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MODELLING OF ASCORBIC ACID CONTENT IN ORANGE JUICE UNDER NON-REFRIGERATED CONDITION

D. ADGIDZI AND S.T. OLORUNSOGO

Abstract

In the course of storing and distributing fruit juice there is an inevitable decline in quality value. The loss in quality occurs because of the sensitivity of the ascorbic acid content of juices to certain storage and environmental conditions. In this paper, the effects of storage temperature (factor X_1), brix value (factor X_2), pH (factor X_3), anti-oxidant (factor X_4), and duration of storage (factor X_5), on the ascorbic acid level under non-refrigeration storage and distribution of Orange juice was investigated. The shelf life and quality values of the juice were estimated, and a model based on the 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. Multi-variate regression analysis was used for relating the variables. The analysis of the experimental data led to the determination of the optimal condition of orange juice under non-refrigeration as: storage temperature 20^0C; 13^0-brix value; a pH of 4.2, anti-oxidant 0.05g/litre and a maximum storage duration of 16 days. At these conditions, the ascorbic acid level was maintained at 22.93mg/100ml.

Introduction

In the course of storage and distribution of orange juice there is an inevitable decline in quality value. The loss occurs because of the sensitivity of the ascorbic acid content of the juice to some storage and environmental conditions; (Heimann, 1980). Ascorbic acid level is usually the criterion for judging fruit juice quality. It is the responsibility of orange juice manufacturers to ensure that quality losses in juices be minimal. The juice manufacturer must seek to control the changes, which influence the quality value of their products. Thus, it is necessary to establish an analytical approach to the chemistry of fruit juice preservation in order to specify the quality value of juices at different storage and distribution conditions.

The problem of fruit juices in the tropics especially sub-Saharan Africa, is inadequate study on quality deterioration from a quantitative, integrated stand point. To predict the extent of deterioration of nutrient value, a knowledge of the loss of important nutritive quality as a function of the critical deteriorative factors are needed (Fennema, 1976). Through integration and/ or modeling of the various deteriorative factors, the juice manufacturer can 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 the products.

Five main factors have been identified as critical to the retention of ascorbic acid in fruit juices during non-refrigeration 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 (Frederick and Albrigo, 1994). Monitoring these factors will bring about satisfactory control of ascorbic acid degradation in orange juice during non-refrigeration storage and distribution. In this paper, the effects of these critical factors on the ascorbic acid level under non-refrigeration storage and distribution of orange juice was investigated, shelf-life and

D. Adgidzi and S. T. Olorunsogo are with the Department of Agricultural Engineering, Federal University of Technology, Minna, Nigeria.

quality value of the juice was estimated, and a model based on these deterioration factors was developed.

Experimental techniques

Experimental Materials

Orange 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. The fruit sample and the initial properties of the juice extracted are presented in Table 1.0 (Olorunsogo, 1998). All chemicals and reagents used for the chemical analysis of the samples are "Analar" produced by BDH chemicals Ltd, Poole England

Table 1. Experimental sample and the initial properties

Experimental Sample	Variety	Properties of freshly extracted juice		
		Vitamin C	Brix value	PH
Orange	Agege 1	36.15mg/100ml.	10^0 Brix	3.2

Determination of quality factors

Determination of Brix Value

Soluble solids may be determined either with a hydrometer or with the use of a refractometer. Brix by a Refractometer (the refractometer with a brix scale calibrated at 20^0C); saturated sugar solution and distilled water were used.

Ascorbic acid determination

The reagents used for ascorbic acid determination are: (i) 2,6-dichlorophenol indophenol (TILLMAN'S Reagent) (ii) Ascorbic acid standard solution (20mg/ml, prepared just before use) (iii) Acetic acid glacial or Trichloroacetic acid (5%) (iv) Chloroform (v) Oxalic acid or metaphosphoric acid (vi) Fruit juice samples. Other materials used for the experiment are: fine textured muslin clothe, conical flasks (100mls), burettes, pipette, measuring cylinder, retort stand, and sartorious top loading balance (BP 310S).

