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Exploratory Analysis of Age-Related Changes in Beef: Bioimpedance and Chemical Composition Perspectives

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Abstract

Bioimpedance-based technologies have found significant utility in categorizing various food types, such as meats, fruits, and beverages. As biological samples, such as chicken, fruits, and vegetables, lose freshness after preservation, their internal structures transform due to distinct biological reactions, resulting in alterations to their internal properties and bonds. This work aimed to study beef's electrical behavior and chemical composition to identify significant variations that occur during the aging process. The transfer impedance values of beef were measured over a frequency range of 100Hz to 100kHz, placing electrodes on the freshly excised beef samples that were frozen for a particular period. Chemical composition analysis, which is known as proximate analysis, was performed in the meantime. Consequently, any changes in the internal properties of the beef sample affect its bioimpedance. It has been seen that over time, among various compositions, the percentage of protein, ash, and moisture is reduced along with bioimpedance, and the rest of the composition, like fat and carbohydrates, is gradually increased. A relation between the bioimpedance and the chemical components of the tissue samples has been predicted. The final relation ignores the dependency of impedance over time on moisture, ash, and fat, as the value of these components changes within a very small scale over time.

Keywords: Bioimpedance, Food freshness, FIM, Food preservation, Proximate analysis

I. Introduction

Cells are the fundamental structural and functional units of biological tissues, which are enclosed by a phospholipid bilayer that acts as an electrical insulator and is covered by semipermeable membranes. In contrast, the ICF and ECF of cells facilitate electrical conduction. Bioimpedance is the term for the phenomenon whereby the resistance and capacitance produced by these components within biological tissues together prevent the flow of electric current[1]. Bioimpedance (Z) is composed of two components: resistance (the real part) and phase (the imaginary part). When current passes through the ICF and ECF, which is an electrolytic solution, in that case, resistance arises, while the dielectric properties of tissues or the temporary accumulation of charges on cell membranes influence the phase. Scientific research has focused on the use of electrical bioimpedance techniques to monitor anatomical structures, physiological processes, and tissue features. Recent studies have highlighted the effectiveness of bioimpedance in characterizing the characteristics of various conditions, including skin cancer analysis[2], assessment of cervical cancer (Homola et al., 2019), characterization of breast cancer, and scrutiny of tongue cancer[3], etc.

With the use of bioimpedance techniques, it is now possible to characterize a variety of foods, including meat[4,5] and fruits[6]. Bioimpedance has been the subject of numerous studies, which have shown its wide range of uses in fields including food science and disease detection. Given that meat's mechanical characteristics vary with time, it makes sense to expect that fruits and vegetables will behave similarly. In this paper, the variation of bioimpedances in beef samples over time and at different frequencies has been examined, and chemical composition analysis, which is known as proximate analysis, was performed in the meantime for the same sample that was taken for bioimpedance measurement. By examining these modifications, important insights into the temporal variations in bioimpedance can be gained, along with a better understanding of how bioimpedance and the chemical composition of the beef sample change over time.

Ensuring the freshness of biological food samples is important for safeguarding consumer health. Fresh foods are less likely to harbor harmful bacteria, pathogens, or toxins that can cause foodborne illnesses. For food manufacturers and suppliers, monitoring freshness is essential to maintain the quality of their products. Fresh food items typically have higher nutrient content than their stale or expired counterparts. Monitoring freshness ensures that consumers receive the maximum nutritional benefits from the food they consume. This study was conducted to explore the utility of electrical bioimpedance analysis for monitoring the freshness of refrigerated biological food samples.

Age is one of the major determinants of beef quality and attributes, among other considerations. To ensure the best possible quality, taste, and nutritional value—all of which are critical for customer satisfaction and market success—it is imperative to understand how beef ages. This thesis investigates how the chemical composition and bioimpedance analysis of beef alter with age.

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It is not surprising that aging causes changes in the mechanical properties of meat. Refrigeration plays a vital role in preserving meat freshness, but its effect on the biochemical properties of beef, particularly with age, remains relatively unexplored. This study aimed to fill this gap by examining how age influences the bioimpedance and chemical composition of refrigerated beef samples.

Bioimpedance

Bioimpedance measurement is a technique used to evaluate the electrical characteristics of a tissue. All approaches that are based on the characterization of the passive electrical characteristics of biological tissue are referred to as bioimpedance measurements. This is accomplished by measuring the voltage potential that results from applying a steady, alternating current below the threshold of perception[7,8].

$$Impedance, Z = \frac{V}{I} \quad \dots\dots\dots (i)$$

$$Z = Impedance\ of\ conductor$$

$$V = Voltage\ across\ electrodes$$

$$I = Current\ between\ electrodes$$

There are several distinct benefits associated with the technology, including the fact that it is inexpensive, that it can be operated at low costs, that it requires only minimal operator training, that it provides continuous monitoring, and that it is rapidly implemented.

Frequency response of bioimpedance

The cell membrane exhibits its mysterious properties as a capacitor in the world of electrical modeling, while the intracellular and extracellular sections take on the roles of resistors. The famed Cole model, first presented by Cole and Cole in 1941[9,10], is a classic electrical model of biological tissue. In Fig. 1, R_e and R_i, the resistances in this model, stand in for the extracellular and intracellular regions, respectively, while C_m represents the capacitance of the membrane.

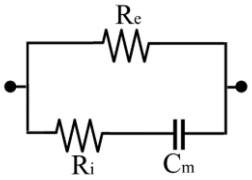


Fig. 1. Electrical equivalent model of biological tissues: Cole model[10]

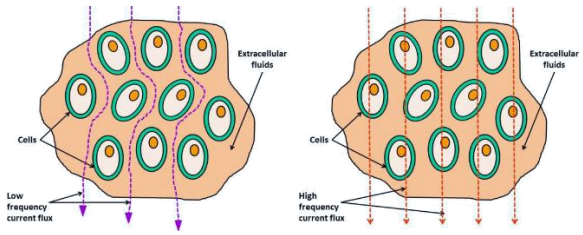


Fig. 2. Current paths through biological tissues both at low and high frequencies of the applied electric field[10]

An intriguing incident occurs at lower frequencies as the current struggles with the capacitive behavior of the cell membrane. The bulk of the current chooses to move via the extracellular spaces as a result. The cell membrane, on the other hand, shows increased permeability to electrical currents as it moves up the frequency spectrum. In Fig. 2, it becomes essential to make a major change to the Cole model to close the discrepancy between modeled impedance values and actual bioimpedance measurements. In particular, a Constant Phase Element (CPE), which was proposed by[11,12,13], is used in place of the model's typical capacitor.

