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## **Research article**

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# Sugar and ethanol production potential of sweet potato (*Ipomoea batatas*) as an alternative energy feedstock: processing and physicochemical characterizations

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#### HIGHLIGHTS

#### G R A P H I C A L A B S T R A C T

- Sweet potato is a cheaper sugarcontaining crop and abundant resource.
  Red-fleshed sweet potato has greater
- sucrose content with higher purity (95.42 g/L).
- Industries can ease the production of sugar & bioethanol using sweet potato.
- Efficiency of studied inoculums is graded as: SPY > LEY > SCY > KEY > SRB.

## ARTICLE INFO

Keywords: Bioethanol Fermentation Pol Saccharimeter Sweet potato Sucrose



## ABSTRACT

This study focuses on the processing, characterization, and sugar and ethanol production potential of red-fleshed sweet potatoes (RFSP) and white-fleshed sweet potatoes (WFSP). These feedstocks were used for the production of sugar; and bioethanol from its pulp by the action of five different microbes. The characterization of raw sweet potatoes and desired products of raw sugar, and bioethanol were carried out through proximate analyses, Fourier transforms infrared spectroscopic (FTIR) method, measurement of pol% by using a Refractometer, Polarimeter, Saccharimeter. The proximate analyses of feedstocks show the presence of a respectable amount of dry solids  $25\pm$ 0.03g/100g with a lower amount of fat ( $0.025 \pm 0.002$ ) and ash ( $0.533 \pm 0.076$ ) contents make them promising crops for the production of sugar and ethanol. Comparatively, RFSP raw sugar (°Z: 95.25  $\pm$  0.05) is considered purer than WFSP raw sugar (°Z: 94.6  $\pm$  0.015). FTIR spectrums of the presently studied raw sugar and bioethanol have characteristic bands. It shows that the raw sugars products are rich in sucrose content, and confirms that the bioethanol was produced from the selected raw materials is at a satisfactory level. The efficiency of microbes was evaluated by taking a sample from the fermented wash to measure the residual sugar in (°Brix). Comparatively, fermented wash with sweet potato extracted yeast was found 14% Brix<sup>o</sup> (consume 86% of pulp) in RFSP, and 17% of Brix<sup>o</sup> (consume 83% pulp) in WFSP within 24 hours of fermentation. The alcohol level of bioethanol's produced from RFSP and WFSP pulps was tested using Ebuliometer and the result was found to be ranged 78 °C - 80 °C which is closer to the boiling point of absolute anhydrous alcohol (78.3 °C). Thus, the results of the present study proved that the sweet potato and its pulp are considered as a potential alternative sugar/energy feedstock.

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#### 1. Introduction

The demand for sugar has extensive applications in human being diet; also, it guarantees a noteworthy part in total energy income and is broadly used in food and food processing industries as sweetener, flavoring agent, preservative, and many multiple purposes like the increasing volume of food products, as well as improving the texture of food or substrate of fermentation. But, surprisingly, only 5% of sugar (sucrose) is utilized for creating non-food items under chemical or biochemical changes. Sugar occurs naturally in every fruit and vegetable in the plant realm (Anna Flavia de Souza and Sandra Helena 2016; Eggleston and Isabel 2015).

Sugar is chiefly used as a sweetener but also used as an additive substitute like fillers and preservatives in bakeries, confectioneries, pharmaceuticals, soft drinks, vegetable canneries, dairy, and related food processing industries, carbonated soft drinks (40 % sugar consumption), and beverage industries (Lewis et al., 2009; Majur et al., 2019). Sugar is believed as one of the global unadulterated nutrition input items (99.95% crystal sugar and remains in a solution which is one of the most normal nourishment utilized by people over hundreds of years that contain the most purified natural mixes on the planet (Ramesh et al., 2017; Endale et al., 2013; Victoria 2013). Sucrose is a special valuable item derived from the plant world mainly from sugar-rich harvests (Motsa et al., 2015), which are excellent, renewable biomass feedstock's useful for the preparation of sugar and energy providing outputs from byproducts.

Worldwide mostly the sugar industries were established based on sugar cane input which is water-intensive and takes at least 12 months of harvesting time, persist the challenges that bring the maximum cost of sugar production may limit the profitability of the industries and business owners. Even nowadays sugar industries located in developing countries face some challenges like a failure of plant and process design, economic loss (deterioration of sugar cane). Hence, the industries facing difficulties to manufacture sugar & its other value-added products from sugar cane. So far no work has been done before regarding alternative raw materials for sugar particularly sweet potato-based investigation is a noble work and therefore the current study was designed to introduce sweet potato (Ipomoea batatas) as a very vital input for the production of sugar since it is highly harvested in some parts of the globe; cultivate around a hundred nations worldwide. Among world crops, it positioned 7th from the observation of entire production after cereals and cassava. Sweet potato has a considerable level of sugar but still, it's not used in sugar industries (Nafeesa et al., 2012; Owusu-Measah et al., 2016) for the production of sugar and co-products of sugar industries like biofuel (bioethanol).

Biofuels are renewable and could reduce petroleum imports, get better regional energy security, and reduce the reliance on petroleum fossils fuels from rural and destabilized parts of the globe. Biofuel may be generated cheaply and has the potential to replace petrol fuels by fulfilling the energy demands. Now a day's actual and usual replaceable form of fuel is ethanol which is prepared using carbohydrate-rich biomass feedstocks like sugar or cereal grain (starch); however, these inputs will not be enough. Ethanol is considered as the gasoline in near future, and many nations in the world pay attention to reducing their dependence on oil-based fuel (Ashok Kumar et al., 2014). Bio-ethanol production plays a vital role in the fuel balance nationwide as an alternative energy source that is also considered an environmentally friendly and renewable energy form (Ashok Kumar et al., 2014). Bio-ethanol is the right type of product for blending renewable fuel with gasoline engines since its maximum octane number value and high heat of vaporization impede self-ignition will enhance quality fuel in the diesel engine. Furthermore, because of its eco-friendly nature, bio-ethanol is considered an alternative fuel that can be used in unmodified petrol engines.