Determination of pH

The reagents for the determination of pH are: (i) Potassium tetroxalate dihydrate (ii) Potassium hydrogen phthalate (iii) Potassium hydrogen phosphate. (iv) Disodium hydrogen phosphate (v) Sodium tetraborate decahydrate. The other equipments used are 100mls beakers, 200mls measuring cylinders, dropping pipettes, pH meter (KENNT EIL 7055).

The total sample size for the experiments was ninety-six (96).

Experimental design method

A five variable two level factorial design ($N=2^k$) (Montgomery, 1991) and (Montgomery and Runger 1994) provides the framework for the orange juice variable experiment. The design matrix for the 2^5 full factorial experiments, which indicates

Table 2. Design matrix (table for 2.5 full factorial experimental design (FFD)

RUN	x_0	x_1	x_2	x_3	x_4	x_5	x_{12}	x_{13}	x_{14}	x_{15}	x_{23}	x_{24}	x_{25}	x_{34}	x_{35}	x_{45}	x_{123}	x_{124}	x_{125}	x_{134}	x_{135}	x_{145}	x_{234}	x_{235}	x_{245}	x_{345}	x_{1234}	x_{1235}	x_{1245}	x_{1345}	x_{2345}	x_{12345}
No.	b_0	b_1	b_2	b_3	b_4	b_5	b_{12}	b_{13}	b_{14}	b_{15}	b_{23}	B_{24}	b_{25}	b_{34}	b_{35}	b_{45}	b_{123}	b_{124}	b_{125}	b_{134}	b_{135}	b_{145}	b_{234}	b_{235}	b_{245}	b_{345}	b_{1234}	b_{1235}	b_{1245}	b_{1345}	b_{2345}	b_{12345}
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.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

the run-by-run experiment is as shown in Table 2. With five variables and two levels, a complete or orthogonalized design leads to a total of 32 experimental runs with each run replicated three (3) times. In the 2^5 full factorial experiment, the low and high levels of the factors were coded as minus (-) and plus (+) respectively.

Conduct of experiment

Data were drawn from 2^5 full factorial experiments conducted in a randomized order in three replicates according to the designed matrix given in Table 2. The values of the varying factors and their coded levels are presented in Table 3.

Table 3. Factors and their coded levels

Level of Factors	Code	Independent Variables				
		X_1	X_2	X_3	X_4	X_5
Base level	0	30^0c	10^0c Brix	3.2	0.08g/lites	12 days
Interval of variation	X_i	10^0c	3^0c Brix	1.0	0.025 " "	4 days
High level	+1	40^0c	13^0c Brix	4.2	0.1 " "	16 days
Low Level	-1	20^0c	7^0c Brix	2.2	0.05 " "	8 days

Multivariate regression analysis was used for relating the variables. The functional relationship between the ascorbic acid level y_u, and the five deteriorative factors x_i (i=1,2,….,5) was presented using a linear model;

$$
\begin{aligned}
{}_u &= b_o \\
&+ b_1x_1 + b_2x_2 + b_3xb_3 + b_4xb_4 + b_5x_5 \\
&+ b_{12}x_{12} + b_{13}x_{13} + b_{14}xb_{14} + b_{15}xb_{15} + b_{23}x_{23} \\
&+ b_{24}x_{24} + b_{25}x_{25} + b_{34}xb_{34} + b_{35}xb_{35} + b_{45}x_{45} \\
&+ b_{123}x_{123} + b_{124}x_{124} + b_{125}xb_{125} + b_{134}xb_{134} + b_{135}x_{135} \\
&+ b_{145}x_{145} + b_{234}x_{234} + b_{235}xb_{235} + b_{245}xb_{245} + b_{345}x_{345} \\
&+ b_{1234}x_{1234} + b_{1235}x_{1235} + b_{1245}xb_{1245} + b_{1345}xb_{1345} + b_{2345}x_{2345} \\
&+ b_{12345} + e
\end{aligned}
$$

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).

