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Nitrite quantification in processed meat using an enzyme biosensor, a portable reader and a smartphone: a facile and accurate approach

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ABSTRACT

The control of nitrite levels in foodstuff is essential for ensuring human safety. However, the protocols are long and require lab equipment (spectrophotometer and centrifuge) not available in industrial or commercial settings. Herein, we adapted an electrochemical nitrite biosensor utilizing the enzyme cytochrome *c* nitrite reductase (ccNiR) previously developed in our lab, to meet the analytical requirements of cured meats, and validated it using real samples from different brands. The enzyme was immobilized on a disposable carbon screen-printed electrode (SPE) modified with multi-walled carbon nanotubes (MWCNTs) and connected to a hand-held potentiostat coupled to a smartphone. The carbon nanotubes suspension was optimized using chemically friendly solvents and the SPE surface was electrochemically characterized using ferricyanide as a redox probe, to fine-tune the modification formulation. The nitrite biosensor delivered a sensitivity of 0.0103 μA/μM, a linear range from 5 to 100 μM, and a lower detection limit (LOD) of 3.2 μM. Subsequently, the biosensor was tested for its ability to detect nitrites in various processed meat samples, considering factors such as type and origin. The validation of the electrochemical biosensor was conducted using the gold standard Griess method and HPLC, although the first one failed to detect nitrites in some samples due to matrix interferences. Accordingly, the nitrite biosensor performed well, providing highly accurate and precise results (smaller SD), even in non-centrifuged samples, making it possible to control the meat quality at any point of the chain distribution.

1. Introduction

Quality control is essential in the food and beverage industry, where evaluating the freshness of perishable foods, such as fish and meat, is vital for ensuring food quality and safety. According to Commission Regulation (EU) No. 1129/2011, nitrites (potassium nitrite, E249; sodium nitrite, E250) are authorized as permitted food additives [1]. They are frequently used in cured meat-based products because they reduce the risk of bacterial contamination, particularly from *Clostridium botulinum*, improve flavor, taste, and aroma, and enhance the red-pinkish color of the meat [2]. Unfortunately, excessive nitrite consumption might have a negative impact on human health [3,4]. Indeed, nitrites and hemoglobin combine permanently to produce methemoglobin, restricting the oxygen amount transported through the blood [5]. The production of *N*-nitrosamines is another adverse consequence of nitrites, which can be produced during meat manufacture, home preparation, and by the digestive system after eating. Many volatile nitrosamines are categorized as group 2B, which may cause human cancer [2]. The maximum allowable added amounts (up to 100 mg/kg of nitrite are permitted in sterilized meat products and 150 mg/kg in other meat products) reflect the ranges referred to in these scientific opinions. Advanced analytical methods, including spectrophotometry [6,7] and chromatography [8–10], are employed to quantify the levels of nitrites in food samples accurately. Despite the high specificity of these methods, they are expensive, technically complex, and require sample pre-treatment. Typically, time to results take hours or even days, and matrix effects can interfere with the results' accuracy. Biosensors, in general, and electrochemical ones, in particular, can play an important

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role here [11]. Electrochemical biosensors are analytical devices based on coupling bioreceptors and transducers. If the bioreceptor is a redox enzyme, the catalytic current produced and recorded at the transducer due to the oxidation or reduction of its substrate is directly proportional to the corresponding concentration. This approach shows considerable advantages over existing analytical methods, including faster response times, higher sensitivity, miniaturization, and lower analytical costs [12]. In our previous works, the enzyme cytochrome *c* nitrite reductase (ccNiR) from *Desulfovibrio desulfuricans* ATCCC 27774, which catalyzes the direct conversion of nitrite to ammonium, was employed as bioreceptor [13,14] (Fig. 1).

