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Effect of variety and influence of stach-hydrolyzing enzyme and yeast on the yield of ethanol generated from sweetpotato flours and starches

[1]Etudaiye H. Adinoyi, [1]Oti E., [2]Sanchez T., [1]Omodamiro R. Majekodunmi, [1]Afuape O. Solomon and [1]Ikpeama A.

[1]National Root Crops Research Institute (NRCRI), Umudike, PMB 7006, Umuahia, Abia State, Nigeria.
[2]International Institute of Tropical Agriculture (CIAT), Palmira, Colombia

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ABSTRACT

Flours and starches were processed from three sweetpotato varieties namely TIS 2352.OP.113, TIS 8164 and TIS 87/0087. The flours and starches were fermented employing the granular starch hydrolyzing enzyme, stargen 00l and the yeast Saccharomyces cereviciae. The flours and starches were analysed for moisture/dry matter, fibre, amylose and amylopectin contents whereas their slurries were analysed for glucose contents, pH, total titratable acidity (TTA) and temperature during the 72hrs of fermentation. The ethanol yield of the flours and starches were determined. Significant differences (P<0.05) were found among the sweetpotato flours and starches with respect to moisture/dry matter, fibre, amylose and amylopectin contents. The ethanol yield of the flours and starches tended to be dependent on their amylose contents. The average yield of ethanol (19.94%) of the sweetpotato starches was higher than the average ethanol yield (15.19%) of the flours. The glucose contents and the pH of the fermenting slurries of the flours and starches decreased as fermentation progressed, whereas their TTA and temperatures generally increased. The sweet potato variety TIS 2352.OP.113 is recommended for exploitation for ethanol production and as a source of renewable energy.

Keywords: Sweetpotato, variety, flour, starch, enzyme, yeast, fermentation, ethanol, yield.

INTRODUCTION

Due to global energy crisis, many countries have initiated an intensive search for alternative liquid fuels particularly those obtained from renewable resource produced within country [1].

The current attention on bio-fuel may have environmental repercussions we are unaware of. Presently, the primary sources of ethanol are corn and sugarcane. Increased commercial production of the petroleum alternative can have the following impact: Firstly poisoning of the soil, water tables and streams due increased use of chemical fertilizers; secondly, reducing food production due to conversion of fertile and prime farmlands to ethanol production and thirdly, encroaching on and clearing of remaining tropical rainforests for growing the crops for bio-fuel production.

Fortunately, sweetpotato (*ipomoea batatas)* may provide a solution to the ethanol fuel dilemma because of its large roots which contains mostly starch and glucose which convert easily to alcohol. The following characteristics make sweetpotato almost perfect for ethanol production: It can be grown in tropical and sub-tropical regions, and indeed in any terrain. The plant can be grown in a variety of soils except for heavy clay-types where the roots do not have much chance of development; it does not require much fertilizer and needs little maintenance. Sweetpotato has a short maturity of 3.5 to 4 months and the roots can be stored for 3 months after a seven-day cure in any open space. Energy security, declining oil reserves and climate change have served as drivers for new governmental initiatives to increase alternative fuel sources, principally ethanol from biological feed-stocks [2]. Cultivation of energy crops under the normal conditions to produce bio-mass energy is thought to be one of the important ways to develop alternative energy.

Ethanol is an important industrial chemical with emerging potential to replace fossil fuels [3]. It is one of the largest volumes of organic chemicals that are industrially produced [4]. Ethanol can be produced by fermentation of sugars from agricultural products or waste materials [5] [6]**,** basically those that contain starch, sugar or cellulose [7] [8].

Starch grains and effluent generated from starch processing units are the cheap feed-stocks and could be used as potential raw materials for ethanol fermentation [9]. Starch consists of two polysaccharides, the linear molecule, amylose and a highly branched molecule, amylopectin.

Starch-hydrolyzing enzymes hydrolyze starches into sugar which are converted into alcohol by yeast enzymes. For starch materials, once simple sugars are formed, enzymes from micro-organisms can readily ferment the sugars to ethanol [10].

The α-amylase that converts starch into dextrin has been used most widely for starch liquefaction and is isolated from the ubiquitous mesophilic soil bacterium *Bacillus licheniformis*. This enzyme operates optimally at 90 to 110^{o}C and pH 6. The glucoamylase that converts dextrin into fermentable sugars is derived from *Aspergillus niger*. The optimal operating conditions for this enzyme are 60 to 70C and pH 4.3. The granular starch-hydrolyzing enzyme (GSHE) is a mixture of α-amylase and glucoamylase which hydrolyzes granular starch directly into fermentable sugars and works at low temperature of 30 to 48C and pH 4.0 to 4.2 [11]

The objectives of this study are to determine the quantity of ethanol produced from starches and flours of different varieties of sweetpotato and the influence of the granular starch-hydrolyzing enzyme and yeast cell *Saccharomyces cereviciae* on the production of ethanol from sweetpotato starch and flour.