Statistical analysis and discussion of experimental data

Statistical analysis

Providing for the number of replicates r = 3, the mean experimental observation, the fitted values, the residuals, the squares of the residuals and the dispersion are presented in Table 4. The mean of the replicated observations was given by.

$$\ddot{y}_u = \frac{1}{r} \sum_{v=1} y_{ur} \qquad (1)$$

where r is replication of the trial, y_{uv} is the running value of the response in the v-th repeat of the u-th.

The dispersion (variance) of the replicated observations was given as:

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

The homogeneity of the dispersion was determined using Cochran criterion G:

$$G = \frac{S^2_{u\,max}}{\sum_{u=1}^{N} S^2_u} = \frac{0.6643}{4.11265} = 0.161526 \quad \text{_____(3)}$$

where $S^2_{u\,max}$ is the maximum dispersion out of N dispersions.

Table 4. The mean experimental observation, the fitted values, the residuals, the squares of the residuals, and the depersion

Run No.	$\ddot{y}_u$	$\ddot{y}_u$	$e_1 = \ddot{y}_u - y_u$	$e^2_1 = (\ddot{y}_u - y_u)^2$	S_u^2
1.	12.52	12.57	-0.05	0.0025	0.10805
2.	22.43	22.41	0.02	0.0004	0.00335
3.	12.60	12.59	0.01	0.0001	0.03125
4.	2.15	2.07	0.08	0.0064	0.03895
5.	23.25	23.15	0.10	0.0100	0.01455
6.	13.88	13.95	-1.07	0.0049	0.14325
7.	18.40	18.33	0.07	0.0049	0.02565
8.	21.67	21.81	-0.14	0.0196	0.00605
9.	15.23	15.25	-0.02	0.0004	0.01585
10.	8.65	8.61	0.04	0.0016	0.10270
11.	9.19	9.31	-0.12	0.0144	0.24375
12.	2.08	2.07	0.01	0.0001	0.02565
13.	12.90	12.91	-0.01	0.0001	0.013045
14.	19.40	19.39	0.01	0.0001	0.03125
15.	21.17	21.17	0.00	0.0000	0.10570
16.	16.79	16.73	0.06	0.0036	0.48015
17.	12.03	12.09	-0.06	0.0036	0.02895
18.	2.92	2.89	0.03	0.0009	0.10805
19.	13.38	13.35	0.03	0.0009	0.06280
20	4.66	4.59	0.07	0.0049	0.23205
21.	4.98	4.99	-0.01	0.0001	0.02645
22.	1.37	0.37	1.38	1.9044	0.02575
23.	22.83	22.93	-0.10	0.0100	0.01030
24.	8.46	8.49	-0.03	0.0009	0.64165
25.	7.48	7.41	1.38	0.0049	0.03040
26.	3.55	3.65	-0.10	0.0100	0.00215
27.	22.11	22.07	0.04	0.0016	0.28375
28.	4.28	4.43	-0.15	0.0225	0.03040
29.	5.36	5.39	-0.03	0.0009	0.42725
30.	3.56	3.55	0.01	0.0001	0.00405
31.	22.19	22.17	0.02	0.0004	0.66430
32.	4.35	4.29	0.06	0.0036	0.02775

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

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

Where, N = number of experimental runs = 32
r = number of replicates = 3
α = level of significance = 0.05

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 attest to the condition of homogeneity of dispersion being met.

