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Commission Regulation (EC) No 1882/2006 of 19 December 2006
laying down methods of sampling and analysis for the official control
of the levels of nitrates in certain foodstuffs (Text with EEA relevance)

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ANNEX

METHODS OF SAMPLING, SAMPLE PREPARATION AND ANALYSIS FOR OFFICIAL CONTROL OF LEVELS OF NITRATES IN CERTAIN FOODSTUFFS

A. GENERAL PROVISIONS

Official controls shall be performed in accordance with the provisions of Regulation (EC) No 882/2004. The following general provisions shall apply without prejudice to the provisions in Regulation (EC) No 882/2004.

A.1 Scope

Samples intended for official control of the levels of nitrate content in foodstuffs listed in Section 1 of the Annex to Regulation (EC) No 1881/2006 shall be taken according to the methods set out in this Annex. Aggregate samples thus obtained, either directly from a field or from a lot, shall be considered as representative of the lots.

Compliance shall be established on the basis of the levels determined in the laboratory samples.

A.2 Definitions

For the purpose of this Annex, the following definitions shall apply:

- A.2.1 'lot' means an identifiable quantity of a food commodity to be harvested at the same time or delivered at one time and determined by the official to have common characteristics, such as origin, variety or soil type within a maximum area of 2 hectares, type of packing, packer, consignor or markings.
- A.2.2 'sublot' means a designated part of a large lot in order to apply the sampling method on that designated part; each sublot must be physically separate and identifiable.
- A.2.3 'incremental sample or unit' means a quantity of material taken from a single place in the lot or sublot. In this case it may be a single lettuce or spinach head, or handful of baby leaf, or one bag of cut leaves.
- A.2.4 'aggregate sample' means the combined total of all the incremental samples taken from the lot or sublot.
- A.2.5 'laboratory sample' means a sample intended for the laboratory.
- A.2.6 'field' means a specified area of land of the same soil type and cultivation practice, containing a single variety of lettuce or spinach at same growth stage. 'Field' may also be referred to as 'lot' in the method of sampling.
- A.2.7 'area under cover' means a specified area of land covered by a glasshouse or a polytunnel (plastic or polyethylene tunnel or greenhouse) containing a single variety of lettuce or spinach at the same growth stage and to be harvested at the same time. 'Area under cover' may also be referred to as 'lot' in the method of sampling.

A.3 General provisions

A.3.1 Personnel

Sampling shall be performed by an authorised person as designated by the Member State.

A.3.2 Material to be sampled

Each lot which is to be examined shall be sampled separately. Large lots (i.e. lots of more than 30 tonnes or larger than 3 hectares) shall be subdivided into sublots to be sampled separately.

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A.3.3 Precautions to be taken

In the course of sampling and preparation of the samples, precautions shall be taken to avoid any changes, which would affect:

- the nitrate content, adversely affect the analytical determination or make the aggregate samples unrepresentative, e.g. the presence of soil on lettuce or spinach during sample preparation,
- the food safety or integrity of the lots to be sampled.

Also, all measures necessary to ensure the safety of the persons taking the samples shall be taken.

A.3.4 Incremental samples

As far as possible incremental samples shall be taken at various places distributed throughout the lot or subplot. Departure from such procedure shall be recorded in the record provided for under part A.3.8. of this Annex.

A.3.5 Preparation of the aggregate sample

The aggregate sample shall be made up by combining the incremental samples.

A.3.6 Replicate samples

The replicate samples for enforcement, defence and reference purposes shall be taken from the homogenised aggregate sample, unless such procedure conflicts with Member States' rules as regards the rights of the food business operator.

A.3.7 Packaging and transmission of samples

Each sample shall be placed in a clean, inert sealed opaque plastic bag to prevent loss of moisture and offering adequate protection against any damage or contamination.

The sample must be transferred to the laboratory within 24 hours of sampling and shall be kept cool during transport. If this is not possible the sample shall be deep-frozen within 24 hours and kept frozen (up to a maximum of six weeks).

All additional necessary precautions shall be taken to avoid any change in composition of the sample, which might arise during transportation or storage.

A.3.8 Sealing and labelling of samples

Each sample taken for official use shall be sealed at the place of sampling and identified following the rules of the Member State.

A record shall be kept of each sampling, permitting each lot to be identified unambiguously and the sampling officer shall record the variety, grower, production method, date, place of sampling, food business operator responsible for consignment, and any other relevant information likely to be of assistance to the analyst.

A.4 Different types of lots

Food commodities may be traded in bulk or in containers, including sacks, bags and crates, or in individual retail packings. The method of sampling may be applied to all the different forms in which the commodities are put on the market.

B. METHOD OF SAMPLING

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As far as possible, incremental samples shall be taken at various places throughout the lot or subplot.

B.1 Sampling in the field

In case the competent authority considers it necessary to sample the lettuce or spinach in the field, the sampling has to be performed as follows:

Incremental samples shall not be collected from areas that appear to be unrepresentative of the field or area under cover. Areas with different soil types, which have been subjected to different cultivation practices or contain different lettuce or spinach varieties, or to be harvested at a different time, shall be treated as separate lots or fields. If the field is larger than 3 hectares, the field shall be divided into sublots of 2 hectares and each subplot shall be sampled separately.

