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Aerobic microbial inactivation kinetics of shrimp using a fixed minimal ozone discharge: A fact or fib during iced storage?

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Abstract

Among researchers worldwide, the combination of preservation methods aimed to achieve improved effects on microbial inactivation of seafood products is an area of research receiving increasing interest. Globally also, the demand for high quality minimally processed food products are on the increase. Ozone treatment, three decade – long declared 'Generally Recognized As Safe' and approved as food contact sanitizing agent has evolved up to recent times where it assumes the likes of domestic food-processing facilities manufactured with environment-friendly status ensuring consumer safety. On the other hand, the subject of inactivation kinetics of seafood microorganisms following ozone treatment is still under debate. Furthermore, kinetic models remain the economical and quick approach to predict the preservation parameters. Nevertheless, there is paucity of information regards aerobic microbial inactivation of crustacean product arising from fixed minimal ozone discharge. Is the phenomenon of aerobic microbial inactivation kinetics of shrimp product subject to a fixed minimal ozone discharge during iced storage a fact or fib? To answer this, the aerobic microbial inactivation kinetics of shrimp during iced storage of up to 11 days was inspected. The process conditions comprised of a fixed ozone concentration of 100 mg/h minimally discharged at wash time of 1 min as well as iced storage of up to 11 days. Minimal ozone treatment was applied either prior to or during iced storage situations. Aerobic microbial inactivation presented significant effects during iced storage ($P<0.05$). Line of fit that could best describe the aerobic microbial inactivation kinetics showed adequacy only at the fourth order of storage time '*x*' variable, which could only but account for between 75 - 96% of explained variance. Overall, aerobic microbial inactivation kinetics of shrimp using a fixed minimal ozone discharge appears quantitatively possible even though it decreases as iced storage progresses.

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Key words: Minimal ozone discharge; Aerobic microbial inactivation kinetics; Shrimp; Storage time; Ozone efficacy

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1. Introduction

Ozone treatment, three decade – long declared 'Generally Recognized As Safe' and approved as food contact sanitizing agent [1, 2] has over the years evolved up to recent times to assume the likes of domestic food-processing facilities manufactured with environment-friendly status ensuring consumer safety. In addition, such facilities are now increasingly commercially available for domestic and home use [3-6, 24-25]. A number of ozone treatment applications have involved seafood products such as mussel, jack mackerel, Japanese flounder, rockfish, salmon, sardine, shrimp, as well as turbot [2-5,7-9]. Health regulatory standards continuously press on manufacturers of ozone generating facilities to authenticate the optimum amounts of ozone required to bring about significant effects on foods to assure that residual quantities by concentration directly in contact with food products remain highly insignificant [2,6]. On the other hand, mathematical models have been used to quantitatively define the different aspects as well as dynamics in food processing with the help of theoretical analysis and experimental data. Moreover, most inactivation kinetic models have aimed to address universal concepts, identify the levels of details, the needed simplifications as well as uncertainties of inactivation [2,10]. However, considering the existence of different food preservation methods, as well as the advances of mathematical models, non-linear description of aerobic microbial inactivation kinetics needs further investigations. Equally, using a range of different parameters, non-linear models are understood to help in estimating microbial inactivation kinetics [2, 11]. Further, the subject of inactivation kinetics of seafood microorganisms following ozone treatment is still under debate [2]. Dynamics of inactivation curves have included linear, linear with tailing, sigmoidal-like, linear with preceding shoulder, biphasic, concave, bi-phasic with shoulder as well as convex. In addition, the use of kinetic models has been a quick and economical approach of predicting ozone treatment control parameters, for example, ozone concentration, gas flow rate, as well as treatment time [2, 12-14].