MATERIALS AND METHODS

Source of materials

Fresh roots of three (3) sweetpotato varieties TIS 2352.OP.113, TIS 87/0087 and TIS 8164 were harvested at about 12 weeks after planting from field trial of Sweetpotato Programme, National Root Crops Research Institute, Umudike.

Processing of sweetpotato roots into starch and flour

Sweetpotato starch was produced using the recommended standard extraction methods described by 12]

Source of Enzymes

Granular Starch Hydrolyzing Enzyme (GSHE) (*Stargen* 001) Genencor International (Palo Alto, CA) and dried from of *Saccharomyces* yeast (BP1422-500, Fischer Scientific, Pittbutgh, PA) were obtained from the International Centre for Tropical Agriculture (CIAT), Palmira, Colombia. The enzyme contains α-amylase from *A. Kawachi* and a glucoamylase from *A. niger* with activity of >456 GSHU/g. BSHE.

Flour and Starch fermentation

The method of **[**13**]** was used. Fermentation was monitored from zero (0) hour to 72hr. About 50g of starch/four from each sweetpotato variety were mixed with hot tap at 35^{o}C to obtain slurries. Different batches of the starch and flour slurries were prepared for ethanol production at different periods. Slurry temperature was monitored and adjusted to 48^{o}C for incubation. The pH of the slurry was adjusted to 4.2 with 5M Sulphuric acid solution. About 140 µL of GSHE and 3g of yeast (*S.cerevisiae*) were added. Free amino acid as urea (0.1% of slurry) was added to supplement the yeast. The slurry was stirred with a glass rod to obtain uniform mixture (mash).

Laboratory analysis
At constant weights (120g) of the fermenting mash, each sample of flour and starch from each variety was analyzed for pH, titratable acidity (TTA) and mash temperature.

Determination of pH and Titratable Acidity (TTA)
pH and TTA of the fermenting sweetpotato mash were determined by the methods described by [14]. About 10mls of homogenized sweetpotato mash was collected in a beaker. The pH of the mash was determined manually at room temperature using Jensway 3016 pH meter.

TTA was obtained from the sample whose pH has been determined. The sample was transferred into 250ml conical flask and 15ml of distilled water was then used to wash out the beaker into the flask. The sample was suspended and titrated with against 0.1M NaOH using phenolphthalein indicator. TTA was calculated as percentage lactic acid.

Determination of slurry temperature, moisture/dry matter and fibre contents
Temperature, moisture/dry matter and fiber of fermented slurries of starch and flour samples were determined using [15] methods.

Determination of amylose and amylopectin
Amylose was determined following the colorimetric standard procedure of [16]. Starch/flour granules were first dispersed with ethanol and then gelatinized with sodium hydroxide. An aliquot was then acidified and treated with an iodine solution, which produced blue-black colour. The colour intensity which relates to amylose content was then measured with a spectrophotometer at 650nm and compared with standard curve obtained using purified amylose (0-40%) concentrations.

Amylopectin content was determined by subtracting amylose content from 100%.

RESULTS AND DISCUSSION

Significant differences ($P<0.05$) occurred among the sweetpotato flours and starches with respect to moisture/dry matter, fibre, amylose and amylopectin contents (Table 1). The moisture contents of the flours and starches were low ensuring long shield life and their high dry matters contributed to production of reasonable quantities of alcohol. The amylose and amylopectin contents of the flour and starch of each sweetpotato variety are comparable. However, TIS 2352.0P.113 produced flour with the highest amylose content while the amylose content of TIS 8164 starch was the highest among the starches. The fibre contents of the flours and starches were very low indicating high starch levels in the products and suggesting their suitability for generating alcohol.

The ethanol yields of the sweetpotato flours ranged from 14.82% to 15.48%, TIS 2352.OP.113 yielding highest; the ethanol yield of the starches ranged from 18.14% to 21.00%, TIS 87/0087 yielding lower than the other two varieties (Table 2). The ethanol yield of the flours and starches tended to be dependent on their amylose contents (Tables 1 and 2). The average yield of ethanol (19.94%) of the sweetpotato starches was higher than the average yield (15.19%) of ethanol from the flours (Table 2).

