OPTIMIZATION OF ASCORBIC ACID CONTENT IN MANGO JUICE

BY

ADGIDZI, D. AND OLORUNSOGO, S.T.

DEPARTMENT OF AGRICULTURAL ENGINEERING
FEDERAL UNIVERSITY OF TECHNOLOGY
MINNA, NIGERIA

ABSTRACT

Fruit juice is assuming a more important role in Nigeria's diversified agricultural industry. However, short shelf life of fresh market fruit juices, an inherent characteristic that tends to impede the growth of the domestic juice industry, is believed to be influenced by many factors in post-processing handling, storage and distribution. In this paper, the effects of storage temperature, brix value, pH, quantity of antioxidant, and duration of storage on the ascorbic acid level under non-refrigeration storage and distribution of mango juice was investigated, the shelf life and quality values of the juice were estimated, and model based on the above mentioned deteriorative factors was developed. Data were drawn from a 2^5 full factorial experiment conducted in three replicates with the order of the replicate experiment randomized Multivariate regression analysis was used for relating the variables. The analysis of the experimental data led to the optimal condition: 40^0C storage temperature, 13^0-brix value, a pH, of 2.3,0.1 g/litre of antioxidant, and a maximum storage duration of 16 days. At this condition, the ascorbic acid level was maintained at 25.89 mg/100ml.

1. INTRODUCTION

In the course of storing and distribution of mango juice there is an inevitable decline in quality value. The loss occurs because of the sensitivity of the ascorbic acid content of the juice Heimann (1980). Ascorbic acid level is usually the criterion for judging fruit juice quality. It is the responsibility of mango juice manufacturers to ensure that quality losses in juices be minimal.

The juice manufacturer must seek to control the changes which reduce the quality value of their products. Thus, it is necessary to establish an analytical approach to the chemistry of fruit juice preservation so as to be able to specify the quality value of juices at different storage and distribution conditions.

The real problem of domestic mango juice industry is inadequate study on quality deterioration of juices and dearth of data on nutrient destruction from a quantitative, integrated standpoint. To predict extent of deterioration of nutrient value, knowledge of the loss of important nutritive quality as a function of the critical deterioration factors is needed Fennewa (1976). Through integration and/or modeling of the various deteriorative factors, the juice manufacturer will be able to specify the quality value of his product at the time of sale which is essential if nutrient claims are to be made on the label or advertising associated with products.

For the domestic fruit juice industry, five main factors have been identified as critical to the retention of ascorbic acid in fruit juices during storage and distribution. These are (a) the level of dissolved oxygen, (b) the storage temperature, (c) the total soluble solids (brix value), (d) the pH, and (e) the duration of storage. Inglet et al, (1979), Davies (1994). Monitoring these factors will bring about satisfactory control of ascorbic acid

degradation in mango juice during storage and distribution. In this paper, the effects of critical factors on the ascorbic acid level under non-refrigeration storage and distribution of mango juice was investigated, shelf life and quality value of the juice was estimated, and a model based on these deterioration factors was developed.

2.0 EXPERIMENTAL TECHNIQUES

2.1 EXPERIMENTAL MATERIALS:

Mango fruit samples were obtained from experimental plots of National horticultural Research Institute (NIHORT), Ibadan. These samples are representatives of the Nigeria fruit market with respect to the variety and cultural condition. All chemicals and reagents used for the chemical analysis of the samples are "Analar" produced by BDH chemicals Ltd, Poole England.

2.2 EXPERIMENTAL DESIGN METHODS:

A five variable two-level factorial design provide the framework for the mango juice variable experiment. The design matrix for the 2^5 full factorial, which indicates the run-by-run experimental design, is shown in Table 1. With five variables, two levels, a complete or orthogonalized design leads to a total of 32 experimental runs Montgomery (1991).

