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SCIENTIFIC PAPER

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AN ARTIFICIAL NEURAL NETWORK AS A TOOL FOR KOMBUCHA FERMENTATION IMPROVEMENT

Article Highlights

- A novel process modeling approach in kombucha production is conducted
- Box-Behnken experimental design was conducted based on three operating factors
- The ANN model showed to be adequate for the prediction of output kombucha factors

Abstract

Kombucha as a tea-based fermented beverage has become progressively widespread, mainly in the functional food market, because of health-improving benefits. As part of a daily diet for adults and children, kombucha was a valuable non-alcoholic drink containing beneficial mixtures of organic acids, minerals, vitamins, proteins, polyphenols, etc. The influence of the specific surface area of the vessel, the inoculum size, and the initial tea concentration as operating factors and fermentation time as output variable on the efficiency of kombucha fermentation was examined. The focus of this study is optimization and standardization of kombucha fermentation conditions using Box-Behnken experimental design and applying an artificial neural network (ANN) predictive model for the fermentation process. The Broyden-Fletcher-Goldfarb-Shanno iterative algorithm was used to accelerate the calculation. The obtained ANN models for the pH value and titratable acidity showed good prediction capabilities (the r^2 values during the training cycle for output variables were 0.990 and 0.994, respectively). Predictive ANN modeling has been proven effective and reliable in establishing the optimum kombucha fermentation process using the selected operating factors.

Keywords: experimental design, fermentation improvement, kombucha production, mathematical modeling.

High beverage consumption worldwide has opened the opportunity to develop different traditional drinks as part of the functional food concept. In recent years, scientific and industrial focus on the extremely valuable functional drink has further developed and improved kombucha fermentation [1]. The worldwide trends in kombucha production have focused on developing health-improving beverages based on different types of tea that contain an advantageous number of promising bioactive compounds. Optimization of the kombucha production process became the main topic of several researchers because of its large importance from the definition of the chemical composition of this functional beverage but also from an industrial point of view [2–5].

Kombucha is typically prepared by fermenting sweetened (with sucrose) black or green tea inoculated with tea fungus pellicle or previously fermented broth at 100-200 mL/L. During kombucha fermentation, the formation of a floating pellicle of microbial cellulose is expected and very typical [3]. As a result, reduced cholesterol levels and blood pressure, influenced weight loss, improved liver and gastric functions, reduced kidney calcification, and increased vitality can be some health-improving benefits attributed to kombucha consumption [4–6].

The tea fungus is a consortium of acetic acid bac-

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teria (AAB) (*Gluconacetobacterxylinum*, previously known as *Acetobacter xylinus* and, more recently, as *Komagataeibacter xylinus*, is the primary and best-studied bacteria in kombucha) and yeasts (species of the genera *Saccharomyces*, *Torulopsis*, *Pichia*, *Brettanomyces*, *Zygosaccharomyces*, *Candida*, and *Saccharomycoides*) [7–11]. It is well known that the microbial community may vary between different kombucha cultures across the globe depending upon the source of the inoculum used. The role of yeasts in kombucha fermentation is to hydrolyze sucrose from the cultivation medium to glucose and fructose and metabolize these monosaccharides to ethanol, which is further oxidized to acetic acid by AAB. However, AAB cannot uptake sucrose alone because of the lack of enzymes for the extracellular hydrolysis of sucrose or its transport into the cell. Also, AAB uses yeast-derived glucose to synthesize gluconic acid and bacterial cellulose in the form of a pellicle, commonly described as the "fungus" [7,12].

Kombucha fermentation requires around seven days statically under aerobic conditions at temperatures between 25 °C and 30 °C [13]. The initial phase of this fermentation process is reflected in high sucrose levels but low acidity. On the other hand, further steps include a gradual decrease in oxygenation. This occurrence results from forming a cellulose layer on top of the cultivation liquid, the oxygen consummation, and the accumulation of organic acids (acetic acid, gluconic acid, etc.) produced by tea fungus [14–15]. At the end of kombucha fermentation, the system can be described with a well-structured cellulose layer on the top, high concentration of yeasts and AAB (106–108 CFU/mL), lower substrate concentration, and high acidity [16]. Creating a more controllable fermentation process and optimizing the operating factors require examining the diversity and role of each microbial group, the dynamics of the microbial population, and all changes in the system reflected through the quality of the final product [3]. Microbial interactions during kombucha fermentation strongly impact substrate consumption and metabolic production (e.g., ethanol produced by yeasts can harm the growth of some microorganisms, organic acids production by AAB can induce acidic stress of other microbiota, the pH changes from 5-7.0 to 2-4.0 after 7 days of fermentation, etc.) [15].

Although the type of tea is recognized as one of the essential factors in kombucha production, the most significant impact of the fermentation process is operating factors. The key factor of kombucha fermentation is assumed to be the oxygen amount in the culture medium, which is necessary for AAB proliferation. The dimensions of the fermentation vessel and the specific interfacial area can influence the oxygen level during fermentation which is a crucial operating parameter [17]. Furthermore, under static conditions, the amount of dissolved oxygen is inevitably the function of the size of the interfacial surface [18]. In the condition of a low oxygen concentration and the acid production by AAB, the pH value is above 4, which can induce lactic acid bacteria (LAB) growth and lactic acid production [3]. De Filippis *et al*. [18] reported that incubation time and temperature also influence the product's microbial activity and chemical characteristics.

