
Textiles — Determination of stable nitrogen isotope ratio in cotton fibres

*Textiles — Détermination du rapport isotopique stable de l'azote
dans les fibres de coton*

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Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular, the different approval criteria needed for the different types of ISO documents should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

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For an explanation of the voluntary nature of standards, the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the World Trade Organization (WTO) principles in the Technical Barriers to Trade (TBT) see www.iso.org/iso/foreword.html.

This document was prepared by Technical Committee ISO/TC 38, *Textiles*.

Any feedback or questions on this document should be directed to the user's national standards body. A complete listing of these bodies can be found at www.iso.org/members.html.

Introduction

Nitrogen (N) is the most essential element for cotton fibre production, since cotton crops require large amounts of N to sustain their growth and productivity. For this purpose, considerable amounts of nitrogen are applied to the soil as chemical fertilizers (e.g. urea) or organic fertilizers (e.g. composted manure).

The nitrogen applied in the form of a chemical fertilizer or a composted manure is absorbed from the soil to cotton crops through their root systems during cultivation, leaving a fertilizer-specific nitrogen isotopic signature in cotton tissues (particularly in cotton fibres). The inerascable nitrogen isotopic fingerprint engraved in cotton fibres remains unchanged during manufacturing of yarns, textiles and fabrics.

This document employs the principle of nitrogen isotope discrimination that fractionates against ^{15}N (heavier nitrogen isotope), resulting in ^{15}N -enriched reactants and ^{15}N -depleted products. In general, composted manure has a much higher $\delta^{15}\text{N}$ (natural ^{15}N abundance, defined hereinafter) than chemical fertilizer due to faster NH_3 (ammonia) volatilization of the lighter nitrogen isotope (^{14}N) than ^{15}N during the composting process. In contrast, chemical fertilizers (e.g. urea) are produced from the atmospheric N_2 via the Haber-Bosch process, resulting in low $\delta^{15}\text{N}$ values close to atmospheric N_2 , which means that similar isotopic nitrogen compositions of chemical fertilizers to the atmospheric N_2 .

Organic farming strictly bans the use of genetically modified crops (GMO), synthetic pesticides, and chemical fertilizers. Therefore, the use of chemical fertilizer during organic cotton production can be detected by determining $\delta^{15}\text{N}$ of cotton fibres, since the difference in $\delta^{15}\text{N}$ values between the two isotopically different nitrogen inputs (chemical vs. organic fertilizers) leaves a fertilizer-specific isotopic fingerprint in cotton fibres that can thereafter provide a forensic evidence for organic cotton fibre production.

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Textiles — Determination of stable nitrogen isotope ratio in cotton fibres

1 Scope

This document specifies the determination of the ratio of nitrogen isotopes in cotton fibres that are used for textile production. It applies not only to cotton textiles but also to raw cotton taken from cotton fields.

2 Normative references

There are no normative references in this document.

3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

ISO and IEC maintain terminological databases for use in standardization at the following addresses:

- ISO Online browsing platform: available at <https://www.iso.org/obp>
- IEC Electropedia: available at <http://www.electropedia.org/>

3.1

stable isotope

atom whose nucleus contains the same number of protons but a different number of neutrons

Note 1 to entry: Stable isotopes do not decay into other elements, while radioactive isotopes are unstable and will decay into other elements.

3.2

isotope-ratio mass spectrometry

IRSM

specialization of mass spectrometry, in which mass spectrometric methods are used to measure the relative abundance of isotopes in given sample

Note 1 to entry: The analysis of isotopes is normally related to measuring isotopic variations caused by mass-dependent isotopic fractionation in natural systems.

3.3

isotopic fractionation

isotopic discrimination

process that affect the relative abundance of isotopes

Note 1 to entry: Normally, the focus is on *stable isotopes* (3.1) of the same element. Isotopic fractionation in the natural environment can be measured by isotope analysis, using isotope-ratio mass spectrometry, to separate different element isotopes on the basis of their mass-to-charge ratios.

