Original Article
Food Science and Technology
ISSN 0101-2061 (Print)
ISSN 1678-457X (Online)
DOI: https://doi.org/10.1590/fst.45822

Optimization of process technology and quality analysis of a new yogurt fortified with *Morchella esculenta*

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Abstract

To optimize the process technology of yogurt fortified with *M. esculenta*, first, the Plackett-Burman experimental design combined with the central composite design (CCD) were used to investigate the effects of four parameters on the sensory score. Then, artificial neural networks (ANN) and genetic algorithm (GA) were used to evaluate the process parameters of the fortified yogurt. Finally, the quality of the yogurt prepared under optimal conditions were observed. The results showed that the optimal parameters from ANN-GA were: 0.2 g of mycelia, 15 g milk powder, 6.4 g sucrose, and a fermentation temperature of 38 °C, with the highest predicted sensory score of 97.1 points, which was more accurate and reliable than CCD. Mycelia of *M. esculenta* gave the yogurt excellent quality, including good acidity (95 ± 2.85° T) and water holding capacity (64.32 ± 4.25%) after 21 days storage at 4 °C; firmness (12.98 ± 1.25) g, consistency (22.85 ± 0.92) g·sec, stickness (-6.16 ± 0.38) g, stringiness (3.53 ± 0.12) mm, and cohesion index (-6.62 ± 1.75) g·sec. Moreover, the living lactic acid bacteria of the yogurt with *M. esculenta* (6.23 ± 0.23) × 10^7 CFU/mL) were significantly higher than that of the control yogurt (5.65 ± 0.31) × 10^7 CFU/mL. This could provide a theoretical basis and parameter guidance for developing a new functional yogurt.

Keywords: *Morchella*; yogurt; central composite design; artificial neural networks; genetic algorithm.

Practical Application: As consumer become more aware of nutritious foods, it was necessary for the food industry to develop food formulations with higher nutritional value and quality attributes. However, there were few scientific studies on the process development of yogurt fortified with *M. esculenta*. Therefore, in order to produce a sweet and sour, smooth and creamy yogurt fortified with *M. esculenta*, response surface modeling and artificial neural network techniques were used to optimize the effect of different factors on product quality. In addition, this systematic approach to food formulation research can save time, resources and effort, and provide theoretical basis and parameter guidance for the industrial production of other new yogurt.

1 Introduction

The fruiting body of *Morchella esculenta*, a rare wild edible and medicinal fungus that gets its name from its resemblance to a sheep's stomach, has a variety of applications and uses in natural medicine, health care products, and cosmetics (Cai et al., 2018; Lee et al., 2018; Martel et al., 2017; Wang et al., 2019). At present, due to its unique flavor and nutritional value, it is in demand both at home and abroad. However, the cultivation of the *M. esculenta* fruiting body is difficult and cannot meet the demand. Therefore, liquid fermentation is used to obtain large amounts of the morel mycelia, which reduces the dependence of *M. esculenta* on the environment and also improves the yield, providing raw materials for the development of new mushroom products (Tietel & Masaphy, 2018).

Yogurt is rich in nutrients and has a unique flavor, and can improve the balance of gastrointestinal flora, promote human digestion and absorption, reduce blood cholesterol, and enhance the immune function of the body (Lisko et al., 2017; Sengupta et al., 2019; Tietel & Masaphy, 2018; Wasilewska et al., 2019). Current research on flavored yogurt mainly focuses on the types of lactic acid bacteria and the effects of added ingredients (Külcü et al., 2021; Corrêa et al., 2018; Chen et al., 2018; Gu et al., 2021; Parvarei et al., 2021). However, the use of *M. esculenta* hyphae in fermented dairy products has not yet been investigated.

To render yogurt fortified with *M. esculenta* acceptable, it is necessary to optimize the process parameters and formulation ratios. There are many factors to consider, for example, the ideal concentrations of *M. esculenta* mycelia, milk powder, and sucrose, as well as factors involved in the preparation such as inoculation size, fermentation temperature, fermentation time, and the culture time of the yogurt starter culture, that are likely to affect the sensory score of yogurt fortified with *M. esculenta* (Wang et al., 2017). The respective influences of the formulation ratios and preparation parameters on the sensory score of the fortified yogurt have a complex nonlinear relationship. At the same time, there are complex interrelationships between the different parameters. Therefore, to optimize the preparation process, it is vital to establish a high-precision prediction optimization model which can synchronize all these factors for the optimal development of the fortified yogurt.

Received 02 May, 2022
Accepted 16 June, 2022
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Conventional single-factor experiments are often used for optimization. However, single-factor experiments are time-consuming and do not take interactions among variables into account (Kavitha et al., 2016; Zhou et al., 2018). Moreover, not every factor has a significant effect on the target value. The Plackett-Burman (PB) design is a cost-effective experimental design method that can filter out the most important factors with fewer experiments (Rahman et al., 2010). Response surface methodology (RSM) is a collection of statistical and mathematical techniques that has been widely used in many biotechnological processes, such as the optimization of culture conditions and production of process technology, which can overcome the shortcomings of traditional methods and achieve good optimization results in various scientific fields, as well as the food industry, agriculture, and bioresource technology (Ekpenyong et al., 2021; Mirzaeinia et al., 2020; Ren et al., 2019). RSM can determine the optimal levels of the selected significant variables from the PB design, and can estimate the interactions between a set of controlled experimental variables (Bezerra et al., 2008; Zhou et al., 2018). The central composite design (CCD) method of the RSM can examine more factors and improve experimental accuracy using a small number of experiments (Zhang et al., 2021).

An artificial neural network (ANN) is a data analysis method that processes data by imitating the behavioral characteristics of neural networks in the brain (Jujjavarapu & Deshmukh, 2018; Mia & Dhar, 2016). Based on real measured data, the neural network can simulate the test process and reduce bias in the analysis caused by human factors, thus compensating for some defects of the RSM method. The genetic algorithm (GA) is a form of stochastic nonlinear optimization based on artificial intelligence. In combination with the ANN design, this can avoid the tendency of RSM to produce local optimal solutions and is thus more suited to achieve the goal of global optimal combination design, resulting in a more extensive and accurate model than RSM (Morgenstern et al., 2014).

To date, there are few reports on the use of ANN combined with GA to optimize the preparation of yogurt fortified with *M. esculenta*. In this study, to evaluate the optimization of the preparation process of the fortified yogurt, *M. esculenta* mycelia obtained from liquid submerged fermentation were used as the raw material, and the PB experimental design combined with CCD was used to customize the experimental scheme and to optimize the process in terms of the optimal amounts of mycelia, milk powder, and sucrose, as well as the fermentation temperature. Using the data obtained from CCD and combined with the GA, the ANN was used to establish the relationship between the yogurt preparation process parameters and the sensory evaluation indicators, and to optimize the process parameters. This resulted in the identification of the best process for preparing yogurt fortified with *M. esculenta*, a healthy lactic acid beverage that not only retained the nutritional attributes of *M. esculenta* and fermented dairy products, but also cost-effective production and the potential for wide industrial application.

