Method for urea and hydrolysed protein content determination in beet molasses urea hydrolysates.

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Introduction

Beet molasses – Urea Hydrolysates have two active substances Urea and Hydrolysed Protein. In case of these products both active substances must specified and not only Urea or Hydrolysed protein. In this kind of natural and complex mixtures the determination that used is total nitrogen and ureic nitrogen determination. Specific methods cannot be applied due to high density and deep reddish brown colour of the product that makes all other methods not applicable.

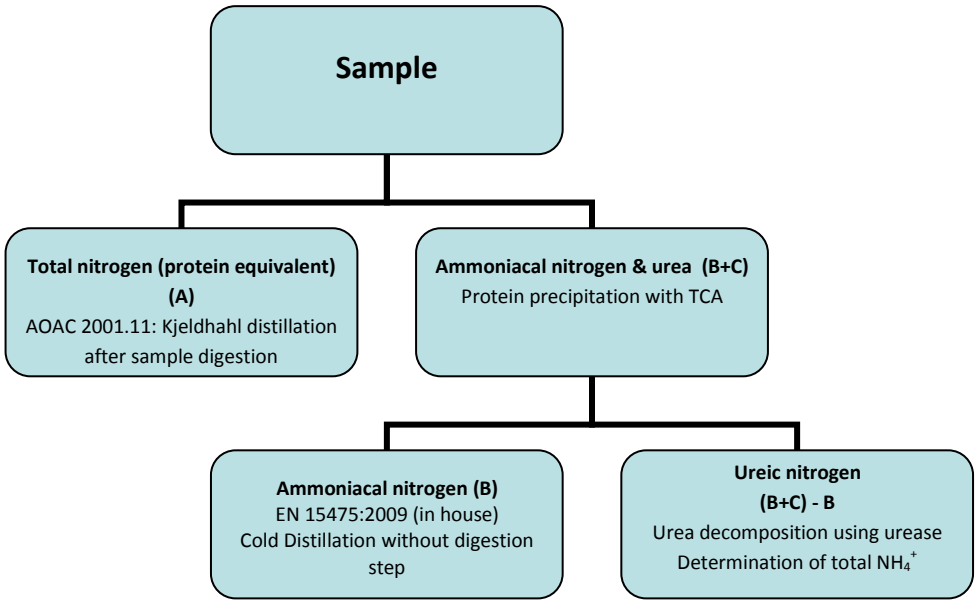
Method

An analytical method has been developed for the determination of the two active substances urea and hydrolysed protein in ENT75 based on determination of different nitrogen types.
The analysis steps which lead us to determine the two types of nitrogen (total and ureic) is given below:

1) Total nitrogen determination (**A).** (Wet digestion, Kjeldahl distillation, Titrimetry)
2) Protein precipitation with TCAA. (This step of protein precipitation with TCAA based on AOAC 991.21).
3) Ammoniacal nitrogen determination **(B)** (Cold distillation, Titrimetry)
4) Urea hydrolysis with urease and nitrogen determination **(B+C)** (Titrimetry, Kjeldahl)
5) Ureic nitrogen **(C)**is calculated as the difference **(C) = (B+C) – (B)**

Below is given the schematic of analysis steps:

Nitrogen analysis steps



Finally the two types of nitrogen are expressed as:

1) Crude protein equivalent = Total nitrogen (A) x 6.25.

2) Urea = Ureic nitrogen (C) x 60/28.

The methods used in nitrogen analysis are all based on AOAC,methods which are acceptable and used in chemical analysis of similar products according to the table:

Test parameter	**Method of analysis**	**Principle**
Total nitrogen (A) - Crude protein equivalent	AOAC 2001.11	Sample digestion, distillation & titrimetry (Kjeldahl)
Ureic nitrogen (C)	AOAC 959.03	Urea decomposition with urease, Titrimetry
Ammoniacal Nitrogen (B)	EN 15475:2009 (in house) Similar method to 2.6.2 section 7.5 EC Reg. 2003/2003	Sample cold distillation & titrimetry (Kjeldahl) without sample digestion

Methods description

Below is the description of these methods used:

1) Total Nitrogen (AOAC 2001.11)

Principle:

The total nitrogen determination according to AOAC 2001.11 method is based on Kjeldahl method. Nitrogen (all forms) derived from the product, is oxidized to ammonium nitrogen using catalyst (copper sulfate – potassium sulfate), sulfuric acid and high temperature for the reaction. After the digestion, a distillation is occurred. Ammonia is liberated using alkaline medium (NaOH sln) and it is captured by a boric acid solution, forming 1:1 complex by ammonia and boric acid:

$$NH_3 + H_3BO_3 \rightarrow NH_4^+:H_2BO_3^- + H_3BO_3 \text{ (excess boric acid)}$$

The boric acid captures the ammonia gas, forming an ammonium-borate complex. As the ammonia collects, the color of the receiving solution changes.

