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Standardization of hydrolysis procedure for saccharification of mango kernel starch.

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Abstract- *Mango processing generates huge quantities of wastes. One of the major problems faced by the factories is the disposal of mango processing waste. Kernel is obtained by breaking the hard seed coat of mango stone. It is a rich source of starch (approximately 60% on dry weight basis). The study was conducted to measure the effect of combinations of different physical, chemical and microbial treatments on starch utilization of mango kernel for total, reducing sugar and ethanol production. 5%, 10%, 15% and 20% w/v each of dried weight samples of mango kernel were treated with 2% H_2SO_4, 2% HCl, Aspergillus niger, 2% H_2SO_4 with Aspergillus niger, 2% HCl with Aspergillus niger, and untreated control. The results indicated that 2% H_2SO_4 with Aspergillus niger exhibited highest hydrolysis in 10% substrate (31.56% total sugar and 21.31 % reducing sugar released in the medium) followed by 2% HCL with Aspergillus niger 27.51 and 17.89 % total and reducing sugars respectively. The released total and reducing sugar was observed in other treatments viz. Aspergillus niger, 2% H_2SO_4, 2% HCl and control (untreated) with 16.48, 19.23, 21.34 % total sugar and 9.79, 12.49, 9.79, 5.14 reducing sugar respectively. Maximum ethanol production was found in the treatment 2% H_2SO_4 with Aspergillus niger (6.62%).*

Key Words: Mango kernel, Waste, Reducing sugar, Total sugar, Mango stone

1. INTRODUCTION

Mango (*Mangifera indica* Linn.) is one of the most important tropical fruits in the world. During processing of mango, by-products such as peel and kernel are generated. By the end of the mango industrial processing, a large quantity of refuse is discarded, 40 and 60% of the material, is composed of mainly peels and seeds. Kernel is obtained by breaking the hard seed coat of mango stone. Kernels take up about 17-22% of the fruit [1]. It is a rich source of starch (approximately 60% on dry weight basis). Starch is a polysaccharide composed of α-glucose units that are linked by α-1,4 and α-1,6 glycosidic bonds, forming two high molecular mass molecules; amylose (15–25%), a linear polymer composed of α-1,4 linked glucopyranose residues, and amylopectin (75–85%), a branched polymer containing α-1,6-glycosidic linkages at the branch points [2,3]. Starch is used in the food industry as a thickener, binder, stabilizer, and emulsifier and as a suspending and gelling agent. Furthermore, it is the primary source of various sugar syrups, which provide the basis of several pharmaceutical and confectionery industries. With industrial development growing rapidly, there is need for environmentally sustainable energy sources [4]. Bio-ethanol is an effective sustain able energy source. Based on the basis that fuel bio-ethanol can contribute to a cleaner environment and with the implementation of environmental protection laws in many countries, demand for efficient bio-ethanol production processes may increase. Amylases are enzymes that convert or breakdown starch into glucose [5, 6].

Amylases are important enzymes employed in processing industries for the hydrolysis of starch into simple sugars [7]. Evidences of amylase production by moulds, yeasts and bacteria have been reported and their properties documented [8, 9]. Fungi among many microbes are good sources of amylolytic enzymes [10, 11]. Amylases hydrolyze the alpha 1-4-glycosidc bonds of amylopectin, glycogen are related compounds [12]. Co-culture or consortia microorganisms are using for hydrolysis of starch or many agricultural wastes. Use of co-cultures of *Aspergillus niger* and *Saccharomyces cerevisiae* for ethanol production from banana peel [13] and tapioca starch [14], on sorghum pomace [15], consortia of *Saccharomyces cerevisiae*,

Trichoderma viride and *Aspergillus niger* have used for the ethanol production from Cassava waste [16]. Bio-ethanol has stimulated worldwide interest due to its utilization as an alternative fuel source and is produced from the renewable and cheap agricultural resources [17]. The aim of this study was to optimize the different substrate concentrations and measure the effect of combinations of different physical, chemical and microbial treatments on the starch utilization of mango kernel for total, reducing sugar and ethanol production.

2. Materials and Methods

2.1 Raw material

Mango stone was obtained from the processing lab of Post Harvest Managment division of Central Institute for Subtropical Horticulture (CISH), Rehmankhera, Kakori, Lucknow. The outer shell was decorticated and kernel was washed thoroughly with the tap water and air-dried under the fan and then dried at 60°C until gaining the constant weight.

2.2 Microorganism

In the present study, the fungal strain *Aspergillus niger* NAIMCCF-02958 was maintained on the potato dextrose agar (PDA) while *Saccharomyces cerevisiae* was maintained on the yeast extract potato dextrose agar media [18]. The culture was routinely reviewed and sub-cultured under the aseptic culture conditions.

