SN

中华人民共和国出入境检验检疫行业标准

SN/T 2115—2008

进出口食品和饲料中总氮及粗蛋白的检测方法　杜马斯燃烧法

Determination of the total nitrogen content and calculation of the crude protein content in food and feed for import and export—Combustion according to the Dumas principle

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前　言

本标准的附录A、附录B和附录C为资料性附录。

本标准由国家认证认可监督管理委员会提出并归口。

本标准起草单位：中华人民共和国山西出入境检验检疫局。

本标准主要起草人：薛平、连庚寅、宋欢、苑利、杜利君、潘雅丽、宋洁、康杰。

本标准系首次发布的出入境检验检疫行业标准。

进出口食品和饲料中总氮及粗蛋白的检测方法　杜马斯燃烧法

1　范围

本标准规定了进出口食品和饲料中总氮的杜马斯测定法，并由此计算粗蛋白含量的方法。

本标准适用于谷物、豆类、碾磨谷物产品、油籽油料、奶及奶制品、动物饲料中总氮含量和粗蛋白含量的测定。

本标准不适用于添加无机含氮物质、有机非蛋白质物质食品的测定。

2　方法提要

在有氧环境下，样品在燃烧管中燃烧加热(约900 ℃)，所生成的干扰成分被一系列适当的吸收剂去除。例如，银毛用于吸收卤素，五氧化二磷用于吸收水分，钨用于吸收二氧化硫和多余的氧等等。样品中含氮的物质定量转化成分子氮，并被热导检测器检测。

3　试剂和材料

3.1　二氧化碳(CO_2)气体：纯度大于等于99.995%。

3.2　氧气(O_2)气体：纯度大于等于99.995%。

3.3　含氮标准物质：冬氨酸($C_4H_7NO_4$)或其他氨基酸，纯度大于等于99%。

4　仪器与设备

4.1　杜马斯定氮仪，配有热导检测器。

4.2　样品粉碎机。

4.3　筛子，孔径0.8 mm～1 mm。

4.4　坩埚。

5　试样制备与保存

5.1　试样制备

5.1.1　粮谷、豆类、油籽油料

取有代表性样品500 g，用粉碎机全部粉碎。混匀并过筛，均分成两份作为试样，分装入洁净的容器中，密闭，标明标记。

5.1.2　奶类或水分高于17%且氮含量低于1%的样品

取有代表性样品500 g，均分成两份作为试样，分装入洁净的容器中，密闭，标明标记。

5.1.3　奶粉、豆粉

取有代表性样品500 g，均分成两份作为试样，分装入洁净的容器中，密闭，标明标记。

5.1.4　饲料

取有代表性样品500 g，用粉碎机全部粉碎。混匀并过筛，均分成两份作为试样，分装入洁净的容器中，密闭，标明标记。

5.2　试样保存

干燥试样于0 ℃～4 ℃保存；水分高于17%的样品和液体样品于－18 ℃以下冷冻保存。抽样及制

样过程中，应防止样品受到交叉污染。

6 测定步骤

6.1 测定

6.1.1 固体样品

根据样品含氮量，称取试样 0.1 g～0.3 g(精确至 0.001 g)，包在适用的锡箔(或无氮纸)中，待测定。

6.1.2 液体样品或水分高于 17%且氮含量低于 1%的样品

根据样品含氮量，称取 1.0 g～3.0 g 样品于仪器配备的锡箔样品舟中，称量(精确至 0.001 g)。于 105 ℃～110 ℃干燥 1 h 以上。直至样品呈固体或半固体状时，包严样品舟，待测定。

6.2 仪器工作条件

6.2.1 燃烧温度

850 ℃～1 200 ℃。

6.2.2 通氧量

一般市售杜马斯仪器可以自行检测最佳通氧量，参见附录 A。对于其他仪器，通氧量应控制在样品燃烧后，保留有 2%～8%的残氧量。

6.2.3 通氧时间

可以根据样品量的不同，以及样品燃烧难易程度不同调节通氧时间，以保证样品完全燃烧，同时不致浪费氧气和还原剂。

6.3 仪器校准

开机，根据各自仪器性能设置适当的条件。待仪器稳定后，用氨基酸类标准物质测定三次，当测定结果稳定并且与仪器校准曲线相当时，用氮含量高于待测样品的标准物质做四次重复测定得到日校正因子。杜马斯仪器都带有氮的积分面积-绝对氮含量校准曲线。但是，如果日校正因子的偏差大于 10%，或是更换了热导检测器，应重新绘制校准曲线(参见附录 C)。

