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Investigation of the Impact of $Na_2S_2O_5$ on the Impedance Spectrum of Pork Loin Tissue in the Low-Frequency Range

Nguyen Phan Kien*, Pham Thi Hong Tham, Tran Bao Tram, Vu Dan Vy, Tran Dinh Hung, Tran Thi Hau
Hanoi University of Science and Technology, Ha Noi, Vietnam
**Corresponding author email: kien.nguyenphan@hust.edu.vn*

Abstract

Food safety, especially concerning fresh meat, has always been a primary concern in the food industry. Pork, due to its high consumption rate, is highly susceptible to spoilage caused by bacterial growth during storage. One of the effective methods to assess the freshness of meat is Electrochemical Impedance Spectroscopy (EIS), a non-destructive technique that allows for the rapid evaluation of meat quality and the detection of chemical preservatives. However, detecting preservatives such as sodium metabisulfite ($Na_2S_2O_5$)–a common additive in food remains limited. This study aims to examine the impact of $Na_2S_2O_5$ on the impedance spectrum of pork loin tissue in the low-frequency range, thereby developing a method to distinguish between three tissue conditions: fresh meat, spoiled untreated meat, and spoiled meat treated with preservatives. Impedance measurements were conducted on three types of meat samples, across frequencies ranging from 20 Hz to 200 kHz. The results revealed that 200 Hz was the most sensitive frequency, allowing clear differentiation between the three sample types. This discovery not only provides insight into the interaction mechanisms between $Na_2S_2O_5$ and biological tissue but also demonstrates the potential of impedance spectroscopy as a fast, non-invasive method for evaluating the quality and degree of degradation of fresh meat in the presence of preservatives.

Keywords: EIS, impedance magnitude, impedance phase, $Na_2S_2O_5$, pork loin.

1. Introduction

Food safety is always a top concern, especially for fresh meat – one of the essential sources of animal protein in the diet. In recent years, Vietnam has ranked 4th in the world in terms of pork consumption. However, fresh meat is highly perishable due to its high-water content and nutrient-rich surface, with bacterial proliferation during storage being the primary cause [1]. This process leads to the development of off-odors, abnormal taste, discoloration, slimy surface formation, and other physicochemical changes, which significantly reduce consumer acceptance [2]. In addition to biological spoilage, pork is also facing the growing issue of being illegally treated with chemical preservatives, artificial colorants, and unauthorized additives to extend shelf life, mask spoilage signs, and maintain a fresher appearance [3]. The abuse of such substances not only distorts sensory quality assessments but also poses serious health risks to consumers, such as allergic reactions, mucosal damage, or toxic accumulation when exceeding permissible limits [4]. Therefore, alongside monitoring conventional biological indicators, it is crucial to develop rapid, non-destructive analytical techniques that can simultaneously assess actual quality and detect the presence of illegally used chemical substances in pork.

Meat freshness can be reflected through various indicators, including physical, physiological, and biochemical properties. Physically, attributes such as color, odor, and texture are often assessed using modern technologies like X-ray imaging [5], thermal imaging [6], NIR - Near-infrared spectroscopy [7], HIS - Hyperspectral Imagery [8], low-field magnetic resonance [9], and electrical conductivity measurements [10]. These techniques are not only applied to meat but are also widely used in evaluating the quality of fruits and seafood. Additionally, methods for assessing protein degradation, enzyme activity, and microbial metabolites (e.g., TAC and TVB-N) also play an essential role in meat quality analysis [11]. Among these, Electrochemical Impedance Spectroscopy (EIS) has emerged as a promising tool due to its capability to analyze the microstructure and composition of biological tissues rapidly and non-invasively [12-15].

Numerous studies have confirmed the efficacy of EIS in monitoring meat freshness. Sooin Huh *et al.* (2021) employed a frequency range from 125 Hz to 128 kHz combined with meat imaging and machine learning, achieving a freshness classification accuracy of up to 91.4% compared to standard indicators such as APC and TBARS [12]. Moreover, the study by Yue Leng [13] demonstrated that bioimpedance measurements in the range of 20 Hz to 200 kHz effectively detected structural alterations in meat following freeze–thaw cycles, with significant changes observed in both resistance and reactance, suggesting the potential of EIS for rapid and

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accurate assessment of quality degradation during cold storage. Nguyen H & Nguyen L [16] found that pork impedance exhibited the most significant changes within the 20 Hz – 2 kHz range, while the influence of frequency diminished above 20 kHz. Low frequencies were more sensitive to cell membrane alterations, whereas higher frequencies correlated better with biochemical spoilage indicators. In a study focusing on EIS for chemical detection in pork loin, Đ. T. Trung [17] utilized a frequency range from 100 Hz to 1 MHz to investigate impedance and phase variations in pork before and after KNO_3 treatment. The results showed distinct differences in both impedance magnitude and phase angle between untreated and KNO_3- treated meat, particularly at low frequencies. Notably, Petrescu *et al.* [18] demonstrated that electrical and dielectric properties of pork changed significantly during 15 days of cold storage at 4°C, with marked signs of quality degradation appearing after 6–8 days. In that study, impedance data were collected in the 20 Hz – 5 kHz range, revealing significant variations in electrical conductivity and dielectric constant over storage time.

