Yuzuncu Yil University
Journal of Agricultural Sciences
(Yüzüncü Yıl Üniversitesi Tarım Bilimleri Dergisi)
https://dergipark.org.tr/en/pub/yyutbd

ISSN: 1308-7576 e-ISSN: 1308-7584

Research Article

Optimization of Media Composition for Maximum Growth of Probiotic *Lactobacillus fermentum* NBC-08 Using Response Surface Methodology

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Article Info

Received: 10.09.2021
Accepted: 10.02.2022
Online Published: 15.03.2022
DOI: 10.29133/yyutbd.993781

Key words

Lactic Acid Bacteria,
Probiotic,
Fermentation,
Food Biotechnology,
Response Surface Methodology

Abstract: In this study, it was aimed to determine the medium where *Lactobacillus fermentum* microorganisms with probiotic properties grow most efficiently by using Response Surface Method (RSM). Studies have been conducted on a 500 ml erlenmeyer scale, the medium optimization of the *Lactobacillus fermentum* strain was carried out according to the Central Composite Design (CCD) included in RSM. The effects of glucose, yeast extract, inorganic salts, and Tween 80 were investigated on the growth rate of the *L. fermentum* NBC-08 strain. Samples were taken at regular intervals from the erlenmeyer flask and the number of viable cells was measured by planting them in petri agar medium. In the study, the number of viable cells $\log_{10}$ (cfu/ml) was chosen as the response variable. As a result of the study, it was concluded that glucose and yeast extract are absolutely essential components in the medium. The optimum medium composition was found as 96.06 g/L glucose, 40.76 g L^{-1} yeast extract, 19.43 g L^{-1} inorganic salts, and 11.01 ml/L Tween 80. The production of the maximum *L. fermentum* strain was determined as 10.75 $\log_{10}$ (cfu ml^{-1}). It is predicted that this study will make positive contributions to the fermentation conditions and medium optimization studies for production of lactic acid bacteria.

To Cite: Kavak, A E, Selen, V, Tamtürk, F, 2022. Optimization of Media Composition for Maximum Growth of Probiotic *Lactobacillus fermentum* NBC-08 Using Response Surface Methodology. *Yuzuncu Yil University Journal of Agricultural Sciences*, 32(1): 69-80. DOI: https://doi.org/10.29133/yyutbd.993781

Cevap Yüzey Yöntemi Kullanılarak Probiyotik *Lactobacillus fermentum* NBC-08 Maksimum Büyümesi İçin Ortam Bileşiminin Optimizasyonu

Makale Bilgileri

Geliş: 10.09.2021
Kabul: 10.02.2022
Online Yayınlanma: 15.03.2022
DOI:10.29133/yyutbd.993781

Anahtar Kelimeler

Laktik Asit Bakterisi,
Probiyotik,
Fermantasyon,
Gıda Biyoteknolojisi,
Cevap Yüzey Yöntemi

Öz: Bu çalışmada probiyotik özelliklere sahip *Lactobacillus fermentum* mikroorganizmasının en verimli olarak büyüdüğü optimimum besi yeri bileşimi Cevap Yüzey Yöntemi (CYY) kullanılarak belirlenmiştir. Çalışmalar 500 ml hacminde erlen ölçeğinde yapılmış olup *Lactobacillus fermentum* suşunun besi yeri optimizasyonu CYY içerisinde yer alan Merkezi Kompozit Tasarım (MKT)'a göre gerçekleştirilmiştir. *L. fermentum* NBC-08 suşunun büyüme miktarı üzerine glikoz, yeast extract, inorganik tuzlar ve Tween-80'in etkisi incelenmiştir. Erlenlerden belirli aralıklarla numuneler alınmış ve petri agar ortamına ekilerek canlı hücre sayılarına bakılmıştır. Çalışmada cevap değişkeni olarak canlı hücre sayısı $\log_{10}$ (cfu/ml) seçilmiştir. Çalışma sonucunda besi ortamında glikoz ve maya ekstraktının kesinlikle bulunması gereken bileşenler

olduğu sonucu ortaya çıkmıştır. Optimum besi yeri bileşimi 96.06 g/L glikoz, 40.76 g/L yeast ekstrakt, 19.43 g/L inorganik tuzlar ve 11.01 ml/L Tween 80 olarak bulunmuştur. Maksimum *L. fermentum* suşunun üretimi ise 10.75 $\log_{10}$ (cfu/ml) şeklinde bulunmuştur. Bu çalışmanın laktik asit bakterilerinin fermantasyon yolu ile üretilmesi ve probiyotik suşların besi yeri optimizasyonu çalışmalarına olumlu katkılar yapacağı ön görülmektedir.