Here, we focus on applying the platform to real samples, aiming to develop an easy, quick and accurate electrochemical biosensor for detecting nitrites in foodstuff, such as cured meat, at the point of testing. To this end, additional adjustments to the analytical performance, namely limits of quantification, were made by integrating nanomaterials (NMs) at the enzyme/electrode interface. Various types of NMs, including metals, metal oxides, and carbon, have been extensively studied and applied in the development of electrochemical sensors and biosensors due to their remarkable characteristics, such as large surface area, high conductivity, electrocatalytic and electroactive properties, biocompatibility, and ability to facilitate system miniaturization [15]. Carbon nanotubes (CNTs) are particularly interesting, due to their electronic, mechanical, and optical properties [16]. However, their high propensity for aggregation due to strong van der Waals interactions and poor solubility in all solvents poses a challenge. The solvent influences the electronic structure of nanotubes, affecting their electrochemical and optical features, as well as the stability of dispersions and biomolecules [17]. Our previous works have shown the usefulness of CNTs in amplifying the current signal while raising the detection limit [18]. However, their application on the electrode surface required prior suspension in an organic solvent. For example, single-walled carbon nanotubes (SWCNTs) were dispersed in tetrahydrofuran (THF) and dimethylformamide (DMF) to create homogeneous suspensions and allow uniform layer deposition on electrodes, significantly improving the biosensor stability [19]. Similarly, MWCNTs were dispersed in water, but to make them hydrophilic, the nanotube surfaces were chemically modified using thermal and chemical methods. Alternatively, surfactants and salts of organic acids can be used to disperse the CNTs. Sodium dodecylbenzene sulfonate (SDBS) and sodium dodecyl sulfate (SDS) are the most used due to their exceptional stability [20]. However, they might interfere with the current intensity in enzymatic electrochemical biosensors. As reported in numerous works in the literature [17,21–23], bile salts, such as sodium cholate and its analogs, can be used instead. In this work, cholic acid was used to enhance the dispersion of carbon nanotubes, which, when integrated with ccNiR, improved enzyme loading and the biosensor's electroanalytical performance towards nitrite detection. This way, we were able to develop, test and validate a bespoke electrochemical biosensor designed to detect nitrites in cured meats sourced from different supermarket brands. Data was acquired and analyzed using a miniaturized potentiostat controlled by a smartphone or tablet. The biosensor exhibited high sensitivity and accuracy in assessing nitrite levels, establishing itself as a straightforward yet effective tool for ensuring immediate food safety and quality assurance.

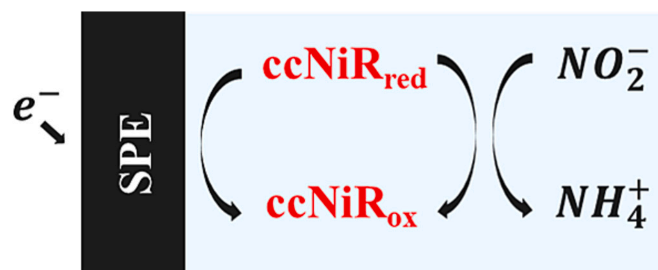

Fig. 1. Schematic representation of the electrocatalytic mechanism of nitrite reduction by ccNiR according to an EC' mechanism (electrochemical reaction followed by an irreversible chemical reaction). In this work, we employed cyclic voltammetry (CV) to trigger the electrochemical reaction and tracking the resulting peak current.

2. Materials and methods

2.1. Reagents and samples

Sodium ascorbate, sodium cholate hydrate, hydrochloric acid, potassium hexacyanoferrate (III), potassium phosphate, sodium phosphate, sodium nitrite, potassium chloride and Trizma were purchased from Sigma-Aldrich. MWCNTs (NC3103) were purchased from Nanocyl SA (Belgium). All solutions were prepared with deionized water (18 MΩ·cm) from a Millipore MilliQ purification system. *cc*NiR (300 U mg^{-1}, 1.2 mg mL^{-1}) was purified from *D. desulfuricans* ATCC 27774 cells, as reported by [24]. All enzyme stock solutions were prepared in 50 mM phosphate buffer, pH 7.6.