Based on the theoretical foundations mentioned above, low-frequency impedance spectra not only accurately reflect microstructural changes but also exhibit sensitivity to biochemical effects on meat tissue. In this context, the present study was conducted to investigate the influence of Sodium Metabisulfite ($Na_2S_2O_5$) - a preservative frequently abused in food -on the impedance spectrum of pork loin tissue in the low-frequency domain. The goal is to enable rapid detection of the presence and effects of chemical additives through the bioelectrical properties of meat tissue.

2. Materials and Methods

The experimental process is illustrated by the block diagram in Fig. 1. The meat samples including fresh meat, spoiled untreated meat, and spoiled meat treated with preservative were measured using an impedance spectroscopy circuit. The acquired data were then collected and analyzed to differentiate between the various sample conditions.

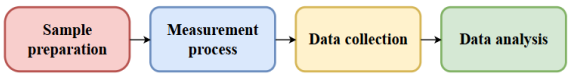

Fig. 1. Block diagram of the experimental procedure

2.1. Sample Preparation

Chemical Reagent: Sodium metabisulfite ($Na_2S_2O_5$), sourced from Esseco Group Italy and purchased from the food additive distributor LabVietChem, is a widely used preservative in food and beverages to inhibit microbial growth [19]. $Na_2S_2O_5$ exhibits cytotoxicity by triggering programmed cell death, promoting the generation of reactive oxygen species (ROS), and activating autophagy mechanisms in normal human cells [20]. Therefore, caution must be exercised in the use of sodium metabisulfite to avoid adverse health effects, including eye, skin, and respiratory tract irritation [21]. Pork loin samples were purchased fresh from a local market early in the morning. The meat surface appeared firm, with a uniform bright pink color and no fat content. Upon sensory evaluation, the meat emitted a mild, characteristic odor typical of freshly slaughtered pork, with no indication of spoilage, unusual odors, or any signs of preservative related contamination. Prior to impedance measurements, a preliminary qualitative screening was performed using litmus paper to check the general acid–base condition of the extracted solution. Approximately 5 g of pork tissue was immersed in 50 mL of distilled water for 15 minutes to extract soluble components. The litmus paper was then dipped into the solution and the color change was observed. It should be noted that litmus paper is a simple qualitative method and does not specifically detect sodium metabisulfite ($Na_2S_2O_5$) or other preservatives. It only reflects general pH changes. Therefore, this step was used for initial screening purposes only and not for accurate identification. More reliable methods, such as pH measurement using a pH meter or other chemical analysis, would be needed for more precise evaluation. This limitation is acknowledged in this study. The chemical principle is as follows:

$$Na_2S_2O_5 + H_2O \rightarrow 2\,Na^+ + 2HSO_3^-$$

$$HSO_3^- \leftrightharpoons H^+ + SO3^{2-}$$

$$H^+ + L^- \rightarrow LH \text{ (light red litmus paper)}$$

Five independent pork loin samples were used in this study (n = 5). Each sample was cut into three sub-samples of similar dimensions (approximately 55 mm × 35 mm × 23 mm), which were assigned to the three experimental conditions: fresh, spoiled untreated, and $Na_2S_2O_5$-treated. This design ensured that all conditions were derived from the same biological origin within each sample while maintaining independence across different samples. To reduce bias from sequential treatment effects, separate sub-samples were used for each condition instead of applying all treatments sequentially on the same piece of tissue. Although five independent samples were used, the sample size remains limited and may not fully represent the variability of pork tissue from different animals. This should be considered a limitation of the study. The experimental procedures for each condition were then conducted independently on the corresponding sub-samples, as described below:

Control sample: Fresh pork, immediately measured for impedance after purchase, served as the control.

Spoiled untreated sample: “For the spoiled condition, the corresponding sub-sample assigned to the spoiled condition was sealed in foodgrade wrap and stored at room temperature (35°C) for 48 hours to induce spoilage. Impedance spectra were then measured again

to assess the impact of biological degradation on tissue structure.

$Na_2S_2O_5$-treated sample: The sub-sample assigned to the $Na_2S_2O_5$-treated condition was immersed in a 0.07% $Na_2S_2O_5$ solution at room temperature for 30 minutes, then gently dried using absorbent paper before performing impedance spectroscopy.

2.2. Sample Holder and Electrodes

To ensure accuracy and stability throughout the impedance spectroscopy measurements, a custom-designed sample holder was manually fabricated.

