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Modeling of UV-C survival of foodborne pathogens and predicting microbial inactivation on fresh-cut 'Tommy Atkins' mango using CFD

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Abstract: Shortwave ultraviolet light (UV-C) disinfection is an emerging technology used to enhance food safety by reducing the pathogen load. Computational fluid dynamics (CFD) served as a numerical simulation tool to calculate the average radiation intensity within a disinfection chamber. The resulting CFD data was employed to estimate the UV-C inactivation kinetic parameters for *Escherichia coli* O157:H7, *Salmonella* Typhimurium, and *Listeria monocytogenes*. Experimental procedures involved irradiating bacterial suspensions with UV-C doses ranging from 0 to 6.028 kJ/m^2. The inactivation of *S.* Typhimurium was described using a log-linear equation, while UV-C survival curves for *E. coli* O157:H7 and *L. monocytogenes* were best fitted to Weibull model. Subsequently, the integration of CFD simulations and kinetic parameters enabled the estimation of UV-C doses approaching 6 kJ/m^2 for the treatment of fresh-cut 'Tommy Atkins' mangoes inoculated with the mentioned microorganisms. This integrated approach partially predicted the inactivation of pathogens on the surface of mango spears.

Keywords: foodborne pathogens; computational fluid dynamics; kinetic modeling; predictive microbiology; fresh-cut

1 Introduction

Foodborne diseases are caused by the contamination of food with bacteria, viruses, parasites, or chemicals at any stage of the production, distribution, or consumption chain [1]. These diseases typically manifest through the consumption of contaminated food, leading to gastrointestinal problems, although more severe outcomes, including fatalities, can also occur [2]. Generally, foodborne pathogens originate from animals and are often linked to poor agricultural practices and subpar processing facilities [3]. The main etiological agents of bacterial origin of outbreaks due to the consumption of contaminated plant foods are *Escherichia coli*, *Salmonella enterica* serovar Typhimurium (not typhoid) and *Listeria monocytogenes* [4, 5]. Previously, U.S. Food and Drugs Administration [6] issued a recall notice for products such as fresh-cut apples, mangoes, pineapples, and cantaloupes sold in markets due to the potential contamination with foodborne pathogens. Fresh-cut products are characterized by having living tissues and the quality of their fresh state, providing convenience to buyers because they are ready for consumption [7]. The primary steps involved in producing pre-cut products include selection, washing, peeling, cutting and disinfection [8]. Therefore, the release of cellular content from wounds and cuts favors the growth of microorganisms [9].

Conventional methods for disinfecting fresh-cut products and for inactivating pathogens involved the use of low-cost chlorinated compounds due to their effectiveness against a broad spectrum of microorganisms. However, the main disadvantage of chlorinated solutions is that promote the formation of carcinogenic and environmentally harmful compounds [10]. As a result, alternative technologies have

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emerged, such as the utilization of short-wave ultraviolet radiation (UV-C). UV-C operates within a wavelength range of 100–280 nm, which is closely aligned with the DNA absorption peak of most microorganisms, resulting in a microbicide effect [11]. Some investigations reported that UV-C was used for the inactivation of foodborne pathogens (*E. coli*, *Salmonella* spp. and *L. monocytogenes*) in bacterial suspensions. The effect of UV-C on survival of these microorganisms was evaluated and allowed to understand the variables involved in irradiation. Kinetics parameters were also considered to calculate UV-C doses required to inactivate microorganisms on different materials [4, 5].

Computational fluid dynamics (CFD) serves as a simulation tool extensively utilized in the food industry to examine various processes, enabling the prediction of intricate phenomena that may not always be feasible on a smaller scale [12]. Previously, some authors have reported the use of CFD to study the intensity distribution of UV-C radiation and the inactivation of *Cladosporium cladosporiodes* and *Penicillium digitatum* on strawberries, both in agar and theoretically [13, 14]. In these investigations, authors only evaluated inactivation of microorganisms responsible for postharvest deterioration and did not compare the theoretically estimated inactivation with experimental tests on the fruit. Furthermore, there have been no studies conducted utilizing CFD to predict the UV-C disinfection treatment of fresh-cut tropical fruits. Therefore, the objective of this study was to determine the UV-C survival curves for *E. coli* O157:H7, *S. enterica* serovar Typhimurium, and *L. monocytogenes*, and to validate the effectiveness of UV-C doses calculated through the integration of UV-C kinetic parameters and CFD for fresh-cut 'Tommy Atkins' mangoes inoculated with these foodborne pathogens.