This change is represented graphically in Fig. 3, which gives expression to the bioimpedance of any biological sample.

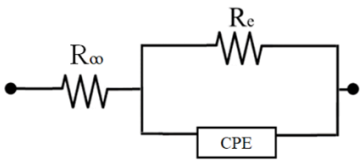


Fig. 3. Electrical equivalent circuit of biological tissue with three elements[11]

$$Z = R_\infty + \frac{\Delta R}{1 + (j2\pi\tau)^\infty} \quad \dots\dots\dots (ii)$$

$$\Delta R = R_0 - R_\infty \quad \dots\dots\dots (iii)$$

Here,

$f = Frequency\ of\ the\ applied\ current$

$\tau = Time\ constant, or\ RC$

$R_0 = Resistance\ at\ zero\ frequency$

$R_\infty = Resistance\ at\ infinite\ frequency$

And R (which indicates the difference between R_0 and R_∞, where R_0 denotes resistance at zero frequency and R_∞ denotes resistance at infinite frequency) is used in this equation. The constant phase element (CPE) notion is closely related to the parameter, which also plays a crucial role. This parameter, which has a value between 0.5 and 1[14], represents the system's intrinsic frequency-dependent capacitance.

A fundamental comprehension of these principles and their implications for physiological processes is essential to accurately interpret BIA results. To offer a straightforward and precise introduction to these notions, they are initially discussed in a broad framework from a physical standpoint. In the subsequent subsection, they will be connected to biological cells and tissues.

II. Background Studies

Various investigations have been carried out on bioimpedance. The 4-electrode Focused Impedance Method (FIM-4) emerges as a simplified method for focused impedance measurements since it only calls for the use of four electrodes[14]. This method is based on a creative

arrangement: while measuring the potential difference across the opposite electrode pair, an electric current is delivered through an adjacent pair of electrodes. The impedance value obtained from this measured potential is designated as Z_1, and the process is then repeated with the setup rotated by 90 degrees. These different configurations are appropriately referred to as I_1, Z_1, and I_2, Z_2, respectively.

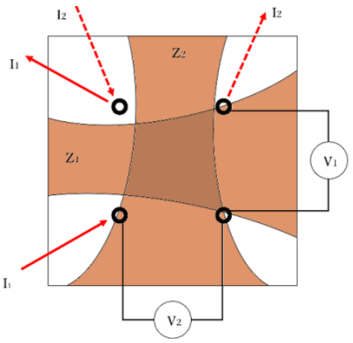


Fig. 4. Bioimpedance measurement technique based on 4-electrode Focused Impedance Method[15]

When the data from these two orthogonal impedance measurements, Z_1 and Z_2, are intelligently integrated, the FIM-4 technique's actual power is revealed. The sections in Fig. 4, which are divided by the proper equipotential lines, demonstrate how their accumulation $(Z_1 + Z_2)$ considerably intensifies sensitivity inside the center region of interest. This center region is given priority, demonstrating the power of the FIM-4 technique in identifying and emphasizing crucial areas of interest for precise impedance evaluation.

Chemical composition profile of beef

The chemical composition of beef is influenced by various factors, including the breed of the cattle, its diet, the cut of the meat, and how it is processed.

The following table presents a typical value of the chemical composition of beef samples in Dhaka City, based on research. The chemical composition of beef can vary due to several factors, including the diet of the animal, age, breed of the animal, **processing methods,** environmental factors, genetics, and preservation procedures. These factors collectively contribute to the variation in the chemical composition of beef, impacting its nutritional value and taste. The typical value of chemical composition, like moisture, fat, protein, and ash, is shown in Table 1[17].

Table 1. Chemical composition (%) of beef sample[17]

Nutrients	Mean (%)	Standard Deviation (SD)
Moisture content	75.56	0.55
Protein content	19.66	0.70
Fat content	3.72	0.38
Ash content	1.06	0.04

Due to the construction of a food composition database for Bangladesh, the chemical composition of beef has been analyzed by students of Dhaka University.

Bioimpedance spectroscopy, which uses electrical signals to evaluate food quality, presents a viable solution to the food industry's need for improved quality control systems. A novel bioimpedance sensor has shown encouraging initial results, but more research is required to validate its dependability and variety of applications[18,19].

Previous research has provided important insights into bioimpedance; however, it is unclear how bioimpedance will change over time for freshly removed tissue. To bridge this gap, the bioimpedance of freshly excised animal, vegetable, and fruit tissues at different frequencies across time is investigated in this work. By establishing a thorough correlation between bioimpedance and aging, our study hopes to offer a dependable way to keep an eye on how fresh these samples are.

In this study, the four-electrode-focused impedance method (FIM-4) was employed for conducting localized impedance measurements[20]. The FIM-4 technique involves the measurement of impedance by positioning current and voltage measurement electrodes in two perpendicular directions. Initially, an alternating current (I) is applied through a pair of electrodes (A, B), while the voltage(V) is concurrently measured across another pair of electrodes (C, D), as illustrated in Fig. 5. The impedance is then determined as the ratio of the measured voltage to the applied current.

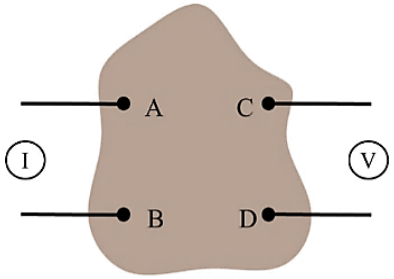


Fig. 5. Electrode placement for electrical impedance measurements from a biological sample[20]

The steps and processes that need to be followed for successful experimental results have been summarized in this chapter. Particular methods and analogies have been proposed at the start of the research, and a proper illustration of a proposed system leads to a successful research outcome. The steps that were precisely followed are: Preparation of test cell, placement of electrodes, collection, and preparation of tissue samples, tissue heating, and measurement of Bioimpedance. With the proper demonstration of these processes, the effects of temperature on bioimpedance were measured. The materials used in this research for impedance measurements are Sciospec ISX-5 (Germany), a Needle electrode, a Digital thermometer, and a Refrigerator. For proximate analysis, the materials used are BUCHI Scrubber B-414, BUCHI K-350 Distillation Unit, BUCHI Digest System K-437, Carbolite ELF, and a Refrigerator.

