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Research Article

Optimization of process parameters for production of Gallic acid by submerged fermentation using Natural tannins of *Anacardium occidentalis* L. testa

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ABSTRACT

Gallic acid has enormous application in many fields and is regarded as a non toxic substance to man. Furthermore, considerable health promoting effects have been ascribed to gallic acid. This work demonstrated the ability of microorganism to utilize cashew testa and crude intermediate product to produce gallic acid in a submerged fermentation system. Under optimal time and temperature tannins experiment was carried out, and estimation of concern byproduct was estimated.

Keywords: Microorganism, Gallic acid, Submerged Fermentation and Tannins.

INTRODUCTION

In developing countries, huge quantities of agro industrial residues are continuously being generated and their disposal is associated with several environmental problems. Utilization of these agro residues and byproducts of agro industries as nutrient sources for microbial tannase production may reduce the final enzyme production cost, which is one of the major challenges affecting the large scale production of enzymes[5]. A number of reports given by different workers showed the use of the liquid surface, submerged (SmF) or solid state fermentation (SSF) for the production of tannase. The submerged fermentation is mostly preferred for sterilization and process-control. But this technique is not only expensive but also energy intensive, hence solid state fermentation (SSF) is the alternative method, since obtained levels of tannase are higher on solid substrates[9]. Phenolic compounds such as gallic acid, pyrogallol, methyl gallate and tannic acid induces tannase synthesis. The induction mechanism has not been demonstrated and there is some controversy about the role of some of the hydrolysable tannins constituents on the synthesis of tannase. For instance, gallic acid, one of the structural constituents of some hydrolysable tannins, such as tannic acid, has been reported as an inducer of tannase synthesis under submerged fermentation[4]. Gallic acid is used in the enzymatic synthesis of gallic acid esters, e.g., propyl gallate, which is used mainly as an antioxidant in fats and oils, as well as in beverages[2]. Ethanolic extracts (gallic acid and ellagic acid) of the leaves and stems of Banaba (*Lagerstroemia speciosa* L.), has been used as traditional medicines and are effective in controlling diabetes and obesity and inhibits HIV-1 infection[6]. The present work is exploring towards the utilization of waste cashew testa into useful industrial product by using microbial fermentation.

Hence the present study was focused on (i) extraction of tannins from industrial waste *Anacardium occidentalis* testa by solvent extraction method (ii) Utilization of tannin extract for production of gallic acid through microbial submerged fermentation.

MATERIALS AND METHODS

Sample Collection:

Anacardium occidentale testae were collected from the cashew industries in Palasa, srikakulam district and Visakhapatnam, Visakhapatnam district, Andhra pradesh, India.

Sample Preparation:

Samples were collected fresh and they were conserved, freeze-drying is the gentlest method of preservation and is recommended instead of freezing and air or oven drying.

Sample handling:

After collecting the samples, Samples were stored in the absence of heat, and placed in a dark container.

Sample extraction:

The dried testa of cashew husk was crushed using ball mill to get the particular size below 5mm. 4g of the powder was added to a bottle and extracted by reflux in 50ml 60% methanol for 2 hours. The mixture was filtered, and the filtrate was collected. The extract was then Concentrated to dryness by rotary vaporization at 30ºC under reduced pressure and a light brown powder was obtained.

Submerged Fermentation:

Obtained extracted powder (sample) was used in submerged fermentation by using modified Czapek's Dox medium.

Composition of modified Czapek's dox medium: (pH 5.5)

Glucose	1% w/v
Pot. Di hydrogen phosphate	0.5% w/v
Pot. Chloride	0.3% w/v
Magnesium sulphate	0.1% w/v
Ferrous sulphate	0.01% w/v
Zinc sulphate	0.1% w/v
Sodium Nitrate	0.1% w/v

Effect of time of incubation:

These experiments were conducted to find out the effect of time of incubation period on production of tannase and gallic acid. After inoculation, the different incubation periods were observed by changing the incubation time between 12 hrs to 84 hrs. The flasks were incubated at 35°C and samples were withdrawn periodically at every 12 hrs[3].

Estimation of Total Tannins:

Tannin estimation was done by following the protein precipitation method for the quantitative determination of tannins as described by Hagerman and Butler[1].

Estimation of Gallic acid:

Spectrophotometic method was used by using methanolic rhodanine for the estimation of gallic acid at 520nm by spectrophotometer (Shimadzu UV-1800)[8].

