Research Article • DOI: 10.2478/bioeth-2013-0003 • BIOETH • 2013 • 20–27

Fermentation of stalk juices from different Nigerian sorghum cultivars to ethanol

Abstract

For improved production of ethanol from sorghum stalk juice fermentation, cultivation location and cultivar type are important factors to consider. In the present study, SSV2 and KSV8 sorghum cultivars were cultivated in Kano and Kaduna states in Nigeria that exhibit notably different rain precipitation and diurnal temperatures. The crude stalk juices (without pre-treatment or nutrient supplementation) were extracted from these sorghum samples and fermented with a distiller's strain of the yeast, *Saccharomyces cerevisiae*. Sugar consumption and alcohol production were determined by HPLC and GC-MS, respectively. When it was grown in the Kaduna site, SSV2 was identified as the highest yielding sorghum cultivar from which we extracted the maximum levels of extractable sugars (161.50 g l^{-1}) that yielded favourable ethanol levels of 80.56 g l^{-1} following fermentation. Our findings show that relatively colder and wetter cultivation sites are preferred for sorghum stalk juice destined for bioethanol production.

Keywords

Sorghum cultivars • Varied climate condition • Juice composition • Fermentation performance • Bioethanol

Muhammad Nasidi[1], Reginald Agu[2], Yusuf Deeni[1], Graeme Walker[1]*

[1]School of Contemporary Sciences, University of Abertay Dundee, Bell street, Dundee, DD1 1HG, Scotland

[2]The Scotch Whisky Research Institute, The Robertson Trust Building, Edinburgh, EH14 4AP, Scotland

Received 09 May 2013
Accepted 09 August 2013

Abbreviations:

NIHORT: National Horticultural Research Institute, Nigeria.
PTDF: Petroleum Technology Development Fund, Nigeria.
Site **B**: Bagauda, Kano. Site **Z**: Zaria, Kaduna.

1. Introduction

As the world approached peak oil production era [1], there is increased uncertainty in predicting future prices of fossil fuels. Concerns on climate change due to continued use of fossil based fuels, has diverted the world attention towards developing renewable and sustainable transport fuels [2,3]. Fossil fuels are reported to account for over 80% of primary energy source globally, of which about 58% is expended as transport fuel [4]. In not too distant future biofuels comprising bioethanol, biobutanol, biodiesel, among others, are envisaged as likely alternatives to fossil fuels in transport sector, this is because of their renewability and sustainability over fossil fuels [5]. Bioethanol is strategically important as transport fuel of the future, because it is an environment friendly energy source which generates relatively acceptable quality exhaust gases leading to reduced GHG emissions [4,6]. Therefore, bioethanol as a plant-based liquid biofuel may be used in automobiles as additive or substitute to petroleum in transportation [7]. Nigeria is the 9th largest oil producing country in the world and largely depends on fossil based fuels as cheap energy source. This has constrained the desired growth in the renewable energy sector in the country [8,9]. However, the rapid depletion of global oil reserves and the spiralling cost of crude oil in global markets will necessitate the search for alternative and sustainable transport fuels in Nigeria. Bioethanol can be produced by bioconversion of plant based sugar-rich crops such as sorghum, sugarcane, cassava, and sugar beet.

Interestingly, sorghum (Sorghum *bicolor (L.) Moench*) is the 5th most important cereal globally and 2nd in Africa. It is a staple food source to over 500 million people and is cultivated in over 45 million hectares of farmland worldwide. Global sorghum production is estimated at over 60 million metric tons annually, where Nigeria is ranked among the top 3 largest sorghum producing countries in the world [10-12]. Sweet sorghum stalk juice contains variable amounts of sugars, proteins and starch depending on the cultivar type, crop harvesting time and cultivation location [13]. The typical sugars are predominantly glucose, sucrose and fructose, while maltose, dextrin, maltotriose and other oligosaccharides may be present in sorghum stalk juice in low concentrations [14]. The stalk may be directly chewed for its sweet juice as a snack, or the juice can be mechanically

*E-mail: g.walker@abertay.ac.uk

extracted and processed into sweet syrup or sugar cake (locally called "mazarkwaila" in Nigeria). High glucose-containing juices with minimal starch content are preferred for syrup production; as this avoids gelling and crystallization problems occurring during juice cooking or during long term storage of the finished syrup [15].

The Nigerian government through its "Biofuel policy statement" of 2007 aspires to achieve self sufficiency in bioethanol supply domestically by the year 2020. Sorghum, among other crops, has been designated as potential feedstock sources for Nigerian bioethanol [9]. Although the grains of some Nigerian local sorghum cultivars have been extensively studied for their potential use in malting and brewing, little or no attention has been given to the potential of these sorghum stalk juices for bioethanol production. Harvesting of sorghum grains before they reach physiological maturity makes them suitable for immediate use in brewing processes, while the stalk juice may be utilised as a fermentation substrate [16-19]. Nigerian SSV2 and KSV8 sorghums are cultivated because of their high grain quality and are regarded as being very tolerant to biotic and abiotic environmental stresses [10,20]. However, despite sorghum's high adaptation to adverse climatic conditions, high productivity output remains constrained by poor soil quality, low and erratic rainfall and low agro-chemical inputs during cultivation. This is even more evident in developing countries where agro-chemicals and irrigation cultivation costs are often beyond the reach of peasant farmers [21,22]. Consequently, it is desirable to investigate the impact of these parameters on sorghum agronomic characteristics and productivity. For example, soil physical quality (such as hydraulic, pH, density, particle size and distribution among others) profoundly effects how best the soil can be managed for optimum crop yields [23]. In this study, the reported [24,25] morphological and physical properties of soils at sites B and Z were summarised in Table 1.

