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ARTICLE

Optimization of saccharification prospective from starch of sweet potato roots through acid-enzyme hydrolysis: structural, chemical and elemental profiling

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ABSTRACT The sweet potato root, a potent source of starch which is being considered as an efficient alternative for fuel ethanol production in recent times. The starchy substrate needs to be subsequently dextrinized and saccharified so as to enhance the utilization of its carbohydrates for ethanol production. In the present investigation, acid-enzyme process was conducted for the dextrinization and saccharification of sweet potato root flour (SPRF). The best optimized condition for dextrinization was achieved with an incubation period of 60 min, temperature 100 ºC and 1M HCl. However, for saccharification, the best result was obtained with an incubation of 18 h, pH 4, temperature 65 ºC and 1000 U concentration of Palkodex®. After the dextrinization process, maximum concentrations of total sugar and hydroxymethylfurfural (HMF) [380.44 ± 3.17 g/kg and 13.28 ± 0.25 mg/g, respectively] were released. Nevertheless, after saccharification, 658.80 ± 7.83 g/kg of total sugar was obtained which was about 73% more than that of dextrinization. After successful dextrinization and saccharification, the structural, chemical and elemental analysis were investigated using techniques such as scanning electron microscopy (SEM), Fourier-transforms infrared spectroscopy (FTIR) and energy-dispersive X-ray fluorescence spectrophotometer (EDXRF), respectively. Effective hydrolysis was demonstrated in thin layer chromatography (TLC) where the HCl was able to generate monomeric sugar such as glucose and maltose. On the other hand, only glucose is synthesized on the mutual effect of HCl and Palkodex®. The SEM findings indicate that the rough structure of both dextrinized and saccharified sample was gained due to the vigorous effect of both acid and enzyme subsequently. The saccharified SPRF when subjected to fermentation with *Saccharomyces cerevisiae* and *Zymomonas mobilis* separately, it was observed that *Z. mobilis* produced more stretching vibration of –OH than *S. cerevisiae*, which evidenced the better production of bioethanol. Additionally, evaluation of the influence of *S. cerevisiae* and *Z. mobilis* through elemental analysis revealed upsurge in the concentrations of S, Cl, Ca, Mn, Fe and Zn and decline in the concentrations of P, K and Cu in the fermented residue of *S. cerevisiae* and *Z. mobilis*, however, *Z. mobilis* showed little more variation than that of *S. cerevisiae.*

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Introduction

Petroleum is the key source of energy for the consumption of automobiles. Above and beyond the adverse global warming effect of fossil fuels, unpredictable oil price and political instability in the oil trading nations ensured a substantial rise in world-wide attention for alternate fuels. Both developed and industrialized countries consider biofuels such as ethanol and hydrogen as appropriate sources for the reason of energy security. Ethanol is one of the finest fluid alternatives of fossil fuel and has emerged as a promising energy substitute (Busic et al. 2018). Meanwhile, it is also reported that the plant biomass containing starch and sugar can be easily fermented to produce alcohol; however, the rate

of production depends upon feedstock availability (Limatainen et al. 2004; Abidin et al. 2014). Bioethanol is manufactured by fermenting sugar with microorganisms, in contrast to synthetically formed ethanol from petrochemical bases. It is produced by distilling the fermented sugars, which can be used as fuel in inner combustion engines, either neat or blended with petrol (Walker 2010). A study on bioethanol production in 2018 has revealed that the worldwide leaders in bioethanol production are USA with a production of about 16.06 billion gallons (primarily from maize and wheat) and Brazil, 7.92 billion gallons (from sugarcane), which accounts for approximately 84% of the total global ethanol (CSS 2019). Asia held the fourth position in the biosphere for ethanol production while China is the largest producer of ethanol in Asia, with an overall alcohol making of nearly 9.770 million liters in 2018 (Patni et al. 2011; USDA FAS 2018). Amongst all the Asian countries, India is having an optimistic viewpoint towards renewable energy technologies and is dedicated for the usage of renewable resources. In Asia, India is ranked as the second largest manufacturer of ethanol, with a yearly production of 2400 million liters in the year 2018 (USDA FAS 2019). In recent times, production of biofuel from tuber crops has been considered for experimental studies due to their high starch content. In India, as compared to yam and other tuber crops, cassava and sweet potato are being considered as the main tuber crops which are cultivated throughout all agro-climatic regions. However, no such exploitation is made for scale-up production of bioethanol by effective implication of sweet potatoes while they are rich in starch particles, the best source of carbohydrates for fermentable microorganisms (Lareo et al. 2013; Thatoi et al. 2014).

