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Kinetics of Inactivation of *Listeria monocytogen*, *Clostridium perfringes*, *Escherichia coli* and *Salmonella* spp. in Ohmic Heated Tomato Juices

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Lycopersicum esculentum **(tomato) were obtained, washed and processed into juice. The juice was assayed for microbial contamination, and then divided into 4 equal parts of 250ml each. The portions were sterilized and inoculated with prepared samples of *Listeria monocytogen, Salmonella* spp, *Clostridium perfringes* and *Escherichia coli* respectively, then allowed to stand for 24 hours. assayed for growth of the inoculated bacteria. The samples with growth were ohmic heated using copper/aluminum electrodes at 70°C and 100°C for periods of 0,5,10,15 and 20 minutes respectively. After each treatment, the bacterial survivors were monitored. Data obtained were used to calculate kinetics parameters of pathogen destruction. Result showed a significant difference in bacterial survival ($P<0.05$) per temperature, heating time and pathogen type. *E. coli* had the less survivors with a z value of 4C, D value of 4 minutes while Salmonella the highest survivors (log 2.590) at 100°C, but at 70°C more Clostridium survived. The D values varied from 3-1mins. for *E. coli,* 8-5 for *Listeria monocytogen*, 5-3.5 for *Salmonella* and 9.5-4.5 for *Clostridium perfringes* at 70-100°C. Various z values indicated significant difference($P<0.05$), where *Salmonella* had 20°C, *Clostridium* and *Listeria monocytogen* -10°C, while the least was 4C in *E. coli* Inactivation energy was least in *Salmonella* (583.84), highest in *E. coli* (2130.2J/mole). The model was effective in total destructions of the pathogens at 20 minutes irrespective of the type.**

Key words: Ohmic heating, Pathogens, Destruction, Kinetics and Tomato-juice.

New processing technologies are constantly being developed which increase the range of options within each unit operation. Major goals of food scientist and processing engineers are to develop new methods, which hold promises for better products, such as Supercritical fluid extraction, Ohmic heating, pulse electric field (PEF), and high pressure processing (HPP) (Potter and Hutchkiss 1995).

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Onwuka *et al.,* (2006) have reported that Electrical heating has taken upper hand in modern processing methods. Ohmic heating, PEF and HPP are mostly geared toward sterilization of liquid foods. This is so because the use of conventional heating methods to destroy microorganism can cause undesirable effects in food texture, flavour and color due to excessive heating for commercial sterilization to be attained. Microbial contamination of food products by pathogens is a major concern of our society (Murphy *et al.,* 2004). The projected cost in relation to *Listeria monocytogens* alone was estimated at 3 million per year in USA (Kanuganti

et al., 2002). Publicity surrounding high-prolific poisoning incidence subjects both government agencies and industries to external pressure to identify and control potential hazards caused by microbial contamination.

Escherichia coli 0157: H7, *Salmonella* and *Listeria monocytogens* are frequently associated with food borne out breaks (Angulo and Swerdlow, 1998; Arnold 1998, Mead *et al.,* 1999, Jay 2000, Bailey *et al.,* 2002) *E. coli* becomes a food safety concern because the pathogencity, the infection dose (< 50 organisms), the severity of the illnesses caused by *E. coli* 0157:H7; and the nature of the susceptible population most affected by its infection (Mackey and Gibson 1997). *Salmonella* is a leading cause of gastroenteritis in human, while *Listeria monocytogen* is accused of *listeriosis* (Jay 2000).

Most often, we are forced by circumstances to take fresh fruit juice. Unpasteurized apply juice, alfa alfa sprout, salmi, lettice, swimming water also have been implicated for *E. coli* outbreak. Apple juices and apple cider, citrus and tomatoes are considered as acidic food with pH values 3.4-4.0. Out breaks of *E. coli* associated with the consumption of fresh apple juice or cider and citrus juice illustrate the acid tolerance of this pathogen (Basser *et al.,* 1993) CDS 1996, 1997). The US food and Drug Administration requires a 5-log, pathogen reduction of *E. coli* 0157. H7 in fibre juices without specifying the use of say particular treatment (FDA 2001).

