Optimization of Fermentation Parameters for "Pupuru" Flour Production

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May 5, 2020

Abstract

The fermentation conditions for the production of "pupuru" are enhanced at optimized conditions using response surface methodology (RSM). A Central Composite Design (CCD) with three independent variables; mass of root (2 to 6 kg), fermentation temperature (28 to 32 oC) and fermentation time (72 to 120 h) are used to study the response variables pH and Total Titratable Acidity (TTA) of fermented cassava. The design gives nineteen runs, 5 centre points, 14 non centre points. A face centred central composite second - order design is used to evaluate the combined effect of the independent variables. The results indicate that the generated regression model represents the relationship between the independent variables and responses. All linear terms, two quadratic terms (fermentation time and fermentation temperature) and all interactive terms had insignificant ($p < 0.05$) effect on pH and TTA. The optimum conditions for fermentation are achieved at 28 oC for 105 h to obtain pH of 3.99 and TTA of 0.25% Lactic acid which shows no significant difference ($p>0.05$) from the response surface methodology predicted pH and TTA of 3.96 and 0.22% Lactic acid.

1. INTRODUCTION

Cassava (*Manihot esculenta)* Crantz is one of the most important root crops in most tropical countries and it is estimated that foods processed from cassava roots are staple for over 500 million people in the tropics [1]. By this fact cassava plays a significant role in alleviating food crises in Africa. Its cultivation and processing provide household with food security, income and employment opportunities for this great number of people in Africa, Asia and the Americas. A wide variety of food products are prepared from cassava, one of such is 'pupuru'. Based on the processing method of 'pupuru' it is different from other cassava fermented product like garri, 'lafun', 'fufu' and 'akpu' [2]. 'Pupuru' is a traditionally fermented, smoked-dried cassava food commonly consumed in some parts of South-Western and Middle belt areas of Nigeria where it is commonly referred to as "Ikwurikwu". It originated from the Ilaje people of the riverine area of Ondo State [3]. The smoking of the fermented cassava mash makes "pupuru" processing unique. The fermented cassava mashed is moulded into balls and dried using smoked heat which is believed to impact some characteristics flavour and aroma to this product [3,4].

Fermentation is an important aspect in the production of cassava product. Study has shown that application of fermentation had reduced anti-nutrients and increased the safety of those products. The activity of lactic acid bacteria on the carbohydrates of the cassava root has been attributed to the acid production during cassava fermentation [5]. As a result of the several microbes and enzymes such as polygalacturonase, pectinase and cellulase with tissue degrading activities associative interaction of tissue softening is produced [5]. The long fermentation time resulted in flavour addition.

Cassava roots contain high level of cyanide depending on the variety, which on consumption is capable of inducing acute poisoning and chronic dietary consumption problems associated with the central nervous system and cardiovascular system. However proper fermentation process is capable of reducing the cyanide content because it ensures efficient breaking away of the cyanogenic glucoside thus releasing cyanogens component and consequently making it safe for consumption. Therefore, there is need for proper fermentation process in order to minimize toxicity of the product. There is dearth information on optimization of fermentation conditions for pupuru.

2. MATERIALS AND METHODS

Materials

Cassava (*Manihot esculenta)* crantz tubers (TMS 07/0593) of 10 months old were obtained from International Institute of Tropical Agriculture (IITA) Ibadan, Oyo State Nigeria.

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The effect of three independent variables A (mass of roots), B (fermentation time) and C (fermentation temperature) on pH (Y_1) and TTA (Y_2) was investigated. A center composite design (CCD) used to study the main and combine effects of fermentation process variables on the pH and TTA in order to create model between the variables; and use variable to optimize cassava fermentation condition for production of pupuru. Nineteen runs based on CCD and five center points with three independent variables were used (Table 1). The independent variables range studied were mass of root (2 to 6 kg), fermentation time (72 to 120 h) and fermentation temperature (28-32 °C). The choice of levels was suggested by [6], based on the fermentation conditions as specified in RSM design (Table 1).