1. Introduction

Probiotics are defined as living microorganisms that cause positive effects on host health when consumed in certain amounts (Anvari et al., 2014). Probiotics affect the host by regulating mucosal and systemic immunity and are thus beneficial microorganisms. Most probiotic bacteria are gram-positive, and their main functions are related to the maintenance of intestinal system health (Marco et al., 2006). Lactic acid bacteria constitute the most important group of probiotic microorganisms. Among the lactic acid bacteria (LAB), *Bifidobacterium* and *Lactobacillus* species stand out as the most widely used as a probiotic (Iranmanesh et al., 2014). LAB is an important group of fermentative bacteria that are widely used as starter cultures for the production of fermented foods both at home and in industry, and it is also part of the normal intestinal microflora (Pithva et al., 2014). Numerous studies have shown that LAB has a wide application area as a probiotic organism and provides beneficial effects to humans and animals (Lee et al., 2010; Shamekhi et al., 2012; Kaewnopparat et al., 2013). The beneficial effects of LAB on human health and intestinal microbial balance stand out as valuable probiotic properties. Lactobacillus strains are characterized by their ability to adhere to and colonize the intestinal mucous layer and to produce antimicrobial agents (Belova et al., 2016; Boricha et al., 2019; Ozkan et al., 2020; Teame et al., 2020). These properties allow probiotic strains to compete with gastrointestinal tract pathogens and food degrading pathogens (Bove et al., 2013). LAB species were listed in the qualified presumption of safety published by the European Food Safety Authority (EFSA) and generally recognized as safe (GRAS) by the US Food and Drug Administration (FDA) (Ricci et al., 2017).

Lactobacillus fermentum is a bacterium with probiotics including health-promoting effects, and it widely inhabits the human gastrointestinal and urogenital tracts. It is present naturally in raw milk, dairy products, and many traditional fermented foods and beverages. *L. fermentum* bacteria is a microorganism whose main task is to produce lactic acid (Ramos et al., 2013). It is considered as beneficial for developing various methods to ensure that large quantities of *L. fermentum* biomass can be produced effectively and cost-effectively. Optimization of the culture medium to obtain high-quality probiotic products is one of the most important steps in improving the efficiency of the production process in terms of both economic and yield-related aims. (Gao et al., 2009).

Many studies have been conducted related to *L. fermentum* strain isolated from breast milk. Also, there have been studies that showed *L. fermentum* strain has various benefits on the gastrointestinal system and cholesterol-lowering effects (Gil-Campos et al., 2012; Maldonado et al., 2012; López-Huertas, 2015; Asan-Ozusaglam and Gunyakti, 2019).

Response Surface Methodology (RSM) is a set of mathematical and statistical techniques useful for analyzing the relationship between several independent variables and one or more responses. (Liew et al., 2005; Kanmani et al., 2012). Compared to traditional methods, RSM has many advantages, such as requiring a minimum number of experiments, lower processing costs, and using fewer medium components (Rodrigues et al., 2006). Additionally, unlike traditional methods, RSM shows the combined effects of all independent variables on response variables (Anvari et al., 2014). Recently, RSM has been widely used in many bioprocessing studies, including modeling and optimization of culture media (Stephenie et al., 2007; Shang et al., 2013; Thite et al., 2020).

The *L. fermentum* NBC-08 strain used in this study was isolated from breast colostrum milk. *L. fermentum* NBC-08 strain is a local probiotic strain originating from Turkey. In addition, the low-cost medium developed in this study can be used on an industrial scale. This aspect makes the study original and significant. In this study, optimization of media components for the maximum growth of *L. fermentum* NBC-08 probiotic strain isolated from breast milk colostrum was performed using the RSM.

2. Material and Method

2.1. Microorganism

The *Lactobacillus fermentum* NBC-08 strain used in this study was isolated from breast colostrum milk by Orzax Pharmaceuticals and Chemical Industry Trade Corporation in a project within the scope of the R&D Support Program supported by the General Directorate of Agricultural Research and Policies (TAGEM). The characterization and reliability tests of this strain were performed by Orzax Company. *L. fermentum* NBC-08 strain was identified on the basis of genus and species by the MALDI-TOF MS method. The *L. fermentum* NBC-08 strain bile tolerance, gastric acid resistance, lysozyme tolerance, and intestinal adhesion tests were applied for the determination of the probiotic character of the bacteria.

