Development of a Functional non-Alcoholic Beverage from Sorghum Stem Sheath and Local Spices

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Abstract: **Optimum conditions for the production of a non-alcoholic beverage from Sorghum Stem Sheath (SSS) and three local spices were investigated. The drink was developed from combinations of important variables, Alligator Pepper, AP (0.00, 0.75, 1.50 g/100 ml), ginger (0.00, 0.75, 1.50 g/100 ml), garlic (0.00, 0.75, 1.50 g/100 ml), Extraction Temperature, ET (80, 90, 100 ^oC) and Time of Extraction, TOE (10, 25, 40 min) based on a modification of fractional central composite rotatable design with 32 runs in three replicates using Design-Expert software (version 6.0.8). Response Surface Methodology (RSM) was applied as an optimization technique over eight response variables: Total Phenols Content (TPC), Vitamin C Content (VCC), 1,1-diphenyl -2-picryl hydrazyl (DPPH), Total Carotenoids Content (TCC), Zinc Content (ZC), Iron Content (IC), Oxalate Content (OC) and Tannin Content (TC). Predictive models for the response variables were developed as a function of the process variables. The conventional graphical method was applied to obtain maximum TPC, VCC, DPPH, TCC and IC, and minimum OC. Second-order polynomials obtained to predict the response variables were all significant ($p<0.05$) with good correlation coefficients (R^2) between 0.895 and 0.937 showing that the models can be used to navigate the design space. Contour plots of each of the response variables were utilized, applying superposition surface methodology to obtain three contour plots for observation and selection of the best (optimum) combination of AP, ginger, garlic, ET and TOE as 1.50 mg/100 ml, 1.49 mg/100 ml, 0.15 mg/100 ml, 80 °C and 40 min, respectively. This combination produced optimized drink with TPC, VCC, DPPH, TCC, IC and OC of 0.22 mg/100 ml, 3.54 mg/100 ml, 61.65%, 699.46 mg/100 ml, 4.16 mg/100 ml and 0.55 mg/100 ml, respectively.**

Keywords**: Optimisation, non-alcoholicbeverage, local spices, sorghum stem sheath, antioxidants, minerals.**

I. INTRODUCTION

Nigeria is the largest sorghum (*Sorghum bicolour*) producer in West Africa accounting for about 71% of the total regional output (Ogbonna, 2011). The production of this cereal has increased over the years in Nigeria, and during its harvest, the enormous volume of sorghum stem sheath (SSS)are being generated as waste. SSS had been in use for centuries as traditional medicine by people of Southwestern Nigeria. The SSS has a high concentration of dimeric 3-deoxy-anthocyanidins (Kayode *et al*., 2011) that have a high content of antioxidants that inhibit the oxidation of other molecules (Devi *et al*., 2011) and are used as a therapeutic cure of anaemia, cancer, and a variety of infectious diseases, including viral diseases (Akande *et al*., 2010). Other plant materials rich in anti-oxidants include spices, roselle calyx among others (Devi *et al*., 2011; Adanlawo and Ajibade, 2006; Ayo *et al*., 2003).

The use of local spices to control the activities of microorganisms in foods has been reported (Aiyeloja and Bello, 2006).According to FAO (2011), spices can be used to preserve food and they have antimicrobial properties. Some antioxidants are found in many spices which can contribute to the body's defence against cardiovascular disease and intestinal cancers. The antimicrobial activity varies depending on the type of spices or herbs. The addition of extracts of spices could also control the microbial activities associated with food samples while retaining the organoleptic properties, nutritive and economic quality of such foods (Ade-Omowaye *et al*., 2015).

Previous attempts have been documented on the use of plant materials to produce health-promoting beverage as a viable alternative to the commonly consumed soft drinks. For example, *zobo*, the extract of roselle calyx has been used as a Non-Alcoholic Beverage (NAB). The nutritional quality of a non-alcoholic beverage produced from sorghum stem sheath (*poporo*) spiced with alligator pepper (Aframomum melegueta) has been reported (Adedeji et al., 2013). Studies showed that both extracts possess antioxidant properties (Adekanye et al., 2018; Ologundudu *et al.*, 2009a, b). However, combined spices and optimum formulation for a beverage from SSS has not been reported. In the same vein, converting SSS into value-added products such as NAB will be a great advantage to the smallholder farmers in terms of improved livelihood, and enhancing food and nutrition security for the populace.

Response surface methodology (RSM) is a collection of statistical and mathematical techniques useful for developing, improving and optimizing processes in which a response of interest is influenced by several variables and the objective is to optimize the response (Manoharan and Prathapkumar, 2012). This study, therefore, investigated the synergy of three spices in producing a health-promoting beverage from SSS using RSM for the research design.

II. MATERIALS AND METHODS

Sorghum stem sheath, alligator pepper, ginger, garlic and food-grade sucrose were procured locallyin Ogbomoso town,Nigeria. All reagents used were of analytical grade.