The effect of many operating factors and conditions and the interaction between them on the efficiency of the fermentation process can be analyzed by different mathematical tools. For example, response surface methodology (RSM) and other experimental designs (e.g., Box-Behnken, Plackett-Burman, Taguchi design, etc.) can be effective tools for optimizing the targeted process and explaining the individual and combined effect of the independent variables [19-20]. Using adequate mathematic analysis can determine and simultaneously explain the optimization of kombucha fermentation and set up further steps during the scale-up of kombucha production [21]. Only a few scientific studies and craft production deal with scale-up and more controllable fermentation processes during kombucha production. It is necessary to optimize operating factors and create the next generation of kombucha as part of the functional food field. In this way, kombucha fermentation will be more predictable and economical for industrial production [3].

The objective of this study was to investigate the possibility of predicting pH value and titratable acidity based on the specific surface area of the vessel (SSAV), the inoculum size (Inn), and the initial concentration of tea (ICT) using artificial neural network modeling. In addition, Time was an additional output, as one of the variables defined after achieving optimal pH value and titratable acidity and used for further mathematical modeling.

MATERIAL AND METHODS

Tea fungus

Fermentation was performed using the local household tea fungus culture. Previous studies showed that it contained at least five yeast strains (*Saccharomycodes ludwigii*, *S. cerevisiae*, *S. bisporus*, *Torulopsis* spp., and *Zygosaccharomyces* spp.) and two bacteria of the *Acetobacter* genera [22–23].

Fermentation conditions

The cultivation medium uses sweetened black tea

(70 g sucrose/L of tap water). Briefly, 0.15%, 0.3%, or 0.45% (w/v) of black tea ("Fructus," Bačka Palanka, Republic of Serbia) was added to boiled tap water and removed after 15 min by filtration. After reaching room temperature, the tea base was inoculated with 2.5%, 5%, or 10% (v/v) of the fermentation broth from the previous fermentation (five-day fermentation until optimal acid content of 4– 4.5 g/L is reached) obtained at 28 ± 1 °C without stirring. The cultivation medium was transferred into cylindrical glass vessels whose geometric characteristics are shown in Table 1. To get different values of the specific surface area of the vessel, the volume of the cultivation medium in the vessels was varied. The specific surface area of the vessel presents the ratio of the free area (cross-sectional area of the vessel) and the volume of the substrate. The sterile gauze was placed on a glass vessel to prevent contamination during cultivation. The medium was incubated at 28 ± 1 °C without stirring. All experiments were performed in three independent replicates, and all obtained values are represented as the arithmetic values of individual measurements.

Table 1. Characteristics of cylindrical glass vessels and specific surface area of the vessel (SSAV)

Vessel characteristics		The volume of cultivation medium	SSAV*
Volume	Diameter		
(L)	(cm)	(L)	(cm^{-1})
0.72	8	0.17	0.30
0.72	8	0.33	0.15
5	16	3.30	0.06

* The ratio of the free area (cross-sectional area of the vessel) and the volume of the substrate.

Sampling

A sampling of the fermentation medium was performed every day until the selected output (pH value and TA) did not show the optimal acid content until the end of fermentation. Sampling was done only once in the specified time to avoid the potential contamination during a further fermentation process. During the fermentation process, pH value and TA were determined.

Methods of Analysis

The pH values were measured using an electronic pH meter (HI 99181, HANNA Instruments, Woonsocket, USA) calibrated at pH 4.0 and 7.0. The titratable acidity (TA) was determined according to Jacobson [24]. After removing CO_2 (during 30-second treatment in an ultrasound bath, B-220, Branson Company, Shelton, USA) from the fermentation broth, an aliquot was taken and titrated with 0.1M NaOH. The TA was expressed in grams of acetic acid per liter of the sample.

Experimental design

The pH value and titratable acidity were predicted based on three operation factors: the SSAV, the Inn, and the ICT. These three operating factors (X1 – SSAV, X2 – Inn, and X3 – ICT) were independent factors in the selected Box-Behnken experimental design. The pH value and titratable acidity were chosen as the dependent factors. The experimental design is given as % in Table 2 with three levels for each independent factor, coded as -1, 0, and +1, corresponding to the lower, middle, and higher levels, respectively. The response surface method was used to evaluate the influence of the MATH operating factors on the kombucha fermentation process. The impact of the examined factors and their interaction was studied using response surface plots to present the influence of fermentation time and initial tea concentration on pH value and TA of kombucha during fermentation. Time was an additional output as one of the variables defined after achieving optimal pH value and titratable acidity and used for further mathematical modeling.

ANN modeling

A multi-layer perceptron model (MLP), which consisted of three layers (input, hidden, and output), was used for modeling an artificial neural network model (ANN) for the prediction of pH value and TA based on three input variables: SSAV, Inn, ICT, as well as on one output, i.e., Time which values defined achieving optimal acidity for kombucha production. In the known literature, the ANN model was proven as quite capable of approximating nonlinear functions [25–27]. Before the calculation, both input and output data were normalized to improve the behavior of the ANN. During this iterative process, input data were repeatedly presented to the network [28–29]. Broyden -Fletcher-Goldfarb-Shanno (BFGS) algorithm was used as an iterative method for solving unconstrained nonlinear optimization during the ANN modeling.

The experimental database for ANN was randomly divided into training, cross-validation, and testing data (with 70%, 15%, and 15% of experimental data, respectively). The training data set was used for the learning cycle of the ANN and the evaluation of the optimal number of neurons in the hidden layer and the weight coefficient of each neuron in the network. A series of different topologies

were used, in which the number of hidden neurons varied from 5 to 20, and the training process of the network was run 100,000 times with random initial values of weights and biases. The optimization process was performed based on validation error minimization. It was assumed that successful training was achieved when learning and cross-validation curves approached zero.

