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Ethyl Carbamate in Burukutu Produced from Different Sorghum Varieties Under Varying Storage Conditions Using Response Surface Methodology

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Abstract: Burukutu was produced using three varieties of sorghum – CSR- 02, S- 17 and S- 44. The ethyl carbamate (EC) content of the burukutu was studied using Response Surface Methodology. A three-level three-factorial Box-Behnken experimental design was adopted to study the effects of storage conditions on the burukutu produced. The ethyl carbamate content was analyzed using standard methods and results obtained were analyzed statistically using the response surface approach. Results showed a significant difference ($p<0.05$) on the effects of storage time, storage temperature and their interactions on the ethyl carbamate content of burukutu and thus the beverage variety type showed non-significant difference ($p>0.05$). When consumed in excess, ethyl carbamate has been implicated in various health complications, including gastroenteric hemorrhages, vomiting, cancer, among others. The EC contents of the beverage varieties ranged from 1.08µg/l to 8.50µg/l; which were within the safe limits set by USDA (125 µg/l) and Canada (150 µg/kg). The R^2 obtained from the statistical analysis of burukutu were all higher than 0.75 (75%) which depicts that the model was best suggested or adequately fits the relationship between the variables under consideration.

Keywords: Burukutu, Ethyl Carbamate, Storage Conditions, Response Surface Methodology, Modeling

1. Introduction

Burukutu originated from the Northern part of Nigeria, from the Hausas from where it spreads to other parts of the country, most especially the Southern and Western parts. It is produced from fermentation of cereals like maize, sorghum, millet, guinea corn, etc. Burukutu is rich in carbohydrates and therefore, it is a satisfying energy giving drink. It is creamy and dirty white in colour [1]. Beverages are consumed mainly either for its thirst-quenching capacity or for its stimulating effect. Many compounds, including ethyl carbamate, may be present in burukutu. In the body, most compounds, such as erythritol, ethyl carbamate, etc. are absorbed into the bloodstream in the small intestine, and then, for the most part, excreted unchanged in the urine [6].

Ethyl Carbamate (EC) with (CAS No 51-79-6) is an ethyl ester of carbamic acid. Ethyl carbamate has the trivial name urethane, which is sometimes also used to describe the unrelated substance polyurethane [21]. It is commonly found in fermented beverages like spirits, wine, beer, thus a by-product of fermentation [10, 19]. There is a general agreement that EC levels in alcoholic beverages should be maintained at the lowest levels technically possible [18]. The mechanism of formation of EC in alcoholic beverages has been investigated, and it has been shown that EC is produced by the chemical reaction of urea with ethanol in alcoholic beverages, and by heating or long periods of storage [9]. The removal of urea, the major precursor from alcoholic beverages by an acid urease has been successful [5]. However, once formed, EC is chemically stable and cannot be easily decomposed [9, 17]. Public health challenges regarding EC presence in alcoholic beverages began in 1985, when relatively high levels of EC were detected by Canadian authorities in imported alcohol products, including German fruit spirits [13]. Urethane has been implicated as a chemical carcinogen and this has led to concern in recent years since it occurs in trace levels (less than 10ppb) in most alcoholic beverages and other food items where fermentation is an integral part of the production process [7, 17].

The response surface methodology approach is a statistical technique that was used by authors in most studies involving optimization. In statistics, response surface methodology (RSM) explores the relationships between several explanatory variables and one or more response variables. The main idea of RSM is to use a sequence of designed experiments to obtain an optimal response, minimizing cumbersome experiments and reducing cost [8]. The objectives of the study are to determine the levels of ethyl carbamate in burukutu, as well as the impacts of storage conditions on the levels of ethyl carbamate in the burukutu made from different sorghum varieties; which are among the cereals used as staple foods in Nigeria [22].

