

Original Research Article

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Characterization of Reducing Sugars of Red Beet (*Beta vulgaris L.*) during Cold Storage through Statistical Modeling

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ABSTRACT

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Formation of reducing sugars during low temperature storage of red beet is detrimental for processing, consumption as vegetable and easily deteriorated when exposed to high temperatures. Most of the analytical methods are time consuming, cost effective and laborious. So, assessing internal red beet quality through statistical methods is alternative as mathematical modeling does not take error in to consideration. These equations contain few parameters and the storability of the product could be predicted before keeping it for storage based on the dependent parameters. An attempt was made by employing Multiple Linear Regression and paired T-test to predict the formation of reducing sugars on dependent variables like moisture content and total soluble solids. Further, the results were confirmed by Shapiro-Wilk's and Run tests. Regression analysis explained that reducing sugars were significantly influenced by selected variables; about 89.5 percent ($R^2 = 0.895$). There was non-significant difference between actual (lab) and predicted (model) reducing sugars as per paired t-test.

Introduction

Beet root or red beet (*Beta vulgaris L.*) is a root vegetable belongs to family Chenopodiaceae. Its wide utilization as salad, cooked vegetable, juice, pickle and in canning encouraged Indian growers to extend the area of cultivation. It has many nutritive properties and the ability of coloured compounds (betalains) with antiradical properties which improves quality of human blood and revives from stress based disorders (Pedreno and Escribano, 2000; Kugler *et al.*, 2007) draws attention of consumers to use it in cuisine

(Kavitha *et al.*, 2014). It's harvesting period extends from January to first week of March. According to econometric analysis at different vegetable markets of West Bengal, the price of vegetables grown in winter was at peak in off season. With the advancement in postharvest technology during past two decades and easy storability of red beet, a deviation from traditional storage had enabled growers and traders to stock in cold storage for 6-8 months (Sealand, 1991). Various physiological processes such as transpiration

and respiration change its biochemical quality during long term storage of red beet. By reducing the storage temperatures to below 4 °C, there is a decrease in respiration rate substantially (Dexter *et al.*, 1969), but there is increase in reducing sugar with the loss of sucrose content (Wyse, 1978). Decrease in the beet quality is attributed to reduction of sucrose levels and increase in the reducing sugars (mainly invert sugars: glucose, fructose and raffinose) (Rosenkranz *et al.*, 2001; Kenter and Hoffman, 2009). Sucrose is the most important respiratory substrate and 70-80 % of it is lost during respiration. During long term cold storage of beet physiological weight loss is observed as a result of transpiration and respiration (Wyse, 1978). This is due to consumption of available moisture during substrate (sugars) break down. There is linear correlation between loss in moisture and increase in total soluble solids (TSS) of beet. If the produce is kept for long term storage, transpiration is high in the beet roots and water loss interferes with total soluble solids, decrease in sugar content and accumulation of reducing sugars (Jaggard *et al.*, 1998). Formation of reducing sugars during low temperature storage is detrimental for processing, consumption as vegetable and easily deteriorated when exposed to high temperatures. Intensive research programmes were carried in European countries like Germany and Sweden to establish various factors effecting quality of beet and sugar losses during storage (Vukov and Hangyal, 1985). Most of the analytical methods are time consuming, costly and laborious. So, assessing internal red beet quality through statistical methods is alternative as mathematical modeling does not take error in to consideration. These equations contain few parameters and the storability of the product could be predicted before keeping it for storage. Multiple Linear Regression (MLR) analysis is a tool for predicting the value of a dependent variable by

incorporating a number of independent variables simultaneously for an effective selection of dependent component characters. Relationships may be nonlinear, independent variables may be quantitative or qualitative, and one can examine the effects of a single variable or multiple variables with or without the influence of other variables taken into account (Cohen *et al.*, 2003). Therefore, an experiment was initiated to forecast the reducing sugars based on moisture content and total soluble solids.

