Novel method for distinguishing the fresh meat and the meat treated by KNO_3 by using automatic measurement of bioimpedance analysis

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ABSTRACT

Pork meat that treated by KNO_3 looks like fresh one that human eyes cannot classify in the real life. The consumption of spoiled meat that treated by KNO_3 will make a higher chance to get cancer in human body because of the changing from Nitrate to Nitrite inside the body. The automatic impedance measurement of meat has been developed and showed that this system can detect the existing of KNO_3 inside the meat. In the system, pork meat is considered an electronics circuit that approved by two models: Fricke and Cole-Cole. By using an inverting amplifier with the piece of meat located at the feedback part of the circuit, the sinusoidal signal generated by the AD9850 module will be passed through the circuit. The signal received from the output of AD9850 will be sent to the oscilloscope and uploaded to the computer, and automatically change the frequency range of the generated signal to investigate the complex impedance of the meat in the frequency range from 10 Hz to 1 MHz. The obtained results will be analyzed by Levenberg-Marquart algorithm, showing that the distinction of fresh meat and meat that has been treated with KNO_3 can be determined by the phase-frequency spectrum and amplitude of impedance of meat at low frequency (100 Hz). In the future, the system will be upgraded and apply for the real life.

Keywords: Pork meat; KNO_3; Bioimpedance measurent; Fresh meat; Spoiled meat.

1. INTRODUCTION

Many methods have been developed to evaluate the freshness of meat in order to differentiate fresh meat from the out-of-date one. The relative permittivity spectra of sample [1] can be obtain by using a Magnetic Induction Spectroscopy system, or using fibre optic probes [2, 3].

The dielectrical properties of meat depend on the state of the cell membranes, the molecular composition, the presence of ions, electrical charges on proteins and pH variations. The electromagnetic waves used to measure the impedance of meat can be in the range from low frequency [4], high frequency, microwaves [5], nuclear magnetic resonance [6], infrared [7, 8], UV light [9], X-ray [10] to DNA technology, including real-time polymerase chain reaction (RT-PCR) and neutral comet assay [11].

However, most of these techniques have significant dis-advantages such as their time consuming, high costs and being destructive to sample. There is clearly a need for an effective, rapid, portable, cost-effective and easy to use method to differentiate such products. Recently, the use of impedance technology has become of interest in the meat processing industry. Bioelectrical impedance analysis is an inexpensive and rapid method for estimating body composition, including fat, muscle, and bone tissues in the human body. The measurement of tissue electrical properties [12] can reveal the quality of meat because tissue has certain electrical characteristics such as impedance and conductivity, which change according to time of post-mortem.

Besides that in the flee market, pork meat is now widely sold in the outdoor and in unpreservation and unprotection conditions. End-of-days, the porcine meat that doesn't sell out will smell. To handle this problem, most sellers often use KNO_3 to handle it. Residual KNO_3 with high concentration when bring into the human body will be one of the causes of cancer [13].

Therefore, in this study, an novel method has been upgraded to distinguish the fresh meat and the meat that treated by KNO_3. The first research [14] to solve this problem is using a functional generator GoldStar FG2002C to create a sin wave then send to measurement circuit. Its frequency is changed to different values during the measurement to see the responsibility of meat impedance at each value. The output is connected to the oscilloscope to measure the output signal. Based on the circuit, the value of amplifier factor K is depended on the time and the frequency. As the received results, it shows that it is possibly distinguish between fresh meat and spoiled meat through impedance changing based on frequency and time. Because of the positive results, the measurement is upgraded to version 2 [15] that automatically measure and can analyze the changing of amplitude-frequency spectrum and phase-frequency spectrum of the meat and then use to do experiment with fresh meat and spoiled meat that treated by KNO_3. In this research, an upgraded system will be use to do experiments with a fresh meat and meat treated by KNO_3 to find out the key parameters for the distinguishes.

2. CONSIDERATION SYSTEM

2.1. Theory and principle of measurement

2.1.1. Tissue Biological Impedance Model

The physicochemical properties of biological tissues depend on their constituents, of which the three most concerned are intracellular fluids (ICF), extracellular fluids (ECF) and cell membranes (Cm). Fricke et al. demonstrated the electrical equivalent of a biological tissue [16]. This model is shown in Fig. 1.

