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PREDICTION OF MICROBIAL INACTIVATION IN UV LIGHT TREATMENT OF WHITE TEA USING MACHINE LEARNING AND NEURAL NETWORKS

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ABSTRACT

The potential of ultra-violet (UV) light to replace the traditional brewing process to make cold tea in terms of inactivation of endogenous microflora has not been explored. Thus, the efficacy of emerging technologies such as UV-C by tea leaves/water ranging from 1 to 3 %, number of lamps ranging from 2 to 8, and number of cycles ranging from 4 to 8 were performed to determine the inactivation of total mesophilic aerobic bacteria (TMAB) and total mold and yeast (TMY) and changes in quality properties in cold drip white tea. The UV-light process was effective to reduce both TMAB and TMY. Increased number of cycles provided a significant amount of inactivation on both TMAB and TMY. The reduction of initial number of TMY was determined as 3.40±0.03 log cfu/mL with the number of lamps of 5, the number of cycle of 4, and tea leaves/water ratio of 1%, whereas TMAB were found as 3.12±0.08 log cfu/ with the number of lamps of 2, the number of cycles of 6 and tea leaves/water ratio of 1%. The resulting datasets were used to predict the inactivation of TMAB and TMY in cold drip white tea using gradient boosting regression tree (GBRT), random forest regression (RFR), and artificial neuron network (ANN) models. The ANN model provided the lowest RMSE and highest R^2 value for predicted inactivation of TMAB. TMY has not been predicted using either machine or neural networks. UV treatment possess a viable alternative for microbial inactivation without adverse effect on the quality properties of cold drip white tea.

Keywords: *Ultraviolet light, Cold drip white tea, Total mesophilic aerobic bacteria, Machine learning.*

INTRODUCTION

Tea, served as hot or ice-cold, is a trendy popular beverage worldwide. Depending on the variations in harvesting, processing, and associated degree of oxidation of fresh tea leaves; white, green, oolong, and black teas are produced from the leaves and buds of the *Camellia sinensis* (L.) (family *Theaceae*) (Unachukwu *et al*., 2010). Among those, white tea, due to being very rare and produced in minimal quantities because the leaves are collected only at dawn during a few days in the spring when the buds are still closed, is a very precious type of tea, and thus, it has been receiving increasing attention in the United States and Europe, recently (Obanda *et al.*, 2004). Ultra-violet (UV) light processing, is currently used to pasteurize food as an alternative non-thermal processing technology. UV-light can eliminate the microbial flora without the sensory quality of the food (Falguera et al., 2011). It is used for various food products to inactivate bacteria, molds, yeast, and protozoa (Guerrero-Beltran and Barbosa-Canovas, 2005)

Machine learning and neural networks can correlate large and complex datasets in solving many complex (non-linear) problems (Torrecilla *et al*., 2004). They have been used as a modeling tool in several food processing applications such as quality control, microbiology inactivation etc. (Goni *et al*., 2008; Yin and Ding, 2009).

To the best of our knowledge, the potential of UV-light to replace the traditional process to make cold tea in terms of the prediction of inactivation of yeast and bacteria using machine learning and neural networks has not been explored. Thus, the objectives of the study were to (i) treat white tea by UV-light as a function of the number of lamps, the number of cycles, and tea leaves/water ratio, and (ii) inactivate total aerobic mesophilic bacteria (TAMB) and total mold and yeast (TMY), (iii) evaluate and compare the several popular machine learning algorithms and neural networks for predicting inactivation of TMAB and TMY in white tea.

MATERIAL AND METHODS

Tea samples. Dry white tea leaves were acquired from the General Directorate of Tea Enterprises (Çay-Kur, Rize Turkey), grounded through one mm sieve, and stored in plastic bags at room temperature (22±2 ^{o}C) until processed.

Cold Drip Tea Preparation. The slower extraction process of cold brewed tea was prepared by using drip cold brew tower. This tower has a ceramic filter. Vessel was loaded by ice and water mixture. Tea leaves put into filter and adding 1L of ice-water mixture at room temperature to 10-30 g of tea leaves. Water dripped over the leaves to brew at room temperature (20-25 ^{o}C) for 8h. Water dripped at 30 drips per minute (1 every 2 seconds).