Nowadays carbohydrate-rich crops and their wastes with the existed value-added technologies applicable for converting the raw inputs to value-added form are most profitable with ethanol production. Sucroserich crops like sugarcane and corn (maize) supply covers almost all the major ethanol that is produced. On the other side, other major starchy crops being used include wheat, sorghum, sugar beet, sweet potato, and cassava and its byproducts like pulp or husks, etc. Tuberous crops like potato and sweet potato can be also used by entrepreneurs as ethanol feedstock because they are prolifically grown; these crops contain mainly starch, sucrose, and glucose, which could be converted into ethanol under the biochemical route. Thus, sweet potato (*Ipomoea batatas L.*) is therefore the other major resource of economical and healthy food crops in developing countries (Nafeesa et al., 2012). Although the sweet potato crop is a cheaper and abundant resource; but, it is not utilized as an energy feedstock in a required manner. Thus, this study positioned forward the utilization of this crop as input for sugar and appropriate sugar co-products like biofuels particularly ethanol.

#### 2. Materials and methods

#### 2.1. Sampling and extraction of sugar

White and red-fleshed sweet potato samples were collected by random sampling method from farmlands of Chano Mile, Arba Minch Zuria Woreda, Gamo Zone; SNNPR, Ethiopia where sweet potato is grown year-round. The fresh, matured and undamaged samples were carefully collected without removing the soil from the farmland for the sake of preservation.

In this present study, sugar was obtained from two varieties such as red and white-fleshed sweet potatoes, and its processes are shown in Figure 1. In the present study, samples of white and red-fleshed sweet potatoes were weighed (3 kg of sliced cossets of sweet potatoes) separately after proper washing and other related sample preparation methods. Extraction was done upon diffusion by employing sweet potato samples which were kept in contact with hot water (3 L) at 85 °C (called hot imbibition's) for 2 hours using a hot-water bath. Upon diffusion the sugar was extracted from sliced cossets of sweet potato; then the pulp of red and white-fleshed sweet potatoes was separated and stored in a moisture-free atmosphere and used as substrate input for ethanol production. Then, pulp-free mixed juice was taken into the next step for liming by the addition of 2.4 g of lime for each variety, and the clarification was carried out on the mixed juice (Mosen 2007). Then, followed by clarification, other operations such as evaporation, crystallization, and centrifugal separation were done according (Hugot, 1986) and obtained the moisture-free raw sugar and further qualitative and quantitative analyses were conducted.

#### 2.2. Production of bio-ethanol

#### 2.2.1. Saccharification of sweet potato pulp

The preserved pulps (3 kg) were mixed with an equal amount (3Litre) of water (as 1:1 w/w ratio); it was kept in a water bath and carried out the Saccharification at 85 °C (by acid hydrolysis) using 50 mL of 5M HCl for an hour; this process was continued for 24 hours. It was cooled, filtered, and isolated the filtrate which contains simple sugars (hydrolysate) substrate (ICUMSA method 2011; Gaily et al., 2012).

#### 2.2.2. Inoculums preparation and activation

In the current study, five different microbes were developed as *Saccharomyces cerevisiae* (SCY), laboratory extracted yeast (LEY), Sweet potato yeast (SPY), Kocho (false banana) extracted yeast (KEY), and from Sheep rumen bacteria (SRB) and used for the fermentation of RFSP and WFSP pulps for the production of bioethanol.

Microbes SPY and KEY were inoculated by the fine powdered (5 g) dry sweet potato and kocho were introduced into potato dextrose agar (PDA) medium, and urea (as a nutrient) was added into each medium to enhance the growth of microbes up to 2 days. Then, 5 % calcium propionate was added to each medium and allowed for additional three days for the growth of microbes. It's were noticed that increased the microbes level and part of each was taken for fermentation. Similarly, the SRB

microbes were prepared by mixing 10 g of water with 5 g of Sheep rumen; agitated the mixer, and filtered through a muslin cloth with pore size 2.78 mm<sup>2</sup>. The obtained solid deposit was removed and the filtrate was serving as SRB inoculated bacteria for the conversion of a hydrolyzed substrate according to Simona et al., (2015).

For sterilization and antiseptic actions, all the microbes were activated before being added to the fermenter that contains the hydrolyzed substrate. Commercial Baker yeast and pure extracted yeast (LEY) and also other microbes (SPY, SRB, and KEY) were activated separately by adding 100 mL of warm tap water, which were taken into a 250 mL conical flask. Further activation was done on inoculated microbes at a controlled condition (at 30 °C) in a water bath which was attached with a mechanical shaker; urea, DAP, and salt were added to each inoculum propagation container to facilitate the nutrients and enhance the growth of microbes.

## 2.2.3. Fermentation and distillation of hydrolysate

Hydrolyzed substrate (1 L) was sterilized in an autoclave at 121 °C for 20 min; it was taken 3-liter capacity laboratory fermenter separately for each case of developed microbes and undertaken the fermentation (shown in Figure 1). Fermentation condition was prepared with pH 5.5, and temperature 30 °C. Fermentation was performed in a laminar flow cabinet for 24 hours and fermented-wash samples were analyzed (in terms of °Brix) at every 8 hours interval to measure the residual sugars (unfermented) left on the fermenter by using a digital automatic refractometer to ensure the efficiency of each inoculum (Simona et al., 2015; Ndams et al., 2011; Jelynne et al., 2014). Then the fermented wash of each substrate was distilled using fractional distillation and re-distillation was done (Jelynne et al., 2014; Mustafa et al., 2008). The produced ethanol was noticed and its alcohol level was measured using an alcoholometer and specific gravity conversion factors.