Globally, there is increasing interest regards combined preservative treatment methods applicable to fishery products to offer improved cold storage capabilities [29]. With regards to ozone treatment, the food technological success of ozone generating facilities especially on microbiological and related qualities as well as impact on food safety require investigations from domestic prior to industrial scale [2-6, 25-26, 28]. If pursued on the domestic scale, ozone discharge levels commence with the lowest / minima prior to the higher levels. It was on this premise that minimal ozone discharge fixed at manufacture of the employed domestic facility was applied to Pacific white shrimp. Significant effects on some characteristic qualities were achieved [3-6]. To date, little to nothing is known about aerobic microbial inactivation kinetics of shrimp using a fixed minimal ozone discharge. Specifically, whether this phenomenon is a fact or fib during iced storage is the object of this communication.

2. Experimental program

Shrimp collection as well as domestic ozone facility for this study has been previously described [3-5,18, 23, 26-27]. Specifically, the two process conditions included ozone concentration of 100 mg/h minimally discharged using wash time of 1 min fixed at manufacture of ozone facility of this work as well as iced storage of up to 11 days.

Standard aerobic plate count adapted followed a previous method [15] but with slight modifications for the minimal ozone-treated shrimp. Representative whole shrimp samples (~15 g) aseptically collected in a vertical laminar-flow cabinet and transferred to sterile plastic stomacher bag containing 135 mL of sterile peptone water (20 g of Buffered Peptone Water [CM0509] to 1 L distilled water mixed and sterilized by autoclaving for 121 °C for 20 min) (BPW CM0509: Oxoid Ltd., Basingstoke, Hampshire UK) were homogenized using a stomacher (BagMixer, Interscience Microbiology International, Frederick MD 21704, USA) for 60 s. A 15 mL aliquot of molten autoclaved commercial Plate Count Agar (PCA) (Becton, Dickson and Co., MD 21152, USA) was poured onto petri dishes and allowed to solidify. Using sterile peptone water, serial 10-fold dilution of shrimp homogenate was prepared and aliquots were spread on the surface of solidified (dry) PCA media. At the end of incubation periods (37 °C for 48 h), the number of colonies of inverted inoculated plates was counted.

Specifically, aerobic microbial inactivation of shrimp was based on minimal ozone treatment applied either prior to (Treatment 1) or sequentially (Treatment 2) during iced storage. Aerobic microbial inactivation of this work is typified using Log (N/N_0) versus iced storage time, where N_0 (CFU/g) is number of microbial numbers in untreated sample and N (CFU/g) is number of survivors determined after minimal ozone treatment, at both situations counted

at the day measured. Aerobic microbial inactivation is dependent variable (*Y*) and iced storage is independent variable (*X*). Data generated was based on repeated measurements using different samples per storage time. The trend of aerobic microbial inactivation is firstly monitored with storage and presented as mean and standard deviations, the latter graphically depicted by error bars. The kinetics of aerobic microbial inactivation was followed using fitted line that would help ascertain the appropriate model.

Statistical computations of aerobic microbial inactivation kinetics employed ANOVA outputs up to coefficients of predicted fit equations with corresponding estimated response variability (R-sq {adj}) at probability level of 5%.

3. Microbial inactivation using fixed ozone discharge by iced storage – fact or fib?

Temperature and solubility of ozone relationship, i.e., ozone solubility decreases with increasing temperature [2] as well as the inevitable increases in microbial proliferation in untreated shrimp during iced storage [16] can be included as contributing factors potentially influencing the efficacy of ozone treatment. Other contributing factors include quantity of ozone applied, residual amounts of ozone present, pH, humility, as well as temperature [2, 17]. Fig. 1 shows the aerobic microbial inactivation kinetics of shrimp using fixed minimal ozone discharge treatment.

