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Evaluation of sugar content and bioethanol production of Ethiopian local varieties "*Nech Tinkish*" and "*Hawaye*" sweet sorghum (*Sorghum bicolor* (L.))

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Abstract

Diversifying the use of climate-smart crops such as sweet sorghum has the potential to solve integrated food, bioenergy, feed, and land management problems. The study's purpose is to quantify the sugar content of *Nech Tinkish* (v1) and *Hawaye* (v2) Ethiopian sweet sorghum varieties and investigate the interaction effect of fermentation parameters to determine their capacity for ethanol production. Sweet sorghum varieties were analyzed to determine their difference in °Brix content by extracting their juices. The juice was clarified using milk lime. Its total soluble sugars, total carbohydrates, and reducing sugars were determined using a digital refractometer, phenol sulfuric acid, and 3,5-dinitrosalicylic acid, respectively. A completely randomized factorial was employed to evaluate ethanol production capacity, and the ethanol content was estimated using a potassium dichromate solution. The °Brix results revealed that v2 had a higher sugar concentration than v1. Additionally, the estimated carbohydrate content of the juice ranged from 37.402 to 157.641 g/L. The estimated reducing sugar also varied from 4.644 to 33.412 g/L. Therefore, the estimated reducing sugar showed the hydrolysis of sweet sorghum juice by invertase and sulfuric acid produced more fermentable sugars. Fermentation at 30 °C with pH 4.5 incubated for 4 days yields the highest ethanol, and *v2* yields higher (15.31%) ethanol, compared to v1 produced 14.85%. This study showed a basis for the existence of two sugar-rich climate smart sweet sorghum varieties with an extraordinary amount of sugar used as a source of biofuel and food simultaneously in a single plot of land.

Keywords Climate-smart, Brix, Ethanol, Sweet sorghum, Sugar, Fermentation

Introduction

By 2050, the world population is anticipated to reach around 9 billion [12], which correlates with the demands for food and energy [30]. On the other hand, the world's population and livestock, which are both expanding quickly, need a guarantee of food security. Although Africa is a source of food and energy resources, the issue of food and energy insecurity remains the continent's biggest challenge [41]. The method in which land is used for both food production and bioenergy requires careful land management on the already existing land. Therefore,

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expanding the use of climate-smart crops like sweet sorghum has the potential to solve issues related to food, biofuel, and land management.

Most grown in Africa (sub-Saharan Africa), Asia, and the Americas, sweet sorghum (*Sorghum bicolor* (L.)) is an annual non-woody stem crop. The grain and fibrous leftovers are used as animal feed, and the dried stalk is combustible [29]. It uses the C4 carbon fixation pathway, which accumulates high levels of readily fermentable in the stalks and has high photosynthetic efficiency with high carbon absorption of 50 g m^{-2} day^{-1}. Sweet sorghum has a unique property that makes it an alternative energy source that is safe, efficient, cost-effective, convenient, renewable, and sustainable [44]. It is a flexible crop that can be grown with low-cost inputs and has high biomass content, making it a promising candidate for the production of bioethanol [11].

Previous studies showed that sweet sorghum is a "climate-smart" multifunctional crop that produces grain for human consumption, stover for use as animal feed, and juicy stalks for use in biofuel production. Sweet sorghum is a drought-tolerant crop that utilizes water efficiently, earning the nickname "camel among crops" from scientists and farmers. In light of this, producing ethanol from sweet sorghum juice does not affect food or feed while enhancing food security [33]. Zhang et al. [48] reported that the replacement of the common grain sorghum with sweet sorghum enables a gain of average ethanol yield of 244.0×10^4 t/year that covers 63.2–84.9% of demand required for E10 (gasoline blend with 10% ethanol) of China. Research on the ethanol production potential from sweet sorghum in Ukraine estimated that about 11,423 L/ha bioethanol can be produced from its juice, grain, and bagasse [37], indicating huge potential for industrial ethanol production.

Sweet sorghum is a versatile crop that has many benefits for human and animal consumption, and industrial applications. It contains high levels of crude protein, fat, carbohydrates, vitamins, minerals, and fiber are abundant. As a result, it is used to produce drinks, fiber, and gluten-free meals. It also has a sweet taste, a rich in dietary fiber, and has a superior nutritional and mineral profile, which makes it suitable for the development and fortification of foods, for example, in bread formulation [2]. According to Australian researchers [33], sweet sorghum is suggested to be used for food, fuel, and animal feed. As a result, the crop may be utilized for food and fuel. Woods [44] also reported an industrial trial for the production of crystal sugar by blending sweet sorghum juice with sugarcane in Zimbabwe, which is an indicator of the huge potential of the sugar-rich crop.

Climate change increasingly affects Ethiopia's agricultural sector, with droughts and precipitation variability challenging farmers' livelihoods and economic prospects. Climatic conditions substantially affect crop production in Ethiopia; the projected changes translate into modeled shifts in suitability patterns for different crops, with net suitability for maize, wheat, and teff decreasing, while the overall suitability to grow sorghum will increase [31]. Sweet sorghum and grain sorghum flourish in regions with little to no irrigation and occasional light rain. In the majority of Ethiopia, sweet sorghum is not as widely grown as grain sorghum. Instead, a small amount of sweet sorghum seeds are planted by mixing with sorghum and maize seeds. Amhara, Tigray, and Oromia are Ethiopia's three major sweet sorghum growing areas [8]. Even though sweet sorghum has huge potential applications, Ethiopian food, and biofuel producers do not take its sugar content into account. Sweet sorghum varieties are being grown in the study area for early consumption, i.e., for chewing at its immature stage. Overall, the potential of sweet sorghum to provide both food and fuel simultaneously is not given due consideration.