Materials and Methods

Roots of red beet were obtained from local cold storage (Amiyo Prabha Das multipurpose cold storage), Hoogly district of West Bengal (Figure 1-7). Nearly 50 tonnes of red beet was stored every year in the cold storage. Red beet with elongated shapes transpires more rapidly than globe shaped ones (Gaweda, 2006). So, local cultivar with globe shaped and medium sized red beets which reached harvest maturity were selected for the experiment. They were washed; air dried in shade and packed in jute bags with polythene lining. They were subjected to low temperature of 3 ± 1 °C and relative humidity of 90-95%. Twenty samples were collected from the cold storage randomly from different lots at monthly intervals. They were analyzed for chemical parameters in the laboratory of department of postharvest technology during the year 2013 to 2014.

Biochemical analysis

Among chemical parameters; Total Soluble Solids (TSS) was analyzed by using an Abbe refractometer (Milton Roy Co., CA and USA), moisture content (MC) and reducing sugars (RS) were examined according to methodologies suggested by Ranganna (1986).

Statistical Analysis

Multiple Linear Regression (MLR)

MLR analysis is the tool available to dependent variable in a simpler and comprehensive manner (Tabachnick and Fidell, 2001). For the estimation of reducing sugars, MC (%), TSS (°Brix) and storage duration (days) are considered as explanatory variables in the present study. The multiple regression model employed here is the extension of simple regression equation such as given below,

$$Y = a + bx$$

Where,

Y = dependent variable;

a = intercept;

b = the slope of the regression line or the amount of change produced in Y by a unit change in x;

x = independent variable

The general form of multiple regression model in which more than one independent variables is included is given by,

$$Y = b_0 + b_1x_1 + b_2x_2 + \dots + b_kx_k + e$$

The dependent variable Y is now regarded as a function of k independent variables (x_1, x_2, \dots, x_k). The coefficients b_1 to b_k are referred to as the regression coefficients which in fact determine the contribution of the independent variables in the equation and b_0 is the y-intercept. The random error term (e) that is a random variable assumed to be normally and independently distributed between a mean of zero and a standard deviation of σ that is added to make the model probabilistic. To

determine the usefulness of the entire regression model as how well a linear model fits a set of data the multiple coefficient of determination (R^2) was used.

$R^2 = SSR/SST$ Where, SSR = regression sum of square or explained variability

SST = total sum of square or total variability

To know if the regression as a whole is significant or it was due to chance only, F-test was used.

$$F = \frac{MSR}{MSE} = \frac{R^2/k}{(1-R^2)/(n-k-1)}$$

Where,

MSR = mean square due to regression

MSE = mean square due to error

With F distribution having k numerator degrees of freedom (k = number of independent variables) and n-k-1 denominator degrees of freedom, F-test was used in the hypothesis test to determine whether there was a significant relationship among the dependent variable Y and all the independent variable involved in the equation (Stevens, 2002).

Paired t- test It is used to test for significant difference of means of two samples. When $n_1=n_2=n$ and the two samples are not independent but the sample observations are paired together, then this test is applicable (Kanji, 2006). Let $(x_i, y_i), i=1, \dots, n$ be a random sample from a bivariate normal population with parameters $(\mu_1, \mu_2, \sigma_1^2, \sigma_2^2, \rho)$. Let $d_i = x_i - y_i$

$$H_0 = \mu_1 - \mu_2 = \mu_0; \quad \text{Test statistic:}$$

$$t = \frac{\bar{d} - \mu_0}{s/\sqrt{n}} \sim t_{n-1}; \quad \text{Where } \bar{d} = \frac{1}{n} \sum_{i=1}^n d_i;$$

$$s^2 = \frac{1}{n-1} \sum_{i=1}^n (d_i - \bar{d})^2$$

Shapiro – Wilk test Normality of the residuals can be tested by Shapiro – Wilk test. In this, data arranged in non – decreasing sequence and is designated by $e_{(1)}, e_{(2)}, e_{(3)} \dots e_{(n)}$. The required test statistic W is defined as

$$W = S^2 / b ; \quad \text{where } b = \sum_{i=1}^n (e_i - \bar{e})^2 ;$$

$$S^2 = \sum a(k)[e(n+1-k) - e(k)].$$

The parameter k takes the values, $K=n/2$ (when data ‘n’ is even); $k=(n-1)/2$ when n is odd. The values of co-efficient “a (k)” for different values of n and k are given in Shapiro – Wilk table. The nonsignificant value is shown to be normality of the residuals (Shapiro–Wilk, 1965).

