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Detection of Spoiled Pork Disguised with Sodium Nitrite in Sausages Using Electrochemical Impedance Spectroscopy

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Abstract

Adding Sodium Nitrite ($NaNO_2$) to spoiled pork to make it look fresh is a major problem for food safety. This study uses Electrochemical Impedance Spectroscopy (EIS) to quickly detect this fraud by measuring the electrical properties (10 Hz – 550 kHz) of sausages made from fresh pork and spoiled pork treated with $NaNO_2$. The results explain how $NaNO_2$ hides the spoiled meat structure. Fresh samples show a sharp drop in total impedance at high frequencies because salt breaks the cell membranes, allowing ions to flow freely. In contrast, spoiled samples treated with $NaNO_2$ maintain a higher impedance and show a phase angle that quickly drops to 0°. This happens because $NaNO_2$ creates strong links between proteins, forming a fake electrical barrier that blocks ion flow. Because the Nyquist plots for both samples look very similar, they are not reliable for practical testing. Instead, the clear differences in impedance magnitude and phase angle on the Bode plots prove that EIS can accurately distinguish naturally fresh meat from chemically hidden spoiled meat.

Keywords: Detecting, EIS, food fraud, impedance, $NaNO_2$.

1. Introduction

In the food processing industry, Sodium Nitrite ($NaNO_2$) is a ubiquitous additive, pivotal in defining the sensory attributes of meat products. Chemically, the role of $NaNO_2$ extends beyond preservation to directly influencing colour and flavour.

The colouring process of $NaNO_2$ is based on its reactions with myoglobin, the main pigment in muscle tissue. Fresh meat has a natural red colour because its Myoglobin (Mb) contains iron in the Fe^{2+} state. However, when meat is stored for a long time or starts to spoil, the iron oxidizes into Fe^{3+}. The myoglobin becomes Metmyoglobin (MetMb), turning the meat a brownish-gray colour [1].

To hide this spoiled colour, $NaNO_2$ is added to create a fake "fresh" look through a chain of reactions. First, when nitrite enters the slightly acidic meat, it changes into nitrous acid (HNO_2):

$$NaNO_2 + H^+ \rightarrow HNO_2 + Na^+ \quad (1)$$

Next, this acid breaks down and releases nitric oxide (NO) gas:

$$2HNO_2 \rightarrow NO + NO_2 + H_2O \quad (2)$$

This NO gas easily binds to the Fe^{2+} in the myoglobin to form a new complex called Nitrosyl-myoglobin:

$$Mb\ (Fe^{2+}) + NO \rightarrow Mb\text{–}Fe^{2+}\text{-}NO \quad (3)$$

This compound has a bright pinkish-red colour [1, 2]. It looks like fresh meat, tricking consumers even though the proteins inside are actually breaking down. When the meat is heated or cooked, this compound turns into Nitroso-hemochrome, which locks in that stable pink colour for products like sausages and hams [3, 4]:

$$Mb\text{-}Fe2\text{+-}NO \rightarrow (Heat) \rightarrow Nitroso\text{-}hemochrome \quad (4)$$

Besides fixing the colour, nitrite also stops fats from oxidizing. This prevents the meat from producing bad smells and off-flavours during storage [2].

However, this artificial freshness capability has led to the abuse of $NaNO_2$ to marinate and disguise spoiled meat, posing severe public health risks. The primary concern is the acute and chronic toxicity of nitrite. At high concentrations, nitrite oxidizes Hemoglobin to Methemoglobin, impairing the blood's oxygen-carrying capacity, leading to "Blue Baby Syndrome" (Methemoglobinemia), which can be fatal, particularly in infants [2]. In the long term, nitrite reacts with secondary or tertiary amines in meat, especially under high heat, to form nitrosamines-a group of potent carcinogens [2]. Approximately 300 types of nitrosamines exist, most of which have been proven to be teratogenic or carcinogenic in laboratory animals [2]. Most volatile nitrosamines are classified as potential human carcinogens. The International Agency for Research on Cancer (IARC) and numerous studies have linked the consumption of nitrite-containing processed

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meats to an increased risk of colorectal, gastric, and esophageal cancers [2].