2 Materials and methods

2.1 Raw materials, microorganisms and medium

Milk powder was brought from Inner Mongolia Yili Industrial Group Company (Yili, China). The freeze-dried yogurt starter culture containing *Lactobacillus bulgaricus* and *Streptococcus thermophilus,* together with 10 probiotic varieties, was brought from Angel Yeast Co., Ltd. (Hubei, China). Sucrose was brought from the local market in Huainan City (China). Other reagents were obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China).

M. esculenta was isolated and preserved in our laboratory

The slant and plate media for *M. esculenta* culture contained (per liter of distilled water): 200.0 g potato, 20 g glucose, 1.5 g KH_2PO_4, 1.0 g $MgSO_4{\cdot}7H_2O$, and 20.0 g agar.

The seed and production media for *M. esculenta* contained (per liter of distilled water): potato 150.0 g, sucrose 30.0 g, peptone 5.0 g, KH_2PO_4 3 g, and $MgSO_4{\cdot}7H_2O$ 0.5 g. The media were fully mixed and then sterilized at 121 °C for 20 min.

2.2 Culture conditions

Isolation of M. esculenta

Normally shaped, healthy, young *M. esculenta* fruiting bodies were selected and placed in sterile petri dishes. After 20 min under ultraviolet irradiation on the ultra-clean table, the mushroom cap was dissected. The internal tissues of the cap were cut into squares of uniform size, placed on plate medium, and cultured at 25 °C for 4 days.

2.3 Preparation of mycelia

The mycelia on the plates were cut under aseptic conditions into blocks with side lengths of 1 cm, then were transferred into 250 mL flasks containing 100 mL liquid seed medium which were then placed on a 160 rpm oscillator at 25 °C for 3 days. Five percent of the inoculum was also added to the production medium under the same conditions. The mycelia-containing solution was filtered and washed with pure water three times until the washing liquid was clear, after which the mycelia were centrifuged at 4500 rpm for 10 min, and supernatant was removed.

2.4 Process technology of yogurt fortified with M. esculenta

Preparation of starter culture for yogurt

Four grams of freeze-dried yogurt starter powder were added to 100 mL of pure milk, mixed well, and transferred into a flask. The culture flask was incubated at 40 °C for 1 h in a constant-temperature incubator, and the product was stored at 4 °C until use as a starter culture for further preparations.

Production of yogurt fortified with M. esculenta

The process flowchart for the preparation of yogurt fortified with *M. esculenta* is presented in Figure 1. The cow's milk powder was blended with the mycelia, sucrose, and water, pasteurized at 80 °C for 30 min and cooled to 40 °C. The mixture was then inoculated with 5% (*v/v*) of yogurt starter culture and incubated in plastic cups (100 mL) for 10 h at 40 °C.

2.5 Experimental design and optimization strategy

Plackett-Burman experimental design

The PB experimental design can screen multiple factors and identity significant factors using a minimal number of runs. The PB experimental design with 12 experiments was constructed

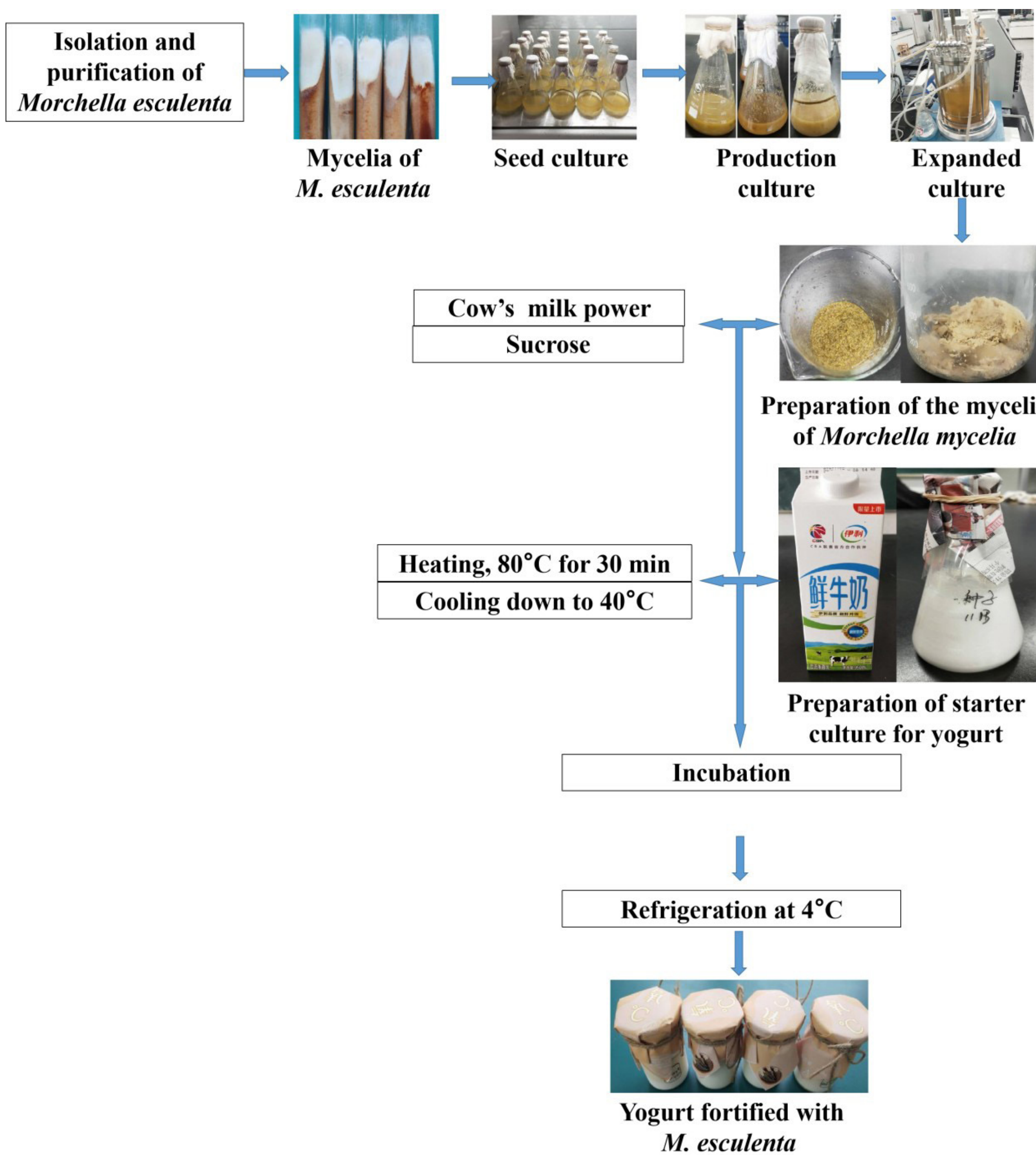

Figure 1. Flowchart of production of yogurt fortified with *M. esculenta*.

using Design Expert version 8.0.6.1 software. The linear equation of the model (Equation 1) was as follows:

$$Y = a_0 + a_1A + a_2B + a_3C + a_4D + a_5E + a_6F + a_7G \quad (1)$$

Where Y is the response of sensory score, a_0 is the constant and a_1, a_2...a_n are the coefficients of factors A, B...G. The seven independent variables selected were the amount of *M. esculenta* mycelia (A), amount of milk powder (B), amount of sucrose (C), inoculum size (D), fermentation temperature (E), fermentation time (F), and the culture time of the starter culture (G). The selection of factors and the two levels used for each factor were based on preliminary experiments. The lower level of the independent factors was denoted by -1, and the higher level was denoted by +1. The dependent variable was sensory score. The results were listed in Table 1.

