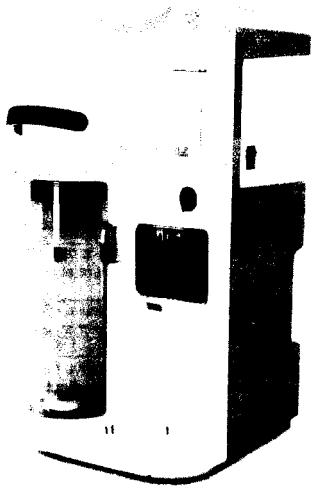
Quick Start Guide

VELP® SCIENTIFICA

UDK Distillation Unit



For more detailed information on installation and use, please refer to the operating manual.

Before turning the instrument on, read the safety warnings and precautions described in the operating manual carefully.

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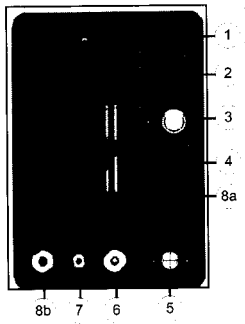
Installation

1. Unpack the UDK and place it on a clean, level and non-flammable surface close to a power socket (min. 16 A) and near (max. 1.5 mt.) a sink/drain (for inlet and outlet). Place the tanks (not included with the unit) below/near the distillation unit.
2. Connect all the adding and discharging reagent tubes to the back of the instrument:



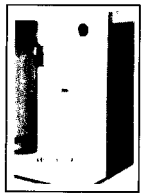
UDK 129	UDK 139	UDK 149	UDK 159/169	
1*	1	1	1	INLET H_2O
-	-	2	2	INLET H_3BO_3
3*	3	3	3	INLET NaOH
-	4	4	4	OUTLET Distillation
5	5	5	5	INLET Cooling Water
6	6	6	6	OUTLET Cooling Water
7	7	7	7	OUTLET Steam
-	-	8a	8b	OUTLET Titration

* without level sensor

INLET H_2O	Connect the **black tube** and the relative reserve connector. Place the other end of the tube with level sensor at the bottom of the **H_2O tank**.
INLET H_3BO_3	Connect the **red tube** and the relative reserve connector. Place the other end of the tube with level sensor at the bottom of the **H_3BO_3 tank**.
INLET NaOH	Connect the **white tube** and the relative reserve connector. Place the other end of the tube with level sensor at the bottom of the **NaOH tank**.
OUTLET Distillation Residues	Connect the **black tube** and the relative full connector. Place the end of the tube with level sensor in the **dedicated tank** and regulate the height of the sensor.
INLET Cooling Water	Connect the unit to the tap water supply using the **red and white tube** with threaded connections.
OUTLET Cooling Water	Push the **PVC tube** supplied onto the corresponding tube holder by hand and place the other end of the tube in a **sink/drain**.
OUTLET Steam Discharge	Push the **silicone tube** supplied onto the corresponding tube holder by hand and place the other end of the tube in the **distilled water tank**.
OUTLET Titration Residues	Push the **PVC tube** supplied onto the corresponding tube holder by hand and place the other end of the tube at the top of the **apposite tank**.

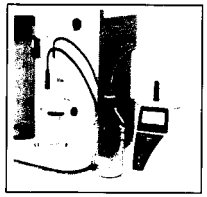
2.A

With UDK 129 ,139 and 149 (without titrator):
Place the collecting flask on the
support on the right of the instrument.
Place the silicone tube in the collecting flask.



2.B

With UDK 149: In order to connect the unit to an external potentiometric titrator (different brands are supported), please refer to the Operating Manual (3.3.5. Connecting to an external titrator).



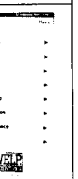
2.C

With UDK 159 / 169: Place the titrant bottle on the support on the right of the instrument. Place the transparent Teflon capillary tube connected to the titrant burette in the titrant bottle making sure it reaches the bottom of the bottle.

3. Open the transparent door, hold the blue handle down, slide the test tube into place making sure that the Teflon tube remains inside the test tube and that the test tube rests on the press test tube.
   Release the blue handle and make sure the test tube is firmly in place.
   Then close the safety guard.

4. Turn the instrument on by pressing the mains switch power on the right of the instrument.

Operation - Programming

	UDK 129	UDK 139	UDK 149	UDK 159 / 169
DISPLAY				
FILLING of REAGENTS Automatic check-up recommended on UDK 139, 149 & 159.	For NaOH > keep pressed **NaOH with +** For water > keep pressed **NaOH with -**	By selecting **System > Check-up**	By selecting **System > Check-up**	By selecting **Check-up**
WASH-DOWN Carry out the wash-down daily before starting sample test and at the end of the daily session.	Add 150ml of H_2O manually, set NaOH at 000ml and 3':00"as distillation time. Press START	By selecting **Analysis > Wash-down**	By selecting **Analysis > Wash-down**	By selecting **Analysis > Wash-down**
BLANK Useful to check the nitrogen in the system (e.g. reagents, tubes).	Manual	Manual	Manual	By selecting **Analysis > Blank**
(SINGLE) DISTILLATION Used whenever you need to test samples using different methods.	By setting: Distillation time, NaOH addition. Press START	By selecting **Analysis > Distillation** and then setting the parameters	By selecting **Analysis > Distiliation** and then setting the parameters	By selecting **Analysis > Distillation** and then setting the parameters
DISTILLATION in SERIES Recommended for samples of the same type that require the same settings.	Perform many Single Distillations with the same parameters	Perform many Single Distillations with the same parameters	By selecting **Analysis > Distillation in Series** and then setting the parameters	By selecting **Analysis > Distillation in Series** and then setting the parameters

UDK 129
Distillation Unit
Operating Manual

Velp Scientifica thanks you for choosing the UDK 129 Distillation Unit. The UDK 129 is designed to perform nitrogen and protein content determination according to the Kjeldahl Method (TKN) in the Food & Feed industry and has several other applications in environmental control (phenols and nitrogen in water, sludge, soil and lubricants) and in the chemical and pharmaceutical industry according to official AOAC, EPA, DIN e ISO procedures.

Safety warnings:


Before using the unit, please read the operating manual supplied with the apparatus carefully.


Warning! Hot surface!


Do not dispose of this equipment as urban waste

This unit must be used for laboratory applications only.

The manufacturer declines all responsibility for any use of the unit that does not comply with the instructions.

SAFETY PRECAUTIONS

In order to prevent the risk of electric shock, fire and personal injury when the unit is in use, basic safety measures must always be taken including:

- Ensure that liquids do not come into contact with the electric power cable or with the electrical parts of the instrument.
- Check that the power supply corresponds to the rating plate on the rear of the unit.
- When replacing the power cable, make sure that the new cable has the same characteristics as the original and that it is earthed.
- Do not use the unit if it is not working correctly. In case of malfunctioning, contact your nearest service centre.
- The personal protective equipment must be compatible with the possible risks posed by the material being processed and the glass of the containers.
- The test tube reaches a temperature of 100°C during the heating phase and also during the cooling phase. Use the pincer supplied to remove the test-tube at the end of distilling.
- The vessels and the products used during the work-cycle must be compatible with the temperatures reached by the unit (approx. 100°C).
- For further information on the handling of the reagents see Chapter 2 "Chemicals".
- Leave the transparent protection in place when the unit is running.
- Follow the cleaning instructions described in this manual

The safety of the instrument is no longer guaranteed in the case of improper use or if the instructions in this manual are not followed.

RÈGLES DE SÉCURITÉ

Pour éviter tout risque de choc électrique, incendie ou blessure corporelle pendant l'utilisation de l'appareil, toujours appliquer les mesures de sécurité de base, y compris les recommandations suivantes :

- S'assurer que les liquides n'entrent pas en contact avec le cordon d'alimentation ou avec toute autre pièce électrique à l'intérieur de l'appareil.
- Vérifier que le cordon d'alimentation est inséré dans une prise électrique (dans une position facilement accessible) correspondant aux valeurs décrites sur la plaque de l'appareil.
- Utiliser uniquement des cordons d'alimentation à trois bornes, c'est-à-dire avec cordon de mise à la terre.
- Ne pas utiliser l'appareil en cas de dysfonctionnement. Si cela se produit, contacter le Service Après-Vente le plus proche.
- Les équipements de protection individuelle doivent être compatibles avec les risques encourus pour les matériaux à traiter et le verre des récipients.
- Le tube à essai atteint une température de 100°C pendant la phase de distillation, mais également pendant la phase de refroidissement. Utiliser les pinces fournies pour retirer le tube à la fin de la distillation.
- Les récipients et les produits utilisés pendant les analyses doivent être compatibles avec la température réglée sur l'appareil (environ 100°C).
- Pour toute autre information sur l'utilisation des réactifs utilisés, voir Chapitre 2 "Produits chimiques".
- S'assurer que la protection transparente est en place lorsque l'appareil fonctionne.
- Respecter les instructions d'entretien de l'appareil décrites dans ce manuel.

Le fabricant décline toute responsabilité pour toute utilisation de l'appareil non conforme à ces instructions.

This unit has been designed and produced in compliance with the following standards:

Safety requirements for electrical apparatus for measurement and control and for laboratory use	**IEC/EN 61010-1** **IEC/EN 61010-2-010** **IEC/EN 61010-2-081** **UL Std. 61010-1 2nd Ed.** **CAN/CSA 61010-1-04 2nd Ed.** **CAN/CSA 61010-2-010-04** **CAN/CSA 61010-2-081**
Electrical equipment for measurement, control and laboratory use - EMC requirements	**IEC/EN 61326-1 (2006)** **FCC CFR 47 Part 15 Sub part B**
Restrictions of the use of certain hazardous substances in electrical and electronic equipment	**2011/65/EU (RoHS)**
On waste electrical and electronic equipment (WEEE)	**2002/96/EC (WEEE)**

The manufacturer is committed to constantly improving the quality of the products and reserves the right to modify the characteristics without prior notice.

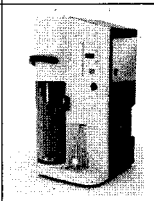
CONTENTS

1. Introduction

Steam distillation is applied in the laboratory for the fractionation of water insoluble liquids or solids according to Dalton's law of partial pressures in a gas mixture.
It is also used for stripping chemicals from mixtures or solutions after displacement of the ionic equilibrium by adding acids or bases, as in the well known Kjeldahl method for total Nitrogen determination.
The Kjeldahl method remains the most used method for determining Nitrogen and protein contents in foods and feeds (official methods).
This is simply due to its precision and reproducibility.
Since its initial design, many improvements have been introduced aimed at reducing energy consumption, space requirements and sample quantities.
The UDK 129 Distillation Unit is electronically controlled by a programmable microprocessor making it possible to vary the duration of distillation and the volume of distillate collected.
The UDK 129 has numerous safety features in order to provide **maximum protection for the user**. Continuous monitoring indicates incorrect tube and handle positioning; the cooling water flow detector provides a high level of safety. With a novel design, a lever is used to displace the tube support enabling sample tubes to be inserted without any effort and clamped in place securely. A range of test tube sizes is accepted.

1.1. Parts included

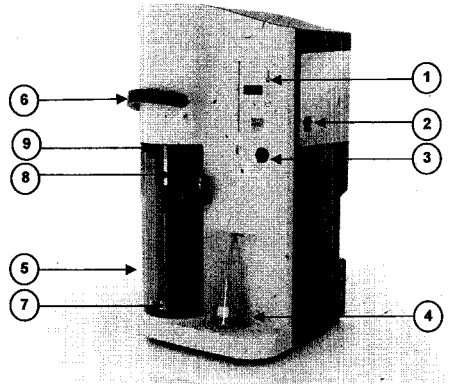
Check that the unit is complete after unpacking.
The table below shows the parts included:

Descrizione	Cod.		Q.tà
UDK 129 Distillation Unit[1]	F30200120 or F30210120		1
Test tube Ø 42 x 300mm[2]	A00001080		1
Collecting flask, 250 ml	10001106		1
Pincer for test tube	10000247		1
Inlet Tube	10000280		1
Tubes for reagents: Inlet tube for H_2O Inlet tube for NaOH	 40000146 40001498		2
Discharge tubes: Silicone tube Ø 6x9 mm PVC tube Ø10x14 mm	10000020 10001087		2

The operating manual is inside the distillation[1] box .
The test tube[2] is not sold separately (see chap. 11 " Accessories").

NOTE: *keep the special shock-resistant packing material for future use.*

1.2. Instrument description



The UDK 129 Distillation Unit uses innovative technology and advanced electronics for the automatic control of the distillation time and the addition of sodium hydroxide.

List of distillation unit components:

1	LCD display
2	On/Off Switch
3	Service door catch
4	Collecting flask, 250 ml
5	Safety guard
6	Lever for insertion of test tube
7	Test tube press
8	Test tube connection
9	Plastic guard for test tube connection

The UDK129 Distillation Unit is fitted with innovative devices and is manufactured using innovative materials and advanced technology:

- The instrument has a **polymer structure** that ensures greater resistance against chemical reagents used during analysis and a high durability.
- The patented Velp Scientifica **steam generator** uses deionised or distilled water and produces a constant and stable quantity of steam ensuring the reproducibility of the analysis without requiring any routine maintenance. The steam flow is controlled by a software which enables the user to choose between rapid or slow distillations according to the product to be analyzed.
- The innovative **titanium condenser** achieves outstanding heat exchange, reduces water consumption, offers higher resistance than glass and is easily and thoroughly cleaned. The display shows the water consumption during each analysis.
- The new technopolymer **splash head** assures optimal resistance to the high temperatures and corrosive chemicals involved during the distillation process.
- The UDK 129 runs automatically, after setting sodium hydroxide addition and distillation time using **the LCD display** in order to get reliable and accurate results.
- The UDK 129 ensures the **highest safety standards**: it signals the absence of the test tube, safety guard open and the absence of cooling water. The use of an innovative system enables the operator to position the test tube in complete safety and means that test tubes of various sizes can be used.
- In a world where **respect for the environment** is increasingly important, the distillation and titration system UDK 159 makes a significant contribution to this cause. The cooling water supply shuts-off automatically during pauses considerably reducing water consumption and the technopolymer housing is 100% recyclable.

The UDK series guarantees unmatched savings whist ensuring an extremely high level of reliability.

The UDK 129 is exceptional in providing savings, by using **TEMS™** technology:

Time: Fast and frequent analyses; no heating delay between runs.

Energy: Cooling water consumption starting from only 0.5 l/min; excellent insulation of internal parts.

Money: Cost reduction is substantial, in line with reduced power consumption.

Space: The extremely compact footprint saves useful laboratory bench space.

2. Chemicals

2.1. Products used

The chemicals used for distillation and titration are potentially dangerous and must be handled with care and with personal protective equipment: gloves, goggles and pincer removal of the reaction hot test-tubes.

1) Boric Acid (H_3BO_3): 4% solution. The low concentration is due to the fact that the boric acid crystallizes at low temperatures and this can cause problems in the hydraulic circuit of the instrument. Ingestion or absorption may cause nausea, vomiting and diarrhea.

2) Sodium hydroxide (NaOH): Velp recommends a 30-35% w/v solution of sodium hydroxide in order to avoid too violent reactions in the test-tubes. Corrosive to all body tissues. Can cause severe burns. Protect the skin and the eyes. If the solution is prepared using pellets, add these to water and not vice versa. Ingestion can cause vomiting, prostration, collapse. Constrictive scarring can occur.
Caution: in case of ingestion DO NOT attempt to evacuate the stomach.

Chemicals used during the digestion phase:

3) Sulphuric Acid (H_2SO_4): concentrated (96-98%). Corrosive for all body tissues. Skin contact can produce necrosis. Protect the skin and the eyes. Dilute by adding the sulphuric acid to the diluent to prevent the generation of excessive heat and the danger of splashes. Neutralize spills using sodium bicarbonate or calcium carbonate powder.

4) Hydrogen peroxide (H_2O_2): 30% w/w. Can cause severe burns. Always wear goggles and rubber gloves. Avoid contact with combustible materials: the drying out of concentrated solutions on paper, clothing, etc. can cause combustion. Heavy metals and their salts, dust and irregular surfaces can cause rapid decomposition resulting in the production of oxygen and increased pressure. Stored hydrogen peroxide solutions decompose slowly but nevertheless bottles must be fitted with vent caps. Empty bottles must be rinsed with clean water. In case of contact, rinse immediately with plenty of water.

2.2. Substances generated by digestion and distillation

1) Sulphur dioxide and sulphur trioxide-containing fumes. During digestion with sulphuric acid the development of fumes occurs which contain mostly sulphur dioxide plus smaller amounts of sulphur trioxide. Both these gases are intensely irritating to eyes and respiratory tract, causing coughing and discomfort. The fumes generated must be removed using efficient devices (i.e. Recirculating Water Pump model JP code F30620198) and preferably neutralized before being released into the atmosphere (i.e. Scrubber model **SMS** code F307C0199).

