ABSTRACT
Ascorbic acid is the least stable of all fruit juice nutrients; it is readily oxidized. Its concentration is an index to the retention of the original nutritive value. In this study, 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 pineapple juices were investigated; maximum shelf life and the quality values of the juice were estimated; and a quality value model 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 conditions: 20°C storage temperature, 18° brix value, pH of 4.5, 0.1g/litre of antioxidant and a maximum storage duration of 16 days during which the ascorbic acid level was 11.68mg/100ml.

Keywords: Ascorbic acid, Brix value, pH, Antioxidant, Shelf life.

INTRODUCTION
Fruit juice is assuming a more important role in Nigeria's diversified food industry. However, short shelf life of fresh market fruit juice, an inherent characteristic that tends to impede the growth of the domestic juice industry, is believed to be influenced by many factors. In the course of storage and distribution of pineapple 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. Ascorbic acid level is usually the criterion for judging fruit juice quality (Heimann, 1980; Philip, 2005).

The problem of fruit juice industry in the tropics, especially sub-Saharan Africa, is inadequate study on quality deterioration from a quantitative, integrated stand point. It is the responsibility of the juice manufacturers to ensure that quality losses in juices be minimal. The juice manufacturer must seek to monitor the factors which influence the quality of their product. To predict the extent of deterioration of quality, knowledge of the loss of important nutritive quality index as a function of the critical deteriorative factors are needed (Owen, 1976; Philip, 2005). Through 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 advertisements associated with the products.

Five main factors have been identified as
Critical to the retention of ascorbic acid in pineapple juice during non-refrigeration storage and distribution. These are: (a) the level of dissolved oxygen, (b) the storage temperature, (c) the total soluble solid (brix value), (d) the pH, and (e) the duration of storage (Frederic et al., 1994). Balancing these factors will bring about satisfactory control of ascorbic acid degradation in pineapple juice during non-refrigeration storage and distribution. In this paper, the effects of these critical factors on the ascorbic acid level under ambient storage and distribution of pineapple juice was investigated, shelf-life and quality of the juice was estimated, and a mathematical model based on these deterioration factors was developed.

**MATERIALS AND METHODS**

**Experimental Materials**

Samples of pineapple juice were manually extracted from fruit samples which were obtained from experimental plots of National Horticultural Research Institute (NIHORT), Ibadan. These samples are representative of the Nigeria pineapple fruit market with respect to the variety and cultural condition. The initial properties of the juice extracted are presented in Table 1 (Olorunsogo, 1998). All chemicals and reagents used for the chemical analysis of the samples are “Analar” produced by BDH chemicals Ltd, Poole England.

**Experimental Design**

A five variable two level factorial design (N = 2^5) provides the framework for the pineapple juice variable experiments. 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 (Olorunsogo, 1998; Douglas, 1991; Douglas, et al., 2003).

**Conduct of Experiment and Data Presentation**

Data were drawn from 2^5 full factorial experiments conducted in a randomized order in three replicates according to the design matrix. The values of the varying factors and

<table>
<thead>
<tr>
<th>Table 1: Experimental Sample and the Initial Properties</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experimental</td>
</tr>
<tr>
<td>-------------------</td>
</tr>
<tr>
<td>Pineapple Juice</td>
</tr>
</tbody>
</table>

Table 2: Factors and Their Coded Levels

<table>
<thead>
<tr>
<th>Level of Factor</th>
<th>Code</th>
<th>Independent Variables</th>
</tr>
</thead>
<tbody>
<tr>
<td>Base level</td>
<td>O</td>
<td>x1 30°C x2 14° Brix x3 3.5 x4 0.08g/litre x5 12 days</td>
</tr>
<tr>
<td>Interval of variation</td>
<td>Δxi</td>
<td>x1 10°C x2 4° Brix x3 1.0 x4 0.025g/litre x5 4 days</td>
</tr>
<tr>
<td>High level</td>
<td>+ 1</td>
<td>x1 40°C x2 18° Brix x3 4.5 x4 0.1g/litre x5 16 days</td>
</tr>
<tr>
<td>Low level</td>
<td>- 1</td>
<td>x1 30°C x2 10° Brix x3 2.5 x4 0.05g/litre x5 8 days</td>
</tr>
</tbody>
</table>

where $x_1$ = storage temperature, $x_2$ = brix value, $x_3$ = pH

$x_4$ = quantity of antioxidant, $x_5$ = duration of storage

Table 3: Mean Ascorbic Acid Level Data for Pineapple Juice (mg/100 ml)