Studies have shown that quantifying the sugar content of sweet sorghum varieties is crucial for selecting the best varieties and exploring potential applications of the crop, improving fermentation efficiency, developing small-scale processing technology that could link with large-scale facilities, evaluating ethanol yield, producing sugar, and integrating sweet sorghum into cropping systems without compromising sustainability or interfering with other crops' ability to produce food [39]. Therefore, this study aims to determine and quantify the sugar content and ethanol production capacity of two vast locally available indigenous sweet sorghum varieties. Using different way of sugar determination methodologies, the °Brix, reducing sugar, and total carbohydrate of sweet sorghum were calculated in this study. Additionally, the effect of pH, temperature, and incubation time on ethanol production from sweet sorghum was determined. This helps design optimal protocol and standard fermentation and maximize ethanol yield from locally available sweet sorghum landraces. Moreover, this study helps provide clues for researchers and investors to produce ethanol from sweet sorghum juice at farms and/or scale up to the industrial level while consuming the grain for food. This will be an essential solution for nations that are affected by climate change and large population size causes land narrowing.

Materials and methods

Description of the study area

This study was conducted in Raya Alamata, Tigray Regional State, which is roughly 600 km from Addis Ababa, the capital of Ethiopia. It is located at 12°15′10N″–12°30′55″N and 39°14′30″E–39°42′15″E.

The study site is lowland with an altitude range from 1450 to 1750 m above sea level. The area has an average annual temperature of 22.3 °C, annual rainfall of 790 mm, and sandy loam soil type. The major crops grown in the study area are sorghum, maize, and *Teff*.

Sample collection and sowing

Two varieties of sweet sorghum known as *Nech Tinkish* (v1) with a white grain (Fig. 1a) and *Hawaye* (v2) with a brown grain (Fig. 1b) were used in this study. These local varieties are indigenous and widely cultivated in the study site. The seed was collected from farmers around the study site, had been sown, and sweet sorghum stalk (Fig. 1c) samples (60 from each variety) were collected at the age of 6 months (May–October) and transported to the Molecular Biotechnology Laboratory for experimental analysis.

Extraction and clarification of sweet sorghum juice (SSJ)

Sweet sorghum was cut into smaller pieces after its peels were removed using a sterilized knife (Fig. 1d). Then its juice was extracted via a roller press/rusher (Linyi Lida milling machine, China) mechanically [18] and then transferred into 1000 ml sterilized Erlenmeyer flasks (Fig. 2).

In the SSJ clarification process, the raw SSJ was clarified by adding 30 ml of filtered milk of lime solution (10% CaO w/v) into one liter of SSJ and heating at 80 °C for 7 min while being constantly mixed. After 24 h of settlement, the clarified juice was separated from the solid debris by decantation and then further filtered via Millipore (Millipore.[R] WP6122050, Germany) following the procedure of Doherty [9] and Kartawiria et al. [19]

Fig. 2 Extracted sweet sorghum juice

Determination of total soluble sugars (Brix) of individual sweet sorghum stalk juice

The total soluble sugar (TSS) of SSJ was determined using a digital ABBE refractometer (A crus optronic,

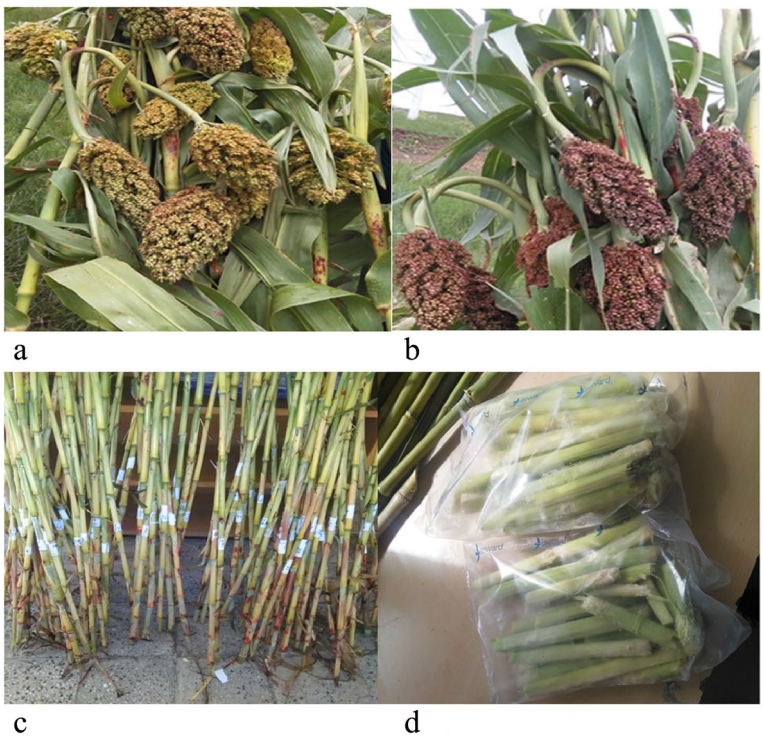

Fig. 1 Sweet sorghum (**a** *Nech Tinkish*; **b** *Hawaye*; **c** stalk; **d** pealed stalk pieces)

Germany). The purified juice extracted from the bottom, middle, and upper nodes of 120 distinct sweet sorghum stalks of the two varieties was measured by adding 1 ml SSJ to the refractometer slide [3].

Determination of total carbohydrate content of SSJ using phenol sulfuric acid method

In the phenol sulfuric acid sugar determination, 80% phenol and 96% sulfuric acid were used in both standard curve preparation and SSJ sample sugar determination.

The standard curve was prepared using a standard glucose solution (100 mg/L), distilled water (DW), phenol, and sulfuric acid. The optical density of the colored solution was measured at 490 nm of the spectrophotometer in comparison to the reagent blank [32].

