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Research article

Bioconversion of mango (*Mangifera indica*) seed kernel starch into bioethanol using various fermentation techniques

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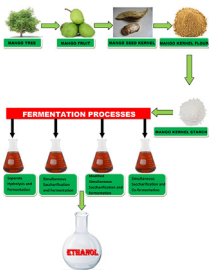
HIGHLIGHTS

- Mango kernels are abundantly generated waste from mango fruit.
- Mango kernel flour was extracted and treated for starch recovery.
- Produced starch was used as feed stock for sugar and ethanol production.
- Varying fermentation techniques were explored for ethanol production.
- Modified simultaneous saccharification & fermentation gave 4.0% ethanol yield.

GRAPHICAL ABSTRACT



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ABSTRACT

The potential of mango seed kernel starch (MKS) as a feedstock for bioethanol production was evaluated in this study. Starch extraction and hydrolysis from mango kernel were studied. Fermentation methods included separate hydrolysis and fermentation (SH&F), simultaneous saccharification and co-fermentation (SS&CF), simultaneous saccharification and fermentation (SS&F), and modified simultaneous saccharification and fermentation (SS&F) techniques. Drying and wet-milling generated 41.2 g of white starch/100 g flour, and processing with alum gave 58.6/100 g MKS. Hydrolysis of 5 g MKS by sulfuric acid, sodium hydroxide, malted "acha", and *Aspergillus niger* amylase for 2 h produced (g/100 mL) 3.97 g, 4.0 g, 4.43 g and 4.24 g of sugar, respectively. Fermentation with 7 g of MKS yielded maximum sugar and ethanol concentrations. Ethanol obtained using SS&CF, SH&F, SS&F and modified SS&F were (v/v); 0.26%, 2.0%, 1.13% and 3.985%, respectively. These results confirmed MKS as a potential feedstock for bioethanol production.

1. Introduction

The dangers of depending on oil revenue as the main source of income for financing the national budget of oil-exporting countries became a reality recently when the oil price crashed due to the coronavirus disease outbreak (COVID-19 pandemic) in 2020. This pandemic affected the economy of many nations, Nigeria inclusive, resulting in significant reductions in the national budget. Though successive governments have advocated for the diversification of the Nigerian economy from oil to non-oil sectors, little has been done because of the steady revenue from fossil oils.

In recent years, the search for alternatives to fossil fuels has increased tremendously due to climate change and the associated problems. From the beginning of the twentieth century the rate at which carbon dioxide and other greenhouse gases (methane and nitrogen oxide) are emitted

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into the atmosphere has been faster than any time in the known history of life on earth (Joanne et al., 2013). Due to industrialization, there has been a massive increase in atmospheric carbon dioxide levels; this is an aftermath of the accelerated burning of fossil fuels for diverse production processes. The transport sector is probably responsible for the highest proportion of carbon dioxide emitted.

The conversion of agricultural and industrial wastes into useful bio-products such as bioethanol has become an increasingly popular alternative to gasoline worldwide (Onwuakor et al., 2017; Ndubuisi et al., 2018; Murata et al., 2021). Ethanol served as a transport fuel in the early years, e.g. Henry Ford's Model T car constructed in 1908 used ethanol. Due to the advantages of ethanol over fossil fuel, such as emission of less carbon dioxide, non-emission of toxic gases, the requirement of less energy for ethanol production and high octane rating than gasoline, many fermentation techniques have been devised for bioethanol production. The economics of bioethanol production by fermentation is highly dependent on the cost of the raw materials used (Lebaka et al., 2011). The cost of procuring the raw materials is often exorbitant and can represent 40–75% of the total ethanol production cost depending on the type of feedstock (Arijina et al., 2018). To reduce the production cost and promote eco-friendliness, the production of value-added products such as bioethanol from agro-industrial wastes is currently of great interest. Adopting wastes as a renewable feedstock for bioprocesses reduces environmental pollution, energy production and revenue generation (Amadi et al., 2020, 2021; Awodi et al., 2021; Onwuakor et al., 2017).

Mango (*Mangifera indica*) is one of the most commercially valuable fruit trees in many tropical countries. It is one of the most extensively exploited fruits for food, juice, flavour, fragrance and colour (Kittiphoom, 2012; Parv and Kalpana, 2018). Mango flesh is usually consumed or processed by the food industries, thus disposing of a large amount of seed as solid waste. Approximately 40–60% of waste products are generated during the processing of mango fruit, predominantly the mango kernel (Manisha and Sikdar, 2015). Mango seed is among the leading agro-industrial wastes worldwide, generating about 123,000 metric tons of seeds annually (Reddy et al., 2016). On a dry weight basis, Mango seed kernels contain 65% starch (Manisha and Sikdar, 2015). Since the starch of mango seed kernels is susceptible to hydrolysis to fermentable sugars, they can be converted into bioethanol applicable as a substitute for gasoline or mixed with gasoline for the internal combustion engine, thereby reducing the over-dependence on fossil fuel.