2) Distillation residues containing toxic catalysts. The use of toxic metals such as mercury, selenium or copper as catalysts for digestion means that these substances are present in the distillation residues. These residues should be collected in a suitable vessel and disposed of in an environmentally friendly way.

3. Assembly and installation

After unpacking the instrument position it on a laboratory bench or on a non-flammable surface at a distance of at least 30 cm from the walls so as not obstruct the air vents on the rear.

NOTE:

- *The instrument weighs 31kg. If you need to move the instrument suitable lifting devices should be used.*
- *Do not remove the protective film from the touch screen.*
- *Position the instrument near the socket power supply because it is considered a means of disconnecting the device*

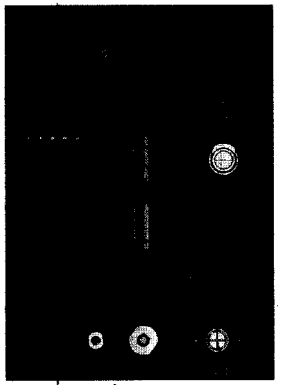
The instrument must be placed near a water tap (necessary for cooling the distillate) with a maximum pressure of 5 bar, and sink in order to discharge the waste water. The instrument requires a tap water flow rate (depending on the temperature of the tap water) of approximately:

- 0.5 l/minute at a temperature of 15°C

- 1 l/minute at a temperature of 30°C

Water is required during the distilling phase only and is interrupted automatically during the other phases.

Position the tanks for NaOH and H_2O solutions below or behind the unit. The tanks are not supplied with the instrument because their volume is chosen according to specific requirements. Usually polythene carboys of 10 or 20 l capacity are adopted.



The hydraulic tubes are supplied with the instrument and the hydraulic connections are clearing identified on the rear of the instrument.

3.1 Hydraulic connections

Connect the tubes supplied to the relative tube holders located on the rear panel following the instructions below:

INLET Cooling Water	Cooling water inlet (tap water). *Connect the unit to the tap water using the red and white tube with threaded connections* Open the tap water in order to obtain a tap water flow rate sufficient to cool the distillate (see chapter 5.4). Tap water is used during the distilling phase only for a considerable reduction in consumption.
INLET H_2O	Distilled water inlet (or deionised, ammonia-free) for supplying the steam generator. *Connect the relative tube to the black tube holder using the rapid connection and push the other end of the tube right to the bottom of the carboy.*
INLET NaOH	Sodium hydroxide inlet (32-35%w/v for Kjeldahl analysis) *Connect the relative tube to the white tube holder using the rapid connection. The H_2O and NaOH tubes have different connections in order to avoid errors with possible damage to tubes and internal components. Push the other end of the tube right to the bottom of the carboy.*
OUTLET Cooling Water	Cooling water outlet. *Push the PVC tube supplied (diameter 10x14 mm) onto the corresponding tube holder by hand and place the other end of the tube in a sink/drain* Check the flow rate of the cooling water discharged from the condenser during the distilling phase - the minimum flow rate using tap water at a temperature of about 15°C should be 0.5 l/min. (see chap.5.4).
OUTLET Steam Discharge	Steam discharge outlet. Discharge of hot water from the steam generator. At the end of each distillation the steam generator discharges the excess hot water to the distilled water tank for re-use. *Push the silicone tube supplied (diameter 6x9 mm) onto the corresponding tube holder by hand and place the other end of the tube in the distilled water tank.*

IMPORTANT

- Place the carboys for H_2O and NaOH solutions on the floor and not at the same level as the unit on the bench in order to avoid air locks in the suction tubes.
- The sodium hydroxide tubes must be kept full or, alternatively, the tube can be emptied and washed at the end of the work cycle. This precaution will avoid the formation of crystals when the solution comes into contact with air which may block the tube and prevent the instrument from working correctly.

3.2 Connection to the electric power supply

Before connecting the instrument to the power supply, make sure that the mains switch is turned off and that the values on the labels correspond to those of the power supply. Make sure the electrical network is earthed. Connect the instrument to the power supply using the cable and plug supplied.
The instrument requires a 220-240V power supply and a frequency of 50 or 60 Hz (cod. F30200183); a 110-120V / 60 Hz version is also available (cod. F30210183).
The instrument is delivered already programmed for 230V/50Hz. If the power supply is 230V/60Hz, the correct values must be selected as described in chap.5.

NOTE: *the mains switch can be used to disconnect the instrument from the power supply when not in use in order to reduce energy consumption.*

NOTE: *when the service door is opened the power supply cuts-off automatically.*

3.3 Preliminary operations

At the end of installation proceed as follows:

- Open the safety guard;
- Position the test tube;

NOTE: *to position the test tube hold the blue handle down, slide the test tube into place making sure that the Teflon tube remains inside the test tube and that the test tube itself rests on the press test tube. Release the blue handle and close the safety guard.*

- Turn on the tap water;
- Place the collecting flask on the flask support;

Turn the instrument on by pressing the mains switch POWER (led on) on the right of the instrument. The instrument automatically carries out the following steps simultaneously:

- Preheating (this takes 3 minutes. The display shows "HEAT" indicating that preheating is in progress.
- Tap water check to ensure the presence of cooling water;

An acoustic signal indicates that pre-heating is complete and that the instrument is ready to start. The display shows the default distillation time: 05':00".

NOTE: *If the tap water flow rate is not sufficient to ensure correct analysis, the instrument sends an acoustic warning signal and the display shows:* ***AL3.*** *This alarm remains displayed when pre-heating is finished. The alarm is deleted by opening the tap water or by pressing*  *at the end of pre-heating.*

IMPORTANT: before beginning analysis fill the NaOH and distilled water tubes as described below.

Filling the tubes

Filling the tubes before starting analysis is very important for the correct running of the instrument. To start the reagent pumps and fill the tubes manually, follow the procedure below:

- To fill the NaOH circuit press the NaOH key and keeping pressed it, press the ⊕ key. In this way the NaOH pump starts to work. Keep the two keys pressed until sodium hydroxide comes out into the glass test-tube. In this way the hydraulic circuit of sodium hydroxide is filled.
- To fill the distilled water circuit press the NaOH key and keeping pressed, press the ⊖ key. In this way the distilled water pump starts to work. Keep the two keys pressed until steam comes out into the glass test-tube. In this way the hydraulic circuit of distilled water is filled.

NOTE: *during pre-heating it is possible to fill the tubes manually as described above.*

4. Set-up

4.1. Distillation time

When the instrument is turned on, the display shows the name of the instrument and the software version for a few seconds, pre-heating is carried out after which the display shows the last distillation time setting.
The last value selected is stored in the memory for the next analysis even if the instrument is switched off. The default value is 05'00".

The distillation time can be set within a range of 3'00" to 10'59" using the ⊕ and ⊖ keys.

The longer the ⊕ key is kept pressed the faster the distillation time increases; the longer the ⊖ key is kept pressed the faster the distillation time decreases.

When a value higher than 10':59" is reached, the display shows --:-- and the instrument runs in continuous mode. The distillation time is displayed and when 99':59" is reached distilling stops automatically. To stop distillation earlier press STOP.
When a distillation time is set (i.e 05':00"), the display shows the distillation time count-down and distilling stops automatically at the end of the set time.

4.2. Sodium Hydroxide volume

When the NaOH key is pressed the display shows the selected NaOH volume for 3 seconds and at the same time a triangular signal lights up.
During these 3 seconds it is possible to change the NaOH volume by pressing the ⊕ and ⊖ keys. If one of the keys is kept pressed, the speed at which the setting increases or decreases will increase.
If no key is pressed for 3 seconds the display shows the last distilling time setting.

4.3. Delay time

When the sodium hydroxide volume is shown on the display, press the NaOH key a second time to display the pause (delay time) "d 00"; this is the time lapse between the entry of reagent and the start of steam production (used for analyses with Devarda alloy).

This time can be programmed (00-99 minutes) using the ⊕ or ⊖ keys.
This parameter is required for various types of analyses, e.g. Devarda's alloy analysis. If no key is pressed for 3 seconds the displayed value is memorized and the display will show the distillation time.
During the reaction time the tap water remains open and water circulates within the titanium condenser.

Velp recommends that the instrument is washed and some blank analyses are carried out before the first distillation of a real sample.

4.4. Wash-down

During wash-down distillation is carried out using distilled water as a reagent in order to "wash" the internal parts of the splash head and condenser. Wash-downs can be carried out at any time.

Fill the glass test-tube with about 150ml of distilled water; position the test-tube and place the collecting flask on the flask support; select 000ml of NaOH and a distillation time of 3':00".

NOTE: *Velp recommends that washing is carried out every day before starting sample analysis and at the end of the daily work-session.*

4.5. Blank analysis

A blank is an analysis carried out without the sample but with the required quantity of reagents in order to correct any possible interference caused by the nitrogen content of the reagents and/or system.

Fill the glass test-tube with about 150ml of distilled water; position the test-tube and place the collecting flask on the flask support; select the NaOH volume indicated in the method used for the sample analysis and a distillation time of 5':00".

NOTE: *at the end of analysis approx. 100 ml of distillate will be collected*

4.6. Frequency and NaOH pump calibration

To select the work frequency keep the two arrow keys ⊕ and ⊖ pressed for at least 5 seconds. The display will show:

FR 50 (frequency setting 50Hz)

Release the keys. Use ⊕ and ⊖ separately to modify the value of the frequency from 50 to 60Hz.

Press START/STOP to confirm the frequency setting.

The display will show:

CAL (NaOH Pump Calibration)

Press START/STOP to exit and return to the main window (distillation time).

To calibrate the NaOH pump, press ⊖. The instrument loads 50ml of NaOH into the glass test-tube after which the display shows: **- 50 -**

Measure the NaOH volume in the test-tube and use ⊕ and ⊖ to adjust the setting so that it corresponds to the volume measured.

Press START/STOP to record the value; the display returns to the main window.

NOTE: *If you wish to double-check correct dosage after calibration, this should be done during a normal work-cycle since during calibration a theoretical volume of 50ml only can be measured.*

NOTE: *the instrument is calibrated before leaving the factory therefore calibration by the user is only necessary if one of the reagent pumps is replaced or if a different concentration of NaOH is used.*

NOTE: *Set-up available only on 230V version (code F30200120). Only 60Hz frequency set on the 115V version (code F30200120).*

5. Operating controls

5.1. Safety devices

The instrument is equipped with various safety devices that can prevent start-up or interrupt a work-cycle to protect the operator and the instrument itself.

Test tube:

If the test tube is not correctly positioned analysis will not start when START/STOP is pressed. An acoustic signal warns the operator of an error and the following warning message appears on the display for a few seconds:
"AL1".
If an attempt is made to remove the test tube during a work cycle an acoustic signal warns of the danger, the above warning message appears and the work cycle is interrupted.

Safety guard:
If the safety guard in front of the test tube is not closed analysis will not start when START/STOP is pressed. An acoustic signal warns the operator of an error and the following warning message appears on the display:
"AL2".
If the safety guard is opened during a work cycle an acoustic signal warns of the danger, the above warning message appears and the instrument continues the work cycle in progress.

Safety lever:

If the lever is not released, analysis will not start when START/STOP is pressed. An acoustic signal warns the operator of an error and the following warning message appears on the display:
"AL2 ".
If the lever is lowered accidentally during a work cycle, an acoustic signal warns of the danger, the above warning message appears and the instrument continues the work cycle in progress.

A flow meter on the tap water inlet measures the flow rate. If water tap is closed or has not been opened far enough, the instrument shows **AL3** at the end of the pre-heating. Open the tap water or open it further. If the tap water is shut off during the cycle, the display will show **AL3** without stopping the cycle.

5.2. Warnings

A flow meter on the tap water inlet measures the flow rate. If water tap is closed or has not been opened far enough, the instrument shows **AL3** at the end of the pre-heating. Open the tap water or open it further. If the tap water is shut off during the cycle, the display will show **AL3** without stopping the cycle.

Insufficient cooling water
The unit is fitted with flow meter that measures the cooling water flow rate.
If the tap has not been turned on or the tap water flow rate is too low:
- when the instrument is turned on, the display shows **"AL3"** to remind the operator to restore a sufficient flow of water:
- in the case of insufficient water supply during the work-cycle, an acoustic signal warns the operator of an error and the display shows the above message whilst continuing the work-cycle.

5.3. Blackout

A power failure during the analysis will result in the analysis in progress being lost. The previously entered parameter will be retained.
When the electricity is restored, the unit will carry out preheating before starting analysis.

***NOTE:** if the display shows the distillation time at the end of the analysis, this indicates that the analysis did not end correctly. When an analysis is completed successfully the display reads END.*

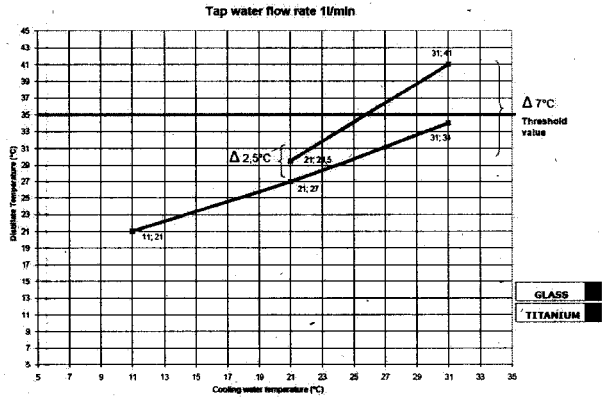
5.4. Graph of tap water consumption related to the flow rate and temperature

The graph below shows the efficiency of the patented Velp titanium condenser compared to the traditional glass condenser .

It is important to underline how the new titanium condenser keeps the temperature of the distillate below the threshold value (35°C) indicated by the Kjeldahl method without increasing the tap water flow rate (1l/min).

***NOTE:** The Kjeldahl method recommends a maximum distillate temperature of 35°C in order to avoid loss of nitrogen.*

As shown in the graph, a distillate temperature of 34°C is obtained by the titanium condenser using tap water at a temperature of 31°C. The temperature of the distillate using a conventional glass condenser would be 41°C under the same conditions.



The titanium condenser is also highly efficiency at low temperatures. With a tap water temperature of 21°C, a distillate temperature of 27°C is obtained using the titanium condenser compared to 29.5°C using a conventional glass condenser.

The new titanium condenser reduces tap water consumption to a minimum. In fact the high thermal exchange means that a tap water flow rate of 0.5 l/min is sufficient for the analyses to be carried out correctly.

6. Work cycle

The unit can perform different types of analysis:

- Analysis
- Devarda's analysis

The steps involved for each method are shown below.

6.1. Analysis

1. Switch on the instrument using the ON/OFF switch on the rh side of the instrument;
2. The instrument carries out preheating lasting 3 minutes;
3. Turn on the tap water;
4. Position the empty test-tube and the empty collecting flask to collect the distillate;
5. Fill the NaOH and distilled water circuits;
6. Carry out one or more wash-downs;
7. Carry out one or more blank analysis;
8. Position the test-tube containing the sample and the correct volume of distilled water, and the collecting flask with the correct volume of boric acid;
9. Set the correct distillation time and NaOH volume on the display of the instrument;
10. Start the analysis by pressing ;
11. The display shows the count-down of the distillation time. An acoustic signal indicates that the analysis is finished and the display shows "END";
12. Remove the test-tube and replace it with a new sample or an empty test tube;
13. Press any key to visualize the distillation time setting;
14. Press to start a new analysis.

6.2. Devarda's analysis

1. Switch on the instrument using the ON/OFF switch on the rh side of the instrument;
2. The instrument carries out preheating lasting 3 minutes;
3. Turn on the tap water;
4. Position the empty test-tube and the empty collecting flask to collect the distillate;
5. Fill the NaOH and distilled water circuits;
6. Carry out one or more wash-downs;
7. Carry out one or more blank analysis;
8. Position the test-tube containing the sample and the correct volume of distilled water, and the collecting flask with the correct volume of boric acid;
9. Set the correct distillation time, NaOH volume and a Pause value >0:00 on the display of the instrument;
10. Start the analysis by pressing ;
11. The display shows the count-down of the distillation time. An acoustic signal indicates that the analysis is finished and the display shows "END";
12. Remove the test-tube and replace it with a new sample or an empty test tube;
13. Press any key to visualize the distillation time setting;
14. Press to start a new analysis.

7. End-of-work operations

Before turning the instrument off at the end of the working day carry out a wash-down (see chap.4).

At the end of the wash cycles:

1. Turn off the tap water;
2. Switch off the unit.

NOTE: *always leave an empty test tube in place to avoid drips.*

8. Maintenance

The front panel (service door) of the instrument opens fully to allow easy and safe access to the internal parts in order to carry out the following operations:

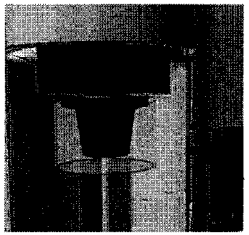
- Simple and immediate inspection of internal parts
- Remove and clean the drip tray
- Remove the test tube connection

Servicing the instrument (routine and extraordinary maintenance) is very important to keep the unit in good working order and to extend its life time. Follow the instructions given below.

