Development and validation of empirical models for the prediction of selected antioxidants in stored oranges

Sunmonu Musliu Olushola[1], Olaniyan Mathew Adesoji[2], Aduba Joseph Junior[3]

(1. *Department of Agricultural & Biosystems Engineering, University of Ilorin, Nigeria;*
2. *Department of Agricultural & Bioresources Engineering, Federal University Oye-Ekiti, Nigeria*;
3. *National Centre for Agricultural Mechanization, PMB 1525, Ilorin, Kwara State, Nigeria*)

Abstract: Empirical models were developed to predict some selected antioxidants present in stored oranges under a modified atmosphere. Three sets of four different types of passive evaporative cooling structures made of two different materials; clay and aluminium were designed and constructed as part of the study. One set consisted of four separate cooling chambers. Two cooling chambers were made with aluminium container (cylindrical and rectangular shapes) and the other two were made of clay container (cylindrical and rectangular). These four containers were separately inserted inside a bigger clay pot inter-spaced with clay soil of 5 cm (to form tin-in-pot, pot-in-pot, tin-in-wall and wall-in wall) with the outside structure wrapped with jute sack. The other two sets followed the same pattern with interspacing of 7 and 10 cm respectively. The set with 7 cm interspace served as the control in which the interspace soil and the jute sacks were constantly wetted at intervals of two to four hours depending on the rate of evaporation with water at room temperature. The other two sets (5 and 10 cm interspaced soil) were constantly wetted with salt solution (Table salt (Nacl)) at the same interval to keep the soil in moist condition. Freshly harvested matured oranges were used for the experiments and the temperature and relative humidity were monitored daily. The vitamin A, C and E, bacterial and fungal counts of this produce were determined at intervals of three days for a period of 21 days. Mathematical models (using essential regression software package) were developed to predict the vitamin A, vitamin C, and vitamin E contents of the stored oranges at various conditions considered in the study. The existence and sufficiency of the regression models given in the equations were also examined using the analysis of variance (ANOVA) of the multiple regression models. The models were found to be at 5% level of significant. The models were validated using pair-wise T-test and the results of the pair-wise shows that there is no significance difference between the mean of observed and the predicted for all the models developed. The R_{adj}^2 value obtained were 82.43%, 86.63% and 76.48% for vitamin A, vitamin C, vitamin E contents respectively for the stored oranges.

Keywords: model, validation, predicted, structures, oranges

Citation: Sunmonu, M. O., Olaniyan, A. M, Aduba, J. J. 2014. Development and validation of empirical models for the prediction of selected antioxidants in stored oranges. Agric Eng Int: CIGR Journal, 16(2): 151－156.

1 Introduction

An orange, specifically sweet orange (*Citrus sinensis*) is the most commonly grown fruit in the world (Morton, 1987). Fruits and vegetables are important to human health. They contain antioxidants, minerals and phytochemicals in their correct combination that help to keep the blood sugar in balance, create energy in the body and build up the immune system (Hartman, 2000). Fruits and vegetables lower incidence of obesity, high blood pressure, type two diabetes, colon and prostate cancer, osteoporoses, asthma, and many more (Barber and Barber, 2002). Antioxidants are nutrients that block some of the damage caused by free radicals, which are by-products that result when our bodies transform food into energy. The build up of these by-products over time is largely responsible for the ageing process and can contribute to the development of various health conditions such as

Received date: 2013-12-06 **Accepted date:** 2014-04-29
Corresponding author: Sunmonu Musliu Olushola, Department of Agricultural & Biosystems Engineering, University of Ilorin, Nigeria. Email: sholams2000@yahoo.co.uk.

cancer, heart disease and a host of inflammatory conditions like arthritis. Antioxidants are very important in maintaining good health and it is the general names for the vitamins, minerals and carotenoids that protect the body from harmful free radicals (Harman, 1992). A large number of antioxidants in food contribute to disease prevention and these include vitamin A, vitamin C, vitamin E and carotenoids as the major nutrients (Padayatty et al., 2003; Brigelius et al., 1999). The major line of defence against free radical damage is the presence of antioxidants which can be found in diets. There are thousands of antioxidants but the best known are vitamin A, vitamin C, vitamin E and lycopene (Coulter et al., 2006). Each of these antioxidant nutrients has specific activities and they often work synergistically to enhance antioxidant capability of the body (Langseth, 1996).