The effectiveness of leaf treatment (restoration) for eliminating *E. coli* 0157: H7 in apple juice have been demonstrated (Splittertoessen *et al.,* 1996). A 5-log reduction of acid -adapted and non-acid adapted *E. coli* 0157: H7 strain was achieved at 65. 1°C for 14 seconds of thermal treatment of apple juice (Mak *et al.,* 2001) but the come up time was high and colour changes and texture deterioration were evident. The temperature of about 96°C have been achieved through pulse electric field pasteurization (Braakman 2003) PEF treatment increases the shelf life of juice products while reducing the loss of their flavor, color and nutrients (Jim and Zhang 1999, Evren Dilek *et al.,* 2000). Investigation on the effect of PEF on the enzymes responsible for quality alteration of food during storage is needed for commercialization of the PEF processing (Raso *et al.,* 1998).

This is because most enzymes withstand the temperatures of 100°C at short time treatment. Ohmic heating uses electric power and transform it into heat energy. When an alternating electric current is passed through a food, it heat up the system due to food's inherent electrical resistance. Here heat penetration is throughout the food is rapid and uniform.

The major advantage of this technology is the much more rapid increase of temperature of the liquid phase compared to the solid phase. This makes it an attraction technology for high-temperature short time sterilization; commercial sterile food with high level of flavour retention and particular integrity has been obtained. The additional advantage of ohmic heating is low frequency (50-60 Hz), which allow cell walls to build up charges and form pores.

The presence of pore-forming mechanism on cellular tissue has been confirmed by recent work (Imai *et al*., 1995; Wang 1995; Kulshrestha and Sastry 1999).

Another recent study (Cho *et al*., 1999), conducted under near identical temperature conditions, indicates that the kinetic of inactivation of *Bacillus subtilis* spores can be accelerated by ohmic treatment. Lee and Yoon (1999) have reported that leakage of intracellular constituents of *Saccharomyces cerevisiae* was enhanced under ohmic heating as compared to conventional heating in boiling water.

Based on these reports, we have considered the inactivation of *L. monocytogens, E. coli, Salmonella spp* and *Clostridium perfringes* in tomato juice using ohmic heating model to determine the kinetics of inactivation of these pathogen for safety of our foods.

MATERIAL AND METHODS

Materials

Good quality ripened fresh tomatoes were obtained from Umuahia main market in Abia State, Nigeria. Other materials were obtained from Microbiological Laboratory of Food Science, Michael Okpara University of Agriculture, Umudike Nigeria.

Methods

Juice Preparation: The tomatoes were washed with potable water, pulped using a kitchen type blender (keen wood), then pressed out manually through a muslin cloth and the juice collected in a measuring cylinder.

Bacterial Preparation

Escherichia coli

Loop full of urine– cathel specimen was cultured on a MacConkey agar for 24 hours at the temperature of 37°C. This developed an impressive number of red-lactose fermenting colonies of Gram-negative rods. Subculture were made into plates of sterile lytophene buffered glucose-protease peptone medium, and then incubated for 24hours at 37°C. This gave a luxuriant yield of *Escherichia coli*, which was then stored in the plate (method adopted from Adams and Moss 1995).

Clostridium perfringes

Lean meat bought from the market was boiled for 30mins, and cooled to room temperature at 27°C. It was kept on the shelve for 48hours, then the meat pieces were spread on a solid sterile MacConkey medium for 24 hours at 37°C in a temperature controlled incubator. A surfacing inoculums build-up of *Clostridium perfringes* were obtained and stored on the plates.