2.1 *Methods*

2.1.1 *Preparation of the sorghum stem sheath beverage samples*

Thoroughly cleaned SSSs, AP, ginger and garlic were ovens dried at 50 ℃ for 48 h, hammer milled to powder and sieved toless than 325 µm granules. Three levels of concentrations (0.00, 0.75, 1.50 g/100 ml) of each of the spices were combined randomly using the Design Expert 6.0.8. The combinations and processing variables are presented in Table 1. The proportion of SSS powder to water that was used for the beverage formulation was 1:30, as reported by Adedeji *et al*. (2013). The samples were extractedbetween 80 and 100 ^oC for 20, 25, 40 min durations. The extract was sweetened with food-grade sucrose to a level of 10% similar to those obtained in local drinks before filtering with a muslin cloth to obtain a clear filtrate. The sweetened beverage samples were dispensed into previously sterilized bottles before pasteurization at 75 ^oC for 15 min. The beverage samples prepared were analyzed for their quality parameters.

2.1.2 *Experimental design*

The effects of the five independent variables;X_1 (AP), X_2 (ginger), X_3 (garlic), X_4 (extraction temperature, ET) and X_5 (time of extraction, TOE) onthe dependent (responses) variables; Y_1, total phenols content (TPC), Y_2, Vitamin C Content (VCC), Y_3, 1,1-diphenyl -2-picryl hydrazyl (DPPH), Y_4, total carotenoids content (TCC), Y_5, zinc content (ZC), Y_6, iron content (IC), Y_7, oxalate content (OC) and Y_8, tannin content (TC) of the SSS drink were evaluated using the RSMas summarized in Tables 2 and 3. Models were generated and fitted to a second-order polynomial (Eqn.1) whileregression analysis and analysis of variance at $p<0.05$ level ofsignificance were used to determine the fitness of the models.Design-Expert (version 6.0.8) (Stat-Ease Inc., Minneapolis,U.S.A) was used for the experimental design and data analysis.

Table 1: Coded level and real values for the modified fractional CCRD experimental design

Independent variable	Coded variable	Coded levels and real values				
		-α	-1	0	+1	+α
AP (g/100 ml)	X_1	0.00	0.00	0.75	1.50	1.50
Ginger (g/100 ml)	X_2	0.00	0.00	0.75	1.50	1.50
Garlic (g/100 ml)	X_3	0.00	0.00	0.75	1.50	1.50
ET (^{o}C)	X_4	80	80	90	100	100
TOE (min)	X_5	10	10	25	40	40

Table 2: Experimental design and results obtained for anti-oxidant properties

Ar un no	Input/independent variables[b]					Response/dependent variables[c]			
	X_1	X_2	X_3	X_4	X_5	Y_1	Y_2	Y_3	Y_4
1	-1	-1	-1	1	-1	0.04	1.89	53.85	480
2	-1	1	1	1	-1	0.09	1.84	53.35	420
3	-1	1	-1	1	1	0.13	1.97	61.14	737
4	-1	-1	-1	-1	1	0.09	1.72	57.96	700
5	-1	1	1	-1	1	0.17	3.31	55.93	812
6	-1	1	1	1	1	0.09	1.89	55.28	800
7	-1	-1	1	1	1	0.08	2.62	52.35	330
8	-1	1	-1	-1	-1	0.09	1.89	55.38	616
9	-1	-1	1	-1	-1	0.09	3.79	51.35	704
10	1	1	1	1	1	0.09	0.73	46.55	164
11	1	0	0	0	0	0.15	1.75	70.16	940
12	1	-1	-1	1	1	0.08	1.14	51.67	680
13	1	-1	-1	1	1	0.03	3.63	42.55	154
14	1	-1	-1	-1	-1	0.11	1.77	48.94	896
15	1	1	-1	1	-1	0.13	2.29	50.05	488
16	1	1	-1	1	-1	0.08	2.69	42.55	360
17	1	-1	1	-1	1	0.12	4.99	49.79	780
18	1	1	-1	-1	1	0.23	5.22	59.23	836
19	0	0	0	0	0	0.16	1.56	66.84	1068
20	0	0	0	0	0	0.16	1.56	66.84	1068
21	0	-1	0	0	0	0.09	3.45	49.14	628
22	0	0	0	-1	0	0.03	4.03	70.18	737
23	0	0	0	0	0	0.16	1.56	67.69	1068
24	0	0	0	0	-1	0.19	4.12	69.12	1376
25	0	0	0	0	0	0.16	1.56	48.94	1068
26	0	0	-1	0	0	0.19	2.91	69.32	1304
27	0	0	0	0	0	0.16	1.56	66.84	1068
28	0	0	0	0	0	0.16	1.56	66.84	1068
29	0	0	0	1	0	0.11	1.39	55.12	876
30	0	0	1	0	0	0.09	1.97	55.12	768

31	0	0	0	0	1	0.16	4.09	57.96	756
32	0	1	0	0	0	0.19	2.55	67.69	1068

[a]Did not necessarily correspond to the order of experiment.

[b]X_1=AP; X_2=Ginger; X_3=Garlic; X_4=ET;X_5=TOE

[c]Y_1=TPC; Y_2=VCC; Y_3=DPPH;Y_4=TCC, and were in the range: 0.03 - 0.23 mg/100 ml, 0.73 - 5.22 mg/100 ml, 42.55 - 70.18% and 154 to 1304 mg/100 ml respectively.

