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Inactivation kinetics of different flexible packing materials for decontamination of yellowfin tuna *Thunnus albacares* using pulsed light technology

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ABSTRACT

Comparative efficiency of three different packing materials for post-packaging decontamination of yellowfin tuna *Thunnus albacares* steaks using pulsed light (PL) technology was investigated during the study. The packing materials used were 300 gauge low density polyethylene (LDPE), 12 µ polyester 300 guage polyethylene (PEST/PE) and 300 gauge cast polypropylene (CPP). Inactivation curves were plotted separately for each material for pulsed light exposure time ranging from 0 to 12 s. The curves were fitted with three different models *viz,* (i) log-linear, (ii) log-linear with Geeraerd and (iii) log-linear with Weibull model and corresponding goodness-of-fit statistics were estimated. Considering the least treatment time for achieving log microbial reduction, CPP was found to be the ideal choice among the three packaging materials. Among the three models, considering the lowest root-mean-square error values (0.0291, 0.0210 and 0.0141 for samples packed in LDPE, PEST/PE and CPP respectively), Weibull model was found to be most appropriate for describing the inactivation curves in all sample cases. Therefore, inactivation curves of steaks packed in CPP was validated with the Weibull model. The corresponding root-mean-square error (0.1036) and correlation coefficient (0.9974) showed that this model can be effectively utilised for modelling the microbial inactivation kinetics using pulsed light technology.

Keywords: Cast polypropylene, Inactivation kinetics, Non-thermal processing, Pulsed light technology, Statistical modelling, Yellowfin tuna

Introduction

Seafood is a highly perishable food commodity and several conventional preservation techniques like chilling, freezing, drying, salting, canning and smoking are being used to minimise spoilage. However, with increasing consumer demands for minimally processed high quality food items, the fish processing industry is witnessing a shift in choice to non-conventional methods for decontamination of fish and fishery products. Pulsed light (PL) treatment is a non-thermal technology for the rapid inactivation of microorganisms on transparent liquids, food surfaces, equipment and packaging materials (Dunn *et al*., 1995). It is a novel technology, in which food is exposed to short time high-peak pulses of broad-spectrum white light (Dunn *et al*., 1989). PL technology has the specific benefits of preserving the nutritional as well as sensory attributes of foods and is found to have good penetration capability in different plastic films (Fernandez *et al.,* 2009; Chen *et al.,* 2015) and hence can also be considered for post-packing decontamination of food.

Light is generally referred to radiations having wavelength ranging from 180 to 1100 nm, which includes ultraviolet rays (UV 180-400 nm, roughly subdivided into UV-A, 315-400 nm; UV-B, 280-315 nm and UV-C, 180-280 nm), visible light (400-700 nm) and infrared rays (IR 700-1100 nm) (Cacace and Palmieri, 2014). The decontaminative effect of PL on foodmatrices is achieved mainly by the photochemical action of the UV radiation which constitutes the major part of PL spectrum to the extent of 40%. UV radiations cause thymine dimerisation in DNA chain of microbes and thus prevent its replication and ultimately lead to cell death (Gomez-Lopez *et al*., 2007). Several studies, have been conducted especially over the past decade to evaluate the effectiveness of PL on various food matrices (Dunn *et al*., 1995; Elmnasser *et al.,* 2007; Fernandez and Hierro, 2016; Heinrich *et al*., 2016; Bhavya and Umesh, 2017). However, very few such studies have been reported in fish and fishery products (Fernandez and Hierro, 2016).

Yellowfin tuna *Thunnus albacares* is a high value pelagic species found in the oceans of tropics and subtropics. Tuna in both fresh and frozen form

find huge market worldwide (Nithin *et al*., 2015). Hence decontamination of raw tuna meat is of extreme importance for enhancement of its keeping quality and shelflife required for commercial purposes. Pulsed light technology has been proven to be effective in preserving the microbiological and physico-chemical characteristics of yellowfin tuna (Ananthanarayanan *et al*., 2019). However, for any new preservation technology to be successfully adopted for specific applications, its potential in decontamination has to be assessed and standardised by characterising the microbial responses. This is generally carried out by modelling the inactivation curves using suitable equations that can optimally represent the decay pattern of the microorganisms. Various useful parameters can be extracted by appropriate modelling of the inactivation curves and these information finds extreme importance in the design and development of industrial or commercial grade decontamination systems. The most general and simplest of all methods is the first order kinetics which represents an exponential decay of microorganisms over time for a constant application of a specific preservation method. This method gives a straight line in a semi-log plot. However, in most cases this has not been obeyed in total and the plots are generally observed to be curved. Hence, more general non-linear modelling has been proposed over the past few years (Van Boekel, 2002). Such non-linear modelling methods are now being used in most cases of microbial inactivation studies (Bialka *et al*., 2008). The inactivation kinetics of PL treatment has also been validated in various food matrices using such non-linear models (Valdivia-Najar *et al*., 2017). However, such studies in fish and fishery products are still lacking. Moreover, considering the effectiveness of PL technology for post-packaging decontamination of foods (Heinrich *et al*., 2015), packaging materials facilitating maximum transmission of UV portion of the light should be used. Therefore, studying the influence of various polymer-based packaging materials on microbial destruction kinetics and appropriate modelling are of paramount importance.

