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The 2nd International Electronic Conference on Foods
Future Foods and Food Technologies for a Sustainable World
15–30 OCTOBER 2021 | ONLINE

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A new approach for nitrite determination from synthetic and natural origin in cured meat

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Introduction

Curing process is a food preservation treatment by the addition of salt, sugar, nitrates or **nitrites**

NITRITE

Nitrite is widely used as curing agent in the production of cooked meat derivatives. However, nitrite and their metabolic compounds are linked to potential adverse health effects

Role of nitrite in meat

- Pink-red color
- Texture and flavor
- Antimicrobial
- Antioxidant

International Agency for Research on Cancer (IARC)

In 2015 nitrite was classified as "probably carcinogenic to humans" under conditions favoring the endogenous nitrosation

Sindelar, J. J., & Milkowski, A. L. (2011). "Sodium nitrite in processed meat and poultry meats: a review of curing and examining the risk/benefit of its use". *AMSA*, *3*, 1-14.
Grosse, Y., Baan, R., Straif, K., Secretan, B., Ghissassi, F. E., & Cogliano, V. (2006). "Carcinogenicity of nitrate, nitrite, and cyanobacterial peptide toxins". *Lancet Oncol.*, *7*(8), 628-629.

Introduction

The European Commission classifies nitrites (potassium nitrite, E249; sodium nitrite, E250) and nitrates (sodium nitrate, E251; potassium nitrate, E252) as permitted food additives under Commission Regulation (EU) No. 1129/2011.

The nitrite amount permitted as an additive in cured meat is currently 150 mg kg^{-1} (expressed as $NaNO_2$).

Although nitrite amount established in cured meat does not involve a risk, excessive and prolonged nitrite consumption can affect human health

Alternative nitrite sources

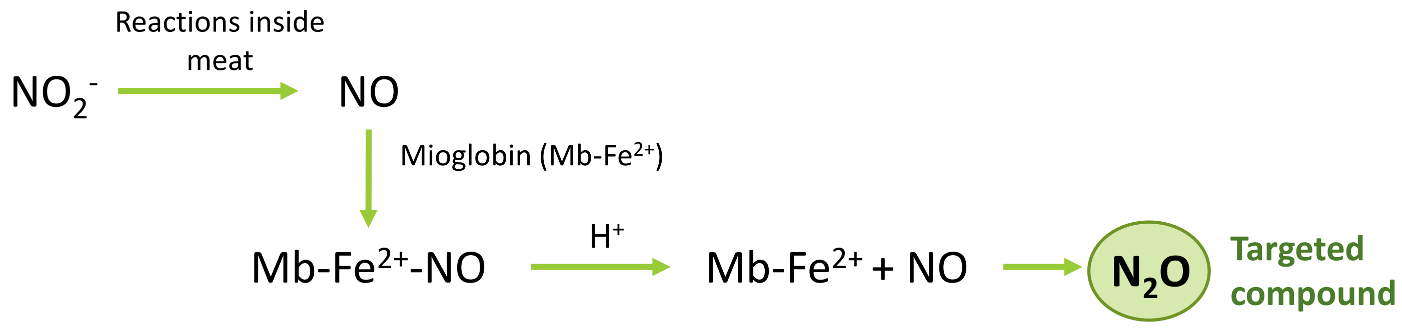
The curing process is usually carried out by adding synthetic nitrite to meat, but the addition of vegetables which are a natural source of nitrite is a currently alternative.

- **Vegetables** are rich in nitrate, which is converted to nitrite inside meat, so is presented as an alternative source
- Plant extracts have **phenolic compounds**, **flavonoids** and **organic acids**, which have antimicrobial and antioxidant properties

Objective

The aim of this study was to detect and quantify both synthetic and natural nitrite in cooked ham using headspace-gas chromatography-mass spectrometry (HS-GC-MS)

- The proposed method determines nitrous oxide (N_2O) as a targeted compound, formed after two reduction steps: from nitrite to nitric oxide (NO), which binds to mioglobin present in meat, and then to nitrous oxide under acidic conditions.
- Cooked hams at different concentrations of synthetic nitrite (from 0.5 to 150 mg kg^{-1}), hams prepared from polyphenol extracts with concentrations of 5, 10 and 20 g kg^{-1} (NATPRE T-10 HT S) and ham samples made with celery at 0.8 and 3 g kg^{-1} were analyzed by the proposed method.