In the 2^5 full factorial experiment, the low and high levels of the factors are coded as "-" and "+" respectively. The levels of the five factors are listed in standard order in the columns X_b X_2....., X_5 in the design table. The sequence of + and – signs in the columns tells us how to combine the observations to get the main effects and their interactions. Columns X_0

Table 1 Design Matrix (table) for a 2^5 full factorial Experimental Design (FFD)

Run No	x_0 b_0	x_1 b_1	x_2 b_2	x_3 b_3	x_4 b_4	x_5 b_5	x_{12} b_{12}	x_{13} b_{13}	x_{14} b_{14}	x_{15} b_{15}	x_{23} b_{23}	x_{24} b_{24}	x_{25} b_{25}	x_{34} b_{34}	x_{35} b_{35}	x_{45} b_{45}	x_{123} b_{123}	x_{214} b_{124}	x_{125} b_{125}	x_{134} b_{134}	x_{135} b_{135}	x_{145} b_{145}	x_{234} b_{234}	x_{235} b_{235}	x_{245} b_{245}	x_{345} b_{345}	x_{1234} b_{1234}	x_{1235} b_{1235}	x_{1245} b_{1245}	x_{1345} b_{1345}	x_{2345} b_{2345}
1	+	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+
2	+	+	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	+	-
3	+	-	+	-	-	-	-	+	+	+	-	-	-	+	+	+	+	+	+	-	-	-	+	+	+	-	+	+	+	-	-
4	+	+	+	-	-	-	+	-	-	-	-	-	-	+	+	+	-	-	-	+	+	+	+	+	+	-	-	-	-	+	-
5	+	-	-	+	-	-	+	-	+	+	-	+	+	-	-	+	+	-	-	+	+	-	+	+	-	+	+	+	+	-	+
6	+	+	-	+	-	-	-	+	-	-	-	+	+	-	-	+	-	+	+	-	-	+	+	+	-	+	+	+	+	-	-
7	+	-	+	+	-	-	-	-	+	+	+	-	-	-	-	+	-	+	+	+	+	-	-	-	+	+	+	-	-	+	+
8	+	+	+	+	-	-	+	+	-	-	+	-	-	-	-	+	+	-	-	-	-	+	+	-	+	+	-	+	-	-	-
9	+	-	-	-	+	-	+	+	-	+	+	-	+	-	+	-	-	+	-	+	-	+	-	+	+	+	-	+	+	-	-
10	+	+	-	-	+	-	-	+	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	+	-	+	-	+	-
11	+	-	+	-	+	-	-	-	-	+	-	+	-	-	+	-	+	-	+	+	-	+	-	-	+	-	+	-	+	+	+
12	+	+	+	-	+	-	+	+	+	-	-	+	-	-	-	-	-	+	-	-	+	+	-	+	+	-	+	-	-	+	-
13	+	-	-	+	+	-	+	-	-	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	+	+	-	+
14	+	+	-	+	+	-	-	+	+	-	-	-	+	+	-	-	-	-	+	+	-	-	-	+	+	+	+	-	-	+	-
15	+	-	+	+	+	-	-	-	-	+	+	+	-	+	-	-	-	-	+	-	+	+	+	-	-	-	-	+	-	-	-
16	+	+	+	+	+	-	+	+	+	-	+	+	-	+	-	-	+	+	-	+	-	-	+	-	-	-	+	-	-	-	+
17	+	-	-	-	-	+	+	+	+	-	+	+	-	+	-	-	-	-	+	-	+	+	-	+	+	+	+	-	-	-	-
18	+	+	-	-	-	+	-	-	-	+	+	+	-	+	-	-	+	+	-	+	-	-	+	+	+	-	-	+	+	-	-
19	+	-	+	-	-	+	-	+	+	-	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	-	-	+	-	+	+
20	+	+	+	-	-	+	+	-	-	+	-	-	+	+	-	-	-	-	+	+	-	-	+	-	-	+	+	-	-	+	-
21	+	-	-	+	-	+	+	-	+	-	-	+	-	-	+	-	+	-	+	+	-	+	+	-	+	-	-	+	+	+	+
22	+	+	-	+	-	+	-	+	-	+	-	+	-	-	+	-	-	+	-	-	+	-	+	-	+	-	+	-	-	+	+
23	+	-	+	+	-	+	-	-	+	-	+	-	+	-	+	-	-	+	-	+	-	+	-	+	-	+	-	+	-	-	-
24	+	+	+	+	-	+	+	+	-	+	+	-	+	-	+	-	+	-	+	-	+	+	-	+	+	-	-	-	-	+	-
25	+	-	-	-	+	+	+	+	-	-	+	-	-	-	-	+	-	+	+	+	+	-	+	+	-	-	+	+	+	-	+
26	+	+	-	-	+	+	-	-	+	+	+	-	-	-	-	+	+	-	-	-	-	+	+	-	-	+	-	+	-	+	-
27	+	-	+	-	+	+	-	+	-	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	-	-	+	-	+	-	+
28	+	+	+	-	+	+	+	-	+	+	-	+	+	-	-	+	-	+	+	-	-	+	-	-	+	+	+	+	-	-	+
29	+	-	-	+	+	+	+	-	-	-	-	-	-	+	+	+	+	+	+	-	-	-	+	+	-	-	-	-	+	-	-
30	+	+	-	+	+	+	-	+	+	+	-	-	-	+	+	+	-	-	-	+	+	+	-	-	+	+	+	+	-	-	-
31	+	-	+	+	+	+	-	-	-	-	+	+	+	+	+	+	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+
32	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