Incremental samples shall be collected by walking a 'W' or 'X' shaped pattern across the field. Crops harvested from narrow beds or area under cover shall be harvested in a 'W' or 'X' shaped pattern from several beds and pooled to form the aggregate sample.

Plants must be cut at ground level.

The sample must contain at least 10 plants, and the aggregate sample of 10 plants must weigh at least 1 kg. Only units of a marketable size shall be sampled⁽¹⁾. Soil, outer non-edible and damaged leaves shall be removed from each unit.

B.2 Sampling of lots of spinach, lettuce, baby foods and processed cereal based food for infants and young children on the market

The sampling method is applicable to lots smaller than or equal to 25 tonnes.

In the case of large lots (lots > 30 tonnes), the lot shall be subdivided into sublots of in principle 25 tonnes on condition that the subplot may be separated physically. Taking into account that the weight of the lot is not always an exact multiple of 25 tonnes, the weight of the subplot may exceed the mentioned weight by a maximum of 20 %. This means that the subplot may have weight ranging from 15 to 30 tonnes. In case the lot is not or cannot be physically separated into sublots, the sample is taken from the lot.

The aggregate sample shall be at least 1 kg, except where it is not possible e.g. when sampling a single head or package.

The minimum number of incremental samples to be taken from the lot shall be as given in Table 1.

TABLE 1

Minimum number of incremental samples to be taken from the lot

Weight of lot (in kg)	Minimum number of incremental samples to be taken	Aggregate sample minimum weight (kg)
< 50	3	1
50 to 500	5	1
> 500	10	1

If the lot consists of individual packages, then the number of packages, which shall be taken to form the aggregate sample, is given in Table 2.

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TABLE 2

Number of packages (incremental samples) which shall be taken to form the aggregate sample if the lot consists of individual packages

Number of packages or units in the lot	Number of packages or units to be taken	Aggregate sample minimum weight (kg)
1 to 25	1 package or unit	1
26 to 100	about 5 %, at least 2 packages or units	1
> 100	about 5 %, at maximum 10 packages or units	1

Each lot or subplot to be checked for compliance, must be sampled separately. However, in cases where such method of sampling would lead to unacceptable commercial consequences resulting from damage to the lot (because of packaging formats, means of transport, etc.) then an alternative method of sampling may be applied, provided that it ensures that the aggregate sample is sufficiently representative of the sampled lot and is fully described and documented. The position from which a sample is taken in the lot shall preferably be chosen randomly but, where this is physically impractical, it shall be from a random position in the accessible parts of the lot.

B.3 Sampling at retail stage

Sampling of foodstuffs at the retail stage shall be done where possible in accordance with the sampling provisions set out in B.2.

Where that is not possible, an alternative method of sampling at retail stage may be used provided that it ensures that the aggregate sample is sufficiently representative of the sampled lot and is fully described and documented⁽²⁾.

B.4 Assessment of compliance of a lot or subplot

- acceptance if the laboratory sample conforms to the maximum limit, taking into account the measurement uncertainty and correction for recovery,
- rejection if the laboratory sample exceeds the maximum limit beyond reasonable doubt taking into account the measurement uncertainty and correction for recovery (i.e. the analytical result corrected for recovery and minus the expanded measurement uncertainty is used to assess compliance).

C. SAMPLE PREPARATION

1. In the case of sampling of fresh produce, sample preparation shall take place within 24 hours of sampling if possible. If not, the sample shall be kept frozen (up to a maximum of six weeks).
2. Soil, heavily soiled and other outer non-edible and damaged leaves shall be removed from each of the individual units. Washing of the samples is not allowed as the content of nitrates can decrease by washing of the samples.
3. The complete sample is to be homogenised (the addition of a known amount of water is optional). Depending upon the size of the blender/macerator/chopper used, one or more individual units may be combined for homogenisation purposes. Blending may be aided by freezing and chopping the units before homogenisation is carried out. It must be demonstrated that the homogenisation process used achieves complete

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homogenisation. Thorough homogenisation is essential for maximum extraction and recovery of nitrate. Samples shall be treated identically in this way irrespective of whether they have been obtained from the field or from retail.

4. One or more analytical samples are taken from the combined slurries for analysis.

D. METHOD OF ANALYSIS, REPORTING OF RESULTS AND LABORATORY CONTROL REQUIREMENTS

D.1 Definitions

For the purposes of this Annex, the following definitions shall apply:

- r = Repeatability, the value below which the absolute difference between two single test results obtained under repeatability conditions, namely same sample, same operator, same apparatus, same laboratory, and short interval of time may be expected to lie within a specific probability (typically 95 %) and hence $r = 2,8 \times s_r$.
- s_r = Standard deviation, calculated from results generated under repeatability conditions.
- RSD_r = Relative standard deviation, calculated from results generated under repeatability conditions ($(s_r / \bar{x}) \times 100$).
- R = Reproducibility, the value below which the absolute difference between single test results obtained under reproducibility conditions, namely on identical material obtained by operators in different laboratories, using the standardised test method may be expected to lie within a certain probability (typically 95 %); $R = 2,8 \times s_R$.
- s_R = Standard deviation, calculated from results under reproducibility conditions.
- RSD_R = Relative standard deviation calculated from results generated under reproducibility conditions ($(s_R / \bar{x}) \times 100$).