Despite the abundance of mango seed kernel and the significant quantity of starch present, information available in the literature is predominantly on mango fruit juice and peels (Veeranjaneya and Vijaya, 2007; Lebaka et al., 2011). *Aspergillus* species are widely exploited in bioprocesses, given their ability to produce a cocktail of amylases for the hydrolysis of starch to simple sugars (Nwagu and Okolo, 2011). The co-fermentation of starch-containing medium for ethanol production using a co-culture comprising *Aspergillus* species and *Saccharomyces cerevisiae* has been reported (Amadi et al., 2016; Awodi et al., 2021). Given the paucity of information on the bioconversion of mango seed kernel into bioethanol, the present paper investigated bioethanol production from mango seed kernel starch using three different methods. The methods are simultaneous saccharification and fermentation, separate hydrolysis and fermentation, and simultaneous saccharification and co-fermentation techniques.

2. Materials and methods

2.1. Collection of mango seeds

"Hindi" mango (*Mangifera indica*) seeds were collected, decorticated, and diced into pieces with a knife.

2.2. Microorganisms

Aspergillus niger isolated from rotten mango seeds was from the Department of Microbiology, University of Nigeria, Nsukka. The mould was cultivated on Sabouraud Dextrose Agar (SDA) and maintained on an agar slant. *Saccharomyces cerevisiae* previously isolated from 'burukutu (an indigenous fermented alcoholic beverage) was from the Department of Microbiology, University of Nigeria, Nsukka. The commercial yeast (instant dry yeast) was a product of STK Industries Limited, Lagos, Nigeria.

2.3. Microbial and plant enzyme production

Amylase production from *A. niger* was by culturing two loops full of *A. niger* spore in a sterilised fermentation medium which consisted of (g/100 mL) MKS, 2g; glucose, 0.1; peptone, 0.1; yeast extract, 0.1; and water, 100 mL. The fermentation was for 24 h; after that, it was centrifuged at 3000 rpm for 10 min in a centrifuge. The supernatant (crude amylase) was stored in a deep freezer until needed for hydrolysis of MKS.

"Acha" (*Digitaria exillis*) grains were spread on a clean cloth (72 × 72) cm wide. Coldwater was sprinkled on the grains at 12 h intervals until the seeds germinated (5 days). The germinated seeds were sun-dried for 48 h and ground into powder. The powder (crude plant enzyme) was stored in a dry place until required for MKS hydrolysis.

2.4. Pretreatment of mango seed kernels and starch extraction

Before starch extraction, fresh mango seed kernels were treated using various methods.

(a) boiling (10 min) and sun-drying
(b) boiling for (10 min) and wet milling
(c) steeping in 95% ethanol for 24h then sun-drying
(d) steeping in 95% alcohol, then wet-milling
(e) wet-milling with alum
(f) wet-milling without alum
(g) sun-drying and dry-milling
(h) sun-dry and wet-milling

Each sample was sieved after milling (dry–mill or wet-mill). Starch was extracted from the samples using a modification of the method by Hassan et al. (2013).

2.5. Hydrolysis of mango kernel starch (MKS)

The starch extraction method, which gave the highest quantity of white starch, was selected for further experiment. The MKS was hydrolysed using 0.1M sulfuric acid, 0.1M sodium hydroxide, malted *Digitaria exilis* (malted acha) and *A. niger* amylase. Before hydrolysis, the gelatinisation of the MKS was by weighing 5 g each into 250 mL conical flasks, followed by the addition of 100 mL of distilled water and boiling for 10 min. After cooling, varied concentrations of 0.1M NaOH (1–10 mL),0.1M H_2SO_4 (0.1–1 mL), malted acha (1–10 g) and *A. niger* amylase (1–10 U/mL) were dispensed into separate conical flasks. The samples were hydrolysed at 28 °C for 4 h. Hydrolysate (10 mL) was withdrawn at 2 h intervals and centrifuged at 3000 rpm for 10 min. The supernatant was collected for fermentable sugar determination using the 3, 5- dinitro salicylic acid method (Miller, 1959).

2.6. Assay of amylase activity

The amylase was assayed by adding 0.5 ml of the crude enzyme to 0.5 ml of soluble starch (1%) in 0.1M citrate phosphate buffer, pH 6 for 30 min at 30 °C. The reducing sugar was evaluated by the DNS method of Miller (1959). One unit of amylase was defined as the amount of enzyme which liberated 1 mg of reducing sugar (glucose equivalent) from the substrate per 30 min under the assay condition.