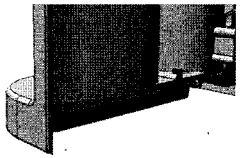
8.1 Routine maintenance

Routine maintenance should be carried out at the end of each use. Follow the instructions below:

- Carry out a wash-down before turning off the unit.



- Clean the test-tube connection, the plastic guard and any trace of sample on the surface of the instrument using a cloth or sponge dampened with plain water



- Rinse or wipe clean the drip tray (to remove the drip tray open the service door and release the catch).

8.2 Extraordinary maintenance

- Extraordinary maintenance operations not included in this manual and the servicing of internal parts, must be carried out by persons expressly authorized by VELP Scientifica.
- In order to reduce the risks arising from contact with reagents or solutions, the use of suitable personal protective equipment (gloves, etc.) is recommended.

Periodic maintenance

- Clean the sodium hydroxide tubes (NaOH). Every 500 analyses.
- Clean the filters. Every 500 analyses.
- Replace the test tube connection. Every 1000 analyses.

Operations to be performed only when necessary:

- Replace fuses
- Clean the condenser
- Clean the inside of the safety guard

Always disconnect the unit from the electric power supply and from the water supply before carrying out extraordinary maintenance.

***NOTE:** due to possible contact with harmful reagents and solutions, the use of personal protective equipment (gloves, etc.) is recommended.*

8.2.1 Periodic maintenance

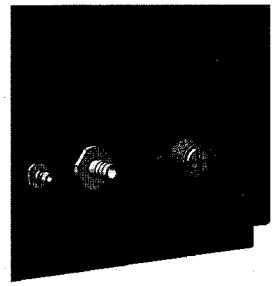
Cleaning the sodium hydroxide tubes
(Frequency: every 500 analyses)

These tubes must be kept full at all times. When the sodium hydroxide solution comes into contact with air, crystals are formed which obstruct the tubes and jeopardize the correct functioning of the internal parts.
The tubes must be cleaned as part of extraordinary maintenance operations as follows:

- Place an empty test-tube in the unit
- Place the end of the sodium hydroxide in a vessel containing warm distilled water (approx. 40°C).
- Following the procedure for filling the tubes manually (see chapter 3.3), fill the circuit with at least 1 liter of water.
- Using the same procedure, fill the tubes with sodium hydroxide solution.

Cleaning the filters
(Frequency: Every month or after 500 analyses)

The unit is fitted with 3 filters to remove particles, sediment or any other foreign bodies from the tap water, NaOH reagent and distilled water for steam generator.



NOTE: *disconnect the unit from the electric power supply and water supply.*

The filters are found on the tap water inlet and on the reagent inlets:

Tap water inlet

Remove the cooling water tube from the unit and pull out the filter using small pincers. Clean the filter under running water or using compressed air.

Reagent inlets

The tubes for NaOH and distilled water are fitted with filters located at the lower extremity. Wearing protective gloves remove the filters by pulling outwards. Open the filters by means of the ridge wash under running water.

During this operation the tubes may empty. After repositioning the filters repeat the manual filling of tubes.

Replacing the test tube connection
(Frequency: Every 1000 analyses)

The test-tube connection must be replaced when it loses its elasticity, when it looks deformed and does not provide a perfect seal, or after 1000 analyses.
For easier removal carry out a wash-down cycle. The splash head can reach high temperatures therefore take care when handling. Open the safety guard.

Remove the test tube, the test tube connection and the safety guard. The following tips will facilitate removal:

1. Wearing a protective glove, grip the splash guard and rotate it whilst pulling downwards.
2. Use a pointed tool or small screwdriver to exert leverage through the special window accessed from the inside of the service door.

Proceed with replacement. The new test tube connection will be easily fitted if it is soaked in warm water beforehand.

The splash guard should be cleaned using soft cloth dampened with warm water. Do not use abrasive detergents or cleaning agents containing solvents.

8.2.2 Operations to be performed only when necessary

Replacing fuses

Fuses should be replaced only when necessary, i.e. when the unit doesn't turn on and the mains switch does not light up. The fuses must only be replaced by qualified personnel and only with fuses supplied by Velp Scientifica Srl.
To replace the fuses disconnect the unit from the power supply, unscrew the fuse caps located on the back of the unit. The unit is fitted with one fuse for each phase: FUSE diam. 6.3x32 mm, 15 A (250V).

Cleaning the condenser
The condenser should be cleaned when it is heavily soiled with salts and organic matter thus reducing the efficiency of heat exchange.

After switching off the unit:

1. Unscrew the water tube from the water tap and place the open end in a receptacle containing water, preferably below the instrument. This triggers the emptying of the hydraulic circuit and the condenser.
2. Open the service door of the instrument
3. Remove the metal clamp that supports the condenser by removing two screws.
4. Detatch the black tube from the condenser inlet and outlet and remove it from the instrument.
5. Unscrew the screw on the bottom flange of the condenser.
6. Unscrew the two white bushings on both flanges of the condenser.
7. Remove the coil. The condenser is now completely dismantled.
8. Wash the parts under running water or diluted hydrochloric acid to remove any deposits.
9. Reassemble the condenser making sure the coil does not touch the walls of the transparent tube and tighten the bushings and screws.

Cleaning the internal parts of the splash head
To clean the internal parts of the splash head and condenser, proceed as follows:

1. Fill a test tube with about 25ml of deionized water and 25ml of concentrated acetic acid and position it in the unit.
2. Select a program with a distillation time of 10 minutes and an NaOH setting of 0 ml.
3. Start analysis.
4. At the end of analysis empty the test tube.
5. Carry out 2-3 distilling cycles with approx. 50ml of distilled water in the test-tube to rinse the internal parts.

8.3 Expedients for transport

The original packaging must be used for transport.

***NOTE**: The tubes of the hydraulic circuit must be emptied and washed in order to avoid reagent residues causing damage to the unit during the transport. The cups must be putted on the output connection on the back panel of the instrument.*

Before disconnecting the unit from the electric power supply and from the water supply, empty the hydraulic circuit of tap water and reagent solutions following the procedure described below

- Place an empty test tube in the unit.
- Remove the inlet tubes from their containers and turn them upside down to prevent the reserve message appearing. Follow the instructions for filling the tubes to empty them completely. Suck warm distilled water (approx. 40°C) into the sodium hydroxide tubes to remove any crystals from the tubes and from the internal parts that come into contact with the NaOH. Empty the circuit by removing the tube from the warm distilled water and turning it upside down.
- Disconnect the reagent tubes from the connectors on the rear panel of the unit (H_2O, NaOH).
- Unscrew the water tube from the water tap and place the open end in a receptacle containing water, preferably below the instrument. This triggers the emptying of the hydraulic circuit and the condenser.

When moving the instrument always use the special handles on the side of the instrument. The instrument must be transported in its original packaging and any indications present on the original packaging must be followed (e.g. palletised). The caps must be placed on the hydraulic connections.

9. Disposing of the unit

For the temporary decommissioning always empty the tubes of reagents and solutions, sodium hydrate in particular, as described in "Expedients for transport".

The instrument is classified as electrical/electronic apparatus and must be disposed of accordingly. The unit is subject to waste separation and cannot be disposed of as urban waste under EEC directive 2002/96/CE. For more information please contact the relative division of your local town council.

10. Accessories

Description:	Code
Test tube Ø 80x300 mm for alcohol determination	A00001083
Test tube Ø 48x260 mm, 300 ml	A00001088
Test tube diam. 42x300mm, 250ml, 3 pcs/box	A00000144
Test tube diam. 26x300mm, 100ml, 6 pcs/box	A00000146
Test tube Ø 50x300mm, 400ml	A00000185
Spacer for test tube Ø 48x260 mm	A00000206
Test tube connection Ø 26 mm, Ø 48 mm and 500 ml Kjeldahl balloon	A00000043
Alcoholic strength kit	A00000285
Kjeldahl ballon, 500ml	A00000082
IQ/OQ/PQ Manual UDK 129	A00000205

11. Spare parts

Description	Code
Positioning disk with tube support	40001706
Standard test tube connection	10002322
Plastic guard for test tube connection	10004708
Inlet tube for H_2O	40000146
Inlet tube for NaOH	40001498
Inlet tube	10000280
PVC tube Ø10x14	10001087
Silicone tube Ø 6x9mm	10000020
Collecting flask 250 ml	10001106
Pincer for test tubes	10000247
* Retarded ceramic fuse 6,3x32mm 15A 250V	10006304

The above mentioned spare parts can be easily replaced by the operator. For other spare parts (and for the complete list) please contact Velp Scientifica Srl nearest Service Centre.

* Replace the fuses only when necessary, i.e. if the unit doesn't turn on and the mains switch does' not light up. The fuses must only be replaced by qualified personnel and only with fuses supplied by Velp Scientifica Srl.

To replace the fuses is needed:
- unplug the unit;
- locate the fuse holders at the back of the instrument;
- unscrew the fuse caps.

12. Technical features

General		F30200120	F30210120
Power supply	V/Hz	230~(+/- 10%)/50-60	115~(+/- 10%)/60
Total electric power	W	2200	1700
Dimensions (WxHxD)	mm	385x780x416	
	Inch	15.2x30.7x16.4	
Weight	kg	25	
	Lb	55	
Display		LCD 4 Digit	
Pollution degree		2	
Overvoltage category		II	
Max altitude	m	4000	
Max. admitted humidity	%	85	
Storage temperature range	°C	+5°C.....+60°C	
	F	+41 +140	
Operating temp. range	°C	+5°C.....+40°C	
	F	+41 +104	
Fuses	A	2x15A 250V	
Sound Level	dBA	35	

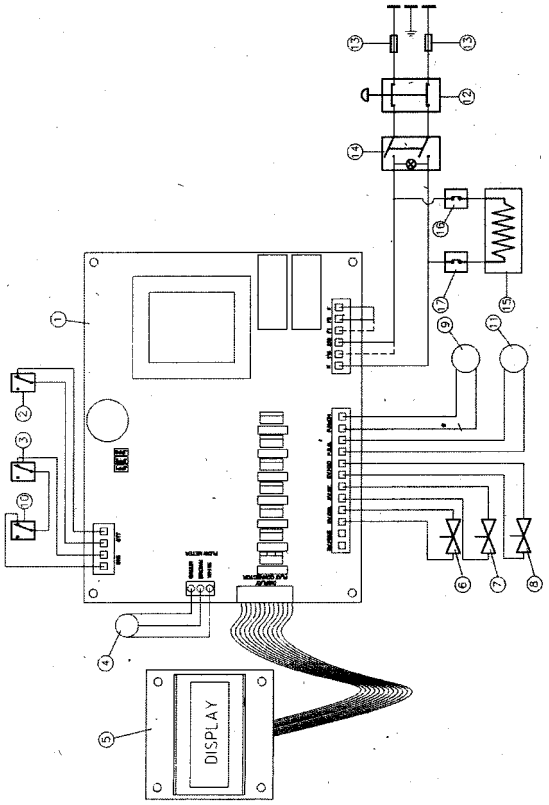
Performances		
Nitrogen amount	mgN	01 - 200
Reproducibility	%	≤ 1
Recovery rate	%	≥ 99.5
Detection limit	mgN	≥ 0,1
Distilling time	min:sec	05':00" (for 100 ml of distillate)

Consumption			
Cooling water	lt/1'	1	30°C only during analysis
		0.5	15°C only during analysis
Steam generator (Deionised water)	ml/1'	40	

Programs	
Number of programs	N° 1
Settable parameters and values:	
Sodium hydroxide	0 - 100 ml
Reaction time	00-99 minutes
Distilling time:	03':00" -10':59" (or continuous)

13. Wiring diagram

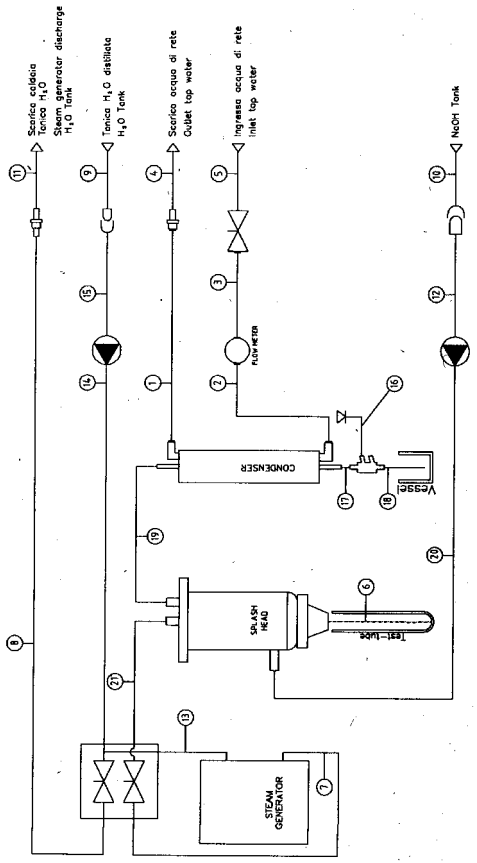
UDK 129 (230V / 50 – 60 Hz):
UDK 129 (115V / 60 Hz):



Note:
Version 115V – Connect (F and F1) and (N and 115)
Version 230V – Connect (F and F2) and (N and 230)

N°	Description
1	Comand electronic board
2	Safety microswitch for test tube
3	Safety microswitch for safety guard
4	Flowmeter
5	Display electrovalve
6	Boiler outlet electrovalve
7	Steam electrovalve
8	Water inlet electrovalve
9	Sodium hydroxide Pump
10	Safety lever
11	Steam power pump
12	Door open trip switch
13	Fuse 6.3x32 15A
14	ON/OFF switch with led
15	Steam generator
16	Safety thermostat
17	Working thermostat

14. Hydraulic scheme



N°	Q.ty	MATERIAL	DIAMETER (mm)	LENGHT (cm)
1	1	LLDPE	6 x 8	103
2	1	LLDPE	6 x 8	60
3	1	LLDPE	6 x 8	5,5
4	1	PVC	10 x 14	190
5	1	NYLON	7 x 14.5	200
6	1	PTFE	6 x 8	58
7	1	PTFE	4 x 6	20
8	1	EPDM	4,8 x 8	65
9	1	EPDM	6 x 10	190
10	1	EPDM	6 x 10	190
11	1	MQ/MVQ	6X9	190
12	1	EPDM	4,8x8	4,5
13	1	PTFE	4 x 6	11
14	1	EPDM	4,8 x 8	41
15	1	EPDM	4,8 x 8	7
16	1	EPDM	4,8 x 8	21
17	1	MQ/MVQ	6 x 9	8
18	1	MQ/MVQ	5x8	33
19	1	EPDM	6,4x11,2	22,5
20	1	EPDM	6,4 x 11,2	33
21	1	EPDM	6,4 x 11,2	55

EPDM = Butil
MQ/MVQ = Silicone
PTFE = Teflon
LLDPE = Low density polyethylene

15. Warranty

The unit is guaranteed against production defects for **25 months** from our invoice date.
Warranty claims can be accepted only if the system has been installed and used as specified on the manual and carried out by qualified service personnel as appointed by VELP Scientifica.

In accordance with this guarantee VELP SCIENTIFICA undertakes to repair any units resulting as faulty due to the quality of the materials used or poor workmanship.
Units rendered faulty due to inexpert handling/use or carelessness will not be replaced or repaired under warranty.

For more details please contact your Distributor.

Exclusions:
The guarantee will be considered null and void for faults resulting from:
- inexperience and carelessness of the operator
- repairs, maintenance or replacement of parts carried out by personnel or Companies not authorized by the manufacturer
- use of the instrument that does not comply to the instructions/recommendations given in the present operating manual
- use of non-original spare parts.

16. Suggestions

We will be pleased to receive any comments and/or suggestions that will help us to improve this operating manual.

e-mail: service@velp.it

17. General description of Kjeldahl method for the measurement of organic Nitrogen

Since its first introduction, more than a century ago, the method underwent different changes in digestion temperature and time, acid concentration and oxidation catalysts. As an example, during years '30 the use of one drop of metallic Mercury as catalyst and of concentrated fuming sulphuric acid (up to 80% Sulphur trioxide added) was common. For practical safety reasons for operators and the environment these prescriptions were modified.
In following paragraphs, the general lines of present Kjeldahl method with the use of a high steam generation capacity apparatus as the model VELP UDK 129 are described.