Coefficients associated with the hidden layer (weights and biases) were grouped in matrices W_1 and B_1. Similarly, coefficients related to the output layer were grouped in matrices W_2 and B_2. It is possible to represent the neural network by using matrix notation (Y is the matrix of the output variables, f_1 and f_2 are transfer functions in the hidden and output layers, respectively, and X is the matrix of input variables [30]:

$$Y = f_1(W_2 \cdot f_2(W_1 \cdot X + B_1) + B_2) \quad (1)$$

Weight coefficients (elements of matrices W_1 and W_2) were determined during the ANN learning cycle, which updated them using optimization procedures to minimize the error between the network and experimental outputs [28, 30–32], according to the sum of squares (SOS) and BFGS algorithm, used to speed up and stabilize convergence [33]. Finally, the coefficients of determination were used as factors to check the performance of the obtained ANN model. Statistical analyses were done using Statistica software v. 13.2 (Dell, Round Rock, Texas, USA).

Sensitivity analysis

Yoon's interpretation method was applied based on the connection weights partitioning of the developed ANN to determine the relative influence (RI) of the SSAV, Inn, ICT, and Time on pH value and titratable acidity. The following equation developed by Yoon et al. [34] was used:

$$RI_{ij} = \frac{\sum_{k=0}^{n}\left(w_{ik} \cdot w_{kj}\right)}{\sum_{i=0}^{m}\left|\sum_{k=0}^{n}\left(w_{ik} \cdot w_{kj}\right)\right|} \cdot 100\% \quad (2)$$

where RI_{ij} is the relative importance of the i-th input variable on the j-th output, w_{ik} is the weight between the i-th input and the k-th hidden neuron, and w_{kj} is the weight between the k-th hidden neuron and the j-th output.

The accuracy of the model

The numerical verification of the developed model was tested using the coefficient of determination (r^2), reduced chi-square (χ^2), mean bias error (MBE), root mean square error (RMSE) and mean percentage error (MPE). These commonly used parameters can be calculated as follows [35]:

$$\chi^2 = \frac{\sum_{i=1}^{N}(x_{\exp,i} - x_{pre,i})^2}{N - n},$$
$$RMSE = \left[\frac{1}{N} \cdot \sum_{i=1}^{N}(x_{pre,i} - x_{\exp,i})^2\right]^{1/2}, \quad (3)$$
$$MBE = \frac{1}{N} \cdot \sum_{i=1}^{N}(x_{pre,i} - x_{\exp,i}),$$
$$MPE = \frac{100}{N} \cdot \sum_{i=1}^{N}\left(\frac{\left|x_{pre,i} - x_{\exp,i}\right|}{x_{\exp,i}}\right)$$

where $x_{exp,i}$ stands for the experimental values and $x_{pre,i}$ are the predicted values calculated by the model, N and n are the number of observations and constants, respectively.

RESULTS AND DISCUSSION

Kombucha fermentation was studied to determine the influence of the SSAV, the Inn, and ICT on the efficiency of kombucha fermentation through pH value and titratable acidity (TA). Additionally, an important factor was also final Time, which was observed after achieving optimal acidity in the system. Namely, to obtain a pleasantly sour beverage, fermentation should be terminated when TA of fermentation broth reaches 4-4.5 g/L, which is confirmed by kombucha consumers [17]. The reason for the selection of incubation time is that an optimum fermentation time is required for the production of kombucha with pleasant flavor and taste, as well as further scaling-up and potential industrialization of kombucha production. Furthermore, longer fermentation produces high levels of acids (like mild vinegar) that may pose potential risks when consumed [36]. For this purpose, Box Behnken design was performed for all mentioned operating factors of the fermentation process. Table 2 summarizes the used experimental design and the obtained results.

Before further mathematical analysis, it can be observed based on the obtained results that the specific interfacial surface area of the vessel has a particular influence on acetic acid synthesis during kombucha fermentation. Furthermore, in the vessels with a specific interfacial surface area is 0.3 cm^{-1}, an optimal acidity of kombucha was achieved practically twice as faster compared to vessels with a specific area of 0.06 cm^{-1} under the same other experimental factors (by comparing experiments 5 and 6, as well as 7 and 8 in Table 2). Cvetković *et al.* [37] previously established a mathematical model to ensure the scaling-up process of kombucha fermentation, which can be quite complex and should consider the specific interfacial area as the main variable. Similar conclusions are reported by Junker [38] and Villarreal-Soto *et al.* [39], which demonstrated the same behavior during the variability

of this operating parameter. Furthermore, without agitation and aeration, the kombucha fermentation process in cylinder vessels strongly depends on the specific surface area, which is indicative of this study's obtained results. Therefore, it can be concluded that an effective process can ensure vessels with a specific surface area greater than 0.15 cm^{-1} provide a sufficient oxygen supply by diffusion through the medium surface area.

The influence of the inoculum size on the fermentation process is primarily observed in the slightly increased acidity of the medium after inoculation. A higher concentration of total acids was recorded in the cultivation medium with a larger inoculum size by studying fermentation processes through the Box-Behnken experimental design. As a result, the time required to obtain the beverage of optimal acidity was shorter. However, slightly higher acidity in the fermentation broth has a beneficial effect on the physiological activity of yeasts and AAB [3]. Acid's presence stimulates yeasts to produce ethanol, which is then used by acetic acid bacteria to grow and produce more acetic acid [40]. Lončar *et al.* [41] reported that fermentation rate was slightly affected by inoculums concentrations ranging between 10 and 15% (v/v). Therefore, although increasing inoculum size can positively affect the productivity of kombucha fermentation, it can be concluded that there is also a negative impact on the economy of kombucha production at the same time. According to the obtained results, it can be summarized that the initial tea concentration had a specific influence since the fermentation will be finalized using any of the tested concentrations of herbal. On the other hand, the fermentation will be finalized at different points in time using a different combination of tested inputs. Briefly, the fermentation time can be shortened on 4 incubation days by applying optimized values of SSAV, Inn, and ICT.

