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Bio-mimic conversion of Maida (polysaccharides) to reducing sugars by acid hydrolysis and its estimation using standard methods

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Abstract

Maida flour is highly refined wheat flour. after the wheat is ground in a roller mill (Chakki), the same is filtered through a fine mesh (600 meshes per square inch) to obtain maida. Maida is a lignocellulosic source that can be converted to reducing sugars. The hydrolysis of maida is carried out using (2M) Sulphuric acid and (2M) hydrochloric acid in a hotplate, equipped with a temperature controller, and continuously shaken during the operation. (i) By varying the concentration of 2M acids ranging as below, where time 60 min and temperature 75°C are kept as constant. (ii) By varying the time 30,60,90 min respectively keeping 75°C and concentration at the ratio of 2M acids (15 mL/100 mL) distilled water as constant. It was observed that the degradation has significant effects with respect to the amount of polysaccharide present and in turn the sugar yield is around 40-50% each which is estimated by Bertrand's, Benedict's and Fehling methods respectively. The quantitative values are tabulated and their values are plotted. Beyond the mentioned conditions charring occurs.

Introduction

Wheat grains are void in shape, rounded at both ends. The components present in Indian wheat are: Moisture, Starch, Sugars, Proteins, Crude fiber, Fat, Ash. In wheat, practically all of the starch is in the endosperm, while the soluble sugars are mostly found in the germ. Carbohydrates of bran are largely cellulose and hemicelluloses. Cereals and cereal products, like semolina (rava), refined flour (maida) and whole-wheat flour (atta), commonly in pre-packed form, are sold in the retail market (Mrozikiewicz *et al*., 1994).

Traditional procedure of milling wheat in India is stone grinding to obtain whole meal flour (atta). In modern milling, wheat is first subjected to cleaning to remove various types of impurities together with damaged, shrunken and broken kernels which are collectively known as 'screenings'. The cleaned wheat is subjected to conditioning. This improves the physical state of the grain for milling. This process involves adjustment of the average moisture content of the wheat. When the moisture content is optimum the bran is toughened and separation of endosperm from the bran becomes easy. The cleaned and conditioned wheat is subjected to milling finally to separate the endosperm from the bran and the germ. The reduced endosperm is known as flour (white flour) and the germ, bran and residual endosperm obtained as by-products are used primarily in animal feeding.

Flour milling is achieved by grinding in roller mills. Grinding is carried out in four or five stages in a gradual reduction process. The different sized particles are sorted by shifting and the coarse particles are conveyed to a subsequent grinding stage. In each grinding stage, endosperm is separated from the bran coats. The percentage of wheat converted into flour from the first grind to the fourth grind will be approximately 30, 66, 78 and 81. Wheat is ground into atta (whole meal), suji or rava (semolina) and maida (flour). Within these there are finer divisions like high gluten and low gluten atta, and low gluten, medium gluten and high gluten maida (Lenzen, 2008).

The lignocellulosic biomasses are hydrolyzed to convert hemi-cellulose and cellulose into sugars (Hahn-Hagerdal *et al.*, 2006). According to Badger (Badger, 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 (Taherzadeh *et al*., 1997, Palmqvist and Hagerdal, 2000). The dilute-acid hydrolysis of lignocellulosic biomass was run with operating condition of 2M sulphuric acid concentration, 65-70 °C, at various amount of maida.

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Materials and Methods

The hydrolysis of maida 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 maida 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 chlorides and activated carbon, followed by filtration. The concentration of reducing sugar was analyzed by Benedict's, Bertrand's and Fehling standard procedures.

The standard methods adopted for estimation are;

(i) Bertrand's method 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.

(ii) In Fehling method (Sausen Silmi *et al.*, 1997) sugar solutions is taken in the burette and known volume of Fehling solution is taken in 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 (Chidan Kumar *et al.*, 2011) and (iii) Benedict 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 (Chidan Kumar *et al.*, 2012).

Results and Discussion

The concentration of the hydrochloric and sulfuric acid solutions used for this study which ranges from 0.3 to 0.5 M. The quantitative values of the reducing sugars obtained by Bertrand's, Fehling's and Benedict's methods at different concentration of sulfuric acid and at constant heating time and at a temperature of 75°C are presented. The same data are graphed in Figure 1. Table 1 shows that increasing the concentration of sulfuric acid from 0.1 to 0.5 M at constant heating time and at 75°C increases the reduced sugar from 0.2700 to 0.569 g from 0.3158 to 0.5714 g and from 0.2873 to 0.5454 g using Bernard's, Benedict's and Fehling's methods respectively. Table 2 shows that by increasing the heating period from 1 hr to 1.30 hr while maintaining the concentration of sulfuric acid and the temperature constant at 0.3 M and 75°C respectively; the amount of reduced sugar increases from 0.4152 to 0.4715 (13.6%) from 0.4454 to 0.4833 (8.5%) and from 0.400 to 0.4985 (19.8%) using Bernard's, Benedict's and Fehling's methods respectively.