***Salmonella* sps.**

Payers' patches from mucosal membrane of bovine small intestine were obtained from butchers at Umugbalu Ikwuano L.G.A of Abia state, Nigeria and cultured in a MacConkey agar for 24 hours at 37°C. A developed floral colony was sub-cultured into plates of Bismuth, Sulfate agar (a method of Wilson and Biar) (Adams and Mass 1995), incubated at 37°C for 24 hours. A moderato growth of *Salmonella* flora was obtained as view on the microscope.

Listeria monocytogen

A milk sample was obtained from weaning goat, then exposed to open air for 8hours and cultured in nutrient agar bacterial colony of human serum enriched MacConkey medium. Incubated at 27°C for 24 hours. This culture yielded a moderate growth of *Listeria monocytogen*.

Inoculation of pathogens into tomato juice samples

Suspensions of different microbial groups were prepared using one loopful of the inocula from different culture plates and 10ml of physicals and 100ml of physiological saline (0.9%Saline) in sterile test tube. 5ml portion of each properly homogenized suspension were taken with sterile strings and transferred with one liter freshly prepared tomatoes juices without any heat treatment or preservative. Each container was labeled appropriately with the name of the microbial flora incubated in it, while sample without any microbe stands as control. Before inoculation, the juices were sterilized and assayed for microbial counts. The inoculated samples were allowed to stand on shelve for 48hours for development and adaptation of the pathogen in their substrate.

Ohmic heating

After 48 hours, the inoculated tomato juice sample for each microflora type was divided into 4equal parts of 250ml each and then subjected to ohmic heating at 90/110volt AC for various temperature of 70°C or 100°C for a period of 5, 10, 15, 20, minutes. In each case, the temperature attained were recorded by a sample laboratory thermometer, the come-up time at each case before attaining the highest temperature was 4 minutes. The samples were immediately placed in an ice water bath and cooled to 25°C.

Bacterial enumeration

The quantity of the different microorganisms present in 1ml portion of each bacterial suspension were determined using counting chamber, subsequently the number of bacterial cell present in 5ml sample of microbial suspension was calculated and recorded at each treatment level or times.

Ohmic apparatus

Ohmic heating apparatus was fabricated on a plastic cylinder with copper and aluminum plate as electrodes and connected through multi tester type Sunwa YX21B and a variable voltage transformer was attached for varying voltages in the system with help of a current regulator for selecting the desired voltage at a time. The variable voltage transformer was locally fabricated and calibrated using a multi tester meter type YX21B (Sunwa Electric Instrument Co. Japan), while the ohmic apparatus was calibrated by measuring the maximum temperature attained with a thermometer and temperatures of 96°C and

above was attained which conforms to the report of Braakman (2003) for an ohmic system.

Data analysis

Data obtained were subjected to 3 Factorial Complete Randomize designed analysis using ANOVA (Excel 2000), means separated by Duncan multiple range test at each maximum temperature attained by each treatment time at a constant voltage of 110Vor 90V AC, $\log_{10}(N_1)$ of *E. coli, Salmonella, L. monocytogen* or *C. perfringes* was plotted against heating times. N_1 represent the colony count in cell/ml for *E. coli. Salmonella, L. monocytogen* or *Clostridium* and t represent treatment time in minutes, $\log_{10}$ (Ni) was the response, pathogen types were the explanatory variable and t was the co-variable. The data was fitted to the linear models (Murphy *et al,* 2004) as follows:

$$\text{Log (Ni)} = a + b\delta + C\,(t) \qquad ...(1)$$

where i= 1,2,3 or 4 Corresponds to the pathogen type *E. coli, Salmonella*, *Listeria* and *Clostridium* respectively and δij = 1 if i = 1 or 0 if i =j.

