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Analysis of Clean and Rotten Squid When Soaked in Hydrogen Peroxide by Using the Impedance Measurement Method

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Abstract

This study presents an analysis of clean and rotten squid samples using the impedance measurement method when soaked in hydrogen peroxide (H_2O_2). The primary objective is to explore the differences in the electrical properties of squid tissue under varying conditions to assess freshness. The research utilizes impedance spectroscopy to monitor the changes in tissue conductivity and permittivity. Clean squid tissues exhibited stable impedance behavior, reflecting intact cellular structures, whereas rotten samples showed marked deviations, likely due to protein denaturation, cell membrane breakdown, and increased ionic activity from decomposition. Portable and automated devices integrated with IoT can be developed for real-time food quality monitoring. The method has potential applications in assessing the freshness of various foods like fish, shrimp, meat, and vegetables. Standardizing this method would support global food quality management and safety. Additionally, optimizing the technology for environmental friendliness and resource efficiency can make it more sustainable.

Keywords: Hydrogen peroxide, impedance, measurement, squid quality.

1. Introduction

In recent years, food safety has become a top concern globally, especially in developing countries like Vietnam. Population growth and abundant seafood resources, increasing demand for food, and a lack of strict control in production, processing, and distribution have led to an increase in contaminated food in the market. This has led to consequences affecting the health of consumers and also undermined confidence in the domestic food supply chain. Vietnam, a coastal country with a long coastline, has an extremely rich source of seafood. Of which, squid is the most popular seafood because of its unique flavor, high nutritional value, and ability to be processed into many dishes. With the sharp increase in consumption demand, the issue of squid quality, especially the distinction between clean and rotten squid, has become a major concern for both consumers and the seafood industry. Therefore, one of the recent difficulties is the use and trade of rotten squid, a type of food that has decomposed or rotted but is still bought and sold by traders after undergoing "recycling" measures such as bleaching, dyeing, or soaking in chemicals (especially hydrogen peroxide [1]). Quality assurance not only plays an important role in protecting public health but also affects transparency and sustainability in the food supply chain.

For respiration, during the decomposition process, "rotten" squid can release compounds such as hydrogen sulfide (H_2S), ammonia (NH_3), or other gases with unpleasant odors. When inhaled for a long time or in high concentrations, these substances can cause: respiratory irritation, headache, nausea, dizziness, and at high concentrations, H_2S can cause suffocation or serious lung damage [2].

For digestion, if you swallow or eat food contaminated with rotten ink and chemicals, causes digestive poisoning, such as nausea, abdominal pain, diarrhea, and some toxic chemicals can also cause liver and kidney damage if accumulated for a long time [3]. In the long term, if rotten squid and cleaning chemicals are used improperly and without safety protection, it will lead to accumulation of toxins in the body, increasing the risk of chronic diseases. Exposure to hazardous chemicals, including formaldehyde and strong oxidizing agents, is recognized as an important environmental risk factor for cancer. Prolonged or repeated exposure to these substances may induce cellular damage, promote genetic mutations, and consequently increase the risk of cancer development [4].

In this context, a controversial issue that has emerged is the use of chemicals. Clean squid is often compared to dirty squid by sensory means, for example, by appearance and color: Fresh squid is usually clear white or slightly pink, the skin is not peeling, and the eyes are bright. Rotten squid is usually opaque gray; the surface is slimy and shows signs of spoilage.

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Sandra Zavadlav *et al.* [5] investigates the use of bioelectrical impedance analysis as a non-destructive method to determine the freshness and quality of European squid during chilled storage. Researchers monitored Loligo vulgaris samples over an 11-day period, measuring electrical parameters alongside traditional indicators such as pH levels, color changes, and nitrogen content. The findings suggest that measuring the phase angle at 5 MHz provides a highly accurate correlation with the length of storage, effectively categorizing samples by their state of decay. Monika Wawerla *et al.* [6] employed impedance microbiology as a rapid method that enables both qualitative and quantitative detection of microorganisms through measurements of changes in electrical conductivity.

In this context, a relevant consideration concerns the use of chemical agents in freshness assessment. The condition of squid is conventionally evaluated through sensory methods, including visual appearance and coloration: fresh squid typically exhibits a clear white or slightly pink coloration, intact skin, and clear eyes, whereas spoiled squid is characterized by an opaque gray appearance, a slimy surface texture, and visible signs of deterioration.