The same experiments were repeated by using hydrochloric acid solutions (0.3-0.5M) at constant heating time and at constant temperature or by using constant acid concentration and constant temperature while changing the heating time. Table 3 and by increasing the concentration of the HCl from 0.3

Table 1. Heating time is kept constant (1 hr) while the concentration of sulfuric acid is varied

Sl.No	Amount of 2M H_2SO_4 / 100mL distilled water	Betrand's method /g of maida	Benedict's method /g of maida	Fehling's method /g of maida	Mean	St dev (s)
1	5.0mL(0.1M)	0.270	0.3158	0.2873	0.291033	0.023127
2	15.0mL(0.3M)	0.415	0.4454	0.4000	0.420133	0.023131
3	25.0mL(0.5M)	0.5685	0.5714	0.5454	0.651767	0.014248

Table 2. Concentration is kept constant (0.3 M) while heating time is varied

Sl.No	Heating time (hr)	Betrand method /g of Maida	Benedict method /g of Maida	Fehling method /g of Maida
1.	1	0.4152 g	0.4454 g	0.4000 g
2.	1.30	0.4715 g	0.4833 g	0.4985 g

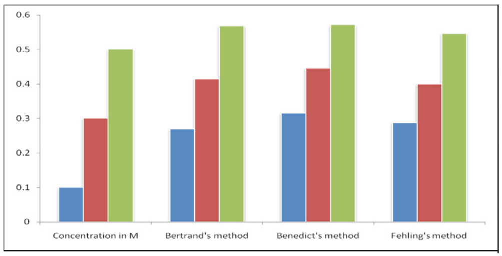

Figure 1. Estimation of reducing Sugar by Bertrand's, Benedict's and Fehling's Method by varying the concentration of sulfuric acid

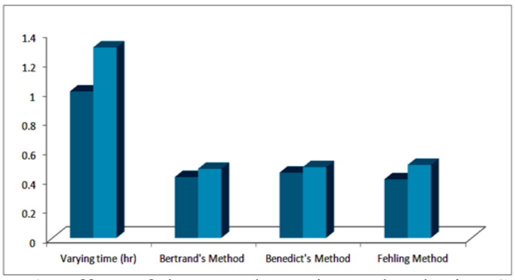

Figure 2. Effect of time on the estimated reducing Sugar by Bertrand's, Benedict's and Fehling's Methods at constant H_2SO_4 concentration (0.3 M).

Table 3. Effect of the concentration of hydrochloric acid on the reducing Sugars using the three different methods, while keeping the heating time constant (1 hr)

Sl.No	Amount of 2M HCl / 100 mL distilled water	Betrand method /g of maida	Benedict method /g of maida	Fehling method /g of maida	Mean	STDEV (s)
1	5.0mL(0.1M)	0.4108g	0.4379g	0.4200g	0.4229	0.013781
2	15.0mL(0.3M)	0.4609g	0.4762g	0.4700g	0.4690	0.007696
3	25.0mL(0.5M)	0.5099g	0.5217g	0.5200g	0.5172	0.006379

Table 4. Effect of heating time on the reducing sugars, while keeping the concentration of sulfuric acid constant (0.3M)

Sl.No	Heating time (in hr)	Betrand method /1g of maida	Benedict method /1g of maida	Fehling method /1g of maida
1.	1	0.3993g	0.4082g	0.4098g
2.	1.30	0.4693g	0.4762g	0.4717g

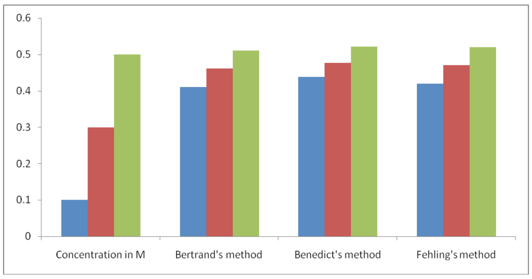

Figure 3. Estimation of reducing Sugar by Bertrand's, Benedict's and Fehling's Method by varying the concentration of hydrochloric acid

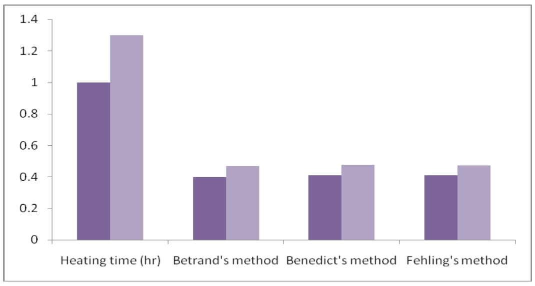

Figure 4. Effect of time on the estimated reducing Sugar by Bertrand's, Benedict's and Fehling's Methods at constant HCl concentration (0.3 M)

to 0.5M, the reduced sugar increases from 0.4108 to 0.5099 (19.4%), from 0.4379 to 0.5217 (19.1%) and from 0.4200 to 0.5200 (23.8%) using Bernard's, Benedict's and Fehling's methods respectively. Table 4 shows that by increasing the heating period from 1hr to 1.30 hr while maintaining the concentration of HCl and the temperature constant at 0.3 M and 75°C respectively; the amount of reduced sugar increases from 0.3993 to 0.4693 (17.5%) from 0.4082 to 0.4762(16.5%) and from 0.4098 to 0.4717(15.1%) using Bernard's, Benedict's and Fehling's methods respectively.

Beyond the mentioned concentration and heating time limit charring occurs. From all the above values it is clear that the value doesn't differ much. After all the analysis the maximum reducing sugar value runs to 40-50% on the whole. There is no absurd difference in the yield of sugars when there is a change of acid whether it is either sulfuric acid or hydrochloric acid. Concentrations of both strong acids say sulphuric and hydrochloric acid chosen and monitored are 0.1M, 0.3M,0.5M which are prepared from 2M stock concentration of the acids where 5 mL, 15 mL,25 mL respectively are made up to 100 mL in a standard flask using distilled water.

Conclusion

Maida 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 the application of various acids like 2M H_2SO_4 and 2M HCl under two conditions 1) By varying the concentration of acid at constant temperature and time of heating and 2) By varying the time of heating at constant concentration and temperature, the amount of reducing sugars are monitored and the yield percent also runs up to 40-50% which is authentically reported by analytical standard procedures in a cost-effective manner.

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