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Modelling for the Survival of *Escherichia coli* in Tender Coconut (*Cocos nucifera l.*) Water by Non-thermal Pulsed Light Treatment

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The effect of pulsed light on the survival of *E.coli* MTCC 433 in tender coconut water was investigated. The sterilized liquid samples were inoculated with *E.coli* @ 10^{10} cfu/ml and treated with pulsed light intensities of 0.18, 2 and 5.6 W/cm^2 for an exposure time between 0 and 15 sec. The results obtained were fitted with three different models such as log linear plus tail, Weibull and biphasic model. Biphasic model showed the best the performance with 7 smaller RSME values of 9 evaluated kinetics followed by Weibull model. Among the three models, biphasic model fitted well with the inactivation of *E.coli* MTCC 433 by using pulsed light treatment.

Key words: *E.coli* MTCC 433, Inactivation, Pulsed light treatment, Modelling

The tender coconut water is one of the nature's most refreshing drinks consumed worldwide for its nutritious and health benefiting properties (Fonseca *et al.,* 2009). Thermal technologies are widely carried out to control microbial spoilage and inactivate microbial pathogens. However, these treatments have negative effects reducing vitamin content, degradation of volatile flavour compounds and other nutrients (Elmnasser *et al.,* 2008).

Non-thermal technologies such as pulsed light are treatment alternatives that is developed to obtain final product with a superior sensory quality, while ensuring microbial safety (FDA/CFSAN report, 2000). Water used in food production has been recently recognized as a vector for the transmission of pathogenic *Escherichia coli* MTCC 433. Hence an objective was taken with modeling for the survival of *E.coli* MTCC 433 in tender coconut water by pulsed light treatment.

Material and Methods

The inoculum was prepared using the *E. coli* MTCC 433 strains as per the procedure described by Sambrook *et al.,* 1989. The fresh liquid foods such as orange, pineapple juice and tender coconut water were autoclaved at 121°C at 1.5 kg/cm^2 for 20 min (Awua *et al.,* 2011).The sterilized liquid samples were inoculated with *E.coli* MTCC 433 of 10^{10} cfu/ml by adding directly in to the liquid foods (Ngadi *et al.,* 2003)

Modelling of E.coli MTCC 433 survival

All the microbial counts cfu per g were log transformed (log10 cfu/ml). The log transformed data were then fitted for modelling of survival of *E.coli* MTCC 433 strains using GInaFIT version 1.6 developed by Dr. Annemie Geeraerd at Katholieke Universiteit, Leuven, Belgium (Geeraerd *et al.,* 2005).

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The log linear plus tail model was used as

$$logN = \log\Big((10^{logN_o} - 10^{logN_{res}})\Big)e^{(k_{max\,d})} + 10^{logN_{res}} \quad \text{.... (1)}$$

Where,

N_{res} = residual population density (log, cfu/ml),

K_{max} = inactivation rate of the log-linear part of the curve (cm^2/J) (Izguier and Gomez-Lopez, 2011).

The weibull survival mode (Mafarat *et al.,* 2002) can be calculated using the following equation

Where,

N = number of survivors (cfu/g),

No = initial inoculum size (cfu/g),

d_{PL} = dosage (W/cm^2),

δ = the time for first decimal reduction of sub-population ,

p = shape parameter (p>1, convex curve is observed; p<1, concave curve is observed; when p=1, a linear curve is observed.

Inactivation curves were also fitted by the biphasic model proposed by Cerf, (1977), which can be formulated as follows,

$$log\left(\frac{N}{N_0}\right) = log_{10}\left(f.e^{-K_{max_1}.t}\right) + \left(1-f\right).e^{-K_{max_2}.t}) \quad \text{.... (3)}$$

Where,

f = fraction of the initial population corresponding to the subpopulation more sensitive to the treatment,

1-f = fraction of initial population corresponding to the subpopulation more resistant to the treatment ,

K_{max1} = Specific inactivation rates of the populations,

K_{max2} = Specific inactivation rates of the populations

Results and Discussion

The sterilized liquid samples were inoculated with *E.coli* of 10^{10} cfu/ml. The inoculated liquid foods were treated with pulsed light intensities of 0.18, 2 and 5.6 W/cm^2 and the exposure time was between 0 and 15 sec. The results obtained from the experiment were fitted with three models such as log linear plus tail, Weibull and biphasic model are presented in the Table 1 and shown in the Fig. 1a, 1b and 1c.