The container was constructed from 3 mm-thick mica sheets, selected for their high mechanical strength, excellent electrical insulation, and ease of fabrication using laser cutting techniques. The structure is a rectangular box with external dimensions of 7.5 cm × 4.5 cm × 2.3 cm (length × width × height), and an internal sample compartment measuring 5.6 cm × 3.5 cm × 2.3 cm (length × width × height). The edges were assembled using specialized adhesive for strong bonding and leak prevention:

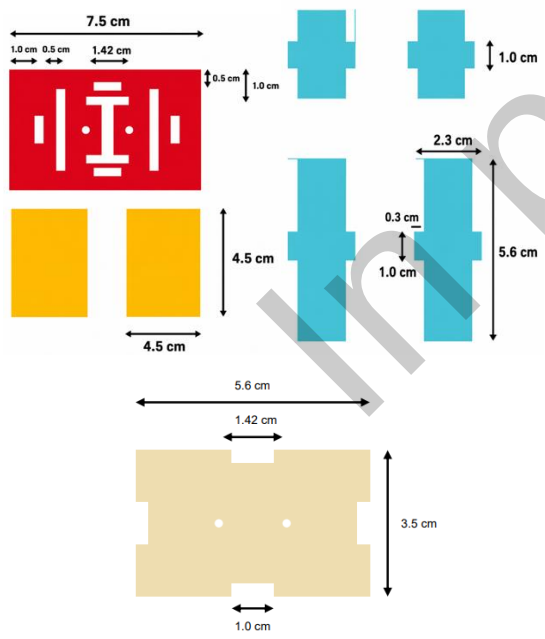

Fig. 2. Dimensions of the box assembly panels

The electrodes (Fig. 3) used in the system were fabricated from a pair of medical-grade stainless steel needles, type 4B3, with dimensions of 0.9 × 36 mm (according to standard CSN 85 59 36). Each electrode had an effective length of 2 cm, with the tip beveled at a 45° angle to facilitate easy penetration into the pork tissue.

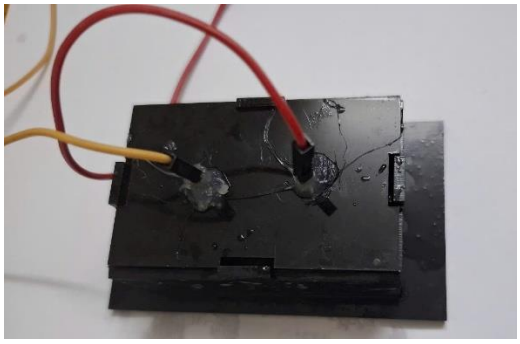

Fig. 3. Sample holder

The two needle electrodes were positioned parallel to each other, spaced 10 mm apart, and inserted perpendicularly to the muscle fibers (i.e., in the transverse direction relative to the fiber orientation). The insertion points were carefully selected to avoid regions containing fat or connective tissue, ensuring consistent and stable electrical signals. Each needle electrode was firmly attached to a female-to-female connector wire, allowing easy and secure connection to the impedance measurement circuit. The connected ends of the needles were inserted through two circular holes in the top panel of the sample holder, as illustrated in Fig. 3. The opposite ends of the needles penetrated through the holes and made direct contact with the pork sample inside the container.

This configuration ensured that the entire electrode tip (approximately 15 mm of the needle length) was fully embedded in the tissue. It provided mechanical stability, prevented electrode movement or vibration, and maintained stable electrical contact, thereby enhancing the accuracy and reliability of the impedance measurements.

2.3. Meat impedance measurement system

From a bioelectrical perspective, biological tissues such as meat are often modeled using equivalent electrical circuits to represent their conductive and dielectric properties. Two commonly used models in tissue impedance analysis are the Fricke model [22] and the Cole–Cole model [23].

The Fricke model (Fig. 4) is based on an idealized structure in which spherical cells are suspended in an extracellular medium. In this model, R_e represents the resistance of the extracellular fluid, R_i is the resistance of the intracellular fluid, and C_m denotes the capacitance of the cell membrane. This configuration highlights the role of the cell membrane as a dielectric barrier that separates two conductive domains intra and extracellular compartments. At low frequencies, the capacitor C_m impedes the flow of current through the membrane, causing the current to flow primarily through the extracellular space. In contrast, at high frequencies, the current can penetrate the membrane, flow into the intracellular compartment, and significantly alter the

total measured impedance. The Cole–Cole model (Fig. 5), on the other hand, is an advanced semi-empirical model developed to describe the frequency-dependent dispersion of bioimpedance - a typical nonlinear behavior of living tissues. Unlike the Fricke model, which uses discrete electrical components, the Cole–Cole model expresses impedance as a continuous complex function of frequency, allowing for a more accurate representation of biological tissue behavior across a wide spectral range:

$$Z^*(\omega) = R_\infty + \frac{R_0 - R_\infty}{1+(i\omega\tau)^\alpha} = R(\omega) + jX(\omega) \quad (1)$$

In this model, R_0 and R_∞ represent the impedance at very low and very high frequencies, respectively; τ is the time constant, characterizing the cellular polarization process; α is the dispersion coefficient ($0 \le \alpha \le 1$), which reflects the non-ideal behavior of the tissue due to structural heterogeneity; and $Z*(\omega)$ is the complex impedance function, consisting of a real part $R(\omega)$ and an imaginary part $X(\omega)$. Thus, biological tissue impedance is a complex quantity expressed as Z = R + jX, where the magnitude indicates the tissue's overall electrical conductivity, while the phase angle reflects its capacitive behavior and the microstructural properties of the biological matrix.