Table 3: Experimental design and results obtained for mineral composition and anti-nutritional components

[a]Run no	Input/independent variables					Response/dependent variables[c]			
	X_1	X_2	X_3	X_4	X_5	Y_5	Y_6	Y_7	Y_8
1	-1	-1	-1	1	-1	3.46	2.88	0.36	1.22
2	-1	1	1	1	-1	3.89	3.37	0.36	1.97
3	-1	1	-1	1	1	3.28	3.58	1.76	2.40
4	-1	-1	-1	-1	1	3.05	2.03	0.81	1.49
5	-1	1	1	-1	1	3.49	2.29	0.81	3.85
6	-1	1	1	1	1	3.38	4.14	0.41	0.41
7	-1	-1	1	1	1	3.18	3.04	1.77	0.38
8	-1	1	-1	-1	-1	3.04	3.25	0.41	4.32
9	-1	-1	1	-1	-1	3.33	3.20	0.63	4.38
10	1	1	1	1	1	4.14	3.43	0.49	1.42
11	1	0	0	0	0	3.84	4.29	0.86	2.64
12	1	-1	-1	1	1	2.39	3.38	0.09	1.06
13	1	-1	-1	1	1	4.22	4.15	1.89	4.93
14	1	-1	-1	-1	-1	2.49	2.59	1.08	2.44
15	1	1	-1	1	-1	3.32	3.26	0.63	1.79
16	1	1	-1	1	-1	3.59	2.72	1.35	1.79
17	1	-1	1	-1	1	3.54	4.96	1.26	3.67
18	1	1	-1	-1	1	4.03	4.19	0.99	1.84
19	0	0	0	0	0	3.46	3.71	1.44	3.12
20	0	0	0	0	0	3.46	3.71	1.44	3.12
21	0	-1	0	0	0	3.94	3.44	0.72	2.78
22	0	0	0	-1	0	3.89	3.58	0.72	3.39
23	0	0	0	0	0	3.46	3.71	1.44	3.12
24	0	0	0	0	-1	3.64	2.89	1.58	3.26
25	0	0	0	0	0	3.46	3.71	1.44	3.12
26	0	0	-1	0	0	3.21	3.95	1.04	2.71
27	0	0	0	0	0	3.46	3.71	1.44	3.12
28	0	0	0	0	0	3.46	3.71	1.44	3.12
29	0	0	0	1	0	3.79	3.07	1.26	1.94
30	0	0	1	0	0	4.12	4.19	0.49	2.88
31	0	0	0	0	1	3.78	4.26	0.72	2.70
32	0	1	0	0	0	4.02	2.89	1.35	3.07

[a]Did not necessarily correspond to the order of experiment.

[b]X_1=AP; X_2=Ginger;X_3=Garlic ; X_4=ET; X_5=TOE

[c]Y_5=ZC; Y_6=IC; Y_7=OC; Y_8=TC

These responses (mg/100 ml) varied as follows: 2.39 to 4.22, 2.03 to 4.96, 0.09 to 1.89 and 0.38 to 4.93 for zinc, iron, oxalate and tannin contents respectively.

2.1.3 Chemical analyses of the beverage samples

The TPC was determined according to the method described by Singleton *et al*. (1999). VCC was determined by titration with 2, 6- dichlorophenol indophenol (Jayaraman, 1992). The free radical scavenging ability of the extracts against DPPH free radical were evaluated as described by Gyamfi *et al*., (1999).TCC was quantified by reversed-phase HPLC (Khachik *et al*., 1992; Mangels *et al*., 1993). IC, ZC, OC and TCwere determined according to the method described byAbulude *et al*. (2006), AOAC(2006), Nwinuka *et al*. (2005), and Markkar and Goodchild (1996) respectively.

2.1.4 Optimization procedure and validation of data

Numerical optimization technique was used on the multiple responses subject tomaximum TPC, VCC, DPPH, TCC and minimum OC and TC as constraints. The criteria for the optimization were selected based on producing a functional beverage with significant antioxidants for potential health enhancement. The best overall desirability which gave the required responses corresponded to the optimum formulation and processing conditions for the beverage. The actual responses of the laboratory-produced beverage were validated with the predicted responses at the optimum conditions.

2.1.5 Sensory Evaluation

The optimized and control beverage (beverage without spice) samples were presented as coded samples to 60 panellists according to the method described by Meilgaard (1991). The samples were evaluated using a 7- point hedonic scale ranked between; like extremely and dislike extremely for 1 and 7 points scale respectively. Each sample was assessed for taste, after taste, flavour/aroma, colour, appearance and overall acceptability in clean transparent bottles at room temperature (30±2 ^{o}C).Each sample assessment by the panellists waspreceded with mouth-watch and rinsing to have unbiased judgement.

2.1.6 Statistical Analysis

All experiments were conducted in three replicates for reproducibility. Statistical analysis of all the experiments carried out in the laboratory was done using Analysis of Variance (ANOVA) in Design-Expert software (version 6.0.8), while sensory evaluation results were analysed using SPSS (version 20).

