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Original Article

Bio-electrical properties of apples in function of sample volume and temperature and probe configuration[1]

Propriedades bioelétricas da maças em função do volume e temperatura da amostra e da configuração da sonda

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HIGHLIGHTS:
The spike method is more stable in acquiring electrical impedance spectroscopy data.
The contact method is prone to data errors due to the influence of the fruit's shape and uneven surface texture.
The contact method is ideal for monitoring individual samples, whereas the spike method is better for large sample sizes.

ABSTRACT: The quality of fruit can be quickly and accurately assessed using bio-impedance spectroscopy, but no comprehensive studies have been conducted on how the detection conditions affect their bio-electrical properties. Accordingly, this study aims to investigate the effects of sample volume, sample temperature, probe depth and probe distance on the bio-electrical properties of apples and uses a controlled bi-variable method to determine the optimal parameter settings, thus providing researchers with reliable references. Two different measurement methods, the spike method and the contact method, are used to measure the electrical properties of apples under various conditions and to compare the results. It is verified that the effects of sample volume, temperature, probe depth, and probe distance on bio-electrical properties follow physical laws when using the spike method. The contact method, however, is prone to errors and is not suitable for large-scale samples. The optimal conditions for the spike method, determined by a bi-variable approach, are sample volume of 20 cm^3 (5 × 2 × 2 cm), probe distance of 2.5 cm at 1 kHz and 1 cm at 20 kHz, and probe depth of 1 cm.

Key words: *Malus domestica*, electrical impedance spectroscopy, bi-variable control method, quality evaluation of fruit

RESUMO: A qualidade das frutas pode ser avaliada de forma rápida e precisa usando a espectroscopia de impedância bioelétrica, mas não foram realizados estudos abrangentes sobre como as condições de detecção afetam suas propriedades bioelétricas. Assim, este estudo visa investigar os efeitos do volume da amostra, temperatura da amostra, profundidade da sonda e distância da sonda nas propriedades bioelétricas das maças e usar um método de controle bivariável para determinar as configurações ideais dos parâmetros, fornecendo assim referências confiáveis para os pesquisadores. Dois métodos de medição, o método de impulso e o método de contato, foram usados para medir as propriedades elétricas das maças sob várias condições e comparar os resultados. Foi verificado que os efeitos do volume da amostra, temperatura, profundidade da sonda e distância da sonda nas propriedades bioelétricas seguem leis físicas ao usar o método de pulso. No entanto, o método de contato é propenso a erros e não é adequado para amostras em grande escala. As condições ideais para o método de impulso, determinadas por uma abordagem bivariável, são volume de amostra de 20 cm^3 (5 × 2 × 2 cm), distância da sonda de 2.5 cm a 1 kHz e 1 cm a 20 kHz, e profundidade da sonda de 1 cm.

Palavras-chave: *Malus domestica*, espectroscopia de impedância elétrica, método de controle bivariável, avaliação da qualidade de frutas

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Introduction

Substantial research has been conducted to accurately assess the quality of fruit, such as magnetic resonance (Qiao et al., 2019), X-ray (Van et al., 2020; Lu et al. 2023), and near infrared spectroscopy (Minas et al., 2021). However, they are costly and require intricate sample preparation and trained personnel. The evaluation of fruit using bio-electrical properties has recently become popular due to its convenience, affordability, and ease of operation. It has been used to characterize the fruit's maturity (Ibba et al., 2019; Li et al. 2019), freshness (Kojić et al., 2022) and quality (Arteaga et al., 2021; Fogué et al., 2022).

The contact method and the spike method are the primary methods to measure the bio-electrical properties of fruit. The former can non-destructively monitor impedance changes during ripening and corruption (Rehman et al., 2011; Li et al., 2019; Ibba et al., 2021). The spike method, despite being invasive, allows for easy control of probe distance and probe depth, ensuring that each sample is tested under consistent conditions (Jackson & Harker 2000; Yang et al., 2013; Qiu et al., 2024).

Despite the widespread use of bio-electrical properties in food research, studies employ varying experimental conditions without explaining their choices. Accordingly, this study aims to investigate the effects of sample volume, sample temperature, probe depth and probe distance on the bio-electrical properties of apples and uses a controlled bi-variable method to determine the optimal parameter settings, thus providing experimenters with reliable references.