2. Materials and Methods

Three varieties of sorghum (CSR-02, S-17 and S-44) used for this research work were obtained from Institute for Agricultural Research Samaru, Ahmadu Bello University, Zaria, Kaduna State, Nigeria. These varieties were certified by the Seed Certification and Quality Control Department, National Agricultural Seeds Council (NASC). All reagents and chemicals used were of analytical grade. The equipment and other materials were obtained from Department of Food Science and Technology.

2.1. Production of Burukutu

The methods used in production of burukutu were similar to the methods used by Igwe et al., 2018. The three varieties of sorghum (CSR- 02, S- 17 and S- 44) were processed into burukutu using the method of [12, 16] with slight modification. The sorghum grains were steeped (200g in 2L of water) for 24 h, drained and allowed to germinate at 25°C for three days. The grains were sprinkled with water every morning and turned over at intervals of 24 h. Kilning was done at 55°C for 24 h using a moisture extraction oven (model PF200), followed by milling with disc attrition mill (Bentall Plate Mill, Model 200 L 090) without removing the sprouts. The malt was mixed with water and boiled for one hour. Cooled and allowed to ferment for 48h at ambient temperature (30 - 32°C).

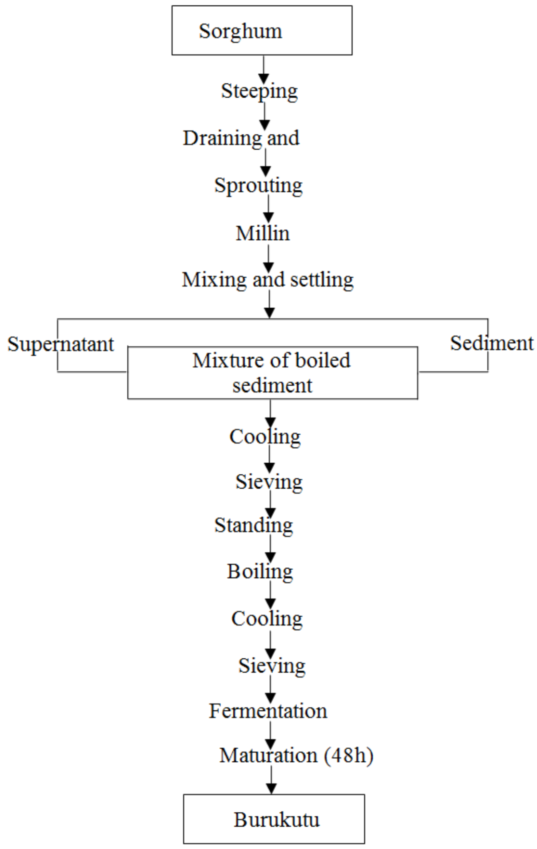

Figure 1. *Flow diagram for production of Burukutu Egbema and Etuk (2007) and Ibrahim and Ierve (2013) with slight modification.*

2.2. Determination of the Chemical Parameters of Burukutu

2.2.1. Ethyl Carbamate

The ethyl carbamate content of the beverages produced from the different varieties of sorghum was determined, according to the method described by [4] using the UV Spectrophotometer (Lasany L1-722). A hundred milliliters (100ml) of distilled water was added to 0.89g (0.1M) of a pure sample of ethyl carbamate (CAS No 51-79-6) in a beaker and stirred continuously using a stirrer until the solution was clear.

Four milliliters (4ml) of the ethyl carbamate solution was pipetted into a cuvette and used to calibrate the spectrophotometer. Thereafter, other cuvettes with burukutu samples were introduced into the sample compartment and readings were taken. Serial dilutions were also made using different concentrations obeying Beer Lambert's law.

The absorbance was measured using the UV-vis spectrophotometer at 340nm for EC. The concentration of ethyl carbamate was obtained by extrapolation from a standard curve using standard ethyl carbamate solution.