Polymers are generally considered to be of great value in packaging applications due to their chemical inertness, light-weight, cost effectiveness and variability in colour, transparency, heat resistance and barrier properties (Heinrich *et al*., 2015). Polypropylene (PP) and polyethylene (PE) are the most common transparent polymers used for food packaging (Marsh and Bugusu, 2007). PP is commonly used for packaging applications requiring resistance to high heat and chemical or electrical stresses, whereas PE is generally chosen for applications requiring outdoor and environmental exposure. These materials also exhibit characteristic differences in terms of UV resistance. PP is generally transparent to UV light, whereas PE is innately UV resistant. The aim of this study was to investigate the inactivation characteristics of PL treatment on yellowfin tuna steaks packed in three commonly used polymer packaging materials namely, 300 gauge low density polyethylene (LDPE), 12 µ polyester 300 guage polyethylene (PEST/PE) and 300 gauge cast polypropylene (CPP). Inactivation curves were plotted separately for each material for exposure time ranging from 0 to 12 s. Packaging material facilitating highest microbial destruction was identified. The curves were fitted with three different models *viz*, (i) log-linear, (ii) log-linear with Geeraerd and (iii) log-linear with Weibull model and corresponding goodness-of-fit statistics were estimated. Microbial destruction kinetics of the best packaging material was validated with the most suitable model.

Materials and methods

Determination of Physico-chemical properties of packaging materials

The packaging materials used for the study were: (i) 300 gauge low densitypolyethylene (LDPE), (ii) 12 µ polyester 300 guage polyethylene (PEST/PE) and (iii) 300 gauge cast polypropylene (CPP). Before testing, all the polymer samples were conditioned at 64% relative humidity at 25±2^{0}C for 24 h using a programmable environmental test chamber (REMI Model No. 412 LAG, Rajendra Electrical Industries, Vasi, India). The mechanical properties like tensile strength and elongation at break were determined on the machine direction and cross direction using Universal Testing Machine (Lloyd instruments LRX plus, UK) as per IS: 2508 (ISI, 1984). Oxygen transmission rate (OTR) was determined using a gas permeability apparatus (Lyssy OPT-5000 PBI Dansensor A/S, Ringsted, Denmark) as per ASTM F2622-08 (ASTM, 2008). Water vapour transmission rate (WVTR) was analysed using Lyssy L80-5000 PBI Dansensor (A/S, Ringsted, Denmark) following ASTM-E398-03 (ASTM, 2020). Samples were analysed in triplicates and compared statistically by multivariate ANOVA (IBM SPSS Statistics version 20).

Pre-processing operations

Fresh yellowfin tuna (*T. albacares*) were purchased from local fish market and brought to the laboratory in iced condition with a fish to ice ratio of 1:1 (w/w) within one hour of purchase. Fishes were beheaded, gutted, de-skinned and washed in potable water. Boneless steaks weighing 80 g with a thickness of 1 cm each were prepared from cleaned fish. The steaks were washed with chilled potable water and allowed to drain on a clean wire mesh screen for 5 min at 4°C. The steaks were then divided into three batches and separately packed and sealed in 14 x 18 cm

pouches made of the three packing materials. The packets were labelled and iced (with a fish to ice ratio of 1:1) in an insulated box and kept in a chill room maintained at 2-4°C for pulsed light (PL) treatment.

Pulsed light treatment

The samples were subjected to PL treatment using pulsed light equipment (XENON steripulse RC847, Wilmington, MA, USA). This model consists of two 16" xenon gas lamps and a quartz table inside a polished stainless steel treatment chamber enclosed in a metal housing. Each lamp is capable of producing flashes of light in the range of 100-1100 nm. The lamp generates 3 pulses per second and delivers a fluence of 1.27 J cm^{-2} per pulse for an input voltage of 3800V at the quartz table (Xenon Corporation, 2016). Samples were separately subjected to pulsed light treatment for 2, 4, 6, 8, 10 and 12 s. Control samples (without PL treatment) were maintained for each of the three packaging materials.