Table 1: Experimental Response Surface Design for Fermentation condition of "Pupuru"

Run	A(Kg)	B(h)	C(°C)	pH	TTA(%)
1	4	96	30	4.00	0.21
2	4	120	30	3.83	0.11
3	6	72	28	4.60	0.19
4	6	96	30	4.00	0.21
5	2	120	28	4.10	0.22
6	6	120	28	3.98	0.11
7	2	120	32	3.89	0.11
8	2	72	28	4.60	0.19
9	6	120	32	3.80	0.11
10	2	96	30	3.90	0.14
11	4	96	28	4.00	0.21
12	4	96	30	4.00	0.21
13	4	72	30	4.60	0.19
14	4	96	32	4.10	0.22
15	4	96	30	4.00	0.21
16	4	96	30	4.00	0.21
17	2	72	32	4.50	0.09
18	6	72	32	4.50	0.09
19	4	96	30	4.00	0.21

A = Mass of root, B= Fermentation time,C= Fermentation temperature

Preparation of Sample

Freshly harvested cassavas tubers were manually peeled washed (based on the quantity specified in RSM design) and steeped in water base. At every 24 hr the water was decanted and replaced with fresh water in order to reduce the odour. At completion of fermentation period for each of the runs as presented in (Table 1) the water was decanted, the soft wet mash was packed into Hessian sack and the water was allowed to drain off using FUTA screw jack to produce a wet '*pupuru'* cake which was pulverized and sifted on a raffia of pore mesh size of 30 mm to remove the shaft and fibre. Another sample was prepared using traditional method of preparing "pupuru" which was used as control, according to the method of [7].

Sample Analyses

The prepared samples were analysed for pH and Total Titratable Acidity (TTA)

pH

The pH was measured by making a 1% (w/v) suspension of the sample in distilled water. The suspension was mixed thoroughly and the pH was measured with a Combo pH meter (Model HI 98129, Hanna Instrument, Italia).

Total Titratable Acidity (TTA)

Total Titratable Acidity was determined using the method of Pearson as reported by [7]. One gram of the each sample was put inside 100 ml conical flask and 10 ml distilled was added; 1ml of phenolphthalein indicator was then added to the mixture. It was titrated with 0.1 M NaOH until pink colour appears the acidity was then calculated as lactic acid (%).

$\text{TTA} = \text{Titre value} \times 0.1M \text{ NaOH} \times \text{acidic factor}$

(Acidic factor $= 0.009008$)

Statistical Analysis

The pH and TTA (response variables) obtained during fermentation were subjected to regression analysis and analysis of variance (ANOVA) to determining regression coefficients and statistical significances of model terms and to fit the mathematical models to the experimental data, aiming at an overall optimal region for the response variable, multiple regression coefficients were determined by employing the least-squares technique to predict linear and quadratic polynomial model for the response variable studied. The behavior of the response surface was investigated for the response function (Y, the predicted response) using the regression polynomial equations. The generalize polynomial model proposed for the predicting the response variable is given as

$$Y=\beta_o+\beta_1x_1+\beta_2x_2+ \beta_3x_3 + \beta_{12}x_1\ x_2+ \beta_{13}x_1\ x_3+ \beta_{23}x_2\ x_3+ \beta_{11}x^2{}_1 + \beta_{22}x^2{}_2 + \beta_{33}x^2{}_3$$

Where β_o is the intercept, β_1,β_2 and β_3 as coefficient. The significance of the equation parameters for each response variable was also assessed by F ratio at a probability (P) of 0.05. The adequacy of the models was determined using model analysis, lack of fit test and coefficient of determination (R^2) analysis as describe by [8, 9], for a good fit of a model R^2 should be at least 0.80 [10, 11]. The experimental design matrix, data and analysis, and optimization procedure were performed using the Design-Expert version 7 (state-Ease, Inc, Minneapolis, MN, USA).