Adequate storage involves proper regulation of temperature, humidity, air circulation, proper stacking pattern, regular inspection, and prompt produce disposal as soon as maximum storage life has been attained. The problems of fruits and vegetables in storage are majorly centered on the environmental condition of the fruits represented by temperature and relative humidity. Therefore, the most important conditions are: temperature of the storage environment must be very low, relative humidity of the storage environment must be very high, initial quality of the fruit should be very high, fruits should be well handled and mechanical damage should be avoided while harvesting as this increases the spoilage risk of the fruits. Once a crop is harvested, it is almost impossible to improve its quality. Proper storage conditions are needed to lengthen storage life and maintain quality once the crop has been cooled to the optimum storage temperature. Fresh fruits and vegetables are living tissues although they are no longer attached to the plant. They breathe, just like humans do, and their composition and physiology continue to ripen and finally, they begin to die as a result of cellular breakdown which are inevitable but can be slowed with optimal storage (Karren, 1991). At high relative humidity, produce maintain saleable weight, appearance, nutritional quality and flavour while wilting, softening and juiciness are reduced. Also low temperature slows down the growth of pathogenic fungi which cause spoilage of fruits and vegetables in storage (Hardenburg, 1986).

Temperature and relative humidity are the most important environmental factors which influence the deterioration rate of harvested perishable crops such as fruits and vegetables. Harvested fruits are living organs; they continue to respire and lose water as if they were still attached to the parent plant. The only difference is that losses are not replaced in the postharvest environment. They therefore change after harvest. These changes include the utilization of energy reserves through respiration, changes in texture associated with both water loss and biochemical change and the increased ethylene production associated with ripening of climacteric fruits (Mitra, 1997). Relative humidity is defined as the amount of water present in air relative to the maximum amount that the air can hold at that particular temperature. It is usually expressed as a percentage; small fluctuations in temperature can cause wide fluctuations in relative humidity.

A predictive model is made up of a number of predictors, which are variable factors that are likely to influence future behaviour or results (Tijskens et al., 2001). This study is focused on empirical models developed from various data generated from the evaporative cooling structures.

2 Materials and Methods

Mature green oranges were obtained from Minna Central Market in Niger State and transported in wooden crates to the laboratory. In the laboratory, mechanically damaged samples were removed and the undamaged samples were washed in running tap water. One hundred and twenty fresh orange fruits were divided into four lots of thirty and put in the designed and constructed passive evaporative coolers. The fresh oranges were stored for a period of 21 days.

2.1 Temperature and relative humidity

Temperature and relative humidity measurements were taken three times daily at 8:00 am, 12:00 at noon and 6:00 pm using HT-6290 humidity/temperature meter (manufactured in Australia by Esis Pty Ltd with sensitivity of ±0.5°C and ±1% for the temperature and relative humidity, respectively). This is a digital instrument that gives accurate humidity and temperature measurements. It has probes for every application (www.esis.cam.au).

2.2 Nutritional values

These were determined in the laboratory using AOAC (1996) nutritional guidelines. The nutritional values determined are vitamin A, vitamin C and vitamin E. They were determined at intervals of three days.

2.3 Preparation of salt solution

About 15000 parts/millions (ppm) solution of sodium chloride (NaCl) was prepared by dissolving 225 g of NaCl in 15 L of water at room temperature and 450 g of NaCl in 30 L of water at room temperature for keeping the four structures in moist condition in the 5 and 10 cm soil inter-spaces, respectively. The four structures in the 7 cm soil inter space were kept in moist condition using 20 L of water.

2.4 Microbial analysis

Ten grams each of fruit samples were suspended in a 90 mL of sterile distilled water and was homogenized. The suspension was filtered through sterile wool and was serially diluted under aseptic solution. The total fungal and bacterial plate counts were determined using the methods of Collins et al. (2004).

