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Original research

Modeling the effect of different infrared treatment on *B. cereus* in cardamom seeds and using genetic algorithm-artificial neural network

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A B S T R A C T

In this study, the effect of infrared (IR) on decontamination of *Bacillus cereus* in cardamom seeds were determined at difference IR radiation powers (100, 200, and 300 W), different sample distances from radiation source (5, 10 and 15 cm) and various holding times. The most successful reduction in *B. cereus* numbers (5.11 log CFU/g) was achieved after a holding time of 8 min at 300 W IR power and 15 cm distance. Data were analyzed to predict antibacterial effects of IR against *B. cereus* in cardamom by artificial neural network (ANN) model. The developed genetic algorithm-ANN (GA-ANN), which included 12 hidden neurons, could predict *B. cereus* population with R^2 = 0.908. The results indicated that GA-ANN model could give good prediction for the population of *B. cereus*. Sensitivity analysis results showed that IR irradiation time was the most sensitive factor for the prediction of *B. cereus* population.

Keywords: *Bacillus cereus,* Cardamom, Genetic algorithm, Infrared heating, Microbial decontamination

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1. Introduction

Spices are extensively used in food preparation and processing because of their distinctive flavor, color, aroma, and functional properties. Spices have an extensive natural microbial flora including mesophilic, soil-borne, and spore-forming bacteria, yeasts and molds (Tainter et al., 2001). Cardamom is a high-value commercial spice extensively used in the world (Nair, 2006). Cardamom, known as the queen of spices, is one of the most expensive spices in the world (Parthasarathy & Prasath, 2012).

The presence of *Bacillus cereus* has been confirmed in cardamom as a contamination with high rates (Klimešová et al., 2015; McKee, 1995). *B. cereus* is a gram-positive and endospore-forming aerobic or facultatively anaerobic bacterium. The extensive presence of *B. cereus* in the air, soil, water, as well as animal and plant materials, and due to its high spore resistance to a number of adverse environmental conditions, has led to the high importance of *B. cereus* in various foods such as spices (Griffiths & Schraft, 2017). Usually, 10^5 Colony-Forming Unit/g of *B. cereus* is necessary to cause illness however European Food Safety Authority (EFSA) (2005), claims that food poisoning prevalence should be given as 10^3 to 10^4 per g.

IR radiation is section of the electromagnetic spectrum between ultraviolet, visible and microwave radiation with wavelength of IR range between 0.76 µm and 1 mm (Ginzburg, 1969). Due to the low penetration depth of IR radiation (about 0.31 to 4.76 mm), this method is used as a surface treatment (Eliasson et al., 2015).

IR treatment was used for decontamination of mung bean at 70°C for 5 min using an IR source with an intensity of 0.299 kW m^{-2}. It was reported that without significantly change in the biochemical and physical attributes of the mung bean, the population of *Aspergillus flavus* and *A. niger* was reduced 5.3 log_{10} CFU/g (Meenu et al., 2018). In other study, Far-IR and ultraviolet radiation was used for surface decontamination of black pepper. It was reported that Far- IR radiation caused a rapid increase in the temperature of black pepper even more than the amount needed to eliminate microorganisms as well as ultraviolet have not a significant effect on the reduction of the microbial population of black pepper but Far-IR radiation was introduced as a suitable method for reducing the microbial population (Erdoğdu & Ekiz, 2013). Furthermore, Staack et al. (2008) reported that for decontamination of *B. cereus* spores in paprika powder by near-IR radiation, the higher temperatures and water activity, the number of *B. cereus* spores decreased significantly. In a similar study, effect of selective IR radiation on the inactivation of *B. cereus* by Response Surface Methodology was investigated. The maximum reduction of *B. cereus* (equal to 4. 34 log CFU/mL) was reported in treatment containing 5.76 µm IR wavelength, 3 cm distance from radiation source, 30 min radiation time and 2 mm sample thickness (Shavandi et al., 2018).