Currently, the degradation of carbohydrates from natural starch is a big challenge for the investigators as this process needs some tricky concepts for the development of simplified form of sugar that can be easily utilised by the microorganism. However, the high cost of the primary investment, involvement of enzymes, requirement of trained man-power and sophisticated laboratories are the limiting factors for the practice of process development (Surmely et al. 2004; Satapathy et al. 2020). The process of acid hydrolysis has several important advantages such as fast reaction rate, simple pre-treatment for starch feedstock, a cheap and easily available acid catalyst and a relatively low reaction temperature with high acid concentration. Inhibitor such as 5-HMF (5-hydroxymethylfurfural) is formed during the reaction of glucose dehydration but the optimization process in terms of temperature, concentration of acid, level of starch and duration of hydrolysis time may overcome this (Bharti and Chauhan 2016). The production of ethanol not only depends on the liquefaction process but also reliably manages as per the nature of the saccharified starch products. For this, a convenient way of enzyme implementation is to simplify the carbohydrates. However, the process of optimization of various starch molecules is a critical factor. The enzyme glucoamylase which is being used on industrial scale is very sensitive and effective for saccharification due to its efficiency of changing the functional properties, viscosity and gelatinization of starch (Cripwell et al. 2019; Strąk-Graczyk and Balcerek 2020). Afterward, ethanol is produced by fermentation of hydrolyzed sugar with the help of desired microorganisms like *Zymomonas mobilis, Saccharomyces cerevisiae, Saccharomyces uvarum, Candida tropicalis, Candida shehatae, Pichia stipites* and *Clostridium* species, etc. However, the yeast, *S. cerevisiae* as well as facultative bacterium, *Z. mobilis* are very promising contenders for the production of alcohol (Delgenes et al. 1996; Yang et al. 2016).

Sweet potato (*Ipomoea batatas* L.) which belongs to the family *Convolvulaceae* is ranked fifth as an essential food crop in developing countries on a fresh weight basis after wheat, rice, cassava and corn (Srinivas 2009). As a starch rich (18-30%) crop and due to its cheaper value and high biomass, it is widely accessed as a substitute of feedstock for grain and also acts as sugar substrate for fuel ethanol production (Qiu et al. 2010). The roots of this herbaceous perennial type of plant species are available throughout all the seasons and having some distinguished characteristics like high multiplication rate, low degeneration rate of the propagule, short life cycle, low illness and plague, and protect from erosive rains and drought stress (Lareo et al. 2013). Emphasizing on the food value of the sweet potato, the Center for Science in the Public Interest, USA ranked it as 'healthiest of all vegetables' due to presence of high value of dietary fiber, protein, vitamins A and C, complex carbohydrates, low levels of saturated fat, and essential elements like potassium, iron, calcium and sodium (Shao et al. 2017). Therefore, the present study is designed to investigate the impact of the hydrolysis process (at structural, chemical and elemental levels) through implementation of acid dextrinization and enzymatic saccharification on sweet potato root starch. This study may act as a baseline report for selecting and comparing the cost-effective strategy for the recovery of reducing and soluble sugar from starches.

Materials and Methods

Substrate and microorganisms

The sweet potato root flour (SPRF; used as substrate) and implemented microorganisms like *S. cerevisiae* MTCC 170 and *Z. mobilis* MTCC 92 used in the present study were obtained and processed as per our previously published paper (Jagatee et al. 2020).

Dextrinization and saccharification of sweet potato

SPRF diluted with distilled water (1:20) was separately treated with sulphuric acid, hydrochloric acid and perchloric acid and further screened for its dextrinization effects. For which, the freshly prepared slurry (5 g of SPRF in 100 ml of distilled water) was hydrolyzed with 1 ml of each acid having 1 M concentration at 100 °C for 60 min. The obtained hydrolysate was cooled and neutralized as per the methods of Koti et al. (2016). The total sugar content was measured using the anthrone reagent method (Hodge and Hofreiter 1962) and the reducing sugar was estimated by using alkaline copper and arsenomolybdate (Somogyi 1952). According to efficiency, further optimization of total sugar and HMF content (following the method of White 1979) was carried out by taking hydrochloric acid. To improve the dextrinization process, various parameters like temperature (80, 85, 90, 95, 100, 105, and 110 °C), incubation time (15, 30, 45, 60, 75, and 90 min), concentration (0.5, 1.0, 1.5, and 2.0 M of HCl) and volume (0.25, 0.5, 1.0, 1.5, and 2.0 ml of HCl) were tried and analyzed. After optimizing the dextrinization, saccharification process was carried out by taking Palkodex® (M/s Maps Enzymes, Ahmadabad, India) a glucoamylase enzyme that competently acts on starch and releases monomeric sugar compounds. To optimize the saccharification, a set of different temperatures (50, 55, 60, 65, and 70 °C), pH (3.5, 4.0, 4.5, 5.0, and 5.5), incubation time (1, 2, 4, 6, 8, 12, 18, and 24 h), enzyme volume (25, 50, 100, 150, 200, 250, and 300 µl) and enzyme concentration (125, 250, 500, 750, 1000, 1250, and 1500 U) respectively were tried with dextrinized SPRF. The dextrinized and saccharified samples were then collected and stored at -20 °C until further investigation.

Sugar analysis using TLC

To evaluate various fermentable sugar from dextrinized and saccharified sweet potato samples, TLC was performed by preparing 0.25 mm thick silica gel plates (20 × 20 cm in size). The dextrinized and saccharified sweet potato slurry was melded with water (1:5) in order to avoid the bulkiness of viscous mash. Separation of sugar was done with the help of butanol, ethanol and water with a ratio of 5:3:2 (v/v), respectively. The spots were developed by the application of 0.2% orcinol mixed with methanol: sulphuric acid (9:1; v/v) followed by incubation at 60 °C for about 2 h (Aquino et al. 2003). The glucose and maltose (1% each) were applied to develop the spot and were considered as standards.

Morphological analysis under SEM

The alteration in morphological appearance of SPRF (before and after dextrinization as well as saccharification) was observed under SEM (FEI Quanta 250, USA). The specimens were fixed on an aluminum stub with carbon tape on each side and the surface morphology was analyzed with a setup of accelerating voltage 10-15 kV (Jagatee et al. 2020).

