SENRA Academic Publishers, Burnaby, British Columbia
Vol. 2, No. 1, pp. 133-137, 2008
ISSN: 1715-9997

Canadian Journal of pure&applied sciences

COMPARISON OF REGIONAL VARIETIES OF SORGHUM FOR ETHANOL, PROXIMATE AND METAL IONS CONCENTRATION

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ABSTRACT

Sorghum [*Sorghum bicolor* (L.) Monech] is the only crop that provides both grain and stem used for sugar, alcohol and syrup etc. In the present study seeds of 10 varieties of sorghum were evaluated for ethanol, starch, fat, fiber and metal ion contents. The simultaneous saccharification and fermentation (SSF) of grain samples were conducted by using Saccharomyces *cerevisiae* and ethanol produced was quantified with the help of gas chromatography (GC). The concentration level of ethanol (7.6-12.5%), starch (63.90-78.11%), protein (9.67-11.23%), fat (5.0-8.4%) and fiber (0.83-1.08%) was obtained from different varieties of sorghum. The study reveals that grains of these varieties can be utilized for the production of ethanol on industrial scale, by improving the existing methods of fermentation process.

Keywords: Sorghum, grains, fermentation, ethanol, gas chromatography.

INTRODUCTION

Pakistan is an agro-based country and has variety of important agricultural crops. Wheat, rice, cotton, sugar cane and maize are major crops and share 24 % in national economy. Sorghum, barley and millet are considered as minor crops, generally used as fodder and grown in the arid zones of country.

Globally Sorghum is very important in the human diet, with over 300 million people dependent on it (Bukantis, 1980). Sorghum (*Sorghum bicolor* [L] Monech) is an important commodity crop in the semi arid regions of the world (Dendy, 1995). The grains of sorghum are rich in starch with similar starch content to maize (Park and Bean, 2003). In some countries of the world sorghum is used primarily as animal feed, but in Africa and India it is a staple food for millions of people (Rooney and Waniska, 2000). Sorghum is a high biomass and sugar yielding crops (Bryan, 1990). Therefore, of many crops currently being investigated for energy and industry, sweet sorghum is one of the most promising, particularly for ethanol production (Gnansounou *et al*., 2005). A strong need exist for efficient ethanol production and production process. Demand of ethanol is growing as a clean substitute for direct use as fuel which can ease both natural resource limitation and environmental pollution (Rehm and Reed, 1996).

Most biological processes concern with the conversion of starchy materials into ethanol through three steps, liquefaction of starch, enzymatic saccharification and fermentation of sugars to ethanol (Laluce and Matton, 1984). Identifying biochemical components of sorghum related to food functionality is very important for improving the quality and yields of industrial materials (such as ethanol, lactic acid, biodegradable film and packaging material etc., (Hames, 2004). However, Rehm and Reed (1996) have described the importance of elemental and proximal analysis for the comparison between total yield, carbon contents, sugars and other parameters for the improvement of a crop.

Therefore keeping in view the importance of sorghum as a valuable energy crop, the study was conducted to evaluated 10 local varieties of sorghum for their protein, fat, starch, ethanol and metal ions contents.

MATERIALS AND METHODS

Experimental Materials

The seed samples of 10 varieties (84-Y-00, 84-Y-01, 85-G-83, 86-G-87, DS-97-1, Mr. Buster, PARC SS1, RARI S-3, RARI S-4 and YSS 9) of sorghum were purchased from Millet Research Station Rawalpindi. These varieties were grown during July-October, 2006 for the purpose of animals feed (All chemicals used in this study were purchased from local dealer of Sigma company).

Biochemical (proximate) analysis

Starch content was measured using a commercial available kit (Megazyme-Ireland).

Protein content was determined by micro-Kjeldahl nitrogen analysis and using the standard method (AACC, 2000). Nitrogen values were converted to protein content

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values multiplying by 6.25. The moisture content of a sample was determined by drying the sample in an oven at 105°C for 24 hours. The weight loss of the sample after drying was considered as the moisture content. Crude fiber, fat, and ash were determined by AOAC standard methods (AOAC, 1995). Reducing sugar was measured by using method of Miller (1959).