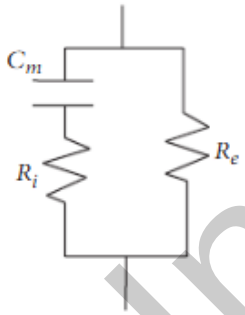

Fig. 4. Fricke Model

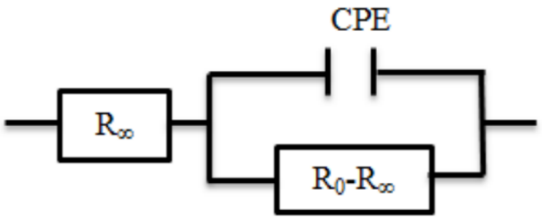

Fig. 5. Cole-Cole impedance model

In this study, the impedance measurement system was designed based on the principle of an inverting amplifier circuit, where the pork tissue sample was placed in the feedback branch and served as the impedance element to be measured. A sinusoidal signal with a fixed amplitude was generated by a function generator and swept across a predefined frequency range. The input signal Vin was applied to the inverting input of the amplifier, while the output signal $Vout$ was the result after the current passed through the tissue sample. Based on the gain and phase-inverting characteristics of the circuit, the impedance of the meat tissue was calculated using the formula:

$$Z_{meat} = \frac{-v_{out}}{v_{in}} \times R_{in} \quad (2)$$

where R_{in} is the known input resistor, and the negative sign indicates the 180° phase inversion characteristic of the amplifier circuit, which does not affect the amplitude value in impedance spectroscopy analysis.

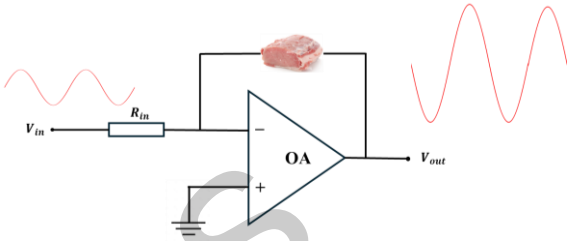

Fig. 6. Inverting amplifier circuit

Our measurement system was constructed based on the inverting amplification principle, with the main components presented in Fig. 7.

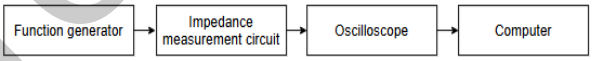

Fig. 7. Diagram of the impedance measurement system

The signal generator used in the system was the HAMEG HM8131-2 manufactured by HAMEG Instruments. This is a precision waveform generator with a frequency range of up to 15 MHz, allowing the generation of sinusoidal signals with adjustable amplitude and frequency. The device also supports automatic frequency sweep mode, making it suitable for biological impedance spectroscopy (EIS) measurements over a wide frequency range. The output signal from the generator was then fed into the inverting amplifier circuit containing the meat tissue sample. The signal acquisition unit was the DSO1012A oscilloscope from Keysight Technologies, which allows simultaneous recording of both the input signal Vin and the output signal Vout of the circuit. Vin is the excitation signal generated by the signal generator, while Vout is the signal obtained after passing through the tissue. The use of a dual-channel oscilloscope allows simultaneous monitoring of waveform and amplitude of both signals, ensuring measurement accuracy in impedance calculation. The signal data from the oscilloscope were exported in digital format and stored on a computer for subsequent data processing. The actual measurement setup is shown in Fig. 8.

Fig. 8. Meat measurement system

2.4. Data Analysis

The measured signal from the oscilloscope included two channels: channel CH2 recorded the input signal $v$in (signal from the generator), and channel CH1 recorded the output signal $v$out (signal after passing through the meat tissue sample). Based on the data from these two channels, the complex impedance of the meat sample at each excitation frequency was calculated using the formula:

$$Z_{meat} = \frac{-v_{out}}{v_{in}} \times R_{in} \quad (3)$$

with:

$$v_{in} = V_1 cos(\omega t + \varphi_1)$$

$$v_{out} = V_2 cos(\omega t + \varphi_2)$$

In this formula R_{in} is the known input resistor, and V_1 and V_2 are the complex amplitudes of the input and output signals, extracted using Discrete Fourier Transform analysis (FFT – Fast Fourier Transform). By applying Euler's formula, each acquired sinusoidal signal can be represented as a complex number, enabling real-time calculation of both the amplitude and the relative phase between the input and output signals.

$$Z_{meat} = \left|\frac{V_2}{V_1}\right| \times R_{in} \quad (4)$$

$$\theta(u) = arctan\frac{sin(\varphi_2 - \varphi_1)}{cos(\varphi_2 - \varphi_1)} = \varphi_2 - \varphi_1 + k\pi \quad (5)$$

The measurement was repeated at multiple excitation frequencies to construct the impedance spectrum of the tissue sample. At each frequency, the amplitude and phase values of the impedance were stored and represented in the form of a Bode plot, including both the magnitude plot and the phase plot (as a function of logarithmic frequency). By analyzing the shape and trends of these plots, the bioelectrical properties of the meat tissue such as electrical conductivity, membrane polarization, and microstructural characteristics were evaluated and compared across different experimental 5 conditions, thereby clarifying the effects of processing agents like $Na_2S_2O_5$ on the tissue in the low-frequency range.