2.6 Modeling and parameter interaction analysis using CCD

CCD was used to optimize the process technology of the *M. esculenta*-fortified yogurt as it is able to predict over the entire design space. The design was constructed using Design Expert version 8.0.6.1 software. Based on the results of the PB experimental design, four independent variables that had significant effects on the sensory score were selected for investigation; these were the amount of the mycelia, amount of added milk powder, amount of added sucrose, and the fermentation temperature. A total of 30 experiments was carried out to estimate the coefficients for yogurt; these results and CCD predictions were listed in Table 2. The fitting surface for the sensory score response and the associated independent variables were expressed according to the quadratic model (Equation 2) as follows:

$$Y = \beta_0 + \beta_1A + \beta_2B + \beta_3C + \beta_4D + \beta_{11}A^2 + \beta_{22}B^2 + \beta_{33}C^2 + \beta_{44}D^2 + \beta_{12}AB + \beta_{13}AC + \beta_{14}AD + \beta_{23}BC + \beta_{24}BD + \beta_{34}CD \quad (2)$$

Where Y is the sensory score response; A, B, C, and D are the four independent variables; β_0 is the model constant; β_i is the linear coefficient; A_{ij} is the cross-coefficients; β_{ii} is the quadratic coefficient.

In silico optimization of the process technology using artificial neural networks modeling and genetic algorithm

The ANN is comprised of three neural structures: the input layer which is determined by the actual process parameters, the hidden layers, and the output layers which are the optimization goals. The same data used for CCD modeling were used to estimate the response output of yogurt sensory evaluation. Here, the ANN modeling was established using the amount of added *M. esculenta* mycelia (g), the amount of added milk powder (g), the amount of added sucrose (g), and the fermentation temperature (°C) as the neurons of the input layer. The modeling had one hidden layer with eight neurons. The final sensory score (Y) of the yogurt fortified with *M. esculenta* was set as the output layer. The topological structure of the BP artificial neural network was 4-8-1.

Since the GA is capable of nonlinear global optimization which the ANN cannot optimize mathematically, the two could be combined to find the best combination of process parameters for the fortified yogurt. In the GA tool, the dependent variable of the sensory score of the yogurt was referred to as a "chromosome". Each chromosome was composed of four genes, i.e., the amount of mycelia, the amount of milk powder, sucrose, and the fermentation temperature, which were the independent input parameters. The inputs were fed in and the resultant sensory score was generated for different combinations. The fitness function was used to select the individual and evaluate the performance of each different individual chromosome. The GA in this study was based on the ANN model as the fitness function of the GA to identify the optimum process parameters of all inputs leading to the highest sensory evaluation score. During the optimization process, the GA parameters were set as follows: population size 20, evolution algebra 100, crossover probability 08, and mutation probability 0.3. Through the basic operations of initialization, selection, crossover, mutation, and evolution, the optimal fitness of each generation of the population was obtained to optimize the preparation process parameters of the fortified yogurt.

Table 1. Plackett-Burman (PB) experimental design matrix for the screening of significant factors for sensory evaluation of yogurt[a].

Run	Amount of the mycelia of *Morchella esculenta* (g), A	Amount of milk powder (g), B	Amount of sucrose (g), C	Inoculum size (%), D	Fermentation temperature (°C), E	Fermentation time (h), F	Culture time of starter culture for yogurt (h), G	Sensory score, Y
1	8	18	1	8	42	10	0.5	60 ± 4
2	8	12	1	2	42	6	1	56 ± 3
3	8	18	1	2	38	10	0.5	43 ± 4
4	8	12	5	8	38	10	1	46 ± 2
5	8	18	5	2	38	6	1	68 ± 2
6	2	12	1	8	38	10	1	46 ± 2
7	2	18	5	8	38	6	0.5	81 ± 3
8	2	18	1	8	42	6	1	58 ± 3
9	2	18	5	2	42	10	1	82 ± 2
10	2	12	1	2	38	6	0.5	49 ± 3
11	2	12	5	2	42	10	0.5	75 ± 3
12	8	12	5	8	42	6	0.5	56 ± 3

[a]The sensory score was the mean ± *SD* of three independent values. Each factor has the low and high levels represented by -1 and 1.

Table 2. Composition of experimental sensory evaluation with the prediction using Central Composite Design (CCD) and Artificial Neural Networks (ANN).

Run	Amount of the mycelia of *Morchella esculenta* (g), A	Amount of milk powder (g), B	Amount of sucrose (g), C	Fermentation temperature (°C), D	Experimental sensory score, Y	CCD predicted	ANN predicted
1	5	21	3	40	70	65	71
2	5	15	3	40	65	71	69
3	2	12	1	42	60	64	61
4	2	18	5	42	85	85	83
5	5	15	3	40	66	71	69
6	5	15	3	40	65	71	69
7	8	12	5	38	44	43	38
8	2	12	1	38	55	46	55
9	8	18	1	38	45	47	40
10	2	18	1	42	75	76	76
11	5	9	3	40	40	46	40
12	5	15	3	44	77	72	69
13	8	12	1	42	50	51	50
14	8	18	1	42	57	58	55
15	2	12	5	42	75	73	78
16	5	15	-1	40	50	49	52
17	5	15	7	40	60	62	57
18	-1	15	3	40	75	78	76
19	2	12	5	38	70	68	70
20	2	18	5	38	81	80	81
21	5	15	3	36	50	56	50
22	8	18	5	42	40	49	42
23	5	15	3	40	78	71	69
24	8	12	1	38	40	39	42
25	11	15	3	40	36	35	39
26	5	15	3	40	75	71	69
27	5	15	3	40	74	71	69
28	8	12	5	42	45	41	45
29	2	18	1	38	55	58	55
30	8	18	5	38	55	50	52

2.7 Sensory evaluation of yogurt fortified with M. esculenta

The sensory evaluation was performed by 10 well-trained participants including five women and five men. All had experience in the evaluation of fermentation products. The sensory evaluation took place in a sensory laboratory with good ventilation and no ambient odors. The scoring standard used a 100-point system. Color and appearance (10 points), taste (30 points), texture (30 points), and aroma (30 points) were used to describe the sensory quality of the yogurt. About 30 mL of yogurt for each sample was served to each member in a transparent glass. The assessment was performed in triplicate.

2.8 Physicochemical analysis

Acidity

Take 10 g yogurt into 150 mL flask, add 20 mL distilled water, and 2 drops of phenolphthalein indicator, titrate yogurt mixture with 0.1 mol/L sodium hydroxide standard solution until it shows reddish color and keeps colorless for 30 s, record the consumption of sodium hydroxide accurately (according to GB 5413.34-2010).