Note 2 to entry: Both heavy and light stable isotopes participate freely in biochemical reactions and in geochemical processes, but the rate at which heavy and light stable isotopes react differs. As a result, the lighter isotopes react faster than the heavier isotopes leading to isotopic fractionation between reactant and product in the reactions.

3.4

natural ^{15}N abundance

relative amount of the isotopes of nitrogen, as it occurs in nature

Note 1 to entry: Natural ^{15}N abundance is expressed in parts per thousand (‰, per mil) deviations from the atmospheric N_2 (a standard).

4 Principle

Isotope-ratio mass spectrometry (IRMS) measures the relative abundance of stable isotopes (e.g. ^{14}N and ^{15}N for nitrogen) in a cotton fibre sample. The abundance of stable isotopes varies according to physical, physiological and biochemical isotopic fractionation processes. Consequently, variations in the isotopic ratio of nitrogen (i.e. $^{15}\text{N}/^{14}\text{N}$) can be used both as fingerprints of the nitrogen source and to track the fate and transformation of nitrogen-containing compounds.

Nitrogen (N) is the most essential element that can increase the production of cotton fibres that are used to manufacture cotton yarns, textiles and fabrics. To ensure cotton fibre production, sufficient amount of nitrogen should be supplied as fertilizers (chemical or organic fertilizers) to the soil to meet the nitrogen demand for the growth and productivity of cotton crops.

Since most of fertilizer-nitrogen released into the soil is absorbed through root systems, translocated, and stored in the cotton fibres during cultivation, the isotopic nitrogen composition of the cotton fibre, expressed as $\delta^{15}\text{N}$, reflects the type of fertilizer from which it originated. In general, $\delta^{15}\text{N}$ of chemical fertilizers is close to 0 ‰, ranging from -3,9 ‰ to +0,5 ‰, while that of composted manure is much higher, ranging between +15,4 ‰ and +19,4 ‰. Cotton fibres harvested are processed to produce cotton yarns, textiles, and fabrics. Therefore, it is believed that $\delta^{15}\text{N}$ of a cotton fibre that contains information about the type of nitrogen fertilizers can serve as an isotopic fingerprint to identify authenticity and trace origin of cotton products, such as yarns, textiles and fabrics.

An accurate measurement of $\delta^{15}\text{N}$ of an aliquot of the dried cotton fibres can be obtained by using a continuous-flow type isotope ratio mass spectrometer linked to an elemental analyser (EA-IRMS). An aliquot of the test sample is washed, dried, ground to very fine powders using a ball-mill, weighed in a tin capsule on a microbalance, and then analysed for cotton- $\delta^{15}\text{N}$.

In particular, the use of chemical fertilizers can be attested by simultaneous measurements of stable nitrogen isotope ratio ($\delta^{15}\text{N}$) of cotton fibre and soil samples at harvest by using the determination process specified herein, and the determination procedure is specified in [Annex A](#). [Annex B](#) shows some main outcomes of feasibility testing performed on cotton fabrics from organic certified textiles (see [Table B.1](#)) and raw cotton fibres obtained from several countries (see [Table B.2](#)).

5 Reagent

5.1 Reference material, nitrogen isotopes in ammonium sulfate (IAEA-N2) provided by the International Atomic Energy Agency (IAEA), necessary to check the accuracy and precision of $\delta^{15}\text{N}$ analysis, and available as NIST RM8548.

The $\delta^{15}\text{N}$ of NIST RM8548 is +20,3 ‰, and the accuracy and reproducibility of $\delta^{15}\text{N}$ analysis checked periodically with this standard should be lower than 0,4 ‰ and 0,2 ‰, respectively.

6 Apparatus

6.1 Drying oven, capable of heating up to around 80 °C.

6.2 Ball-mill, capable of grinding to final size smaller than 5 µm, and vibrational frequency ranging 3 Hz to 30 Hz (180 ~1 800) r/min are at least required.