Ultra-Violet (UV) Light Processing. The UV light system was constructed in the Food Engineering Dept. at Bolu Abant İzzet Baysal University, Bolu, Turkey. The UV light system was consisted of an annular tube made from quartz glass and 12 (254 nm) UV lamps. UV lamps were placed around the outer cylinder of the quartz tube at an equivalent distance. 12 lamps are controlled independently on the panel and the desired lamp is turned on and off alone or together with other lamps. The diameter of the area created by UV lamps was 15 cm. The UV system consists of 12 UV lamps, each with 65 W power measuring 740 mm in size, which can see the products equally. Samples for microbial analysis were taken by using glass sample taps after each cycle.

Microbial inactivation. Inactivation of total mesophilic aerobic bacteria (TMAB) and total mold and yeast (TMY) were performed with the appropriate dilutions prepared by 0.1 % (w/v) peptone (Fluka, Seelze, Germany). TMAB samples were surface plated on plate count agar (PCA, Fluka, Seelze, Germany) and TMY samples on potato dextrose agar (PDA, Fluka, Seelze, Germany) acidified with 10 % (w/v) tartaric acid (Sigma Chemical Co., Stockholm, Sweden). PCA plates were incubated at 35±2 °C for 24-48 h, whereas PDA plates were incubated at 22±2 °C for 3-5 days, respectively. Results were reported as log cfu/mL.

Construction of machine learning algorithms and ANN. Two supervised machine learning (ML) algorithms including gradient boosting regression tree model and random forest regression and ANN model were evaluated to predict the inactivation of TMAB and TMY in cold drip white tea by controlling input variables such as number of lamps, number of cycle, and tea leaves/water ratio. Parameters for the gradient boosting regression tree model (GBRT-M) algorithm are the number of trees, the number of splits in the trees, the learning rate and minimum number of observations in nodes of trees. The random forest regression model (RFR-M) creates predictions by generating many decision trees and combining their predictions in a weighted average giving the final prediction. ANN models with one and two hidden layers and two activation functions such as hyperbolic tangent (TanH) and Gaussian were investigated. For all machine learning and ANN models, the dataset was randomly split into training (65% of the initial dataset), validating (25% of the initial dataset) and testing (10% of the initial dataset) sets. An optimal hyperparameter for each model was selected based on a trained model with a small RMSE value. All the models were performed by JMP Pro software package. The performance of each model was evaluated on the test data sets with RMSE (root mean square error) and the coefficient of determination (R^2) to measure the difference between the observed and predicted values of the selected model.

Data analyses. Box-Behnken experimental design was applied with total of 15 runs using explanatory variables of number of lamps, number of cycle, and tea leaves/water ratio (Table 1). Each run was conducted in triplicate.

Table 1. Sample codes, variables and their levels for cold drip white tea.

Sample code	Tea leaves/water ratio (%)	Number of lamps	Number of cycle
UV1	2	5	6
UV2	1	8	6
UV3	1	5	4
UV4	1	5	8
UV5	1	2	6
UV6	2	8	4
UV7	3	5	4
UV8	3	5	8
UV9	2	8	8
UV10	3	8	6
UV11	3	2	6
UV12	2	2	4
UV13	2	2	8

RESULTS AND DISCUSSION

Control cold drip white tea samples had the initial TAMB and TMY count of 2.54±0.18 and 2.77±0.86 log cfu/mL, respectively. UV process with different number of lamps, number of cycles, and tea leaves/water ratio was effective to reduce both TAMB and TMY (Fig.1). Generally, the higher number of lamps, number of cycles, and tea leaves/water ratio were more effective for microbial inactivation (Fig. 1).

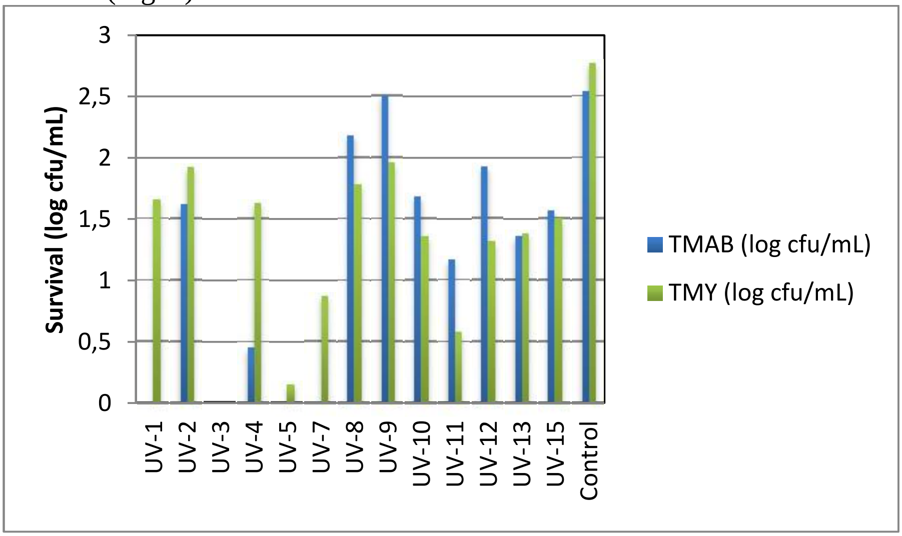

Figure 1. Inactivation of total mesophilic aerobic bacteria and total mold and yeast in cold drip white tea processed by UV