2.3 Hydrolysis of mango kernel for reducing sugar

Took the 5%, 10%, 15% and 20% w/v each of dried weight samples of mango kernel and mixed with 100 mL distilled water) in sterilized 250 mL cononical flasks. The samples were treated with 2% H_2SO_4, 2% HCl, 2% inoculum of *A. niger*, 2% H_2SO_4 with *A niger,* 2% HCl with *A. niger.* One sample used untreated as a control. The pH of samples was maintained at 5.6 ± 0.5 and fermented at the 35°C for 10 days. After completion of fermentation, samples were centrifuged and released amounts of TSS (total soluble solid) total sugars and reducing sugars were determined in each supernatant.

2.4 Alcohol production

After completion of fermentation and reducing sugar filtration; each sample was inoculated with *Saccharomyces cerevisiae* (3.42 X10^4 cfu/mL) and incubated at 37°C for 4 days for ethanol production.

2.5 Analysis

Filtrate was considered for the estimation of the reducing sugars by DNS method [19] 3 mL of DNS reagent was added to 3 ml of glucose sample in test tubes in three replications. Heated the mixture at 90º C for 10 min to develop the red-brown colour, and added one mL of 40% potassium sodium tartrate [20] solution to stabilize the colour. After being cool up to the room temperature in a cold water bath, absorbance was measured with a spectrophotometer at 575 nm [19]. Alcohol was determined by method of Caputi *et al.,* (1968) [21] by spectrophotometric methods. All the statistical analysis was performed to test the Null hypothesis by considering the probability values at 5%.

3. Results and Discussion

The results showed that the fermented mango kernel produced significant amounts of the total carbohydrate, reducing sugar and bio-ethanol. The volumetric production of total, reducing and bio-ethanol was varied significantly with various treatments; HCl, H_2SO_4, fungal strain and acids with fungal strain.

3.1 Effect of substrate concentration

Moisture is the key element for regulating and optimizing the solid state fermentation process. Intermediate moisture content is required for an efficient solid state fermentation process [22]. Concentration of the substrates were varied and allowed to saccharify with different treatments (Fig.1).

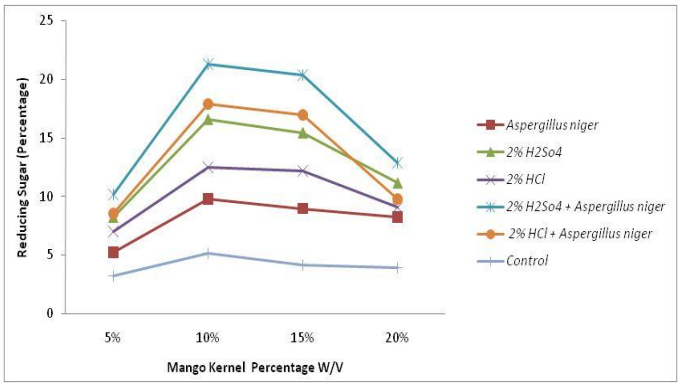

Fig-1. Effect of substrate concentration on reducing sugar.

Maximum yields of total and reducing sugars was obtained when the substrate concentration increased from 5 % to 10 %. However, with increasing the substrate concentration up to 20%; the total and reducing sugar yield gradually decreased (Fig.1). Higher concentration of substrate decreased the saccharification rate as evidenced from lower amount of sugars, providing key indication of substrate inhibiting nature of that enzyme [23].

3.2 Effect of *A. niger*

α- Amylase enzymes are important enzymes employed in the starch processing industries for hydrolysis of polysaccharides such as starch into simple sugar [24, 25, 26]. Most of the fungus are capable of producing high amounts of amylase; *Aspergillus niger* is used for the commercial production of α-amylase [27] using the substrate wheat bran and potato peel, banana peel [28. 29]. The TSS, total, reducing sugar and alcohol production from mango kernel inoculated with *Asperigillus niger* was incubated up to 4 days at 35°C. The highest TSS, total sugar, reducing sugar and alcohol production was obtained by *Aspergillus niger* strain at the substrate concentration 10 %-, followed by substrate concentration15%, 20% and 5% respectively (Fig. 2).