Preparation of electrodes

Cylindrical needle electrodes (Fig. 6) have been chosen to conduct the Focused Impedance Method.

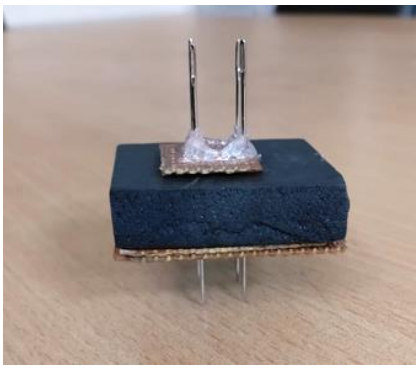


Fig. 6. FIM-4 needle electrode

Collection and Preparation of Tissue Samples

It has been used in a wide range of biological beef samples in the course of this research. These products were carefully chosen from the neighborhood market to guarantee their freshness and appropriateness to the experimental goals. It has taken the precautionary step of preserving these samples within a refrigerated unit, maintaining a constant temperature of 15°C, to preserve their integrity and to defy the natural degradation processes.

To preserve the biological materials' natural properties for research, this regulated environment within the refrigerator provided the critical function of slowing down the degradation of the materials. By maintaining this temperature, it has been possible to increase the shelf life of the samples and reduce any potential environmental changes.

However, it is important to remember that the actual experimentation phase was conducted in a distinct setting. The studies have been carried out at a constant temperature of 20°C to accurately mimic real-world situations or controlled conditions. This particular temperature setting was chosen because it supports the study goals and offers a consistent starting point for this investigation.

In conclusion, the method of sample preparation and collecting entailed carefully choosing a variety of biological samples from the local market and then storing them at 15°C to prevent decomposition. To be sure that the results were representative of the anticipated circumstances, the additional trials were conducted at 20°C.

III. Methodology

Impedance measurement

$Scale$: $Logarithmic\ Scale$
$Precision$: 0.01
$Electrode\ (Needle)$: 4
$Frequency$: $100Hz - 100KHz$
$Temperature$: $18 - 20°\ C$
$Duration$: 5 $days$

Measurements were performed using a bioimpedance analyzer (Sciospec ISX-5, Germany). Metallic electrodes made of copper were placed on the surface, touching the biological samples while measuring the impedance. But there may be an air gap between the surface electrode and the sample to avoid this consequence electrolyte gel was used between them.

Initially, impedance measurements were obtained at multiple frequencies on a specific biological sample (freshly excised) at room temperature (25°C). Subsequently, the sample was stored in a household refrigerator. To track the variation in impedance with storage time, measurements were repeated the following day at the same time and room temperature. This process was repeated daily, with samples being preserved in the refrigerator after each reading.

The measurements spanned six consecutive days for all biological samples under investigation. Bioimpedance measurements were conducted in the frequency range of 100Hz to 100kHz, with readings taken at 50 different frequencies in logarithmic increments. This comprehensive approach allowed for a detailed analysis of the impedance changes in the biological samples over the specified storage period. The experimental flow chart illustrating the sequential steps of this study is presented below in Fig. 7.

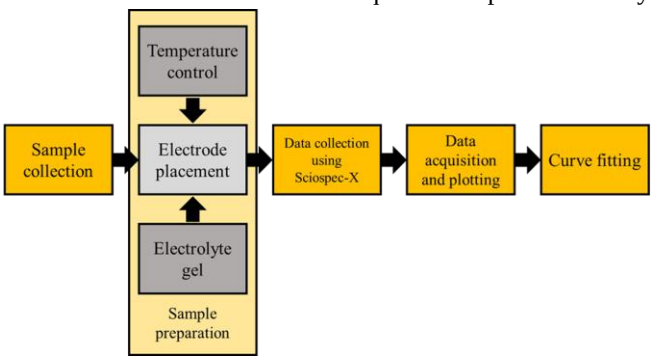


Fig. 7. Flow chart of the experimental procedure

Measurement of Bioimpedance

The bioimpedance is the ratio of measured voltage to injected current. For measuring the bioimpedance of tissue samples, Sciospce ISX-5 software and instruments were used.

The Sciospec ISX-5 is a sophisticated impedance spectroscopy device used for a variety of applications, including material characterization, bio-impedance measurements, and electrochemical analysis. Below is a detailed working procedure and the necessary precautions to ensure the safe and effective operation of the Sciospec ISX-5.

Proximate analysis

Proximate analysis of beef is a common method used to determine the composition of beef in terms of moisture, protein, fat, ash, and carbohydrates. This analysis helps in assessing the nutritional value of the beef and ensuring quality control in the meat industry. Here is a step-by-step outline of the proximate analysis process[21,22,23].

