Characterization of Fruit juices treated with Electrical Pulses

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***Abstract* - Non-thermal pasteurization of fruit juices, such as orange juice using electrical pulses is gaining momentum due to their attractive attributes, such as less heating and more retention of taste, flavor and color. Using electrical pusles of suitable intensity (V/cm) and duration or pulsewidth (milli/micro/nano seconds), it is possible to pasteurize the juices to enhance their longevity (at room temperatures). This paper presents the results of a juice study using milli and micro pulses. Multiple pulses with optimal intervals are in general used in these applications to reduce the heat generated. Various juices including orange and lime were tested in this study. Analyses were made using microbial study and impedance spectroscopy measurements of the pulsed and the unpulsed (control) juices. Preliminary results indicate that there is a reduction in the amount of Lactic acid bacterial count of the pulsed orange juice compared to the unpulsed orange juice. Impedance spectroscopy measurements also showed difference in impedance values between the pulsed and unpulsed juices. This study will shine more light on the characteristics of electrically pulsed juices and extending their shelf-life.**

I. INTRODUCTION

The application of alternative techniques to process liquid food, such as fruit juices is gaining momentum. This is due to the drawbacks of the conventional thermal processing, such as generation of heat that destroys the true nature of the juice, its taste, color, and flavor [1-8]. Low energy techniques, such as pulsed electric field (PEF) are attractive due to their low energy use, less heat generated and retention of original taste, color, and flavor. In PEF technique, high intensity, short duration pulses are applied to inactivate the microbes in the juice. Various electric field intensities are used by several researchers. Inactivation of naturally grown microorganisms in orange juice was studied using 16.7 to 46.7kV/cm pulses by El-Hag et al [2]. Field strengths of 25 t 35kV/cm for durations up to 1000us square wave pulses of 4us width at 200Hz frequency were used by Elez-Martinez et al [3]. These high electric fields generate heat which could be used for further inactivation of the microbes. Also, they are costly. In this study, we intend to use low electric field intensities, such as 1250V/cm pulses for longer durations and still effectively inactivate the microbes. This paper furnishes the results obtained so far.

II. MATERIALS & METHODS

Milli and micro second pulses were used in our study at very low field strengths. For this purpose a commercial BTX ECM 830 pulser was used. The main process parameters that determine pulse electric treatments are electric field strength, shape and width of the pulse, treatment time, frequency, specific energy and temperature [1-5]. In our study, two different pulses were applied to treat fresh lime and orange juice. The parameters of applied pulses are shown in Table I. The electrode gap was 0.4cm. In addition, some samples were pulsed with 20 pulses of 500V, 5ms pulses and their microbial counts were studied.

Fig.1: BTX ECM 830 Pulser used in the research

TABLE I
PULSE PARAMETERS APPLIED

	# Pulses	Pulse Length	Voltage (V)	Electric Field (V/cm)
BTX01	30	100μs	1000	2500
BTX02	30	1ms	500	1250

III. RESULTS & DISCUSSION

A. Microbial count study

Freshly squeezed orange juice were pulsed with twenty 500V, 5ms pulses and were analyzed for microbial counts in the Food Science Dept. Table II gives the results.

"Lactic acid bacteria" or "lactics" are Gram-positive (having a thicker cell wall (peptidoglycan laers) than gram-negative), non-sporeforming microaerophilic bacteria that produce mainly lactic acid from fermentable carbohydrates (Fig. 2) [8]. Lactic acid bacteria are ubiquitous in nature and can grow in foods with available carbohydrates, vitamins, minerals and amino acids, and low oxygen tension. They are important in the manufacture of fermented foods or in the spoilage of foods due to metabolic activity. Lactic acid bacteria are normal flora in the orange juice, and it has no health hazards. Their metabolic activity of converting glucose to lactic acid through homofermentation, bifidus pathway and heterofermentation causes the spoilage of juice and lead to the end of shelf-life. In the juice, lactic acid is the metabolic product of lactic acid bacteria. It is a normal composite (0.5g/l [9]) in orange juice.