Preparation of fermentation medium

For the preparation of 1 l of the medium, 50 g of saccharified SPRF was initially diluted with 500 ml of distilled water, supplemented with 2% $(NH_4)_2SO_4$ and 10% inoculums of *S. cerevisiae* was allowed to incubate for 48-120 h at 30 °C with pH 5.0. Similarly, for *Z. mobilis*, the fermentation was processed by taking the same 2% $(NH_4)_2 SO_4$ and 10% inoculum but incubated at 35 °C for 48-120 h at pH 5.5. To carry out the whole fermentation process, individual set up for *S. cerevisiae* and *Z. mobilis* were established under separate stationary conditions.

FTIR spectroscopy analysis

The fermented sample of saccharified SPRF was diluted with hexane and scanned in FTIR spectrophotometer (Nicolet iS5 spectrometer, Thermo-Fisher, Chicago, USA). With a resolution of 1.0 cm^{-1}, the spectra were processed through a scanning range of 1500-1800 cm^{-1} and 3000-3700 cm^{-1} and the compounds were quantified by using OMNIC software (Thermo Fisher, Chicago, USA). Hexane was used as a control for generating the data.

Elemental analysis using EDXRF spectrometer

Elemental profiling of both the saccharified and saccharified fermented SPRF was estimated using EDXRF spectrometer (Jordan Valley Ex-3600 EDXRF). The oven dried samples (150 mg/each pellet) were pressed to make the pellets with the help of table-top pelletizer (pressure: 100-110 kg/cm^2 for 5 min). The EDXRF instrument consisting of Rh anode X-ray tube (voltage 50 kV, current 1 mA) radiates signature X-rays for each element (Na-U) upon bombardment of X-rays on the samples. The elemental concentration is measured as the intensity of characteristic X-rays. The measurements were carried out in a vacuum condition and different filters were used (between the source and sample) for optimum identification of various elements. For detecting the P, S, Cl, K and Ca, no filter was used but a voltage of 6 kV and a current of 200 µA were applied, whereas for Mn, Fe, Cu and Zn, a 0.05 mm thick Ti filter was used with an applied voltage

Table 1. Screening of acids for dextrinization of sweet potato root flour (SPRF).

Acid	Total sugar (g/kg)	Reducing sugar (g/kg)
Control (as per normal procedure)	326.14 ± 3.87	190.91 ± 2.41
Sulphuric acid	298.42 ± 3.77	183.26 ± 1.78
Hydrochloric acid	340.63 ± 3.87	197.80 ± 3.22
Perchloric acid	201.28 ± 2.43	144.64 ± 2.04

of 14 kV and a current of 900 μA. The resulted X-ray fluorescence were detected by Si (Li) semiconductor detector (resolution 150 eV at 5.9 keV) and the quantification of the spectra was done by the in-built EX-WIN software (Rout et al. 2017).

Results

Screening of various acids for dextrinization of SPRF

The SPRF was initially treated with three significant acids such as sulphuric acid, hydrochloric acid and perchloric acid to release simple form of sugar, and the results are represented in Table 1. Among all treated acids, it was observed that the hydrochloric acid performed better dextrinization process as the concentration of total sugar (340.63 ± 3.87 g/kg) and reducing sugar (197.80 ± 3.22 g/kg) was yielded which is significantly improved than that of the control (326.14 ± 3.87 and 190.91 ± 2.41 g/kg of total and reducing sugar, respectively).

Standardization of the optimum conditions for acid dextrinization and enzyme saccharification

To obtain the non-reducing ends of starch molecule, hydrochloric acid and Palkodex® (glucoamylase) were used in the present experiment for evaluating the hydrolysing properties of SPRF. After initial screening, HCl was applied for better optimization of dextrinization process by interchanging different parameters like temperature, incubation time, concentration and volume of HCl. The results of HMF and total sugar content indicate the improved liquefying characteristic of SPRF, which facilitate the hydrolysis of complex carbohydrates. The environmental condition comprising of incubation time of 60 min, temperature of 100 °C, 1 M concentration of 1 ml HCl was found to be more suitable for better yielding of total sugar (380.44 ± 3.17 g/kg), which is comparatively better (16%) than that of the control one (326.14 ± 3.87 g/kg). Although, a significant variation in concentration of HMF (ranges from 2.74 ± 0.56 to 15.39 ± 0.23 mg/kg) was detected but in an optimum condition of dextrinization, 13.28 ± 0.25 mg/kg of HMF was achieved (Table 2). In case of saccharification, the efficiency of starch degrading glucoamylase enzyme (Palkodex®) was assessed by considering a different set of parameters like pH, temperature, incubation period, enzyme concentration and volume for releasing of monomeric sugar compounds. The best ideal condition for saccharification was noticed at pH 4.0, temperature 60 °C, incubation period of 18 h and 1000 U (200 μl) of enzyme. Under this condition more amount of total sugar (658.80 ± 7.83 g/kg) was released as compared to the optimized condition of dextrinized process (380.44