Similarly $\log_{10}$ (D) of *E. coli,* and *Salmonella*, *L. monocytogen* or *Clostridium* was plotted against heating temperatures. D represents the decimal reduction time (mins) for the pathogens and T represents the temperature (C). Log_{10}(D) was the response, the pathogen type was the explanatory variable and T was the co-variable. The data was fitted to the linear model;

$$\log 10\,(D) = a + b\delta ij + c(T) \qquad ...(2)$$

The results obtained by these plots were compared with the result obtained using first order kinetics of degradation ln

$$Ni = -K_E t \qquad ...(3)$$

Where Ni = N/No.

N – final count of pathogens after treatment. No – initial count at zero time. K_E – first order kinetic.

$$D = 2.2003/K_E \qquad ...(4)$$

D-one log reduction time for the pathogen type at a particular temperature.

The activation energy (Ea)(J/mol) for each pathogen type was calculated from the relation

$$\ln (K_E) = - Ea/R\,(1/T) \qquad ...(5)$$

where R – is the universal gas constant (8.3144 $J/molk^{-1}$) and T is the ohmic temperature (k). Z value for each pathogen was derived from the

Table 1. Effect of time of heating, temperatures of heating and pathogen type on survivors

Pathogens	Temp. (°C)	Heating time/survivors (log) (mins.) 0	5	10	15	20
Listeria monocytogen	70	4.262^{c}	3.453^{e}	3.0500^{w}	2.425^{j}	0.00^{m}
	100	4.262^{c}	3.346^{f}	2.105^{k}	0.00^{m}	0.00^{m}
Escherichia coli	70	4.420^{b}	2.572^{c}	0.00^{m}	0.00^{m}	0.00^{m}
	100	4.420^{b}	1.125^{b}	0.00^{m}	0.00^{m}	0.00^{m}
Salmonella spp.	70	4.835^{a}	3.060^{h}	2.590^{j}	0.00^{m}	0.00^{m}
	100	4.835^{a}	3.050^{h}	2.590^{i}	0.00^{m}	0.00^{m}
Clostridium perfringes	70	4.405^{b}	3.550^{d}	3.385^{f}	2.895^{l}	0.00^{m}
	100	4.405^{b}	3.202^{g}	1.102^{b}	0.00^{m}	0.00^{m}
LSD	0.06391					

Data are means of duplicate determination, a.b.c.- means with the same superscript along rows and across columns are significant different at P<0.05.

graph plot of $\log_{10}$ D against temperature.
F value was calculated using the formula

$$F = D \log {}^{No}/_{Ni} + \text{"} \, dt \quad ...(6)$$

RESULTS AND DISCUSSION

Result of the analysis conducted on the log survivors showed a significant difference ($P<0.05$) per temperature, treatment time, pathogen type and interaction between temperature and pathogen types, and the interaction of the three sources of variation. Table 1 shows the effect of heating time, temperature of heating and pathogen type on log survivors.

At zero treatment time, survivors were maximum, indication of no treatment, while at 20 minute the survivors were zero, indication of total destruction of microbes. *E.coli* was the least resistant because by 10 minutes, it survivor was zero while *Clostridium perfringes* proved the most resistant microbe to the treatment when it had survivors at 15 minutes treatment.

The table further revealed that the actual population of microbe was significant different for pathogen type with *Salmonella* the highest (log 4.835) and *Listeria monocytogen* the least (log 4.262) at zero time.

The log survivor of pathogen varied per temperature of treatment for all pathogens. *Listeria monocytogen* was destroyed more at 100°C then at 70°C, destruction of *E. coli* was more then 50% at 100°C than at 70°C as shown in Table 1, but *Salmonella* did not change with