Analysis of metal ions

The analysis of metal ions from grains of sorghum was conducted by using wet digestion method using HNO_3 – $HCLO_4$. About 0.5 gram powdered grain material was taken in 100 ml pyrex digestion tube and digested with 10 ml of nitric and perchloric acid (2:1) mixture and allowed to stand overnight. The tubes were placed in a cold block digester, heated up to 235°C for complete digestion of reaction mixture. The temperature of tubes were reduced to normal and final volume was adjusted 100 ml with deionized water. Sodium and Potassium were analyzed by Flame photometer (Jenway PFP-7), while the remaining elements were analyzed by Atomic Absorption Spectrophotometer (GBC 932 plus, Australia). All analysis were conducted in triplicate.

Fermentation and measurement of ethanol content
Media

A standard strain of *Saccharomyces cerevisiae* was obtained from Biotechnology Lab., Islamabad. The medium used to maintain *S. cerevisiae* contained 0.5% yeast extract, 3 % peptone, 2 % glucose and 2% agar, pH 5.5. YM broth medium which contained yeast extracts 0.3%, malt extract 0.2%, peptone 0.5%, glucose 1%, pH 5.5 was used for yeast inoculum preparation. The growth medium used for preparing the fungal inoculum, contained soluble starch 1%, peptone 0.2 %, yeast extract 0.2 %, $MgSO_4.7H_2O$ 0.1 %, $(NH_4)_2PO_4$ 0.2 %. The fermentation medium used for the ethanol production from starch was identical to the growth medium except that the substrate used (3-10 g/100 ml). 1 N HCl or 1N NaOH was used to obtain the desired pH for testing the effect of pH on fermentation. For the sporulation McClary medium (McClary et al., 1959) was used.

Simultaneous saccharification and fermentation

The saccharification and fermentation of grain powder proceeded simultaneously in one vessel. Erlenymer flask (500 ml) containing 200 ml of fermentation medium were sterilized by autoclaving for gelatinization at 121°C for medium. Experiment was conducted with different starch concentrations (3-15%) of grain powder with shaking (150 rpm) at 30°C for 3 days. Since starch is insoluble in water at room temperature the initial broth gave a viscous slurry. The viscosity of the medium decreased rapidly due to the saccharification and fermentation. The experiment was replicated twice and the average values are presented.

Analytical methods

After fermentation, the samples (10 ml) were collected and centrifuged at 5000 x g for 15 min at 4 ˚C to remove cells and the supernatant was used for determining of ethanol, sugar and amylase activity. The activity of enzyme (amylase) was determined in terms of mg of glucose released from starch. The reaction mixture, containing 2 ml of 1 % starch in deionized water, 1 ml of 0.1 M acetate buffer (pH 5.0) and 1 ml of enzyme solution was incubated at 30 ˚C in a water bath for 10 min. The amount of reducing sugar was determined by the method described by Miller (1959). One unit of amylase activity was determined by making a pellet of cells by centrifugation, drying them at 70 ˚C and expressing dry weight as g/100 ml of growth medium. The ethanol was determined by GC with a flame ionization detector. The internal standard method was applied. Chromatogram column and samples injection were stainless steel column and auto headspace samples injection. Ethanol (GR) and normal propyl alcohol (GR) were used for the standard curve and internal standard substance, respectively. The column temperature was controlled at 200 ˚C. N_2 was used as a carrier gas (40 mL min^{-1} and 500 mL^{-1}), respectively. Samples of 20 µl were injected directly into column. All determinations were done by means of standard curves and results were mean of two replications. The concentration of ethanol (%) was determined from peak areas obtained for each analysis.