Material and Methods

The experiment used Fuji apples that had a consistent shape without mechanical damage. Fifty apples, purchased at Yonghui Superstores in Xiamen (Geographic coordinates: 24.4798° N, 118.0894° E; Altitude: about 7 meters above sea level), China, were used in the experiments. The weights of the apples range from 190 to 240 g, with an average of 225 g. The maximum diameter ranged from 7 to 8.8 cm, with an average of 8.1 cm. To ensure the freshness of the samples, they were promptly refrigerated at 8 °C after purchase.

In the experiments several pieces of equipment were used, including the LRH-150 biochemical incubator from Shanghai Yiheng Scientific Instrument Co. (China), the TH2816A LCR digital bridge from Changzhou Tonghui Electronics Co. (China), copper probes, copper sheet electrodes and a self-made measuring apparatus for controlling the probe depth and the distance between the probes (Figure 1).

In the contact method experiment, copper sheet electrodes were firmly attached directly to the surface of the apple to measure its bio-electrical properties (Figure 1A). To investigate the effect of the distance between the copper sheet electrodes on the bio-electrical properties, this electrode distance was reduced by applying more pressure on the sample.

The measuring apparatus for the spike method experiment consisted of a square base 5 cm high and a side length of 16 cm, with three square sample holders of varying heights (1, 2, and 3 cm) and a side length of 15 cm (Figure 1B). Uniformly-spaced holes with diameter of 0.1 cm were positioned along the diagonal of the base, and copper probes with a diameter of 0.1 cm and a length of 8 cm were inserted into these holes to control the distance between the two probes. The sample holders of different heights were placed on the base to adjust the probe depth into the sample.

To investigate the effects of sample volume on the bio-electrical properties of apples in the spike method experiment, rectangular samples were obtained and their volumes calculated. The samples were then placed in the biochemical incubator with a temperature range of 8-80 °C. During this experiment, which studied the sample volume, probe depth and probe distance, the temperature was maintained at 8 °C in a constant humidity environment of approximately 40%. Another experiment involved the sample temperature. In this, the temperature of the incubator was set to 3, 6, 9, 12, 15 18, 21 and 24 °C, and measurements would only begin after the sample temperature reached that of the incubator. To minimize the effects of oxidation and moisture loss, the samples were wrapped in plastic wrap. The resistance and capacitance of the samples were measured using an LCR meter with an alternating voltage of 1 V at frequencies of 1 and 20 kHz respectively, to determine their bio-electrical properties at