The mean squared error was determined by;

$$S^2_y = \frac{1}{N} \sum_{u=1}^{N} S_u^2 = \frac{1}{32}(4.11265) = 0.1285203 \quad \text{_____(5)}$$

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

$$S(y) = \sqrt{S^2_{y.}} = \sqrt{0.11285203} = 0.33593 \quad \text{_____(6)}$$

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

$$b_o = \frac{1}{N} \sum_{u=1}^{N} (x_0 \ddot{y}_u) \quad \text{_____(7)}$$

where x_0 are the coded signs in the x column of the design matrix. The main effect were estimated by:

$$b_i = \frac{1}{N} \sum_{u=1}^{N} (x_i \ddot{y}_u) \quad \text{_____(8)}$$

where x_i are the coded signs in the x_i columns of the design matrix
The k – factor interactions are estimated by:

$$b_{ij.....k} = \frac{1}{N} \sum_{u=1}^{N} (x_{ij..k} \ddot{y}_u); i \neq j \neq .. \neq k \quad \text{_____(9)}$$

where $x_{ij....k}$ are the coded signs in the $x_{ij....k}$ columns of the design matrix. The quantities in brackets in equations (7), (8) and (9) are called contrast in the treatment combinations.

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:

$$b \pm t_{[\alpha, N(r-1)]} S_b \quad \text{_____(10)}$$

where S_b is the estimated standard error in regression coefficient b and t [α, N (r-1)] 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:

$$Sb_o = Sb_i = Sb_{ij} = Sb_{ij..k} = \frac{S_y}{\sqrt{N.r}} = \frac{0.333593}{\sqrt{32x3}} = 0.034 \quad \text{_(11)}$$

where S_y = the experimental error.

The statistical significance of the regression coefficients were tested by:

$$t_{i....k} = \frac{|b_{i...K}|}{Sb_{i...K}} \quad \text{_____(12)}$$

where $|bi....k|$ 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;

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

For any coefficient that was statistically insignificant, such a coefficient was left out of the regression model. The summary of the estimated effects, that is, the coefficients of the response function, the calculated t – values and the confidence intervals are given in Table 5.

The adequacy of the model was evaluated using the null hypothesis (H_0: bi =O) on the individual regression coefficients. The analysis of variance is very useful in confirming the significance of the coefficients (Montgomery, 1991). In the 2^k factorial design with replicates, the regression sum of squares for any effects is:

$$SS_R = \frac{r}{N}\ (\text{Contrasts})^2 \quad \text{_____(14)}$$

and has a single degree of freedom. The total sum of squares was calculated by:

$$SS_T = \sum_{U=1}^{Nr} y^2_{uv} - \frac{[\sum_{u=1}^{Nr}(y_{uv})^2]}{Nr} = 5149.03 \quad \text{_____(15)}$$

The error of squares was given as

$$SS_E = SS_T - \Sigma SS_R = 7.04 \quad \text{_____(16)}$$

i.e $SS_E = SS_T - (S_{Sbi} + ... + S_{Sbij...k})$ _____(17)

Testing the significance of individual coefficient was carried out by the Fisher's test (F-test)

$$F_{cal} = \frac{MS_R}{MS_E} = \frac{SS_R/df_R}{SS_E/N_{(r-l)}} \quad \text{_____(18)}$$

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:

$$F_{cal} > F_{[\alpha,dfR,\, N(r-1)]} \quad \text{_____ (19)}$$

with the conclusion that the coefficient contributes significantly to the regression (Montgomery, 1991). The complete analysis of variance is summarized in Table 6.

The adequacy of the model was further validated by calculating the dispersion of adequacy for the replicated experiments and comparing the magnitude with the variance estimate given by the mean squared error. The dispersion of adequacy is given by:

$$SS^2_{(ad)} = \frac{r}{N-\lambda\sum_{U=1}^{N}(\ddot{y}-\ )^2} = \frac{r}{df_{(ad)}}\frac{N}{\Sigma(\ddot{y}_u-\ _u)^2} = \frac{3}{30}(2.0388) = 0.20388 \quad \text{___(20)}$$

where λ = number of inadequate coefficients = 2
$_u$ = the design estimate of the response value in the u-th trial,
$df_{(ad)}$ = the degree of freedom of adequate values
calculated from the fitted model.