Run test Run test is used for examining whether a set of observations constitutes a random sample from an infinite population. Test for randomness is of major importance because the assumptions underlie statistical inference. In addition, tests for randomness are important for time series analysis (Sidney and Castellan, 1988).

H_0 : Sample values come from random sequence

Test statistic: let r be the number of runs. For finding number of runs, observations are listed in their order of sequence. Each observation denoted by a ‘+’ sign if it is more than the previous observation and by a ‘-’ sign if it is less than the previous observation. r is the sum of ‘+’ and ‘-’.

When sample size is more than 25, then critical value r_c can be obtained using a normal distribution approximation.

The critical values for two sided test at 5% level of significance are

$$r_c \text{ (lower)} = \mu - 1.96 \sigma; \quad r_c \text{ (upper)} = \mu + 1.96 \sigma;$$

$$\text{Where } \mu = \frac{2n-1}{3} \text{ and } \sigma = \sqrt{\frac{16n-29}{90}}$$

Decision is based on, $r_c \text{ (lower)} \leq r \leq r_c \text{ (upper)}$; then accept H_0 .

Results and Discussion

The details of the changes in the biochemical parameters and prediction of reducing sugars with statistical modeling for different storage periods were discussed in the following subheadings. Before applying the statistical model, the selected variables were subjected to descriptive statistics which gives basic idea on nature of variables. The variables were studied individually at monthly intervals to know the pattern of variation.

Biochemical parameters

TSS An increase in TSS was recorded during storage of beetroot for the first 60 days (Table 1). The increment was 3.4% with CV (%) at 60 days was 3.14. There was a decrease in TSS from 60 days to 90 days, though it was only 0.7% and from 90 to 120 days the increment was 0.9%. An overall raise (4.21%) from beginning of the storage to end of storage of red beet could be observed. Similar trend was observed by Gaweda (2006) was observed while working on red beetroot quality with different storage conditions. Vitti *et al.* (2005) reported same trend but the difference in TSS from initial to final storage conditions was only 1% during cold storage of minimally processed beetroot. The final increase in soluble solids might be due to decrease in moisture content and increase in

dry weight of the stored product (Zwart-Roodzant, 1989; Kaur, 2014; Gaweda, 2006).

Moisture content (MC) There was a decrease in the MC during the storage of red beet (Table 1) and the difference from initial to final storage period is (13.4%). Initial decrement of roots stored from 30 days to 60 days period was highest (3.8%). These findings were in line with the reports from Gaweda (2006) on cold storage of red beet and Hailu *et al.* (2012) cold storage of banana. Decrease in MC might be due to liberation of heat and gaseous exchange of the produce as a result of metabolic processes like transpiration and respiration (Paull, 1999 and Hailu, 2014).

Reducing sugars (RS) showed an increasing trend during 180 days of storage. Overall elevation of reducing was 4.05% (Table 1). The increment in RS was mainly due to breakdown of sucrose by acid invertase. Almost 70-80% losses in sucrose were observed by Wyse (1970) due to respiration. Findings of Kenter and Hoffmann (2009) strengthen the above findings while working on quality of beet during storage. They also stated that formation of raffinose during storage causes deterioration in quality.

Multiple Linear Regression (MLR) RS were regressed on MC, TSS and duration of storage and estimated parameters were given in table 2. Regression analyses showed that all the components contributing to reducing sugars were negatively affected except for storage duration. All the variables have significant effect on dependent variables such as MC and TSS. Dahal and Routray (2011) applied MLR to predict yield of paddy by taking soil variables like soil reaction, organic matter, total nitrogen, phosphorus, potassium and soil texture. Similar prediction studies were prepared by Attua (2008) and Hamjah (2014) and estimated carbon stock and crop production respectively.