Previous research on improving sorghum juice fermentation has focused on sorghum stalk juice pre-treatment e.g. enhanced juice clarification and pH optimisation [26], supplementing juice substrates with commercially available yeast nutrients [27,28], enriching juice sugar levels with e.g. cane molasses for very high gravity (VHG) fermentation [29,30], immobilizing fermenting yeast cells e.g. on corncob, sorghum stalks or entrapment in sodium alginate gel beads among others [31-33]. Hence, the identification of highly fermentable sorghum juice producing cultivars that require lesser nutritional supplements is highly desirable, especially in the context of effective and manageable local bioresource utilisation, technological viability and economic sustainability. Limited attention has been given to the effect of climatic and environmental conditions on raw sorghum juice fermentation performance [26,31,34-36]. We therefore investigated the influence of environmental cultivation conditions (rainfall and temperature) on fermentable juice yield among locally grown Nigerian sorghum cultivars. We identified a cultivar (SSV2) that demonstrated superior potential for bioethanol production.

2. Methods

2.1 Sorghum cultivation and harvesting

Nigerian sorghum cultivar seedlings SSV2 and KSV8 were cleaned and treated with metalaxyl fungicide chemical (Apron Star™, Nigeria) and planted in Kano (site B) and Kaduna (site Z) in Northern Nigeria (Figure 1). The crops were cultivated under rain-fed conditions and only cow dung manure was applied. The SSV2 sorghums were harvested 11 weeks after planting when their grains were observed to reach soft-dough stage, while the KSV8 sorghums were harvested 16 weeks after planting when their grains also reached their soft-dough stage. The crops were harvested manually by cutting stalks above ground. One hundred stripped and cleaned stalks of SSV2 and KSV8 (randomly selected from each corner of fields) were roller-milled to extract the stalk juices (Ohaus®, Switzerland). The freshly extracted crude juices were clarified by gravity settling in holding tanks prior to filtration through polyester filter bags™ (SS Bolting Cloth, China) and pasteurization at 65°C for 2 h [15,18] using juice steriliser™ (Dacheng, China). Juice samples were stored at -20 °C until further analysis.

2.2 Compositional analysis of sorghum juice

Total starch contents of juices were determined using an enzyme assay with Megazyme™ K-TSTA kits according to AACC (American Association for Clinical Chemistry) standard method 76.13. Total free amino nitrogen (FAN) was determined by K-Large 02/11™ (yeast available nitrogen, YAN) and K-PANOPA 02/11™ (primary amino acid nitrogen, PAN) assay kits (Megazymes, Northern Ireland). Crude protein contents were

Table 1. Soils physical and morphological properties of crop cultivation location.

Parameters	Site B	Site Z
pH	5.0	5.2
Org. C (g kg^{-1})	0.38	3.3
Total N (g kg^{-1})	0.08	0.53
Avail. P (mg kg^{-1})	0.56	1.8
Exchangeable bases (C mol kg^{-1})		
Ca	0.27	1.80
Mg	0.08	0.36
Na	0.30	0.05
K	0.19	0.33
Exch. Acidity (Al^{3+} H^{+})	0.24	0.10
CEC	1.08	4.0
Soil physical properties (g kg^{-1})		
Sand	78	46
Silt	12	40
Clay	10	14

Source: [23-25].