7 结果计算和表述

7.1 氮含量

用仪器数据处理机或式(1)计算试样中氮含量：

$$W_N = \frac{m_N}{m} \times 100\% \quad \cdots\cdots(1)$$

式中：

W_N——样品中总氮含量，%；

m_N——仪器显示测试样品的绝对氮量，单位为克(g)；

m——样品的质量，单位为克(g)。

7.2 粗蛋白含量

按式(2)计算，计算结果表示为三位有效数字：

$$W_p = W_N \times F \quad \cdots\cdots(2)$$

式中：

W_p——样品中粗蛋白含量，%；

W_N——样品中总氮含量，%；

F——蛋白质因子，氮换算为蛋白质的系数(参见附录 B)。

8 回收率及检测限

8.1 检测限

本标准方法检出限为0.02%含氮量。测定范围0.1 mg～200 mg绝对氮。

8.2 回收率

本方法总氮含量回收率范围见表1。

表1 本方法总氮含量回收率范围

样品名称	添加范围/%	回收率范围/%
牛奶	0.02	96.3～98.0
酱油	0.02	95.2～99.1
奶酪	0.02	98.2～101.5
奶粉	0.05	95.4～100.5
黄豆	0.05	99.1～99.6
小麦粉	0.05	97.5～103.1
豆粕	0.02	98.6～99.0
鱼粉	0.02	97.0～98.6

附　录　A
（资料性附录）
杜马斯仪器基本流程

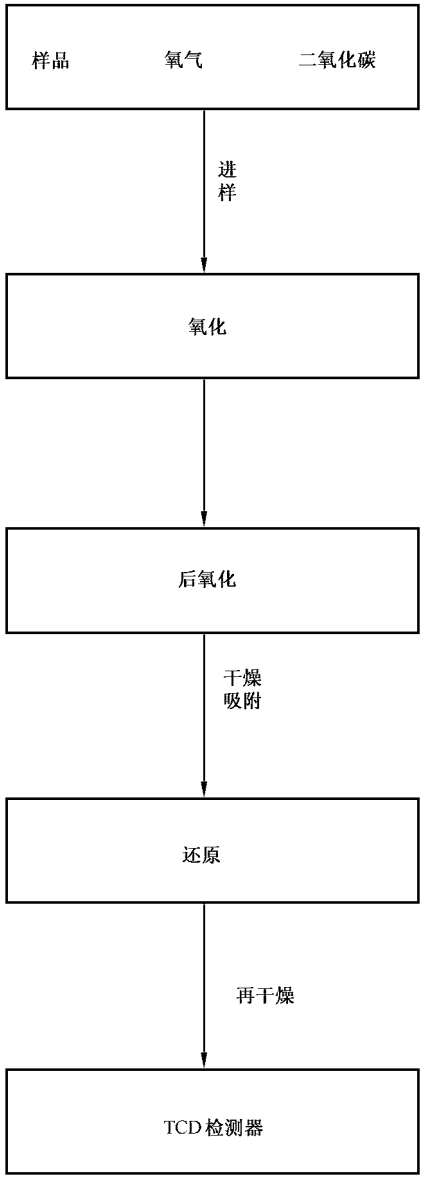

图 A.1　杜马斯仪器工作流程

附 录 B
（资料性附录）
蛋白质因子

表 B.1 部分食品和饲料的蛋白质因子

样 品 名 称	蛋白质因子 F
一般食品	6.25
乳、乳制品	6.38
面粉	5.70
玉米、高粱	6.24
花生	5.46
米	5.95
大豆及其制品	5.71
肉、肉制品	6.25
大麦、小米、燕麦、裸麦	5.83
芝麻、向日葵、棉籽	5.30
饲料	6.25

附 录 C
（资料性附录）
校准曲线

C.1 校准曲线的绘制

当仪器需要更换热导检测器或发生其他重大变化时，应重新做仪器校准曲线。在仪器适当的条件下，根据各个用户不同的测定范围，以冬氨酸（含氮量：10.52%）和尿素（含氮量：46.65%）为标样，测定仪器的校准曲线。以下为测量实例：称取 22 个冬氨酸标样，质量分别为 0.1，0.2，0.5，0.8，1，2，3，5，7，10，15，20，25，30，50，75，100，125，150，175，200 和 250 mg；再称取 13 个尿素标样，质量分别为 30，45，60，80，100，125，150，175，200，250，300，350，429 mg，测量以上样品中的绝对氮含量，得到的仪器校准曲线如图 C.1。图中 X 轴为 TCD 检测器测量的峰面积，Y 轴为标样中的绝对氮含量。这样得到的校准曲线最大可以测量含 200 mg（429 mg×46.65%尿素）氮的样品。