<table>
<thead>
<tr>
<th>Run No</th>
<th>Experimental Mean $\hat{y}$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>6.43</td>
</tr>
<tr>
<td>2</td>
<td>1.61</td>
</tr>
<tr>
<td>3</td>
<td>4.74</td>
</tr>
<tr>
<td>4</td>
<td>3.71</td>
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<tr>
<td>5</td>
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<tr>
<td>6</td>
<td>3.51</td>
</tr>
<tr>
<td>7</td>
<td>4.49</td>
</tr>
<tr>
<td>8</td>
<td>4.17</td>
</tr>
<tr>
<td>9</td>
<td>3.66</td>
</tr>
<tr>
<td>10</td>
<td>2.23</td>
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<td>4.69</td>
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<tr>
<td>15</td>
<td>4.98</td>
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<tr>
<td>16</td>
<td>4.29</td>
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</table>

<table>
<thead>
<tr>
<th>Run No</th>
<th>Experimental Mean $\hat{y}$</th>
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</thead>
<tbody>
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<td>11.57</td>
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<td>31</td>
<td>11.65</td>
</tr>
<tr>
<td>32</td>
<td>4.42</td>
</tr>
</tbody>
</table>
Multivariate regression analysis was used in relating the variables (Douglas, et al., 2003; Klaus et al. 2005; Robert et al., 2003; Maxino et al., 1984; Zivorad, 2004). The mean of the replicated observations were given by:

$$\bar{y}_u = \frac{1}{r} \sum_{u=1}^{r} y_{uv}$$  \hspace{1cm} (1)

where \( r \) is replication of the trial, \( y_{uv} \) is the value in the \( u \)-th repeat of the \( r \)-th. The dispersion (variance) of the replicated observation were given as:

$$S_u^2 = \frac{1}{r-1} \sum_{u=1}^{r} (y_{uv} - \bar{y}_u)^2$$  \hspace{1cm} (2)

The sum of the dispersion =

$$\sum_{u=1}^{N} S_u^2;$$

where, \( N = \) number of experimented runs (\( u = 1, 2, \ldots, 32 \)).

The maximum dispersion is designated as \( S_{u,max}^2 \). The homogeneity of dispersion of the replicate experiments were verified using the Cochran G-criteria (G-test). The calculated G - Value is given as:

$$G_{cal} = \frac{S_{u,max}^2}{\sum_{u=1}^{N} S_u^2}$$  \hspace{1cm} (4)

The calculated G - value was compared with an appropriate table value. The condition of homogeneity is given as:

$$G_{cal} < G_{\alpha < 1}$$

where, \( \alpha = \) level of significance. If this condition is satisfied then we can proceed with regression analysis. The mean-square-error is given as:

$$S^2_y = \frac{1}{N} \sum_{u=1}^{N} S_u^2$$  \hspace{1cm} (6)

It is the average sample variance estimate. The experimental error is given as
The effects and the sum of squares for each factor were estimated through the contrast associated with effects. The mean effect was given as:

\[ u = 1, 2, \ldots, 32 \] ..........................(8)

where \( X_0 \) are the coded signs. The main effects were estimated by:

\[ b_i = \frac{1}{N} \hat{\alpha}^N_{u=1} (X_{i,u}) \]

\[ i = 1, 2, \ldots, 5; \quad u = 1, 2, \ldots, 32 \] ..............................(9)

where \( X_i \) are the coded signs.

The \( k \)-factor interactions were estimated by:

\[ b_{i,j,\ldots,k} = \frac{1}{N} \hat{\alpha}^N_{u=1} (X_{i,j,\ldots,k,u}) \]

\[ i = 1, 2, \ldots, 5; \quad u = 1, 2, \ldots, 32; \quad i \neq j \neq \ldots \neq k \] (10)

where \( X_{i,j,\ldots,k} \) are the coded signs in the \( X_{i,j,\ldots,k} \) columns of the design matrix.

The quantities in brackets in equations (8), (9) and (10) are called contrast in the treatment combinations.