2.7. Determination of fermentable sugar concentration

The test solution consisted of 0.1 mL of sample and 0. 1 mL DNS solution in a test tube, mixed and boiled in a water bath for 10 min. The reaction sample was cooled, distilled water (0.8 mL) was added, and the optical density (OD) was read at 540 nm using a Spectrophotometer. The absorbance values obtained from the spectrophotometer were converted into glucose concentration using the glucose standard curve.

2.8. Fermentation medium for simultaneous saccharification and fermentation of MKS

The fermentation medium for ethanol production consisted of (g/100 mL): MKS, 7; glucose, 0.1; peptone, 0.2; and yeast extract, 0.2 (Awodi et al., 2021). For the control samples, 7g glucose was used in place of MKS. The medium was sterilised in an autoclave at 121 °C for 15 min. After cooling, the medium was inoculated with 5 mL of *A. niger* spores (2.0×10^4 SFU/mL) and 5 mL of *S. cerevisiae* cells (2.5×10^5 CFU/mL) and incubated at 28 °C for 24 h. Samples (10 mL) were withdrawn from the fermenting medium at eight hourly intervals and centrifuged at 3000 rpm for 10 min. After 24 h of fermentation, the supernatant of the hydrolysate was used for fermentable sugar determination by the DNS method (Miller, 1959) and for ethanol determination. The ethanol concentration was determined using high-performance liquid chromatography (HPLC) Cecil Instruments, UK.

2.9. Optimisation of culture condition for increased bioethanol production using SSF

The concentrations of all the media components were optimised using the one variable at a time (OVAT) technique.

2.9.1. Effects of media components

The fermentation medium for evaluating the effect of glucose concentrations on sugar generation comprised MKS, 7; peptone, 0.1g; yeast extract, 0.1 g, and glucose (0.1–0.5 g) in 100 mL distilled water. After medium inoculation and fermentation (24 h), the hydrolysate (10 mL) was centrifuged at 3000 rpm for 10 min. The supernatant was used for sugar analysis by the DNS method (Miller, 1959).

A similar procedure was used to determine the effect of peptone (0.1–0.5 g) and yeast extract (0.1–0.5 g) concentration on the sugar generation. However, all parameters remained constant at 0.1 g, while the parameter under investigation was varied.

2.9.2. Influence of pH on sugar and bioethanol production

Citrate phosphate buffer 0.2 M (100 mL) of varying pH values (3–7) was dispensed into 250 mL conical flasks each, containing (g/100 mL): peptone, 0.1, yeast extract, 0.1, glucose, 0.1 and MKS, 7. The fermentation medium was sterilised in an autoclave at 121 °C for 15 min. After cooling, 5 mL of *A. niger* spores (2.0×10^4 SFU/mL) and 5 mL of *S. cerevisiae* cells (2.5×10^5 CFU/mL) were inoculated into the fermentation medium and incubated (28 °C) for 24 h. After fermentation, an aliquot (10 mL) was withdrawn and centrifuged at 3000 rpm for 10 min. The fermentable sugar and ethanol analysis were done as described above.

2.9.3. Time course of sugar and bioethanol production

The fermentation medium (g/100 mL) consisted of 100 mL of 0.2 M citrate phosphate buffer (pH4), peptone 0.2, yeast extract 0.2, glucose 0.2 and MKS, 7. The medium was sterilised in an autoclave at 121 °C for 15 min. After cooling, 5 mL of *A. niger* spores (2.0×10^4 SFU/mL) and 5 mL of *S. cerevisiae* cells (2.5×10^5 CFU/mL) were inoculated into the medium. Fermentation was for 32 h, and samples (10 mL) were collected at eight hourly intervals and centrifuged at 3000 rpm for 10 min. The fermentable sugar and ethanol analyses were as described above.

Table 1. Effect of processing methods on mango seed kernel.

Method	Starch produced (g/100 g)	Color	Alum Test	Iodine Test
Boil, dry-mill.	56	light brown	blue-black	Blue-black
Boil, wet-mill.	Nil	-	-	-
Dehydrated, dry- mill.	58.1	Light Brown	Blue-black	Blue-black
Dehydrated, wet-mill.	34.6	Light brown	Blue-black	Blue-black
Wet-mill, with alum.	58.6	Blue-black	Blue-black	Blue-black
Wet-mill without alum.	57.0	dark brown	blue-black	blue-black
Dried seeds, dry-mill.	56.5	light brown	Blue-black	Blue-black
Dried seeds, wet-mill.	41.2	White	Blue-black	Blue-black

2.9.4. Influence of MKS concentration on sugar and ethanol production

The content of the fermentation medium was the same as described above. Varying concentrations (1–10 g) of mango kernel starch were added to the medium. After sterilisation and cooling, 2.0×10^4 SFU/mL of *Aspergillus niger* spores and 5 mL of *S. cerevisiae* cells (2.5×10^5 CFU/mL) were inoculated into the medium. Fermentation was for 24 h; later, 10 mL samples were withdrawn and centrifuged at 3000 rpm for 10 min.

