

# Nitrate surveillance: Methodology

## Sampling Schedule

In April 2021 a sampling schedule was prepared by ADAS and agreed by the Food Standards Agency (FSA). The schedule was developed to ensure that samples were representative of the wider UK production. The schedule ensured that the sampling of fresh produce complied with the guidelines given in EU retained law Regulation EC/1882/2006 and met with the requirement to spread the sampling over representative geographical regions throughout the UK. Whilst the sampling period coincided with the ongoing covid-19 pandemic, the sampling activities were not disrupted.

The sampling schedule covered the period from 20th April 2021 to the 28th March 2022 and involved the collection of lettuce, rocket, spinach and other leafy green vegetables from domestic sources. Geographic representation and seasonal growing trends were maintained and it was left to the discretion of the Sample Officer to ensure that appropriate numbers of samples from within each category were collected from a representative cross section of growers. A total of 202 samples were collected across the lettuce, rocket, spinach and other leafy green vegetable categories (**Table 1**). The range of samples included in the other leafy green vegetable category is given in **Table 2**.

**Table 1. Summary figures for nitrate samples taken between 20th April 2021 and 28th March 2022.**

Crop Type	Count
Lettuce	130
Rocket	9
Spinach	26
Other green leafy vegetables	37
Total	202

**Table 2: Summary figures for nitrate samples taken between 20th April 2021 and 28th March 2022 – numbers of “Other Green Leafy Vegetables”**

Crop Type	Count
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Chinese leaves	4
Bulls Blood	1
Cannabe	6
Celery	2
Kale	5
Komatsuna	1
Mizuna	4
Multi-leaf salad	1
Mustard	3
Pak Choi	3
Red Batavia	1
Red Chard	4
Red Mizuna	2
Total	37

## Sampling Collection

### Sampling Strategy

Samples were collected by trained Sample Officers, in accordance with Standard Operating Procedure (SOP) 'Field sampling and transportation of lettuce and spinach samples for the UK Nitrate Monitoring Programme' (see Appendix) and EU retained law Regulation EC/1882/2006.

Prior agreement was obtained from the grower before a sample was taken. A minimum of 10 heads of lettuce or 1.0 kg of spinach, rocket and other leafy green vegetables was randomly collected from various points within the lot. Where samples were collected from the field or glasshouse the sample points were, as far as possible, evenly distributed across the area by walking a 'W' pattern back and forth. Lot size did not exceed 2.0 ha and samples were not taken from the field edges. Plants were not collected from patches within the lot which appeared unrepresentative and material that was obviously damaged or diseased was avoided.

## **Sample Labelling and Documentation**

The sampling schedule assigned a unique identification number to each sample, along with details of the Sample Officer, month of collection and region. All samples were anonymised. A copy of the schedule was sent to each Sample Officer and the laboratory to ensure that all parties were fully informed and prepared. Pre-printed labels were provided by NRM and sent directly to the Sample Officers. Samples were sealed and labelled by the Sampling Officer, immediately following collection. The Project Manager held a master copy of the schedule and tracked sample collection, analysis and reporting of results throughout the year. Crop husbandry details were collected by the Sample Officers to accompany each sample. Details included grower, date and time sample was collected, variety or type, location, lot size and fertiliser input.

## **Transportation of Samples to the Laboratory**

Each sample was carefully placed into a clean polythene bag which was subsequently placed into polystyrene insulated box, provided by NRM. Ice packs were placed in the base of the box, as appropriate, to ensure the sample remained below 10°C during transit. The containers provided were inert and offered adequate protection for samples against water loss, deterioration, contamination, damage, heat and significant changes in nitrate content during transportation to the laboratory. Samples were dispatched to the laboratory to arrive before 10.30am on the day after harvest. Samples from Scotland and Northern Ireland were placed in sealed plastic bags and transported in insulated containers at <10°C, which arrived at the laboratory within two days of harvest.

## **Sample Preparation in the Laboratory**

Samples were checked upon receipt to ensure they met the requirements of EU retained law Regulation 1882/2006. Basic checks were carried out to ensure that the temperature upon arrival was below 10°C and that samples were intact and had not begun to degrade during transportation. Associated documentation was checked against the sample and each sample was assigned a unique NRM laboratory number, which was later reported alongside the unique identification number.

Samples were prepared in accordance with the requirements of EU retained law Regulation 1882/2006 and the quality assurance procedures meet the requirements of the Joint Code of Practice for Quality Assurance in Research and are in Compliance with the provisions of items 1&2 of Annex III to Regulations (No 882/2004). The whole sample was homogenised using a protocol developed by NRM Ltd which has been demonstrated to produce suitably homogenous samples. Four representative sub-samples were taken, (A, B, C and D). Sub-sample A was used immediately for analysis. Sub-sample B was kept refrigerated in case of a requirement for repeat analysis when exceedance occurred. Sub-samples C and D were frozen and will be kept in storage for 12 months following the reporting of results.

## **Analytical Analysis**

Analysis commenced immediately after preparation and initial analysis of all samples was completed within five days of sampling. Analysis was undertaken using a UKAS accredited method which fully meets the requirements of EU retained law Regulation 1882/2006. The method is accredited to BS EN ISO 17025: 2005 and has been since 2000. The method uses an extraction procedure which has been shown to be reliable and robust and involves freezing in liquid nitrogen prior to homogenisation. Detection is based on flow injection colorimetry and is currently used by NRM Ltd for analysis of all commercial samples.