Sandra Zavadlav *et al.* [5] used electrical impedance spectroscopy to determine electrode impedance using an Agilent 4294A precision impedance analyzer controlled by TCP/IP protocols (Santa Clara, CA, USA). A custom- designed needle-type multi-electrode array consisting of two rows with six parallel, electrically connected, gold- plated needles was connected to the Agilent 16047E test fixture to provide a bipolar measurement set-up.

L. Narasimha Murthy *et al.* [8] have shown that careful handling of fish and shellfish on board is extremely important to maintain freshness. Mud ice was used for storing fish on board, which allowed rapid heat transfer as well as minimizing product deterioration caused by conventional ice. Samples stored at different time intervals (7th and 11th days) were collected and analyzed for their biochemical, textural, and microbiological quality characteristics. The authors determined the squid using the AOAC method. That is, the pH of the homogenate was determined using a glass electrode digital pH meter [7].

K. R. Remyakumari *et al.* studied the biochemical and nutritional composition of Indian squid. The approximate composition of Uroteuthis duvauceli showed 80.47% moisture, 17.5% protein, 0.52% fat, and 1.13% ash. Amino acid analysis showed higher content of glutamine followed by aspartine, tryptophan, leucine, alanine, and glycine [9].

In the study by Nguyen Phan Kien *et al.* [10], it was demonstrated that Electrochemical Impedance Spectroscopy (EIS) is also an effective method for distinguishing clean meat from contaminated meat or meat adulterated with chemicals. Therefore, in this study, we aim to utilize this method for differentiating spoiled squid treated with chemicals, with the goal of developing a system capable of assessing the quality of squid, similar to previous studies conducted on meat.

Currently, the issue of using hydrogen peroxide, for the treatment and cleaning of squid. Hydrogen peroxide is known for its whitening and antibacterial properties, but its use in food, especially in treating rotten squid, raises many questions related to food safety and business ethics. Many countries have strict regulations on the use of hydrogen peroxide in the food industry, but abuse still exists in some places, raising great concerns in the community.

Among the modern methods, we choose impedance analysis to measure the quality of squid. This method is based on measuring the electrical properties of squid samples, thereby detecting the difference between clean squid and rotten squid based on the level of protein decomposition or the presence of unusual chemical compounds (eg, Hydrogen peroxide). With the ability to be fast, accurate, cheaper, and non-destructive, impedance analysis is increasingly widely used in food quality research and control.

This article will focus on analyzing the current methods of measuring clean squid and rotten squid in the world, emphasizing the challenges associated with the use of hydrogen peroxide in cleaning squid, and evaluating the potential of impedance analysis technology as an effective solution to control squid quality scientifically and sustainably.

2. Materials and methods

2.1. Electrical Properties of Squid

The Electrical Fricke Model is an extension of the Fricke model used to describe the electrical propertiesof tissues. Originally, the Fricke model, named after physicist Hugo Fricke, was developed to understand the dielectric behavior of biological cells. The model considers cells as spherical structures consisting of a conductive interior (cytoplasm) surrounded by a resistive and capacitive membrane within an external conductive medium (extracellular fluid).

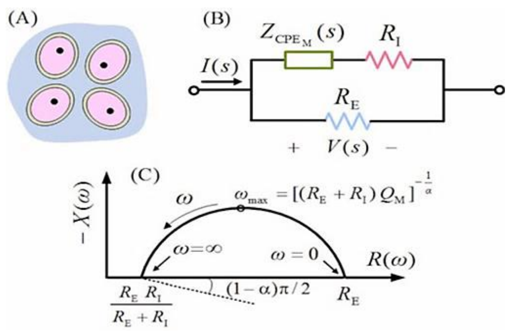

Fig. 1. The fractional-order Fricke–Morse EEC (B) representing the electrical bioimpedance of the biological tissue (A) and the impedance Nyquist plot (C)

Fig. 1 illustrates the fractional-order Fricke–Morse equivalent electrical circuit [10]. After consulting some studies on meat impedance measurement, in these researches, Fig. 2 replaces aresistor R1 with a capacitor to approximate the value of the data we collected in practice. This demonstratesthat the Fricke model can be combined with a Cole- Cole model to study ageing in meat, which is ananisotropic medium [11, 12].