Table 5. The estimated effects, confidence intervals and calculated t-values

Regression coefficient	Estimated effect	Confidence interval	t-values
B_0	11.76	±0.07	294.00
B_1	-2.97	±0.07	74.25
B_2	1.14	±0.07	28.50
B_3	2.05	±0.07	51.25
B_4	-0.61	±0.07	15.25
B_5	-2.76	±0.07	69.00
b_{12}	-1.87	±0.07	46.75
b_{13}	0.40	±0.07	10.00
b_{14}	-0.34	±0.07	8.50
b_{15}	-1.83	±0.07	45.75
b_{23}	2.04	±0.07	51.00
b_{24}	0.49	±0.07	12.25
b_{25}	2.65	±0.07	66.25
b_{34}	0.02	±0.07	0.50
b_{35}	-1.86	±0.07	46.50
b_{45}	0.73	±0.07	18.25
b_{123}	0.28	±0.07	7.00
b_{124}	-0.72	±0.07	18.00
b_{125}	-0.67	±0.07	16.75
b_{134}	0.70	±0.07	17.50
b_{135}	-0.28	±0.07	7.00
b_{145}	-0.0013	±0.07	0.033
b_{234}	-0.78	±0.07	19.50
b_{235}	-0.55	±0.07	13.75
b_{245}	-0.16	±0.07	4.00
b_{345}	-0.46	±0.07	11.50
b_{1234}	-1.06	±0.07	26.50
b_{1235}	-1.14	±0.07	28.50
b_{1245}	-0.48	±0.07	12.00
b_{1345}	-0.61	±0.07	15.25
b_{2345}	-0.45	±0.07	11.25
b_{12345}	1.65	±0.07	41.25

* Insignificance at 5 percent

TABLE 6. 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	-2.97	846.81	1	846.81	7698.27
b_2	1.14	124.35	1	124.35	1130.46
b_3	2.05	404.43	1	404.43	3676.64
b_4	-0.61	36.09	1	36.09	328.09
b_5	-2.76	732.95	1	732.95	6663.18
b_{12}	-1.87	335.48	1	335.48	3049.82
b_{13}	0.40	14.88	1	14.88	135.27
b_{14}	-0.34	11.14	1	11.14	101.27
b_{15}	-1.83	322.15	1	322.15	2928.64
b_{23}	2.04	397.80	1	397.80	3616.36
b_{24}	0.49	22.93	1	22.93	208.46
b_{25}	2.65	674.80	1	674.80	6134.55
b_{34}	0.02	0.036	1	0.036	0.3273
b_{35}	-1.86	322.35	1	322.35	3021.36
b_{45}	0.73	51.16	1	51.16	465.09
b_{123}	0.28	7.56	1	7.56	68.73
b_{124}	-0.72	49.08	1	49.08	446.18
b_{125}	-0.67	43.58	1	43.58	396.18
b_{134}	0.70	46.45	1	46.45	422.27
b_{135}	-0.28	7.43	1	7.43	67.55
b_{145}	-0.0013	0.00015	1	0.00015	0.0014
b_{234}	-0.78	58.97	1	58.97	536.09
b_{235}	-0.55	29.31	1	29.31	266.46
b_{245}	-0.16	2.33	1	2.33	21.18
b_{345}	-0.46	19.98	1	19.98	181.64
b_{1234}	-1.06	108.12	1	108.12	982.91
b_{1235}	-1.14	123.67	1	123.67	1124.27
b_{1245}	-0.48	22.52	1	22.52	204.73
b_{1345}	-0.61	35.58	1	35.58	323.46
b_{2345}	-0.45	19.28	1	19.28	175.27
b_{12345}	-1.65	260.77	1	260.77	2370.64
Error		7.04	64	0.110	
Total		5149.03	95		

** Insignificant at 5 percent

The adequacy of the model is confirmed by the Fisher's test:

$$F_{cal} = \frac{SS^2_{R\,(ad)}}{S^2_{(y)}} = \frac{0.20388}{0.12852} = 1.5864 \qquad \text{(21)}$$

where $S^2_{(y)}$ is the variance estimate given by the mean sugared error. The calculated F – value was compared with the appropriate table value, $F_{(0.05,30,64)} = 1.6433$. The condition of adequacy is:

$F_{cal} \leq F_{[\alpha,\ N\text{-}\lambda,\ N(r\text{-}1)]}$

Since this condition was satisfied, the fitted model is considered adequate.