III. RESULTS AND DISCUSSION

3.1 Total phenols of the beverage

The relationship between the independent variables and total phenols content (Table 2) is described by Eqn.2.Analysis of variance for each variable showed that a significant effect (p<0.05) was found for total phenol.The significant model terms were X_2 (ginger), the quadratic term of extraction temperature (X_4^2) and interaction terms for the alligator pepper and garlic (X_1X_3).

$$Y_{Phenolcoded} = +0.15 + 0.02X_2 - 0.05{X_4}^2 - 0.02X_1X_3 \quad (2)$$

X_2 exhibited a positive linear effect while a negative quadratic effect was seen for X_4 from the equation. These observationssupportedthe initial analysis of the raw materials which showed that ginger and alligator pepper had high total phenols content. The negative quadratic effect of temperature implies that the maximum increase in totalphenol content will occur within the range of extraction temperature used in this study. Also, the interaction of alligator pepper and garlic reduced the phenol content of the beverage. A previous study demonstrated that the addition of ginger increased the phenol content of a non-alcoholic beverage from sorghum stem sheath which is consistent with this present finding (Adedeji *et al*., 2013).The phenol content ranged from 0.03 to 0.23 mg/100 ml. This observation confirms the earlier report that spices possess significant antioxidant capacity (Wood and Pittler, 2000). Phenolics were identified as components with the strongest antioxidant capacity in fruits, vegetables and mushrooms (Heo *et al*., 2007).

3.2 Vitamin C content of the beverage

The vitamin C content of the beverage ranged from 0.73 to 5.22 mg/100 ml. The maximum value at coded point 1, 1, -1, -1, 1 was about 7 times more than the lowest value at coded point 1, 1, 1, 1, 1. Eqn. 3 describes the relationship between the independent variables and vitamin C.The selected model showed that the quadratic term of extraction time (X_5^2) exhibited a positive effect while extraction temperature (X_4) and the interaction of ginger and garlic exhibited a negative effect. This suggests a minimum increase in vitamin C will occur within the extraction period in the study. The elevated temperature will reduce vitamin C content. This observation agrees 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 (Spinola *et al*., 2013; Paul and Ghosh, 2012).

$$Y_{VitCcoded} = 2.09 - 0.80X_4 + 0.70X_5^2 - 0.62X_2X_3 \quad (3)$$

This result shows the high potency of garlic over ginger in vitamin C retention in the beverage. USDA National Nutrient database of 2009showed that the vitamin C content of garlic was higher than ginger. The range recorded is lower than the value (9.33 mg/100 g) reported by Amoo (2006) for *zobo* drink. This is evident from the report of the USDA Nutrient database of 2014 that sorghum stem sheath does not contain vitamin C. Therefore, the vitamin C content measured from the non-alcoholic beverage was a result of the inclusion of the spices. Ali *et al*. (2005) reported that the vitamin C status of beverages (such as *zobo*) can be improved by blending with spices.Vitamin C is a powerful water-soluble antioxidant that boosts the immune system and helps prevent cancer and heart disease (Rai and Anand, 2008).

3.3 Antioxidant activity of the beverage by DPPH scavenging

The relationship between the independent variables and DPPH scavenging ability indicated that all the raw materials have the potential of increasing the scavenging activity of the beverage but they were not significantly different from one another as shown in Equation 4.

$$Y_{DPPHcoded} = 55.40 \quad (4)$$

Spices, especially garlic have been said to have essential oil which is effective in scavenging free radicals and has the potential to be a powerful anti-oxidant (Ana *et al*., 2012).

3.4 Total carotenoids of the beverage

The model showing the relationship between the independent variables and total carotenoids is expressed in Eqn. 5.The effects of ginger (X_3) and the quadratic term ofextraction temperature (X_4) were the only significant model terms butthe interactions were not significant (p<0.05).The selected model showed that both the linear term of ginger (X_3) and the quadratic term of extraction temperature (X_4^2) exhibited negative effects. This observation suggests that total carotenoids may be negatively affected by increasing ginger in the formulation. Furthermore, a maximum increase in the total carotenoids may occur within the temperature range used in this study.

$$Y_{Carotenoidcoded} = 1003.43 - 124.72X_3 - 61.50X_4 + 16.72X_5 - 438.04X_4^2 - 37.19X_4X_5 \quad (5)$$

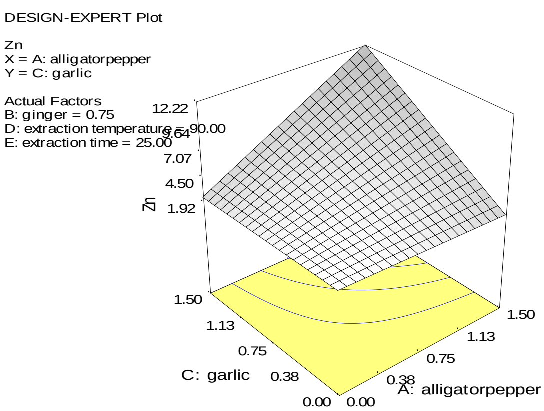
The total carotenoids (154-1304 mg/100 ml) was found to be higher than the carotenoids content reported by Adedeji *et al*. (2013) for sorghum stem sheath beverage spiced with ginger.The difference might be associated with the raw materials used. Carotenoids present in sorghum bicolour stem sheath include 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). Epidemiological studies provide information that carotenoids and other antioxidants may protect humans against certain types of cancer and cardiovascular diseases thus suggesting the potential of this beverage in promoting the health of the Nigerian populace if adopted as an indigenous beverage (Adedeji *et al*., 2013).

