APPLICATION OF PLANT OILS AND SURFACTANTS AS STIMULATING AGENTS FOR OPTIMUM CITRIC ACID PRODUCTION FROM CASSAVA BAGASSE

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Abstract: This study investigated the stimulatory effect of plant oils (castor oil and olive oil) and surfactants (tween 20 and tween 80) on citric acid production from cassava bagasse using *Aspergillus niger*. The fermentation process was designed using Box-Behnken design while the effect of the oils and surfactants was optimised using response surface methodology (RSM) and artificial neural network (ANN). RSM analysis yielded a statistically significant quadratic model ($p<0.05$) which was used to predict an optimal citric acid concentration of 4.82 g/L at a castor oil concentration of 3% w/w, olive oil concentration of 2.45% w/w, tween 20 concentration of 1.5% w/w and tween 80 concentration of 0.8% w/w. ANN analysis showed that a multilayer full feed forward (MFFF) network with quick propagation (QP) and hyperbolic tangent transfer function (Tanh) yielded the best model for predicting citric acid production. The optimal ANN model predicted a citric acid concentration of 4.76 g/l at a castor oil concentration of 3% w/w, olive oil concentration of 1.84% w/w, tween 20 concentration of 1.5% w/w and tween 80 concentration of 0.67% w/w. The oils and surfactants were beneficial to citric acid production with both enhancing citric acid production by 23.7 and 9.8%, respectively. The predictive capacity of the RSM and ANN models was assessed based on their respective coefficient of determination (R^2) and root mean square error (RMSE) values. These values were obtained as 1.00 and 0.005 for ANN and 0.99 and 0.018 for RSM, respectively. The higher R^2 value and lower RMSE value of the ANN model shows that it is a better predictive tool compared to RSM.

Keywords: Citric acid, Box-Behnken design, artificial neural network, cassava bagasse

Introduction

Citric acid is a multipurpose tricarboxylic acid which is naturally found in fruits such as orange, pineapple, lemon, pear etc (Betiku and Adesina, 2013). It is a commercially valuable product which has a lot of applications domestically and industrially. It is widely used in the food, pharmaceutical and beverage industries as an acidifying, preserving and flavour-enhancing agent. In the chemical industry, it is used as an antifoam agent and for the treatment of textiles. It is also used in cosmetics and toiletries as a pH stabiliser, antioxidant and buffering agent (Kuforiji *et al*., 2010).

There has been a rapid increase in the global production of citric acid with an estimated annual production of about 1.7 million tons recorded in 2008. This figure has been projected to increase by as much as 5% annually in order to meet the growing demand for citric acid. Microbial fermentation appears to be the most viable route for citric acid production compared to chemical synthesis (Hayder, 2012). Citric acid has traditionally been produced commercially via submerged fermentation of molasses.However, most industrial scale production of citric acid is achieved by solid state fermentation (SSF) using *Aspergillus niger* because this option has lower energy and water requirements, less possibility of bacterial contamination, reduced wastewater generation, etc. (Imandi *et al*., 2008).Amongst the microorganisms that have been investigated for producing citric acid, *Aspergillusniger* is preferred because its usage is characterised by easy handling, high citric acid yields and the microbe has the ability to ferment a wide range of substrates (Schuster *et al*., 2002).The cost of citric acid production could be reduced by using cheap and abundantly available agricultural wastes as feedstock. Several researchers have adopted SSF to produce citric acid from solid waste materials such as grape pomace (Hang and Woodams, 1985), kiwi fruit peel (Hang *et al*., 1987), kumara (Lu *et al*., 1995), okara (Khare *et al*., 1995), carob pod (Roukas, 1999), date palm (Assadi and Nikkhah, 2002), sugarcane bagasse (Kumar and Jain, 2008), pineapple peels (Amenaghawon *et al*., 2014; Imandi *et al*., 2008), apple pomace (Baei *et al*., 2008), banana peels (Amenaghawon *et al*., 2015; Kareem and Rahman, 2013), etc.

Nigeria is by far the world's largest producer of cassava with an annual production rate of 45 million tonnes per annum (FAO, 2009). In Nigeria, cassava is processed into food materials such as garri, fufu, starch, cassava flour, pudding, etc. However, of the huge quantities of cassava bagasse generated in the process, only a small fraction is utilised,particularly as animal feed and the rest discarded (Amenaghawon *et al*., 2013). However, as a result of its relatively high cellulose and low ash content as well as containing other nutrients, cassava bagasse has been identified as a potentially ideal substrate for the production of value-added products such as citric acid (Prado *et al*., 2005).