2 Materials and methods

2.1 Equipment for UV-C treatment

The UV-C radiation treatment employed a compact, cost-effective, and practical prototype for food preservation research. This disinfection chamber had dimensions of 200 mm in height, 190 mm in width, and 460 mm in length. It featured a 15 W UV lamp (G15T8, Lumiaction Co., Taipei, TW), positioned 146 mm from the base (Figure 1) [15].

2.2 CFD simulation

2.2.1 Geometry: Two different geometries were created using DesignModeler (Academic Student version 2020 R1, ANSYS Inc., San Jose, CA, USA). The first geometry was utilized for validating the radiation model and establishing the UV-C survival curves (Figure 2A). In the second geometry, three mango spears were included inside the chamber. Each mango spear had dimensions of 10 mm in height, 60 mm in length, and

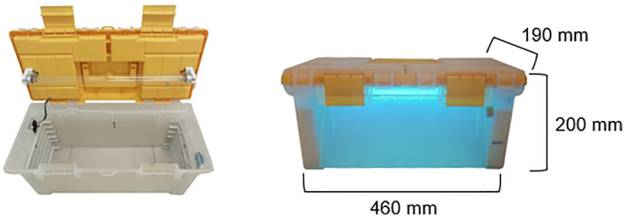

Figure 1: Disinfection chamber.

20 mm in width. These spears were positioned at the center of the disinfection chamber, with a separation of 100 mm between them and from the ends of the smaller side walls of the equipment. These were oriented perpendicular to the lamp, situated on top of two square aluminum bars with a radius of 12.7 mm. The spacing between these bars was 34.6 mm. The distance from the bars to the radiation source was 75 mm (Figure 3).

2.2.2 Meshing: The mesh of geometries was generated in Meshing (Academic Student version 2020 R1, ANSYS Inc., San José, CA, USA) with the automatic meshing method. The quality of the mesh was chosen as high. Tetrahedral discretization was applied to all surfaces of the disinfection chamber, while face meshing was utilized for the larger area walls. The number of mesh elements was 130,379 and 138,433 for the geometry of disinfection chamber empty (Figure 2B) and the geometry that included the mango spears (Figure 4), respectively.

2.2.3 Radiation model: The discrete ordinate (DO) radiation model was employed to calculate the average UV-C radiation intensity within the disinfection chamber. This method involves solving the Radiative Transfer Equation (RTE) within a discrete solid angle domain, considering aspects such as absorption, scattering, emission, and the incorporation of radiative energy from all directions and various wavenumbers. It operates under the guiding principle of conserving radiative energy [16, 17]. The formula is represented as follows:

$$\nabla.(I(r,s)s) + (\alpha+\sigma_s)I(r,s) = \alpha\eta^2(\sigma T^4/\pi) + (\sigma_s/4\pi)\int_0^{4\pi} I(r,s')\Phi(s,s')\mathrm{d}\Omega' \quad (1)$$

where the symbols in the equation denote the following:

I is the radiation intensity that depends on the position (r) and direction (s); s' is the scattering direction vector; α is the scattering direction vector; η is the refractive index; σ_s is the scattering coefficient; σ is the Stefan–Boltzmann constant; T corresponds to the local temperature; Φ refers to the phase function, and Ω' is the solid angle.

2.2.4 Computational domain and boundary conditions: In Fluent (Academic Student version 2020 R1, ANSYS Inc., San José, CA, USA), the DO radiation model was used to solve the RTE by a solid angle discretization with theta and phi divisions of 10 × 10 and theta pixels and phi of 3 × 3. The continuity, momentum and energy equations for an incompressible laminar flow were solved for the geometries of the system. The parameters of boundary conditions and computational domain are presented in Table 1. According to Trivittayasil et al. [14], volumes inside the lamp and mango spears were left blank. All walls were considered opaque, except for the walls of the lamp. The semi-transparent wall of the lamp had a diffuse fraction of 1, while the other walls had a diffuse fraction of 0.5.