TABLE II
MICROBIAL CONTENT OF FRESHLY SQUEEZED ORNAGE JUICE

Item	Unpulsed juice	Pulsed juice
General microbial count (acid tolerant bacteria, molds and yeasts)	8.0 x 10^8	7.7 x 10^8
Lactic acid bacterial count	1.7 x 10^7	1.1 x 10^3

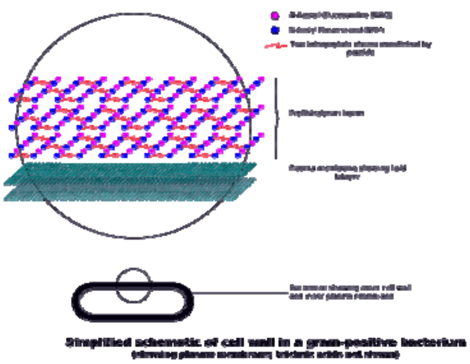

Fig. 2: Simplified schematic of a gram-positive bacterium cell wall [9]

The pulsed juice has less lactic acid bacterial count than the unpulsed juice by five orders of magnitude while the general microbial count (acid tolerant bacteria, molds and yeasts) is almost the same for both juices, pulsed and unpulsed. This indicates the significant effect of the low intensity electrical pulses on the lactic acid bacteria in the fresh orange juice. The juice was pulsed at room temperature immediately after squeezing. The samples were analyzed after 5 days for their microbial count.

When exposed to electric field, transmembrane potential of the cell membranes increase because more free charges accumulate at the membrane surfaces. These charges are opposite and attract each other resulting in membrane compression and therefore, membrane thickness is reduced. When the transmembrane potential reaches a special voltage (about 1V), the electrical membrane breakdown occurs. The threshold electric field intensity at which membrane breakdown occurs is called critical electric field. Due to an external electric field close to or greater than the critical electric field, harmful microorganisms are destroyed without major changes in the chemistry of the food. The treated juice samples keep it fresh longer than the unpulsed.

B. *Effect on physical parameters of juice*

The pulsed electric field treatment of juice is to inactivate microorganism at non lethal temperatures to avoid thermal effects on the juice properties. However, pulsed electric field also increases the temperature of the juice slightly, since there is energy absorbed in these treated juice. The various physical parameters including temperature were measured immediately after pulsing the juice. The other properties measured are pH, conductivity, and salinity. Table III shows the results. The temperature of the juices treated by the electric fields is slightly higher than the temperature of juice control. This increase could be beneficial as it would work synergistically in inactivating the microbes without affecting the characteristics of the juice. The pH of the orange juice decreased slightly enhancing the acidic nature of the juice, while the conductivity and salinity also reduced slightly. They were slightly increased for lime. Similar results were observed by previous researchers [4].

Table III
Measurements of Temperature, PH, Conductivity (σ), and Salinity

	Temperature, °C	**pH**	**σ (mS)**	**Salinity (S)**
Lime Control	22.6	1.96	4.23	2.11
Lime BTX01	22.9	2.11	4.27	2.10
Lime BTX02	27.1	2.17	4.12	2.04
Orange Control	22.5	3.79	3.19	1.57
Orange BTX01	22.9	3.68	3.02	1.56
Orange BTX02	27.5	3.61	2.57	1.39

C. *Impedance Spectroscopy study*

Impedance Spectroscopy (IS) is an ac measurement technique in which the ratio of voltage and current is measured over a range of frequencies. It is a non-invasive, nondestructive test technique in which very little energy need be dissipated by the system under test, leaving it virtually unaffected. This method is useful to characterize cellular changes quantitatively. It can be used as a method of identifying and following detectable cellular responses, in ex vivo, in vivo and in vitro [10-12]. IS measures the electrical properties of any material, i.e. the conductance (or resistance) and the reactance as a function of applied alternative current (ac) frequency. Cole-Cole plot (resistance vs reactance) is used to arrive at RC equivalent circuits and their values [10]. The main advantage of this technique is that it can provide information on the state of material.

In this study, it was used to characterize the impedance changes due to applied pulses. Autolab PGSTAT 12 impedance analyzer was used. It gives 40 data points for a frequency range from 0.5Hz to 100kHz. A sine voltage of 25mV is applied and the data were collected from 46Hz to 10kHz. A 0.4cm electrode gap was used for this measurement and the volume of the juice was about 7ml. Figs. 3 and 4 show the variation of the resistance and reactance of pulsed fresh lime and orange juices with respect to the unpulsed juice (control). The pulsed lime juices have higher resistance than the control (unpulsed, c) (Fig. 3a). Here, 01 and 02 correspond to pulses BTX01 and 02 in Table 2. Both the pulsed and the unpulsed samples follow the inverse power law characteristic of dielectric samples [11]. Similar results were obtained for reactance too except that sample 02 has magnitudes almost same as control. However, the orange juice samples showed a lower resistance than the control and almost the same reactance as the control, while still exhibiting the inverse power law. This variation in trends of the lime and orange juices will be studied further to understand the mechanism behind it.