Several studies have been reported on citric acid production from cassava bagasse using different fermentation methods. For example, Vandenberghe *et al*. (2000) produced citric acid from cassava bagasse in solid state fermentation using *Aspergillus niger*. They reported that *Aspergillus niger* had good adaptation to the substrate (cassava bagasse). In another study, Prado *et al*. (2005) investigated the effect of cassava bagasse loading on citric acid production. Nevertheless, none of the authors applied statistical experimental design approach in their work and the production of citric acid from cassava bagasse was not optimised.

The production of citric acid has been reported to be influenced by fermentation conditions such as type of bacterial strain, composition of fermentation medium, substrate type and concentration, agitation rate, aeration, temperature, pH etc. Furthermore, certain substances have been known to enhance citric acid production. Amongst these are low molecular weight alcohols, trace metals, oils, surfactants etc (Max *et al*., 2010). The efficiency of citric acid production can be improved by modelling and optimisation of the process (Betiku and Taiwo, 2015). The traditional method of optimisation is the one-factor-at-a-time method which involves varying one factor at a time while keeping the other factors constant. However, this method is time consuming, does not explain the interaction between factors and does not identify the true optimum point (Imandi *et al*., 2008). Application of response surface methodology (RSM) is one way of overcoming these shortcomings. RSM is an experimental design and empirical modeling tool which is useful in establishing the relationship between a set of experimental factors and some observed response. It reduces the number of experiments needed to obtain statistically acceptable results, can elucidate the interaction between factors and can identify the true optimum point. It has been extensively applied in optimising many bioprocesses such as citric acid production (Imandi *et al*., 2008; Betiku and Adesina, 2013), biodiesel production (Mostafaei *et al*., 2016), bioethanol production (Tamayo and Migo, 2014), xylanase production (Kumar *et al*., 2017), etc. Despite the advantages of RSM, it might not be suitable for optimising all bioprocesses. The quadratic model generated may not be able to adequately model the actual behaviour of the real system. For instance, Bas and Boyaci (2007) reported that it could not explain the effects of pH and substrate concentration on the initial rate of an enzymatic reaction. Similar observations have also been reported by other researchers (Beg *et al*., 2002; Senanayake and Shahidi, 2002). Moreover, the RSM optimisation is a local optimisation technique and it can only identify the local optimum (Chen *et al*., 2005). Artificial Neural Networks (ANNs) which are modelled after the biological nervous system have emerged as the most popular artificial learning tool in biotechnology. ANN is an attractive option for developing nonlinear empirical models particularly when it is not possible to develop conventional empirical models or when such models are inadequate in describing real life systems (Velu *et al*., 2016).

This study focused on modelling and optimisation of the stimulatory effect of plant oils and surfactants on citric acid produced from cassava bagasse with *Aspergillus niger*. A mathematical model was developed to predict the production of citric acid and the suitability of RSM and ANN as optimisation tools were then assessed.

Materials and Methods

Substrate and microorganism

Fresh cassava bagasse was obtained from a local cassava processing facility in Agenebode, Edo State, Nigeria. It was sundried to constant weight, milled to a particle size of about 1.5 mm and then homogenized in a single lot. The milled bagasse was then stored under ambient conditions prior to use.

Microorganism and inoculum preparation

Aspergillus niger ATCC 9167, obtained from Microbiology Department of the University of Benin, Benin City, Edo State, Nigeria was used throughout the study as the fermenting organism. Conidia suspensions of fungal strains were obtained from cultures grown on potato dextrose agar slants at 30ºC for 5 to 7 days. The spores were washed with sterilised 0.8% tween 80 solution by shaking vigorously for 1 minute before it was used for fermentation (Amenaghawon *et al*., 2014).

Media preparation

The substrate was wetted with a supplemental salt solution to the desired moisture level in a 250 ml Erlenmeyer flask. The composition of the salt solution was as follows (%w/w): $FeCl_3{\cdot}6H_2O$, 0.015; $ZnSO_4{\cdot}7H_2O$, 0.002; $CaCl_3$, 0.015; $MgSO_4{\cdot}7H_2O$, 0.15; $MnSO_4{\cdot}H_2O$, 0.006. Yeast extract served as a source of nitrogen. The composition of the plant oils (olive oil and castor oil) and surfactants (tween 20 and tween 80) were set as per the experimental design. The content of the flask was mixed and autoclaved at 121°C and 15 psi for 15 min for sterilisation (Imandi *et al*., 2008).