2.5 Bulk density and moisture content

The bulk density and moisture contents of the soil were determined in the laboratory using AOAC (1979) instruction guidelines.

2.6 Fitting data

Experimental data were fitted to the mathematical models developed using Essential regression (software package). The degree of fitness of the model was determined using (R^2), thereafter the models were validated using pair-wise t-test to compare the mean values of the observed and predicted data.

3 Results and discussion

The data generated from the experiment is presented in Table 1. From the Essential regression analysis, best models were selected for vitamin A, vitamin C and vitamin E contents of stored oranges based on their higher values of R^2 as is shown in Equations (1) to (3).

$$\text{VIT\_A} = 3.18\text{E}{+}01 - 2.62\text{E}{-}04T^2R - 2.17\text{E}{-}03\sigma MB + 1.53\text{E}{-}01S\gamma F,\ R^2 = 82.43\% \quad (1)$$

$$\text{VIT\_C} = 2.06\text{E}{+}01 + 2.75\text{E}{-}04T^2M + 5.15\text{E}{-}02\gamma TF - 5.52\text{E}{-}05R^2\sigma,\ R^2 = 86.63\% \quad (2)$$

$$\text{VIT\_E} = 3.23\text{E}{+}00 - 6.86\text{E}{-}05T^2\sigma + 1.33\text{E}{-}03TSF,\ R^2 = 76.48\% \quad (3)$$

where, T = temperature, °C; R = Relative Humidity, %; S = Soil Inter-space, cm; ?? = Storage Structure (1 = Tin in pot or Pot in pot, 2 = Tin in wall or wall in wall); γ = Material Component (1 = Aluminium component, 2 = Clay component), F = Fungal Count(ppm/ml), σ = Bacterial Count, cfu/mL; M = Soil Moisture Content, %; B = Bulk Density, g/cm^3.

Table 1 Experimental data for stored oranges

SP	SI	SS	M	T	RH	FC	BC	SMC	BD	VIT_A	VIT_C	VIT_E
1	1	1	1	30.20	70.80	2.10	22.00	78.40	0.98	11.76	34.51	1.60
5	1	1	1	29.50	68.60	1.60	21.00	81.58	1.16	10.98	41.44	2.00
8	1	1	1	29.60	66.30	1.80	17.00	82.56	0.91	12.14	39.70	2.30
11	1	1	1	29.30	56.90	2.40	14.00	79.12	0.98	16.74	38.74	2.20
15	1	1	1	29.40	65.10	1.80	20.00	79.68	0.99	18.12	38.86	1.88
18	1	1	1	25.70	72.80	2.20	24.00	79.74	0.97	12.54	31.64	1.90
21	1	1	1	28.70	75.80	1.80	20.00	79.74	0.92	11.30	32.42	2.60
1	1	1	2	29.90	68.90	1.70	23.00	78.40	0.98	14.62	36.72	1.70
5	1	1	2	29.90	63.40	1.80	24.00	81.58	1.16	12.62	42.33	1.00
8	1	1	2	29.30	69.00	2.50	19.00	82.56	0.91	11.42	39.42	2.30
11	1	1	2	29.20	59.10	2.20	17.00	79.12	0.98	18.63	39.04	2.40
15	1	1	2	29.50	67.10	2.70	24.00	79.68	0.99	14.56	45.98	1.74
18	1	1	2	25.70	73.80	1.80	17.00	79.74	0.97	14.76	34.54	2.60
21	1	1	2	28.90	77.00	2.40	24.00	79.74	0.92	12.52	39.68	1.80
1	1	2	1	30.40	63.90	1.60	24.00	78.40	0.98	11.56	34.32	2.10
5	1	2	1	30.30	66.90	2.10	22.00	81.58	1.16	12.32	39.56	2.60
8	1	2	1	28.40	70.10	2.40	18.00	82.56	0.91	11.12	40.04	2.60
11	1	2	1	29.30	61.00	2.40	14.00	79.12	0.98	16.98	34.32	2.10
15	1	2	1	29.50	63.60	2.20	18.00	79.68	0.99	18.14	36.54	2.60