6.3 Microbalance, with readability of 1 µg.

6.4 Continuous-flow elemental analyser-isotope ratio mass spectrometer (EA-IRMS).

7 Preparation

7.1 Sampling of test specimen

Cotton fibre samples are taken from fabricated textiles and prepared as test specimens.

7.2 Pre-treatment of test specimen

Cotton fibre samples are immersed with 100 ml distilled waters for 3 min and oven-dried at 80 °C for 24 h. Dried samples are chopped, homogeneously ground into a very fine powder using a ball-mill and used in triplicate for ¹⁵N analysis.

8 Test procedure

8.1 Weighing of aliquot

8.1.1 Determination of weighing amount needed

Each finely ground test specimen is weighed with a tin capsule (or can) using a microbalance (readability: at least 1 µg).

Provide between (100 ~1 200) µg of nitrogen for each aliquot of the test specimen to meet EA-IRMS detection limits.

However, the minimum amount needed for accurate and precise analysis of δ¹⁵N is typically about 100 µg of nitrogen.

A simple formula to determine the amount of an aliquot to weigh for the EA-IRMS measurements is given as [Formula \(1\)](#):

$$m_a = \frac{m_N}{1000} \times \frac{100}{w_p} \quad (1)$$

where

m_a is the dry mass of an aliquot needed for EA-IRMS measurements (mg);

m_N is the dry mass of nitrogen needed (µg);

w_p is the mass fraction percentage of nitrogen in the test specimen (%).

For example, if the test specimen has 1 % nitrogen and 100 µg of nitrogen for the IRMS measurements is provided, then an aliquot that weighs 10 mg shall be prepared. For samples with unknown % nitrogen, it is recommended to determine the % nitrogen of the test specimen before making isotope analysis.

In general, the optimal mass of an aliquot for the IRMS measurements is 33 mg to 56 mg for cotton products (cotton fibres in yarns or textiles), since cotton products typically contain between 0,18 and 0,30) % nitrogen.

8.1.2 General weighing procedure

a) Clean the plate and tools using lint-free papers or equivalents moistened with alcohol or acetone.

- b) Clean the microbalance with a brush.
- c) Turn on the microbalance and wait for the microbalance to stabilize.
- d) Use the tweezer to grab an empty tin capsule by the edge and place on top of the balance.
- e) Push the “TARE” button to re-zero the mass.
- f) Carefully scoop out a small aliquot of test specimen into the tin capsule by adding in small increments.
- g) Remove the tin capsule from the microbalance.
- h) Fold the tin capsules several times into a small cube or sphere with the tweezers to make sure the openings are closed.
- i) Place the cube into your sample tray such as 96-well tray for delivery or shipping.
- j) Clean the spatula between each weighing to avoid contamination.

When finished, clean the microbalance and tools before turning off the microbalance.

8.2 Natural ^{15}N abundance analysis

8.2.1 Instrumentation

Continuous-type EA-IRMS is used for the determination of ^{15}N in a gas sample that is introduced into IRMS through the elemental analyser (EA). The EA-IRMS is equipped with an ion-source for ionizing the incoming sample gases, a permanent magnet and an electromagnet for steering the ions, and the Faraday cups collecting N_2 gas travelling with the different mass-to-charge ratios.

The isotopic nitrogen composition of the sample gas is compared to that of standard gas (atmospheric N_2) during each sample run, and the resulting sample's $\delta^{15}\text{N}$ value is calculated using [Formula \(1\)](#).

8.2.2 General analytical procedure

- a) Place packed tin capsules continuously into a carousel (auto-sampler unit) for automatic analysis.
- b) Drop packed tin capsules sequentially into the oxidation reactor (980 approximately 1 080) °C and then combust them with excess O_2 to complete oxidation.
- c) Perform EA-IRMS measurements and check the isotopic nitrogen composition of gas relative to that of the standard gas (atmospheric N_2) from a compressed cylinder with known or predetermined isotope ratio at every measurement.
- d) During each run, check the precision of isotope analysis with an internationally accepted reference material.
- e) Calculate and report the final isotopic value ($\delta^{15}\text{N}$) relative to that of atmospheric N_2 .