Solid state fermentation

The sterilised substrate with the media was cooled to room temperature and then inoculated with 2 mL of inoculum. It was subsequently incubated at 30°C for 5 days. All the experiments were carried out in triplicate.

Citric acid analysis

Citric acid produced was extracted from the broth by diluting with 100 mL of distilled water. The resulting mixture was then filtered and the filtrate was used for subsequent analysis. The concentration of citric acid produced during fermentation was determined using the pyridine-acetic anhydride method (Marrier and Boulet, 1958).

Experimental design

A three-level-four-factor Box-Behnken design was used for the fermentation process and this resulted in 29 experimental runs. The factors chosen for optimisation were olive oil, castor oil, tween 20 and tween 80. The coded and actual levels of the factors are shown in Table 1. Equation 1 is a quadratic response model which was used to fit the experimental data and this was achieved by using multiple regression analysis to estimate the values of the coefficients of the model. Analysis of variance (ANOVA) was then used to assess the quality and significance of the model.

$$Y_i = b_o + \sum b_i X_i + \sum b_{ij} X_i X_j + \sum b_{ii} X_i^2 + e_i \quad (1)$$

Where: Y_i is the predicted response or dependent variable, X_i and X_j are the independent variables, b_o is the offset term, b_i and b_{ij} are the single and interaction effect coefficients and e_i is the experimental error term.

The low, middle, and high levels of each variable were coded as -1, 0, and +1, respectively. The factors were coded according to Equation 2;

$$x_i = \frac{X_i - X_o}{\Delta X_i} \quad (2)$$

Where x_i and X_i are the coded and actual values of the factors, respectively; X_o is the actual value of the factors at the centre point and ΔX_i is the step change in the actual value of the factors. Design Expert® 7.0.0 (Stat-ease, Inc. Minneapolis, USA), a statistical software used for the experimental design, regression analysis and analysis of variance (ANOVA).

Table 1: Coding of factors used for Box-Behnken design

Variables	Symbols	Coded and actual levels		
		-1	**0**	**+1**
Olive oil(% w/w)	X_1	0.00	1.50	3.00
Castor oil(% w/w)	X_2	0.00	1.50	3.00
Tween 20(% w/w)	X_3	0.00	0.75	1.50
Tween 80(% w/w)	X_4	0.00	0.75	1.50

Artificial neural network design

Commercial ANN Software, NeuralPower, version 2.5 (C.P.C-X Software USA) was used to model and optimize the fermentation of citric acid production. Citric acid concentration produced was predicted using the multilayer full feed forward (MFFF) and the multilayer normal feed forward (MNFF) neural networks. Both network architectures were trained using different learning algorithms including incremental back propagation (IBP), batch back propagation (BBP), quick propagation (QP), generic algorithm (GA), and Levenberg-Marquadt (LM) algorithms. Each of these learning algorithms used 70% of the experimental data for training the network, 15% for validating and the remaining 15% for testing. The training algorithm that best described the fermentation process was selected based on itscoefficient of determination (R^2) and root mean square error (RMSE) value. The network topology contained a single hidden layer while the number of neurons in this layer, the transfer function of the hidden and the output layers were determined iteratively by developing several neural networks with transfer functions of Sigmoid, Hyperbolic-tangent, Gaussian, Linear, Threshold, Linear and Bipolar Linear. Each of the network was trained using a stopping criterion of an RMSE less than 0.0001.

RSM and ANN data verification

The predictive capacity of both RSM and ANN were evaluated using the RMSE and R^2 values and these are defined as follows;

$$RMSE = MSE^{1/2} \quad (3)$$

$$MSE = \frac{1}{n}\sum_{i=1}^{n}\left(y_{pred} - y_{\exp}\right)^2 \quad (4)$$

$$R^2 = 1 - \sum_{i=1}^{n}\frac{\left(y_{pred} - y_{\exp}\right)^2}{\left(y_{exp} - y_{ave,exp}\right)^2} \quad (5)$$

Where: n is the number of points; y_{pred} is the predicted value obtained from the model; y_{exp} is the actual value; $y_{ave,exp}$ is the average of the actual values.

The mean square error (MSE) and RMSE are used as an indication of the error between the predicted response and the actual response and both terms are also useful indication of a model's predictive capacity (Ghaffari *et al*., 2006). The R^2 value is an indication of the fit of a mathematical model. An R^2 value close to unity is indicative of a good fit between model and experimental data (Qi *et al*., 2009).