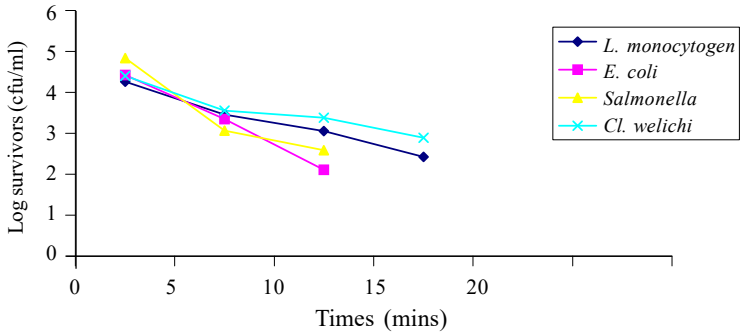

Fig. 1. Survival profile of pathogens during ohmic heating at 70°C

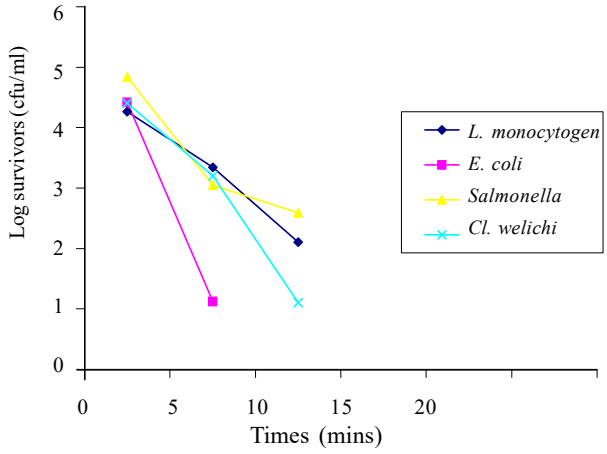

Fig. 2. Survival profile of pathogens during ohmic heating at 100°C

Table 2. D values of *E. coli, Salmonella, L. monocytogen* and *Clostridium perfringes* in tomato juice at 70/100°C.

Temperature (°C)	Pathogen	D (min)	SD (min)
70	(R^2=0.95,DF3)		
	L. monocytogen	8[a]	1.0
	E.coli	3[b]	0.5
	Salmonella	5[b]	0.8
	Clostridium perfringes. i	9.5[a]	1.0
100	(R^2=0.91,DF3)		
	L. monocytogen	5[a]	0.5
	E.coli	1[b]	0.25
	Salmonella	3.7[b]	1.0
	Clostridium perfringes	4.5[a]	0.55

Data are means of triplicate measurements a,b:- means with the same superscript along the columns per given temperature are not significant different at 5%.

temperature.

At each heating temperature, the survivor of *E. coli, Listeria monocytogen,* and *Salmonella* or *Clostridium perfringes* linearly decreased with heating time. (Fig 1) shows a combined heating curve for the pathogens at 70C (ie log survivors against heating time (min), similar plots at 100°C is shown in Fig 2. The D values were measured from inverse negative slops of the model at each temperature and recorded in table 2. At 70°C -100°C the D values for *Listeria monocytogen* were 8-5mins, the D values for *E. coli* were 3-1 min, for *Salmonella* 5- 3.7 mins, while the D value for *Clostridium perfringes* ranged from 5.5-4.5 mins. In Fig 1 there was evidence of shoulder at 70°C and tail at 100°C for *E. coli* and *Clostridium perfringes* Fig. 2. The regression (Table 4) showed that log 0.09l of *listeria monocytogens* survived per minute at

Table 3. Z values, F values and Energy of inactivation of *L. monocytogen, E. coli*, *Salmonella* and *Clostridium* spp at 70 to 100°C

Pathogens	EA/mole	F values/Temperature		Z values
		70	100°C	(°C)
L. monocytogen	911.33[b]	9.64[a]	4.41[b]	10[b]
E.coli	2130.2[a]	5.11[b]	4.03[b]	4[c]
Salmonella spp.	583.84[c]	7.94[ab]	6.03[a]	20[a]
Clostridium perfringes	1448.85[b]	12.00[a]	6.2[a]	10[b]

a, b, c means with the same superscript along the column are not significantly difference a P<0.05

Table 4. Regression of Pathogen Survival Versus time of heating at 70 and 100°C.

