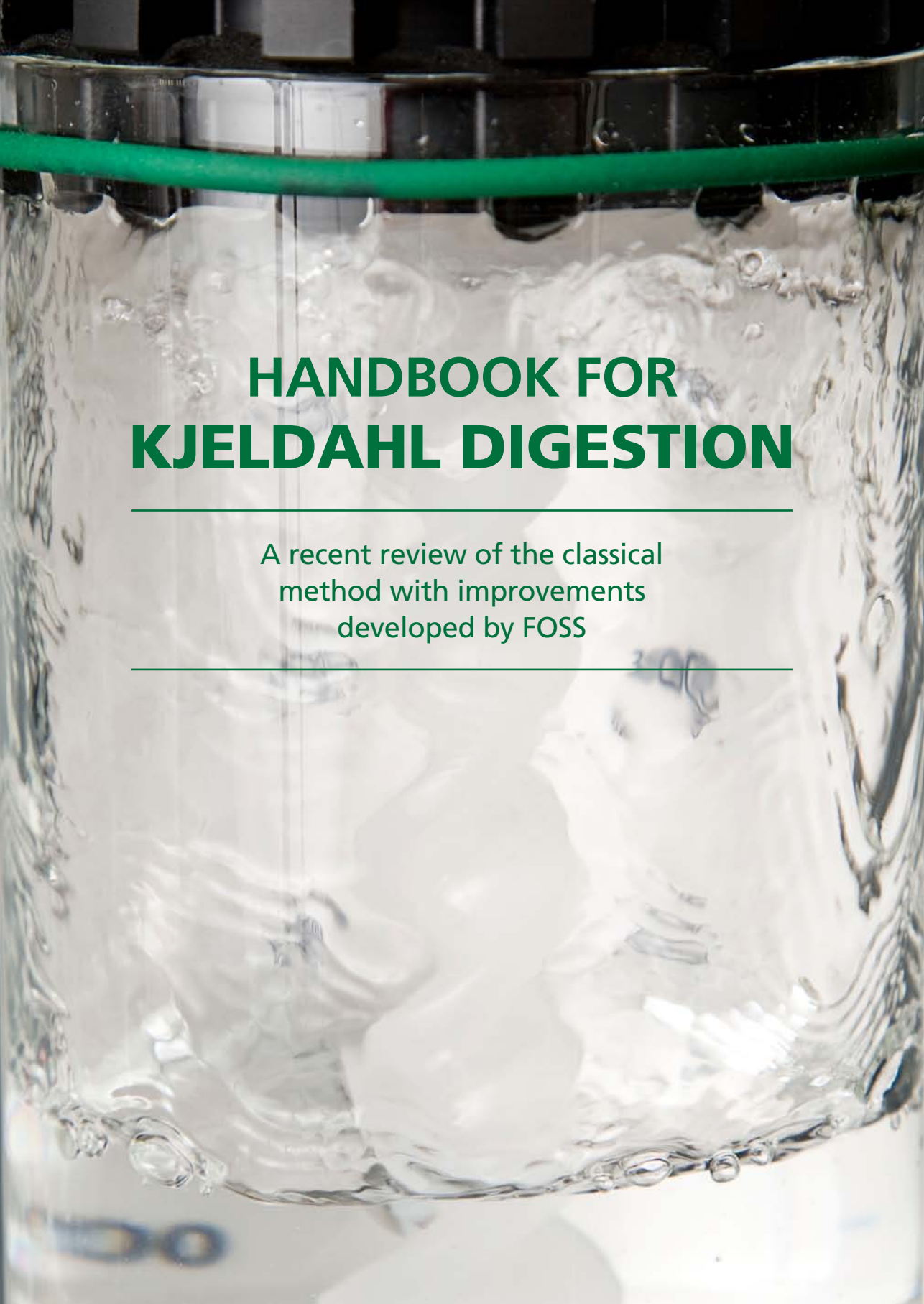

HANDBOOK FOR KJELDAHL DIGESTION

A recent review of the classical method with improvements developed by FOSS

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Foreword

FOSS is pleased to present this handbook for Kjeldahl digestion. The aim of this handbook is to provide a deeper knowledge about the Kjeldahl digestion procedure and to supply information that can be used in optimising the method for various types of samples.

This book is the result of the collective experiences and efforts done by our customers and distributors. We felt that it was important to compile available information into a format that was accessible to all.

The handbook is divided into separate sections covering different aspects of the digestion process. In order to simplify the reading of this book separate “conclusions” have been included in appropriate places.

For essential reading on the basic Kjeldahl digestion procedure, see Chapter 2.

We hope that this handbook will enable you to improve your Kjeldahl digestions and also provide you with a deeper insight into the chemistry of the digestion process.

Yours sincerely,
FOSS

Camilla Alvesson
Product Manager – Kjeldahl

Content

Digestion of samples for nitrogen analysis by the Kjeldahl method

1 Introduction

The Danish chemist Johan Kjeldahl, 1849 – 1900, developed what today is well known as the Kjeldahl method for analyzing nitrogen in organic substances[1]. He worked at the Carlsberg Laboratory where one of his tasks was to find a fast and reproducible nitrogen method that could determine the protein content in grains. This was to overcome the effects caused for the brewery by the varying protein contents in the grains. The lower the protein content in the mash, the higher return volume of beer is produced.

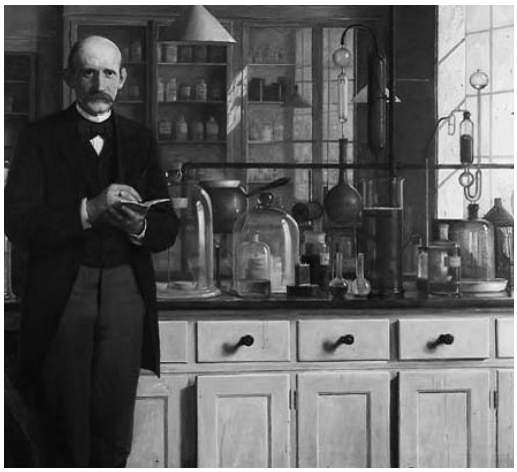


Figure 1. Johan Kjeldahl.

The original method as it was presented by Kjeldahl has been continuously improved. These developments have improved environmental and personal safety aspects, increased the speed and versatility of the method, and simplified the entire analytical procedure.

The Kjeldahl method has three different steps, digestion, distillation and titration. Of these, the initial digestion procedure, the most important and complex step is covered in this handbook.

Due to it's many areas of application, it is not practical to look at a single Kjeldahl digestion as a procedure to handle every sample type. However by utilising a simple non-optimised procedure as a starting point, it is relatively easy to develop the modifications required to achieve the necessary performance of the method for most, if not all, samples. For your convenience this basic procedure is included in chapter 2 of this handbook.

A consideration in developing a suitable modification of the method for a particular laboratory is the actual needs of that laboratory. For example, because many laboratories are required to perform Kjeldahl analysis on a wide range of different sample types, it may be neither desirable nor practical to use an optimised procedure for each type. Very often it is more appropriate to have a single robust procedure which can be applied to all samples passing through the laboratory. The benefit of this being that staff are required to use only one method which gives satisfactory precision in a reasonable timespan.

2 The Standard Kjeldahl Procedure

To simplify the Kjeldahl procedure FOSS has developed a robust and simple digestion method for use with nearly all sample types. More than 90 % of protein containing samples and over 60 % of all other nitrogen containing samples can be analysed using this basic procedure. This basic method might not be the fastest method to use for each sample type but will work as a straightforward solution that always will give satisfactory results. This method can be summarized as follows:

Sample amount:	1 g
H_2SO_4 volume:	12 ml
Salt/Catalysts:	2 Kjeltabs Cu/3.5 (3.5 g K_2SO_4 + 0.4 g $CuSO_4$ × 5 H_2O)
Digestion temperature:	420°C
Digestion time:	60 minutes

The actual amount of acid required will be dependent on variables such as the efficiency of the exhaust system, the amount of non-protein content in the sample and the degree of salting out effect experienced. In most laboratories the above procedure can be used without any type of modification.

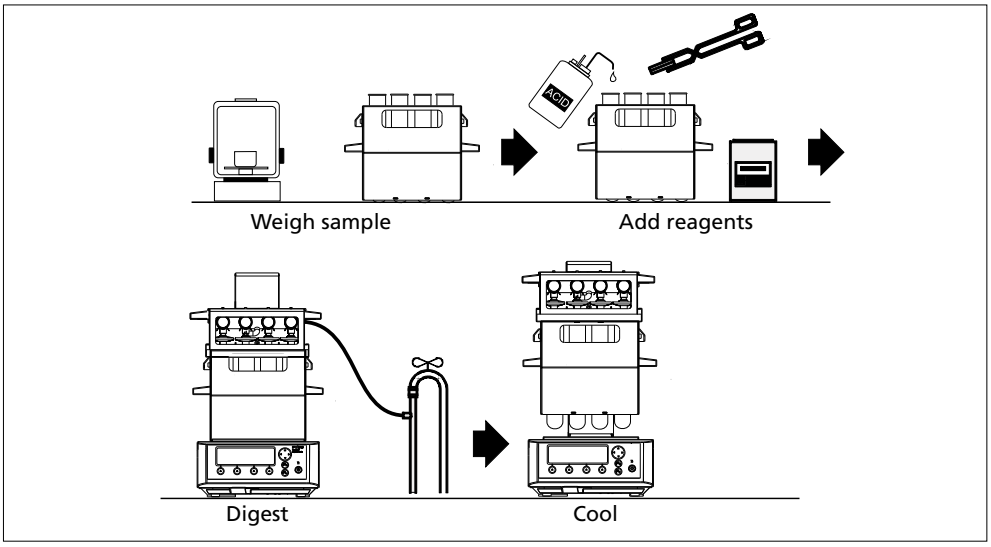


Figure 2. The Kjeldahl digestion procedure.

If you want help to optimize your application, contact a FOSS* representative. In order to support you properly FOSS has an extensive application library and a computerized Application Support System.

In FOSS Application Note AN 300, information about all the basic chemistry and the actual practical handling of the digestion systems and distilling units can be found. In order to find detailed information related to a specific sample type the corresponding Application Sub Note (ASN) system can be consulted. See chapter 8.

Based on the standard procedure described here internationally validated, global standard methods have been issued, see references 10 and 11, as well as Application Notes 300 and 3001.

* FOSS sales and service companies are located in 24 countries and represented around the world by more than 75 dedicated distributors.

3 Optimizing the Kjeldahl digestion

3.1 Sample preparation

Sample preparation for Kjeldahl analysis should be carefully performed to avoid errors in the final result. This procedure must involve one or more treatments to homogenize the sample, i.e. the particle size of the sample should ideally be reduced to a size < 1 mm. Homogeneity of the analytical sample improves the reproducibility of the method and also offers the possibility to reduce the sample size used without sacrificing the quality of the final results. Usually the speed of the digestion will be improved when small particle sizes are used.

Figure 3. Samples commonly analysed by the Kjeldahl method.

A good sample blender is necessary for the sample preparation. An overview of the possibilities offered by FOSS is given in section 7.1.

The Cyclotec™ mill is generally the most suitable choice for dry products such as grains and feed samples. The Cemotec™ mill offers the possibility to do accurate moisture tests on the ground sample, whereas the Knifetec™ mill is suitable for samples with a high moisture and/or fat content.

As a general rule the aim of the sample preparation step should be to obtain a particle size less than 1 mm.

3.1.1 Sample weight – Solids

For Kjeldahl analysis an analytical balance accurate to 0.1 mg should be used for weighing samples.

The actual weight of sample required for analysis depends primarily on the homogeneity of the sample. For non-homogenous samples high precision of the measurements can not be obtained using small sample sizes. For homogenous samples, weight is not as critical and can be optimized to give a suitable final titration volume, 2 – 20 ml.

Using a titrant concentration of 0.1 – 0.2 N acid the rough guide below can be used for selecting the sample size.

Protein content	**mg sample**
< 5 %	1000 – 5000
5 – 30 %	500 – 1500
> 30 %	200 – 1000

The analytical sample should ideally contain 30 – 140 mg N.

In order to be able to compare results with other laboratories it is also important to know the moisture content of the sample. This can either be done by correcting for the moisture content and reporting results on a dry basis or by always analysing predried samples.