Microbiological analysis

Total viable counts (TVC) were determined as per AOAC (2002). Briefly, 10 g portion of the fish was aseptically weighed and transferred to a stomacher bag and 95 ml of sterile physiological saline was added. The suspension was homogenised for 30 s in a stomacher blender (Lab Blender 400, Seward Medical, UK). Serial dilutions of the samples were made using normal saline. Appropriate dilutions were plated on 3M™ Petrifilm™ aerobic count plates and incubated for 48 h at 37°C. Values were recorded as log cfu ml^{-1}. Initial microbial load was obtained from control samples.

Model fitting of bacterial inactivation curves

Variations in microbial counts with respect to time of exposure to PL were plotted for each packing materials. Resultant microbial inactivation curves were analysed using the freeware tool GInaFIT (Geeraerd *et al*., 2005). The models were developed as function of time. The curves were fitted with three different models: (i) log-linear, (ii) log-linear with Geeraerd model and (iii) log-linear with Weibull model.

The log linear model (Bigelow and Esty, 1920) is expressed as:

$$\log N = \log N_0 - \frac{k_{max}\, t}{D} \quad \text{.....(1)}$$

where N_0 (cfu g^{-1}) represents the initial microorganism count, N (cfu g^{-1}) represents the count of survivors at time (t) and the inactivation rate (cm^2 J^{-1}) is represented by k_{max}. D is the decimal reduction value, which is the time required for attaining 1-log reduction in the population.

The log-linear model by Geeraerd *et al.* (2000) is expressed as:

$$\log N = \log[(10^{\log(N_0)} - 10^{\log(N_{res})}).e - k_{max}\, t \frac{e^{k_{max} Si}}{1 + (e^{k_{max} Si} - 1).e^{-k_{max} t}} + 10^{\log(N_{res})}] \quad \text{.....(2)}$$

where N_0 (cfu g^{-1}) represents the initial microorganism count, N (cfu g^{-1}) represents the count of survivors at time (t) and the inactivation rate (cm^2 J^{-1}) is represented by k_{max}. N_{res} is the residual population and S_i is the parameter representing shoulder effect.

The Weibull model by Mafart *et al*. (2002) is expressed as:

$$\log_{10}(N) = \log_{10}(N0) - \left(\frac{t}{\delta}\right)^p \quad \text{.....(3)}$$

where N (cfu g^{-1}) represents the count of survivors, N0 (cfu g^{-1}) represents initial population at time (t), δ corresponds to time taken for the first decimal reduction (10-fold reduction of the surviving population) and p (dimensionless) is a parameter describing concavity or convexity of the curve.

Performance of the models was compared on the basis of root mean sum of squared errors (RMSE) to assess the goodness-of-fit. Experiments were conducted in triplicates and the mean values from two data sets were used for modelling. The third data set was used for validation of the results.

For model validation, the third data set was back-predicted and a linear regression was performed with the estimated *versus* the experimental data (Bialka *et al*., 2008). The corresponding correlation coefficient and the slopes were evaluated for assessing the performance of the predicted model.

Results and discussion

Physico-chemical properties of the packaging materials

Physico-chemical properties of the packaging materials are shown in Table 1. Significant difference was observed between the packaging materials in terms of all the analysed parameters ($p<0.05$). PEST/PE exhibited highest tensile strength and least OTR and WVTR. CPP showed lowest tensile strength and moderately high OTR and WVTR. Highest OTR and WVTR values were recorded in LDPE.

Model fitting of bacterial inactivation curves

Fig. 1 shows the microbial inactivation curve of PL treated yellowfin tuna steaks packed in LDPE and Fig. 1b to d depicts results of curve fitting with log-linear, Geeraerd and Weibull models, respectively. Similarly Fig. 2a to d and Fig. 3a to d show the inactivation curves and corresponding curve fitting for the sample in PEST/