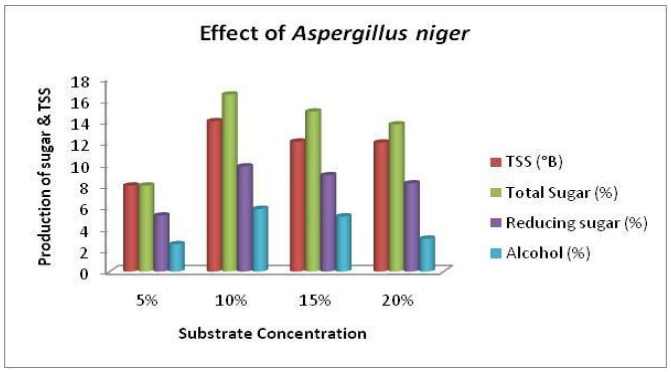

Fig-2. Effect of Aspergillus niger on TSS, Total and Reducing sugar production.

3.3 Effect of sulphuric acid

The release of TSS (total soluble and solid), total sugars, reducing sugar and bio-ethanol from the mango kernel after H_2SO_4 treatment is shown in Fig.3. During acid hydrolysis, increase in the acid concentration, time and temperature favoured the increase in the yields of glucose. That is, the complete breaking of α – 1,4 – and α – 1,6 – glycosidic bonds to glucose (fermentable sugar) occurred after the 60 min. of operation at 2% acid concentration and at 100 °C. Maximum TSS, total suagr, reducing sugar and bio-ethanol were found at the substrate concentration 10 %, followed by the substrate concentration 15%, 20% and 5% respectively.

Miller and Cantor (1952) reported that D-glucose formed during starch hydrolysis process by acid, is dehydrated to the 5-hydroxymethyl furfural as the main product, and lesser amounts of 2-hydroxymethyl furan. Therefore, after 12 h of hydrolysis at 40 °C and 50 °C, there was no sign of decrease in the sugar concentration, probably showing that there was no significant evaporation, at such low temperatures to cause noticeable dehydration. Rather, hydrolysis progressed slowly up to the 12 h.

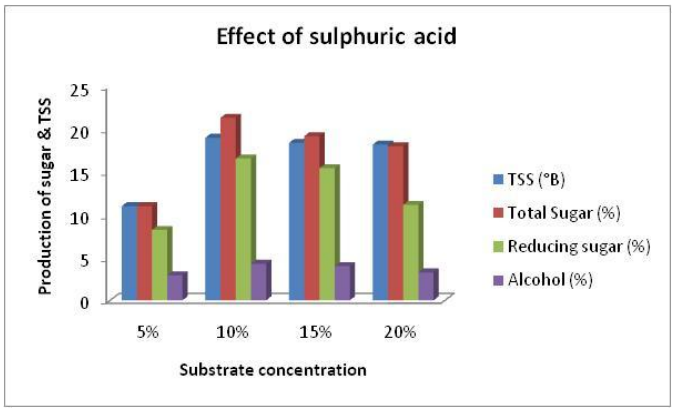

Fig-3. Effect of sulphuric acid on TSS, Total and Reducing sugar production.

3.4 Effect of 2% hydrochloric acid

The results obtained from mango kernel hydrolysis with HCl showed that TSS, total sugar, reducing sugar and bio-ethanol production varied with substrate concentration 10%, followed by 15%, 20%, and5% respectively. Figure 4 show that optimal percentage of TSS, Total and reducing sugar yields on 10% substrate concentration were obtained 17 °B, 19.23% and 12.49% using 2% HCl and hydrolysis at 100°C for 60 min with 2 % HCl.

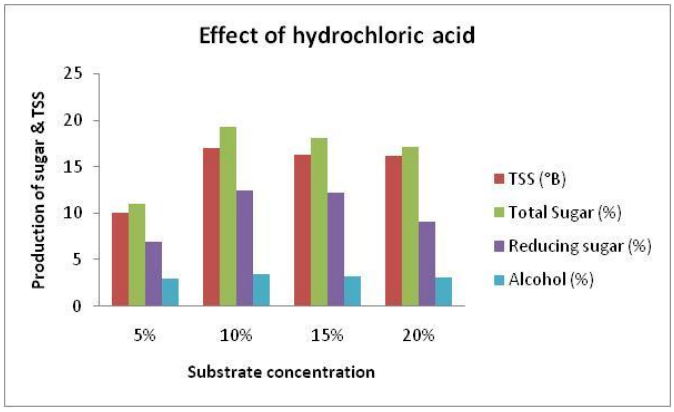

Fig-4. Effect hydrochloric acid on TSS, Total and Reducing sugar production.

3.5 Effect of H_2SO_4 with *A. niger*

To maximize the hydrolytic process *A. niger* was tasted along with the 2% H_2SO4 at pH 5.5 and at 35°C for four days; TSS, total sugar, reducing sugars were determined. Results showed that the combination of H_2SO_4 and *A. niger* was found to be best amongst all the treatments Fig.5.