3. RESULTS AND DISCUSSION

Fitting of the models

The pH and TTA of cassava mash obtained from the 19 runs during various fermentation conditions is presented in Table 2. The pH of the cassava mash ranged between 3.8 to 4.60 to a range of 3.80 (run 9) to 4.60 (run 3, 8, 13). The fresh cassava with pH 6.5 has been shown to have reduced; this indicated that fermentation reduced the pH of the cassava mash. This agrees with the report of [8] for *gari* production which reduced from 6.7 to 4.0; Oyewole and Ogundele [11] for *fufu*which reduced from 5.8 – 4.1; Oyewole and Ogundele [11] had reported that fermentation process was characterized by increased acid production.

While the total titratable acidity (TTA) ranged from 0.09 (run 17, 18) to 0.22% (run 5) lactic acid. The TTA obtained were higher than those reported by Osundahunsi [2] for "pupuru" (0.09 – 0.12) and Awolu *et al* [12] for *Abacha* (0.047 to 0.073%) but within the range reported by [11] for *fufu* (0.08 – 0.22) % lactic acid. The activity of lactic acid bacteria on the carbohydrates of the cassava root has been attributed to the acid production during cassava fermentation [5]. This may be as a result of the several microbes and enzymes such as polygalacturonase, pectinase and cellulase with tissue degrading activities associative interaction of tissue softening produced [5]. The long fermentation time resulted in flavour addition to the product by the increase in total titratable Acidity (TTA) and decrease in pH level.

Table 2: Experimental values of responses for Fermentation condition of "Pupuru"

Run	A(Kg)	B(h)	C(°C)	pH	TTA(%)
1	4	96	30	4.00	0.21
2	4	120	30	3.83	0.11
3	6	72	28	4.60	0.19
4	6	96	30	4.00	0.21
5	2	120	28	4.10	0.22
6	6	120	28	3.98	0.11
7	2	120	32	3.89	0.11
8	2	72	28	4.60	0.19
9	6	120	32	3.80	0.11
10	2	96	30	3.90	0.14
11	4	96	28	4.00	0.21
12	4	96	30	4.00	0.21
13	4	72	30	4.60	0.19
14	4	96	32	4.10	0.22
15	4	96	30	4.00	0.21
16	4	96	30	4.00	0.21
17	2	72	32	4.50	0.09
18	6	72	32	4.50	0.09
19	4	96	30	4.00	0.21

A = Mass of root; B = Fermentation time**;** C= Fermentation temperature

The experimental equation deduced for fermentation of cassava root for '*pupuru* ' production is given by:

$$\text{pH} = 4 - 0.020A - 0.35B - 0.04C - 0.037\text{AB} + 0.038\text{AC} - 0.012\text{BC} - 0.045A^2 + 0.2B^2 + 0.055C$$

$$\text{TTA} = 2.19 + 0.039A + 0.064B + 0.29C - 0.11\text{AC} - 0.15\text{BC} + 0.22A^2 + 0.45B^2 - 0.051C$$

The analysis of variance (ANOVA) of the regression parameters for the response surface model is presented in Table 3. The ANOVA obtained showed that the regression model obtained was significant ($P < 0.05$) for the response variable with $R^2 = 0.9674$ and insignificant lack of fit ($P = 0.05$). The goodness of fit of the mathematic model was validated by the determination coefficient (R^2) and adjusted R^2 is 0.9674 and 0.9349 respectively. In this case, the value of R^2(96.7%) indicated that the sample variation of 96.7% for pH was attributed to the independent variables and that 3.3% of the total variation could not be explained by the model. The $R^2$and the adjusted R^2 for pH are highly adequate because they have satisfactory levels of R^2 of more than 80% with no significant Lack of Fit [11, 13]. The model shows that TTA had a coefficient of determination (R^2) of 0.7846 and the adjusted R^2 of 0.5692. which means that 78.46% of the variability in the response could be explain by the model, This might be due to experimental errors, handling errors etc.