Water holding capacity

The mass of the centrifugal tube was denoted as m_0, and the mass of the yogurt was denoted as m_1. The centrifuge tube was centrifuged at 3500 r/min for 10 min, and after abandoning the supernatant, its mass was recorded as m_2 (Equation 3).

$$\text{Water holding capacity} = \frac{m_2 - m_0}{m_1 - m_0} * 100\% \quad (3)$$

The enumeration of lactic acid bacteria

The enumeration of lactic acid bacteria was done according to GB 4789-2010 using MRS agar.

2.9 Texture profile

Texture profile analysis of the yogurt was asserted by a texture analyze (TA-XT plus, Stable Micro Systems Ltd.) equipment with a 45-mm cylindrical probe. The pre-test speed and the test speed was 1 mm/s, the post-test 5 mm/s. The test distance was 3 mm. The trigger force was auto-2.0 g. The firmness, consistency, stickness, stringiness and cohesion index were analyzed by the Texture Exponent 32 software (Stable Micro Systems, version 6.1.17.0). The texture profile of yogurt was evaluated 3 times within 24 h after the yogurt fermentation completed.

2.10 Statistical analysis

The ANN modeling and GA optimization were statistically analyzed using Matlab R2014a. The software SPSS 23.0 was used for single factor analysis to test the significance of differences between samples, $P < 0.05$, the difference was significant.

3 Results and discussion

3.1 Analysis of variables using the Plackett-Burman design

The sensory score of the fortified yogurt was influenced by various factors, including ingredients such as the concentrations of mycelia, milk powder, and sucrose, together with preparation parameters such as inoculum amount size, fermentation temperature, fermentation time, and the culture time of the starter culture. In order to get a better sensory evaluation, single-factor experiments are often utilized for this purpose. However, single-factor experiments are both time-consuming and do not evaluate interactions among the variables. Also, when there are many variables, the results tend to be highly unreliable (Lim et al., 2007). So, based on our previous one-way experiments, a PB-design experiment using a set of 12 runs was used to select the significant factors from the original seven factors. The PB design and results are shown in Table 1 and the analysis of variance (ANOVA) of the results is presented in Table 3. The sensory scores of the yogurt showed marked increases from 43 to 82 points at different levels of each combination. Design-Expert Version 8.0.6.1 software was used to analyze the experimental results, and the multivariate linear equation (Equation 4) was obtained.

$$Y = -60.06 - 1.72*A + 1.78*B + 4*C + 2.25*E \quad (4)$$

The decision coefficient (R^2) was used to evaluate the fitted model; the closer the R^2 value to 1, the better the predictive ability of the model. R^2 in this study was found to be 0.9276, indicating that 92.76% of the expected value change could be explained by the established model. *P*-values were used to evaluate the significance level of each factor in the F test, with $P < 0.05$ considered statistically significant. The *P*-value was found to be 0.01, indicating that this model was highly reliable, and could describe the relationship between the experimental factors and response values well. The most influential variables identified by the PB design, namely, the mycelia concentration, amounts of milk powder and sucrose, and the fermentation temperature, were chosen for further evaluation at various levels of actual and coded values using CCD. According to Equation 4, the coefficients of the amounts of milk powder and sucrose, and the fermentation temperature were positive, showing that they had positive effects on the response value. On the other hand, the mycelia concentration had a negative effect on the response value. These results revealed that increasing the amounts of milk powder and sucrose as well as the fermentation temperature, or decreasing the amount of mycelia, could raise the response value and thus improve the sensory points.

Mondragón-Bernal et al. (2017) used a PB experimental design to select sucrose and soy extract as the significant factors associated with synbiotic soy foods fermented by lactic acid bacteria from probiotic mixtures, sucrose, soy extract, calcium lactate, fructooligosaccharides, and polydextrose. Ma et al. (2020) also utilized the PB experimental design to demonstrate that potato extract, glucose, and peptone were the most important factors when optimizing the medium to improve the yield of fermented *Tremella* polysaccharides. These results indicate that the PB design is an effective tool to identify factors having significant impacts from a variety of factors.

3.2 Optimization of process technology for yogurt using central composite design

CCD, as a type of response surface methodology, has often been used to statistically and mathematically model the optimal conditions for the process technology of products (Chaharbaghi et al., 2017). CCD can not only precisely decrease the number of experiments required, but can also enhance our understanding of the interactions among different variables under different conditions (Ahsan et al., 2017; Tang et al., 2019). This study dealt with the optimization of the most significant process parameters in the preparation of yogurt fortified with *M. esculenta* using CCD to produce a better sensory score of the yogurt. The experimental design was conducted with Design Expert software.

Table 3. ANOVA results for the Plackett-Burman design.

Source	Sum of squares	df	Mean square	F value	*P*-value (Prob > F)
Model	1903.33	6	317.22	10.67	0.01
X_1	320.33	1	320.33	10.77	0.0219
X_2	341.33	1	341.33	11.48	0.0195
X_3	768	1	768	25.83	0.0038
X_5	243	1	243	8.17	0.0355
K-K	225.33	1	225.33	7.58	0.0402
L-L	5.33	1	5.33	0.18	0.6895
Residual	148.67	5	29.73		
Cor Total	2052	11	R^2 0.9276		

The experimental results and the predicted values obtained from the fitted equation by the Design Expert software are shown in Table 2. The fitted second-order polynomial equation explaining the sensory score using response surface analysis is shown in Equation 5.

$$Y = -937.15 + 15.41A + 14.57B + 43.54C + 37.46D - 0.125AB - 0.77AC - 0.27AD - 1.70{*}10^{-15}BC - 1.44{*}10^{-14}BD - 0.81CD - 0.40A^2 - 0.41B^2 - 0.93C^2 - 0.40D^2 \quad (5)$$

ANOVA results (Table 4) verified the suitability of the model and clarified the significance of each of the parameters that affected the sensory score of the yogurt. The model F-value was 10.89 and the *P*-value < 0.0001 for sensory evaluation, indicating the significance of the model (Lakshmi et al., 2020; Lang et al., 1998). Moreover, the linear terms (A, B, C, D), interaction terms (AC, CD), and quadratic terms (A^2, B^2, and C^2) were found to be significant as the *P*-values were less than 0.05. The *P*-value for *M. esculenta* was less than 0.0001, indicating that this factor had the greatest influence on sensory evaluation, followed by milk powder, temperature, and sucrose. The *P*-values of AC, CD, A^2, B^2, and C were all less than 0.05, also implying a significant influence on sensory evaluation. The *P*-values of AC and CD were 0.0072 and 0.0451, respectively, indicating a strong interaction. The coefficient of variation (CV), a measure of precision, was found to be 9.84%, indicating the accuracy and reliability of the experimental value (Ghribi et al., 2012). In addition, the decision coefficient R^2 was 0.9104, indicating a good fit of the regression equation (He et al., 2013). The obtained adjusted R-squared (Adj. R^2) of 82.68% indicated that the regression equation could explain 82.68% of the data variation. The *F*-value (1.07) of lack of fit was not significant (*P* = 0.5036).

The 3D response surface plots and the 2D contour plots are used to determine the optimal values of the independent variables and the interactive effects of each independent variable on the response. The steeper the curve in the plots, the greater the influence of the corresponding factors on the response value, and the greater the change in the response value. The 3D response surface figures can directly reflect the influence of various factors on the sensory scores as well as the interactions among the various factors. The 2D contour shape can intuitively reflect the interaction between two factors. An elliptical contour shows significant interaction between two factors, while a circular contour indicates weak interaction. The overall trend of the two kind of graphs was consistent (Zhou et al., 2017).