Natural ^{15}N abundance of a given sample is expressed in parts per thousand (‰, per mil) deviations from the atmospheric N_2 (a standard), and this precisely small difference in the abundance of isotopes can be measured by using isotope-ratio mass spectrometers. The deviation in natural ^{15}N abundance of a sample from that of the standard (atmospheric N_2) is referred to as $\delta^{15}\text{N}$ which is defined as the following [Formula \(2\)](#):

$$N_a = \left(\frac{R_{sa}}{R_{st}} - 1 \right) \times 1\,000 \quad (2)$$

where

N_a is $\delta^{15}\text{N}$ (‰);

R_{sa} is the ratio of sample;

R_{st} is the ratio of standard.

where the ratio is the isotopic ratio of the heavier isotope (^{15}N) to lighter isotope (^{14}N). Atmospheric N_2 having the ratio of 0,366 3 atom % ^{15}N is chosen as the standard for this ^{15}N analysis of samples.

9 Precision

The accuracy and reproducibility of the analytical procedure can be checked by reference materials.

10 Test report

The test report shall at least include the following items:

- a) a reference to this document, i.e. ISO 20921:2019;
- b) sample number;
- c) cotton- $\delta^{15}\text{N}$ and nitrogen content;
- d) mean and standard deviation of the reference material.

Annex A (normative)

Identification procedure of organic raw cotton fibres by using stable nitrogen isotope ratios

A.1 General

Natural ^{15}N abundance ($\delta^{15}\text{N}$) of chemical fertilizers is close to 0 ‰, ranging from $-3,9$ ‰ to $+0,5$ ‰, while composted manure has much higher $\delta^{15}\text{N}$ than chemical fertilizers, ranging between $+15,4$ ‰ and $+19,4$ ‰.

Therefore, the addition of composted manure increases cotton- $\delta^{15}\text{N}$ compared with soil- $\delta^{15}\text{N}$ at harvest, while that of chemical fertilizer lowers cotton- $\delta^{15}\text{N}$. This contrasting pattern of cotton- $\delta^{15}\text{N}$ versus soil- $\delta^{15}\text{N}$ makes it possible to use $\delta^{15}\text{N}$ signal stored in cotton fibres as a potential marker for identifying organic cotton fibres from their conventionally grown counterparts. Consequently, this nitrogen isotopic fingerprinting can provide a forensic evidence in tracing cotton products from the origins (cotton fibres) to processing of cotton yarn, textile and fabrics.

A.2 Preparation

A.2.1 Sampling of test specimen

Follow the general procedures and protocols for cotton fibres and soil sampling in principle.

A.2.2 Cotton fibres

Sampling of cotton fibres (test specimen 1) is made at harvest from each cotton field before further shipping and processing. At least two cotton crop (cotton fibre) samples are randomly collected in $1\ 000\ \text{m}^2$.

A.2.3 Soil

Sampling of soil (test specimen 2) at harvest time is mandatory particularly at the early identification of cotton fibres; otherwise, can be omitted. In each cotton field, at least two composite soil samples were randomly collected in $1\ 000\ \text{m}^2$. Each composite soil sample was made using five sub-samples taken individually from five root zones (10 cm approximately 30 cm) around each representative crop chosen. Sample size could vary depending on soil heterogeneity, crop history, fertilization history, and so forth.