ANN modeling

Based on experimental results obtained from the experimental design, the final fermentation time to reach optimal acidity was included as one of the data for further statistical analysis. This step provided another aspect of examination of operating factors of kombucha fermentation. There have been no published results on the application of ANNs for predictive modeling of kombucha production based on the pH and TA values. Despite the lack of results in this field, ANN models are recognized in bioprocesses as a good modeling tool. They offer an empirical explanation of the problems from experimental data and can conduct complex systems with nonlinearities and interactions between decision variables [20]. The ANN model is developed to accurately predict pH and TA values based on the selected factors. The obtained number of hidden neurons in the network was 8 (network MLP 4-8-2). In this way, very high values of r^2 (during the training cycle, the r^2-values for the output variables were: above 0.99) were gained, shown in Table 3. The acquired optimal neural network model showed a good generalization capability for the tested experimental data. However, the obtained ANN model for the prediction of output variables was complex (58 weights-biases) because of the high nonlinearity of the observed system [42].

The three-dimensional surface plots were created (Figure 1) to present the influence of fermentation time and initial tea concentration on pH value and TA of kombucha fermentation. It can be observed that minimal initial tea concentration (0.15%) provided sufficient nitrogen compounds and mineral elements necessary for kombucha fermentation under stationary fermentation conditions. However, further tests should confirm whether this concentration of tea is the final minimal amount that ensures the efficiency of kombucha fermentation. As the time and temperature of fermentation are used in kombucha production, the tea and sugar proportions can vary according to each region or consumer preferences (2017).

According to Jayabalan *et al.* [4], the standard procedure for kombucha production implies the use of 50 g sucrose and 5 g tea leaves with 1 L boiled tap water. The tea is removed by filtration after 5 min, and after cooling to room temperature, the medium is inoculated with 24 g of the tea fungus culture. Kallel *et al.* [42] used even 12 g/L of green or black tea with 5 min of infusion to prepare the kombucha cultivation medium. After 15 days of fermentation, the TA for green tea kombucha was 5.4 g/L and 8.0 g/L for black tea kombucha. Generally, the same differences in TA, process duration, and cell counts in kombucha beverages obtained in different studies are expected because of inoculums (tea fungus culture) from other locations. The variations could be due to geographic, climatic, and cultural conditions and local species of wild yeasts and bacteria or, possibly, cross-contamination between cultures [8]. Based on the unattainable adequate TA and pH values, it can also be concluded that, despite the high contents of C and N sources, the fermentation process with a high tea concentration can be slower and, therefore, economically less acceptable.

The fermentation time in kombucha production is a variable parameter demonstrated in this study (Table 2, Figure 1). Based on the obtained results (Figure 1a), it is evident that the adequate pH value was achieved

Table 2. Box-Behnken design with three independent factors (SSAV - Specific surface area of the vessel (cm^{-1}), Inn - Inoculum size (v/v), and ICT - Initial concentration tea (w/v) and the obtained results for pH and titrable acidity (TA, g/L)