The danger lies in the inability of consumers to distinguish between naturally fresh meat and chemically treated spoiled meat through sight or smell alone. Therefore, developing a rapid, non-destructive method to detect the presence of $NaNO_2$ and assess the true quality of the meat tissue structure is urgent.

2. Related Work

Traditional physicochemical analysis methods such as High-Performance Liquid Chromatography (HPLC) or UV-Vis spectroscopy, while ensuring high accuracy in additive quantification [2,5], exhibit significant limitations for real-time monitoring due to high operational costs, complex sample preparation, and their destructive nature [5]. Recently, several studies have developed smartphone-integrated electrochemical sensors to improve measurement speed [4], yet these still do not fully resolve chemical contact issues. To address these drawbacks, Electrochemical Impedance Spectroscopy (EIS) has emerged as a superior solution due to its rapid, non-invasive, and cost-effective capabilities [6].

The principle of EIS relies on equivalent circuit models such as Fricke or Cole-Cole, which describe the electrical properties of intra/extracellular fluids and cell membranes [3]. Previous studies have confirmed a strong correlation between impedance variations and meat quality. Specifically, Trung *et al.* [11] and Kien *et al*. [12] demonstrated that post-mortem decomposition ruptures cell membranes, leading to a significant decrease in impedance modulus ($|Z|$). Expanding on applications, Huh *et al.* [13] integrated EIS with machine learning to predict freshness based on bacterial indices, while studies by Damez *et al.* [14] exploited electrical anisotropy to monitor meat aging.

While advanced electrochemical methods, such as nanostructured ZrO2@MWCNTs sensors, excel at quantifying free nitrite molecules in liquid food extracts [8], they cannot detect the 'structural masking' effect in solid, processed matrices like cooked sausages. In contrast, EIS serves as a non-destructive biophysical tool that captures structural and phase angle variations, offering a vital complementary approach to distinguish true biological freshness from chemically simulated integrity.

However, the application of EIS to detect commercial fraud via chemical reprocessing remains novel. Although a preliminary study by Trung *et al.* [15] on raw pork noted that washing spoiled meat with KNO3 restored colour but reduced impedance due to increased ionic conductivity, no publications have focused on cooked sausages—a complex material system with thermally destroyed cell structures and high background conductivity under the influence of $NaNO_2$ to mask spoilage. This paper aims to fill this research gap by defining the electrochemical discrimination threshold between fresh sausages and adulterated spoiled sausages.

3. Methodology

3.1. Sample Preparation

The study was conducted on heat-treated (cooked) sausages to evaluate electrical impedance characteristics under varying additive concentrations and storage conditions. The samples were categorized into two main groups: Fresh Sausages and Sausages made from naturally spoiled pork (48 hours) (Fig. 1).

To investigate the effect of $NaNO_2$ on spoiled meat and compare its impedance differences with fresh meat, the grouping protocol was as follows:

Standard Group (Fresh): Fresh sausages prepared with NaCl at concentrations of 1.5%, 2.0%, 4.0%, and 5.0%. This group serves as a baseline for evaluating standard conductivity.

Spoiled Group: Samples were allowed to decompose naturally under ambient conditions for 48 hours. These samples were prepared with a base of 1.5% NaCl, followed by the addition of $NaNO_2$ at concentrations of 1.5%, 2.0%, 3.0%, and 5.0% to assess the impact of tissue degradation on impedance parameters.

Regarding sample size: The study performed measurements on 4 replicates for each group, total 34 samples to ensure data reliability.

