International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 8, Issue 6, 2016

Original Article

UTILIZATION OF UNSERVICEABLE STRAWBERRIES FOR PRODUCTION OF ELLAGIC ACID AND ITS ENHANCEMENT BY ASPERGILLUS NIGER

AKSHAY S. DANGE[1], J. W. EKNATH[1], K. S. PALEKAR[1], S. S. PATEL[1], D. R. SAPKAL[1], MADHU SHARMA[3], ANUPAM BANSAL[2], MONIKA BANSAL[3]

[1]Department of Biochemistry and Molecular Biology, K. K. Wagh College of Agricultural Biotechnology, Nasik, [2]SGT University, Gurgaon, Haryana, [3]School of Agriculture, Lovely Professional University, Phagwara 144411, Punjab
Email: monphd@gmail.com

Received: 07 May 2015 Revised and Accepted: 20 Apr 2016

ABSTRACT

Objective: Use of A*spergillus Niger* (616) for the fabrication of ellagic acid from unserviceable strawberry as a substrate which is readily and chiefly available from Agro-industries and farm waste.

Methods: The ellagic acid content was determined by HPLC method which shows a higher concentration of ellagic acid (143.085+1.669 ppm) after fermentation of 96 h at 35 °C. DPPH assay was carried out for antioxidant activity of ellagic acid.

Results: Results designated that ellagic acid has higher antioxidant activity as compared with ascorbic acid.

Conclusion: Existing study exposes that this agro waste and farm waste can be used at commercially for the production of ellagic acid which has enormous medicinal properties.

Keywords: Ellagic acid, Raw strawberry, DPPH assay, *Aspergillus Niger*, Fermentation, and HPLC

INTRODUCTION

Plant polyphenols are rich sources of different antioxidant, anti-inflammatory and anticancer activities. Strawberry contains high concentrations of antioxidants, such as ascorbic acid (vitamin C) and polyphenols such as ellagic acid ([1-5]). Ellagic acid is a dimeric derivative of gallic acid commonly found in grapes, strawberries, raspberries, pomegranate and walnut and acts as strong chemopreventive agents. Ellagic acid provides numerous health benefits by providing antimutagenic and anticarcinogen responses [6-10]. Most of the ellagic acid in plants is present within the vacuoles, as water-soluble ellagitannins rather than as the free acid. It has very good antioxidative and antiviral properties. Also, it prevents carcinogens from binding to the cellular DNA, reduces the risk of heart attack and birth defects and promotes wound healing. EA helps to regulate growth and seed germination in plants and protects them from microbial infections and heavy metal poisoning. It also regulates glucose levels and reduces blood pressure. Industrially, EA is used in skin clearing creams and other cosmetic products. This study tries to understand the possibility of using this agroindustrial from strawberries for commercial production of ellagic acid, which has immense medicinal benefits.

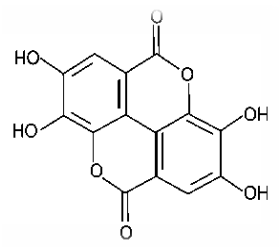

Fig. 1: Structure of ellagic acid

MATERIALS AND METHODS

Microorganism strain and plant material

The pure cultures of *Aspergillus niger* (616) were obtained from National Chemical Laboratory, Pune. *Aspergillus niger* spore inoculums were prepared by adding 2.5 ml of sterile distilled water containing 0.1 % Tween 80 to a fully sporulated culture. The spores were dislodged using a sterile inoculation loop under strict aseptic conditions and kept for incubation at 37 °C for 48 h by using the protocol of [11], 0.1 ml of inoculums is spread on plates by the spreader and loopful of suspension, is streaked on slants and kept for incubation at 37 °C for 3 d (72 h). The plant material used here is unprocessed strawberries, which were collected from local agro-industries in Nashik, India. After washing with water, it is kept in the hot air oven at 60 °C for 3 d for removal of moisture and ground to fine powder and stored at room temperature.

Culture medium and conditions

Czapek-Dox medium having the following composition in (g/l) $NaNO_3$ (7.65), KH_2PO_4 (3.04), $MgSO_4$ (1.52) and KCl (1.52) was prepared. After that, about 8-gram substrate of strawberry was moistened with 18.6 ml of Czapek-Dox medium. Later on, about 1 ml of spore inoculums of *A. niger*.($23x10^7$spores) was added to this medium. After inoculation of the substrate with the organism, it was properly mixed and then kept for solid state fermentation for 96 h at 30 °C, 35 °C and 40 °C in the incubator, observations were recorded after every 24 h, after incubation, the solid culture was washed with pre-cooled and preheated distilled water.

Solid state fermentation

The content of Ellagic acid present in strawberry was determined by High-Performance Liquid Chromatography (HPLC) method. The method for extraction and analysis of crude enzyme was adopted from Paranthaman *et al.*, [10]. Crude ellagic acid was then filtered through 0.45 µm nylon membrane and injected into an HPLC column and quantified. The DPPH (1, 1-Diphenyl-2-pycrylhydrazyl free radical scavenging activity) assay is standard procedure to determine the antioxidant activity of ascorbic acid [4]. In this method, 500 µl of methanolic DPPH solution (0.2 mM) was added to 500 µl of the sample at different concentrations (5-25 µg/ml) and to 500 µl of methanol as the control. After 30 min, the absorbance was measured at 520 nm. An ascorbic acid was used as positive control. The radical scavenging activity of DPPH was calculated using the following equation:

DPPH* scavenging effect (%) = [(A0-A1)/A0] × 100

(1, 1-Diphenyl-2-pycrylhydrazyl free radical scavenging activity)

Where, A0 is the absorbance of the control reaction and A1 is the absorbance in the presence of methanol or Ascorbic acid.