2.2. MWCNTs preparation

1.5 mg of MWCNTs were weighed and dispersed in 2 mL of ethanol and water in a 1:1 ratio, in which 18 mM of cholic acid was dissolved as a surfactant. Next, the dispersion was sonicated for 20 min in an ultrasound bath with ice to keep a low temperature.

2.3. Electrochemical apparatus

All CV measurements were performed using a portable potentiostat, the BT Smart potentiostat by PalmSens (**Fig. S1**), controlled by PSTouch (v2.8) on a smartphone or tablet (Android system). The experiments were carried out using a three-electrode miniaturized cell composed of a screen-printed electrode (SPE) from Nitrogen Sensing Solutions (NS2) including a working electrode (WE) with a diameter of 4 mm, a pseudo-reference electrode (PRE) made of silver, and an auxiliary electrode made of carbon ink, and a 50 μL drop deposited directly on the electrode. The WEs were modified with a 5 μL suspension of MWCNTs (0.75 mg/mL,) which was allowed to dry for 10 min., at 37 °C. Scanning electron microscopy (SEM) and Energy-dispersive X-ray spectroscopy (EDS) were performed to characterize the electrode surface morphology using a Dual Beam Auriga Zeiss instrument located at the Sapienza Nanoscience & Nanotechnology Labs (SNN-Lab).

2.4. Sample preparation and treatment

In this study, four types of cured meat were selected, namely bacon, ham, sausage and salami, from the labels of two local supermarkets (Pingo Doce and Continente). Nitrites were extracted from food with the following protocol [25]: 10 g of food was weighed and transferred into a blender cup. Then, 100 mL of deionized water was added to the meat sample. The meat sample was liquified in the blender for 1 min. The liquified sample was heated and maintained at 80 °C, for 15 min. The sample was then allowed to cool to room temperature. It was centrifuged at 4960 xg, for 10 min. The supernatant was removed and filtered using a Whatman No. 1 filter. All samples were analyzed in duplicate, at room temperature.

2.5. Biosensor fabrication

On top of the MWCNTs layer deposited onto the WE, as described in section 2.3, a 5 μL of a ccNiR solution was dropped cast and left to dry, at room temperature. Given that dissolved oxygen interferes with electrochemical measurements in the potential window used (0 to −0.8 V vs. pseudo ref. Ag), before each assay, it was removed using the mono-

enzymatic method described in [26].

2.6. Electrochemical measurement

The characterization and optimization of the MWCNTs preparation were performed by CV, using a 1.1 mM $Fe(CN)_6^{3-/4-}$ solution, with 0.1 M KCl as supporting electrolyte, within a potential range from −0.4 V and +0.6 V vs Ag/AgCl reference electrode, at a scan rate of 5 mV/s.

The biosensor assays using the SPE/MWCNTs/ccNiR bioelectrodes, including the system calibration and real sample measurements, were carried out by CV, in a 50 μL drop solution of 0.1 M Tris-HCl buffer, pH 7.6, with or without nitrite standard, respectively. Scans were registered at 20 mV/s, within the potential window 0 V to −0.8 V vs Ag/AgCl reference electrode.

All experiments were carried out at room temperature.

2.7. Griess method

The Griess method was employed as a reference method to measure the total nitrite content in food samples. This colorimetric assay uses the Griess reagent, composed of sulphanilamide and *N*-(1-naphthyl) ethylenediamine (NED), which reacts with nitrites to form an azo product that causes the solution to turn pink. The intensity of the pink color is directly proportional to the concentration of nitrites in the sample. For this purpose, a calibration curve was plotted using standard solutions of sodium nitrite prepared at known concentrations (ranging from 2.5 to 25 μM). Specifically, 500 μL of each standard solution was first mixed with 250 μL of sulphanilamide and then 250 μL of NED, resulting in a total volume of 1 mL. After a 10 min reaction-time, the absorbance of the standard solutions was measured using a ThorLabs (UV–Visible) Spectrophotometer at a wavelength of 450 nm. The same procedure was followed for the food samples with unknown nitrite concentrations.