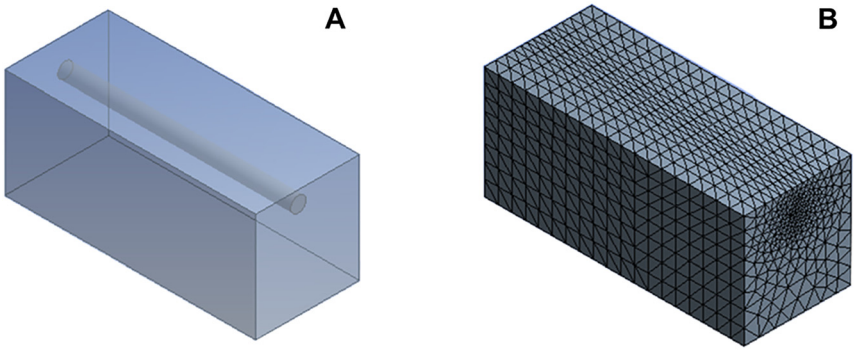

Figure 2: This figure illustrates how the equipment and CFD pre-processing were represented to validate the radiation model. Geometry (A) and meshing (B) of the disinfection chamber empty.

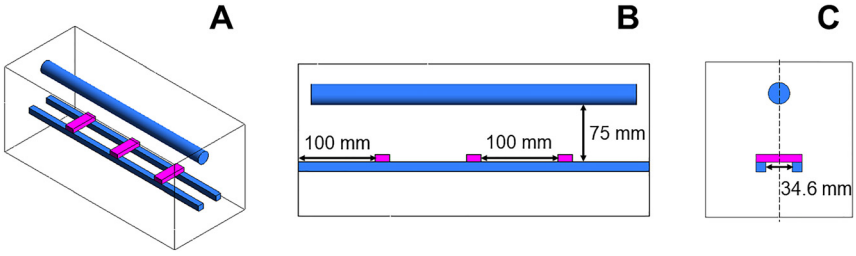

Figure 3: Geometry of the disinfection chamber with the mango spears. Isometric (A), front (B) and side (C) view.

2.2.5 Solution setup: The SIMPLE algorithm was used to solve the steady-state equation, while spatial discretization was solved by a first order upwind for the DO model. The fluid temperature was reduced to 10 K to minimize the effect of thermal radiation. The number of iterations was 18. The average time to reach convergence was 276.598 and 362.986 s for the first and second geometry, respectively. The simulation was performed on an Intel Core TM i5-1035G1 CPU (1.19 GHz) with 8 GB RAM.

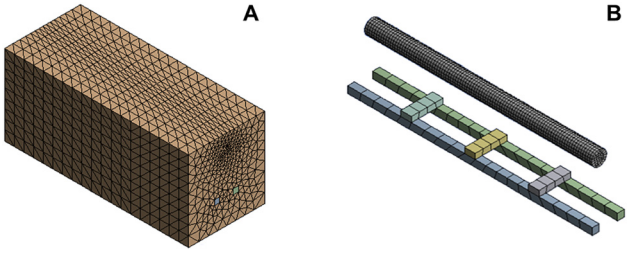

Figure 4: This figure illustrates how the equipment and mango spears were represented during CFD pre-processing to simulate UV-C irradiation, enabling the calculation of the average radiation on the surface of the mango spears. Meshing the disinfection chamber with the mango spears. External (A) and internal (B) view.

Table 1: Specifications for the boundary conditions and the computational domain for the CFD simulation.

Parameter	Value
Air density (kg/m^3)[a]	1.225
Aluminum density (kg/m^3)[a]	1190
Emissivity of the walls of the equipment, bars of aluminum and mango[b]	0.95
Emissivity of the lamp wall[c]	0.89
Average radiation intensity in the lamp tube (W/m^2)[d]	30.5

[a]Fluent; [b][14]; [c][13]; [d]Measurement with UV meter (UVC-254, Lutron Electronic Enterprise Co., Taipei, TW).