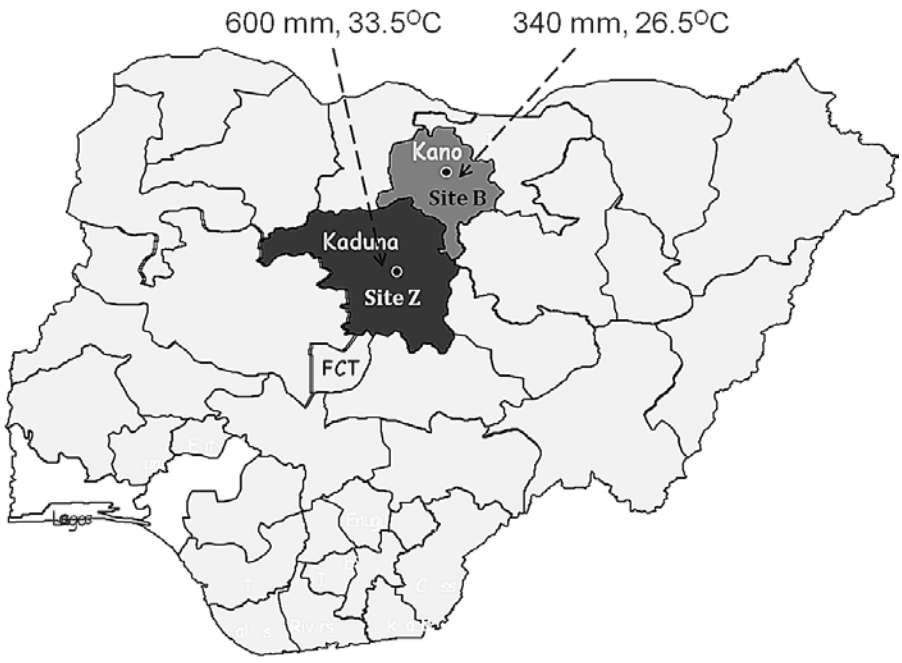

Figure 1. Map of Nigeria showing site locations B (coordinates: 11.33 °N, 8.23 °E) and Z (coordinates: 11.10 °N, 7.38 °E) as well as the climatic conditions where SSV2 and KSV8 sorghum cultivars were grown for the purpose of this study.

determined by Bradford's reagent (Sigma-Aldrich, UK) using recommended 3.1 ml protocol and the absorbance read with a Genesys® 10 s spectrophotometer (Thermo spectronic, USA). Total amino acid concentrations were determined courtesy of Heriot-Watt University Edinburgh by gradient elution method using HPLC equipment [37]. Briefly, fresh juice (2 ml) were filtered through 0.22 µm filters into HPLC-grade vials and placed in Gilson 231 autosampler with 40 l dilutor (Gilson, USA), the juice amino acids components were separated with a 150 mm × 4.6 mm phenosphere NEXT, 5u, C18 column™ (Phenomenex, UK) and detected by FP-1520 fluorescent detector (Jasco, USA), Gilson 715 data handling package was used to quantified amino acids. To determine the major fermentable sugars in juices (i.e. glucose, sucrose and fructose), 1 ml aliquots of sorghum juices (at 1:10 dilution) were filtered through 0.22 µm micro syringe filters into 2 ml vials containing 1ml *meso*-erythritol solution (internal standard sugar). The final solutions were vortexed and placed in an HPLC auto sampler (Spectra-physics, USA) and the sugars separated with a 300 mm × 7.8 mm REZEX RPM-monosaccharide pb+2 (8%) column™ (Phenomenex, USA) and quantified using HPLC software (CSW32 version v.1.4 chromatogram software from DataApex®, USA).

2.3 Yeast seed culture preparation

Yeast seed cultures were prepared by firstly inoculating two loop fulls of *Saccharomyces cerevisiae* (DCLM distillers' yeast strain, courtesy of Kerry Biosciences, Menstrie, Scotland) into 400 ml YEPD media comprising of 2.0% (w/v) yeast extract, 4.0% (w/v) bacteriological peptone, and 4.0% (w/v) glucose. Cultures were incubated at 32 °C with orbital shaking at 150 rpm for 20 h.

2.4 Fermentation and alcohol analysis

Frozen crude juices were thawed to room temperature and filtered through glass fibre filters (Millipore®, Sigma Aldrich). Appropriately washed cell pellets of *S. cerevisiae* yeast from the prepared YEPD culture were inoculated into 100 ml of raw juice at pitching rate of 10×10^6 cell ml^{-1}. Fermentation was then conducted at 32°C with orbital shaking at 130 rpm. Samples were withdrawn every 24 h for alcohol determination by gas chromatography using a GC-column (ZB-AAA; Phenomenex Inc, USA) on Agilent GC-MS model 6890GC (Agilent, Palo Alto, USA) equipped with MSD model 5975 Inert XL, PTV injector (Gerstel, Muehlheim, Germany).

2.5 Statistical analysis

Analysis of variance (ANOVA) by a GLM model using Minitab™ 16 statistical software (MINITAB©, USA) was used to test for significant differences in compositions of stalk juices of SSV2 and KSV8 sorghum cultivars grown in sites B and Z, respectively. Significant differences were tested at 95% confidence level and results with p values < 0.05 were considered significantly different.

3. Results and discussion

The mean recorded diurnal temperatures and rainfall for site locations in Kano (site B) were respectively 33.5 °C and 340 mm, and in Kaduna (site Z) were 26.5 °C and 600 mm (NIHORT, Nigeria). The sorghum crops were harvested before grains reached physiological maturity to avoid juice sugars getting converted to starch; allowing the sorghum grains to grow beyond soft-dough stage would result to conversion of the juice sugars predominantly to starch. The SSV2 sorghum cultivar showed

higher total stalk juice yield despite having less cultivation duration relative to KSV8 (Table 2). Whilst significant differences ($p < 0.05$) were observed in juice yields between SSV2 and KSV8 sorghums and accounted for about 22% in favour of SSV2, the observed significant differences ($p < 0.05$) in juice yields of SSV2 and KSV8 due to varied climatic conditions between sites B and Z appeared to account for over 10% differences in favour of site Z (Table 2). This suggests that sorghum cultivar selection is a very important factor to consider prior to site selection when cultivating sorghum crops for stalk juice production. In addition to quantitative stalk juice yields of sorghum cultivars from the present study [38,39], have considered the qualitative composition of juices as important criteria for sorghum cultivar selection destined for bioethanol production.