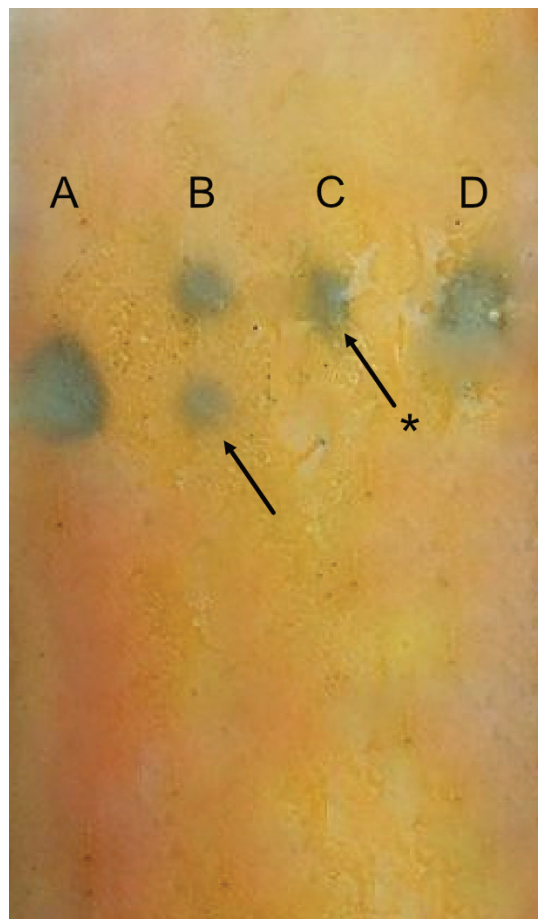

Figure 1. TLC chromatogram of untreated and treated SPRF. **A**: standard maltose; **B**: dextrinized SPRF; **C**: saccharified SPRF; **D**: standard glucose. The normal arrow and arrow with asterisk mark indicate maltose and glucose, respectively.

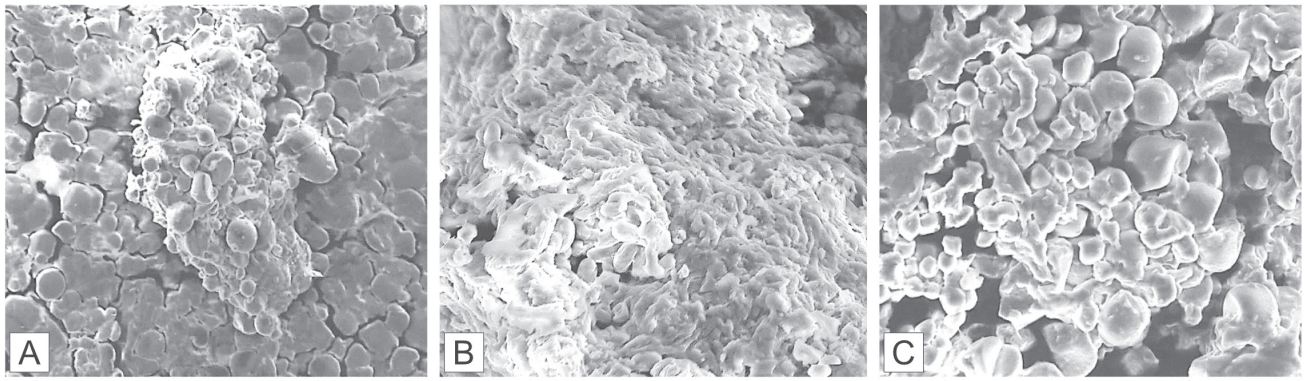

Figure 2. Scanning electron micrograph of untreated and treated SPRF. **A**: untreated SPRF; **B**: dextrinized SPRF; **C**: saccharified SPRF.

± 3.17 g/kg), and the increase is 73% (Tables 2,3).

TLC results of dextrinized and saccharified SPRF

For transformation of complex sugar into a simple form, HCl and glucoamylase were applied to carry out the dextrinization and saccharification process. TLC analysis revealed two fermentable sugars from dextrinized and saccharified sweet potato with maltose and glucose as standards (Fig. 1). The presence of both maltose (as compared with standard maltose) and glucose (as compared with standard glucose) was confirmed from the developed spot of acid hydrolyzed SPRF whereas, only glucose was detected from samples of saccharified SPRF. These results advocate that maltose and/or glucose are the main fermentable sugar components of sweet potato.

SEM analysis of dextrinized and saccharified SPRF

Certain acids and/or enzymes act upon the complex starch through which the complex structure breaks down into simple forms and can be noticeable under microscopic observation. A remarkable structural difference was witnessed among raw, dextrinized and saccharified SPRF samples when studied under SEM. An even granule surface of SPRF starch appeared in the untreated samples whereas both the dextrinized and saccharified SPRF samples exhibited an uneven surface of starch granules, indicating the breakdown of starch due to the vigorous action of HCl and glucoamylase. However, in comparison to the dextrinized sample, the saccharified sample has produced significant structural changes which indicate active involvement of both HCl and glucoamylase (Fig. 2).

