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Full Length Research Paper

Predicting α-amylase yield and malt quality of some sprouting cereals using 2nd order polynomial model

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Alpha amylase yield in sprouting Maize, Acha, Rice and Sorghum were studied for 180 h. The result was analyzed using 2nd order polynomial model. The result showed that the rate of α- amylase secretion with growth period is significantly high ($p < 0.05$) and the R^2 for each ranged from 67 - 90%, while the R^2 for sprouting vigour ranged within 99% for all the cereals studied. The prediction for amylase activity from sprouting vigour was significant ($p < 0.05$) for all the cereals studied, the R^2 for all the cereals ranged between 63 - 91%. The results conclude that α-amylase and malt quality can be predicted in sprouting cereals from the growth vigour.

Key words: Cereals, amylase, growth vigour, model.

INTRODUCTION

Alpha amylase is synthesized during cereal development and stored in matured endosperms (Evans, et al, 2003). Alpha-amylase, as other amylases increase markedly during germination. It has been shown that alpha amylase yield will peak within 3 - 4 days of cereal germination (Egwim and Oloyede, 2006).

George-Kraemer et al. (2001) have shown that amylase activity is a good predictor of Diastatic Power (DP) which is required in brewing processes and an important characteristic for estimating the quality of malt for beer production (Evans, et al, 1995).

Malting forms a critical stage in the production of cereal-based-beverages in which amylase and proteases inherently embedded in the cereal grain are activated for the purpose of hydrolysis of starch and protein into sugars and amino acids respectively (Okafor, 1987). The determination of alpha-amylase yield in sprouting cereals is a rigorous one involving several stages of chemical reactions and calculations. A quick method of predicting alpha-amylase yield from sprouting cereals is investigated using 2nd order polynomial model.

The goal of prediction is to determine either the value of a new observation of the response variable, or the values of a specified proportion of all future observations of the response variable (www.iti.nist.gov/div898/handbook).

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In this paper, this goal will be achieved using polynomial model. Polynomials are particularly important in the experimental sciences since they often give a simple theoretical description of experimental results (Eason et al., 1989).

MATERIALS AND METHODS

Cereals (Maize, Acha, Rice and Sorghum) used were obtained from National Cereal Research Institute (NCRI) Badeggi, Niger State, Nigeria.

The cereals were sprouted for 180 h. Cereal ascrospire was measured with a meter rule while alpha amylase activity was assayed as reported earlier (Egwim and Oloyede, 2006). Briefly, an aliquot (0.1ml) of crude enzyme was pipetted into clean test tube, and 0.9ml of 2% starch solution was added and incubated in a shaking water bath at 50℃ for 30 min. The reaction was stopped by adding 3 ml of DNSA reagent and boiled for 3 min for colour development. Absorbance was read at 550 nM against reagent blank. Enzyme activity was thereafter computed from a standard glucose curve (0.1 – 1 mM glucose mL^{-1}). The data mean was analyzed using the 2nd order polynomial regression model.

MODEL SPECIFICATION

The polynomial response model $y = \beta_o + \beta_1 x + \beta_2 x^2 + \beta_3 x^3 + \ldots$ is another example of linear model despite the fact that y is described by non-linear function of the explanatory variable x (Everitt and Dunn, 1991). A polynomial model may be a 0, 1, 2, 3, . . . etc order, in this present study; a 2nd order polynomial model was adopted given by

$$y = \beta_o + \beta_1 x + \beta_2 x^2 \text{ , where } \beta_2 \neq 0.$$

The method of least squares is used to estimate the model coefficients. The deviations around the regression line (ε- error term) are assumed to be normally and independently distributed with mean of 0 and a standard deviation sigma which does not depend on x (www.iti.nist.gov/div 898/handbook).

The estimate of the model coefficient is obtained from the normal equations:

$$\sum Y = n\hat{\beta}_o + \hat{\beta}_1 \sum X + \hat{\beta}_2 \sum X^2 \qquad \text{. . . (i)}$$

$$\sum XY = \hat{\beta}_o \sum X + \hat{\beta}_1 \sum X^2 + \hat{\beta}_2 \sum X^3 \qquad \text{. . . (ii)}$$

$$\sum X^2 Y = \hat{\beta}_0 \sum X^2 + \hat{\beta}_1 \sum X^3 + \hat{\beta}_2 \sum X^4 \qquad \text{. . . (iii)}$$