2.2. Substrates and Chemicals

In this study, glucose as carbon source, yeast extract as organic nitrogen source, and inorganic salts (sodium acetate, potassium dihydrogen phosphate, magnesium sulphate, and manganese (II) sulphate) which are thought to be needed for the growth of microorganisms and Tween 80, were used to provide a rich culture growth medium. All chemicals used in the experiments are of analytical purity.

2.3. Microorganism growth medium and conditions

Lactobacillus fermentum strain was taken from 20% (v/v) glycerol stock and first inoculated into MRS broth (Table 1) medium, and its growth was achieved in a shaking incubator at 37 °C and 200 rpm agitation speed for 24 hours. Bacterial cultures that completed their pre-development were cultivated in MRS Agar (Table 1) culture storage medium by spreading with drigalski spatula and kept in an incubator at 37 °C for 48 hours. *L. fermentum* strain in petri dishes was inoculated into 500 ml erlenmeyer flasks containing 100 ml of bacteria growth medium with the help of a sterile spatula (loop). At the end of the incubation period, petri dishes were kept at +4 °C, and the cultures were renewed once every 15 days. The *L. fermentum* strain used in each stage of the study was inoculated in two stages. First, the *L. fermentum* strain taken from the culture storage medium was inoculated in the bacteria growth medium and then *L. fermentum* strain taken from bacteria growth medium was inoculated in the production medium.

Table 1. MRS (De Man, Rogosa and Sharpe) broth medium composition

Component	Storage Medium (g L^{-1})	Growth medium (g L^{-1})
Glucose	20	20
Peptone	10	10
Meat Extract	8	8
Yeast Extract	4	4
Sodium Acetate ($C_2H_3NaO_2$)	5	5
Dipotassium hydrogen phosphate (K_2HPO_4)	2	2
Triammonium citrate ($C_6H_{17}N_3O_7$)	2	2
Magnesium sulphate ($MgSO_4$)	0.2	0.2
Manganese sulphate($MnSO_4$)	0.05	0.05
Tween 80	1	1
Agar	15	

MRS broth medium of 100 ml was put into 500 ml erlenmeyer flasks. The initial pH of the bioprocess media was adjusted to a value of 6.0 by using 0.1 N HCl and 0.1 N NaOH. The brim of the erlenmeyer flasks was sealed with a plug made of cotton and gauze, and the top of the plug was covered with aluminum foil. The medium prepared in this way was sterilized with an autoclave (Alp CL40, Japan) for 20 minutes at 121 °C and 1.2 bar pressure. The sterilized media was cooled to ambient

temperature in laminar flow. 1% of the *L. fermentum* strain in the growth medium was inoculated into growth media of different compositions. The growth of bacteria was ensured for 18 hours in the culture inoculated into the erlenmeyer flasks at 37 °C and in a shaker with an orbital incubator (Stuart SI600, United Kingdom) with an agitation speed of 200 rpm.

2.4. Experimental Design

Four of the medium components (glucose concentration (g L^{-1}), yeast extract concentration (g L^{-1}), inorganic salts (sodium acetate (50%, w/w), potassium dihydrogen phosphate (20%, w/w), magnesium sulphate (20%, w/w), and manganese (II) sulphate (10%, w/w mixture) and Tween 80 (ml L^{-1}) were selected as an independent parameter for the RSM. Above mentioned medium components are also present in the MRS broth medium and are thought to be effective in the production of *Lactobacillus fermentum* strain. The value ranges of the independent variables were determined by preliminary experiments. In the study, all experiments were carried out in 3 parallel, and the arithmetic mean of the obtained results was used.

The experimental design was carried out according to Central Composite Design (CCD), which is included in RSM and is widely used in optimization processes, and the number of live cells $\log_{10}$ (cfu/ml) was selected as the response variable. The independent variables selected for the medium composition and examined ranges are given in Table 2.

Table 2. Experimental values and levels of independent variables for CCD

Independent variables	Code	-2.0 (-α)	-1.0 (low)	0.0 (central)	+1.0 (high)	+2.0 (+α)
Glucose (g L^{-1})	A	50	75	100	125	150
Yeast extract (g L^{-1})	B	15	30	45	60	75
Inorganic salts (g L^{-1})	C	5	10	15	20	25
Tween 80 (ml L^{-1})	D	10	15	20	25	30

In the full factorial design for CCD, an experimental matrix consisting of 16 factorials, 8 endpoints, and 6 center points were used, and the total of the experiments was equal to 30. The total number of experiments was calculated using the equation given below.

$$\text{Total Number of Experiments} = 2^n + (2)(n) + 6 = 2^4 + (2)(4) + 6 = 30 \quad (1)$$

Here, n represents the number of independent variables.