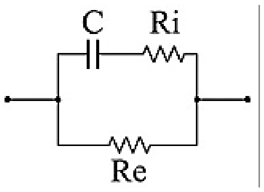

Fig. 2. The Cole-Cole model combines with Fricke model

2.2. Circuit Design

Fig. 2 shows the experiment setup to measure the impedance. This is a simple negative amplifier circuit. In which the value of resistance R_{in} is known, V_{in} is a voltage source.

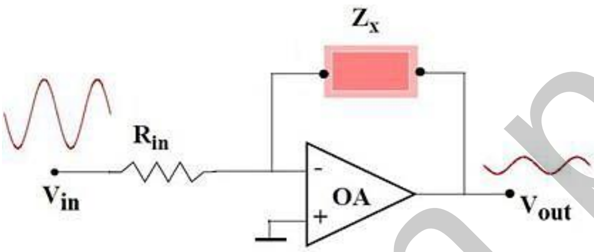

Fig. 3. Designed circuit to measure the electrical impedance

By measuring the output voltage V_{out}, we can interpret the impedance Z_x by the following equation:

$$Z_X = -\frac{V_{out} \times R_{in}}{V_{in}} \quad (2)$$

In which the input impedance (R_{in} = 1000) and the impedance of the squid are not pure resistances, but they also have complex values depending on the frequency (capacitive reactance, inductive reactance). The purpose of the experiment is to measure the complex electrical impedance of the squid. That means we want to find the relationship between impedance and frequency along with time: Z_x=K(ω).

3. Experiment Results and Discussion

3.1. Process

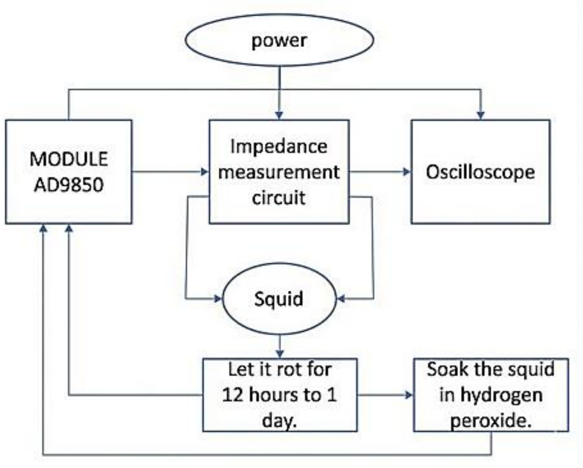

Fig. 4. Experimental process

The impedance measurement circuit designed to distinguish between fresh and spoiled squid is based on an inverting amplifier configuration. An AD9850 module is employed to generate the excitation signal, such as a sinusoidal waveform, which is applied to the squid sample. The resulting signal is then processed by the impedance measurement circuit for further analysis.

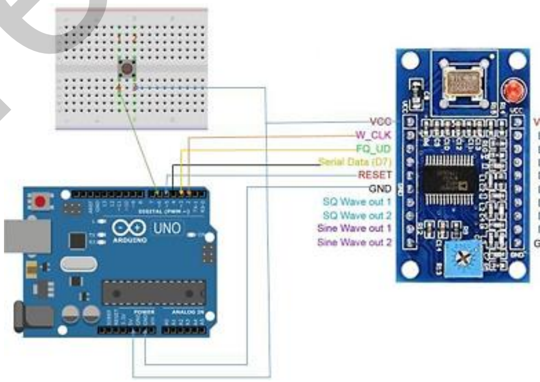

Fig. 5. Module AD9850

After the AD9850 module is connected to the impedance measurement circuit (IC LM741). This circuit is used to measure the impedance of the squid sample. The squid sample is placed at a distance of 2.5 cm for the 2 electrodes of the clamp to perform the measurement and we perform the measurement experiment at a position about 2.5 cm from the head with the upper left side of the squid. We perform the experiment through three measurements, which is when the fresh squid is the squid we bought at a frozenseafood restaurant, then brought it home to defrost for 1-2 hours and then performed the measurement. Whilemeasuring, to see the difference, we will connect the circuit to the output of the Oscilloscope, which helps us to process and analyze the signal (Impedance, phase shift or time delay...). Then, we let the sample rot from1 to 2 days in a food box until it smells rotten. This is the step to change the chemical properties of the sample because then the cells in the sample will gradually die, leading to a decrease in the impedance of the sample (the phase shift is different). Then it willbe easy to see the difference between fresh squid and rotten squid. Finally, after measuring the rotten squid,we use Hydrogen Peroxide (50%) to bleach the squid, it is soaked in hydrogen peroxide for about 3 - 5 minutes in the food box then rinsed with cold water and continue to measure again in the same position as when measuring the marked fresh squid. Hydrogen Peroxide helps the squid remove the bad smell and then the impedance of the squid increases compared tothe rotten squid but is still smaller than the fresh squid. Our entire system is powered by a ±9V power source(2 electrodes) to create a source for the circuit to run stably.