Results and Discussion

Modelling and optimisation using RSM

The results of the 29 BBD experiments are shown with the observed and predicted responses in Table 2. Equation 6 is the model equation which relates the response (citric acid concentration) to the factors in terms of actual values. The equation represents citric acid concentration (Y) as a function of castor oil (X_1), olive oil (X_2), tween-20 (X_3) and tween-80 (X_4).

$$\begin{aligned} Y = {} & 3.66 + 0.14X_1 + 0.50X_2 - 0.19X_3 + 0.28X_4 - 0.032X_1X_2 \\ & + 0.075X_1X_3 - 0.021X_1X_4 + 0.0066X_2X_3 + 0.0053X_2X_4 \\ & + 0.022X_3X_4 - 0.0087X_1^2 - 0.085X_2^2 + 0.070X_3^2 - 0.16X_4^2 \end{aligned} \quad (6)$$

Table 2: BBD matrix showing actual and coded values along with the experimental values and predicted citric acid concentration

Run	Actual and coded values				Citric acid concentration (g/L)		
	X_1	X_2	X_3	X_4	Observed	RSM predicted	ANN predicted
1	0.0 (-1)	1.5 (0)	0.00 (-1)	0.75 (0)	4.32	4.34	4.32
2	1.5 (0)	1.5 (0)	0.75 (0)	0.75 (0)	4.42	4.43	4.43
3	1.5 (0)	3.0 (1)	0.75 (0)	1.50 (1)	4.49	4.49	4.49
4	1.5 (0)	1.5 (0)	0.75 (0)	0.75 (0)	4.43	4.43	4.43
5	1.5 (0)	0.0 (-1)	0.75 (0)	1.50 (1)	3.84	3.86	3.84
6	0.0 (-1)	0.0 (-1)	0.75 (0)	0.75 (0)	3.68	3.68	3.68
7	1.5 (0)	0.0 (-1)	0.00 (-1)	0.75 (0)	3.97	3.94	3.97
8	1.5 (0)	1.5 (0)	1.50 (1)	1.50 (1)	4.45	4.46	4.46
9	1.5 (0)	0.0 (-1)	0.75 (0)	0.00 (-1)	3.83	3.83	3.83
10	0.0 (-1)	1.5 (0)	0.75 (0)	1.50 (1)	4.22	4.21	4.22
11	3.0 (1)	1.5 (0)	0.75 (0)	0.00 (-1)	4.47	4.49	4.47
12	0.0 (-1)	1.5 (0)	0.75 (0)	0.00 (-1)	4.11	4.12	4.11
13	1.5 (0)	1.5 (0)	0.75 (0)	0.75 (0)	4.43	4.43	4.43
14	3.0 (1)	1.5 (0)	1.50 (1)	0.75 (0)	4.76	4.74	4.76
15	3.0 (1)	3.0 (1)	0.75 (0)	0.75 (0)	4.64	4.62	4.64
16	1.5 (0)	3.0 (1)	0.00 (-1)	0.75 (0)	4.55	4.54	4.55
17	1.5 (0)	3.0 (1)	1.50 (1)	0.75 (0)	4.61	4.64	4.61
18	1.5 (0)	3.0 (1)	0.75 (0)	0.00 (-1)	4.45	4.43	4.45
19	3.0 (1)	0.0 (-1)	0.75 (0)	0.75 (0)	4.18	4.15	4.18
20	3.0 (1)	1.5 (0)	0.75 (0)	1.50 (1)	4.48	4.48	4.48
21	1.5 (0)	1.5 (0)	0.00 (-1)	1.50 (1)	4.36	4.35	4.36
22	3.0 (1)	1.5 (0)	0.00 (-1)	0.75 (0)	4.45	4.49	4.46
23	1.5 (0)	0.0 (-1)	1.50 (1)	0.75 (0)	3.99	4.01	3.99
24	0.0 (-1)	1.5 (0)	1.50 (1)	0.75 (0)	4.29	4.25	4.29
25	1.5 (0)	1.5 (0)	1.50 (1)	0.00 (-1)	4.40	4.39	4.40
26	1.5 (0)	1.5 (0)	0.75 (0)	0.75 (0)	4.44	4.43	4.43
27	1.5 (0)	1.5 (0)	0.00 (-1)	0.00 (-1)	4.35	4.34	4.35
28	1.5 (0)	1.5 (0)	0.75 (0)	0.75 (0)	4.45	4.43	4.43
29	0.0 (-1)	3.0 (1)	0.75 (0)	0.75 (0)	4.43	4.44	4.43