having only plus signs, represents the average of the entire experiment. Column X_1, X_2, ..., X_5 are the main effects while columns X_{12} through X_{12345} are the interactions.

2.3 CONDUCT OF EXPERIMENT:

Data were drawn from 2^5 full factorial experiments conducted in a randomised order in three replicates according to the design matrix. The values of the varying factors and their coded levels are presented in Table 2.

Table 2: Factors and their coded levels

Level of factors	Code	Independent Variables				
		x_1	x_2	x_3	x_4	x_5
Base level	$\Delta 0$	30^0C	10^0C Brix	3.3	0.08 g/litre	12days
Interval of variation	x_i	10^0C	3^0C Brix	1.0	0.025 g/l	4 days
High level	+1	40^0C	13^0C Brix	4.3	0.1 g/l	16 days
Low level	-1	20^0C	7^0C Brix	2.3	0.05 g/l	8 days

Multivariate regression analysis was used for relating the variables. The relationship between the ascorbic acid level, y, and the five deteriorative factors x_i (i = 1,2, ...,5) is formulated as a linear model:

$$\wedge = b_0x_0 + b_1x_1 + b_2x_2 + b_3x_3 + b_4x_4 + b_5x_5 + b_{12}x_{12} + b_{13}x_{13} + b_{14}x_{14} + b_{15}x_{15}$$
$$Y_u$$
$$+ b_{23}x_{23} + b_{24}x_{24} + b_{25}x_{25} + b_{34}x_{34} + b_{35}x_{35} + b_{45}x_{45} + b_{123}x_{123} + b_{124}x_{124} + b_{125}x_{125} + b_{134}x_{134} + b_{135}x_{135} + b_{145}x_{145} + b_{234}x_{234} + b_{235}x_{235} + b_{245}x_{245} + b_{345}x_{345} + b_{1235}x_{1235} + b_{1245}x_{1245} + b_{1345}x_{1345} + b_{2345}x_{2345} + b_{12345}x_{12345}$$

Where the b's are the regression coefficients of the model, the x's are the coded variables and "e" measures the discrepancy in the functional relationship and is a random error with zero mean and constant variance Montgomery, (1991).

2.4 STATISTICAL ANALYSIS OF EXPERIMENTAL DATA

Providing for the number of replicates r = 3, the mean value of y and the dispersion of the replicated observations in the u-th trial are presented in table 3.

