A new method for detecting ammonia in the Kjeldahl's protein analysis.

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Protein content of meat and meat products is generally determined by the Kjeldahl's method. It is a fundamental part of the method the rapid determination of nitrogen in ammonia originating from the digestion.
A new method and instrument are suggested here which provide a more rapid and sensitive detection of ammonia.
This method based on pH measurement is suitable for serial determinations, and the instrument with a little modification for automatization.

Neues Verfahren zur Bestimmung des Ammoniaks in der Eiweissanalytik nach Kjeldahl.

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Der Eiweissgehalt des Fleisches und der Fleischprodukten wird auch heuzutage durch Kjeldahl-Methode bestimmt. Der Hauptpunkt der Analyse nach Kjeldahl ist die Schnellbestimmung des beim Aufschluss entstehenden Ammoniaks.
Die Verfasser proponieren ein von den klassichen Methoden abweicheindes Bestimmungsverfahren und ein Gerät, wodurch der Ammoniakgehalt wesentlich schneller und empfindlicher gemessen wird.
Das auf pH-Messung gegründete Bestimmungsverfahren ist anwendbar zur Serienmessung beim hohen Probenzahl und durch Modifizierung des dargelegten Gerätes zur Automatisierung.

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Une méthode nouvelle pour la perception de l'ammoniaque dans l'analyse Kjeldahl de la protéine.

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Le dosage de la teneur de la protéine vient réalisé en général par la méthode Kjeldahl.Un point fondamental de l'analyse Kjeldahl est le dosage rapide de l'ammoniaque dégagée pendant le cours de la minéralisation.
Les auteurs proposent un'instrument et une méthode différée des méthodes classiques avec lesquelles on peut doser la teneur de l'ammoniaque plus rapidement et plus sensiblement.
Cette méthode basée sur la mesure de pH est propre au mesurage à série et à l'automatisation par la modification de l'instrument écrit sur cette notice.

Новый метод измерения аммиака в белковом анализе Кьелдаля

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Определение белков в мясе и мясопродуктах чаше всего происходит с помощью метода Кьелдаля.
В этом методе важным требования является быстро определить аммиак,который образуется при разрушении в виде ионов аммония.

В отличие от метода Кьелдаля автор предлагает новый способ и прибор с помощью которого измерение аммиака может быть значительно более быстрым и чуствительным.

Метод,основанный на измерении "p_H",пригоден для анализа многочисленных проб,и при некотором изменением прибора может исползоваться для автоматизирования.

New method for detecting ammonia in the Kjeldahl's protein analysis.

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The protein determination with Kjeldahl method has been and to all probability remains a fundamental procedure for meat and meat products qualification.
The Danisch researcher Kjeldahl worked out a method for determining organic nitrogen in 1883.He studied the protein changes in grain.Since the first publication of Kjeldahl,the methods has undergone many changes and it has been used for examination of different foods containing protein.
As it's known in the Kjeldahl method the sample is heated in sulfuric acid and digested till the carbon and hydrogen are oxidized,and the protein nitrogen is reduced and transformed into ammonium sulfate.Then concentrated sodium-hydroxide is added to it and the digest heated to drive off the liberated ammonia into a known volume of a standard acid solution.The unreacted acid is determined and the results are transformed by calculation into protein percentage of the original sample.
Several methods are available to determine the ammonium-sulfate in the digest,either acidimetry or colorimetry.The colorimetric method is based on the procedure of Van Slyke and Hiller.In this method the ammonium ions react with alkaline phenol and hypochlorite.On heating the solution an intensive blue color is produced,which is closely related to that of the indophenol.Because of its high sensitivity and simplicity Nessler's colorimetric method directly applied to Kjeldahl digests can be used for determination of nitrogen in foods.However,the conditions for the optimum color reaction and the stability are rigorous,therefore the distillation of ammonia is widely used in Kjeldahl analysis.
For the ammonia distillation generally the steam-vapor method is used.The Parnas-Wagner and the Schulek-Vastagh instruments have spread in Hungary.In the Parnas-Wagner method / figure 1./ the stream is lead from a reservoir into the alkalized digest while the Schulek-Vastagh method / figure 2./ we direct distil the diluted and alkalized digest to drive off the ammonia.In both methods the problem is the significant dilution of the distillate.

