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Mesocarp of *Terminalia catappa* L.-A Potential and Cost Effective Source for the Production of Alpha 1, 5- L Endo- Arabinase

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Abstract : The fruit of *Terminalia catappa* L. is used as a nutrient source in the formulation of the production media for Alpha 1, 5- L Endo- Arabinase**.** The mesocarp promoted the production of the enzyme (% activity of 71%) when compared to the whole fruit (60.6%) and the other epicarp layers-Endocarp (26%), Kernel (28%). The incubation time required for peak production also varied with the fruit and the epicarp layers, with the kernel peaking at the earliest in the fifth day of production. The presence of endo-arabinase activity was confirmed with the azurin cross linked (AZCL) debranched arabinan assay. The study proposes a value addition to the mesocarp which is otherwise considered to be a waste material.
Keywords : Alpha 1, 5- L Endo- Arabinase, *Terminalia catappa* L, AZCL debranched arabinan, epicarp, mesocarp, kernel, % activity.

Introduction

The substrate of alpha 1, 5-L-endo-arabinase (endo-arabinase) is arabinan. Arabinan consists of a backbone of α-1, 5-linked L-arabinofuranosyl residues which are substituted with α-1, 2- and α-1, 3-linked L-arabinosyl. Alpha 1, 5-L-endo-arabinase acts on the alpha-1, 5 linkage of arabinan liberating arabinose. Arabinan and hemicelluloses together constitute the major part of plant cell wall. This is a subset of heterogeneous mixture of polysaccharides present in the cell wall which includes xylans, glucans, mannans, galactans, and arabinans[1].

Endo-arabinase is used in different application due to its specific action on the substrate such as bio fuel production, feed processing, juice clarification, bio film removal etc[2-4]. The production of enzyme using specific substrate is cost prohibitive. So, in order to reduce the production cost of the endo- arabinase, formulation of cost effective production media is the need of the hour. The reported use of soya chunk powder as a nutrient source in the media formulation is a step in that direction[1]. In the described method the use of *Terminalia catappa* L. (Indian almond) fruit is used as a nutrient source for the enzyme production. The rational in using this plant is based on the fact that it belong to the same class *Rosids* to which Prunis dulcis (Almond) belongs, whose composition is well studied[5]. Arabinan rich pectic polysaccharide is isolated from the cell wall material of *Prunis dulcis* with a molecular mass of 762kDa. The arabinan rich isolated substrate showed (1-5) ara*f* backbone[6-9]. So for the present study ripened red colored *Terminalia catappa* L. fruit is used as a nutrient source.

Terminalia catappa L. is grown in tropical and subtropical regions of India and Pacific oceans. It is mainly cultivated for its timber and nuts[10]. It is known in different names such as tropical almond, Malaysian almond etc. Flavanoids and numerous volatile compounds are isolated from fried nut that are used in different folk medicines. Fruit meal fermented by *Aspergillus niger* is used as feed in poultry. The phenolic compounds present in the plant is used for treatment of cardiovascular, diabetics, cancer and Alzheimer's diseases[11-13]. Fruit of *Terminalia catappa* are composed of three layers namely, fleshy mesocarp, middle hard endocarp and inner edible kernel that have a nut with thin seed coat (Fig.1.). For the present study, initially, the whole fruit was used as a nutrient source to check its usefulness. Subsequently, different parts of the fruit were assessed for their utility as a nutrient source. Different parts of the fruit owing to their difference in chemical composition (such as carbohydrates) are used in various applications[14].

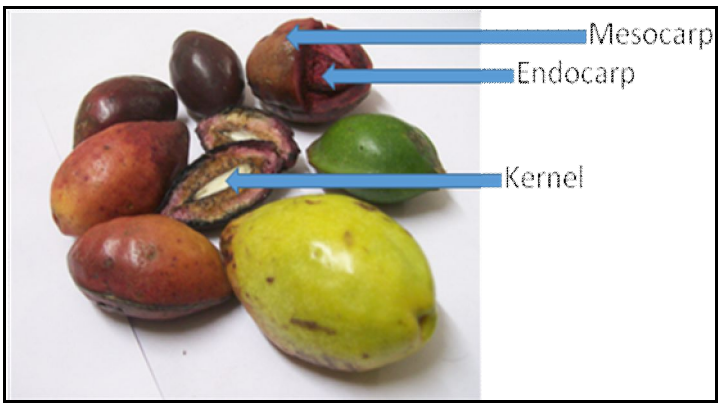

Fig.1. *Terminalia catappa* L. fruit and its pericarp layers.
Source: - https://upload.wikimedia.org