Table 1. Physico-chemical properties of the packaging materials

Parameters (Units)	LDPE	PEST/PE	CPP
Tensile strength (kg cm^{-2}) MD	265±2.2[a]	313.12±2.35[b]	163.42±1.94[c]
Tensile strength (kg cm^{-2}) CD	249.15±1.49[a]	290.79±1.88[b]	142.58±1.79[c]
Elongation at break (%) MD	80.11±1.8[a]	71.28±1.51[b]	76.44±1.66[c]
Elongation at break (%) CD	75.22±1.44[a]	65.92±1.35[b]	68.26±1.54[c]
OTR (ml m^{-2} day^{-1} at 1 atm.)	2605.1±0.53[a]	101.3±0.29[b]	1346.6±0.34[c]
WVTR (g m^{-2} day^{-1})	2.21±0.51[a]	1.65±0.11[b]	1.89±0.23[c]

Different superscripts (a, b, c) in the same row indicate significant difference between treatments means ($p<0.05$). MD-Machine direction, CD-Cross direction

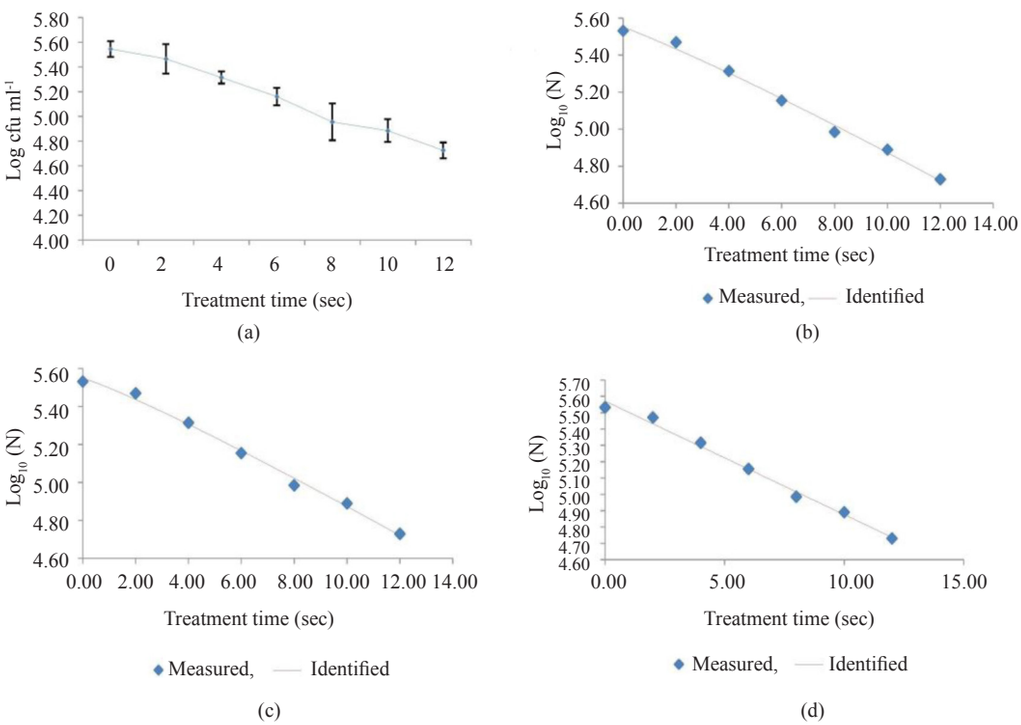

Fig. 1. (a) Microbial inactivation curve of yellowfin tuna steaks packed in LDPE. Results of curve fitting with (b) Geeraerd (c) Weibull and (d) Log-linear models

PE and CPP, respectively. Curve fitting parameters of the three models for each of the sample cases are given in Table 2.

All the inactivation curves showed a downward concavity and none of the curves exhibited considerable shouldering. From the observed downward concavity of these curves, it can be inferred that the microbial inactivation of PL on these three packaging materials are directly proportional to treatment time. Fitting parameter "δ" which represents the decimal reduction time was further compared for selecting the best among the three tested packing materials for post-packaging PL treatment. "δ" represents the time required for first decimal reduction in microbial count and serves as a measure of effectiveness of the inactivation method adopted. The value of "δ" indicates how fast the method can achieve microbial reduction. From Table 2, it can be understood that, the lowest value of δ (12.63±0.14 S) was obtained for CPP. This suggests that CPP offers the most suitable packaging option by providing better effectiveness of PL treatment on the samples. Thus yellowfin tuna samples packed in CPP help PL treatment to achieve greater log reduction compared to the other two materials for any given time of exposure. The better decontamination observed in the case of samples packed in CPP can be attributed to the higher UV transmission. These results are in concurrence with Keklick *et al*. (2010) wherein it was reported that plastic packaging made of PP exhibited significantly higher transmittance percentage for UV radiation compared to that of LDPE.

