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Effect of Storage Conditions on the Methanol Content of Burukutu Produced from Different Sorghum Varieties; A Response Surface Methodology Approach

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Abstract: Methanol content of burukutu made from different sorghum varieties was studied using Response Surface Methodology. A three-level three-factorial Box-Behnken experimental design was adopted to study the effects of storage conditions and chemical properties on burukutu produced from different sorghum varieties. The methanol content was determined 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 physicochemical parameters (methanol, pH) of burukutu. There was no significant difference ($p>0.05$) due to different sorghum varieties. Increase in storage time showed a significant decrease in pH (6.21-2.88). The R^2 obtained from the statistical analysis of burukutu were all higher than 0.75 (75%) which depicts that the model used adequately fits the relationship between the variables under consideration. Exposure to high concentration of methanol has numerous health consequences.

Keywords: Burukutu, Sorghum, Methanol, Modeling, Response Surface Methodology

1. Introduction

Burukutu is a traditional cereal-based fermented beverage [1]. Cereals are important in many parts of the world as food sources, and starches from them differ in chemical properties and molecular structures [15]. The main chemical component of millet and sorghum grain is starch. Burukutu is an alcoholic beverage contains ethanol (commonly known as alcohol). Alcoholic beverages that have lower alcohol content are produced by fermentation of sugar or starch containing plant materials. Beverages with higher alcohol content (spirits) are produced by fermentation followed by distillation. The major local alcoholic beverages produced in Nigeria are Burukutu, palm wine, pito, and Ogogoro [1].

Methanol is ubiquitously present in the human body and it also appears in human blood and breath. It is produced by bacterial fermentation or by decomposition of pectin contained in grains [14]. Sorghum contains part per million levels of methanol [9]. The presence of methanol in alcoholic beverages is a well-known problem [10]. When grains are fermented to obtain alcohol, methanol is also formed. In the process of distillation of fermented grains, methanol is

distilled together with ethanol due to their similar chemical properties. That is the reason why all alcoholic beverages obtained from fermented grains will contain methanol [9]. Methanol poisoning outbreak has been implicated in the consumption of adulterated/ counterfeit or informally-produced spirit drinks. There have been numerous outbreaks in recent years, including in Cambodia, Czech Republic, Ecuador, Estonia, India, Indonesia, Kenya, Libya, Nicaragua, Norway, Pakistan, Turkey and Uganda [21]. In Nigeria, methanol poisoning has so far killed more people than Ebola did during the outbreak last year. Sixty-six people reportedly died after the consumption of locally brewed gin in four districts of Rivers State, Nigeria [13].

The incidence of methanol contamination of traditionally fermented beverages is increasing globally resulting in the death of a number of individuals [16]. The presence of Methanol content is not fully elucidated in burukutu and thus its presence is yet to be fully known. Burukutu, on the other hand, is stored locally hence may be susceptible to microbial attack at storage. The presence of Methanol Content in burukutu may not show forth presently and on excessive consumption, they have the tendency to bio-accumulate in the body since it has no pathway in human system, is capable of damaging vital organs in the body since fermented beverages and foods are therefore the most susceptible sources in terms of methanol formation. Besides being a matter of public health concern, methanol in a concentration higher than the permitted limit by international standards could be a barrier to exportation [12]. Response surface methodology (RSM) is a statistical experimental design that enables simultaneous varying of process variables, unlike what obtains in the conventional experimentation, thereby eliciting the interaction between such variables [2]. It is a faster and more economical method for gathering research results than the classic one-variable at a time or full-factor experimentation.

The objective of the study is to determine the effect of storage conditions on the methanol content of burukutu made from different sorghum using Response Surface Methodology.

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 departments of Food Science and Technology.

2.1. Production of Burukutu

The three varieties of sorghum (CSR- 02, S- 17 and S- 44) were processed into burukutu using the method of Egbema and Etuk (2007) and Ibrahim and Ierve (2013) 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°C - 32°C).