3.5 Zinc content of the beverage samples

All the interactions of each of the independent variables and zinc content (Table 3) followed the same trend as reflected in Figure 1. Therefore, two of the figures were selected as representatives. A significant effect (p<0.05) was found for zinc, with regards to all the five independent variables. All the variables exhibited positive linear and interactive effects (Eqn. 6).

$$\begin{aligned} Y_{Zncoded} = 4.74 &+ 2.33X_1 + 2.53X_2 + 2.54X_3 + 2.19X_4 \\ &+ 2.24X_5 + 2.66X_1X_2 + 2.61X_1X_3 \\ &+ 2.42X_1X_4 + 2.55X_1X_5 + 2.46X_2X_3 \\ &+ 2.51X_2X_4 + 2.54X_2X_5 + 2.55X_3X_4 \\ &+ 2.40X_3X_5 + 2.39X_4X_5 \qquad (6) \end{aligned}$$

Increasing AP, ginger, garlic, and extraction temperature and time may increase the zinc content in the beverage samples. The zinc content of the beverage ranged from 2.39 to 4.22 mg/100 ml (Table 3). The mean value (3.53 mg/100 ml) of zinc in the beverage was comparable to the values (2.70-3.51 mg/100 ml) obtained by Oluwalana *et al*. (2013) for sorghum stem sheath drink, *kunnu* (3.30 mg/100 ml) and *zobo* drink (2.68 mg/100 ml) (Adetuyi *et al*., 2007). SSS powder, garlic, ginger and alligator pepper had zinc content of 7.15 mg/100 ml (Adetuyi *et al*., 2007), 1.16 mg/100 ml, 0.34 mg/100 ml, 0.2 mg/100 ml, respectively (USDA, 2009). All the materials used in the beverage formulation contributed to the content of zinc in the beverage.



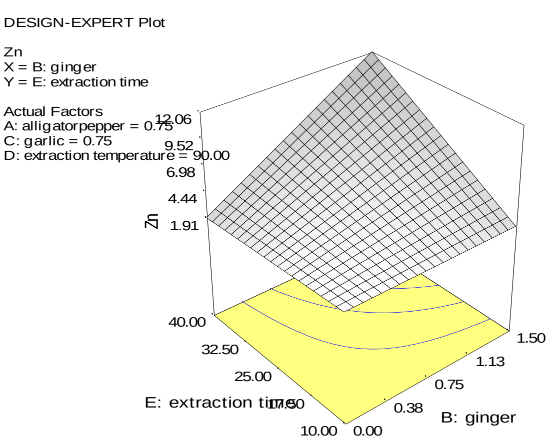


Figure 1: Effect of the independent variables on zinc content of the beverage.

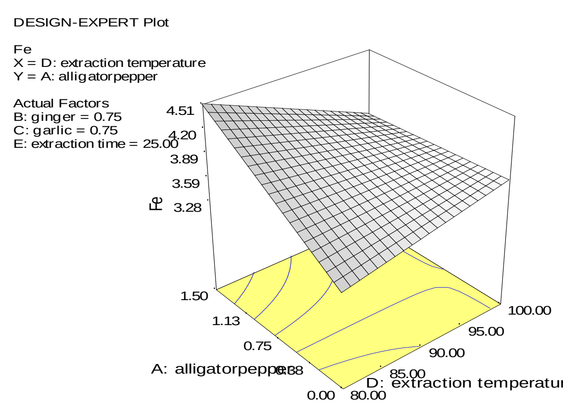
3.6 *Iron content of the beverage samples*

A significant effect (p<0.05) of the independent variables was found for iron content. X_1, X_2, $X_3$and X_1X_5exhibited a positive effect while the interactions of X_1X_4, X_2X_3, X_3X_4 and the quadratic effect of X_2^2exhibited a negative effect as seen in Eqn. 7.

$$\begin{aligned} Y_{Fecoded} = 3.79 &+ 0.29X_1 + 0.12X_2 + 0.12X_3 - 0.11X_4 \\ &+ 0.16X_5 - 0.53X_2^2 - 0.33X_1X_4 \\ &+ 0.31X_1X_5 - 0.26X_2X_3 - 0.16X_2X_5 \\ &- 0.19X_3X_4 \qquad (7) \end{aligned}$$

