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CONVERSION OF RAGI HUSK (*POLYSACCHARIDES*) TO REDUCING SUGARS BY ACID HYDROLYSIS AND ITS ESTIMATION BY DIFFERENT STANDARD VOLUMETRIC METHODS

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ABSTRACT

Ragi husk is a lignocellulosic source that can be converted to reducing sugars. It was hydrolyzed using sulphuric acid (2M) and hydrochloric acid (2M) at a constant temperature of 65-70 °C by varying time and concentration. It was found that the degradation has significant effects with respect to amount of polysaccharide present and in turn the sugar yield is around 45-50% each which is estimated by Bertrand's, Benedict's and Fehling's methods respectively.

INTRODUCTION

Ragi (Eleusine coracana), known also as finger millet, is an important stable food in certain parts of India (Hilu KW and Wet JM J, 1976). Among the lower socio-economic groups in India, finger millet is the staple cereal. We reported the beneficial effects of including ragi husk at a level of 8% in semi synthetic diets on growth and body composition of albino rats fed ad lib (Hilu KW and Amer. J. Bot., 1979). However the feed intake of animals fed ragi husk was higher than the control animals. To eliminate the possibility of the beneficial effect being due to the high feed intake and also to determine the level of protein in the diet at which addition of ragi husk shows this growth promoting influence, further studies were conducted on albino rats (Wet JMJ and Amer. J. Bot.,1984).

The lignocellulosic biomasses are hydrolyzed to convert hemi-cellulose and cellulose into sugars (Hahn-Hagerdal B *et al*., 2006). According to Badger (Badger PC, 2002) there are two types of hydrolysis, i.e. enzymatic and chemical hydrolysis. Chemical hydrolysis was selected because it is relatively low cost and fast (Palmqvist E *et al*., 2000 and Taherzadeh MJ *et al*., 1999). The dilute-acid hydrolysis of lignocellulosic biomass was run with operating condition of 2M sulphuric acid concentration, 65-70 °C, at various amount of ragi husk.

Experimental

Ragi (Eleusine coracana), was soaked in water for 48 h at 54°C and was flaked in a flaking machine. The flaked material was agitated with water for one hour and passed through 300 mesh sieve. The hydrolysis of ragi husk was carried out at constant stirring using 2M sulphuric acid in a hotplate, equipped with a temperature controller, and continuously shaken during the operation. Initially, measured volumes of water, sulphuric acid or hydrochloric acid with 1g husk sample were put into the beaker and kept under hot plate as well as the temperature controller was adjusted such that the temperature of the mixture is about 65-70°C.

The reaction was expected to be at constant temperature (isothermal), but before that temperature was achieved, reaction has occurred. The hydrolyzed was neutralized to bring the pH to 7, the addition of calcium carbonate to neutralize the excess sulphates and precipitated as calcium sulphate, the addition of lead carbonate to neutralize the excess chlorides and precipitated as lead chloride and activated carbon, followed by filtration. . The concentration of reducing sugar was analyzed by Benedict's, Bertrand's and Fehling's standard procedures.

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The standard methods adopted for the estimation are

1. Bertrand's method (http://www.rakuto-kasei.net/textile/8.index.htmL) is based on the reducing action of sugar on the alkaline solution of tartarate complex with cupric ion; the cuprous oxide formed is dissolved in warm acid solution of ferric alum. The ferric alum is reduced to $FeSO_4$ which is titrated against standardized $KMnO_4$; Cu equivalence is correlated with the table to get the amount of reducing sugar.
2. In Fehling's method (Sausen S *et al*., 1997) sugar solutions is taken in the burette and known volume of Fehling's solution is taken in a conical flask. This is titrated at a temperature 65-70°C. Titration is continued till it acquires a very faint blue color; add 3 drops of methylene blue indicator. The dye is reduced to a colorless compound immediately and the color changes from blue to red (at the end point) (Chandraju S *et al*., 2011) and
3. Benedict's quantitative reagent gives a visual clear end point which turns blue to white by using potassium thiocyanate which converts the red cuprous oxide to white crystals of cuprous thiocyanate; it helps in visual view (Chandraju S *et al*., 2011 and Chandraju S *et al*., 2012)

RESULT AND DISCUSSION

(i) By varying the amount of 2M H_2SO_4 and 2M HCl ranging as below, at constant time (60') and temperature (75°C).