Analytical procedure

Sample treatment

15 g of cooked ham
(hams produced at PROSUR Meat Laboratory)

+ 5 g crushed ice
+ 12 mL water

Homogenized at 7000-9000 rpm
and
Centrifuged
(5 min at 2500 rpm at 10 °C)

Supernatant (8 mL)

+ 8 µL 1-octanol
+ 4 mL sulfuric acid (20 % v/v)

Sealed and gently shaken by hand for 30 s

HS-GC-MS analysis

HS-GC-MS conditions

Table 1. HS-GC-MS conditions

Injection mode	Split 1:10
Incubation time	45 min
Incubation temperature	30 °C
Stirring rate	750 rpm
Capillary column	HP-MOLSIV (30 m x 0.32 mm x 12 µm)
Oven programme	70 °C (5 min) to 200 °C at 30 °C min^{-1} (3 min) and then to 220 °C at 3 °C min^{-1} (5 min)
Ionization source (EI)	70 eV
Temperature ionization source	230 °C
MS mode	SCAN (from 10 to 100 *m/z*) SIM (*m/z* 43.8; 29.8; 28.0)

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Evaluation of the cured meat color

In the first place, the color of the produced cooked hams was evaluated. Three color replicate measurements were performed for each one comparing hams made with synthetic nitrite and hams prepared from vegetable material.

The parameters L*, a* and b* were determined and the variable a* (red pigmentation) was used for comparison purposes.

Figure 1 indicates that values of the variable a increased for nitrite concentrations added to the cooked ham up to about 80 mg kg^{-1}, while the signal remained practically constant for higher concentrations. However, the variable a* gave constant values for the uncured ham samples treated with NATPRE T-10 HT S.* ***These results indicate that the color of the product made with nitrite is similar to the color of the final product made with the NATPRE T-10 HT S.***

The color measurements did not permit the differentiation of the cured and uncured ham samples, therefore, a new procedure is mandatory making possible to discriminate between both type of samples.

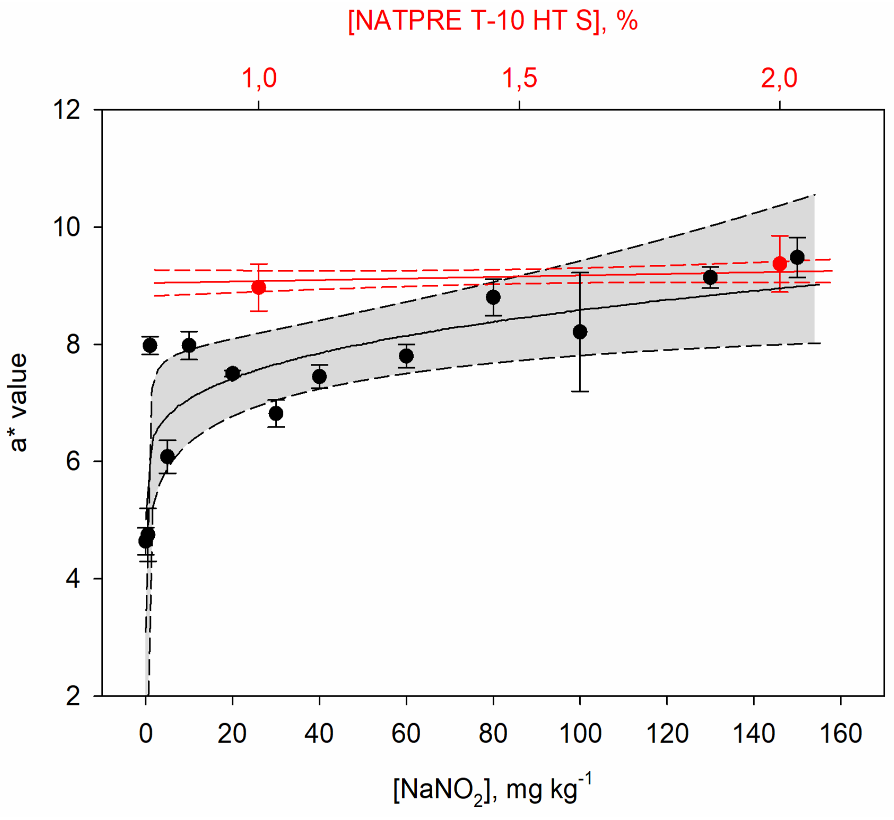

Figure 1. Influence of the presence of both sodium nitrite and NATPRE T-10 HT S on the color of cooked ham samples

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Validation of the method

As a result, a good correlation was obtained between nitrous oxide detected by the prpoposed HS-GC-MS method and nitrite added to meat.