A positive sign in front of the coded factors in Eqn. 7 signifies a synergistic effect, while a negative sign signifies an antagonistic effect. The information contained in the selected model indicates that the iron content of the non-alcoholic beverage may increaseat varying degrees with an increase in the addition of the three spices and extraction time, while a decrease may occur with interactions of some of the factors(AP and extraction temperature, ginger and garlic; and garlic and extraction temperature). The negative quadratic effect of garlic indicated that maximum increase in iron may occur within the range of garlic concentration used in the study. The surface responses of the iron content of the beverage to the local spices are displayed in Figure 2. The iron content in the samples ranged from 2.03 to 4.96 mg/100 ml. This is, however, lower than the value (5.20-7.75 mg/100 ml) obtained by Oluwalana *et al*. (2013) for *poporo* drink. The iron content of *kunnu*, 70.02 mg/100 ml,is higher than that of the stem

sheath beverage (Agarry *et al*., 2010), while the iron content of the *zobo* drink, 2.40 mg/100 ml (Oboh *et al*., 2004) is comparable to that of the stem sheath beverage. The iron content in this study is higher than the values reported by Adeyeye (1997) for *Pyrus communis*, *Irvingia gabonesis* and *Mangifera indica* fruits consumed in Nigeria (1.86-4.49 mg/100g). Iron is important in many biological processes because it is an ideal oxygen carrier and because it can function as a protein-bound redox element (Obuzor and Ajaezi,2010).



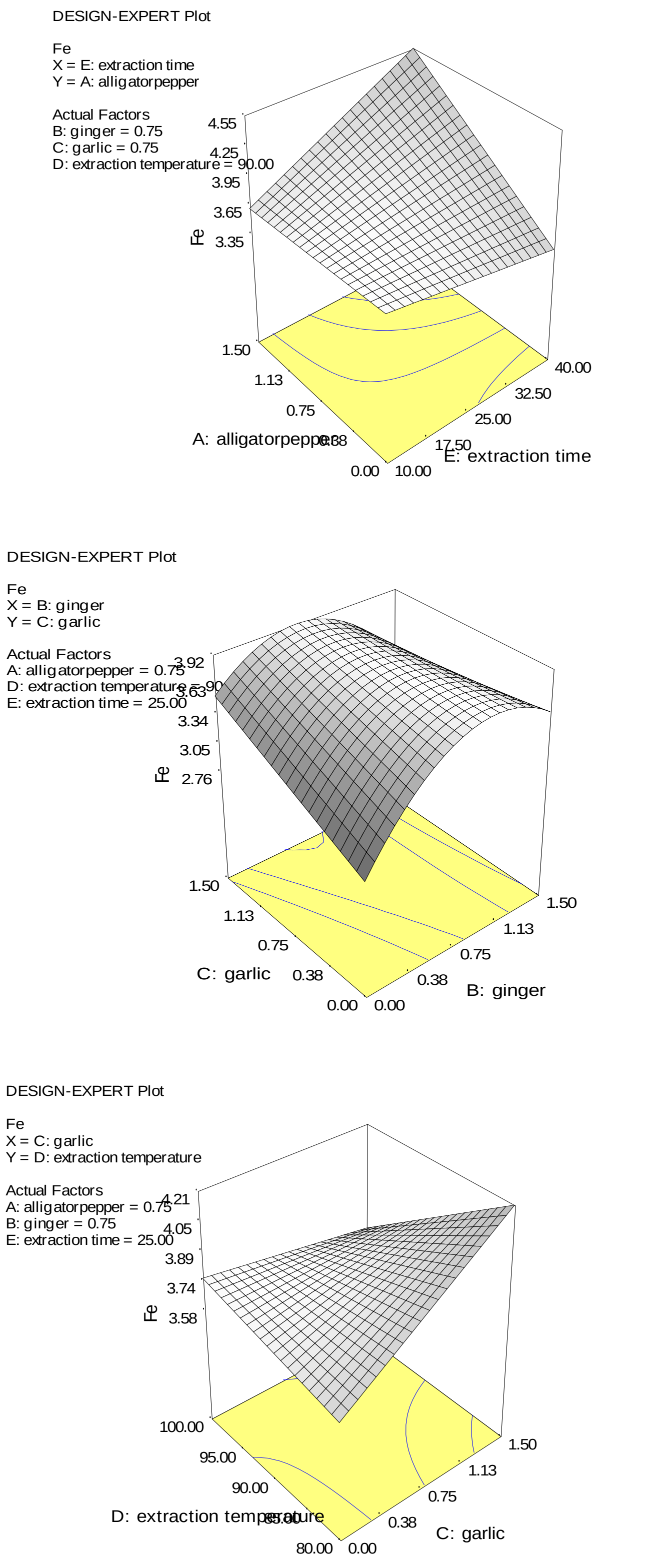


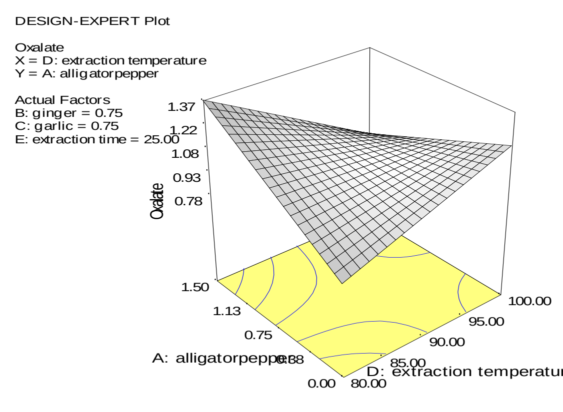
Figure 2: Effect of the interaction of variables on the iron content of the beverage: (a) alligator pepper and extraction temperature (b) alligator pepper and extraction time (c) ginger and garlic (d) garlic and extraction temperature