2.9.5. Influence of inoculum concentration on sugar and ethanol production

The inoculum was prepared by culturing *A. niger* and *S. cerevisiae* (two loops each) in peptone water (10 mL). Different inoculum concentrations (1–5 mL) were transferred into the fermentation medium containing (g/100 mL) 0.2 M citrate phosphate buffer (pH 4) 100 mL, peptone 0.2, yeast extract 0.2, glucose 0.2 and MKS, 7. After 24 h of fermentation, the sample (10 mL) was centrifuged at 3000 rpm for 10 min and used for analysing sugar and ethanol.

2.10. Modified simultaneous saccharification and fermentation (MSS&F) of MKS

The fermentation medium consisted of (g): peptone 0.2, yeast extract 0.2, glucose 0.2, MKS 7, chloramphenicol, 0.2 suspended in 100 mL of 0.2M citrate phosphate buffer (pH 5) in 250 mL flat-bottom conical flask. The medium was sterilised in an autoclave at 121 °C for 15 min. After cooling, 5 mL of *A. niger* spores (2.0×10^4 SFU/mL) and 5 mL of *S. cerevisiae* cells (2.5×10^5 CFU/mL) were inoculated into the medium. Fermentation was for 32 h; however, after 8 h, 5 mL of *S. cerevisiae cells* (2.5×10^5 CFU/mL) was added again (modified SS&F). Samples (10 mL) were withdrawn every 8 h, centrifuged at 3000 rpm for 10 min, and the supernatant was analysed for ethanol using HPLC.

2.11. Separate hydrolysis and fermentation (SH&F) of MKS

The fermentation medium was prepared as described above and then sterilised. After cooling, the medium was inoculated with 5 mL of *A. niger* spores (2.0×10^4 SFU/mL) and incubated for 24 h at 28 °C. After 24 h, 5 mL of *S. cerevisiae* cells (2.5×10^5 CFU/mL) was introduced, followed by fermentation for 32 h. The sample (10 mL) was withdrawn every 8 h and centrifuged at 3000 rpm for 10 min, and the supernatant was analysed using HPLC.

2.12. Simultaneous saccharification and co-fermentation of mango kernel starch

The fermentation medium was prepared as described above, followed by sterilisation. After cooling, 5 mL of *A. niger* spores (2.0×10^4 SFU/mL), *S. cerevisiae* cells (2.5×10^5 CFU/mL) and 2 g of commercial *Saccharomyces cerevisiae* strain were inoculated into the medium and fermented for 32 h, 10 mL was withdrawn and centrifuged at 3000 rpm for 10 min. The supernatant was analysed using HPLC.

2.13. Statistical analysis

The results of the fermentable sugar produced from mango seed via hydrolysis and fermentation are means of duplicate experiments. All the data were subjected to analysis of variance (ANOVA) using the Statistical Package for the Social Sciences (SPSS) version 17.0.

3. Results and discussion

3.1. Pretreatment of mango seed kernel and starch extraction

Dried and wet-milled seeds produced 41.2 g starch per 100 g MK. It had the best starch colour, while dried seeds and the dry-mill method yielded 56.5 g starch per 100 g MK processed (Table 1). Parv and Kalpana (2018) reported that dried mango seed kernels yielded significantly higher starch than fresh mango seed kernels. The lower concentration of starch recovered from the wet-mill method may be due to the loss of starch granules while removing excess water from the sedimented starch granules. The dried and wet-mill method produced white starch, while the dried seeds and dry-mill method produced cream colour starch. The dry milling process encourages starch fragmentation and generates more damaged starch, while wet milling achieves starch of purer quality than dry milling due to repeated washing and filtration (El Halal et al., 2019; Kringerl et al., 2020). Blue-black coloured starch was obtained by adding alum (potassium aluminium dodecahydrate) to the wet-mill method. This observation suggests that alum may be used for determining the presence of starch since both alum and iodine tests gave a blue-black colour when reacted with starch.

3.2. Hydrolysis of MKS

Hydrolysis of mango seed kernel starch by sulfuric acid, sodium hydroxide, malted "acha", and *A. niger* amylase for 2 h (Figure 1) produced (g/100 mL MKS slurry) 3.97, 4.00, 3.55 and 4.26 fermentable sugar, respectively. According to Kapdan et al. (2011), starch hydrolysis generally occurs in two phases; the first phase is rapid, involving the hydrolysis of the amylose component of starch, while the second stage is a slow modification of the semi-crystalline and crystalline regions of starch molecules. Kapdan et al. (2011) reported that acid concentration was a significant factor during the hydrolysis of ground wheat starch. The fermentable sugar produced increased with increasing sulfuric acid concentration, reaching a peak at 0.6 ml of 0.1M sulfuric acid. This observation is in line with Kaushlesh et al. (2016), where an increase in the acid concentration, time and temperature employed during acid hydrolysis of MKS favoured the increase in glucose production. The result showed that the highest acid concentration did not give the optimum sugar yield, similar to earlier findings by Kapdan et al. (2011). Utilizing a relatively low acid concentration for starch modification is more favourable to reducing the quantity of alkali required for neutralization (Kapdan et al., 2011).