Microbial behavior was predicted for microbial inactivation using machine learning and ANN. The relationships between independent variables and explanatory variables can be determined empirically from the data processing approach using machine learning and ANN instead of a statistical approach. Different numbers of the hidden layers (one and two), number of neurons, and activation functions of the hidden layer (Gaussian and Tan H) were tested for ANN models of TMAB and TMY inactivation values. The number of hidden neurons is one of the crucial parameters of ANN. Thus, the number of neurons in the hidden layer was determined by the trial-and-error method. Several ANN models with different network topologies were trained, tested, and validated to select the best network topology. The R^2 and RMSE from training, validating, and testing data for different ANN topologies were summarized in Table 2.

Table 2. Characteristics of ANN for TMAB inactivation

Index	**No. of hidden layer**	**ANN network model**	**Training set**		**Validating set**		**Testing set**		**Activation function in hidden layer**
			RMSE	$\boldsymbol{R^2}$	**RMSE**	$\boldsymbol{R^2}$	**RMSE**	$\boldsymbol{R^2}$	
TMAB inactivation	1	3-3-1	0.89	0.49	0.88	0.62	0.87	0.56	Tan H
	1	**3-4-1**	**0.002**	**0.99**	**0.002**	**0.99**	**0.003**	**0.99**	**Tan H**
	1	3-5-1	0.96	0.59	0.75	0.74	0.96	0.52	Tan H
	2	3-2-3-1	0.80	0.68	0.73	0.66	0.91	0.63	Tan H
	2	3-2-4-1	0.53	0.48	0.85	0.75	1.04	0.68	Tan H
	2	3-3-3-1	0.80	0.61	0.74	0.78	0.06	0.79	Tan H

The 3-4-1 topology (Fig.2) was the best with minimum RMSE and maximum R^2 values for TMAB inactivation (Table 2). The best fit ANN model with one hidden layer was tested for TMAB inactivation. ANN model for TMAB inactivation was used as the first hidden layer by TanH as an activation function. The highest R^2 of 99.9% for TMAB belonged to the best-fit ANN models based on the TMAB inactivation testing data (Table 2). RFR-M, GBRT-M, and ANN-M were evaluated for TMAB and TMY inactivation. The ANN model provided the lowest RMSE and highest R^2 value for the predicted inactivation of TMAB (Table 2). TMY was not predicted using either machine or neural networks.

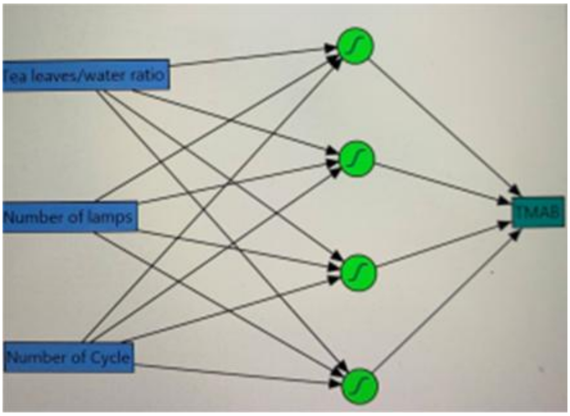

Figure 2.Architecture of ANN model used in the present study.

TMAB inactivation for all test data was predicted and plotted by ANN model against the observed values (Table 2). The models revealed R^2 values of 0.99, 0.99, and 0.99 for training, validation, and testing data of ANN-M with the RMSE values of 0.002, 0.002, and 0.003 (Table 2). ANN does not require a standard experimental design to build the model, and it is flexible and permits to addition of new experimental data to build more trustable models. This may be why ANN models can handle nonlinear responses better than the others (Chau *et al*., 2018).

CONCLUSIONS

Two machine learning algorithms (GBRT and RFR) were performed to predict the inactivation of TMAB and TMY. ANN-M predicted better inactivation of TMAB than the machine learning algorithms. However, TMY was not predicted using either machine or neural networks.

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