FTIR spectra results of saccharified fermented SPRF

Two ethanologenic organisms, *S. cerevisiae* and *Z. mobilis* were subjected to ethanol production with the application of saccharified products. By maintaining the optimized fermentation condition i.e. incubation for 96 h at 30 °C and pH 5.0 (for *S. cerevisiae*) and incubation for 96 h at 35 °C and pH 5.5 (for *Z. mobilis*), the maximum production of ethanol [data not given] was separately standardised for both the microorganisms. To characterise the variation in functional group among the fermented samples, fermented SPRF samples were evaluated through FTIR spectroscopy. Significant variation of spectra was observed with respect to change of the functional components which also differed with the involvement of microorganisms tested (Fig. 3). The saccharified SPRF fermented with *S. cerevisiae* exhibited numerous significant peaks with respect to their specific functional groups such as 1512.26 cm^{-1} (N-O), 1676.12 cm^{-1} (C-N), 1716.11 cm^{-1} and 1772.28 cm^{-1} (C-O) due to variant compound development. Further, a varied range of band peaks between 3234.10 cm^{-1} to 3614.72 cm^{-1} were noticed due to bending of the O-H group (Fig. 3a,b). Similarly, due to N-O, C-C and C-O stretching, the saccharified SPRF fermented with *Z. mobilis* present a diverse range of peaks at 1530.17 cm^{-1}, 1641.78 cm^{-1}, 1646.69 cm^{-1} and 1783.57cm^{-1}. The absorption bands between 3263.37 cm^{-1} to 3608.95 cm^{-1} when taken into consideration for *Z. mobilis* fermented samples, contributed several peaks which is attributed to the stretching vibrations of -OH and water molecules (Fig. 3c,d). Such observations indicate the smooth conversion of ethanol by interchanging the functional complexity of starch of SPRF.

EDXRF results of saccharified fermented residues of SPRF

Elemental constituents of saccharified (control) and saccharified fermented SPRF residues were assessed to find out the differential impact of *S. cerevisiae* and *Z. mobilis* on substrate simplification during fermentation. Essential elements like potassium (K), sulphur (S), chlorine (Cl), calcium (Ca), manganese (Mn), iron (Fe), zinc (Zn), copper

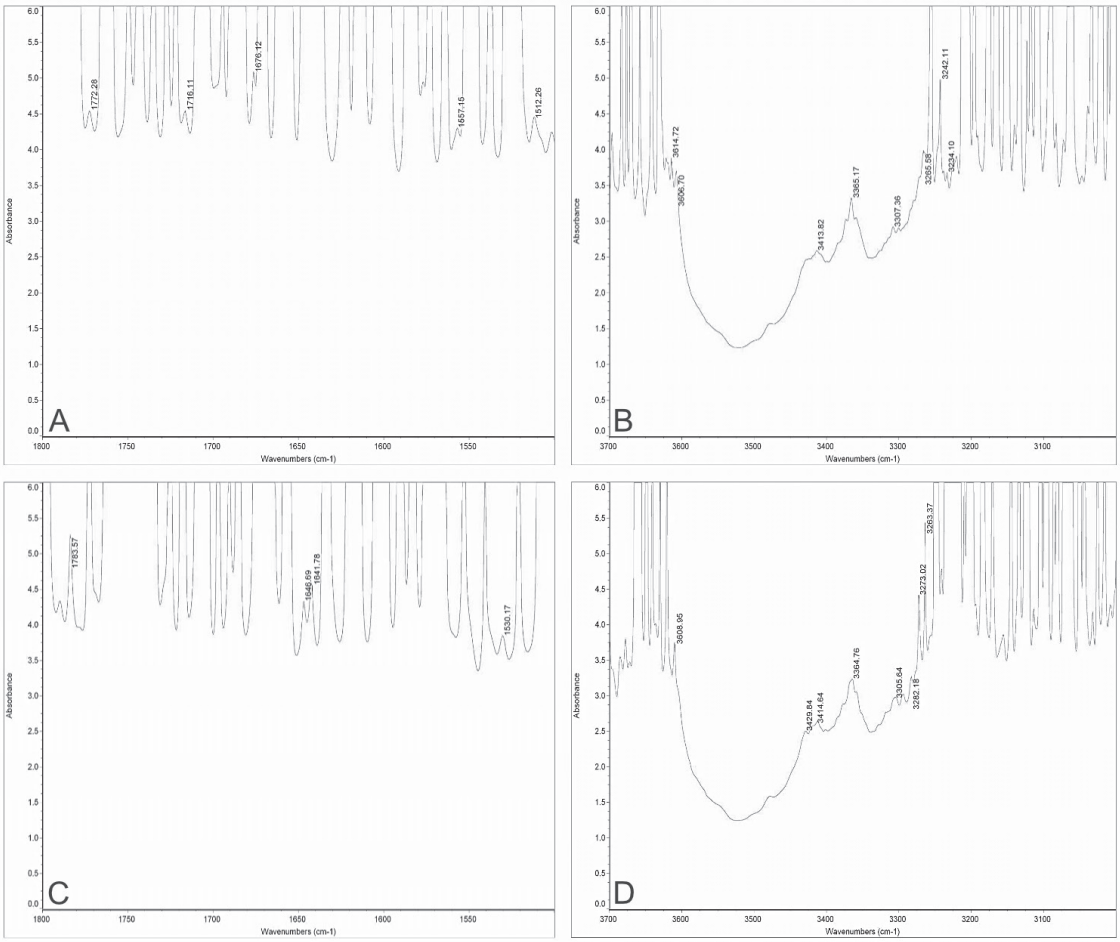

Figure 3. FTIR analysis of saccharified SPRF fermented with *S. cerevisiae* (A, B) and *Z. mobilis* (C, D). **A**: between 1500-1800 cm-1 spectral range of *S. cerevisiae*; **B**: between 3000-3700 cm-1 spectral range of *S. cerevisiae*. **C**: between 1500-1800 cm-1 spectral range of *Z. mobilis*; **D**: between 3000-3700 cm-1 spectral range of *Z. mobilis*.