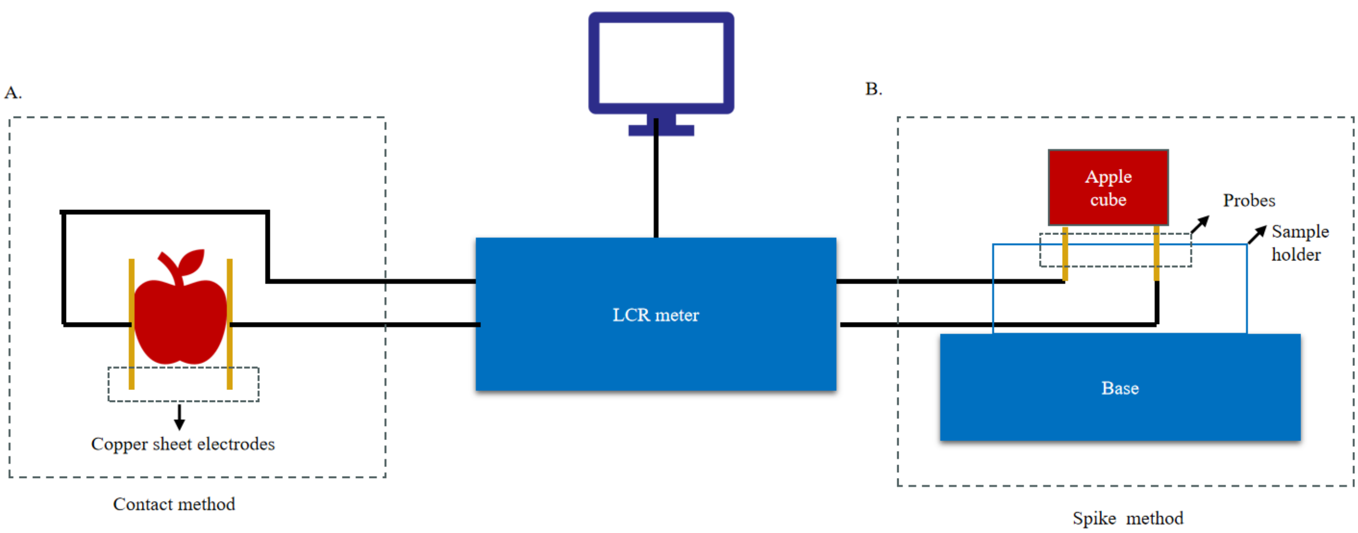


Figure 1. A system for measuring the bio-electrical properties of apples

low and high frequencies. The main reason for choosing these two frequencies is that the frequency range of the LCR meter used in this experiment is limited to 0.1 to 25 kHz. Each data point was obtained by measuring the average of three samples, and the value is represented as mean ± SD.

The bivariate control method (Chong et al., 2015) is a commonly used statistical method in data analysis to control for the mutual influence between two variables, to assess their effects more accurately on other variables. It is often employed to investigate whether a causal relationship exists while excluding other possible confounding factors.

This study involved the selection of the probe depth and probe distance as the two correlated variables of interest and examined them as independent variables based on previous research (Gao et al., 2024; Qiu et al., 2024). Then, by controlling one of these variables and keeping it constant, the impact of the second variable on the bio-electrical properties (capacitance and resistance in this study) was determined. Bio-impedance spectroscopy was fitted using the least square method to obtain the slope. A smaller slope indicates more stable data. The probe depth and probe distance with the smallest slope were the optimal measurement conditions (Chong et al., 2015).

Results and Discussion

The results of measurements of the bio-electrical properties of apples using the contact method are presented in Figure 2. Because the apple has a curved shape and its surface is somewhat elastic, when the distance between the copper sheet electrodes is reduced, the contact area between the sheets and the sample increases. Therefore, according to the formulas for capacitance (C) and resistance (R), C should increase, and R should decrease with a decrease of the distance. Specifically, the capacitance is given by $C = \varepsilon S/4\pi kd$ and the resistance is given by $R = \rho L/S$. However, due to the irregular shape of the sample, the contact area does not always increase with decreasing distance during the measurement in Figure 2. Thus, there are significant variations in C and R during the measurement. In addition, as the distance of the copper sheet electrodes decreases, the moisture content of the apple begins to leak out due to the pressure exerted by the electrode sheets, which also affects the bio-electrical properties. The results can be unreliable due to the influence of factors such as the fruit's varying volume and shape. Therefore, the contact method is more commonly used for monitoring a single sample (Rehman et al., 2011; Li et al., 2019) and is not suitable for measuring large-scale samples.

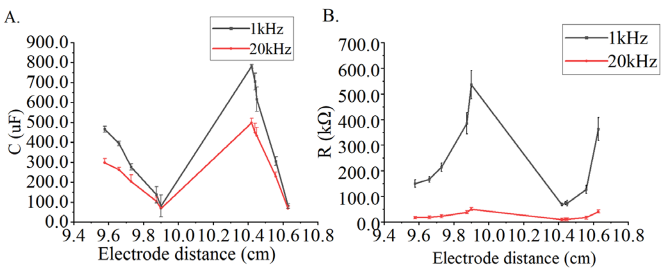
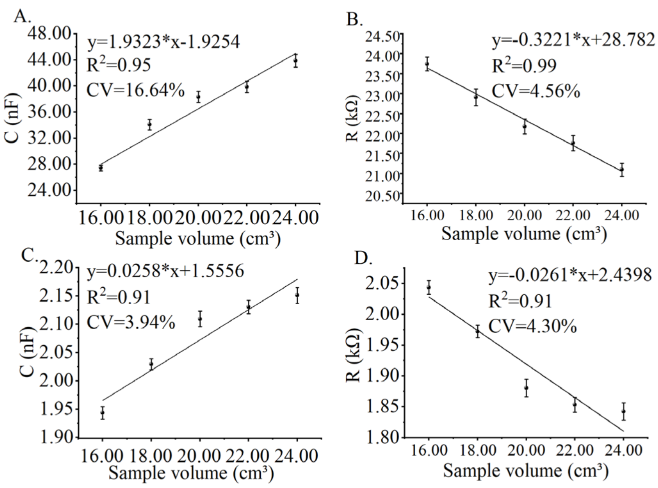


Figure 2. Capacitance (C) of apple at 1 kHz and 20 kHz as a function of electrode distance (A); Resistance (R) at 1 kHz and 20 kHz as a function of electrode distance (B)

Based on the above, when conducting the experiment using the contact method, it is susceptible to uncontrollable factors that might introduce errors. This limitation lowers the applicability of the contact method when multiple samples are involved. So, the controlled variable method cannot be used to investigate the effects of experimental conditions on the bio-electrical properties. Thus, this study does not investigate the effects of experimental conditions on the bio-electrical properties of apples using the contact method.