When the moisture content is known the following formula can be used to calculate the result to a dry basis:

Content on dry basis = A × 100/ (100 - B)
where A = Sample content in % Protein
B = Moisture content in % Moisture

3.1.2 Sample volume – Liquids

For most of the liquid samples that are tested for nitrogen in food and feed the guidelines regarding sample weights for solid samples are valid. When analysing Total Kjeldahl Nitrogen (TKN) in water/wastewater where nitrogen content can be quite low, larger sample amounts need to be used. If a Kjeltec™ system is used for analyses, the following guideline can be used:

Nitrogen conc. mgN/l ml	**Volume of sample**
< 20	100
20 – 50	50
50 – 100	25

To obtain low detection limits a titrant concentration of 0.01 N should be used. Analysing the digest using a flow analysis technique smaller sample amounts can be used.

3.2 The digestion process

The aim of the digestion procedure is to break all nitrogen bonds in the sample and convert all of the nitrogen into ammonium ions. For this purpose sulphuric acid was originally used by Johan Kjeldahl and still remains the acid of choice. However, the sole use of sulphuric acid for digestion is not practical due to the slow speed of the digestion process. The reason for this is that the speed of the digestion and the breakdown of the sample depends not only on the properties of the acid, but also on the actual temperature used during digestion. The higher the temperature used, the faster digestion can be obtained. Using only sulphuric acid, the digestion temperature will mainly be limited by the boiling point of the sulphuric acid which is 338°C. It should be noted that the critical temperature for decomposition is as high as 373°C. The speed of the digestion can be greatly improved by the addition of salt and catalysts.

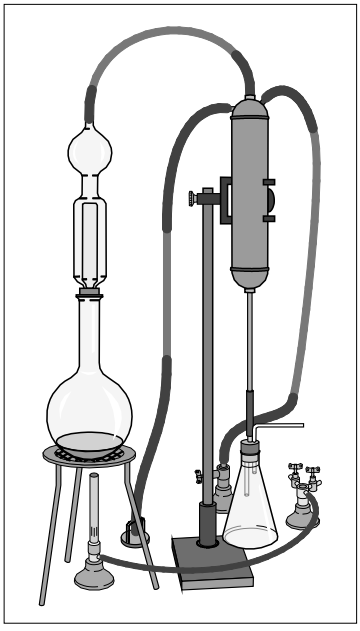


Figure 4. A classical Kjeldahl apparatus.

3.2.1 Acid requirements

In a classical Kjeldahl system, usually 25 ml acid per gram of sample is used whereas in FOSS Tecator digestors block the volume have been optimized so that most commonly only 12 ml acid is used.

The volume of sulphuric acid used in the digestion process can be optimized for a specific sample type. In order to do this we have to have an understanding of the action of the acid during the digestion, i.e. we have to look at the amount of acid that is lost by evaporation, reacts with other reagents and reacts with the sample.

In reality we can easily control evaporation losses in the digestion system and also the amount of reagents used. This simplifies our optimization as the main factor to consider is the properties of the sample.

The total amount of acid needed during a digestion can vary quite a lot from one sample type to another, i.e. in comparison with a wheat sample up to 4 ml more acid may be consumed by a sample rich in fat.

NOTE! If the acid amount used in the digestion is increased, the amount of alkali used in the distillation should be increased proportionally.

3.2.1.1 Acid consumption by the sample

In order to understand this process we have to consider the composition of each sample. In Table 1, the acid consumption of various sample constituents is given[2].

Table 1. Consumption of acid by constituents in samples.

Sample Component	**Acid consumption ml H_2SO_4 / g component**
Soil, organic C	10.0
Fat	9.7
Protein	4.9
Salicylic acid	6.8
Carbohydrate	4.0
Clay	0.6
Sand	0.0

From this Table we can see that samples high in fat content will consume more acid than those that are high in protein or carbohydrate content.

As an example, we can calculate the acid consumption obtained by a wheat sample.

Assume that the wheat sample has the following composition:

Protein	12.5 %
Carbohydrate	66.5 %
Fat	3.5 %

For a digestion of this wheat sample with a weight of 1.0 gram, the acid consumed by the sample would then be as follows:

Protein	12.5 % × 1.0 × 4.9 = 0.61 ml
Carbohydrate	66.5 % × 1.0 × 4.0 = 2.66 ml
Fat	3.5 % × 1.0 × 9.7 = 0.34 ml
	3.61 ml

In total, this wheat sample would consume a volume of 3.61 ml sulphuric acid during the digestion.

As a comparison a sample such as a sausage, rich in fat, would consume 6.9 ml sulphuric acid during the digestion. To compensate for this and obtain the same digestion conditions as for the wheat sample, an initial volume which is 3 ml larger than for the wheat sample has to be used. Similar calculations can be made for other samples with differing contents and the results obtained can be used to modify already proven applications into new areas of usage.

3.2.1.2 Acid consumption by the reagents

Potassium sulphate is added in all Kjeldahl digestions and some sulphuric acid reacts to form potassium hydrogen sulphate according to the following formula:

$K_2SO_4 + H_2SO_4 \rightarrow 2\ K_2HSO_4$

One gram of potassium sulphate consumes about ~0.3 ml sulphuric acid, i.e. using 2 Kjeltabs (7g K_2SO_4) about 2.1 ml acid is consumed.

In all ASN's this consumption has been accounted for since the salt addition is always constant.

However, in some applications special reagents are added to increase the scope of the Kjeldahl method. The most commonly used are sodium thiosulphate or salicylic acid which can be added to include nitrate and nitrite in the measurements.

Sodium thiosulphate reacts with sulphuric acid such that the final product is sulphur dioxide. In this process 1 g of sodium thiosulphate pentahydrate consumes 0.5 ml sulphuric acid.

Salicylic acid is decomposed to carbon dioxide and water when it reacts with sulphuric acid. During this process 1 g salicylic acid consumes 5.6 ml sulphuric acid.

Sucrose is decomposed to carbon dioxide and water. During this process 1 g sucrose consumes 1.9 ml sulphuric acid.

The consumption of sulphuric acid has to be accounted for in digestions when these reagents are used.

3.2.1.3 Acid losses by evaporation

Another factor to consider is the loss of acid that occurs due to evaporation through the exhaust system used. In conventional classical Kjeldahl systems quite large volumes of acid are evaporated into the environment.

Studies conducted to measure the total evaporation loss of acid during a digestion have given some important facts. With a classical digestion setup about 5 % of the total amount of acid is lost during the first 15 minutes of digestion. The loss becomes lower as the digestion goes on but still as an average 3 to 5 % of the sulphuric acid is lost per 15 minutes. Using an acid volume of 25 ml and a digestion time of 2 h as in AOAC 920.87 this corresponds to an evaporation loss of 7.2 ml acid per sample analysed.

In the FOSS Tecator Digestion systems the combined function of the heat shields and the exhaust system acts to control acid loss. During the initial part of the digestion process, when excessive fumes are formed due to the

high reaction rate with the acid, the exhaust is operated at full flow rate for 5-10 minutes. The flow rate should then be decreased so that fumes are retained in the digestion tube throughout the rest of the digestion period. In order to minimize losses into the environment it is important to decrease the aspirating effect of the exhaust to the correct level. An automatic digestor combined with scrubber can ensure correct conditions are achieved time after time. Using this system ~8 % of the acid is lost during the first 15 minutes but for the remaining part of the digestion when the exhaust is on low, only 0.8 % of the acid is lost per 15 minutes digestion time. In total this corresponds to a total acid loss through evaporation of only 1.2 ml acid when using 12 ml acid during a 60 minute digestion[3].

By replacing a classical Kjeldahl system with a FOSS Tecator Digestion system the evaporation loss per sample can be reduced from 7.2 to only 1.2 ml acid per sample, i.e. a total saving of 6.0 ml/test. During a year the difference will be substantial, for each 20 samples analysed a saving of 120 ml acid is obtained. From an environmental point of view this is definitely a substantial benefit.

Avoid using digestors without exhaust systems. This will dramatically shorten the life of the digestor and cause expensive damage to your fume cupboard.

3.2.1.4 Conclusion, acid requirements

By replacing classical Kjeldahl digestion systems with aluminium block digestors and efficient exhaust heads most of the environmental issues related to the Kjeldahl method can be minimized. In Table 2, an overview of the total acid consumption during the digestion is given.

Table 2. A comparison of acid consumption between a classical Kjeldahl system and Tecator block digestor system.

	Block Digestor	**Classical Kjeldahl**
Acid volume used	12 ml	25 ml
Loss by evaporation	1.2 ml	7.2 ml
Consumption by 1 g sample	3.6 – 7 ml	3.6 – 7 ml
Consumption by reagents	2.1 ml	4.2 ml
Remaining in digestion tube	1.7 – 5.1 ml	6.6 – 10.0 ml
Alkali volume used	50 ml	100 ml

This table shows that we gain two major environmental advantages by using a block digestor, i.e. the acid loss by evaporation is minimized and the volume of sodium hydroxide that needs to be added during the distillation is reduced.

From an environmental point of view the acid remaining in the tube is not really critical because it is converted to sodium sulphate by the alkali added during the distillation.The major concern refers to the amount that evaporates into our environment. To eliminate this a Scrubber system that neutralizes the fumes should be used.

A problem that may occur during the digestion is drying out of the digest, a process called “salting out effect”. The common cause of this is that the exhaust has been operated at too high a flow rate so that excessive evaporation loss has taken place. The optimal way of preventing this is to readjust the exhaust level to proper conditions or to initially add 2 – 3 ml extra acid to compensate for this loss.

3.2.2 Salt requirements

The speed of the digestion process is dependent on the temperature used. By increasing the temperature the time needed to complete a digestion can be decreased. However, only increasing the temperature of the digestor cannot increase the actual digestion temperature since this is controlled by the boiling point of the acid. The digestion temperature can never exceed the boiling point of the acid.

The boiling point can be increased by adding a salt to the acid. For Kjeldahl digestions potassium sulphate is the most suitable salt to use because of it’s high solubility in sulphuric acid. The first use of potassium sulphate was reported already in 1889 and it has since proved to be the most efficient salt to use. Several times in the history of the Kjeldahl method, salts other than potassium sulphate have been tested but these attempts have generally been unsuccessful.

When potassium sulphate is added part of the acid will be consumed by reactions with the salt. From a practical point of view, this is of minor importance and can be compensated for.

3.2.2.1 Acid salt ratio

One of the keys to a successful digestion is the acid salt ratio. The ratio between acid salt determines the boiling point of the acid and hence also the digestion time needed. The acid salt ratio is determined by dividing the millilitres of sulphuric acid by the grams of potassium sulphate salt added. There are practical limits to which ratios may be used. Reaction temperature which is too high can result in loss of nitrogen in the form of nitrogen gas. However, with the precise temperature control of Tecator digestors block this is unlikely to happen. The upper temperature limit that will be reached is either determined by the boiling point of the acid: salt mixture or by the setting on the block digestor if the boiling point exceeds that of the block setting.

The choice of the ratio between the acid and the salt depends on factors such as, acid consumption by salts added, acid consumption by the constituents in the sample, digestion time used and the digestion equipment used. In practice all of these variables can be kept constant. It is only the sample content that has to be considered.

Typical initial acid: salt ratios range from 1.4 to 2.0. For samples where acid consumption is higher, i.e. samples with high fat content, initial acid salt ratios may be in the range 2.5 to 2.8.

From an environmental point of view it is also important to use an optimal ratio since both chemicals and digestion time needed can be minimized.

3.2.3 Conclusion, salt requirements

The digestion time can be decreased by increasing the boiling point of the sulphuric acid. This is done by optimizing the acid/salt ratio. Different sample types require different initial acid/salt ratios due to the variations in acid consumption caused by the samples. If optimized procedures are used it is important to keep close control of this ratio if stable and repeatable results are to be obtained.

The best way to avoid variations in the salt addition is to use a standardized product such as Kjeltabs.

3.3 Catalysts

The speed and efficiency of the digestion is not only influenced by the temperature used but can also be improved by the addition of a suitable catalyst. In 1885, only 2 years after the original method was published, papers appeared where catalysts were added to increase the speed and efficiency of the digestion procedure. During the years many systematic studies on the efficiency of various catalysts have been made. A conclusion from all of these studies is that mercury, selenium and copper are the Catalysts of choice. For certain applications also titanium has found some usage. In Table 3, a comparison of digestion efficiency for the most commonly used catalysts can be found[4].

Table 3. The influence of catalysts on the digestion time needed.