2.2.6 Validation of the radiation model: To validate the computational simulation methods, the radiation intensity was estimated using the UV meter and the first geometry in CFD-Post software (Academic Student version 2020 R1, ANSYS Inc., San José, CA, USA). Various points positioned at the central region of the equipment, spanning distances from 14 to 122 mm from the lamp, and at intervals of 60 mm within the middle section of the equipment, specifically at 122 mm from the radiation source, were considered. The fit of the DO radiation model was assessed by computing the mean absolute error (MAE) and root mean square error (RMSE) between the experimental data and the simulation results, according to He et al. [18].

$$\mathrm{MAE} = \frac{1}{m}\sum_{i=1}^{m}|x_{\mathrm{si}} - x_{\mathrm{mi}}| \tag{2}$$

where the symbols in the equation denote the following:

m is the number of determinations, x_{si} is the value found with the simulation and x_{mi} is the value measured with the UV meter.

2.3 Bacterial strains and media

E. coli O157:H7 (ATCC 43,895), *S. enterica* serovar Typhimurium (ATCC 14,028) and *L. monocytogenes* (ATCC 7644) strains were used in this study. According to Ruiz-Cruz et al. [19], bacterial cultures were individually cultivated in 3 mL of tryptic soy broth (Difco, Sparks, MD, USA) and left to incubate overnight at 37 °C (Incubator IC400, Yamato Scientific, Tokyo, KNTO, JPN). A loop of *E. coli* O157:H7 was streaked on Sorbitol MacConkey agar (Difco, Sparks, MD, USA), *S.* Typhimurium on Bismuth Sulfite agar (Difco, Sparks, MD, USA), and *L. monocytogenes* on Oxford agar (Condalab, Madrid, CM, ES). The plates were then incubated

for 24 h at 37 °C. For each microorganism, a colony from the plates was selected and inoculated in 9 mL of peptone water (2.3 %, PW; Difco, Sparks, MD, USA). The population of initial suspension was adjusted to 0.5×10^8 cells/mL with McFarland turbidity standard.

2.4 Modeling of UV-C survival of foodborne pathogens

For each pathogen, a working suspension of approximately 5×10^5 cells/mL was prepared by adding 0.5 mL of the initial bacterial suspension to 150 mL of 2.3 % PW. This working suspension was evenly distributed into six Petri dishes with a 90 mm diameter, each filled to a depth of 50 mm. Subsequently, plates were exposed to UV-C treatment individually for durations of 0, 3, 6, 9, 12, and 15 min, at 75 mm of the lamp and in the center of the disinfection chamber. Following the treatments, decimal dilutions of the samples were prepared using 2.3 % PW, and 1 mL aliquots of these dilutions were plated onto MacConkey Sorbitol agar for *E. coli* O157:H7, Bismuth Sulfite agar for *S.* Typhimurium, and Oxford agar for *L. monocytogenes*. The plates were then incubated at 37 °C for 24–48 h, and the bacterial populations were counted. This experimental procedure was conducted with two replicates for each pathogen and three plates for each dilution.

2.4.1 UV-C inactivation kinetic models: The log-linear equation is the simplest model that describes inactivation kinetics when microorganism populations exhibit equal treatment sensitivity, and there exists a negative and linear correlation between cell count and the inactivation rate [19, 20]. The formula is as follows:

$$\log_{10}(N/N_0) = -kt/2.303 \tag{3}$$

where the symbols in the equation denote the following:

N and N_0 are respectively population count over time and the initial population count, k is the inactivation rate (s^{-1}), t is the treatment time (s), and the value of 2.303 corresponds to ln(10), which refers to an estimated in 1 logarithmic cycle.

According to Trivittayasil et al. [13], the microbial inactivation as function of UV-C dose is considered in terms of radiation intensity and exposure time. Consequently, equation (3) incorporates the average radiation intensity on the surface of the bacterial suspension, and it is represented as follows.

$$\log_{10}(N/N_0) = -k' It/2.303 \tag{4}$$

where the symbols in the equation denote the following:

k' is the modified inactivation rate (m^2/kJ) and I represents the average radiation intensity on the surface of the bacterial suspension determined through CFD simulations (kW/m^2).

Another equation that characterizes microorganism inactivation is the Weibull model, which is derived from the cumulative form of the asymmetric Weibull probability density function. It indicates the number of cells that can adapt to stress, or those that have a damage with a microbicide treatment [21]. The formula is expressed as follows:

$$\log_{10}(N/N_0) = -(1/2.303)(t/\alpha)^{\beta} \tag{5}$$

where the symbols represent the following:

α modifies the slope and is a scale parameter (s). β describes shape of the curve ($\beta < 1$ refers to a concave curve and $\beta > 1$ suggests a convex curve).