For the optimization of medium components, the equation of full factorial design given by Eq. (2) was used (Liu et al., 2020);

$$Y = \beta_0 + \sum_{i=1}^{n}\beta_i X_i + \sum_{i=1}^{n}\beta_{ii} X_i^2 + \sum_{i=1}^{n-1}\sum_{j=2}^{n}\beta_{ij} X_i X_j \quad (2)$$

Where Y; the number of viable cells ($\log_{10}$ (cfu ml^{-1})), β0; a constant coefficient, Xi and Xj are independent variables that affect the response, βi; linear effect, βii; second degree effect, βij; binary interaction between independent variables. Analysis of variance (ANOVA) and three-dimensional surface graphs for CCD were obtained using Design Expert 10.0 (Stat-Ease) software.

3. Results and Discussion

One of the most important parameters affecting the production of primary and secondary metabolites produced by microorganisms is the medium composition. Among the medium components, the carbon source accounts for approximately 60-80% of the medium cost (Rehm et al., 1987). The maximum availability of valuable metabolites obtained as a result of microbial activities is closely related to the carbon/nitrogen (C/N) ratio in the growth medium where the production takes place (Farezvidal et al., 1992). In previous studies conducted it is stated that microbial cells and metabolite

production will reach maximum values when the C/N ratio is selected as optimum (Ülger, 1997). In addition, with the thought that inorganic salts and Tween 80 would positively affect the microbial cell formation, experiments were carried out within the framework of the experimental plan given in Table 3. Cultivation was carried out using the medium compositions in Table 3, and at the end of the 18th hour, the samples taken from the Erlenmeyer flasks were diluted as required and inoculated in petri dishes containing MRS broth solid medium with agar. After the petri dishes were incubated for 24 hours in an incubator at 37 °C, the colonies formed (Figure 1) were counted, and the results were multiplied by the dilution factor, and thus the number of viable cells formed under each experiment was determined. Actual numbers of living cells obtained in the experiment and the living cell numbers estimated by the model are given in Table 3 as cfu/ml on a $\log_{10}$ basis.

Table 3. Observed and predicted responses for CCD in experimental design matrix prepared with real and coded values of independent model parameters

	Experiment No	(A) Glucose g L^{-1}		(B) Yeast extract g L^{-1}		(C) Inorganic salts g L^{-1}		(D) Tween 80 ml L^{-1}		Observed $\log_{10}$ (cfu ml^{-1})	Predicted $\log_{10}$ (cfu ml^{-1})
Factorial design points, 2^n	**1**	75	-1	30	-1	10	-1	15	-1	10.544	10.460
	2	125	+1	30	-1	10	-1	15	-1	10.380	10.360
	3	75	-1	60	+1	10	-1	15	-1	10.114	10.120
	4	125	+1	60	+1	10	-1	15	-1	9.954	9.950
	5	75	-1	30	-1	20	+1	15	-1	10.505	10.470
	6	125	+1	30	-1	20	+1	15	-1	10.342	10.300
	7	75	-1	60	+1	20	+1	15	-1	10.431	10.420
	8	125	+1	60	+1	20	+1	15	-1	10.255	10.180
	9	75	-1	30	-1	10	-1	25	+1	10.462	10.470
	10	125	+1	30	-1	10	-1	25	+1	10.230	10.250
	11	75	-1	60	+1	10	-1	25	+1	10.176	10.220
	12	125	+1	60	+1	10	-1	25	+1	9.954	9.930
	13	75	-1	30	-1	20	+1	25	+1	10.000	10.000
	14	125	+1	30	-1	20	+1	25	+1	9.778	9.710
	15	75	-1	60	+1	20	+1	25	+1	10.079	10.040
	16	125	+1	60	+1	20	+1	25	+1	9.602	9.680
End points r, 2n	**17**	50	-2	45	0	15	0	20	0	9.954	9.980
	18	150	+2	45	0	15	0	20	0	9.477	9.520
	19	100	0	15	-2	15	0	20	0	10.279	10.360
	20	100	0	75	+2	15	0	20	0	10.000	9.980
	21	100	0	45	0	5	-2	20	0	10.462	10.460
	22	100	0	45	0	25	+2	20	0	10.146	10.210
	23	100	0	45	0	15	0	10	-2	10.532	10.630
	24	100	0	45	0	15	0	30	+2	10.176	10.140
Central point	**25**	100	0	45	0	15	0	20	0	10.690	10.640
	26	100	0	45	0	15	0	20	0	10.568	10.640
	27	100	0	45	0	15	0	20	0	10.699	10.640
	28	100	0	45	0	15	0	20	0	10.556	10.640
	29	100	0	45	0	15	0	20	0	10.672	10.640
	30	100	0	45	0	15	0	20	0	10.663	10.640