The determination of nitrate-N is based on the formation of a diazo compound between nitrite and sulphanilamide. This compound is then coupled with N-1-Naphthylethylenediamine dihydrochloride to produce a red azo dye. The colour is measured at a light wavelength of 540 nm in a spectrophotometer. Nitrate is reduced quantitatively to nitrite by cadmium metal in the form of an open tubular cadmium reactor (OTCR). The nitrate content of the sample was calculated from the analysed nitrate-N value. Nitrite-N was monitored and was quantified if it was present. The measurement of Nitrite was not part of the accredited Nitrate method and was dealt with, when required, outside of the accredited system.

If any value was  $\geq$  90% of the maximum Nitrate level (**Table 3**) for a particular product then this triggered a requirement for a repeat extraction and analysis of refrigerated Sample B to confirm the high value. This repeat confirmatory analysis was carried out within two days of the initial analysis and both results were reported on the same day.

**Table 3. Maximum permitted level of nitrates in lettuce, spinach and rocket.**

Product Type	Cultivation	Harvest Date	Maximum permitted level (NO <sub>3</sub> mg/kg)
Fresh spinach	Any	Any	3500
Preserved, deep-frozen or frozen spinach	Any	Any	2000
Fresh lettuce Non-iceberg type	Protected	1st October – 31st March	5000
Fresh lettuce Non-iceberg type	Protected	1st April – 30th September	4000
Fresh lettuce Non-iceberg type	Open Air	1st October – 31st March	4000
Fresh lettuce Non-iceberg type	Open Air	1st April – 30th September	3000
Fresh lettuce Iceberg type	Protected	Any	2500
Fresh lettuce Iceberg type	Open Air	Any	2000
Rocket	Any	1st October – 31st March	7000

Product Type	Cultivation	Harvest Date	Maximum permitted level (NO <sub>3</sub> mg/kg)
Rocket	Any	1st April – 30th September	6000
Other Leaf Green Vegetables	Any	Any	n/a

## Quality Control

All quality control (QC) information was recorded on the laboratory worksheets. Routinely an in-house reference material is included with every batch of samples at a frequency of at least one QC sample in every batch of twenty samples. A spiked sample may also be included at the same frequency if required. A reagent blank is prepared with each batch of samples. A mid-range standard is included at the end of each batch to ensure any drift over the run is within acceptable limits (+/- 5%). All QC results are plotted on Shewhart Charts and monitored to ensure they conform to NRM's policy on Quality Control (i.e. precision, accuracy, 9 point bias, ascending or descending trends etc).

In-house reference materials are routinely used. These are prepared in-house from material obtained from growers or retailers. These materials are typical of produce entering the retail chain and therefore contain nitrate levels typical of those encountered in the marketplace. New materials are run alongside existing materials to obtain reference values for the new material.

The value obtained for the reagent blank must be less than 0.2 mg/l NO<sub>3</sub><sup>-</sup>N. This equates to 8.9 mg/kg. One QC value at ± 2 standard deviations = Warning. Two consecutive QC values at ± 2 standard deviations = Action. One QC value at ± 3 standard deviations = Action.

A QC Failure Record is generated when an Internal QC falls outside the required criteria. This initiates a documented investigation into the cause of the failure under NRM's Non-Conforming Work policy. This typically results in the retained sample being re-extracted and re-analysed from the start.

New in-house reference materials and standard solutions are crossed over against the current reference material or standard solution prior to use. Documented evidence of this cross-over is retained. Control materials are included in every batch at a frequency of at least one QC sample in every batch of twenty samples (5%). LOQ = 50 mg/kg, LOD = 6 mg/kg, Blanks = generally less than 2 mg/kg. Precision values over the relevant concentration range expressed as relative standard deviations; 4.4% at approx. 2000 mg/kg, 8.9% at approx. 450 mg/kg, 11.3% at approx. 100 mg/kg. IHRM: Currently Spinach, mean = 314 mg/kg, SD = 15.6 mg/kg, RSD = 5%.

Recovery was determined on five batches of triplicate samples spiked at three levels. Approx. 2000 mg/kg average recovery = 98%, range = 94% - 105%, approx. 450 mg/kg average recovery = 102%, range = 85% - 114%, approx. 100 mg/kg average recovery = 104%, range = 80% - 117%. Reporting limit = 50 mg/kg. Recovery: acceptable between 90% and 110%.

Measurement uncertainty is estimated using precision and bias data.

## Reporting of Results

Analysis commenced immediately after preparation and initial analysis of all samples was completed within five days of sampling. Where nitrate levels exceeded the limits the frozen sub-sample was re-analysed within two days of the initial analysis, in all cases. Results were received by ADAS within five days of sample receipt. Nitrate concentrations are expressed in milligrams of nitrate per kilogram of sample fresh weight (mg/kg).

## **Communication of Results to FSA**

Results were reported to the FSA on a monthly basis. Individual data were reported in an Excel spreadsheet and filters were added to the column headings to enable the FSA to search for and group results, as appropriate. Monthly mean values and running totals of maximum, minimum and mean nitrate levels, grouped according to category, were tabulated (Appendix 1 and Appendix 2).

## **Communication of Results to Growers and Wholesalers**

A template letter was produced by the FSA and forwarded to ADAS for use when reporting results (Appendix 3). When the nitrate level of a sample was within the maximum permitted level, as described in retained law Regulation (EC) No 1881/2006 or Regulation (EC) No 1258/2011 (from 02 December 2011 onwards), ADAS reported the results directly to the grower/wholesaler. A copy of the letter was also sent to the FSA. If the nitrate level in a sample exceeded the maximum permitted limit then, following confirmation of the result by NRM, ADAS informed the responsible person at the Agency before reporting the result to the grower/wholesaler (Appendix 4).

## **Long term sample storage in case of dispute**

Sub-samples 'C' and 'D' (see above) from each sample have been frozen and will be stored by NRM for a period of 12 months after the reporting of results.