#### 2.3. Physicochemical characterization of sweet potato raw sugar (SPRS)

Physicochemical attributes including moisture (dampness), reducing sugar, and ash content are the main parameters that could be affected much among the sample of raw sugar (Zia-Ud-Din and Rasool, 2015).

#### 2.3.1. Determination of moisture on SPRS by dry base

The oven-drying method was applied to all kinds of raw sugar and the moisture level was determined according to ICUMSA's official method of the raw sugar analysis of this study (ICUMSA 2011).

Moisture (%) of sugar = Loss in a mass of on drying x 100 / Mass of sugar taken(1)

#### 2.3.2. Determination of sulphated Ash of SPRS

The sulfated ash was determined gravimetrically and the result provides the total of water-soluble and insoluble ashes. Successive incineration was carried out at 550  $^{\circ}$ C and 650  $^{\circ}$ C with sulphuric acid, by double Sulphitation that ensures complete conversion of the ash into the sulfate form (ICUMSA method 2011).

Sulfated ash (%) Weight of residual ash (100) / Weight of sample (2)

## 2.3.3. Quantification of reducing sugars on SPRS

Lane and Eynon's consistent amount technique of ICUMSA GS1/3/7-3, (2011) was used to find quantification of reducing sugar on the study sample.

#### 2.3.4. Polarization of SPRS

This method is applicable for specialty sugars that may need clarification and for white sugars, which need further clarification (ICUMSA method 2011). Here in this study, optical rotation of solution of produced sugar that measured by using Saccharimeter (model: AUTOPOL 980) at 589.3 nm with D-line of Na lamp. The measurement is represented in  $^{\circ}Z$  of the international sugar scale.

#### 2.3.5. Estimation of sucrose concentration of SPRS using polarimetry

Polarimetry is one of the best methods for the estimation of sucrose at a fixed wavelength of the light source and a fixed temperature (usually 20 °C). In the present study, an experiment was performed for two varieties of raw sugars such as RFSP and WFSP. The optical property of 100 mL of standard sucrose solutions were prepared by dissolution of 5, 10, 15, 20,



Figure 1. Process flow diagram for production of sugar and ethanol from sweet potato.

and 25g of sucrose. The concentration and the amount of sucrose present in studied samples were calculated according to the literature using a linear equation (Yixiang and MilfordLoren, 2008).

#### 2.3.6. Measurement of purity (pol) of SPRS by using Saccharimeter

Accurately weighed 26 g of SPRS samples added into 100 mL volumetric flask containing 2 g of neutral lead acetate and this mixture was diluted to 100 mL by adding water. The purity of SPRS samples was measured by employing the Saccharimeter model AUTOPOL 880 at 589.3 nm wavelength of D-line Na light source by taking the filtrate of the sample solution. Purity of the samples was represented in pol % and it's calculated as follows (Starzak and Mathlouthi, 2010; Chen and Chou, 1993):

$$Pol\% = pol reading x pol factor$$
 (3)

#### 2.3.7. FTIR study of SPRS

Dried ground fine powder of the SPRS sample was mixed with KBr (FT-IR grade) at an appropriate ratio of 1:100 and pressed as a pellet. The pellet was kept on the sample holder of Spectrum 65 FTIR (PerkinElmer) and operated in the range of wave number 4000 - 400 cm<sup>-1</sup> at room temperature.

### 2.4. Analysis of bioethanol

#### 2.4.1. Functional studies of bioethanol

A bio-ethanol sample (5 mL) was taken into a test tube and drops of potassium dichromate solution with some drops of  $H_2SO_4$  were added to it. The appearance of pink color on sample solution was observed (Thenmozhi and Victoria 2013). The laboratory-produced ethanol was also confirmed by employing a combustion test (AHPA 2017).

#### 2.4.2. FTIR study

FTIR analysis of ethanol obtained from sweet potato pulp was performed by employing Spectrum 65 FTIR (PerkinElmer), operating in the range of wave number 4000-400 cm<sup>-1</sup> using proper condition at room temperature. The scanned spectrum of the samples and their data were obtained by using the software called essential FTIR.

#### 2.4.3. Quantitative estimation of ethanol

Under this current study, the specific gravity of ethanol was measured using a specific gravity bottle after the obtained measured data was calculated using the AOAC table (Shinde and Patil 2016). The alcohol level of Ethanol was also measured using an alcoholmeter and the result was compared with the result obtained from a specific gravity table (U.S. Pharmacopia, 2010).

### 2.4.4. Bioethanol analysis by using Ebuliometer

Bioethanol analysis was concerned to measure the alcohol value of the corresponding biofuel and it was determined by using an electronic automatic Ebuliometer by taking the boiling point of water as a reference at Wonji-Shoa sugar factory research laboratory, Ethiopia. The liquid is heated under equilibrium conditions at atmospheric pressure until it is boiling and then alcohol level can be easily determined.

#### 2.5. Numerical analyses

All data were analyzed by using SPSS software version 23 for windows using one-way ANOVA among treatments at a 5 % level of significance.

#### 3. Results and discussions

### 3.1. Physico-chemical analysis of SPRS

#### 3.1.1. Proximate composition of SPRS

The proximate composition represents the content of some significant characteristics such as moisture, sulfated ash, reducing sugar; pol %, and Brix % of evaluated samples. These physicochemical parameters are very

useful in analyzing the quality of sugar. Each analysis performed according to ICUMSA and Ethiopian sugar development agency research directorate ESDARD) laboratory manual (Chauhan et al., 2011; Hamerski et al., 2011).

The moisture of the sample of RFSP (0.077  $\pm$  0.002) and WFSP (0.075  $\pm$  0.003) varied extensively (shown in Table 1). According to the literature (Oliveira et al., 2007; Aquinoo and Franco, 2008), the moisture content of sugar samples which is below 0.15% is considered satisfactory. The results of ash in crude sugar show that the values affected much. Data are given in Table 1 shows the greater value of ash found in the sample of WFSP raw sugar that's higher (0.077 %) than the RFSP raw sugar (0.0737 %) sample.

