

## Optimization of important production factors of a non-alcoholic beverage from roselle calyx, sorghum stem sheath and local spices

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### Abstract

RSM was used to optimize the important processing variables for a non-alcoholic beverage (NAB) from roselle calyx, sorghum stem sheath and two local spices. A CCRD consisting of six variables reduced to five for mathematical convenience [Roselle Calyx/Sorghum Stem Sheath, (RC/SSS, 0-75/25-100 g), ginger (0-1.50 g/100ml), Alligator Pepper (AP, 0-1.50 g/100ml), Extraction Temperature (ET, 80-100°C) and Time of Extraction (TOE, 20-40 min)] with five coded levels (-2, -1, 0, +1, +2) were studied with two replications, making 54 experiments. Mathematical models were developed for the responses [Vitamin C (VC), Total Carotenoids (TC), 1, 1-Diphenyl-2-picrylhydrazyl (DPPH), Total Phenols (TP), pH, Total Titratable Acidity (TTA)] for making a prediction during NAB production. Storage stability indices [pH, VC, TTA, Total Viable (TVC) and coliform counts] of the beverage were monitored for 28 days. The optimum processing variables were RC/SSS, 66.67 g/33.33 g; ginger, 0.375 g/100ml; AP, 0.375 g/100ml; ET, 85°C; and TOE, 35 min, yielding actual values of 15.33, 1757.2, 0.306 (mg/100ml), 89.21% for VC, TC, TP and DPPH respectively. The developed mathematical models for the measured responses could be successfully used for their prediction during non-alcoholic beverage production with a correlation coefficient ( $R^2$ ) ranging from 0.84-0.99. Reduction in pH (4.98-2.66) and VC (15.33 -12.69 mg/100 ml) were observed while TTA increased (1.70-5.02%) during storage. There was no coliform growth throughout the storage period, however, after 7 days, TVC was  $7 \times 10^2$  CFU/ml.

## 1. Introduction

Beverages are consumed to provide refreshment, hydration, increase well-being and to prevent the occurrence of nutrition-related disorders (Tenge and Geiger, 2001). Non-alcoholic beverages (NABs) are recognized because they contribute to household nutrition and they perform a special role in hydrating the body (EFSA, 2011). NABs are also rich in essential amino acids, vitamins and minerals, therefore they are used in nutrition-enhancing programs (Dharmasena, 2010). In the Western markets, beverage consumers prefer drinks that have healthful benefits to the commonly consumed soft drinks. The human health depends on the quality of the consumed beverages (WCRF, 2011). Consumers are increasingly aware of the impact of diet on their health and well-being. The

increase in health awareness has resulted in a shift from consumption of soft drinks which have been linked to certain diseases to health-promoting beverages.

The imbalance between pro-oxidants and antioxidants in an organism that causes oxidative stress is considered to play a very important role in the pathogenesis of several chronic diseases, such as diabetes, cancer, and cardiovascular diseases, including arterial sclerosis, aging and neurodegenerative diseases (Bagchi *et al.*, 2000). However, the natural defense in the human body may not be enough for severe oxidative stress (Tedesco *et al.*, 2000). Hence, certain amounts of external antioxidants are constantly required to maintain an adequate level of antioxidants in order to balance the Reactive Oxygen Species-scavengers (ROS) (Tedesco *et al.*, 2000). These antioxidants can be obtained from plant

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food materials such as roselle calyces, sorghum stem sheath, and spices.

Roselle calyx has been used by people in Africa either directly or indirectly in the treatment of several diseases such as abscesses, bilious conditions, cancer, cough, debility, dyspepsia, fever, hangover, heart ailments, hypertension, and neurosis (Tom *et al.*, 2013). The calyx extracts are used for the treatment of several complaints, including high blood pressure and liver diseases. The pharmacological actions of the calyx extracts include strong *in vitro* and *in vivo* antioxidant activity (Ali *et al.*, 2005). Sorghum bicolor leaf sheaths (SBLF) has a high concentration of dimeric 3-deoxyanthocyanidins (Geera *et al.*, 2012). An aqueous extract from sorghum leaf sheaths has hepatoprotective and hematopoietic effects (Akanke *et al.*, 2010). In addition, it supports blood cell generation and hemoglobin-content of erythrocytes, and the leaf base also has central antinociceptive properties (Ogwumike, 2002). Spices inhibit or kill bacteria and fungi that either spoil food or harm humans and also provide micronutrients. They can be used to preserve food and they have antimicrobial properties. Some antioxidants are also found in many spices which can contribute to the body's defense against cardiovascular disease and intestinal cancers. Therefore, the addition of extracts of spices could also control the microbial activities associated with food samples while retaining the nutritive and economic quality of such foods (FAO, 2011).

NABs have been produced from plant materials having high anti-oxidant substances and significant nutrient profiles which include *kunnu*, sorghum stem sheath, roselle calyx and others (Devi *et al.*, 2011). This study, therefore, adopted Response Surface Methodology (RSM) to develop and optimize the processing variables for a functional non-alcoholic beverage from roselle calyx, sorghum stem sheath, and two local spices.

## 2. Materials and methods

The raw materials used were mature reddish purple Sorghum stem sheath (*poporo*), roselle calyx, ginger, alligator pepper and food grade sucrose. All the materials were procured locally in Ogbomoso, southwestern Nigeria. All the reagents used for the study were of analytical grade.

### 2.1 Sample preparation

The roselle calyx and sorghum stem sheath were dusted to remove dirt and dried in the locally fabricated cabinet drier at 50°C for 6 h for moisture uniformity

(Adedeji *et al.*, 2013). Ginger and alligator pepper were dried under the same condition for 48 h to a moisture content of between 10 and 12%. The dried roselle calyx, sorghum stem sheath, ginger and alligator pepper were pulverized/milled into fine powdery form using a locally fabricated hammer mill. The powdered samples were packed in polythene bags before storing in airtight opaque plastic containers until when needed for analysis.