To evaluate SSJ carbohydrate, the sample from a mixture of v1 and v2 SSJ was diluted to 1:1000 dilutions in a flask containing DW. First, 0.05 ml phenol was added to each tube containing a total volume of 2 ml (DW + SSJ). Second, 5 ml sulfuric acid was added to each tube and thoroughly shaken in a vortex (Clifton™ cyclone, England). The tubes were then incubated in a water bath at 25 °C for 10 min. The reagent blank was used to calibrate the spectrophotometer to zero. After the samples were mixed via vortex, the absorbance was measured at a 490 nm spectrophotometer [32, 42]. The experiment was replicated six times. Finally, the unknown concentration of total carbohydrates in SSJ was calculated using Eq. (2).

The linear regression equation for phenol–sulfuric acid the standard curve is:

$$\text{Absorbance} = -0.1240 + 0.008716 * \text{Carbohydrate conc.}(\mu g/\text{ml}) \tag{1}$$

Rearrangement of the equation for the estimated carbohydrate concentration and considering a dilution factor (DF) gives:

$$\text{Carbohydrate conc.}(\mu g/\text{ml}) = \frac{\text{absorbance} + 0.1240}{0.008716}(DF) \tag{2}$$

Determination of SSJ-reducing sugar using the dinitrosalicylic acid (DNS) method

The reducing sugar of SSJ was calculated using a dinitro salicylic acid solution that contained g/L (3,5-dinitrosalicylic, 1; NaOH, 1; Na–K-Tartarate, 2; Na_2HSO_4, 0.05 and phenol, 0.2). In this experiment, a reagent blank was prepared by mixing an acetate buffer of 150 g of sodium acetate and 15 ml of glacial acetic acid in 1 L of DW with a pH of 4.5.

To create a standard curve, 100 mg of anhydrous glucose was dissolved into l liter of DW. Appropriate amounts of glucose solution, DNS, and DW were then added to each clean test tube and subjected to a spectrophotometer following the protocol described by Miller [28] with some modifications.

To determine SSJ-reducing sugar, an SSJ sample from a mixture of v1 and v2 was diluted to 1:1000 (SSJ: DW) dilutions before the recommended volume of samples was added to each test tube. This was followed by adding 1 ml of invertase (1:1000 diluted) to each test tube, placing it in a 30 °C water bath for 10 min, and chilling in an ice bath. Then, 2 ml of DNS was added to all test tubes and placed in a boiling water bath for 5 min. The test tubes were then each filled with 7 ml of DW after being chilled in an ice bath. Finally, the absorbance of the colored solution was measured at a 540-nm spectrophotometer against the reagent blank [17, 28]. The experiment was replicated six times. With a standard curve prepared via the DNS method, the concentration of SSJ reduced sugar was estimated using Eq. (4).

The linear regression equation for the DNS standard curve is:

$$\text{Absorbance} = -0.01848 + 0.01453 * \text{reducing sugar conc.}(\mu g/\text{ml}) \tag{3}$$

Rearrangement of the equation and considering a dilution factor (DF), we get the estimated reducing sugar concentration:

$$\text{Reducing sugar conc.}(\mu g/\text{ml}) = \frac{\text{Absorbance} + 0.01848}{0.01453}(DF) \tag{4}$$

Experimental design for ethanol production from v1 and v2 mixed SSJ fermentation

A completely randomized factorial design (CRD factorial) experimental design was employed to examine the effect of fermentation factors in the ethanol production from the mixed SSJ of v 1 and v2 which its °Brix was 15.5%. Accordingly, the fermentation was carried out at the temperatures of 26 °C, 30 °C, and 37 °C; the incubation period of 48 h, 72 h, and 96 h; and pH of values of 3.5, 4.5, and 5.5 each in three factors. The experiment was conducted in di replicates in 1000-ml flasks to which 250 SSJ, 2.5 g of $(NH_4)_2SO_4$, and 10 ml of (1×10^7 CFU/ml) yeast cells were added. Before fermentation, high ethanol and sugar concentration tolerant ethanol-producing SJU14 yeast was used for fermentation of SSJ [20]. To examine ethanol production from purified SSJ, ethanol-producing SJU14 was prepared and propagated to obtain a sufficient amount of yeast [36, 40].

Comparison of the ethanol production from SSJ of v1 and v2

The ethanol production capacity of v1 and v2 was compared through the fermentation of SSJ. Before fermentation, °Brix of v1 and v2 SSJ was recorded at 19% and 21%, respectively, and was pasteurized at 70 °C for 15 min [22]. Then, 500 ml of SSJ from each variety, where its pH adjusted to 4.5 via 10N HCl and 5N KOH, was added to each 1000 ml Erlenmeyer flask, followed by the inoculation of 5 g of $(NH_4)_2SO_4$ and 20 ml of (1×10^7 CFU/ml) yeast cells into the flask. Finally, fermentation was carried out at 30 °C incubated for 96 h under the anaerobic condition at 150 rpm in a shaking incubator and the produced ethanol was checked for its flammability (THZ-300C, China) [6, 14, 43]. The comparison experiment was replicated six times.

Estimation of unknown ethanol content produced from SSJ of v1 and v2 using potassium dichromate solution

To estimate the SSJ ethanol, absolute ethanol (97%) [Desta alcohol and Liquor factory P.L.C, Ethiopia] was used to construct a standard curve following standard protocols. Firstly one ml ethanol sample was added to nine ml of distilled water (1:10 dilution). Secondly, two ml of potassium dichromate solution was added to each tube and heated in a water bath at 60 °C for 20 min. Then the absorbance of the mixtures was recorded at 600 nm after cooling [4]. Finally, the unknown ethanol sample concentration was estimated using Eq. (6) generated from a standard curve.

The linear regression equation for the ethanol standard curve gives:

$$\text{Absorbance} = -0.09700 + 0.3691 * \textit{Ethanol conc.}(\%v/v) \quad (5)$$

Rearrangement of the equation and considering a dilution factor (DF) for the estimated ethanol concentration gives:

$$\textit{Ethanol conc.}(\%v/v) = \frac{\text{absorbance} + 0.09700}{0.3691}(DF) \quad (6)$$

Data analysis

The data were analyzed using Minitab software version 20. Tables and bar charts were used to display results. °Brix of SSJ is presented via bar charts and ANOVA was used to determine if the two varieties have significant differences in their sugar concentration. Additionally, the *Z*-test was used to compare the means of v1 and v2. Ethanol concentration produced from SSJ was presented via graphs.