Comparatively the sample of RFSP raw sugar may be considered purer than WFSP raw sugar. But, it's recommended by various researchers and evident the amount of ash in sugar must not exceed 0.5 %. The results of the current research agree with the values reported (Zia-Ud-Din and Rasool, 2015). Greater conductivity ash in sugar is an attribute of inferior clarity and hence small profitable value (Hamerski et al., 2011; Oliveira et al., 2007) because further purification beyond laboratory scale is developed. Likewise, high estimation of ashes is a sign of excess minerals that hinders crystallization of sugar, moreover promoting amendments in sensory features like inappropriate and unwanted flavor (Aquinoo and Franco, 2008; Cecchi 2003; NEPA 2011). Thus, the values of sulfated ash content of the samples (shown in Table 1) allow us to find whether the samples are in harmony with the standard recognized by some researchers (NEPA 2011; Trott 1988; Wilson 2000). Further, produced raw sugar from both varieties of sweet potato has been considered well enough for consumption.

Thus, the concentration of reducing sugar (RS) in the samples WFSP and RFSP has  $0.059 \pm 0.009\%$  RS, which implies that these are raw materials matured fully and the processing temperature was appropriately causing the monosaccharides destruction. This at last brought about great quality sugar. It's examined that whether the diminishing sugar contents were related to the amount of sucrose or not. It's discovered that concerning their percentage, both have consequences for one another (Zia-ud-din and Rassol (2015); Ingabire and Hilda (2011); Wireko-Manu et al. (2010).

#### 3.1.2. Amount of sucrose and purity of SPRS

The purity of raw sugar and sucrose part of RFSP and WFSP samples were determined by employing a refractometer, polarimeter, and Saccharimeter. By employing refractometry, Brix or degree Brix of RFSP and WFSP measured and obtained (shown in Table 1) as  $8.467 \pm 0.01$  and  $8.40 \pm 0.02$  respectively. Ghana standard board specifies that for non-alcoholic beverages, the sugar samples should have are a refractive value of greater than or equal to  $8^{\circ}$ brix (8% w/w) (Wireko-Manu et al., 2010). Accordingly, the Brix values obtained in the present study confirm that the raw sugar obtained from sweet potato varieties is a useful

Table	1. Some	proximate	composition	of RFSP	and	WFPS	raw	sugar
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Compositional	parameters	Sweet potato raw sugar				
		Red fleshed sweet potato (RFSP)	White fleshed sweet potato (WFSP)			
Moisture %		$0.077\pm0.002$	$0.075\pm0.003$			
Sulphated ash (	%)	$0.737 \pm 0.05$	$\textbf{0.770} \pm \textbf{0.01}$			
Reducing sugar	(g)	$0.059\pm0.009$	$0.059\pm0.009$			
Solids (Bx %) b	y Refractometric method	$\textbf{8.467} \pm \textbf{0.01}$	$\textbf{8.40} \pm \textbf{0.02}$			
Amount of sucrose and purity	Polarization (°Z) by Saccharimeter of 26 g of SPRS samples	$95.25\pm0.05$	$94.6\pm0.015$			
	In 26 g of raw sugar sample (g of sucrose in terms of pol %)	24.03	23.87			
	Purity of sugar to pol %	92.42	91.81			

alternative to the sugar harvests (sugar cane and sugar beet). Refractive apparent purity (otherwise called polarization or purity) depicts the ability to improve sweeten, other than being a part that speaks to the quality of the item (Damodaran et al., 2008). This reason might be comprehended as the noticeable percent weight of sucrose. This could be examined from the divergence of light in the polarized plane (Damodaran et al., 2008; CTC/Copersucar 2002).

Sucrose concentration (standard) that guarantees the base an incentive to refined sugars meant for straight utilization is 99.7Z (that implies 99.7%), though the remains are comprised by impurity (Damodaran et al., 2008). Pol values of the presently studied samples are  $95.25 \pm 0.05$  (RFSP) and  $94.6 \pm 0.015$  (WFSP) which differ in purity. Similar is the case when comparing the data with the ICUMSA standard. But these sugars have more than 90°Z (pol %) and are considered in good agreement with pol values of demarara and brown sugar variety according to Brazilian standards (Damodaran et al., 2008).

RFSP sample is much greater than WFSP raw sugar. Higher values are might be the presence of a greater amount of sucrose. Raw sugar despite everything desires to handle to make it satisfactory refined sugar for human utilization (Zia-Ud-Din and Rasool, 2015). But, in health aspects, the consumption of the presently studied raw sugar is reliably good for edible, because it may have some useful nutrients like reducing sugar (glucose like), minerals, fibers, fat, vitamins, etc. Since the physical meaning of pol % corresponds to the plain purity of sugar, comparatively the sugar sample produced from red flesh sweet potato is purer and this means that it is the one with the greatest percentage of desired product, the sucrose (crystal sugar). The results (Table 1) show that the 92.42% (24.03  $\pm$  0.005 g sucrose/26 g sample) on RFSP and 91.81% (23.87  $\pm$  0.022 g sucrose/26 g sample) on WFSP were found as sucrose, and remaining compounds are other solids such as reducing sugars, insoluble solids, impurities, minerals, fibers, fats, etc.

#### 3.1.3. FTIR characterization of SPRS

FTIR spectra (shown in Figure 2) of the presently studied samples are given with their corresponding peak picking spectrums. Maximum absorption between 3700 -  $3000 \text{ cm}^{-1}$  with a broad peak was observed in samples of RFSP and WFSP. It showed the presence of –OH stretching, which well agrees with the literature reported (Medhat et al., 2006). Sharp peaks at 2934 and 2937 cm<sup>-1</sup> were found on both samples that have been associated with the CH- stretching of CH<sub>2</sub>. Combination bands such as –OCH and C–OH deformations were noticed at 1526 - 1347 cm<sup>-1</sup>;

CH & OH (in-plane) deformations were noticed at 1362-1191 cm<sup>-1</sup> and a peak of CO & C–C stretching was recorded at 1191 - 995 cm<sup>-1</sup>. All these could be observed on both RFPS and WFPS raw sugar samples.