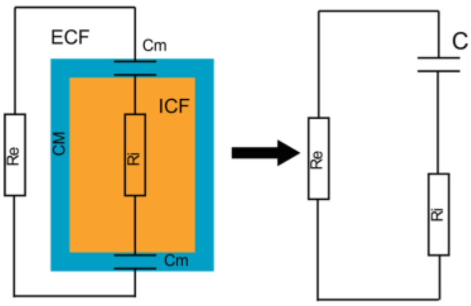

Figure 1. *Fricke equivalent impedance model.*

Based on Fig. 1, the complex impedance of a tissue is calculated using Eq. (1).

$$Z = Re(Z) + jIm(Z) = |Z|ej\theta \quad (1)$$

In which, Re(Z) and Im(Z) are real and imaginary parts, |Z| is the amplitude of the impedance, h is the phase displacement angle. Parameters Re, Ri and Cm are the typical parameters to evaluate the tissue structure electrically.

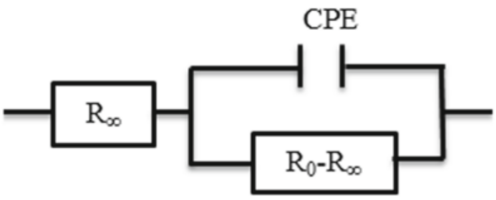

Figure 2. *Cole-Cole impedance model.*

In addition to the Fricke model, Cole-Cole is a simple model and is widely used for the description of the electrochemical properties of biological [17, 18]. The model consists of three components: impedance at very low frequency (characteristic of the extracellular medium), impedance at high frequency (representing both intracellular and extracellular media) and a constant component phase (CPE - Constant Phase Element); is shown in Fig. 2.

Equations of the Cole-Cole model are shown in the equation (2).

$$Z^* = R_\infty + \frac{R_0 - R_\infty}{1+(iw\tau)^\alpha} = Z' + i.Z'' \tag{2}$$

In which:

- R0 and R∞ are the impedances at low frequencies and high frequencies in the Cole-Cole model.
- τ is a characteristic time constant
- α is within (0,1), related to the heterogeneity of cell size and morphology of living
- tissue.
- Z* is the complex impedance, Z' is the real part and Z" is the virtual part.

The characteristic parameters for the research object (R0, R∞, s and a) can be found when "matching" experimental data to Eq. 2. Based on these parameters, it is possible to monitor, evaluate any changes in biological tissue.

2.1.2. Measurement Principle

The inverting amplifier circuit is used to measure the impedance of meat, where the test sample replaces the OpAmp feedback resistance as in Fig. 3.

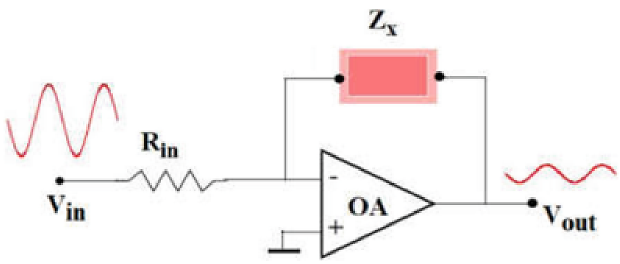

Figure 3. *Principle of measurement.*

The input signal is a sine wave source with a fixed amplitude and a fixed frequency range. The output is a sinusoidal signal that has been phase inverted in comparison to the input signal. This principle was described in detail in the study [19].

2.1.3. System Design

The block diagram of the measuring system is shown in the Fig. 4 and also describe in detail in the study [18].

The signal from the oscillator will be received at channel 1 of the Oscilloscope, the controller generates the control signal, providing the power for amplifier 1 to operate, along with the controller to transmit the signal to connect the output to the channel 2 of the Oscilloscope. After the frequency sweep from 10 Hz to 1 MHz is terminated at amplifier 1, the controller sends a signal to amplifier 2 to begin measuring. The data acquisition process was similar to that in study [15, 19]. Every hour the measurement is repeated, this is done for 24 h.

