

# Nitrite in Meat Products

## Reflectometric determination after reaction with Griess reagent

### Introduction

Nitrites, primarily in the form of sodium nitrite (E250) and potassium nitrite (E249), have been widely used as curing agents in meat production due to their multifunctional role in ensuring product quality and safety. They are directly responsible for the development of the characteristic flavor, stabilization of the cured red color, and inhibition of lipid oxidation. More importantly, nitrites exert antimicrobial effects, creating conditions unfavorable for the growth of spoilage organisms and foodborne pathogens, including *Clostridium botulinum*.

Despite their functional importance, nitrites are associated with toxicological concerns. Under certain conditions, they can contribute to the formation of *N*-nitroso compounds, a group of substances classified by the International Agency for Research on Cancer (IARC) as probable human carcinogens.<sup>1</sup> The regulation of nitrite use has therefore focused on achieving a balance between ensuring microbiological safety and minimizing the formation of harmful nitrosamines. This balance is reflected in strict maximum levels set by the European Union [Regulation (EC) No 1333/2008 and its subsequent amendments],<sup>2</sup> and other international authorities which continue to guide the use of nitrites in cured and dried meat products.

Given these safety considerations, accurate monitoring of nitrite levels in meat products is essential. Photometric determination using the Griess method provides a simple, sensitive, and reliable analytical tool for this purpose, ensuring compliance with food safety standards while supporting routine quality control in the meat industry.

### Experimental

This application note describes the rapid quantification of nitrite in meat products using the RQflex® 20 alongside the Reflectoquant® Nitrite Test (EM1.16973.0001).

### Method

In the presence of an acidic buffer nitrite ions react with an aromatic amine to form a diazonium salt, which in turn reacts with *N*-(1-naphthyl)-ethylene-diamine to form a red-violet azo dye that is determined reflectometrically.

### Measuring Range

- Test kit: 0.5–25.0 mg/L NO<sub>2</sub><sup>-</sup> (0.2–7.6 mg/L NO<sub>2</sub>-N)
- Method: 1.5–75 mg/kg NO<sub>2</sub><sup>-</sup>

### Applicable Sample

Chopped meat

### Influence of Foreign Substances

Foreign substances in the sample solution can

- Increase the measurement value because of an amplification of the reaction
- Lower the measurement value because of a prevention of the reaction

A quantification of these effects is stated in tabular form in the respective package insert for the most important foreign ions and substances. The tolerance limits have been determined for the individual ions and substances; they may not be evaluated cumulatively.

In the case of samples with a complex, in many cases inexact known composition (matrix) it is particularly difficult to estimate the potential influence of the foreign substances on the analysis (matrix effect). The following instructions describe a method by means of which the user can test whether a matrix effect is present or not.

- Interference check by dilution
- Interference check by spiking

## Reagents, Instruments and Materials

### Test/Reagents Kit

Reflectoquant® Nitrite Test (EM1.16973.0001)

### Instrument(s) & Devices

Reflectometer RQflex® 20,  
Reflectoquant® (EM1172460001)

### Other Reagents and Accessories

- MQuant® Nitrite Test 2–80 mg/L NO<sub>2</sub><sup>-</sup> (0.6–24 mg/L NO<sub>2</sub><sup>-</sup>N) (EM1.10007.0001)
- Distilled water (e.g., from a suitable Milli-Q® system) or water for analysis (EM1.16754.4000)
- Balance
- Beaker
- Blender
- Heating plate
- Folded filter

### Products for AQA

- RQcheck set for RQflex® 20 Reflectometer (78542-302)
- Recalibration Set for RQflex® 20 Reflectometer (EM1.16954.0001)
- Nitrite standard solution certified reference material, 1000 mg/L (NO<sub>2</sub>), Certipur® (EM1046590500)

## Analytical Procedure

### Sample Preparation

- Weigh approximately 50 g chopped meat exactly into a beaker, add 150 mL distilled water and homogenize in a blender.
- Heat this mixture to 80 °C and filter through a folded filter. Allow the solution to cool down to room temperature before starting the analysis.
- Check the nitrite content with the MQuant® Nitrite Test. Samples containing more than 25.0 mg/L NO<sub>2</sub><sup>-</sup> must be diluted with distilled water.