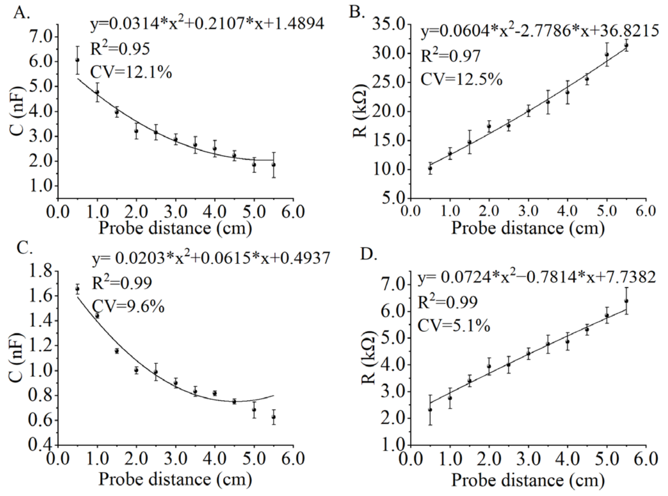
The results of the measured bio-electrical properties for different sample volumes are presented in Figure 3. As the sample volume increases, the cross-sectional area (S) increases, which, according to the formula $C = \varepsilon S/4\pi kd$, resulting in an increased C. Additionally, due to the increase in sample volume, the area through which the current flows increase as well, causing S to increase and R to decrease, as indicated by $R = \rho L/S$. Linear regression analysis is performed on the curves presented in Figure 3 and the slope of the fit indicates that the bio-electrical properties change more drastically at low frequency (Figures 3A and B) than at high frequency (Figures 3C and D). This is due to the fact that high frequency current can directly flow through the cells while low frequency current can only flow between them. In larger samples, the electrical properties become more complex due to variations in cell density and arrangement at different locations, along with a significant increase in the total number of cells. In larger samples, the interactions between cells are more intricate, potentially leading to the formation of diverse resistive and capacitive networks. Additionally, differences in tissue structure and cell arrangement in various parts of the apple may cause an increase in the irregularity of the current flow pathways. Therefore, a smaller sample can lead to a simpler equivalent electrical model at low frequency. Although a smaller sample volume produces more reliable data, a medium sample volume is required for fruit characterization, as a sample volume that is too small may not accurately represent the overall quality of the fruit.

Figure 4 presents the results of bio-electrical property measurements at various probe distances, with quadratic regressions employed to elucidate the specific relationships



* - Significant at $p \leq 0.05$ by t-test

Figure 3. Capacitance (C) and resistance (R) of apple at 1kHz (A, B) and at 20 kHz (C, D) as a function of sample volume

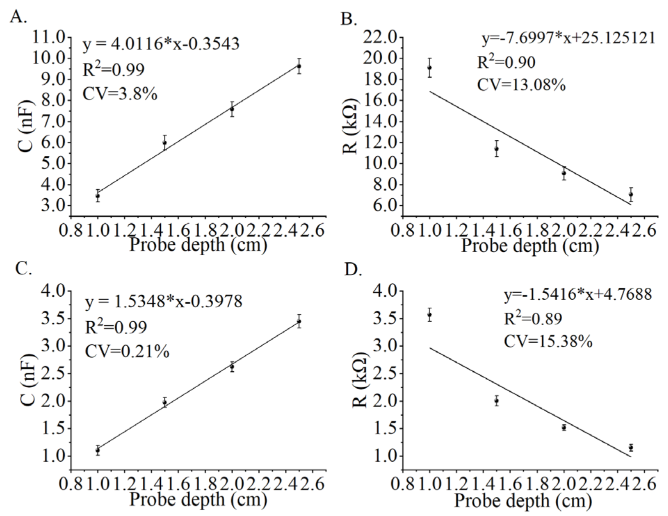


* - Significant at $p \leq 0.05$ by t-test

Figure 4. Capacitance (*C*) and resistance (*R*) of apple at 1kHz (A, B) and at 20 kHz (C, D) as functions of probe distance when probe depth is 1 cm