2.8. HPLC method

Nitrites analyses were carried out using a The HPLC used was an ion chromatography system model ICS3000 (Dionex, Sunnyvale, CA, USA) equipped with a UV detector PDA-100 (Dionex). The data were processed with the software Chromeleon 6.8 (Dionex, Sunnyvale, CA, USA). In all analyses, an IonPac AS11-HC column (4 mm I.D. × 250 mm) with a guard column (4 mm × 50 mm) was used (Thermo Scientific, Waltham, Massachusetts, EUA). Analyses were conducted at 25 °C, under isocratic conditions, with a mobile phase composed of 5 mM sodium hydroxide at a flow rate of 1 mL/min.

2.9. Validation for nitrite quantification in processed meat

The system suitability was evaluated by measuring the relative standard deviation (RSD) of the current peak area from multiple measurements of nitrite reduction using the electrochemical biosensor. The linearity was determined by constructing calibration curves from nine standard solutions (5–100 μM), which were analyzed in a randomized sequence. The matrix effect was also considered. Precision was assessed through repeatability tests, expressed as RSD%, while accuracy was evaluated by comparing the biosensor results with those from the Griess method and HPLC. The limit of detection (LOD) and limit of quantification (LOQ) were established based on signal-to-noise ratios of 3:1 and 10:1, respectively.

3. Results and discussions

3.1. MWCNTs dispersion and characterization

Water and ethanol were utilized as solvents to disperse multi-walled carbon nanotubes (MWCNTs), followed by a five-minute sonication step. The temperature rise led to the aggregation of MWCNTs in water and the evaporation of ethanol. To mitigate this issue, ice was added to the sonicator to maintain a low temperature and prevent aggregation. Additionally, a 1:1 mixture of ethanol and water was tested, resulting in an acceptable, albeit not optimal, dispersion. To improve the MWCNTs dispersion, the sodium salt of cholic acid was added to the ethanol/water solution, proving to be an effective agent for the MWCNTs resuspension (**Fig. S2**). Since the addition of cholic acid efficiently dispersed the MWCNTs, this combination of water/ethanol/cholic acid was chosen for further experiments. Then, CVs were traced with different volumes (5 and 10 μL) and concentrations of MWCNTs (0.3, 0.75, and 1.25 mg/mL) in a solution of potassium ferricyanide (III) with 0.1 M KCl as supporting electrolyte. As shown in Fig. 2A, there is an improvement in both reversibility and current intensity with MWCNTs compared with blank. In the following experiments, a concentration of 0.75 mg/mL and a volume of 5 μL were selected.

The electrochemical performance enhancement by the simple modification of the electrode surface with MWCNTs was further demonstrated by assessing the anodic electroactive area (A_{an}). CVs were performed at various scan rates, from 5 to 500 mV/s (Fig. 2B) to determine the A_{an}, which was calculated to be 5.65 mm^2 using the Randles-Sevcik equation:

$$Ip = (2.99x10^{-5})n^{3/2}A_{an}D_0C_0v^{1/2}$$

where n = number of electrons transferred; D_0 = diffusion coefficient

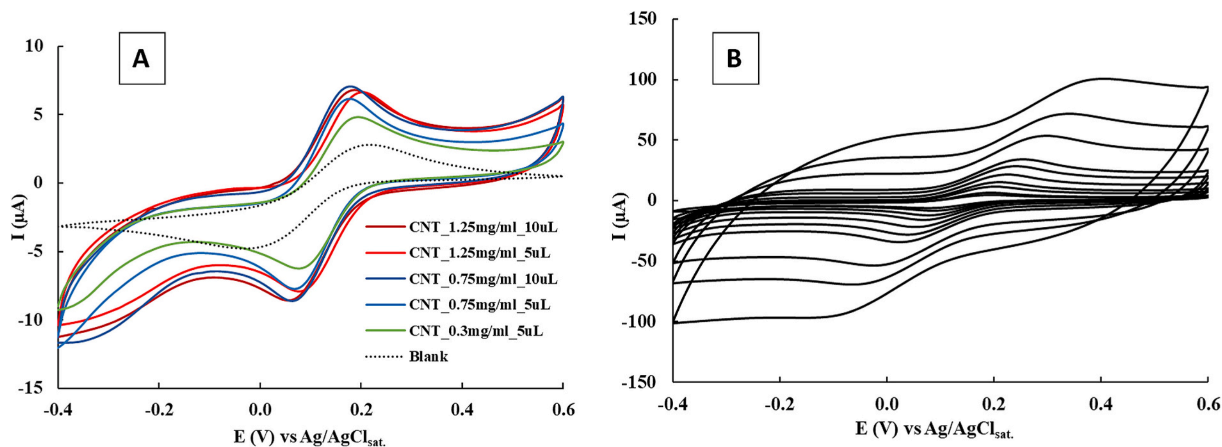

Fig. 2. A) CVs measurements carried out in a solution of 1.1 mM ferricyanide, 0.1 M KCl, scan rate 5 mV/s; B) CVs obtained at various scan rates, from 5 to 500 mVs, in a 1.1 mM potassium ferricyanide, 0.1 M KCl solution.

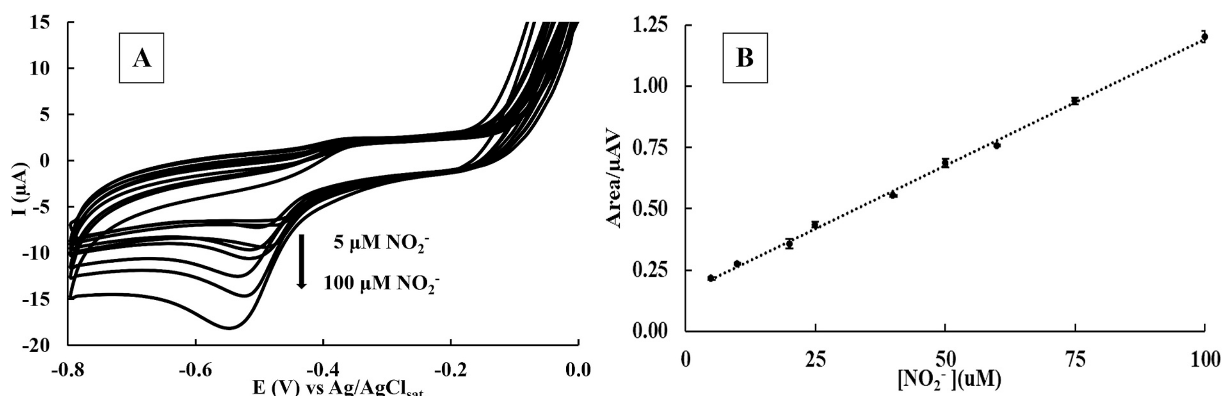

Fig. 3. A) Selection of CVs registered in a 0.1 M Tris-HCl pH 7.6 buffer, 0.1 M KCl, as a supporting electrolyte, and increasing concentrations of nitrite (5–100 μM), scan rate 20 mV/s; B) Calibration curve, y = 0.0103× + 0.1613 (R^2 = 0.998).

Table 1
Analytical features provided by the biosensor developed in this work and other sensors available in the literature.