		% protein			
	Min.	**Hg**	**Se**	**Cu**	**Ti**
Dog food	10	–	–	–	–
	20	25.6 ± 0.07	25.1 ± 0.10	24.8 ± 0.19	25.0 ± 0.17
	30	25.6 ± 0.14	25.4 ± 0.15	25.2 ± 0.22	25.3 ± 0.27
	45	25.7 ± 0.13	25.4 ± 0.16	25.4 ± 0.11	25.5 ± 0.25
	60	25.7 ± 0.07	25.6 ± 0.11	25.5 ± 0.12	25.6 ± 0.22
Meat	10	–	–	–	–
	20	18.0 ± 0.26	17.7 ± 0.33	17.4 ± 0.23	17.2 ± 1.07
	30	18.0 ± 0.15	17.7 ± 0.27	17.8 ± 0.22	17.9 ± 0.37
	45	18.0 ± 0.13	17.9 ± 0.17	17.8 ± 0.24	18.0 ± 0.32
	60	18.2 ± 0.09	17.9 ± 0.24	18.1 ± 0.23	18.0 ± 0.06
Fishmeal	10	69.1 ± 1.62	65.0 ± 0.75	64.7 ± 1.21	64.8 ± 1.15
	20	72.4 ± 0.22	70.1 ± 0.66	72.6 ± 2.83	69.5 ± 0.54
	30	73.0 ± 0.31	71.0 ± 0.23	70.6 ± 0.74	70.4 ± 0.34
	45	72.7 ± 0.14	71.7 ± 0.19	71.4 ± 0.39	71.4 ± 0.43
	60	72.7 ± 0.28	72.2 ± 0.15	72.3 ± 0.21	72.3 ± 0.45
Wheat	10	11.4 ± 0.31	11.2 ± 0.24	1.0 ± 0.19	11.0 ± 0.29
	20	11.7 ± 0.06	11.6 ± 0.07	11.6 ± 0.06	11.0 ± 0.03
	30	11.7 ± 0.08	11.6 ± 0.03	11.6 ± 0.07	11.7 ± 0.22
	45	11.7 ± 0.07	11.6 ± 0.04	11.6 ± 0.05	11.7 ± 0.06
	60	11.7 ± 0.05	11.7 ± 0.07	11.7 ± 0.04	11.7 ± 0.05
Lysine-HCl	10	–	–	–	–
	20	14.8 ± 0.18	13.0 ± 1.00	12.6 ± 0.60	12.5 ± 0.32
	30	15.3 ± 0.12	13.3 ± 0.72	13.0 ± 0.19	12.9 ± 0.48
	45	15.3 ± 0.16	13.9 ± 0.56	13.4 ± 0.56	13.7 ± 0.32
	60	15.3 ± 0.09	14.2 ± 0.27	13.8 ± 0.27	14.2 ± 0.55

As can be seen from this table, mercury is the most efficient catalyst. The effect is most pronounced on lysine and fishmeal. However, in the majority of cases the same results can be obtained using other catalysts although often longer digestion times have to be used.

As protein determinations often are performed for trading purposes, it is important that both parties involved base their result evaluation from tests using similar analytical procedures.

The use of copper as catalyst is becoming more common, as it is recognized to be more environmentally friendly. Today selenium or copper are used as catalysts in more than 90 % of the Kjeldahl digestions being performed all over the world. Mercury together with mixed catalysts are declining in usage.

3.3.1 Mercury

Mercury was initially discarded for usage as a catalyst as it formed a complex with ammonia that prevented ammonia being distilled. However, further research showed that this complex could be destroyed by the addition of alkali sulphide or thiosulphate.

After this modification was introduced it could be shown that mercuric oxide, HgO, was the most efficient catalyst available. It can be used for a wide range of applications, covering industrial samples like coal, leather, feed, food and wastewater.

Due to the negative environmental effects of mercury, usage has declined. In nearly all applications it has now been replaced by other catalysts such as selenium or copper. As can be seen in table 3, these catalysts are not as efficient as mercury but by using a slightly longer digestion time similar results can be obtained.

When mercury is used, collect all waste for safe disposal.

3.3.2 Copper

Copper was the first metal that was used as a catalyst. In most cases copper is a satisfactory catalyst, although it usually gives a slower digestion process than mercury and selenium. Copper sulphate, $CuSO_4$, has been shown to be the most effective copper catalyst and this salt also meets the requirements of a modern Kjeldahl procedure, i.e. speed, efficiency and environmental safety.

Intensive tests have been performed for many types of samples, comparing mercury and copper catalysts. In most cases similar results can be obtained and the effect of these studies have been that mercury has started to be replaced by copper as the catalyst of choice also in official procedures by ISO, AOAC etc.[5]

Today copper is the most frequently used catalyst and has replaced mercury and selenium as catalyst in many different types of applications.

3.3.3 Selenium

Selenium was first used as a catalyst 1931 and has since then been the subject of many studies. It is a controversial catalyst generating many reports about erratic results, incomplete recovery or loss of nitrogen.

Losses of nitrogen can take place especially when larger amounts of the catalyst are used, > 0.25 g, or when long digestion times are used. During the digestion losses can start to occur at ~390°C. The actual temperature where losses begin depends on the conditions in the reaction mixture and are therefore hard to predict. From a general point of view selenium is more difficult to work with than other catalysts as the function is much more sensitive to the acid: salt ratio obtained. If unfavourable conditions are created losses will occur.

Due to all of these effects many basic studies have been conducted. This research has given valuable insight into the kinetics and chemistry of the Kjeldahl digestion process.

3.3.4 Mixed catalysts

Mixed catalysts have been tested in search of a catalytic activity higher than that obtained by single catalysts. However, in most applications no significant advantage over a single catalyst has been achieved.

Investigations on single and mixed catalysts using single catalysts such as selenium, copper, mercury, vanadium or tellurium and mixtures of them have been made. In these tests also varying levels of potassium sulphate have been used to evaluate all different aspects of the digestion process. The conclusion of these tests was that no mixture could show a better performance than a single catalyst.

Today the only mixed catalyst that has found usage is a 1:1 mixture of copper and titanium oxide. This catalyst is officially accepted by AOCS, AOAC & AACC for applications in oilseed testing, and by ISO for grain testing.

3.3.5 Conclusion

Environmentally the pressure to replace mercury as a catalyst with more harmless substances has been strong since the seventies. Today copper and to some extent selenium are clearly the dominating catalysts. One restraint in this process has been the fact that most of the protein testing done is regulated by official methods. It has been important to work according to these procedures as the results obtained usually have been used in trading of raw materials or for labelling purposes.

The use of mercury catalysts can nearly always be replaced by copper without sacrificing the analytical accuracy although a longer digestion process is necessary.

In recently published or updated official procedures the trend is clearly towards usage of copper.

3.4 Reducing agents

Originally Kjeldahl's method was designed to determine protein. The addition of salt and catalyst improved the method and widened the range of sample types possible to analyse. However, many compounds were still classified as refractory as their nitrogen could not be recovered even by these modifications. In order to include these compounds in the measurement, it became necessary to reduce them prior to the actual digestion.

The reduction can be effected in two ways, either by pretreatment before the digestion or during the digestion itself. Practically, it is preferable to do it during digestion but many compounds cannot be reduced by this procedure and must be reduced by other means. Common areas of usage are fertilizers, soil and water. All applications where nitrate should be included in the test. For further information it is recommended to use the Official **Methods** of Analysis of **AOAC** INTERNATIONAL (OMA), 18th Edition, Revision 2, 2007 published by the Association of Official Analytical Chemists (AOAC).

Commonly used reducing agents are chromium, zinc, iron sulphate, salicylic acid and sucrose.

See respective ASN for specific information regarding the usage of these compunds.

Sucrose or glucose is also commonly included in digestions of blank samples in order to get an acid consumption similar to the samples.

3.5 Oxidizing agents

Oxidizing organic material in boiling sulphuric acid is normally a rather slow process. It can be accelerated by addition of salt and catalysts but oxidizing agents can also be added to improve the speed even further. Even the original Kjeldahl method used powdered potassium permanganate to complete the oxidation process. However, this procedure was quite controversial as large variations in the recovery of nitrogen occurred. The use of permanganate is now discontinued.

Today the only commonly used oxidizing agents are hydrogen peroxide and potassium persulphate.

Of these two hydrogen peroxide has the widest usage and it has two functions:

1. As an oxidizing agent to accelerate the decomposition of organic material.
2. As an antifoaming agent to control foaming during the digestion.

Use of hydrogen peroxide which is **extremely reactive** in the presence of sulphuric acid can cause loss of nitrogen as N_2 gas and in most cases no appreciable improvement of the digestion time can be obtained[6].

Undoubtedly there are benefits as an antifoam particularly when the sample contains fats and / or carbohydrates. If peroxide is used it should be added in small quantities < 5 ml slowly down the inner walls of the digestion tube. As a general rule, unless experience shows an improvement in digestion conditions, it is preferrable not to use peroxide.

Although improvements of the digestion time can be obtained by using hydrogen peroxide as an oxidizing agent it is better to depend on the higher temperatures obtained by salt addition. In general the risk for nitrogen losses is too high to justify the time that can be gained.

3.6 Boiling time

The term “boiling time” should be divided in two parts. First, the time it takes until the digestate has cleared or become colourless, usually called “digestion time”. Next, the “after boil” time, to convert the last part of the nitrogen into a form that can be distilled, usually called “boil period”.

The intermediate compounds formed during digestion and especially during the early stage of the digestion, can be more resistant to decomposition than the original sample. If the temperature is at or near the decomposition point of the intermediate, an extended boiling time is necessary as the decomposition proceeds slowly. In theory the boiling time should be determined for each compound under the specific conditions used. In practice, this is not possible, however a “boiling time” two to three times the clearing time is usually sufficient to achieve complete recovery.

The total time needed for digestion depends on many factors:

- Type of sample
- Amount of salt
- Oxidizing agent
- Temperature of block digestor
- Volume of acid
- Catalyst used
- Reducing agent

The time needed for boiling after clearing is strongly dependent on the digestion temperature, i.e. if too much acid is used, it will take time to evaporate enough acid to achieve a proper acid: salt ratio.

The progress of the digestion can easily be followed by stopping the digestion at various time intervals and determining the nitrogen. By plotting nitrogen recovery vs digestion time, a graph can be constructed.

Figure 5 illustrates the fact that different sample types behave in different ways. For some, a complete digestion is obtained at the point of clearing (substance B) but for others a prolonged digestion time is needed (substance A). For substance A, the recovery of nitrogen increases slightly with pro-

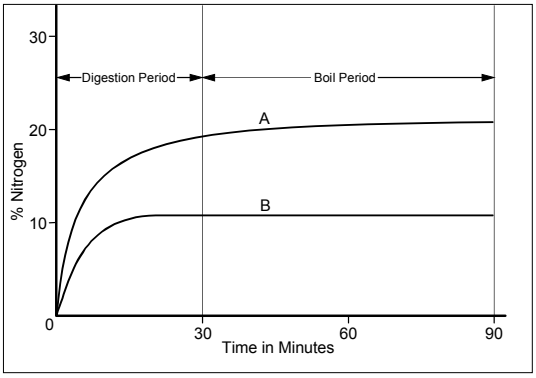


Figure 5. Recovery of nitrogen as a function of digestion time.

longed digestion time and in total about 60 minutes digestion is needed to get > 99 %recovery.

At the point of clearing, recoveries in the range 95 – 100 % are commonly obtained. By extending the digestion time with a "boil period", recoveries can easily be optimized for each individual compound.

Using optimal conditions, digestion times can be dramatically reduced in comparison with commonly used procedures. An example is given in Table 4. These data were obtained by using an acid: salt ratio of 1:3 using mercury as the catalyst. Samples were only heated to the point of clearing with no boil period. Recoveries were compared with theoretical values[7].

Table 4. Recovery of nitrogen at the point of clearing for pure test substances.

Compound	**Digestion time (min) Point of clearing**	**Recovery%**
Acetanilide	11	99.8
Alanine	9	99.7
Arginine	9	100.5
Barbituric acid	11	99.4
Glycine	15	101.5
Hydroxyproline	10.5	99.5
l-Lysine	15	100.3
Methionine	14	98.8
Nicotinic acid	18	100.1
Tryptophan	16	100.1
Tyrosine	12	99.5
Urea	11	100.1

As can be seen, time to reach clearing varies. Tryptophan needs 80 % longer time than alanine. Tryptophan is also sensitive to the temperature used, i.e. if an acid: salt ratio of 1.1 is used, recovery decreases with 5 %.

A similar study on some natural products is shown in Table 5. Conditions were the same as above and results were compared with determinations using official procedures[7].

Table 5. Recovery of nitrogen at the point of clearing for natural products.

Compound	**Digestion time (min)**	**Recovery %**
Casein	23	99.9
Egg albumin (soluble)	23	99.9
Flour	14	100.0
Hide powder	21	100.3
Leather	16	100.7
Oats	26	100.7

Large variations in the required digestion time can be seen. Although satisfactory results were obtained with these short digestion times, close control of digestion conditions is needed.

To obtain a digestion procedure which can be used for reliable routine analysis, safety margins with regard to time used should be used. Usually a time period of 10 – 20 minutes "after boil" time is sufficient.