Experimental

Materials and Methods

Debranched arabinan from sugar beet and AZCL debranched arabinan were purchased from Megazyme. Bovine serum albumin was purchased from Himedia. The fungal culture used for the study is isolated from the precincts of Amrita School of Biotechnology, Amritapuri, Kollam, Kerala, India[1]. The organism was sequenced and identified as *Aspergillus niger* and the sequence is deposited in GenBank (Accession No.KU987575).

Preparation of the Substrate.

T. catappa fruits were collected from the vicinity of the school. Fully ripened, matured red coloured fruits were chosen for the study. The fruit was washed primarily with distilled water and then surface sterilized by using 70% ethanol. It is then washed immediately with distilled water to remove the ethanol content which may interfere with the growth of organism.

Production Media Preparation

Initially, whole fruits were supplemented as nutrient source in the minimal media[15]. The fruits were crushed using mortar and pestle and then ground to small pieces. As in the previous study on soya chunk powder[1] the substrate concentration of 1.5% was used in the media formulation. Initial pH of 4 was kept constant for all the production media. To the autoclaved media, chloramphenicol was added at a concentration of 35µg/mL to inhibit the growth of bacteria. *Aspergillus niger* spores were inoculated in the media and kept for incubation at 200rpm in 30^0c. The set up was prepared in triplicate. The enzyme activity and specific activity was determined after 7 days of incubation. The presence of endo-arabinase activity was confirmed with the AZCL debranched arabinan assay[1].

Second set of production media was prepared by separately using the mesocarp, endocarp and kernel of the fruit. Each part of the fruit is supplimented at a concentration of 1.5% in the minimal media and the initial

pH was set at 4 for all the three production media. Inoculation and conditions of incubation are as mentioned above. Productions were carried out in triplicates. The enzyme activity and specific activity were determined from the 4th day of incubation extending till 11th day.

Determination of Enzyme Activity.

To determine the endo- arabinase activity Dinitro Salicilic Acid (DNS) assay was performed with L-arabinose as standard at a concentration ranging from120 µg/mL to 600µg/mL. Debranched arabinan was used as the substrate which is specific for endo-arabinase. Three sets of controls, DNS control, substrate control and enzyme controls were also run simultaneously to account for the presence of other reducing sugars that could be expected in a non specific substrate. To determine the specific activity Bradford assay was performed by keeping the BSA as the standard at a concentration ranging from 200 µg/mL to 1mg/mL. The specific activity was expressed in U/mg.

Results and Discussion

***T. catappa* Fruit as a Nutrient Source**

In preliminary investigation whole fruit was used as nutrient source and compared with the enzyme activity of the crude preparation of the enzyme using debaranched arabinan. The Specific activity of the crude enzyme was found to be 1.32U/mg and 2.1U/mg for fruit as a whole and debranched arabinan as nutrient source respectively (Fig.2.). In the case of whole fruit enzyme activity peaked after 8 days of incubation and on the contrary, debranched arabinan peaked on 5th day.

