Cole Bio-Impedance Model Variations in *Daucus Carota Sativus* Under Heating and Freezing Conditions

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ABSTRACT This paper reports on the variations in the parameters of the single dispersion Cole bio-impedance model of Daucus Carota Sativus (carrots) under heating and freezing conditions. Experiments are conducted on six samples with recorded live bio-impedance spectra versus temperature. The Cole model parameters are extracted from the measured data using the *Flower Pollination Algorithm* (FPA) optimization technique and their variations are correlated with well-known bio-chemical and bio-mechanical variations. This represents a non-invasive method for characterizing and measuring the degree of change in biological cellular morphology and composition.

INDEX TERMS Bio-impedance, cole model, optimization, electrochemistry.

I. INTRODUCTION

Assessment of the environmental effects on agricultural crops as well as the effects of food preservation methods, commonly employed in the food industry, are very important from economical and health perspectives. Identification of crops types and condition is a prime goal for recent research efforts as attempted by [1]–[3] and [4]. Several studies have investigated the changes in biological plant tissues, specially the alterations on the cellular level, due to temperature variations when freezing or heating is used such as the studies in [5]–[7] and [8]. Most of these studies use the changes in the cellular composition of the plant cell to assess the damage resulting from temperature variation. This is clearly an invasive method of testing. The plant cell anatomical structure, shown in Fig. 1(a), consists of a nucleus storing the DNA in it's nucleolus surrounded by the cytoplasm, which is a semi-fluid, within the cellular membrane. The vacuole store water, salt, protein bodies and various types of granules

or fibrous materials. The cell wall is a rigid and complex structure surrounding the plasma membrane of the cell [9].

The cell wall structure, shown in Fig. 1(b) consists of pectin and cellulose [10], while the middle lamella consists of pectic polysaccharides cross-linked with Calcium ions [11]. In the primary wall, there are similar percentages of pectic substances, hemicelluloses and cellulose, where the cellulose has the function of giving rigidity and resistance to tearing, while the pectic substances (and hemicelluloses) confer plasticity and the ability to stretch [12]. The middle lamella may be considered an extension of the matrix material of the primary cell wall without the cellulose fibrils, and being at the outermost portion of the plant cell, it plays the primary role in inter-cellular adhesion [13] that keeps the cell intact. Pectic substances make up about one-third of the dry substance of the primary cell walls, and a greater proportion of the dry substance of the middle lamella. Thus, they contribute to the mechanical strength of the wall and to the adhesion between cells [14]. Textural changes that happen during ripening, storage or cooking are accompanied by changes in the characteristics of pectic substances. Water is known
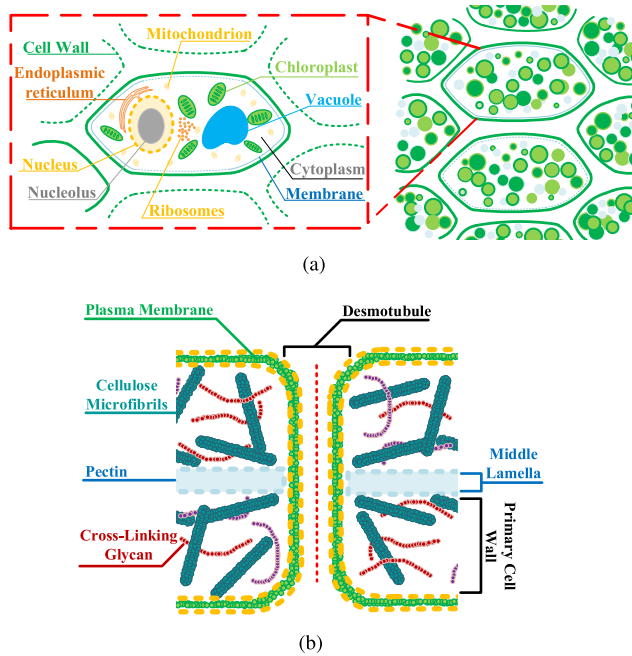