Results

Determination of total soluble sugars (°Brix) of individual sweet sorghum stalk juice

The average yield of SSJ from one kg of peeled sweet sorghum stalk was found to be 0.8 L. Sugar determination revealed that the average °Brix of the middle and upper levels of the SSJ juice did not differ significantly when 120 stalks from both varieties were combined or mixed. This indicates that there was no significant difference between the average °Brix of the middle and upper levels, which had values of 16.595% and 16.144%, respectively. However, the bottom level with a °Brix value of 13.783% showed statistically significant differences with the middle and upper values (Table 1).

Analysis of variance (ANOVA) performed on the °Brix value of SSJ extracted from the stalks of the two varieties at a 95% level of confidence showed that F computed (50.40) was greater than the F critical (3.021). In addition, the probability ($P=5.241\times10^{-20}$) is extremely low compared to the level of significance ($p<0.05$) (Table 2). Thus, this indicates the presence of highly significant variation in sugar content among the different levels/nodes (lower, middle, and upper) of sweet sorghum stalks.

The average °Brix of the bottom, middle, and upper levels of the juice extracted from the stalks of *Nech Tinkish* (v1) and *Hawaye* (v2) was recorded. The middle level of sorghum had the °Brix, measuring 16.18% in v1 and 17.01% in v2, followed by the upper level, measuring 15.695% in v1 and 16.593% in v2. The bottom level of sorghum, however, had the lowest °Brix, which was 13.26% in v1 and 14.307% in v2. The mean °Brix was higher in the middle and upper levels compared to the lower levels in both varieties (Fig. 3). However, the sugar content of the two varieties was statistically insignificant.

Comparison of Brix means from v1 and v2 via Z-test

Z test was used to compare the means of the two varieties (v1 and v2) to determine whether there is a significant difference between them or not. Accordingly, *Z* computed (2.376) was found to be greater than *Z* critical (1.960). In comparison to the level of significance

Table 1 Comparison of °Brix (%) of juice of sweet sorghum stalk levels

Groups	Count	Sum	Average	Variance
Bottom	120	1654	13.783 [b]	6.518
Middle	120	1991	16.595 [a]	5.130
Upper	120	1937	16.144 [a]	4.639

Means that do not share the same letter are significantly different

Table 2 One-way ANOVA

Source of variation	SS	DF	MS	*F*	*P*-value	*F* crit
Between groups	547.290	2	273.645	50.404	5.241×10^{-20}	3.021
Within groups	1938.180	357	5.429			
Total	2485.470	359				

°Brix values in the table are in %

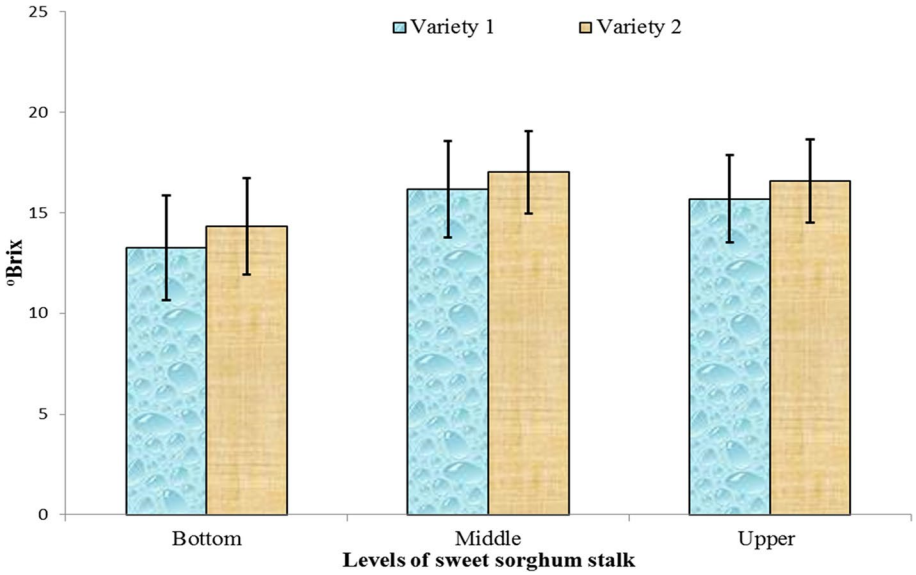

Fig. 3 Average °Brix (%) at different levels (node) of sweet sorghum stalk. A significant difference observed between the varieties at $P < 0.05$ or $P = 0.0175$

Table 3 Comparison between the means of v1 and v2 via *Z*-test

***Z*-test: two sample for means**		
	Variety 1	**Variety 2**
Mean	15.045	15.97
Known variance	4.982	4.113
Observations	60	60
Hypothesized mean difference	0	
z (computed)	− 2.376	
$P(Z \leq z)$ two-tail	0.0175	
z Critical two-tail	1.960	

°Brix values in the table are in %

($p < 0.05$), the probability of 0.0175 is quite low. Therefore, a variation in °Brix between the sorghum varieties was found to be significant ($P = 0.0175$). According to the two-mean comparison, v2 has a higher °Brix percentage than v1 (Table 3).

The respective °Brix of the lower, middle, and upper levels of v1 and v2 were compared to determine which level (either of the bottom, middle, or upper) produced a difference of deference for means between v1 and v2. According to the results of the *z*-test of two means comparison in all levels of the °Brix content, i.e., bottom, middle, and upper, the absolute value of *Z* computed was greater than *Z* critical (1.960) with the probability of 0.022, 0.042, and 0.020, respectively (Table 4). Therefore, the difference between the °Brix of v1 and v2 was due to the difference in the °Brix of all levels/nodes of the sweet sorghum stalk.