(Cu) and phosphorus (P) were measured by using EDXRF spectrometer and the obtained results are presented in Table 4. It was observed that the acid-enzyme saccharified SPRF contains both macro and micronutrients such as P, S, Cl, K, Ca, Mn, Fe, Zn and Cu (2.12 ± 0.04, 0.75 ± 0.01, 3.37 ± 0.12,14.21 ± 0.36, 2.21 ± 0.04, 0.02 ± 0.003, 0.33 ± 0.001, 0.01 ± 0.002 and 0.03 ± 0.001 mg/g, respectively). However, a significant alteration of elemental constitution is noticed from both the fermented residues of *S. cerevisiae* and *Z. mobilis*. When the saccharified SPRF was utilized by *S. cerevisiae*, concentration of elements like P, K and Cu decreased to 1.80 ± 0.03, 13.14 ± 0.08 and 0.01 ± 0.003 mg/g whereas the concentration of S, Cl, Ca, Mn, Fe and Zn increased to 2.14 ± 0.28, 4.70 ± 0.07, 3.02 ± 0.02, 0.04 ± 0.005, 0.40 ± 0.01 and 0.16 ± 0.01 mg/g, respectively, after fermentation. Similar trends were also observed with respect to *Z. mobilis,* i.e. the concentration of P, K and Cu was slightly declined to 1.77 ± 0.02, 12.48 ± 0.10 and 0.01 ± 0.003 mg/g as compared to control. The increasing trend of S, Cl, Ca, Mn, Fe and Zn to 1.84 ± 0.03, 4.32 ± 0.10, 3.12 ± 0.03, 0.05 ± 0.001, 0.58 ± 0.004 and 0.17 ± 0.001 mg/g, respectively, as compared to control was also detected. However, the rate of decrease in concentration for all most all the elements was preferably higher in the fermented residues of *Z. mobilis* with respect to *S. cerevisiae* (Table 4).

Discussion

Acid-enzyme hydrolysis optimization

The SPRF slurry when exploited with acids for the liquefaction, yielded release of reducing sugar due to the cleavage of α and β glycosidic bonds. A comparative analysis of the liquefaction with different acids indicated greater release of reducing sugar with HCl than other acids (Putri and Rizaldi 2010). Similarly, Putri et al. (2012)

Table 2. Effect of temperature, incubation period, concentration and volume of HCl on acid hydrolysis (dextrinization) of sweet potato root flour (SPRF).

Concentration of HCl (M)	Volume (ml)	Temperature (°C)	Incubation period (min)	HMF (mg/kg)	Total sugar (g/kg)
Effect of temperature					
1.0	1.0	80	30	4.25 ± 0.17	295.21 ± 2.48
1.0	1.0	85	30	5.44 ± 0.19	310.34 ± 3.59
1.0	1.0	90	30	6.78 ± 0.20	328.45 ± 2.61
1.0	1.0	95	30	7.27 ± 0.26	340.26 ± 3.74
1.0	1.0	100	30	9.16 ± 0.29	354.15 ± 2.78
1.0	1.0	105	30	7.94 ± 0.28	348.73 ± 2.98
1.0	1.0	110	30	7.96 ± 0.28	347.64 ± 3.76
Effect of incubation time					
1.0	1.0	100	15	6.44 ± 0.12	334.23 ± 2.63
1.0	1.0	100	30	9.34 ± 0.14	354.14 ± 2.78
1.0	1.0	100	45	11.88 ± 0.17	371.42 ± 3.91
1.0	1.0	100	60	13.17 ± 0.27	380.14 ± 2.92
1.0	1.0	100	75	15.39 ± 0.23	380.31 ± 2.96
1.0	1.0	100	90	15.35 ± 0.29	380.40 ± 3.07
Effect of concentration					
0.5	1.0	100	60	7.92 ± 0.24	349.10 ± 3.18
1.0	1.0	100	60	13.28 ± 0.43	380.25 ± 2.94
1.5	1.0	100	60	13.28 ± 0.45	373.18 ± 2.91
2.0	1.0	100	60	13.28 ± 0.33	370.32 ± 3.09
Effect of volume					
1.0	0.25	100	60	2.74 ± 0.56	198.98 ± 2.19
1.0	0.5	100	60	3.53 ± 0.18	253.47 ± 2.34
1.0	1.0	100	60	13.28 ± 0.25	380.44 ± 3.17
1.0	1.5	100	60	13.29 ± 0.42	378.95 ± 2.97
1.0	2.0	100	60	13.39 ± 0.28	380.13 ± 3.10