Table 1. Estimated parameters of models for survival of *E.Coli* MTCC 433 in tender coconut water

Treatments (w/cm^2)	Log linear plus tail model			Weibull model			Biphasic model			
	K_{max}	$LogN_{res}$	4D	δ	p	4D	f	k_{max1}	k_{max2}	4D
0.18	0.61± 0.18^a	-2.98± 0.35^b	nc	1.20± 0.54^a	0.44± 0.07^a	nc	0.94± 0.02^{ab}	9.77± 0.02^c	0.29± 0.03^d	nc
2	3.37± 0.28^c	-4.28± 0.06^{cd}	±3^b	0.22± 0.14^a	0.24± 0.13^a	nc	0.99± 0.0001^{abc}	5.20± 1.76^b	0.07± 0.01^a	±2.4^a
5.6	3.42± 0.31^c	-4.75± 0.10^e	±2.85^a	0.26± 0.23^a	0.12± 0.01^a	±2.25^c	0.99± 0.0001^{abc}	3.71± 0.11^{ab}	0.13± 0.01^b	±2.7^c

Column values (a,b,c,d,e) with different letters indicates significant effect (P< 0.01) K_{max} – Specific inactivation rate (cm/J), LogNres – Residual population density (log, cfu/ml), δ- dose for the first decimal reduction (J/cm^2), p- shape parameter (dimesionless), f- fraction of the initial population in a major subpopulation, K_{max1}, K_{max2}- inactivation rates of the two populations respectively. 4D- 4 log reduction, nc- not calculated by GInaFit program.

In the present study, *E.coli* reductions in sample did not fit for the first order kinetics models due to a tailing effect (Fig.1). The log linear plus tail model was appropriate for representing survival of *E.coli* in liquid foods in which estimated parameters showed a significant effect (p<0.01). The model showed that survival curve of *E.coli* with tails exhibited a sigmoidal shape with tail (CFSAN-FDA, 2006).

Table 2. Evaluation of the models for estimating the reductions of *E.Coli* MTCC 433 in tender coconut water after pulsed light treatment

Treatments (W/cm^2)	Statistical indices	Log linear plus model	Weibull model	Biphasic model
Tender coconut water				
	RMSE	0.4042	0.1718	0.1149
0.18	R^2	0.8969	0.9814	0.9938
	R^2 adj	0.8454	0.9721	0.9875
	RMSE	0.1313	0.9348	0.0299
2	R^2	0.9956	0.7753	0.9998
	R^2 adj	0.9933	0.6629	0.9997
	RMSE	0.2285	0.055	0.0286
5.6	R^2	0.989	0.9994	0.9999
	R^2 adj	0.9836	0.999	0.9997

RMSE – Root mean squared error, R^2- Co-efficient of determination, R^2 adj – adjusted coefficients of determination

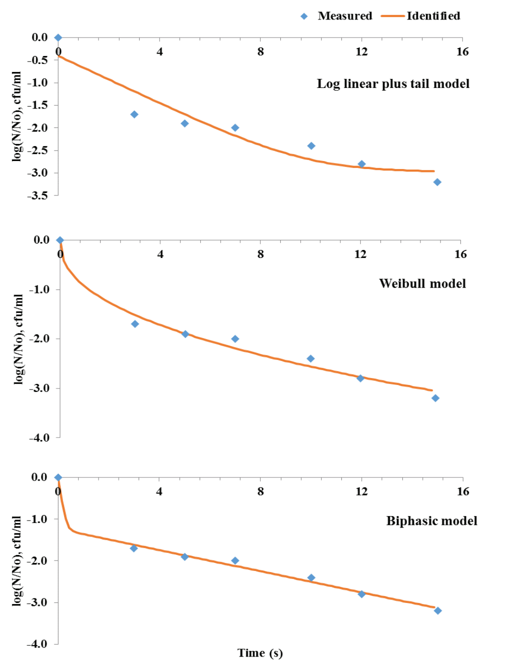

Fig. 1a Survival curve of *E.coli* MTCC 433 in pulsed light treated tender coconut water at an intensity of 0.18 W/cm^2

