Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 7, Issue, 10, pp.21043-21045, October, 2015

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

ISSN: 0975-833X

RESEARCH ARTICLE

PRELIMINARY STUDIES ON *BETA*-AMYLASE FROM *SYZYGIUM CUMINI* FRUITS OF SOUTH INDIA

***Jyothilekshmi, S. and Aswathy J Panicker**

Affiliation Biotechnology Division, Union Christian College, Aluva, Kerala, India

ARTICLE INFO

Article History:
Received 08th July, 2015
Received in revised form
26th August, 2015
Accepted 21st September, 2015
Published online 20th October, 2015

Key words:

Beta amylase,
Syzygium cumini,
Underutilized fruit,
Kinetic parameters,
Immobilization.

ABSTRACT

New usages of old crops hold promise to restore the balance of trade reduce our dependence on imports and to meet the growing needs of industrial products of human population. Fruit plants are playing a vital role in providing nutritional and economical security to the poor mass in rural areas. There are many plants still underexploited and unexplored. Jamun (*Syzygium cumini*) is an important fruit crop belongs to family Myrtaceae. It is an underutilised indigenous fruit tree of South India. The present study was an attempt to exploit *Syzygium cumini* as a new plant source of choice for beta amylase enzyme for industrial production because the enzyme is abundant in the fruits, which allows for relatively low cost production. Beta amylase was extracted from *Syzygium cumini* fruits and was successfully immobilised in calcium alginate beads. The pH optima for free and immobilised beta amylases were found to be 6.5 and 6.8 respectively, time of incubation in the range of 15-20 minutes, optimum substrate concentration in the range of 0.5 % - 1.25% and optimum temperature were 400C for free and 450 C for immobilised beta amylases activities.

***Citation*:** Jyothilekshmi, S. and Aswathy J Panicker, 2015. "Preliminary studies on beta-amylase from *Syzygium cumini* fruits of South India", *International Journal of Current Research*, 7, (10), 21043-21045.

INTRODUCTION

India is a rich resource of wild/ underutilized fruits due to the diversity in climate, soil, altitudes and other eco-geographical conditions. These fruits are chiefly used by the tribals/ local inhabitants as a natural source of treatment for curing various diseases and ailments. (Rao, Palada, and Becker, 2004) Since they possess an array of chemical diversity they provide unlimited opportunities for screening of new bioactive compounds. The post harvest technology of these crops is poor. Since the farmers do not get sufficient returns from the fruits of these crops, they do not bother even to harvest and are removing plants of these nutritionally rich crops. The present study is an attempt to explore *Syzygium cumini* fruits of South India as a new source of β -amylase enzyme for industrial use. Further studies can also be designed to strengthen the post harvest handling of this fruit so as to provide sufficient amounts of this enzyme to industries and hence it can be an additional source of income for farmers of Kerala.

Amylases constitute a class of industrial enzymes which alone form 25% of the enzymes market covering industrial processes such as brewing sugar, textile, paper, distilling industries and pharmaceuticals and are employed in the conversion of starch into different sugar solutions (Olufunke *et al*., 2012), (Mamo *et al*., 1999), (Pandey *et al*., 2000), (Oudjeriouat *et al*., 2003). Amylases originate from different sources such as plants, animals and microorganisms (Olufunke *et al*., 2012). In plant, β – amylase is distributed in higher plants such as soybean, sweet potato, barley etc., (Chang *et al*., 1996; Oudjeriouat *et al*., 2003). The properties of the β - amylase varies from one source to the other (Olufunke *et al*., 2012). Unlike other members of the amylase family, only a few attempts have been made to study β– amylases of plant origin. The fruits of *Syzygium cumini* – a tree which is on verge of elimination because of underutilisation and deforestation have not been considered for β– amylase study. Hence the present study.