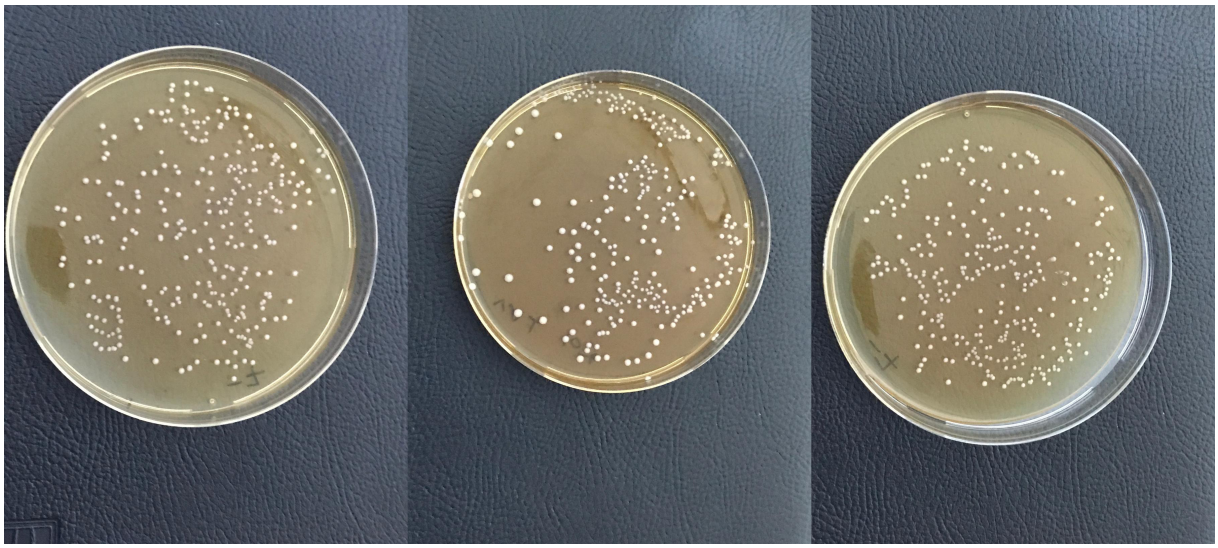

Figure 1. *Lactobacillus fermentum* colonies formed in petri dishes after 10^9 dilution at the end of the 24^{th} hour.

In order to understand which model (linear, 2FI, quadratic, and cubic) better fit the experimental data, Design Expert 10 software was used in the analysis of statistical results, and the findings are given in Table 4. By looking at both the program and statistical coefficients, it was observed that the experimental data fit the quadratic model equation better.

Table 4. Statistical results of suggested models after ANOVA analysis

Source	**Sequential p-value**	**Lack of fit p-value**	**Adjusted R^2**	**Predicted R^2**	**Result**
Linear	0.0373	0.0010	0.2168	0.0937	
2FI	0.6969	0.0007	0.1429	0.0985	
Quadratic	< 0.0001	0.3679	0.9516	0.8840	Suggested
Cubic	0.5134	0.2298	0.9513	0.2373	

As a result of the ANOVA, the quadratic model *Probe* > F value was found to be significant with <0.0001 at a 95% confidence level. It has been determined that the model terms A, B, C, D, BC, CD, A2, B2, C2, D2 where the *probe*>F value is less than 0.05 are effective medium composition parameters in the production of *Lactobacillus fermentum* strain. *Probe*>F value is used to control the effect of coefficients on the result of each component. By using these coefficients, the interaction between independent variables can be determined. *Probe*> F values of linear, quadratic, and interaction terms and other statistical results are presented in Table 5.

The model equation proposed as a result of ANOVA analysis for the production of *Lactobacillus fermentum* strain is given in Equation 3 and Equation 4 in terms of actual and coded values, respectively.