Sample range
2 - 150 mg of organic Nitrogen
15 - 1000 mg of proteins

Nitrogen standard
If the control of only the distillation steps is required, ammonium salts (chloride, sulphate) are used. For example:
153 mg of ammonium chloride reagent grade are dissolved to 100.0 ml with ammonia free water.
25.0 ml of this solution and 10 ml of 1 N sulfuric acid free of ammonia are diluted to 1000 ml with ammonia free water.
1 ml of the final solution contains 0.01 mg of N - NH_3.
This solution is very diluted (1 mg of N-NH_3 corresponds to 100 ml) and therefore can be useful to use less diluted solutions. For example bringing the indicated amounts to 100 ml (instead of 1000 ml) one obtains a final content of 0.1 mg N-NH_3 per ml of standard solution.
When the control of oxidative digestion is also required, pure Nitrogen containing chemicals are used previously desiccated to constant weight.
- Glycine (glycocoll or aminoacetic acid) 18.66% Nitrogen
- Sulfamic acid (amidosulfonic acid) 14.43% Nitrogen
- Acetanilide 10.36% Nitrogen
- Cystine 11.66% Nitrogen
- Nicotinic acid 11.38% Nitrogen

For the analysis of inorganic nitric or ammonia Nitrogen the following standards can be used:
- Ammonium dihydrogen phosphate ($NH_4H_2PO_4$) 12.15% Nitrogen
- Potassium nitrate (KNO_3) 13.85% Nitrogen

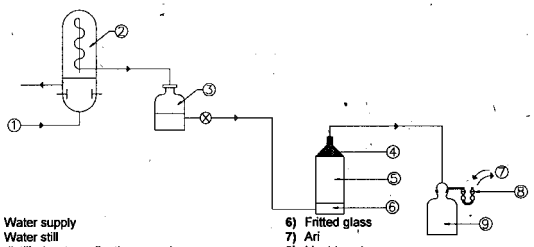
Digestion acid
Concentrated sulphuric acid (H_2SO_4) is used, reagent or technical grade, with a reduced Nitrogen content (ammonia and nitrate), 98%, d 1.84 at 20 °C.
This acid is also specified "for Kjeldahl analysis". It is possible to use also concentrated sulphuric acid not specified "for Kjeldahl", but in this case a regular control by blanks is recommended.
There are recipes for the preparation of catalyst containing (Copper and Selenium) sulphuric acid. For example: 10 g of cupric sulphate ($CuSO_4 \cdot 5\ H_2O$) are dissolved by 500 ml of ammonia free water and then 500 ml of concentrated sulphuric acid are slowly added while slowly shaking (do not add the water to acid to avoid dangerous

splashing). As an alternative, 2 g of cupric sulphate ($CuSO_4 \cdot 5\ H_2O$), 2 g of Selenium dioxide (SeO_2) and 100 g of anhydrous Sodium sulphate (Na_2SO_4) are dissolved by 500 ml of ammonia free water and then 500 ml of concentrated sulphuric acid are added.

Ammonia free distilled water for dilutions

Add 0.1 ml of concentrated sulphuric acid (H_2SO_4) to one liter of distilled water and distill again.
It is also possible to use deionized water with very low conductivity, obtained by a mixed bed of strong anionic and cationic resins, and tested for the absence of ammonia and of Nitrogen containing organic matter (biofouling).

A complete equipment for the production of ammonia free distilled water is shown in the following scheme:



1) Water supply
2) Water still
3) distilled water collecting vessel
4) Glass fiber
5) Ion exchange column. Strongly acidic cation exchange resing mixed with a strongly basic anion exchange resin
6) Fritted glass
7) Ari
8) Liquid seal
9) Ammonia-free distilled water collecting vessel

Sample to be analyzed

Liquid, semisolid or solid samples can be submitted to analysis. If a liquid is measured by volume the result will be referred to volume and not to weight if the density is different from 1.
When the result is to be referred to dry weight, solid or semisolid samples containing water must be desiccated to constant weight. Grinding of samples is aimed to obtain an easier digestion but is mostly aimed to homogenize the matter in order to obtain significant results. Weighing accuracy is 0.1 mg.
To allow a quantitative transfer of a solid or semisolid sample into the digestion tube, it is recommended to use weighing boxes made with Nitrogen free parchment paper (Code CM0486000). These boxes will be introduced with the sample into the tubes and digested without modifying the result. Sample dimensions are related to Nitrogen content and must give at least 10-15 mg of ammonia Nitrogen. If a sample is evaluated as poorly homogeneous, amounts larger than normal (e.g. 3-4 g) are to be analyzed.

Catalysts

Mercury is considered the best catalyst for Kjeldahl oxidative digestion, but its toxicity for man and the environment brought to dismiss its use. The same can be said for Selenium. To-day Copper sulphate ($CuSO_4 \cdot 5\ H_2O$) with lower environmental damage is commonly used. It is previously mixed with anhydrous Potassium sulphate (K_2SO_4) with a weight ratio of 9 K_2SO_4 to 1 $CuSO_4 \cdot 5\ H_2O$. Each sample is added with 7.5 g of this mixture (Missouri catalyst). There are commercially available tablets containing anhydrous Potassium sulphate and different types of catalysts in pre-dosed amounts, easily used (for example packing of 1000 tablets by Velp Scientifica).

Other additions

A rise of boiling temperature of the digestion acid is obtained by adding mineral salts, mostly Potassium sulphate (K_2SO_4). The use of Hydrogen peroxide (H_2O_2) 35% (130 volumes) is aimed to assure a complete mineralization of samples.
This reagent is to be added to the cold mixture (sample + acid) before digestion in order to avoid violent reaction and dangerous splashing.
Do not ever start heating if no acid is still added, because Hydrogen peroxide in contact with a sample can give rise to explosions.

Digestion temperatures and times

Normal digestion temperature is 420°C. Lower temperatures increase digestion time. At 420°C digestion is completed in 20-45 minutes.
A completed digestion gives raise to a clear colourless solution that crystallizes on cooling. If this crystallization is not wanted it is enough to lower a little the amount of the salt added for raising the boiling point of the acid.
Special samples as steel or coal may require a digestion time of many hours (up to 24).

Other reagents

An alkaline reaction of digested samples, before distillation, is obtained by adding a concentrated (32-35%) solution of Sodium hydroxide (NaOH), ammonia free (50 ml of NaOH for 7 ml of concentrated H_2SO_4).
1 ml of concentrated sulfuric acid 98% d. 1.84 contains 36.6 milliequivalents of acid.
1 ml of sodium hydroxide solution 32% w/w d.1.35 contains 10.8 milliequivalents of alkali. Thus 1 ml of concentrated sulphuric acid is stoichiometrically neutralized by 3.39 ml of sodium hydroxide 32% solution. The distillation of ammonia requires a highly alkaline environment which means that ammonia is completely in the so called "free" form. This is obtained adding a 100% excess of alkali.
We suggest to use 50 ml of sodium hydroxide 32% solution for a sample digested with 7 ml of concentrated sulphuric acid (70 ml for 10 ml or 100 ml for 15 ml acid).

Steam distilled ammonia can be collected with the condensate from the water cooled condenser in two different ways:

a) in a classical solution of sulphuric acid of known titre and following back-titration.
b) more recently, by a solution 4% of boric acid (H_3BO_3). Boric acid is practically un-dissociated and allows direct titration of alkali (ammonia) by strong acids of known titre.

Indicator solutions
A volume of 125-130 ml of solution to be titrated requires 10 drops about of indicator solution.
a) for a back-titration of sulphuric acid with Sodium hydroxide: 20 - 30 mg of methyl red in 100 ml of water; colour change from red to yellow at pH 4.9.
Or: 100 mg of bromocresol green and 30 mg of methyl red in 100 ml ethyl alcohol 96%; colour change from red to green at pH 4.5.
b) titration by sulphuric or hydrochloric acid of ammonia absorbed by boric acid solution.
Tashiro's indicator: 0.6 g of methyl red are dissolved by 50 ml of 95% ethyl alcohol and then added to a methylene blue solution (0.1 g in 50 ml of distilled water). The colour is green in alkaline range and gray to pink (pH 4.9) in acid medium to red with an excess of acid.

Potentiometric titration
It can be performed by using N/50 HCl and an end point of pH 4.7. The low dissociation of boric acid allows direct titration of alkali (ammonia).

Colorimetric determination
Besides titration, ammonia nitrogen determination can be performed by colorimetric methods, for example:

1. Method 417 B. Nesslerization or Method 417 C. Phenate: Standard Methods for the Examination of Water and Wastewater. 16th Edition.
APHA-AWWA-WPCF. Washington D.C. 1985.

2. Method D 1426. Ammonia nitrogen. Book of Standards ASTM. Part. 23. Water and Atmospheric Analysis. Philadelphia. 1983.

3. Analytical kits for water. Available on request.

In any case the ratio between weight or volume of sample and volume of distillate used for ammonia determination must be taken into account.

Calculation
It is commonly agreed that protein content is obtained multiplying by 6.25 the weight of Nitrogen obtained by the Kjeldahl method. This corresponds to consider all proteins as composed by 16% Nitrogen.
As a matter of fact protein composition ranges from 15 to 18% Nitrogen. This accounts for other factors, sometimes used, different from 6.25, generally lower. AOAC suggests for specific products the following factors: 5.18 for almonds; 5.30 for coconuts and other tree nuts, 5.46 for peanuts and Brasil nuts; 5.70 for wheat flour; 6.38 for milk and dairy products (official Methods of Analysis (1984). Association of Official Analytical Chemists. Arlington, VA-USA). FAO-WHO (1973) propose factor 5.70 for wheat meal and soy.

Definitions
Total Kjeldahl nitrogen (TKN) is defined as the sum of ammonia nitrogen and organic nitrogen converted to ammonium sulphate in the mineralization conditions adopted by the method. Organic Kjeldahl nitrogen is given by the difference between the value of total Kjeldahl nitrogen and the value of ammonia nitrogen.
If a direct determination is wanted, ammonia nitrogen is to be removed from the sample before mineralization.

Digestion procedure
The weighed sample is quantitatively transferred into a digestion tube (1 g about) and 7-15 ml of digestion acid and then, after placing on the digester two heat shields which make the heating of the digestion tube more homogeneous, heating is started (420°C) and continued till white fumes of Sulphur trioxide (SO3) are produced (20 - 30 minutes).
The apparatus should be under a hood or with a disposing mean for fumes in operation with neutralization by concentrated solutions of strong alkalies
(e.g. SMS Scrubber Velp Scientifica, Code F307C0199). Sulphur trioxide fumes are strongly irritant and aggressive for mucous membranes at concentrations as low as 1 ppm. If the liquid is not clear, one drop of Hydrogen peroxide solution (H_2O_2 30% 130 vol.) is added and boiling is continued for 10 minutes.
When digestion is finished the tubes are left to cool. Cooling time can be shortened by blowing air with a fan. When the tubes are cool, 50 ml of ammonia free distilled water are added and the tubes are shaken.

NOTE!
It's strongly recommended NOT to introduce in the test tubes, during sample digestion or distillation, boil-reducers (small glass balls, small pieces of ceramic material) which could damage the distillator.

Distillation procedure
A cool digestion tube is placed into position in the steam distilling unit.
The same is done with an empty Erlenmeyer flask for distillate collection.
The automatic distillation program is set for the addition of boric acid 4% solution (usually 25 ml) to the 250 ml Erlenmeyer flask for distillate collection; of an adequate volume of sodium hydroxide 32% solution for neutralization and alkalinisation (from 50 to 100 ml) of the sample and of distilled or deionized water for its dilution (usually 50 ml). The end of the silicone tube from the condenser must be under the surface of boric acid solution to avoid ammonia losses. Usually 100 ml of distillate are collected.

NOTE!
It's strongly recommended NOT to use the distillation residues aspiration if the sample to be distillate contains the solid residues, to avoid damages to the distillation unit.

18. AOAC, method 960.52, Microchemical determination of nitrogen- Micro-Kjeldahl method

AOAC International 1990. Official Methods of Analysis of AOAC International , 15th ed., The Association , Arlington, VA.

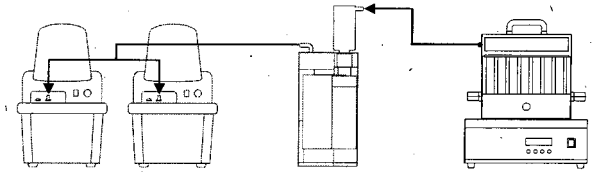
This method ISN'T suitable for sample materials having N-N or N-O bonds.

Equipment:
- digester DK 20/26 code F30100185, or DK 42/26 code F30100186
- glass test tubes diam. 26mm x 300 mm, code A00001091
- adapter A00000043

- necessary accessories for DK 20/26:
  glassware handle with heat shields, code A00001110
  suction cup, code A00109626
  support system, code A00001206

- necessary accessories for DK 42/26:
  glassware handle with heat shields, code A00001109
  suction cup, code A00109326
  support system, code A00001204

- JP pump, code F30620198.When using DK 42/26 digester, Velp suggests to use 2 JP (for the connection refer to the below scheme) in order to guarantee an effective fumes removal:



- SMS Scrubber, code F307C0199

- Steam distillator UDK129

Procedure:

1) Sample: weigh 10-30mg of sample, nitrogen free weighing boats can be used, code CM0486000, and introduce it into the test tube.
Use for analysis an amount of sample which needs 3-10ml of 0.01 or 0.02N HCl (containing 0.85-1.70mg of nitrogen).

2) Reagents for digestion: for each sample add in the test tube:
- 1 catalyst tablets ST (code CT0006609) (containing Selenium as catalyst)
- 2,0ml of sulphuric acid concentrated, 96-98%

Normally, the sample weight to analyse is 15mg about; add 0.1ml of acid more for every 10mg of dried organic substance >15mg.
Halve the amount of reagents above, if the sample is < 7mg, except when using the weighing boats.

3) Digestion: heat at 420°C for 1 hour, 1 hour and a half.

4) Cooling: cool down test tubes to 50-60°C

5) Distillation: place in position in the distilling unit the digested sample.

Program:
Select a method with the following volumes of the reagents:
dilution water, 10 ml
Boric acid, 25 ml
Sodium hydroxide, 35% (W/V), 10 ml

Distillation time: 5 min

Push START to initiate the analyses.

Titrate the distillate by HCl or H_2SO_4 0,01 N or 0,02 N programming an end point titration to pH 4.7.

19. Analytical procedure
Typical analytical scheme for organic Nitrogen

A	Grind or homogenize the sample
B	Weight or pipette the sample
C	Transfer quantitatively the measured sample to digestion tube
D	Add digestion acid and salts
E	Add catalysers if not present in the acid
F	Place the digestion tube in the digestion unit: i.e. DK6 or DK20
G	Digest the samples
H	Cool the digestion tubes
I	Place a digestion tube with sample in UDK 129 unit
L	Start the programmed automatic distillation cycle
M	Titrate distillate
N	Compute the results

20. Analytical Methods

1 - Kjeldahl method to determine the protein content on milk and derived products

The protein content of raw cow milk is meanly 3,2 g/100 ml (512 mg of nitrogen). Human milk is poorer in proteins (1 g/100 ml), while the milk of other animals is richer than cow milk (sheep 5,6 g/100 ml).

Procedure

1 Sample:
a_ Milk sample (cow's, goat's and sheep's milk)
Place the test sample in the water bath (e.g. OCB, code F40300240) at 38 °C to 40 °C. Mix gently by inversion without causing frothing or churning. Once the sample is mixed thoroughly, cool to room temperature and add 5 ml of sample in the test tube.

b_ Cheese sample (cow's, goat's and sheep's cheese)
Remove the rind, smear or mouldy surface layer of the cheese. Homogenize the cheese manually with a spoon or a knife.
Weigh 1,000 g of sample into a nitrogen-free weighing boat (code CM0486000) and place it the test tube.

2 Reagents for digestion
a_ For milk sample add in the test tube:
2 catalyst tablets CM (code CT0006650)
20 ml concentrated sulphuric acid (96-98%)
5 ml hydrogen peroxide (H_2O_2) 35% (130 voll.)

b_ For cheese sample add in the test tube:
2 catalyst tablets TCT (code CT0006621)
12 ml concentrated sulphuric acid (96-98%)

3 Digestion: heat for 15 min at 150 °C,
15 min at 250 °C,
40 min at 420 °C.

4 Cooling: let cool the digestion tubes to 50-60 °C.

5 Distillation-titration: place in position the test tube with the digested sample in the unit UDK 129.

Factor: 6.38
Addition of reagents:
dilution water= 50ml
H_3BO_3= 30ml
NaOH= 70 ml
Titrant: HCl 0.1N
Distillation time= 5 min

Press the button starting distillation cycle.

References: AOAC method 991.20. ISO 8968-1:2014.

2 - Kjeldahl method to determine the protein content on almonds, nuts, hazelnuts

Procedure

1 Sample: grind the sample by a suitable device. Weigh 0.5-0.8 g of sample with an accuracy of ± 0.1mg.

2 Reagents for digestion: for each sample add in the test tube:
2 catalyst tablets CM (code CT0006650)
12 ml concentrated sulphuric acid (96-98%)
2 antifoam tablets S (code CT0006600)

shake gently the test tubes.

3 Digestion: 60 min at 420 °C.

4 Cooling: let cool the test tubes to 50-60 °C.

5 Distillation-titration: place in position the test tube with the digested sample in the unit UDK 129.

Factor: 5.18

Addition of reagents:
dilution water= 50ml
H_3BO_3= 30ml
NaOH= 50 ml

Titrant: HCl 0.2 N
Distillation time= 5 min

Press the button starting distillation cycle.