The mean of the replicated observations is given by:

$$\bar{y}_u = \frac{1}{r} \sum_{r=1} y_{uv} \qquad (1)$$

Where, r= number of replicates

Y_{uv} = replicate observations

The dispersion of the replicated observations is given by

$$S^2_u = \frac{1}{r-1} \sum_{r=1} (y_{uv} - \bar{y}_u)^2 \qquad (2)$$

The homogeneity of the dispersion was determined using Cochran criterion:

$$G_{cal} = \frac{S^2_{u\,max}}{\sum_{u=1}^{N} s^2_u} = \frac{0.61245}{4.3963} = 0.1393 \qquad (3)$$

The G-test was used to check if the output factors of the replication have maximum accuracy of the replication. It ascertains the possibility of carrying out regression analysis. The condition of homogeneity is

$$G_{[\alpha(r-1),N]} > G_{cal} \quad (4)$$

Where, N = number of experimental runs = 32

r = number of replicates

α= level of significance

The critical value of the criterion $G_{(0.05,2,32)} = 0.1884$ (table value.) this critical value of the criterion exceeds the calculated one, that is, $G_{(0.05,\ 2,\ 32)} > G_{cal}$ which attests to the condition of homogeneity of dispersion being met.

The mean squared error was determined by:

$$S^2_{(y)} = \frac{1}{N}\sum_{i=1}^{N} S^2_{(y)} = \frac{1}{32}(4.3963) = 0.13738 \quad (5)$$

It is the average sample variance estimate. The experimental error was given as:

$$S_{(y)} = \sqrt{S_{(y)}^2} = \sqrt{0.13738} = 0.37065 \quad (6)$$

The effects and the sum of squared for each effect were estimated through the contrast associated with that effect. The mean effect is given as:

$$b_0 = \frac{1}{N}\sum_{u=1}^{N}(x_0\bar{y}_u) \quad (7)$$

Where x_0 are the coded signs in the x column of the design matrix.

The main effect were estimated by:

$$\frac{1}{N}\ N\ =$$

$$b_i = \frac{}{N} \sum_{u=1} (x_i y_u) \qquad (8)$$

Where x_i are the coded signs in the x columns of the design matrix

The k – factor interactions are estimated by:

$$b_{ij...k} = \frac{1}{N} \sum_{u-1}^{N} (x_{ij..k} \bar{y}_u); \; i \neq j \neq \ldots \neq k \qquad (9)$$

Where x are the coded signs in the x columns of the design matrix.

The quantities in brackets in equations (7), (8) and (9) are contrasts in the treatment combinations. The summary of the estimated effects (i.e. the coefficients of the response function) is presented in table 3.

Construction of confidence interval and testing of hypothesis about individual regression coefficients are frequently used in assessing their statistical significance. Confidence intervals for the regression coefficients with confidence coefficient α are of the general form:

$$b \pm t_{(\alpha,N(r-1))} S_b$$

Where s is the estimate standard error in regression coefficient b and is an appropriate standard t – value with N(r - 1) degrees of freedom. For full factorial experiments, error in each regression coefficient is the same and is determined by:

$$S_{b0} = S_{bi} = S_{b\,ij...k} = \frac{S_{(y)}}{\sqrt{N\,r}} \qquad (11)$$

Where S_b = the experimental error.

The statistical significance of the regression coefficients were tested by: $\frac{|b_{i.....k}|}{}$

The statistical significance of the regression coefficients were tested by:

$$t_{i.....k} = \frac{|b_{i.....k}|}{S_{bi.....k}} \quad (12)$$

Where $|b_{i....k}|$ is the absolute value of the estimate of the coefficient being checked. The calculated t – values are compared with the appropriate critical value found from standard t – tables.

A coefficient is considered significant if and only if

$$t_{cal} > t_{[C, N(r=1)]} \quad (13)$$

For any coefficient that was statistically insignificant, such a coefficient was left out of the regression model. The summary of the calculated t – values are presented in table 3.

The adequacy of the model was evaluated using the null hypothesis () on the individual regression coefficients. The analysis of variance is very useful in confirming the significance of the coefficients (Douglas, 1991) in the 2^k factorial design with replicates, the regression sum of squares for any effect is:

$$SS_R = (\text{Contrasts})^2 \quad (14)$$

And has a single degree of freedom.