Platform	Linear range (μM)	Sensitivity	LOD (μM)	LOQ (μM)	RSD (%)	Ref.
MWCNTs/ccNiR	5.0–100	0.037 μA/μM	3.2	4.1 μM	0.5–3.1	This work
Griess	2.5–25	0.047 μM^{-1}	–	–	1.3–26.3	This work
HPLC	1.1–217	0.0675 Area·μM^{-1}	0.93	1.2 μM	0.6–6.3	This work
Hb-poly(nBA)-rGO	1.09–108.6	–	0.65	–	–	[30]
G/My-SWCNT/Nafion	10–10,000	0.001 μA/μM	3800	–	–	[28]
Au-NPs/PPyC/SrTiO3 NC/GCE	150–1500	0.063 μA/μM	20 ± 1	–	–	[27]
MXene/MWCNTs-VB_{12}	10–20,000	0.01053 μA/μM	4.22	–	4.0–4.8	[31]

(cm^2/s); v = scanning velocity (V/s); C_0 = substrate concentration in solution (mol/cm^3).

The A_{an} values were calculated with the corresponding roughness factors (ρ), defined by the electroactive/geometric area ratio (A_{an}/A_{geo}). The A_{an} and ρ values increase as follows: MWCNTs (A_{an} = 5.65 mm^2, ρ = 0.45) > blank (A_{an} = 1.57 mm^2, ρ = 0.12). Furthermore, it is also noted that with the MWCNTs application, the redox peak separation (ΔEp) decreases to 103 mV, compared to 273 mV without MWCNTs, suggesting an enhanced electron transfer rate. This improvement in electrochemical response highlights the conductive and catalytic properties of MWCNTs, facilitating faster electron transport and more efficient interfacial interactions.

3.2. *Calibration curve with nitrite standards*

The detection of nitrite was carried out using SPEs modified with one layer of MWCNTs and a second layer of ccNiR. CV measurements were performed in a 50 μL solution of sodium nitrite from 5 to 100 μM, prepared in Tris-HCl buffer 0.1 M, pH 7.6, from 0 V to −0.8 V vs AgPRE. The biosensor demonstrates a sensitivity of 0.0103 μAV/μM a linear response from 5 to 100 μM, a lower detection limit (LOD) of 3.2 μM a limit of quantification (LOQ) of 4.1 μM and an R^2 = 0.998. The experimental results were highly reproducible, with low error observed across measurements. Fig. 3A, B shows the CVs and the respective linearity range based on the peak area.

The surface modification of SPE electrodes was investigated through impedance spectroscopy (EIS) (Fig. S3), SEM (Fig. S4) and Energy-dispersive X-ray spectroscopy (EDS). Specifically, EIS measurements have been performed using a 5 mM $Fe(CN)_6^{3-/4-}$ solution, with 0.1 M KCl as the supporting electrolyte. EIS measurements revealed a high charge transfer resistance for the bare SPE (Rct = 2322 Ω). Following the modification of the SPE with MWCNTs, a significant decrease in resistance was observed (Rct = 151 Ω), attributable to the excellent electrical conductivity and high surface area of MWCNTs. Subsequent modification with the ccNiR enzyme led to a slight increase in resistance (R_{ct} = 342 Ω), likely due to the non-conductive nature of the enzyme, which reduces the exposed conductive surface area. The fitting of the impedance curves was performed using the Randles equivalent circuit model (Circuit: R(Q[RW])). SEM analysis revealed that the presence of cholic acid promotes a more uniform dispersion of the MWCNTs, resulting in a smoother and more homogeneous surface morphology compared to the MWCNTs without the cholic acid, which exhibited a rough, uneven topography with “peaks and valleys.” Furthermore, the presence of the enzyme is also confirmed in the SEM image by the increased opacity of the surface, as commonly reported in the literature. EDS analysis (Fig. S5) performed after the enzyme immobilization showed the presence of iron, originating from the heme group of the ccNiR enzyme, confirming its adsorption and homogeneous distribution across the electrode surface.