observed. As the probe distance increases, C decreases and R increases. As the probe distance increases, d increases and the capacitance decreases. Apple tissue could be regarded as a resistance: the resistance increases as the probe distance increases. As *L* increases, the resistance also increases. Figure 5 illustrates the relationship between the probe depth and bio-electrical properties, with linear regressions applied to precisely characterize this correlation. As the depth increases, C increases and R decreases. With a larger probe depth, more capacitors are connected in parallel. Thus, using the formula, $C = C_1 + C_2 + ... + C_n$, a larger probe depth leads to a larger capacitance. Similarly, with a larger probe depth, more resistances are connected in parallel. Thus, according to the formula $1/R = 1/R_1 + 1/R_2 + ... + 1/R_n$, it can be concluded that a larger probe depth results in a lower resistance.

When evaluating many samples, it is important to minimize variations in bio-electrical properties due to the conditions of the measurement process, ensuring that the bio-electrical



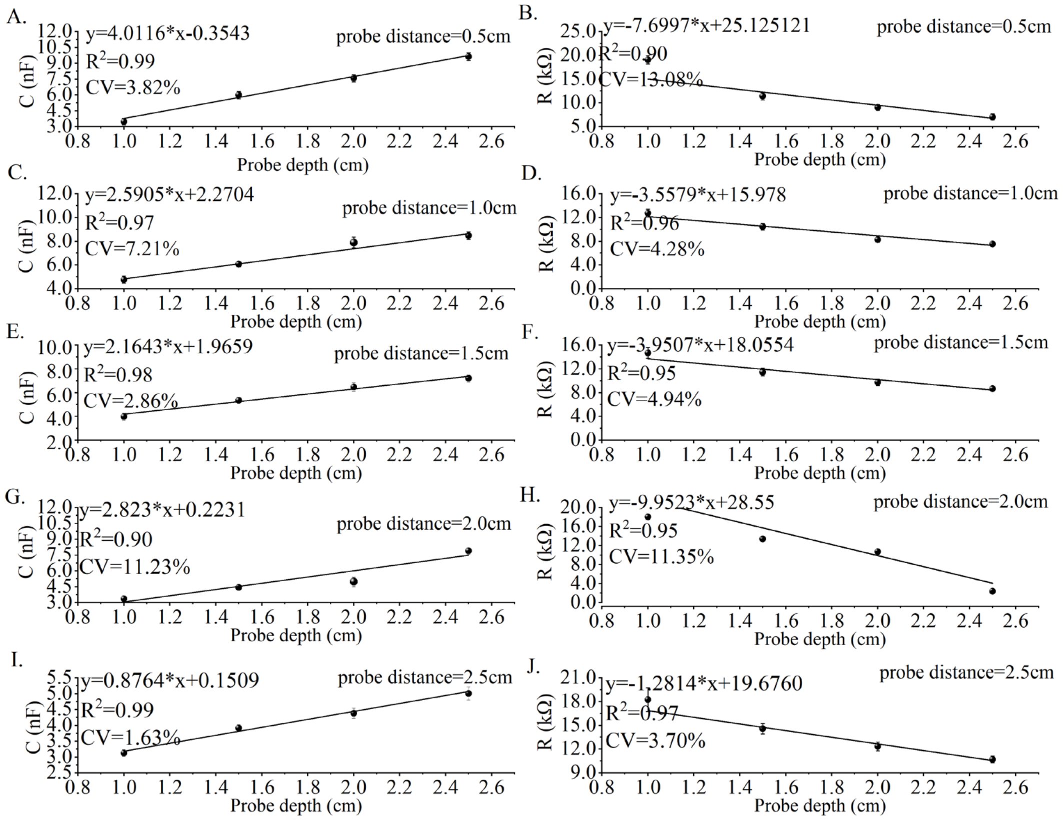
* - Significant at $p \leq 0.05$ by t-test