The total sum of squares was calculated by:

$$SS_T = \sum y^2_{uv} - \frac{(\sum_{u=1}^{Nr} (y_{uv})^2}{N.r} \quad (16)$$

The error sum of squares was given as

$$SS_E = SS_T - \sum SS_R$$

$$\text{i.e. } SS_R = SS_T - (Ss_{b1} + ... + Ss_{bij...k}) \quad (17)$$

Testing the significance of individual coefficient was carried out by the fisher's test (f - test).

$$F_{cal} = \frac{MS_R}{Ms_E} = \frac{SS_R \mid Df_R}{SS_E \mid N_{(r=1)}} \quad (18)$$

Where dF_r = degree of freedom regression = 1.
The calculated F – value are compared with the appropriate critical table value. The null hypothesis was rejected using:

$$F_{cal} > F_{[cl,Dfr,\ N(r=1)]} \quad (19)$$

With the conclusion that the coefficient contributes significantly to the regression (Douglas, 1991). The complete analysis of variance is summarised in Table 4.

3. RESULTS AND DISCUSSIONS

The 2^5 full factorial experimental design technique led to the optimal non-refrigeration storage/distribution conditions and models for predicting the ascorbic acid content:

40c storage temperature, 13^0 brix value, pH of 2.3, o.1 g/litre of antioxidant, and a maximum storage duration of 16 days. At this condition, the ascorbic acid level was maintained at 25.89 mg/100ml.

$$\hat{y}_u = 17.18 + 1.49x_2 - 3.04x_3 - 1.10x_5 + 1.02x_{12} + 0.66x_{14} - 0.50x_{34} + 0.85x_{124} + 0.96x_{125} + 1.28x_{135} + 0.70x_{145} + 0.57x_{2355} - 0.67x_{1345} + 0.76x_{1235} + 0.67x_{1245} - 0.70x_{12345}$$

Where, x_1 = Storage temperature, ^0c

Table 3: The dispersion S_u^2 of the replicate observations, the estimated effects (b's), and the calculated t – values.

S^2_u	b's		t – values	
0.41045	b_0	17.18	t_0	429.00
0.18895	b_1	0.39	t_1	9.75
0.10270	b_2	1.49	t_2	37.25
0.10405	b_3	-3.04	t_3	76.00
0.27125	b_4	0.30	t_4	7.50
0.10405	b_5	-1.10	t_5	27.50
0.29790	b_{12}	1.02	t_{12}	25.50
0.08320	b_{13}	-0.008	t_{13}	0.20*
0.01080	b_{14}	0.66	t_{14}	16.50
0.11205	b_{15}	0.08	t_{15}	2.00
0.09615	b_{23}	-0.45	t_{23}	11.25
0.61245	b_{24}	-.27	t_{24}	6.75
0.43960	b_{25}	-0.41	t_{25}	10.25
0.03040	b_{34}	-0.52	t_{34}	13.00
0.02965	b_{35}	-0.20	t_{35}	5.00
0.13860	b_{45}	0.41	t_{45}	10.25
0.03125	b_{123}	0.30	t_{123}	7.50
0.10990	b_{124}	0.85	t_{124}	21.25
0.03825	b_{125}	0.96	t_{125}	24.00
0.00730	b_{134}	0.22	t_{134}	5.50
0.02425	b_{135}	1.28	t_{135}	32.00
0.08890	b_{145}	0.70	t_{145}	17.50
0.17920	b_{234}	0.016	t_{234}	0.40
0.04645	b_{235}	-0.57	t_{235}	14.25
0.43680	b_{245}	-0.22	t_{245}	5.50
0.02830	b_{345}	-0.67	t_{345}	16.75
0.03040	b_{1234}	-0.18	t_{1234}	4.50
0.10270	b_{1235}	0.76	t_{1235}	19.00
0.00030	b_{1245}	0.67	t_{1245}	16.75
0.19695	b_{1345}	0.07	t_{1345}	1.75
0.01745	b_{2345}	0.12	t_{2345}	3.00
0.02565	B_{12345}	-070	T_{12345}	17.50