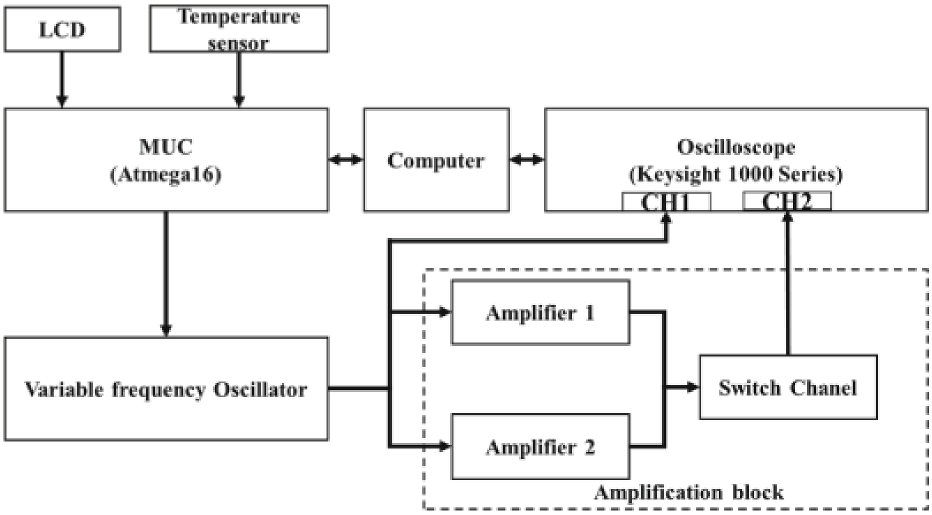

Figure 4. *Block diagram of measurement system.*

Raw data after the measurement is processed and obtained results include phase and impedance components. After that, data were matched into models by CNLS Fitting [20] method on EIS Spectrum Analyzer software. The data matching process is described in the study [15].

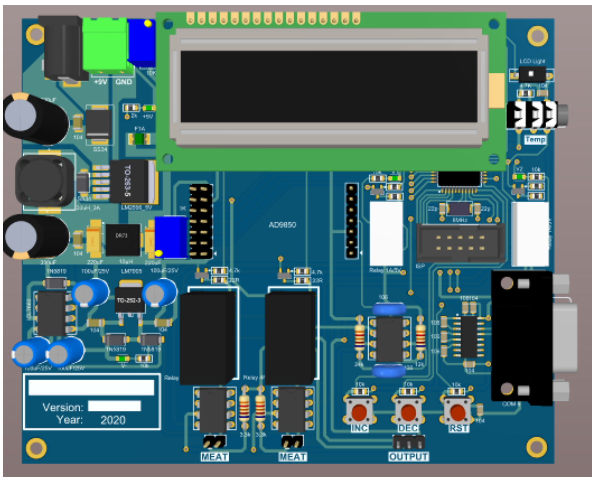

Figure 5. *The final measurement circuit.*

3. EXPERIMENTS

Meat samples during the measurement were purchased at supermarket. The meat sample belongs to the type of tenderloin without the fat. Sample of meat will be cut into 2 small samples of equal size (5 cm long, 3 cm wide, 2.5 cm high). If two samples of freshly cut meat are considered to be rectangular, the longitudinal grain of the meat will be on the 2 small sides (width, height) of the rectangle.The electrode used in the measurement is made from a pair of medical sewing needles. -stainless steel (type 4B-3, size 0.9x36 mm, Czech standard CSN 85 59 36). They are initially curved, then straightened provided there are no scratches during bending. Two parallel needles, separated by a distance of 2 cm, were attached to a transparent mica plate as the electrode base with a length of 2,3 cm inserted into the meat sample as shown in figure 6.

Figure 6. *Electrodes using for measurement the impedance of pork meat.*

After preparing the meat sample, it will be put in a mica box (shown in figure 7) with dimensions of 50 x 30 x 25 mm (length x width x height). It is also slightly pressed on the four corners of the meat sample so that the meat sample is evenly spread across the bottom four corners of the box. Plug the electrode into the box and fix the electrode by 2 pieces of tape to the box wall. Finally, 2 sample containers were placed in 2 plastic containers, covered with food wrap. In order to avoid the influence of insects and/or bacteria during measurement.