Jamun (*Syzygium cumini*) is an important minor fruit of Indian origin commonly known as Black plum, found growing widely in different agro-climatic conditions. (Patel and Rao, 2012). *Syzygium cumini* (Family Myrtaceae) is also known as *Syzygium jambolanum* and *Eugenia cumini*. Other common names are Jambul, Black Plum, Java Plum, Indian Blackberry, Jamun etc. The plant is a large evergreen tree found in plains and hills throughout India. It is an important medicinal plant in various traditional systems of medicine. (Swami *et al*., 2012). The ripe fruits are used for health drinks, making preserves, squashes, jellies and wine (Warrier *et al*., 1996).The blooming period is from March to April and the tree fruits once in a year (May- June) (Ramya *et al*., 2012).

****Corresponding author: Jyothilekshmi, S.***
Affiliation Biotechnology Division, Union Christian College, Aluva, Kerala, India.

The fruits are largely eaten raw, the purplish coloured flesh of the fruit is sweetish sour to taste and leaves the tongue (and the lips) dark purple-tinged for several hours. The supply for β - amylase enzyme has been limited to edible plants such as barley, wheat and soya bean until now. Considering the worldwide food crisis, it is necessary to explore alternative sources for β- amylase supply. The present work was an attempt to extract, β- amylases from fruits of *Syzygium cumini*, to immobilise and then compare the characters of crude and immobilised.

MATERIALS AND METHODS

Collection and preliminary studies of plant materials

Fresh fruits of *Syzygium cumini* were collected during March – May 2011 from the premises of Union Christian College, Aluva, Ernakulam District, Kerala, South India. The pericarp of fruits was taken and preliminary experiments were conducted for presence of β-amylase activity.

Extraction of enzymes

The fruits were washed with distilled water, cut opened and fresh pericarp was collected. Then 1g of pericarp was taken and grind thoroughly in a mortar and pestle with 10 ml of 0.02M phosphate buffer of pH 7, containing 0.85 % NaCl as stabilizing agent. The extract is filtered through a muslin cloth and then through filter paper and kept in the freezer stored at 4^o C as the enzyme source. The extraction of enzyme was done in ice cold condition.

Enzyme Immobilization

Calcium alginate beads were prepared with an equal volume of crude enzyme solution and sodium alginate solution to form a 2% (w/v) final concentration of sodium alginate in the mixture. The mixture of enzyme and alginate was taken into a syringe (0.8 mm diameter), and beads were formed by dropping the solution into $CaCl_2$ (5% w/v) solution with gentle stirring at 4^oC for 1 h. The formed beads were recovered by filtration and were thoroughly washed with distilled water to remove excess $CaCl_2$. The beads were dried using filter paper (Whatman no. 1) followed by exposure to the open air for 1 h before use (Demirkan *et al*., 2011).

Study of kinetic parameters of the free and immobilised β-amylases

Determination of β -amylase activity

The activity levels of β-amylases were determined by measuring the maltose liberated in micro mole/ min/ g tissue when treated using 3-5 dinitrosalicylic acid. One unit of beta amylase activity was defined as the amount of enzyme required to produce one micromole of maltose from starch under the assay condition (Bernfeld, 1955). The protein content was determined by Lowry method (Lowry *et al*., 1951).

Optimum pH

To determine the optimum pH, the enzymes were incubated in a set of different concentrations of phosphate buffers of pH ranging from 6 to 8 and the reaction was performed at 40°C for 15 minutes

Optimum temperature

The optimum temperature for maximum enzyme activity was determined by varying incubation temperature of the reaction mixture from 30°C to 60°C.

Optimum substrate concentration

To determine the optimum substrate concentration, various amount of starch ranging from 0.5% - 1.25% were used and the reactions were carried out 40^oC and 45^oC for 15 min for free and immobilized enzyme respectively.

Optimum incubation time

The optimum incubation time for maximum enzyme activity for both the free and immobilized enzymes were determined by varying incubation time of the reaction mixture from 5 to 30 minutes.

RESULTS AND DISCUSSION

Results obtained during the study takes the form of a table below (Table 1)

Table 1.