Hydrolysis of MKS by 1.0 mL of 0.1 M sulfuric acid for 2 h produced more sugar than 4 h. On the contrary, Ayodeji et al. (2013) obtained the highest glucose concentration using 1.0 M sulfuric acid after 4 h. The observation of low sugar concentration beyond 2 h of treatment suggests its conversion to inhibitors such as 5-hydroxymethylfurfural or furfural compounds (Timung et al., 2016). During a two-step acid hydrolysis of starch, treatments above 20 min reduced the reaction efficiency owing to product degradation Choi and Mathews (1996).

It is worthy to note that the hydrolysis with *A. niger* amylase derived the highest sugar concentration. Amylases are enzyme proteins and are, therefore, more advantageous to other treatments since the use of acid or alkali for hydrolysis generates toxic by-products. Also, following starch hydrolysis with acid or alkali treatment, product neutralization is required; this involves more chemicals and is not eco-friendly. Production of microbial enzymes is relatively easy, fast and achievable with cheap renewable resources. As *A. niger* amylase concentration increased

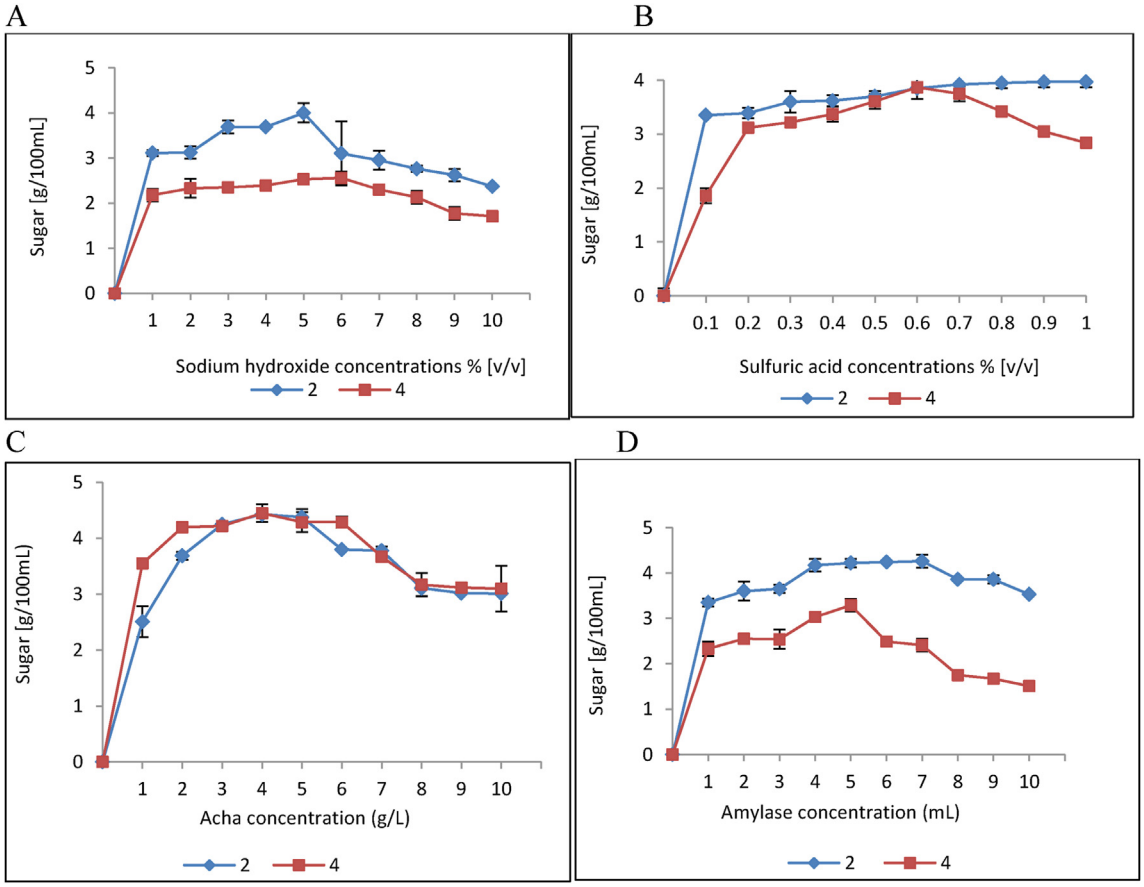

Figure 1. Hydrolysis of MKS by (a) 0.1 M H_2SO_4, (b) 0.1 M NaOH, (c) Acha, and (d) Amylase at 2 h and 4 h.