Table 3: Analysis of variance and regression coefficients of independent variables for fermentation condition: Response pH

Term Regression coefficient F-value P value

B_0 4.00 29.72 0.011

A - 0.20 0.72 0.4184

B - 0.35 220.22 0.0001

C - 0.040 2.88 0.1241

Quadratic effects

A^2 -0.045 1.01 0.3110

B^2 + 0.2 20.57 0.0014

C^2 + 0.055 1.47 0.2567

Interaction effect

AB - 0.037 2.02 0.1887

AC + 0.038 2.02 0.1887

BC - 0.012 0.22 0.6468

Lack of fit 0.05

A = Mass of root; B = Fermentation time**;** C= Fermentation temperature

Effect of Independent Variables on the Responses

The significance of each term was determined using the F ratio and P value as shown in Table 3. The significant and magnitude of the coefficients indicated the effect of the variables on the response and the negative coefficient means a decrease in response when the level of variable increased while a positive coefficient signifies an increase in the response [14]. Furthermore, a significant interaction indicates that the level of one interactive variable may increase while the other may decrease for a constant value of the response [15]. Tables 3 and 4 showed that all linear terms, two quadratic terms (fermentation time and temperature and all interactive terms had significant ($P < 0.05$) effect on the pH and TTA. In Figure 1, the 3D response surface plot of pH and fermentation time displayed a linear effect on the response where pH decreases with increase in fermentation time. This was evident in the regression equation where fermentation time had a negative coefficient which indicated a decrease in the response. The 3D response surface plots of TTA during fermentation are shown in Figure 2. The TTA increased to an optimum level when the fermentation time and mass of root individually increased to 105 h and 3kg respectively, followed by a decline with further increase in each of these variables (Figure 1). The decrease in TTA as fermentation increased above 30°C could be due to the fact that the optimum fermentation time have exceeded for maximum TTA. It could be that microorganism was not active enough to produce more acid. This is in accordance within the range reported by Oyewole and Ogundele [11] for fufu (0.08 – 0.22)% lactic acid.

Table 4: Analysis of variance and regression coefficients of independent variables for fermentation condition: Response TTA