also detected the reducing sugar from the sweet potato by implementing HCl, however a combination of HCl and *Aspergillus niger* has improved the properties of sugar synthesis. Additionally, Bharti and Chauhan (2016) and Azhar et al. (2017) stated that starch when hydrolyzed with a combination of acids resulted the release of compounds like HMF and 2-furaldehyde indicating a better dextrinization process. These furan derivatives pose a hindrance to ethanol production by inhibiting cell growth, so the production of HMF needs to be examined very carefully during the optimization process. In the present investigation, a significant upturn in the level of total soluble sugar along with HMF was obtained from the slurry of SPRF, through optimization of suitable environmental parameters involving temperature, incubation period and concentration of HCl. Temperature higher than 100 °C showed a momentous increase in HMF with minimum variation in the level of total sugar (Table 2). Kim and Hamdy (1985) have also observed increase in 5-HMF concentration at temperature above 100 °C, which on reaching the critical point inhibits the alcoholic fermentation. Increase in incubation period resulted an enhanced total sugar production till 60 minutes, after which the sugar level remained constant. This can be due to the polymerization of simple monosaccharides into polysaccharides. Whereas, optimizing the acid concentration, no such variation in total sugar and HMF was observed, and a higher level of sugar value at 1M concentration of HCl was detected in comparison to that of other strengths of HCl. Under acid hydrolysis, starch molecules randomly break down into simple sugar by dislocating the hydrogen bonds between starch chains, altering it to an entirely amorphous state, thereby developing a homogenous gelatinous substance (Judoamidjojo et al. 1992). However, during acid hydrolysis, starch usually breaks down into glucose and maltose and the ethanologenic organisms like *Z. mobilis* and *S. cerevisiae* cannot efficiently utilize the maltose as a substrate. Therefore, the acid hydrolyzed SPRF product needs to be carried out for the conversion of simple sugars with the application of moderately thermostable glucoamylase enzyme. Saccharification of starch with the help of glucoamylase enzyme is well

Table 3. Effect of pH, temperature, incubation period, enzyme concentration on saccharification of sweet potato root flour (SPRF).

Enzyme volume (µl)	Enzyme concentration (U)	pH	Temperature (°C)	Incubation period (h)	Total sugar (g/kg)
Effect of pH					
50	250	3.5	65	1	500.22 ± 6.31
50	250	4.0	65	1	515.14 ± 6.46
50	250	4.5	65	1	478.23 ± 7.35
50	250	5.0	65	1	451.54 ± 6.29
50	250	5.5	65	1	449.39 ± 5.92
Effect of temperature					
50	250	4.0	50	1	430.63 ± 5.28
50	250	4.0	55	1	478.58 ± 6.22
50	250	4.0	60	1	527.88 ± 4.33
50	250	4.0	65	1	515.12 ± 6.14
50	250	4.0	70	1	485.24 ± 6.08
Effect of incubation time					
50	250	4.0	60	1	527.90 ± 7.11
50	250	4.0	60	2	547.57 ± 6.19
50	250	4.0	60	4	557.26 ± 5.07
50	250	4.0	60	6	568.53 ± 6.13
50	250	4.0	60	8	580.91 ± 6.24
50	250	4.0	60	12	594.52 ± 7.10
50	250	4.0	60	18	611.46 ± 7.32
50	250	4.0	60	24	608.62 ± 6.26
Effect of enzyme concentration					
25	125	4.0	60	18	581.63 ± 6.34
50	250	4.0	60	18	611.42 ± 7.12
100	500	4.0	60	18	628.15 ± 7.34
150	750	4.0	60	18	642.36 ± 6.65
200	1000	4.0	60	18	658.80 ± 7.83
250	1250	4.0	60	18	648.49 ± 8.04
300	1500	4.0	60	18	647.94 ± 7.93

known for the development of glucose as its end product catalysing the α-(1-4) and α-(1-6) glycosidic linkages from the non-reducing terminus of starch molecule (Riaz et al. 2012). The potent application of glucoamylase from various sources have also been applied for the simplification of various carbohydrates where the rate of conversion is varied as per the nature of applied starches like raw starch (Cripwell et al. 2019), maize starch (Li et al. 2018), cassava starch (Pervez et al. 2014) rye starch (Strąk-Graczyk and Balcerek 2020) and raw flours starch (Xu et al. 2016).

Hydrolysis product analysis through TLC

Both the process of dextrinization and saccharification split into sugar consisting of reducing form the slurry form of SPRF starch. TLC analysis of the hydrolysis product confirmed the presence of maltose and glucose as the end products of the process. Particularly the treatment with acid is able to enhance the porosity of starch and then modify the granules, but this property may interact with the solubility and thermal properties of starch through which the starchy environment may generate some low weigh molecular product by rupturing some hydrogen bonds (Ulbrich et al. 2019; Vargas-León et al. 2019). Further, the combined treatment with enzymes like glucoamylase and HCl which is very common for the saccharification process was noted to simplify the sugar products. The release of glucose molecules from the slurry of acid treated SPRF typically depends on several factors like pH, temperature, incubation period, enzyme concentration and the maximum glucose is formed through break down of α-(1-4) and α-(1-6) chemical bonds (Riaz et al. 2012; Lincoln et al. 2019; Strąk-Graczyk and Balcerek 2020). Moreover, the glucoamylase-maltose complex (maltose first produced after acid hydrolysis) also insists on the rate of formation of glucose with interacting the non-reducing end side of the substrate (Chiba 1997; Salimi et al. 2019).

Table 4. Elemental analysis of saccharified and saccharified fermented residues of SPRF.