SP	SI	SS	M	T	RH	FC	BC	SMC	BD	VIT_A	VIT_C	VIT_E
18	1	2	1	25.90	72.70	1.80	22.00	79.74	0.97	14.54	32.78	2.70
21	1	2	1	28.40	74.20	2.60	24.00	79.74	0.92	12.30	33.98	2.00
1	1	2	2	30.80	65.10	2.00	22.00	78.40	0.98	14.76	46.31	1.60
5	1	2	2	30.40	56.20	2.00	24.00	81.58	1.16	13.14	39.50	1.40
8	1	2	2	28.30	73.90	1.80	21.00	82.56	0.91	12.32	43.32	2.10
11	1	2	2	28.00	63.00	2.20	22.00	79.12	0.98	16.94	36.66	2.30
15	1	2	2	29.40	67.30	2.00	14.00	79.68	0.99	16.52	49.47	2.20
18	1	2	2	26.00	78.20	2.60	19.00	79.74	0.97	16.72	32.98	2.00
21	1	2	2	28.40	80.00	1.80	22.00	79.74	0.92	11.54	34.94	2.40
1	2	1	1	29.30	68.70	1.80	22.00	80.47	0.99	10.98	35.98	2.10
5	2	1	1	30.70	65.80	2.00	22.00	79.87	0.88	11.06	41.53	2.40
8	2	1	1	29.90	66.20	2.20	19.00	79.28	1.00	12.08	39.68	2.20
11	2	1	1	29.00	61.20	2.40	16.00	82.74	0.88	16.66	38.12	2.40
15	2	1	1	29.20	67.60	2.00	19.00	79.92	1.00	17.98	38.76	2.10
18	2	1	1	25.90	74.50	2.40	22.00	80.49	0.78	16.24	34.84	2.10
21	2	1	1	28.30	80.00	2.00	21.00	79.98	0.98	11.42	31.78	2.60
1	2	1	2	29.30	67.00	1.60	23.00	80.47	0.99	12.78	34.33	1.90
5	2	1	2	29.60	69.60	2.10	23.00	79.87	0.88	13.04	41.76	1.80
8	2	1	2	29.70	71.00	1.80	21.00	79.28	1.00	10.98	39.61	2.70
11	2	1	2	28.70	62.60	2.00	18.00	82.74	0.88	17.98	39.62	2.60
15	2	1	2	28.70	68.70	2.60	26.00	79.92	1.00	15.06	44.72	1.98
18	2	1	2	25.80	75.30	2.20	19.00	80.49	0.78	15.04	38.61	2.60
21	2	1	2	28.40	83.00	2.40	22.00	79.98	0.98	12.32	30.80	2.20
1	2	2	1	29.10	66.40	2.10	23.00	80.47	0.99	13.33	32.62	2.60
5	2	2	1	29.30	67.90	2.30	24.00	79.87	0.88	12.46	40.72	1.90
8	2	2	1	30.00	63.90	2.60	21.00	79.28	1.00	11.62	41.62	2.70
11	2	2	1	28.10	60.00	2.60	21.00	82.74	0.88	16.78	35.54	2.10
15	2	2	1	28.80	67.20	2.40	19.00	79.92	1.00	17.88	35.76	2.70
18	2	2	1	25.90	73.80	2.00	24.00	80.49	0.78	15.00	33.34	2.60
21	2	2	1	28.20	77.90	2.40	26.00	79.98	0.98	12.32	32.94	2.10
1	2	2	2	29.70	69.10	2.10	22.00	80.47	0.99	14.10	45.14	1.60
5	2	2	2	29.90	67.80	2.10	21.00	79.87	0.88	13.16	39.32	1.20