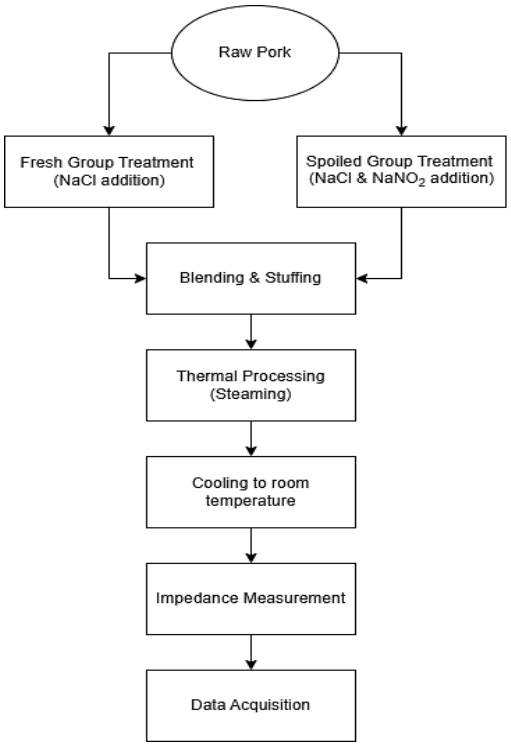

Fig. 1. Sample preparation and impedance data collection workflow

3.2. Experimental Setup

The impedance data acquisition system utilized a custom-designed two-electrode probe. The electrodes were fabricated from medical-grade stainless steel needles to minimize rusting, electrode polarization, and chemical corrosion when in contact with electrolytes.

Electrode Spacing: Two parallel needles fixed at a distance of 1.5 cm.

Penetration Depth: Needles were inserted into the sample at a controlled depth of 1.0 cm to ensure a consistent measurement volume.

Measurement Chamber: During measurement, samples were placed in an insulating plastic box to isolate them from conductive surfaces and the surrounding environment.

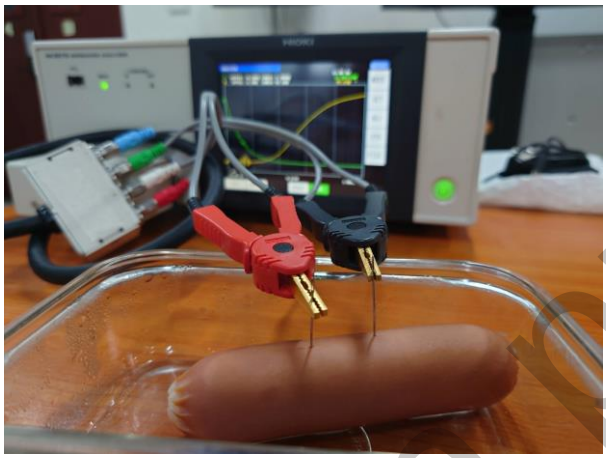

Fig. 2. Experimental setup for measurement

3.3. Impedance Measurement Procedure

Bio-impedance spectra (EIS) were collected using a HIOKI IM3570 Impedance Analyzer. Measurements were performed in frequency sweep mode from 10 Hz to 550 kHz using a low excitation voltage of 10 mV to ensure system linearity and prevent sample alteration.

3.4. Data Processing and Analysis

To optimize accuracy, raw impedance data (Z, θ) collected from samples in each group were averaged before analysis. This process helps eliminate noise and inherent variations in biological samples. The averaged dataset was then fitted to the Modified Cole-Cole model to extract characteristic bio-electrical parameters.

This model is an improved equivalent circuit that describes the bio-electrical properties of meat tissue over a wide frequency range more accurately than the traditional Cole-Cole model by adding Electrode Polarization in the low-frequency region as a Constant Phase Element (CPE).

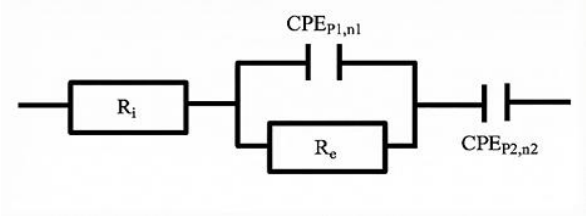

Fig. 3. Modified Cole-Cole equivalent circuit model

The total impedance Z(ω) is defined as follows:

$$Z(\omega) = R_\infty + \frac{(R_0 - R_\infty)}{(1 + (j\omega\tau)^\alpha)} + \frac{1}{(P(j\omega)^n)}$$

Where:

R_∞ and R_0: Impedance at infinite frequency and low frequency, respectively.