3.2. Electrode Clamp

The system consists of a clamp with two stainless poles taken from the negative feedback position in the acoustic amplifier circuit in Fig. 4. Those electrodes are in contact with the squid at a fixed distance of 2 poles (2.5 cm). The input signal is a sine wave voltage, taken from the AD9850 module. When we fix the input impedance (R in), the frequencies are changed to different values during the measurement to calculate the impedance of the squid. The output is connected to an oscilloscope to measure the output signal. Based on the measurement circuit, we can calculate the value of K depending on time and frequency.

About the squid clamping device, we chose a clamp that can be easily stretched and is wide enough to firmly clamp the squid body. To increase convenience and efficiency, we also installed a handle made of mica sheet, creating a stand that makes clamping the squid easier and safer. In particular, this mica sheet not only supports the grip but also helps to position the squid stably during the process.

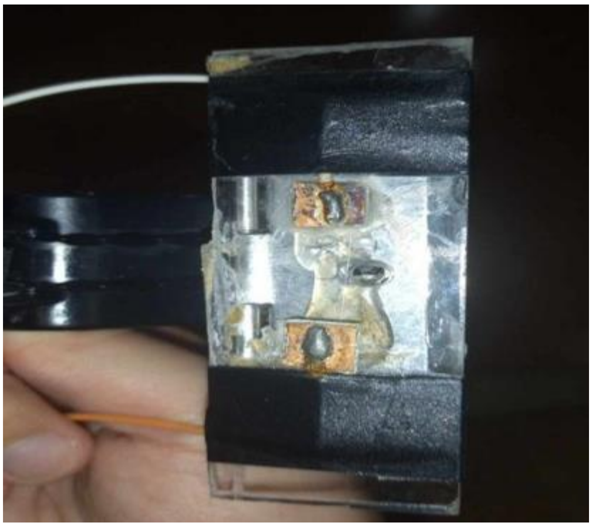

Fig. 6a. Electrod

Fig. 6b. Electrode clamp

For the electrodes, we used two small copper pieces, firmly attached to the mica sheet. Then, we soldered the conductive wires to the copper piece andconnected it to the power source, forming a resistancesystem that can directly affect the squid. This system helps to measure the resistance of the squid easily.

In addition, to ensure flexibility and easy adjustment, we used additional hinges, attached to themica sheet and two electrodes. This allows the electrode to move flexibly and be easily adjusted, so that it can clamp and handle ink of different sizes, from small to large squid. All of these parts work together to optimize the ink clamping process, creatingconvenience and efficiency in work.