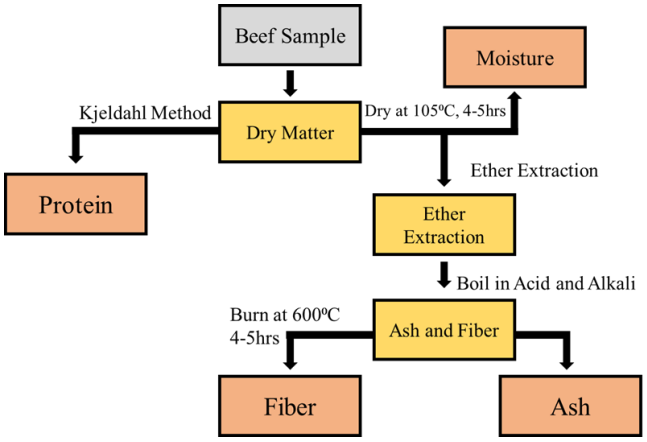


Fig. 8. Proximate analysis flow chart of beef[21,22,23]

Calculation of moisture content

Moisture content from any sample was determined by a method called the oven drying method. First of all, an amount of 3~5 g of samples was weighed (W_1), which should be a well-mixed crucible and cleaned. Then the crucible was started to heat an oven at $105°C$ for approximately $5\ hrs$ up to a constant weight was reached. Finally, a desiccator was used to cool the crucible for 25~30 min. Now, the cooling crucible is weighted as W_2. The following formula was introduced to calculate the percent of moisture content of any sample, like beef[21,22,23].

$$Moisture(\%) = \frac{(W_1 - W_2) \times 100}{Weight\ of\ sample} \dots\dots\dots (iv)$$

$W_i = Initial\ weight\ of\ crucible + Sample$

$W_f = Final\ weight\ of\ crucible + Sample$

Calculation of ash content

Ash content from any sample was determined by an oven drying method, as like as the determination of moisture content. First of all, a $1g$ sample was collected that should be placed in a cleaned, empty crucible. After that heating procedure was performed for the crucible with a muffle furnace at $600°C$ for $1\ hr$. After $1\ hr$, the crucible should be cooled in a desiccator and then weighed as a weight of (W_1). Each $1\ g$ of the sample is weighted as (W_2). Now, it's time the ignition the sample with a burner. The ignition procedure was continued until the sample was charred, and again the crucible should be placed in a muffle furnace at $550°C$ for approximately $5\ hr$. During the heating, a gray-white appearance of ash indicates the completeness of oxidation of all organic substances in that sample. Now, the ashing furnace should be turned off for this moment. The final cooled crucible was weighed as (W_3).

The following formula was introduced to calculate the percent of ash content of any sample, like beef[21,22,23].

$$Ash(\%) = \frac{(W_3 - W_1) \times 100\%}{Weight\ of\ sample} \dots\dots\dots (v)$$

$$The\ difference\ in\ weight\ of\ Ash\ is\ (W_3 - W_1)$$

Calculation of crude protein content

The Kjeldahl method is used to determine the amount of crude protein from a sample like beef. First of all, the samples were mixed with concentrated sulphuric acid (H_2SO_4) in the presence of a digestion mixture, and then the samples were digested by a heating procedure. After that, an alkaline was made from that mixture, and then Ammonium sulfate $(NH_4)_2SO_4$ was formed, which released ammonia (NH_3) as a by-product. Finally, ammonia (NH_3) was collected in 2% boric acid (H_3BO_3) solution and then introduced to a titration procedure against $0.1N\ HCl\ (standard)$, which was collected in 2% boric acid solution and titrated against $standard\ HCI$. The following equation is introduced to find out the amount of protein by multiplying the nitrogen by an appropriate factor (6.25) known as the correlation factor[23].

The reagents are essential to determine crude protein are Standard hydrochloric acid (0.1N HCl), Copper sulfate ($CuSO_4$), Concentrated sulphuric acid (1N H_2SO_4), Sodium hydroxide (NaOH) solution of 40% concentration, Potassium sulfate (K_2SO_4) as a digestion mixture, and the final reagent is Boric acid (H_3BO_3); dissolved 40 g of boric acid in sufficient distilled water, and then the volume should be up to 100 ml Methyl red as an indicator.

$$Crude\ protein\ (\%) = 6.25^{*} \times \%N\ \frac{(S - B) \times N \times 0.014 \times D \times 100}{Weight\ of\ sample \times V} \dots\dots\dots (vi)$$

$$* (Correlation\ factor)$$

$S = Sample\ titration\ reading$

$B = Blank\ titration\ reading$

$N = Normality\ of\ HCI$

$D = Dilution\ of\ sample\ after\ digestion$

$V = Volume\ taken\ for\ distillation$

$0.014 = Milli\ equivalent\ weight\ of\ Nitrogen$

Calculation of fat content

A method known as dry extraction is applied for the determination of crude fat. Crude fat means all the fat-type materials like fats, sterols, fatty acids, pigments, carotenoids, phospholipids, chlorophyll, etc. Crude fats were determined by the dry extraction method, but finally, pure fat was separated from crude fat using a Soxhlet extraction apparatus. At first, $1g$ of the moisture-free sample(beef) was taken and placed in a fat-free thimble that was wrapped in filter paper, and then also introduced into the extraction tube chamber. It was performed, then the sample was weighed, dried, and cleaned, and was filled with petroleum ether, which fitted into the Soxhlet extraction apparatus. Now, it's time to turn on the water heater to start the extraction procedure. Then, it should be allowed for the ether to evaporate up to 4-6 siphoning, and also remove the beaker at the very last moment of final siphoning. Now, the extracted materials are introduced into a clean glass dish with ether washing, and then they start to evaporate in a water bath. Finally, it's time to heat the extracted materials in an oven at $105°C$ for $2\ hrs$ and then cool them in a desiccator. The following formula has been introduced to determine the percentage of fat[24].

$$Fat(\%) = \frac{Weight\ of\ ether\ extraction \times 100}{Weight\ of\ sample} \quad \ldots\ldots\ldots (vii)$$

Calculation of carbohydrate

It will be simpler to determine the amount of carbohydrates if the amounts of moisture, ash, protein, and fat can be determined.

$$Carbohydrate(\%) = 100\% - (Moisture + Ash\ + Protein + Fat\ )\% \ldots\ldots\ldots (viii)^{24}$$

Buchi B-414 Scrubber Unit

The Buchi B-414 Scrubber Unit is employed in laboratory settings to neutralize and remove harmful acidic or basic fumes, particularly those produced during chemical procedures such as Kjeldahl digestion. The unit is placed on a stable surface and connected to the fume-generating equipment using appropriate tubing. The absorption bottle is filled with a suitable neutralizing solution, such as sodium hydroxide for acidic fumes or sulfuric acid for basic fumes, and the wash bottle is filled with distilled water to eliminate residual vapors. A vacuum source may be connected if required to enhance fume extraction. During operation, the unit is monitored for effectiveness, with changes in the absorption solution's color serving as an indicator of its neutralizing capacity. Adjustments are made as needed to maintain optimal performance. After use, the unit is powered down, waste solutions are exposed by laboratory protocols, and all components are cleaned, rinsed, and dried before being stored or reused.