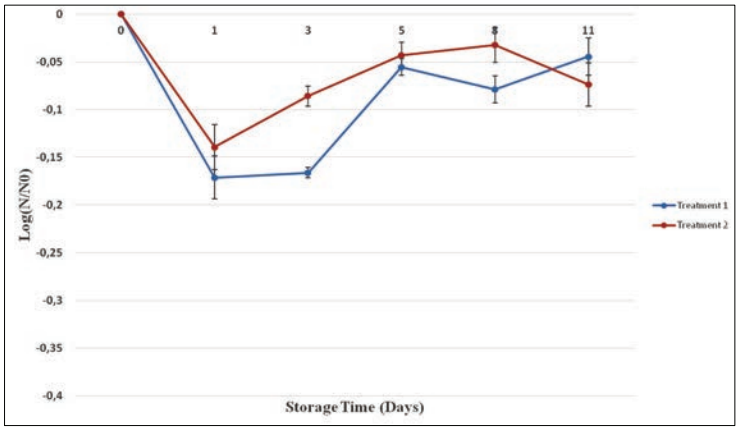

Figure 1: Aerobic microbial inactivation kinetics of shrimp subject to fixed minimal ozone treatments. Treatment 1 refers to minimal ozone treatment of fresh shrimp prior to iced storage. Treatment 2 refers to minimal ozone treatment sequentially applied to ice-stored shrimp with storage.

From ANOVA results, aerobic microbial inactivation during iced storage showed significant effects ($P<0.05$). At day 1, Treatment 1 (Trt1) specifically showed slightly below 2-log microbial reduction compared with Treatment 2 (Trt2), depicting the former with somewhat better performance over the latter. Conceivably, the performance of sanitizing action of quantities of ozone molecules sequentially supplemented on the surface of ice-stored shrimp might be less effective given the presumed temperature influence of iced storage within the flesh and tissues of shrimp meat at point of ozone discharge and over storage time of this study. On the other hand, the performance of sanitizing action of quantities of ozone molecules supplemented on the surface of fresh shrimp might be somewhat improved because of the direct contact with the flesh but would be to some degree at storage, possibly attributable in part to the complete absence of presumed temperature influence of ice at point of application of ozone discharge [24].

Given that microbial inactivation showed significant effects with storage, to ascertain fitted lines that best described these trends is necessary. Regression coefficients of predicted aerobic microbial inactivation models with corresponding response variability (R-sq {adj}) values, degree of freedom (DF), F-value as well as p-value are shown in Table 1 below.

Table 1: Regression coefficients of predicted aerobic microbial inactivation of minimal ozone-treated shrimp during iced storage

Source	Linear[1]	Linear[2]	Non-linear[1]	Non-linear[2]
Intercept	-0.104004***	-0.0658231***	-0.08612***	-0.062422***
x	0.003831	0.0007288	0.07242***	0.013777
x^2	-	-	0.11853***	0.002689
x^3	-	-	-0.16605***	-0.150425***
x^4	-	-	0.22034***	0.136131***
R-sq (adj)	0.05296	0.00363	0.9557	0.7474
DF	22	22	19	19
F_{stat}value	1.23	0.08019	125.00	18.01
P- value	0.2793	0.7797	$2.295e^{-13}$	$2.922\ e^{-06}$

Significant values *** P<0.0001; DF = Degree of Freedom; R-sq (adj) = Coefficient of determination; [1]Coefficients of model generated from minimal ozone treatment of fresh shrimp prior to iced storage; [2]Coefficients of model generated from minimal ozone-treated sequentially during iced storage.

Firstly, we performed the linear model to see whether it fits the data. The result showed no significant effects (P>0.05) as exemplified by the poor determination coefficients (R-sq {adj.} values). Accordingly, the use of linear model could not be applied even with significant terms of intercept (Table 1). The solution to this rests on the use of non-linear modelling and this would require achieving higher coefficient orders via regression analysis. Besides, the non-linear Log behavior of microbial inactivation has been previously described. Specifically, the difficulties to fit microbial inactivation by first order kinetics has been associated with either evolving shoulder(s) or tail(s) during microbial inactivation [19-20]. Of the present work, it was only at fourth order (x^4) of independent variable of storage time (x) that the fit of model appeared adequate and depicted in Fig. 2 below. Along the trends both shoulders and tails seemed apparent.