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FIGURE 1. (a) Typical plant cell and its different components and (b) plant cellular wall structure.
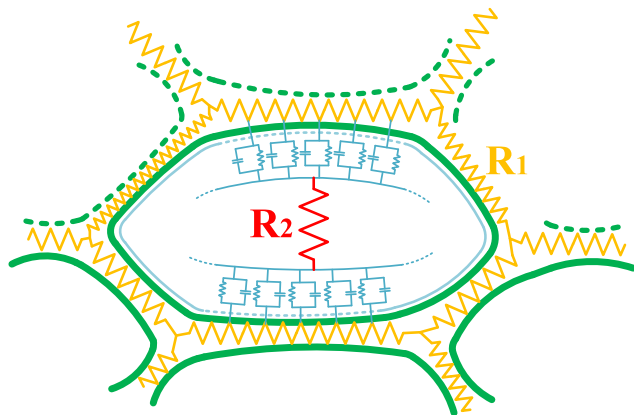
FIGURE 2. Plant cellular electrical circuit equivalent.

FIGURE 3. (a) Plant cellular circuit representation with cellular membrane transmission line impedance Z_1 and (b) cole equivalent circuit model.
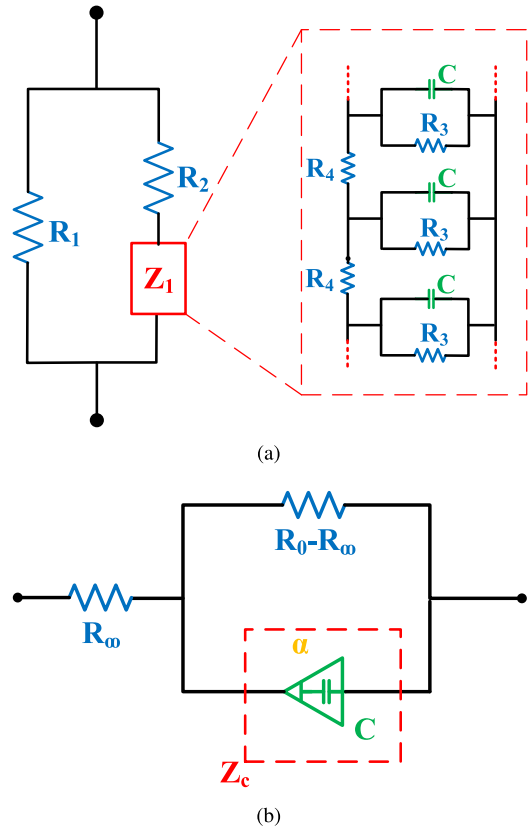
to constitute a large part of young cell walls [11], as it can serve as a wetting agent and a stabilizing agent [15]. Many experimentations showed that the bio-impedance attribute of plant cells is mainly affected by the cellular wall structure, cellular membrane and middle lamella [11] in addition to the concentration of water, cellulose and pectin.

The electrical impedance of the cellular structure [16] can be portrayed as resting cell membrane, where the ion permeability is represented by electrical resistors, and the impermeable structure is represented by capacitances. As shown in Fig. 2 [17] the electrical modeling of the cellular structure usually represents the extra-cellular resistance by R_1, the intra-cellular resistance by R_2 and the cell membrane by a transmission line impedance, depicted in Fig. 3(a) as Z_1. Z_1 is formed of an infinite number of interconnected sections composed of R_3 and C (the specific membrane resistance and capacitance) and R_4 (the lateral resistance of water film in a

unit area of cell surface) [17]. The model in [17] provides a comprehensive way to relate bio-impedance data to biological changes as per the testing conducted on scots pine needle considering the Cole bio-impedance model

Over the years, many equivalent circuits for bio-impedance data using integer and/or fractional order circuit elements have been proposed [18]. Integer order techniques can be employed to characterize uniform tissues, while fractional order models better describe heterogeneous tissues [19]. The Cole impedance model is one of the preferred fractional-order models [16] widely used to fit bio-impedance data [20] measured over a specific frequency range of interest. This model, shown in Fig. 3(b), consists of low frequency resistance R_0, a high frequency resistance R_∞ and a fractional-order capacitor with dispersion coefficient α [20]. The impedance model is given by [21]

$$Z(f) = R_\infty + \frac{R_0 - R_\infty}{1 + (j\frac{f}{f_c})^\alpha}, \tag{1}$$

where f_c is the characteristic frequency of the tissue related to its pseudo-capacitance C as $(R_0 - R_\infty)(2\pi f_c)^\alpha = \frac{1}{C}$. Alternatively, the model can be re-written as

$$Z(s) = R_\infty + \frac{R_0 - R_\infty}{1 + s^\alpha(R_0 - R_\infty)C} \tag{2}$$