2.2.2. Alcohol Content

The percentage of alcohol by volume was determined according to [3]. The samples were distilled using a glass distillation apparatus to recover the alcohol-water mixture. An alcoholmeter was used to determine the percentage alcohol content of the distillate obtained.

2.2.3. Specific Gravity Determination

Specific gravity was determined using a density bottle.

Each sample was poured into a 50ml density bottle and weighed. Specific gravity was obtained by dividing the density of a substance to the density of water.

2.3. Experimental Design and Data Analysis

The three-level three-factorial Box–Behnken experimental design [14] was adopted to study the effect of storage time (X_1), storage temperature (X_2) and product type i.e. beverage variety (X_3) on the chemical compositions of the beverage. Response surface plots associated with the results were also developed as well as response surface regressions of dependent data (including ANOVA of the model) was performed using the Design expert software version 10.0.2 (StatSoft, Inc.), and model significance ($p<0.05$), lack of fit and adjusted regression coefficients (R^2_{adj}) which indicate the model fitness was determined from the analysis. A quadratic polynomial regression model was assumed for predicting individual responses [14].

$$Y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \beta_{11} {x_1}^2 + \beta_{22} {x_2}^2 + \beta_{33} {x_3}^2 + \beta_{12} x_1 x_2 + \beta_{13} x_1 x_3 + \beta_{23} x_2 x_3 \quad (1)$$

***Table 1.** Independent Variable and Levels used for Box-Behnken Design.*

Coded variable level (x_i)				
Variable	**Symbol (x_i)**	**-1**	**0**	**1**
Storage time (h)	X_1	0	72	144
Storage temperature (°C)	X_2	25	30	35
Beverage variety type	X_3	1	2	3

Transformation of coded variable (x_i) levels to un-coded variables (X_I) levels could be obtained from $X_1=72x_1 + 72$; $X_2= 5x_2 + 30$; $X_3= x_3 + 2$. Where -1 = minimum values, +1 = maximum values, and 0 = centre points

***Table 2.** Experimental design of samples in their coded and natural units.*

Sample	Coded units			Natural units		
	X_1	X_2	X_3	X_1	X_2	X_3
1	-1	0	1	0	30	3
2	0	0	0	72	30	2
3	-1	0	-1	0	30	1
4	0	-1	-1	72	25	1
5	0	-1	1	72	25	3
6	1	1	0	144	35	2
7	1	-1	0	144	25	2
8	-1	1	0	0	35	2
9	0	0	0	72	30	2
10	0	1	-1	72	35	1
11	0	0	0	72	30	2
12	0	0	0	72	30	2
13	0	1	1	72	35	3
14	1	0	1	144	30	3
15	-1	-1	0	0	25	2
16	1	0	-1	144	30	1
17	0	0	0	72	30	2

Samples (2, 9, 11, 12, 17) are centre points. X_1- storage time (h); X_2- storage temperature (°C) and X_3- beverage variety type (g). $X_1=72x_1 + 72$; $X_2= 5x_2 + 30$; $X_3= x_3 + 2$.

3. Results and Discussion

The results in Table 3 shows the mean results of ethyl carbamate, alcohol content and specific gravity.

***Table 3.** The mean results of the chemical analysis of burukutu.*

Runs	ST (h)	STT (°C)	BVT (g)	EC (µg/L)	AC (%)	SPG
1	0	30	3	1.20[f]	1.05[d]	1.038[b]
2	72	30	2	3.75[e]	3.53[bc]	1.025[c]
3	0	30	1	1.19[f]	0.99[d]	1.040[b]
4	72	25	1	3.58[e]	3.49[c]	1.025[c]
5	72	25	3	3.60[e]	3.50[c]	1.025[c]
6	144	35	2	8.50[a]	4.45[a]	1.018[c]
7	144	25	2	7.00[d]	4.40[a]	1.018[c]
8	0	35	2	1.25[f]	1.05[d]	1.05[ab]
9	72	30	2	3.73[e]	3.53[bc]	1.025[c]
10	72	35	1	3.83[e]	3.51[c]	1.028[b]
11	72	30	2	3.73[e]	3.53[bc]	1.025[c]
12	72	30	2	3.75[e]	3.53[bc]	1.025[c]
13	72	35	3	3.85[e]	3.50[c]	1.028[b]
14	144	30	3	7.80[c]	4.47[a]	1.015[c]
15	0	25	2	1.08[f]	0.99[d]	1.052[a]
16	144	30	1	8.00[b]	4.47[a]	1.017[c]
17	72	30	2	3.75[e]	3.53[bc]	1.025[c]
LSD				0.179	0.106	0.011