A matrix was then formed

$$\begin{bmatrix} n & \sum X & \sum X^2 \\ \sum X & \sum X^2 & \sum X^3 \\ \sum X^2 & \sum X^3 & \sum X^4 \end{bmatrix} \begin{bmatrix} \hat{\beta}_o \\ \hat{\beta}_1 \\ \hat{\beta}_2 \end{bmatrix} = \begin{bmatrix} \sum X \\ \sum XY \\ \sum X^2 Y \end{bmatrix}$$

$$let\ A = \begin{bmatrix} n & \sum X & \sum X^2 \\ \sum X & \sum X^2 & \sum X^3 \\ \sum X^2 & \sum X^3 & \sum X^4 \end{bmatrix}$$

The determinant method can be used to estimate the model parameters

$$\hat{\beta}_0 = \frac{\begin{vmatrix} \sum X & \sum X & \sum X^2 \\ \sum XY & \sum X^2 & \sum X^3 \\ \sum X^2 Y & \sum X^3 & \sum X^4 \end{vmatrix}}{|A|}, \quad \hat{\beta}_1 = \frac{\begin{vmatrix} n & \sum X & \sum X^2 \\ \sum X & \sum XY & \sum X^3 \\ \sum X^2 & \sum X^2 Y & \sum X^4 \end{vmatrix}}{|A|}$$

$$\hat{\beta}_2 = \frac{\begin{vmatrix} n & \sum X & \sum X \\ \sum X & \sum X^2 & \sum XY \\ \sum X^2 & \sum X^3 & \sum X^2 Y \end{vmatrix}}{|A|}$$

The correlation coefficient can be obtained using the formular below

$$r = \frac{n\sum XY - \sum X \sum Y}{\sqrt{\left(n\sum X^2 - (\sum X)^2\right)\left(n\sum Y^2 - (\sum Y)^2\right)}}$$

While the coefficient of determination is given as $R^2 = (r)^2 \times 100\%$ and the

Adjusted $\bar{R}^2 = \left[1 - \left[(1 - R^2)\left(\frac{n-1}{n-k}\right)\right]\right] \text{x } 100\%$

The t statistic can be used to test whether or not the coefficient is significantly different from 0. The t-statistic is given as:

$$t_c = \frac{\hat{\beta}_i - \beta_i}{S(\hat{\beta}_i)}$$

The hypothesis of interest

H_o: Coefficient equal 0 verses H_1: Coefficient not equal 0.

We reject H_o if $t_c > t^{n-k}_{1-\alpha/2}$.

Lastly, the F-test is used to test how suitable is the model obtained. It is given as

$$F_c = \frac{R^2/(k-1)}{(1-R^2)/(T-k)}, \quad F_c > F_{0.05,(k-1)(T-k)}$$ where T is the number of observation, k is the number of parameter estimated. The model obtained is suitable for forecasting if $F_c > F_{0.05,(k-1)(T-k)}$ (Omotosho, 2000). For this study SPSS software, version 10.0 was employed for polynomial regression analysis.

RESULTS AND DISCUSSION

The summary of cereal acrospire vigour in relation with time is shown in Table 1. The result showed that time was significant to the acrospire vigour; this implies that the growth of acrospire increases significantly with time. The relationship between vigour and time is linear as shown in Figure 1. The degree of relationship between acrospire vigour and time is very strong (correlation (r) is within 0.998). The R^2 for the relationship is within 99% for all the cereals studies, which further reveals that the models can be used to predict future value of acrospire vigour for any known time.

The summary of alpha amylase yield in relation with time is shown in Table 2. The result shows that time was significant to alpha amylase yield for all cereals studied, that is, as time increases, alpha-amylase yield also increase. It further shows that amylase yield can be predicted using 2nd order polynomial model since all (t * * 2) were significant in the models for all the cereals studied, this finding is further expressed in Figure 2. The

Table 1. Summary of Results on the Acrospire Vigour with Respect to Time (Polynomial Regression Model, n = 10).

Cereal Acrospire (mm)	Rate of Increase t	Rate of Change t**2	R^2	R^2(Adjusted)	r
Maize	1.062882	-0.00175	0.99701	0.99651	0.99850
Acha	0.423099	0.000495	0.99241	0.99114	0.99620
Rice	0.575556	0.000101	0.99591	0.99523	0.99795
Sorghum	1.253353	0.000140	0.98584	0.98348	0.99290

Table 2. Summary of Results on the Amylase Activity in sprouting Cereal with Respect to Time (Polynomial Regres-sion Model, n = 10).