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According to reports, in the last years ANN has offerred more real advantages than the conventional modeling (Funes et al., 2017). ANN in a parallel structure is made of nodes or artificial neuron's. ANN for each independent variable has one input layer containing one node, one or more hidden layers, where the data are processed, and one output layer, including one node for each dependent variable (Gonçalves et al., 2005). Many reports have said the advantages of the application of technical ANN compared to other statistical methods (Atsamnia et al., 2017; Kavuncuoglu et al., 2018; Marzouk & Elkadi, 2016; Soleimanzadeh et al., 2018; Yamamura et al., 2008; Yolmeh et al., 2014). ANNs are helpful tools for food safety and quality analyses, such as predicting microbial growth, spectroscopic data, and predicting physical, chemical, and functional properties of food (Kavuncuoglu et al., 2018).

Cardamom is very important in terms of consumption, the most expensive and having economic and export value. There is no study available in the literature relating to the use of computing technology for prediction of *B. cereus* inactivation in cardamom by IR. Therefore, the goal of this study was to investigate the effect of IR on the inactivation of *B. cereus* in cardamom to study the performance of GA-ANN model to simulate this decontamination of *B. cereus* in cardamom.

2. Material and Methods

2.1. Preparation of the raw material

The purple grade cardamom (*Elettaria cardamomum*) was prepared from emperor akbar natural green cardamoms (India) with a moisture content of 9.86% and water activity of 0.409 and stored at 20°C in plastic zip lock bags. Food borne indicator *B. cereus* bacterium (PTCC 1015) was purchased as lyophilized from Iran's culture collection and activated by calturing in BHI broth (Merck, Germany) at 37°C for 24 h. Bacterial concentration of about 10^8 CFU/mL was determined with spectrophotometer (PG Instruments LTD T80, England) and in comparing with McFarland standards. Bacterial solution was added to cardamom seeds by spotting and mixing (Shavandi et al., 2020b).

2.2. IR treatments of the cardamom

An experimental stainless steel chamber (45×45×40 cm) IR heating system was designed and aluminum waveguide with an IR lamp (1000 w, 350 mm) was used in this study (Shavandi et al., 2020a). The effect of IR radiation power at three levels of 100, 200, and 300 W, different sample distance from radiation source at three levels of 5, 10 and 15 cm and various holding times (depending on treatment conditions from 0 to 15 min) on the microbial inactivation kinetics was investigated.

2.3. Microbiological analysis

The effect of IR treatments on the population of *B. cereus* in cardamom seeds were determined by spread-plating method. Cardamom seeds were sterilized before analyzing. Following IR treatments cardamom seeds, serial dilutions were spread on BHI agar (Merck, Germany) in triplicate. After incubation at 37°C for 24 h, the colonies that appeared were enumerated. Microbial enumerations were expressed as log of reduction (CFU/g). The survival (N) was also calculated, where N is the number of surviving bacteria after treatment.

2.4. Statistical analysis

Results were analyzed for the determination of antimicrobial effects of IR against *B. cereus* in cardamom using one-way analysis of variance (ANOVA) by SAS software version 9.3 (SAS Institute Inc.). Significant differences between the means were further analyzed using the Duncan Test at a significance level of 0.05.

2.5. GA-ANN model

ANN is a popular type from artificial intelligence and the multi-layer feed-forward neural network is the most popular, where the neurons are arranged into three layers of input, hidden and output (Yolmeh et al., 2014). A schematic description of the 3-layers network structure used in this study is shown in Fig. 1. The performance of an ANN depends severely on its topology. The number of input neurons and the number of output neurons corresponds to the number of input variables into the neural network and the number of target output variables, respectively. At least one hidden layer that can have any number of neurons, there is between the input and the output layers and depends on the application of the network. Usually by trial and error method, determination of optimum number of hidden layer neurons is done (Bahram Parvar et al., 2013; Salehi et al., 2012).

