



## Biochemical Analysis of Ash Guard Peel Optimization for a Noble Substrate for the Growth of *Bacillus Subtilis*

### KEYWORDS

Zoya Javed

Department of Biotechnology,  
Meerut Institute of Engineering and  
Technology, Meerut (India)

Shailendra Kumar Srivastva

Department of Biochemistry and  
Biochemical engineering, Jacob  
School of Biotechnology and  
Bioengineering, Sam Higginbottom  
institute of agriculture technology  
and sciences, Allahabad (India)

Gyan Datta Tripathi

Department of Biochemistry and  
Biochemical engineering, Jacob  
School of Biotechnology and  
Bioengineering, Sam Higginbottom  
institute of agriculture technology  
and sciences, Allahabad (India)

**ABSTRACT** *Ash Guard (Benincasa hirsuta) found all over India and having medicinal and economic importance. In order to optimize an alternative substrate for Bacillus subtilis, a proximate analysis of ash guard peel has been carried out which is a solid waste. Ash guard peels were found to contain 93.1% moisture content, 0.62 mg/g organic content, total soluble sugar 1.1 mg/g, reducing sugar 0.81 mg/g, total lipid 0.3 mg/g, total protein content 12.5 mg/g, and total phenol 5.17 mg/g. The results indicate that the ash guard peels are rich in proteins, phenol and sugar content. So due to these contents the ash guard peel can be a good substrate for growth of microorganisms like Bacillus species.*

### Introduction

Ash Guard found all over India and having medicinal and economic importance. A delicious sweet named petha is prepared by ash guard and its peel becomes useless and waste. Since a very large industry of petha sweet is situated in Allahabad. Everyday approx 500-700 kg of ash gourd peel is thrown as waste, which causes a major problem of solid waste management. The peels of ash guard are rich in protein, carbohydrates, lipid and phenolic components in large amount.

Various species of microorganism require artificial growth medium which contain majority of carbon sources like sugars or carbohydrates and protein content besides this a little amount of trace element are also needed. Since ash guard peels having these contents can be provided as a substrate for growth of the various microorganism which are able to produce some industrially important products like enzyme, organic acid, vitamins etc. So by this study we try to standardize a new substrate to produce for growth *Bacillus* sp. In this study ash gourd peel was provided as a substrate to *Bacillus subtilis* for growth.

### Materials and methods

#### Collection of Ash Guard peels:

A well established small scale industry of petha sweet is situated in Rambag market Allahabad. Peels were collected from Rambag market Allahabad from local sources.

**Total moisture content:** The total moisture content was estimated by the method of **A.O.A.C., (1965)**

**Ash content (organic portion):** The total ash content was estimated by the method of **A.O.A.C., (1965)**.

**Determination of total Soluble sugar:** The determination of total sugar was elucidated by the method of **Hedge et al., 1962** and measured the green to dark green colour at 630 nm using glucose as standard.

**Determination of reducing sugar:** The determination of reducing sugar was estimated by the method of **Samogyi M., 1952**, taking glucose as standard (working standard 100 µg/ml) at 630 nm.

**Protein estimation:** The total protein content was esti-

mated by the method of **Lowry's et al., 1951**, Taking the reading of sample at 660 nm against standard Bovine Serum albumin (BSA) curve.

**Extraction of Lipids:** Extraction of total lipid from fresh sample by the method of **Jayarrman, J., 1981**. The tissue is extracted in 3:1 ether and ethanol mixture followed by centrifugation and separation by adding KCl solution which helps in layer separation and salts prevent emulsification. The lipid layer is then dried, weighed and the amount of the lipid is calculated.

#### Phenols:

The total phenol content was estimated by method of **Bray and Thorpe, 1954**. Standard curve using different concentration of catechol measure the absorbance at 650 nm.

#### Bacterial Strain and Growth Conditions (standard media)

(**Yavuz, et al., 2004**): The bacterium, thermophilic *Bacillus* sp., that was used in this study, was collected from Dept. of Microbiology and Fermentation Technology (J.S.B.B., SHIATS). Thermophilic *Bacillus* was cultivated overnight in 2xYT media, at 55°C and 200 rpm. 2xYT (Yeast Extract Tryptone) medium consisted of 1.6g Tryptone, 1.0g yeast extract and 0.05 g NaCl per 100 ml of water with a final pH of 7.0.