Data analysis

Data analysis was performed using one way ANOVA variance and expressed in the form of mean, standard deviation, significance or non significance values

RESULTS AND DISCUSSION

Proximate analysis

A variation in protein concentration (9.67 to 11.23 %) was found in different varieties of sorghum (Table 1). The highest concentration of protein (11.23%) was found in variety 84-Y-00 and lowest in DS-97-1 (9.67 %). Fat content show less variation among the varieties, however, 86-G-87 (8.4 %) and 85-G-83 (8.2%) has a higher fat contents where as DS-97-1 exhibited the lowest (5.0 %) fat contents. The less variation was found in crude ash, fiber and moisture contents of sorghum varieties (Table 1). The starch content was higher (78.11 %) in variety (Mr. Buster) and lowest 63.90 % in variety PARC SS 1. The presence of significant amounts of starch and protein in these sorghum varieties indicates their suitability for food and animal feed (Table 1).

Table 1. Proximate analysis (%) of 10 different varieties of Sorghum bicolor.

Varieties	Protein	Fat	Starch	Reducing sugar	Fiber	Ash	Moisture
84-Y-00	11.23±0.12*	6.8 ± 0.13	75.46 ± 3.14	6.5±0.02	0.83 ± 0.29	1.6± 0.20*	10.16± 0.12
84-Y-01	10.40± 0.10^{cd}	7.0 ±1.30	77.55 ±1.07*	7.8±0.01*	1.00 ± 0.00	1.6± 0.17*	10.26 ± 0.22
85-G-83	10.67 ± 0.29	8.2± 1.39*	73.89 ± 0.79	6.8±0.03*	0.93 ± 0.12	1.4 ± 0.06	10.13± 0.22
86-G-87	10.50± 0.20	8.4± 0.56*	70.20 ± 0.54	6.4±0.02	0.93 ± 0.13^{a}	1.3± 0.29	10.11±0.06
DS-97-1	9.67 ± 0.05	5.0 ± 0.47	67.48 ± 2.06	6.2±0.03	1.03 ± 0.06	1.7 ± 0.21	11.37± 0.71^{a}
Mr. Buster	10.57± 0.40	6.9 ± 0.99	78.11 ±1.87*	6.3±0.01	1.00 ± 0.00	1.5 ± 0.06	9.14 ± 0.21^{d}
PARC SS 1	10.57±0.12*	6.5 ± 0.63	63.90 ± 0.40	5.4±0.02	0.86 ±0.04	2.0 ±0.00	10.06±0.05
RARI S-3	10.97± 0.15*	6.5 ± 1.27	74.87 ±2.78	6.2±0.03	0.83 ±0.29	1.4 ± 0.12	9.93 ± 0.13
RARI S-4	9.92 ± 0.50	6.5 ± 0.62	75.09 ±2.40*	6.3±0.02	0.97 ± 0.06	1.2 ±0.29	9.89 ± 0.10
YSS 9	10.13± 0.06	6.5 ± 0.70	65.67 ± 2.91	5.8±0.01	0.93 ±0.12	1.8 ±0.15	9.32 ± 0.27

Means± SE of three replication on dry weight basis. * Significant values ($p \leq 0.01$) with reference to other values.

Morphological and physiological characteristics of *S. cerevisiae*

The vegetative cells of *S. cerevisiae* were reproduced by multilateral budding and four ascospores were observed per ascus, when it was grown on sporualtion medium. It was able to ferment glucose, galactose, sucrose and maltose but unable to ferment xylose and lactose. The variation in biomass was observed depending on substrate (7.0±0.3x 10^8 cells/ml), when *S. cerevisiae* was grown in pure culture

Utilization of starch for ethanol production

The higher concentration of ethanol (12.5 %) was obtained from variety, Mr. Buster with 92% recovery, where as lowest level of ethanol (7.6%) was obtained from variety 84-Y-00 with 71.2% recovery. However, no significant variation was observed in other varieties of sorghum for their ethanol contents (Table 2). It was observed that increased in ethanol yield was due to increase in substrate concentration for all varieties studied. Furthermore, more biomass and less reducing sugars were also observed at the end of fermentation with the strains of *S. cerevisiae.* The different concentrations of substrate tested was found optimum for producing maximum yield of ethanol in 70 h (Fig. 1).

