Extraction of Bromelain from pineapple waste

N. Lakshminarasimaiah, RajaRajeshwari B Vibhuti , Barnali Ghosh

Abstract— The Pineapple is most popular of all tropical fruits. It is a non citrus fruit and is grown in hot regions all around the world. Pineapple is the leading edible member of the family Bromeliaceae. In the present study the main focus is on utilizing the waste i.e., the Core, Crown, and peel to extract "Bromelain" which is proteolytic enzyme and has great value in the pharmaceutical industry.

Index Terms—Bromelain, Core, Crown, peel, Pineapple ,Proteolytic enzyme, tropical fruits

1.INTRODUCTION:

India ranked fifth with a share of 8.2% of the world production of pineapples. (K.L. Chadha, B.M.C. reddy 1998, "Pineapple", 96-97). Kew and Mauritius are the two varieties of pineapple grown in India. The major pineapple producing states in India are Assam, Karnataka, Kerala, Meghalaya and West Bengal. Pineapple in the leading edible member of the family Bromeliaceae. It is a popular ingredient in fruit drinks and Juice products such as juice concentrates, Jaws, squash, jellies, essence, pickles ect . In the processing units only the pulp part of the fruit is used which contains large quantity of Juice. The other part of the fruit such as Crown, bottom leaves, core ,peel etc goes as solid waste.

After juice in extracted around 60% of fruit goes as waste (Eckenfelder W.W.et al 1958)
and in offer diluted with wash water and in disposed into sewer or effluent treatment plant. Solid waste obtained are either subjected for composting or dumped in an open site, which again causes nuisance to the environment. The waste in very wet and apparently useless and it is uneconomically to dry in order to use the fibrous residues as boiler fuel. It is also unsuitable to use it as cattle feed, because they induce diarrhea and other disorders. The high moisture content and development of acidity during the decomposition of thin wet waste prevented its utilization as compost or its direct return to the soil as an organic fertilizer. Hence value addition for these solid waste is done by extracting proteolytic enzyme. Bromelain obtained from the solid waste can be used in medical therapy (H.R. Maurer. 2001 BROMELAIN :)

Heinicke introduced Bromelain as a therapeutic Compound. It is a general name for a family of Sulfahydryl protolytic enzymes obtained from Ananas Comosus, the pineapple plant. Bromelain also contains a peroxidase, acid phosphatase, several protease inhibitors, and organically bound calcium. Research on bromelain apparently was first conducted in Hawaii but more recently has been conducted in countries in Asia, Europe and Latin America. Germany has recently taken a great interest in bromelain research where bromelain in currently the 13th most widely used herbal medicine. Bromelain in present in all parts of the pineapple plant (Ananas Comosus) but the stem is the most common commercial source, presumably because it readily available after the fruit has been harvested. Pineapples have a long tradition as a medical plant among the natives of south and Central America.

Bromelain can be used in a vast array of medical conditions. It is an anti-inflammatory agent and so can be used for sports injury, trauma, arthritis, and other kinds of swelling. Its main uses are athletic injuries, digestive problems, phlebitis, Sinusitis, aiding and healing after surgery.

It has also been proposed in the use of arthritis, chronic venous insufficiency, easy bruising, gout hemorrhoids, menstrual pain, auto immune disorders and ulcerative colitis ,studies have shown that bromelain can be also be useful in the reduction of platelet clumping and blood clots in the blood stream, especially in the arteries. It may even be useful in the treatment of AIDS to stop the spread of HIV. It has no major side effects, except for possible allergic reactions. (William V. et.al)

1. *N. Lakshminarasimaiah ,Professor, Email: laknar_05@yahoo.com*
2. *RajaRajeshwari B Vibhuti ,Assisstant Professor, email:rajbvibhuti@gmail.com*
3. *Barnali Ghosh ,Associate Professor , email:barnalighosh08@gmail.com*

Department of civil Engineering, East Point college of Engineering and Technology, East Point Campus, Jnana Prabha, Virgo Nagar Post, Bangalore 560-049

2.MATERIALS AND METHODOLOGY:

To carry out protein concentration by Bradford method Comassie Brilliant Blue (CBB) was purchased from Sisco research laboratories private limited. Photophoric acid and ethanol were procured from Qualigens Fine chemicals, Mumbai and Hayman limited, Eastways Park, England respectively. BSA (Bovine serum albumin) was obtained from sigma chemical laboratories. Extraction Buffer chemicals (laboratory grade) such as Di-sodium orthophosphate dehydrate from Glaxo India Limited, Mumbai and sodium di Hydrogen orthophosphate were purchased from Ranbaxy Chemicals, India. Polyvinyl pyrrolidone was obtained from Himedia laboratories private limited, Mumbai. Assay chemicals such as Ethylene diamine-tetra-acetic acid and L-Cystine Hydrochloride, monohydrate were obtained from Himedia Laboratories private limited, Mumbai. Tri-chloro acetic acid was obtained from Ranbaxy chemicals, India. Sodium acetate trihgdrate was obtained from Glaxo India Limited, Mumbai. Casein was obtained from Loba chemical Private Limited. The solutions were prepared using demonized water from a Milli-Q system (Millipore, USA). Centrifuge TC 4100D was used which had maximum speed of 20000 rpm. UV-visible shimadgu and spectrophotometer was used to measure the absorbance which operated in UV range and visible range. For real system experiments fresh pineapples were , collected from interstate fruit market at Huskur near electronic city, Hosur Road,Bangalore Karnataka.

2.

2.1SAMPLE PREPARATION:

Extraction using deionized water fresh pineapple were taken and washed with water to take off the dust particles, sand and other foreign or Extraneous matter from the fruit. The waste parts were separated according to the requirement (crown, peel and core) care should be taken that the pulp part is excluded while removal. The wastes are taken separately and weighed and is crushed using phorphate buffer with pH 6.0 in ratio 1:1.5. The mixture is filtered twice using same muslin cloth. The filtrate is centrifuged for 15 min at 10000 rpm to remove all suspended impurities. The obtained extract is freeze, dried for 24hrs at 20° C to obtain crude powdered extract which was stored in airtight polyethylene covers at 4° C freezer for further analysis.

2.2ANALYTICAL METHODS:

The analysis of protein concentration and enzyme activity was done by Bradford method and enzyme activity assay. All the samplers were taken in duplicates and average values were taken for the calculations.

2.2.1Enzyme Concentration (mg/ml)

The primary measurement is of protein concentration, i.e., mg/ml, which is obtained using protein assay.

Concentration (mg/ml) = (Absorbance-0.0947)/0.0132 x 100

2.2.2Enzyme Activity (CDU/ML)

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The activity in CDU\ml, is obtained from activity assay which is calculated using the following activity (CDU\ml) = ((Enzyme sample-Enzyme blank x 11 x 100 Dilution factor)/ std tyrosine x 10

2.2.3. SPECIFIC ACTIVITY (CDU/mg):

Specific activity is defined as the ratio of activity of enzyme to the protein concentration.

Specific Activity (CDU/mg) = activity /enzyme concentration.

3.RESULTS AND DISCUSSION:

The study was carried out to estimate the protein content and bromelain activity in the wastes obtained from pineapple fruit.

3.1 VARYING pH OF EXTRACT:

The stability of the enzyme depends on the pH of the media in which it is present. To study the stability of the enzyme in different pH, the extract pH was altered using dilute NaOH solution. The pH was varied from 5.0 to 8.0 in case of core and peel. And extract from the crown, it was varied from 7.0 to 8.0, the crude extract itself had a pH of 6.0. The enzyme activity, protein concentration and specific activity is measured in all the three extract.