ANOVA test was carried out to evaluate the quality and statistical significance of the model equation and the results are shown in Table 3. The model F-value of 199.87 and p-value of <0.0001 indicate that the model is statistically significant at the 95% confidence level. All the model terms were significant except the interaction terms of olive oil and tween-80, castor oil and tween -20, castor oil and tween-80 as well tween 20 and tween 80. The model terms with positive coefficients indicates a favourable effect for citric acid production while model terms with negative coefficient indicates an antagonistic effect on citric acid production. The "lack of fit" test compares the residual error to the pure error from replicated design points. The lack of fit value of the model was 0.0734. As this value is greater than 0.05, it implies that the lack of fit was not significant. The coefficient of determination (R^2) was obtained as 0.995 indicating that 99.5% of the variability observed in citric acid concentration could be attributed to the independent factors. The R^2 value indicates the degree to which the model is able to predict the response. The closer the R^2 value is to unity, the better the model can predict the response (Qi *et al*., 2009). The R^2 value of 0.995 obtained in this study shows that there was significant fit between the observed and predicted values of citric acid concentration. Furthermore, the predicted R^2 and the adjusted R^2 were within 0.20 of each other as is commonly desired. A low standard deviation of 0.026 means that there was very little deviation of the individual values of the response from the mean, further confirming the fit of the model. The coefficient of variation (CV) is the standard deviation expressed as a percentage of the mean. The experimental data is usually considered reproducible if the CV is not greater than 10%. A value of 0.590% obtained in this case, indicates reliability of the experiments. The adequate precision value measures signal to noise ratio and a ratio greater than 4 is desirable. A value of 57.638 obtained in this case indicates an adequate signal meaning that the model can be used to navigate the design space (Montgomery, 2005).

The Design-Expert software was used to numerically optimise the statistical model (Equation 6) to determine the optimum citric acid production conditions. The results showed that a maximum citric acid concentration of 4.82 g/l was obtained. The corresponding values of the independent factors were castor oil (3% w/w), olive oil (2.45% w/w), tween 20 (1.5% w/w) and tween 80 (0.8% w/w). the optimum citric acid concentration predicted by the model was validated by carrying out repeated experiments at the optimum conditions. The mean of the observations was obtained as 4.82 g/L.

Table 1: Statistical test of significance and ANOVA results

Source	Sum of squares	df	Mean square	F value	p value
Model	1.83305	14	0.13093	199.87	<0.0001
X_1	0.31942	1	0.31942	487.61	<0.0001
X_2	1.13042	1	1.13042	1725.61	<0.0001
X_3	0.01969	1	0.01969	30.06	<0.0001
X_4	0.00503	1	0.00503	7.68	0.0150
X_1X_2	0.02107	1	0.02107	32.17	<0.0001
X_1X_3	0.02822	1	0.02822	43.08	<0.0001
X_1X_4	0.00223	1	0.00223	3.40	0.0863*
X_2X_3	0.00022	1	0.00022	0.33	0.5728*
X_2X_4	0.00014	1	0.00014	0.21	0.6515*
X_3X_4	0.00060	1	0.00060	0.91	0.3565*
X_1^2	0.00247	1	0.00247	3.77	0.0727*
X_2^2	0.23467	1	0.23467	358.23	<0.0001
X_3^2	0.01061	1	0.01061	16.19	0.0013
X_4^2	0.05324	1	0.05324	81.27	<0.0001
Residual	0.00917	14	0.00066		
Lack of fit	0.00846	10	0.00085	4.75	0.0734
Pure error	0.00071	4	0.00018		
Cor Total	1.84222	28			
		ANOVA			
R^2			0.995		
Adjusted R^2			0.990		
Predicted R^2			0.973		
CV			0.590		
Std dev.			0.026		
Adeq. Precision			57.638		

*not significant

Modelling and optimisation using ANN

It is usually difficult to determine apriori, which ANN learning algorithms or transfer functions will be suitable for a particular process (Saracoglu, 2008). Hence it was necessary to train and test several networks and architectures to determine the one most suitable for the present study. The results presented in Table 4 shows that QP was the best training algorithm for predicting citric acid concentration. This was chosen because it had the highest R^2 value (0.99981) and the lowest RMSE value (0.00505).The optimum network topology was determined by assessing different transfer functions and a range of number of neurons (1 to 7). The Hyperbolic-Tangent function gave the highest R^2 values compared to other transfer functions. The optimum number of neurons was determined to be five based on the R^2 value (Fig. 1). Thus, the optimum network topology with five neurons was 4-5-1 i.e. four input factors in the input layer, five neurons in the hidden layer, one output layer and a transfer function of hyperbolic-tangent for the hidden and output layers (Fig. 2). The high R^2 value and low RMSE value obtained for the optimum ANN topology indicates that the ANN model can be used to adequately predict citric acid concentration from the input factors.