	Coded factor level			Varied factor value			Output	Fermentation time, days								
	X_1	X_2	X_3	SSA	Inn	ITC		0	1	2	3	4	5	6	7	8
1	-1	-1	0	0.06	2.5	0.3	pH	6.05±0.01	5.08±0.02	4.26±0.0	3.46±0.01	3.17±0.00	3.14±0.0	3.04±0.00	-	-
							TA	0.00±0.0	0.15±0.0	0.55±0.07	1.83±0.07	3.27±0.12	4.18±0.04	5.06±0.01	-	-
2	1	-1	0	0.3	2.5	0.3	pH	5.86±0.03	5.34±0.01	4.51±0.01	3.94±0.00	3.34±0.0	3.16±0.1	-	-	-
							TA	0.00±0.0	0.04±0.2	0.14±0.04	0.57±0.03	1.96±0.04	2.77±0.3	-	-	-
3	-1	1	0	0.06	10	0.3	pH	4.61±0.03	3.93±0.04	3.34±0.0	3.14±0.06	2.99±0.00	2.90±0.01	-	-	-
							TA	0.00±0.0	0.43±0.01	1.91±0.04	3.53±0.00	4.86±0.00	6.56±0.03	-	-	-
4	1	1	0	10	0.3	10	pH	4.5±0.0	4.21±0.0	3.42±0.0	2.99±0.01	2.78±0.02	-	-	-	-
							TA	0.00±0.0	0.12±0.03	1.23±0.01	3.47±0.11	8.58±0.04	-	-	-	-
5	-1	0	-1	0.06	5	0.15	pH	4.70±0.01	4.27±0.01	4.20±0.01	3.61±0.2	3.38±0.10	3.13±0.00	-	-	-
							TA	0.00±0.0	0.04±0.2	0.26±0.31	1.06±0.01	1.97±0.09	3.94±0.01	-	-	-
6	1	0	-1	0.3	5	0.15	pH	5.15±0.06	4.56±0.01	3.88±0.0	3.29±0.00	2.89±0.02	-	-	-	-
							TA	0.00±0.0	0.10±0.04	0.49±0.27	2.65±034	6.99±0.81	-	-	-	-
7	-1	0	1	0.06	5	0.45	pH	4.70±0.04	4.09±0.01	4.26±0.0	3.79±0.00	3.46±0.01	3.12±0.00	-	-	-
							TA	0.00±0.0	0.12±0.14	0.26±0.06	0.89±0.31	1.98±0.22	3.99±0.01	-	-	-
8	1	0	1	0.3	5	0.45	pH	5.14±0.11	4.57±0.0	3.88±0.07	3.28±0.00	2.89±0.10	-	-	-	-
							TA	0.00±0.0	0.10±0.13	0.49±0.11	2.65±0.06	6.99±0.81	-	-	-	-
9	0	-1	-1	0.15	2.5	0.15	pH	5.53±0.01	5.17±0.0	435±0.0	3.44±0.0	3.10±0.00	2.84±0.00	2.72±0.03	-	-
							TA	0.00±0.0	0.03±0.2	0.15±017	0.72±0.2	2.53±1.24	6.84±0.51	9.94±0.67	-	-
10	0	1	-1	0.15	10	0.15	pH	5.53±0.04	5.31±0.0	4.81±0.0	4.37±0.0	3.55±0.04	3.29±0.00	3.17±0.00	3.00±0.00	2.94±0.00
							TA	0.00±0.0	0.10±0.09	0.16±0.01	0.47±0.02	1.10±0.07	2.65±0.03	2.69±0.16	4.11±0.01	5.78±0.23
11	0	-1	1	0.15	2.5	0.45	pH	6.05±0.01	6.02±0.00	5.08±0.01	4.43±0.0	3.99±0.06	3.09±0.07	2.86±0.00	-	-
							TA	0.00±0.0	0.002±0.0	0.04±0.2	0.14±0.04	0.44±0.07	3.44±0.10	6.91±0.40	-	-
12	0	1	1	0.15	10	0.45	pH	4.46±0.03	4.32±0.01	3.56±0.03	2.89±0.06	2.81±0.09	2.74±0.00	2.60±0.05	-	-
							TA	0.0±0.0	0.033±0.1	0.62±0.27	2.03±0.1	5.15±0.01	8.69±0.21	11.09±0.2	-	-
13	0	0	0	0.15	5	0.3	pH	4.70±0.11	4.24±0.05	4.35±0.0	3.81±0.0	3.48±0.03	3.13±0.0	-	-	-
							TA	0.00±0.0	0.12±0.02	0.24±0.07	0.69±0.11	1.89±0.02	3.05±0.02	-	-	-
14	0	0	0	0.15	5	0.3	pH	4.70±0.01	4.24±0.0	4.35±0.0	3.81±0.1	3.48±0.00	3.13±0.01	-	-	-
							TA	0.00±0.0	0.12±0.06	0.24±0.1	0.69±0.03	1.89±0.13	3.05±0.06	-	-	-
15	0	0	0	0.15	5	0.3	pH	4.70±0.04	4.24±0.01	4.35±0.01	3.81±0.05	3.5±0.01	3.13±0.00	-	-	-
							TA	0.00±0.0	0.12±0.02	0.24±0.03	0.69±0.05	1.9±0.03	3.05±0.07	-	-	-

Table 3. Artificial neural network model summary (performance and errors)

Network name	Performance*			Error			Training algorithm	Error function	Hidden activation	Output activation
	Train.	Test.	Valid.	Train.	Test.	Valid.				
MLP 4-8-2	0.992	0.902	0.990	0.053	0.723	0.052	BFGS 56	SOS	Tanh	Logistic

* Performance term represents the coefficients of determination, while error terms indicate a lack of data for the ANN model.

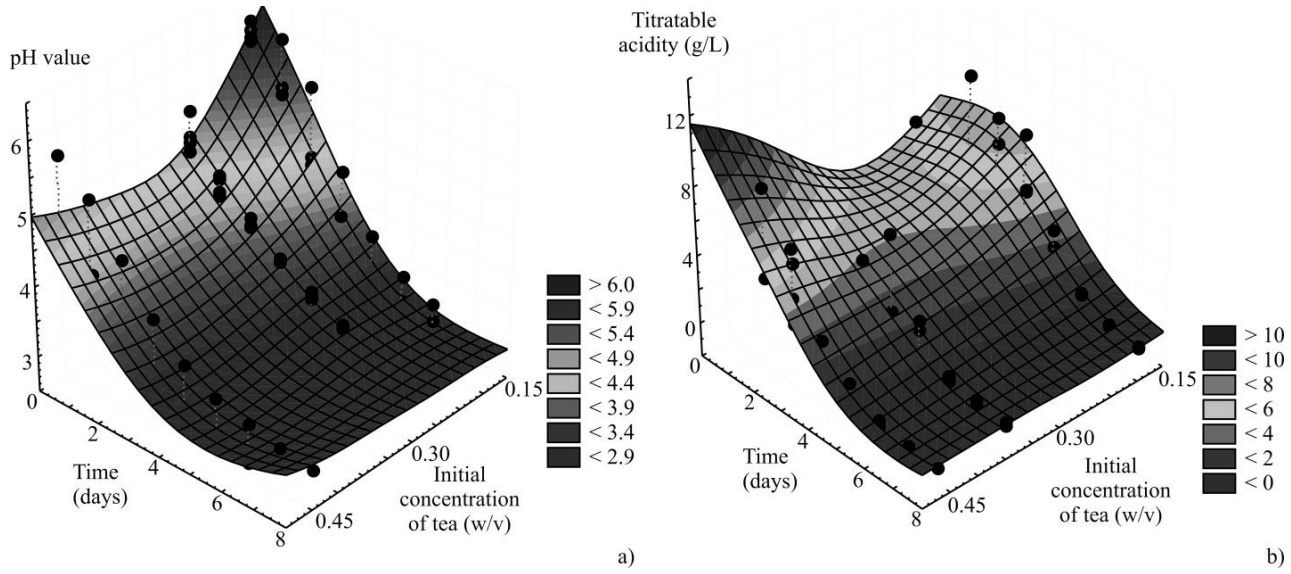

Figure 1. The influence of time (days) and initial concentration of tea on the pH value and titratable acidity (g/L) (3D surfaces - predicted values, black dots - experimental values).