Both plots are presented in Figure 2. As shown in Figure 2b and 2f, there were significant interactions between the *M. esculenta* mycelia and the sucrose, and between the sucrose and the fermentation temperature on the sensory scores of the yogurt (*P* < 0.05). As shown in Figure 2a, 2c, 2d, and 2e, the effects of interactions between the mycelia and the milk powder, the mycelia and the fermentation temperature, the milk powder and the sucrose, and the milk powder and the fermentation temperature, on the sensory scores were not significant (*P* > 0.05); this was consistent with the results of ANOVA analysis shown in Table 4.

To validate the established model, Figure 3 presents the graphical summaries. The Box-Cox plots (Figure 3a) showed that the model was sufficient, the lambda (lambda = 1) was within the 95% confidence range, requiring no additions. The predicted vs. actual plot (Figure 3b) demonstrates that the actual values were close to the predicted ones (Ibrahim et al., 2019). The residuals vs. run plot (Figure 3c) showed that the model was consistent with the data (El-Housseiny et al., 2016). The normal plot of the residuals (Figure 3d) showed a straight line, indicating a normal distribution.

Table 4. ANOVA results for the central composite design.

Source	Sum of squares	df	Mean square	F value	*P*-value (Prob > F)
Model	5390.78	14	385.06	10.89	< 0.0001
X_1	2773.5	1	2773.5	78.41	< 0.0001
X_2	541.5	1	541.5	15.31	0.0014
X_3	253.5	1	253.5	7.17	0.0172
X_4	384	1	384	10.86	0.0049
X_1X_2	20.25	1	20.25	0.57	0.461
X_1X_3	342.25	1	342.25	9.68	0.0072
X_1X_4	42.25	1	42.25	1.19	0.2917
X_2X_3	0	1	0	0	1
X_2X_4	0	1	0	0	1
X_3X_4	169	1	169	4.78	0.0451
X_1^2	352.19	1	352.19	9.96	0.0065
X_2^2	377.19	1	377.19	10.66	0.0052
X_3^2	377.19	1	377.19	10.66	0.0052
X_4^2	68.76	1	68.76	1.94	0.1835
Residual	530.58	15	35.37		
Lack of Fit	361.08	10	36.11	1.07	0.5036
Pure Error	169.5	5	33.9		
Cor Total	5921.37	29			

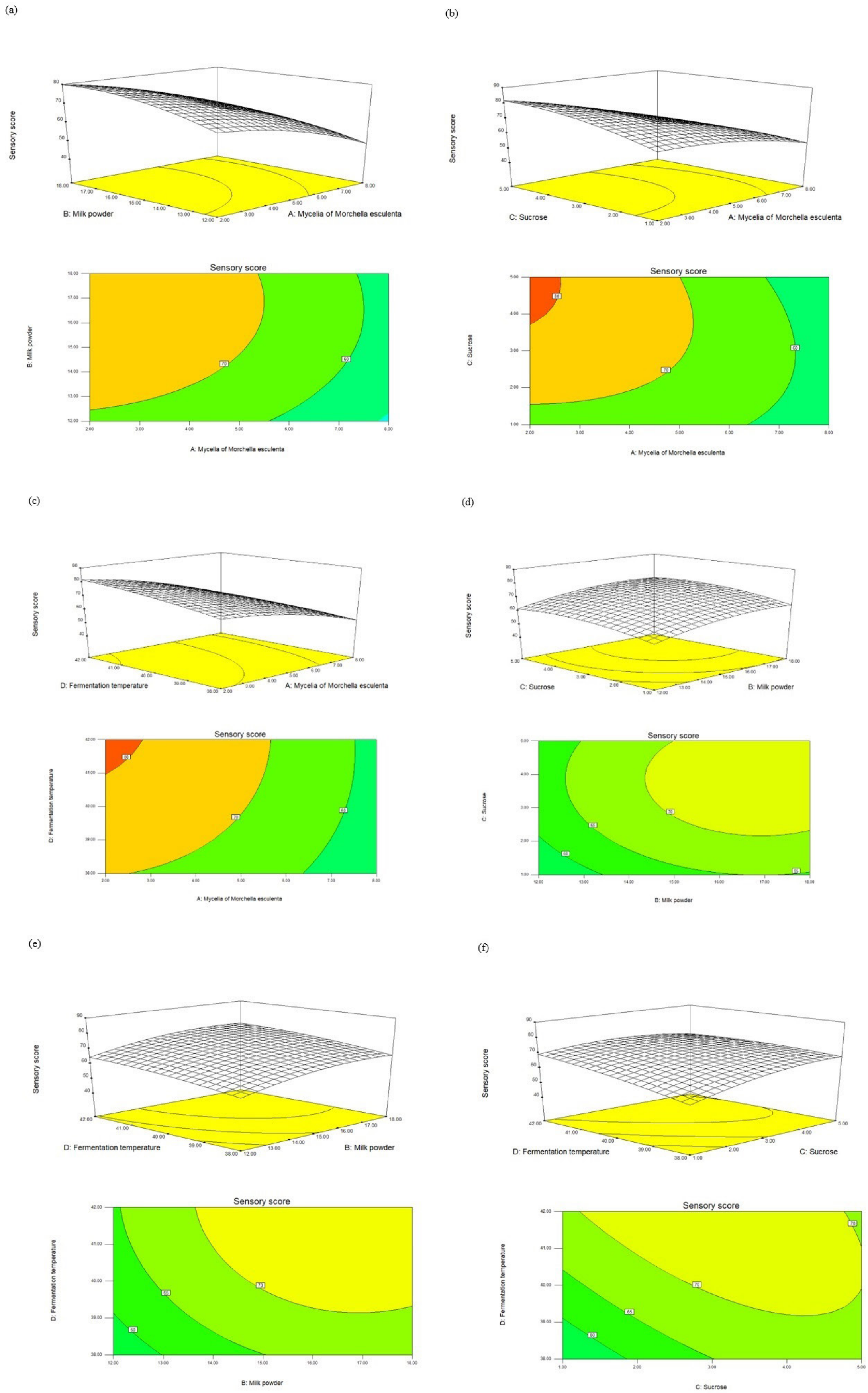

Figure 2. Three-dimensional (3D) response surface and contour plots.