A.2.4 Pre-treatment of test specimen

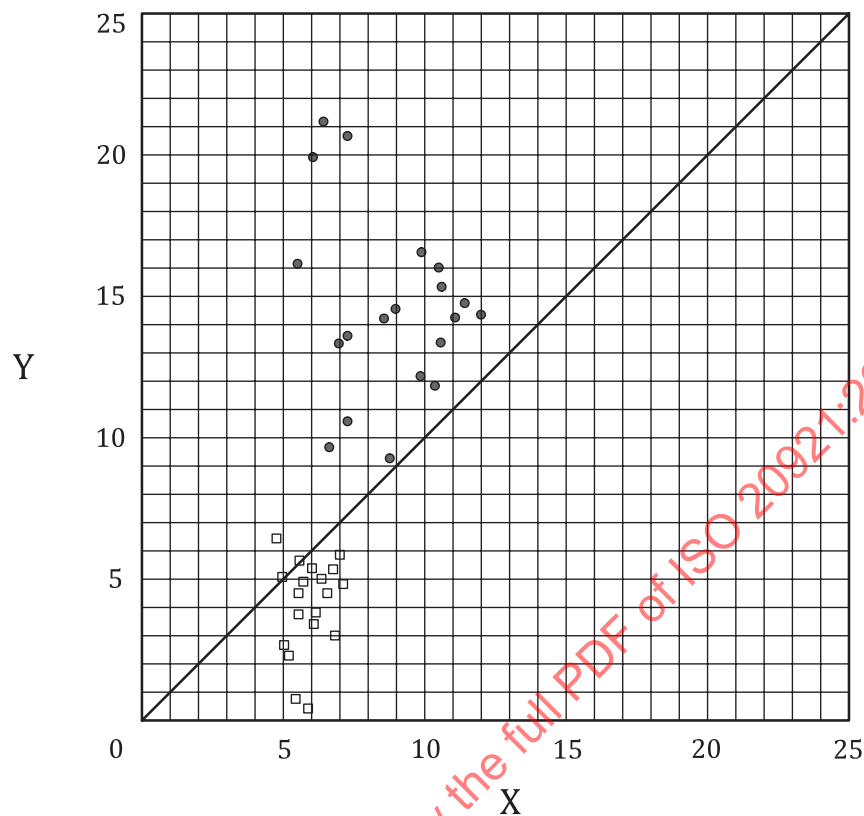
A.2.4.1 Cotton fibres

Cotton fibre samples are immersed with 100 ml distilled waters for 3 min and oven-dried at $80\ ^\circ\text{C}$ for 24 h. Dried samples are chopped homogeneously ground into a very fine powder using a ball-mill.

A.2.4.2 Soil

Soil (test specimen 2) samples are oven-dried at $105\ ^\circ\text{C}$ for 24 h, passed through a 2 mm sieve before grinding. Dried samples are homogeneously ground into a very fine powder using a ball-mill.

A.3 Comparison between cotton and soil results



Key

- X $\delta^{15}\text{N}$ of total soil-N (‰)
- Y $\delta^{15}\text{N}$ of total plant-N (‰)
- compost-applied
- fertilizer-applied

Figure A.1 — Cross-plot of $\delta^{15}\text{N}$ values of total soil-N versus $\delta^{15}\text{N}$ values of total plant-N from fertilizer-applied and compost-applied upland fields

Figure A.1 shows that plants grown in compost-applied fields were enriched in ^{15}N , while those grown in fertilizer-applied fields were depleted relative to soils. The 1:1 line of plant- $\delta^{15}\text{N}$ versus soil- $\delta^{15}\text{N}$ is indicated. In summary, the application of compost resulted in a significant ^{15}N enrichment of total soil-N and total plant-N relative to fertilizer amendment.

Annex B (informative)

Analytical results of cotton fibres of various origin and process stage

B.1 Example of test results for cotton fabrics from organic certified textiles

Table B.1 — Nitrogen content and its natural abundance ($\delta^{15}\text{N}$) of white and coloured cotton fabrics of organic certified textiles

Sample	Sampling part	N content g/kg	$\delta^{15}\text{N}$ ‰
	white	2,17	+1,79
	violet	2,56	+2,01
	white	2,77	-3,74
	sky blue	1,28	-4,01
	white	3,13	-2,38
	navy blue	1,26	-2,34

[Table B.1](#) shows the results of a test analysis of cotton fabric samples taken from commercially available infant garments. The garments used as samples were certified organic cotton, and the cotton fibres used were subjected to a general dyeing process. The result shows that nitrogen content and natural ^{15}N abundance ($\delta^{15}\text{N}$) contained in cotton fibres can be measured even if they are used in manufacturing textile products.