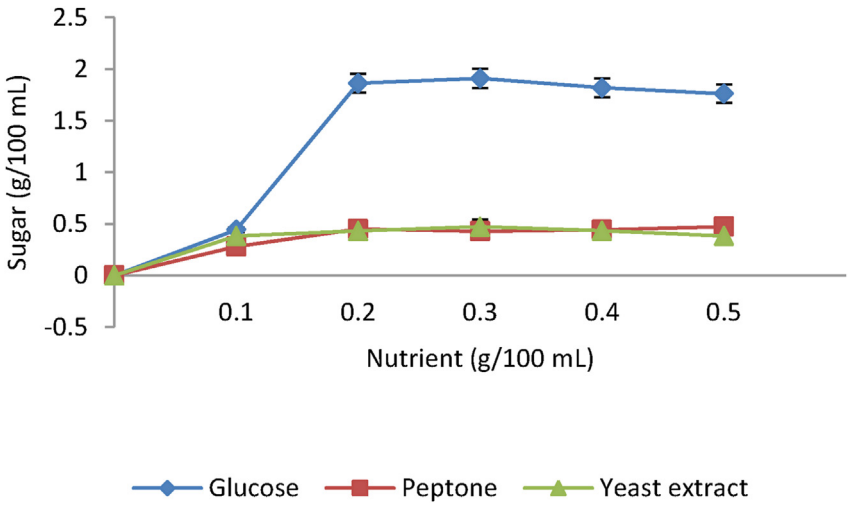

Figure 2. Influence of nutrient addition on fermentable sugar production from MKS.

from 1.0 – 9.0 mL, the sugar produced changed from 3.35 to 4.26 (g/100 mL). Abalaka and Adetunji (2017) reported that the utilization of starch by the crude enzyme of *A. niger* is dependent on the incubation time.

3.3. Production of fermentable sugar from MKS

Utilization of whole-cell rather than enzyme extract for substrate hydrolysis during fermentation is often more efficient and cost-effective and facilitates a one-step bio-process. Therefore, during fermentation using *Aspergillus* species as the source of enzyme, we observed the influence of some culture variables on sugar production. There was increased fermentable sugar production as glucose concentration increased (Figure 2) from 0.1 to 0.3 g/100 mL. Beyond 0.3g initial glucose concentration, there was a decrease in the sugar produced. Glucose addition at the beginning of fermentation provided the initial carbon source for yeast growth and subsequent metabolism. This study suggests that fermentation with a relatively high glucose concentration negatively influenced fermentable sugar production.