Figure 7. *Meat sample and covered for the experiment.*

To confirm the existance of KNO_3, the samples before measurement and after 24 hours were tested again with Diphenylamine ($(C_6H_5)_2NH$) in a cold and solid H_2SO_4. The reaction occurred to produce a compound Nitrosobenzene ($(C_6H_5)NO$) which is dark blue in solid state and light yellow in dillute state. The reaction is given by equation (3)

$$2(C_6H_5)_2NH + 2KNO_3 + H_2SO_4 \rightarrow K_2SO_4 + 2H_2O + 4(C_6H_5)NO \tag{3}$$

Based on the changing in the color of the reaction so we can confirm that the meat have KNO_3 or not.

The research has been done with 3 steps of experiment such as:

1. Calibration system: Test complex impedance by using real value of resistors, capacitors as model of Fricke and Cole-Cole that mentioned in the study [15] to confirm that the system is precision.
2. Measure the impedance of the fresh meat sample during 24 hours and get data every hours with the confirmation of existing KNO_3 described above.
3. Take out the measured meat, treated by KNO_3 then measure again to get data after that.

The raw data obtained after the measurement process will be processed through a program on the Matlab. This program will perform the impedance and phase calculation.

Experiments have been done over 100 meat samples and some of data shown in figure 8, 9.

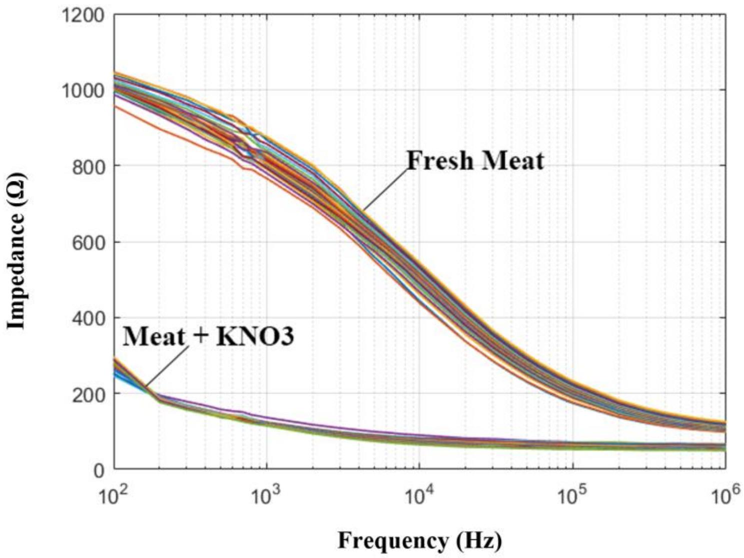

Figure 8. *Amplitude-frequency spectrum of fresh meat and meat treated with KNO_3 measured in 24 hours.*

As shown in figure 7, impedance amplitude of meat samples before and after treated by KNO_3 both decreased with increasing frequency. At 100 Hz, the impedance amplitude of the experimental samples is usually in the range of 500 Ω to 1200 Ω and decreases sharply after treated by KNO_3, the value is only in the range of 200 to 300 Ω. At 1 MHz, the impedance amplitude of the meat samples is in the range of 50 Ω to 150, the deviation is not significant for the samples before and after washing KNO_3.

Phase-frequency spectrum of the meat samples before treated by KNO_3 has 2 extreme points, the minimum point is in the frequency range 20 KHz – 60 KHz with a value in the range -0.8 rad to -0.6 rad and the maximum point is in the frequency range 100 Hz – 700 Hz with a value from -0.2 rad to -0.4 rad. The phase-frequency spectrum before and after treated by KNO_3 is completely different both in value at the frequencies as well as in the shape of the graph, especially in the frequency ranges of 100 Hz to 1 KHz and 10 KHz to 100 KHz. From there, we can distinguish pork before and after using KNO_3 solution based on the shape of the graph.