### 2.2 Experimental design

RSM was used to investigate the combined effects of significant variables (roselle calyx, sorghum stem sheath, ginger, alligator pepper, extraction temperature and time) in the production of the non-alcoholic beverage. Based on the previous studies on local non-alcoholic beverages, desirable ranges of the independent variables (factors) were selected at five coded levels (-2, -1, 0, +1, +2). For roselle calyx/sorghum stem sheath ( $X_1$ ), two equations (Equations 1 and 2) were formulated to give the quantity of the plant materials at each of the coded levels. For ginger ( $X_2$ ) and alligator pepper ( $X_3$ ), the values ranged from 0.0 - 1.5 g/100 ml, while extraction temperature ( $X_4$ ) and extraction time ( $X_5$ ) ranged from 80 - 100°C and 20 - 40 min respectively.

$$\text{Roselle calyx} = \frac{X \times 100}{X + 1} \quad (1)$$

$$\text{Sorghum stem sheath} = \frac{100}{X + 1} \quad (2)$$

Where X = The five coded levels (-2, -1, 0, +1, +2)

The experimental design adopted was a fractional Central Composite Rotatable Design (CCRD). Table 1 gives the summary of the coded levels and real values for the fractional CCRD. The experimental runs totaling twenty-seven (27) as obtained from the design is presented in Table 2. The experiments were replicated twice for reproducibility.

The dependent variables (Y) measured were VC, TC, DPPH, TP, pH, and TTA describing the quality characteristics of the non-alcoholic beverage. These dependent variables were individually expressed as a function of the independent variables known as response functions.

### 2.3 The non-alcoholic beverage preparation

Five levels were used for each spice; ginger and alligator pepper (0, 0.375, 0.75, 1.125, 1.5 g/100 ml) using the RSM. The processing conditions studied include extraction temperature (80-100)°C and time (20-40) min for the production. The various spice combinations and processing variables are presented in Table 2. The proportion of roselle calyx and sorghum

Table 1. Coded level and real values for the CCRD experiment

Independent variable	Coded variable	Coded levels and real values				
		-a	-1	0	+1	+a
Roselle calyx (g) sorghum stem sheath (g)	X <sub>1</sub>	0.000 (0/100)	0.750 (42.86/57.14)	1.500 (60/40)	2.250 (69.23/30.77)	3.000 (75/25)
Ginger (mg/100 ml)	X <sub>2</sub>	0.000	0.375	0.750	1.125	1.500
Alligator pepper mg/100 ml)	X <sub>3</sub>	0.000	0.375	0.750	1.125	1.500
Extraction temperature (°C)	X <sub>4</sub>	80	85	90	95	100
Time of extraction (min)	X <sub>5</sub>	20	25	30	35	40

Table 2. Chemical properties of the beverage samples as affected by the processing variables

<sup>a</sup> Run no	Input/independent variables <sup>b</sup>					Response/dependent variables <sup>c</sup>					
	X <sub>1</sub>	X <sub>2</sub>	X <sub>3</sub>	X <sub>4</sub>	X <sub>5</sub>	Y <sub>1</sub>	Y <sub>2</sub>	Y <sub>3</sub>	Y <sub>4</sub>	Y <sub>5</sub>	Y <sub>6</sub>
1	-1	-1	-1	-1	1	11.50	367	75.79	0.11	4.25	0.68
2	1	-1	-1	-1	-1	9.52	1216	80.85	0.14	4.30	1.31
3	-1	1	-1	-1	-1	8.86	472	68.39	0.11	4.50	0.99
4	1	1	-1	-1	1	13.74	1456	64.14	0.18	4.35	1.85
5	-1	-1	1	-1	-1	8.33	1124	68.00	0.13	4.05	1.04
6	1	-1	1	-1	1	8.59	1368	74.42	0.15	4.45	1.35
7	-1	1	1	-1	1	9.65	328	78.28	0.11	4.15	1.03
8	1	1	1	-1	-1	10.44	1160	69.28	0.14	4.05	0.86
9	-1	-1	-1	1	-1	10.31	740	75.71	0.12	5.60	2.48
10	1	-1	-1	1	1	7.54	1184	72.50	0.14	4.45	2.39
11	-1	1	-1	1	1	9.65	240	71.85	0.10	4.20	0.90
12	1	1	-1	1	-1	9.25	201	74.50	0.09	4.15	1.36
13	-1	-1	1	1	1	8.73	1064	60.64	0.13	4.30	1.08
14	1	-1	1	1	-1	12.56	480	74.78	0.15	4.20	0.90
15	-1	1	1	1	-1	11.10	1296	67.71	0.14	4.30	1.22
16	1	1	1	1	1	8.86	1127	72.49	0.13	4.40	1.04
17	-2	0	0	0	0	10.44	724	46.79	0.12	4.50	0.99
18	2	0	0	0	0	7.40	856	81.49	0.14	4.40	1.13
19	0	-2	0	0	0	9.91	1344	64.14	0.15	4.10	1.89
20	0	2	0	0	0	8.86	752	85.70	0.12	4.50	1.62
21	0	0	-2	0	0	11.24	880	74.78	0.13	4.30	1.04
22	0	0	2	0	0	15.99	528	79.56	0.11	4.10	1.76
23	0	0	0	-2	0	10.71	140	76.35	0.08	5.70	1.67
24	0	0	0	2	0	9.91	424	64.78	0.11	4.50	1.22
25	0	0	0	0	-2	10.31	440	73.16	0.11	4.30	0.99
26	0	0	0	0	2	11.89	608	63.50	0.12	4.20	1.71
27	0	0	0	0	0	9.91	1401	68.00	0.16	4.40	1.13

<sup>a</sup>Did not necessarily correspond to the order of experiment.

<sup>b</sup>X<sub>1</sub> = Roselle calyx/sorghum stem sheath, X<sub>2</sub> = Ginger, X<sub>3</sub> = Alligator pepper, X<sub>4</sub> = Extraction temperature, X<sub>5</sub> = Extraction time

<sup>c</sup>Y<sub>1</sub> = Vitamin C, Y<sub>2</sub> = Total carotenoids, Y<sub>3</sub> = DPPH, Y<sub>4</sub> = Total phenols, Y<sub>5</sub> = pH, Y<sub>6</sub> = TTA

These responses were in the range: 7.40-15.99 mg/100 ml, 140-1456 mg/100 ml, 46.79-85.70%, 0.08-0.18 mg/100 ml, 4.05-5.70 and 0.68-2.48% for vitamin C, TC, DPPH, TP, pH and TTA respectively.

stem sheath powder to water used for the beverage formulation was 1:30, based on chemical and sensory evaluation reported by Adedeji *et al.* (2013).