In the context of the effect of temperature and rainfall, the aggregate compositions of SSV2 and KSV8 stalk juices between sites B and Z shown in Table 2 were significantly different ($p < 0.05$). Site B favoured higher juice starch accumulation, whilst site Z favoured higher total contents of fermentable sugars (Table 2). These results were consistent with the C-4 agronomic characteristics of sorghum crops [13]. For example, the observed warmer and drier climatic condition of site B appeared to favour relatively higher biomass yield as observed from the bagasse yield shown in Table 2. Furthermore, higher protein content of juice was observed to correspond to higher FAN and amino acids concentrations in juice and vice versa as shown in Tables 2 and 3.This was suggested to be related to cultivation soil quality rather than rainfall or temperature variation effect as discussed by Roland and Gene [40].

The primary extracellular nitrogen sources for yeast biosynthetic activities during fermentation are individual amino acids, ammonium ions (NH_4^+) and small peptides. The NH_4^+ and small peptide molecules are principally derived from proteolytic catabolism of proteins and FAN available in juice. In addition to the extracellular free amino acids, *S. cerevisiae* cells are also able to utilised endogenous amino acids such as arginine as sole nitrogen sources [41,42]. Due to specificity and sensitivity limitations of Megazyme K-LARGE/K-PANOPA assay kits in detecting specific amino acids [43], individual amino acid concentrations in sorghum juices were determined by Gas chromatography-mass spectrophotometry. Data in Table 3 show SSV2 and KSV8 sorghum juices from sites B and Z contain all amino acids necessary for efficient yeast metabolism during fermentation. These amino acids were broadly classified into 3 groups based on *S. cerevisiae* cells orderly preferential uptake during fermentation [42]. Thus, group 1 amino acids were assimilated by yeast within 24 h of the onset of fermentation, and this mirrors the observed fast fermentation rates of SSV2Z juice (Figure 2) which has higher levels of group 1 amino acids along with higher FAN and glucose contents compared with SSV2B, KSV8B and KSV8Z juices (Tables 2 and 3). This nutritionally favourable combination of readily available nitrogen plus fermentable sugar in the SSV2Z juices explains the faster overall fermentation rates observed with this particular sorghum cultivar, as shown in Figure 2. The KSV8Z juice showed similar fast fermentation rates compared with SSV2Z juice in the first

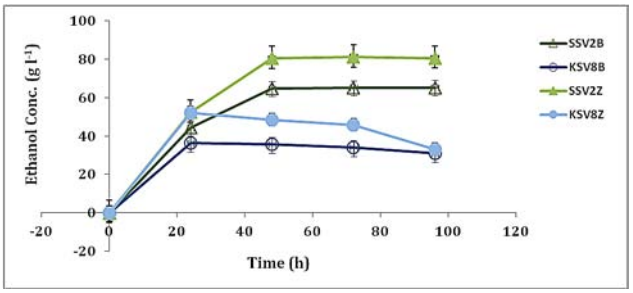

Figure 2. Ethanol concentration profile of sorghum stalk juice fermentation. Stalk juices fermentation performance for SSV2 and KSV8 sorghum cultivars fermented with S. cerevisiae at 32 °C and 120 rpm. Sorghum crops were grown in Kano (site B) and Kaduna (site Z), Nigeria. Std mean of duplicate experiments.

Table 2. Compositional analysis of SSV2 and KSV8 sorghum stalk juices.

cultivar	SSV2		KSV8	
	Site B	Site Z	Site B	Site Z
Dry bagasse (t ha^{-1})	35.60 ± 1.17^{a}	29.31 ± 1.92^{b}	39.72 ± 1.86^{c}	33.49 ± 1.24^{d}
Juice yield (l ha^{-1})	25024 ± 20.43^{a}	25596 ± 13.32^{b}	23304 ± 4.93^{c}	24536 ± 9.07^{d}
Total starch (g l^{-1})	0.97 ± 0.01^{a}	0.64 ± 0.01^{b}	0.51 ± 0.01^{c}	0.37 ± 0.01^{d}
Total protein (g l^{-1})	1.58 ± 0.01^{a}	1.82 ± 0.01^{bc}	1.08 ± 0.01^{d}	1.03 ± 0.01^{d}
Total FAN (mg L^{-1})	224 ± 1.14^{a}	325 ± 3.22^{b}	191 ± 1.43^{c}	134 ± 1.52^{d}
Sucrose (g l^{-1})	102.71 ± 3.76^{a}	113.93 ± 1.88^{b}	36.41 ± 2.11^{c}	55.67 ± 1.39^{d}
Glucose (g l^{-1})	27.58 ± 2.03^{a}	32.07 ± 1.14^{bc}	19.73 ± 0.83^{d}	21.76 ± 1.18^{d}
Fructose (g l^{-1})	13.69 ± 1.54^{ab}	15.50 ± 0.34^{ab}	9.67 ± 0.13^{cd}	10.52 ± 0.96^{cd}
Total sugars (g l^{-1})	143.99 ± 3.27^{a}	161.50 ± 3.36^{b}	65.81 ± 2.81^{c}	87.96 ± 3.53^{d}

The composition of crude stalk juices of SSV2 and KSV8 sorghum cultivars grown in Kano (site B) and Kaduna (site Z), Nigeria, under rain fed conditions and without application of chemical fertilizer. SSV2 and KSV8 were harvested 11 and 16 weeks after planting dates respectively. Results are Mean of triplicates ± SD. Results on the same row followed by different superscript letter (a-d) indicate significant difference ($p \leq 0.05$) by GLM (ANOVA) test.