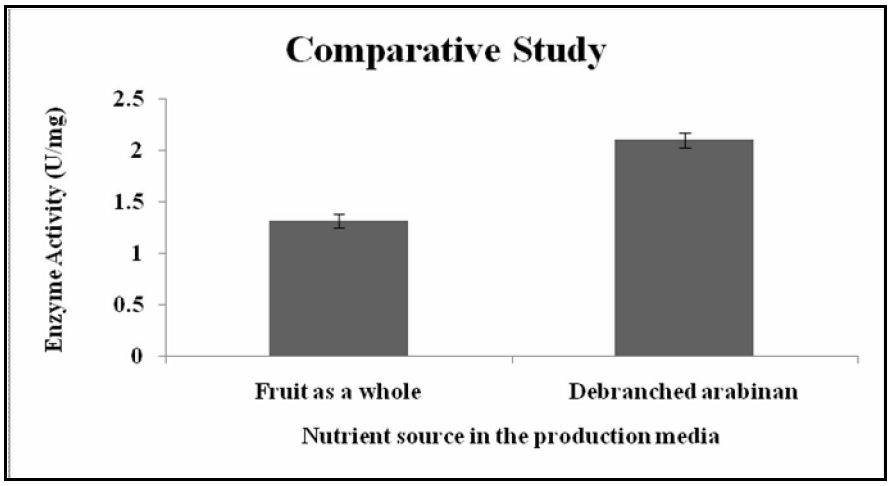

Fig.2. Comparative study of the fruit and debranched arabinan as nutrient source.

Epicarp Layers of the Fruit as a Nutrient Source

Mesocarp

Specific activity of the enzyme was checked from fourth day of incubation, as the enzyme activity showed a steady increase from the fourth day and peaked on seventh day of incubation. On seventh day enzyme activity showed maximum of 1.55U/mg, and on eighth day enzyme activity decreased and maintained a steady activity on continued incubation (Fig.3.).

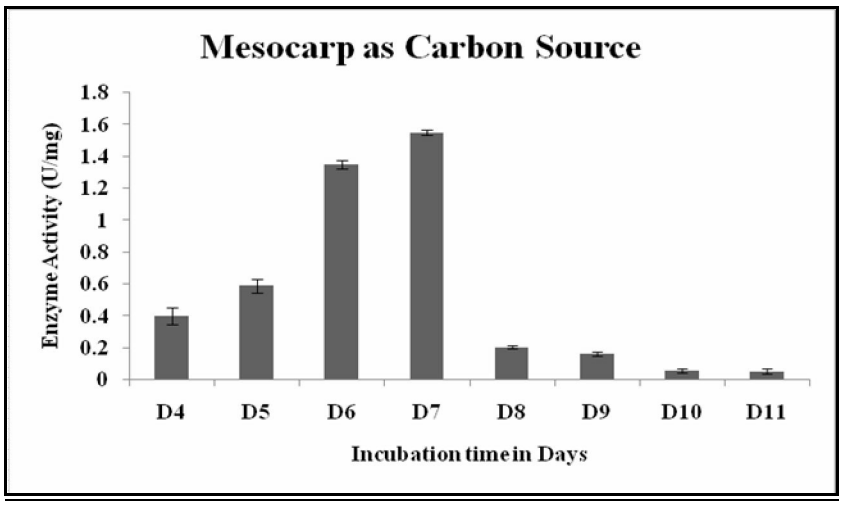

Fig.3. Specific activity of the enzyme in the presence of Mesocarp as a nutrient source.

Endocarp

Specific activity of the enzyme in the production media where endocarp is used as the carbon source peaked in a relatively long incubation time. The enzyme activity showed an increase on sixth and seventh day but showed a reduction on the eighth day. It again peaked and reached its maximum 0.57U/mg on tenth day (Fig.4.). The reduction of the enzyme activity might be due to the degradation of the enzyme or due to the production of other protein[16]. It can be also due to the hard endocarp.

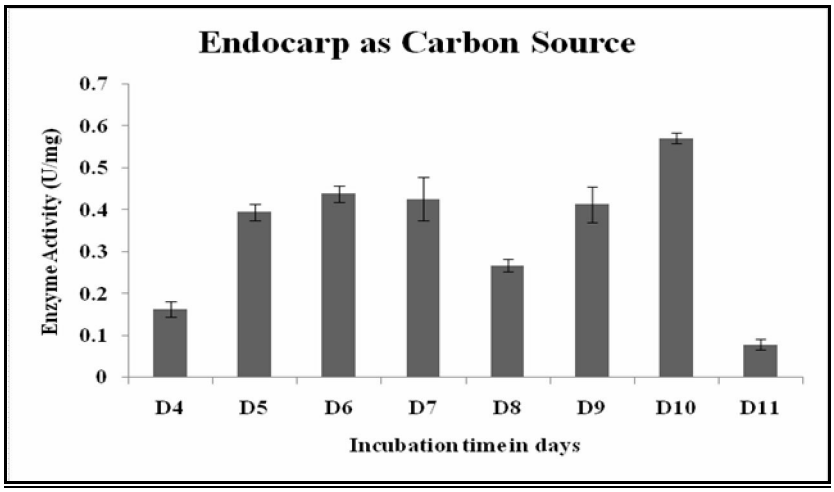

Fig.4. Specific activity of the enzyme in the presence of Endocarp as a nutrient source.