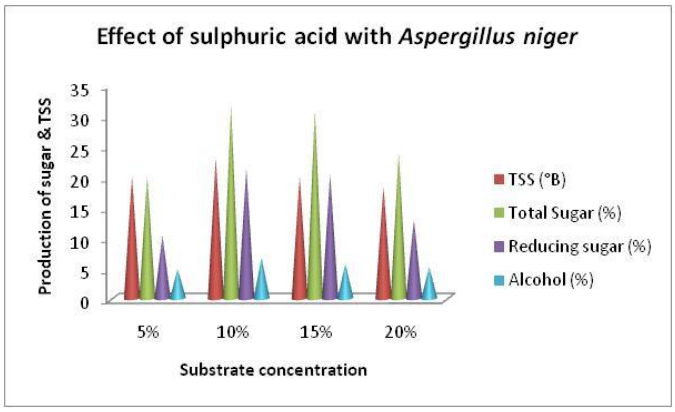

Fig-5. Effect of sulphuric acid with *Aspergillus niger* on TSS, Total and Reducing sugar production.

3.6 Effect of HCl with *A. niger*

The effect of HCl and *A niger* on mango kernel at pH 5.5 was tasted for four days, and after fermentation TSS, total sugar and total reducing sugar were determined. Maximum production was observed at the substrate concentration of 10%, followed by 15%, and 20% while minimum production was observed at the 5% substrate concentration (Fig.6).

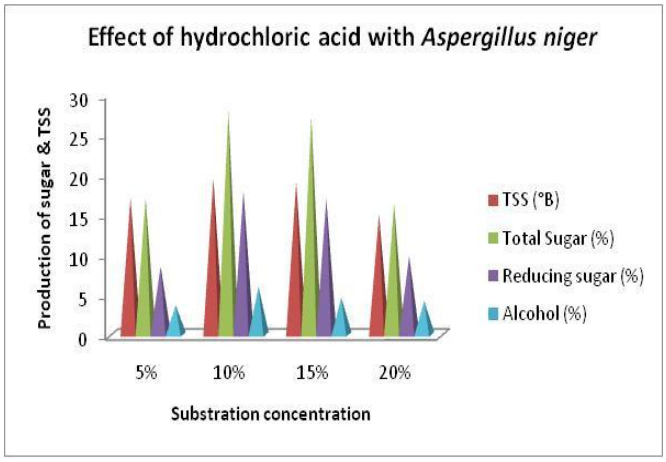

Fig-6. Effect of hydrochoric acid with *Aspergillus niger* on TSS, Total and Reducing sugar production.

4. Conclusion

Present results showed that the combinatorial treatments of 2% H_2SO_4 acid with *A. niger* was most effective among all the treatments, followed by HCL with *A.niger*, 2% $H_2SO_{4,}$ and 2% HCl, and *A.niger* respectively. Therefore, enzymatic hydrolysis was found to be better than acid hydrolysis. So, it can be hypothesized that utilizing both fungus and acid in appropriate combination could hydrolyse the maximum amounts of kernel into reducing sugars. In

addition, there is a dire need to further optimize the mixed effects of HCl, H_2SO_4, and *A. niger* towards hydrolysis of mango kernel. It could be a possible way to reach the industrial scale reality of reducing sugar production from mango kernel.

Fig.7 shows that control (without treated) was found very less hydrolysis and production of TSS, total, reducing sugar, alcohol in comparatively to treated.

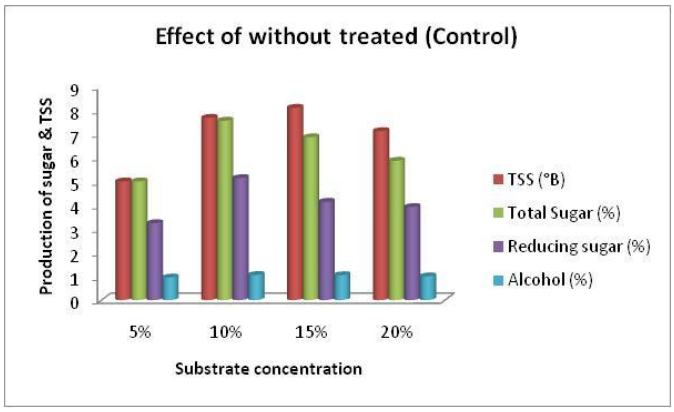

Fig-7.Effect of without treated (control) on TSS, Total and Reducing sugar production.

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