D.2 General requirements

Methods of analysis used for food control purposes must comply with the provisions of items 1 and 2 of Annex III to Regulation (EC) No 882/2004.

D.3 Specific requirements

D.3.1 Extraction procedure

Particular attention must be paid to the extraction procedure applied. Several extraction procedures have proven to guarantee an effective extraction of the nitrate, such as hot water or methanol/water (30/70) extraction method. Cold water extraction may only be used if the analytical sample has been frozen prior to sample extraction.

D.3.2 Performance criteria

The specific criteria for methods of analysis used in the monitoring of nitrate levels shall be:

Criterion	Concentration range	Recommended value	Maximum permitted value
Recovery	< 500 mg/kg	60-120 %	

Precision RSD_r may be calculated as 0,66 times Precision RSD_R at the concentration of interest.

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	≥ 500 mg/kg	90-110 %	
Precision RSD _R	All	As derived from Horwitz Equation	2 × value derived from Horwitz Equation

Precision RSD_r may be calculated as 0,66 times Precision RSD_R at the concentration of interest.

Notes to the performance criteria

- Concentration ranges are not stated, as the precision values are calculated at the concentrations of interest,
- the precision values are calculated from the Horwitz equation, i.e.:

$$RSD_R = 2^{(1-0,5\log C)}$$

where:

- RSD_R is the relative standard deviation calculated from results generated under reproducibility conditions ($(s_R / \bar{x}) \times 100$)
- C is the concentration ratio (i.e. 1 = 100 g/100 g, 0,001 = 1 000 mg/kg).

D.4 Reporting of results, estimation of measurement uncertainty and recovery calculation⁽³⁾

The analytical result must be reported corrected or uncorrected for recovery. The manner of reporting and the level of recovery must be reported. The analytical result corrected for recovery shall be used for checking compliance.

The analytical result must be reported as $\times \pm U$ whereby \times is the analytical result and U is the expanded measurement uncertainty.

U is the expanded measurement uncertainty, using a coverage factor of 2 which gives a level of confidence of approximately 95 %.

The present interpretation rules of the analytical result in view of acceptance or rejection of the lot apply for the analytical result obtained on the sample for official control. In case of analysis for defence or referee purposes, the national rules apply.

D.5 Laboratory quality standards

Laboratory must comply with the provisions of Article 12 of Regulation (EC) No 882/2004.

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- (1) The marketable size for lettuces, curled leaved and broad leaved endives is provided for in Commission Regulation (EC) No 1543/2001 of 27 July 2001 laying down the marketing standard for lettuces and curled leaved and broad-leaved endives ([OJ L 203, 28.7.2001, p. 9](#)), as last amended by Commission Regulation (EC) No 6/2005 of 4 January 2005 ([OJ L 2, 5.1.2005, p. 3](#)).
- (2) In case the portion to be sampled is so small that it is impossible to obtain an aggregate sample of 1 kg, the aggregate sample weight might be less than 1 kg. Also in case of sampling of processed cereal-based foods and baby foods for infants and young children, the aggregate sample weight might be 0,5 kg.
- (3) More details on procedures for the estimation of measurement uncertainty and on procedures for assessing recovery can be found in the report 'Report on the relationship between analytical results, measurement uncertainty, recovery factors and the provisions of EU food and feed legislation' — http://ec.europa.eu/food/food/chemicalsafety/contaminants/report-sampling_analysis_2004_en.pdf

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Changes and effects yet to be applied to the whole legislation item and associated provisions

- Signature words omitted by [S.I. 2019/639 reg. 26](#)
- Annex para. A.3.1 words omitted by [S.I. 2019/639 reg. 27\(a\)](#) (This amendment not applied to legislation.gov.uk. Reg. 27 substituted immediately before IP completion day by S.I. 2020/1504, regs. 1(2), 8(9))
- Annex para. A words omitted by virtue of S.I. 2019/639, reg. 27(a) (as substituted) by [S.I. 2020/1504 reg. 8\(9\)](#)
- Annex para. A.3.6 words substituted by [S.I. 2019/639 reg. 27\(b\)](#) (This amendment not applied to legislation.gov.uk. Reg. 27 substituted immediately before IP completion day by S.I. 2020/1504, regs. 1(2), 8(9))
- Annex para. A.3.8 words substituted by [S.I. 2019/639 reg. 27\(c\)](#) (This amendment not applied to legislation.gov.uk. Reg. 27 substituted immediately before IP completion day by S.I. 2020/1504, regs. 1(2), 8(9))
- Annex para. A words substituted by S.I. 2019/639, reg. 27(b) (as substituted) by [S.I. 2020/1504 reg. 8\(9\)](#)
- Annex para. A words substituted by S.I. 2019/639, reg. 27(c) (as substituted) by [S.I. 2020/1504 reg. 8\(9\)](#)