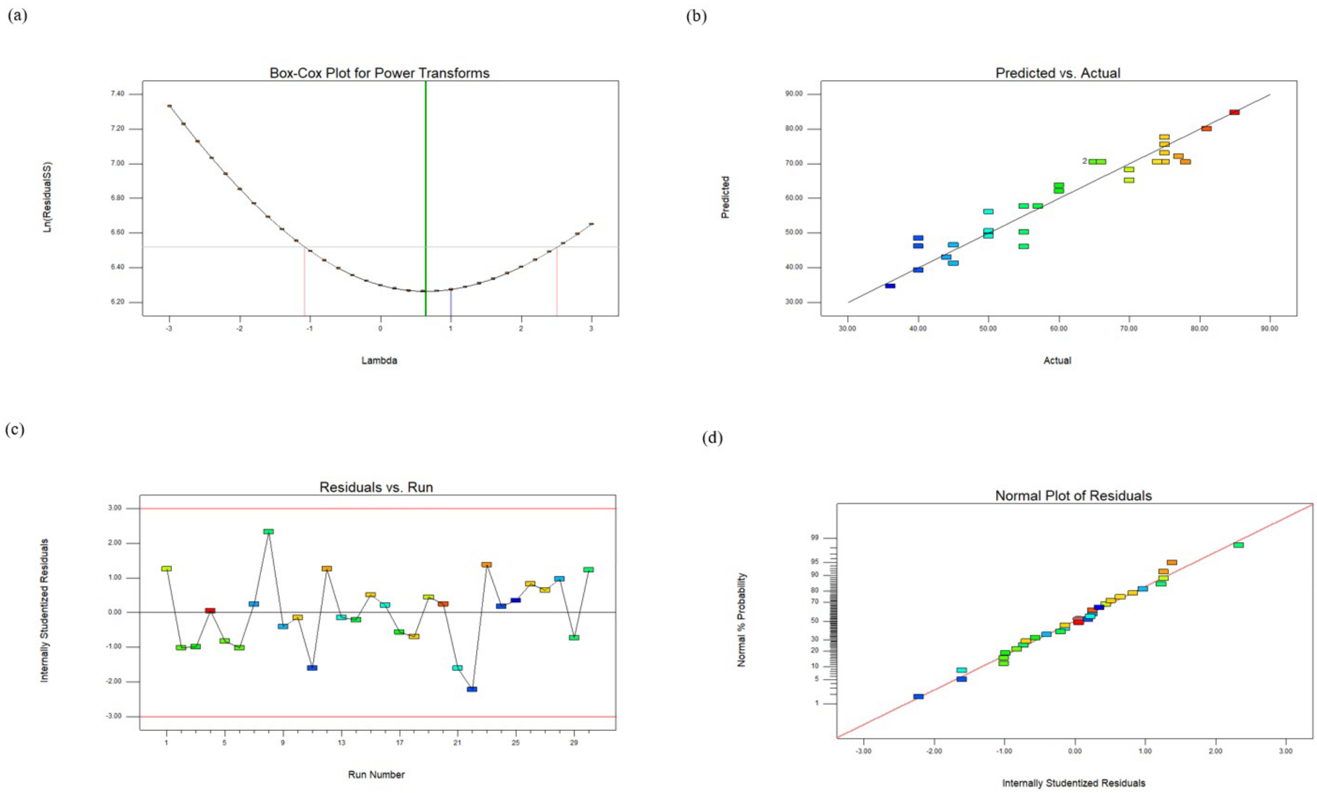

Figure 3. Model diagnostics of response surface design from Design Expert software. (a) Box-Cox plot for power transforms; (b) predicted vs. actual plot; (c) residuals vs. run plot; (d) normal plot of residuals.

The optimum process parameters for the best sensory score were found to be 1.0 g of *M. esculenta* mycelia, 17.5 g of milk powder, 4.5 g of sucrose, and a fermentation temperature of 42.3 °C. The model predicted that the highest score of the yogurt fortified with *M. esculenta* using these conditions was 88.3.

3.3 The establishment of artificial neural networks and optimization of the genetic algorithm for yogurt fortified with M. esculenta

ANN is an effective nonlinear regression tool for establishing a connection between input and output variables through simulating the neurological processing behaviors of the human brain (Haykin, 2007). The mapminmax function was chosen to normalize the processing of the data obtained from the response surface experiments. These data were trained and learned automatically through ANN, and, when the optimal parameters of the model were obtained, the training was completed. The mean square error (MSE) of the system was set at 10^{-8}, which was able to measure the mean error of the data based on the degree of variability. The smaller the MSE value, the better the accuracy of the model in describing the test results (Sanz-González et al., 2002). Figure 4 shows the variation in MSE during the training phase of the ANN. The horizontal axis represents the number of training steps, and the vertical axis represents the MSE of the data. Each index decreased continuously as the number of training steps increased, and, when the iteration reached the fifth step, the accuracy no longer decreased, indicating that the neural network training was completed. The convergence speed of neural network training was fast and stable, indicating that the model could meet the experimental requirements well. The best performance of the MSE was 0.0359, close to the real data, indicating that the neural network model could be applied to the subsequent experimental analysis.

The fitting regression coefficient R value of the model trained by the ANN indicated the degree of correlation between the target data and the output results. As shown in Figure 5, the R values of the training, test, and validation were 0.96075, 0.96775, and 0.96884, respectively. This indicated that the model had a strong explanatory ability and low simulation error, and could be used to train, predict, and perform overall fit well. The trained model could simulate and express the mapping relationship between the input and the output to ensure the simulation effect, and then predict the test results accurately.

The GA can further refine the optimized preparation process for the experiments. After 100 iterations of global optimization, the fitness curve converged to around 100 in the 80^{th} interaction. The optimal process parameters were: the amount of mycelia, 0.1950, the amount of milk powder, 15.0088, the amount of sucrose, 5.9561, and the fermentation temperature, 39.2266, with the optimal sensory score of 99.9969 points. Considering both the product quality and practical operation, the following conditions were selected as the optimal process parameters:

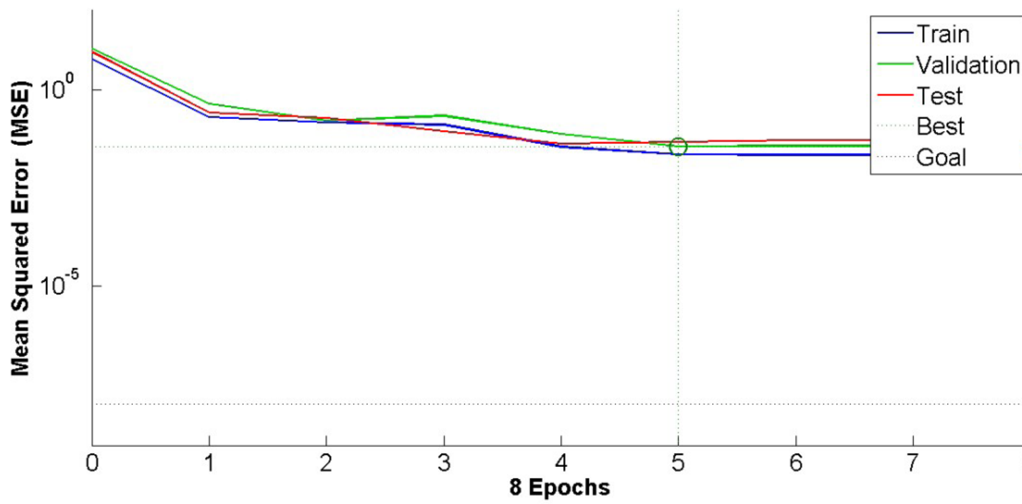

Figure 4. Variation of the Mean Square Error (MSE) plot of the Artificial Neural Network (ANN).