The powdered roselle calyx, sorghum stem sheath and local spices at different proportions were mixed with an appropriate volume of water and extracted at varying temperatures and times as described in Table 2. The extract was filtered with a clean muslin cloth to obtain a clear filtrate. The filtrate was sweetened with food grade sucrose to a Brix level of 10 °. The sweetened beverage was then dispensed into previously sterilized bottles before pasteurization at 75°C for 15 min. The beverage samples were stored in the freezer (-18°C) for about 8 days before analysis.

## 2.4 Chemical analyses of the samples

### 2.4.1 Ascorbic acid determination

The determination of the vitamin C content of the sample was carried out using the technique of iodometric titration (AOAC, 2005). This involved adding about 20 ml of 0.5 ml of H<sub>2</sub>SO<sub>4</sub> solution to 50 ml of the sample, followed by the addition of the standard potassium iodide. Thereafter, about 25 ml of the standard potassium trioxiodate solution was pipetted into the sample solution. The ascorbic acid in the sample was analyzed by generating excess water-iodide solution. The iodine was obtained from a standard solution of potassium trioxiodate and potassium iodide. After the ascorbic acid has reacted, the remainder was titrated with a standard thiosulphate solution. The concentration of the ascorbic acid in the sample was expressed as

$$\frac{\text{mg ascorbic acid}}{100 \text{ ml of sample}} \quad (3)$$

### 2.4.2 Determination of carotenoids by HPLC

Carotenoids were quantified by reversed – phase HPLC (Khachik *et al.*, 1992; Mangels *et al.*, 1993). About 20 ml of ethanol was added to 2 ml of the sample already diluted with 1.8 ml of water. After vortexing for 30 sec, the samples were extracted twice with n-hexane (1 ml each time, stabilized with 0.05% butylated hydroxytoluene (BHT) and vortexed for 30 min. The supernatant was removed, cooled and evaporated under nitrogen and reconstituted with isopropanol and injected into the HPLC system.

### 2.4.3 Determination of DPPH radical scavenging ability

The free radical scavenging ability of the extracts against DPPH (1, 1- Diphenyl -2- picrylhydrazyl) free radical was evaluated as described by Gyamfi (1999).

Appropriate dilution of the extracts (1 ml) was mixed with 1 ml, 0.4 ml methanolic solution containing DPPH radicals, the mixtures were left in the dark for 30 min and the absorbance measured at 516 nm in a spectrophotometer. The percentage DPPH free radical scavenging ability was subsequently calculated.

### 2.4.4 Determination of total phenol content

This was determined according to the method described by (Singleton *et al.*, 1999). Appropriate dilution of the aqueous extracts was oxidized with 2.5 ml of 10% Folin-Ciocalteau's reagent (v/v) and neutralized by 2.0 ml of 7.5 ml sodium carbonate. The reaction mixture was incubated for 40 min at 45°C and the absorbance was measured at 765 nm in the UV-Visible spectrophotometer (JENWAY 6405 Model, UK).

### 2.4.5 pH measurement

About 10 ml of distilled water was added to 5 ml of the sample and stirred. The pH was measured using a pH meter, digital model EA513 – 055, ELE, England standardized with a buffer solution of 4.0 and 7.0 (AOAC, 2005). The glass electrode of pH meter was dipped in about 30 ml of the beverage sample measured into a cuvette at ambient temperature and was allowed to stabilize for sometimes after which the reading was taken.

### 2.4.6 Total titratable acidity

About 10 ml of the beverage sample was mixed with 90 ml of distilled water and about 0.3 ml of indicator solution (1% phenolphthalein in 95% solution of ethanol) and this was titrated against 0.1 M sodium hydroxide solution until a constant pink colour was observed. The amount of acid was calculated using the formula below.

$$\text{Percentage of acid} = \text{number of ml of sodium hydroxide} \times \text{conversion factor} \quad (4)$$

## 2.5 Optimization procedure

Optimization was performed on the basis of a multiple response methods called desirability. VC, TC, DPPH, and TP were maximized, while pH and TTA were set in ranges. The criteria for the optimization were selected on the basis of producing a beverage with high antioxidants for health promotion. The best overall desirability which gave the expected responses corresponded to the optimum processing conditions for the beverage. The measured responses of the produced beverage in the laboratory were validated with the predicted responses at the optimum conditions.

## 2.6 Storage stability

Samples were stored at ambient temperature ( $30 \pm 2^\circ\text{C}$ ) for 28 days and analyzed at 1-week interval for the following parameters: vitamin C, pH and TTA, Total Viable Count (TVC) and Coliform Count, CC [the method described by Harrigan (1998) was used for TVC and CC].

## 2.7 Statistical analysis

Experimental data from the fractional CCRD were analyzed by Analysis of Variance (ANOVA) at 95% confidence interval using RSM to fit the quadratic polynomial equation with the Design Expert software (version 6.0.8, State-Ease, Inc., Statistics Made Easy, Minneapolis, MN).

## 3. Results and discussion

The experimental design and responses for VC, TC, DPPH, TP, pH and TTA are presented in Table 2. Vitamin C content was highest (15.99 mg/100 ml) in the beverage formulation with  $X_1 = 1.5$  g (RC, 60 g, SSS, 40 g),  $X_2 = 0.75$  g/100 ml,  $X_3 = 1.5$  g/100 ml,  $X_4 = 90^\circ\text{C}$  and  $X_5 = 30$  min, while it was lowest (7.40 mg/100 ml) in the beverage formulation of  $X_1 = 2.25$  g (RC, 69.23 g, SSS, 30.77 g),  $X_2 = 0.375$  g/100 ml,  $X_3 = 0.375$  g/100 ml,  $X_4 = 95^\circ\text{C}$  and  $X_5 = 35$  min and  $X_1 = 3.0$  g,  $X_2 = 0.75$  g/100 ml,  $X_3 = 0.75$  g/100 ml,  $X_4 = 90^\circ\text{C}$  and  $X_5 = 30$  min. The high value of vitamin C in the first beverage might be attributed to relatively high vitamin C content of RC, ginger and alligator pepper. Whereas, the low concentration of ginger and alligator pepper with high extraction temperature and time could have contributed to the reduction in vitamin C in the second beverage sample.