Key: EC = Ethyl carbamate content; AC = Alcohol content; SPG = Specific gravity; ST=Storage time; STT = Storage temperature; BVT = Beverage variety type. Means with the same superscript in the same column are not significantly different ($P <0.05$) at 5% level of significance.

***Table 4.** Coefficient for significant and non-significant terms for chemical properties of burukutu.*

Coefficient	EC	AC	Spg
Linear			
β_0	3.74	3.53	1.02
A	3.32*	1.71*	-0.014*
B	0.27*	0.016*	5×10^{-4}
C	-0.019	7.5×10^{-3}	-5×10^{-4}
Quadratic			
A^2	0.77*	-0.78*	5.2×10^{-3}*
B^2	-0.059	-0.026*	4.2×10^{-3}*
C^2	0.032	-3.7×10^{-3}	-2.7×10^{-3}
Interaction			
AB	0.33*	-2.5×10^{-3}	5×10^{-4}
AC	-0.052	-0.015	2.4×10^{-19}
BC	2.38×10^{-18}	-5×10^{-3}	-6×10^{-19}
R^2	0.9980	0.9999	0.9677
Adj R^2	0.9954	0.9998	0.9261
Lack of fit	NS	NS	NS
Model (Prob>F)	<0.0001*	<0.0001*	0.0002*

Key: A – Storage time; B – Storage temperature; C – Beverage variety type; EC – Ethyl carbamate content; MC=Methanol content; AC=Alcohol content; SPG = Specific gravity; NS – Not significant. *Significant at the 5% level ($P < 0.05$).

3.1. Ethyl Carbamate Content

The EC content of the beverage varieties ranged from 1.08µg/L to 8.50µg/L as shown in Table 3. The result showed that storage time of the beverage varieties type was directly related to the EC content and to the storage temperature. Iwouno and Igwe (2013) reported similar results for different spirits.

Table 4 shows the estimated response surface regression coefficient for EC content of burukutu made from different sorghum varieties. The values obtained showed that these responses were adequately described by the factors in the polynomial model. There were significant differences ($p < 0.05$) in the quadratic effect and linear effect, i.e. storage time (A), storage temperature (B), the interaction between storage time and storage temperature (AB) and the square of storage time (A^2) or significant with a probability of 95%. As seen in Table 4, the model was significant ($p < 0.05$) and the result of the polynomial after removing the non-significant terms in Table 4 gives

$$EC = 3.74 + 3.32A + 0.27B + 0.77A^2 + 0.33AB \qquad (2)$$

The response surface plot in Figure 2 indicated a progressive linear increase in EC content as storage time increased from 0 to 144h. The coefficient of determination (R^2) of the model was 0.998 (Table 4) which indicated that the model adequately represented the real relationship between the variables under consideration. The R^2 value indicated that 99.8% of the variability was explained by the model and only 0.2% was as a result of chance or accounts for factors not included in the model. The coefficient of variation (C.V.) obtained was 3.98%. From equation 2 it was observed that for every given storage time and storage temperature the EC concentration is increased by 0.33µg/L.

The presence of EC in beverages is an indicator of poor storage conditions or product that were exposed to excessive heat stress [17]. Therefore increasing the temperature during storage or exposing them to heat also increases the rate of EC formation, thus reduction in thermal load during storage at lower temperatures are also ways to mitigate EC in beverages [10].