Source of Amylase	Rate of Increase t	Rate of Change t**2	R^2	R^2 (Adjusted)	r
Maize-amy	0.002924	-1.54667×10^{-5}	0.87716	0.85668	0.93657
Acha-amy	0.004150	-2.1634×10^{-5}	0.90099	0.88449	0.94921
Rice-amy	0.001142	-7.5078×10^{-6}	0.67693	0.62309	0.82276
Sorghum-amy	0.002374	-1.386×10^{-5}	0.78354	0.74746	0.88518

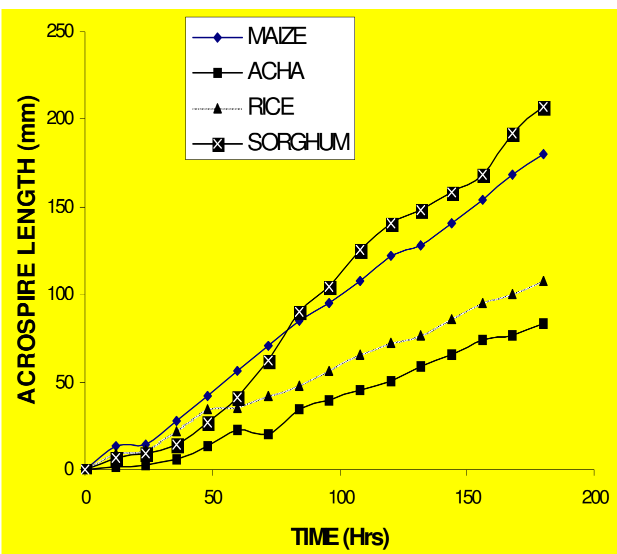

Figure 1. Acrospire vigour with time.
Model: Maize=-6.19086* + 1.0629t** - (0.00175t**2)*.
Acha= -6.802154** + 0.423099t** + (0.00495t**2)*.
Rice= 1.068879* + 0.57556t** + (0.000101t**2)*.
Sorghum= -22.37833** + 1.253353t** + (0.000140t**2)*.
Remark: ** - Significant, * - Not Significant at 5% level of Significance.

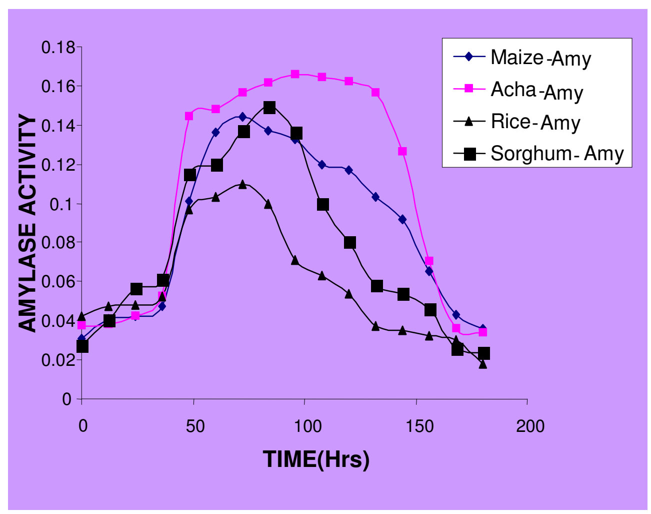

Figure 2. Amylase activity in sprouting cereals with time.
Model
Maize-amy = -0.00622* + 0.002924t** - (1.54667×10^{-5}t**2)**.
Acha-amy = -0.03065** + 0.004150t** - (2.1634×10^{-5}t**2)**.
Rice-amy = 0.0395* + 0.001142t** - (7.5078×10^{-6}t**2)**
Sorghum-amy = 0.017316* + 0.002374t** - (1.38608×10^{-5}t**2)**.
Remark: ** - Significant, * - Not Significant at 5% level of Significance.

result also reveals that the degree of relationship between amylase yield and time is very strong (correlation (r) ranges from 0.823 - 0.937). The R^2 for each cereal ranges from 67 - 90%, which further reveals that the models can be used to predict future value of amylase yield for any known time. The implication of this finding is quite interesting because it give predictive information of the yield of alpha amylase with time in sprouting cereal.

The summary of alpha amylase yield in relation with acrospire vigour is shown in Table 3. The result shows that amylase yield is significant with acrospire vigour for all the cereals studied, that is, as acrospire vigour increases, amylase yield also increase. The result further reveals that the degree of relationship between amylase

Table 3. Summary Results on the Amylase Activity in Sprouting With Respect to Acrospire Vigour of the Cereals (Polynomial Regression Model, n = 10).