TABLE 6. 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	-2.97	846.81	1	846.81	7698.27
b_2	1.14	124.35	1	124.35	1130.46
b_3	2.05	404.43	1	404.43	3676.64
b_4	-0.61	36.09	1	36.09	328.09
b_5	-2.76	732.95	1	732.95	6663.18
b_{12}	-1.87	335.48	1	335.48	3049.82
b_{13}	0.40	14.88	1	14.88	135.27
b_{14}	-0.34	11.14	1	11.14	101.27
b_{15}	-1.83	322.15	1	322.15	2928.64
b_{23}	2.04	397.80	1	397.80	3616.36
b_{24}	0.49	22.93	1	22.93	208.46
b_{25}	2.65	674.80	1	674.80	6134.55
b_{34}	0.02	0.036	1	0.036	0.3273
b_{35}	-1.86	322.35	1	322.35	3021.36
b_{45}	0.73	51.16	1	51.16	465.09
b_{123}	0.28	7.56	1	7.56	68.73
b_{124}	-0.72	49.08	1	49.08	446.18
b_{125}	-0.67	43.58	1	43.58	396.18
b_{134}	0.70	46.45	1	46.45	422.27
b_{135}	-0.28	7.43	1	7.43	67.55
b_{145}	-0.0013	0.00015	1	0.00015	0.0014
b_{234}	-0.78	58.97	1	58.97	536.09
b_{235}	-0.55	29.31	1	29.31	266.46
b_{245}	-0.16	2.33	1	2.33	21.18
b_{345}	-0.46	19.98	1	19.98	181.64
b_{1234}	-1.06	108.12	1	108.12	982.91
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b_{2345}	-0.45	19.28	1	19.28	175.27
b_{12345}	-1.65	260.77	1	260.77	2370.64
Error		7.04	64	0.110	
Total		5149.03	95		

** Insignificant at 5 percent

The adequacy of the model is confirmed by the Fisher's test:

$$F_{cal} = \frac{SS^2_{R\,(ad)}}{S^2_{(y)}} = \frac{0.20388}{0.12852} = 1.5864 \qquad \text{(21)}$$

where $S^2_{(y)}$ is the variance estimate given by the mean sugared error. The calculated F – value was compared with the appropriate table value, $F_{(0.05,30,64)} = 1.6433$. The condition of adequacy is:

$F_{cal} \leq F_{[\alpha,\ N-\lambda,\ N(r-1)]}$

Since this condition was satisfied, the fitted model is considered adequate.

Discussion

The 2^5 full factorial experimental design technique led to the optimal non-refrigeration storage/distrution conditions and model for predicting the ascorbic acid content: 20^0C storage temperature, 13^0 brix value, pH of 4.2, 0.05 g/litre of antioxidant, and a maximum storage duration of 16 days. At this condition, the ascorbic acid level was maintained at 22.93 mg/100ml.

$$\begin{aligned} {}_u = {} & 11.76 - 2.97X_1 + 1.14X_2 + 2.05X_3 - 0.61X_4 \\ & - 2.76\,X_5 - 1.87X_{12} + 0.40X_{13} - 0.34X_{14} - 1.83X_{15} \\ & + 2.04\,X_{23} + 0.49X_{24} + 2.65X_{25} - 1.86X_{35} - 0.73X_{45} \\ & + 0.28\,X_{123} - 0.72X_{124} - 0.67X_{125} + 0.70X_{134} - 0.28X_{135} \\ & - 0.78\,X_{234} - 0.55X_{235} + 0.16X_{245} - 0.46X_{345} - 1.06X_{1234} \\ & - 1.14\,X_{1234} - 0.48X_{1245} + 0.16X_{1345} - 0.45X_{2345} - 1.65X_{12345} \end{aligned} \quad \text{\_\_\_\_\_(22)}$$

where, x_1 = Storage temperature, ^{0}C

x_2 = Total soluble solid, brix

x_3 = pH

x_4 = Quantity of antioxidant, g/litre

x_5 = Duration of storage, days.