The regression equation obtained is

$$EC = 3.74 + 3.32A + 0.27B - 0.019C + 0.33AB - 0.052AC - 2.546\,E017BC + 0.77A^2 - 0.050B^2 - 0.032C^2 \qquad (3)$$

The above equations in terms of coded factors can be used to make predictions about the responses for given levels of each factor. The coded equation is useful for identifying the relative impact of the factors by comparing the factor coefficients. EC content which ranged from (1.08µg/L – 8.5µg/L) were within the range of maximum allowed level of EC in fermented beverages [2]. These levels were lower than the maximum permissive level of FDA (1.5mg/L) [23] and the Canadian limit of 0.15mg/L [2].

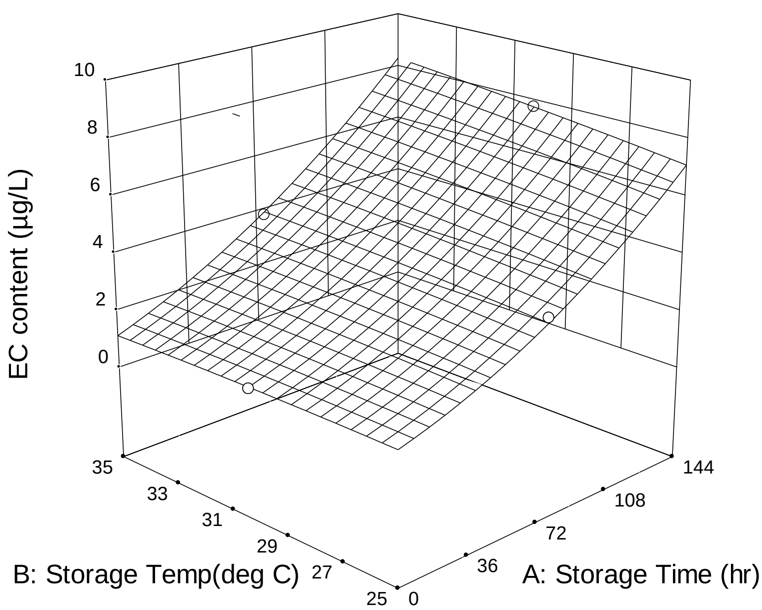

Figure 2. *Effect of Storage time and Storage temperature on EC content of burukutu.*

3.2. Alcohol Content

The Alcohol content of burukutu ranged from 0.99% to 4.47% from (Table 3), thus storage time had a highly positive linear effect and negative quadratic effect on the alcohol content of the burukutu samples (Table 4). This is similar to the results of the work done by Igwe et al., 2018. This implies that the alcohol content of the burukutu samples produced from different sorghum varieties increased with increase in storage time. Thus there exist a direct relationship between alcohol content and storage time.

In 2004, World Health Organization reported that the percentage alcohol content of burukutu ranged from 1 – 6%; this is in line with results of samples obtained. Hence (Figure 3) showed a progressive increase in the alcohol content of burukutu sample as storage time increased from 72 to 144h. There was a significant difference ($P<0.05$) in the quadratic and linear effect of the model i.e. storage time (A), storage temperature (B), square of storage time (A^2) and the square of storage temperature (B^2) or significant with a probability of 95%.

$$AC = 3.53 + 1.71A + 0.016B - 0.78A^2 - 0.026B^2 \quad (4)$$

The coefficient of determination (R^2) of the model was 0.9999 (Table 4) which indicated that the model adequately represented the real relationship between the variables under consideration. An R^2 value means that 99.99% of the variability was explained by the model and only 0.01% was as a result of chance or accounts for factors not included in the model. From equation (4) it was observed that for every given storage time and storage temperature, the alcohol content decreased by 0.026%.