Source of amylase	Rate of increase t	Rate of change t**2	R^2	R^2 (Adjusted)	r
Maize-amy	0.002751	-1.495×10^{-5}	0.90717	0.89170	0.95246
Acha-amy	0.007155	-8.8226×10^{-5}	0.92649	0.91424	0.96254
Rice-amy	0.001943	-2.1167×10^{-5}	0.68399	0.63132	0.82704
Sorghum-amy	0.001594	-9.1850×10^{-6}	0.75156	0.71016	0.86693

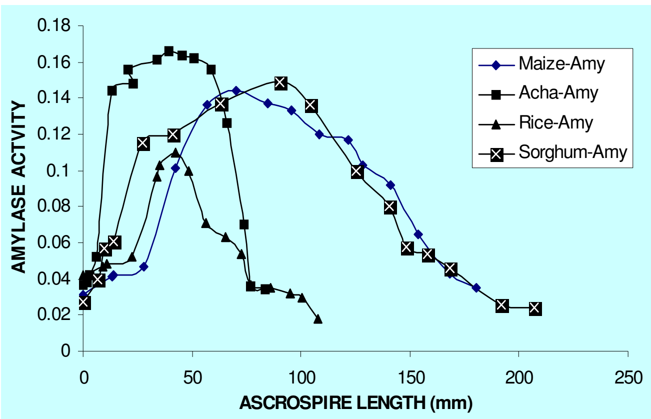

Figure 3. Amylase activity with acrospire length.

Model

Maize-amy = -0.006628* + 0.00275Maize** - (1.49508 x 10^{-5}Miaze**2)**

Acha-amy = 0.029563** + 0.007155Acha** - (8.82226 x 10^{-5}Acha**2)**

Rice-amy = 0.038269** + 0.001943Rice** - (2.11668 x 10^{-5}Rice**2)**

Sorghum-amy = 0.053624** + 0.001594Sorghum** - (9.1850 x 10^{-6}Sorghum**2)**

Remark: ** - Significant, * - Not Significant at 5% level of Significance

yield and acrospire vigour is very strong (correlation (r) ranges from 0.827 to 0.963). The predictions model for amylase yield from acrospire vigour was significant ($p < 0.05$) for all the cereals studied, and the result further reveals that 2nd polynomial model is very suitable for modeling amylase yield from acrospire vigour because the model relating alpha amylase yield with acrospire vigour was significant ($p < 0.05$) for both 1st and 2nd order polynomial models. The 2nd order polynomial model therefore would be a good predictive model. The R^2 for all the cereal studied ranges between 63 - 91% which reveal that the models obtained can be used to predict future value of amylase yield from any known acrospire vigour in the cereal studied (Figure 3).

The present finding suggests that the 2nd order polynomial model is suitable for predicting amylase yield in sprouting cereals. The implication of this finding is that the yield of alpha-amylase is a good indicator of malting quality. This finding agrees with the report of Jin-Xin et al. (2006) who have shown a strong relationship between amylase activity and malting quality.

The present work therefore conclude that it is possible to predict alpha-amylase yield as well as malting quality of sprouting cereals by measuring the acrospire vigour using a 2nd order polynomial model.

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Appendix:

Amylase activity in sprouting cereals (nM glucose min^{-1})				
Time(h)	**Maize-Amy**	**Acha-Amy**	**Rice-Amy**	**Sorghum-Amy**
0	0.031	0.037	0.042	0.027
12	0.041	0.038	0.047	0.04
24	0.042	0.042	0.048	0.057
36	0.047	0.052	0.052	0.061
48	0.101	0.144	0.097	0.115
60	0.136	0.148	0.103	0.12
72	0.144	0.156	0.11	0.137
84	0.137	0.161	0.1	0.149
96	0.133	0.166	0.071	0.136
108	0.12	0.164	0.063	0.1
120	0.117	0.162	0.054	0.08
132	0.103	0.156	0.037	0.058
144	0.092	0.126	0.035	0.054
156	0.065	0.07	0.032	0.046
168	0.043	0.036	0.03	0.026
180	0.035	0.034	0.018	0.024

Acrospire vigour				
Time(hrs)	**Maize**	**Acha**	**Rice**	**Sorghum**
0	0	0	0	0
12	13.1	2	9.3	6.8
24	14.1	2.9	10.9	9.6
36	28.1	6.1	22.2	14
48	42.4	13.2	34.1	27
60	56.7	22.9	35.3	41.1
72	70.5	20.6	42.3	62.7
84	84.7	34.1	48.4	90.1
96	95.5	39.2	56.3	104.1
108	108.1	45.3	65.4	125.2
120	121.9	50.8	72.8	140.2
132	128.1	58.8	76.9	148.1
144	140.8	65.8	86.1	158.2
156	153.9	73.7	94.9	168.4
168	168.09	77.02	100.39	191.64
180	180.45	83.24	107.53	207