$\ddot{y}_u$ = Ascorbic acid level, mg/100ml

Equation (22) expresses the fitted model for predicting ascorbic acid level in orange juice under non-refrigerated storage and distribution conditions. To assist in the practical interpretation of the experiment, the plots of the full main effects are presented in Fig. 1. From this figure,

a_L = experiment 31 and a_H = experiment 2,

b_L = experiment 30 and b_H = experiment 3,

c_L = experiment 28 and c_H experiment 5

d_L = experiment 24 and d_H = experiment 9,

e_L = experiment 16 and e_H experiment 17.

From Tables 2 and 4 the above experimental observations can be explained as follow:

Experiment 31 puts the temperature at low level while other factors are at their high levels and the fitted value Y_{31} = 22.17mg/100ml.

Experiment 30 puts brix value at low level while other factors are at their high levels and the fitted value Y_{30} = 3.55mg/100ml.

Experiment 3 puts brix value at high value while other factors are at their low levels and the fitted value Y_3 = 12.59mg/100ml.

Experiment 28 puts pH at low level while other factors are at their high levels and the fitted value Y_{28} = 23.15mg/100ml.

Experiment 24 puts anti-oxidant at low level while other factors are at their high levels and the fitted value Y_{24} = 8.49mg/100ml.

Experiment 9 sets anti-oxidant at high level while other factors are at their low levels and the fitted value Y_9 = 15.25mg/100ml.

Experiment 16 sets duration of storage at low level while other factors are at their high levels and the fitted value Y_{16} = 16.73mg/100ml.

Experiment 17 sets duration of storage at high level while other factors are at their low levels and the fitted value Y_{17} = 12.09mg/100ml.

From the given fitted model all the main effects and the interactions, have significant influence on the level of the ascorbic acid of orange juice under non-refrigerated storage and distribution conditions. However, storage temperature (with coefficient b_1= -2.97), duration of storage (with coefficient b_5=-2.76) and pH (with