Determination of total carbohydrate content of SSJ using phenol sulfuric acid method

The total carbohydrate concentration of of SSJ sample was estimated using the standard curve (Fig. S1). The estimated total carbohydrate increased by more than four times when the SSJ sample increased from 0.4 to 2 ml. Consequently, the estimated total carbohydrate concentration increased from 37.402 to 157.641 g/L as the absorbance increased from 0.202 to 1.250 (Fig. 4).

Table 4 *Z*-test: comparison of two samples for means of the bottom, middle, and upper levels of respective v1 and v2

	Variety 1			Variety 2		
	Bottom	**Middle**	**Upper**	**Bottom**	**Middle**	**Upper**
Mean °Brix (%)	13.26	16.18	15.695	14.307	17.01	16.593
Known variance	6.800	5.761	4.654	5.789	4.237	4.291
Observations	60	60	60	60	60	60
Hypothesized Mean Difference	0	0	0			
Z (computed)	−2.285	−2.033	−2.327			
$P(Z \leq z)$ two-tail	0.022	0.042	0.020			
z Critical two-tail	1.960	1.960	1.960			

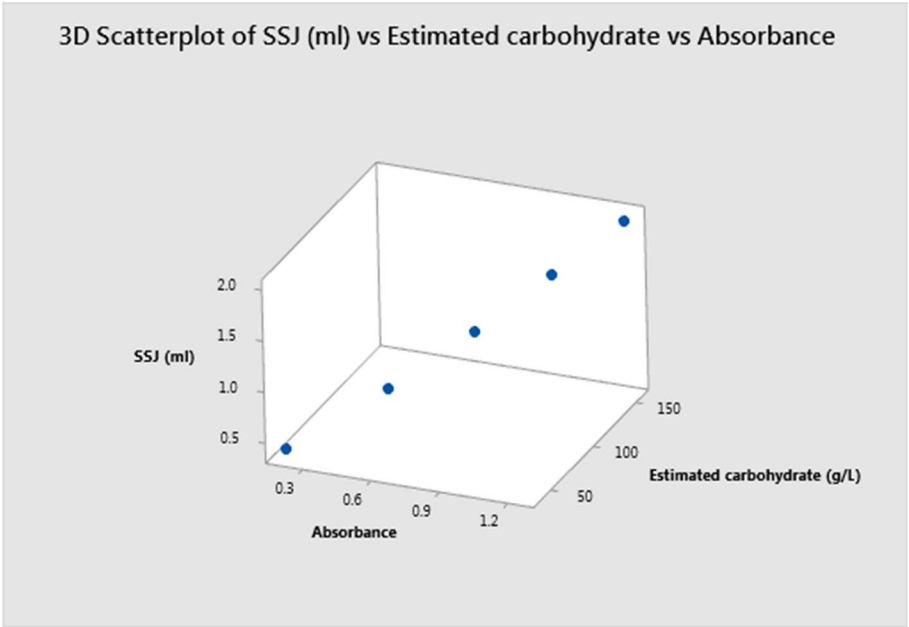

Fig. 4 The relationship between absorbance and estimated total carbohydrate of SSJ. Positive significant relation ($p < 0.05$) exit between the variables

Determination of SSJ-reducing sugar using the dinitrosalicylic acid (DNS) method

The reducing sugar content of the SSJ samples was estimated using the standard curve (Fig. S2), revealing that the estimated reducing sugar increased by more than eightfold (from 4.644 to 33.412 µg/ml) as the amount of SSJ increased from 0.2 to 1 ml confirmed by the respective absorbance increase. Initially, the amount of estimated reducing sugar was also almost doubled when the amount of SSJ doubled (Fig. 5).

Experimental design for ethanol production from v1 and v2 mixed SSJ fermentation

The result for the effect of pH, temperature, and incubation time in ethanol production from SSJ using novel yeast (16% ethanol and 60% glucose tolerant), SJU14, demonstrated how the parameters significantly affect the fermentation process as presented in detail in the following sections:

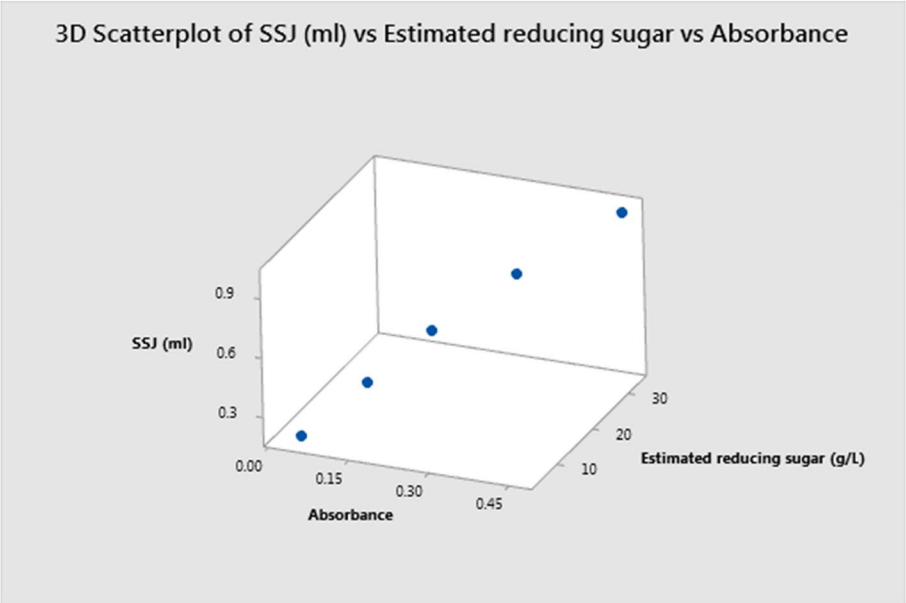

Fig. 5 Relationship among sweet sorghum juice absorbance and estimated reducing sugar reducing sugar determination. Significant relation ($P < 0.05$) among SSJ absorbance and estimated reducing sugar