τ: Relaxation time, representing the capacitive nature of the cell membrane.

α: Dispersion coefficient, representing the homogeneity of the cell structure ($0 < \alpha < 1$).

P: Magnitude coefficient of the CPE, representing admittance.

n: Characterizes the electrode surface properties ($0 < n < 1$).

4. Results and Discussion

4.1. Analysis: Effect of Salt (NaCl) Concentration on Sausage Impedance

4.1.1. Impedance changes with varying salt concentrations

On the Bode (Magnitude) plot, samples with higher salt content consistently exhibit curves positioned lower, with the most distinct separation occurring in the high-frequency region. At this point, the electrical current can pass through the entirety of both intra- and extracellular fluids, causing the total impedance |Z| to drop sharply from 160.57 Ω (1.5% NaCl sample) to only 74.21 Ω (5.0% sample).

When more salt is added, the quantity of free ions (Na^+ and Cl^-) in the meat surges. These ions act as "charge carriers," facilitating easier current transmission through the sample. Additionally, salt draws water from inside the cells into the extracellular space, increasing the volume of conductive extracellular fluid, thereby reducing the total impedance of the entire system.

4.1.2. Phase angle analysis reflecting cell membrane rupture

The Bode (Phase) plot shows that the most negative phase angle only shifts slightly from -65.27° at 1.5% NaCl concentration and gradually decreases in value as the salt concentration increases, reaching -67.33° at 5% NaCl concentration. This level is quite far from the -90° mark of a perfect capacitor.

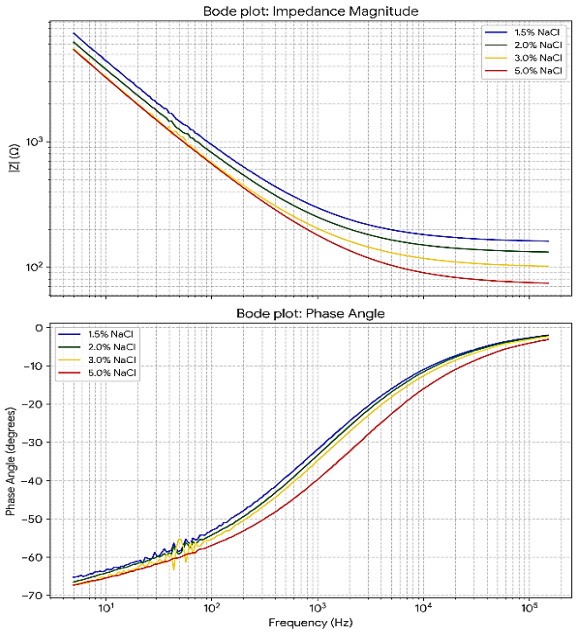

Fig. 4. Bode plots of sausages with varying NaCl concentrations

If the cell membranes were intact, they would act as the insulating shell of a capacitor. However, under the effect of temperature (during sausage steaming) and the strong protein-dissolving ability of NaCl, these cell membranes have been ruptured. When the membrane is torn, the insulating barrier disappears, and free ions easily leak back and forth. This reduces the capacitive nature of the meat tissue, turning it into a regular conductive medium.

4.2. Analysis: Effect of $NaNO_2$ Concentration on Sausage Impedance

4.2.1. Impedance magnitude changes and saturation phenomenon

Based on impedance measurements at the high-frequency region (550 kHz), representing the resistance of intra- and extracellular fluids (R_∞) we observed the variation of magnitude $|Z|$ with salt concentration as follows.