Table 1 Concentrations of strong acids chosen and monitored

Amount of 2M H_2SO_4 / 100[[mL]] distilled water	Amount of 2M HCl / 100[[mL]] distilled water
5.0[mL] (0.1M)	5.0[mL] (0.1M)
15.0[mL](0.3M)	15.0[mL](0.3M)
25.0[mL](0.5M)	25.0[mL](0.5M)

(ii) By varying the time 30, 60, 90 minutes respectively at a temperature 75°C and concentration at the ratio of 2M H_2SO_4 and 2M HCl 15mL / 100mL distilled water as constant, the quantitative values are tabulated in Tables - 2, 3, 4 and 5 respectively and their values are plotted in the figures.1-8. Beyond the mentioned concentration and heating time limit charring occurs.

Table 2 Estimation of sugars by different methods (time is constant (1 h), $[H_2SO_4]$ is varied.

Sl.No	Amount of 2M H_2SO_4 / 100[mL] distilled water	Betrand's method [g]	Benedict's method [g]	Fehling's method [g]
1	5.0[mL] (0.1M)	0.3671	0.3582	0.3573
2	15.0[mL] (0.3M)	0.4522	0.4454	0.4412
3	25.0[mL] (0.5M)	0.5664	0.5725	0.5692

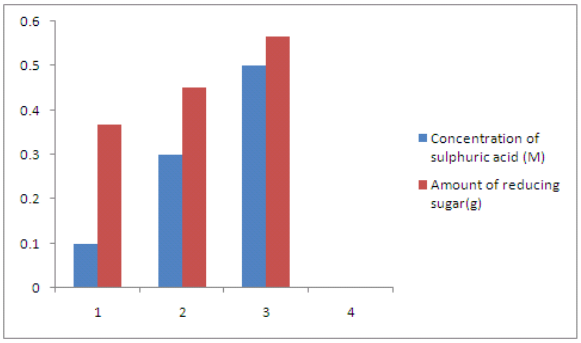

Fig 1 Estimation of reducing Sugar by Bertrand's Method by varying $[H_2SO_4]$.

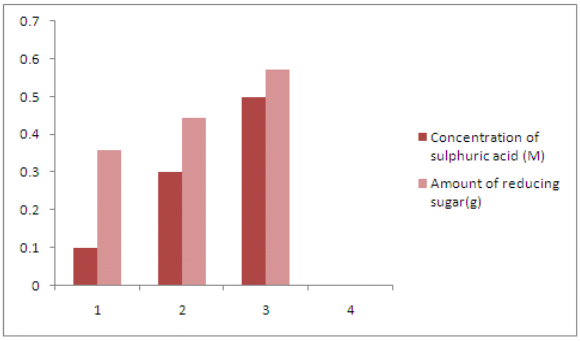

Fig 2 Estimation of reducing Sugar by Benedict's Method by varying $[H_2SO_4]$.

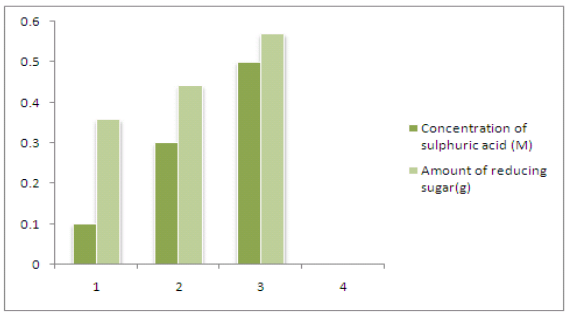

Fig 3 Estimation of reducing Sugar by Fehling's Method by varying $[H_2SO_4]$.

Table 3 Estimation of sugars by different methods at constant acid concentration (15 mL of 2M H_2SO_4 / 100mL water), heating time is varied.

Sl.No	Heating time [h]	Betrand's method [g]	Benedict's method [g]	Lane-Eynon method [g]
1.	1.0	0.4432	0.4449	0.4399
2.	1.30	0.4715	0.4738	0.4753

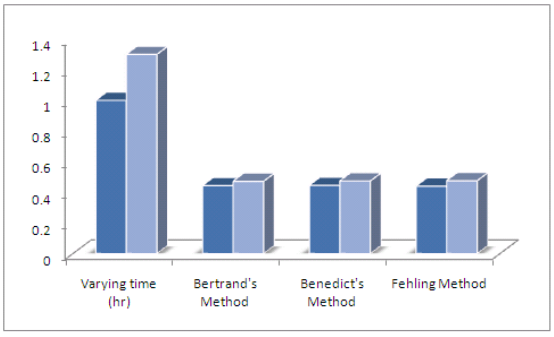

Fig 4 Estimation of reducing Sugar by Bertrand's, Benedict's and Fehling's Method by variation of heating time, ($[H_2SO_4]$ = constant).