Table 2. Specification of Scrubber Unit B-414

Scrubber Unit B-414	
Dimensions (Length x Breadth x Height)	260 x 450 x 480 mm
Weight of the machinery	13Kg
Power system voltage /frequency range	230 V ± 10 % / 50 Hz 120 V ± 10 % / 60 Hz 100 V ± 10 % / 50/60 Hz 140 W100/ W100 /W100
Overvoltage category of the pump	II
Current consumption (230 V)	1.5A
Power consumption	200W
Ambient temperature	For indoor use only, altitude up to 2000 m above sea level, maximum relative humidity 80 %, 5 - 40 °C
Suction capacity of the pump	34 l/min
Degree of pollution	2

Büchi K-350 is a distillation unit

The Büchi K-350 Distillation Unit is utilized for Kjeldahl nitrogen determination, wherein nitrogen in organic samples is digested, distilled as ammonia, and substantially titrated. Initially, the sample is digested with concentrated sulfuric acid and a catalyst to convert organic nitrogen into ammonium sulfate. The Büchi K-350 is then prepared and assembled, ensuring all components are secured. A receiving flask containing a boric acid solution is positioned under the condenser to capture distilled ammonia. The digested sample is transferred into the distillation flask, and sodium hydroxide is added to alkalize the solution, enabling the release of ammonia gas. Upon activation, steam is generated to drive the distillation, transporting ammonia into the boric acid, forming ammonium borate. After the predetermined distillation time, the unit is stopped, and the receiving solution is titrated with a standard acid, such as hydrochloric or sulfuric acid, to quantify nitrogen content. Following the procedure, the unit and all associated glassware are cleaned, and chemical wastes are disposed of in compliance with laboratory safety protocols.

Table 3. Specification of Distillation Unit K-350

Distillation Unit K-350	
Dimensions (Length x Breadth x Height)	400×660×360 mm
Current consumption	(230 V) 8.5 A
Frequency	50/60 Hz
Power consumption	Max. 2.2 kW
Mains connection	3-pole (P, N, E) via power cord
Pollution degree	2
Reproducibility	(RSD) ≤ 1 %
Detection limit	≥ 0.1 mg Nitrogen
Environmental conditions: Temperature, Altitude, Humidity	for indoor use only - 35 °Cup to 2000m maximum relative humidity 80 % for temperatures up to 31 °C decreasing linearly to 67 % relative humidity at 35°C
Weight	kg
Recovery rate	≥ 99.5 %
Connection voltage	± 10 % / 200 V ± 10 %

Büchi K-437

The Büchi K-437 is used for nitrogen determination through the Kjeldahl method, automating the digestion process to ensure consistent and accurate results. The unit must be cleaned and inspected for any residues from

previous operations, with all connections, including gas and reagent supply lines, verified for proper function. The sample is refused and placed in a Kjeldahl digestion tube, to which concentrated sulfuric acid and a suitable catalyst (such as selenium, copper, or mercury) are added. The digestion tubes are then placed into the unit's digestion block, ensuring the fume removal system is operational. Digestion parameters, including temperature and time, are set through the control panel or selected from pre-programmed methods. Once the process begins, the unit automatically heats the samples, maintaining the set temperature for the required duration. The digestion process is monitored periodically through the unit's display, and the fume removal system is verified for efficiency. After digestion, the tubes are allowed to cool, and the digested samples are transferred for further analysis. The unit is cleaned thoroughly to prevent contamination, and the power is turned off when not in use, with waste containers employed as necessary.

Table 4. Specification of the digestion system K-437

Digest System K-437	
Dimensions (Length x Breadth x Height)	435 x 560 x 780 mm
Net Weight(including digestion tubes)	35 kg
Power consumption capacity	Max. 2200 W
Voltage range	200-230 V ± 10 %
Frequency	50/60 Hz
Degree of pollution	2
Temperature range	50 °C to 420 °C (450 °C)
Installation category	II
Temperature drift	± 5 °C > 200 °C / ± 2 °C < 200 °C
Environmental conditions: Temperature, Altitude, Humidity	for indoor use only - 40 °Cup to 2000 m maximum relative humidity 80% for temperatures up to 30 °C

Carbolite ELF

The Carbolite ELF furnaceis utilised for various laboratory applications such as ashing, heat treatment, and material testing. The furnace must be placed on a stable, heat-resistant surface and verified to be clean and free of residues. All connections, including power supply and accessories, should be checked for integrity. The sample is prepared according to the specific application requirements and placed in a crucible or container capable of withstanding high temperatures. The sample container is then carefully loaded into the furnace chamber, ensuring it does not touch the furnace walls or heating elements, and the door is securely closed. The furnace is powered on, and the temperature and time are set using the control panel. During the heating process, the temperature and time are monitored, and the furnace is periodically checked for stability and proper function. Once the set time has elapsed, the furnace will either cool automatically, or the heating process will need to be manually stopped. After the furnace cools to a safe temperature, the sample container is removed using appropriate tongs or gloves, with caution to avoid burns or thermal shock. The furnace is then allowed to cool completely before cleaning, and the chamber and accessories are cleaned to remove any residues. The furnace is powered off, and the power supply is disconnected if it is not to be used for an extended period.

IV. Results and Discussion

Four-electrode FIM

The following formula was used to determine the absolute value of impedance based on the real and imaginary portions of the recorded data file:

$$|Z| = \sqrt{R_e^2 + I_m^2} \ \ldots\ldots\ldots (ix)$$

Where,

$|Z| = \ Absolute\ value\ of\ Impedance$

$R_e \ = \ Real\ value\ or\ resistive\ part\ of\ complex\ Impedance$

$I_m = Imaginary\ value\ or\ reactive\ part\ of\ complex\ Impedance$

Bioimpedance vs. Time for different samples

The experiment was conducted between 100 Hz to 100 kHz on beef samples. All samples showed a decreasing bioimpedance for increasing frequency. As capacitive reactance decreases with increased frequency, the lowest bioimpedance shows at 100 KHz. It has taken 7 points between 100 Hz to 100 KHz; they are 100Hz, 237Hz, 1000Hz, 3162Hz, 10000Hz, 31622Hz, and 100000Hz, and the data have been taken over 5 consecutive days.