Model equation for the actual values;

$$Y\left(log^{10}\left(\frac{cfu}{ml}\right)\right) = 10.64 - 0.12(Glucose) - 0.093(Yeast\ extract) - 0.061(inorganic\ salts) - 0.12(tween\ 80) - 0.016(glucose)(yeast\ extract) - 0.016(glucose)(inorganic\ salts) - 0.031(glucose)(tween\ 80) + 0.073(yeast\ extract)(inorganic\ salts) + 0.022(yeast\ extract)(tween\ 80 - 0.12(inorganic\ salts)(tween\ 80) - 0.22 * (glucose)^2 - 0.12(yeast\ extract)^2 - 0.076(inorganic\ salts)^2 - 0.064(tween\ 80)^2 \quad (3)$$

Model equation for the coded values;

$$Y\left(log10\left(\frac{cfu}{ml}\right)\right) = 4.325 + 0.0757\ (A) + 0.0246\ (B) + 0.1443\ (C) + 0.1604\ (D) - 4.24E - 5(AB) - 1.30E - 4(AC) - 2.45E - 4(AD) + 9.67E - 4(BC) + 2.98E - 4(BD) - 4.76E - 3(CD) - 3.58E - 4(A^2) - 5.23E - 4(B^2) - 3.06E - 3(C^2) - 2.56E - 3(D^2) \quad (4)$$

Table 5. ANOVA results of regression coefficients in RSM

Source	Sum of squares	DF	Mean square	*F*-value	*P*-value
Model	2.940	14	0.210	41.73	< 0.0001
A-Glucose	0.320	1	0.320	63.51	< 0.0001
B-Yeast extract	0.210	1	0.210	41.32	< 0.0001
C-inorganic salts	0.088	1	0.088	17.52	0.0008
D-Tween 80	0.360	1	0.360	72.30	< 0.0001
AB	4.044E-3	1	4.044E-3	0.800	0.3841
AC	4.239E-3	1	4.239E-3	0.840	0.3732
AD	0.015	1	0.015	2.990	0.1045
BC	0.084	1	0.084	16.74	0.0010
BD	7.993E-3	1	7.993E-3	1.590	0.2268
CD	0.230	1	0.230	45.10	< 0.0001
A^2	1.370	1	1.370	272.4	< 0.0001
B^2	0.380	1	0.380	75.42	< 0.0001
C^2	0.160	1	0.160	31.82	< 0.0001
D^2	0.110	1	0.110	22.34	0.0003
Residual	0.075	15	5.032E-3	-	-
Lack of fit	0.056	10	5.578E-3	1.420	0.3679
Pure error	0.020	5	3.939E-3		
Core total	3.020	29			
Mean	10.26				
R^2	0.975				
R^2_{Adj}	0.952				
R^2_{Pre}	0.884				
Std. Dev.	0.071				
C.V. %	0.690				
PRESS	0.350				
Adequate precision	22.43				

The compatibility of the model with experimental data is evaluated based on the value of the multiple correlation coefficient, R2 (Yang et al., 2020). R2 shows that suggested linear models can define the changes in the response variable as high as 97.5% and only 2.5% of the total variation cannot be explained by the model. Similarly, the realistically corrected R2Adj term value was determined as 95.2% (Table 5).

High R^2 and R^2_{Adj} values are an indicator of a close interaction between the experimental results and the values obtained from the model (Wu and Ahn, 2018). From the high R^2 and R^2_{Adj} values obtained in the study, it is possible to say that experimental and predicted values are very close to each other, indicating the success of the established model. , The adequate precision value was found to be 22.43. This value shows the signal to noise ratio, and this value is expected to be above 4 (Venkateswarulu et al., 2017). Lack of Fit value (0.3679) was found to be insignificant for the model. The low CV (CV = 0.690%) value clearly demonstrates that the deviations between experimental and predicted values are low, and there is a high degree of precision in the experiments performed, and also the model is reproducible (Long et al., 2018). All descriptive statistical data obtained show that the models proposed in this study are significant.

Studentized residues and a normal % probability plot are given in Figure 2.a. Studentized residues measure the number of standard deviations that distinguish actual and estimated values. The normal % probability checks whether the residues are in a normal distribution. The straight line between these two variables shown in Figure 2.a determines whether a response transformation is required (Mona et al., 2011). Experimentally obtained log^{10} (cfu ml^{-1}) values and estimated log_{10} (cfu ml^{-1}) values are given in Figure 2.b. As it can be understood from Figure 2.b, it is clearly seen that the estimated log_{10} (cfu ml^{-1}) values suggested by the created model and the experimentally obtained log10 (cfu ml^{-1}) values conform and can be defined by the model.