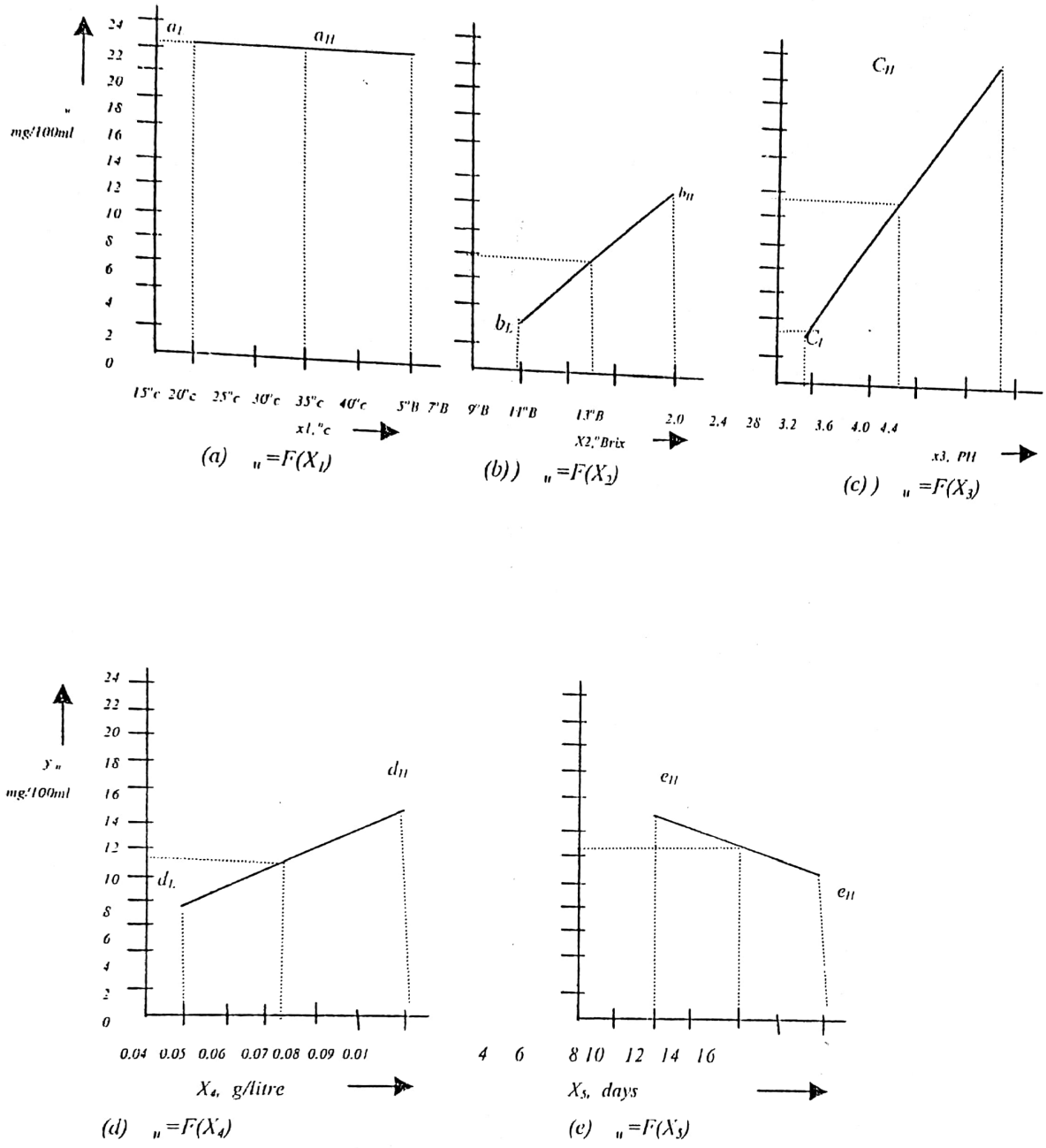

Fig. 1. Main effects plot for Orange Juice Experiment.

coefficient b_1=-2.05) have higher influence on ascorbic acid of the juice. High levels of each of these factors will lead to drastic reduction in the ascorbic acid level of the juice under non-refrigerated storage condition. On the other hand, the interactions of brix value and duration of storage (with coefficient b_{25}=2.65) and the brix value/pH (with coefficient b_{23}=2.04) have positive influences on the ascorbic acid level.

Comparing the predicted values based on the fitted model with the mean experimental value for the thirty-two experimental runs, as shown in Table 4, it can be seen that storage and distribution condition of experiment 5 with predicted value Y_5 =23.15, maintains the ascorbic acid level of the juice at the highest level. However, storage and distribution conditions of experiment 23 (with predicted value Y_{23} =22.93), and experiment 27 (with predicted value Y_{27} =22.07) all still meet the minimum quality standard (Inglet and Chara,aribous1979). Since the goal of the research includes the determination of the maximum shelf life, under non-refrigerated storage condition, the optimum condition will be that of experiment 23, that is: 20^0C storage temperature (factor X_1); 13^0 Brix value (factor X_2); a pH of 4.2 (factor X_3), 0.05g/litre of anti-oxidant (factor X_4) and a maximum storage duration of 16 days (factor X_5). At these conditions the ascorbic acid level is Y_{23} =22.93mg/100ml.

CONCLUSION

The results of the orange 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, that fall within the intervals of values used in producing it. It is purely for non-refrigeration storage and distribution of orange juice.

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