Regression Coefficient	Pathogen type			
	L. monocytogen	*E. coli*	*Salmonella* spp.	*C. perfringes*
Bo_1	4.549	3.682	4.643	4.74
$_2$	4.318	3.102	4.639	4.74
B_1	-0.91	-0.228	-0.255	-0.189
$_2$	-0.237	-0.199	-0.254	-0.189
R^2_1	0.91	0.80	0.928	0.91
$_2$	0.944	0.75	0.928	0.91

Bo = constant of the linear equation: logN=Bo+Bt
B=contribution of time at constant temperature
N=Pathogen survival
R^2=coefficient of Regression.

70°C, while log 0.255 *Salmonella* and log 0.228 *E.coli* survived at the same time. At 100°C Fig. 2. Survival of pathogen stood 0.199 and 0.198 per minute. For *listeria monocytogen* and *Salmonella* log pathogen survival stood at 0.24 and 0.25 per minutes with R^2 -0.944 and 0.928. The curve and regression lines showed that *Salmonell*a had the same pathogen at 70°C and 100°C, indication that it does not change much with change in temperature. This was confirmed by the earlier observation in table 1 that at 70°C and 100°C for 5 or 10 mins heating time, survival of S*almonella* did not change.

Limited information was found on thermal inactivation of *E.coli, Salmonella, L. monocytogen* and C*lostridium perfringes* in tomato juice. Murphy *et al* (2004) have reported higher D value in pork ranging from 45.87-1 min. for salmonella at 55-70°C,while this work has D values of 9 mins. or less at 70°C .Different organisms exhibits different D values which are affected by critical factors such as temperature, pH and the heating medium. Implication of different D values shows different method of heating.

The report by Murphy *et al.,* (2004) are based on conventional heating method while here ohmic heating is considered and holding time and come up time where included in D values. In addition to this, meat have been reported (Stirling 1987) with a higher conductivity rate (0.8-1.2) Sm^{-1} , than in fruits or vegetable pieces (0.05-015)Sm^{-1}). Sastry (1994) found that conductivity of materials affect heat penetration rate and hence inactivation of microorganisms. This means lower D values in meats pieces than in fruits. Kotrola and Corner (1997) studied the effect of some preservatives on D value of *E.coli* in ground pork and found D value 7.7 to 27. 2minutes at 55 C, 2.7-13 minutes at 57°C and 0.7 to 4.8 minutes at 60°C. This study revealed a D value of less than 5minutes at 70°C in *E. coli* and in seconds at 100°C, which conforms to the findings of Kotrola and Corner (1997). The effectiveness of heat treatment (pasteurization) to elimination of *E. coli* in apple juice have been demonstrated (Splittstoesser *et al.,* 1996; Mak *et al.,* 2001). 5-log reduction of acid adapted and non-acid adapted *E. coli* strains was achieved at 68.°1C for 14 seconds of thermal treatment. Their report agrees with the finding of this research that the D value; of *E.coli* was in seconds at 70°C and 5 log reductions was achieved after 5 minutes of heating or less than 30 seconds of holding time.

Literature report has indicated that temperatures of 121.1C at Z value of 10°C *Clostridium botulium* has a D value of 0.3 minutes and F value of 3 minutes, but revelation from this work reports that *Clostridium perfringes* has a D value of 5-1 minutes at 70 to 100°C with a Z value of 10°C. The variation was due to strains and heating temperature. Statistical analysis on D values presented in Table 2 showed the significant