The preceding **Table 5** indicates that, as a result of the destruction of the beef cell membrane during chilling, impedance decreases over time for different frequencies.

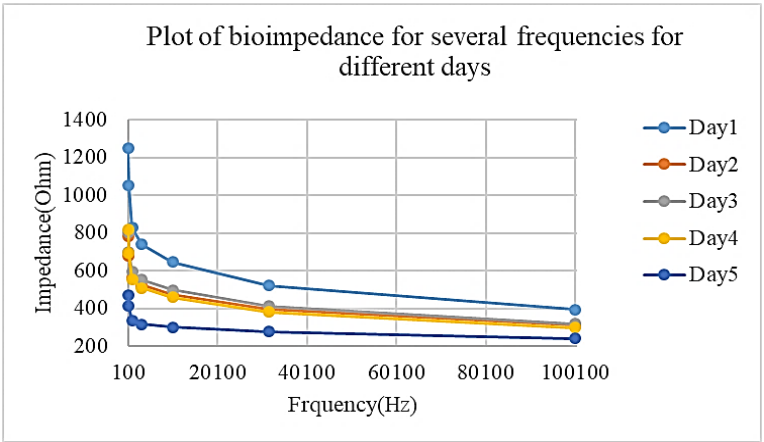


Fig. 9. Bioimpedance plot for several frequencies for the following days

For beef, bioimpedance varies from 1248Ω (100Hz, Day 1) to 242Ω (100000Hz, Day 5). So, it should be concluded after observing the dataset that during the aging process, bioimpedance of beef change at high frequency is very low (at 74989Hz impedance was 252Ω but slightly reduced to 242Ω at 100000Hz), but it shows a significant change of Bioimpedance at low frequency during aging (at 100Hz impedance was 1248Ω but reduced to 1051Ω at 237Hz which is significant).

Data Interpretation

For further inspection and predicting the behavior between impedance and time, values of impedance of the sample have been taken over the first day to the fifth day for four frequencies: 100 Hz, 1000 Hz, 10000 Hz, and 100000 Hz.

Table 5. Impedance at different frequencies over five days

Impedance at 100 Hz (Ω)	Impedance at 1000 Hz (Ω)	Impedance at 10000 Hz (Ω)	Impedance at 100000 Hz (Ω)
1248.65	827.07	646.74	395.66
784.29	564.21	473.37	312.52
804.34	593.74	499.68	318.63
820.28	553.41	459.51	298.01
473.40	336.17	302.59	242.04

Using the data, individual graphs have been formed for four different frequencies, and analyzing those graphs, the mathematical relationship between impedance and time has also been predicted.

It maintains an exponential relation like $Z(t) = a\, e^{-mt}$, where $t = time\ in\ days$, $Z(t)$ = impedance at time t, and a and m are constant parameters dependent on frequency. So, the equations can be built as follows,

$$Z_{100Hz}(t) = 1405\, e^{-0.1891t}$$

$$Z_{1000Hz}(t) = 939\, e^{-0.1734t}$$

$$Z_{10000Hz}(t) = 724\, e^{-0.1463t}$$

$$Z_{100000Hz}(t) = 423\, e^{-0.1036t}$$

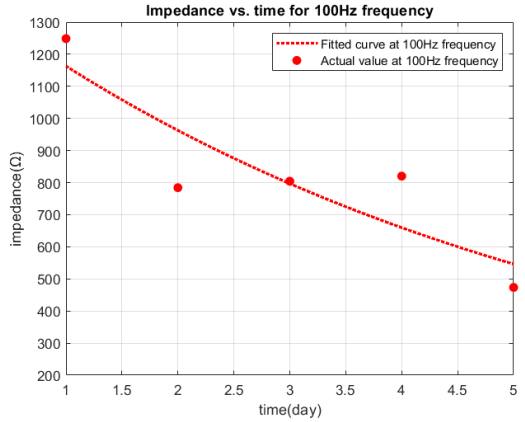


Fig. 10. Relation between Impedance and Time (Days) for 100Hz frequency, where $R^2 = 0.7697$

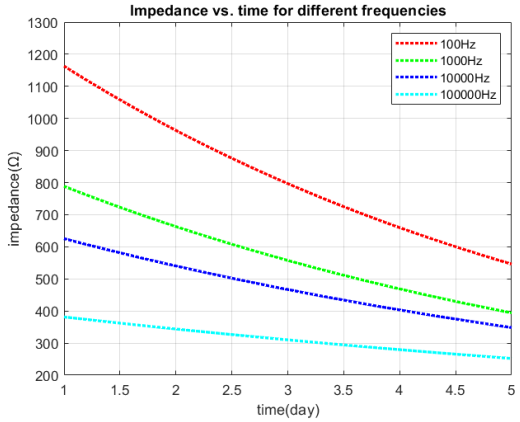
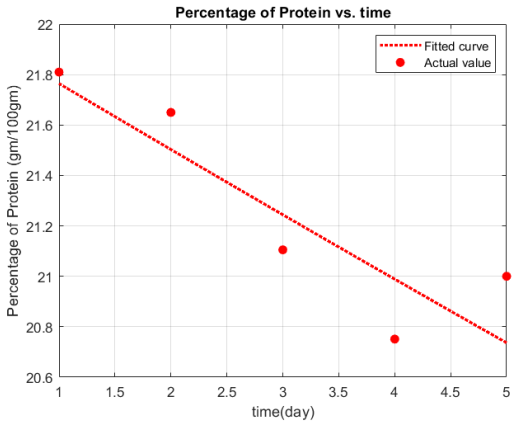
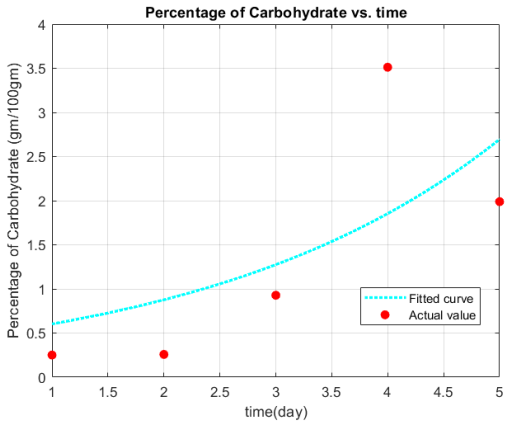


Fig. 11. Relation between Impedance and Time (Days) for different frequencies

Similarly, the relationship of variation of protein and carbohydrate over time also maintains a similar kind of exponential behavior.