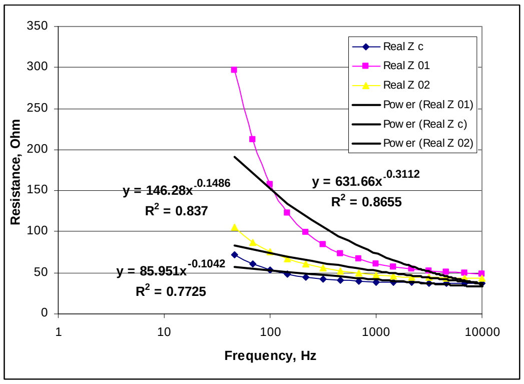

Fig. 3a: Resistance variation with frequency – Fresh Lime juice

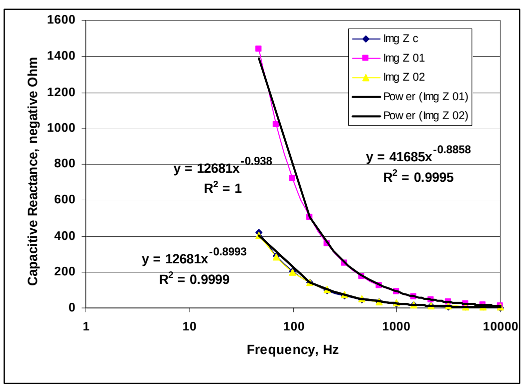

Fig. 3b: Reactance variation with frequency – Fresh Lime juice

Figs. 5 and 6 show the resistance vs reactance plots for various frequencies (Cole-Cole plot) for the first 5 days of juice pulsing. Each point in the graph indicates a frequency. Normally, the impedance magnitudes are lower at high frequencies and larger at low frequencies [10, 11]. Thus, the points on the left correspond to data at high frequencies where there is not much change.

The trend of variation of the control and the pulsed juices is similar except for those samples pulsed with BTX02 pulses for both lime and orange juice. In this case, one ms pulses at 1250V/cm used compared to 100μs pulses 2500V/cm in the case of BTX01. Both the lime and orange juice samples show a significant reduction (from 400Ω to 250Ω (Fig. 5c, lime) and from 650Ω to 400Ω (Fig. 6c, orange) when BTX02 pulses were used. BTX01 pulsed lime sample shows an increase in Xc (Fig. 5b). These data also show the difference in the lime and orange juice impedance magnitudes illustrating the difference in the cellular structure and content of these juices. Further work is in progress to better understand the underlying mechanisms.

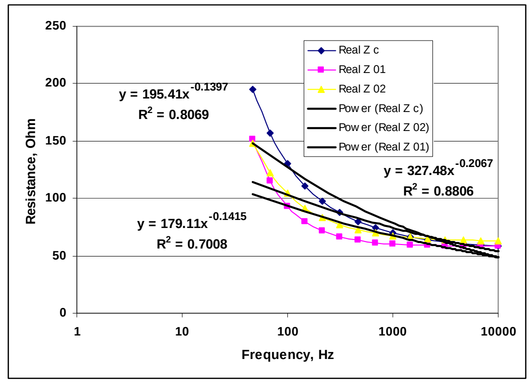

Fig. 4a: Resistance variation with frequency – Fresh orange juice

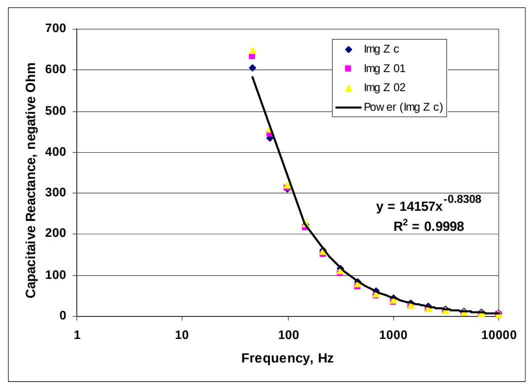

Fig. 4b: Reactance variation with frequency – Fresh orange juice

IV. CONCLUSIONS

The inactivation of naturally occurring microorganisms in freshly squeezed orange and lime juices using low intensity, low energy PEF treatment at 1250V/cm and 2500V/cm at 1ms and 100μs respectively was studied. There was a 5 log decrease in the lactic bacteria content in one of the orange juice samples, pulsed at 1250V/cm, 5ms (20 pulses). Impedance Spectroscopy data showed reduction in the resistance and reactance magnitudes in all the cases except one (Lime BTX01). While more work needs to be done, the results show the promise of the use of low voltages for inactivation of naturally occurring microbes in juices.