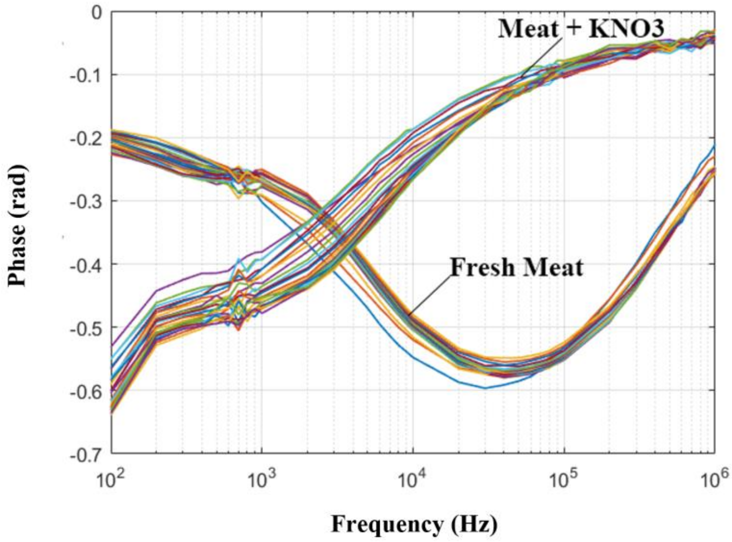

Figure 9. *Phase-frequency spectrum of fresh meat and meat treated with KNO_3 measured for 24 hours.*

4. CONCLUSIONS

In research [14], experiment with meat that treated by KNO_3 showed that customers cannot distinguish fresh meat and meat that treated by KNO_3 by normal eyes. The manual measurement shows the positive results for distinguish them. However, it is impossible to recognize quality of meat in the research. So, research [15] has been done to upgrade the system from the manual up to automatically measurement with capacity of analysis of phase-frequency spectrum and amplitude-frequency spectrum of the meat. The ugraded system can measure from 10 Hz to 1 MHz but to detect and distinguish the fresh meat and meat treated by KNO_3 have no signification at that frequency so data of this paper just focus on the range of frequency that useful to detect the different between two kind of meat only.

In conclusion, with the range of impedance of fresh meat at low frequency (100 Hz) is easy to distinguish the fresh one and meat treated by KNO_3 but not at high frequency (1 MHz). Besides, phase-frequency spectrum is shown much easier to distinguish between the fresh meat and meat treated by KNO_3 because of the shape of the plot. In the future, the system will be upgraded by building a server that can store a big data of measurement and can apply Artificial Intelligent (AI) technology to continue to find out important keys to assess quality of the meat.

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TÓM TẮT

Phương pháp mới phân biệt thịt tươi và thịt được xử lý bằng KNO_3 sử dụng phép đo phân tích trở kháng sinh học

Thịt lợn được xử lý bằng KNO_3 không thể phân biệt được bằng mắt thường. Việc sử dụng thịt hư hỏng đã qua xử lý bằng KNO_3 sẽ làm cho con người có nguy cơ mắc bệnh ung thư cao hơn do cơ thể chuyển từ Nitrat thành Nitrit ở bên trong cơ thể. Do đó, một hệ thống đo trở kháng tự động cho thịt lợn đã được phát triển. Kết quả thực nghiệm cho thấy rằng hệ thống này có thể phát hiện sự tồn tại của KNO_3 bên trong thịt. Trong thiết kế của hệ thống đo này, thịt lợn được coi là một mạch điện tử với các mô hình đã được chứng minh bởi Fricke và Cole-Cole. Khi đó, miếng thịt sẽ được đặt tại phần hồi tiếp âm trong mạch khuếch đại đảo. Tín hiệu hình sin tạo bởi mô-đun AD9850 sẽ được đưa vào đầu vào khuếc đại đảo. Tín hiệu đầu ra từ mạch khuếch đại đảo sẽ được đưa đến ô xi lô và truyền lên máy tính, đồng thời sau khi dữ liệu ghi lại thì máy tính sẽ tự động ra lệnh thay đổi dải tần của tín hiệu tạo ra để khảo sát trở kháng phức của thịt trong dải tần từ 10 Hz đến 1 MHz. Kết quả thu được sẽ được phân tích bằng thuật toán Levenberg-Marquart trên Matlab, cho thấy sự phân biệt giữa thịt tươi và thịt đã được xử lý bằng KNO_3 có thể được xác định bằng đồ thị phổ pha-tần số hay giá trị biên độ tại tần số thấp. Trong thời gian tới, hệ thống sẽ được nâng cấp và áp dụng cho đời thực.

Từ khoá: Thịt lợn; KNO_3; Đo trở kháng sinh học; Thịt tươi; Thịt hư.