Table 1: Study on enzyme activity and protein concentration in core extract by varying pH of the extract

pH	Enzyme activity (CDU\ml)	Protein concentration (mg\ml)
5.0	217.67	0.209
6.0	172.77	0.214
7.0	142.02	0.221
8.0	161.92	0.232

Table 2: Study on enzyme activity and protein concentration in peel extract by varying pH of the buffer

pH	Enzyme activity (CDU\ml)	Protein concentration (mg\ml)
4.0	450.94	0.273
5.0	368.62	0.332
6.0	433.77	0.340

7.0	423.80	0.341
8.0	373.14	0.321

Table 3: study on enzyme activity and protein concentration in crown extract by varying pH of the buffer

pH	Enzyme activity (CDU\ml)	Protein concentration (mg\ml)
6.0	583.21	0.331
7.0	501.98	0.327
8.0	512.50	0.274

It could be inferred from the above study that the enzyme obtained from three wastes were more stable at pH 6.0 to 7.0 and showed relatively higher specific activity. Among the three wastes, crown gave higher specific activity as compared to other two wastes.

3.2 STORAGE STUDIES:

The extract obtained should be stable for a certain period ,if they have to be used for further downstream processing to get a higher purification or concentration. Hence, storage stability of the crude extract was carried out for all the three extracts. The crude extract obtained with phosphate buffer of pH 6.5 was stored at 4° C for 4 days to study the stability during storage.

Table 4: Variation in enzyme activity and protein concentration on storage for crown

Day	Enzyme activity (CDU\ml)	Protein concentration (mg\ml)
1	476.00	0.363
2	421.85	0.354
3	390.26	0.333
4	317.06	0.279

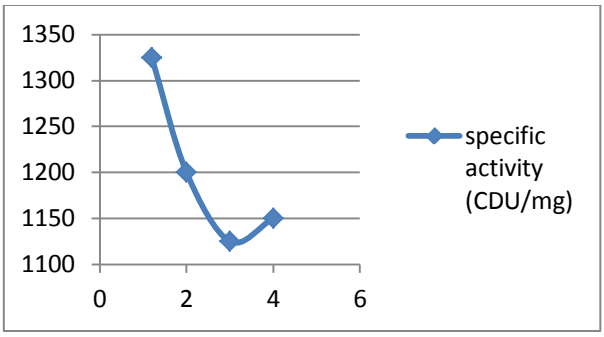

Figure 1: Variation in specific activity for crown sample with storage

4.CONCLUSIONS:

From the above study it could be concluded that the enzyme present in the crude extract looses it activity if it is stored for longer duration. Storage upto 1 or 2 days is possible under cold storage condition (4° C). Hence, it is recommended that extract is to be immediately taken for further processing to preserve its activity. This study also showed that bromelain content was high in crown part of the fruit and then peel and core. The study also showed that activity decreases with storage. Extraction of bromelain increases the economy of industry as it is in demand.100g of bromelain costs about $25-150

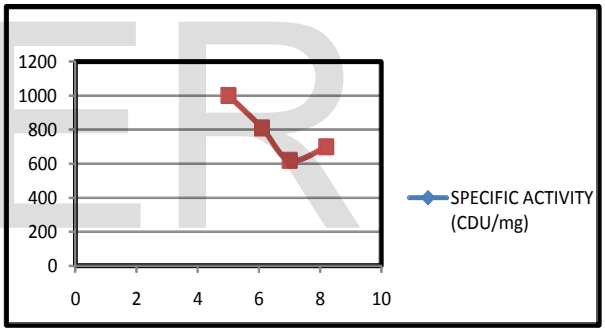

Fig.2 Variation in specific activity with PH of the core extracts

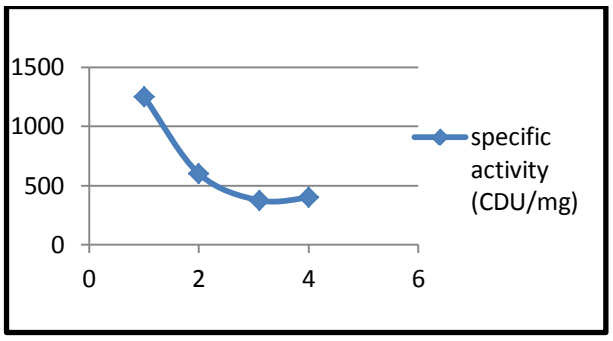

Fig.3 Variation Of Specific Activity For Peel Sample With Storage

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