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

\[ b_i \pm t_{\frac{\alpha}{2}, N-r} S_{b_i} \] .................................(11)

where \( S_{b_i} \) is the estimated standard error in regression coefficients \( b_i \)’s,

\[ t_{\frac{\alpha}{2}, N-r} \text{ is an appropriate tabulated } t \text{- criterion with } N(r-1) \text{ degree of freedom.} \]

For full-factorial experiments error in each regression coefficient is the same and is determined by:

\[ S_{b_0} = S_b = \ldots = S_{b_{i,j,k}} = \frac{S_y}{\sqrt{N\cdot r}} \] ..........................(12)

where, \( S_y \) = the experimental error.
The statistical significance of the regression coefficients are tested by:

\[ t_{i,j,\ldots,k} = \frac{|b_{i,j,\ldots,k}|}{S_{b_{i,j,\ldots,k}}} \]  

(13)

where, \( |b_{i,j,\ldots,k}| \) is the absolute value of the estimate of the coefficient being checked. The calculated t-values were compared with the appropriate critical value found from standard t-tables. A coefficient is considered significant if:

\[ t_{cal} > t_{\alpha, N(r-1)\text{df}} \]  

(14)

For any coefficient that was statistically insignificant, such a coefficient was left out of the regression models. The summary of the estimated effects, confidence interval and the t-values are presented in Table 5. Using only the statistically regression coefficients, the fitted models were then used to generate the predicted values, and the residuals which are used to examine the adequacy of the models.

The adequacy of the fitted models were evaluated using the null hypothesis (H₀: \( b_{i,\ldots,k} = 0 \)) on the individual regression coefficients. The analysis of variance (ANOVA) was used 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 determined by:

\[ SS_{b_{i,\ldots,k}} = \frac{r}{N} (\text{contrast})^2 \]  

(15)

and has a single degree freedom. The regression sums of squares for the models is the summation of the sums of squares for the individual effects:

\[ SS_r = SS_{b_1} + SS_{b_2} + \cdots + SS_{b_{i,\ldots,k}} \]  

(16)

The total sum of squares were calculated by:

\[ SS_T = \sum_{u=1}^{N} y_{uv}^2 - \frac{(\sum_{u=1}^{N} y_{uv})^2}{N \cdot r} \]  

(17)

The error sums of squares were given as

\[ SS_E = SS_T - \hat{\alpha} \quad SS_{b_{i,\ldots,k}} = SS_T - SS_r \]  

(18)
The calculated F-values are given by:

\[ F_{cal} = \frac{MS_R}{MS_E} = \frac{SS_R/dF_R}{SS_E/dF_E} \]  

(19)

where, \( dF_R \) = the degree of Freedom regression = 1, \( dF_E \) = the degree of Freedom error = \( N(r-1) \). The calculated F-values are compared with the appropriate critical table value. The null hypothesis was rejected if:

\[ F_{cal} > F_{[dF_R, dF_E, N(r-1)]} \]  

(20)

with the conclusion that the coefficient contributes significantly to the regression (Douglas, 1991; Olorunsogo, 1998). The complete analysis of variance (ANOVA) is summarized in Table 5.

The adequacy of the models were further validated by calculating the dispersion of adequacy for the replicated experiments and comparing the magnitudes with the variance estimates given by the mean squared error. The dispersion of adequacy is given by:

\[ SS_{ad} = \frac{r}{N-l} \sum_{u=1}^{N} (\hat{y}_u - \bar{y}_u)^2 \]  

(21)

where \( l \) = number of inadequate regression coefficients. The adequacy of the models are confirmed by the Fisher’s test:

\[ F_{cal} = \frac{SS_{ad}}{S_y^2} \]  

(22)

where \( S_y^2 \) = variance estimate given by the mean squared error (i.e. eqn. 6). The calculated F-values were then compared with the appropriate table values.

The condition of adequacy is

\[ F_{cal} \leq F_{[r, N-l, N(r-1)]} \]  

(23)

If this condition is satisfied then we conclude that the fitted models are adequate.