The study conducted by [17] found that the relation between the model parameters R_0, R_∞ and the extra and
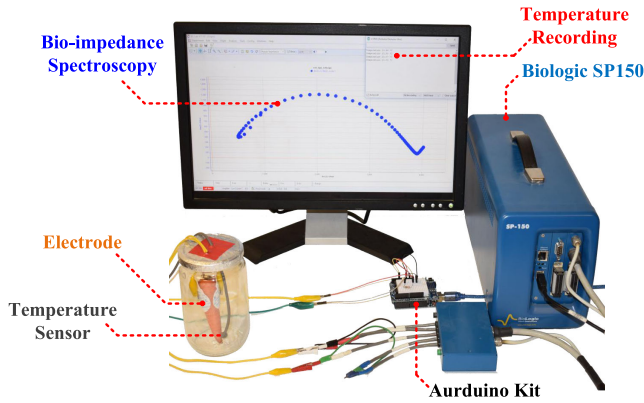
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FIGURE 4. Experimental setup.

intra-cellular resistances R_1, R_2 is

$$R_\infty = \frac{R_1 R_2}{R_1 + R_2} \quad and \quad R_0 = R_1. \tag{3}$$

Also, the relation between the fractional order capacitive impedance $Z_C = \frac{1}{s^\alpha C}$ in Fig. 3(b) and the membrane transmission line impedance Z_1 shown in Fig. 3(a) was given in [17] as:

$$Z_c = \frac{Z_1}{(1 + \frac{R_2}{R_1})^2}. \tag{4}$$

The techniques employed to fit the Cole model parameters to the measured data can be classified into deterministic techniques (also known as gradient-based techniques) and stochastic methods [21]. One of the conventional optimization techniques widely employed is the non linear-least square (NLS) technique [22], [23]. However, NLS fitting is not capable of handling outliers in the measured data, which affects the accuracy of the extracted parameters [21]. Meta-heuristic algorithms appear to be more suitable to solve optimization problems [24] particularly biologically inspired algorithms such as the Genetic Algorithm (GA) and Particle Swarm Optimization (PSO) [21]. The accuracy and effectiveness of a number of meta-heuristic optimization algorithms was tested in [25] particularly targeting the problem of bio-impedance parameters extraction. It was concluded in [26] that the Flower Pollination Algorithm (FPA) is the most efficient and outperforms all other tested algorithms.

The dispersion coefficient α is proportional to the quantity of air space within the cell such that α increases with the increase of inter-cellular air spaces [17].

In this paper, we study the effects of heating and freezing on the cole bio-impedance parameters. The paper is organized as follows: Section II describes the experimental setting and conditions of heating and freezing. Section III is dedicated to the heating effects on the bio-impedance studied via fitted impedance spectra and error diagrams where the resulting variation in the Cole model parameters are correlated the bio-chemical and bio-mechanical changes in the cellular strcuture. Section IV studies the effect of freezing on the parameter variation and corresponding cellular deformation.
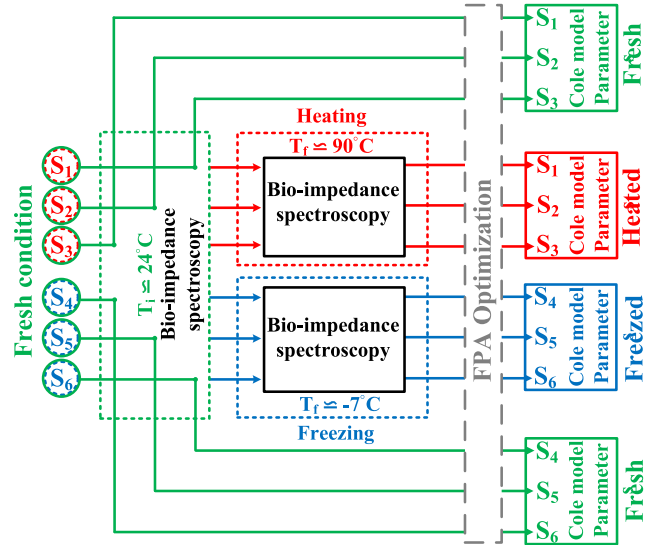