Tables 3 and 4 present the changes which occurred in some of physico-chemical parameters of the flours and starches from the sweetpotato varieties during the 72hrs they underwent fermentation. The glucose and pH of the fermenting slurries of the flours decreased as fermentation progressed while the TTA and slurry temperature increased except for mash temperature of TIS/0087 where the temperature did not change from the 0-hr to the 72-hr of fermentation (Table 3). The glucose and pH of the fermenting starch slurries also decreased from the 0-hr to the 72-hr, while TTA and slurry temperature of the starches increased as fermentation progressed (Table 4).

Table 1: Some biochemical contents of flours and starches from sweetpotato varieties used for ethanol production.

	Biochemical Contents									
	Sweetpotato flours					**Sweetpotato Starches**				
	Moisture (%)	Dry Matter (%)	Fibre (%)	Amylose (%)	Amylopectin (%)	Moisture (%)	Dry Matter (%)	Fibre (%)	Amylose (%)	Amylopectin (%)
TIS 2352.OP.113	9.28^{a}	90.72^{c}	1.25^{c}	17.32^{a}	82.68^{c}	6.72^{b}	93.28^{b}	0.52^{b}	18.24^{b}	81.76^{b}
TIS 8164	8.90^{c}	91.10^{a}	1.32^{b}	16.25^{c}	83.75^{a}	6.45^{c}	93.55^{a}	0.48^{c}	19.60^{a}	80.40^{c}
TIS 87/0087	9.16^{b}	90.84^{b}	1.36^{a}	16.64^{b}	83.36^{b}	7.24^{a}	92.76^{c}	0.60^{a}	17.50^{c}	82.40^{a}

Values with the same letters are not significantly different (P>0.05).

Table 2: Ethanol yield of flours and starches from sweetpotato varieties after 72hrs fermentation period

	Ethanol Yield (%)	
Sweetpotato Varieties	Sweetpotato Flours	Sweetpotato Starches
TIS 2352.OP.113	15.48^{a}	21.00^{a}
TIS 8164	14.82^{c}	20.68^{a}
TIS 87/0087	15.27^{b}	18.14^{b}
LSD (0.05%)	**0.05**	**1.15**

Values with the same letters are not significantly different (P>0.05)

Table 3: Some changes in physico-chemical parameters during 72-hrs fermentation of sweetpotato flours.

	0-hr and 72-hr values of parameters/changes											
	Glucose (%)			**pH**			**Total-titratable acidity (%)**			**Slurry Temperature (°C)**		
Sweetpotato varieties	0-hr	72-hr	Changes	0-hr	72-hr	Changes	0-hr	72hr	Changes	0-hr		Changes
TIS 2352.OP.113	6.8	2.5	(-)	5.2	4.5	(-)	0.4	0.6	(+)	28	30	(+)
TIS 8164	7.1	2.7	(-)	5.1	4.6	(-)	0.4	0.6	(+)	29	30	(+)
TIS 87/0087	6.8	2.4	(-)	5.1	4.1	(-)	0.4	0.6	(+)	28	28	(0)

(-) = decrease in level of parameter,
(+) = increase in level of parameter,
(0) = no change in level of parameter.

Table 4: Some changes in physico-chemical parameters during 72-hrs fermentation of sweetpotato starches.

	0-hr and 72-hr values of parameters/changes											
	Glucose (%)			**pH**			**TTA(%)**			**Slurry Tempt. (°C)**		
Sweetpotato varieties	0-hr	72-hr	Changes	0-hr	72-hr	Change	0-hr	72hr	Change	0-hr	72-hr	Change
TIS 2352.OP.113	8.2	3.4	(-)	4.9	4.5	(-)	0.4	0.7	(+)	28	30	(+)
TIS 8164	7.5	2.3	(-)	4.9	4.3	(-)	0.5	0.8	(+)	28	29	(+)
TIS 87/0087	7.3	3.2	(-)	5.2	4.4	(-)	0.5	0.7	(+)	28	30	(+)

(-) = decrease in level of parameter
(+) = increase in level of parameter.

CONCLUSION

Sweetpotato flours and starches are suitable for producing ethanol and higher levels of ethanol could be generated from the starches. Ethanol yield from the flours and starches were higher than the values reported by earlier workers and this is probably due to the higher activity of the granular starch-hydrolyzing enzyme (*stargen* 001). The sweetpotato variety TIS 2352.OP.113 stands out as its flour and starch have shown very high potential for ethanol production. Sweetpotato has proved to be a dependable source of renewable energy.

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