Weibull model was also rearranged in terms of UV-C dose adjusting the units of scale parameter [13].

$$\log_{10}(N/N_0) = -(1/2.303)(It/\alpha')^{\beta} \tag{6}$$

where α' is the modified scale parameter (kJ/m^2).

2.4.2 Calculation of UV-C doses for kinetic modeling of foodborne pathogens: The UV-C treatment doses were computed by multiplying the treatment times with the average radiation intensity on the surface of the bacterial suspension. The average radiation intensity was determined through post-processing of the simulation using CFD-Post software (Academic Student version 2020 R1, ANSYS Inc., San José, CA, USA), which involved incorporating a circular plane within the geometry of the chamber (Figure 5).

2.5 Predicting of foodborne inactivation on fresh-cut mango

2.5.1 Calculation of UV-C doses for fresh-cut mango: To estimate the UV-C treatment times of fresh-cut mangoes, the best-fitting UV-C kinetic model for each microorganism was utilized. Theoretical reductions were considered based on those achieved with the bacterial suspensions of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes*. The average radiation intensity on the surface of the mango spears was determined using CFD-Post. Finally, the UV-C doses were calculated using the equation expressed as follows:

$$D = It/1000 \tag{7}$$

where the symbols in the equation denote the following:

D is the UV-C dose applied to fresh-cut mango (kJ/m^2), I is the average radiation intensity on the surface of the mango spears (kW/m^2), and t is the UV-C treatment time of the mango spears estimated through kinetic models (s).

2.5.2 Inoculation and UV-C treatment of fresh-cut mango: Mangoes (*Mangifera indica* L.) cv. 'Tommy Atkins' were purchased in a local market in Hermosillo, Sonora, Mexico. Those were subsequently stored under refrigeration (5 °C) until they presented a stage 3 of maturity according to Kader [22]. Both the whole mangoes and the equipment employed for minimal processing and UV-C treatment were disinfected

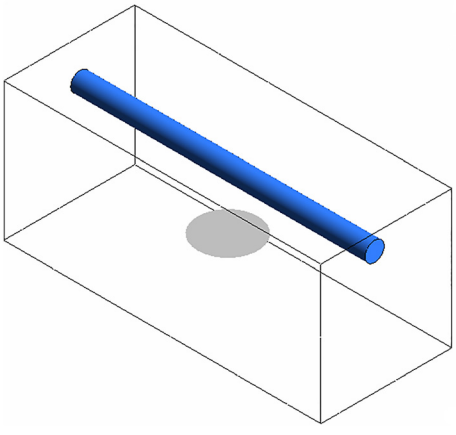

Figure 5: Disinfection chamber during post-processing simulation with a circular plane for calculation of the average radiation intensity on the upper of the bacterial suspension.

with a 200 ppm aqueous chlorine solution. The peel was removed from the fruit and six spears of approximately 10 mm in height, 60 mm in length and 20 mm in width were obtained for the experimental procedures with each pathogen.

The spears were immersed in individual inoculums of approximately 10^6 cells/mL prepared by adding 3 mL of the initial suspensions of *E. coli* O157:H7, *S.* Typhimurium and *L. monocytogenes* to 300 mL of 2.3 % PW. Following the methodology proposed by Ruiz-Cruz et al. [23], the mango spears were kept in the inoculums for 30 min under manual agitation. After inoculation, the fruit spears were drained one by one for 10 s and then left in a laminar flow cabinet for 30 min (Horizontal Clean Bench, Labconco Corporation, Kansas City, MO, USA). Subsequently, for each microorganism, three inoculated mango spears were introduced into the disinfection chamber and subjected to UV-C treatment at an estimated dose. The amount of inoculum remaining on the surface of mango spears without UV-C treatment served as the control, representing the initial microbial population.

The control and irradiated samples were separately placed in sterile homogenizer bags (Nasco, Whirl-Pak, Madison, WI, USA) containing a 2.3 % peptone water solution in a 1:10 ratio (w/v). The aqueous solution with the samples were manually agitated for 1 min. Decimal dilutions were prepared with 2.3 % PW for microbial enumeration. For each dilution, 1 mL aliquots were plated in triplicate on MacConkey Sorbitol, Bismuth Sulfite, and Oxford agar for *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes*, respectively, and then incubated at 37 °C for 24–48 h.