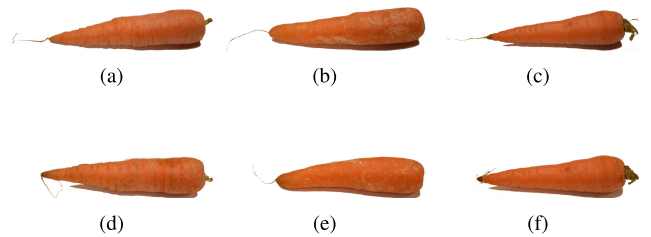**FIGURE 5. Illustration of the experimental procedure.**

FIGURE 6. Tested samples at fresh condition (a) sample 1, (b) sample 2, and (c) sample 3. Vegetables after being heated for 1 hour (d) sample 1, (e) sample 2, and (f) sample 3.

II. EXPERIMENTAL PROCEDURE

The effect of heating and freezing was conducted on six fresh carrot samples bought from the local market, with the same ripening time. Starting at room temperature, three samples were heated to approximately $90°C$ for 1 hour and the other three were frozen till reaching approximately $-7°C$ for 5 hours. The time period difference between the two tests is because the samples take low time period to reach the targeted high temperature, and a longer time period to reach a considerable low temperature. The impedance data was collected using a Biologic SP-150 electrochemical station (Manufacturer: Biologic) configured in potentiostatic mode with a sinusoidal excitation voltage of amplitude $500\ mV$ and no DC offset. The station was used to log the impedance real and imaginary parts over the frequency range 0.001–$500\ kHz$; which is a sufficiently wide range for this application. Considering magnitude representation of the bio-impedance, high impedance magnitudes are experienced at low frequencies as $1\ Hz$. Silver chloride electrodes with hydro-gel (Ag/AgCl) (Manufacturer: TopTrace) were applied to the samples in a non-penetrative way placed around the largest diameter of each sample to improve the measurement accuracy, with no special preparation conditions to measure the biological degradation experienced nominally. Each sample was kept in a cylindrical glass compartment, as shown in Fig. 4, which housed the electrodes of the device and a temperature sensor
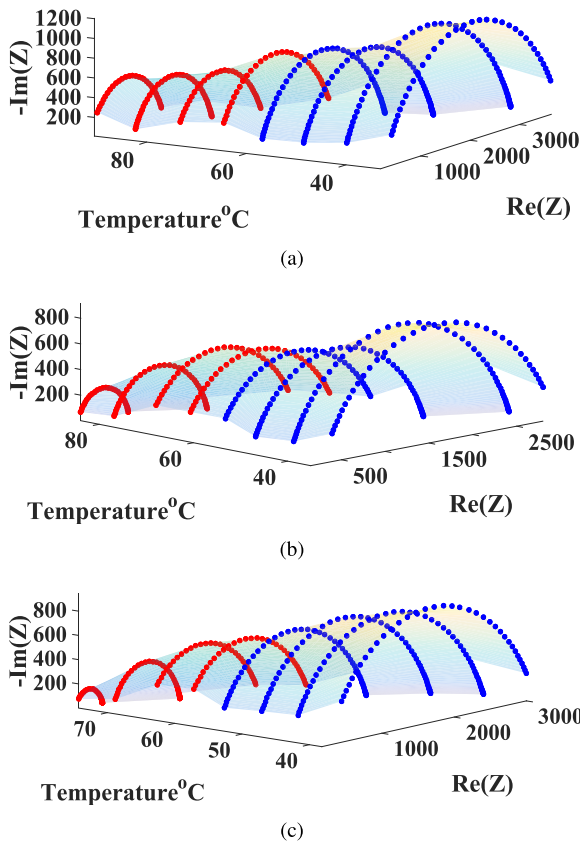
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FIGURE 7. Bio-impedance variation versus temperature increase during heating for (a) sample 1, (b) sample 2, and (c) sample 3.
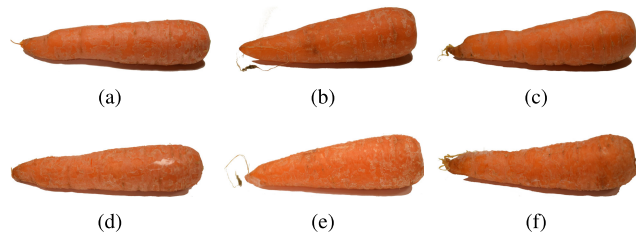

FIGURE 8. Fresh condition for (a) sample 4, (b) sample 5, and (c) sample 6 versus frozen condition for (d) sample 4, (e) sample 5, and (f) sample 6.