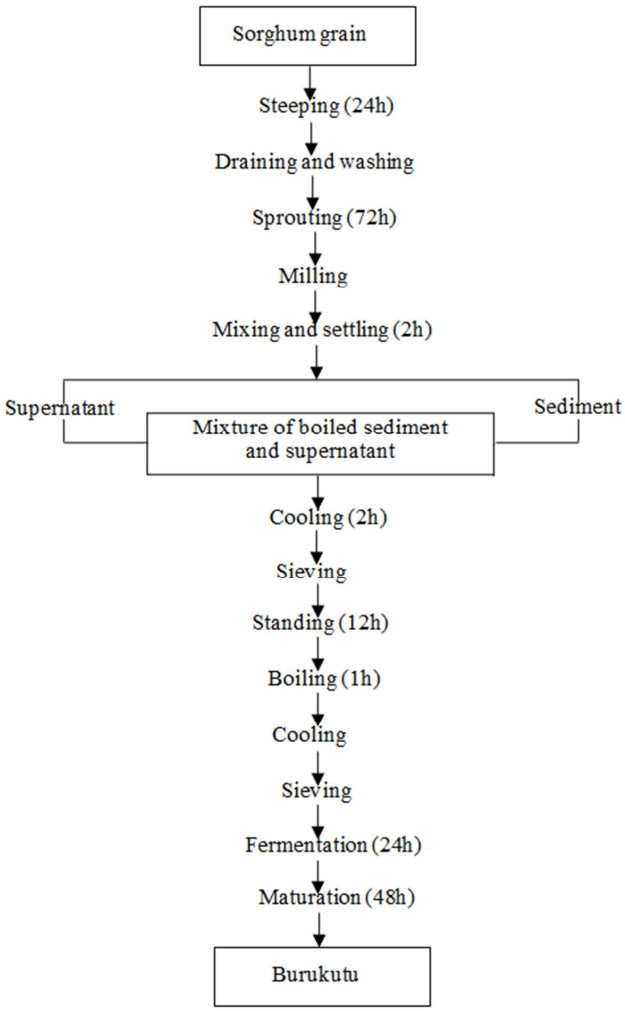

Figure 1. *Flow diagram for production of Burukutu*

Egbema and Etuk (2007) and Ibrahim and Ierve (2013) with slight modification

2.2. Determination of the Methanol and pH Content of Burukutu

2.2.1. Methanol Content

Methanol concentration was determined using the modified method of Wang *et al.*(2004) and Okunowo and Osuntoki (2007). The methanol content of the beverages produced from the different variety of sorghum was determined using the UV-vis Spectrophotometer (Lasany l1-722). Four milliliters (4ml) of the methanol solution was

pipetted into a cuvette and used to calibrate the spectrophotometer before another cuvette with the 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 205nm for MC. The concentration of methanol was obtained by extrapolation from a standard curve using standard methanol solution.

2.2.2. pH

Ten milliliters (10ml) of the fermenting medium was poured into sterile bottles and pH was measured with a Jenway pH meter equipped with a glass electrode. Buffer 4 and buffer 9 were used for calibration of the pH meter before readings were taken. AOAC (1990).

2.3. Experimental Design and Data Analysis

The three-level three-factorial Box–Behnken experimental design (Filli *et al.,* 2011) 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 (Filli *et al.,* 2011).

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

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

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 were obtained from the equations: $X_1 = 72\,x_1 + 72$; $X_2 = 5\,x_2 + 30$; $X_3 = x_3 + 2$
Where; -1 = minimum values; +1 = maximum values; 0 = centre points.

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

Sample	Coded units X_1	X_2	X_3	Natural units 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, and 17) are center points. X_1 = storage time (h); X_2 = storage temperature (°C) and X_3 = beverage variety type (g).
$X_1 = 72\,x_1 + 72$; $X_2 = 5\,x_2 + 30$; $X_3 = x_3 + 2$.

3. Results and Discussion

Results in Table 3 show the Mean results for the methanol content and pH of burukutu

Table 3. *Mean results for the methanol content and pH of burukutu.*

Runs	ST (h)	STT (°C)	BVT (g)	MC (µg/L)	pH
1	0	30	3	0.139^{g}	6.18^{ab}
2	72	30	2	1.753^{e}	3.17^{e}
3	0	30	1	0.135^{g}	6.17^{b}
4	72	25	1	1.640^{f}	3.12^{f}
5	72	25	3	1.643^{f}	3.12^{f}
6	144	35	2	2.405^{a}	2.92^{g}

Runs	ST (h)	STT (°C)	BVT (g)	MC (µg/L)	pH
7	144	25	2	2.299^{c}	2.88^{h}
8	0	35	2	0.143^{g}	6.21^{a}
9	72	30	2	1.750^{e}	3.17^{e}
10	72	35	1	1.798^{d}	3.68^{d}
11	72	30	2	1.750^{e}	3.17^{e}
12	72	30	2	1.748^{e}	3.17^{e}
13	72	35	3	1.809^{d}	3.66^{d}
14	144	30	3	2.398^{b}	2.93^{g}
15	0	25	2	0.128^{g}	6.13^{c}
16	144	30	1	2.392^{b}	2.94^{g}
17	72	30	2	1.753^{e}	3.17^{e}
LSD				0.013	0.03

Key: MC=Methanol content; 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.