Using classical Kjeldahl digestion apparatus boiling periods in the range of 0 – 235 hours have been reported. Rice, wheat flour, oat meal and bran are completely decomposed after the clearing time, while certain types of coal require up to 235 hours boiling period after clearing.

The digestion is not complete if the digest is cloudy or hazy, or if black carbonized particles are floating in the acid or clinging to the side walls of the digestion tube. Basically a complete digestion must be clear although it might contain a light colour. This colour can range from a pale blue-green to a pale yellow-orange depending on the catalyst used.

In order to minimize the digestion time, it is important to use an acid salt ratio that is suitable to the sample type analysed.

Using classical Kjeldahl apparatus, a general digestion procedure for routine

use requires a minimum of 2 hours total digestion. With the improved sample to sample temperature control obtained with Tecator digestion blocks and the improved control of the other digestion parameters, the "after boil" period has been significantly reduced. Most agricultural materials can be digested in 30 – 60 minutes. See ASN's for further information.

3.7 Foaming during digestion

One common problem in Kjeldahl digestions is foaming that can occur during the initial part of the digestion. This tendency is strongest for samples high in fat, oil, carbohydrates or for samples containing high amounts of surface active agents. Usually foaming can be controlled but it can also be a limitation, especially for samples with low nitrogen contents where the problem is accentuated as large sample volumes have to be used, i.e. foaming is more difficult to control when the liquid level is high in the tube.
In order to handle a large volume, > 40 ml of liquid in the digestion tube the use of boiling rods is recommended. The rods often give a superior effect to boiling stones or chips.

Another way of controlling foaming is to add hydrogen peroxide. This reagent has two effects as it acts both as an oxidizing agent and as an antifoaming agent. Hydrogen peroxide can usually give substantial improvements with regard to foaming but it's use is controversial as losses of nitrogen has been reported.

If foaming is the only problem it is better to use 1 – 3 drops of octanol or a proprietary antifoam emulsion.

Commonly the addition of antifoaming substances is combined with the use of slow heating during the initial part of the digestion. Using an automatic digestor, gradual heating of the block digestor can be automated. One procedure is to place the tubes in the digestor at ~200°C, slowly raising the temperature to 420°C and then digest for the time required.

Another option to reduce foaming is to add all the reagents to the sample in the digestion tube and let the tubes stand overnight at room temperature before digestion. Although this procedure limits the sample turnaround time in the laboratory it is a very effective way to reducing foaming problems.
The extent of foaming is directly related to the amount of sample used. It is

always possible to minimize foaming by reducing the sample amount used. Limitations to this strategy can include sample nonhomogeneity or low nitrogen content in the sample, both conditions require the sample size to be kept large.

Generally the 250 ml tubes make for easier sample handling than the 100 ml tubes. The larger tubes provide the flexibility to handle the broadest range of samples, sample sizes and applications. They also handle foaming problems better than the 100 ml tubes.

For Applications where larger sample sizes are required, such as in water analysis, or where foaming can present problems, such as beer analysis, 400 ml tubes are available.

The additional capacity helps to overcome some of the issues arising.

4 Limitations of Kjeldahl digestion

The Kjeldahl method was originally designed for the determination of proteins. Modifications to the original method have increased versatility so that many different sample types can be determined.

However, there are still many compounds that are classified as refractory since their nitrogen can not be easily recovered.

Refractory compounds are those containing the nitrogen in an oxidized form or as heterocyclic compounds. Examples of such substances are nitrate, nitrite, alkaloids, pyridine, quinoline derivates, triazoles, pyrazolones, aminopyrine and antipyrine.

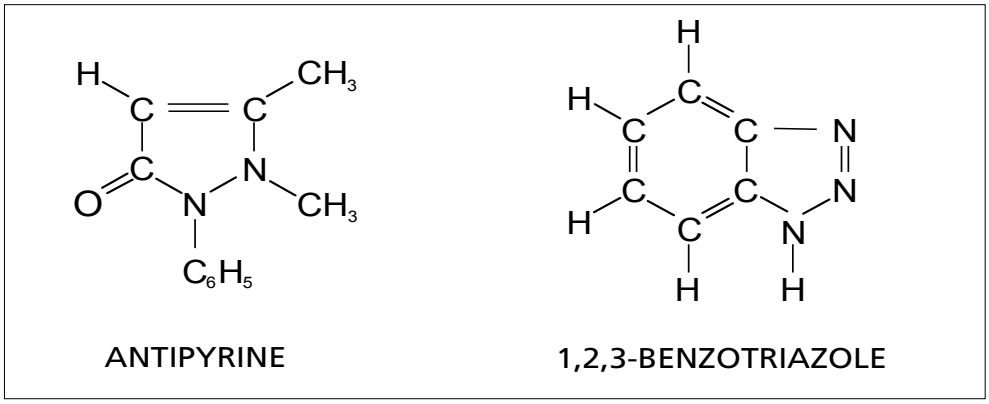


Figure 6. Examples of refractory compounds.

Samples containing refractory compounds need to be pretreated before the Kjeldahl digestion. For example, by adding reducing agents, nitrate can be included in the analysis.

Where applicable, specific information is given in respective ASN.

5 Digestion procedure using Tecator™ digestion systems

5.1 General

The Kjeldahl method received attention in the beginning of 1883. During the years much effort has been made in developing the technique but basically little has been changed after the initial development phase.

It was not until 1970 when a revolution for Kjeldahl laboratories occurred. The first block digestors were developed by Roger Mossberg at Tecator, now FOSS. The block digestors made it possible to improve the speed and accuracy of the digestion procedure as well as save space, chemicals and energy.

The digestion system has since been improved by the addition of exhaust systems, heat shields, reagent handling systems, tube racks, etc. The system of today represents an efficient analytical system with a high degree of user friendliness. Ease of use and sample throughput have been maximized but laboratory requirements and reagent usage has been minimized. As a result of this, todays digestion systems offers safety both from a personal perspective and from an environmental point of view.

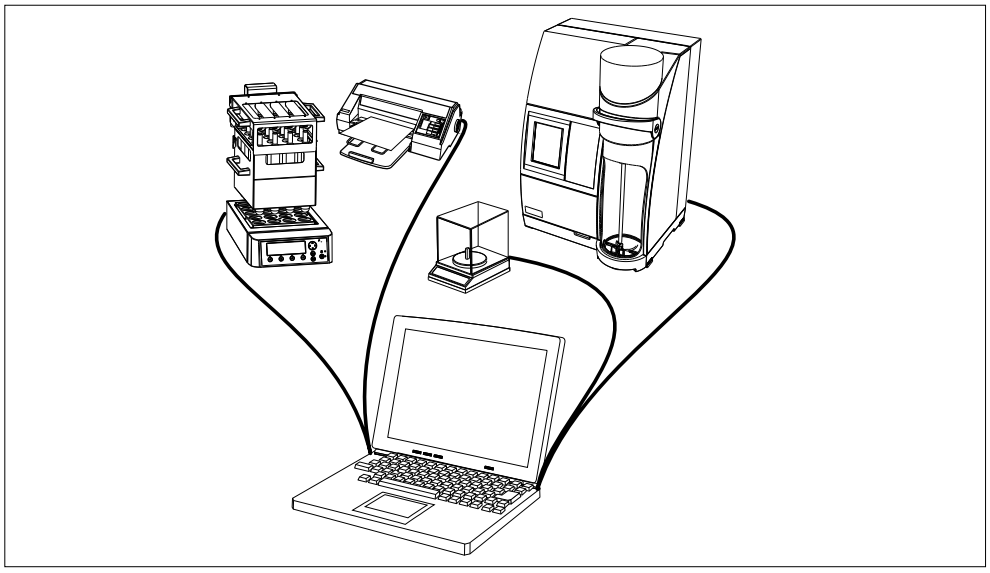


Figure 7. A complete Kjeltec™ System for Nitrogen determination.

A comparison of the classical Kjeldahl digestion procedure vs the Tecator digestion block procedure is given in table 6.

Table 6. The Kjeldahl digestion method, an overview.

	Classical	**Kjeltec™**
Space	5 – 6 units/2 m (100/day)	1.5 m (100/day)
Sample Size	0.1 – 5 g	0.1 – 5 g
Acid Consumption	25 ml 12 ml	
Alkali Consumption	100 ml 50 ml	
Heating	Individual heaters without close control of temperature	Block digestor with close control of temperature
Digestion Time	2 – 3 hours	45 – 60 minutes
Distillation Time	20 minutes	4 minutes
Fume Control	Often problematic	Efficient exhaust systems
Catalyst	Salt & catalyst addition (14 g)	2 Kjeltabs (7 g)
Heat Generation	High heat radiation	Small heat radiation
Power Consumption	~2.5 kWh / sample	~0.1 kWh / sample

As can be seen from the Table, by using the Tecator block digestion system, the total time required for digestion has been reduced by a factor of three, power consumption reduced 95 % and chemical consumption 50 %. All are substantial improvements to the classical procedure.

The Tecator digestion systems of today are of the most advanced design to be found in a modern Kjeldahl laboratory. They cover a range from manual to totally automated systems. Accessories such as Lifts, Exhausts, Reflux heads and Scrubbers enable flexible automation of the systems.

5.2 Block digestor

Block digestors were developed by Tecator to overcome limitations in traditional Kjeldahl digestion but also to optimize the digestion procedure.

The use of block digestors introduced the possibility to optimize the Kjeldahl digestion with regard to time and chemicals needed. Block digestors are easily adapted to the needs of different laboratories. Units are available

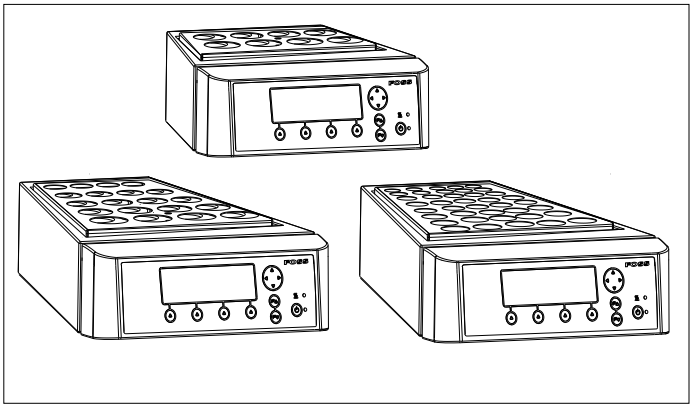


Figure 8.
A. Digestor 2508 Auto
B. Digestor 2520 Auto
C. Digestor 2540 Auto

in 8, 20 and 40 place format. The latter taking 100 ml tubes only whilst the 8 & 20 place version can be combined with 100, 250 and 400 ml tubes.

Heating elements inside the block make it possible to control the temperature with a high degree of precision. To enhance safety all digestors are equipped with over-temperature protection circuits.

Major advantages are the small size of the systems and the high degree of analytical accuracy that can be obtained. These systems also generate much less excess heat and waste than conventional digestion systems.

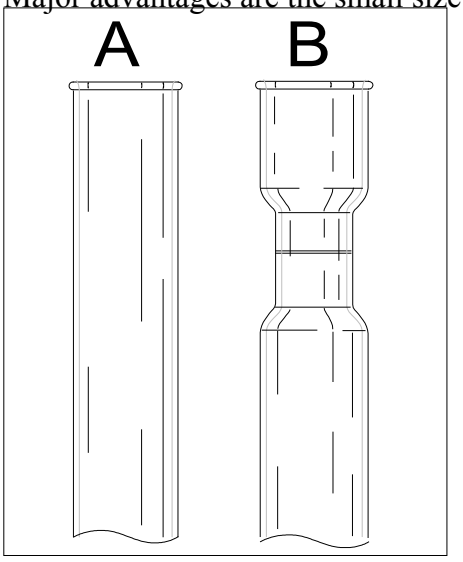


Figure 9.
A. Digestion tube, straight model.
B. Digestion tube with constriction and volume mark.

With block digestors the volume of acid can be reduced compared to a classical digestion. A traditional method recommends use of 20 to 30 ml H_2SO_4, however it is usually sufficient to use 10 to 15 ml in a modern Tecator digestion system using a 250 or 400 ml tube. For a micro digestion, with 100 ml tube only 2 to 5 ml need to be used.

The larger size tubes are usually preferred due to their broader versatility. In situations where automatic flow analysis systems are used for the analysis step, it

is practical to use constricted volumetric digestion tubes. Using such tubes, the final dilution to volume can be made directly in the tube.

The new generation of digestors are simpler to use and safer compared to a traditional Kjeldahl apparatus and in 1982 the first official approvals were obtained by AOAC[8].