C.2 校准曲线的类型

根据某一台仪器经常测量的样品的氮含量范围，可以选择不同类型的校准曲线：

C.2.1 线性校准曲线

$$y = a + bx$$

这种类型的校准曲线适用于所测样品的氮含量集中在一个很窄的范围，例如，0.02%～8%。

C.2.2 四次非线性曲线

$$y = a + bx + cx^2 + dx^3 + ex^4$$

这种类型的校准曲线适用于所测的样品的氮含量集中在一个很宽的范围内，例如，1%～60%。

C.2.3 混合型：线性＋非线性

这种类型的校准曲线适用于以上两种情况的混合。图 C.1 中所示的是非线性校准曲线：$y=a+bx+cx^2+dx^3+ex^4$。

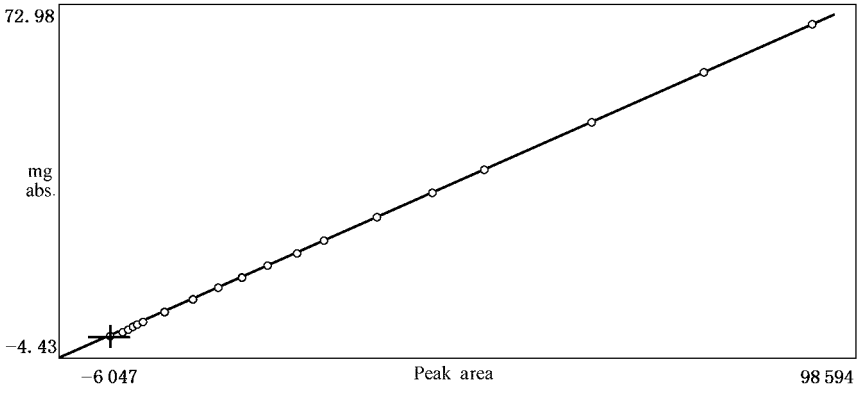

图 C.1 校准曲线

Foreword

Annex A，Annex B and Annex C of this standard are informative annexes.

This standard was proposed by and is under the charge of the National Regulation Commission for Certification and Accreditation.

This standard was drafted by the Shanxi Entry-Exit Inspection and Quarantine Bureau.

This main drafters of this standard are：Xue Ping，Lian Gengyin，Song Huan，Yuan Li，Du Lijun，Pan Ya-li，Song Jie，Kang Jie.

This standard is inspection and quarantine professional standard promulgated for the first time.

Determination of the total nitrogen content and calculation of the crude protein content in food and feed for import and export— Combustion according to the Dumas principle

1 Scope

This standard spicifies a method for the determination of the total nitrogen content and the calculation of crude protein content of food, animal feeding stuffs.

This standard is applicable to the determination of the total nitrogen content and the crode protein content of cereals, pulses, milled cereal products, oilseeds and feeding stuffs.

This standard is not applicable to the determination of the food which have been added inorganic nitrogen, or have been added organic nitrogen but not protein.

2 Principle

Samples are converted to gases by heating in a combustion tube. All interfering components are removed from the resulting gas mixture. The nitrogen compounds in the sample are converted to moiecular nitrogen, which is quantitatively determined by a thermal conductivity detector. The nitrogen content is calculated by a microprocessor.

3 Reagents and materials

3.1 Carbon dioxide(CO_2), as pure as possible and not less than 99.995%.

3.2 Oxygen(O_2), as pure as possible and not less than 99.995%.

3.3 Aspartic acid($C_4H_7NO_4$)or others amino acid standard reference materials with known constant certified nitrogen content. Minimum assay should be amass fraction of 99%.

4 Apparatus and equipment

4.1 Dumas apparatus, with a thermal conductivity detector and suitable device for signal integration.

4.2 Grinding device.

4.3 Sieve, of aperture size 0.80 mm or 1 mm, made of iron-free material.

4.4 Crucibles, made of stainless steel or ceramic.

5 Preparation and storage of test sample

5.1 Preparation of test sample

5.1.1 Cereals, pulses, milled cereal products, oilseeds

Quarter the sample 500 g, grind thoroughly in a Grinding device. Pass through the sieve. Mix thoroughly and divide into two equal portions as test sample. Place in clian containers, seal and label them.