Genetic algorithm (GA) optimization technique is a method for overcome this inherent limitation of ANN. GA, mimicking the mechanism of biological evolution and is a search technique for optimal value (Morimoto, 2006).

The net input (xj) to node j in the hidden and output layers, is of the form:

$$X_j = \sum_{i=a}^{n} W_{ij} y_i + b_j \quad (1)$$

where y_i is the inputs, w_{ij} is the weights associated with each input/node connection, n is the number of nodes and b_j is the bias associated with node j (Soleimanzadeh et al., 2018).

In this study, because of Levenberg–Marquardt technique is more powerful and faster than the conventional gradient descent technique, for ANN training was used (Sagdic et al., 2012). All data were randomly divided into 3 partitions: training (60%), validating (15%), and testing data (25%). For designing the GA-ANN model was used the Neurosolution software (release 6.01, NeuroDimension, Inc., USA).

3. Results and Discussion

3.1. Microbial decontamination

The differences between inhibitory effects of various IR powers at constant distances of the IR lamp during the time, as well as different distance from IR lamp at fixed IR power during the time are shown in Fig. 2. A, and B, respectively.

The final bacterial concentration in the cardamom after contamination was 6.32 $\log_{10}$ CFU/g. In the present study, the treatments were continued until the apparent stability of the samples against the produced heat. According to the results, the highest rate decontamination of cardamom was at 300 watts IR power, 15 cm distance and 8 min time with -5.11 log CFU/g and the minimum decontamination of cardamom was obtained at 100 watts IR power, 15 cm distance and 10 min time with -2.035 log CFU/g. It was found that with increasing of the lamp power, the amount of decontamination in cardamom seeds was significantly increased ($p < 0.05$). According to the findings of the present study, the removal rate of *B. cereus* in the cardamom seeds was significantly increased ($p < 0.05$) by decreasing the sample distance from the IR lamp.

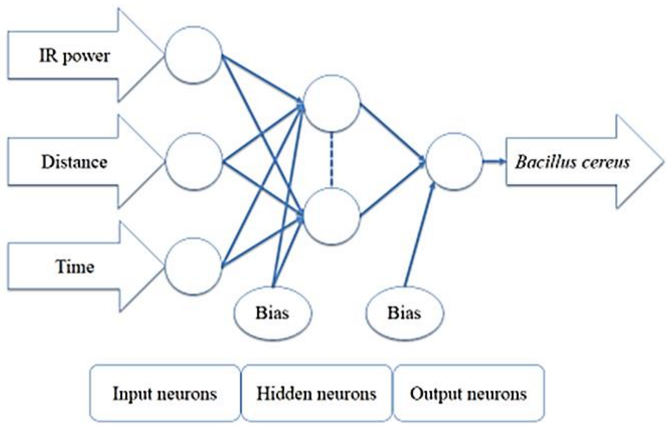

Fig. 1. ANN architecture with one hidden layer for prediction the reduction of the *B. cereus* population.