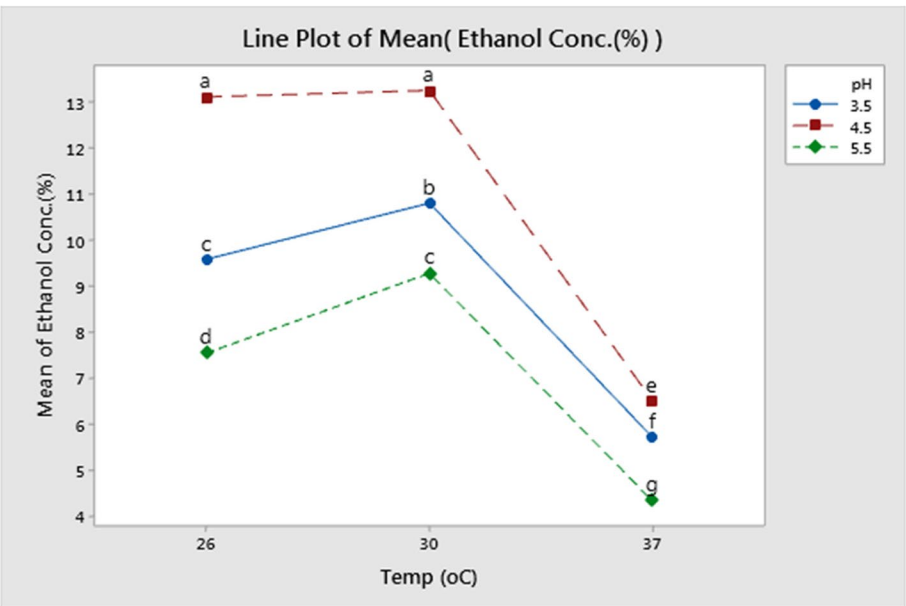

Fig. 6 Interactive effect of pH and temperature on ethanol content (%). Means that do not share a letter are significantly different. temp, temperature; significant interaction between pH and temperature ($p < 0.05$)

Interactive effect of pH and temperature

Based on the interaction effect of pH and temperature, maximum ethanol content (13.255%) was obtained at pH 4.5 and 30 °C it is non-significance with ethanol produced at 26 °C of the same pH. In contrast, minimum ethanol content (4.333%) was recorded at pH 5.5 and 37 °C. At a constant temperature of 30 °C, the ethanol content decreased at pH greater and less than pH 4.5 (Fig. 6).

Interactive effect of pH and incubation period

The highest ethanol yield (15.125%) resulted from the interaction effect of pH and the incubation period was found at pH 4.5 incubated for four days. There was no

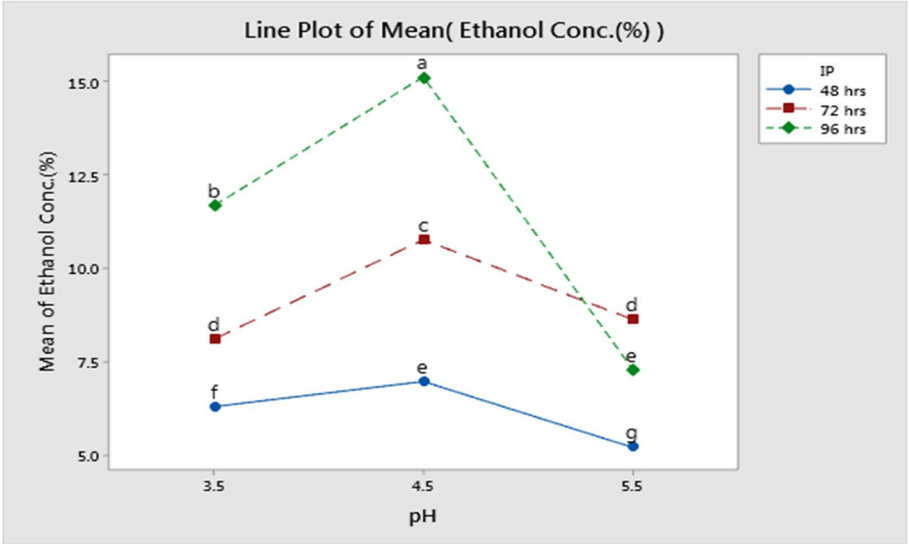

Fig. 7 Interactive effect of pH and incubation period on ethanol content (%). Means that do not share a letter are significantly different. IP, incubation period; hrs, ours; significant interaction effect between pH and IP ($P < 0.01$)

significant difference between the ethanol content produced at a constant 48 h incubation period with low pH 3.5, and high pH 5.5 where the least ethanol content was obtained at this interaction point. Statistically similar ethanol content was also recorded at pH 4.5 and 48 h, and pH 5.5 and 96 h. At constant pH of 3.5 and 4.5, ethanol production was increased as the incubation period increased from 48 to 96 h. At constant all of the incubation days, the ethanol production was increased as the pH was increased from 3.5 to 4.5. Significantly higher ethanol concentration (11.680%) was also produced at an acidic 3.5 pH. However, pH has no effect on the fermentation of SSJ for three days (Fig. 7).

Interactive effect of temperature and incubation period

In the temperature and incubation period interaction effect, maximum ethanol content (14.230%) was obtained when the fermentation was carried out at 30 °C and for 96 h. Conversely, the least ethanol content (4.787%) was recorded at 37 °C and a low incubation period (48 h). That is statistically the same as the ethanol content (5.313%) obtained at 37 °C and 72 h incubation period. similarly, the same amount of ethanol content was obtained when the fermentation was carried out at 26 °C and 30 °C incubated for 72 h. At constant any of the temperatures, an increment of ethanol content was observed as the incubation period was increased to 96 h (Fig. 8).