3.3. Experiment and Results

Fig. 7a. Fresh squid

Fig. 7b. Fresh squid

Fig. 7c. Rotten squid

Fig. 7d. Rotten squid

Fig. 7e. Squid after using hydrogen peroxide

Fig. 7f. Squid after using hydrogen peroxide

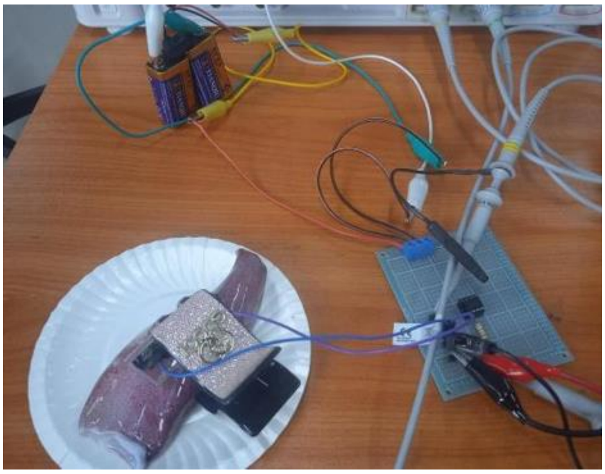

Fig. 8. Preliminary circuit

Fresh squid specimens were procured from a local seafood market and prepared by soaking in water, followed by air-drying prior to measurement. Impedance measurements were conducted at defined time intervals–specifically at 24 and 48 hours–to evaluate the temporal variation in impedance characteristics of the samples stored under ambient conditions. In parallel, a separate set of measurements was performed on squid specimens maintained under refrigerated conditions at equivalent time intervals of 1, 2, and 3 days. Throughout all measurements, the input signal amplitude was held constant while the excitation frequency was systematically varied over the range of 100 Hz to 20 kHz.

As shown in Fig. 7a and Fig. 7b, the fresh sample (FS) exhibited a characteristic pink coloration upon initial preparation. Following approximately 24 to 30 hours of storage under ambient conditions, the spoiled sample (SS) displayed visible signs of deterioration, including color fading and the appearance of white discoloration patches, accompanied by a pronounced malodor, as illustrated in Fig. 7c and Fig. 7d. The spoiled sample was subsequently immersed in a hydrogen peroxide solution to neutralize the malodor, yielding the hydrogen peroxide-treated sample (HTS). As observed in Fig. 7e and Fig. 7f, the treatment resulted in the formation of extensive white patches on the skin surface, attributable to the degradation of skin cells induced by the oxidative effects of hydrogen peroxide.

The experiment results are shown in figures below.