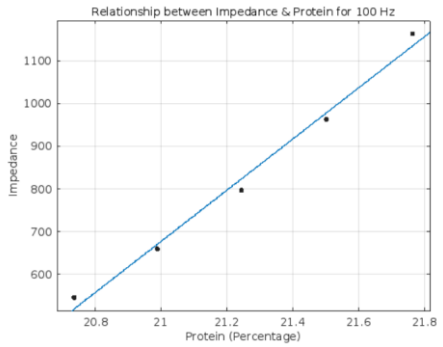
$P(t) = (22.0296)e^{-0.0121t}$, where $R^2 = 0.7687$



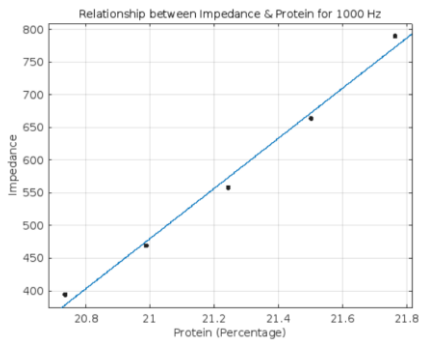
$C(t) = 0.4148\, e^{0.3743t}$, where $R^2 = 0.4743$

Fig. 12. Relation of Protein and Carbohydrate over Time (Days)

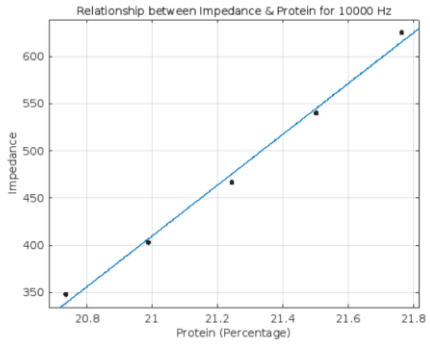
Then, two relations of impedance over the variation of protein and carbohydrate have been predicted by inspecting the data from the previously predicted equation, eliminating the variable time (t). By these two relations, some ideas have been developed about how the impedance may change with the variation of protein and carbohydrate. The value of impedance increases linearly with the variation of protein, maintaining an equation like $Z = aP + b$, where a and b are the constant parameters dependent on different frequencies. Here, the value of a, which represents the slope of the equation, is very high, eventually meaning that the impedance tends to increase highly with a very small change in protein variation.



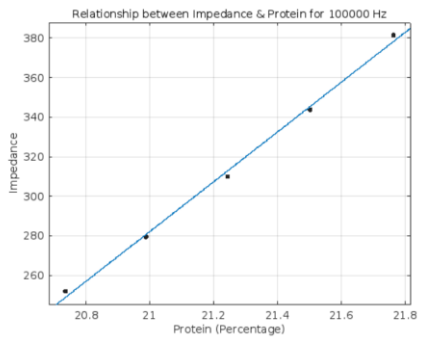
$Z_{100\ Hz} = 599P - 11894$, where $R^2 = 0.9893$



$Z_{1000\ Hz} = 383P - 7565$, where $R^2 = 0.9911$



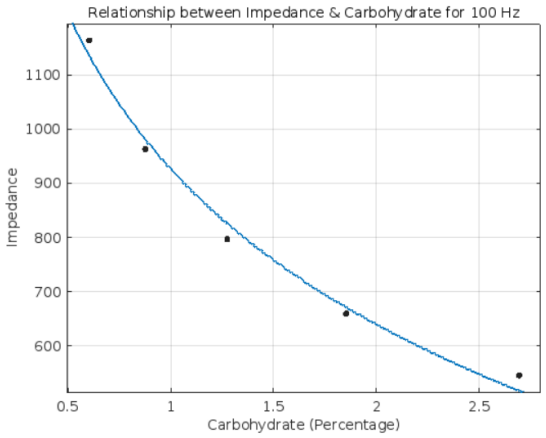
$Z_{10000\ Hz} = 269\ P - 5237$, where $R^2 = 0.9938$



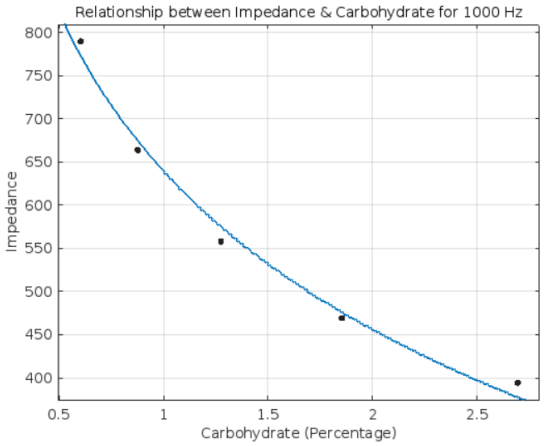
$Z_{100000\ Hz} = 126\ P - 2358$, where $R^2 = 0.9971$

Fig. 13. Relation between Impedance and Protein (percentage) for different frequencies

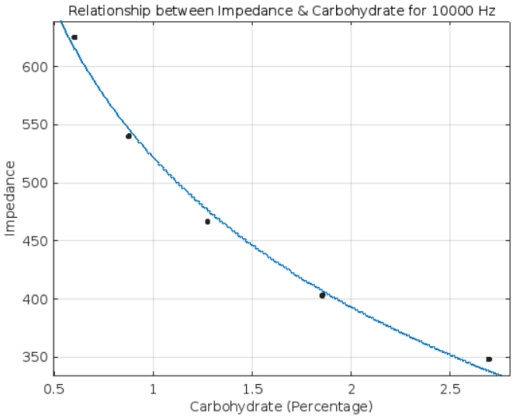
Whereas, the relation between impedance and variation of carbohydrate is a highly decaying logarithmic behavior, maintaining an equation like $Z = -m\ln(C) + n$, where m and n are also dependent on different frequencies.