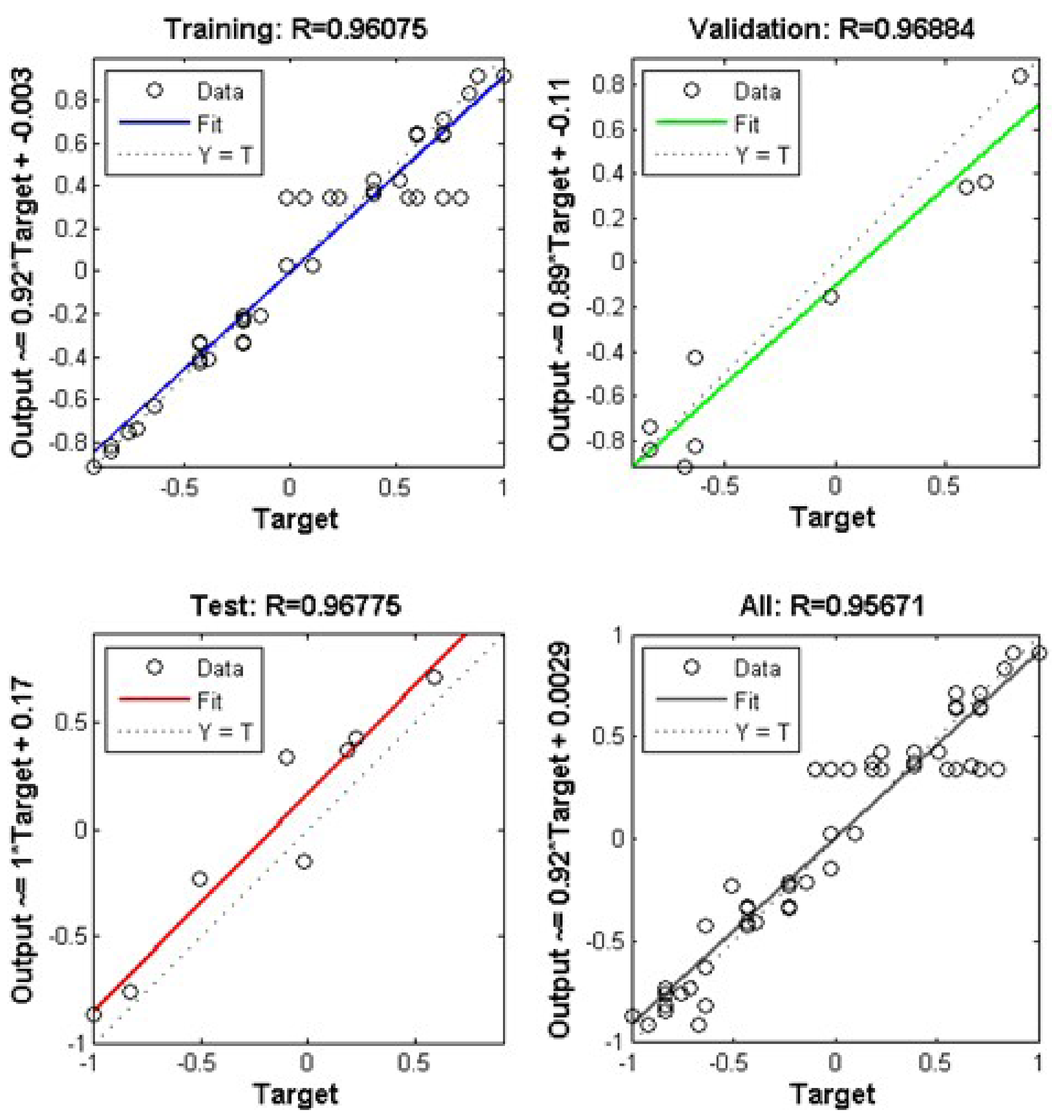

Figure 5. Neural network model with training, validation, test, and all prediction sets.

the amount of *M. esculenta* mycelia, 0.2 g, the amount of milk powder, 15.0 g, the amount of sucrose, 6.4 g, and the fermentation temperature 38.0 °C, with the optimal predicted sensory score of 97.1 points.

3.4 Comparison of the optimum process parameters from ANN, GA, and CCD models

The optimal process parameters determined by ANN-GA and the CCD model were examined separately. Table 5 shows the comparison between the optimized process conditions at the highest sensory scores selected for the preparation of the fortified yogurt. The highest sensory score predicted by the CCD model was 88.3, while under the same conditions, the experimental value was 84.5. Similarly, the highest sensory score predicted by ANN-GA was 97.1, compared to the experimentally obtained score of 94.2. The percentage standard deviations were 2.7% and 2.1%, respectively, which were small and acceptable. The results showed that the ANN was better compared with CCD as a modeling tool for the nonlinear data of sensory score of the fortified yogurt. An earlier study by Cheok et al. (2012) also reported that the ANN modeling technique had superior predictive ability compared to RSM (Cheok et al., 2012). The ANN can deal with nonlinear relationships more effectively (Musa et al., 2016) and can adopt the experimental results better than the RSM model (Mondal et al., 2021). Many studies have confirmed that sweetness is one of the most important attributes of yogurt, and sucrose concentration directly affects the sweetness of yogurt (Costa et al., 2020; Souza et al., 2021). In this experiment, the sucrose concentration in the optimal recipe derived from the ANN model (6.4 g/100 mL) was 1.9 g/mL higher than that derived from the CCD model (4.5 g/100 mL). Souza et al. (2021) found in the strawberry yogurt study that sensory scores would not be high when the sucrose concentration was equal to or less than 6.36%. This finding was similar to the results of this experiment. Fresh *Morchella* mycelium has a special unacceptable taste to consumers, and the higher the amount added, the less acceptable the yogurt. The amount of mycelium added from the ANN model (0.2 g) was 0.8 g lower than that from the CCD model (1.0 g), and the lower amount of mycelium added greatly improved the acceptability of the yogurt. These might be the reasons why the yogurt prepared from the optimal recipe obtained from the ANN model was more popular than that from the CCD model.

Table 5. The optimized process parameters for the sensory scores from the ANN-GA and CCD models.

Factors	CCD model	ANN-GA model
Amount of the mycelia of *Morchella esculenta* (g)	1.0	0.2
Amount of milk powder (g)	17.5	15.0
Amount of sucrose (g)	4.5	6.4
Fermentation temperature (°C)	42.3	38
Predicted sensory scores	88.3	97.1
Experimental sensory score	84.5	94.2
Deviation (%)	2.7	2.1

3.5 Quality analysis of yogurt

The best formula of yogurt with *M. esculenta* obtained by ANN-GA was used to prepare yogurt, as shown in Figure 6. During storage at 4 °C, the acidity of yogurt gradually increased with increasing storage time. The titrated acidity of the two kinds of yogurt changed almost in the same way. By 21 d of storage, the acidity of control yogurt increased from 76.4 ± 5.76° T to 88 ± 5.58° T, and that of *M. esculenta* fortified yogurt increased from 84 ± 6.36° T to 95 ± 2.85° T. Thus, the acidity of *M. esculenta* fortified yogurt varied less than that of control yogurt during storage at 4 °C. The variation in yogurt acidity during the storage period might be related to the post-acidification of yogurt, the extent of which depends on the concentration of D-lactic acid, which is mainly produced by lactic acid bacteria (Beal et al., 1999). The number of lactic acid bacteria in yogurt fortified with *M. esculenta* ((5.65 ± 0.31) × 10^7 CFU/mL) was higher than that in control yogurt [(6.23 ± 0.23) × 10^8 CFU/mL], which corroborated the previous statement (Table 6). This may be because the mycelia of *M. esculenta* contains not only fungal polysaccharides, but also small molecular compounds such as amino acids, peptides and nucleotides required for the growth of lactic acid bacteria, thus promoting the growth of lactic acid bacteria. Similar conclusions were reached by Rosyidi et al. (2021) in their study on the effect of *Pleurotus ostreatus* aqueous extract on yogurt, β-glucan, a water-soluble dietary fiber contained in the extract, was able to provide carbon source and energy for the growth of lactic acid bacteria, thus increasing the number of lactic acid bacteria and reducing acidity.