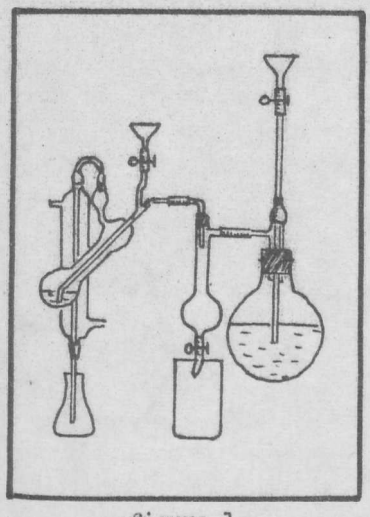

figure 1.
Parnas-Wagner instrument

Many electrometrical instrumental methods have been worked out for the ammonia determination.We can classify these methods into two parts.In one respect these methods are based upon the end point determination as potentiometric,high frequency and colorimetric one,on the other hand the ammonia is determined with direct potenciometric manner.In the later case we can use ionic selective electrodes as ammonium and ammonia selective one.The ammonium selective electrodes can be used in the digest,however it is necessary to keep the conditions as rigorous as in the Nessler's method.The ammonia selective electrode can be applyed in the distillated ammonia,however volatility of the ammonia causes errors.
We worked out a new method based on pH measuring in

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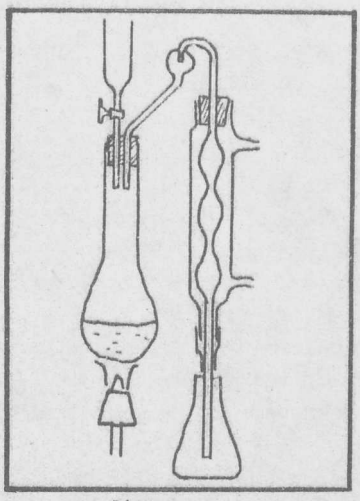


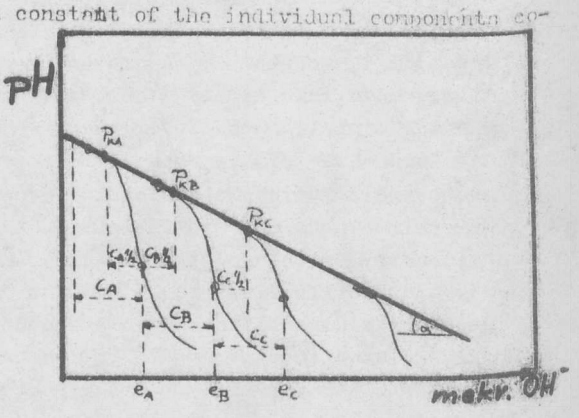
figure 2.
Schulek-Vastagh instrument

the distillated ammonia. The new method is quick and exact. In this work it became necessary to work out a new distillation technic, because we wanted to avoid the extensive dilution of the distillate.

The acid-base determination based upon the pH measuring was found in the article by Ochne and Dolezalova and in another one by Domokos and Havas. In these methods we measure the acid and base quantities in a no acidic or basic buffer solution, in which the pH changing depends on the acid or base concentration linearly.

The buffer solution consists of several components. A buffer solution is suitable when the negative logarithm of the dissotiation constant of the individual components co-

figure 3.
principle of acid-base determination



uld be arranged in line and their pK values stand near to each other. In this case during the neutralization process here isn't a pH jumping, but the change in pH appears linearized. We show the principle of the function of the reagent in the figure 3. In this figure it can be seen, that the pH changes along a line determined by the indivi-

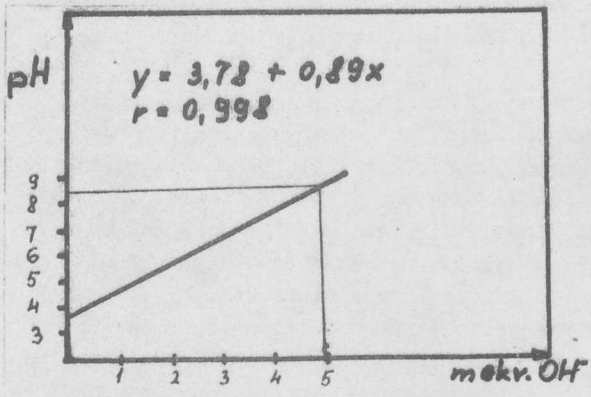


figure 4.
pH changing on the base quantity

dual components. Naturally similar connection can be stood with the basical reagents.

Consequently the pK values the individual components are important, but the ratios of the individual components are similarly important. It is typical for the reagents found in the technical literature, that the extension of their linearized parts comparatively short, therefore their applications are limited.

