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Research Article

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Estimation of reducing sugar by acid hydrolysis of black grape (*Vitis vinifera* L.) peels by standard methods

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ABSTRACT

Black grape peels (Vitis vinifera L.) are polysaccharide source that can be converted to reducing sugar. Black grape peels was hydrolyzed using (0.2N) sulphuric acid at temperature of 65-70˚C. It was observed that the degradation has significant effect with respect to amount of peel taken and in turn sugar yield is around 40-50%, each which is estimated by Bertrand's, Benedict's and Lane-Eyon method.

Keywords: Degradation, hydrolysis, black grape peels, reducing sugar, estimation.

INTRODUCTION

Black grape (*Vitis vinifera* L.) is one of the world's largest fruit crops. World production of grape was 67 million metric tonnes from 7.3 million hectares in 2007. Black grape contains the solid waste with extreme waste production as high as 20% generally consists of skins and seeds. One product produced from black grape waste is the black grape seed oil, which has many nutritional properties like Cholesterol free, low in saturated fats, contains linoleic acid and high-density lipoprotein, and rich in Vitamin E and antioxidants [1]. In India, black grapes are seasonal and extensively available during summer. It contains flavonoids and they are phytonutrients that give the vibrant purple color to black grapes, grape juice and red wine; the stronger the color, the higher the concentration of flavonoids [2].

Polyphenol compounds are of great importance as food and drink products derived from plant, since these compounds are responsible for their organoleptic properties, makes their analysis of considerable interest. The consumption of foods rich in polyphenols helps in the prevention of cardiovascular diseases (CVD), certain types of cancer and other diseases related to aging, because of their antioxidant properties [3]

Black grapes has excellent sources of manganese and good sources of vitamin B6, thiamin (vitamin B1), potassium, and vitamin C. Health benefits of black grapes include its ability to treat constipation, indigestion, fatigue, kidney disorders, macular degeneration and prevention of cataract. Black grapes, is one of the most delicious fruits, which contain rich sources of vitamins A, C, B6 and folate in addition to essential minerals like potassium, calcium, iron, Phosphorus, magnesium and selenium.

Black grapes contain flavonoids that are very powerful antioxidants, which can reduce the damage caused by free radicals and slacken ageing. Good blood and body builder, it is also a quick source of energy [4].

The great advantage of wine as a matrix for polyphenols in the diet is that in wines they are present in soluble state and are hence more biologically available, in contrast with plant foods that contain their polyphenol compounds in polymeric, insoluble or strongly bonded forms and are thus less available for absorption. Most of the past research dealing with the analysis of polyphenols [5]

The black grape peels suppress the immune system [6], it upsets the mineral relationship in the body [7] sugar can increases fasting levels of glucose [8], and also sugar contribute to the reduction in defense against bacterial infection [9].

The importance of estimation of reducing sugar from black grape peel was various kinds of sugars and compound sugars are involved in bodily functions and as source of energy. Sugars are used as raw materials in food processing and pharmaceutical industries. More over utilization of the agro-industrial wastes for sugar production will be of immense benefit at preventing the pollution hazards associated with these waste.

EXPERIMENTAL SECTION

REAGENTS USED

- Fehling A,
- Fehling B,
- Methylene Blue indicator.
- Benedict's quantitative reagent
- Ferric Alum: KMnO4
- Oxalic acid (AR)

The hydrolysis of black grape peels was carried out with constant stirring using 50 ml of 0.2N sulphuric acid in a hot plate, equipped with a temperature controller, and continuously shaken during the operation. Initially, 50mL of 0.2 N sulphuric acid solution (20 mesh) black grape peels were put into a beaker and kept on hot plate and the temperature of the mixture was maintained to about 65-70˚C. The reaction was expected to be at constant temperature (isothermal), but before that temperature was achieved, reaction has occurred. It was neutralized to bring the pH to 7 by the addition of calcium carbonate and activated carbon, followed by filtration. The concentration of reducing sugar was analyzed by the following standard procedures (i, ii and iii).

(i) Bertrand's method [10] is based on the reducing action of sugar on 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. This is based on the alkaline solution of tartarate complex of cupric ion.