The addition of an acid solution by titration (as HCl 0,1N) exactly neutralizes the ammonium borate complex, and a reverse color change is produced.

$$2NH_4^+:H_2BO_3^- + HCl \rightarrow (NH_4)_2Cl + 2H_3BO_3$$

Equivalent protein is determined by multiplying total nitrogen with a factor 6,25 as method refers.

Apparatus:

Buchi digestion block B-426

Buchi distillation apparatus B-323

Reagents:
As referred in AOAC method 2001.11

Preparation of analytical sample:
No preparation is needed

Determination:
As referred in AOAC method 2001.11

Calculation:
As referred in AOAC method 2001.11

2) Ammoniacal nitrogen (EN 15475:2009 (in house) or similar method to 2.6.2 section 7.5 EC Reg. 2003/2003).

Principle:
The ammoniacal nitrogen determination according to EN 15475:2009 in house method is based on ammonia no thermal distillation in samples free from proteins and without any digestion step.
A primary step of protein precipitation using trichloroacetic acid (TCA) occurs. A sample solution portion free from protein is transferred to a distillation apparatus and after the addition of sodium hydroxide solution ammonia is liberated and it is captured to an acid media (boric acid). A final step of titration (with HCl) occurs similar to total nitrogen determination (AOAC 2001.11) but also other combinations of acid media (sulphuric acid) & indicators could be used e.g. as those referred in EN 15475.

Apparatus:
Buchi distillation apparatus B-323

Reagents:
As referred in method EN 15475:2009
Trichloroacetic acid (TCA)
Boric acid sln (according to AOAC method 2001.11)
Mix of bromocresol green sln & methyl red sln (according to AOAC method 2001.11)

Preparation of analytical sample:
No preparation is needed

Determination:
In a centrifuge tube of 50ml is added 10g of sample (where contains approximately 1,23g N or 7,7g protein equivalent) and TCA solution for protein precipitation. After solution vortex, the sample is centrifuged for 10min at 3000rpm. After precipitation and centrifugation of sample, 10ml of supernatant is transferred to Buchi distillation tube and 40ml of water is added. A no thermal distillation occurs according to 2.6.2 section 7.5 EC Reg. 2003/2003 and the liberated ammonia is captured by boric acid (as AOAC 2001.11 method, but also other compinations of acid media & indicators could be used e.g. those that are referred in EN 15475)

Calculation:
Nitrogen is determined from the titration as in AOAC method 2001.11 (expressed as %w/v) and the results is reduced to the original sample
Ammoniacal nitrogen is expressed as ammonium chloride using a factor 53.5/14 that is the molecular weight ratio for nitrogen of ammonium chloride (Ammonium chloride = Ammoniacal nitrogen X 53.5/14.

3) Ureic nitrogen (AOAC 959.03).

Principle:
The urea nitrogen determination according to AOAC 959.03 is based on urea hydrolysis by urease and the determination of ammonia liberated in a slightly acid environment according to Kjeldhahl method. The samples tested are free from proteins that previously precipitated with trichloroacetic acid. After precipitation and centrifugation of sample, the supernatant is transferred to a distillation apparatus and after the addition of sodium hydroxide solution ammonia is liberated and it is captured to an acid media (boric acid). A final step of titration (with HCl) occurs similar to total nitrogen determination (AOAC 2001.11).

Results are corrected from free ammoniacal nitrogen of samples that is determined by EN 15475 in house method as previously described.

Apparatus:
Buchi distillation apparatus B-323

Reagents:
As referred in AOAC method 959.03

Preparation of analytical sample:
No preparation is needed

Determination:
In a centrifuge tube of 50ml is added 2-3g of sample (where contains approximately 1,23g N or 7,7g protein equivalent) and TCA solution for protein precipitation. After solution vortex, the sample is centrifuged for 10min at 3000rpm. After precipitation and centrifugation of sample, 25ml of supernatant is transferred and the sample solution is handled as AOAC 959.03 referred

Calculation:
Nitrogen is determined from the titration (expressed as %w/v) and the result is reduced to the original sample.
Urea is determined after the subtraction of ammoniacal nitrogen that was previously determined (according to EN 15475:2009 in house method) and using a factor 60/28 that is the molecular weight ratio for nitrogen of urea (Urea=Ureic nitrogen X 60/28)

Refefences Table

1. AOAC official method 2001.11. Protein (crude) in animal feed, Forage (plant tissue), grain and oilseeds.

2. AOAC official method 959.03. Urea in fertilizers.
3. EN 15475:2009 Fertilizers. Determination of ammoniacal nitrogen
4. EC Reg. 2003/2003 2.6.2 section 7.5.
5. AOAC official method 991.21 Non protein nitrogen in whole milk.