Table 4: R^2 and RMSE values of MNFF and MFFF using different training algorithms

Learning algorithm	MNFF		MFFF	
	R^2	RMSE	R^2	RMSE
IBP	0.9961	0.00724	0.99980	0.00507
BBP	0.99965	0.00676	0.99963	0.0070
QP	0.99973	0.00602	0.99981*	0.00505*
GA	0.99736	0.01883	0.99660	0.02129
LM	0.99712	0.01957	0.99921	0.01023

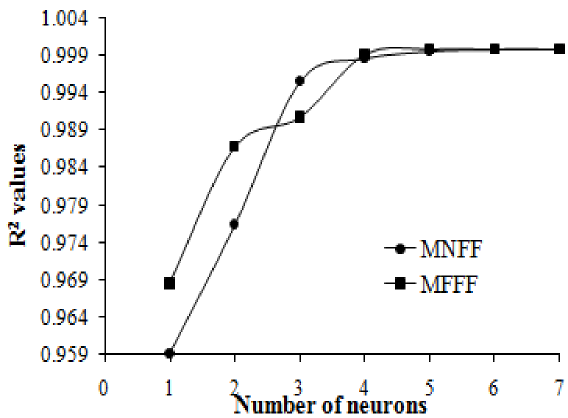

Fig. 1: R^2 values and corresponding number of neurons for MNFF and MFFF using hyperbolic-tangent function

The ANN model predicted an optimum citric acid concentration of 4.76 g/L and this was achieved with a castor oil concentration of 3.00%w/w, olive oil concentration of 1.84%w/w, tween 20 concentration of 1.5%w/w and tween 80 concentration of 0.67%w/w. Repeated experiments at the identified optimum point were used to validate the prediction of the ANN model. An actual citric acid concentration of 4.66 g/L was obtained demonstrating the impressive predictive capacity of the ANN model.

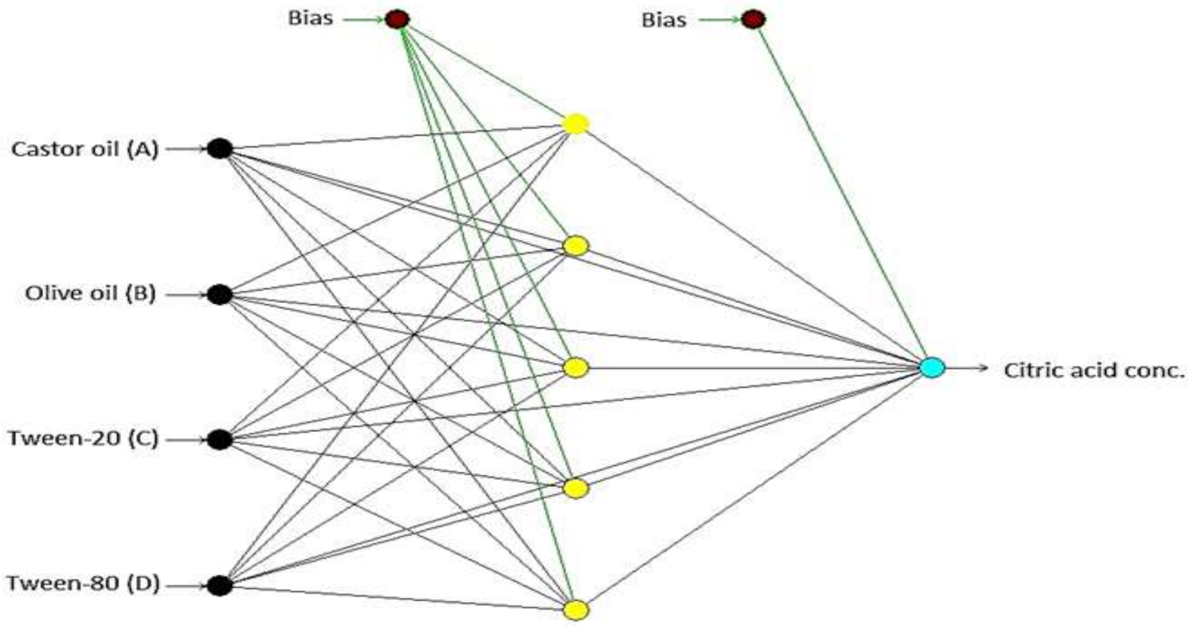

Fig. 2: Architecture of the optimal ANN for predicting citric acid concentration

Table 5: Comparison of RSM and ANN performance

Model	Castor oil (w/w%)	Olive oil (w/w%)	Tween 20 (w/w%)	Tween-80 (w/w%)	Citric acid (g/L)	R^2	RMSE
RSM	3.000	2.450	1.500	0.800	4.820	0.990	0.018
ANN	3.000	1.844	1.500	0.667	4.764	1.000	0.005