F ratio was significant (Table 2) indicating the overall explanatory power of the equation and regression line explaining about 89.5 percent ($R^2 = 0.895$) variations in reducing sugars by independent variables taken together all of these coefficients were significant (t-value in parentheses) as shown in table 3.

From table 3, fitted regression was calculated as below

Equation 1

$$RS = 27.894 - 0.261 * \text{Moisture content} - 0.079 * \text{TSS} + 0.005 * \text{Days}$$

$$(R^2 = 0.895)$$

Shapiro Wilk and Run Test Normality and randomness of residual assumptions were tested by Shapiro Wilks and Run test respectively. From table 4, it was clear that residuals were distributed normally and independently as probability values of both tests were non-significant. Shapiro Wilk test was further verified by normal probability and histogram plot (Figure 8). So, it was confirmed that selected model was normally distributed and there were no extreme residuals.

Two samples were randomly selected for each storage interval for both TSS and MC from 140 samples for each parameter. Prediction of reducing sugar (RS) was made by the regression equation (stated earlier in equation 1). The predicted values were depicted in table 5. Actual and predicted values for RS were given in table 5 and could be concluded that the values were close.

Furthermore, a paired samples t-test approach was used to compare the RS values predicted by using RS-(MC, TSS and SD) model and RS value measured by laboratory test. The RS values of both predicted (from model) and determined (from lab) were depicted in table.

Table.1 Descriptive statistics of biochemical parameters during cold storage of red beet

DAYS	Parameters	Mean	Standard Deviation	CV%	Minimum	Maximum
0	RS (%)	4.74	0.41	8.67	4.1	5.7
	MC (%)	84.66	0.87	1.03	83.2	86
	TSS (° Brix)	14.26	0.55	3.89	13.2	15.2
30	RS (%)	5.14	0.41	7.99	4.5	6.1
	MC (%)	81.86	0.87	1.06	80.4	83.2
	TSS (° Brix)	15.86	0.55	3.5	14.8	16.8
60	RS (%)	6.24	0.41	6.59	5.6	7.2
	MC (%)	78.06	0.87	1.11	76.6	79.4
	TSS (° Brix)	17.66	0.55	3.14	16.6	18.6
90	RS (%)	7.94	0.41	5.18	7.3	8.9
	MC (%)	75.26	0.87	1.15	73.8	76.6
	TSS (° Brix)	16.96	0.55	3.27	15.9	17.9
120	RS (%)	7.64	0.41	5.38	7	8.6
	MC (%)	74.06	0.87	1.17	72.6	75.4
	TSS (° Brix)	17.86	0.55	3.11	16.8	18.8
150	RS (%)	8.23	0.38	4.6	7.6	8.9
	MC (%)	72.52	0.88	1.21	71.1	73.9
	TSS (° Brix)	17.55	0.55	3.11	16.5	18.5
180	RS (%)	8.79	0.42	4.78	8.1	9.7
	MC (%)	71.2	0.93	1.3	69.7	72.8
	TSS (° Brix)	18.47	0.55	2.96	17.4	19.4
Average	RS (%)	6.95	1.52	21.89	4.1	9.7
	MC (%)	76.8	4.7	6.12	69.7	86
	TSS (° Brix)	16.94	1.44	8.51	13.2	19.4

MC- moisture content TSS- Total soluble solids RS- Reducing Sugar

Table.2 ANOVA for multiple regression

	df	SS	MS	F-stat	P-value
Regression	3	288.479	96.159	387.499	< 0.00
Residual	136	33.749	0.248		
Total	139	322.223			

df- degree of freedom; SS-Sum of Square: MSS-Mean Sum of Squares

Table.3 Estimated parameters of linear regression model

Variables	Coefficients	Standard Error	t Stat	P-value
Intercept	27.894	3.302	8.446	< 0.00
MC	-0.261	0.035	-7.440	< 0.00
TSS	-0.079	0.005	-15.012	< 0.00
Days	0.005	0.003	2.034	0.044