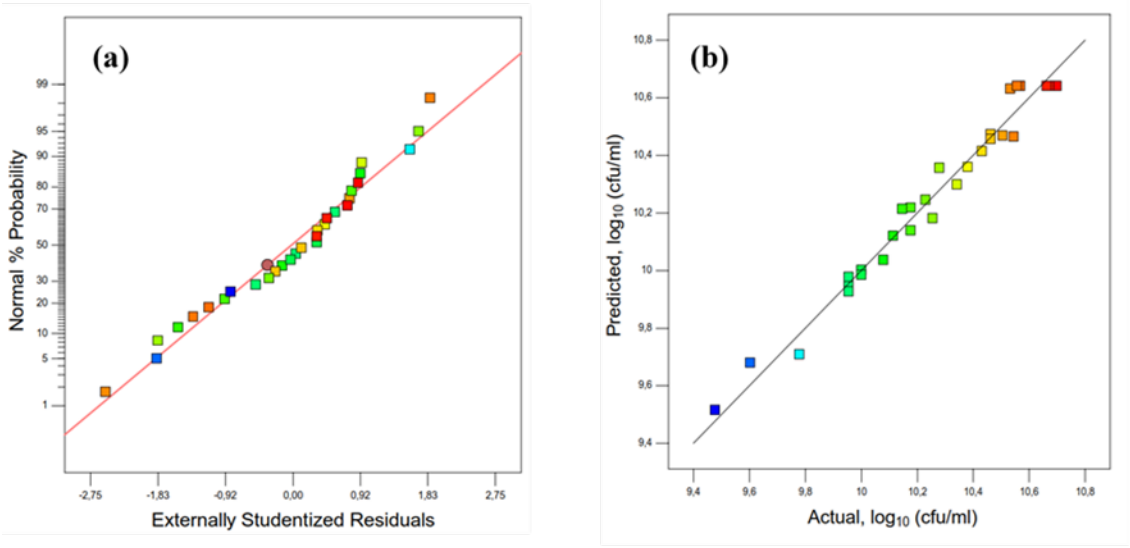

Figure 2. (a) Studentized residues and a normal % probability plot, (b) Experimentally obtained log_{10} (cfu/ml) values and estimated log_{10} (cfu ml^{-1}) values plot.

Four independent parameters, namely, glucose concentration (g L^{-1}), yeast extract concentration (g L^{-1}), inorganic salts, and Tween 80 (ml L^{-1}), appear to significantly affected the production of the *Lactobacillus fermentum* strain. It is extremely important to determine the interaction of components with each other while modeling studies when parameters are affected by each other. RSM can reveal these interactions between parameters with the help of stratified graphics and 3D graphics. The 3D response surface graphics obtained as a result of the study are given in Figure 3.