3. Experimental Procedure

3.1. Sample Measurement Procedure

Sample preparation: as described in Section 2.1. The image of the measurement sample is shown in Fig. 9 below.

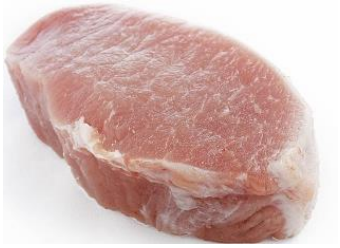

Fig. 9. Initial fresh meat sample

The experimental procedure was recorded as follows:

Experiment 1: Measurement at the fresh stage.

Date of measurement: April 25, 2025

Each pork loin sample (n = 5, each processed independently) with approximate dimensions of 5 cm × 3 cm × 2 cm, after being inspected and confirmed to be free of chemicals, was fitted with electrodes placed horizontally along the muscle fibers.

Both electrode tips were inserted vertically at a 90° angle, with the entire length of each electrode embedded within the sample.

After connecting the electrodes to the circuit board, the measurement process was carried out manually, sequentially measuring five frequency bands ranging from 20 Hz to 200 kHz (20 Hz, 200 Hz, 2 kHz, 20 kHz, 200 kHz). For each frequency band, data were recorded 10 consecutive times, repeating this process until all five frequencies were measured.

Experiment 2: Measurement at the spoiled stage

Date of measurement: April 27, 2025

For each sample, the corresponding sub-sample assigned to the spoiled condition was tightly sealed in FSSC 22000 - certified plastic food wrap and stored at a temperature of 35°C for 48 hours.

Upon unwrapping, each spoiled sub-sample exhibited sensory characteristics commonly associated with spoilage, including an unpleasant odor, yellowish surface slime, gas formation, paler color, softened texture, and the release of exudate. These characteristics are commonly associated with meat spoilage under practical conditions.

Based on these observable changes, the sample was regarded as being in a sensory-spoiled condition.

However, no quantitative measurements (such as pH, microbial count or TVB-N (Total Volatile Basic Nitrogen)) [24] were performed, and this should be considered a limitation of the study.

(a) (b)

Fig. 10. (a) Meat sample after 2 days (b) Slimy texture with yellowish exudate

The sample was then rinsed with water, gently dried using a paper towel, and measured for impedance to compare with the initial fresh condition.

For the treated condition, the corresponding sub-sample from each independent sample was soaked in a $Na_2S_2O_5$ solution prepared at a ratio of 14 mL distilled water to 0.01 g $Na_2S_2O_5$ for 30 minutes. After soaking, the sample was rinsed again and dried with a paper towel. The texture and color became firmer, denser, and redder compared to before soaking.

The measurement process was carried out using five frequency bands (20 Hz, 200 Hz, 2 kHz, 20 kHz, and 200 kHz) for comparison with the reference sample. These five frequency bands were selected based on previous studies and are broadly consistent with the reported bioelectrical behavior of biological tissues. For each frequency and sample condition, impedance measurements were repeated ten times under the same experimental setup. The results are presented as mean values with corresponding standard deviations (mean ± SD) to describe the repeatability of the measurements within the same biological specimen. At low frequencies (around tens of Hz), the current mainly flows through the extracellular fluid, at medium frequencies (kHz), the cell membrane begins to influence the current and at high frequencies (hundreds of kHz), the current can penetrate the cell membrane, reflecting both extracellular and intracellular components. Therefore, selecting an exponentially spaced frequency range may help provide a broader characterization of the tissue's electrical properties and potentially facilitate the differentiation of samples with varying freshness conditions.

The sample shown in Fig. 11(a) corresponds to untreated fresh pork, characterized by a relatively uniform light pink color, a naturally dry surface, and clearly distinguishable muscle fibers. In contrast, the sample in Fig. 11(b), which had previously exhibited sensory signs associated with spoilage and was subsequently soaked in the chemical solution, appeared visually more similar in color and surface appearance to fresh meat, with a glossier and more uniform surface.

(a) (b)

Fig. 11. (a) Fresh meat sample (b) Soaked spoiled meat

These visual observations suggest that the chemical treatment may have altered certain surface characteristics of the sample, potentially reducing the visibility of some sensory spoilage signs.

3.2. Data Collection and Analysis

Impedance measurements were conducted over a frequency range from 20 Hz to 200 kHz, as previously described, in order to characterize the electrical response of the meat samples across different frequency bands. In each measurement cycle, the signal was sequentially adjusted to five fixed frequencies: 20 Hz, 200 Hz, 2 kHz, 20 kHz, and 200 kHz. At each frequency, the corresponding phase and amplitude values were recorded. Each measurement was repeated ten times under identical experimental conditions to evaluate technical repeatability and reduce the influence of random measurement variations. The results at each frequency and sample condition were summarized as mean values with corresponding standard deviations (mean ± SD). All measurements were carried out under stable and consistent environmental conditions across sample groups, including room temperature, time since sample preparation, and electrode contact method.