Amylase activity at 10 % concentration of all substrates was found to be 18 U/ml at the end of fermentation and hydrolyzed more than 90 % of the starch present in the grains of different sorghum varieties. The ethanol productivity was studied by varying the pH of the fermentation medium from 4-7 and maximum production of ethanol was obtained at pH 5.0 for all 10 varieties of sorghum.

It was reported earlier by Yeon *et al.* (1994) that 64.3g/l of ethanol was produced by utilizing 94 % of 150g/l soluble starch with a mixed cultured of mutant M-6 Sawanniomyces castelli and *S. cerevisiae*. The starch content in the different varieties of sorghum was 63.90-78.11%, as compared to other varieties reported earlier (Rehm and Reed, 1996). Meanwhile these varieties were local and were grown as fodder for animals feed but not for commercial purposes. These substrates were cheap and easily available for ethanol production in this culture media at 10 % substrate concentration. However, it can improve by using better strains of *S. cerevisiae* to utilize more than 10 % substrate concentration and to reduce the duration of fermentation.

These results indicates that simultaneous saccharification and fermentation of starch from sorghum grains to ethanol can be conducted efficiently by using non-amylolytic sugar fermented by *S. cerevisiae.*

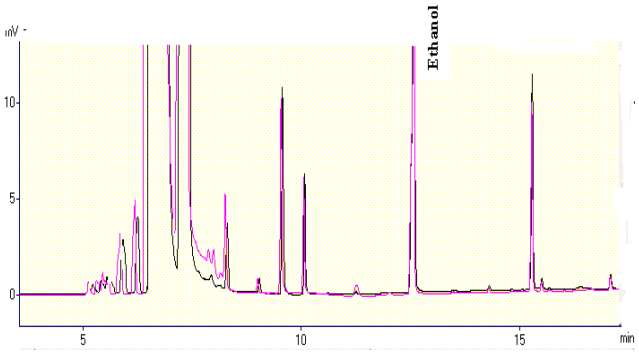

Fig. 1. GC peaks showing analysis of ethanol from sorghum.

Table 2. Analysis of ethanol obtained from different varieties of sorghum.

Varieties	Left over sugar g/100 ml	Ethanol found (% v/v)	% Recovery	Biomass (g/100ml)
84-Y-00	0.16	7.6±0.21	71.2	1.0±0.0
84-Y-01	0.24	10.8± 0.52	78.4	1.2±0.01
85-G-83	0.23	10.5± 0.31	87.5	1.25±0.02
86-G-87	0.18	9.5±0.42	86.3	1.05±0.01
DS-97-1	0.17	8.6± 0.32	87.1	1.22±0.02
Mr. Buster	0.45	12.5± 0.41	92.0*[r]	1.35±0.01*[b]
PARC SS 1	0.17	9.5± 0.35	87.7	1.32±0.03*[b]
RARI S-3	0.18	11.1±0.31	88.2	1.21±0.02
RARI S-4	0.17	9.5±0.42	84.2	1.22±0.02
YSS 9	0.16	8.2±0.0.21	85.3	1.24±0.01

*Significant level of ethanol analyzed with GC; *b Significant biomass obtained after fermentation
*r Significant recovery of ethanol from substrate

Table 3. The concentration level (mg/g) of selected metals analyzed from 10 varieties of sorghum.