Table 4 Estimation of sugars by different methods at constant heating time (1h), [HCl] is varied

Sl.No	Amount of 2M HCl/ 100[mL] distilled water	Betrand's method [g]	Benedict's method [g]	Fehling's method [g]
1	5.0[mL](0.1[M])	0.4308	0.4394	0.4297
2	15.0[mL](0.[3M])	0.4625	0.4700	0.4654
3	25.0[mL]([0.5M])	0.5199	0.5221	0.5233

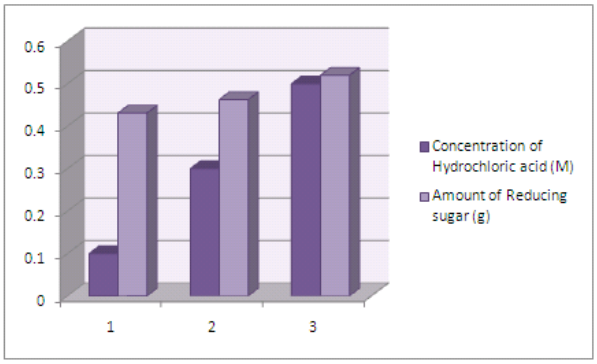

Fig 5 Estimation of reducing Sugar by Bertrand's Method by varying [HCl].

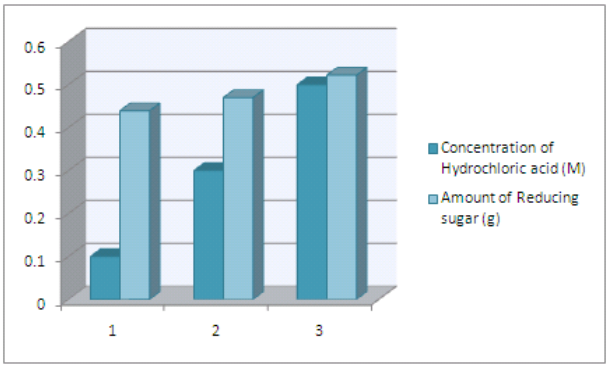

Fig 6 Estimation of reducing Sugar by Benedict's Method by varying [HCl].

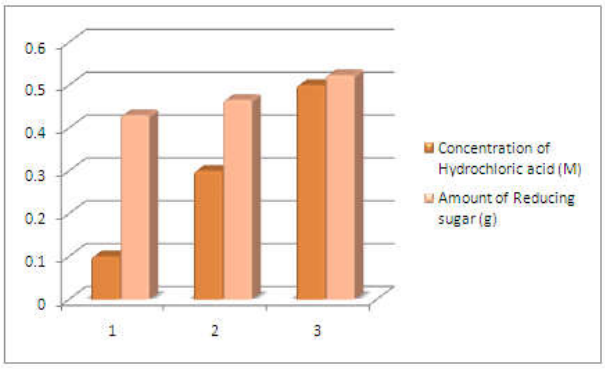

Fig 7 Estimation of reducing Sugar by Fehling's Method by varying [HCl].

Table 5 Estimation of sugars by different methods at constant acid concentration (15 [mL] of 2M H_2SO_4 per 100[mL] water) by varying heating time

Sl.No	Heating time [h]	Betrand's method [g]	Benedict's method [g]	Fehling's method [g]
1.	1.0	0.3995	0.4020	0.4003
2.	1.30	0.4710	0.4778	0.4757

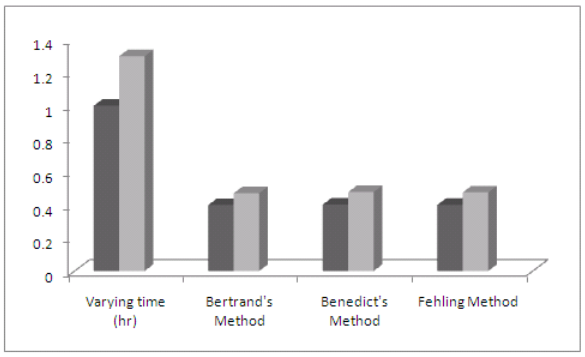

Fig 8 Estimation of reducing Sugar by Bertrand's, Benedict's and Fehling's Method by variation of heating time ([HCl] =Constant).

CONCLUSION

Ragi is used in many Indian dishes and the polysaccharide is hydrolyzed enzymatically to get glucose which gives energy. In the present work, the mimicking conversion is exhibited through a simple acid hydrolysis process by using different acids like 2M H_2SO_4 and 2M HCl are employed to obtain reducing sugars and the yield also runs up to 45-50% which is authentically reported by analytical standard procedures in an economical way.

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