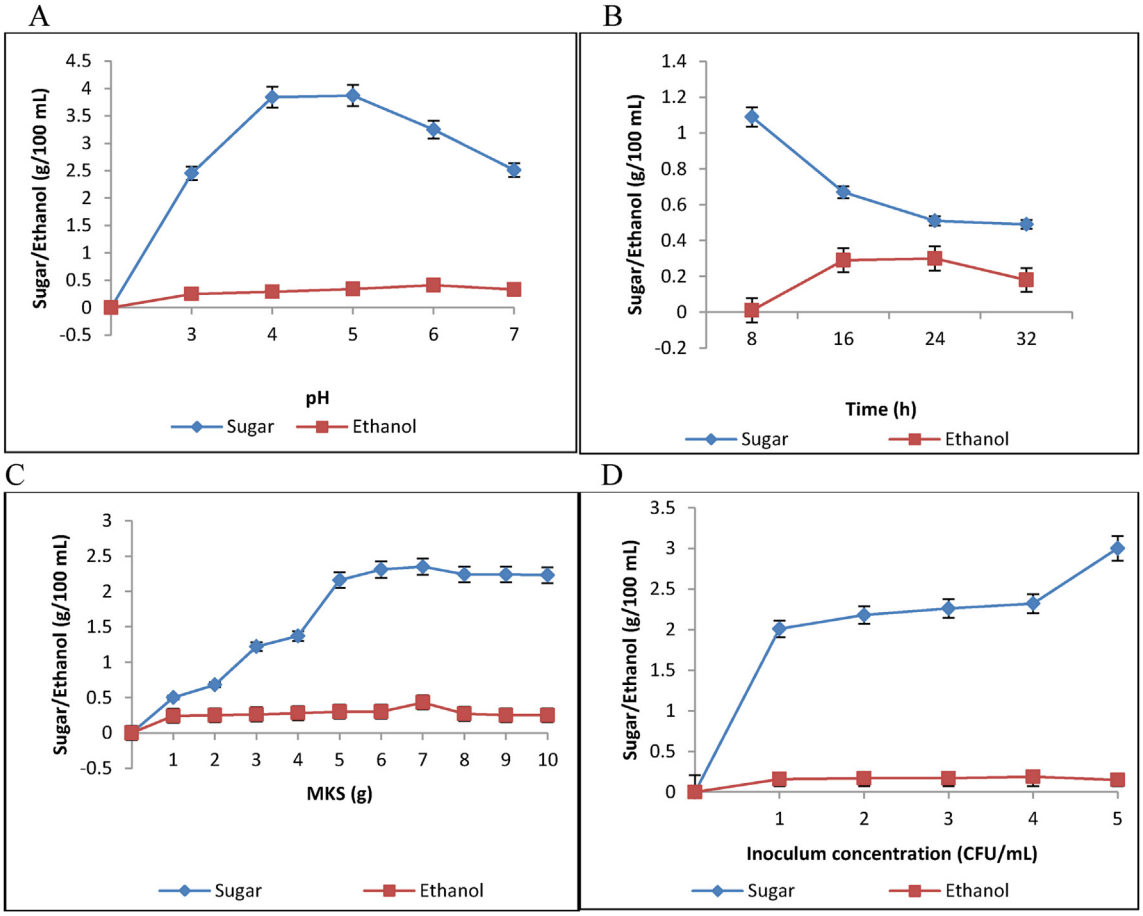

Figure 3. Influence of fermentation parameters on sugar and bioethanol production from MKS. (a) pH, (b) Time course, (c) MKS concentration, and (d) Inoculum concentration; MKS conc. was 7% w/v.

There was increased fermentable sugar production from 0.28 g to 0.51 g per g/100 mL of MKS slurry when peptone concentration rose from 0.1 g to 0.2 g (Figure 2) but decreased as peptone concentration increased from 0.3 g to 0.5 g. Also, the increase in yeast extract concentration from 0.1 to 0.3 (g) increased the fermentable sugar production from 0.38 g to 0.47 g (Figure 2).

The percentage of sugar generated is directly proportional to the amylase secreted by the *A. niger*. Studying *Fusarium oxysporum* and *Aspergillus nidulans*, da Silva et al. (2001) concluded that the nitrogen source and structure strongly influence the fungal synthesis of hydrolytic enzymes. Nitrogen sources facilitate cell growth and product development; however, excessive concentration inhibits both processes (Clarke, 2013).

3.4. Influence of pH on sugar and bioethanol production

The effect of pH on MKS saccharification and fermentation is in Figure 3a. The fermentable sugar production during SS&F increased with increasing pH, from 3.0 to 4.0. Above this value, there was a decrease in the fermentable sugar concentration showing that pH influenced the saccharification of MKS by *A. niger* amylase. Betiku and Ajala (2010) reported a progressive increase in ethanol concentration as pH increased from 3.0 to 6.0. The optimum pH for ethanol production was pH 6.0, according to earlier reports by Obueh et al. (2015); in this study, the optimum pH observed was 4.0–5.0.

3.5. Time course study of MKS hydrolysis and ethanol production

The maximum sugar production occurred after 8 h of fermentation, followed by a reduction in sugar concentration until 32 h. The decrease in sugar concentration corresponded with a gradual increase in ethanol concentration from 8 h to 24 h (Figure 3b). The highest ethanol concentration of 3.9 % (v/v) was observed after 16 h fermentation. Modupe et al. (2018) reported maximum ethanol production after 48 h of fermentation.

3.6. Effect of starch concentration on sugar and ethanol production

The fermentable sugar production increased with increasing MKS concentration from 1 g to 7 g (Figure 3c). As earlier stated, the quantity of sugar produced from MKS is a factor of the amount and properties of amylase synthesized by the *A. niger*. The increased sugar production at relatively high MKS implies the copious secretion of amylase in the fermentation medium. When MKS is low, and amylase concentration is high, only a few enzyme molecules will participate in amylolysis. Increased MKS will facilitate more amylase-MKS complexes and, therefore, higher product formation. This phenomenon likely explains the consistent increase in sugar generation as starch concentration increased. This trend is expected to continue until the saturation of enzyme molecules with MKS or catabolite repression occurs due to product accumulation in the fermentation medium. Using 7 g MKS for fermentation yielded the highest ethanol concentration, beyond which the sugar and ethanol concentrations decreased. Working on wild cocoyam as a fermentation substrate, Amadi et al. (2020) observed that 5% gave maximum sugar production.

Table 2. Simultaneous Saccharification and fermentation of MKS using *A. niger* and *S. cerevisiae*.

Fermentation time (h)	Temperature (° C)	pH	Starch (%)	Glucose % (v/v)	Ethanol % (v/v)
0	28	4.5	7.00	0.000	0.00
8	28	4.5	3.83	0.013	0.06
16	30	4.1	2.67	0.014	0.67
24	29	3.6	2.45	0.028	1.03
32	29	3.1	2.24	0.013	1.13

3.7. Effects of inoculum concentration on sugar and bioethanol production

The influence of inoculum concentration on sugar and ethanol production is in Figure 3d. There was an increase in fermentable sugar concentration from 2.01 to 3.0 (g/7 g MKS), while the ethanol concentration increased from (v/v) 0.15%–0.20% per 7 g MKS with increasing inoculum volume.