Compared to previous studies, such as [19], which reported a LOD of 2.1 μM and a linear range of 2.1–150 μM using single-walled carbon nanotubes (SWCNTs) and ccNiR, our biosensor shows a comparable linear range with a slightly higher LOD of 3.2 μM. However, our approach benefits from a simpler and chemically friendly protocol for CNTs suspension and a low-cost design using screen-printed electrodes, making it more practical for scaling up and real-world applications.

The reproducibility of the experimental procedure is remarkable, as highlighted by the small error bars associated with each standard (RSD 0.5–3.1 %), further strengthening its reliability and suitability for deployment. Moreover, the repeatability of the system was evaluated by performing 10 measurements, using different SPE, all with the same concentration of nitrites, giving a very low standard deviation (RSD) of 1.4 %. Also, the stability over time of the biosensor was evaluated giving a 102 % retention of the response after 21 days (Fig. S6). The electrodes were stored at refrigerator temperature before each measurement.

Compared to previous studies listed in Table 1, such as [27,28], our biosensors exhibit a markedly lower limit of detection (LOD) and improved sensitivity. While these studies report LODs of 20 μM and 3.8 mM, our biosensor achieves an LOD of 3.2 μM, ensuring more accurate nitrite detection in products such as sausage and ham. This demonstrates that our approach delivers superior performance in direct nitrite detection in processed meat samples, simplifying the analytical process and reducing sample preparation costs. Additionally, as thoroughly

demonstrated in previous works [13,14,29], the ccNiR selectivity has been crucial in distinguishing nitrites from potential interfering species, such as commonly co-occurring nitrates.

3.3. Application in real samples and method validation

The optimized nitrite biosensor was tested in four types of cured meat (ham, salami, sausage, and bacon) from two supermarket brands, Pingo Doce and Continente, designated as Brand 1 and 2, respectively. The samples were prepared according to the procedure described in section 2.6. The initial results indicated that no nitrites were detected in the salami and ham samples from Brand 1, i.e., Sa1 and Ha1. To evaluate the functionality of the biosensor in these matrices, the samples were fortified with nitrites. The results are summarized in Table 2.

According to the obtained data (recovery between 95 and 103 %), the nitrite biosensor demonstrated an effective performance in these matrices. Furthermore, the Griess method faced issues with the formation of a white precipitate, especially in ham (Fig. 4).

Consequently, it was not possible to validate the sensor across all matrix types using this colorimetric method. Therefore, ionic-exchange (IEX) chromatography HPLC was employed as an additional validation method, following the procedure described in Section 2.8. The results are reported in Table 3 (the concentration units were converted into mg/kg, so the nitrite levels could be interpretated in the context of quality control). An immediate output is that the nitrite biosensor approach had an excellent correlation with the IEX-HPLC reference method. Additionally, the biosensor provided highly accurate results for most samples, with a very low standard deviation, indicating good reproducibility. However, compared to IEX-HPLC, the detection limit is

Table 2
Nitrites quantification in spiked meat samples from Brand 1.

Sample	Brand	Foodstuff	Spiked (μM)	Recovery (μM)
Ha1	1	Ham	20	18.95 ± 0.03
Sa1	1	Salami	20	20.7 ± 0.3

Fig. 4. Formation of a white precipitate in the meat extracts (bacon, on the left; ham on the right) after the addition of the Griess reagents.

Table 3
Nitrites quantification in cured meat samples from two different suppliers using three different analytical techniques. All samples were tested in duplicate (ND, not determined; C/F sample centrifugation and filtration).