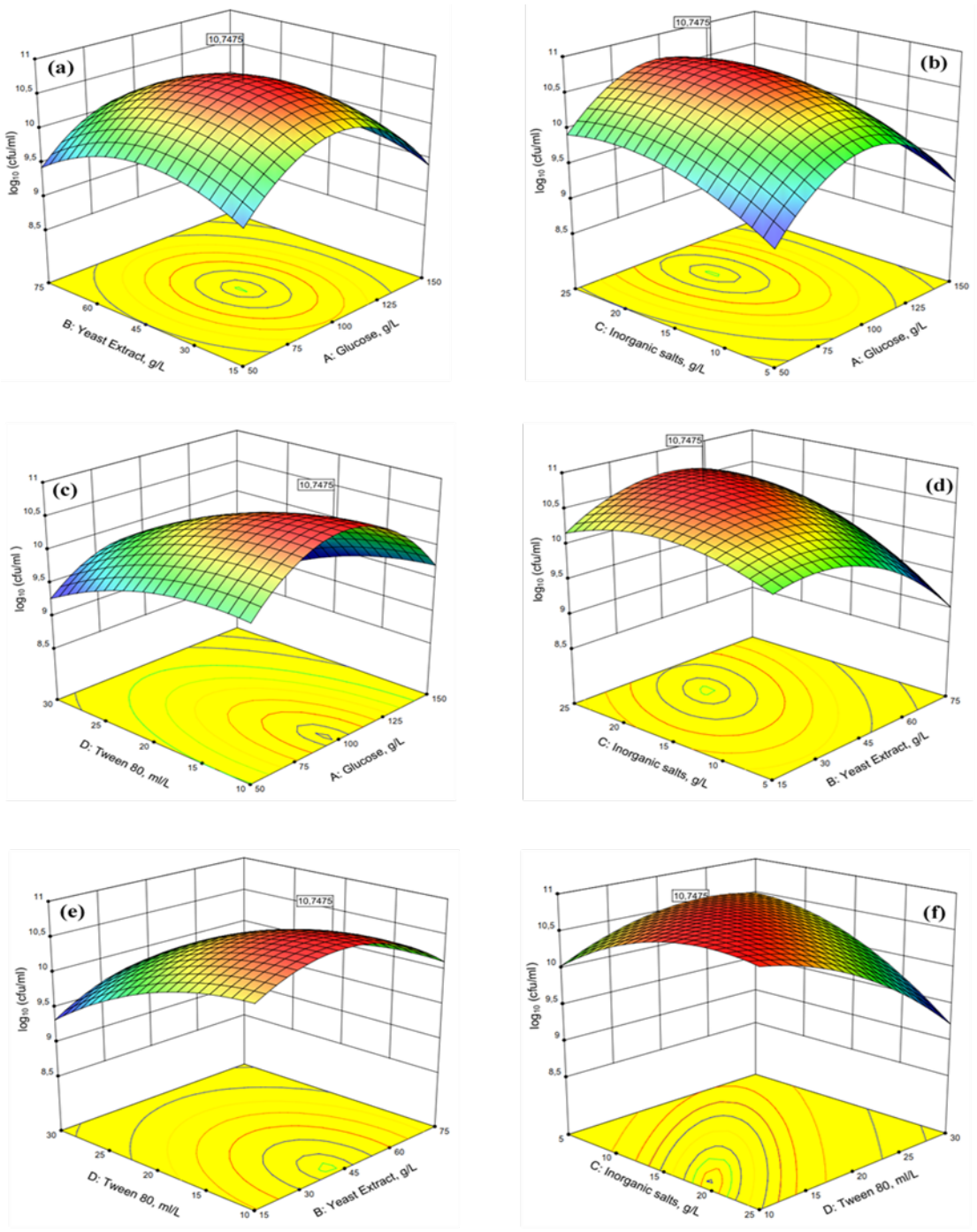

Figure 3. 3D response surface graphics obtained for production of *Lactobacillus fermentum* strain depending on medium composition, **(a)** Glucose and Yeast extract, **(b)** Glucose and inorganic salts, **(c)** Glucose and Tween 80, **(d)** Yeast extract and inorganic salts, **(e)** Yeast extract and Tween 80, **(f)** inorganic salts and Tween 80.

As can be seen from Figures 3.a, 3.b, 3.c, 3.d and 3.e, the production level of *Lactobacillus fermentum* strain decreases in media with low and high concentrations of glucose and low and high yeast extract concentrations. In other current studies, this observation has been expressed as a decrease in microbial activity due to the repression effect of some carbon sources at high concentrations in the growth medium (Yun and Ryu, 2001; Myers and Montgomery, 2002; Geckil et al., 2004). It is reported that microbial activity will be negatively affected due to the absence of a sufficient concentration of nitrogen source in the growth medium. However, it is stated that if the nitrogen source at high concentrations is in the growth medium, the microbial product production efficiency will be inhibited

(Avonts et al., 2004). In this context, a similar decrease was observed in low and high yeast extract concentrations. In Figure 3.b, 3.d, and 3.f it is seen that the production decreases in the medium where inorganic salts at higher and lower concentrations are present. This can be interpreted as a requirement for ensuring the stability of enzymes that catalyze the reactions necessary for microbial cell formation (Djekrif-Dakhmouche et al., 2006). The same is also valid for Tween 80 containing growth medium (Figures 3.c, 3.e and 3.f).

4. Conclusion

As a result, optimization of the media components for the production of *Lactobacillus fermentum* strain by using Design Expert 10 software has revealed the conditions by which the maximum production of *L. fermentum* NBC-08 strain biomass is possible. Confirmation experiments were carried out under these conditions, and the production levels of *L. fermentum* strain in the medium composition suggested by the model were examined. The optimum medium composition suggested by the model is 96.06 g L^{-1} glucose, 40.76 g L^{-1} yeast extract, 19.43 g L^{-1} inorganic salts, and 11.01 ml L^{-1} Tween 80. This medium composition created as a result of this study was coined as NBC media. The production of the maximum *L. fermentum* strain is 10.75 $\log_{10}$ (cfu ml^{-1}). By using the medium composition suggested by model 3 parallel validation experiments were performed, and the production of *L. fermentum* strain as the result of these experiments was 10.79 $\log_{10}$ (cfu ml^{-1}). These values, which are quite close, indicate that the optimization work is successful and reliable. As a controlled study, the *L. fermentum* NBC-08 strain was grown in MRS Broth medium. As a result of this study, cell viable count in MRS Broth was reached 9.43 $\log_{10}$ (cfu ml^{-1}). A significant difference was found when comparing viable cell counts between MRS broth and the medium suggested by the model. In this case, it can be achieved a more viable cell count by using less medium composition.

It is concluded that this study will make important contributions to the literature and industry for optimizing nutrient composition media for producing maximum lactic acid bacteria.

Acknowledgments

This study; it has been prepared by making use of a part of the public project numbered TAGEM-18/ARGE/24 supported by TAGEM.

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