8	2	2	2	30.20	65.80	2.00	20.00	79.28	1.00	12.18	44.16	2.40
11	2	2	2	28.10	64.50	2.20	26.00	82.74	0.88	16.84	37.78	2.70
15	2	2	2	28.60	71.50	2.40	21.00	79.92	1.00	16.42	42.06	2.60
18	2	2	2	26.00	77.20	2.40	24.00	80.49	0.78	16.16	32.78	2.10
21	2	2	2	28.20	83.60	2.00	19.00	79.98	0.98	11.08	33.32	2.70
1	3	1	1	30.50	60.90	2.10	23.00	78.73	1.42	12.32	36.62	1.40
5	3	1	1	30.70	68.20	2.00	22.00	79.17	1.21	11.12	41.21	1.80
8	3	1	1	29.70	65.80	2.40	21.00	78.18	1.34	11.74	39.66	2.60
11	3	1	1	28.60	61.00	2.60	14.00	78.34	1.00	17.04	39.12	2.40
15	3	1	1	28.60	70.90	2.20	19.00	82.54	0.98	18.14	39.04	2.40
18	3	1	1	26.00	74.60	2.40	22.00	81.79	0.98	16.66	33.78	2.20
21	3	1	1	28.40	78.60	2.20	22.00	81.36	0.97	11.34	31.54	2.70
1	3	1	2	30.40	66.60	2.10	24.00	78.73	1.42	13.88	36.34	2.10
5	3	1	2	29.90	63.90	1.60	22.00	79.17	1.21	12.40	42.08	1.30
8	3	1	2	29.80	69.00	2.40	22.00	78.18	1.34	11.52	39.48	3.00
11	3	1	2	30.00	60.30	2.00	22.00	78.34	1.00	18.03	39.10	2.70
15	3	1	2	28.40	72.40	2.20	24.00	82.54	0.98	14.66	44.02	2.00
18	3	1	2	25.90	76.00	2.00	21.00	81.79	0.98	14.98	34.12	2.40
21	3	1	2	28.30	84.90	2.20	26.00	81.36	0.97	12.40	31.16	1.90
1	3	2	1	29.70	65.30	2.20	24.00	78.73	1.42	12.21	34.59	1.90
5	3	2	1	30.40	64.90	2.10	23.00	79.17	1.21	12.72	40.82	1.60
8	3	2	1	30.00	63.80	2.80	18.00	78.18	1.34	11.14	42.94	2.70
11	3	2	1	28.20	63.50	2.40	22.00	78.34	1.00	16.88	36.32	2.20
15	3	2	1	28.40	71.00	2.60	16.00	82.54	0.98	18.10	36.44	2.40
18	3	2	1	25.80	78.50	2.20	21.00	81.79	0.98	14.64	33.84	2.40
21	3	2	1	28.20	81.60	2.20	21.00	81.36	0.97	12.32	33.42	2.00
1	3	2	2	30.10	65.70	1.80	23.00	78.73	1.42	13.22	43.16	2.40
5	3	2	2	29.90	65.00	2.10	23.00	79.17	1.21	13.18	39.60	1.10
8	3	2	2	30.00	65.80	2.40	19.00	78.18	1.34	12.21	45.32	2.20
11	3	2	2	28.20	61.80	2.20	20.00	78.34	1.00	17.06	37.90	3.62
15	3	2	2	28.30	73.30	2.00	20.00	82.54	0.98	15.89	44.12	2.40
18	3	2	2	25.90	78.10	2.60	20.00	81.79	0.98	16.22	33.12	2.10
21	3	2	2	28.30	83.00	1.80	20.00	81.36	0.97	11.54	33.54	2.30