after 4 days of fermentation and at any value of initial tea concentration. According to the results presented in the three-dimensional plot (Figure 1b), it can be concluded that the TA value is equally achieved at medium values of fermentation time and any values of tea concentration. The obtained result for pH and TA correlated with the results of Cvetković *et al.* [37], which suggested that after three days of incubation, the pH value of 3.21 and TA value of 4.32 g/L can be achieved. The accuracy of the ANN model could be visually assessed by the dispersion of points from the diagonal line in the graphics presented in Figure 2. For the ANN model, the predicted values were very close to the measured values in most cases, in terms of r^2 values. Therefore, SOS obtained with the ANN model were of the same order of magnitude as experimental errors for the pH value and TA. Table 4 presents the elements of matrix W_1 and vector B_1 (shown in the bias column) and the elements of matrix W_2 and vector B_2 (bias) for the hidden layer used for Eq. (2). The goodness of fit between experimental measurements and model-calculated outputs, represented as ANN performance (sum of r^2 between measured and calculated output variables), during training, testing, and validation steps, are shown in Table 5.

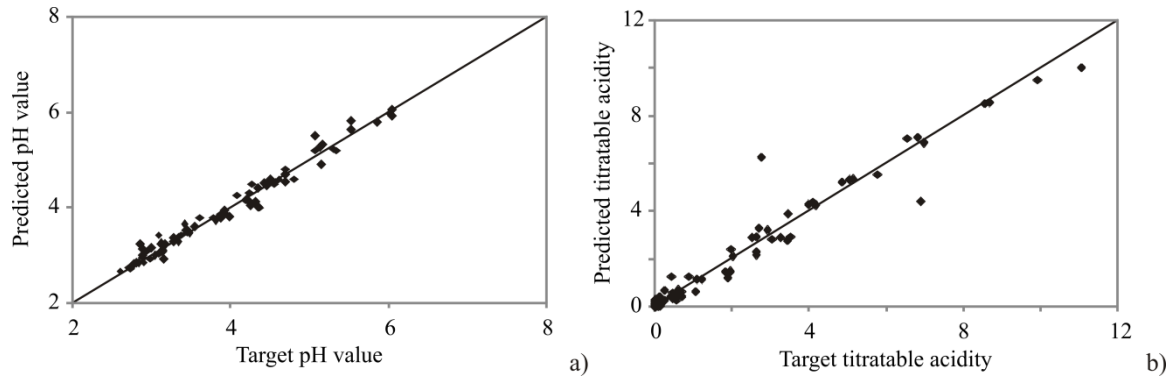

Figure 2. Comparison between experimental and calculated pH values and titratable acidity (g/L).

Table 4. Elements of matrix W_1 and vector B_1 (presented in the bias column)

Inputs	1	2	3	4	5	6	7	8	
SSAV	-0.912	2.235	-0.674	0.279	1.357	-5.377	-0.295	0.545	
Inn	-0.538	-3.939	-1.484	6.261	-3.636	1.385	1.628	0.344	
ICT	1.231	-0.655	-0.325	-1.273	0.818	2.114	-1.333	0.690	
Time	0.242	-3.318	0.025	4.048	-2.839	-1.876	0.176	-2.758	
Bias	-0.297	0.046	-0.272	0.786	3.328	3.201	-1.161	0.457	
Outputs	1	2	3	4	5	6	7	8	Bias
pH value	0.381	1.503	-1.098	-3.438	2.105	1.092	3.240	0.808	2.962
Titratable acidity	0.808	-3.533	0.334	1.703	-2.594	-2.677	-3.506	-1.880	-5.347

Table 5. The "goodness of fit" tests and residual analysis for the developed ANN model

Output variable	χ^2	RMSE	MBE	MPE	SSE	AARD	r^2	Skew	Kurt	Mean	StDev	Var
pH value	0.02	0.15	-0.004	2.73	1.96	13.05	0.97	0.06	1.08	-0.004	0.15	0.02
Titratable acidity	0.29	0.53	0.007	38.75	26.66	25.51	0.96	-1.91	23.88	0.007	0.54	0.29

A high r^2 is indicative that the variation was accounted for and that the data fitted the proposed model satisfactorily [43]. The residual analysis of the developed model is presented in Table 5. The ANN model reasonably predicted experimental variables for a broad range of process variables. For the ANN model, the predicted values were very close to the measured values in most cases, in terms of r^2 values. SOS values obtained with the ANN model were of the same order of magnitude as experimental errors for output variables reported in the literature [28, 32]. The ANN model had an insignificant lack of fit tests, which means the model satisfactorily predicted output variables. A high r^2 indicates that the variation was accounted for and that the data adequately fitted the proposed model [44–45].

Global sensitivity analysis- Yoon's interpretation method

This section studied the influence of SSAV, Inn, ICT, and Time on the relative importance (RI) of pH value and TA. According to Figure 3, Time and Inn were the most influential factors with the relative importance of -47.26% and -44.08%, respectively, for the pH value calculation. In comparison, the relative influence of the mentioned factors was 55.32% and 35.59%.