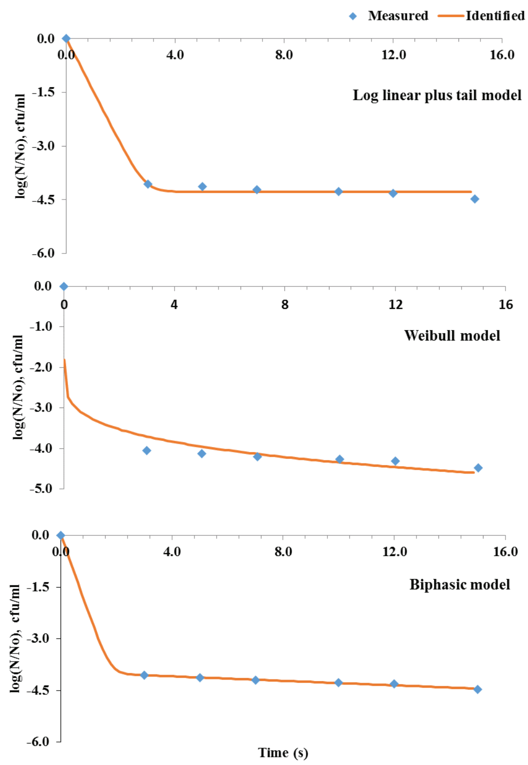

Fig. 1b Survival curve of *E.coli* MTCC 433 in pulsed light treated tender coconut water at an intensity of 2 W/cm^2

The log linear plus tail model exhibited a sigmoidal shape with tail. Inactivation rate K_{max} of the log linear plus tail increased with intensity of pulsed light and were found as 0.61, 3.42, 3.37 for tender coconut water. Weibull model, scale parameter δ represents the dose for the first decimal reduction and found to be 1.20, 0.22, and 0.26 for tender coconut water. The required pulsed light dose to achieve an inactivation of 4 log units was found as 2 and 5.6 W/cm^2.

Pulsed light treatment of 0.18W/cm^2 resulted in lowest f value (sensitive population after treatment) in tender coconut water of biphasic model. The kinematic parameter K_{max1} was always greater than K_{max2} indicating higher inactivation rate during first

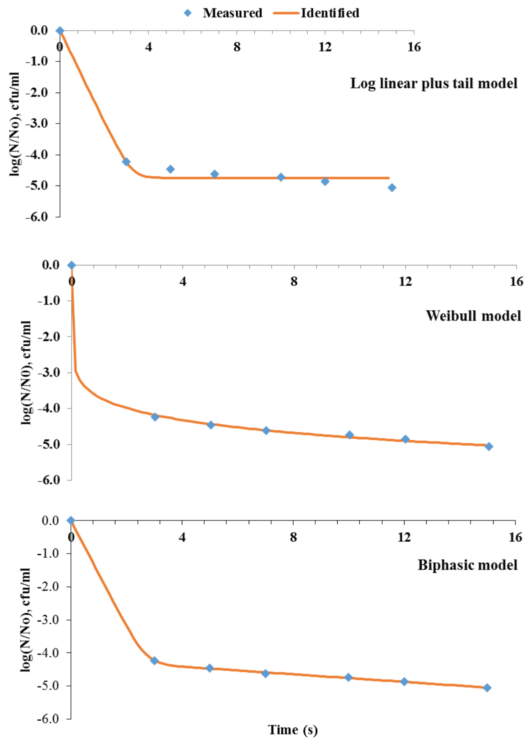

Fig. 1c Survival curve of *E.coli* MTCC 433 in pulsed light treated tender coconut water at an intensity of 5.6 W/cm^2

seconds of treatment. The pulsed light intensity of 2 and 5.6 W/cm^2 were required to achieve an inactivation of 4 log units in all the three models.

Based on the root mean squared error (RSME) which is shown in the Table 2, biphasic model showed best performance with 7 smaller RSME values of 9 evaluated kinetics followed by weibull model. Among the three models, biphasic model fitted well with the *E.coli* MTCC 433 inactivation of pulsed light treated liquid foods. Similar kind of result was reported by Ferrario *et al.* (2013) for apple, orange and melon juice treated with pulsed light.

Conclusion

Among the three models, biphasic model fitted well with the inactivation of *E.coli* MTCC 433 by pulsed light treatment of tender coconut water. This technology could be an alternative treatment to thermal treatment. The results showed biphasic model had a good fit for the inactivation. The future study should investigate the quality characteristics of tender coconut water after pulsed light treatment.

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