Figure 5. Capacitance (*C*) and resistance (*R*) of apple at 1kHz (A, B) and at 20 kHz (C, D) as functions of probe depth when probe distance is 0.5 cm

properties effectively reflect the quality of the fruit. The controlled bi-variate method is frequently used to control multiple other factors while concentrating on the influence of two specific factors on the physical quantities. This study aims to determine the most reliable experimental parameters for the measurement of the bio-electrical properties by adjusting the probe distance and the probe depth. So, the controlled bi-variate method is applied to determine the optimal probe distance by changing both it (0.5-3.0 cm) and the probe depth (1.0-2.5 cm). The changes of C and R with increasing probe distance are studied under different probe depths. Linear regression analysis is performed on the curve presented in Figure 6 and 7. Analyzing the fitted slopes enabled us to discern the trend in bio-electrical properties concerning various probe distance, while maintaining consistent probe depth variations (ranging from 1.0 to 2.5 cm). When using the controlled bi-variate method, keeping one variable constant while varying the other results in a smaller change in the bio-electrical property, which in turn leads to a smaller slope of the fitted line on the graph. A smaller slope indicates that, under the given measurement conditions for the controlled variable, the bio-electrical property is less affected by changes in other variables. Specifically, a smaller slope at the probe distance reflects improved stability in the bio-electrical property, suggesting greater immunity to errors from fluctuating conditions. The results indicate that the most stable measurements occurred when the probe distance was 2.5 cm at 1 kHz and 1 cm at 20 kHz. The lowest slopes of the curves are attributed to different probe distance at different frequencies as the current can easily pass through the cells at high frequency, while this is not the case at low frequency. By rearranging the figure displays in Figures 6 and 7 (i.e., changing the x-axis from probe depth to probe distance) and comparing the slopes, the optimal measurement probe depth is concluded to be 1 cm.

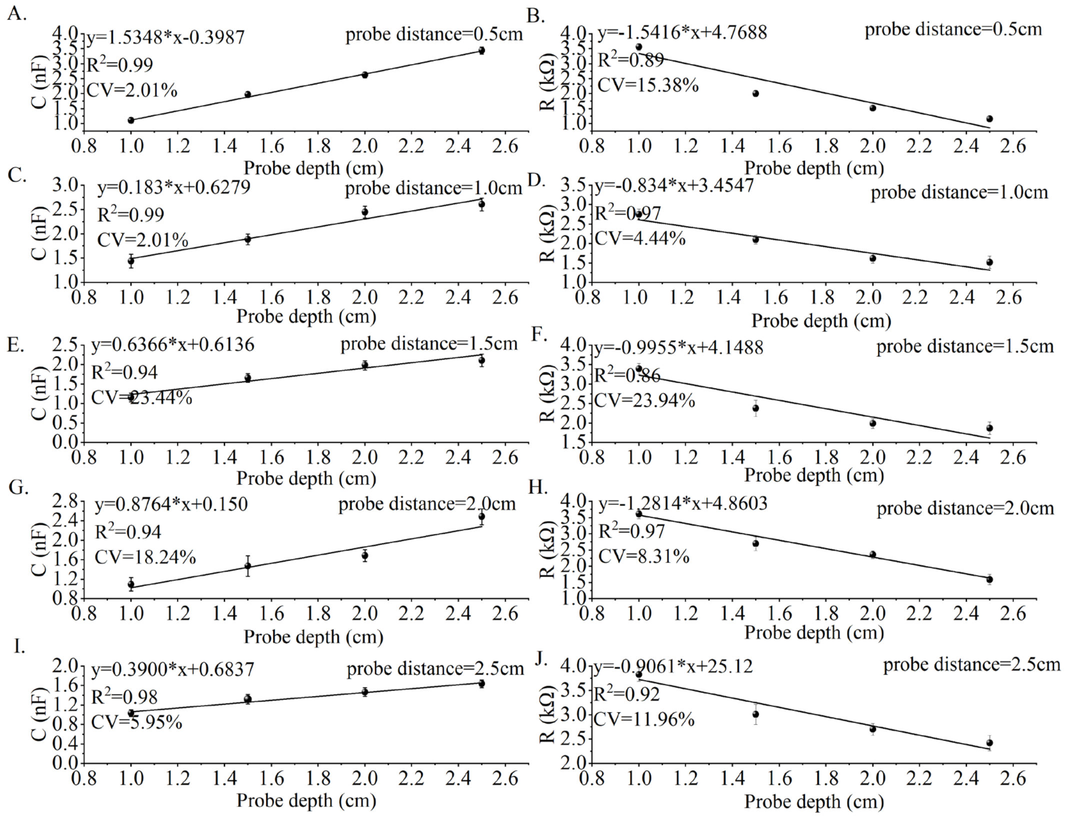
Through the above experiments, the medium volume 20 cm^3 (5 × 2 × 2 cm), the optimal probe distance (2.5 cm at 1 kHz and 1 cm at 20 kHz) and the optimal probe depth (1.0 cm) were selected as the experimental conditions and then the sample temperature experiment was conducted. The results of the bio-electrical properties measured under different sample temperatures are presented in Figure 8. As the temperature increases, *C* increases and *R* decreases. According to a previous study (Jha et al., 2011), it is expected that as the temperature increases, the dielectric constant (ε) will increase and the resistivity (ρ) will decrease, leading to an increase in C and a decrease in *R*. In Figure 8, it can be observed through linear fitting that with every degree increase in temperature, C increases by 1.36% at 1 kHz and 1.52% at 20 kHz, while R decreases by 1.68% at 1 kHz and 1.29% at 20 kHz. Thus, maintaining a stable temperature is crucial in reducing measurement errors during large-scale measurements.