In our experiments we prepared such a buffer solution, in which the pH changing depending on the ammonia quantity is linear over 4 pH value. The buffer consists of four componets, but we took it into consideration, that we received the distillated ammonia in 4% boric-acid and the boric-acid has also buffer quality, therefore this buffer solution is useful only when we mixed the buffer solution in the given ratio with the distilled ammonia received in 4% boric-acid.

The buffer solution consists of potassium-dihydrogenephosphate, citric-acid, phosphoric-acid and oxalic-acid. We dissolved each material in distillated water in 0,1 normale concentration, and mixed them with each other in equal ratio. After mixing we set its pH for 4. In the figure 4 we show the pH changing depending on the ammonia quantity. As it can be see the linearized interval lasts from 1 to 4 pH value and the correlation-coeffitient is very good. The solve the distillation problem we set out the Schulek-Vastagh method. We found such a solution, in which the volume of the receiver solution grows hardly during the distillation of the complette quantity of the ammonia. At present the distil-

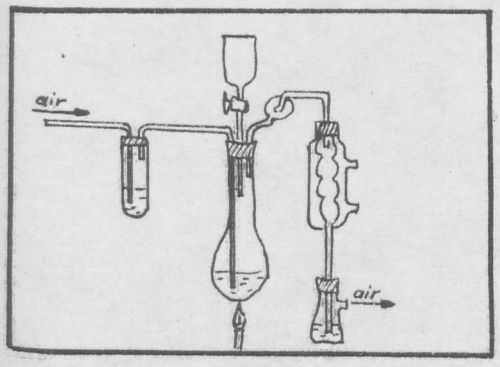


figure 5.

distillation instrument

lation instrument in the figure 5. As it can be see we distillate the ammonia with an air flow made with an ejector jet pump. The air bubbles through a sulfuric acid washing in order to avoid the ammonia pollution from the air.

On the basis of the experiments we worked out the following method for the ammonia determination in the Kjeldahl nitrogen analysis.

We digest the meat and meat products with Kjeldahl procedure then we pour the digests into a 400 cm^3 normal flask and fill it up to sign with distillated water. We use this solution in the ammonia determination.

We pipette 20 cm^3 of aliquot part from the solution, in the distillation vessel and 25 cm^3 of 4% boric-acid in the receiver. We close the apparatus drop 20 cm^3 of 33% sodium-hydroxide solution into the distillation vessel and beside air flow and intensive boiling we begin the distillation. After 10 minute of distillation the ammonia distils totally.

We pour the distillated material in a 100 cm^3 normal flask and fill up till sign with distillated water. From this solution we pipette 9 cm^3 into a beaker, add 1 cm^3 buffer solution and measure the ammonia content.

For the pH measuring we use a special instrument made by the Hungarian analytical instrument society RADELKIS, type: OP-213. This apparatus is named acid-base instrument and it is suitable for measuring the acid or base content on the basis of pH. The instrument has a special scale in potential units, from 0 to 2000. We make a calibration line from which

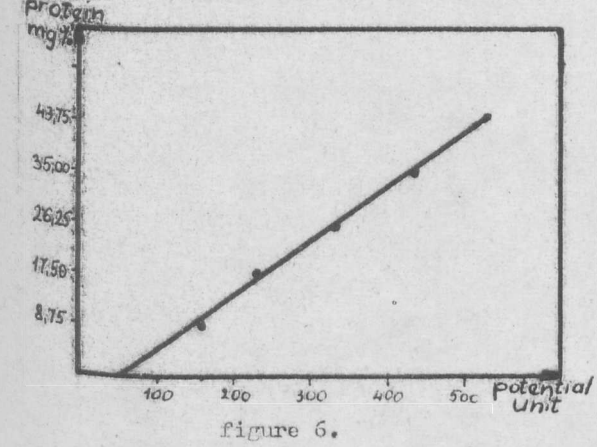


figure 6.

calibration line

we get the ammonia content upon the read value. In our experiments we made a calibration line in which the interval of the ammonia content corresponds to the protein content of the meat and meat products. We present this calibration line in the figure 6. As it can be see the protein content can be measured from 0 to about 40%.

We carried out experiments for the reproducibility of the methods. We measured with different ammonia content and we found that the reproducibility was $\pm$ 5 potential unit when measured directly, and $\pm$ 8 potential unit when measured after distillation. This corresponds to about 0,5% or 0,8% of protein.

We can measure protein content of meat and meat products with this method quickly and exacly. This experiments showed us that this method can be automatized. We can automatize all the operations / distillation included / or only the pH measuring. The latest can be solved by using a sample changer.

Summarizing: we worked out a new method for ammonia determination. This method is quick and exact that's why we recommend it to the analysts working with Kjeldahl method.