5.3 Integrated Controllers

Most digestions require only the control of a single temperature and time, for example 420°C for 60 minutes. However where foaming, or larger volume, samples are used it is often desirable to ramp the temperature in two or more temperature/time sequences. Using such sequences dramatically reduces foaming and enables large volume samples such as water to be evaporated without risk of boil over and loss of sample. The exhaust is operated at high flow rate during this initial evaporation.

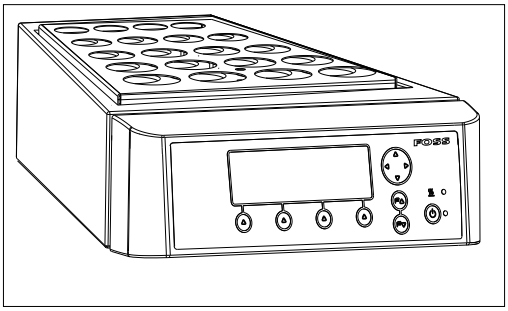


Figure 10. Digestor with built in controller.

All FOSS Tecator digestors have integrated controllers. The Basic units have simple, single temperature and time control. The Auto units have multi-functional programmable control as standard.

NOTE! Some official water testing standards specify 380°C. This may lengthen the digestion time, but is acceptable.

The Auto systems also enable control of a Lift system and/or Scrubber when connected. In the case of the Scrubber the program will automatically turn on, adjust and turn off according to the selected application. Additional features that maintain precise and repeatable analytical conditions independent of the operator.

5.4 Fume removal systems

In all Kjeldahl digestions some acid fumes will be generated during heating of the sample. In order to control the emission of these fumes to the environment an exhaust system should be used. An exhaust system efficiently controls fumes, but systems should nonetheless be installed in a fume cupboard or fume hood as a safety precaution. The exhaust head sits on top of the tubes within the tube rack and vapours are transferred and diluted to drain or alternatively to a closed neutralising scrubber.

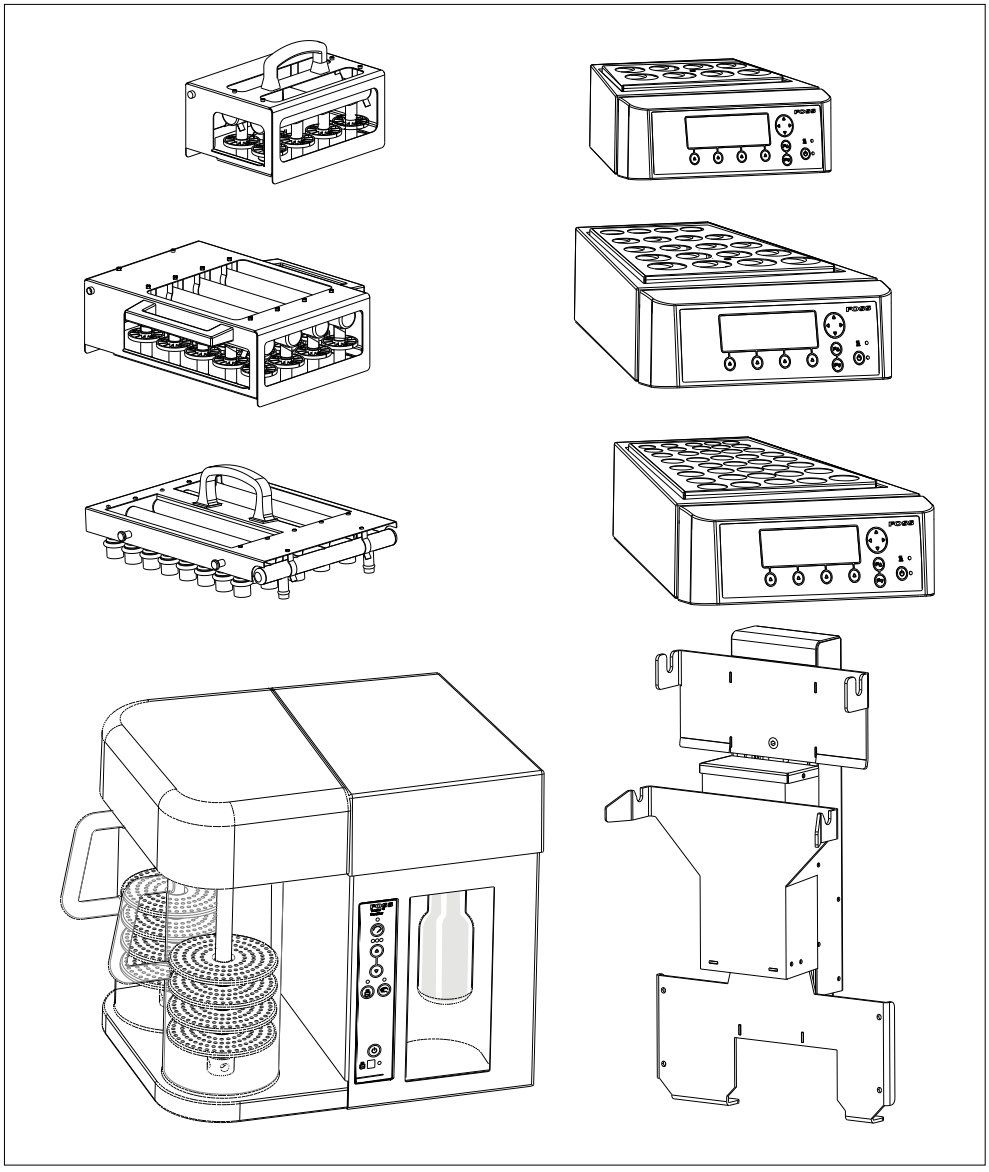


Figure 11.
A-C. Exhaust systems for 8, 20, 40 place units
D-F. Digestors in 8, 20 & 40 place versions
G. 2501 Scrubber Unit
H. 2515 Lift System

5.4.1 Exhaust systems

During the initial part of the digestion larger volumes of fumes are generated and the need for efficient exhaust is greater. This is achieved by operating the water aspirator at full flow during the initial 5 minutes of the digestion. After 5 – 10 minutes, it is essential to decrease the aspirating effect to a minimum to prevent excess acid losses. The correct mimimum level of aspiration is obtained when the fumes are retained within the tube. A “cloud” can be seen in the tube, but no fumes escape out of the tube.

If large sample volumes of water are used, the exhaust should be used at full aspirating effect to the point where the water has evaporated, and then a reduction of the aspiration flow can be made.

With the combined action of the exhaust system and the heat shields, the use of excessive acid can be eliminated.

In these systems ~8 % of the acid is lost during the initial 15 minutes. However, during the remaining part of the digestion when the exhaust is on low only 0.8 % of the acid is lost per 15 minutes digestion time. In total, this corresponds to a total acid loss through evaporation of only 1.2 ml acid when using 12 ml acid and a digestion time of 60 minutes.

Always use an exhaust to minimize evaporation of acid to the environment. The exhaust also helps to keep the temperature in the digestion tube at the correct level. Without the use of an exhaust and heat shields, draughts in a fume hood can cool the tubes. The result is prolonged digestion times and inconsistent digestion.

An automatic digestor with integrated multi function control can automate exhaust control and ensure correct operation. Digestion time, temperature and exhaust conditions will be consistent and repeatable. These options also save water in comparison with the use of a regular water suction pump.

The small amount of acid that is actually evaporated from the digestion tubes to the environment can effectively be collected by the exhaust system and then be adsorbed and neutralized using a Scrubber system.

If a Flow Injection (FIA) or Segmented Flow (SFA) method is used for detection it is often important to have close control of the final acid concentration in the digest. The use of a Scrubber system for aspiration often helps in creating repeatable digestion conditions. The use of volumetric digestion tubes simplifies these analytical procedures.

NOTE! In laboratories with large day to day variations in water pressure it is recommended to control aspirator settings and make adjustments if necessary.

5.4.2 Scrubber

A Scrubber unit is used to eliminate emission of exhaust to the atmosphere. The Tecator systems also have the advantage that they can be operated without a separate water supply. The Scrubber units circulate the water in a closed system. The additional advantage being that variations in the water supply have no influence on the fume removal. The fumes are collected in a tank and neutralized by alkali. The final waste product is sodium sulphate in an alkaline solution.

The 2501 Scrubber from FOSS can be connected to any exhaust system from any manufacturer. When operated in conjunction with a FOSS Basic, or non-FOSS digestor, control is executed by the panel on the side of the unit.

When the 2501 Scrubber is connected to a FOSS Auto digestor it is fully controlled by the application selected in the digestor.

It is recommended that indicator is added to the neutralising (right hand) flask so that the efficiency can be monitored. When the indicator starts to become acidic the neutralising solution, normally 15-20%NaOH, should be replaced.

Further automation is achieved by use of an integrated programmable Lift system.

5.5 Reagent addition

5.5.1 Acid and liquid reagents

The high density of sulphuric acid makes it both difficult and hazardous to add precise amounts using a manual pipette. Automatic bottletop dispensors help to avoid unnecessary contact with acids and ensure accuracy in delivery.

5.5.2 Kjeltabs

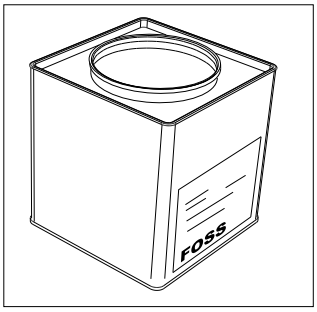


Figure 12. Kjeltab tablets.

The addition of salts and catalysts can be done manually by directly weighing the amounts needed into each digestion tube. This procedure has two major drawbacks, it requires extensive manual work and increases the risk for adding incorrect amounts. To obtain comparable and repeatable results it is crucial to use controlled digestion conditions, i.e. the acid: salt ratio must be controlled.

In order to address these problems, Tecator developed the Kjeltab tablets with a preweighed amount of both salt and catalyst in tablet form. Kjeltabs are available in two basic sizes, 1.5 and 3.5 g, and can be obtained with Selenium, Mercury or Copper catalysts. Mixed Copper/Titanium catalyst is also available. The smaller dimension is designed for the 100 ml test tubes.

Using Kjeltabs saves labour time but first of all ensures that correct results are obtained throughout.

The following are the most commonly used Kjeltabs:

15270001 Selenium tablets with 1.5 g K_2SO_4 + 7.5 mg Se
15270034 Copper tablets with 1.5 g K_2SO_4 + 0.15 g $CuSO_4 \times 5\ H_2O$
15270003 Selenium tablets with 3.5 g K_2SO_4 + 3.5 mg Se
15270018 Copper tablets with 3.5 g K_2SO_4 + 0.4 g $CuSO_4 \times 5\ H_2O$
15270020 Copper/Titanium tablets with 3.5 g K_2SO_4 + 0.105 g $CuSO_4 \times 5\ H_2O$ + 0.105 g TiO_2
15270010 Copper/Titanium tablets with 5.0 g K_2SO_4 + 0.15 g $CuSO_4 \times 5\ H_2O$ + 0.15 g TiO_2

5.6 Simplified handling

To improve the overall efficiency of the system a number of accessories aimed at simplifying the operators daily routines were developed. These include tube racks, Kjeltabs, heat shields and the lift system.

5.6.1 Tube racks

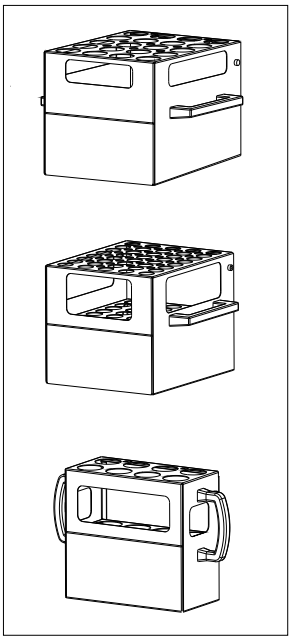


Figure 13. Available tube racks.

Tube racks are used to simplify the overall handling of digestion tubes. Weighing of samples can be done directly into the digestion tubes which are placed in the tube rack. The tube rack with tubes can be transferred directly to the block digestor. After digestion the rack is moved to a separate stand for cooling and finally to the distiller. Using a Kjeltec Autosampler System the tube rack can be placed directly into the sample changer from where the tubes will automatically be transferred to the analyser for processing as programmed.

NOTE! Do not place hot tubes directly on a cold surface. This may cause “star fractures” in the bottom of the tube. Such tubes can easily break. Always use the separate stand for cooling of the tubes.