Comparison of the ethanol production from SSJ of v1 and v2

The ethanol concentration produced from the SSJ sample was approximated using an ethanol-potassium dichromate solution generated standard curve. Accordingly, variety two with 21% °Brix resulted in 15.31% ethanol content which is higher than the ethanol concentration (14.85%) produced from variety 1 which had lower (19%) °Brix. The variation in their ethanol concentration was confirmed by absorbance (Table 5). The ethanol produced in this study was found to be flammable when struck by a match indicating that the ethanol produced was one of the fuel grades.

Discussion

The average yield of SSJ from one kg of peeled indigenous sweet sorghum stalk was found to be 0.8 l or a Quintal of pealed SSJ can yield 80 kg of SSJ which is a huge amount of sugar-rich juice for the food formulation and bioethanol production. The middle and upper levels of both kinds of sweet sorghum stalk juice had higher mean °Brix values compared to the bottom level/node, which indicates the presence of a high accumulation of sugar at these levels. This may be due to the presence of lower water content and high sugar accumulation in the middle and the upper position of the sweet sorghum stalk. The current finding is similar to a previous study by Disasa et al. [7] showed that the mean °Brix was lowest at the bottom compared to the middle, and upper levels of the stalk. Due to the stem's rapid sugar accumulation efficiency in the middle position, the sugar concentration increased at this position [21]. Researchers Freeman et al. [13] and Holou and Stevens [15] stated that the sugar of sweet sorghum at the maturity stage gives a sufficient amount of sugar that can be used for the production

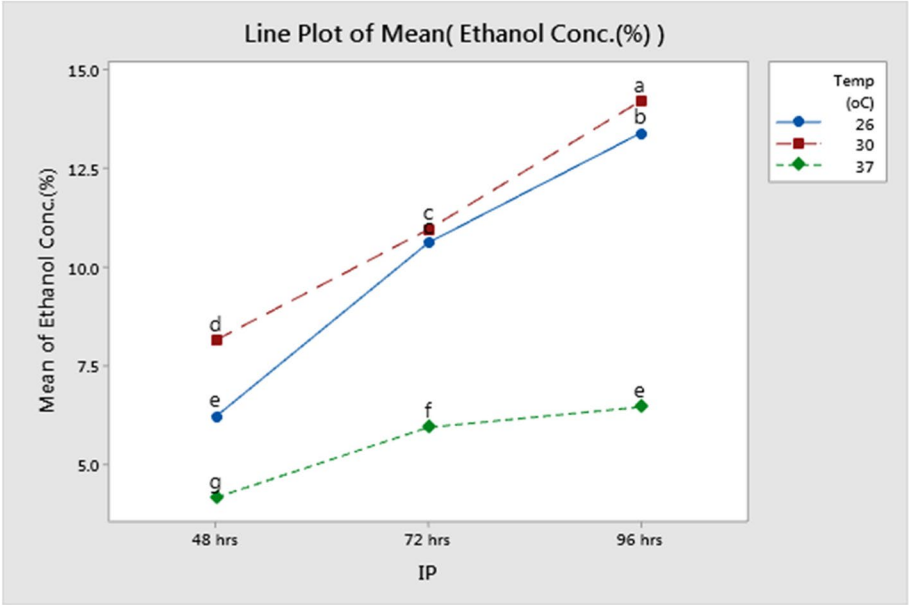

Fig. 8 Interactive effect of temperature and incubation period on ethanol content (%). Means that do not share a letter are significantly different. IP, incubation period; temp, temperature; significant interaction effect between temp and IP ($P < 0.01$)

of valuable products. In contrast, the mean °Brix at the bottom level was the lowest in the present study. If non-sugar tolerant yeast is used, lowering °Brix at the lower part of the sweet sorghum, which contains more water, may be crucial in lowering the amount of distilled water required to dilute the SSJ during fermentation to produce bioethanol.

The data analysis of the °Brix of SSJ showed that v2 had a higher sugar percentage at all levels of the sweet sorghum stalk compared to v1. Even though both varieties have remarkable sugar concentrations, v2 produced more bioethanol and may produce other sugar-based food items due to v2's greater sugar concentration than v1. This may be due to v2's higher rate of carbohydrate accumulation. According to Rao et al. [38], the high photosynthetic efficiency nature of sweet sorghum enables it to convert H_2O and CO_2 into carbohydrates. Consequently, variety 2 yields higher ethanol concentration compared to the ethanol produced from variety 1. However, a previous study by Prasad and Dhanya [36] reported that 14.7% sugar present in juice can give an ethanol yield of 6.9% ethanol which is less than the ethanol concentration produced from both varieties in this study. Ethanol produced in this study has the flammability capacity to be used as fuel for different propose either independently or blending with other fuels. Based on the mean °Brix result of the current study, the two varieties have sufficient potential for sugar that can be used for the production of bioethanol and other sucrose-based processed foods stuff.

Table 5 Comparison of °Brix, mean absorbance, and estimated ethanol produced from v1 and v2

Varieties	°Brix (%)	Mean absorbance	Mean estimated ethanol	Ethanol content conc. (%v/v)	Flammability
v1	19	0.451	1.485	14.85	Flammable
v2	21	0.468	1.531	15.31	Flammable

v1, variety 1; *v2*, variety 2

In the current study, phenol sulfuric acid treated SSJ resulted in the highest total carbohydrate, 157.402 g/L, which was higher compared with the findings of Cséfalvay and Bakacsi [5], 118.4 ± 5.9 g/L though it varies annually due to different factors. To produce this range of sugar, the non-simple sugars in SSJ were hydrolyzed by sulfuric acid, which has the potential to degrade polysaccharides into simple sugars. Sucrose is the main sugar component in SSJ [25].