#### Synthetic Media for Bacterial Growth (Maria et al., 1981):

The Synthetic media was prepared for the growth of *B. subtilis* according to the % content of Ash gourd peel which determined in lab 0.87% protein content, 0.8% sugar, 0.84% phenol, 0.3% lipid, was cultivated overnight at 55°C and 200 rpm.

#### Submerged Fermentation:

The peel of substrate was grinded and powder of peel was autoclaved and then hydrolysed for 2 hours. Filter the hydrolysed sample, aliquots of the hydrolysed sample was taken in the ratio of 1:1, 1:2, 1:3 with water in a 100 ml flask and inoculated one loop full culture of *B. subtilis*. Incubated the flask in shaker for 48 hrs. the best growth observed in the sample 1:1 followed by 1:2 dilution ratio.

#### Results and discussion

##### Proximate analysis of Ash Gourd peel:

The study on the proximate composition/contents, of the Ash

Gourd peel has been carried out using recommended method of analysis. The result of the analysis shows that the moisture content 93.1%, ash 0.62 mg/gm, protein 12.4mg/gm., Reducing sugar 0.81 mg/gm., Lipid 0.3mg/gm., total phenol 5.17 mg/gm. respectively. Standard graphs are given from figure 3.1 to 3.4. Earlier these findings were coined by Chinedu and Nwinyi, (2012). The results shown in the Table No 1.

Table No. 1: Proximate analysis of Ash Gourd peel

S. No	Particulars	Results
1	Moisture content (%)	93.1 %
2	Ash/Organic (mg/gm)	0.62 mg
3	Total soluble sugar(mg/gm)	1.1 mg
4	Reducing Sugar (mg/gm)	0.81 mg
5	Total Lipid Content(mg/gm)	0.3 mg
6	Total Protein (mg/gm)	12.4 mg
7	Total Phenol (mg/gm)	5.17 mg