Table 3. Amino acids profile of SSV2 and KSV8 sorghum stalk juices.

Cultivar	SSV2		KSV8	
Amino acids	Site B	Site Z	Site B	Site Z
Group 1: (μmole ml^{-1})				
aspartic acid	1.141 ± 0.011^{a}	0.730 ± 0.009^{b}	0.631 ± 0.007^{c}	0.530 ± 0.006^{d}
glutamic acid	0.444 ± 0.080^{ad}	0.538 ± 0.008^{bc}	0.403 ± 0.005^{ad}	0.462 ± 0.007^{ad}
asparagine acid	6.410 ± 0.120^{a}	10.580 ± 0.020^{b}	6.705 ± 0.070^{c}	3.885 ± 0.070^{d}
glutamine	3.145 ± 0.090^{a}	2.690 ± 0.080^{b}	1.545 ± 0.070^{c}	1.595 ± 0.070^{d}
serine	0.956 ± 0.007^{a}	0.750 ± 0.005^{b}	0.396 ± 0.007^{c}	0.345 ± 0.006^{d}
arginine	0.093 ± 0.004^{a}	0.082 ± 0.005^{b}	0.055 ± 0.006^{c}	0.030 ± 0.006^{d}
threonine	0.391 ± 0.007^{a}	0.293 ± 0.004^{b}	0.196 ± 0.007^{c}	0.125 ± 0.003^{d}
lysine	0.086 ± 0.005^{a}	0.076 ± 0.006^{b}	0.048 ± 0.003^{bc}	0.014 ± 0.003^{d}
Sub-Total	12.664 ± 0.234^{a}	15.738 ± 0.103^{b}	9.977 ± 0.025^{c}	6.985 ± 0.157^{d}
Group 2: (μmole ml^{-1})				
histidine	0.071 ± 0.007^{a}	0.033 ± 0.002^{bc}	0.061 ± 0.003^{a}	0.023 ± 0.004^{d}
methionine	0.027 ± 0.004^{a}	0.020 ± 0.004^{a}	0.013 ± 0.001^{bc}	0.007 ± 0.003^{d}
isoleucine	0.264 ± 0.006^{a}	0.293 ± 0.004^{b}	0.181 ± 0.006^{c}	0.133 ± 0.004^{d}
leucine	0.205 ± 0.008^{a}	0.275 ± 0.005^{b}	0.147 ± 0.005^{c}	0.105 ± 0.007^{d}
phenylalanine	0.192 ± 0.008^{a}	0.085 ± 0.004^{b}	0.086 ± 0.005^{b}	0.037 ± 0.007^{cd}
valine	0.644 ± 0.003^{a}	0.674 ± 0.008^{a}	0.354 ± 0.005^{bc}	0.241 ± 0.007^{d}
Sub-Total	1.402 ± 0.008^{a}	1.380 ± 0.003^{a}	0.841 ± 0.005^{bc}	0.544 ± 0.001^{d}
Other Groups: (μmole ml^{-1})				
glycine	0.109 ± 0.006^{a}	0.165 ± 0.006^{b}	0.064 ± 0.005^{cd}	0.076 ± 0.007^{cd}
alanine	0.967 ± 0.004^{a}	1.063 ± 0.008^{b}	0.434 ± 0.008^{c}	0.389 ± 0.005^{d}
proline	0.034 ± 0.008^{ad}	0.134 ± 0.008^{bc}	0.031 ± 0.005^{ad}	0.027 ± 0.005^{ad}
tryptophan	0.226 ± 0.006^{a}	0.070 ± 0.004^{b}	0.095 ± 0.004^{c}	0.042 ± 0.004^{d}
tyrosine	0.450 ± 0.005^{a}	0.181 ± 0.008^{bd}	0.231 ± 0.007^{c}	0.184 ± 0.010^{bd}
Sub-Total	1.786 ± 0.007^{ab}	1.613 ± 0.002^{ab}	0.853 ± 0.019^{cd}	0.717 ± 0.009^{cd}
Grand Total	15.852 ± 0.055^{a}	18.731 ± 0.046^{b}	11.671 ± 0.114^{c}	8.246 ± 0.231^{d}

Amino acid concentration (μmole ml^{-1}) in crude stalk juices of SSV2 and KSV8 sorghum cultivars grown in sites B and Z under rain fed condition and without chemical fertilizer application. Results are Mean of duplicates ± SD. Results on the same row followed by different superscript letter (a-d) indicate significant difference ($p \leq 0.05$) by GLM (ANOVA) test.

