotics
- Alternative plant-derived protein sources
- Biofortification
- GMO foods

English translation prepared from the Hungarian laboratory manual.