References: AOAC, "Official methods of analysis", method 950.48

3 - Kjeldahl method to determine the protein content on coconuts

Procedure

1 Sample: grind the sample by a suitable device. Weigh 0.5-0.8g of sample with an accuracy of ±0.1mg.

2 Reagents for digestion: for each sample add in the test tube:
2 catalyst tablets CM (code CT0006650)
12 ml concentrated sulphuric acid (96-98%)
2 antifoam tablets S (code CT0006600)

shake gently the test tubes

3 Digestion: 60 min at 420 °C.

4 Cooling: let cool the test tubes to 50-60 °C.

5 Distillation-titration: place in position the test tube with the digested sample in the unit UDK 129.

factor: 5.30

Addition of reagents:
dilution water= 50ml
H_3BO_3= 30ml
NaOH= 50 ml

Titrant: HCl 0.2N
Distillation time= 5 min

Press the button starting distillation cycle.

References: AOAC, "Official methods of analysis", method 950.48

4 - Kjeldahl method to determine the protein content on peanuts and Brazil nuts

Procedure

1 Sample: grind the sample by a suitable device. Weigh 0.5-0.8g of sample with an accuracy of ±0.1mg.

2 Reagents for digestion: for each sample add in the test tube:
2 catalyst tablets CM (code CT0006650)
12 ml concentrated sulphuric acid (96-98%)
2 antifoam tablets S (code CT0006600)

shake gently the test tube

3 Digestion: 60 min at 420 °C.

4 Cooling: let cool the test tubes to 50-60 °C.

5 Distillation-titration: place in position the test tube with the digested sample in the unit UDK 129.

factor: 5.46

Addition of reagents:
dilution water=50ml
H_3BO_3=30ml
NaOH=50 ml

Titrant: HCl 0.2N

Distillation time= 5 min

Press the button starting distillation cycle.

References: AOAC, "Official methods of analysis", method 950.48

5 - Kjeldahl method to determine the protein content on beer

Among components of soluble extract of beer there are proteins and aminoacids.

Soluble extractives represent from 3% to 12% of the weight of beer.

Procedure:

1) Sample: 25 ml of beer (from which carbon dioxide was previously removed by introducing a proper volume in a big flask and shaking a long time) in a test tube. Concentrate it in the digester at 100 °C to sirupy consistency.

2) Reagents for digestion: for each sample add in the test tube:
2 catalyst tablets ST (code CT0006609)
10 ml concentrated sulphuric acid (96-98%)
10 ml hydrogen peroxide (H_2O_2) 35% (130 voll.)
2 antifoam tablets S (code CT0006600)

3) Digestion: mix well the test tube content.
Heat for 30 min at 100 °C, 30 min. at 200 °C, 30 min. at 300 °C and then for 40 min at 420 °C.

4) Cooling: let cool the test tubes to 50 - 60°C.

5) Distillation-titration: place in position the test tube with the digested sample in the unit UDK 129.

factor: 6.25

Addition of reagents:
dilution water = 50 ml
H_3BO_3 = 30 ml
NaOH = 50 ml

Titrant: HCl 0.1 N
Distillation time= 5 min

Press the button starting distillation cycle.

References: AOAC *Official Methods of Analysis.* Method 920.53.

6 - Kjeldahl method to determine the protein content on barley malt

Procedure

1) Sample: 1 g of malt finely grinded and sieved to 2 mm, previously dried in oven at 105°C up to constant weight, accurately weighed and transferred quantitatively in a test tube.

2) Reagents for digestion: for each sample add in the test tube:
2 catalyst tablets ST (code CT0006609)
12ml sulphuric acid concentrated (96%)

3) Digestion: set on the digester the following temperature ramps:
20 min at 220°C and then 40 min at 420 °C.

4) Cooling: let cool the test tubes to 50-60°C.

5) Distillation-titration: place in position the test tube with the digested sample in the unit UDK 129.

factor: 6.25

Addition of reagents:
dilution water=50ml
H_3BO_3=30ml
NaOH=50 ml

Titrant: HCl 0.1N
Distillation time= 5 min

Press the button starting distillation cycle.

References: Official Methods of Analysis , AOAC (ed.1990), vol.2 (method n. 950.09).

7 - Kjeldahl method to determine the protein content on feed

Procedure

1) Sample: 2 g about of grinded product, sieved to 2 mm and dried at 105 °C up to constant weight, accurately weighed and transferred quantitatively in a test tube. In the case of products rich in proteins (meat or fish flour) the sample can be reduced to 1 g.

2) Reagents for digestion: for each sample add in the test tube:
1 catalyst tablet W (code CT0006613)
12 ml concentrated sulphuric acid (96-98%) 96%
5 ml hydrogen peroxide (H_2O_2) 35% (130 vol.)

3) Digestion: heat for 60 min at 420 °C.

4) Cooling: let cool the test tubes to 50-60 °C.

5) Distillation-titration: place in position the test tube with the digested sample in the unit UDK129.

factor: 6.25

Addition of reagents:
dilution water=50ml
H_3BO_3=30ml
NaOH=50 ml

Titrant: HCl 0.2N
Distillation time= 5 min

Press the button starting distillation cycle.

References: AOAC, "Official methods of analysis", method 984.13

8 - Kjeldahl method to determine the protein content on wheat

Procedure

1) Sample: grind the sample by a suitable device. If the result is to be expressed on dried sample, perform a separate determination of the humidity on the ground sample. Weight 1g of sample with an accuracy of ±0.1mg.

2) Reagents for digestion: for each sample add in the test tube:
2 catalyst tablets CM (code CT0006650)
2 Antifoam-tablets S (code CT0006600)
12 **ml** concentrated sulphuric acid (96-98%)

shake gently the test tubes

3) Digestion: heat for 40 min at 300 °C and 90 minutes at 420 °C.

4) Cooling: let cool the test tubes to 50-60 °C.

5) Distillation-titration: place in position the test tube with the digested sample in the unit UDK 129.

factor: 5.70

Addition of reagents:
dilution water=50ml
H_3BO_3=30ml
NaOH=50 ml

Titrant: HCl 0.2N
Distillation time= 5 min

Press the button starting distillation cycle.

References: AOAC, "Official methods of analysis", method 979.09

9 - Kjeldahl method to determine the protein content on oats, barley, corn, rice, rye

Procedure

1) Sample: grind the sample by a suitable device. If the result is to be expressed on dried sample, perform a separate determination of the humidity on ground sample. Weigh 1g of sample with an accuracy of ±0.1mg.

2) Reagents for digestion: for each sample add in the test tube:
2 catalyst tablets CM (code CT0006650)
2 Antifoam tablets S (code CT0006600)
12 ml concentrated sulphuric acid (96-98%)

shake gently the test tubes

3) Digestion: heat for 40 min at 300 °C and 90 minutes at 420 °C.

4) Cooling: let cool the test tubes to 50-60 °C.

5) Distillation-titration: place in position the test tube with the digested sample in the unit UDK 129.

factor: 6.25

Addition of reagents:
dilution water=50ml
H_3BO_3=30ml
NaOH=50 ml

Titrant: HCl 0.2N
Distillation time= 5 min

Press the button starting distillation cycle.

References: AOAC, "Official methods of analysis", method 979.09

10 - Kjeldahl method to determine the protein content on soya beans and lupins

Procedure

1) Sample: grind the sample by a suitable device. If the result is to be expressed on dried sample, perform a separate determination of the humidity on ground sample. Weigh 1g of sample with an accuracy of ±0.1mg.

2) Reagents for digestion: for each sample add in the test tube:
2 catalyst tablets CM (code CT0006650)
12 ml concentrated sulphuric acid (96-98%)

shake gently the test tubes

3) Digestion: heat for 60 min at 420 °C.

4) Cooling: let cool the test tubes to 50-60 °C.

5) Distillation-titration: place in position the test tube with digested sample in the unit UDK 129.

factor: 6.25

Addition of reagents:
dilution water=50ml
H_3BO_3=30ml
NaOH=50 ml

Titrant: HCl 0.2N
Distillation time= 5 min

Press the button starting distillation cycle.

References: AOAC, "Official methods of analysis", method 979.09

11 - Kjeldahl method to determine the protein content on canned cat/dog food

Procedure

1) Sample: homogenize the sample by a suitable device. For samples having a humidity content up to 60%, weigh 2g of sample with an accuracy of ±0.1mg, for samples having a humidity content higher than 60% weigh 3g of sample with the same accuracy.

2) Reagents for digestion: for each sample add in the test tube:
2 catalyst tablets CM (code CT0006650)
15 ml concentrated sulphuric acid (96-98%)

shake gently the test tubes.

3) Digestion: heat for 70 min at 420 °C.

4) Cooling: let cool the test tubes to 50-60 °C.

5) Distillation-titration: place in position the test tube with digested sample in the unit UDK 129.

factor: 6.25

Addition of reagents:
dilution water=50ml
H_3BO_3=30ml
NaOH=60 ml

Titrant: HCl 0.2N
Distillation time= 5 min

Press the button starting distillation cycle.

References: AOAC, "Official methods of analysis", method 984.13

12 - Kjeldahl method to determine the protein content on forage and straw

Procedure

1) Sample: cut the sample by a pair of scissors to a length of 2-3 cm about. Dry the sample and finally grind by a suitable device. Weigh 1g of sample with an accuracy of ± 2mg.

2) Reagents for digestion: for each sample add in the test tube:
2 catalyst tablets ST (code CT0006609)
12 ml concentrated sulphuric acid (96-98%)

shake gently the test tubes.

3) Digestion: heat for 60 min at 420 °C.

4) Cooling: let cool the test tubes to 50-60 °C.

5) Distillation-titration: place in position the test tube with the digested sample in the unit UDK 129.

factor: 6.25

Addition of reagents:
dilution water= 50ml
H_3BO_3= 30ml
NaOH= 50 ml

Titrant: HCl 0.2N
Distillation time= 5 min

Press the button starting distillation cycle.

References: AOAC, "Official methods of analysis", method 978.04

13 - Kjeldahl method to determine the protein content on bacon, ham, hot dog, salami, sausage

Procedure

1) Sample: 2g of sample weighed with an accuracy of ±0.1mg after homogenizing.

2) Reagents for digestion: for every sample add in the test tube:
2 catalyst tablets CM (code CT0006650)
15 ml sulphuric acid concentrated (H_2SO_4)

shake gently the test tube with the sample.

3) Digestion: heat for 75 min at 420 °C.

4) Cooling: let cool the test tubes to 50-60 °C.

5) Distillation-titration: place in position the test tube with digested sample in the unit UDK 129.

factor: 6.25

Addition of reagents:
dilution water= 75ml
H_3BO_3= 30ml
NaOH= 50 ml

Titrant: HCl 0.2N
Distillation time= 5 min

Press the button starting distillation cycle.

References: AOAC, "Official methods of analysis", method 981.10

14 - Kjeldahl method to determine the protein content on meat and derived products

The protein content in fresh bovine meat is about 20% (3% of proteic nitrogen about). Dried salted meats contain about 35% proteins, while salted meat show values between11 and 17%, depending on fat and water content.

Procedure

1) Sample: 1 g about of ground product, accurately weighed (60 mg about of proteic nitrogen for fresh meat, interval 40÷100) and quantitatively transferred in the test tube.

2) Reagents for digestion: for each sample add in the test tube one of the following catalysts:

2 catalyst tablets ST (code CT0006609)
20 ml concentrated sulphuric acid (96-98%)
5 ml hydrogen peroxide (H_2O_2) 35% (130 vol.)

3) Digestion: heat for 30 min at 250 °C,
30 min at 350 °C
60 min at 420 °C

4) Cooling: let cool the test tubes to 50-60 °C.

5) Distillation-titration: place the test tube with digested sample in the unit UDK 129.

factor: 6.25

reagents addition:
dilution water= 50ml
H_3BO_3= 30ml
NaOH= 70 ml

Titrant: HCl 0.2N
Distillation time= 5 min

Press the button starting distillation cycle.

References: AOAC, "Official methods of analysis", method 981.10

15 - Kjeldahl method to determine the protein content on bread and baked products

Procedure

1) Sample: grind the sample by a suitable device. Weigh 2g of dried sample with an accuracy of ±0.1mg.

2) Reagents for digestion: for each sample add in the test tube:
2 catalyst tablets TCT (code CT0006621)
12 ml concentrated sulphuric acid (96-98%)

shake gently the test tube with the sample.

3) Digestion: heat for 60 min at 420 °C.

4) Cooling: let cool the test tubes to 50-60 °C.

5) Distillation-titration: place in position the test tube with digested sample in the unit UDK 129.

factor: 5.7

Reagents addition:
dilution water= 75ml
H_3BO_3= 30ml
NaOH= 50 ml

Titrant: HCl 0.2N
Distillation time= 5 min

Press the button starting distillation cycle.

References: AOAC, "Official methods of analysis", method 950.36

16 - Kjeldahl method to determine the protein content on compressed and granular yeast

Procedure

1) Sample: grind, if necessary, and make the sample homogeneous by suitable devices; weigh 0.5-0.7 g of sample with an accuracy ± 0.1mg.

2) Reagents for digestion: for each sample add in the test tube:
2 catalyst tablets CM (code CT0006650)
12 ml concentrated sulphuric acid (96-98%)

shake gently the test tube with sample.

3) Digestion: heat for 70 min at 420 °C.

4) Cooling: let cool the test tubes to 50-60 °C.

5) Distillation-titration: place in position the test tube with digested sample in the unit UDK 129.

factor: 6.25

Reagents addition:
dilution water=75ml
H_3BO_3=30ml
NaOH=50 ml

Titrant: HCl 0.2N
Distillation time= 5 min

Press the button starting distillation cycle.

References: AOAC, "Official methods of analysis", method 962.10

17 - Kjeldahl method to determine the protein content on liver patè

Procedure

1) Sample: 2g of sample weighed with an accuracy of ±0.1mg after homogenizing by suitable device.

2) Reagents for digestion: for each sample add in the test tube:

2 catalyst tablets CM (code CT0006650)
12 ml concentrated sulphuric acid (96-98%)

shake gently the test tube with sample.
For samples having high fat content, use 15ml of acid and then 5ml of hydrogen peroxide.

3) Digestion: heat for 45 min at 420 °C.

4) Cooling: let cool the test tubes to 50-60 °C.

5) Distillation-titration: place in position the test tube with sample in the unit UDK 129.

factor: 6.25

reagents addition:
dilution water= 50ml
H_3BO_3= 30ml
NaOH= 50 ml

Titrant: HCl 0.2N
Distillation time= 5 min

Press the button starting distillation cycle.

References: AOAC, "Official methods of analysis", method 981.10

18 - Kjeldahl method to determine the protein content on sugar, syrup, molasses

Procedure

1) Sample: weigh 1g of sample with an accuracy of ± 0.1mg (2g for molasses with the same accuracy).

2) Reagents for digestion: for each sample add in the test tube:

2 catalyst tablets CM (code CT0006650)
12 ml concentrated sulphuric acid (96-98%)

shake gently the test tube with sample.

3) Digestion: heat for 60 min at 420 °C.

4) Cooling: let cool the test tubes to 50-60 °C.

5) Distillation-titration: place in position the test tube with digested sample in the unit UDK 129.

factor: 6.25

Reagents addition:
dilution water= 75ml
H_3BO_3= 30ml
NaOH= 50 ml

Titrant: HCl 0.2N
Distillation time= 5 min

Press the button starting distillation cycle.

References: AOAC, "Official methods of analysis", method 945.23

19 - Kjeldahl method to determine the protein content on wheat spaghetti and macaroni, egg pasta

Procedure

1) Sample: break the pasta in small pieces by hands or suitable device and mix well. Grind finally the sample by a suitable device. Weigh 1g of sample with an accuracy of ±0.1mg.

2) Reagents for digestion: for each sample add in the test tube:

2 catalyst tablets CM (code CT0006650)
12 ml concentrated sulphuric acid (96-98%)

shake gently the tube with sample.

3) Digestion: heat for 60 min at 420 °C.

4) Cooling: let cool test tubes to 50-60 °C.

5) Distillation-titration: place in position the tube with digested sample in the unit UDK 129.

factor: 5.7

Addition of reagents:
dilution water= 50ml
H_3BO_3= 30ml
NaOH= 50 ml

Titrant: HCl 0.1N
Distillation time= 5 min

Press the button starting distillation cycle.

References: AOAC, "Official methods of analysis", method 930.25

20 - Kjeldahl method to determine the protein content on grain spaghetti, macaroni

Procedure

1) Sample: break the pasta in small pieces by hands or suitable device and mix well. Grind finally the sample by suitable device. Weigh 1g of sample with an accuracy of ± 0.1mg.

2) Reagents for digestion: for each sample add in the test tube:

2 catalyst tablets CM (code CT0006650)
12 ml concentrated sulphuric acid (96-98%)

shake gently the tube with sample.

3) Digestion: heat for 60 min at 420 °C.

4) Cooling: let cool test tubes to 50-60 °C.