This observation corroborated with the report of Ashaye *et al.* (2006) that vitamin C is unstable at elevated temperature. Also, vitamin C in solution degrades at high temperature (Spínola *et al.*, 2013) to 3-deoxythreosone. The observed decrease in vitamin C content may indicate degradation. The vitamin C content of the non-alcoholic beverage increased significantly ( $p < 0.05$ ) from 7.40 – 15.99 mg/100 ml, with an average value of 10.75 mg/100 ml. The average vitamin C content of the non-alcoholic beverage was higher than the value (9.33 mg/100g) reported by Amoo (2006) for *zobo* drink. Ali *et al.* (2005) reported that the vitamin C status of *zobo* beverages can be improved by blending with spices such as ginger. Vitamin C also plays an important role in immune function and improves absorption of non-heme iron (Jacob and Stoudeh, 2009).

It is involved in protein metabolism and it is an important physiological antioxidant which helps to prevent molecular changes caused by oxidation (Li and Schellhorn, 2007; Jacob and Stoudeh, 2009).

The total carotenoids content of the non-alcoholic beverage produced ranged from 140 - 1456 mg/100 ml, with an average value of 1065.78 mg/100 ml. It was observed that the total carotenoids were at the highest value of 1456 mg/100 ml in the beverage with formulation of  $X_1 = 2.25$  g (roselle calyx, 69.23 g, Sorghum stem sheath, 30.77 g),  $X_2 = 1.125$  g/100 ml,  $X_3 = 0.375$  g/100 ml,  $X_4 = 85^\circ\text{C}$ ,  $X_5 = 35$  min. The lowest value of the total carotenoids was 140 mg/100 ml in the beverage with formulation of  $X_1 = 1.5$  g (roselle calyx, 60 g, sorghum stem sheath, 40 g),  $X_2 = 0.75$  g/100 ml,  $X_3 = 0.75$  g/100 ml,  $X_4 = 80^\circ\text{C}$ ,  $X_5 = 30$  min. The results showed that the total carotenoids content was enhanced at moderate extraction temperature ( $85^\circ\text{C}$ ) and extraction time (35 min). Previous studies have shown the presence of carotenoids in sorghum stem sheath beverage (Rock *et al.*, 2001; Adedeji *et al.*, 2013).

The average carotenoids content was found to be higher than the carotenoids content reported by Adedeji *et al.* (2013) for sorghum stem sheath beverage spiced with ginger. Carotenoids present in sorghum stem sheath include red, yellow and orange pigments which are widely distributed in nature and serve many functions in plants life (essential for photosynthesis) and play a major role in the photo-protection of cells and tissues by neutralizing oxidant (Rock *et al.*, 2001). The beta-carotene in the sorghum stem sheath plays an essential role as the main dietary source of vitamin A, which helps eyesight (El-Sohemmy *et al.*, 2002). Epidemiological studies provide information that carotenoids and other antioxidants may protect humans against certain types of cancer and cardiovascular diseases thus revealing its potential in promoting the health of the Nigerian populace if adopted as an indigenous beverage (Adedeji *et al.*, 2013).

DPPH had peak value of 85.70% in the sample with  $X_1 = 1.5$  g (roselle calyx, 60 g, Sorghum stem sheath, 40 g),  $X_2 = 1.5$  g/100 ml,  $X_3 = 0.75$  g/100 ml,  $X_4 = 90^\circ\text{C}$  and  $X_5 = 30$  min, while the lowest DPPH value of 46.79% was recorded in the sample with  $X_1 = 0$  g (roselle calyx, 0 g, sorghum stem sheath, 100 g),  $X_2 = 0.75$  g/100 ml,  $X_3 = 0.75$  g/100 ml,  $X_4 = 90^\circ\text{C}$  and  $X_5 = 30$  min. This low value could be attributed to the non-inclusion of roselle calyx, (which was zero) in the beverage. The DPPH radical scavenging activity of the beverage samples ranged from 46.79-85.70%, with an

average value of 67.97%. The DPPH of the beverage samples were much higher than that of soymilk 7.2% (Wiwat, 2008). This observation agrees with the report of Wood and Pitler (2000) that spices have superior antioxidant capacity than many plant materials. Spices also have essential oil which is effective in scavenging free radical and has the potential to be powerful antioxidant (Colin-González *et al.*, 2012).

Total phenols was highest value (0.18 mg/100 ml) in sample with  $X_1 = 2.25$  g (roselle calyx, 69.23 g, Sorghum stem sheath, 30.77 g),  $X_2 = 1.125$  g/100 ml,  $X_3 = 0.375$  g/100 ml,  $X_4 = 85$  °C and  $X_5 = 35$  min while the lowest value (0.08 mg/100 ml) was observed in sample with  $X_1 = 1.5$  g (roselle calyx, 60 g, Sorghum stem sheath, 40 g),  $X_2 = 0.75$  g/100 ml,  $X_3 = 0.75$  g/100 ml,  $X_4 = 80$  °C and  $X_5 = 30$  min. The presence of antioxidants in roselle calyx, Sorghum stem sheath, ginger and alligator pepper must be responsible for the relatively high value.

A significant increasing trend in the total phenol content with increasing concentration of alligator pepper and ginger at moderate extraction temperature and time was observed. This observation confirmed earlier report that spices have the superior antioxidant capacity to berries, other fruits, vegetables and nuts (Wood and Pitler, 2000). Phenolics were identified as components with the strongest antioxidant capacity in fruits, vegetables, and mushrooms (Heo *et al.*, 2007). Jacob – Velzquez and Isneros- zevallos (2009) reported that total phenolic contents of carrots under different storages had a linear relationship with the anti-oxidant activities.

The pH value was highest (5.7) in the beverage formulation with  $X_1 = 1.5$  g (RC, 60 g, SSS, 40 g),  $X_2 = 0.75$  g/100 ml,  $X_3 = 0.75$  g/100 ml,  $X_4 = 80$  °C and  $X_5 = 30$  min, while the sample with the lowest pH (4.05) was noted in the formulation with  $X_1 = 2.25$  g (RC, 69.23 g, SSS, 30.77 g),  $X_2 = 1.125$  g/100 ml,  $X_3 = 1.125$  g/100 ml,  $X_4 = 85$  °C and  $X_5 = 25$  min. It was observed that the pH of the beverage samples decreased with increase in the concentration of RC, ginger, and AP, while the increase in the concentration of SSS increased the pH of the beverage. A decrease in pH is synonymous to increase in acidity (Ade-Omowaye *et al.*, 2015). The low pH could be attributed to the effect of RC extract which has been reported to be very acidic (Oluwaniyi, 2009).