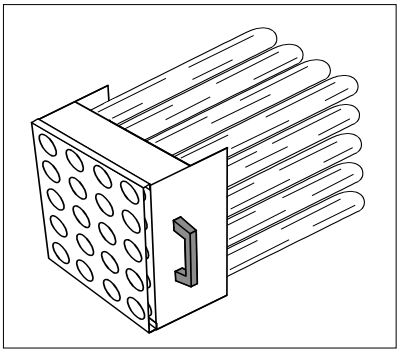
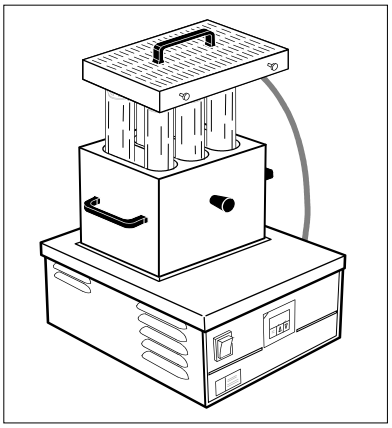


Figure 14. A retainer plate in use.

Tube racks allow batch handling of the tubes whether cold or hot A retainer plate locks the tubes in position in the tube rack so that all tubes can be simultaneously washed and dried after analysis is done.

The tube rack is also an important item in the quality system of the laboratory. Eliminating the risk for mixing up tubes during the analytical procedure, reliability is increased.

5.6.2 Heat shields



Traditionally the heat shield was a removable plate that was placed on the the tube rack. Today the heat shield is normally integrated into the tube rack. This simplifies handling, and also ensures the correct reflux conditions occur within the digestion tubes. The integrated tube rack and heat shield ensure that a condensation ring occurs near the top of the tube. The condensing acid then washes sample to the bottom of the tube where most efficient digestion takes place.

Figure 15. The use of heat shields.

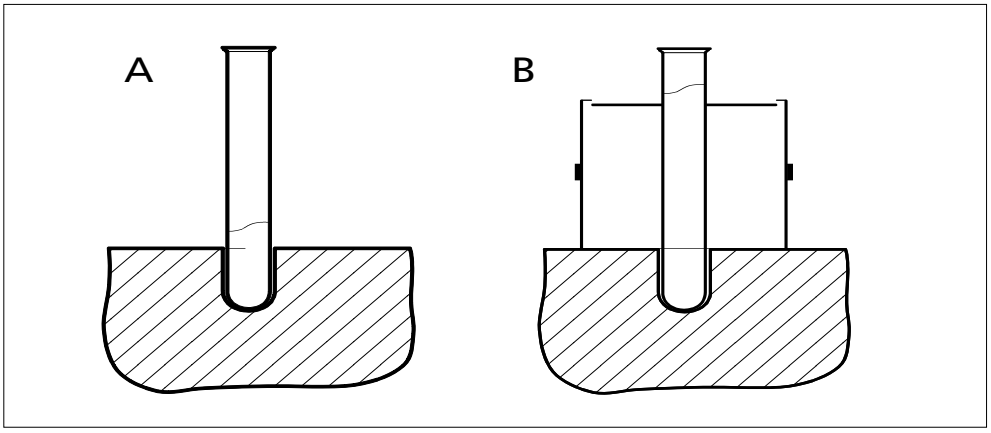


Figure 16. The effect of heat shields.

A. Condensation ring low due to cooling of the test tube (no heat shield in position).

B Correct position of condensation ring. (Heat shield in position.)

From an environmental point of view heat shields also have a positive effect as they act to retain the acid within the tube. This means that the digestion can be optimised so that a minimal amount of acid can be used. Excessive acid volumes need not be added in order to compensate for evaporation losses.

5.6.3 Lift System

The Lift system automates the digestion procedure by eliminating heavy and risky manual handling of hot chemicals. The Lift is controlled by the digestor according to the selected application.

Lift systems are available for 8, 20 and 40 place auto digestors. Tube racks are placed on the lift and the rack rises to the upper position and docks with the exhaust manifold. The rack and exhaust moves down into the preheated digestor. After the preset digestion time, tubes and exhaust rise to the cooling position. A drip tray is inserted before the tube rack is removed in order to eliminate possible dripping of acid from the exhaust manifold down onto the digestor.

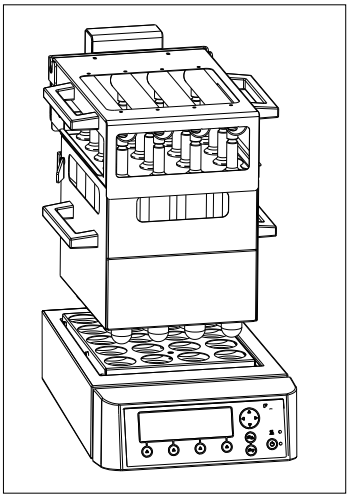


Figure 17. The 2520 Auto Lift System.

A Lift system in combination with an exhaust head and 2501 Scrubber enables full automation of the digestion procedure. As an example, the system can be set up to perform unattended digestions during non-working hours to increase laboratory productivity.

Improved control of digestion conditions is a major advantage offered by the automation. All digestions will be done using exactly the same conditions. Any dependence on individual operator skill is minimized.

5.7 Conclusion

Tecator Digestion systems offer both higher sample throughput and easier handling than the classical Kjeldahl equipment while improving environmental and safety aspects.

Digestion systems are available in 8, 20 and 40 place versions. There is a choice of two integrated controllers for the 8 and 20 place units. 40 place units come only as auto versions with or without a lift. Basic units have a simple temperature and time controller built in. Auto models have an integrated multifunctional controller. This enables fully automated control of the digestor and exhaust, together with Lift and Scrubber. Auto models have the additional feature of memory capable of holding an application database of both FOSS and User applications.

Various accessories are available to make routine analysis safe and simple. They include a range of test tubes, tube racks, retainer plate for washing, fume removal systems, reflux condensors, catalyst tablets and lift system.

The Lift contributes to safety and convenience by removing the need to handle hot chemicals and tubes. It also saves space as the exhaust manifold can be mounted on top of it.

6 Quality Control in Kjeldahl procedures

It is not always easy to perform a separate quality control of only the digestion procedure. The final analytical result is always dependent on the quality of the total analytical process. Both the digestion and the method of detection must be verified. The final analysis step is most commonly a steam distillation/titration, however automatic flow analysis systems can be used. The quality control routines for a combination of a block digestor and distilling system are given below. These principles can also be used with other methods of detection.

In order to verify the accuracy of the digestion process it is important to have access to a detection procedure that performs with an acceptable recovery. For distillation/titration systems usually recoveries better than 99.5 % should be achieved. If the detection system performs with the proper recovery it can be used to control the digestion procedure.

6.1 Verifying the distillation system

The distillation principle is to convert ammonium (NH_4+) into ammonia (NH_3) by adding alkali (NaOH) and steam distil into a receiver flask containing boric acid with mixed indicators. Titration with standard acid solution using colour sensing titrimetric end-point detection is officially approved for the final determination.

Since all nitrogen in the samples after digestion form ammonium sulphate $(NH_4)_2SO_4$, it can be used as a standard to check the recovery of the distilling unit.

Application Note AN 300 “The determination of Nitrogen according to Kjeldahl using block digestion and steam distillation” gives a complete description on how the recovery is verified in the distillation/titration step. One of the following procedures should be used:

1. Use ammonium sulphate $(NH_4)_2SO_4$ > 99.5 %
   Mol. weight = 132.14 g/mol.
   % Nitrogen in ammonium sulphate (99.5 %) = 21.09
   Weigh 0.15 g ammonium sulphate into a tube.
   Add 75 – 100 ml distilled water and 50 ml 40 % NaOH and distil

$$\% \text{ Nitrogen} = \frac{(\text{ml- blank}) \times N \times 1.401}{\text{g sample}}$$

N = Normality of titrant to 4 places of decimal.

$$\% \text{ Recovery} = \frac{\text{Actual \% Nitrogen}}{21.09} \times 100$$

2. Use ammonium iron (II) sulphate $(NH_4)_2\ Fe(SO_4)_2 \times 6H_2O$
   Mol. weight = 392.14 g/mol.
   % Nitrogen in ammonium iron (II) sulphate = 7.145
   Weigh 0.5 g ammonium iron (II) sulphate into a tube.
   Add about 75-100 ml distilled water, and 50 ml 40 %
   NaOH and distil

   $$\% \text{ Nitrogen} = \frac{(\text{ml- blank}) \times N \times 1.401}{\text{g sample}}$$

   N =Normality of titrant to 4 places of decimal.

   $$\% \text{ Recovery} = \frac{\text{Actual \% Nitrogen}}{7.145} \times 100$$

3. Alternatively the recovery can be tested using this simplified procedure:

   Prepare an ammonium solution which has the same normality (N) as the titrant acid. Then pipette 10 ml of the ammonium solution into a digestion tube and distil. The titrant volume multiplied by 10 will equal the recovery percent. Always use calibrated volumetric glassware in order to obtain highest possible accuracy.

   Using ammonium sulphate with a purity of 99.5 % the following formula can be used to prepare the ammonium solution:

   $$g(NH_4)_2SO_4 \text{ / } 1000 \text{ ml} = \frac{N \times 132.14}{2 \times 0.995}$$

   where N = Normality of titrant

Example: With a titrant acid with a strength of 0.2000 N, 13.280 g ammonium sulphate should be diluted to a final volume of 1000 ml.

NOTE! Please also note that the above calculations must be adjusted if other purity levels of ammonium salts are used.

The above recovery test procedures verify the function of the distillation unit including the burette and the titration system and can be done without involving the digestion procedure.

If the obtained recovery is acceptable proceed to further tests designed to verify the function of the digestion procedure.

6.2 Verifying the digestion procedure

In order to ensure high quality of the analytical results produced in routine work, it is essential to include a check sample in all batches digested and analysed. By monitoring the performance of this sample over time, it is very easy to note if performance starts to degrade.

Basically four procedures can be used for verifying accuracy of the digestion procedure:

1. Digestion of a standard substance with known nitrogen content. Glycine or acetanilide are commonly used.

   Basically a procedure similar to the one described in 6.1 can be used. The only exception is that the test substances is digested prior to distillation/titration.

   Nitrogen content of pure glycine is 18.66 % N.
   Nitrogen content of pure acetanilide is 10.36 % N.

   A sample weight of 500 mg can be used for the tests.

   The recovery including the digestion should be within ± 1 % relative. The quality and purity of the test substance should be considered.

This procedure verifies the digestion procedure, provided that the distilling unit/titration system works according to specifications.

2. Digestion of certified reference sample.

3. Participation in proficiency testing schemes is recommended, as comparisons with other laboratories are obtained at regular intervals.

4. Prepare a reference sample within the laboratory and verify content versus other laboratories. Always use this sample as an internal reference in daily routine use.

6.3 Equipment

From a user point of view there must be a demand that the equipment purchased actually performs according to specifications. One way is to demand from the manufacturer that approved quality routines are used in the production and development of the equipment.

The Quality System at FOSS is certified according to ISO 9001:2000. This means that both development and production of analytical equipment is done according to clearly documented routines. For the production of equipment specified procedures for both assembly and test routines are followed. This ensures that the equipment meets instrument specifications provided in the Data Sheet.

Basically also the function of the digestion block is tested when the digestion procedure is tested. If the block digestor malfunctions it should be noted by poor quality of the analytical results.

To further improve safety in the laboratory all digestors are equipped with overheat protection circuits.

In Tecator Digestors Block it is also possible to verify actual temperature versus set temperature. Using a calibrated and approved thermometer, actual temperature of the digestion block can be verified by measurement in the small opening in the front of the block. If the temperature reading differs from that stated in the digital display, suitable adjustment of the temperature setting can be made to achieve the preferred temperature.

Accuracy in temperature measurements can at best be ± 2°C.

The temperature difference between different holes in the block should be within 3°C provided that the surrounding environment offers stable conditions. A strong airflow as in a fume cupboard might influence temperature stability so that larger variations can occur.

Balances used for weighing samples should be calibrated with regular intervals.

7 Application guidelines

7.1 Introduction

The applications are an essential part of any analytical procedure. For the Kjeltec™ systems application support is extensive. The applications are divided between Application Note (AN) and Application Sub Notes (ASN). See chapter 8.

The general procedure of Kjeldahl analysis including sample preparation, digestion, preparation of reagents, distillation and titration is given in AN 300 “The determination of Nitrogen according to Kjeldahl using block digestion and steam distillation”.