At low concentrations (1.5% – 2%), a significant drop in impedance was observed. Specifically, $|Z|$ decreased from 169.65 Ω (at 1.5%) to 126.10 Ω (at 2%). This aligns with electrolytic theory: adding $NaNO_2$ increases the density of free conductive ions Na^+, NO_2^-within the tissue structure, thereby increasing bulk conductivity [15] and reducing total system impedance.

At high concentrations (3% – 5%), this decreasing trend plateaus, showing a distinct saturation state. When the concentration increased from 2% to 3%, $|Z|$ continued to drop to 90.08 Ω. However, increasing the concentration further to 5% resulted in a negligible change, with impedance slightly shifting to 90.79 Ω.

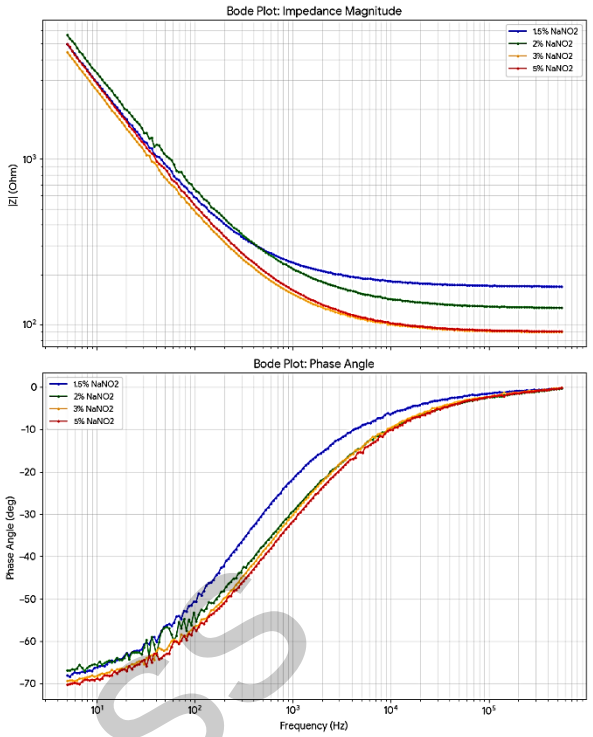

Fig. 5. Impedance spectra and phase angle of sausage samples with varying $NaNO_2$ concentrations (1.5% – 5%)

4.2.2. Phase angle analysis and tissue heterogeneity

Through phase angle analysis in Fig.4, the group determined experimental values for the parameter *α.*

At low concentrations (1.5% – 2%), the lowest phase angle reached approximately 67° −69°, corresponding to α ≈ 0.75.

This value reflects a highly electrochemical rough electrode-sample interface and a Constant Phase Element (CPE) system that is not yet fully stable.

At high concentrations (3%–5%), the lowest phase angle increased slightly to about 69°–71°, corresponding to α≈ 0.78. This indicates that at high salt concentrations, the system becomes more stable and less influenced by diffusion compared to low-concentration samples.

The fact that α is always less than 1 is explained by the heterogeneous structure of the sausage (comprising unevenly distributed meat, fat, and other components), causing the sample to behave not as an ideal capacitor but as a complex distributed system.

4.3. Comparison of Cl^- vs.NO_2^-: Membrane Integrity and Diffusion Mechanisms

The difference in electrochemical nature between fresh sausage (processed with 3% NaCl) and sausage made from spoiled meat (treated with 1.5% NaCl + 1.5% $NaNO_2$) reveals an interesting paradox, clarified through two mechanistic aspects:

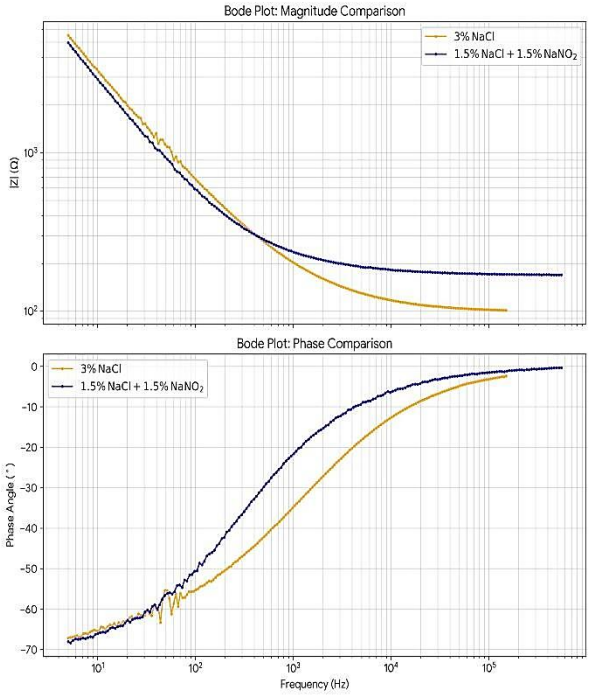

Fig. 6. Bode plots showing impedance magnitude and phase angle between fresh samples with $Cl^-$and spoiled samples with $NO_2{}^-$

4.3.1. Impedance characteristics

At f < 400 Hz, the fresh meat sample (3% NaCl) maintains a higher impedance compared to the spoiled sample. This is because, in this frequency range, the insulating property of the cell membrane plays the dominant role. Despite osmotic pressure, the cell membranes of fresh samples retain better physical integrity than the completely degraded membrane structures in spoiled samples, creating a larger capacitive barrier against current.

At higher frequencies (f > 400 Hz), the spoiled sample exhibits higher impedance than the fresh sample. At this stage, membrane capacitive reactance is negligible, so bulk conductivity dominates. The fresh sample sees a sharp drop in impedance due to the phenomenon of ionic flooding by mobile Cl^-ions. Conversely, the spoiled sample maintains an artificially higher impedance due to the structural masking effect [16] of $NaNO_2$: the formed protein cross-links act as steric hindrances, restricting ion flow more effectively than the fresh sample's environment.

4.3.2. Phase angle characteristics spoiled sample

Although biologically composed of decomposed meat tissue, this sample shows a phase angle converging to 0° faster with a steep slope. This proves the structural masking capability [16] of $NaNO_2$. This substance creates cross-linking [15] with the disintegrating protein network, erecting an artificial barrier that functions like a stable capacitor, blocking leakage current more effectively than living cells.

In contrast, for the fresh meat sample, the phase spectrum shows a slower and less steep transition. Although cells were initially intact, the high salt concentration (3%) caused osmotic shock and ruptured the cell membrane structure. Consequently, the cell no longer retains the properties of a sealed biological capacitor but transforms into a leaky dielectric structure. Conductive ions create a conduction effect through the damaged membranes, reducing capacitance and preventing the phase angle from converging to 0 as quickly as the $NaNO_2$ - containing sample.

4.3.3. Nyquist mechanism

On the Nyquist plot, the $NaNO_2$ sample displays a tail with a steep vertical slope, approaching ideal capacitor behaviour (n→1). Meanwhile, the NaCl sample has a more gradual slope, clearly exhibiting strong ionic diffusion (Warburg impedance, n→ 0.5). Furthermore, at high frequency, the $NO_2{}^-$ ion has lower mobility than Cl^-combined with the intact cell membrane, forming a more effective resistive barrier against intracellular fluid.

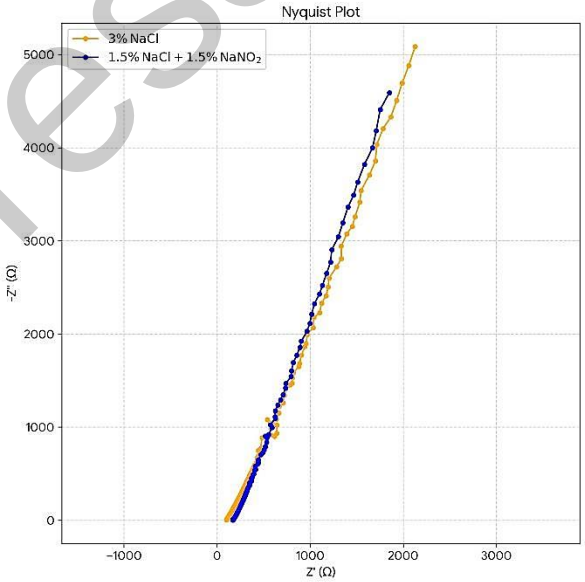

Fig. 7. Nyquist plot of fresh sausage (3% NaCl) and spoiled sausage (1.5% NaCl + 1.5% $NaNO_2$)