(ii) In Lane-Eynon method [11] sugar solution is taken in a 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. At this stage 3 drops of methylene blue indicator is added. The dye is reduced to a colorless compound immediately and the end point is changing of blue to red. In this method it is susceptible for interference from other type of molecules that act as reducing agent.

(iii) In Benedict's method pipette out benedict's quantitative reagent in to a clean conical flask. The contents were heated to a temperature of 60-70ºC. Then it is titrated against unknown sample solution till the appearance of chalky white precipitate. 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 [12].

CHEMICAL REACTIONS

Cu^+ (Red precipitate) →CuSCN (White precipitate)

Ferro cyanide helps to prevent the deposition of cuprous oxide and dissolve and thiocyanate helps to convert red cuprous oxide to white crystals of cuprous thiocyanate.

$R\text{-}CHO+Cu^{2+} + 5OH^{-} \rightarrow Cu_2O$ + {Different species of oxidized sugar}

The cuprous oxide thus formed is dissolved in warm acid solution of ferric alum. The ferric alum is reduced to $FeSO_4$ and is titrated against $KMnO_4$.

$Cu_2O + Fe_2(SO_4)_3 + H_2SO_4 \rightarrow 2CuSO_4 + 2FeSO_4 + H_20$

$10FeSO_4 + 2KMnO_4 + H_2SO_4 \rightarrow K_2SO_4 + 2MnSO_4 + 5Fe_2(SO_4)_3 + 8H_2O$

RESULTS AND DISCUSSION

By varying the amount of black grape (*Vitis vinifera* L.) peels (1, 2, 3, 4 and 5g) at constant temperature (65-70˚C) and concentration of sulphuric acid is 0.2N is fixed constant. The experiment resulted in the data of reducing sugar concentrations at 3 hour from Benedict's method(0.521,1.042,1.562,2.082 and 2.606g), Bertrand's method(0.502,1.004, 1.542, 2.064, and 2.512g) and Lane-Eynon method(0.501,1.002,1.506,2.005and 2.512g) respectively. Were reported below **Table 1** and there corresponding data are plotted which are shown in **figures 1, 2 and 3**.

Table 1: Amount of Reducing Sugar Estimated by different methods

Weight of Black grape (Vitus Vinifera. L) Peels (g)	Benedict's Method(g)	Bertrand's Method(g)	Lane-Eynon method(g)
1.01	0.521	0.502	0.501
2.002	1.042	1.004	1.002
3.021	1.562	1.542	1.506
4.004	2.082	2.064	2.005
5.002	2.606	2.512	2.512