* Statistically insignificant

Table 4: Analysis of variance for replicated 2^5 full factorial experiment,

Source of variation	Effect	Sum of squares (SS)	Degree of freedom (dF)	Mean squares (MS)	F - ratio
B_1	0.39	14.60	1	14.60	2.49**
B_2	1.49	212.40	1	212.40	36.18
B_3	-3.04	891.21	1	891.21	151.82
B_4	0.30	8.89	1	8.89	1.51**
B_5	-1.10	111.07	1	111.07	18.92
B_{12}	1.02	99.27	1	99.27	16.91
B_{13}	-0.08	0.006	1	0.006	0.001**
B_{14}	0.66	42.06	1	42.06	7.17
B_{15}	0.08	0.63	1	0.63	0.11
B_{23}	-0.45	19.66	1	19.66	3.35**
B_{24}	-0.27	7.13	1	7.13	1.22**
B_{25}	-0.41	16.43	1	16.43	2.80**
B_{34}	-0.52	24.52	1	24.52	4.35
B_{35}	-0.20	3.77	1	3.77	0.64**
B_{45}	0.41	16.38	1	16.38	2.80**
B_{123}	0.30	8.46	1	8.46	1.44**
B_{124}	0.85	69.26	1	69.26	11.80
B_{125}	0.96	88.82	1	88.82	15.13
B_{134}	0.22	4.44	1	4.44	0.76
B_{135}	1.28	158.21	1	158.21	26.95
B_{145}	0.70	47.38	1	47.38	8.07
B_{234}	0.016	0.03	1	0.03	0.01**
B_{235}	-0.57	31.19	1	31.19	5.31
B_{245}	-0.22	4.43	1	4.43	0.75**
B_{345}	-0.67	43.17	1	43.17	7.35
B_{1234}	-0.18	3.09	1	3.09	0.53**
B_{1235}	0.76	54.18	1	54.18	9.34
B_{1245}	0.67	42.85	1	42.85	7.30
B_{1345}	0.07	0.42	1	0.42	0.07**
B_{2345}	0.12	1.44	1	1.44	0.25**
B_{12345}	-0.70	46.62	1	46.62	7.94
Error		375.97	64	5.87	
Total		2449.62	95		

** Insignificant at 5 percent.

B_{2345} B_{12345}	0.12 -0.70	1.44 46.62	1 1	1.44 46.62	0.25** 7.94
Error		375.97	64	5.87	
Total		2449.62	95		

** Insignificant at 5 percent.

On the basis of the experimental results and the above fitted model, for values of x_1 (1 = 1,2 ....., 5) that falls within the intervals used in producing the model, the following observations were made.

1. Raising the pH value from its low-level (i.e. 2.3) to its high-level (i.e. 4.3) leads to reduction in the ascorbic acid level in the juice. This is explained by the negative sign against the coefficient of the factor (i.e. $b_3 = -3.04$) explains this fact.

2. Raising the brix value from its low level (i.e. 7^0 brix) helps in maintaining a high ascorbic acid level in the juice. This is explained by positives sign against the coefficient of this factors (i.e. $b_2 = 1.49$).

3. Increasing simultaneously the storage temperature, pH and duration of storage from their low levels to their high levels maintains a high ascorbic acid level in the juice. This is explained by the positive sign against the coefficient of the interaction between these two actors. (I.e. $b_{135} = 1.28$).

4. Raising the duration of storage from its low level (i.e. 8 days) to its high level (16 days) leads to a reduction in the ascorbic acid level in the juice. This is explained by the negative sign against the coefficient of this factor (i.e. $b_{135} = -1.10$).

It can be seen that the pH, with coefficient $b_3 = -3.04$, has the highest influence on the ascorbic level of the ascorbic acid level of the juice.

To assist in the practical interpretation of the fitted model the plots of the main effects in the model are presented in figure 1.

4 CONCLUSIONS

The results of the mango juice experiments and the developed model confirms that storage temperature, brix value, pH, quantity of antioxidant and duration of storage govern the shelf life and are important for characterizing the quality of fruit juices. These quality variables enable the prediction of shelf – life of the juices under the non-refrigeration storage and distribution. The developed model is valid only for values of x_1 that fall within the intervals of values used in producing it. It is purely for non-refrigeration storage and distribution of mango juice.