24 h, this was despite the former having lower glucose and group 1 amino acids contents than the latter (Tables 2 and 3). The KSV8Z juice may be richer in vital minerals and vitamins necessary to facilitate yeast cellular adaptation to early efficient fermentations. Although SSV2Z juice has high sucrose contents similar to that of SSV2B juice, fermentation kinetics of both juices was different. However, the comparatively higher concentrations of group 1 amino acids, FAN and fermentable sugars present in the SSV2Z juice mean that yeast cells exhibit better fermentation performance in this medium compared with SSV2B which is comparatively lower in these nutrients. For bioethanol production, the correct nutritional balance for yeast in fermentation media is crucial in dictating ethanol yields [44-46]. Failure to sustain and maintain fast fermentation rates in KSV8Z after 24 h (see Figure 2) may be due to depletion of both fermentable sugar and assimilable nitrogen. Of the 4 juice substrates studied, SSV2B had the highest total group 2 amino acid content (as well as other amino acid groups), followed by SSV2Z and KSV8B juices (Table 3). The group 2 amino acids are assimilated at a steady rate by yeast during fermentation, whereas the other amino acid groups are normally assimilated towards the latter stages of fermentation. Regarding proline, the uptake of this amino acid may be virtually negligible depending on the "substrate's nutrient worth" [44].

Fermentation of the crude juice from SSV2 sorghum stalks (from site Z) resulted in the highest ethanol yield of over 80 g l^{-1} (Table 4). This compares favourably with the figure of 73 g l^{-1} reported by Gyalai-Korpos et al. [35] for crude juice from another

Table 4. Ethanol yield and residual FAN/sugars from fermented sorghum stalk juices.

Cultivar	SSV2		KSV8	
Location	Site B	Site Z	Site B	Site Z
Ethanol (g l^{-1})	65.26 ± 1.43^{a}	80.56 ± 2.17^{b}	36.31 ± 1.66^{c}	52.07 ± 0.81^{d}
FAN (mg L^{-1})	76.34 ± 1.46^{a}	89.13 ± 1.46^{b}	32.84 ± 1.46^{c}	17.83 ± 1.46^{d}
Sucrose (g l^{-1})	11.35 ± 1.04^{ab}	6.98 ± 0.31^{cd}	0	0
Glucose (g l^{-1})	0	0	0	0
Fructose (g l^{-1})	0	0	0	0
TOTAL sugars (g l^{-1})	11.35 ± 1.04^{ab}	6.98 ± 0.31^{cd}	0	0

Ethanol concentration yields and total residual sugars of sorghum stalk juice after fermentation. Results are Mean of triplicates ± SD. Results on the same row followed by different superscript letter (a-d) indicate significant difference ($p \leq 0.05$) by GLM (ANOVA) test.

sorghum cultivar, and the 86 g l^{-1} yield reported by Zhao et al. [39] for crude sorghum juices supplemented with additional nutrients (urea, DAP, and $MgSO_4$). In addition, the ethanol yields of all SSV2 and KSV8 crude juices, irrespective of cultivation site, also compared well within the range of 24-68 g l^{-1} reported by Widianto et al. [47] for a variety of crude juices from different sorghum cultivars fermented without nutrient supplementation. However, when compared to juices of other sorghum cultivars whose crude juices were pre-treated and enriched with cane molasses and other commercial yeast nutrients, ethanol from SSV2 juice fermentation did not compare favourably. For example, ethanol yields of 94 g l^{-1} to 130 g l^{-1} were reported by Nuanpeng et al. [29] and Yue et al. [38] in sugar-supplemented very high gravity (VHG) sorghum stalk juice fermentations.

Overall, fermentation of the SSV2 stalk juices, particularly those extracted from the cultivar grown in site Z (exemplified by colder and wetter conditions) resulted in the highest ethanol yields. This work therefore points to differences in the climatic conditions of sorghum cultivation significantly impacting on fermentation performance of stalk juices.

When ethanol yields are projected per hectare, the SSV2 sorghum cultivar crude juices in sites B and Z were estimated to reach 2062 l ha^{-1} and 2595 l ha^{-1} respectively (from Tables 2 and 4). These results compare well with the 2100-2345 l ha^{-1} results reported by Kothari et al. [48] and Serna-Saldivar et al. [49] for crude juices of various sorghum cultivars grown under rain-fed conditions and with agrochemical applications (in contrast to this study where SSV2 was cultivated under rain-fed conditions and no agrochemical application).The reported ethanol yields of 2062 l ha^{-1} and 2595 l ha^{-1} of crude juices of SSV2 sorghum in this study are lower than previously reported values of 3450-4132 l ha^{-1} for juices of sorghum crops cultivated under fertilizer and other agrochemical applications [13,49]. However, taking into account the potential economic and environment costs of the use of fertilizers and agrochemicals, ethanol yields reported in the present study appeared to be favourable in the wider context of responsible and manageable local bioresource utilisation and economic and environmental sustainability.

4. Conclusions

Choosing the right sorghum cultivar and suitable cultivation location may lead to significantly improved stalk juice ethanol yields. We recommend that sorghum crops destined for stalk juice fermentation should be cultivated in locations with good rain precipitation and moderate diurnal temperature. For example, the Nigerian SSV2 sorghum, despite grown under rain fed condition and without agrochemical applications, showed better ethanol yield potential in site Z with 600mm mean precipitation and 26.5^{o}C diurnal temperature. KSV8 appeared not to be economically viable juice source for bioethanol production. The potential of several other sorghum crops cultivated in Nigeria for biofuels deserves further attention.