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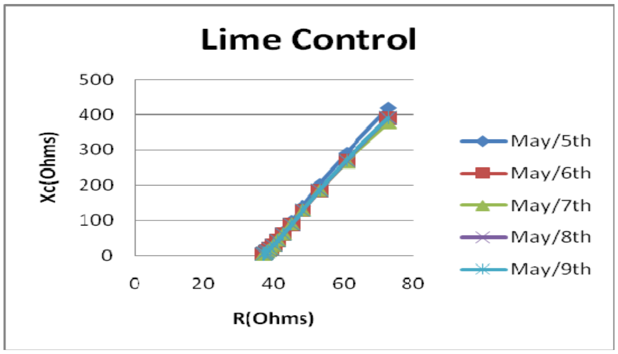

Fig. 5a: Cole-Cole plot comparison of Lime control for different days

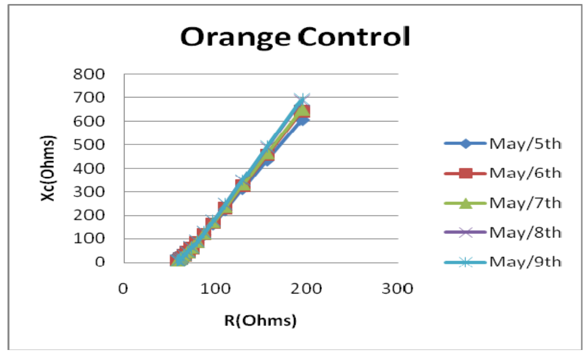

Fig. 6a: Cole-Cole plot comparison of Orange control for different days

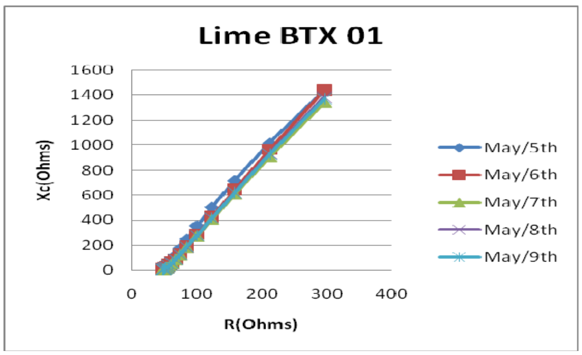

Fig. 5b: Cole-Cole plot comparison of Lime 01 for different days

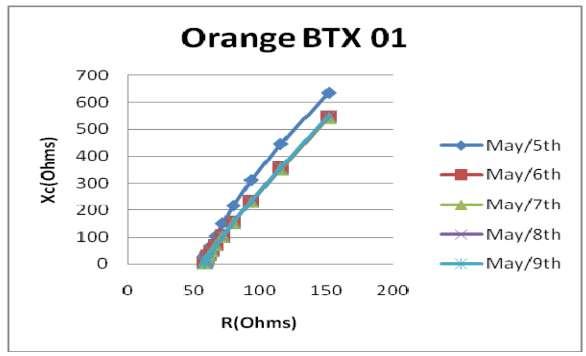

Fig. 6b: Cole-Cole plot comparison of Orange 01 for different days

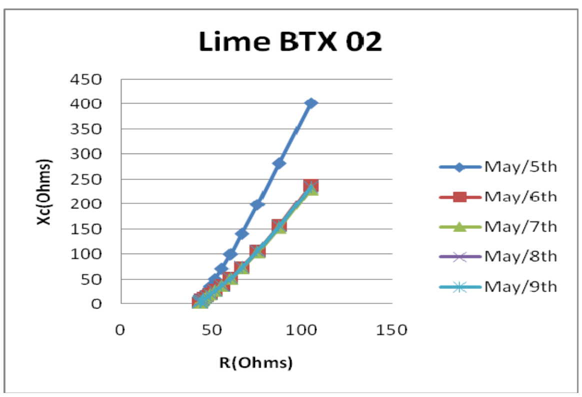

Fig. 5c: Cole-Cole plot comparison of Lime 02 for different days

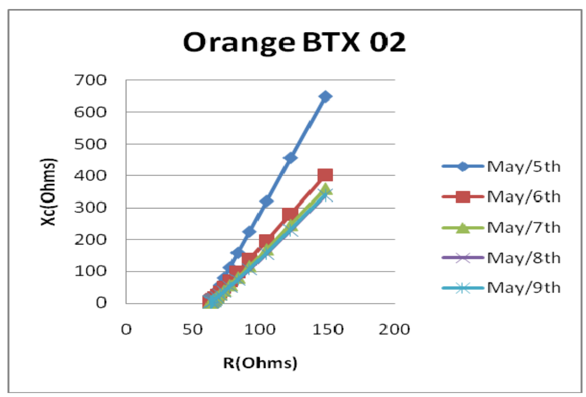

Fig. 6a: Cole-Cole plot comparison of Orange 02 for different days

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