In the ASN’s detailed information on how to perform analysis of specific sample types is given. The major application areas for the Kjeldahl method are already covered by the existing application note system. However, all individual sample types are not covered. One of the major issues in all applications is the sample preparation step. In order to obtain accurate results it is important to select the proper mill for the purpose. A guideline on the use of Tecator mills is given below:

Performance data:

Feature	**Cemotec™**	**Cyclotec™**	**Homogenizer**	**Knifetec**
Sample type	Dry samples prior to moisture analysis, up to 20 % moisture and/or 20% fat	Dry samples prior to wet chemistry or IR analysis, up to 15% moisture and/or fat content up to 20%	High-moisture, high-fat and fibrous samples	High-moisture, high-fat and fibrious samples
Applications	grains, seeds, feed, beans, dry granular foods, fertilizer, tablets	grains, seeds, cereals, forage, feed, leaves, tablets, tobacco, lime, coal	forage, dry food, meat, fish, vegetables, prepared foods, chemical and pharmaceutical formulations	oilseeds, prepared foods, meat products, fruit, vegetables and feed

Feature	Cemotec™	Cyclotec™	Homogenizer	Knifetec
Sample size	up to 14 mm	up to 10 mm, large inlet up to 40 mm	0.2 kg up to 2.0 kg (up to 2.5 litres)	50 up to 150 grams
Grinding principle	two discs, one rotating one stationary	turbine + sieve	rotor knives	rotor blade
Grinding rate/ time	appr. 3 g/s	over 4 g/s	10 to 50 seconds	2 to 10 seconds
Grinding speed	grinding disc 3 000 rpm	impeller 10 000 rpm	blade 1 500 / 3 000 rpm	blade 20 000 rpm
Particle size	coarse grist	fine and uniform grist 0.5 mm screen: max 0.45 mm 1.0 mm screen: max 0.75 mm	depending on sample	depending on sample
Timer	no	no	yes	yes
Safety	microswitch	microswitch	magnetic safety switch	microswitch
Cleaning	minimal	minimal	manual	manual

Contact either FOSS or your local representative for further information on available application support.

It is also recommended to use the Official **Methods** of Analysis of **AOAC** INTERNATIONAL (OMA), 18th Edition, Revision 2, 2007, or later revisions, published by the Association of Official Analytical Chemists (AOAC) as a reliable source of additional application information[9].

In order to further simplify the development of methods not covered by the present ASN library, some comments to keep in mind are given below.

7.2 Food

Nitrogen determinations are very common in all types of food products. The large variety of sample types that are analysed makes it impossible to give a complete coverage with detailed ASN's. Below you will find some guidelines that can be useful when developing your own applications.

7.2.1 Vegetables/Fruit

These products are rather simple to analyse with the Kjeldahl method. The commonly used “Standard Kjeltec™ Procedure” can usually be used without any modifications.

The problems that can occur with vegetables and fruits are mainly related to the sample preparation step. Many of these samples have a high moisture content and a high fibre content. The high fibre content especially where fibres are strong can present difficulties during the grinding step. Commonly used procedures utilize food choppers or the Knifetec mill. For further information see AOAC 920.149.

For reliable and repeatable results, it is extremely important to control the moisture content in each sample. A commonly used procedure is always to analyse predried samples.

7.2.2 Meat/Fish

The main problem with these sample types is related to the lack of homogeniety of the analytical sample. Ordinary mills are usually insufficient and conventional meat grinders or the Homogenizer should be used. The non-homogeniety of the sample means that a large sample size needs to be used during sample preparation to get a representative analytical sample.

For fish the analytical sample should include all components which are normally consumed, i.e. skin. Instructions may be modified in accordance with the purpose of the examination. For further information see AOAC 937.07 (Fish) & 983.18 (Meat & meat products).

The high fat content found in some sample types may also cause foaming during the digestion.

7.2.3 Dairy

One common problem with dairy samples is the natural separation of fat from the rest of the sample, especially noted in raw milk samples. Appropriate procedures for homogenization of the samples must be used. Commonly the sample is heated to 38°C in a water bath while mixing to disperse lumps of fat. If possible the homogenized sample should be cooled to 20°C be-

fore transferring to test tube. See AOAC 925.21 (Milk), 925.26 (Cream) or 955.30 (Cheese) for further information. Many of these samples are unstable so they need to be analysed quickly.

The high fat content that can be found in some sample types may also cause foaming during the digestion.

7.2.4 Prepared foods

The digestion step is ususally straightforward and the "Kjeltec™ Standard Procedure" can be used. Sample preparation is commonly the critical step due to the nonhomogenous nature. Large sample sizes may have to be used in the sample preparation to get a representative analytical sample. The Homogenizer can be a useful tool.

7.3 Beverages

The "Kjeltec™ Standard Procedure" can usually be used for the digestion step.

Beverages usually are readily homogenized, so representative samples can be obtained. The most common exception to this is raw milk samples.

The low nitrogen content in many beverages makes it necessary to use large sample volumes. During the digestion, the water must be initially evaporated, and boiling rods or other means to avoiding bumping have to be used.

High contents of surface active agents can also cause foaming during the initial part of the digestion.

7.4 Agriculture

These products are mostly rather simple to analyse with the Kjeldahl method. The commonly used "Standard Kjeltec™ Procedure" can usually be used without any modifications.

The problems that can occur are mainly related to oilseeds and their high fat content.

7.4.1 Oilseeds

For most commonly analysed oilseeds, detailed descriptions can be found in the corresponding Application Sub Notes. With these samples the main problem is to obtain a homogenous and representative sample. During the sample preparation the oil tends to separate from the rest of the sample and oil residues are retained in the mill. Samples of this type are best homogenized using the Knifetec mill.

7.4.2 Soil

Soil samples are simple to analyse with the Kjeldahl method. The commonly used “Standard Kjeltec™ Procedure” can usually be used without any modifications.

To get reliable and repeatable results, it is extremely important to control the moisture content in each sample. The samples should not be dryed due to the risk of evaporating ammonia.

Foaming can also occur during digestion especially for samples with a high organic content.

7.4.3 Fertilizers

For sample preparation step the Cyclotec™ or the Cemotec™ mill can be used. Some sample types can be hygroscopic so that care should be taken to control moisture content.

Regarding the digestion procedures, different techniques are used depending on the aim of the analysis. Further information can be found in ASN’s or in AOAC 955.04, 970.02, 978.02 & 970.03 all for total Kjeldahl Nitrogen. AOAC 920.03 (Ammonia), 829.01 (Ammonia & Nitrate), 930.01& 930.02 (Nitrate) give further information.

7.4.4 Grain

For sample preparation the Cyclotec™ or the Cemotec™ mill can be used. Cemotec is preferred when simultaneous moisture determinations have to be made.

Two different digestion procedures are commonly used:

a) the standard digestion procedure as described in AOAC official method 2001.11[10] or EN ISO 5983-2:2005[11] using copper catalyst, or
b) a digestion procedure according to EN ISO 20483:2006[12] using a copper/titanium oxide catalyst

See also Application Notes AN 300 and 3001.

7.4.5 Feed

For many feed products, detailed descriptions can be found in the corresponding Application Sub Notes. In most cases the "Standard Kjeltec™ Procedure" can be used for the digestion step. Sample preparation is straightforward for dry feeds and either the Cemotec™ or Cyclotec™ mill can be used. For canned pet foods, the high moisture content limits the use of conventional mills. For these samples, the Knifetec mill can be the best choice.

To get reliable and repeatable results for such samples it is extremely important to control the moisture content in each sample.

7.5 Water/wastewater and sludge

The properties of these samples can vary considerably depending upon the source. The choice of analytical methods is also regulated by official agencies since they form an integral part of environmental protection programs.

Low nitrogen levels in unpolluted waters make it necessary to use large sample volumes. During the digestion, the water is initially evaporated and use of boiling rods or other means of avoiding bumping are needed.

High concentrations of surface active agents in some wastewaters can also cause foaming during the initial step of the digestion, see references [10] and [11] as well as AN 300 and AN 3001.

Where an automatic flow analysis system is used for detection, it is important to control the acid strength in the final digests. Variations in acidity from sample to sample and also versus standards can cause errors in many currently used flow methods. Verify the influence of acidity variations in the method.

Digests for nitrogen are also commonly used for phosphorus analysis.

7.6 Industrial

Nitrogen determinations are quite common in various types of raw materials and industrial products. The large variety of sample types makes it impossible to cover all aspects with detailed Application Sub Notes. Below you will find guidelines that can be useful in developing your own applications.

Due to commodity-trade reasons, methods are usually regulated by official methods.

7.6.1 Petrochemicals

These sample types can be relatively difficult to handle. The high oil content causes foaming during the initial part of the digestion. Usually quite long digestion procedures with slow heating of the sample are required.

The foaming problems can be difficult to control as nitrogen levels usually are low so that large sample amounts have to be used.

7.6.2 Rubber/Plastics/Polymers

These products are simple to analyse with the Kjeldahl method. The commonly used “Standard Kjeltec™ Procedure” can usually be used without any modifications.

The major problems with these products can be found in the sample preparation step. These samples can be difficult to grind in mills due to the heat generated which can “melt” the samples in the mill. An alternative can be to cool the samples with liquid nitrogen so that they become hardened and then rapidly perform the milling. This procedure is not easy, as the heat generated in the mill rapidly makes the samples soft again. If so, liquid nitrogen has to be poured into the mill during the milling.

Quite often samples are cut into small pieces using an ordinary knife. This procedure is time consuming, but necessary to obtain an homogenous sample.

7.6.3 Textiles/Leather

These products are simple to analyse with the Kjeldahl method. The commonly used “Standard Kjeltec™ Procedure” can usually be used without any modifications.

Usually a knife or scissors are used to reduce the size of the sample into a particle size suitable for analysis.

7.6.4 Paper/Pulp

These products are simple to analyse with the Kjeldahl method. The commonly used “Standard Kjeltec™ Procedure” can usually be used without any modifications.

Usually a knife or scissors are used to reduce the size of the sample into a particle size suitable for analysis. Nitrogen levels can be quite low so that larger sample amounts have to be used.

7.6.5 Conclusion

The Kjeldahl method is versatile and can handle a very wide range of samples. It is very rare that the method is unsuitable to use for nitrogen testing.

In most cases the key to successful Kjeldahl analysis can be found in the sample preparation step. For some sample types quite large sample amounts have to be used in order to get a representative sample. Whereas other samples, especially those with a high fat and/or moisture content, can separate into two phases during sample preparation. For such samples special care has to be taken in order to get a representative sample for testing.

For the digestion, the basic procedure given in chapter 2 can always be used for the initial tests when developing a new method. This procedure covers more than 90 % of all protein containing samples and over 60 % of the other nitrogen containing samples. To optimize a specific method use the procedures outlined in chapter 3.6.

8 FOSS Application Note System; Kjeltec™ systems

AN Application Note:
General method information on all steps in the analytical method

No.	Title
300	Determination of Nitrogen according to Kjeldahl using block digestion and steam distillation
303	Determination of Nitrogen according to Direct Distillation (DD) using steam distillation
3001	Determination of Crude Protein (Kjeldahl nitrogen) in Animal Feed, Forage, Plant Tissue, Grain & Oilseeds
3002	Determination of Nitrogen in milk and milk products – Kjeldahl method, *According to ISO 8968-3/ IDF 20-3*
3003	Determination of Nitrogen in milk and milk products – Kjeldahl method, *According to ISO 8968-2/IDF 20-2, but with lower reagent volumes and reduced digestion time*

ASN Application Sub Note:
Detailed information for the analysis of a specific sample type

No.	Title
3100	Determination of Nitrogen according to Kjeldahl in Cereals
3102	Determination of Nitrogen according to Kjeldahl in Hamburgers
3103	Determination of Nitrogen according to Kjeldahl in Beefburgers
3104	Determination of Nitrogen according to Kjeldahl in Oats
3105	Determination of Nitrogen according to Kjeldahl in Oilseeds
3106	Determination of Nitrogen according to Kjeldahl in Meat and Meat Products
3107	Determination of Nitrogen according to Kjeldahl in Barley
3108	Determination of Nitrogen according to Kjeldahl in Wheat
3109	Determination of Nitrogen according to Kjeldahl in Corn (Maize)
3110	Determination of Nitrogen according to Kjeldahl in Liver Pate
3111	Determination of Nitrogen according to Kjeldahl in Sausage and Sausage Meat