Applying eqns. (1) - (23) to the ascorbic acid level data for the fruit juices (Table 4), the fitted model was found to be:
Table 4: The Estimated Effects, Confidence Interval and t-values

<table>
<thead>
<tr>
<th>Regression coefficient</th>
<th>Estimated Effect</th>
<th>Confidence Interval</th>
<th>t-value (Calculated Table value/1.670)</th>
</tr>
</thead>
<tbody>
<tr>
<td>b0</td>
<td>4.47</td>
<td>±0.05</td>
<td>149.00</td>
</tr>
<tr>
<td>b1</td>
<td>-1.15</td>
<td>±0.05</td>
<td>38.33</td>
</tr>
<tr>
<td>b2</td>
<td>0.245</td>
<td>±0.05</td>
<td>8.00</td>
</tr>
<tr>
<td>b3</td>
<td>2.66</td>
<td>±0.05</td>
<td>22.00</td>
</tr>
<tr>
<td>b4</td>
<td>-0.11</td>
<td>±0.05</td>
<td>3.67</td>
</tr>
<tr>
<td>b5</td>
<td>0.40</td>
<td>±0.05</td>
<td>13.33</td>
</tr>
<tr>
<td>b12</td>
<td>0.16</td>
<td>±0.05</td>
<td>5.33</td>
</tr>
<tr>
<td>b13</td>
<td>-0.13</td>
<td>±0.05</td>
<td>4.33</td>
</tr>
<tr>
<td>b14</td>
<td>0.11</td>
<td>±0.05</td>
<td>3.67</td>
</tr>
<tr>
<td>b15</td>
<td>-0.43</td>
<td>±0.05</td>
<td>13.33</td>
</tr>
<tr>
<td>b23</td>
<td>-0.08</td>
<td>±0.05</td>
<td>2.67</td>
</tr>
<tr>
<td>b24</td>
<td>0.75</td>
<td>±0.05</td>
<td>25.00</td>
</tr>
<tr>
<td>b25</td>
<td>-0.08</td>
<td>±0.05</td>
<td>2.67</td>
</tr>
<tr>
<td>b34</td>
<td>-0.07</td>
<td>±0.05</td>
<td>2.33</td>
</tr>
<tr>
<td>b35</td>
<td>0.51</td>
<td>±0.05</td>
<td>17.00</td>
</tr>
<tr>
<td>b45</td>
<td>0.13</td>
<td>±0.05</td>
<td>4.33</td>
</tr>
<tr>
<td>b123</td>
<td>0.18</td>
<td>±0.05</td>
<td>6.00</td>
</tr>
<tr>
<td>b124</td>
<td>-0.70</td>
<td>±0.05</td>
<td>23.33</td>
</tr>
<tr>
<td>b125</td>
<td>-0.23</td>
<td>±0.05</td>
<td>7.67</td>
</tr>
<tr>
<td>b134</td>
<td>-0.28</td>
<td>±0.05</td>
<td>9.33</td>
</tr>
<tr>
<td>b135</td>
<td>-0.39</td>
<td>±0.05</td>
<td>3.00</td>
</tr>
<tr>
<td>b145</td>
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<td>±0.05</td>
<td>7.33</td>
</tr>
<tr>
<td>b234</td>
<td>0.48</td>
<td>±0.05</td>
<td>16.00</td>
</tr>
<tr>
<td>b235</td>
<td>0.01</td>
<td>±0.05</td>
<td>0.33*</td>
</tr>
<tr>
<td>b245</td>
<td>0.40</td>
<td>±0.05</td>
<td>13.33</td>
</tr>
<tr>
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<td>±0.05</td>
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<td>±0.05</td>
<td>4.67</td>
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<td>b2345</td>
<td>0.43</td>
<td>±0.05</td>
<td>14.33</td>
</tr>
<tr>
<td>b12345</td>
<td>-0.22</td>
<td>±0.05</td>
<td>7.33</td>
</tr>
</tbody>
</table>

*Statistically insignificant
Table 5: ANOVA for Replicated 2-Factorial Pineapple Juice Experiment