Sample	Brand	Biosensor (mg/kg)	Griess (mg/kg)	HPLC (mg/kg)	Recovery
Ham	1	<LOD	ND	<LOD	–
Salami	1	<LOD	<LOD	0.097 ± 0.002	–
Sausage	1	0.4116 ± 0.0006	0.28 ± 0.03	0.410 ± 0.001	100.3 %
Bacon	1	0.553 ± 0.004	0.459 ± 0.01	0.56 ± 0.01	95.7 %
Ham	2	4.90 ± 0.04	ND	4.93 ± 0.01	99.5 %
Salami	2	<LOD	<LOD	0.09 ± 0.01	–
Sausage	2	3.91 ± 0.03 3.92 ± 0.05 (no C/F)	3.91 ± 0.08	3.93 ± 0.02	99.4 % 99.8 %
Bacon	2	0.298 ± 0.003	0.18 ± 0.04	0.30 ± 0.02	98.2 %

higher, failing to detect the nitrite presence in three samples (Ha1, Sa1, and Sa2), whereas the chromatographic method only failed one sample (Ha1). Nonetheless, the non-detected nitrite concentrations are well below the maximum allowable levels.

The Griess method generally provided lower nitrite concentrations, except for sample Sa2, where the values were comparable to the other two methods. Furthermore, it failed to detect any nitrites (ND, not detected) in both ham samples due to the formation of a white precipitate that interfered with the UV analysis. This suggests that the Griess method may not be suitable for specific types of cured meat such as ham.

Another conclusion is that despite significant variations in the nitrite content (0.097–4.93 mg/kg), depending on the brand or product, the values found are well below the legal maximum admissible levels.

A test with the "Sausage 2" sample, without centrifugation and filtration, gave a result of 3.92 ± 0.05 mg/kg, closely matching the HPLC method's result (3.93 ± 0.02 mg/kg). Moreover, we obtained a recovery ranging from 95.7 % to 100.3 % between the biosensor and HPLC. This shows the biosensor can accurately detect nitrites even without complex preparation. Simplifying the process saves time and costs, making it ideal for routine use in industrial settings. The biosensor's accuracy in unfiltered samples highlights its robustness and potential for on-site nitrite detection in food products like processed meats.

4. Conclusions

Nitrites are essential food additives due to their preservative properties, but excessive consumption can adversely affect human health. Consequently, accurately measuring nitrite levels in meat products is crucial. Although the gold standard Griess method is frequently used, it is not always suitable for analyzing such samples because of matrix effects. Conversely, the enzyme-based nitrite electrochemical biosensor is effective across various meat samples, regardless of matrix composition, as validated by the HPLC analysis. Contrasting to optical methods, the electrochemical transducing mode is not influenced by sample turbidity or colored compounds. The optimal dispersion of MWCNTs enhanced the biosensor's sensitivity to nitrite within a convenient concentration range.

Overall, the innovative biosensor here applied for the first time to food samples, proved to be accurate, user-friendly, and quick, thereby eliminating the need for sample clarification, and making it a practical option for nitrite analysis in these products. The technology behind the biosensor's operation is fully miniaturized, utilizing disposable SPEs and a portable potentiostat that can connect to a smartphone or tablet. This biosensor technology offers a practical solution for ensuring food safety and monitoring public health at the point-of-measurement, circumventing the need for laboratory settings.

Novelty statement

For the first time, this study presents the successful application of an electrochemical enzymatic biosensor based on ccNiR for the determination of nitrites in processed meat products. Unlike conventional detection methods, our sensor enables rapid, selective, and cost-effective analysis in complex food matrices, at the point-of-measurement. This innovative approach represents a significant advancement in food quality control, offering a reliable tool for ensuring compliance with safety regulations and improving consumer protection.

CRediT authorship contribution statement

Luca Surace: Writing – original draft, Investigation, Formal analysis, Conceptualization. **Rosaceleste Zumpano:** Writing – review & editing, Investigation, Conceptualization. **Francesca Polli:** Formal analysis, Data curation. **Július Gajdar:** Investigation. **Franco Mazzei:** Writing – review & editing, Supervision. **M. Gabriela Almeida:** Writing – review & editing, Supervision, Methodology, Conceptualization.

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work, the author(s) used ChatGPT to improve readability and language. After using this tool/service, the author(s) reviewed and edited the content as needed and take(s) full responsibility for the content of the publication.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.microc.2025.114841.

Data availability

Data will be made available on request.

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