Intense distinctive bands were found in the fingerprint area, 900 - 1400 cm<sup>-1</sup> of the middle IR series. This shows the combinations of carbohydrates in the sample. Bands in 900–1153 cm<sup>-1</sup> consigned to stretching of CO & C–C bonds, whilst frequencies ranged 1400 - 1199 cm<sup>-1</sup> shown the availability of –OCH, –CCH & –COH of bending vibrations corresponds to carbohydrates (Leopold et al., 2011). It's understandable from the spectra that some carbohydrates were there in the same sample. A superior sucrose level was observed in this studied spectrum that was revealed due to the powerful band of sucrose in 990 cm<sup>-1</sup> and this result is with acceptable range and in fine agreement with the literature (Leopold et al., 2011).

IR spectra of the current studied sample do show no signal of free H<sub>2</sub>O molecules owing to nearby no supportive frequencies at 2130 cm<sup>-1</sup>, 1650 cm<sup>-1</sup>, and 700 cm<sup>-1</sup>. It reveals that laboratory-produced sweet potato raw sugar is considerably free from moisture content, which is clear through the polarization (<sup>o</sup>Z) of the samples such as RFSP and WFPS are 95.25  $\pm$  0.05 and 94.6  $\pm$  0.015 respectively.

#### 3.2. Analysis of bioethanol

#### 3.2.1. Identification of bioethanol

About 5 mL of distilled and re-distilled ethanol was taken into a long test tube. A pinch of  $K_2Cr_2O_7$  and some drops of  $H_2SO_4$  were poured into it. The color appearance of the sample turns pinks to a green that shows the prevalence of bioethanol (Thenmozhi and Victoria 2013). This was also confirmed by a combustion test (AHPA 2017) as 5 mL of produced ethanol was added into a 15 mL test tube containing boiling chips and subjected for heating. The open end of the test tube was shown to the flame and the ethanol vapors were ignited. Ethanol burns with a pale blue flame with no smoke; it's further confirmed that the obtained product is ethanol.

## 3.2.2. FTIR characterization of bioethanol

FTIR characterization of distilled bioethanol (96 %) was carried out and the corresponding peak picking spectrum is given in Figure 3. Ethanol has characteristic IR absorptions related to O–H, C–O, and C–H stretching vibrations. The studied bioethanol shows strong broadband at  $3403 \text{ cm}^{-1}$  in the region of  $3700-3000 \text{ cm}^{-1}$  that confirmed the O–H



Figure 2. FTIR spectrum of RFSP and WFSP raw sugars.

stretching of alcohols, while the bands at 1085 cm<sup>-1</sup> and 1046 cm<sup>-1</sup> might confirm the C–O stretching vibrations. The bands at 3000 and 2840 cm<sup>-1</sup> (2978 cm<sup>-1</sup> is C–H stretching) were assigned to the symmetric stretching modes of –CH<sub>2</sub> and –CH<sub>3</sub> groups, respectively (Bodirlau and Teaca 2007).

Furthermore, a strong peak at  $1644 \text{ cm}^{-1}$  appeared in the spectrum of ethyl alcohol that represents the absorbed water (Yusuf Muhammed 2016). The intensity of this peak noticeably increases when compared with the IR spectrum of raw sugar. This shows that some water has been absorbed during the processing of alcohol. Also, compare the FTIR spectrum (Figure 2) of raw sugar samples with distilled bioethanol spectrum (Figure 3). It revealed that there is no peak in the fingerprint regions, at 900 - 1400 cm<sup>-1</sup> on later; but obtained a plain broad band spectrum for ethanol (Figure 3). This shows that almost the entire amount of fermentable sugars such as glucose, fructose, and sucrose were converted into alcohol with the action of efficient sweet potato extracted yeast (SPY) strain, during the 24 h of fermentation. Thus, this FTIR spectroscopic study concludes that the product obtained from sweet potato under acid hydrolysis followed by fermentation and distillation is ethyl alcohol.

#### 3.2.3. The efficiency of microbes culture and fermentation

In the case of sugar solution, the Brix % measured using a Refractometer refers to the sugar content by mass and it's a measure of total solids. Substrate concentrations (Brix %) in the fermented wash greatly depend on the type of microbes utilized during fermentation. Microbial fermentation and distillation of ethanol may characterize by the maximum range of selectivity, low accumulation of byproducts, yield, and fast rate of fermentation rate, etc.

The activity of microbes was analyzed (using a Refractometer) every 8 h through the measurement of Brix % of fermented wash. The results are correlated with the specific gravity (obtained from brix% specific gravity conversion table) and alcohol level (in %) obtained from distilled bioethanol (shown in Table 2).

Results revealed that yeasts have higher efficiency than bacteria (Table 2). This is due to the viability and genetic stability of yeast cells, which have greater efficiency than bacteria. Also, yeasts can survive with or without oxygen and microbial fermentation is possible no matter how fast it is taking place. The Brix % was measured at an interval of every 8 hours for both varieties of sweet potatoes. The Brix % was found more in WFSP on every measurement during the fermentation. This shows that the activity (in terms of alcohol conversion rate) of microbes in WFSP is lower than in RFSP. It can be attributed to the fact that WFSP pulp has

lower amounts of total fermentable solids (initial Brix % on WFSP is 13.54 %) than RFSP (initial Brix % is 14.38 %).