RESULTS & DISCUSSION

Capable of moving with high speed industrialization and increasing population in India have contributed to an increased generation of variety of solid waste. Currently in India, about 960 million tones of solid waste is being generated annually as by-products during industrial, mining, municipal, agricultural and other processes. In India more than 40% of solid waste generated annually is from organic and agricultural sources. There is a growing concern for these accumulating wastes as they are either being dumped in landfills, burnt, or left to rot in the open, leading to severe environmental pollution. A sustainable solution would be to utilize these solid wastes as an 'economical' alternative to costly raw materials and produce industrially important products of practical utility[7]. Tannase has now been extensively used in different biochemical industries. The selected bacterium used in this study is able to synthesize high amounts of tannase through fermentation of crude tannin of *A. Occidentale*. Exploitation of these plant extracts could be a source of cheaper substrate for industrial production of microbial tannase.

By studying the parameters time and temperature that enhancing the extract of tannins from testa, we found that the maximum tannins was extracted from solvent extraction method (5.83 mg/ml) in the presence of 60% methonol.

The optimum time period for maximum tannin extraction was 40 min (Table 1). Extraction of tannins has increased with increase in time up to 40 minutes, with further increase in time decreased tannins content.

Temperature 50ºC was optimum for extraction of 5.83mg/ml concentration of tannin with the optimum temperature condition (Table 2). High temperature results less concentrations of tannin and it promotes hydrolysis and unwanted extraction of hemicelluloses and cellulose materials. Obtained tannin extract was used for the production of gallic acid by using microbial fermentation. By using this method 30.12mg/ml (Table 3) gallic acid was obtained for 48 hours of fermentation with 8% substrate concentration, 120 rpm agitation speed at room temperature.

The optimum period 48hrs was found for the production of gallic acid (Table 3). Further extension of time, the minimum production of gallic acid concentration was observed at 84hrs incubation

(Graph 1). The production of gallic acid has increased with increase in incubation time up to 48hrs, with further increase in the incubation time, decrease production was observed. Decreased yield on prolonged incubation could also be due to inhibition and denaturation of the intermediate product in microbial submerged fermentation system.

Thus this gallic acid production from this waste cashew testa would help the country to produce gallic acid from cheep resources. Plenty of such efficacious raw materials ensured the possibility for exploitation of this microbial fermentation in large-scale production of gallic acid.

Table 1
Optimisation of the time period for the extraction of tannins from cashew testa.

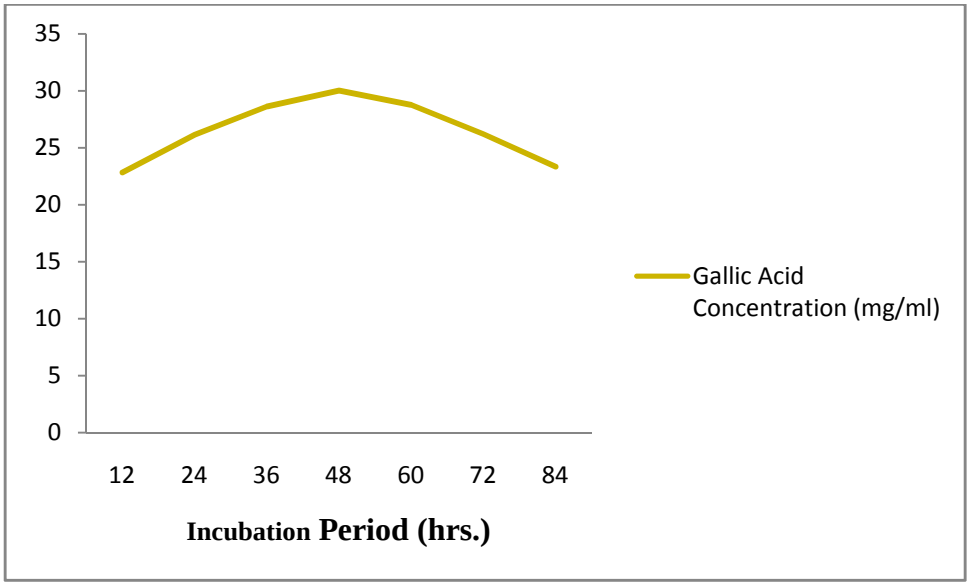
Time period (min.)	Tannin (mg/ml)
15	4.79
20	4.82
25	4.99
30	5.14
35	5.47
40	5.82
45	5.65
50	5.41

Table 2
Optimisation of temperature for extraction of tannins from cashew testa.

Temperature (ºC)	Tannin (mg/ml)
30	5.12
35	5.23
40	5.53
45	5.62
50	5.83
55	4.89
60	4.61

Table 3
Effect of incubation period on production of gallic acid.

Incubation period (hrs.)	Gallic acid con. (mg/ml)
12	22.82
24	26.15
36	28.62
48	30.12
60	28.77
72	26.19
84	23.34



Graph 1
Effect of incubation period on gallic acid concentration.

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