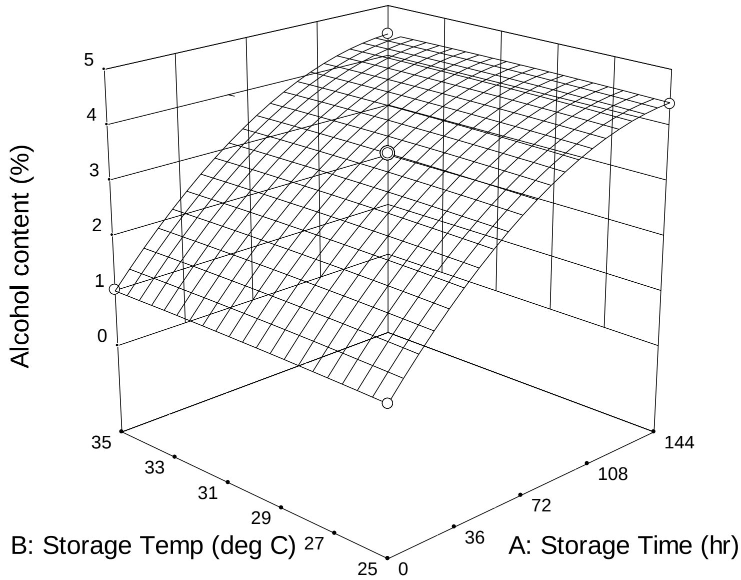

Figure 3. *Effect of Storage time and Storage temperature on the Alcohol content of burukutu.*

3.3. Specific Gravity

The specific gravity of samples ranged from 1.015 to 1.052 (Table 3). The results showed that storage time of the beverage variety type was indirectly related to the specific gravity. Specific gravity is defined as the ratio of a substance to that of a standard (water). From Table 4, the storage time had a highly negative linear effect and also a positive quadratic effect on the specific gravity of samples while the storage temperature had a positive quadratic effect on the specific gravity of samples. This, therefore, implied that the specific gravity of burukutu samples decreased with increase in storage time and also decreased with a decrease in storage temperature (Figure 4). There were significant differences ($P<0.05$) in the linear and quadratic effects of the model i.e. storage time (A), square of storage time (A^2), square of storage temperature (B^2) or significant with a probability of 95%.

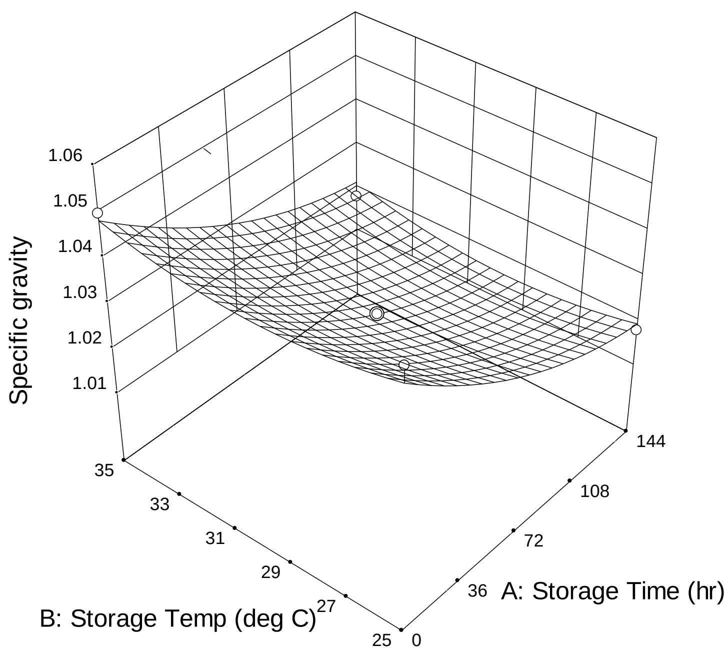

Figure 4. *Effect of Storage time and Storage temperature on the Specific gravity of burukutu.*

From equation (5) it was observed that for every given storage time and storage temperature, the specific gravity is increased by 0.0005. The regression equation obtained was as follows

$$Spg = 1.02 - 0.014A + 0.0005AB \quad (5)$$

The coefficient of determination (R^2) of the model was 0.9677 which indicated that the model adequately represented the real relationship between the variables under consideration. An R^2 value means that 96.77% of the variability was explained by the model and only 3.23% was as a result of chance or accounts for factors not included in the model. The coefficient of variation (C.V.) obtained was 0.28%.