5.1.2 Milk, samples which moisture higher than 17% and nitrogen content less than 1%.

Quarter the sample 500 g, mix thoroughly and divide into two equal portions as test sample. Place in clian containers, seal and label them.

5.1.3 Milk powder, soybean powder

Quarter the sample 500 g, mix thoroughly and divide into two equal portions as test sample. Place in clian containers, seal and label them.

5.1.4 Feeding stuffs

Quarter the sample 500 g, Grind thoroughly in a Grinding device. Pass through the sieve. Mix thoroughly and divide into two equal portions as test sample. Place in clian containers, seal and label them.

5.2 Storage of test sample

The dry test sample should be stored between 0 ℃～4 ℃. The samples which moisture higher than 17% and liquit test sample should be stored bilow－18 ℃. While sampling and sample preparation, precaution must be taken to avoid contamination.

6 Procedure

6.1 Determination

6.1.1 Solid samples

Weigh test samples of food, feeding stuff 0.1 g～0.3 g to the nearest 0.001 g. Into a crucible or tin capsule to waiting for determination.

6.1.2 Liquid samples, and samples which moisture higher than 17% and nitrogen content less than 1%.

Depending on the nitrogen content of samples, Weigh 1.0 g～3.0 g (to the nearest 0.001 g) of the prepared test sample into a boat or crucible and dry for at least 1 h in an oven set between 105 ℃ and 110 ℃. Untill become solid or semisolid, determinate according to the operating condition.

6.2 Operating condition

6.2.1 Heating temperature

850 ℃～1 200 ℃.

6.2.2 Oxygen demand

Generic Dumas equipment require an estimation of the oxygen demand of the test portion. Annex A For instruments with a self-optimizing oxygen control, the mass fraction of the residual oxygen content shall be between 2%～8%.

6.2.3 The time of the oxygen provide: The time and flow rate of the oxygen can be adjusted according to the sample weight and combustibility to ensure the complete combustion.

6.3 Calibration

Operate the instrument and introduce the test portion according to manufacturer's instructions. Check the instrument response by running at least three nitrogen standards of known content. When the response is constant and the valres obtained correspond to the long-term calibration as established above, proceed with the determination of the daily calibration factor by analysing at least four standard nitrogen compounds representing a nitrogen mass higher than the test samples to be analysed.

Full range re-calibration will become necessary if the daily calibration factor deviates from its expected value by more than 10% relative, or if thermal conductivity detector have been replaced (Annex C).

7 Calculation and expression of the result

7.1 Calculate the content of nitrogen in the test sample by Dumas equipment data processor or according to the formula(1).

$$W_N = \frac{m_N}{m} \times 100\% \qquad \cdots\cdots(1)$$

where:

W_N —the nitrogen content in the test sample, %;

m_N —the weigh of nitrogen in the test sample, g;

m —the weigh of sample, g.

7.2 Crude protein content

The crude protein is obtained by the formula(2).

$$W_p = W_N \times F \qquad \cdots\cdots(2)$$

where:

W_p —the crude protein content in the test sample, %;

W_N —the nitrogen content in the test sample, %;

F —the agreed ratio factor between the protein and nitrogen content(see annex B).

8 Limit of determination and recovery

8.1 Limit of determination

The limit of determination of this method is 0.02%(content of nitrogen). The determination scope is 0.1 mg～200 mg nitrogen.

8.2 Recovery

The recovery of this method see table 1.

Table 1—The recovery of this method

Samples	Fortified range/%	Recovery range/%
milk	0.02	96.3～98.0
sauce	0.02	95.2～99.1
cheese	0.02	98.2～101.5
milk powder	0.05	95.4～100.5
soybean	0.05	99.1～99.6
wheat powder	0.05	97.5～103.1
bean cake	0.02	98.6～99.0
fish meal	0.02	97.0～98.6