3.8. Bioconversion of mango kernel starch into bioethanol

During bioconversion of MKS into ethanol by simultaneous saccharification and fermentation, the maximum fermentable sugar 0.028% v/v was observed at 24 h (Table 2), while the maximum ethanol concentration of 1.13% v/v was obtained at 32 h. A gradual reduction in the MKS concentration from 2.8% v/v at 8 h to 2.24% v/v at 32 h was observed.

Incomplete conversion of MKS to fermentable sugar and incomplete conversion of the sugar to bioethanol were observed, hence, the low ethanol concentration. Modification of SS&F (Table 3) revealed an increase in the ethanol concentration (3.98%/7 g MKS) at 16 h. The modified SS&F suggests that the re-addition of *S. cerevisiae* to the fermentation broth at 8 h of fermentation enhanced further conversion of the fermentable sugar to ethanol. The re-addition of *S. cerevisiae* at 8 h fermentation led to a 3-fold increase in the ethanol produced after 16 h. The decrease in ethanol concentration at 32 h may be due to the conversion of the ethanol earlier produced into ethanoic acid and other organic acids. Therefore, using MKS as the carbon source for bioethanol production, SS&F can be modified favourably by adding *Saccharomyces cerevisiae* in two stages.

Separate hydrolysis and fermentation of MKS (Table 4) resulted in 0.57% ethanol per 7g MKS after 24h. The low ethanol concentration obtained may be due to the disadvantages associated with SH&F, such as enzyme inhibition by the sugar produced during starch hydrolysis (Devarapalli and Atiyeh, 2015). Therefore, the production of high concentrations of bioethanol from MKS by SH&F may not be possible.

The data obtained from simultaneous saccharification and co-fermentation of MKS by commercial yeast, *S. cerevisiae* and *A. niger* (Table 5) showed that 0.267% v/v ethanol per 7 g MKS was produced. The lower concentration of ethanol obtained shows that the commercial yeast (bakers' yeast) used in this study is not a good fermenter. From the data, it is evident that producing high concentrations of bioethanol from

Table 3. Modified simultaneous saccharification and fermentation of MKS using *A. niger* and *S. cerevisiae*.

Fermentation time (h)	Temperature (° C)	pH	Ethanol % (v/v)
0	28	4.7	0.000
8	28	4.7	0.309
16	28.2	4.1	3.986
24	29.1	3.9	3.870
32	29.0	3.7	0.411

NB: SS&F was modified by re-addition of *S. cerevisiae* cells after 8h fermentation.

Table 4. Modified Separate hydrolysis of mango kernel starch using *A. niger* amylase and fermentation using *S. cerevisiae*.

Fermentation time (h)	Temperature (° C)	pH	Ethanol % (v/v)
0	28	4.9	0.000
8	28	4.9	0.4681
16	28	4.2	0.4960
24	28.1	3.9	0.5708
32	28.2	3.9	0.5309

NB: SH&F was modified by re-addition of *S. cerevisiae* cells at the 8 h during fermentation.

Table 5. Simultaneous saccharification and co-fermentation of mango kernel starch.

Fermentation time (h)	Temperature° C	pH	Glucose % (v/v)	Fructose % (v/v)	Maltose % (v/v)	Ethanol % (v/v)
0	30.0	5.0	0.001	0.000	0.000	0.000
8	30.0	5.0	0.008	0.097	0.004	0.267
16	30.0	5.0	0.007	0.079	0.003	0.224
24	30.2	4.8	0.002	0.084	0.004	0.224
32	30.2	4.6	0.002	0.091	0.004	0.264

MKS by SS&CF is also not feasible. These results indicate that SS&F, which gave the highest ethanol concentration of 3.98% v/v, is the best method for MKS fermentation to ethanol.

4. Conclusion

Efficient starch extraction from mango seed kernel and its susceptibility to hydrolysis for sugar and subsequent fermentation to bioethanol by *S. cerevisiae* has demonstrated that mango seed is a potential raw material for bioethanol production. In many tropical countries, mango trees and fruits abound; if not channelled into value-added products, they will constitute an environmental nuisance. The mango seed kernels are cheap waste sources, and their application as the substrate for bioethanol production should reduce the cost of production, a deterrent to adopting this technology. SS&F optimization and a slight modification of the conventional method gave the highest ethanol concentration from MKS. Emphasis should, therefore, be on modifying the existing fermentation processes for maximum bioethanol production.

Declarations

Author contribution statement

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

J. C. Ogbonna: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.

T. N. Nwagu: Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper.

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

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The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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