Note: SP = Storage Period (Days), T = temperature (^oC), Rh = Relative Humidity (%), FC = Fungal Count (ppm/mL), BC = Bacterial Count (cfu/mL), SMC = Soil Moisture Content (%), BD = Bulk Density (g/cm^3). SI = Soil Inter-space (1, 2 and 3 represent 5, 7and 10 cm respectively), SS = Storage Structures (1 represents tin-in-pot and pot-in-pot which are cylindrical in shape while 2 represents tin-in-wall & wall-in-wall which are rectangular in shape), M = Material Component for the Storage Structures (1 represents tin component made of aluminium material while 2 represents pot /wall components made of clay material).

The existence and sufficiency of the regression models given in the equations above were also examined using the analysis of variance (ANOVA) of the multiple regression models shown in Table 2. The analysis was carried out using Essential regression computer software package. Regression models are sometimes examined or tested with Analysis of Variance (ANOVA). This is because ANOVA tests the acceptability of the model from a statistical perspective, i.e., it supports the argument that the model fits well. Also the analysis of variance table (Table 2) which tests the acceptability of the model from a statistical perspective was seen to be significant at 1% for all the selected models. By substituting different values of predictor variables/factors (materials, temperature, relative humidity, storage structures, fungal and bacterial counts) into the model equations, the expected values of vitamin A, vitamin C and vitamin E contents of stored oranges were predicted. The predicted and measured (observed) values were plotted (Figures 1 to 3) for stored oranges. The graphs suggest a very close relationship between the observed and the predicted- an indication of a good fit.

Table 2 Analysis of variance of the multiple regressions on nutritional parameters as a function of stored oranges

Variable	Source	Df	SS	MS	F	F Sig.
VIT_ A	Regression	3	245.91	81.97	28.77	0.001*
	Residual	80	227.96	2.850		
	Total	83	473.88			
VIT_C	Regression	3	746.20	248.73	27.85	0.001*
	Residual	80	714.40	8.930		
	Total	83	1460.6			
VIT_E	Regression	2	3.612	1.806	11.45	0.001*
	Residual	81	12.78	0.158		
	Total	83	16.39			

Note: *significant at 5% level.