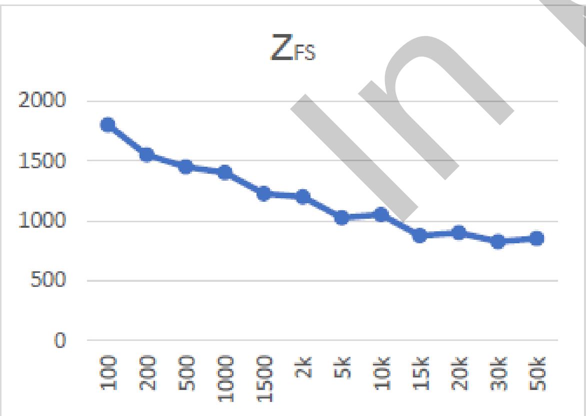

Fig. 9. Impedance of squid to frequency when freshly thawed

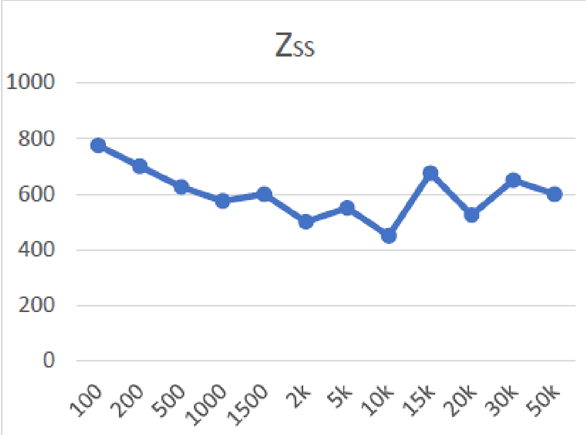

Fig. 10. Impedance of squid to frequency when left to rot after 1 day

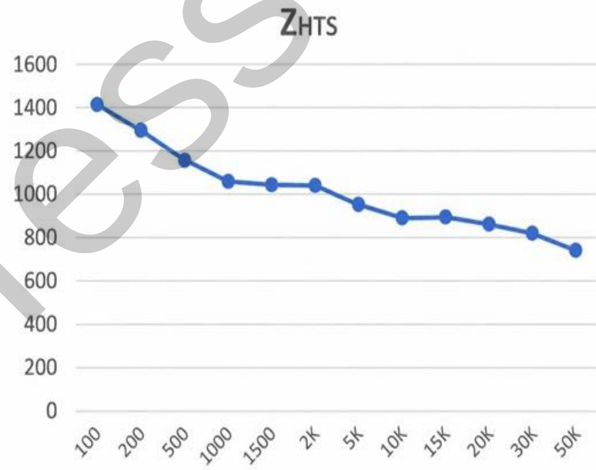

Fig. 11. Impedance of squid to frequency after using Hydrogen Peroxide

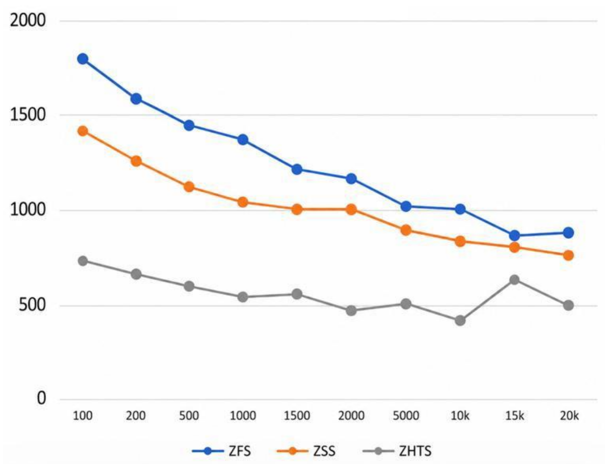

Fig. 12a. 1st squid impedance measurement result

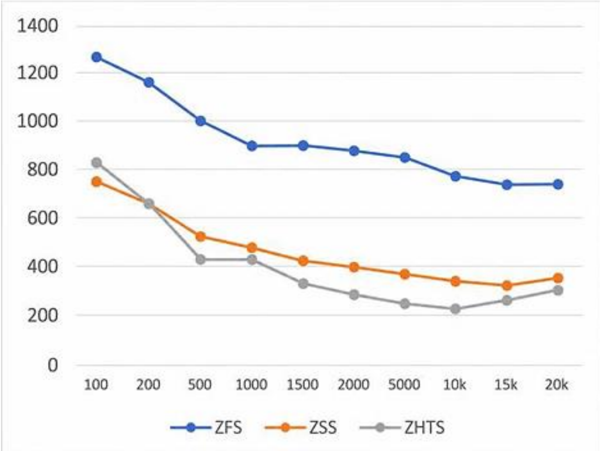

Fig. 12b. 2nd squid impedance measurement result

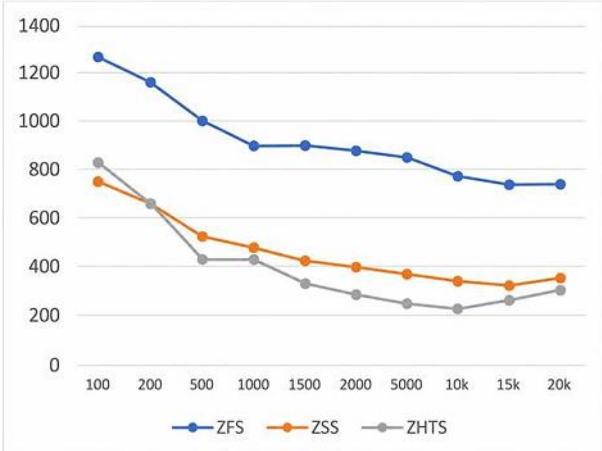

Fig. 12e. 5th squid impedance measurement result

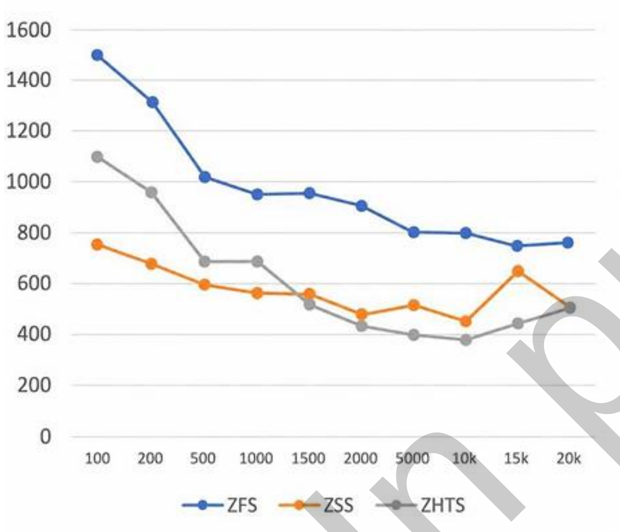

Fig 12c. 3rd squid impedance measurement result

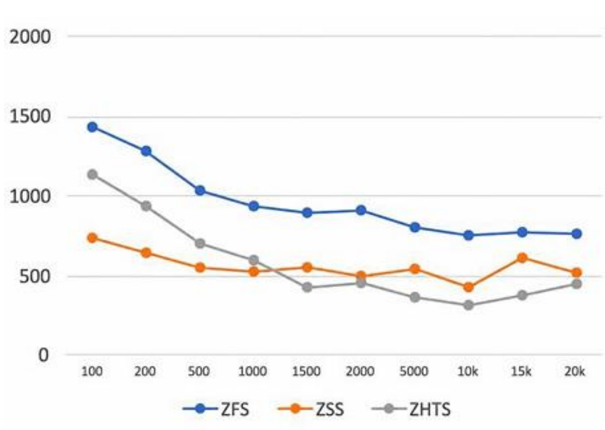

Fig. 12d. 4th squid impedance measurement result

Fig. 12a to Fig. 12e show the impedance of the squid at different frequencies f = 100 Hz, 200 Hz, 5000 Hz, 1000 Hz, 2000 Hz, 5000 Hz, 10 KHz 15kHz, 20kHz, 30kHz and 50kHz. We can see that after the squid is thawed and we leave it outside for a day, the impedance decreases. This phenomenon can be attributed to the structural degradation of squid cells over time. As the cell membranes deteriorate, the resistive components associated with both the intracellular and extracellular regions – denoted as Re and Ri, respectively – are reduced, resulting in a decrease in the overall impedance of the tissue. As illustrated in Fig. 11, the application of hydrogen peroxide as a cleaning agent partially restored the impedance of the squid sample; however, the measured values remained significantly lower than those obtained immediately following the thawing process.