(Omega ON-909-44004). The heating process was performed by placing the samples while in the glass container into a heated water bath. Freezing was also done using a refrigerating facility in the same manner. The impedance data was imported into MATLAB to apply the optimization algorithm where 1000 independent runs were executed to extract the parameters of the Cole model. This was done on a PC with core i5-4590 CPU running at 3.30 *GHz* with 12 *GB* of memory. For the optimization routine to work properly, it is essential to define an objective function, the vector of optimized variables (X), the lower and upper boundaries of the search space, the population size and the iteration number [26]. The search vector is defined as $X = [R_0, C, R_\infty, \alpha]$, where the lower and upper limits are chosen to be $[1, 10^{-10}, 0, 0.4]$ and $[10^6, 5 \times 10^{-7}, 10^6, 0.95]$ respectively.
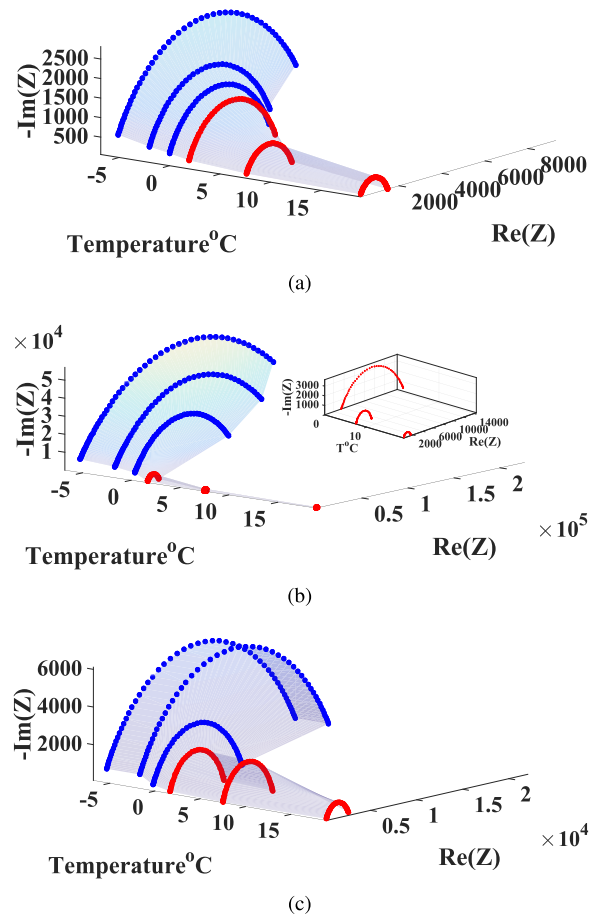
FIGURE 9. Bio-impedance variation versus temperature decrease during freezing for (a) sample 4, (b) sample 5, and (c) sample 6.

Selecting an optimization algorithm is crucial in order to have confidence in the extracted impedance model parameters. Therefore, five different meta-heuristic optimization algorithms namely the Flower Pollination Algorithm (FPA), Whale Optimization Algorithm (WOA), Grasshopper Optimization Algorithm (GOA) and the Grey Wolf Optimizer (GWO) were first tested on the fresh samples samples. The results were inline with our previous finding in [26] asserting the superiority of the FPA which has thus been adopted in this work. A complete schematic of the experimental process is shown in Fig. 5 while details of the FPA algorithm and code can be found in [25] and the references therein.

III. EFFECT OF HEATING

The three samples used in the heating experiment are shown in Fig. 6 before and after the experiment. When fresh, their weights were respectively 44, 38 and 53 grams while they were 43, 37 and 52 grams respectively at the end of the 1 hour heating process. The loss in water content is therefore in-significant. Table 1 shows the Cole model parameters obtained after applying the FPA optimization at nine different temperatures for each sample. The accuracy of the model compared to the measured data is shown in Tables 2, 3 and 4

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TABLE 1. Extracted cole model parameters for samples 1, 2, and 3 during the heating process.