To the best of our knowledge, there are currently no studies on the impact of different measurement conditions on electrical properties. Thus, there is much room for improvement and further investigation in this area. For example, due to instrument limitations, this study only examined frequencies up to 20 kHz, while bio-electrical impedance spectroscopy can typically reach frequencies of 1-3 MHz. Additionally, this



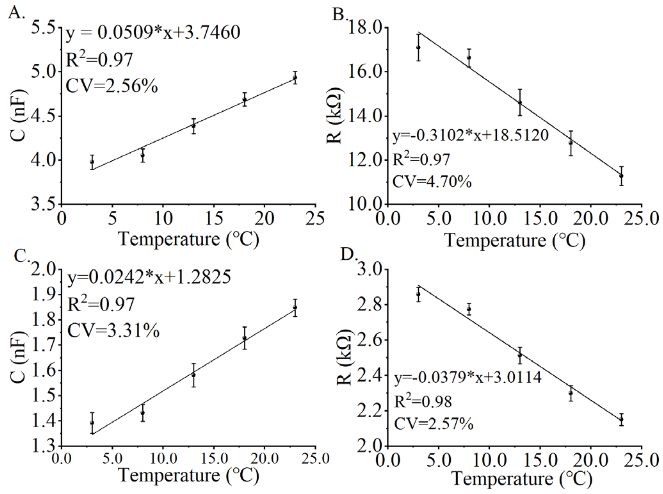
* - Significant at $p \leq 0.05$ by t-test, *C*=Capacitance, *R*=Resistance

Figure 6. Effects of probe depth at various probe depths on the bio-electrical properties of apple at 1 kHz: (A), (B) probe distance is 0.5 cm; (C), (D) probe distance is 1 cm; (E), (F) probe distance is 1.5 cm; (G), (H) probe distance is 2.0 cm; (I), (J) probe distance is 2.5 cm



* - Significant at $p \leq 0.05$ by t-test, C - Capacitance, R - Resistance

Figure 7. Effects of the probe depth and probe distance on the bio-electrical properties of apple at 20 kHz: (A), (B) probe distance is 0.5 cm; (C), (D) probe distance is 1 cm; (E), (F) probe distance is 1.5 cm; (G), (H) probe distance is 2.0 cm; (I), (J) probe distance is 2.5 cm



* - Significant at $p \leq 0.05$ by t-test

Figure 8. Effects of sample temperature on the bio-electrical properties of apple: (A), (B) capacitance (C) and resistance (R) at 1 kHz; (C), (D) capacitance (C) and resistance (R) at 20 kHz

study focused solely on Fuji apples to determine the optimal measurement conditions. Future work could address these limitations by conducting experiments at higher or lower frequencies and on different varieties of apple. Future studies should also develop quantitative regression models between various experimental parameters related to bio-electrical properties using machine learning algorithms. Moreover, statistical methods can be employed to analyze the correlation between crucial quality indicators (firmness, taste, and flavor) of apples and their bio-electrical properties. Applying these techniques can enhance the comprehension of the factors that affect the bio-electrical properties of fruits.

Conclusions

1. The effects of sample volume, sample temperature, probe depth and probe distance on bio-electrical properties follow physical laws when using the spike method.

2. By using the controlled bi-variate method, the most reliable conditions for measuring the bio-electrical properties of fruit are a volume of 20 cm^3 (5 × 2 × 2 cm), probe distance of 2.5 cm at 1 kHz and 1cm at 20 kHz and probe depth of 1 cm.

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