The Brix % of fermented wash of RFSP and WFSP ranged: 7.5-10.90 (at 8 h), 3.52-6.74 (at 16 h), and 0.4-1.35 (at 24 h) respectively. SPY has the highest fermentation efficiency for the conversion of substrate faster into alcohol which corresponds to the lowest Brix values (Brix %: at 8 h are 8.08 & 7.5, at 16 h: 3.03 & 3.52 and 24 h: 0.4 & 0.6) for both substrates, whereas SRB has the highest Brix % values during fermentation (i.e. 10.91 & 10.25 at 8 h; 6.23 & 6.74 at 16 h; and 1.18 & 1.35 at 24 h which shows that SRB has the lower fermenting efficiency.

Brix % (residual sugar) of fermented wash used for the determination of sugar consumption shows the rate is lower at the beginning stage of substrate conversion with aid of microbes and this is because of the saturated sugar level. Further, the ethanol yield and alcohol level was increased with an increase in conversion time, showing that with the elapse of time, consumption of sugar increases in the cases of all inoculums for both substrates (such as RFSP and WFSP). This reveals that due to the lack of substrate, more microbes are available in the medium to facilitate the fermentation of the substrate.

Comparatively, SPY can consume more substrates in RFSP around 86 % than in WFSP (83 %). That is, residual sugar of about 0.4 °Brix remains after 24 h of fermentation in the case of RFSP, and 0.6 °Brix remains in the case of WFSP when SPY is used. Secondly, LEY has a good efficiency that consumes 84 % of substrates in RFSP (0.62 °Brix remains after 24 h) and 81 % sugars in WFSP (0.78 °Brix remains after 24 h). Similarly, *S.cerevisiae* and kocho extracted yeasts have moderate fermentation efficiency, around 82–83 %. Finally, fermentation of RFSP and WFSP pulps with sheep rumen bacteria resulted in the least fermentation efficiency, showing 80 % consumption and conversion of the substrate in the case of RFSP (5 °Brix remains after 24 h) and a corresponding 79.8 % in the case of WFSP (5.38 °Brix remains) which shown in Table 2.

Specific gravity values of the fermentation wash were obtained (using Brix % and specific gravity conversion table), in the case of each inoculum according to °Brix - specific gravity conversion table, the samples containing zero and closer to zero, show some considerable percentage of alcohol. The samples containing some °Brix  $\geq 1$  show that there is no considerable amount of alcohol in them. It may have some by-products formed during the conversion of sugar. The values of specific gravity were found to be in the range of 1.001–1.18 (in RFSP) and 1.002–1.45 (in WFPS).

The specific gravity values of the fermented wash show that there is not an applicable yield of ethanol produced from the beginning of fermentation by the action of inoculums. After 24 h of fermentation, except SRB, all other microbes have the potential to produce the



Figure 3. FTIR spectrum of distilled bioethanol produced from RFSP pulp using SPY.

Table 2. Measurement Brix %, specific	gravity, and sugar co	onsumption of fermented v	vash at different time	intervals and in different microbes.
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Types of Microbes	Properties of fermented wash	RFSP (Init	RFSP (Initial Brix - 14.5)			WFSP (initial Brix - 13.4)			Alcohol level (%) by Ebuliometry	
		8 h	16 h	24 h	8 h	16 h	24 h	RFSP	WFSP	
SPY	Specific gravity	1.032	1.012	1.001	1.030	1.014	1.002	96	95	
	Brix %	8.08	3.03	0.4	7.50	3.52	0.6			
	Sugar consumed	6.30	5.05	2.63	5.9	3.98	2.92			
LEY	Specific gravity	1.036	1.016	1.002	1.033	1.016	1.003	94	94	
	Brix %	8.90	4.08	0.62	8.32	3.94	0.73			
	Sugar consumed	5.48	4.82	3.46	5.22	4.38	3.21			
SCY	Specific gravity	1.037	1.015	1.002	1.034	1.017	1.004	93	93	
	Brix %	9.12	4.00	0.65	8.45	4.40	0.88			
	Sugar consumed	5.26	5.12	3.35	5.09	4.05	5.52			
KEY	Specific gravity	1.039	1.021	1.004	1.035	1.019	1.004	92	92	
	Brix %	9.67	5.42	0.94	8.66	4.87	0.97			
	Sugar consumed	4.71	4.25	4.48	4.88	3.79	3.97			
SRB	Specific gravity	1.044	1.025	1.005	1.041	1.027	1.006	88.5	88	
	Brix %	10.91	6.23	1.18	10.25	6.74	1.45			
	Sugar consumed	3.47	4.08	5.00	3.29	3.51	5.38			

maximum possible amount of alcohol as shown by the reduction of specific gravity nearing zero and <sup>o</sup>Brix of fermented wash lower than 1. Furthermore, the efficiency of all microbes was also confirmed by the comparison of alcohol level (in %) or concentration found in distilled alcohol after fermentation by the action of all kinds of inoculums. This was measured by Ebuliometer and the results show that the range of alcohol level is between 96 - 88 % in the case of RFSP and WFSP pulps. The alcohol level of distilled alcohol produced from RFSP and WFSP by the action of SPY was found to be 96 % and 95 % respectively. The SRB inoculums have produced lower alcohol levels and were found to be 88.5 % (in RFSP) and 88 % for WFSP at the end of fermentation (after 24 h). Hence the fermentation efficiency of the microbes are graded as SPY > LEY > SCY > KEY > SRB.

Swain et al., (2007) reported that the quantity of total sugar left after fermentation (measured by Brix %) was found low as the fermentation period is prolonged since an increase in the multiplication of microbes consumed complete biomass and therefore ethanol production is enhanced (shown in Table 2). This could be due to the rapid increase in biomass after appreciable microbial growth leads to maximum conversion of substrate to ethanol. The results show that the concentration of residual sugars reduced rapidly and consistently during fermentation, and mostly fermentation period of 24 h was used. According to Swain et al., (2007) fermentation media, moisture, fermentation temperature, the number of inoculums, and other related condition has defined impact on the yield of ethanol and fermentation rate, and their research was concluded that efficient fermentation was achieved at 72 h. This current research also found the same scenario in evaluating fermentation conditions as the maximum concentration of 96 % of ethanol was achieved by the consumption of 86 % of sugar that converted into alcohol by the action of SPY; however, the maximum concentration (88.5 %) of ethanol was achieved upon the conversion of 80 % of sugar into alcohol by employing SRB. All the microbes used in this study are efficient for the production of ethanol, as proven from the currently studied results. This observation is in agreement with the earlier reports of bioethanol production.