2.6 Statistical analysis

Statistical analyses were conducted in Minitab 17 (Minitab Inc., State College, PA, USA). For UV-C kinetic modeling of foodborne pathogens, were employed to assess whether significant there were significant differences in the population of pathogens under varying UV-C doses ($p < 0.05$). The UV-C inactivation kinetic parameters were determined by averaging the logarithmic reductions of microbial population data. The estimation process involved the utilization of both linear and non-linear least squares methods for the log-linear equation and Weibull model, respectively. In the case of non-linear least square method, the resolution of the Gauss–Newton algorithm was considered, specifically with a maximum allowable number of iterations set at 200 and a convergence tolerance of 0.00001. The models' goodness of fit was assessed through the calculation of the mean square error (RMSE), the coefficient of determination (R^2) and the adjusted coefficient of determination ($\overline{R}^2$).

To predict foodborne inactivation on fresh-cut mango, the logarithmic reduction achieved for each microorganism on the surface of the mango spears after UV-C treatment was determined by calculating the mean of the differences between the population counts of the samples subjected to UV-C irradiation and the average microbial population of the control samples, both values expressed in logarithmic units. The formula is presented below:

$$\left|\overline{\log_{10}\left(\frac{N}{N_0}\right)}\right| = \left|\frac{1}{9}\sum_{i=1}^{9}\left[\log_{10}(N_i) - \overline{\log_{10}(N_0)}\right]\right| \tag{8}$$

where the symbols in the equation denote the following:

N_0 represents the population count in the control sample (without UV-C irradiation), while N is the remaining population count after UV-C treatment, respectively. $\overline{\log_{10}(N_0)}$ is computed with $\frac{1}{9}\sum_{i=1}^{9}\log_{10}(N_{0i})$. These means were calculated based on nine determinations because the microbial population count was conducted in triplicate for each control sample and each sample subjected to UV-C irradiation.

A one-sample *t*-test was utilized to evaluate whether there were significant differences between the values of the theoretical reduction and the experimental reduction of each microorganism on the surface of the mango spears ($p < 0.05$). Additionally, the mean absolute percentage error (MAPE) was calculated concerning the theoretical reduction values. The formula is expressed as follows:

$$\text{MAPE}(\%) = \frac{100}{n}\sum_{i=1}^{n}\left|\frac{x_i - \widehat{x}_i}{x_i}\right| \tag{9}$$

where the symbols in the equation denote the following:

n represents the number of experimental determinations conducted, x_i is the value of the experimental logarithmic reduction, and $\widehat{x}_i$ is the value of the theoretical logarithmic reduction.

3 Results and discussion

3.1 Validation of the radiation model

The validation of the radiation model involved a comparison between the radiation intensity values predicted by the CFD simulation and the measurements obtained from the UV meter inside the disinfection chamber. Both horizontally and vertically, the experimental values were slightly higher than the simulation predictions (Figure 6). This difference could be attributed to the limited precision of decimal values displayed and potential rounding by the UV meter. The MAE, RMSE, and R^2 for the horizontal estimates were 0.099, 0.118, and 0.975, respectively. For the vertical estimations, these values were 0.371, 0.508, and 0.999, respectively. The results indicated that the MAE and RMSE were lower for the data points located horizontally within the equipment, while the R^2 was higher for the data from the vertical determinations. However, all R^2 values were close to 1, and the MAE and RMSE approached 0. This suggested that the simulation values closely approximated the measurements. Therefore, DO model was effective in predicting the behavior of UV-C radiation in the disinfection chamber.

Trivittayasil et al. [13] theoretically validated the discrete ordinate radiation model and utilized it to simulate the UV-C inactivation of *C. cladosporiodes* and *P. digitatum* on agar at different distances from a lamp (50, 100, 150 and 200 mm) and times of irradiation (0, 5, 10, 15, 20, 25 and 30 min). Trivittayasil et al. [14] employed UV meter measurements and mathematical equations to validate the DO model. They also conducted CFD simulations to analyze radiation intensity distribution and mold inactivation on the surface of strawberries arranged on a tray.