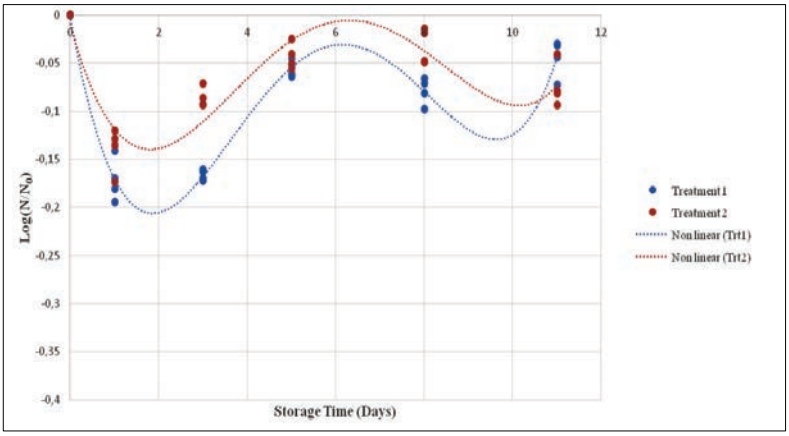

Figure 2: Non-linear Log (N/N_0) inactivation curves of ozone-treated shrimp with storage time. Treatment 1 (Trt1) refers to minimal ozone treatment of fresh shrimp prior to iced storage. Treatment 2 (Trt2) refers to minimal ozone treatment sequentially applied to ice-stored shrimp with storage.

As depicted in Table 1 and Fig. 2, the regression model of four order independent variables (x^n) of aerobic microbial inactivation of Treatment 1(Trt1) showed high statistical significance with the intercept (P<0.0001). On the contrary, Treatment 2 (Trt2) appeared somewhat contradictory because the first (x) and second orders (x^2) showed no statistical effects (P>0.05). Undoubtedly, the non-significant first (x) and second (x^2) order coefficients of Treatment 2 are corroborated by the fairly reduced P- and F-values, which might also depict somewhat reductions in the evolving efficacy of minimal ozone treatment. Besides, both Treatments 1 and 2 produced determination coefficients (R-sq{adj} values) equaling 0.96 and 0.75 with corresponding P-and F-values of $2.295e^{-13}$ and 125.00 as well as $2.922e^{-06}$ and 18.01. Based on experimental data therefore, the model values would suggest some aerobic microbial inactivation, since it could account for 96% and 75% of response variability, respectively. Detectably, the performance of aerobic microbial inactivation kinetics of Treatment 1 (Trt1) would be rather improved compared to Treatment 2 (Trt2) except at approaching day 11 (Fig. 2). Besides, sequential minimal ozone treatment has been shown to potentially reduce microbial numbers of ice-stored shrimp later at storage much less earlier [3]. Moreover, the generated radical-reactive molecular-decomposed molecule of ozone acting directly on the microbe unswervingly affects the microbial inactivation after ozone treatment [2, 21-22].

4. Conclusion

Authors of this work are of the opinion that the aerobic microbial inactivation of shrimp during iced storage appears quantitatively possible, factual and convincing even though the kinetics would unstably decrease in the progress of ice storage time. However, to appreciate the performance of fixed minimal ozone discharge on aerobic microbial inactivation kinetics particularly during iced storage could be challenging indeed. This perhaps substantiates why explorations that reported microbial inactivation kinetics of ozone treated food products have involved different treatment concentrations, exposures, times and so on, and far much less the use of storage time. Subsequently, to establish which direction aerobic microbial inactivation kinetics of shrimp under increasing ozone exposures specifically discharged by a domestic ozone facility would follow would be worthwhile, also incorporating other existing robust models to enable comparisons so as to supplement existing information.

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