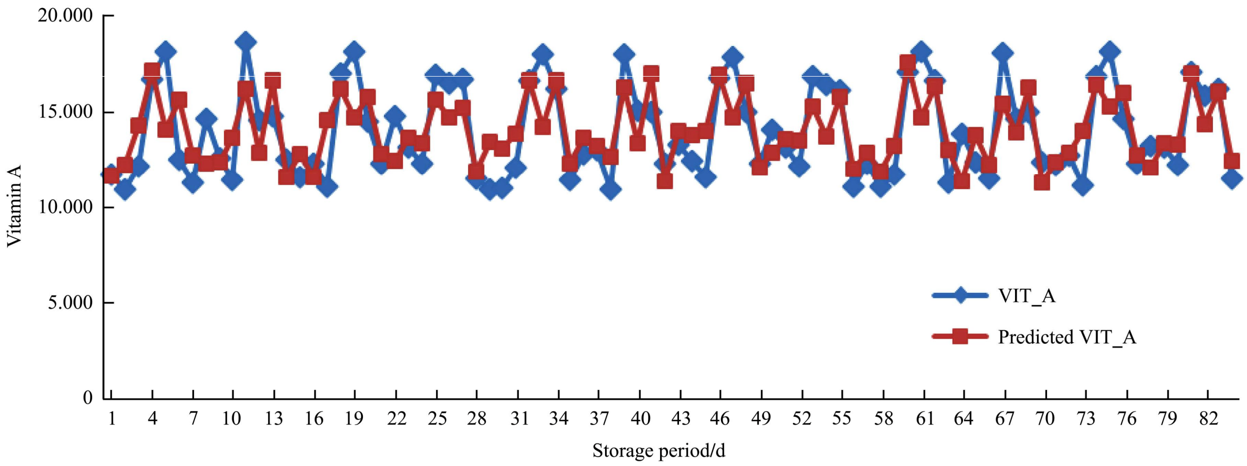

Figure 1 Predicted and observed values of vitamin A for stored orange

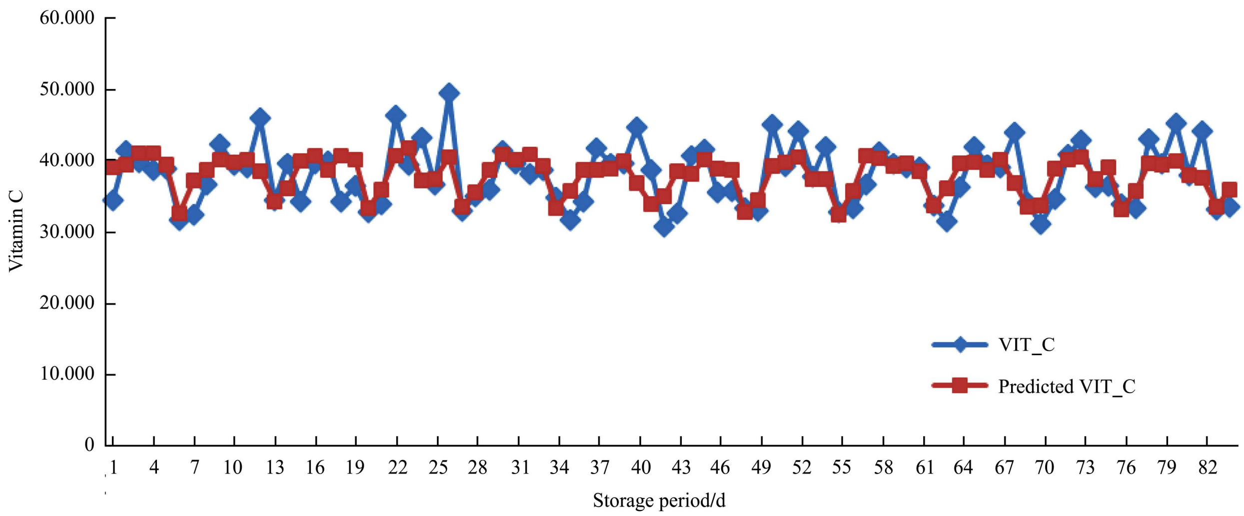

Figure 2 Predicted and observed values of vitamin C for stored orange

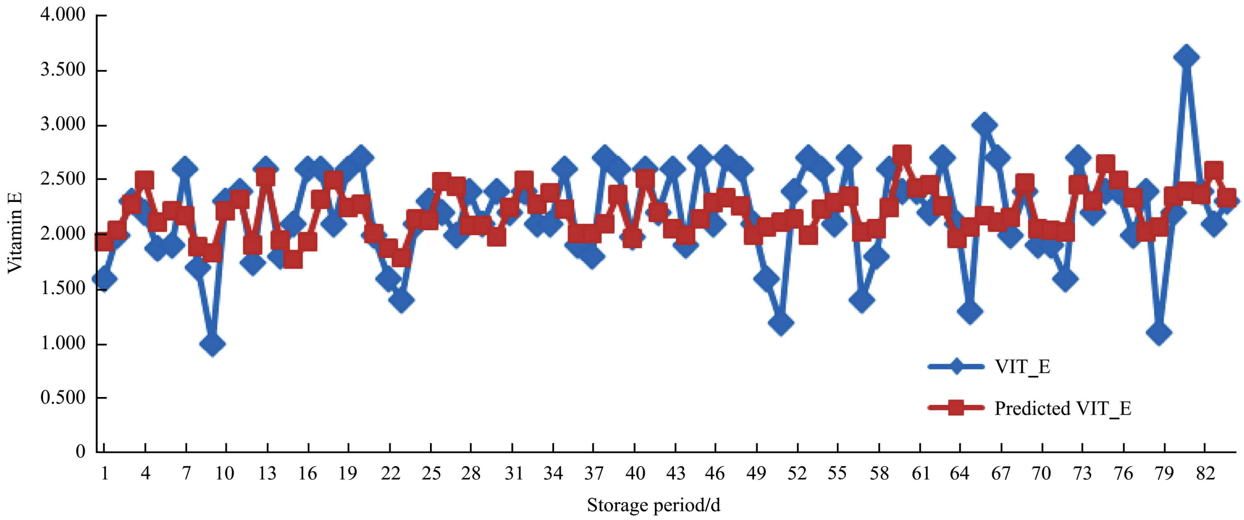

Figure 3 Predicted and observed values of vitamin E for stored orange

4 Conclusions

This research focused on the development and validation of an empirical model for the prediction of selected antioxidants present in stored oranges. The mathematical models developed are reasonably accurate to predict the storability of fruits and vegetables in passive evaporative cooling structures. Also, the model performance was found to be satisfactory and showed good predictability.

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