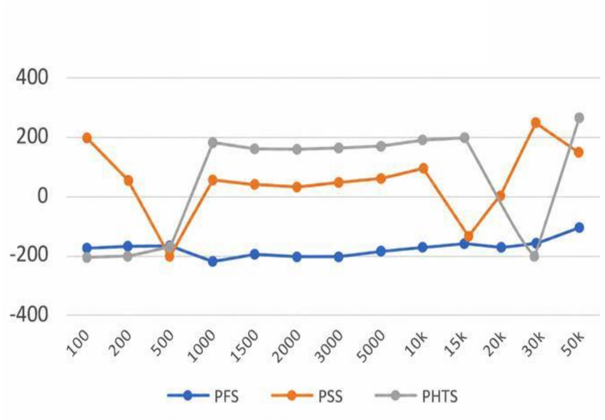

Fig. 13a. 1st squid phase spectrum measurement results

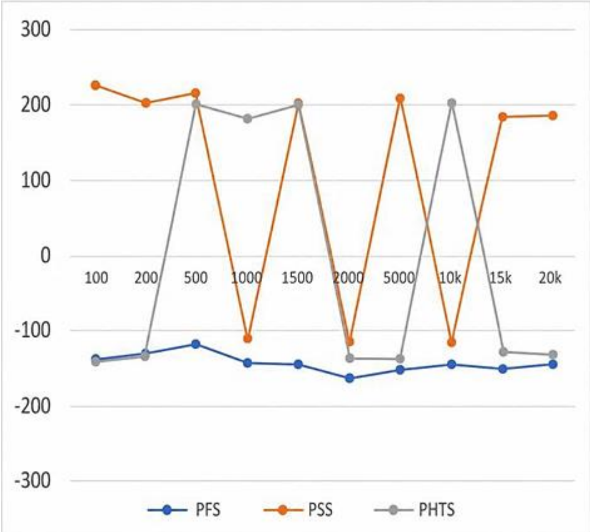

Fig. 13b. 2nd squid phase spectrum measurement results

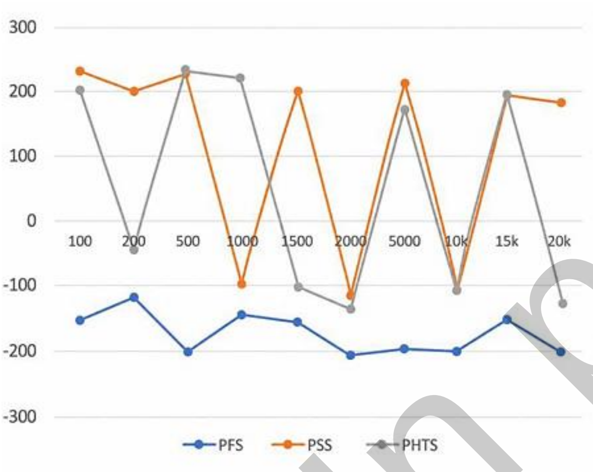

Fig. 13c. 3rd squid phase spectrum measurement results

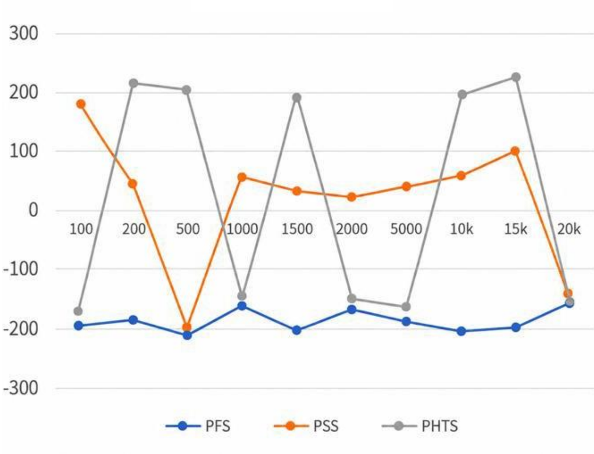

Fig. 13d. 4th squid phase spectrum measurement results

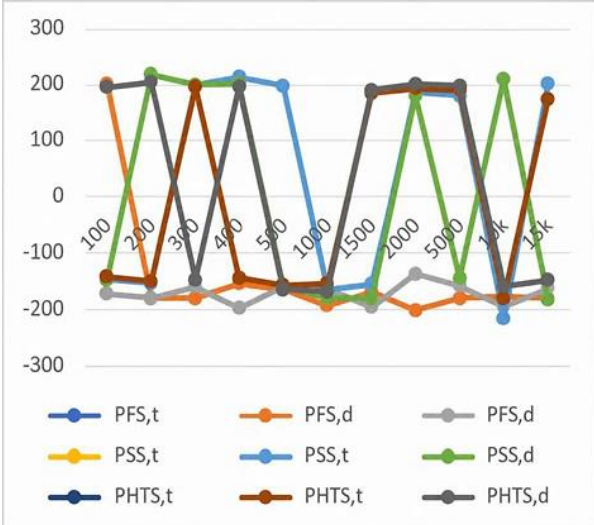

Fig. 13e. 5th squid phase spectrum measurement results

Fig. 13a to Fig. 13e show the phase spectrum of three squid measurements during the process. From the graphs, we can clearly see that when the squid is fresh, the phase spectrum will always be a negative line, but when measuring the phase spectrum of rotten squid and squid after soaking in hydrogen peroxide, the graph will often change unevenly.

A clear comparison of the squid's condition across three experimental stages can be established: immediately after thawing, following one day of post-thaw storage, and after treatment with hydrogen peroxide subsequent to one day of storage. The results indicate that impedance measurements in the low-frequency range of 100–500 Hz provide a reliable and distinguishable parameter for differentiating between fresh and spoiled squid samples.

4. Conclusion and Discussion

This study demonstrated the effectiveness of using impedance measurement methods to distinguish between clean and rotten squid soaked in hydrogen peroxide. The results revealed significant differences in the electrical properties of the samples, reflecting the chemical and structural changes associated with the soaking process and varying freshness levels. The findings highlight the potential of impedance spectroscopy as a non–destructive, rapid, and accurate technique for evaluating seafood quality and detecting adulteration. Future research could further refine this approach, integrating it with advanced data analysis techniques and exploring its application across other seafood types to ensure broader applicability in quality assurance processes.

Despite the promising initial findings, this study has several limitations that should be addressed in future research. First, the preliminary nature of the experiment resulted in a limited sample size, which constrains the evaluation of repeatability and reliability. Consequently, the current impedance analysis lacks deep quantitative statistical validation–such as specific thresholds, standard deviations, and *p*-values–making the findings primarily qualitative. Furthermore, the interpretation of the phase spectrum remains preliminary; the observed variations have not yet been fully correlated with the underlying biophysical and cellular mechanisms. Future studies will focus on expanding the sample size to conduct rigorous quantitative analyses and deeply investigate the physical mechanisms driving these bioimpedance changes.

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