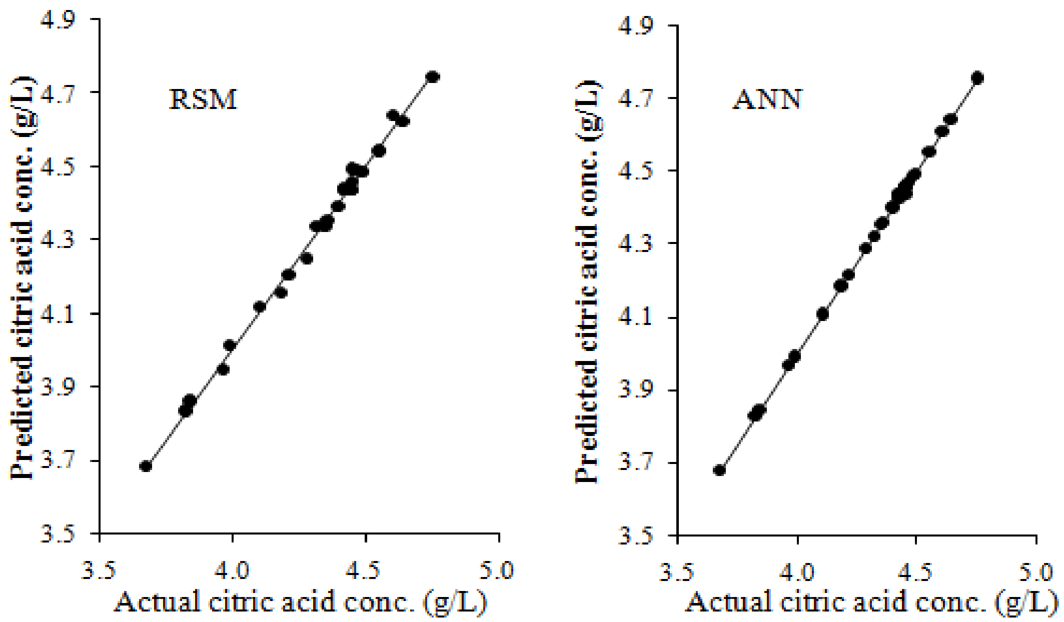

Fig. 3: Parity plots of predicted values against actual values for ANN and RSM models

Comparison of RSM and ANN performance

Table 5 shows a comparison between the predictions of the RSM and ANN models. The quality of the prediction of both models were evaluated using their respective R^2 and RMSE values. The results revealed that both models performed well with respect to their high R^2 value and low RMSE value. However, the ANN model was found to have better predictive capability because of its higher R^2 value and lower RMSE value. This observation was further supported by the parity plots between the experimental observations and the prediction of the RSM and ANN models (Fig. 3). Furthermore, the ANN model predicted slightly lower values for some of the factors (olive oil and tween 80) compared to the RSM model. This could become important in terms of material conservation. In summary, it can be inferred that the ANN model performed better in terms of data fitting and predictive capacity compared to the RSM model.

Effect of input factors on citric acid production

Three-dimensional (3D) response surface plots were generated to analyse the effect of the input factors (castor oil, olive oil, tween 20 and tween 80)on citric acid production. These plots were generated by keeping two factors at their center point and varying the other two factors within their experimental range. Fig. 4 shows the interactive effect of olive oil and castor oil on citric acid concentration. The plot shows that castor oil and olive oil had significant effects on citric acid production. This fact is corroborated by the fact that the model terms representing these factors were significant ($p<0.05$) as shown in Table 3. Both plant oils appear to enhance citric acid production as seen in the positive correlation between their concentrations and citric acid production. Some reports have highlighted the stimulatory effect of plant oils on the production of fungal metabolites like citric acid (Fukushima *et al*., 1991; Yang *et al*., 2000). Oils could be used as an alternative carbon source and are then broken down to glycerol and fatty acids with the latter entering the citric acid cycle and the former resulting in the formation of acetyl-CoA, which contributes to improving citric acid yield (Grewal and Karlra, 1995). Other reports have attributed the stimulatory effect of plant oils to the fact that oils like castor oil and olive oil serve as alternate hydrogen acceptors instead of oxygen during fermentation (Ethiraj, 1996). Maximum citric acid concentration was obtained at castor oil and olive oil concentration of 3.0% w/w and 3.0% w/w respectively and this represents an enhancement of about 23.7% compared to the case when no oils were added to the medium.