	T °C	R_0 (Ω)	C (nF)	R_∞ (Ω)	α
Sample 1	24.86	3826.3566	62.696	251.8507	0.7542
	33.06	3672.9843	75.319	233.6227	0.7400
	41.26	2926.7799	78.197	207.7492	0.7515
	49.46	2771.9881	77.985	204.5997	0.7543
	57.67	2530.0207	72.292	191.1059	0.7646
	65.87	1955.5525	79.384	143.9506	0.7654
	74.07	1584.0120	83.386	106.8486	0.7658
	82.27	1317.8133	51.752	123.9984	0.8102
	90.47	664.7055	61.192	89.9357	0.8056
Sample 2	28.14	2934.7379	84.172	291.2503	0.7708
	35.11	2798.1976	85.414	281.2371	0.7707
	42.08	2195.1270	96.383	222.0268	0.7653
	49.08	2038.0131	114.527	191.5906	0.7520
	56.02	1939.7889	124.30	160.1759	0.7436
	62.99	1835.0568	126.72	137.0895	0.7394
	69.97	1217.7111	56.201	124.7658	0.8212
	76.94	690.5266	47.664	122.1269	0.8450
	83.91	407.3425	63.860	98.2246	0.8261
Sample 3	32.99	3124.3038	77.151	243.5483	0.7403
	38.15	2899.2524	85.420	218.7412	0.7328
	43.31	2646.2752	79.893	206.1627	0.7412
	48.47	2232.7722	77.566	176.4641	0.7474
	53.63	1922.0068	82.594	145.9747	0.7471
	58.79	1707.0273	93.855	114.4438	0.7375
	63.96	1093.1468	30.528	152.1518	0.8401
	69.12	499.3974	63.766	124.3499	0.8086
	74.28	315.1841	84.149	100.0134	0.7907

TABLE 2. Extracted cole model parameters for sample 1 at varying temperatures.

for the three sample respectively. An interesting "nonlinear" behavior is observed in all samples at higher temperatures (roughly above 58°C) in the very low frequency end of the spectrum. This nonlinear behavior results in the semi-circle behaviour shown, where the Cole model is in-adequate at these frequencies and hence increasing the fitting error. In all cases, the lowest error is obtained in the frequency range 1–100 kHz.

Figure 7 is a plot of the fitted spectra of the bio-impedance curves versus temperature increase, from which it is seen that the heating effect clearly decreases the overall impedance in all samples, which also reflects in the component values of the model given in Table 1. In particular, the low frequency resistance R_0 and high frequency resistance R_∞ both decrease significantly with increasing temperature. The parameter which is least affected by the temperature increase is the dispersion coefficient although it slightly increased towards higher temperatures.

The hardness of the thermally treated samples in Fig.6 experienced a decrease with heat increase. This softening

is a consequence of increased cell separation and reduced inter-cellular adhesion, which is justified by the degradation of the pectic polysaccharides [27] and rise in water

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TABLE 3. Extracted cole model parameters for sample 2 at varying temperatures under heating treatment.

	T °C	Graph	
		Fitted Spectra	Error%
Sample 2	28.14		
	35.11		
	42.08		
	49.05		
	56.02		
	62.99		
	69.97		
	76.94		
	83.91		

TABLE 4. Extracted cole model parameters for sample 3 at varying temperatures under heating treatment.

	T °C	Graph	
		Fitted Spectra	Error%
Sample 3	32.99		
	38.15		
	43.31		
	48.47		
	53.63		
	58.79		
	63.96		
	69.12		
	74.28		

TABLE 5. Extracted cole model parameters for samples 4, 5 and 6 using FPA during freezing treatment.