The beverage samples had an average pH of 4.4. Fasoyiro *et al.* (2005) reported that the pH of RC drink ranged from 2.19 to 3.62. Oluwaniyi (2009) also reported that the pH of *zobo* drink ranged from 1.6 to 2.7, while SSS drink ranged from 6.2 to 8.4. Majority of

NABs contain high concentrations of simple carbohydrates, preserving agents, which make the product acidic; which could afterward exact a negative impact on health and well-being. The pH of *zobo* drink was reported to range from 3.13 to 3.28 (Bolade *et al.*, 2009), while carrot – orange drink was 2.5 to 5.0 (Karangwa *et al.*, 2010). However, the pH of the formulated beverage sample showed that the beverage could be a suitable alternative or substitute for acidic NABs.

TTA was highest (2.48%) in the beverage formulation with  $X_1 = 0.75$  g (RC, 42.86 g, SSS, 57.14 g),  $X_2 = 0.375$  g/100 ml,  $X_3 = 0.375$  g/100 ml,  $X_4 = 95$  °C and  $X_5 = 25$  min. It was very low (0.68%) in the beverage formulation with  $X_1 = 0.75$  g (RC, 42.86 g, SSS, 57.14 g),  $X_2 = 0.375$  g/100 ml,  $X_3 = 0.375$  g/100 ml,  $X_4 = 85$  °C and  $X_5 = 35$  min, depicting increase in TTA with increased extraction temperature. An average TTA of 1.24% was obtained, which was slightly higher than the prescribed range (0.39-1.1%) for NABs. The TTA of cooked ginger drink was reported to be 0.52%, while that of uncooked ginger drink was 0.21% (Hazarika *et al.*, 2013).

Tables 3 -7 show the ANOVA results to establish predictive models (equations 5 - 10) for the responses measured in the non-alcoholic beverage obtained from the various levels of the processing variables. The response surface plots in 3 dimensions showing the effect of roselle calyx/sorghum stem sheath and extraction time on the non-alcoholic beverage is shown in Figures 1 and 2.

Positive sign in front of the factors in equations 5 - 10 signifies a synergistic effect, while negative sign signifies an antagonistic effect. The positive factors are important parameters responsible for increasing each of the responses and vice versa.

### 3.1 Optimization

The optimum conditions for independent variables and the predicted values of the responses were as follows: RC/SSS, 66.67 g/33.33 g; ginger, 0.375 g/100 ml; AP, 0.375 g/100ml; ET, 85°C; and TOE, 35 min, and 11.32, 1472, 0.16 (mg/100ml), and 73.24% for VC, TC, TP and DPPH respectively. A verification experiment at the optimum conditions, consisting of 3 runs, was performed and the measured responses were 15.33, 1757.2, 0.31 (mg/100 ml) and 89.21% for VC, TC, TP, and DPPH respectively. These results of the conducted experiments showed that these optimized conditions can be used for the beverage production yielding high values

Table 3. Analysis of variance for vitamin C

Variable	Sum of Square	D O F	Mean Square	F value	Prob. > F
Model	172.31	25	6.89	26.78	<0.0001*
X <sub>1</sub>	3.25	1	3.25	12.61	0.0014*
X <sub>2</sub>	3.41	1	3.41	13.25	0.0011*
X <sub>3</sub>	5.24	1	5.24	20.38	0.0001*
X <sub>4</sub>	0.38	1	0.38	1.46	0.2368
X <sub>5</sub>	1.51	1	1.51	5.86	0.0222*
X <sub>1</sub> X <sub>2</sub>	1.71	1	1.71	6.63	0.0156*
X <sub>1</sub> X <sub>4</sub>	3.84	1	3.84	14.93	0.0006*
X <sub>1</sub> X <sub>5</sub>	1.98	1	1.98	7.67	0.0098*
X <sub>2</sub> X <sub>4</sub>	3.16	1	3.16	12.26	0.0016*
X <sub>2</sub> X <sub>5</sub>	5.45	1	5.45	21.19	< 0.0001*
X <sub>3</sub> X <sub>4</sub>	15.39	1	15.39	59.79	< 0.0001*
X <sub>3</sub> X <sub>5</sub>	15.39	1	15.39	59.79	< 0.0001*
X <sub>4</sub> X <sub>5</sub>	27.36	1	27.36	106.31	< 0.0001*
Residual	7.21	28	0.26		
Lack of Fit	6.3	1	6.3	186.7	< 0.0001
Pure Error	0.91	27	0.034		
Cor Total	179.52	53			

DOF = Degrees of Freedom

R<sup>2</sup> = 0.9599; Adj R<sup>2</sup> = 0.9240 ; Pred R<sup>2</sup> = 0.8598

For coded factors: X<sub>1</sub> = *Hibiscus sabdariffa* and Sorghum Stem Sheath, X<sub>2</sub> = Ginger, X<sub>3</sub> = Alligator pepper, X<sub>4</sub> = Extraction temperature, X<sub>5</sub> = Extraction time

\*Significant at 95% confidence level

Table 4. Analysis of variance for total carotenoids

Variables	Sum of Square	D O F	Mean Square	F value	Prob. > F
Model	8021000	25	320900	8.59	< 0.0001*
X <sub>1</sub>	655600	1	655600	17.55	0.0003*
X <sub>3</sub>	652900	1	652900	17.48	0.0003*
X <sub>4</sub>	231400	1	231400	6.19	0.019*
X <sub>1</sub> X <sub>3</sub>	458400	1	458400	12.27	0.0016*
X <sub>1</sub> X <sub>4</sub>	1327000	1	1327000	35.52	< 0.0001*
X <sub>1</sub> X <sub>5</sub>	1722000	1	1722000	46.1	< 0.0001*
X <sub>3</sub> X <sub>4</sub>	160500	1	160500	4.3	0.0475*
X <sub>4</sub> X <sub>5</sub>	228500	1	228500	6.12	0.0197*
Residual	1046000	28	37358.8		
Lack of Fit	1011000	1	1011000	787.37	< 0.0001
Pure Error	34681	27	1284.48		
Cor Total	9067000	53			

DOF = Degrees of Freedom

R<sup>2</sup> = 0.8846; Pred. R<sup>2</sup> = 0.7816; Adj. R<sup>2</sup> = 0.6001