Elements tested	Control/saccharified sample (mg/g)	Saccharified fermented residue (mg/g)	
		S. cerevisiae fermented residue	*Z. mobilis* fermented residue
P	2.12 ± 0.04	1.80 ± 0.03	1.77 ± 0.02
S	0.75 ± 0.01	2.14 ± 0.28	1.84 ± 0.03
Cl	3.37 ± 0.12	4.70 ± 0.07	4.32 ± 0.10
K	14.21 ± 0.36	13.14 ± 0.08	12.48 ± 0.10
Ca	2.21 ± 0.04	3.02 ± 0.02	3.12 ± 0.03
Mn	0.02 ± 0.003	0.04 ± 0.005	0.05 ± 0.001
Fe	0.33 ± 0.001	0.40 ± 0.01	0.58 ± 0.004
Zn	0.01 ± 0.002	0.16± 0.01	0.17 ± 0.001
Cu	0.03 ± 0.001	0.01 ± 0.003	0.01 ± 0.003

Structural property of treated SPRF

The structural changes of SPRF starch obtained from the hydrolysis due to the combination of acid and enzyme are clearly viewed through scanning electron micrography. The structural appearance of starch granules with an irregular matrix as evidenced through SEM is a good indication of hydrolysis due to the simultaneous action of acid and enzyme. The action of hydrolysis by HCl alone (dextrinization) indicates starch degradation from roots of sweet potato due to its emulsification activity (Babu et al. 2015). The dextrinized sample then allowed for saccharification, in which glucoamylase is used for further hydrolysis to produce glucose. The glucoamylase consists of a catalytic domain associated with a starch-binding molecule linked with O-glycosylated linker region that vigorously acts upon the partly treated starch molecules to release monomeric form of sugar and resulted in the formation of rough surface of the substrate as compared to the dextrinized sample (Sauer et al. 2000; Betiku et al. 2013). A similar kind of experiment was also conducted to digest the complex starch molecules by the action of acids and some significant starch hydrolyzing enzymes (Zhang et al. 2010; de Souza et al. 2019; Strąk-Graczyk and Balcerek 2020).

Functional property of saccharified fermented SPRF

The efficacy of sweet potato as a substrate for the production of ethanol and the differences in the starch biodegradation involving both *S. cerevisiae* and *Z. mobilis* fermented SPRF were examined through FTIR analysis within the spectral range of 1500-1800 and 3000-3700 cm^1. Between the spectral range of 900-1200 cm^{-1}, no absorbance peak was detected from both the fermented microorganisms which indicated the utilization of resulted carbohydrates (Vodnar et al. 2010; Warren et al. 2016; Jagatee et al. 2020). However in the spectral range of 1500-1800 cm^{-1}, both *S. cerevisiae* and *Z. mobilis* fermented SPRF pronounced shifting of peak position corresponding the various functional groups which revealed the formation of aromatic C-C stretching, carbonyl C-O stretching, alkene C-C stretching, esters functional groups. Such stretching may be attributed to lipids and fatty acids with C-O stretching (Nandiyanto et al. 2019). Moreover, in the spectral range of 3000-3700 cm^{-1}, various absorbance shifting of peaks appeared which is a strong indication of polymeric, dimeric and internally bonded -OH stretch and such stretch in the acid-enzyme hydrolyzed slurry of SPRF corresponds to the better ethanol production potential (Nieuwoudt et al. 2006; Jivan et al. 2014; Nandiyano et al. 2019).

Elemental variation of fermented residue of SPRF

Sweet potato is being considered as an economically important food crop for its substantial level of starch as well as for the bioavailability of soluble sugars, vitamins, minerals and other nutrients. Additionally, the root parts of sweet potato have significant amount of essential elements like Ca and Fe (Senanayake et al. 2013). Hence, an effort was made to simplify and change the constituents of some valuable elements by implementing *S. cerevisiae* and *Z. mobilis* individually to the saccharified SPRF product and a considerable change of the constituents was achieved after the fermentation. The applied acid-enzyme hydrolysis method not only successfully synthesizes the simple form of sugar but also resulted in better bioavailability of some essential elements within the residue of fermented SPRF which is a good sign of further utilization of that type of waste biomaterials. As the fermented SPRF residues having various nutritional values along with good source of pectin and dietary fiber, the product can be used as the cattle and fish feed (Murugan et al. 2012; Jagatee et al. 2020). Moreover, the residual product may act as potential organic fertilizer for sustainable agriculture and improve the growth and metabolism of plants (Varzakas et al. 2016; Akoetey et al. 2017). The availability of specific carbohydrates is the key biomolecules for regulating the

fermentation process, however, the deployed microorganisms and producing certain enzymes play a central role by regulating the whole metabolic activity. The fluctuation of trace elements in the residue of fermented SPRF maybe an indication of their utilization by certain enzymes as a cofactor for better production of bioethanol (Choong et al. 2016; Nimbalkar et al. 2018).

Conclusion

The study concludes that the acid-enzyme hydrolysis can be an alternative and sustainable technology for the production of bioethanol. The experimental outcome obtained from the study validates the activation of hydrochloric acid and glucoamylase (Palkodex®) for the production of maltose and glucose from sweet potato starch. The saccharified substrate subjected to fermentation and the fermented residues of *Z. mobilis* and *S. cerevisiae* showed the presence of several absorbed peaks for ethanol functional group (-OH), indicating a more suitable substrate for the production of bioethanol. Moreover, the variation of elemental components of control as well as fermented sweet potato starch may act as a possible bioindicator for utilization of specific nutrients corresponding to the deployed microorganisms. These observations advocated further need of research on subcellular and molecular level to understand the role of individual element during each step of the fermentation process of bioethanol production.

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