The data were then stored on a USB drive and exported in digital format for processing using the Python programming language in the Visual Studio Code environment. The analysis steps included calculating the impedance magnitude and phase at each frequency using the NumPy and SciPy libraries and visualizing the results in the form of Bode plots using the Matplotlib library. The use of Python enhanced flexibility in signal processing, ensured analytical accuracy, and enabled high reproducibility throughout the entire data analysis process.

4. Results and Discussion

4.1. Results

In this study, the electrical impedance characteristics of pork loin tissue were investigated at five excitation frequencies: 20 Hz, 200 Hz, 2 kHz, 20 kHz, and

200 kHz. Three sample conditions were considered, including fresh meat, spoiled untreated meat, and spoiled meat treated with sodium metabisulfite ($Na_2S_2O_5$). Each condition was evaluated across five independent samples (n = 5), with ten repeated measurements per sample in order to obtain technical replicates and enable statistical evaluation of measurement repeatability and discrimination capability.

In this study, the electrical impedance characteristics of pork loin tissue were evaluated at five excitation frequencies: 20 Hz, 200 Hz, 2 kHz, 20 kHz, and 200 kHz. Measurements were conducted on five independent pork samples. For each independent sample, three corresponding sub-samples were assigned to the three experimental conditions: fresh, spoiled untreated, and spoiled meat treated with sodium metabisulfite. At each frequency, impedance measurements were repeated ten times for each sample to ensure measurement stability. The repeated measurements were averaged to obtain a representative value for each sample. The final reported results are expressed as mean ± standard deviation across five independent samples.

Fig. 12 shows the variation of impedance magnitude |Z| as a function of frequency for the three meat conditions. A consistent decreasing trend of impedance magnitude with increasing frequency was observed across all samples. Fresh meat generally exhibited the highest impedance magnitude, followed by spoiled untreated meat, while $Na_2S_2O_5$-treated meat showed the lowest values. At low frequencies, particularly at 200 Hz, a clear separation trend among the three conditions was observed. However, some variability across samples was present, reflecting biological differences between tissue samples. At higher frequencies (20 kHz and 200 kHz), the impedance values tended to converge, resulting in reduced discrimination capability.

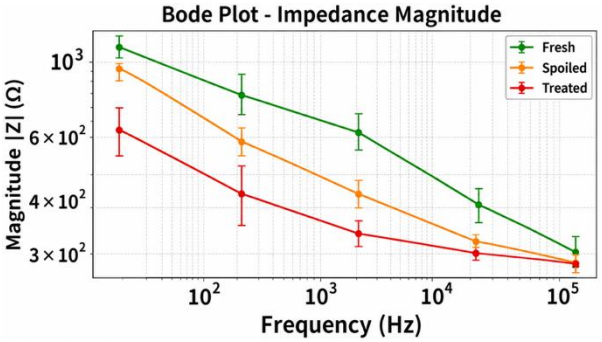

Fig. 12. Meat impedance magnitude

The phase characteristics are presented in Fig. 13. Fresh meat generally exhibited more negative phase values at low frequencies, indicating stronger capacitive behaviour associated with intact cell membranes. In contrast, spoiled untreated meat and treated meat showed less negative phase angles, suggesting progressive degradation of membrane integrity and increased dominance of resistive conduction. Similar to the magnitude results, the differences in phase among conditions became less distinct at higher frequencies. In addition, inter-sample variability was observed, particularly at low frequencies.

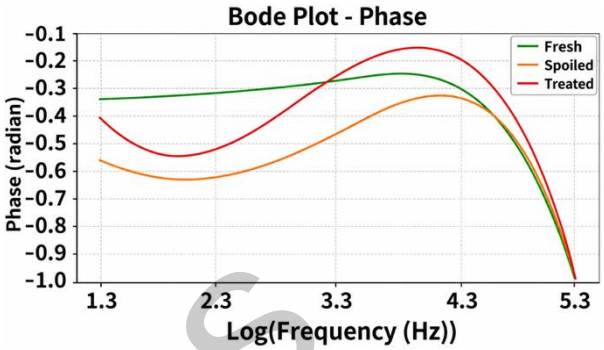

Fig. 13. Impedance phase as a function of frequency for fresh, spoiled untreated, and $Na_2S_2O_5$-treated pork samples

The mean and standard deviation values of impedance magnitude across the five samples are summarized in Table 1. Statistical analysis was performed using one-way ANOVA based on biological replicates. The ANOVA results (Table 2) indicated statistically significant differences among the three meat conditions at all investigated frequencies ($p < 0.05$). However, at 20 Hz, the difference between fresh and spoiled untreated meat was not statistically significant, indicating limited sensitivity of this frequency for distinguishing spoilage alone. At 200 Hz, significant differences were observed among all sampleforr fo pairs, confirming this frequency as the most sensitive point for discrimination. Although statistically significant differences were also observed at 2 kHz and 20 kHz, the contrast between groups decreased progressively with increasing frequency. At 200 kHz, impedance values showed strong convergence, and only weak statistical differences were observed, indicating Table 2. One-way ANOVA results for impedance magnitude across biological replicates at each excitation frequency reduced sensitivity of high-frequency measurements to microstructural chantges.