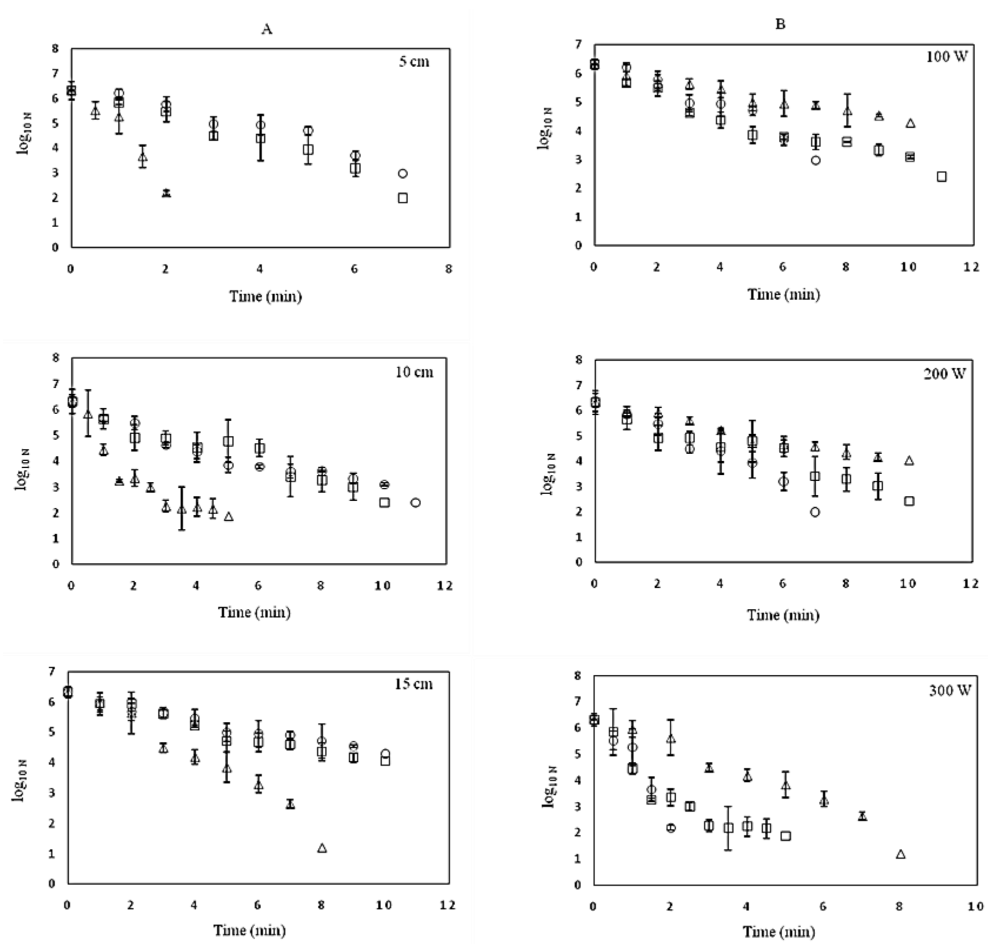

Fig. 2. The effect of different IR power- 100 W (○), 200 W (□) and 300 W (∆)- at constant distance from IR lamp (A) and the effect of different distances from IR lamp- 5 cm (○), 10 cm (□) and 15 cm (∆)- at constant IR power (B) on Log N of *B. cereus* in cardamom seeds.

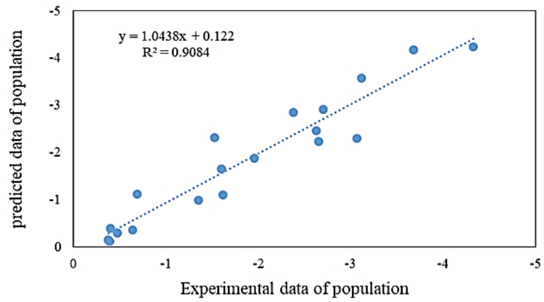

Fig. 3. Experimental versus predicted values of log N/N_0 of *B. cereus* using GA-ANN model for the test data set (r = 0.935).

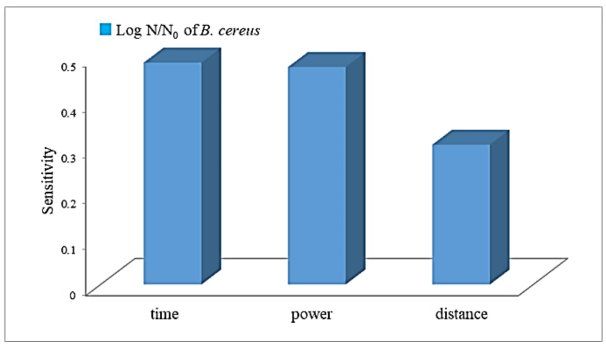

Fig. 4. Sensitivity analysis of optimized GA-ANN (3/12/1) for prediction of *B. cereus* population.