Acknowledgements

This work was graciously supported by funding from Petroleum Technology Development Fund (PTDF), Nigeria. We would like to thank Idris Giginyu (NIHORT, Nigeria), Heriot-Watt University Edinburgh and Kerry Bioscience, Menstrie, Scotland.

References

[1] Aleklett K., Peeking at Peak Oil, 1st ed., Springer sciences, New York, 2012

[2] Olsson L., Ahring B.K., Biofuels: advances in biochemical engineering/ biotechnology, 1st ed., Springer, New York, 2007

[3] Shiva V., Soil not oil: Climate change, peak oil and food insecurity, 1st ed., Zed Books, London, 2009

[4] Nigam P.S., Singh A., Production of liquid biofuels from renewable resources, Prog. Energy Comb. Sci., 2011, 37, 52-68.

[5] Dennis J.S., Scott S.A., Stephenson A.L., Improving the sustainability of the production of biodiesel from oilseed rape in the UK, Process Saf. Environ. Protect., 2008, 86, 427-440.
[6] Chiras D.D., Environmental science: creating a sustainable future, 1st ed. Jones Bartlett, London, 2009
[7] Pandey A., Handbook of plant-based biofuels, CRC, New York, 2009
[8] Abila N., 2010. Biofuels adoption in Nigeria: A preliminary review of feedstock and fuel production potentials, Management of Environmental Quality: An International Journal, 2010, 21, 785-795.
[9] Ishola M.M., Branberg T., Sanni S.A., Taherzadeh M.J., Review: Biofuels in Nigeria: A critical and strategic evaluation, Renew. Energ., 2013, 55, 554-560.
[10] Etuk E. B., Ifeduba A. V., Okata U. E., Chiaka I., Okoli, Ifeanyi C., et al., Nutrient composition and feeding value of sorghum for livestock and poultry: a review, J. Anim. Sci. Adv., 2012, 2, 510-524.
[11] Nasidi M., Akunna J., Deeni Y., Blackwood D., Walker G., Bioethanol in Nigeria: comparative analysis of sugarcane and sweet sorghum as feedstock sources, Energy Environ. Sci., 2010, 3, 1447-1457.
[12] Zegada-Lizarazu W., Zatta A., Monti, A., Water uptake efficiency and above- and below ground biomass development of sweet sorghum and maize under water regimes, Plant soil., 2012, 351, 47-60.
[13] Almodares A., Hadi M.R., Production of bioethanol from sweet sorghum: a review, Afr. J. Agric. Res., 2009, 4, 772-780.
[14] Massoud M.I., Abd Razek A.M., Suitability of ***Sorghum bicolor*** L. stalks and grains for bioproduction of ethanol, Annals Agric. Sci., 2011, 56, 83-87.
[15] Mazumdar D., Poshadri S., Srinivasa Rao, P., Ravinder Reddy C.H., Reddy B.V.S., Innovative use of sweet sorghum juice in the beverage industry, International Food Research, 2012, 19, 1361-1366.
[16] African Agricultural Technology Foundation (AATF), Feasibility Study on Striga Control in sorghum, African Agricultural Technology Foundation, 2011, http://aatf-africa.org/userfiles/Striga-in-Sorghum.pdf
[17] Ayoola A.A., Adeeyo O.A., Efeovbokhan V.C., Ajileye O., A comparative study on glucose production from ***Sorghum bicolor*** and ***Manihot esculenta*** Species in Nigeria, Inter. J. Sci. Tech., 2012, 2, 353-357.
[18] Blitzer M.J., Fox J.D., Processing sweet sorghum for syrup, Agricultural and Natural Resources, Dept. Of Agriculture, University of Kentucky, 2000, http://www. ca.uky.edu. Issued 6-1987
[19] Agu R.C., Bringhurst T.A., Brosnan J.M., Production of grain whisky and ethanol from wheat, maize and other cereals, J. Inst. Brew., 2006, 112, 314-323.
[20] Ogbonna A.C., Current developments in malting and brewing trials with sorghum in Nigeria: A Review, J. Inst. Brew., 2011, 117, 394-400.
[21] Erpelding J.E., Field evaluation of foliar anthracnose disease response for sorghum germplasmfrom the metabeleland North province of Zimbabwe, Afr. J. Agric. Res., 2008, 3, 792-796.
[22] Rao S.P. Kumar C.G., Reddy B.V.S., Sweet sorghum-from theory to practice, In: Rao S.P., Kumar C.G. (Eds.), Characterization of improved sweet sorghum cultivars, Springer Briefs in Agriculture, Springer, New Delhi, 2013.
[23] Owonubi A., Olowolafec E.A., Joshua V.I., Implication of soil physical properties for agriculture and environmental sustainability: A case study of an arable land in the guinea savanna area of Nigeria, Journal of Education and Social Research, 2012, 2, 155-161.
[24] Giginyu B.M., Fagbayide J.A., Effect of Nitrogen fertilizer on the growth and calyx yield of two cultivars of Roselle in Northern guinea savanna, Middle-East J. Sci. Res., 2009, 4, 66-71.
[25] Oduze A.C., Kureh I., Land use limitations and management option for a savanna zone Alfisol. Journal of Agriculture and Environment for International Development, 2009, 103, 321-335.
[26] Andrzejewski B., Eggleston G., Lingle S., Powell R., Development of a sweet sorghum juice clarification method in the manufacture of industrial feedstock for value-added fermentation products, Ind. Crop. Prod., 2013, 44, 77-87.
[27] Liu R., Shen F., Impacts of main factors on bioethanol fermentation by immobilized ***Saccharomyces cerevisiae*** (CICC1308), Bioresour. Technol., 2008, 99, 847-854.
[28] Liu R., Li J., Shen F., Refining bioethanol from stalk juice of sweet sorghum by immobilized yeast fermentation, Renew. Energ., 2008, 33, 1130-1135.
[29] Nuanpeng S., Laopaiboon L., Srinophakun P., Klanrit P., Jaisil P., Laopaiboon P., Ethanol production from sweet sorghum juice under very high gravity conditions: batch, repeated batch and scale up fermentation. Electron. J. Biotechnol., 2011, 14, 1-12.
[30] Sridee W., Laopaiboon L., Jaisil, P., Laopaiboon P., The use of dried spent yeast as a low-cost nitrogen supplement in ethanol fermentation from sweet sorghum juice under very high gravity conditions. Electron. J. Biotechnol., 2011, 14, 1-15.
[31] Ariyajareanwong P., Laopaiboon L., Jaisil P., Laopaoboon P., Repeated- batch ethanol fermentation from sweet sorghum juice by ***Saccharomyces cerevisiae*** immobilized on sorghum stalks NP01, Energies, 2012, 5, 1215-1228.
[32] Laopaiboon L., Laopaiboon P., Ethanol production from sweet sorghum juice in repeated-batch fermentation by ***Saccharomyces cerevisiae*** immobilized on corncob, World J. Microbiol. Biotechnol., 2012, 28, 559-566.
[33] Jin H., Liu R., He Y., Kinetics of batch fermentations for ethanol production with immobilized ***Saccharomyces cerevisiae*** growing on sweet sorghum stalk juice, Procedia Environmental Sciences, 2012, 12, 137-145.
[34] Jia F., Chawhuaymak J., Riley M.R., Zimmt W., Ogden K.L., Efficient extraction method to collect sugar from sweet sorghum, J. Biol. Eng., 2013, 7, 2-8.