#### 3.2.4. Determination of alcohol level of bioethanol using Ebuliometer

The alcohol level (%) of the produced ethanol was measured concerning the boiling point of medium (water) as the standard as given in Figures 4 and 5 in the case of RFSP and WFSP respectively. The results show that the maximum alcohol level of about 96 % ethanol was produced from RFSP. This was obtained by employing the yeast which is extracted from sweet potato, and the lower level of alcohol was produced (about 88.5 %) by employing sheep rumen bacteria. Therefore, the study reveals that the yeast extracted from sweet potato is highly efficient for the production of ethanol and it also has a high degree of selectivity. The boiling temperature shows that the maximum alcohol-containing bioethanol boils at 78 °C. This is relatively closer to the boiling point of absolute alcohol/anhydrous ethanol/fermentation alcohol (78.3 °C). Also, Zeinelabdeen et al., (2014) stated that the boiling point of alcohol in the range of 78.3–79.45 °C is considered denatured fuel alcohol. Accordingly, all the bioethanol produced in the present study by the action of different yeast/microbes (except SRB, which has a boiling point of 80 °C) have a boiling point within the appreciable range and they are all produced from RFSP and WFSP pulps under fermentation. Thus, all these produced bioethanol samples can be considered as fuel ethanol.

Here, the comparative efficiency of microbes varies widely and the production of ethanol has a significant difference (shown in Figures 4 and 5) on yeast strains and the sheep rumen bacteria. At the end of fermentation (at 24 h), the highest alcohol level was found (96 % in RFSP and 95 % in WFSP) by the action of SPY, moderate and equivalent alcohol level was found (94 %–92 %) by employing other yeast strains such as LEY, SCY, and KEY and the least alcohol level was noticed (88.5 % in RFSP and 88 % in WFSP) by the action of SRB strain. Hence, the efficiency of microbes (based on alcohol level) on the production of bioethanol from sweet potato pulps could be classified as SPY > LEY  $\cong$  SCY  $\cong$  KEY > SRB.

Atiyab and Duvanjak (2001) reported 84.6 % ethanol yield for sucrose media with the beginning of hydrolyzed and prepared substrate of 257.4 g/L using *S.cerevisiae* as fermentation strain. In the present study, the yield of ethanol was found to be 88 % and 88.5 % by the action of SRB strain for the respective sweet potato pulp. Hence, SRB microbe is also to be considered as a potential strain that can be used for the fermentation of starchy substrates into ethyl alcohol.

#### 3.2.5. Quantitative estimation of bioethanol from sweet potatoes pulp

3.2.5.1. Quantification of ethanol by direct weighing method. Ethanol produced from two varieties such as red-fleshed and white-fleshed sweet potato pulps were collected after the extraction of sugars under diffusion. Then, about 1 kg of each variety of pulp was subjected to the process of saccharification (under acid hydrolysis) and then fermentation was performed on the substrate by the action of five different microbes. The fermentation process was performed for about 24 h at 30 °C the completion of the process was ensured by measuring the <sup>o</sup>Brix of the sample. The quantity of ethanol produced was measured by weighing the product directly (after cooling) and comparing it with the initial amount of substrate taken and thus measured the yield. The yield of the product



Figure 4. The boiling temperature (°C) and alcohol level (%) of bio-ethanol produced from RFSP pulp by different microbes.



Figure 5. The boiling temperature (°C) and alcohol level (%) of bio-ethanol produced from WFSP pulp by different microbes.

Table 3. The amount of ethanol, % yield, and alcohol level (%) of distilled alcohol of RFSP and WFSP pulps produced after 24 h of fermentation time at 30 °C and pH: 5.5

S.No.	Types of microbes	Quantity of bioethanol obtained (g/kg)	Alcohol level (%) by Ebuliometry				
		Amount of RFSP ethanol (g/1kg)	% Yield	Amount of WFSP ethanol (g/1kg)	% Yield	RFSP	WFSP
1	SPY	$800\pm0.002$	80	$780\pm0.003$	78	96	95
2	LEY	$675\pm0.017$	67.5	$650\pm0.021$	65	94	94
3	SCY	$650\pm0.003$	65	$600\pm0.005$	60	93	93
4	KEY	$600\pm0.049$	60	$585\pm0.022$	58.5	92	92
5	SRB	$560\pm0.030$	56	$500\pm0.027$	50	88.5	88

obtained was then compared with the % alcohol level of the product achieved by the action of different microbes (which was measured by using an Ebuliometer) as shown in Table 3.

The results obtained reveals the amount of alcohol produced and it ranged between 800  $\pm$  0.002 g/kg - 560  $\pm$  0.030 g/kg (% yield 80 - 56 & alcohol level 96 %–88.5 %) 780  $\pm$  0.003–500  $\pm$  0.027 (% yield 78-50 & alcohol level 95 %–88 %) of RFSP and WFSP pulps respectively by the action of different microbes (Table 3). The rate of production of alcohol from RFSP pulp was comparatively higher than that produced from WFSP. This may be due to the fermentable substrate called carbohydrates, which is more in RFSP than in WFSP. While analyzing the efficiency of microbes, it can be found that the higher amount of ethanol produced is 800  $\pm$  0.002 (in RFSP) and 780  $\pm$  0.003 (in WFSP) by the action of SPY with greater % yield and alcohol level in it (shown in

Table 3). It is a fact that by using SRB, only a lower amount of ethanol was produced, viz.  $560 \pm 0.030$  (in RFSP) and  $500 \pm 0.027$  (in WFSP) with a considerably lower % yield (56 % in RFSP and 50 % in WFSP) and alcohol level (88.5 % in RFSP and 88 % in WFSP). The other microbes are performing moderately and the range is between 58.5 - 67.5 % yields with 92–94 % of alcohol for both the varieties of sweet potato pulp substrates. Hence, the efficiency of microbes is in the order SPY > LEY > SCY > KEY > SRB.