3.7 *Oxalate content of the beverage samples*

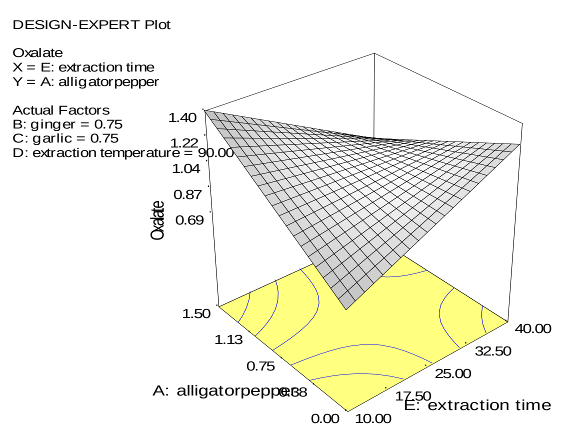
Eqn. 8 summarises the relationship between the studied independent variables and oxalate content. The interaction terms, X_1X_4, X_1X_5 and X_2X_3exhibited a negative effect as seen inthe selected model.

$$Y_{Oxalatecoded} = 1.03 - 0.24X_1X_4 - 0.30X_1X_5 - 0.23X_2X_3 \quad (8)$$

The interactions of alligator pepper and extraction temperature, alligator pepper and extraction time, and garlic and ginger may reduce the oxalate content in the beverage which is desired since oxalate is an anti-nutritional component. Initial analysis showed that all the raw materials used in the non-alcoholic beverage production had appreciable oxalate contents, but, processing temperature and time are known to reduce the anti-nutritional factors present in some plant materials. The oxalate content (0.09-1.89 mg/100 ml) of the non-alcoholic beverage was found to be comparable to the oxalate content of *the zobo* drink (0.615 mg/100 ml) (Adanlawo and Ajibade, 2006). The oxalate content was within the safe level of oxalate consumption in humans as reported that the lowest lethal dose is 5 g (about 70 mg/kg) (Tsai, 2005) and 40 – 50 mg/day is recommended (UPMC, 2017).



a



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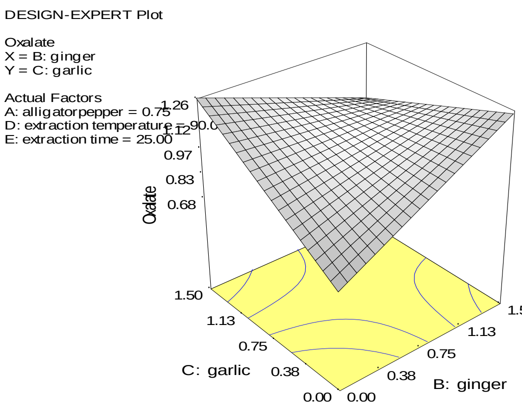


c

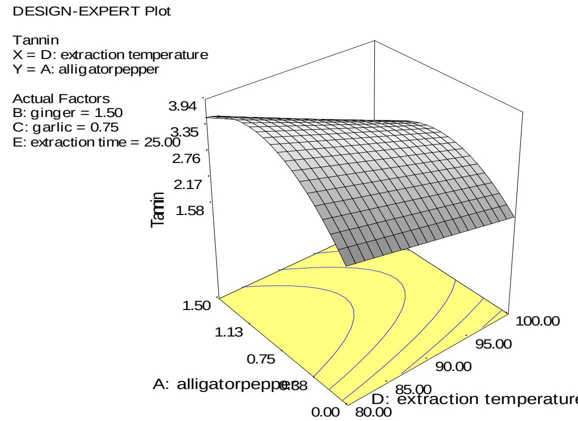
Figure 3: Effect of the interaction of variables on the oxalate content of the beverage (a) alligator pepper and extraction temperature (b) alligator pepper and extraction time (c) ginger and garlic.

3.8 Tannin content of the beverage samples

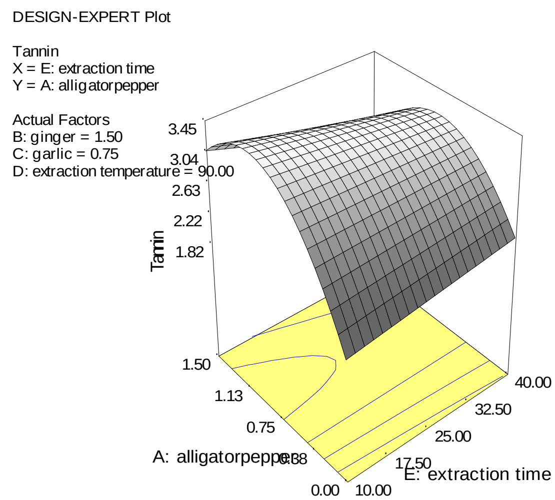
The variation of tannin with the independent variables is presented in Table 3. ANOVA analysis showed a significant effect (p<0.05) of alligatorpepper (X_1), ginger (X_2), garlic (X_3), extraction temperature (X_4), quadratic term of alligator pepper (X_1^2) and interaction terms for alligator pepper and extraction temperature (X_1X_4), alligator pepper and extraction time (X_1X_5), garlic and extraction time (X_3X_5). X_1, $X_2$and X_3 exhibited a positive effect while X_4, the quadratic term of alligator pepper and the interaction terms of alligator pepper and extraction temperature, alligator pepper and extraction time, garlic and extraction time exhibited a negative effect as seen in Eqn. 9.