4. Conclusion

The study showed that storage time and storage temperature significantly influenced the chemical properties of the burukutu made from different sorghum varieties especially for ethyl carbamate since the storage conditions (storage time and temperature) are the major precursors responsible for its formation in burukutu. Increase in storage time caused a significant decrease in the specific gravity (1.015 to 1.052), whereas the increase in storage time caused a significant increase in the following chemical properties: Ethyl carbamate content (1.08μg/L to 8.50μg/L) and alcohol content (0.99% to 4.47%). The R^2 obtained from statistical analysis for the chemical properties (ethyl carbamate, alcohol content and specific gravity) of each of the beverages was higher than 0.75 (75%) which means that the model was adequate in predicting the effects of storage time, storage temperature and their interactions on the chemical properties of burukutu made from different varieties of sorghum. Burukutu stored at lower temperatures ($\leq 25^{\circ}C$) and a short storage time ($\leq$ 72h) gave least values based on EC and AC levels thus lesser effects on other chemical properties. The response surface methodology has been effectively employed to predict the models for the chemical properties of burukutu made from different raw material sources and thus predict the influence of storage time and temperature on the chemical properties of burukutu.

References

[1] Alais, C. and Linden, G. (2009). *Food Biochemistry*. Aspen Publishers Inc. Gaithersburg, Maryland, USA. Pp 140-143.

[2] Alexander, J., Auounsson, G., Bentford, D., and Cockburn, A. (2007). Ethyl carbamate and hydrocyanic acid in food and beverages. Scientific opinion of the panel on contaminants. *European Food Safety Authority Journal,* 551: 1-44.

[3] AOAC (1990). Official methods of Analysis. Association of Official Analytical Chemists, Washington DC.

[4] AOAC (2006). Official methods of Analysis. Association of Official Analytical Chemists, Washington DC.

[5] Aresta, M., Boscolo, M., Franco, D. W. (2001). Copper (II) catalysis in cyanide conversion into ethyl carbamate in spirits and relevant reactions. *Journal of Agricultural Food Chemistry* 49 (6): 2819–2824.

[6] Awuchi, Chinaza G. (2017). Sugar Alcohols: Chemistry, Production, Health Concerns and Nutritional Importance of Mannitol, Sorbitol, Xylitol, and Erythritol. *International Journal of Advanced Academic Research (IJAAR),* 3 (2); 31 – 66. ISSN: 2488-9849.

[7] Aylott, R. I., McNeish, A. S., and Walker, D. A. (2000). Ethyl carbamate formation in grain-based spirits. *Journal of Institute of Brewing*. 93 (1): 382-388.

[8] Box, E. P., Hunter, J. and Hunter, G. 2005. *Statistics for Experiments*. New Jersey: John Willey and Sons, Inc.

[9] Butzke, C. E. and Beland, F. A. (2007). *Ethyl carbamate preventative action manual*. U.S Food and Drug Administration, Washington D. C, USA. Available at URL: http://Vm.cfsan.fda.gov/Afrf/ ecaction.html [Accessed February 2nd, 2015].