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>Effect</th>
<th>Sum of Squares (SS)</th>
<th>Degree of Freedom (df)</th>
<th>Mean Squares (MS)</th>
<th>F-ratio</th>
<th>Calculated Value</th>
<th>Table values (5%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>b1</td>
<td>-1.15</td>
<td>126.75</td>
<td>1</td>
<td>126.75</td>
<td>2018.31</td>
<td>3.9947</td>
<td>3.9947</td>
</tr>
<tr>
<td>b2</td>
<td>0.24</td>
<td>5.57</td>
<td>1</td>
<td>5.57</td>
<td>88.69</td>
<td></td>
<td></td>
</tr>
<tr>
<td>b3</td>
<td>0.66</td>
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<td>41.78</td>
<td>665.29</td>
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</tr>
<tr>
<td>b4</td>
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<td>1.09</td>
<td>1</td>
<td>1.09</td>
<td>17.36</td>
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<td></td>
</tr>
<tr>
<td>b5</td>
<td>0.40</td>
<td>15.05</td>
<td>1</td>
<td>15.05</td>
<td>239.65</td>
<td></td>
<td></td>
</tr>
<tr>
<td>b12</td>
<td>0.16</td>
<td>2.55</td>
<td>1</td>
<td>2.55</td>
<td>40.61</td>
<td></td>
<td></td>
</tr>
<tr>
<td>b13</td>
<td>-0.13</td>
<td>1.52</td>
<td>1</td>
<td>1.52</td>
<td>24.20</td>
<td></td>
<td></td>
</tr>
<tr>
<td>b14</td>
<td>0.11</td>
<td>1.17</td>
<td>1</td>
<td>1.17</td>
<td>18.63</td>
<td></td>
<td></td>
</tr>
<tr>
<td>b15</td>
<td>-0.43</td>
<td>17.52</td>
<td>1</td>
<td>17.52</td>
<td>278.98</td>
<td></td>
<td></td>
</tr>
<tr>
<td>b23</td>
<td>-0.08</td>
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<td>1</td>
<td>0.57</td>
<td>9.08</td>
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</tr>
<tr>
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<td>0.75</td>
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<td>1</td>
<td>53.87</td>
<td>857.80</td>
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<td>1</td>
<td>0.65</td>
<td>10.35</td>
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</tr>
<tr>
<td>b34</td>
<td>-0.07</td>
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<td>1</td>
<td>0.50</td>
<td>7.96</td>
<td></td>
<td></td>
</tr>
<tr>
<td>b35</td>
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<td>1</td>
<td>25.37</td>
<td>403.98</td>
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<tr>
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<td>0.13</td>
<td>1.63</td>
<td>1</td>
<td>1.63</td>
<td>25.96</td>
<td></td>
<td></td>
</tr>
<tr>
<td>b123</td>
<td>0.18</td>
<td>3.06</td>
<td>1</td>
<td>3.06</td>
<td>48.73</td>
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</tr>
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<td>46.75</td>
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*Insignificant at 5%
DISCUSSION AND INTERPRETATION OF MODEL

Equation (24) expresses the fitted model for predicting ascorbic acid level in pineapple juice under non-refrigerated storage and distribution conditions. From the statistical analysis, only the regression coefficient \( b_{235} \) was found statistically insignificant at confidence coefficient \( \alpha = 0.05 \). All the main effects and the other interactions have significant influence on the level of the ascorbic acid of pineapple juice under non-refrigerated storage and distribution conditions. However, storage temperature (with coefficient \( b_1 = 1.5 \)) and pH (with coefficient \( b_3 = 0.66 \)) have higher influence on ascorbic acid level of the juice. High level of storage temperature will lead to drastic reduction in the ascorbic acid level of the juice whereas pH has positive influence on the ascorbic acid level. The interaction of brix value and quantity of antioxidant (with coefficient \( b_{24} = 0.75 \)) has positive influence on the ascorbic acid level whereas the interaction of storage temperature, brix value and quantity of antioxidant (with coefficient \( b_{124} = -0.70 \)) has negative influence.

Comparing the predicted values based on the fitted model with the mean experimental value for the thirty-two experimental runs, it can be seen that storage and distribution condition of experiment 31 (with predicted value, \( \hat{Y}_{31} = 11.69 \) mg/100 ml) maintains the ascorbic acid level at the optimal level. The condition is: 20°C storage temperature, 18° Brix value, a pH of 4.5, 0.1g/litre of antioxidant, and a maximum storage duration of 16 days.

CONCLUSION

The results of the pineapple experiments and the developed model confirm 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 pineapple fruit juice. These quality variables enable the prediction of shelf-life of the juice under non-refrigeration storage and distribution. The developed model is valid only for values of variables that fall within the intervals used in producing it. It is purely for non-refrigeration storage and distribution of pineapple juice.
REFERENCES


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