Varieties	Zinc	Copper	Iron	Manganese	Sodium	Potassium
84-Y-00	0.016 ±0.01*	0.002 ±0.0	0.027 ± 0.02	0.004 ± 0.01*	0.051 ± 0.03	0.106 ± 0.05
84-Y-01	0.014 ±0.02	ND[e]	0.033 ± 0.07*	0.004 ± 0.01*	0.055 ± 0.04	0.107 ± 0.01*
85-G-83	0.014 ±0.01	ND[e]	0.032 ± 0.01	0.006 ± 0.0	0.057 ± 0.01	0.113 ± 0.05
86-G-87	0.182 ±0.021*	ND[e]	0.029 ± 0.02	0.007 ± 0.0	0.053 ± 0.09*	0.116 ± 0.02
DS-97-1	0.019 ±0.0	0.035 ± 0.0	0.008 ± 0.0	0.009 ± 0.0	0.067 ± 0.03	0.155 ± 0.05
Mr. Buster	0.012 ±0.0*	0.001 ± 0.0	0.029 ± 0.03*	0.006 ± 0.01	0.056 ± 0.05	0.111 ± 0.03
PARC SS1	0.009 ±0.0	0.003 ± 0.0	0.026 ±0.02[b]	0.004 ± 0.0	0.052 ± 0.03*	0.065 ± 0.0
RARI S-3	0.008 ±0.0	0.041 ± 0.01	0.052 ± 0.0	0.047 ± 0.0	0.054 ± 0.003	0.047 ± 0.05*
RARI S-4	0.007 ±0.0	0.002 ± 0.0	0.013 ± 0.006	0.003 ± 0.0	0.07 ± 0.02	0.058 ± 0.02
YSS 9	0.01 ± 0.0	0.02 ± 0.0	0.007 ± 0.001	0.005 ± 0.0	0.117 ± 0.06	0.073 ±0.02

Mean ± SE of three replications expressed on dry weight basis
*Significance ($p \leq 0.01$) with reference to others and samples used ND Not Detected

Metal ions analysis

Data in Table 3 shows metal ion concentration in all 10 varieties of sorghum. Potassium concentration (0.155 mg/g) was highest in variety DS-97-1 and lowest in RARI S-3, (0.047 mg/g) The concentration level of potassium in other varieties were in the order of 86-G-87 >85-G-83 >Mr. Buster >84-Y-01 >84-Y-00 >YSS-9 > PARC-SS1 > RARI S-4 (Table 3). Potassium is necessary for formation of sugars, starches, carbohydrates and protein synthesis and also for cell division in roots and other parts of the plant (McClary *et al*., 1959). The concentration of sodium was less variable among all the varieties (Table 3). Iron concentration was 0.007 to 0.033 mg/g as compared to copper 0.002 to 0.0041 mg/g was observed (Table 3). Zinc level was high in variety 86-G-87 (0.182 mg/g). Whereas concentration of manganese was highest (0.009 mg/g) in DS-971 and lowest in RARI S-4 (0.003 mg/g). It was observed that some varieties e.g. 84-Y-01, 85-G-83, 86-G-87 and Mr. Buster were lower or deficient in copper ions concentration. Possibly due to the reason that copper is bound tightly in organic matter, not readily lost from soil but may often be unavailable for plants. Micro-nutrients assist many biochemical reactions that ensure the growth and survival of plants. For example zinc (Zn) is required for the production of the growth hormone auxin (Skoog, 1940) and is needed for optimal photosynthesis. Iron (Fe) plays critical role in processes such as DNA synthesis, respiration and photosynthesis (Krishnasamy *et al*., 2005). Manganese (Mn) acts as an enzyme activator for nitrogen assimilation (Przemeck and Schrader, 1981). Copper (Cu) plays a part in nitrogen

metabolism (Llorens *et al*., 2000). Zinc and copper belong to the group of elements whose minimal doses are indispensable for the proper functioning of organisms. A number of studies also indicate the uptake of trace metals by *S. cerevisiae* during fermentation processes. Zinc, copper and manganese are important because they have a positive effect on the respiratory process and growth rate of *S. cerevisiae* for the higher production of ethanol (Yeon *et al*., 1994). As sorghum is one of the important agricultural crop that can be utilized for the production of bio-ethanol, must have reasonable amount of micro nutrients for proper function. Furthermore there is no or little need to add addition amount of trace metals in broth media, when sorghum is used as substrate for fermentation. The results revealed that these varieties of sorghum have full potential for production of ethanol on industrial scale through fermentation process by using *S. cerevisiae*

CONCLUSION

Significant level of ethanol, macro and micronutrients were observed in these varieties of sorghum, those were not evaluated earlier. The results of this study would be beneficial for production of ethanol through fermentation process from grains of these local varieties.

ACKNOWLEDGEMENT

Authors acknowledge the financial support of HEC for the research project "Production and utilization of bio fuel from sweet sorghum".

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