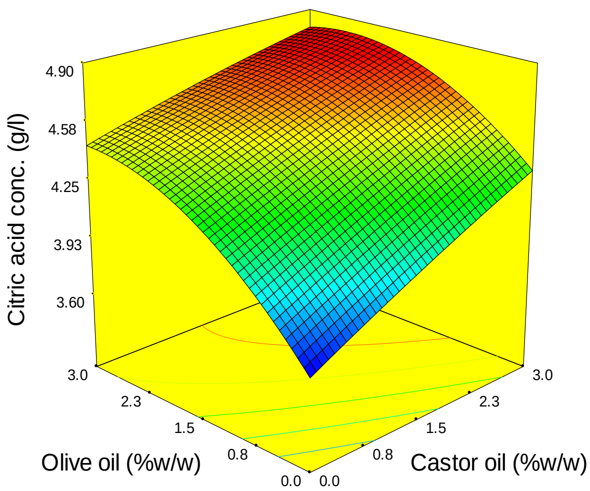

Fig. 4: Response surface plot showing the effect of olive oil and castor oil on citric acid production

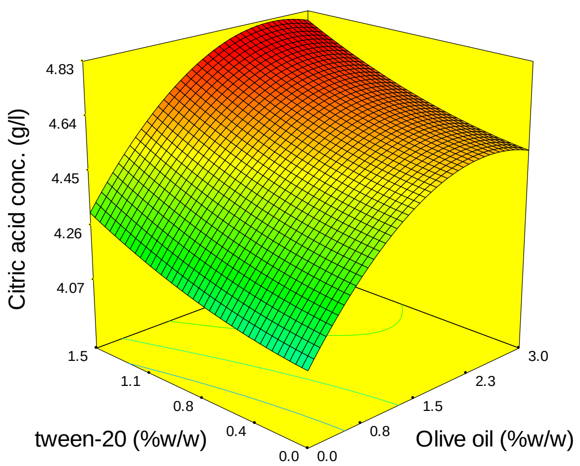

Fig. 5: Response surface plot showing the effect of tween 20 and olive oil on citric acid production

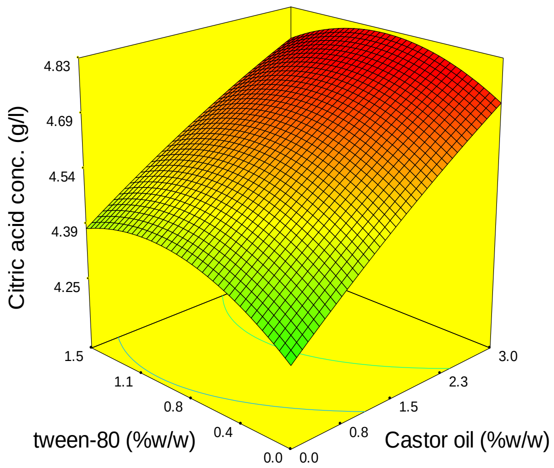

Fig. 6: Response surface plot showing the effect of tween 80 and castor oil on citric acid production

The introduction of the surfactants (tween 20 and tween 80) also enhanced citric acid production as seen in the increase in citric acid concentration when the levels of the surfactants were increased (Figs. 5 and 6). Citric acid production was favoured at tween 20 and tween 80 concentrations of 1.5% w/w and 0.8% w/w, respectively. The use of surfactants improved citric acid production by as much as 9.8% compared to the case when no surfactants were used. Similar positive influences of surfactants have been reported by previous researchers (Goes and Sheppard, 1999; Pardo, 1996).

Conclusion

The effects of plant oils (castor oil and olive oil) and surfactants (tween 20 and tween 80) on citric acid production was investigated. Introduction of the oils and surfactants in the fermentation medium was beneficial to citric acid production. Both oils and surfactants enhanced citric acid production by 23.7 and 9.8%, respectively. A quadratic model developed with RSM predicted optimum citric acid concentration as well as the optimum concentration of the stimulants. A multilayer full feedforward ANN with quick propagation algorithm and hyperbolic tangent transfer function showed better predictive qualities compared to RSM because of its higher R^2 value and RMSE value.

Conflict of Interest

Authors have declared that there is no conflict of interest reported in this work.

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