	$T\,^{\circ}C$	$R_0\,(\Omega)$	$C\,(nF)$	$R_\infty\,(\Omega)$	α
		Extracted Cole model parameters			
Sample 4	24	1493.6072	187.4183	148.6727	0.7542
	18.94	2467.0427	170.5614	222.2640	0.7465
	7.45	4569.7533	104.2005	348.1004	0.7600
	1.45	5385.5050	92.6522	478.2280	0.7502
	−0.83	6566.2340	97.0016	581.9604	0.7336
	−3.13	9880.4858	104.8756	790.3482	0.7069
	−6.81	28707.3643	93.0018	1928.7849	0.6710
Sample 5	24.43	1625.5275	123.8805	135.9591	0.7840
	18.94	3288.0373	80.6043	244.6213	0.7972
	7.45	14699.6385	92.4889	1970.5120	0.6654
	1.47	125209.8107	62.9286	11278.9266	0.5879
	−0.83	200760.5555	64.3288	12767.0455	0.5529
	−3.13	270463.8374	64.3741	12576.1135	0.5307
	−6.81	385602.3539	67.8282	8436.5779	0.4963
Sample 6	26.06	2739.5302	80.9763	132.1599	0.7813
	18.94	5705.6060	61.3464	235.2491	0.7914
	7.45	6314.4717	60.2446	372.4826	0.7676
	1.45	10620.7726	86.7979	679.0244	0.6964
	−0.83	22732.7484	84.9857	1304.0516	0.6605
	−3.13	63676.1726	64.3205	3131.6690	0.6364
	−6.81	254935.2078	48.3268	5534.8939	0.5856

soluble pectin [8]. The softening effect is however not associated with cell breakage because the forces holding the cells together are still strong [8]; which is reflected in bio-impedance by the relatively unchanged value of α. However, thermally treated samples experience a substantial increase in the amount of water soluble pectin [28], [29]. This fact is the main reason behind the over-all decrease in impedance as temperature increases.

It is also observed that the decline in low frequency resistance R_0 is more steady than the decline in the high frequency resistance R_∞. This can be explained by the fact that the intra-cellular resistance is more subtable to changes induced by heat than the extra cellular resistance. The cellular membrane increased solubility [28] accompanied by cell wall thickness deformation [30] affect the value of the pseudo capacitance C which seems to increase with temperature up till a certain point after which it drops significantly. The point at which a significant drop in capacitance is observed is also the point at which α shows an increased value. From Table 1, this is seen to happen between $74^{\circ}C$ and $82^{\circ}C$ for sample 1, between $63^{\circ}C$ and $70^{\circ}C$ for sample 2 and between $59^{\circ}C$ and $64^{\circ}C$ for sample 3. Based on the relation between the dispersion coefficient α and the inter-cellular air space experimented by [17], the slight increase in the dispersion coefficient α at higher temperatures is attributed to the increase of air spaces within the cellular structure due to the increased cellular separation [8].

TABLE 6. Extracted cole model parameters for sample 4 using FPA under freezing treatment.

	$T\,^{\circ}C$	Fitted Spectra	Error%
		Graph	
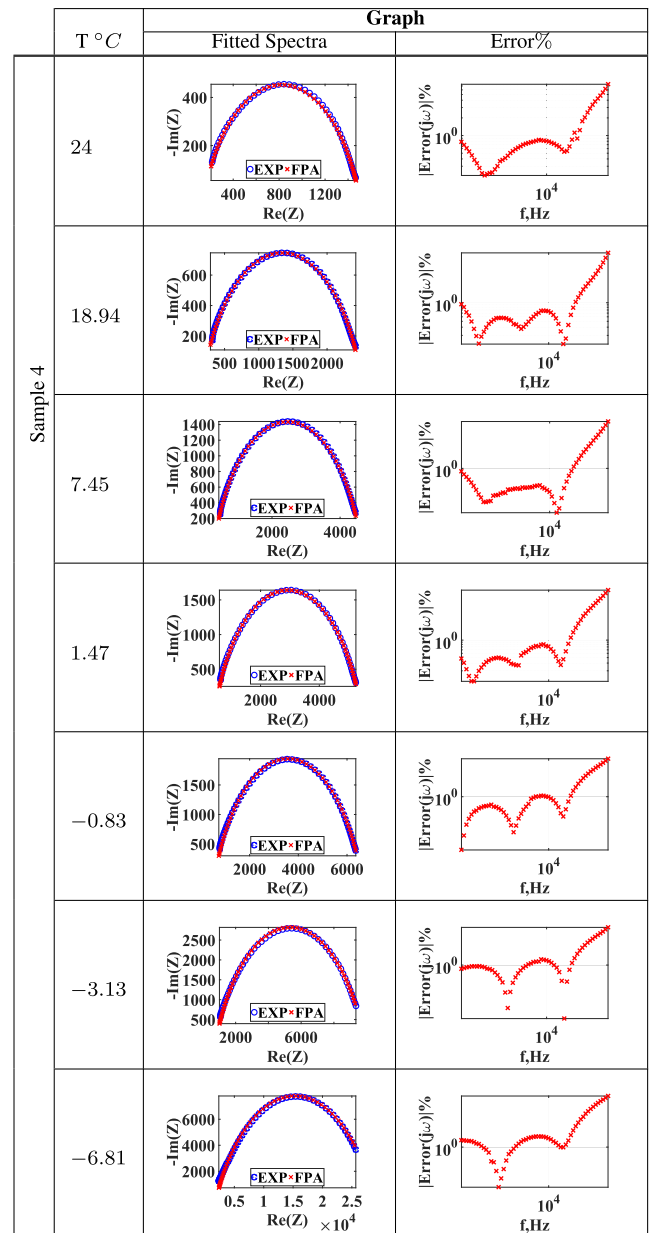
IV. EFFECT OF FREEZING