Parameters	Crude	Immobilised
Activity	2.22U/g tissue	2.00U/g tissue
pH	6.5	6.8
Temperature	40^o C	45^o C
Incubation time	15 minutes	20 minutes
Substrate concentration	1.25 %	1.25 %

During the study it was observed that β-amylase was highly active at 35^o C to 40^o C with maximum activity at 40^o C for free enzyme and 45^o C for immobilised enzyme. Similar work by (Rani, 2012) in pulses justifies the result. In this study the pH optimum for beta amylase activity was found at pH 6.5 for free enzyme and 6.8 for immobilised enzyme. These are pretty comparable to earlier reports (Rani, 2012) in sprouted pulses. The immobilized amylase activity was higher than the free enzyme at these temperatures and pH conditions. During the study of effect of substrate concentration, both free and immobilised enzyme activities were progressively increased with the increase in starch concentration from 0.25 up to 1.25%. These results were agreed with previous reports (Rani, 2012). Our present study also showed that both the β -amylase had maximum time of incubation in the range 15-20 minutes with an optimum time of incubation for free enzyme was at 15 minutes and for immobilised one it was at 20 minutes. These results were comparable to earlier reports by Rani (2012).

Conclusion

Hence, β -amylase was extracted from *Syzygium cumini* fruits and was successfully immobilised in calcium alginate beads. Studies on their kinetic parameters showed that the immobilisation do not interfere with the β amylases activities.

Since the supply for β - amylase enzyme has been limited to edible plants such as barley, wheat and soya bean, it is worthwhile to explore the *Syzygium cumini* fruits, as an alternative potential source for supply of β- amylase industrially.

Acknowledgement

The authors wish to thank the management of Union Christian College, Aluva, Kerala for providing necessary facilities for carry out research on this medicinally important tree and Dr. Sandhya C, Assistant professor, K. E. College, Mannanam, Kerala for the support provided for this study.

REFERENCES

Bernfield, P. 1955. Amylases: α and β. *Methods in Enzymology*, 1, 147-154.

Demirkan, E., Dincbas, S., Sevinc, N., and Ertan, F. 2011. Immobilization of B. amyloliquefaciens - amylase and comparison of some of its enzymatic properties with the free form. *Romanian Biotechnological Letters*, 16(6), 6690–6701.

Mamo, G., Gashe, B. A. and Gessesse, A. 1999. A highly thermostable amylase from a newly isolated thermophilic Bacillus sp. WN11. *Journal of Applied Microbiology,* 86(4), 557–560.

Olufunke, F.O. T. and Azeez, I.I. 2012. Purification and characterization of beta-amylase of Bacillus subtilis isolated from kolanut weevil. *Journal of biology and life science,* 4(1), 68–78.

Oudjeriouat, N., Moreau, Y., Santimone, M., Svensson, B., Marchis-Mouren, G. and Desseaux, V. 2003. On the mechanism of α-amylase. Acarbose and cyclodextrin inhibition of barley amylase isozymes. *European journal of biochemistry,* 270(19), 3871–3879.

Pandey, A., Nigam, P., Soccol, C. R., Soccol, V. T., Singh, D. and Mohan, R. 2000. Advances in microbial amylases. Biotechnology and applied biochemistry, 31(2), 135–152.

Patel, P. R and Rao, T.V.R. 2012. Antibacterial Activity of Underutilized Fruits of Jamun (*Syzygium cumini* l. skeels). *International journal of current pharmaceutical research,* 4(1), 36–39.

Ramya, S., Neethirajan, K., and Jayakumararaj, R. 2012. Profile of bioactive compounds in *Syzygium cumini* – a review. *Journal of pharmacy research,* 5(8), 4548-4553.

Rani, K., 2012. Extraction and study of kinetic parameters of variety of sprouted pulses β-amylases. *International journal of pharmacy and life sciences,* 3(8), 1893–1896.

Rao, M. R., Palada, M. C. and Becker, B. N. 2004. Medicinal and aromatic plants in agroforestry systems. Agroforestry systems, 61-62(1-3), 107–122.

Warrier, P. K., Nambiar, V. P. K. and Ramankutty, C. 1996. Indian medical plants, 5: 225-228.

****