Kernel

Kernel is composed of a seed with a membranous seed coat and is used for the enzyme production. In this case, specific activity peaked on the fifth day of incubation and showed a maximum of 0.63U/mg. Then the activity of enzyme decreased and maintained a steady production till the eleventh day (Fig.5.).

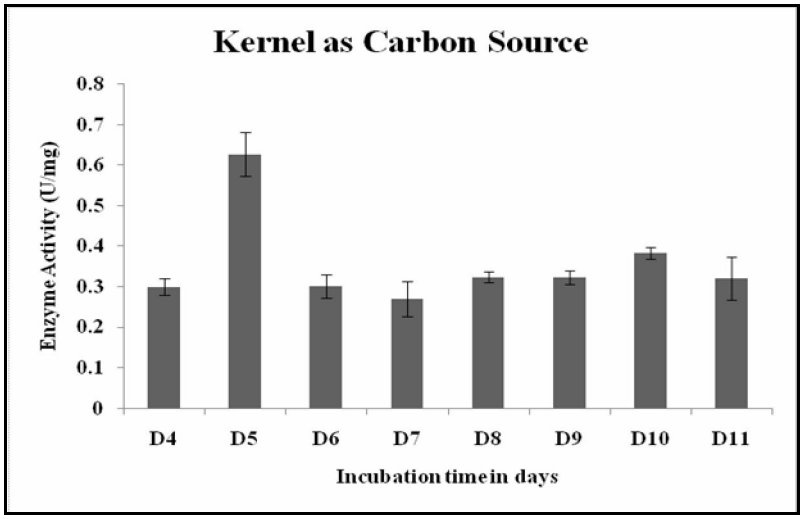

Fig.5. Specific activity of the Enzyme in the presence of Kernel as a nutrient source.

Comparative Study of the Different Nutrient Source on Enzyme Production.

Comparative study of the whole fruit and the epicarp layers- mesocarp, endocarp, kernel of *T. catappa* with the specific substrate debranched arabinan was carried out. The mesocarp showed higher activity (71%) when compared to the whole fruit (60.6%) and other epicarp layers (endocarp-26%, kernel-29%) (Fig.6.). The %activity of the substrate was deduced from the value during their peak production.

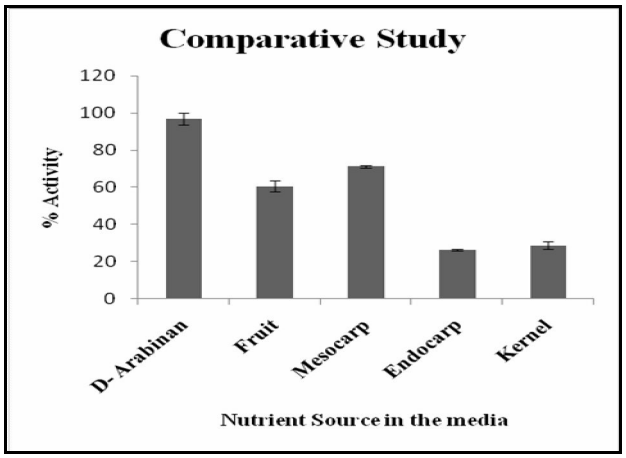

Fig.6. Comparative study of the specific activity in the presence of different nutrient source. (D-Debranched)

Conclusion

The described study establishes the suitability of *Terminalia catappa* L. fruit and its epicarp layers as nutrient source for the cost effective formulation of production media for Endo-arabinase. The study also indirectly underlines that the fruit of *T. catappa* has sufficient arabinan to sustain the enzyme production. This has not been reported till date. Mesocarp when compared to the whole fruit and other epicarp layers was efficient as a nutrient source for the Endo-arabinase production. Hence this study proposes value addition to the fruit mesocarp which is considered to be a waste material. Further, for the optimisation of the substrate, Response Surface Methodology (RSM) would be carried out. A pre-treatment strategy also needs to be incorporated in the current protocol to increase the enzyme production.

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