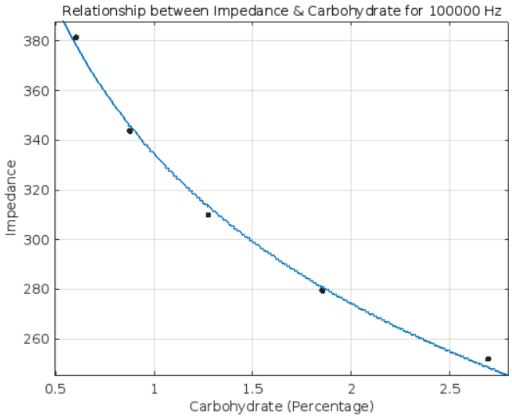
$Z_{100\,Hz} = -411\ln(C) + 926$, where $R^2 = 0.9878$



$Z_{1000\,Hz} = -263\ln(C) + 639$, where $R^2 = 0.9897$



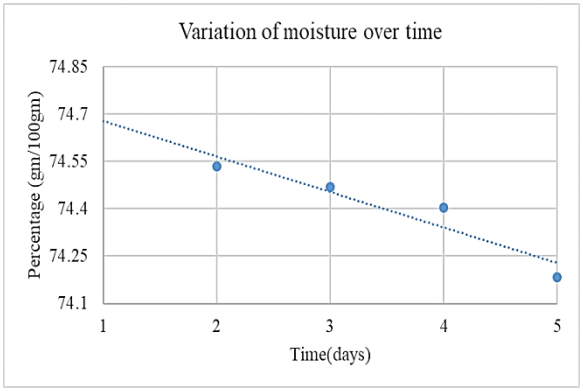
$Z_{10000\,Hz} = -185\ln(C) + 521$, where $R^2 = 0.9926$



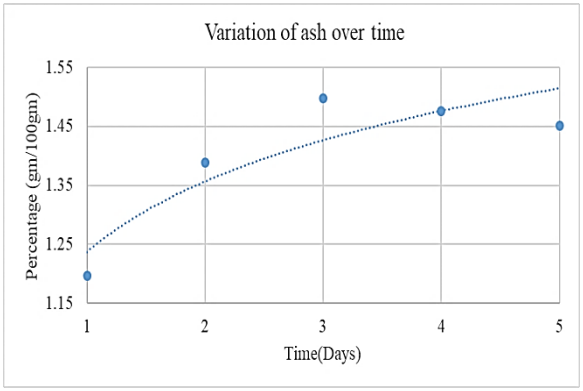
$Z_{100000\,Hz} = -86\ln(C) + 334$, where $R^2 = 0.9963$

Fig. 14. Relation between Impedance and Carbohydrate (percentage) for different frequencies

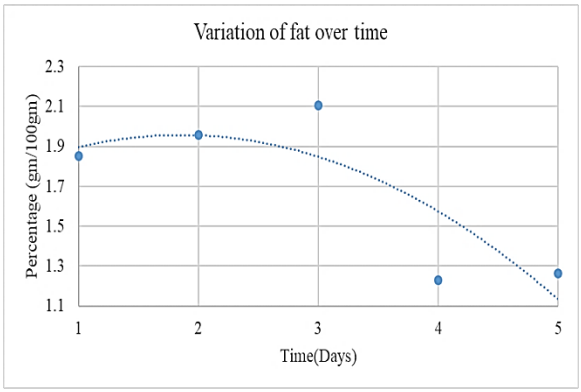
Variation of moisture, ash, and fat has also been measured over time, but the dependency of impedance over these three factors is ignored because of very small-scale changes over time (shown in Fig. 14).



(a)



(b)



(c)

Fig. 15. Variation of (a) moisture, (b) ash, and (c) fat over time

As impedance is dependent on both protein and carbohydrate at a very significant amount of changes over time, so the impedance (Z) at a certain frequency f will be $Z_f = aP + bln(C) + d$, where P is the percentage of protein, C is the percentage of carbohydrate, $a, b,$ and d are arbitrary constants.

V. Conclusion

The exploratory analysis of age-related changes in beef through bioimpedance and chemical composition perspectives has provided insightful findings into how beef quality and characteristics evolve with aging. Bioimpedance analysis techniques have proven to be effective in identifying variations in beef muscle properties as it ages. The key outcomes from this analysis include electrical conductivity and resistance: Changes in bioimpedance parameters, such as electrical conductivity and resistance, were observed as the beef aged. These changes are indicative of alterations in muscle fiber structure and water content. There was a notable correlation between bioimpedance measurements and the tenderness of beef. As the meat aged, it became more tender, which was consistently reflected in the bioimpedance readings.

The chemical composition analysis highlighted several age-related transformations in beef, including protein degradation. Proteolysis increased with aging, leading to the breakdown of muscle proteins into smaller peptides and amino acids. This process contributes to the enhancement of flavor and tenderness in aged beef. The degree of lipid oxidation was found to increase over time, affecting the beef's flavor profile. While some level of oxidation contributes to desirable flavors, excessive oxidation can lead to off-flavors and decreased quality.

There was a gradual reduction in moisture content as the beef aged, which was correlated with changes observed in the bioimpedance measurements. Combining bioimpedance and chemical composition analyses offers a comprehensive understanding of how beef quality changes with age. Bioimpedance provides a non-destructive, rapid assessment tool, while chemical composition analysis offers detailed insights into the molecular transformations occurring within the meat. Together, these methods can be used to optimize aging processes, ensuring the production of high-quality beef with desirable sensory attributes.

In conclusion, this exploratory analysis underscores the importance of using both bioimpedance and chemical composition perspectives to monitor and understand age-related changes in beef. This integrated approach can lead to better quality control and optimization of aging practices in the beef industry, ultimately enhancing the consumer experience.

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