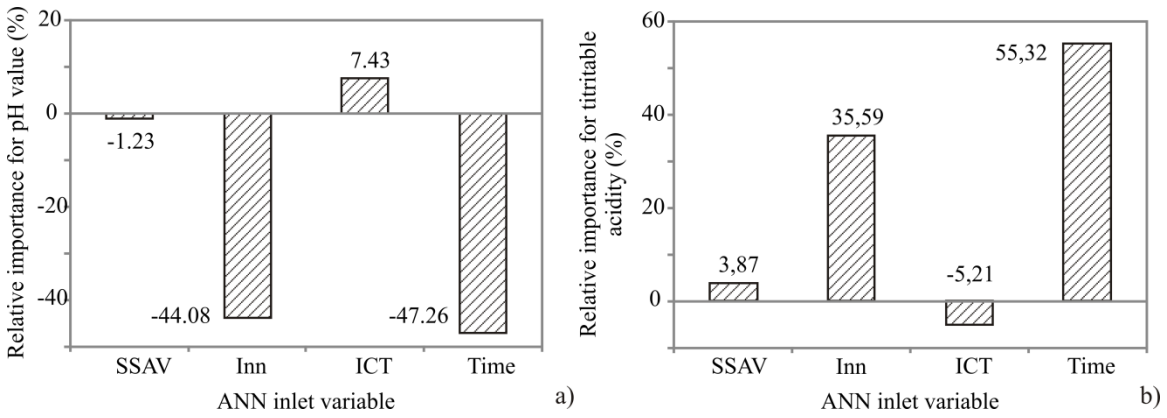

Figure 3. The relative importance of operating parameters on pH value and titratable acidity determined using the Yoon interpretation method.

CONCLUSION

An investigation of the kombucha fermentation optimization was performed to determine the possibility of predicting the pH value and TA based on three input variables: SSAV, Inn, ICT, as well as one output, i.e., Time which was defined as achieving the optimal acidity in the system, using the ANN model. The ANN model was shown to be adequate for predicting output variables (the r^2 values during the training cycle for these variables were: 0.990 and 0.994, respectively). In summary, Box Benhken's experimental design was applied, and predictive ANN modeling was developed to establish the optimum kombucha fermentation process to achieve the effective fermentation of the kombucha beverage. Effective kombucha fermentation can be performed using vessels with a specific surface area greater than 0.15 cm^{-1}, which provides sufficient oxygen supply by diffusion through the medium surface area. The final product can be obtained after 4 days of fermentation and at any tested value of initial tea concentration. Furthermore, the scale-up process from a laboratory scale to a commercial product is a challenge because of the difficulty of optimizing the factors which may influence the scaling process during fermentation. Therefore, more scientific research in the optimization of the operating factors should be done to establish effective fermentation and production of a functional beverage.

In the following steps of this investigation, other essential factors for monitoring kombucha fermentation have to be tested in the same way as reported in this study. For example, the selection of additional factors can be directed to a concentration of residual sugar and alcohol and the evolution of carbon dioxide. In addition, testing as many characteristics of the fermented product and determining their correlation with operation factors leads to obtaining functional beverages that consumers will quickly accept. Furthermore, understanding the type and number of microbiota present in the tea fungus culture can explain specific influence on kombucha fermentation and tested factors in this research.

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REFERENCES

[1] J. Kim, K. Adhikari, Bevarages 6 (2020) 1–19.

[2] J. Islam, Y. Kabir, Yects and Mechanisms of Antioxidant-Rich Functional Beverages on Disease Prevention, Woodhead Publishing: Duxford, UK (2019) p. 118.

[3] D. Laureys, S. Britton, J. De Clippeleer, J. Am. Soc. Brew. Chem.78 (2020) 165–174.

[4] R. Jayabalan, R.V. Malbaša, M. Sathishkumar, Kombucha Tea: Metabolites. In Fungal Metabolites. Springer International Publishing (2017) p. 965–978.

[5] M. Coton, A. Pawtowski, B. Taminiau, G. Burgaud, F. Deniel F, FEMS Microb. Ecol.93 (2017) 1–16.

[6] J.M. Kapp, W. Sumner, A. Ann. Epidemiol.30 (2019) 66–70.

[7] C.J. Greenwalt, K.H. Steinkraus, R.A. Ledford, J. Food Prot.63 (2000) 976–981.

[8] Al. Teoh, G. Heard, J. Cox, Int. J. Food Microbiol.95 (2004) 119–126.

[9] S.C. Chu, C. Chen, Food Chem.98 (2006) 502–507.

[10] Y. Yamada, P. Yukphan, Int. J. Food Microbiol.125 (2008) 15–24.

[11] Y. Yamada, P. Yukphan, H.T. Lan Vu, Y. Muramatsu, D. Ochaikul, S. Tanasupawat, Y. Nakagawa, J. Gen Appl. Microbiol.58 (2012) 397–404.

[12] C.P. Kuerzman, C.J. Robnett, E. Basehoar-Powers, FEMS Yeast Res.1 (2001) 133–138.

[13] S. Chakravorty, S. Bhattacharya, D. Bhattacharya, S. Sarkar, R. Gachhui, Kombucha: A Promising Functional Beverage Prepared from Tea, Sawston, Cambridge (2019).

[14] Z.W. Yang, B.P. Ji, F. Zhou, B. Li, Y. Luo, L. Yang, T. Li, J. Sci. Food Agric.89 (2009) 150–156.

[15] K. Neffe-Skocinska, B. Sionek, I. Scibisz, D. Kolozyn-Krajewska, Cyta J. Food 15 (2017) 601–607.

[16] A.J. Marsh, O. O'Sullivan, C. Hill, R.P. Ross, P.D. Cotter, Food Microbiol.38 (2014) 171–178.

[17] D. Cvetković, S. Markov, M. Djurić, D. Savić, A. Velićanski, J. Food Eng.85 (2008) 387–392.

[18] F. De Filippis, A.D. Troise, P. Vitaglione, D. Ercolini, Food Microbiol.73 (2018) 11–16.