The results in Table 4 show Regression equation coefficients for significant and non-significant terms for methanol content and pH of burukutu.

Table 4. *Regression equation coefficient for significant and non-significant terms for methanol content and pH of burukutu.*

Coefficient	MC	pH
Linear		
β0	1.75	3.17
A	1.12*	-1.63*
B	0.056*	0.15*
C	3×10^{-3}	-2.5×10^{3}
Quadratic		
A^2	-0.48*	1.26*
B^2	-0.025	0.10
C^2	-3.02×10^{-3}	0.12
Interaction		
AB	0.023	-0.010
AC	5×10^{-4}	-5×10^{-3}
BC	2×10^{-3}	-5×10^{-3}
R^2	0.9995	0.9958
Adj R^2	0.9988	0.9903
Lack of fit	NS	NS
Model (Prob>F)	<0.0001*	<0.0001*

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

3.1. Methanol Content

The methanol content ranged from 0.128µg/L to 2.405µg/L (Table 3).

The results showed that methanol content increases with increase in storage time from 0h – 144h; therefore there exist a direct relationship between methanol content and storage time. The maximum storage time (144h) had the highest methanol content, this could be due to the fact that during fermentation of burukutu to obtain alcohol, methanol may also be formed; hence, methanol and ethanol have similar chemical properties. This is the reason why all alcoholic beverages obtained from fermented fruits will contain methanol [5]. Methanol is a potent toxicant in humans and occurs naturally at a low level in most alcoholic beverages without causing harm. However, illicit drinks made from "industrial methylated spirits" [5% (v/v) methanol: 95% (v/v) ethanol] can cause severe and even fatal illness [19]. Ellis *et al* (2017) demonstrated that it is possible to detect a total of 10 denaturants/additives in extremely low concentrations without any contact with the sample; discriminate between and within multiple well-known Scotch whiskey brands, and detect methanol concentrations well below the maximum human tolerable level.

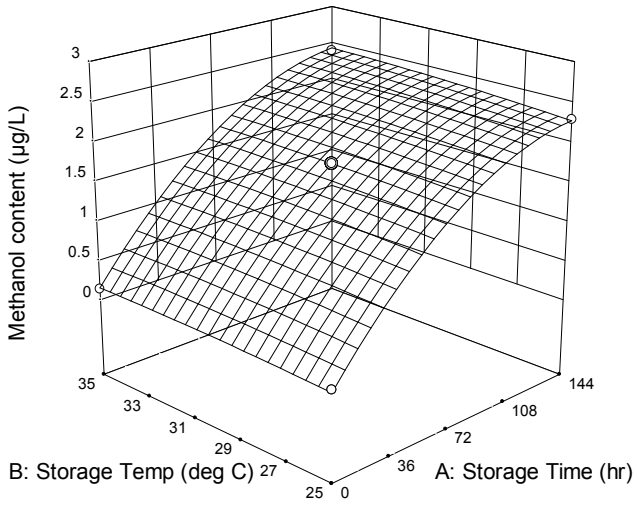

Figure 2. *Effect of Storage time and storage temperature on methanol content of burukutu.*

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

The methanol content levels fell within the maximum permissive level set by National Agency for Food Drug Administration and Control, NAFDAC (0.05mg/L) [20]. Also, the Australian New Zealand Food Authority (ANZFA) permitted the maximum concentration of methanol in spirit beverages at 8g/L of ethanol in the beverage. Methanol may be found as an adulterant of alcoholic drinks [20].

Methanol is also presented as a consequence of enzymatic degradation of pectin. The values obtained (Table 4) showed that these responses were adequately described by the factors in the polynomial model. There were significant differences ($p < 0.05$) in both the quadratic and linear effects i.e. storage

time (A), storage temperature (B) and the square of storage time (A^2) or significance with a probability of 95%. Methanol production in traditionally fermented beverages can be linked to the activities of pectinase producing yeast, fungi, and bacteria [16].