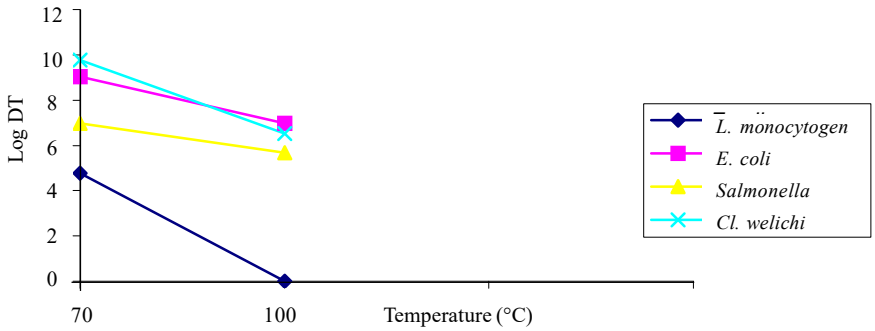

Fig. 3. Regression line of log DT against temperature for *L. monocytogen., E.Coli, Salmonella* and *Cl. welichi*

difference between *L. monocytogen* and *Clostridium perfringes* at 70°C, while *E.coli* and *Salmonella* did not differ at the same temperatures. At 100C, D value for *L. monocytogen*, *Salmonella* and *Clostridium perfringes* did not differ significantly ($P<0.05$), while *E coli* had the least D value. No significant difference in D value or equal log reduction times indicate equality in resistance to heat treatments

By conducting the regression of $\log_{10}$ (D minutes) against heating temperatures, the Z value of *E.coli, L. monocytogen Salmonella and Clostridium* were obtained from a negative inverse slope of the plot (Fig 3). The result of various Z value are presented in Table 3. This result showed that Z value is function of heating temperature and pathogen type. Various Z values indicated significant difference ($P<0.05$), where *Clostridium* had the highest Z value of 20°C, *Salmonella* and *L. monocytogen* did not differ at 10°C each, while *E.coli* the least at 4°C. A high Z value means a slower temperature response to 1 $\log_{10}$ increase in the decimal reduction time (D value).

Therefore the pathogen (*Clostridium*) in this case with the highest Z value is the most tolerant to change in temperature. This is confirmed in Table 1 and Fig.1, 2. Where temperature did not bring significant change in survivors of *Clostridium*

In an attempt to compare the energy of activation (Ea) of each pathogen, it was found from table 3 that the inactivation energy of *E. coli* was highest while the least-*Salmonella*. High activation energy for microbial inactivation permits rapid commercial sterilization of food at UHT conditions (Foegeding and Busta, 1981). This is so in this work since the destruction of *E. coli* was fastest among the pathogens; hence achievement of 5-log reduction in *E. coli* was at split of seconds.

Statistically, the energy of inactivation did not differ between *L.monocytogen* and *Clostridium perfringes* at LSD of 583.53 J/mol. Ea for *E.coli* differed significantly from all other pathogens. In all the cases, the activation energy varied inversely to F value and Z value for each pathogen type irrespective of temperature of the process.

A review of literatures describing the application of ohmic heating to foods revealed that there are no particular pathogen strains with a unique resistance to the technology (Palananippan *et al.*, 1990). This is confirmed in this report where all the pathogens met their death at different death times. More recent studies suggest that a mild electroporation mechanism may contribute to cell inactivation during ohmic heating. That is why Cho *et al.,* (1999) reported a 1.5 mins. difference in D values for *Bacillus subtilis* at 92.3°C between ohmic and conventional heating, indicating that ohmic heating has additional effect, which had been attributed to electoporation mechanism due to low frequencies of the electric current used. Cho *et al* (1999), further found that the D value for inactivation of *Bacillus subtilis* spore at 90°C during ohmic heating was 14.2 mins. This shows that at 20 minutes treatment time, the total elimination of even spore forming pathogens are possible as was confirmed in this work, when all the microbes were eliminated at 20 minutes. Ttable 1.

CONCLUSION

This study showed that the use of ohmic heating at 70 and 100°C using Copper/Aluminum electrodes with electric source of 90V or 100V AC was highly effective in the destruction of commercially important pathogens. There were variations in the rate of destructions, which were dependent on pathogen strains and the heating medium. Further work is now in progress on the toxicology of the treated juices and other electrodes combination.

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