For coded factors: X<sub>1</sub> = *Hibiscus sabdariffa* and Sorghum Stem Sheath, X<sub>2</sub> = Ginger, X<sub>3</sub> = Alligator pepper, X<sub>4</sub> = Extraction temperature, X<sub>5</sub> = Extraction time

\*Significant at 95% confidence level

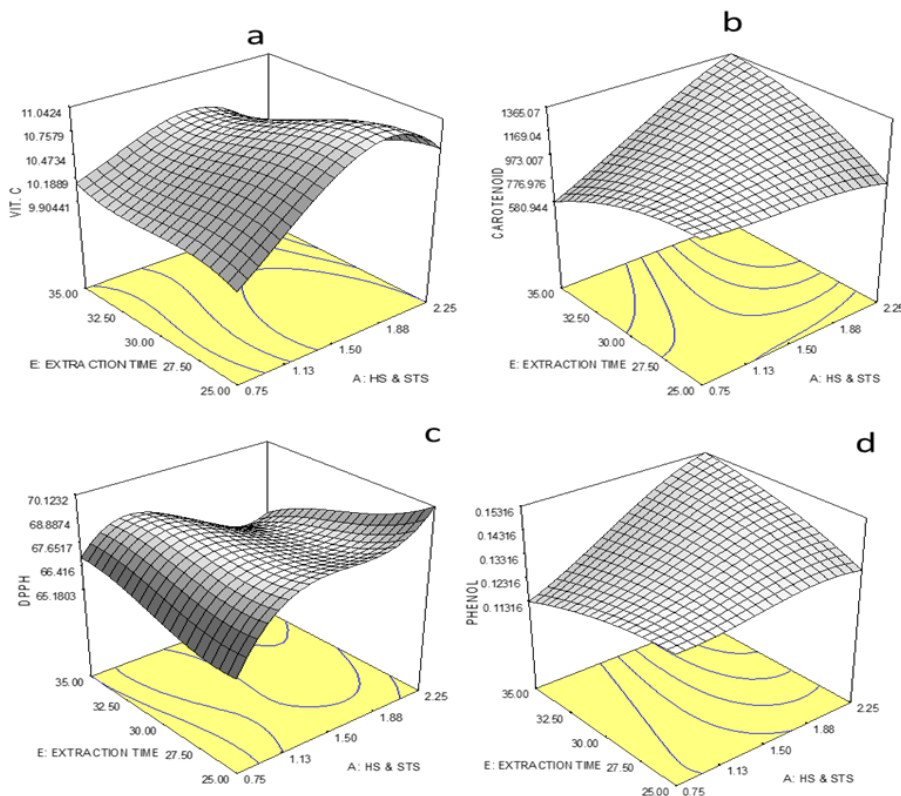


Figure 1. Response surface plots for the interaction of Roselle calyx/Sorghum stem sheath and extraction time (X<sub>1</sub>X<sub>5</sub>) on: (a) Vitamin C, (b) total carotenoids, (c) DPPH, and (d) total phenols

Table 5. Analysis of variance for DPPH

Input variable	Sum of Squares	D O F	Mean Square	F value	Prob. > F
Model	3203.54	25	128.14	349.69	< 0.0001*
X <sub>1</sub>	36.14	1	36.14	98.62	< 0.0001*
X <sub>2</sub>	156.38	1	156.38	426.74	< 0.0001*
X <sub>3</sub>	57.91	1	57.91	158.04	< 0.0001*
X <sub>1</sub> X <sub>2</sub>	100.15	1	100.15	273.29	< 0.0001*
X <sub>1</sub> X <sub>3</sub>	32.10	1	32.10	87.60	< 0.0001*
X <sub>1</sub> X <sub>4</sub>	50.18	1	50.18	136.93	< 0.0001*
X <sub>1</sub> X <sub>5</sub>	63.42	1	63.42	173.08	< 0.0001*
X <sub>2</sub> X <sub>3</sub>	160.43	1	160.43	437.80	< 0.0001*
X <sub>2</sub> X <sub>4</sub>	59.49	1	59.49	162.34	< 0.0001*
X <sub>2</sub> X <sub>5</sub>	65.87	1	65.87	179.75	< 0.0001*
X <sub>3</sub> X <sub>4</sub>	49.08	1	49.08	133.93	< 0.0001*
X <sub>3</sub> X <sub>5</sub>	56.84	1	56.84	155.13	< 0.0001*
X <sub>4</sub> X <sub>5</sub>	56.42	1	56.42	153.96	< 0.0001*
Residual	10.26	28	0.37		
Lack of Fit	0.017	1	0.017	0.045	0.8334
Pure Error	10.24	27	0.38		
Cor Total	3213.80	53			

DOF = Degrees of Freedom

R<sup>2</sup> = 0.9968; Pred. R<sup>2</sup> = 0.9940; Adj. R<sup>2</sup> = 0.9876

For coded factors: X<sub>1</sub> = *Hibiscus sabdariffa* and Sorghum Stem Sheath, X<sub>2</sub> = Ginger, X<sub>3</sub> = Alligator pepper, X<sub>4</sub> = Extraction temperature, X<sub>5</sub> = Extraction time

\*Significant at 95 % confidence level

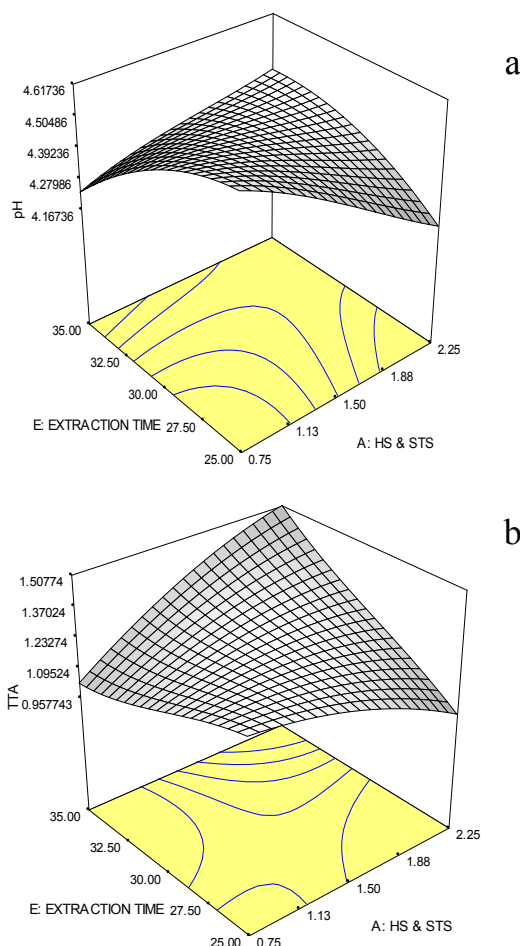
Table 6. Analysis of variance for total phenols