[35] Gyalai-Korpos M., Feczak J., Reczey K., Sweet sorghum juice and bagasse as a possible feedstock for bioethanol production, Hungar. J. Ind. Chem., 2008, 36,, 43-48.

[36] Imam T. And Capareda S., Fermentation kinetics and ethanol production from different sweet sorghum varieties, Int. J. And Biol. Eng., 2011, 4, 33- 40.

[37] Chiba Y., Bryce J.H., Goodfellow V., MacKinlay J., Agu R.C., Brosnan J.M., et al., Effect of germination temperatures on proteolysis of the gluten-free grains sorghum and millet during malting and mashing, J. Agric. Food. Chem., 2012, 60, 3745-3753.

[38] Yue G.,Yu J., Zhang X., Tan T., The influence of nitrogen sources on ethanol production by yeast from concentrated sweet sorghum juice, Biomass Bioenerg., 2012, 39, 48-52.

[39] Zhao S., Wang B., Liang X., Enhanced ethanol production from stalk juice of sweet sorghum by response surface methodology, Afr. J. Biotech., 2012, 11, 6117-6122.

[40] Holou R., Stevens G., Impact of nitrogen fertilization and the soil type on the quality and yield of sweet sorghum juice in the UC Davis, California, In: The Proceedings of the International Plant Nutrition Colloquium XVI (2008, Missouri, USA), 2009.

[41] Lekkas C., Stewart G.G., Hill A.E., Taidi B., Hodgson J., Elucidation of the role of Nitrogenous wort components in yeast fermentation, J. Inst. Brew., 2007, 113, 3–8.

[42] Walker G.M., Yeast-Physiology and Biotechnology, 1st ed., John Wiley, London, 1998

[43] Megazyme, Primary amino nitrogen assay procedure booklet: K-PANOPA 02/11. Megazyme International, Ireland., 2011, http://secure.megazyme.com/files/BOOKLET/K-PANOPA_1207_DATA.pdf

[44] Feldmann H., Yeast-molecular and cell biology. 2nd ed., Wiley-VCH Verlag, Amsterdam, 2012.

[45] Nie C., Wang C., Zhou G., Dou F, Huang M., Effects of malting conditions on the amino acid compositions of final malt, Afr. J. Biotech., 2010, 9, 9018-9025.

[46] Walker G.M., 125th anniversary review: Fuel alcohol: Current production and future challenges, J. Inst. Brew., 2011, 117, 3–22.

[47] Widianto D., Arofatallah A., Yuwono T., Dwidya I., Ethanol production by fermentation of various sweet-sorghum juices using various yeast strains, Indonesian J. Biotech., 2010, 15, 86 - 92.

[48] Kothari D.P., Singal K.C., Ranjan R., Renewable Energy Sources and Emerging Technologies, 2nd ed., PHI Learning, New Delhi, 2012.

[49] Serna-Saldivar S.R.O., Cereal grains: properties, processing and nutritional attribute-Food preservation technology, The Food Preservation Technology Series, 1st ed., CRC, New York, 2011.