RESULTS AND DISCUSSION

A. niger (NCIM 616) degraded the ellagitannins present in unprocessed strawberries and produced ellagic acid under solid state fermentation at different temperatures namely 30, 35 and 40 °C. Whereas optimum Incubation temperature was found to be 35 °C, at which the ellagic acid production was the highest as shown in table 2. With further increase in temperature, ellagic acid production was found to decrease. The time duration of 96 h was proved to be the optimum incubation period required for maximum ellagic acid production, i.e. (143.085+1.669 ppm).

Thereafter, the ellagic acid production started decreasing. Previous reports also indicated the decrease in ellagic acid accumulation after a definite incubation period of fermentation [12]. The decrease in phenolics is due to the response of the fungus to nutrient depletion, which activates the lignifying and tannin forming peroxidase enzymes. The free radical scavenging activity of ellagic acid was confirmed in the present investigation. DPPH free radical scavenging activity of four samples was evaluated with ascorbic acid as a standard compound. The percentage inhibition was calculated for four different concentrations as well as ascorbic acid as standard. The percentage of inhibition was increased with increasing concentration of test compound. It showed that ellagic acid is equally effective as antioxidant compared to ascorbic acid. In earlier reports, also, it had been shown that different fungal strains showed distinct abilities to ferment ellagitannin sources. *Aspergillus niger* GH1 has also been reported as being fungi with great ability to hydrolyze ellagitannins into ellagic acid [13].

In 2009 Cuccioloni *et al.*, [14] explained that pomegranate wastes contain hydrolyzable compounds like ellagic acid and phenolic compounds, which are also comparable with ellagic acid. Fermentation of pomegranate husk to produce ellagic acid has been recorded by [15]. In the present study, a trial aims to enrich unprocessed strawberries with phenolic antimicrobials via solid state fermentation is achieved. Plants produce a large amount of bioactive elements of a wide variety, which can be significantly utilized for health care issues and food arena [16].

Such health benefits are mainly attributed to the content of ellagic acid, anti-oxidant compound, and polyphenols, [17]. Although ellagic acid is believed to function as both *in vitro* and *In vivo* antioxidants, their efficiency depends on their chemical structure, mostly the number of hydroxyl groups [18], also ellagic acid had the highest antioxidant activity of all the ellagic acid measured by its ability to neutralize the free radical DPPH assay, and they attributed this to the fact that free ellagic acid has two dihydroxyl groups [19].

In strawberry and boysenberry ellagic acid content was in polymeric form while in plum it was in the free form, so the increasing percentage of dehydroascorbic acid indicates that enhanced transformation of ascorbic acid in its oxidative degradation product together with stable ellagic acid levels [20].

Table 1: Effect of incubation temperature on ellagic acid production

Incubation temperature (°C)	Ellagic acid content (ppm)
30	65.7+0.820
35	143.69+1.654
40	137.65+0.608

ppm= Parts per Million, (Values are the means of three replicates±SD)

Table 2: Effect of incubation period on ellagic acid production

Incubation temperature (35 °C)	Ellagic acid content (ppm)
After 48 h	70.32+2.06128
After 96 h	143.085+1.669

ppm= Parts per Million, (Values are the means of three replicates±SD)

Table 3: Antioxidant activity of ascorbic acid (standard) with different concentrations

Ascorbic acid (µg/ml)	% Inhibition
5	29.725+0.3889
10	37.27+1.499
15	39.06+1.329
20	40.945+1.011
25	43.475+1.675

µg/ml=microgram per millilitre, (Values are the means of three replicates±SD)

Table 4: Antioxidant activity of ellagic acid (sample) with different concentrations at 35 °C (96 h)

Ellagic acid (µg/ml)	% Inhibition
5	23.475+0.912
10	25.31+1.145
15	34.335+1.407
20	35.64+0.678
25	42.47+2.863

mg/ml=microgram per millilitre, (Values are the means of three replicates±SD)

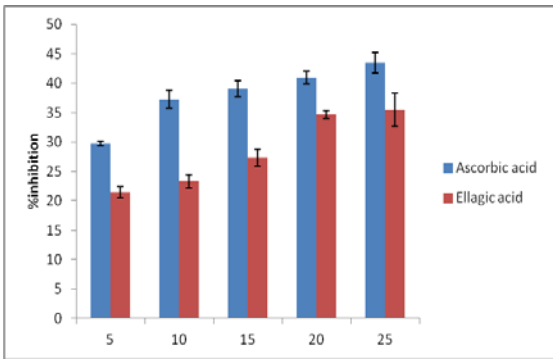

Fig. 2: Antioxidant activity of ellagic acid determined by DPPH assay

CONCLUSION

The present research indicated that *A. Niger* could substantially enhance production levels of ellagic acid in unserviceable strawberry. The optimum temperature and incubation period were also observed, which results in maximum ellagic acid production. So this source of ellagic acid proves to be an economically viable and unexplored substrate for maximum commercial output.

CONFLICT OF INTERESTS

Declared none

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