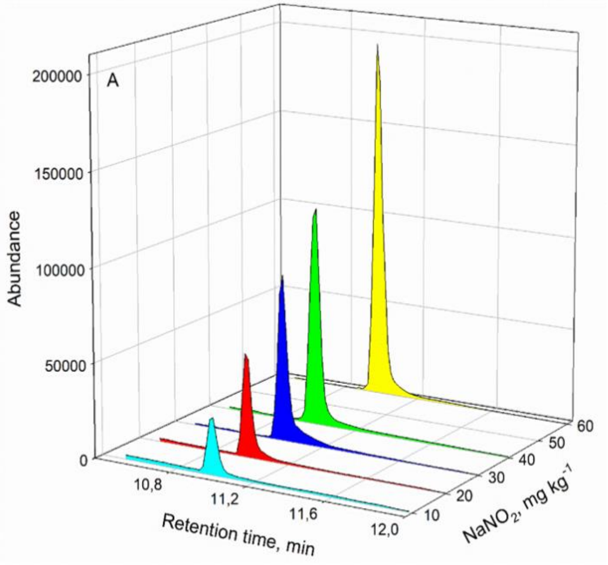

Figure 2. Chromatographic profiles for different amounts of nitrite added to ham samples

Table 2. Recovery study for sodium nitrite ham samples

Concentration $NaNO_2$ added ($mg\ kg^{-1}$)	Concentration $NaNO_2$ found ($mg\ kg^{-1}$)
10	13±5
20	17±6
40	40±4
80	89±4
100	118±5

Values are mean concentration ± standard deviation (n=3)

LOD= 2.7 $\mu g\ g^{-1}$

LOQ= 9.0 $\mu g\ g^{-1}$

These results ensure the accuracy of the proposed method.

The precision (repeatability and intermediate precision) was evaluated by analyzing the samples at three concentration levels (k=3) in triplicate (n=3) over 3 non-consecutive days (p=3).

Table 3. Trueness and precision (RSD %) (k=3, n=3, p=3) studies

Level (mg $NaNO_2$ /kg ham)	Relative bias (%)	Repeatability (%)	Intermediate precision (%)
10	-20	3.6	7.3
30	14	5.1	10
60	4	5.2	7.8

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Analysis of real samples

To study the applicability of the method, different commercial samples of meat products obtained from local supermarkets were analyzed, corresponding to four samples of cooked ham and two samples corresponding to a loin and a serrano ham.

Table 3. Sodium nitrite content in meat products determined by HS-GC-MS

Meat product	Concentration $NaNO_2$ (mg kg^{-1})
NATPRE T-10 HT S (5 g kg^{-1})	NQ[1]
NATPRE T-10 HT S (10 g kg^{-1})	NQ
NATPRE T-10 HT S (20 g kg^{-1})	NQ
Celery (0.8 g kg^{-1}) ham 1	105±3
Celery (0.8 g kg^{-1}) ham 2	106±4
Nitrite free ham 1	NQ
Nitrite free ham 2	NQ
Cooked ham 1	14±1
Cooked ham 2	20±5
Cooked ham 3	14±6
Cooked ham 4	13±1
Loin	NQ
Serrano ham	NQ

[1]NQ means not quantifiable (below LQ at 9 mg kg^{-1})

- ***NATPRE T-10 HT S*** (hams prepared from polyphenol extracts) as well as nitrite free hams, had a not measurable level of nitrite as it was expected.
- In the case of ***celery hams***, the nitrate content is reduced to nitrite inside meat, which was measured by this methodology.
- ***Cooked ham 1*** and ***2*** did not show information about the nitrite content, however, nitrite appeared in both samples, one near the LQ and the other at a high nitrite level.
- ***Cooked ham 3*** and ***4*** were labelled as "nitrite free", and the levels of nitrite found using the proposed method was very low, near the LQ level.
- ***Loin*** and a ***serrano ham*** samples did not show information about the nitrite content, and the method revealed not measurable levels of nitrite.

The detection of nitrous oxide as a target compound using the proposed method provides an efficient way to identify nitrite treated meats from different sources

Conclusions

- The described method presents a sensitive and selective approach for the quantification of nitrous oxide as a target compound, which is suitable for identifying nitrite treated hams.
- A linear relationship was obtained between nitrite concentration in the sample injected in the headspace and the area of the nitrous oxide peak generated.
- The analysis of commercial cooked ham and studies about intermediate precision and repeatability demonstrated the applicability and reproducibility of the proposed method.

The HS-GC-MS method is presented as an efficient approach to ensure the quality and safety of these products.

THANK YOU FOR YOUR ATTENTION

Acknowledgements

The authors acknowledge the financial support of the Comunidad Autónoma de la Región de Murcia (CARM, Fundación Séneca, Project 19888/GERM/15), the Spanish MICINN (PGC2018-098363-B-100), and PROSUR.