Annex A
（Informative annex）
The flow of Dumas apparatus

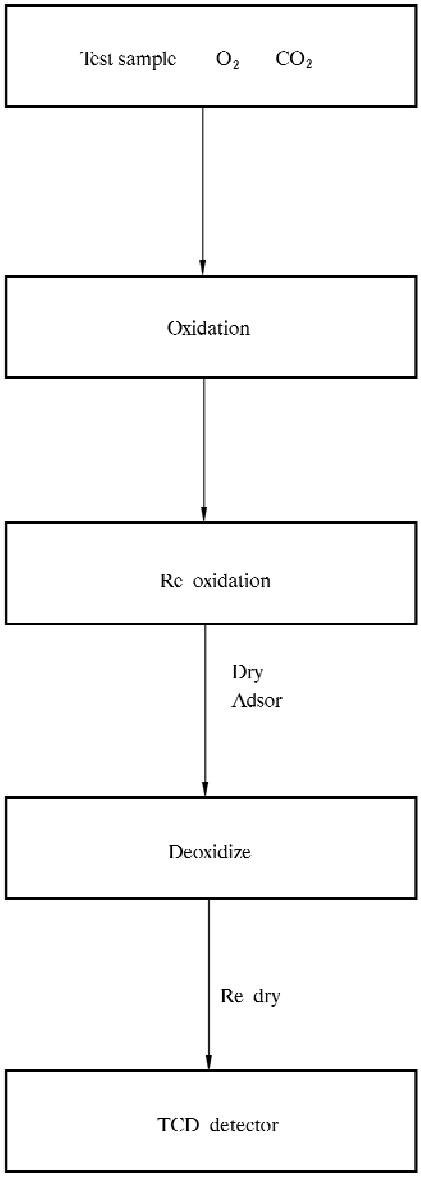

Figure A. 1 —The flow of Dumas apparatus

Annex B
（Information annex）
Factors for converting nitrogen content to protein content

Table B. 1—Factors for converting nitrogen content to protein content

Sample	the agreed ratio factor F
General food	6. 25
Milk and mild product	6. 38
Wheat powder	5. 70
Corn and broomcorn	6. 24
Peanut	5. 46
Rice	5. 95
Soybean and bean product	5. 71
Meat and meat product	6. 25
Foodstuff	5. 83
gingili、sunflower seed、cottonseed	5. 30
feedstuff	6. 25

Annex C
(Information annex)
Catibration curve

C.1 Draw catibration curve

It is necessary to set up an instrument calibration if the daily calibration factor deviates from its expected value by more than 10% relative,or if thermal conductivity detector have been replaced. Operate the instrument and introduce the test portion according to manufacturer's instructions. Pure standard nitrogen compounds (aspartic acid or urea) with a known constant nitrogen content are used for instrument calibration. According to the different measuring ranges,use aspartic acid(N:10.52%)and urea(N:46.65%)as calibration samples to measure the calibration curve. The following is a real example:Weight 22 aspartic acid samples with suitable balance,the weights are 0.1,0.2,0.5,0.8,1,2,3,5,7,10,15,20,25,30,50,75,100,125,150,175,200,250 mg. Then weight 13 urea samples,the weights are 30,45,60,80,100,125,150,175,200,250,300,350,429 mg. The calibration curve is as below illustration. In the illustration,the X axis is peak area of TCD signal,Y axis is the absolute N content in the samples. By using this curve,the highest absolute N content in the samples can be measured is 200 mg(429 mg×46.65% urea).

C.2 Kind of calibration curve

For different N concentration's measurements,the different mode of calculation can be selected:

C.2.1 Linear

$$y = a + bx$$

Applicable in a narrow concentration range N(for instance,0.02%～8%).

C.2.2 Non-linear(4 th order polynom)

$$y = a + bx + cx^2 + dx^3 + ex^4$$

Usually evaluation for the entire range N(1%～60%).

C.2.3 Mixed linear+non-linear

Combination of the 2 cases above.

The below illustration is a non-linear(4 th order polynom)calibration curve：

$$y = a + bx + cx^2 + dx^3 + ex^4$$

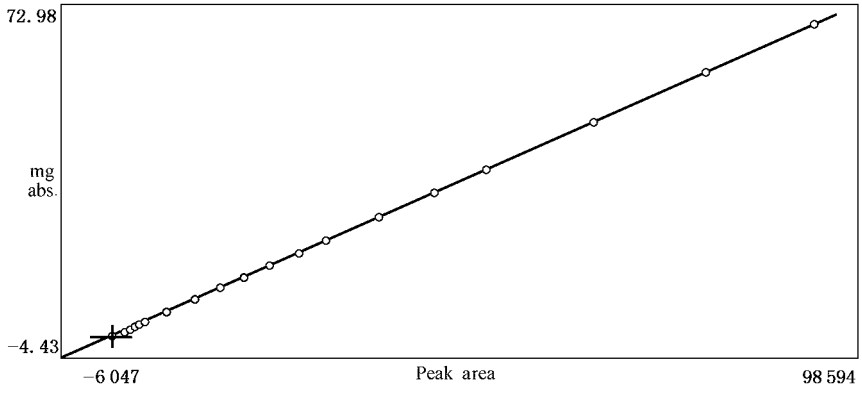

Figure C. 1 —Calibration curve

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