Term Regression coefficient F-value P value

B_0 2.19 3.64 0.0338

A 0.039 0.18 0.6814

B 0.064 0.48 0.5046

C 0.29 9.89 0.0118

Quadratic effects

A^2 0.22 1.14 0.2475

B^2 0.45 1.14 0.0328

C^2 - 0.051 2.08 0.7785

Interaction effect

AB - 0.11 1.53 0.3139

AC + 0.11 6.35 0.3139

BC - 0.15 0.084 0.1827

Lack of fit 0.05

A = Mass of root; B = Fermentation time; C= Fermentation temperature

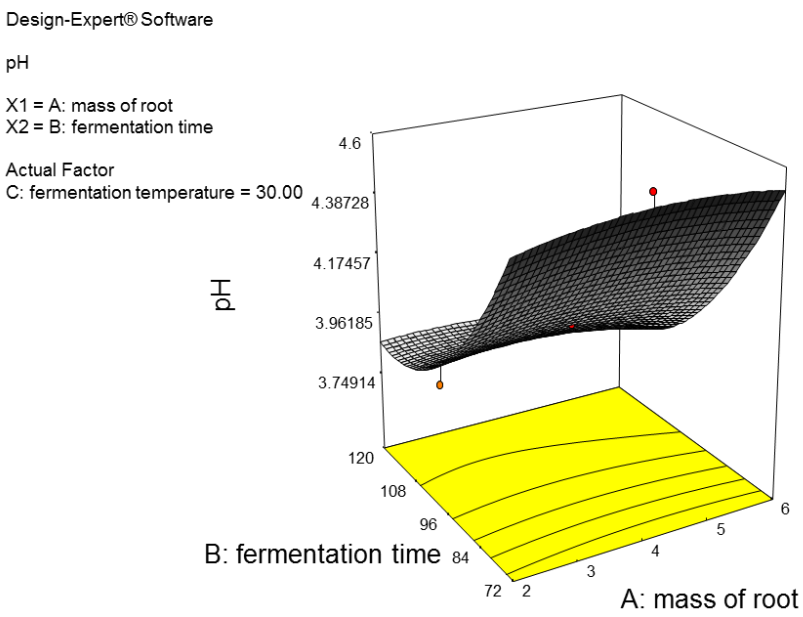

Figure 1: Response surface graphs showing the combine effect of fermentation time and mass of root on the pH

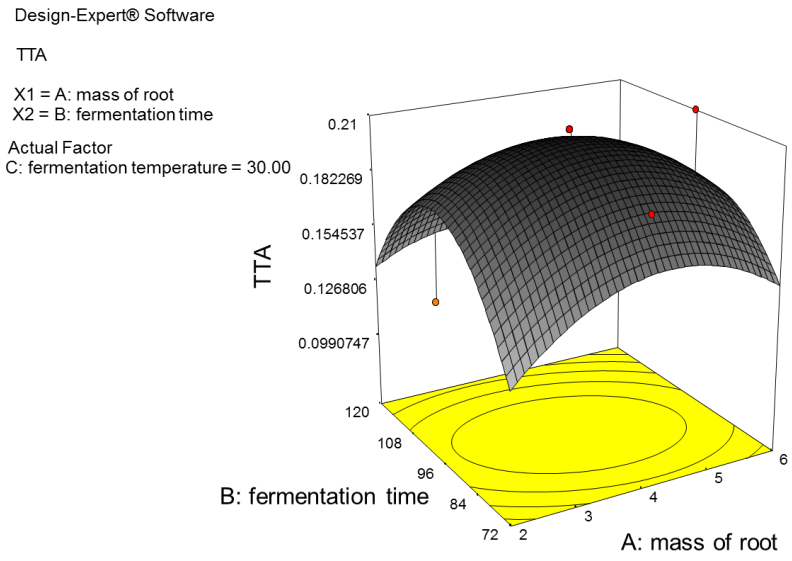

Figure 2. Response surface plots showing the combined effects of fermentation

time and mass of root on the TTA.

Optimum conditions and verification of model

The optimum conditions for fermentation condition were achieved at the optimum mass of 4 kg at 28 °C for 105 h. The pH and TTA were 3.99 and 0.25% respectively at optimum conditions which shows no significant ($p > 0.05$) difference from the RSM predicted value (3.96 and 0.22). On the other hand, the pH and TTA of the traditional prepared "pupuru" had 4.5 and 0.35

In addition, the closeness between experimental and predicted values confirmed the adequacy of the corresponding response surface model used for describing the pH and TTA as a function of fermentations. The pH of the fresh cassava 6.5 has been shown to have reduced; this indicated that fermentation reduced the pH of the cassava mash. This agrees with the report of Lee et al [10] for gari production which reduces from 6.7 to 4.0; Oyewole and Ogundele [11] for *fufu* which reduces from 5.8 to 4.1.

4. CONCLUSION

The use of response surface methodology was successful in optimizing cassava fermentation for the production of "pupuru" flour. The optimum fermentation conditions resulted in pH and TTA which was not significantly different from the RSM predicted ones.This implies that resulted "pupuru" flour will have low cyanide content when compared with that of traditionally prepared "pupuru" .

Author Contributions: The laboratory work was carried out by J. O. Alaba, supervision of the laboratory work and write was done by J. A. V. Olumurewa and review of the article was done by M. O. Oluwamukomi.

Conflicts of Interest: "The authors declare no conflict of interest."

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