The distribution of impedance magnitude across samples is further illustrated using boxplots in Fig. 13. The clearest separation among the three conditions is observed at 200 Hz, whereas increasing overlap between groups is observed at higher frequencies.

Table 1. Mean ± standard deviation of impedance magnitude across five independent samples

Frequency	Fresh	Spoiled	Treated
20 Hz	1642.59 ± 99.97	1560.54 ± 72.76	947.80 ± 187.71
200 Hz	1156.98 ± 111.64	805.93 ± 38.56	532.36 ± 121.96
2 kHz	846.56 ± 100.75	490.51 ± 18.35	358.67 ± 9.95
20 kHz	446.20 ± 38.70	323.64 ± 4.30	293.18 ± 5.37
200 kHz	263.95 ± 4.81	264.69 ± 2.18	269.25 ± 1.68

Table 2. One-way ANOVA results for impedance magnitude across biological replicates at each excitation frequency

Frequency	F value	P value	Statistical interpretation
20 Hz	85.6	< 0.001	Overall significant difference: fresh vs spoiled not significant
200 Hz	102.03	< 0.001	Significant difference among all meat conditions
2 kHz	180.50	< 0.001	Significant difference among all meat conditions
20 kHz	127.39	< 0.001	Significant difference: reduced contrast between spoiled and treated
200 kHz	8.07	0.0018	Weak statistical difference:impedance values converge

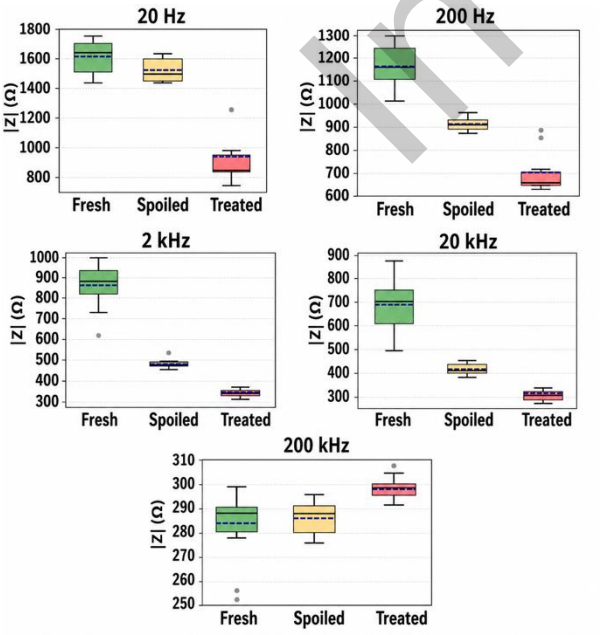

Fig. 14. Boxplot comparison of impedance magnitude across biological replicates for fresh, spoiled untreated, and $Na_2S_2O_5$-treated pork samples at different excitation frequencies

To further illustrate the impedance behaviour at the most sensitive frequency, an additional independent pork sample was measured at 200 Hz. Fig. 15 presents the impedance magnitude of this independent sample under the three conditions. A similar trend was observed, with fresh meat exhibiting the highest impedance, followed by spoiled and treated conditions. Fig. 16 shows the corresponding phase values at 200 Hz for the same sample, demonstrating consistent phase behaviour with the main dataset.

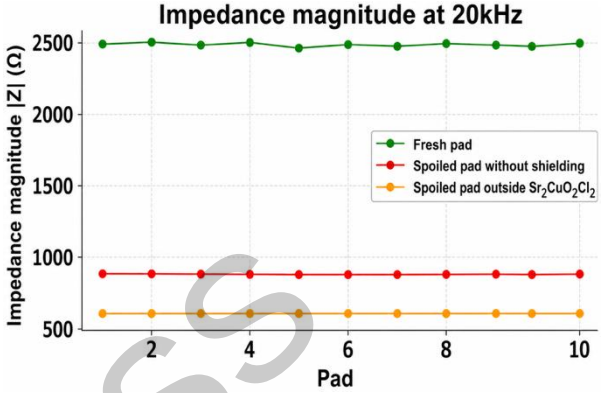

Fig. 15. Impedance magnitude at 200 Hz for an independent pork sample

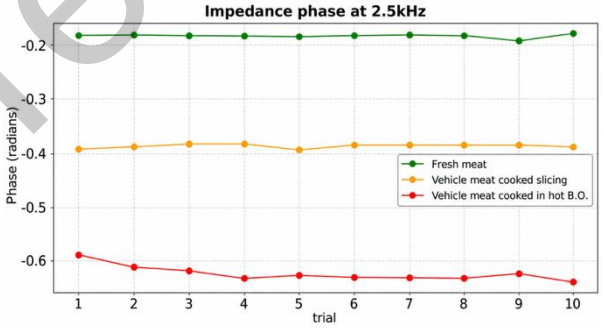

Fig. 16. Impedance phase at 200 Hz for an independent pork sample

4.2. Discussion

The statistical results presented in Section 4.1 confirm that impedance magnitude differences among meat conditions are most pronounced at low frequencies. The changes in impedance amplitude and phase in meat are sensitive indicators that directly reflect structural, chemical, and biological alterations occurring during spoilage and treatment. In fresh muscle tissue, cells remain intact, with phospholipid membranes serving as effective dielectrics that hinder current flow at low frequencies. This results in high impedance amplitude and a strongly negative phase angle due to charge accumulation across the membrane. However, as the meat begins to spoil, microbial activity and endogenous enzymes degrade the cell membranes. This damage causes electrically conductive ions and molecules to leak from inside the cells into the extracellular environment, significantly increasing the tissue's overall conductivity. As a result, the impedance amplitude sharply decreases,