### Measurement

- Press the START button of the reflectometer and - this is imperative - at the same time immerse both reaction zones of the test strip in the pretreated sample (15–30 °C) for 2 seconds.
- Carefully allow excess liquid to run off via the long edge of the strip onto an absorbent paper towel.
- Immediately insert the strip all the way into the strip adapter with the reaction zones facing the display.
- After the end of the reaction time (15 sec), read off the result from the display in mg/L NO<sub>2</sub><sup>-</sup>. The result is automatically stored.

### Notes on the measurement:

For detailed operating procedures, consult the operating instructions for RQflex® instrument and the package insert of the Reflectoquant® Nitrite Test.

### Calculation

$$\text{Nitrite content} = \frac{\text{Measured value [mg/L]} \times 150 \text{ [mL]}}{\text{Weight of sample [g]}} \text{ [mg/kg]}$$

### Analytical quality assurance

Analytical Quality Assurance (AQA) is recommended before each measurement series. Check the instrument using the RQcheck. If RQcheck failed, perform a recalibration using the recalibration set and repeat the RQcheck. For more details see RQflex® 20 manual.

To check test strips, test reagent, measurement device, and handling (recommended before each measurement series): Dilute the nitrite standard solution with distilled water to 10.0 mg/L NO<sub>2</sub><sup>-</sup> and analyze as described in the section measurement.

### Results and Discussion

Here in this study, four different samples of chopped meat were used as test samples for measurement and comparison. The results were verified using ion chromatography as orthogonal method to the Reflectoquant® test kit.

**Table 1.** Results – comparison with ion chromatography (IC)

| Sample | Reflectoquant® [mg/kg] | IC [mg/kg] |
|--------|------------------------|------------|
| 1      | 0.8                    | 0.9        |
| 2      | 6.0                    | 6.8        |
| 3      | 3.2                    | 2.8        |
| 4      | 2.2                    | 2.0        |

### Conclusion

- Rapid testing of nitrite content in meat products can be performed with Reflectoquant® Nitrite Test (EM1.16973.0001) with RQflex® 20 reflectometer.
- Orthogonal method using ion chromatography has provided evidence that experimental results from both methods have differences within ±10 %.

### References

1. dos Santos LF, Lima PA, de Oliveira CA, de Souza PH, Pereira FR, Ribeiro GB, Goddard CL, Valladão SA. Validation of a quick and effective method for determining nitrate and nitrite in meat products. *Brazilian Journal of Development*. 2021;7(1):3416-24. DOI:10.34117/bjdv7n1-230
2. Regulation (EC) No 1333/2008 on food additives <https://eur-lex.europa.eu/eli/reg/2008/1333/oj/eng>

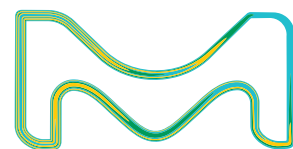
## Featured Products

| Description                                                                                     | Cat. No.       |
|-------------------------------------------------------------------------------------------------|----------------|
| <b>Reflectoquant® instruments and strips</b>                                                    |                |
| Nitrite Test, reflectometric, 0.5–25.0 mg/L NO <sub>2</sub> <sup>-</sup> , Reflectoquant®       | EM1.16973.0001 |
| Reflectometer RQflex® 20, pkg of 1 unit, Reflectoquant®                                         | EM1172460001   |
| <b>Products for sample preparation</b>                                                          |                |
| Nitrite Test, colorimetric, 2–80 mg/L NO <sub>2</sub> <sup>-</sup> , MQuant®                    | EM1.10007.0001 |
| Water for analysis                                                                              | EM1.16754.4000 |
| <b>Products for AQA</b>                                                                         |                |
| Recalibration Set for RQflex® 20 Reflectometer, 1 unit                                          | EM1.16954.0001 |
| RQcheck Set for RQflex® 20 Reflectometer, 1 set                                                 | 78542-302      |
| Nitrite standard solution certified reference material, 1000 mg/L (NO <sub>2</sub> ), Certipur® | EM1046590500   |

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