The results of the polynomial after removing the non-significant terms in Table 4: $MC = 1.75 + 1.12A + 0.056B - 0.48A^2$

The response surface plot in Figure 2 indicated a progressive linear increase in MC content of burukutu samples as storage time increased with slight increase in temperature. The coefficient of determination (R^2) of the model was 0.9995 which indicated that the model adequately represented the real relationship between the variables under consideration. An R^2 value means that 99.95% of the variability was explained by the model and only 0.05% was as a result of chance or accounts for factors not included in the model.

The regression model obtained

$$MC = 1.75 + 1.12A + 0.056B + 0.003C + 0.023AB + 0.0005AC + 0.002BC - 0.48A2 - 0.25B2 - 0.0003C2$$

3.2. pH

The pH of the beverage (burukutu) variety type ranged from 2.88 to 6.21 as shown in (Table 4). Storage time had a highly negative linear and quadratic effect on the pH of the samples (Table 4). This implies that pH of the burukutu samples produced from different sorghum varieties decreased with increase in storage time. The decrease in pH with an increase in storage time is attributed to the production of organic acid during storage by the activities of the lactic acid bacteria and *Saccharomyces spp* on the carbohydrate content of the cereal grain (sorghum) [18].

Burukutu with the highest pH value showed that acid content was very close to neutrality (pH tending to 7). The short shelf life may be due to the low lactic acid content, low titrable acidity, low alcohol content, a high concentration of fermentable sugars and the presence of lipoxidation product. There were significant differences ($p < 0.05$) in the quadratic and linear effect i.e storage time (A), storage temperature (B), square of storage time (A^2) or significance with a probability of 95%. The results of polynomial after removing the non-significant terms in Table 3: $pH = 3.17 - 1.63A + 0.15B + 1.26A^2$

The coefficient of determination (R^2) of the model was 0.9958 which indicated that the model adequately represented the real relationship between the variables under consideration. An R^2 value means that 99.58% of the variability was explained by the model and only 0.42% was as a result of chance or accounts for factors not included in the model.

The regression model obtained is

$$pH = 3.17 - 1.63A + 0.15B - 0.0025C - 0.001AB - 0.005AC - 5E - 003BC + 1.26A2 + 0.1B2 + 0.12C^2$$

The above equations in terms of coded factors can also be used to make predictions about the response for a given level in each factor.

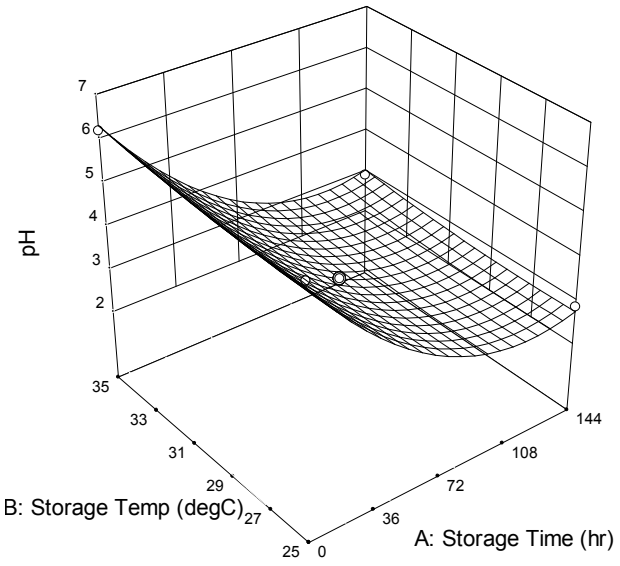

Figure 3. *Effect of Storage time and Storage temperature on pH of burukutu.*

4. Conclusion

The study showed that storage time and storage temperature significantly influenced the chemical properties of the burukutu made from different sorghum varieties. Increase in storage time caused a significant decrease in the pH (6.21–2.88), whereas the increase in storage time caused a significant increase in the following chemical properties: methanol content (0.128µg/L – 2.405µg/L). The R^2 obtained from statistical analysis for the chemical properties (methanol content, pH) 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 (≤ 25°C) and a short storage time (≤ 72h) gave least values based on MC levels and lesser effects on other chemical properties. The response surface methodology has been effectively used 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.

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