Table 1. The weights and bias values of optimized GA-ANN model.

Hidden neurons	Bias	Input neurons			Output neurons
		Time	Power	Distance	Log N/N_0 of *B. cereus*
1	-3.443	-2.868	0.238	-0.417	1.527
2	0.505	-0.344	0.446	0.353	-0.035
3	-0.181	0.334	0.185	0.386	0.355
4	-0.089	0.469	-0.324	0.301	-0.273
5	0.140	-0.164	-0.221	-0.293	-0.199
6	-0.016	-1.049	-0.088	0.805	-1.316
7	-1.292	0.607	1.215	-0.362	-1.509
8	-0.575	-0.016	-1.026	-0.652	-0.355
9	-0.742	1.244	0.272	0.146	-0.903
10	0.517	0.249	0.878	0.387	0.302
11	-0.502	0.169	0.085	0.648	0.505
12	-0.565	1.488	0.498	0.659	0.996
Bias					0.۱۹۰

Gain in the power of IR heating source generates more energy and energy absorbed by microorganisms increases that leading to microbial inactivation (Krishnamurthy et al., 2008).

The results of this study were in accordance with other studies, for example IR used for decontamination of *A. flavus* and *A. niger* in mung been. It was reported that increse of IR temperature, decontamination rates of mung been was increased. Furthermore, scanning electron microscopy showed that surface disorders and physical disruption of spores coat are the main causes behind the inactivation of IR treated fungal spores (Meenu et al., 2018). In other study, IR used for sterilization of wheat surface and reported that surface temperature increased with gain IR power as well as decontamination of wheat increased. IR radiation may be has a mechanism similar to ultraviolet radiation, with effect on nucleic acids or similar to microwave waves, by induction heat generation leading to inactivation microorganism (Hamanaka et al., 2000). In a study, IR was used in order to reduction of *Cladosporium sp*. and *Penicillium sp*. isolated from peaches, in culture media at different distance of the sample (90, 110, 130, 150 and 170 mm). It was reported that decreasing of sample distance from the IR lamp due to rapid heating sample, the amount of fungal spores removal increased (Trivittayasil et al., 2013). Furthermore, ultraviolet radiation and IR were used for decontamination of *S. aureus* and *B. subtilis* in milk and water. It was reported that with reduction the distance of IR lamp from sample, decontamination of samples was increased (Krishnamurthy, 2006).

3.2. GA-ANN

In this study, GA-ANN model was developed for prediction of *B. cereus* population in cardamom and ANN with 2-25 neurons was trained using GA to find out the optimal network configuration. It was found that GA-ANN with 12 neurons in one hidden layer could predict *B. cereus* population with high correlation coefficient (R^2 = 0.935). The prediction efficiency of the GA-ANN model for experimental and predicted value the reduction of *B. cereus* population is presented in Fig. 3. The weights and bias values of the optimized network showed in Table 1 that could be applied in a computer program for estimation of *B. cereus* population in cardamom. Based on the results, GA-ANN model had a good performance in prediction of the inactivation of *B. cereus* in the cardamom by IR.

Fig. 4 illustrates the sensitivity analysis of neural network models than different inputs. Among the input variables, IR irradiation time was the most sensitive factor, followed by IR power and finally sample distance from IR lamp for prediction the reduction of *B. cereus* population by the selected GA-ANN.

4. Conclusion

In this study, the decontamination of *B. cereus* in cardamom by IR treatment were modeled by GA-ANN as a function of IR power and sample distance from IR lamp was investigated. The IR treatments decreaced the *B. cereus* count in cardamom seeds to the acceptable levels. Moreover, the study results indicated that the GA-ANN with 1 hidden layer comprising 12 neurons with an acceptable correlation coefficient (0.935) could successfully be used for the prediction of the bacteria counts in cardamom.

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