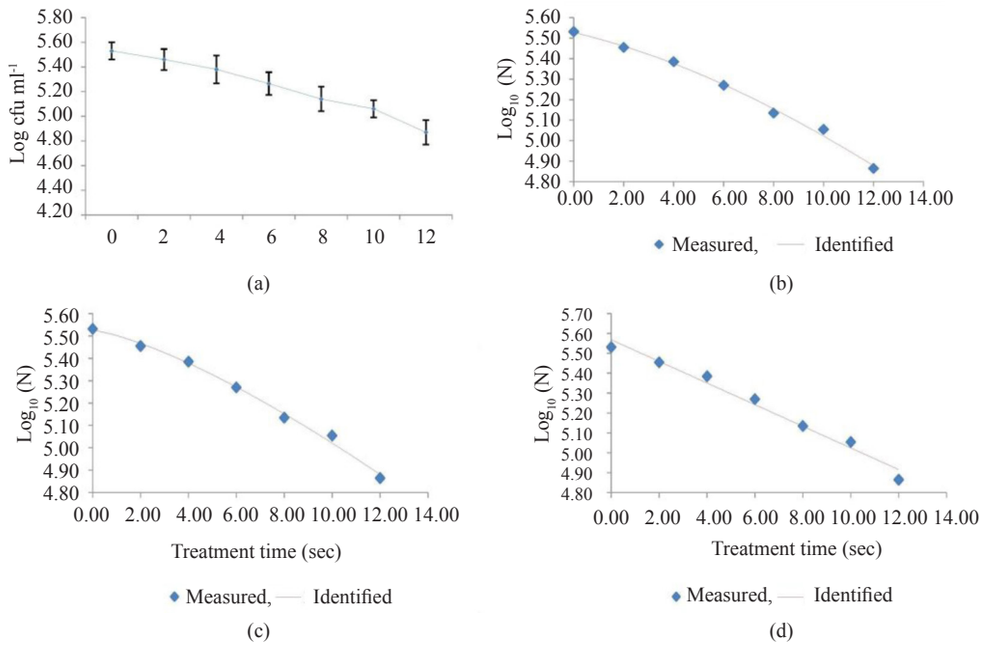

Fig. 2. (a) Microbial inactivation curve of yellowfin tuna steaks packed in PEST/PE. Results of curve fitting with (b) Geeraerd, (c) Weibull and (d) Log-linear models

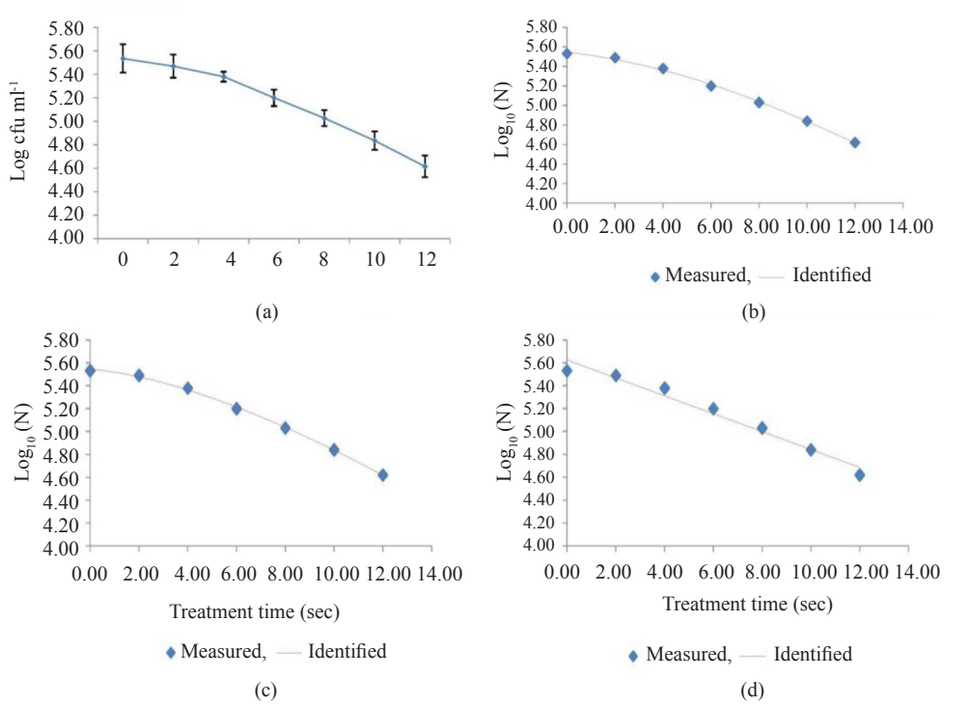

Fig. 3. (a) Microbial inactivation curve of yellowfin tuna steaks packed in CPP. Results of curve fitting with (b) Geeraerd, (c) Weibull and (d) Log-linear models