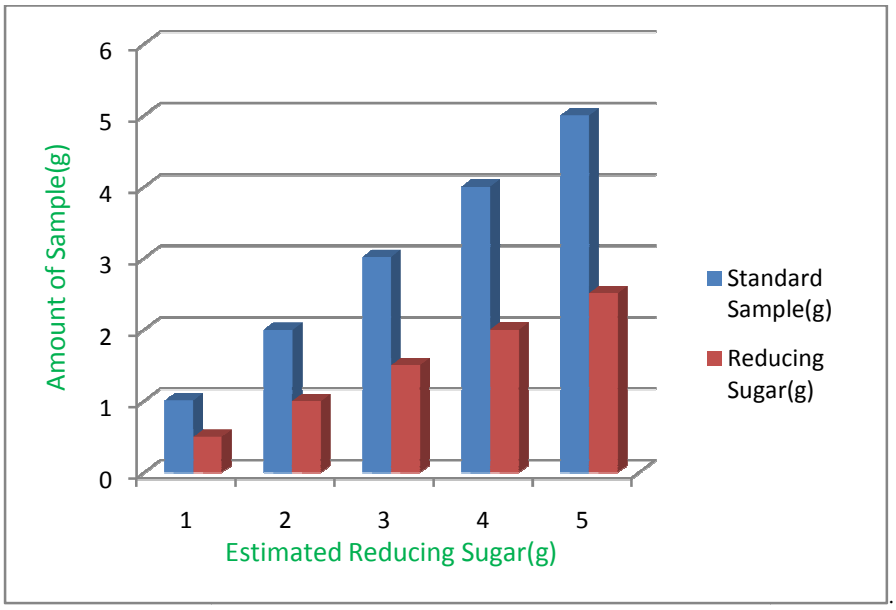

Figure 1: Estimation of reducing sugar by Benedict's method

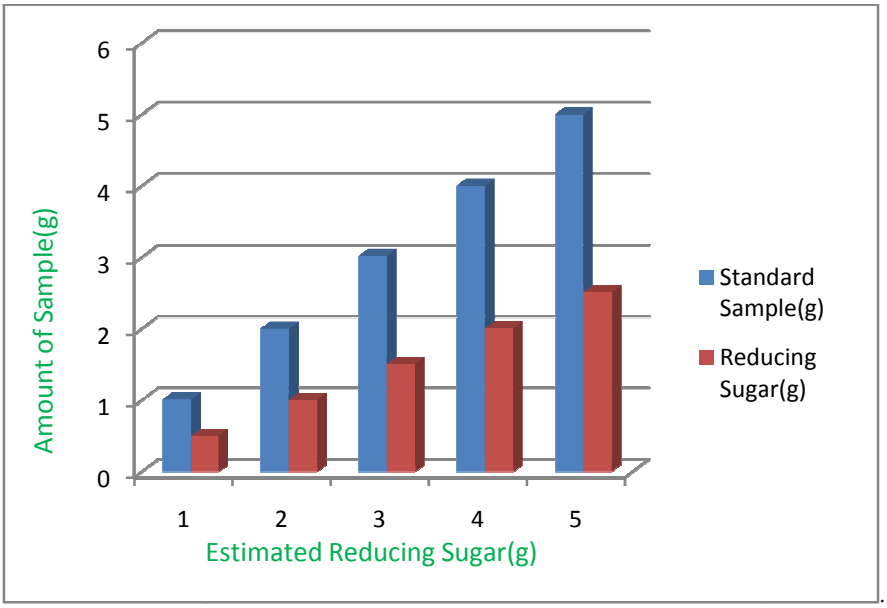

Figure 2: Estimation of reducing sugar by Bertrand's method

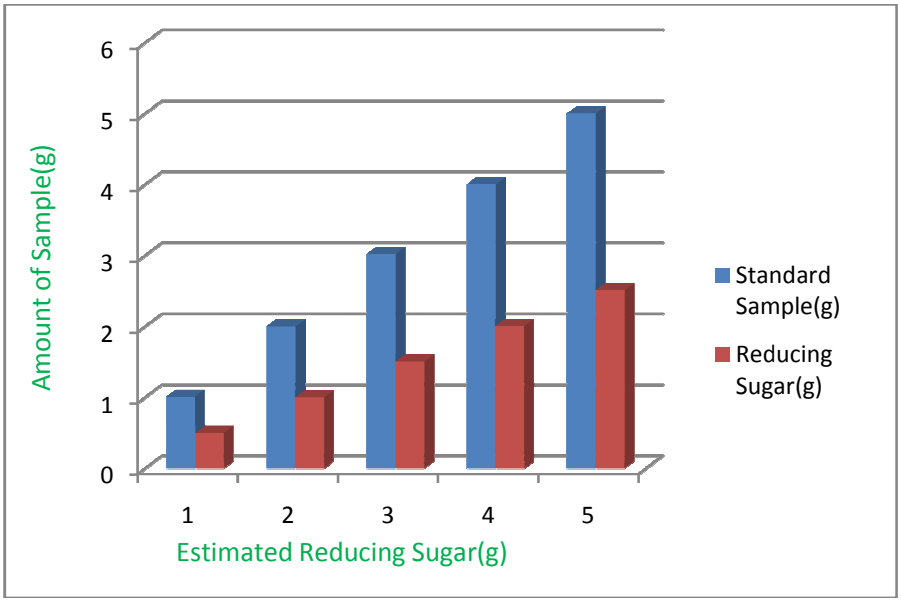

Figure 3: Estimation of reducing sugar by Lane-Eynon's method

CONCLUSION

A fruitful and economic industrial application was applied in this current work. Based on the above studies, a standard method for the estimation of reducing sugar has been developed.While there are some uses generally, in the production of wine but still it is considered a waste product. In this work, we have applied simple hydrolysis process to obtain reducing sugar which is very good consumable source of energy and runs up to 40-50% which is authentically reported by analytical standard procedures in an economical way [13, 14].

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