Input variable	Sum of Squares	Degrees of Freedom	Mean Square	F value	Prob. > F
Model	0.020	25	7.977E-004	6.01	<0.00016*
X <sub>1</sub>	3.403E-003	1	3.403E-003	25.63	< 0.0001*
X <sub>3</sub>	1.736E-003	1	1.736E-003	13.08	0.0012*
X <sub>1</sub> X <sub>4</sub>	1.953E-003	1	1.953E-003	14.71	0.0007*
X <sub>1</sub> X <sub>5</sub>	2.628E-003	1	2.628E-003	19.80	0.0001*
X <sub>2</sub> X <sub>4</sub>	7.031E-004	1	7.031E-004	5.30	0.0290*
X <sub>3</sub> X <sub>4</sub>	1.653E-003	1	1.653E-003	12.45	0.0015*
X <sub>3</sub> X <sub>5</sub>	1.128E-003	1	1.128E-003	8.50	0.0069*
Residual	3.717E-003	28	1.328E-004		
Lack of Fit	3.117E-003	1	3.117E-003	140.28	< 0.0001
Pure Error	6.000E-004	27	2.222E-005		
Cor Total	0.024	53			

R<sup>2</sup> = 0.8429; Pred. R<sup>2</sup> = 0.7026; Adj. R<sup>2</sup> = 0.4513

For coded factors: X<sub>1</sub> = *Hibiscus sabdariffa* and Sorghum Stem Sheath, X<sub>2</sub> = Ginger, X<sub>3</sub> = Alligator pepper, X<sub>4</sub> = Extraction temperature, X<sub>5</sub> = Extraction time

\*Significant at 95 % confidence level

Figure 2. Response surface plots for the interaction of Roselle calyx/Sorghum stem sheath and extraction time (X<sub>1</sub>X<sub>5</sub>) on the (a) pH (b) TTA



$$\begin{aligned} \text{Vitamin C (Y}_1\text{)} &= 10.75 + 0.45X_1 + 0.46X_2 - 0.57X_3 - 0.15X_4 - 0.31X_5 + 0.23X_1X_2 + 0.18X_1X_3 \\ &- 0.35X_1X_4 - 0.25X_1X_5 - 0.05X_2X_3 - 0.31X_2X_4 + 0.41X_2X_5 + 0.69X_3X_4 \\ &- 0.69X_3X_5 - 0.92X_4X_5 \end{aligned} \tag{5}$$

$$\begin{aligned} \text{Total carotenoids (Y}_2\text{)} &= 1065.78 + 202.42X_1 - 55.92X_2 + 202X_3 - 120.3X_4 + 23.1X_5 + 40.88X_1X_2 \\ &- 19.69X_1X_3 + 203.6X_1X_4 + 232X_1X_5 + 63.31X_2X_3 + 3.38X_2X_4 - 25X_2X_5 \\ &+ 70.8X_3X_4 - 49.44X_3X_5 + 84.5X_4X_5 \end{aligned} \tag{6}$$

$$\begin{aligned} \text{DPPH (Y}_3\text{)} &= 67.95 - 1.50X_1 - 3.13X_2 - 1.90X_3 + 0.22X_4 + 0.04X_5 - 1.77X_1X_2 + 1.00X_1X_3 \\ &+ 1.25X_1X_4 - 1.41X_1X_5 + 2.24X_2X_3 + 1.36X_2X_4 + 1.43X_2X_5 - 1.24X_3X_4 \\ &+ 1.33X_3X_5 - 1.33X_4X_5 \end{aligned} \tag{7}$$

$$\begin{aligned} \text{Total Phenols (Y}_4\text{)} &= 0.14 + 0.02X_1 - 2.5E - 003X_2 + 0.01X_3 - 5.8E - 003X_4 + 1.25E - 003X_5 \\ &- 9.38E - 004X_1X_2 - 2.8E - 003X_1X_3 - 7.8E - 003X_1X_4 + 9.1E - 003X_1X_5 \\ &+ 3.13E - 004X_2X_3 - 4.69E - 003X_2X_4 + 3.44E - 003X_2X_5 + 7.19E - 003X_3X_4 \\ &- 5.94E - 003X_3X_5 - 9.38E - 004X_4X_5 \end{aligned} \tag{8}$$

$$\begin{aligned} Y_{pH} &= +4.44 - 0.087X_1 - 0.15X_2 - 0.15X_3 + 0.22X_4 - 0.042X_5 + 0.038X_1X_2 + 0.094X_1X_3 \\ &- 0.094X_1X_4 + 0.16X_1X_5 + 0.081X_2X_3 - 0.094X_2X_4 + 0.044X_2X_5 - 0.038X_3X_4 \\ &+ 0.13X_3X_5 - 0.075X_4X_5 \end{aligned} \tag{9}$$

$$\begin{aligned} Y_{TTA (coded)} &= +1.24 + 0.04X_1 - 0.06X_2 - 0.26X_3 + 0.14X_4 + 0.04X_5 - 0.057X_1X_2 - 0.19X_1X_3 \\ &- 0.04X_1X_4 + 0.2X_1X_5 + 0.16X_2X_3 - 0.23X_2X_4 + 0.1X_2X_5 - 0.21X_3X_4 + 0.11X_3X_5 \\ &- 0.14X_4X_5 \end{aligned} \tag{10}$$