[19] A. Vidaković, O. Šovljanski, A. Ranitović, D. Cvetković, S. Markov, Acta Period. Technol.48 (2017) 295–305.

[20] O. Šovljanski, A. Tomić, L. Pezo, A. Ranitović, S. Markov, J. Serb. Soc. Chem.85 (2020) 1417–1427.

[21] P. Manivasagan, J. Venkatesan, K. Sivakumar, S. Kim, Microb. Res.169 (2020) 262–278.

[22] S. Markov, R. Malbaša, M. Hauk, D. Cvetković, Acta Period. Technol. 32 (2001) 133–138.

[23] A. Velićanski, D. Cvetković, S. Markov, Rom. Biotechnol. Lett. 18 (2012) 8034–8042.

[24] J.L. Jacobson, Introduction to Wine Laboratory Practices and Procedures. Springer Science, New York (2006).

[25] D.P. Johnson, A. Stanforth, V. Lulla, G. Luber, Appl. Geogr. 35 (2012) 23–31.

[26] T.S. Yun, Y.J. Jeong, T.S. Han, K.S. Youm, Energ. Buildings 61 (2013) 125–132.

[27] J.P.C. Kleijnen, Design and Analysis of Simulation Experiments. Springer Proceedings in Mathematics and Statistics, Italy (2018).

[28] T. Kollo, D. von Rosen, Advanced Multivariate Statistics with Matrices, Springer, Berlin (2005).

[29] L. Pezo, B.Lj. Ćurčić, V.S. Filipović, M.R. Nićetin, G.B. Koprivica, N.M. Mišljenović, Lj.B. Lević, Hem. Ind.67 (2013) 465–475.

[30] C.I. Ochoa-Martínez, A.A. Ayala-Aponte, LWT40 (2007)

638–645.
[31] L.A. Berrueta, R.M. Alonso-Salces, K. Héberger, J. Chromatogr. A1158 (2007) 196–214.
[32] M. Doumpos, C. Zopounidis, Eur. J. Oper. Res. 209 (2011) 203–214.
[33] B.J. Taylor, Methods and Procedures for the Verification and Validation of Artificial Neural Networks, Springer Science and Business Media, New York (2006).
[34] Y. Yoon, G. Swales, T.M. Margavio, J. Oper. Res. Soc.44 (1993) 51–60.
[35] O. Šovljanski, L. Pezo, A. Tomić, A. Ranitović, D. Cvetković, S. Markov, J. Basic Microb.61 (2021) 835–848.
[36] G. Sreeramulu, Y. Zhu, W. Knol, J. Agri. Food Chem.48 (2000) 2589–2594.
[37] D. Cvetković, A. Ranitović, D. Savić, N. Joković, A. Vidaković, L. Pezo, S. Markov, Pol. J. Food Nutr. Sci. 69 (2019) 407–415.
[38] B.H. Junker, J. Biosci. Bioeng. 97 (2004) 347–364.
[39] S. Villarreal-Soto, S. Beaufort, J. Bouajila, J.P. Souchard, T. Renard, S. Rollan, P. Taillandier, Process Biochem. 83 (2019) 44–54.
[40] M.J. Santos, Kombucha: Caracterizaçao da microbiota e desenvolvimento de novos produtos alimentares para uso em restauraçao. Universidade Nova de Lisboa, Lisboa (2016).
[41] E. Lončar, K. Kanurić, R. Malbaša, M.S. Đurić, S.D. Milanović,.CICEQ 20 (2014) 345–352.
[42] L. Kallel, V. Desseaux, M. Hamdi, P. Stocker, E.H. Ajandouz, Food Res. Int. 49 (2012) 226–232.
[43] D.C. Montgomery, Design and Analysis of Experiments, John Wiley and Sons, New York (1984).
[44] T. Turanyi, A.S. Tomlin, Analysis of Kinetics Reaction Mechanisms. Springer, Berlin (2004).
[45] Z. Erbay, F. Icier, J. Food Eng. 91 (2009) 533–541.

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NAUČNI RAD

VEŠTAČKA NEURONSKA MREŽA KAO ALAT ZA POBOLJŠANJE KOMBUHA FERMENTACIJE

Kombuha je fermentisani napitak na bazi čaja, koji postaje sve rasprostranjeniji uglavnom na tržištu funkcionalne hrane, zbog koristi za poboljšavaju zdravlja. Kao deo svakodnevne ishrane odraslih i dece, kombuha se izdvojila kao bezalkoholno piće koje sadrži smešu korisnih sastojaka: organskih kiselina, minerala, vitamina, proteina, polifenola i dr. U radu je ispitan uticaj specifične površine suda, veličine inokuluma i početne koncentracije čaja kao radnih parametara, i trajanja fermentacije kao izlazne promenljive na efikasnost kombuha fermentacije. Fokus ovog rada je optimizacija i standardizacija uslova kombuha fermentacije korišćenjem Box-Behnken eksperimentalnog dizajna i primenom modela predviđanja veštačke neuronske mreže (ANN) za proces fermentacije. Za unapređenje proračuna korišćen je iterativni algoritam Broiden-Fletcher-Goldfarb-Shanno. Dobijeni ANN modeli za pH vrednost i titrabilnu kiselost pokazali su dobre mogućnosti predviđanja (vrednosti r^2 tokom ciklusa treninga za izlazne varijable bile su 0,990 i 0,994, respektivno). Prediktivno ANN modelovanje pokazalo se efikasnim i pouzdanim u uspostavljanju optimalnog procesa kombuha fermentacije koristeći odabrane radne parametre.

Ključne reči: eksperimentalni dizajn, poboljšanje fermentacije, proizvodnja kombuhe, matematičko modelovanje.