The carrot samples in the glass container were placed in a refrigerating facility with continuous data acquisition for 5 hours. Temperature measurement was recorded versus bio-impedance for each sample while its temperature declined till reaching approximately $-7^{\circ}C$. The bio-impedance was acquired at seven descending temperatures and then processed by the FPA optimization technique to extract the Cole impedance parameters given in Table 5. The accuracy of the fitting is depicted in Tables 6, 7 and 8 for the three samples respectively. Below $-3.13^{\circ}C$, all samples tend to exhibit a second-dispersion at high frequencies reflected as a second semi-circle in the impedance Nyquist plot. This second dispersion effect can be addressed by using a double dispersion

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TABLE 7. Extracted cole model parameters for sample 5 using FPA under heating treatment.

		Graph	
T °C	Fitted Spectra		Error%
24.43			
18.94			
7.45			
1.47			
−0.83			
−3.13			
−6.81			

(Sample 5)

TABLE 8. Extracted cole model parameters for sample 6 using FPA funder heating treatment.

		Graph	
T °C	Fitted Spectra		Error%
26.06			
18.94			
7.45			
1.47			
−0.83			
−3.13			
−6.81			

(Sample 6)

Cole model despite the single Cole model presented. The fitted spectra show the lowest error in frequency range 100 Hz to 10 kHz. The plot in Fig. 9 show the fitted bio-impedance curves versus temperature decrease. Overall, freezing is shown to increase the impedance of all samples with a sharp increase in impedance.

The frozen state of the three samples is shown in Figs. 8(d), 8(e) and 8(f) exhibiting hardened texture, where plant cells freeze when they cannot avoid nucleation [7]. The cell structure disintegrates during freezing, where the membrane structure is damaged when freezing-induced dehydration exceeds the dehydration-tolerance of a cell [6]. The decline in temperature causes the low frequency resistance R_0 to surge as a result of the extracellular resistance experiencing nucleation of ice causing hardening and damage

to the tissue. The high frequency resistance R_∞ also experiences a significant increase signaling the hardening of the intracellular resistance. Due to the intracellular structure solubility, it is affected earlier than the extracellular structure. The decline in the capacitance with temperature signifies the freeze damage to the cellular structure through the deformation in the cellular membrane [17]. Meanwhile, the steady decrease in the value of the dispersion coefficient α reflects the decrease in the air space within the cellular structure due to the nucleation of water content and hardening of cellular walls [6].

V. CONCLUSION
The samples undergoing heating process experienced a gradual decrease in impedance versus increasing temperature.

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The extracted parameters of the Cole model experienced changes corresponding to the amount of cellular damage, where the low frequency resistance R_0 and high frequency resistance R_∞ decrease showning decrease in extracellular and intracellular resistance which reflects increased pectin solubility. The increase in the dispersion coefficient α is related to the increase in inter-cellular air spaces. The increase and then sudden decrease in capacitance signals an important point of transformation in the specific membrane and its soluble content. The samples experiencing freezing damage, showed increasing impedance in visible jumps to large values, depicting cellular damage and related to formation if ice. The extracted low frequency resistance R_0 and high frequency resistance R_∞ both sharply increase depicting an increase in extracellular and intracellular resistance. The delayed increase in R_∞ compared to R_0 reflects the fact that intracellular resistance is more subtable to low temperatures. The steady decline in the value of α is related to the decrease in inter-cellular air spaces with a corresponding decrease in capacitance.

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