$$Y_{Tannincoded} = 2.96 + 0.35X_1 + 0.26X_2 + 0.42X_3 - 0.62X_4 - 0.12X_5 - 0.91X_1^2 - 0.24X_1X_4 - 0.27X_1X_5 - 0.58X_3X_5 - 0.09X_4X_5 \quad (9)$$

In the selected model, increasingalligator pepper,ginger and garlicmay increase the tannin content of the beverage while an increase in extraction temperature and time may reduce the tannin content of the beverage samples. The beverage samples had tannin content in the range of 0.38-4.93 mg/100 ml. The tannin content of the non-alcoholic beverage was found to be much higher than that of *zobo* drink, 0.035mg/100ml (Osuntogun and Aboaba, 2004) and SSS beverage samples containing ginger extract, 1.190-1.520 mg/100ml (Adedeji *et al.*, 2013). The high content of tannin in the beverage could be attributed to the inclusion of alligator pepper and garlic in the beverage. The tannin content of the non-alcoholic beverage was within the safe level.The tannin content of up to 15 mg/kg is allowed in foods(EFSA, 2014; Ghosh *et al.*, 2014). Tannins have been shown to possess extensive health benefits and therapeutic potentials for treating some disease conditions (Ghosh, 2015).



a



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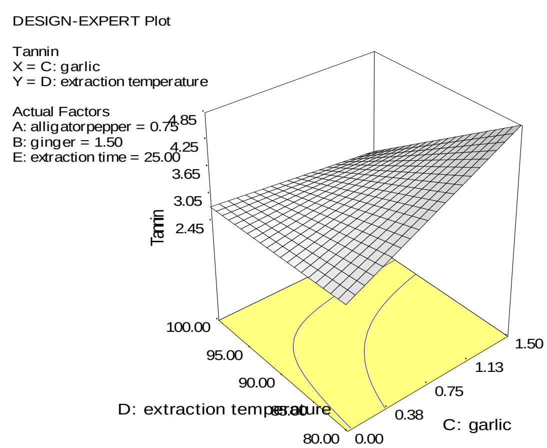

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Figure 4: Effect of the interaction of variables on the iron content of the beverage (a) alligator pepper and extraction temperature (b) alligator pepper and extraction time (c) garlic and extraction temperature.

3.9 Optimization

Applying the desirability function to maximize responses of interest and minimize the ones with an adverse effect on the beverage quality,the optimum drink was produced from the combination of the following variable parameters;1.50 g/100 ml AP, 1.49 g/100 ml ginger, 0.15 g/100 ml garlic, 80 °C ETand 40 minTOE. The responses of the predicted optimized beverage to the actual optimized beverage are presented in the graph of predicted optimised beverage against actual experimental values (Figure 5). The alignment of the values to the 45^{o} line shows that the models used are predictive and are comparable to the experimented optimized beverage. Also, the high correlation coefficients obtained (R^2 = 0.999) show that the observationsfrom the predicted and actual areclose.

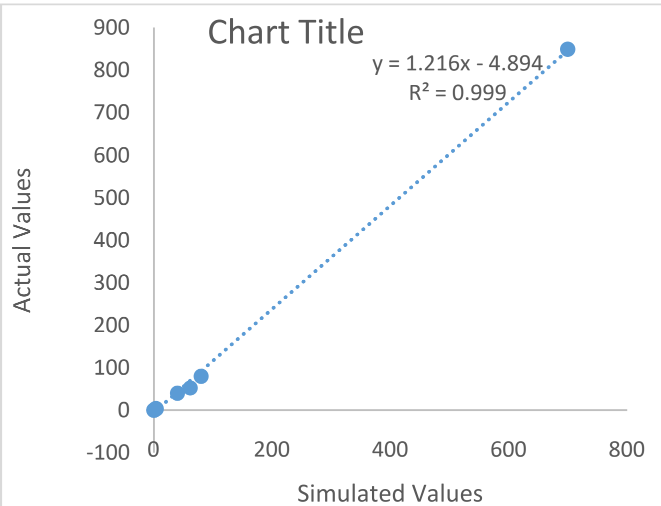

Figure 5: Graph of a simulated optimized sample against actual experimental values

IV. CONCLUSION

The adopted RSM in this research has made it possible to simultaneously study interaction effects of alligator pepper, ginger, garlic, extraction temperature and extraction time. The study, therefore, established the optimum processing variables and conditions for producing a non-alcoholic beverage from sorghum stem sheath and three local spices with optimum contents of total phenols, ascorbic acid, DPPH, total carotenoids and iron that could serve as a health-promoting beverage for the consuming populace. Industries may adopt these optimal extraction conditions for the production of SSS beverage to maximize the health benefits of SSS.

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CONFLICT OF INTEREST

There is no conflict of interest

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