5) Distillation-titration: place in position the test tube with sample in the unit UDK 129.

factor: 6.25

Reagents addition:
dilution water= 50ml
H_3BO_3= 30ml
NaOH= 50 ml

Titrant: HCl 0.1N
Distillation time= 5 min

Press the button starting distillation cycle.

References: AOAC, "Official methods of analysis", method 930.25

21 - Kjeldahl method to determine the protein content on plants (vegetable)

Procedure

1) Sample: mince and homogenize the sample by suitable devices, then weigh 0.5-1.5 g in the test tube.

2) Reagents for digestion: for each sample add in the test tube:

2 catalyst tablets ST (code CT0006609)
13 ml concentrated sulphuric acid (96-98%)

shake gently the tube with the sample.

3) Digestion: heat for 60 min at 420 °C.

4) Cooling: let cool test tubes to 50-60 °C.

5) Distillation-titration: place in position the test tube with digested sample in the unit UDK 129.

factor: 6.25

Reagents addition:
dilution water=75ml
H_3BO_3=30ml
NaOH=50 ml

Titrant: HCl 0.2N
Distillation time= 5 min

Press the button starting distillation cycle.

References: AOAC, "Official methods of analysis", method 978.04

22 - Kjeldahl method to determine the protein content on mushrooms

The average protein content on fresh mushrooms is 2-4%.

Procedure

1) Sample: 10g of ground dried sample.

2) Reagents for digestion: for each sample add in the test tube:

2 catalyst tablets ST (code CT0006609)
15 ml concentrated sulphuric acid (96-98%)

shake gently the tube with the sample.

3) Digestion: heat for 60 min at 420 °C.

4) Cooling: let cool the tubes to 50-60 °C.

5) Distillation-titration: place in position the test tube with digested sample in the unit UDK 129.

factor: 6.25

Reagents addition:
dilution water=50ml
H_3BO_3=30ml
NaOH=50 ml

Titrant: HCl 0.2N
Distillation time= 5 min

Press the button starting distillation cycle.

References: AOAC, "Official methods of analysis", method 976.06

19.2 Pre-defined Methods for different samples

23 - Kjeldahl method to determine total nitrogen on crude oil and fuels (ISO n. 333)

Procedure

1) Sample: homogenize the sample by shaking before analysis. Weigh 1g of sample with an accuracy of ± 0.1 mg.

2) Reagents for digestion: for each sample add in the test tube:

2 catalyst tablets ST (code CT0006609)
20 ml concentrated sulphuric acid (96-98%)
add carefully 10ml of hydrogen peroxide to avoid foaming.

Shake gently the test tube with sample.

3) Digestion: start from room temperature and use heating ramps up to 420 °C ; overall time:4 hours.
Maintain at 420 °C for 30 min. Overall digestion time: 4 hours and 30 min.

4) Cooling: let cool the test tubes to 50-60 °C.

5) Distillation-titration: place in position the test tube with the digested sample in the unit UDK 129.

factor: 0.00

Reagents addition:
dilution water= 70ml
H_3BO_3= 30ml
NaOH= 70 ml

Titrant: HCl 0.1N
Distillation time= 5 min

Press the button starting distillation cycle.

References: ISO norm n. 333

24 - Kjeldahl method to determine total nitrogen on ABS, SAN, rubber

Procedure

1) Sample: RUBBER: the sample is cut in small pieces and then ground.
0.5 g of sample accurately weighed are used for the analysis.

SAN: cut in small pieces the sample. Grind it. Pellets of 1x3 mm can be directly analysed. Weigh 0.50 g of sample with an accuracy of ± 2 mg.

ABS: grind the sample. Weigh 0.50g with an accuracy of ± 2 mg. Pellets of 1x3 mm can be directly analysed.

2) Reagents for digestion:

2 catalyst tablets CM (code CT0006650)
2 Antifoam tablets S (code CT0006600)
15 ml concentrated sulphuric acid (96-98%)
5 ml Hydrogen peroxide 30-32 % (added 1 by 1 ml).

after the addition of reagents shake to wet the sample.
In some cases it could be necessary to pre-digest the samples overnight at room temperature.

3) Digestion:

30 min at 250 °C
30 min at 350 °C
60 min at 420 °C.

4) Cooling: let cool the test tubes to 50-60°C.

5) Distillation-titration: place in position the test tube with digested sample in the unit UDK 129.

factor: 6.25
Reagents addition:
dilution water= 50ml
H_3BO_3=30ml
NaOH (40% w/v) = 60 ml

Titrant: HCl 0.1N
Distillation time= 5 min

Press the button starting distillation cycle.

References: ISO norm n. 1656

25 - Kjeldahl method to determine total nitrogen in urea

The method is suitable for the determination of urea content in aminoplastics, i.e. urea-formaldehyde resins.

Procedure
1) Sample: the sample is ground in order to obtain a powder very fine and homogeneous .
1g of sample accurately weighed is quantitatively transferred in a test tube.

2) Reagents for digestion. for each sample add in the test tube:
2 catalyst tablets CM (code CT0006650)
12ml concentrated sulphuric acid (96-98%)

after the addition of reagents shake to wet the sample.

3) Digestion: 60 min at 420 °C.

4) Cooling: let cool the test tubes to 50-60 °C.

5) Distillation-titration: place in position the test tube with digested sample in the unit UDK 129.

factor: 0.00
Addition of reagents:
dilution water= 50ml
H_3BO_3= 30ml
NaOH= 50 ml

Titrant: HCl 0.2N
Distillation time= 5 min

Press the button starting distillation cycle.

The urea content of the sample is calculated considering that the nitrogen content of urea is 46.65%.
The quantity of distilled ammonia from 0.1g of urea requires 16.64ml of 0.2N HCl to be titrated.

References: ISO norm n. 1292.

26 - Kjeldahl method to determine total nitrogen on water

The value obtained by this method corresponds to the sum of organic nitrogen (proteins, nucleic acids, urea, synthetic organic chemicals) and ammonia nitrogen.
The measurement of this parameter is required by Italian laws 319/76 and 650/79 for the discharge of wastewaters in lakes, direct or indirect, included in a band of 10 km from the coast line (organic + ammonia + nitrous + nitric = 10 mg N/l maximum).
Kjeldahl method doesn't measure, or measures partly, some nitrogen compounds: azides, azocompounds, hydrazones, hydrazine and hydroxylamine, nitrite and nitrate.

Procedure

1) Sample: 50-200 ml of water (0,1 - 0,5 mg of nitrogen in most cases) in a standard test tube.
With DKL12 it is possible to use the Jumbo test tube 400 ml (code A00000185) with the dedicated sample rack (code A00000181).

2) Reagents for digestion: for each sample add in the test tube:
2 catalyst tablets CM (code CT0006650)
10 ml concentrated sulphuric acid (96-98%)

3) Digestion: mix well the test tube content.
Heat for 30 min at 150°C, 30 min. at 250 °C and then for 1 hour at 420 °C.
The initial heating is used to evaporate water.

4) Cooling: let cool the tubes to 50-60 °C.

5) Distillation-titration: place in position the tube with digested sample in the unit UDK 129.

factor: 0.00
Addition of reagents:
dilution water = 50 ml
H_3BO_3 = 30 ml
NaOH = 50 ml

Titrant: HCl 0.01N
Distillation time= 5 min

Press the button starting distillation cycle.

References: Environmental Protection Agency, EPA PAI-DK01, AOAC 973.48, ISO 5663-1984, ISO11732

27 - Kjeldahl method to determine total nitrogen on soil

A soil of good fertility contains 2-3% of organic matter mainly humus which is composed by 4 - 6% of nitrogen.
There is also some ammonia nitrogen (some ppm) which is to be determined separately and substracted from the value of Kjeldahl nitrogen obtained,if organic nitrogen is needed.

Procedure

1) Sample: 1 g about of air dried soil, sieved to 2 mm (3÷12 mg of organic nitrogen), dried at 105 °C up to constant weight, accurately weighed and quantitatively transferred in a test tube.

2) Reagents for digestion:

2 catalyst tablets ST (code CT0006609)
12 ml concentrated sulphuric acid (96-98%).

3) Digestion: heat for 60 min at 420 °C.

4) Cooling: let cool the test tubes to 50-60°C.

5) Distillation-titration: place in position the test tube with digested sample in the unit UDK 129.

factor: 0.00
Addition of reagents:
dilution water = 50 ml
H_3BO_3 = 30 ml
NaOH = 50 ml

Titrant: HCl 0.2 N
Distillation time= 5 min

Press the button starting distillation cycle.

References: "Methods of soil analysis" part 2 – Chemical and microbiological properties, 2ª ed.

28 - Kjeldahl method to determine gelatin on paper

During paper manufacture animal glue or gelatin, casein and soya proteins are used as bonding agents. Kjeldahl method gives reliable results for the protein content if other nitrogen compounds are not present (e.g. synthetic resins for improving resistance to humidity).

Procedure

1) Sample
2 g about of paper cut in pieces of 1 cm^2

2) Reagents for digestion
To each sample add in the test tube:
2 catalyst tablets ST (code CT0006609)
15 ml concentrated sulphuric acid (96-98%).

3) Digestion
Heat at 200 °C for some minutes to let the foam develop, then go to 420 °C for 30 minutes.

4) Cooling
Let cool test tubes to 50-60 °C

5) Distillation-titration: place in position the test tube with the digested sample in the unit UDK 129.

factor: 5.60
Addition of reagents:
dilution water= 50ml
H_3BO_3= 30ml
NaOH= 50 ml

Titrant: H_2SO_4 0.1 N
Distillation time= 5 min

Press the button starting distillation cycle.

References:
TAPPI Standard T418 05-61. Organic Nitrogen in Paper, modified by reducing to the half the quantity of mercury catalyst.
TAPPI Standards and Suggested Methods. Technical Association of the Pulp and Paper Industry. Atlanta, Georgia, USA.

29 - Kjeldahl method to determine casein on paper

During paper manufacture animal glue or gelatin, casein and soya proteins are used as bonding agents. Kjeldahl method provides reliable results for the protein content if are not present other nitrogen compounds (e.g. synthetic resins for improving resistance to humidity).

Procedure

1) Sample
2 g about of paper cut in pieces of 1 cm^2

2) Reagents for digestion
To each sample add in the test tube:
2 catalyst tablets ST (code CT0006609)
15 ml concentrated sulphuric acid (96-98%).

3) Digestion
Heat at 200 °C for some minutes to let the foam develop, then increase the temperature to 420 °C for 30 minutes

4) Cooling
Let cool test tubes to 50-60 °C

5) Distillation-titration: place in position the test tube with digested sample in the unit UDK 129.

factor: 6.30
Addition of reagents:
dilution water= 50ml
H_3BO_3= 30ml
NaOH= 50 ml

Titrant: H_2SO_4 0.1 N
Distillation time= 5 min

Press the button starting distillation cycle.

References: TAPPI Standard T418 05-61. Organic Nitrogen in Paper, modified by reducing to the half the quantity of mercury catalyst.
TAPPI Standards and Suggested Methods. Technical Association of the Pulp and Paper Industry. Atlanta, Georgia, USA.

30 - Kjeldahl method to determine total nitrogen on sludges from wastewater treatment plants

The final use of sludges from municipal wastewater treatment plants should be on agricultural land for the recovery of useful nutrients. This is suggested also by the Council of European Economic Community (EEC Directive 86/278) related to the agricultural use of sludges from wastewater treatment if the level of contained toxic elements (Cd, Cu, Ni, Pb, Zn, Cr, Hg) is not too high.
The total nitrogen content ranges from 2,2 to 4,2%, dry matter, and is represented by ammonia, nitrite, nitrate, urea and organic nitrogen.
The method operates first a reduction of nitrite and nitrate to ammonia with metallic chromium in acid solution and then the digestion of organic nitrogen to ammonia with concentrated sulphuric acid and catalysts.
Finally ammonia is steam distilled and titrated.

Procedure

1) Sample: about 0,5 g of sludge dried at 105 °C up to constant weight.
(10 - 20 mg of N) or a corresponding amount of wet sludge, accurately weighed and quantitatively transferred in a test tube.

2) Reagents for the reduction: for each sample add in the test tube:
0,5 g chromium powder (Cr)
20 ml hydrochloric acid 7% about (HCl) (200 ml of concentrated acid, 36% , to 1 liter by ammonia free distilled water).

3) Riduction: let reaction run at room temperature for 5 min, shaking at intervals.
Heat to starting boiling for 4 minutes and let cool.

4) Reagents for digestion: for each sample add in the test tube:
2 catalyst tablets ST (code CT0006609)
10 ml concentrated sulphuric acid (96-98%)

5) Digestion: heat for 60 min at 420 °C.

6) Cooling: let cool the tubes to 50-60 °C.

7) Distillation-titration: place in position the test tube with digested sample in the unit UDK 129.

factor: 0.00
Addition of reagents:
dilution water=50ml
H_3BO_3=30ml
NaOH=50 ml

Titrant: HCl 0.2 N

Distillation time= 5 min

Press the button starting distillation cycle.

References: method CNR – IRSA, Rome, Italy

31 - Method to determine the alcohol strength on wines, musts and spirits by steam distillation and volume

Steam distillation is a method to determine the alcoholic strength in wine, musts and spirits: the distillate obtained is an ethanol – water mixture and, using a measurement of density by a pycnometer and expressing the results through the official tables, the alcoholic strength can be calculated.

1) Chemicals and Equipment

- Suspension of calcium hydroxide ($Ca(OH)_2$) 2 M: 120 grams of CaO in 1 liter of water at 60-70 °C (for wine)
- 1 l test tube (code A00001083)
- 200 ml graduated flasks with stopper
- Pycnometer (volume 100 ml or 50 ml)
- Cooled Incubator (VELP Scientifica FOC Series)
- Analytical balance

2a) Procedure for wine

To remove the carbon dioxide, stir gently the sample (250-300 ml in a 1000 ml flask) with a VELP magnetic stirrer.
Measure out 200 ml of the wine using a graduated flask and let the sample adjust to temperature 20 °C, this will take about 15 minutes.
Bring the volume of the sample to the mark (exactly 200 ml) by taking away excess sample by a small pipette.
Transfer the wine to the 1-liter test tube. Rinse the graduated flask four times with successive 5 ml washings of water added to the test tube.
Then, add 10 ml of the suspension of calcium hydroxide. The color of the wine must change, for example from red to deep grey. For very acid wines, it's suggested to add some drops of phenolphthalein in the test tube, until the color of the indicator changes.

2b) Procedure for spirits

Measure out 200 ml of the spirits drinks using a graduated flask and thermostat it at 20°C for 15 minutes.

Let the sample adjust to temperature, this will take about 15 minutes. Bring the volume of the sample to the mark (exactly 200 ml) by taking away excess sample by a small pipette. Transfer the liquids to the 1-liter test tube (Code A00001083).
Rinse the volumetric flask 3 times with 20 ml of distilled water (3 x 30 ml if liqueur and crèmes).

3) Distillation
place in position the test tube with digested sample in the unit UDK 129.
factor: 0.00
Addition of reagents:

- H_2O (dilution water): 0 ml
- NaOH (32%): 0 ml
- Distillation Time: 8-10 minutes *
- H_3BO_3 (4% with indicators): 0 ml

Collect the distillate in the 200 ml graduated flask used to measure the wine quantity.

*set a distillation time to obtain a maximum of 200 ml of distillate.
After the distillation, position the receiving flask in the thermostat for about 15 min. and finally bring up to volume (200 ml) using distilled water at 20 °C.

4) Density of the Distillate

Measure the density of the distillate through a pycnometer:
1. Weigh the empty pycnometer, clean and dry, with all parts in place (M_{pyc} in g)
2. Weigh the pycnometer filled with the distillate at 20 °C (M_{dist} in g)
3. Calculate the density of the distillate at 20 °C ($D_{dist\ 20°C}$) following the formula:

$$D_{dist\ 20°C} = (M_{dist} - M_{pyc}) / (V_{pyc} \times 1000)$$

V_{pyc} (m^3) is the volume of the empty pycnometer and is calculated as follows:

$$V_{pyc} = (M_{pyc,H_2O} - M_{pyc}) / (\rho_{H2O,20°C} \times 1000)$$

Where:

$M_{pyc,H2O}$ = pycnometer weight filled with water at 20 °C (g)
$\rho_{H2O,20°C}$ = density of the water at 20 °C (0.99823 g/ml)

4. Use the density table of the official method to express the results:
   for wine: OIV method - MA - AS312 - 01A
   for spirits: Recommendation n°22 of the International Legal Metrology Organization suggested by Reg. EC 2870/2000
5. Take care during the entire procedure: avoid fat from fingertips, temperature changes when holding the pycnometer and air bubbles in it.

Reference:

For wine: Official analytical method OIV - MA - AS312 - 01A - Alcoholic strength by volume

For spirits: COMMISSION REGULATION (EC) N° 2870/2000

32 - Method to determine the residual urease activity in soya beans

To evaluate if a soya bean sample, submitted to thermal treatment (toasting), has been treated in a correct way, it is possibile to measure the residual activity of the enzyme urease. This enzyme is present in uncooked soya bean and is progressively destroyed by heat.
The result is expressed as milliequivalents of ammonia produced from urea by 1 g of grinded soyabeans during 1 hour at 30 °C (Rassmussen's method).
In uncooked soyabean the urease activity is 5 milliequivalents/g about, the correctly roasted soyabean shows values between 0,5 and 1 milliequivalents.
A too strong heat treatment lowers values under 0,2 milliequivalents.