MC- moisture content; TSS- Total soluble solids



Fig. 1 Cold storage (view from outside)



Fig. 2 Walk way in the cold storage



Fig. 3 Room in cold storage



Fig. 4 Packaging of beetroot in cool store



Fig. 5 Arrangement of packaged beet root



Fig. 6 Inner view of package



Fig. 7 Beet root

6 months after storage



Cross section

Fig.8 Probability plot and Histogram of residuals

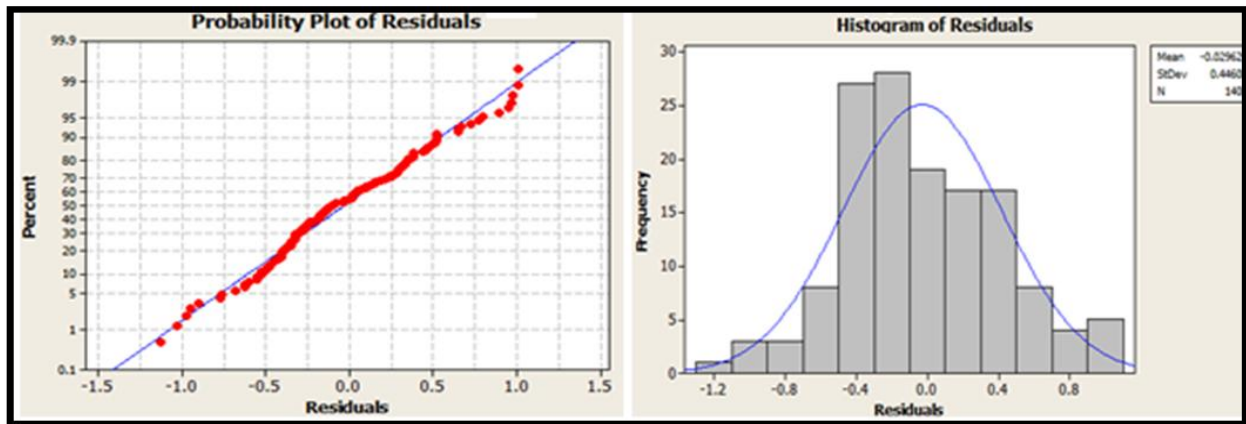


Table.4 Test for normality and randomness of residuals

Prob. Value of Residuals	Shapiro Wilks Test	Run Test
	0.132	0.077

Table.5 Actual and predicted values of reducing sugars for randomly selected samples

DAYS	MC	TSS	Actual RS	Predicted RS for Randomly Selected Samples
0	85.4	14.1	4.3	4.474
0	84.8	14.5	4.6	4.592
30	81.8	15.8	5.1	5.425
30	81.0	15.9	5.5	5.618
60	78.0	17.6	6.2	6.418
60	79.4	16.6	6.4	6.148
90	75.0	17.9	7.7	7.333
90	74.5	16.7	7.3	7.557
120	72.9	17.0	8.1	8.119
120	72.6	18.0	8.2	8.113
150	72.3	18.5	8.0	8.332
150	71.1	17.7	8.8	8.699
180	70.3	18.5	9.1	9.020
180	69.7	18.6	9.3	9.163

MC- moisture content TSS- Total soluble solids RS- Reducing Sugar

The paired samples t-test results demonstrate that the RS values predicted with the RS- (MC, TSS and SD) model were not significantly different than that measured with laboratory tests as the calculated value 0.972 was lesser than tabulated value 2.160.

In conclusion, biochemical analysis suggested that there were changes in selected parameters

i.e., total soluble solids, moisture content and reducing sugars during cold storage of red beet. Predicted model obtained from multiple linear regression suggested that the laboratory values and the predicted values for reducing sugars were non-significantly different. So far, statistical modeling was used to forecast yield, weather and soil fertility. In conclusion, such models could be applied for various

postharvest quality attributes to predict storage life and changes that occur in produce.

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