The water holding capacity of yogurt is one of the important indicators of yogurt stability (Farinde et al., 2009; Gheshlaghi et al., 2021). Figure 6 showed the changes of water holding capacity of yogurt during storage, from Figure 6, it could be seen that the water holding capacity of both yogurts changed significantly during storage, where the water holding capacity of the control yogurt [66.23 ± 0.51%] was lower than that of the yogurt with *M. esculenta* (68.73 ± 3.47%) on the first 14 days, but on the 21st day, the yogurt with *M. esculenta* (64.32 ± 4.25%) had a lower

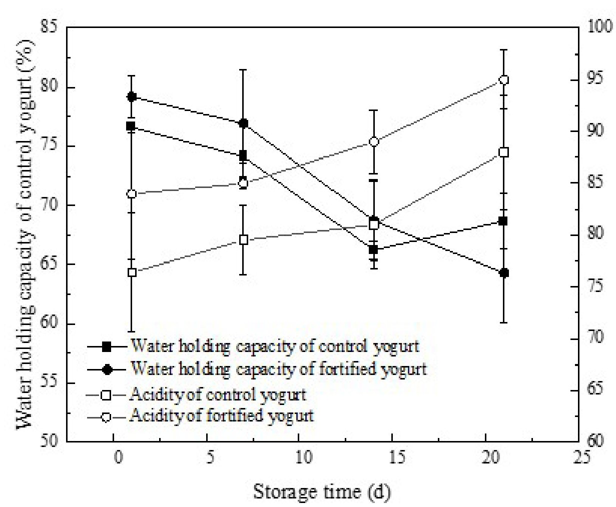

Figure 6. Water holding capability and acidity changes of yogurt during storage.

Table 6. Quality analysis between control yoghurt and yogurt fortified with *M. esculenta*.

	The enumeration of lactic acid bacteria/ (CFU/mL)	Frimness/g	Consistency/ (g·sec)	Stickness/g	Stringiness/mm	Cohesion Index/ (g·sec)
Control yogurt	$(5.65 \pm 0.31) \times 10^{7a}$	11.88 ± 0.22^{a}	23.50 ± 0.07^{a}	-6.59 ± 0.22^{a}	2.96 ± 0.02^{a}	-6.40 ± 0.25^{a}
Yogurt fortified with *M. esculenta*	$(6.23 \pm 0.23) \times 10^{8a}$	12.98 ± 1.25^{a}	22.85 ± 0.92^{a}	-6.16 ± 0.38^{a}	3.53 ± 0.12^{b}	-6.62 ± 1.75^{a}

There are significant differences among the mean values that do not share the same letter in the same column (P < 0.05).

water holding capacity than the control yogurt (68.7 ± 2.37%). This might be because, in the first 14 days of storage, the addition of mycelium of *M. esculenta* could strengthen the stability of the gel network in yogurt. In a study by Wang et al. (2022) on *Auricularia cornea* var. Li polysaccharide (ACP) fortified yogurt came to a similar conclusion that the addition of ACP improved the WHC of yogurt during storage and it was speculated that the improvement of WHC by ACP might be due to the content of hydroxyl groups and anionic properties in ACP, which could form complexes with protein clusters in yogurt, thus improving the structure of protein gels. In the later stages of storage, due to soaking in yogurt for too long, resulting in softening of mycelia, thus losing the role of reinforcement, and might probably release water or some other substances. This lead to a reduction in water holding capacity of yogurt with *M. esculenta.*

The texture profile of yogurt were evaluated in terms of firmness, consistency, stickness, stringiness and cohesion index. Table 6 showed the texture profile of yogurt with or without *M. esculenta*. The firmness, consistency, stickness and cohesion index did not change significantly, only stringiness changed significantly. This indicated that the mycelia of *M. esculenta* might improve the surface viscosity of yogurt. This might be caused by the long, slender filaments of the mycelium itself.

4 Conclusion

In the preparation of yogurt fortified with *M. esculenta*, the PB experimental design was used to select the most significant factors (the amount of *M. esculenta*, amount of milk powder, amount of sucrose, and the fermentation temperature) from among seven factors, including ingredients such as the concentrations of mycelia, milk powder, and sucrose, and yogurt preparation technology parameters such as inoculum amount, fermentation temperature, fermentation time, and the culture time of the yogurt starter culture. Then, the CCD response surface design was utilized for the optimization of the fortified yogurt, and, based on the CCD results, the ANN with a 4-8-1 topological structure was established on the Matlab R2014a platform to realize the simulation of the product preparation and model prediction, combined with GA for the optimization of the process parameters. Ultimately, the following parameters were selected for the optimization of yogurt fortified with *M. esculenta*: 0.2 g *M. esculenta* mycelia, 15.0 g milk powder 15.0, 6.4 g sucrose, and a fermentation temperature of 38.0 °C. Overall, both CCD and ANN-GA proved to be effective tools for solving the multi-objective synchronization optimization problem of the process technology in the preparation of the fortified yogurt. Compared with the CCD model, the optimization results of the ANN-GA model were more accurate and reliable, which could provide both a theoretical basis and parameter guidance for the industrial production of yogurt fortified with *M. esculenta*. The yogurt with *M. esculenta* had an impact on acidity, the amount of lactic acid bacteria, water holding capacity and texture characteristics. The addition of mycelia was able to promote the growth of lactic acid bacteria; during storage, the acidity of yogurt fortified with *M. esculenta* was higher than that of the control yogurt. In the first 14 days of storage, the water holding capacity of both yogurts decreased continuously, but the water holding capability of yogurt with *M. esculenta* was higher than that of the control yogurt; but in the 21st day, the water holding capacity of yogurt with *M. esculenta* was lower than that of the control yogurt. The addition of mycelia was able to significantly improve the stringness of texture property, but had insignificant effects on other textural properties, and these studies laid some theoretical foundation for the development of new functional yogurts.

Acknowledgements

The authors would like to thank all the reviewers who participated in the review and MJEditor (www.mjeditor.com) for its linguistic assistance during the preparation of this manuscript.

Conflict of interest

The authors declare that they have no conflicts of interest.

Funding

This study was financially supported by the general program in the youth excellent talents supporting plan in universities of Anhui province (grant gxyq2020046) and natural science foundation of Anhui (grant 2108085QC146).

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