\*all the experiment performed in triplets and results are in average value

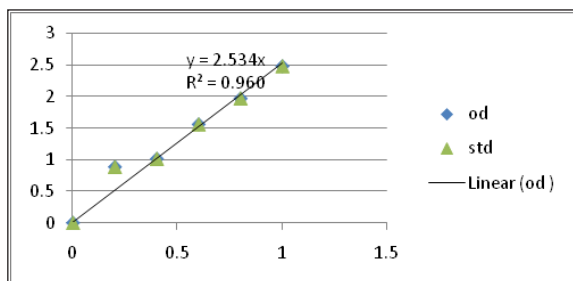


Figure 1: Standard curve of total soluble sugar

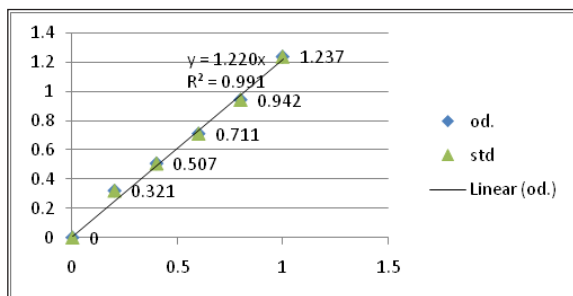


Figure 2: Standard curve of reducing sugar

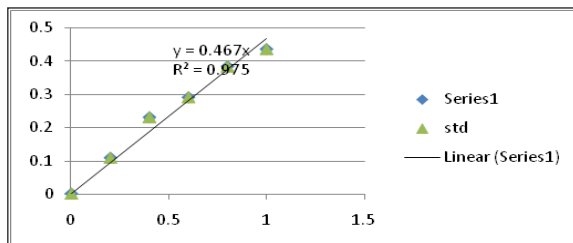


Figure 3: Standard curve of Bovine Serum Albumin

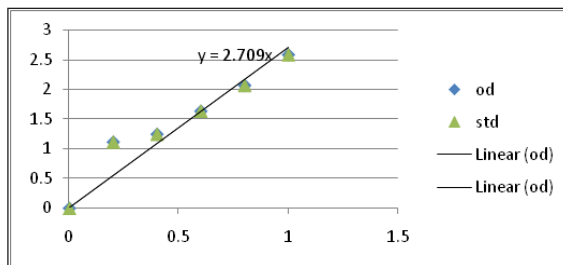


Figure 4: Standard curve of Phenol

Synthetic media for bacterial growth

The growth of micro organisms is greatly influenced by media components, especially carbon and nitrogen sources, and physical factors such as temperature, pH, and incubation time and inoculums density. It is important to produce the enzyme in inexpensive and optimized media on a large scale for the process to be commercially viable; hence the studies on the influence of various physico-chemical parameters such as incubation periods, inoculum size, temperature, pH, carbon, and nitrogen sources. Agricultural byproducts rich in cellulosic biomass can be exploited as cheap raw material for the industrially important enzymes and chemicals. The fermentation medium was inoculated with the bacterial strain and incubated for certain time (1-3days). Growth curve of the *Bacillus subtilis* is given comparatively in Figure 3.5 this indicated that ash gourd peels can be used for growth and production media substrate without any other external content for growth.

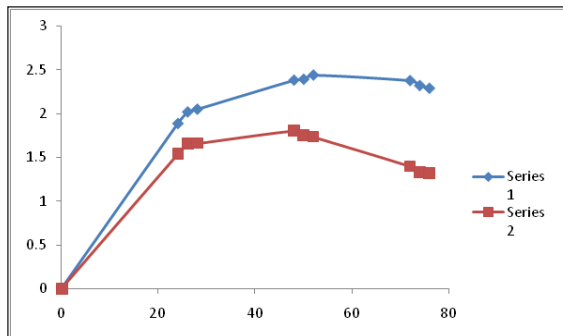


Fig 5: The figure indicate that comparative growth curve of *Bacillus subtilis* between Hydrolyzed sample (series 2) and standard media (series 1).

Conclusion

Peel of Ash Gourd is became a waste after its usage . The peel of Ash Gourd contain sufficient amount of protein approximately 12.4 mg.and Sugar 1.1 mg. Phenol 5.17 mg, Lipid 0.3mg., etc.Polyphenol oxidase is an important enzyme which responsible for formation of brown pigment to the wounded tissue. It is also used in various industrial applications. Poly phenol oxidase can be produced by various microorganisms by providing appropriate substrate eg. *Bacillus subtilis*. Ash Gourd peel having significant amount of protein, sugar and other growth supplements for growth of *Bacillus subtilis*. Since a high phenolic and protein content it may influence the use of production of poly phenol oxidase and other enzymes like protease and vitamins like riboflavin. The process may be screen for other microorganisms like fungi etc. for the production of useful products.

REFERENCE

1. A.O.A.C. (1965) Official methods of analysis of the association of Official Agricultural Chemists, 10th edn., Washington, D.C. | 2. Bray H. and thorte W.V (1954) Meth. Biochem. Anal.1: 27-53. | 3. Chinedu S. N. and Nwinyi C. O.(2012) Proximate analysis of *Sphenostylis stenocarpa* and *Voadzea subterranean* consumed in South –Eastern Nigeria. Journal of Agricultural Extension and Rural Development. 4(3): 57 –62. | 4. Hedge J.E. and Hofreiter B.T. (1962) Carbohydrate Chemistry. In: 17(Eds. Whistler R.L. and Be Miller, J.N.). Academic Press. New York. | 5. Lowry O.H., Rosebrough N.J., Farr A.L and R.J Randall (1951) J.Biol.Chem.193-265. | 6. Somgyi M. (1952) J.Biol.Chem.200-245. | 7. Jayaraman J. (1981) In :Laboratory manual in biochemistry,Wiley Eastern Limited,New Delhi p.96. | 8. Yavuz E., Gunes H., Harsa S. and Yenidunya A. F. (2004) Identification of extracellular enzyme producing thermophilic bacilli from Balçova (Agamemnon) geothermal site by ITS rDNA RFLP. Journal of Applied Microbiology, 97 (4):810-817. |