3112 Determination of Nitrogen according to Kjeldahl in Root Vegetables
3113 Determination of Nitrogen according to Kjeldahl in Rice
3114 Determination of Nitrogen according to Kjeldahl in Rye
3115 Determination of Nitrogen according to Kjeldahl in Soya Beans
3116 Determination of Nitrogen according to Kjeldahl in Rapeseed
3117 Determination of Nitrogen according to Kjeldahl in Cottonseed
3118 Determination of Nitrogen according to Kjeldahl in Lupin
3119 Determination of Nitrogen according to Kjeldahl in Sunflower Seed
3137 Determination of Total Nitrogen in Butter
3138 Determination of Total Nitrogen in Cream
3139 Determination of Total Nitrogen in Cheese
3140 Determination of Total Volatile Basic Nitrogen (TVBN) in Fresh and Frozen Fish
3164 Determination of Nitrogen in Dietary Fibre procedures
3169 Determination of Protein in Meat and Meat products using Direct Distillation (DD)
3170 Determination of Protein in Cheese using Direct Distillation (DD)
3172 Determination of Nitrogen in Sugar and Syrup
3175 Determination of Protein in Compressed Yeast
3176 Determination of Protein in Granular Yeast
3182 Kjeldahl Nitrogen in Bacon
3183 Kjeldahl Nitrogen in Hot Dog
3184 Kjeldahl Nitrogen in Bratwurst
3185 Kjeldahl Nitrogen in Salami, Hard Sausage
3186 Kjeldahl Nitrogen in German Sausage
3187 Kjeldahl Nitrogen in Smoked Ham
3188 Nitrogen according to Kjeldahl in Egg Noodles
3189 Nitrogen according to Kjeldahl in Spaghetti
3190 Nitrogen according to Kjeldahl in Macaroni
3200 Determination of Nitrogen according to Kjeldahl in Beer
3201 Determination of Total Nitrogen in Milk
3203 Determination of Total Sulphite in Beer
3204 Determination of Total Sulphite in Dried Apricots

3205 Determination of Total Sulphite in Celery
3206 Determination of Total Sulphite in Jams or Preserves
3207 Determination of Total Sulphite in Dried Vegetables
3208 Determination of Total Sulphite in Sausage
3209 Determination of Total Sulphite in Fruit Juices
3210 Determination of Total Sulphite in Wine
3211 Determination of volatile Acids in Wine
3214 Determination of Alcohol content in Wine using the 1026 Distilling Unit
3215 Determination of Protein in Milk using Direct Distillation (DD)
3300 Determination of Total Nitrogen in Fertilizer according to Kjeldahl
3301 Determination of Ammonium Nitrogen in Inorganic Fertilizers according to Kjeldahl
3302 Determination of Ammonium Nitrogen in Organic Fertilizers according to Kjeldahl
3303 Determination of Urea-N & Ammonium-N in Fertilizer – Enzymatic method
3311 Determination of Protein in Barley using Direct Distillation (DD)
3312 Determination of Protein in Wheat using Direct Distillation (DD)
3313 Determination of Nitrogen according to Kjeldahl in Soil
3314 Determination of Nitrogen according to Micro Kjeldahl in Grain
3315 Determination of Cation Exchange Capacity
3316 Nitrogen according to Kjeldahl in Almond
3317 Nitrogen according to Kjeldahl in Hazelnut
3318 Nitrogen according to Kjeldahl in Maize Starch
3319 Nitrogen according to Kjeldahl in Wheat Starch
3401 Determination of Nitrogen according to Kjeldahl in Animal Feed
3402 Determination of Nitrogen according to Kjeldahl in Soya
3403 Determination of Nitrogen according to Kjeldahl in Rapesee Meal
3404 Determination of Nitrogen according to Kjeldahl in Peanut Meal
3405 Determination of Nitrogen according to Kjeldahl in Blood Meal
3406 Determination of Nitrogen according to Kjeldahl in Fish Meal
3407 Determination of Nitrogen according to Kjeldahl in Pet Food

3408 Determination of Nitrogen according to Kjeldahl in Poultry Feed
3409 Determination of Nitrogen according to Kjeldahl in Pig Feed
3410 Determination of Nitrogen according to Kjeldahl in Cattle Feed
3411 Determination of Nitrogen according to Kjeldahl in Sheep Feed
3412 Determination of Nitrogen according to Kjeldahl in Shrimp Feed
3413 Determination of Nitrogen according to Kjeldahl in Fish Feed
3423 Determination of Nitrogen according to Kjeldahl in Cottonseed Meal
3424 Determination of Nitrogen in Forage according to Kjeldahl
3425 Determination of Nitrogen in Straw according to Kjeldahl
3438 Determination of Nitrogen according to Kjeldahl in Molasses
3439 Determination of Nitrogen according to Micro Kjeldahl in Feed
3448 Protein According to Kjeldahl in Dry Cat Food
3449 Protein According to Kjeldahl in Dry Dog Food
3450 Protein According to Kjeldahl in Canned Cat Food
3451 Protein According to Kjeldahl in Canned Dog Food
3452 Determination of Non-Protein Nitrogen in liquid Milk products
3453 Determination of Protein Nitrogen in liquid Milk products
3502 Determination of Ammonium Nitrogen in Water
3503 Determination of Kjeldahl Nitrogen (TKN) in Water
3506 Determination of Chemical Oxygen Demand (COD) in Water and Wastewater
3507 Determination of Ammonium and / or Total Oxidized Nitrogen (T.O.N.) in Water
3508 Determination of Weak Acid Dissociable and Total Cyanide in Waste Water, Soil and Sludges
3509 Determination of Phenol in Water
3510 Determination of Total Nitrogen in Water after Reduction/Digestion with Devardas Alloy: Range 3 – 30 mg N/l
3511 Determination of Total Nitrogen in Water after Reduction/Digestion with Devardas Alloy: Range 10 – 200 mgN/l
3512 Determination of Kjeldahl Nitrogen (TKN) in water – EPA: Range 0.4 – 10 mg N/l
3514 Low Level Kjeldahl Nitrogen (TKN) in water – EPA: Range 0.5 – 2 mg N/l

3515 Low Level Kjeldahl Nitrogen (TKN) in water – EPA: Range 0.08 – 0.5 mg N/l

3600 Determination of Nitrogen in Crude & Refined Oil according to Kjeldahl

3609 Determination of Nitrogen in Melamine according to Kjeldahl

3610 Determination of Nitrogen in Urea according to Kjeldahl

3620 Determination of Nitrogen in Styrene Plastic (SAN) according to Kjeldahl

3621 Determination of Nitrogen in Styrene Plastic (ABS) according to Kjeldahl

9 Troubleshooting

As for all types of analysis some questions are more frequent than others. Below the 12 most common questions related to the performance of the digestion procedure can be found.

Problem	Possible cause	Solution	Section
Low result.	**Poor digestion**		
	Too little acid.	Try new digestion with ~2 ml additional acid.	6.1; 3.2.1.1; 3.2.1.3
	Too low temperature in the block.	Increase temperature 10 - 15°C.	6.1; 6:2
	Temperature too low due to improper acid/salt ratio.	2 Kjeltabs & 12 ml acid should normally be used.	3.2.2.1; 6.1; 6:2
	Nitrogen losses during digestion.	Check digestion procedure for recovery rate.	3.3.3; 3.5; 6.2
	Digestion time too short.	Increase digestion time 15 minutes.	3.6
	Sample weight.	Check balance for correct sample weight.	6.3
	Distillation step malfunctioning.		
	Poor distillation recovery.	Increase amount of alkali.	3.2.1
	Reagents & chemicals.	Check distillation unit and reagents used for proper recovery.	6.1
High results.	Reagents & chemicals.	Check titration acid and verify recovery on distillation unit.	6.1
	Sample weight.	Check balance for correct sample weight.	6.3
Poor repeatability.	**Poor digestion.**		
	Sample preparation.	Poor representativity of sample: Improve sample preparation or use larger sample size.	3.1

Problem	Possible cause	Solution	Section
	Digestion conditions.	Uneven digestion. Check if temperature of block digestor is correct. High draught in fume cupboard can decrease temperature of outer digestion tubes.	6.2; 6.3
	Exhaust rate malfunctioning due to uneven water pressure.	Manual inspection and corrections if necessary. The use of a Scrubber eliminates the dependency of a separate water supply.	5.4.1; 5.4.2
	Digestion conditions.	Use Kjeltabs to get a controlled acid/salt ratio.	3.2.3
	Digestion time too short.	Increase digestion time with 15 minutes.	3.6
	Distillation step malfunctioning		
	Poor distillation recovery.	Increase amount of alkali.	3.2.1
	Reagents & chemicals.	Uncontrolled blank contribution. Check for Nitrogen contribution from reagents used.	6.1
	Adsorption of nitrogen in distilling unit.	Clean distillation unit by distilling acetic acid, see manual for distilling unit. Check condition of splash head, replace if faulty. At high workload, 100-120 samples/day, the splash head have to be replaced 1-2 times/year.	6.0
Poor accuracy.	Nonhomogenous sample.	Improve sampling. Improve sample preparation.	3.1
	Distillation step malfunctioning.	Check distillation unit and reagents for proper recovery. Increase amount of alkali.	6.1; 6.2; 6.3; 3.2.1
Foaming during digestion.	High fat content or surface active agents in the sample.	Change digestion conditions or use antifoaming agents	3.7

Problem	Possible cause	Solution	Section
Bumping during digestion.	Acid drops from exhaust head directly down into acid in the bottom of the tube.	Tilt the digestor by putting a piece of wood (5 - 10 mm) under the front feet. This allows the condensed acid to slide along the tube wall, bumping will be avoided.	5.4.1; 5.6.2
No condensation ring.	No heat shields used.	Use heat shields.	5.6.2
Acidic fumes escape from the tubes.	Exhaust capacity insufficient.	Poor teflon seals in exhaust head. Too low setting on water suction pump/scrubber unit. Check water supply for day to day variations in water pressure.	5.4
Crystallization during digestion.	Too much acid consumed/evaporated during the digestion.	Too much salt. Too little acid. Too high exhaust level Too much sample. Too high temperature.	3.2.1; 3.2.1.1; 3.2.1.3; 3.2.1.4
Crystal formation after digestion.	Low volume of acid remaining in the digestion. tube. Solubility of salt decreases when the acid gets cool, crystals are formed.	Gently warm tube in the block prior to dilution. Crystal formation can be prevented by adding some water to the tube ~10 minutes after the digestion is completed.	3.2.1; 3.2.1.1; 3.2.1.3; 3.2.1.4
Uneven results within batch.	Uneven temperature in digestion block.	Can be caused by cooling of outer tubes from high airflow in the fume cupboard.	6.2; 6.3
Uneven results between batches.	Sample preparation.	Poor repeatibility of digestions. Uncontrolled sample weights. Samples have lost moisture.	3.1; 3.1.1; 3.1.2
	Laboratory routines.	Variations between operators. Different balances used, different digestion conditions used etc.	6.1; 6.2

10 Questions and answers

Question	Answer
What is a blank sample?	A blank sample is a digested sample that contains all the reagents as used for the digestion of the sample. It contains no sample and should reflect the nitrogen contribution from the reagents and procedures used. In situations where the final acidity of the blank sample can be of interest, sugar free of nitrogen may be added to the blank prior to digestion. The sugar will consume some acid and acidity of the blank will be quite close to that of the samples. This can be of importance, for example when automated colorimetric flow analysis procedures are used for the detection. The blank obtained during the colorimetric titration after distillation of distilled water is caused by the pH adjustment of the boric acid. The boric acid should always be adjusted so that a positive reading of 0.05 - 0.15 ml titration acid is obtained.
How can throughput in Kjeldahl digestions be increased?	Using an auto digestor together with Exhaust, Lift and Scrubber digestions can be fully automated. This enables digestions to be done during nonworking hours. By combining an efficient digestion system with a fully automated Kjeltec Auto Sampler System, operators are not locked to the equipment during analysis. Their time can be efficiently used for preparing new samples for analyses, quality control work etc. Higher productivity can also be obtained during working hours by using the integrated controller to start and preheat the digestion block in the morning prior to work.
How should digestion tubes be cleaned?	In ordinary Kjeldahl analysis it is sufficient to rinse the tubes with deionized water after analysis. The tube rack together with a retainer plate can be used so that all tubes can be washed and handled simultaneously.

Question	Answer
How should digestion tubes be inspected?	Inspect tubes for any visible damages. If a tube is cracked it should be discarded. Also inspect the bottom part of the tube for any "star fractures". If such appear the risk for tube breakage during digestion/distillation increases. Such tubes should be discarded. "Star fractures" in the bottom of the tube is usually caused by placing hot tubes directly on a cold surface. Use a separate stand to hold the tubes during cooling.
How does one know that the digestion is completed?	For new unknown sample types it is advisable to verify digestion efficiency according to 3.6. A valuable source of information is Official **Methods** of Analysis of **AOAC** INTERNATIONAL (OMA), 18th Edition, Revision 2, 2007, or later revisions, published by the Association of Official Analytical Chemists (AOAC).[9] In this manual official procedures for a wide range of materials can be found. This information together with own experimental tests can be used to validate the digestion procedure. For routine laboratory work it is advisable to follow one or more of the routines in 6.2. Contact FOSS Application Laboratory for the latest information on application development.

11 References

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