Table 7. Analysis of variance for TTA

Input variable	Sum of Squares	D OF	Mean Square	F value	Prob.> F
Model	11.48	25	0.46	43.34	< 0.00018
X <sub>1</sub>	0.028	1	0.028	2.62	0.1166
X <sub>2</sub>	0.057	1	0.057	5.36	0.0281*
X <sub>3</sub>	1.11	1	1.11	104.37	< 0.0001*
X <sub>4</sub>	0.33	1	0.33	30.84	< 0.0001*
X <sub>1</sub> X <sub>2</sub>	0.064	1	0.064	6.03	0.0205*
X <sub>1</sub> X <sub>3</sub>	1.17	1	1.17	110.82	< 0.0001*
X <sub>1</sub> X <sub>4</sub>	0.049	1	0.049	4.61	0.0406*
X <sub>1</sub> X <sub>5</sub>	1.31	1	1.31	123.45	< 0.0001*
X <sub>2</sub> X <sub>3</sub>	0.79	1	0.79	74.61	< 0.0001*
X <sub>2</sub> X <sub>4</sub>	1.70	1	1.70	160.19	< 0.0001*
X <sub>2</sub> X <sub>5</sub>	0.33	1	0.33	30.77	< 0.0001*
X <sub>3</sub> X <sub>4</sub>	1.39	1	1.39	131.20	< 0.0001*
X <sub>3</sub> X <sub>5</sub>	0.41	1	0.41	38.43	< 0.0001*
X <sub>4</sub> X <sub>5</sub>	0.64	1	0.64	59.98	< 0.0001*
Residual	0.30	28	0.011		
Lack of Fit	0.12	1	0.12	17.94	0.0002
Pure Error	0.18	27	6.602E-003		
Cor Total	11.78	53			

DOF = Degrees of Freedom

R<sup>2</sup> = 0.9748; Pred. R<sup>2</sup> = 0.9523; Adj. R<sup>2</sup> = 0.9098

For coded factors: X<sub>1</sub> = *Hibiscus sabdariffa* and Sorghum Stem Sheath, X<sub>2</sub> = Ginger, X<sub>3</sub> = Alligator pepper, X<sub>4</sub> = Extraction temperature, X<sub>5</sub> = Extraction time

\*Significant at 95% confidence interval

for the responses. A correlation of the predicted and measured responses gave a high R<sup>2</sup> of 0.9999 (Figure 3).

### 3.2 Storage stability of the beverage samples

The result of the storage study is presented in Table 8. The value of TTA increased progressively while pH and vitamin C content decreased gradually as storage period progressed. There was a reduction in vitamin C from 14.82 to 12.69 mg/100 ml during the 4th week of the storage period. This reduction could be attributed to the nutrient depletion and the effect of a by-product of metabolism on food items as reported by Nwafor and Ogiehor (2003) and possible utilization by associated microorganisms, bioconversion into organic acids and other organic compounds. Similar observations have been reported for related food items by Omobuwajo *et al.* (1993). These may partly explain the significant decrease in pH as storage period progressed which is also an indication of increased acidity. A similar trend was reported for TTA in SSS beverage by Ade-Omowaye *et al.* (2015).

The results of the microbial analysis showed that there were no observable bacteria and coliform growths in the fresh beverage. This is an indication that the beverage was produced hygienically. Bacterial growth was detected in the beverage after the first two weeks (1.0x10<sup>2</sup> CFU/ml) and it increased (7.0 x 10<sup>2</sup> CFU/ml) after the 4th week. The microbial load obtained after the 4th week of storage is within the safe limits of 1.0 x 10<sup>5</sup> CFU/ml (Ogbuile *et al.*, 2005).

Samples of roselle calyx liquor and sorghum bicolor treated with 1.0% *Aframomum melegueta* had average microbial loads of 3.70 and 4.10 x10<sup>2</sup> CFU/ml, respectively (Oluwalana and Adedeji, 2014). Result obtained has shown a clear trend of microbial growth

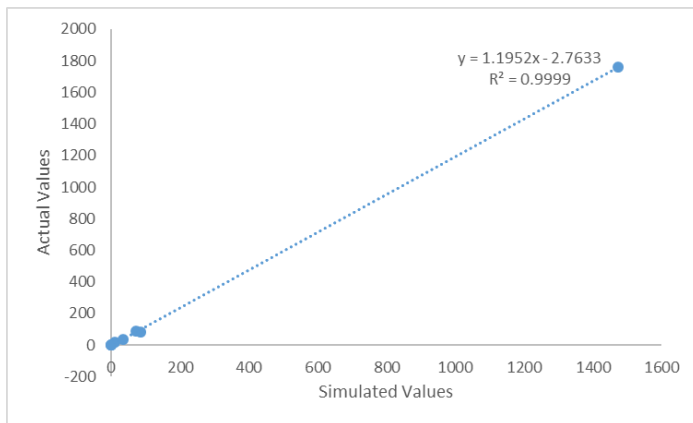


Figure 3. Graph of predicted optimized sample against actual experimental values

Table 8. Effect of storage time on the optimized beverage sample

Parameter	Week 0	Week 1	Week 2	Week 3	Week 4
pH	4.19	4.19	3.11	2.81	2.66
Vit C (mg/100 ml)	14.82	13.21	13.09	12.92	12.69
TTA (%)	1.70	1.98	2.97	3.50	5.09
Total viable count (CFU/ml) x 10 <sup>2</sup>	-	-	1	2	7
Total coliform count (CFU/ml) x 10 <sup>2</sup>	-	-	-	-	-

suppression with an increase in the percentage of inclusion of natural preservatives to the beverage samples (Power *et al.*, 1995). The addition of ginger and alligator pepper to the beverage produced showed that the spices have antimicrobial activity. Ginger extract has been shown to elongate the shelf life of roselle calyx and sorghum bicolor liquor from two to four weeks (Adegoke and Skura, 1994). Ginger has been shown to possess anti-tumor, anti-proliferative, antihypertensive, bactericidal and nematocidal properties due to its 6-gingerol, 6-paradol, shagaols and zingiberene contents (Ben-Nwadia *et al.*, 2005).

Similar findings have been documented for roselle calyx drink (Ogiehor *et al.*, 2008) in which shelf-life was extended by a period of two weeks with the inclusion of 0.2% ginger extract. There was no coliform growth in the beverage throughout the period of storage.

#### 4. Conclusion

RSM was successfully applied to optimize the processing variables for the production of a non-alcoholic beverage from roselle calyx, Sorghum stem sheath, and two local spices. The processing variables employed in this study had a great effect on the quality

of the beverage. To a certain level, the vitamin C, total carotenoids, DPPH and total phenols increased. Modeling of experimental data allowed the generation of useful equations for later usage, in order to predict the behavior of the system under different factor combinations.

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