From the curve fitting results in Table 2, it can be observed that Weibull model showed lowest RMSE values and highest R^2 values compared to other two models. RMSE values of Weibull model for steaks packed in LDPE, PEST/PE and CPP were 0.0291, 0.0210 and 0.0141, respectively. R^2 values for the Weibull model was 0.9938, 0.9948 and 0.9989 for LDPE, PEST/PE and CPP, respectively. From these values, it can be inferred

Table 2. Parameters obtained during model fitting of bacterial inactivation curves

	LDPE			PEST/PE			CPP		
Parameters (Units)	Log-linear	Geeraerd	Weibull	Log-linear	Geeraerd	Weibull	Log-linear	Geeraerd	Weibull
RMSE	0.0305	0.0307	0.0291	0.037	0.0211	0.0210	0.0650	0.0184	0.0141
R^2	0.9915	0.9931	0.9938	0.9398	0.9948	0.9949	0.9701	0.9981	0.9989
Log_{10} No. (log cfu g^{-1})	5.57±0.02	5.55±0.03	5.55±0.03	5.57±0.03	5.53±0.02	5.52±0.02	5.63±0.04	5.55±0.02	5.54±0.01
K_{max} (s^{-1})	0.16±0.01	0.18±0.03	-	0.13±0.91	0.20±0.02	-	0.18±0.01	0.29±0.02	-
Delta (δ) (s)	-	-	14.21±0.52	-	-	16.64±0.63	-	-	12.63±0.14
p	-	-	1.11±0.1	-	-	1.35±0.12	-	-	1.49±0.06

that the inactivation curves are most appropriately described by the Weibull model in all the sample cases. Therefore, inactivation curve of steaks packed in CPP was validated with the Weibull model by comparing bacterial counts predicted by this model with those of the counts obtained experimentally. For this, reduction in bacterial counts with varying exposure time ranging from 0 to 12 s, were estimated using the Weibull model equation. These values were plotted against corresponding experimentally obtained values. This plot was used to qualitatively evaluate the agreement between the predicted and experimental values. Results of the validation study is shown in Fig. 4. The X axis represents experimentally obtained values of bacterial counts for the time of exposures 0 to 12 s and Y axis represents the corresponding values predicted by the model equation. The RMSE and R^2 values between these sets of predicted and experimental values can quantitatively assess the extent of agreement between these values and thereby serve as an indicator of the appropriateness of the selected model. The RMSE (0.1036) and R^2 values (0.9974) between the predicted and experimental data showed that Weibull model can be effectively utilised for modelling the microbial inactivation kinetics using pulsed light technology.

Results obtained during the study indicate that, 300 gauge cast polypropylene pouches will be a suitable packaging material for post-packaging decontamination using PL technology. Weibull model was identified most suitable for statistical modelling of the microbial inactivation using PL. Despite of widespread application

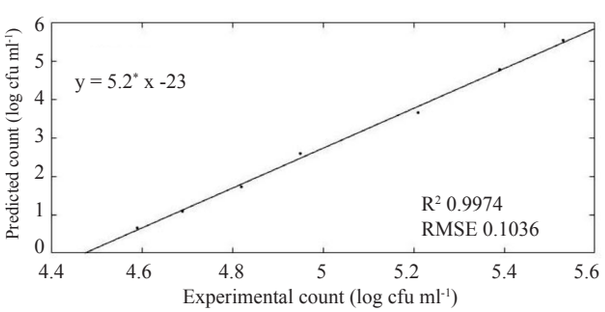

Fig. 4. Validation result of inactivation curve obtained from CPP packed steaks with Weibull model

in liquid foods, PL technology is yet to prove its capability for preserving solid foods. Thickness and composition of the materials are two major factors that affect the treatment efficiency in solid foods. Limited penetration power and higher cost of equipment are two important hurdles to be crossed before commercialisation of the technology. These results provide a preliminary insight into the process conditions with respect to minimum time required for achieving a reasonable reduction in the microbial load of packaged fishery products. Findings of the present study are expected to pave way for focused research on fine-tuning the PL technology for preservation of fish and fishery products.

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