The estimated reducing sugar in the SSJ glucose determination performed using the DNS technique ranged from 4.644 to 33.412 g/L. The calculated reducing sugar showed the hydrolysis of SSJ sucrose by invertase. This was slightly greater than the finding of Yuvraj et al. [46] who reported that the reducing sugar in stalk juice was recorded up to 33.25 g/L. Nevertheless, the current findings are better compared to those of Wu et al. [45], who

found 16 to 18% fermentable sugars in sweet sorghum. Hence, the enzyme invertase catalyzes the hydrolysis of sucrose yielding glucose and fructose named invert sugar [1]. According to the current study, sweet sorghum contains a sufficient quantity of total carbohydrates that may be utilized to produce ethanol and other foods using sugars. A significant quantity of ethanol may be produced if invertase from yeasts can break down the SSJ sucrose into glucose and fructose in the range of 4.644 g/L to 33.412 g/L or more during fermentation and this results in a large amount of ethanol can be produced from fermented SSJ. Unless it is treated with sulfuric acid and invertase, the result of the phenol-suluric acid treatment and DNS methods of sugar estimation revealed a limited quantity of reducing sugar in raw SSJ. However, it gives confidence to conclude that the sulfuric acid method of hydrolysis produced more fermentable or simple sugars than invertase hydrolysis. This indicates that SSJ juice has a significant quantity of disaccharides or polysaccharides that need external action for hydrolysis to yield more simple sugar that may be used as raw material to produce food products and ethanol for use as a fuel or beverage.

The most important point that needs to be addressed is the relationship of the temperature, pH, and incubation period during their interaction in the fermentation of SSJ from the indigenous varieties by SJU14 yeast. In the current study, the highest ethanol was produced when the fermentation was adjusted to pH 4.5 at 30 °C. However, the interaction of low pH vs. low temp, and high pH vs. high temperature were unfavorable conditions for the SJ14 yeasts involved in the fermentation of SSJ that resulted in significantly less ethanol. Nevertheless, the former interaction yield higher ethanol content compared to the last interaction. Though the current work is similar to [27] who found maximum ethanol content at pH 4.5 and 30 °C, it is different from the finding of Ortiz-Muñiz et al. [35] reported maximum concentration of ethanol was obtained at fermentation pH 3.5 and 30 °C using yeast isolated from sugar cane molasses. Even though Kundiyana et al. [24] reported that fermentation of SSJ by reducing the pH from 5.4 to pH 4.3 remained constant, the current study demonstrated that ethanol concentration was increased as the pH was decreased from 5.5 to 3.5 indicated that the possibility of higher ethanol production at low pH values using low pH resistant SJU14 yeast. Unlike the current finding, Ebrahimi-aqda and Ogden's [10] pH value of 5.5 and 28 °C was the optimal fermentation condition at which the highest ethanol was produced. During fermentation, pH affects the H^+ concentrations of yeast cells which pushes them to change the total charge of the plasma membrane interfering with the permeability of some essential nutrients into the cells [26].

In the present study, maximum ethanol concentration (15.125%) was produced at pH 4.5 incubated for 96 incubation period. However, lower ethanol production at high (5.5) pH and low (3.5) pH with a low incubation period (48 h) interaction. In the current study, high pH with high temperature, low pH, and high pH with low incubation period resulted in low ethanol concentration. Onoghwarite et al. [34] reported that at a pH lower than pH level 4, the incubation time to get maximum ethanol concentration was prolonged. Yeasts thrive in acidic environments compared to basic ones. This is because increases in pH can disrupt the hydrogen bonds holding protein structures together, altering their shape and potentially hindering their function [23]. Zabed et al. [47] reported that a pH range of 4.0–5.0 is suitable for the ethanol production process, and short fermentation time leads to insufficient microbial growth which affects negatively the fermentation and results in incomplete conversion of sugars into ethanol.

In the temperature vs. incubation period interactive effect of the current study, maximum ethanol (14.23%) was produced at 30 °C incubated for four days which is consistent with the finding Dash et al. [6] found the highest ethanol concentration at fermentation of 30 °C but 84 h. The current fermentation of SSJ gave remarkable ethanol yield at 26 °C and 30 °C incubated for four days while Kundiyana et al. [24] demonstrated that two strains of *S. cerevisiae* (Fermax and Superstart yeast) yield maximum ethanol of 7.9% in the range of 10–25 °C incubated for five days. Consequently, the production of ethanol near room temperature is consistent with the finding of Kundiyana et al. [24], whereas the ethanol produced at 30 °C in the current study and at 10 °C in the finding of Kundiyana et al. [24] contradicted. In this case, the SJU14 was the right choice for good fermentation performance in tropical areas. *S. cerevisiae GC-IIB31* produced maximum ethanol at 30 °C incubated at 120 h [16] which is one day longer than the current maximum ethanol production incubation period though both had the same optimum temperature. Less amount of ethanol (4.787%) was produced at an interaction of 37 °C and 48 h of incubation period might be due to the specified temperature causing denaturation of the enzyme of the yeasts used for fermentation.

Conclusion

Generally, the locally available sweet sorghum has an adequate amount of carbohydrates, and reducing sugars from variety two has a large amount of sugar compared to variety 1. These have a remarkable quantity of sugar/fermentable reducing sugar that can be used to produce bioethanol. The interaction effect demonstrated that effect that pH 4.5 and at 30 °C temperature incubated

for four days was the optimum fermentation condition at which the highest ethanol was produced. Hence, sweet sorghum juice is found to contain easy ferment by yeasts with slight pre-treatments best choice for household-level and large-scale ethanol production for vehicle and cooking fuels. Additionally, huge amounts of sugar may be used to produce crystal sugar, alcoholic beverages, syrup, and other sugar-made food products that help enhance food security. Therefore, it is advisable to conduct extensive research on sweet sorghum-based food and fuel products that have uses beyond using them for simple chewing, combustion, and animal feed. This will be an essential solution for nations that are affected by climate change and large population size causes land narrowing.

Supplementary Information

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Supplementary Material 1.

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Availability of data and materials
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Declarations

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