4.4. Limitations

The study notes two primary barriers affecting the method's accuracy:

Conductive Saturation: When $NaNO_2$ concentration increases from 3% to 5%, impedance magnitude varies very little (from 102Ω to 106Ω). This is caused by excessive ion density creating electrostatic interactions, reducing mobility and limiting quantification capabilities at high concentrations.

Sample Heterogeneity: The low alpha values (0.75-0.78) reflect the nature of sausages as a minced mixture of lean meat and insulating fat. Unlike an intact, uniform muscle or fat, this uneven distribution creates

local electrical barriers, causing signal noise and making the stability of the measurements highly dependent on the exact placement of the electrodes.

4.5. Comparisons with Previous Researches

Recent studies have successfully used EIS to monitor the natural aging and freshness of raw meat tissues by correlating impedance with storage time. However, our research uniquely applies EIS to detect deliberate food fraud in cooked sausages, where $NaNO_2$ is maliciously used to mask the structural degradation of spoiled meat.

To demonstrate the distinct positioning of our work, a comprehensive comparison with other non-destructive and analytical methods is summarized in Table 1.

Table 1. Comparison of research analysis methods for meat quality and additive detection

Method	Target	Sample	Range/LOD
EIS (Modified Cole-Cole)	Detecting fraud via structural masking and protein cross-linking	Cooked Sausages	1.5% – 5.0% (Fraud range)
EIS (Traditional/Hayden Models) [7]	Assessing natural freshness degradation and post-mortem aging	Raw pork and beef	Non-destructive monitoring over storage time
Amperometry / Voltammetry (ZrO2) [8]	Quantitative evaluation of free nitrite ions	Water and Food extracts	Linear Range: 5.0 – 100 uM; Limit of Detection (LOD): 0.94 uM
Colorimetric / Smartphone [4, 9]	Monitoring color and preservation	Processed cured meat products	Color intensity screening range: 1.0 – 50 mg/kg

4.6. Future Directions

To overcome errors due to sausage structural heterogeneity (lean/fat ratio), developing calibration algorithms using high-frequency impedance as a reference parameter is essential. At high frequencies, cell membrane capacitance is shorted, and impedance reflects only the total amount of electrolyte fluid. Normalizing low-frequency impedance will help eliminate background noise from structural variations, highlighting electrochemical changes caused by additives.

Additionally, constructing machine learning models can address the issue of ionic saturation at high concentrations. Classification algorithms such as Support Vector Machine (SVM) [17] or Random Forest [18] will be trained to recognize complex nonlinear patterns, thereby automatically and accurately classifying three states: "Fresh Meat", "Spoiled Meat", and "Spoiled Meat Disguised with Nitrite".

5. Conclusion

This study demonstrates that Electrochemical Impedance Spectroscopy (EIS) offers a practical, non–destructive way to catch food fraud–specifically where spoiled pork sausages are chemically masked with $NaNO_2$.

While Nyquist plots proved unreliable because of overlapping curves and the natural, uneven mix of lean meat and fat in sausages, Bode plots provided a much clearer diagnostic signal. In particular, the distinct gap in total impedance ($|Z|$) at high frequencies ($f > 400$Hz and the rapid drop of the phase angle to 0 serve as the most reliable markers for spotting disguised, spoiled meat.

Although challenges like structural background noise and ionic saturation still need to be addressed, they can be overcome by combining high-frequency normalization with machine learning models. Ultimately, this approach paves the way for low-cost, handheld screening devices that can help regulators and consumers alike ensure food safety.

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