Procedure

1) sample: introduce into a test tube a 1 g soya sample finely grinded, add 0,20 g of urea and 150 ml of distilled water.
Close immediately the test tube.

2) Incubation: shake the closet test tube and bring to 30 °C (water bath). Allow the enzymatic reaction develop for 60 min, shaking at intervals.

3) Distillation:
analytical parameters:

H_2O=0ml. H_3BO_3=0 ml. NaOH=0 ml.
Distillation time = 5 min

Introduce 30,0 ml of titrated 0,1 N solution of hydrochloric acid in the Erlenmeyer flask used to collect the distillate; place it in position with the tip of the silicone tube dipped in the acid.
If it is not dipped add 30-50 ml of ammonia free distilled water. At the end of the 60' min of reaction add in the test tube about 2 g of magnesium oxide (MgO) (pH 10,3), place in position in the steam distillator and start distillation to obtain 100 ml of distillate.

4) Titration: titrate the residual acid in the flask by an alkalyne solution of known titre and a suitable indicator (eg. Sodium hydroxide 0,1 N and methyl red).

5) Computation: the result is expressed as milliequivalents of ammonia produced from urea per each gram of soyabean:

$$\frac{\text{ml HCl 0,1N neutralized}}{\text{soya weight g} \times 10} = \text{meq } NH_3$$

6) Note: a strong foaming can develop during distillation, particularly with poorly roasted soyabeans. Control distillation and if necessary stop it at intervals.

33 - Method to determine urea nitrogen in feed and roughages

During ensiling of green forages some urea can be added. Some feed can be supplemented with urea in order to increase raw protein content. Urea content can be determined separately from other nitrogen compounds by the enzyme urease which hydrolizes urea to ammonia and carbon dioxide. The method is based on steam distillation of ammonia before and after enzymatic treatment with urease and its determination by titration.

Procedure

1) Reagents for anzymatic incubation:

- Urease 1 International Unit (I.U.) produces 1,0 mg of ammonia nitrogen from urea in 5 min at pH 7,0 at 20 °C. Commercial urease powders show activities between 17000 and 200000 units per gram. It's necessary to prepare a standardized solution of enzyme containing 0,5-1 I.U./ml, able to free completely ammonia from 100 mg of urea (46,6 mg as N) in 1 hour at room temperature.
- Silicone antifoam.
- Calcium chloride solution: 25 g of $CaCl_2$ in 100 ml of water.

2) Standardization of urease solution.
Urease solutions are alkaline and should be neutralized to pH 7 after dilution in water of a known amount (e.g. 0,1 g in 50 ml) using hydrochloric acid 0,1 N and methyl red indicator. The solutions cannot be stored.
The determination of the enzymatic activity is performed by adding increasing known volumes of neutralized urease solution to 0,1 g of pure urea. After 1 hour incubation at room temperature, ammonia is steam distilled.
It is to be used the volume of urease solution which frees completely ammonia from 0.1g of urea during 1 hour.

3) Incubation: 2 g of homogeneous sample finely grinded and weighed are introduced into a test tube with 100 ml of water and the adopted volume of urease solution, close and incubate 1 hour at room temperature shaking at intervals. If the urea content of the examined material is more than 5% use more urease.

4) Distillation and titration: at the end of incubation introduce in the test tube 2 g of magnesium oxide, 5 ml of calcium chloride solution and some drops of antifoam. Place in position in the steam distilling unit the test tube with the sample.

Set the following analytical parameters:

H_2O=50ml. H_3BO_3=30ml. NaOH=50 ml.
Titrant = HCl 0,2 N

Distillation time = 5 min
The nitrogen content of urea is 46,65%. The amount of ammonia freed from 0,1 g of urea requires 16,64 ml of acid of this normality (1 ml HCl 0,2 N = 2,803 mg N - NH_4).
If the original sample contains ammonia , it is to be determined separately and substracted from the value obtained after incubation with urease.

References: AOAC. Official Methods of Analysis (1984).

34 - Determination of the volatile acidity of tomato paste

The volatile acids are measured ,in the presence of phenolphthalein indicator, in the distillate obtained by steam distillation of a proper amount of sample.

NOTE: *in order to peform this kind of analysis it is necessary to substitute the techno-polimer splash-head on the distillator with the new glass splash-head, code A00000238.*

Procedure

1) Reagents:
For the steam generator use distilled or deionised water boiled for some minutes in order to eliminate the carbon dioxide. Use the same kind of water to dilute the sample and for the distillate collecting flask.

- crystallized tartaric acid
- phenolphthalein, 1% hydro-alcoholic solution
- sodium hydroxide (NaOH), 0.1N solution
- VELP weighing boats, code CM0486001

2) Sample:
weigh 20g of sample by the VELP weighing boats and introduce in the test tube. The use of the weighing boats avoids errors during the sample weighing, permitting to transfer completely the sample to the test tube for the analysis.

As an alternative, the following amount of sample can be used:

600/R g, weighed with a tolerance margin of 0.01g; R is the content of NTSS (natural total soluble solids, or soluble dry matter of the sample).

Afterwards introduce in the test tube containing the sample 0.1g of tartaric acid and then place in the distillator the test tube.

3) Distillation:
analytical parameters:

H_2O = 50ml (distilled or deionized and boiled). NaOH = 0ml. H_3BO_3 = 0ml.

Take off the distillate collecting tube from the condenser and place a new silicon tube in the same position.
Place in position on the mobile round stand of the distillator an Erlenmeyer flask (250ml) containing 40ml of deionised or distilled water boiled for some minutes. Be sure that the end of the silicon tube, which is connected to the condenser, is completely dipped in the water.

Distillation time = 5 minutes

Push START to initiate the cycle

Collect 150ml of distillate.

4) Titration: titrate the distillate, containing 4 drops of phenolphthalein 1%, up to a persisting pink colour, using the 0.1N NaOH solution.

5) Computation:
the sample volatile acidity is expressed as acetic acid in 100g of sample; 1ml of NaOH 0.1N corresponds to g 0.006 of acetic acid:

g of acetic acid/100 g of sample = 0.6 x ml NaOH 0.1N / g of sample

NOTE: *at the end of the analyses, perform 3 washing cycles of the distillator. Introduce in the test tube 150-200ml of distilled or deionised water and set for each washing cycle 5 min for the distillation time. The aim is the complete removal of traces of sample from the glass splash-head.*

References:
- Commission Reg. (EEC) No 1764/86, "Minimum quality requirements for tomato-based products eligible for production aid".
- SSICA-Stazione Sperimentale per l'Industria delle Conserve Alimentari-Parma-Italy,
monography "The tomato", pag. 156 "Quality control of the tomato preserves" (Italian language)
- Books of Food Chemistry, R. Giuliano and M.L. Stein, Bulzoni Ed.-Roma-Italy, 5° vol. ALIMENTARY PRESERVES, pag. 119 "Analyses of the tomato preserves" (Italian language).

35 - Method to determine the volatile acidity of wines

The volatile acidity is due to the acids belonging to the acetic series which are present in the wines as free or salt forms.
The method is based on the titration of the volatile acids separated from the wine by a steam distillation.
The wine to be analysed is previously freed from the carbon dioxide.
It is also necessary to subtract from the distillate acidity the acidity of the sulphur dioxide free or combined which have been distilled.
It is also necessary to subtract the acidity due to the sorbic acid added in the wine. The salicylic acid added in some countries before the analyses in order to stabilise the wines is present , in some part, in the distillate. It is then necessary to dose it and subtract from the volatile acidity.

Reagents

- Crystallised tartaric acid ($C_4H_6O_6$), analytical purity
- solution 0.1M of sodium hydroxide (NaOH), analytical purity
- solution of phenolpthalein 1% in neutral ethanol 96% vol.
- hydrochloric acid (ρ_{20} = 1.18-1.19 g/ml), analytical purity, diluted 1/4 (v/v)
- solution 0.005M of iodine (I_2), analytical purity
- crystallised potassium iodide (KI), analytical purity
- starch-water 5g/l: dissolve 5g of starch in about 500ml of water. Bring to boiling ,stir, and maintain 10 minutes; add 200g of sodium chloride. After cooling down, bring the volume to 1l.
- saturated solution of sodium tetra-borate ($Na_2B_4O_7$-10 H_2O), analytical purity, about 55g/l at 20°C.

Procedure

1) Sample preparation: elimination of the carbon dioxide (CO_2)

Bring the sample to room temperature. The dissolved carbon dioxide is removed by stirring the sample or by using an ultra-sonic bath.
As an alternative: pour about 50ml of wine in a vacuum flask; stir and create vacuum at the same time using a pump; stirring must take 1 or 2 minutes.

2) Distillation:

pour in a test tube 20ml of wine freed from the carbon dioxide.
Add about 0.5g of tartaric acid (used to make free the salified volatile acids which, in the international methods, must be included in the volatile acidity) and place the test tube in its site in the distillator.
Analytical parameters:
H2O = 0ml. NaOH = 0ml. H3BO3 = 0ml.

Take off the tube used to collect the distillate from the end of the condenser and place a new silicon tube.

Place in position on the round stand of the distillator an empty Erlenmeyer flask and collect at least 250ml of distillate.

3) Titration:

titrate using the solution 0.1M of sodium hydroxide in presence of 2 drops of phenolpthalein solution, being n the volume in ml used.
Add 4 drops of diluted hydrochloric acid 1/4 , 2ml of starch-water, and some crystals of potassium iodide (in order to make more visible the colour change). Titrate the free sulphur dioxide using the solution 0.005M of iodine. Being n' the volume in ml used.
Add the saturated solution of sodium tetra-borate until the pink colour appears again. Titrate the combined sulphur dioxide using the solution 0.005M of iodine . Being n'' the volume in ml used.

4) Results:

- the volatile acidity expressed as milli-equivalents per liter, with 1 decimal, is:

A = 5 (n 0.1 n' 0.05 n'')

- the volatile acidity expressed as grams of acetic acid per liter with 2 decimals , is:

0.300 (n 0.1 n' 0.05 n'')

5) Wines containing sorbic acid:

inside the first 250ml of distillate is included also 96% of the sorbic acid present; for this reason it is necessary to subtract its acidity from the volatile acidity considering that 100mg of sorbic acid correspond to an acidity of 0.89 milli-equivalents or to 0.053g of acetic acid, knowing the amount of sorbic acid (mg/l) determined separately.

6) Determination of the salycilic acid present in the distillate of the volatile acidity:

After the determination of the volatile acidity and after the correction for the sulphur dioxide, the presence of salyciiic acid is revealed, after the addition of acid, by the presence of a violet colour forming after addition of an iron III salt.The determination of the salycilic acid ,present in the distillate of the volatiie acidity, is to be performed in a second distillate having the same volume of the distiliate used for the dosage of the voiatile acidity.
In such a distillate the salycilic acid is determined by a comparative colorimetric method. It is then subtracted from the distillate acidity.

a) Reagents:

- hydrochloric acid (HCl) (ρ_{20} = 1.18-1.19 g/ml)

- sodium thio-sulphate ($Na_2S_2O_3$-5 H2O) in solution 0.1M

- solution of ammonic ferric sulphate $[Fe_2(SO_4)_3 \times (NH_4)_2 SO_4 \times 24 H_2O]$ 10% (w/v).

- solution of sodium salycilate 0.01M: solution containing 1.60 g/l of sodium salycilate ($Na C_7H_5O_3$).

b) Procedure:

-characterisation of the salycilic acid in the distillate of the volatile acidity:

immediately after having determined the volatile acidity and the correction for the free and combined sulphur dioxide, add in the flask 0.5ml of hydrochloric acid, 3ml of solution 0.1M of sodium thio-sulphate and 1ml of solution of ammonic ferric sulphate. If salycilic acid is present a violet colour will appear.

-dosage of the salycilic acid:

In the flask described above mark the level of the distillate volume. Empty the flask and wash it. Distill a new sample aliquot of 20ml of wine and collect the distillate in the flask filling it up to the level mark. Add 0.3ml of hydrochloric acid and 1ml of solution of ammonic ferric sulphate. The liquid in the flask will turn violet. Pour in a flask ,equal to the flask with the level mark, some distilled water up to the same distillate level. Add 0.3ml of hydrochloric acid and 1ml of solution of ammonic ferric sulphate . Add by a burette the solution of sodium salycilate 0.01M ,until a violet colour is obtained, having the same intensity of the colour in the flask containing the wine distillate. Being n''' the used ml.

-correction of the volatile acidity:

subtract 0.1 x n''' ml from the volume of n ml of solution of sodium hydroxide 0.1M used to titrate the distillate acidity during the dosage of the volatile acidity.

References: EEC Reg. n. 266/90 , 17 Sept. 1990, determining the methods to be used for the analyses in the wine sector.

36 - Kjeldahl method to determine total nitrogen in crude oil, lubricants and fuel oils (ASTM, D3228-96)

Combined nitrogen in petrochemical fuels gives origin by combustion to nitrogen oxides whose emission in the atmosphere is subjected to limitations.
Combustions occurring at relatively low temperatures (1400-1700 °F, 750-950°C) produce nitrogen oxides mostly from fuel-bound nitrogen and not from atmospheric nitrogen. The considered group of products does contain nitrogen compounds with N-N and N-O linkages, which are difficult to be destroyed by the commonly used conditions of mineralization. Moreover, oil products are characterized by strong foaming capacity.
Both kinds of problems are overcome by the use of heating blocks with ramps heating and by long digestion times in the order of many hours.

Procedure

1) Sample
1-1,5 g of homogeneous sample, weighed with a proximation of 0,1 mg.
It's suggested to use nitrogen free weighing boats
(Cod. Art. CM0486000, CM0486001). It's suggested furthermore to introduce the boat in the test tube immediately after weighing, to avoid the raise of oil by capillarity along the folded sides of the boat.
The sample containing boat is slipped along the side of the digestion tube, in order to avoid its dispersion before reaching the bottom.

2) Reagents for mineralization:
Add to each sample in the test tube one of the following group of reagents:
2 catalyst tablets ST (code CT0006609)
18 ml concentrated sulphuric acid (96-98%)
10 ml hydrogen peroxide (H_2O_2) 35% (130 Vol.)

3) Procedure for the mineralization:
Shake each tube to mix the content, better if before the addition of hydrogen peroxide.
Start heating increasing the temperature of 100°C every hour during 4 hours, up to 420 °C.
Use temperature ramps.
Maintain the temperature at 420 °C during 30 min and then let cool the tubes to 50-60°C or better up to room temperature.

4) Distillation and titration

Place in position the test tube with digested sample in the unit UDK 129
Set the following analytical parameters:

Factor: 0.00
Addition of reagents:
H2O: 70 ml
H_3BO_3: :30 ml
NaOH: 50 ml

Titrant: HCl or H_2SO_4 0,1 N.
Distillation time = 5 min

Push START to start the analysis.

References: American Society for Testing and Materials. Standard Test Method D 3228-96 Total Nitrogen in Lubricating Oils and Fuel Oils by Modified Kjeldahl Method.

37 - Method to determine nitric nitrogen on water after reduction to ammonia nitrogen (Devarda's alloy method)

Nitric nitrogen content on municipal wastewaters treated in wastewater treatment plant can vary between 0 and 15 mg/l $N\text{-}NO_3$ depending on nitrification systems and on water consumption of the served population.
High concentrations permit to perform the reduction of nitrate and nitrite to ammonia by Devarda's alloy powder (45% Al, 50% Cu, 5% Zn) in alkaline solution.
From the amount of ammonia nitrogen determined after steam distillation will be subtracted the amount of ammonia nitrogen and nitrite nitrogen present in the sample, determined separately, to obtain nitrate nitrogen in the sample.

Procedure

1) Sample: 50 ml of water (or between 20 and 70 ml depending on the nitrate content) accurately measured and introduced in a test tube.

2) Reagent for the reduction of nitrates (and nitrites) to ammonia for each sample:
2 g Devarda's alloy powder

3) Reduction and distillation/titration: introduce Devarda's alloy in the tube and place it in the unit UDK 129.

Set the following analytical parameters:
Reagents addition:
H_2O = 0 ml
H_3BO_3 = 25 ml
NaOH = 50 ml

Pause= 20-30 min
Titrant: H_2SO_4 0,01 N or H_2SO_4 0,001 N

Push START to begin NaOH addition and then (after the pause) distillation and titration.
Distillation time= 5 min

References: ISO, norm n.10048

38 - Method to separate ammonia in water from interfering substances

Ammonia nitrogen is an important parameter for the management of a water treatment plant.
Its direct determination in turbid waters, coloured or rich in magnesium, ammines and other organic substances is subjected to large errors.
In these cases it's better to proceed with a separation of the ammonia by steam distillation.