[10] Butzke, C. E. and Bisson, L. F. (2007). *Ethyl carbamate preventative action manual*. U.S Food and Drug Administration, Washington D. C, USA. Available at URL: http://Vm.cfsan.fda.gov/^frf/ecaction.html

[11] Dennis, M. J., Howarth, N., Key, P. E., Pointer, M., and Massey, R. C. (2008). Investigation of ethyl carbamate levels in some fermented foods and alcoholic beverages. *Food Additives and Contaminants*. 6 (3): 383-389.

[12] Egbema, K. C., and Etuk, V. E. (2007). A kinetic study of Burukutu fermentation. *Journal of Engineering and Applied Science*. 2 (7): 1193-1198.

[13] FAO/WHO, (2006). Food and Agriculture Organization of the United Nations/World Health Organization. In: Safety evaluation of certain contaminants in food. Prepared by the sixty-fourth meeting of the joint FAO/WHO Expert Committee on Food Additives (JECFA). *Food Nutrition Paper*. 82: 1-778.

[14] Filli, K. B., Nkama, V. S., Jideani, A. Z. and Abubakar, U. M. (2011). Application of response surface methodology for the study of the composition of extruded millet-cowpea mixtures for the manufacture of fura: A Nigerian food. *African Journal of Food Science*, 5 (17): 884-896.

[15] Ibrahim, J. S., and Ierve, I. A. (2013). Development of Equations for Estimating Energy Requirements in Processing Local Alcoholic Beverage (Burukutu) In Nigeria. *International Journal of Engineering Research and Applications* (IJERA). 4 (3): 648-654.

[16] Igwe Victory Somtochukwu, Omeire Gloria Chinenyenwa, Awuchi Chinaza Godswill, Kwari Mercy Ibrahim, Oledimma Ngozi Uchenna, Amagwula Ikechukwu Otuosorochi. Effect of Storage Conditions on the Methanol Content of Burukutu Produced from Different Sorghum Varieties; A Response Surface Methodology Approach. American Journal of Food, Nutrition and Health. Vol. 3, No. 3, 2018, pp. 42-47.

[17] Iwouno, J. O., and Igwe, V. S. (2013). Prevalence of ethyl carbamate in spirits from different sources. *African Journal of Food Science and Technology*. 4 (2): 25-28.

[18] JECFA, (2006). Joint FAO/WHO Expert Committee on Food Additives. In: *Evaluation of certain food contaminants. WHO Technical Report Series 930*. Sixty-fourth report of the JECFA. Available from: http://whqlibdoc.who.int/trs/WHO_TRS_930_eng.pdf [Accessed on 2nd February, 2015].

[19] Kim, Y. K. L, Koh, E., Chung, H. J and Kwon, H. (2000). Determination of ethyl carbamate in some fermented Korean foods and beverages. *Food additives and contaminants*. 17: 469-475.

[20] Norman, F. H., Cherl-Ho, L. and Quintero-Ramirez, R. (2009). Fermented cereals; A global perspective. *Food and Agricultural Organization* (F.A.O) Bulletin No. 38.

[21] NTP, (2011). National Toxicology Program. Urethane Report on Carcinogens. National Toxicology Program 12th ed., pp. 1–3.

[22] Udeogu, E; Awuchi, C. G. (2016). Effect of Processing On Hemagglutinin Activity of Lectin Extracts from Selected Cereals and Legumes. *International Journal of Advanced Academic Research (IJAAR*), 2 (12); 24 – 59. ISSN: 2488-9849. *http://ijaar.org/articles/Volume2-Number12/Sciences-Technology-Engineering/ijaar-ste-v2n11-nov16-p5.pdf*

[23] Vallejo-Cordoba, B., Gonzalez-Cordoba, A. F. and Estrada-Montoya, M. C., (2004). Tequila Volatile characterization and ethyl ester determination by solid-phase micro extraction gas chromatography-mass spectrometry analysis. *Journal of Agricultural and food chemistry*, 52: 67-5571.

[24] WHO (World Health Organization) (2004). Global Status report on Alcohol. *African Journal of Drug and Alcohol Studies,* 1 (2): 3-4.