and the phase angle shifts toward zero, indicating the loss of dielectric properties from the membranes and the dominance of resistive behavior. When spoiled meat is soaked, particularly in a solution containing $Na_2S_2O_5$, this process is further intensified. Soaking increases the amount of free water and facilitates the diffusion of ions, including those from $Na_2S_2O_5$, into the tissue. The increase in free ion concentration further enhances the electrical conductivity of the meat, causing the impedance amplitude to drop even more. At the same time, the impedance phase angle continues to approach zero because the tissue becomes a more homogeneous conductive medium, with little to no dielectric properties remaining from intact cell structures. In summary, the changes in dielectric impedance in meat result from a complex series of events, including cell membrane integrity, biological degradation, and variations in ionic concentration and distribution within the tissue.

The frequency range of 20 Hz to 200 kHz used in this study covers the entire spectrum previously applied in numerous studies evaluating meat quality and freshness using electrochemical impedance spectroscopy (EIS). According to published research, changes in the electrical properties of meat are most pronounced in the low to mid-frequency range where interactions between electrical current and tissue structure are most sensitive. Notably, the 200 Hz to 2 kHz range, identified in this study as the most stable and discriminative band, was also emphasized in the work of Nguyen H & Nguyen L. [16] where pork impedance was also recorded to change most significantly in the 20 Hz to 2 kHz range. Additionally, the measurement results at 200 Hz and 2 kHz in this study clearly demonstrated the separation between different meat states (fresh, spoiled untreated, and spoiled treated), with high repeatability consistent with the observations reported in the study by D.T. Trung [17] where the pronounced changes in impedance magnitude and phase between unadulterated meat and meat treated with KNO3 were also concentrated in the low-frequency range. Although a wide frequency range up to 200 kHz was investigated, like the study by Petrescu *et al.* [18], the signals in the high-frequency region in this study exhibited weaker differentiation between the samples, reflecting the recognized trend that the ability to detect microstructural changes and cellular degradation is most prominent at low to mid frequencies. These findings suggest the potential feasibility of low-frequency impedance measurements as a rapid screening tool for meat condition assessment.

This study has several limitations that should be acknowledged. The classification of spoiled meat was primarily based on sensory evaluation, which may lack objectivity and does not provide a quantitative assessment of biochemical spoilage processes such as microbial growth or protein degradation. In addition, the detection of sodium metabisulfite was performed using pH indication with litmus paper, which is not a specific analytical method and only reflects general acidity changes rather than the presence of the compound itself. Stainless steel needle electrodes were selected due to their mechanical stability and ease of implementation. However, it is well established that metal electrodes can introduce electrode polarization and contact impedance effects, particularly at low frequencies, which may influence the measured impedance response of biological tissues [25].

Furthermore, sodium metabisulfite was only evaluated at a single concentration of 0.07%, so the detection limit of the proposed method and its sensitivity at lower concentrations could not be determined in the present study. In addition, because all measurements were performed under controlled laboratory conditions, the impedance response, including the sensitivity observed around 200 Hz, may vary under practical market or retail environments where temperature and humidity fluctuate.

5. Conclusion

This study has demonstrated that Electrical Impedance Spectroscopy (EIS) is an effective and non-invasive tool for distinguishing between fresh, spoiled, and chemically treated spoiled pork tenderloin tissue, particularly in the low-frequency domain. The measurement system, based on an inverting amplifier circuit, enables accurate acquisition of input and output voltage signals, from which the amplitude and phase of the tissue impedance can be calculated across multiple frequency bands. Measurements conducted at five frequencies ranging from 20 Hz to 200 kHz revealed distinct impedance characteristics among the three sample groups, with 200 Hz standing out as the optimal frequency at which both amplitude and phase differences between tissue states were most clearly observed.

The fact that spoiled meat soaked in $Na_2S_2O_5$ exhibited a distinctly different impedance profile from fresh meat despite their similar appearance demonstrates that EIS can detect chemical adulteration agents that conventional sensory evaluation methods may fail to identify. These findings clarify the impact of $Na_2S_2O_5$ on the bioelectrical properties of tissue and affirm the potential of EIS as a rapid, objective, and practical method for food quality monitoring.

In the future, expanding the study to include various meat types, incorporating statistical analyses, and applying machine learning algorithms could further enhance the accuracy and real-world applicability of EIS-based systems for large-scale detection of chemically treated meat. In addition, machine learning approaches could be employed to automatically classify impedance patterns instead of relying on visual observation.

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