Procedure

1) Sample: 50 ml of water (or between 20 and 70 ml depending on ammonia nitrogen content) accurately measured and introduced in the test tube.

2) Reagents: the sample to be distilled must be alkalinized (indicator phenolphthalein) with alkali or simply adding:
1,2 g magnesium oxide (MgO)
A saturated solution of magnesium oxide shows pH = 10,3.

3) Distillation e titration:

Set the following analytical parameters:

Addition of reagents:
H_2O= 50ml.
H_3BO_3= 30ml (4% p/v)
NaOH= 0ml

Titrant: H_2SO_4 0,01 N or H_2SO_4 0,001 N
Distillation time= 5 min

Press the button starting distillation cycle.

References: ISO, norm n.o. 5664

39 - Determination of phenols in drinking water and in industrial wastes

The determination of phenols is one of the routine analysis in order to assess the quality in drinking and ground water and in domestic and industrial wastes.

Procedure

1) Sample:
If the sample to be analyzed contains oxidizing agents (e.g. chlorine), oils and tars or sulphur compounds, it must be treated at sampling or before distillation according to the official method prescription.

2) Reagents and equipment:

- Phosphoric acid (H_3PO_4) diluted (1 : 10). 100 ml of phosphoric acid concentrated (85%, d 1,68) mixed with 900 ml of distilled water.
- Copper sulphate ($CuSO_4 \cdot 5 H_2O$) solution 10%. 100 g of salt dissolved in distilled water for a final volume of 1000 ml.
- Distilled water, previously boiled and cooled (Type II)
- Aminoantipyrine solution: dissolve 2 g of 4-aminoantipyrine in 100 ml of distilled water
- Potassium ferricyanide solution: dissolve 8 g of $K_3Fe(CN)_6$ in 100 ml of distilled water
- Ammonium chloride solution 2%: dissolve 20 g of NH_4Cl in 1000 ml of distilled water
- Ammonia 25 %
- Concentrated phenol solution (1000 mg/l): dissolve 250 mg of phenol in 250 ml of freshly boiled and cooled distilled water
- Diluted phenol solution (10 mg/l): dilute 2.5 ml of the concentrated phenol solution to 250 ml
- Chloroform, analytical grade

- Photometer for use at 460 nm
- 50 mm glass cuvette
- pH meter
- 500 ml volumetric flasks
- 500 or 1000 ml separatory funnels
- Filter papers

3) Sample preparation:
before distillation the water sample is to be acidified to pH 4 by phosphoric acid. Add 1 ml of solution 10% of copper sulphate to 100 ml of sample and introduce in the test tube.

4) Distillation:

Set the following analytical parameters:
Addition of reagents:
H2O=0
H3BO3=0
NaOH=0

Distillation time: 8 min

Position the test tube containing 10 ml of sample into the distillation unit UDK and start the distillation.

Push START to begin the distillation.
About 200 ml of distillate are collected into a 500 ml volumetric flask.

Blank: 10 ml of distilled water Type II

Dilute the distillation solution to 500 ml using water type II and transfer the solution into a 1000 ml becker.

Add 25 ml of ammonium chloride solution 2 % by using a pipette and adjust the pH to 10.0 ± 0.2 with some drops of ammonia 25 %.

Transfer the solution into a 500 or 1000 ml separatory funnel and add 3.0 ml of Aminoantipyrine solution and 3.0 ml of potassium ferricyanide solution. Shake the separatory funnel and after 5 minutes add 25 ml of chloroform with a pipette.

Shake vigorously for about 2 minutes and let the organic phase settles.

Filter chloroform extract through a filter paper and collect the filtrate in a 100 ml flask.

Transfer the filtrate solution in a 50 mm glass cuvette and read the extinction values at 460 nm, against distilled water.

Note:

In order to calculate the phenol concentration, create a calibration curve by using phenols solution diluted in the following range: 0.005-0.100 mg /l phenols.

Dilute different amounts of the phenols diluted solutions to 500 ml using water type II and proceed the chloroform extraction as indicated above. Record the extinction value corresponding to the different concentrations.

References:
- APAT CNR IRSA (Water Research Institute), Italy, method n° 5070A1
- EPA method n° 9065

40 - Separation of hydrocyanic acid from wastewaters

Heavy metal complexes, sulphides, aromatic amines, oxidants and dyes interfere with colorimetric or titrimetric determination of cyanides. In these cases hydrocyanic acid is to be separated from water sample by a distillation. Steam distillation at 95-100°C gives results 0,5-1% lower than exact value, due to HCN hydrolysis to ammonium formiate. Take into account that hydrocyanic acid is a very weak acid (dissociation constant $7,2x10^{-10}$); its solutions at a pH lower than 12 release acid by the action of atmospheric carbon dioxide.

Procedure

1) Water sample
The sample should be processed as soon as possible after sampling. If it is to be stored, sodium hydroxide should be added to bring the pH to at least 12, kept in a completely filled bottle, well closed and maintained at low temperature (+4°C).

2) Reagents for pre-treatment and distillation
- lead carbonate ($PbCO_3$), powder
- Ascorbic acid ($C_6H_8O_6$), powder
- Sodium hydroxide(NaOH), about 1 N, 40 g/l
- Sulphuric acid (H_2SO_4), concentrated d.1,84
- Mercury chloride ($HgCl_2$), 68 g/l in water
- Magnesium chloride hexahydrate ($MgCl_2 \cdot 6H_2O$) 510 g/l in water
- Silver nitrate ($AgNO_3$) dried at 140 °C, 3.2467 g/l in water (0.0192 N),
- Rhodanine indicator, 0,02 g/100 ml in acetone.

3) Pre-treatment of the sample before distillation
- Sulphides are removed at pH 11 by adding lead carbonate powder up to a complete precipitation of lead sulphide which is removed by filtration.
- Oxidizing agents are destroyed by adding ascorbic acid powder.

4) Distillation
200 ml of sample are introduced in 1 liter test tube (code A00001083). Add 10 ml of mercury chloride solution and 5 ml of magnesium chloride solution. 10 ml of concentrated sulphuric acid are added in the test tube immediately before to start steam distillation, and test tube with sample is introduced in the unit UDK129.

Set the following analytical parameters:
Reagents addition:
H_2O = 0 ml.
H_3BO_3 = 0 ml.
NaOH = 0 ml.

The distillate is collected into a flask containing 50 ml of sodium hydroxide solution 1 N about. The tube collecting the distillate is to be taken off from the condenser and substituted with a new silicone tube, which is introduced into the flask.

Begin the distillation by START.

100-120 ml of condensate are collected and the flask content can be adjusted to the volume with the washings in a volumetric flask 200 ml (the same volume of the sample). The quantitative determination of the cyanides can be performed by titrimetry: add 10 drops of Rhodanine indicator into the flask and titrate by silver nitrate.

The concentration of cyanides can be calculated as follows:

$$CN\ (\mu g/L) = \frac{(A-B)}{C} * D * \frac{2\ mole\ CN^-}{1\ eq.\ AgNO_3} * \frac{26.02\ g\ CN^-}{1\ mole\ CN^-} * \frac{(1*10^6\ \mu g)}{1\ g}$$

A = ml AgNO3 for titration of sample
B = ml AgNO3 for titration of blank
C = ml of original sample before distillation (200 ml recommended).
D = actual normality of $AgNO_3$ (0.0192 N recommended)

References: ENVIRONMENTAL PROTECTION AGENCY : Test method n. 9010. APAT Agenzia per la protezione dell'ambiente e per i servizi tecnici, IRSA-CNR Istituto di Ricerca sulle Acque - Consiglio Nazionale delle Ricerche n° 4070.

41 - Method to control the efficiency of an anaerobic digester by determination of volatile acids content in digesting sludge

The volatile acids content in a slurry submitted to anaerobic digestion is considered the best index of process performance when compared to alkalinity level.
If the ratio by weight between volatile acids (C2 - C6) content, expressed as acetic acid, and total alkalinity expressed as calcium carbonate is maintained lower than 0,2 (eg. 300/2000), the digestion proceedes regularly. Values of 0,3 - 0,4 show some malfunction and the need of intervenction. At values higher than 0,8 methane production is inhibited.
A quick method for determining volatile acids (acetic, propionic, butirric, valerianic, etc.) in digesting sludge is based on steam distillation and titration in the distillate.

Procedure

1) Reagents:
• sulphuric acid 1 : 1. Dilute 1 volume of concentrated sulphuric acid (H_2SO_4) slowly added to the same volume of water, stirring at intervals.
Do not add water in the acid to avoid dangerous splashings.
• Sodium hydroxide (NaOH), titrated 0,04 N solution.
• Phenolphthalein indicator 0,5 g in 100 ml of distilled water
• Methyl orange indicator solution. 0,05 g in 100 ml of distilled water
• Acetic acid (CH_3COOH), 2000 mg/l. Dilute 1,9 ml glacial acetic acid to 1000 ml by deionized or distilled water. Titrate by sodium hydroxide 0,04 N. 10 ml of the solution (0,33 meq) require 8,3 ml of sodium hydroxide 0,04 N (phenolphthalein indicator).

2) Sample: 150 : 200 ml of digesting slurry are centrifuged to remove solids and avoid a possible production of volatile acids by hydrolysis. 5 min at low speed. The sample is the supernatant liquid.

3) Distillation: 100 ml of sample in a 250 ml test tube are acidified by sulphuric acid 1 : 1, added shaking in 1 ml volumes up to the change of methyl orange indicator from red to yellow (pH 2,7).

Place the tube with acidified sample in the unit UDK129.
Select the mode "analysis without titration".

Place in position in the steam distillator UDK129 an Erlenmeyer flask empty; take off the distillate collecting tube from the condenser and place a new silicone tube, introduced in the flask.

Set the following parameters:

Reagents addition:
H_2O=0.
H_3BO_3=0.
NaOH=0.

Distillation time = 5 min

Push START to begin the distillation.

Avoid collection of hydrogen sulphide and carbon dioxide present in the sample discarding the first 10 ml of condensate. Collect 100 ml of distillate.

4) Titration: after the addition of phenolphthalein indicator (10 drops) titrate by sodium hydroxide 0,04 N, until pink colour persists.

5) Computation: the result is obtained by the formula:

$$\text{mg/l volatile acids (as acetic acid)} = \frac{\text{used ml NaOH} \times N \times 6005 \times 1000}{\text{ml of used sample} \times F}$$

F represents a recovery factor of the distillator used, which is obtained by distilling known volumes (eg. 10 ml) of acetic acid solution 2000 mg/l and computing:

$$\text{factor} = \frac{\text{ml NaOH used} \times 100}{\text{ml NaOH theoretical}}$$

References:
- Lombardo, J.B. (1973). *Improved distillation method for volatile acid analysis.* Journal Water Pollution Control Federation. 45:1046-1051.
- Buswell, A.M. (1945). *A note on the determination of volatile acids in digester sludge.* Sewage Works Journal. 20: 845-850.

42 - Determination of ammonia nitrogen in organic fertilizers according to the Kjeldahl method

Procedure

1) Sample: the sample must be ground in order to obtain an homogeneous powder (particles dimension 1mm). 0.7-3.5g of sample are then accurately weighed (with a precision of 0.1 mg) and quantitatively transferred in a test tube.

2) Distillation and titration: the sample to be distilled must be alkalized (phenolpthalein indicator) by adding 2g or a higher quantity of Magnesium oxide (MgO) free from carbonates. Place the test tube containing the alkalized sample in the UDK 129 unit.
Select the following analytical parameters:

Factor: 0.00
Addition of reagents:
H_2O: 50 ml
H_3BO_3: 30 ml
NaOH: 0 ml.

Titrant: HCl 0.2N

Push START to start the analysis.

References:
AOAC, Official Methods of Analysis, method 920.03.

43 - Total sulphite determination in foods by steam distillation and titration

Allergenic reactions due to Sulphur dioxide or sulphite in foods were recently reported. This fact lowers the concentration which was considered safe for human consumption and increases the number of control analyses to be performed.
One of the most commonly used methods for this analysis is the Monier-Williams (a) which isolates Sulphur dioxide from complex matrixes by distillation before measuring it by titration. This distillation performed by steam in a automatic apparatus shortens considerably the required time not modifying the results.

Procedure

SO_2 is stripped using phosphoric acid and water steam, received in a hydrogen peroxide solution and then, determined titrimetrically.

1) Apparatus:

A) Steam distilling unit UDK.

B) Magnetic stirrer and stirring bar.

C) Burette, 25 ml,

D) Erlenmeyer flask, wide neck opening 300 ml.

2) Reagents:

A) Phosphoric acid w = 80 % about (H_3PO_4),
B) Methyl orange indicator (alternatively bromocresol blue or bromocresol pink) 0.1 % in alcoholic solution, the pH value should be between 3.3 and 3.4.

C) Sodium hydroxide (1 N) (NaOH),

D) Hydrogen peroxide w = 30 % about (H_2O_2).

3) Sample storage and preparation:

Samples should be stored in tightly closed containers. If samples are not analyzed within 24 hours, they are to be kept frozen. Liquid or wet samples must be homogenized. Dry samples are to be grinded to pass 0.4 mm (40 U.S. mesh) sieve.

4) Distillation and titration:

Place distillate collecting flask under the prolonged condensate outlet, the collecting tube tip should be always dipped in the liquid; with 20 ml deionised water, 2.5 ml about of methyl orange indicator and 5 ml of hydrogen peroxide. Prepare the collecting flask immediately before starting the distillation, because the hydrogen peroxide is instable in water solution.

Add manually in the test tube 20 ml of water and 10 ml of phosphoric acid.

Set the UDK program:
NaOH = 0 ml.
Time of distillation: 5 minutes
Press the button starting distillation cycle.

At the end of the distillation, introduce the stirring bar into the flask and place it on a Magnetic stirrer (i.e. VELP MST code F203A0160).

Record the volume of NaOH solution consumed for titration.

Blank value: is made with all reagents, except the sample.

5) Calculations:

The quantity of sulphur dioxide in the analyzed sample is calculate as follow:

$$m\ (SO_2) = \frac{(V-V_{Bl}) * c_{NaOH} * M_{SO2}}{2}$$

$m\ (SO_2)$ = mass SO_2 (mg)
V = Consumption of sodium hydroxide solution (ml)
V_{Bl} = Blank value consumption of sodium hydroxide solution (ml)
c_{NaOH} = concentration of sodium hydroxide solution (mol/l)
M_{SO2} = molar mass sulphur dioxide (64.06 g/mol)

The sulphite concentration in the analyzed sample is given by (expressed as Sulfur dioxide):

$$SO_2\ ppm = \frac{m\ (SO_2) \times 1000}{W}$$

W = weight of the sample as grams.

6) Comments:
The described method proved its validity in determining Sulphur dioxide and sulphites in a variety of foods. It is simple and rapid and showed a very good reproducibility.

7) Literature:
A) Monier-Williams method. Official Methods of Analysis (1984) n. 962.16 A.O.A.C. Association of Official Analytical Chemists. Arlington. VA. U.S.A.

44 - Determination of the total volatile basic nitrogen (TVBN) in fresh and frozen fish

The controls required by EEC aimed at preventing fish and seafood not suitable for human consumption from being put on the market, can include some chemical controls, one of them being the TVBN determination.
The TVBN, expressed as mg/100g of sample, is the content of nitrogen from ammonia and volatile amines, produced from the proteins during the fish perishing.
The Reg. EEC 2074/2005 fixes the TVBN concentrations for some kinds of fish and the related analytical methods.
Among the commonly used methods suitable to control the limit value of TVBN, the EEC Reg. describes also the method from Conway and Byrne (1933).
The fish products (raw material) included in the categories detailed in the above Reg., are to be considered as "not suitable for human consumption" should there be any doubt concerning the freshness of the product from the sensorial control, or if the chemical control indicates that the limit for TVBN (25/30/35 mg/100g depending on the species of fish) has been exceeded.
The described method is suitable also for meat analysis.

Procedure

1) Sample: prepare a representative sample by a suitable homogenizer. Bigger skin and bone pieces should be removed before homogenization. The sample must be analysed within 4 hours from the preparation. If not analysed immediately, the sample should be stored into a refrigerator.
Weigh 10.0 g of sample with an accuracy of ± 0.1g and introduce it into a test tube.

2) Distillation: add manually into the test tube 2 g of magnesium oxide (MgO) and 1 antifoam tablet S (code CT0006600).
Place the test tube in the distillator and set the following parameters:
H_2O= 50 mL
H_3BO_3= 45 ml (use 500 ml flask)
NaOH= 0 ml
Distillation time: 10 min
Titrant solution: HCl or H_2SO_4 0,01 N.
Start the distillation.

For this application it's recommended the use of the glass splash head (code A00000238).

4) Result:

mg TVBN / 100g = (T-B) x 14.007 x N x 100 / sample weight (g)

T = ml of titrant used for sample titration
B = ml of titrant used for blank titration
N = titrant normality

5) References:
Pearson D. (1973), Laboratory techniques in food analysis (Conway method).