The maximum rate of formation of ethanol (800  $\pm$  0.002 g/kg substrate) with 96 % concentration was obtained in RFSP substrate by the action of SPY strain. Under the current study, the rate of ethanol distillation yield was in the range of 800  $\pm$  0.002–500  $\pm$  0.027 g/kg on both cases of sweet potato pulps (Table 3). In earlier studies, Swain et al., (2013) described that maximum ethanol yield (using sweet potato flour inoculated with *S.cerevisiae and Trichoderma sp.*) around  $154 \pm 4$  g of ethanol per kilogram of the substrate with 95 % concentration after 72 h of incubation. According to Kiren Sree et al. (1999), the high yield of ethanol was found as 50 g/kg of the substrate (sweet sorghum and sweet potato) under SSF at 37 °C using a thermo-tolerant strain of *S.cerevisiae*. The yield obtained was informative as about 558 g ethanol/kg starch; with a high fermentation efficiency of 98.4 % (Shanavas et al., 2011) was reported. Therefore, some possible reasons for these differences, including the nature of strain used the biochemical composition of the substrate, the fermentation system used, and the condition under which the fermentation took place has a significant impact on fermentation efficiency that described earlier (Chen and Chou, 1993; Henk and Linden, 1996).

Thus, in the present study, the production of bioethanol from RFSP and WFSP pulp was performed at 30 °C for 24 h of fermentation at pH 5.5 of the medium. Five different types of microbes such as SPY, LEY, SCY, KEY, and SRB were employed. In all the cases of microbes, better performances were noticed compared to the previously reported results (Nurhayati et al., 2016; Muruga et al., 2016; Grahovac et al., 2011; Nibedita et al., 2012; Sanat et al., 2014; Shanavas et al., 2011).

*3.2.5.2. Quantification of ethanol by specific gravity method.* The substrates were allowed to keep in a laminar incubator at 30 °C and samples were taken out of the incubation after completion of fermentation (24 h) and distillation was done immediately. The concentration of ethanol was estimated using a specific gravity method by employing a specific gravity conversion table (US NBS Bulletin 2018). The study found that SPY strain was used efficiently (at 30 °C upon 24 h) to convert substrates into bioethanol. The substrate of RFSP pulp showed the highest ethanol production (95.32 %) with a specific gravity of 0.801 g/cm<sup>3</sup>, whereas WFSP pulp gave only 88.62% of ethanol with a specific gravity of 0.831 g/cm<sup>3</sup>. RFSP pulp has more efficiency due to the available carbohydrate percentage which is higher than the WFSP. According to the literature, there was a report (Zeinelabdeen et al., 2014), where a higher amount of alcohol was produced on both varieties of sweet potatoes by employing SPY strain as the microbe.

Hence, this quantification method and previous analyses such as the measurement of pol % and alcohol value that was estimated by Ebuliometer, polarimetry, and quantification of ethanol (by direct method) confirms the greater potential to ferment the RFSP (used as a raw material) into bioethanol under feasible conditions are temperature (30 °C), pH of the medium 5.5 and fermentation period of 24 h.

#### 4. Conclusions

This study is particularly concerned with the production and analyses of sugar and bioethanol produced from red and white-fleshed sweet potatoes (*Ipomoea batatas*). Raw sugar of RFSP and WFSP consists of lower moisture ( $0.077 \pm 0.002g \& 0.075 \pm 0.003$ ), ash content ( $0.737 \pm 0.05 \& 0.77 \pm 0.01$ ), and reducing sugars ( $0.059 \pm 0.009$ ), but it has a higher amount of sucrose ( $95.25 \pm 0.05$  in RFSP and  $94.6 \pm 0.015$  in WFSP). Upon polarimeter measurement, it was found that RFSP raw sugar has greater sucrose content and higher purity (95.42 g/L) than WFSP (which has 94.05 g/L of sucrose). FTIR results of raw sugars provide the characteristic absorption peak at  $990 \text{ cm}^{-1}$  confirms the higher sucrose level reported in RFSP and WFSP.

The produced bioethanol is confirmed by the peaks that appeared at 3403 cm<sup>-1</sup> (-OH), at 1085 cm<sup>-1,</sup> and 1046 cm<sup>-1</sup> (for C–O). The efficiency of all the five different inoculums (used for fermentation) was studied through the measurement of Brix values, specific gravity, sugar consumption, % yield of ethanol, and alcohol concentration. Five different inoculums were used for the fermentation of raw materials, and the fermentation was performed at 30 °C for 24 h at pH 5.5. In all the cases of microbes, respectable performances were observed. The efficiency of inoculums was graded as SPY > LEY > SCY > KEY > SRB.

In sugar extraction and distillation of bioethanol, sweet potato can be found as an alternative crop like sugarcane, sugar beet, and sweet sorghum, etc. that are commonly used, and out of the two varieties of sweet potatoes which were used in this study, red-fleshed sweet potato is rich in sucrose with the highest level of total dry solids confirms this study concludes the sweet potato is one of the alternative harvests for sugarproducing industries.

#### Declarations

#### Author contribution statement

Ketemaw Salelign: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Ramesh Duraisamy: Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper.

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Data included in article/supp. material/referenced in article.

#### Declaration of interests statement

The authors declare no conflict of interest.

#### Additional information

No additional information is available for this paper.

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