International Journal of Industrial Engineering *Vol. 1, No. 4, 2017. Page 135-140.*
http://ijie.gjpublications.com/ ***ISSN: 2456-8449.***

Research Article

Microscopic and Spectroscopic analysis of asparaginase treated fried tapioca chips

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Abstract

The effect of mitigation of acrylamide by using fungal asparaginase on physicochemical characteristics of fried tapioca chips on was studied in the present work. The small and uniform sized tapioca chips were soaked in asparaginase solution to mitigate the acrylamide formation during frying. The presence of residual acrylamide in fried cassava chips with and without fungal asparaginase treatment was analyzed using LC-MS and FT-IR. Acrylamide content is fried chips was estimated using liquid chromatography with mass spectroscopy with a retention time of 13.32 min in chromatogram. The FT-IR spectrum analysis proved that the asparaginase treated samples were found to have low acrylamide content and reduction in other toxic impurities. The SEM results revealed that the formation of aggregates was less in asparaginase treated fried tapioca chips and the surface becomes microgranular structure. Thus the asparaginase pretreatment tapioca chips slices has changed the physicochemical characteristics of fried chips and makes the tapioca chips are more porous and crispy for human consumption.

Keywords: Fried foods; Tapioca Chips; Scanning Electron Microscope; Fourier Transfer Infra-Red Spectroscopy; Mass Spectrometry.

Introduction

Foods processed at high temperature contain high levels of acrylamide. Long-term exposure to acrylamide may cause damage to the nervous system both in humans and animals to a certain extent. Acrylamide in fried animal foods and specific hemoglobin adducts was demonstrated on rates [1,2]. The risk assessment of acrylamide evaluated by the Scientific Committee on Toxicity Ecotoxicity and the Environment (CSTEE) of the European Union (EU) reported the exposure of acrylamide to humans should be kept low due to the inherent toxic properties of acrylamide such as neurotoxicity, genotoxicty to both somatic and germ cells, carcinogenicity and reproductive toxicity [3-6]. The conventional frying process increases the intensity of drying with decreased moisture content in cereals and enhances the acrylamide formation [7,8].

Liquid Chromatography with Mass Spectrometry (LC-MS) is as simple analytical methods have brought attention to researchers for qualitative and quantitative analysis of acrylamide in fried foods such as cocoa and coffee. Solid phase extraction was used for the elimination of interfering compounds before LC-MS analysis. The triple quadrople mass spectrometers for LC-MS was used for the analysis of acrylamide in water extracts of food with high sensitivity where the ions observed for acrylamide are m/z 72 (Protonated molecular ions), 55 (ions of amino) and 27 (Subsequent loss of ions). Food products such as breast milk substitute, canned baby foods was analyzed and found at 0.0005 mg of acrylamide/kg for liquids and 0.002 mg of acrylamide/kg for other foods [9-11]. The foodstuffs mashed potato and cookies were analyzed by LC-MS and reported the acrylamide content. HPLC-MS is a sensitive method for the analysis of acrylamide content in foods. The quantification limits for foods and drinks analyzed by HPLC-MS method was found at 0.004 mg of acrylamide/kg of food products. The detection limits of acrylamide in solid food are in the range of 1.480 mg/kg and for drinks and records with the highest value of 29 µg/l [12].

The pretreatment of food products before frying alters its physicochemical characteristics of fried foods which makes the fried foods are more attractive and safer to eat. Though the various methods of acrylamide mitigation in

Received: 15.07.2017; Received after Revision: 26.07.2017; Accepted: 26.07.2017; Published: 31.07.2017

fried and baked foods are well studied, the changes in physicochemical characteristics of fried foods due to the pretreatment are less explored so far. Hence in the present work, the effect of mitigation of acrylamide by using fungal asparaginase on physicochemical characteristics of fried tapioca chips on was studied in the present work. Physiological and surface characteristics of asparaginase treated and untreated fried chips was studied using Scanning Electron Microscope (SEM) and The presence of acrylamide in asparaginase treated and untreated fried chips were analyzed by Fourier Transfer InfraRed Spectroscopy (FT-IR) and Liquid Chromatography with Mass Spectrometry (LC-MS) analysis.

Material and methods

Materials used

Tapioca (*Manihot esculenta)* used in this study was purchased from local vegetable market, Chennai, Tamilnadu. L-asparagine and L-proline used in the production were purchased from HiMedia laboratories Pvt. Ltd., Mumbai, India. *Aspergillus terreus* MTCC 1782 was obtained from Institute of Microbial Technology, Chandigarh, India. The stock culture was cultivated in their respective growth medium and stored at 4°C and they are sub cultured monthly. Asparaginase was produced by *Aspergillus terreus* MTCC 1782 using modified Czepek-Dox media [13].

Sample preparation and pretreatment

The tapioca root was collected and made into slices pieces of uniform size. Slices were immediately rinsed in distilled water to eliminate free starchy materials adhered on the surface of the slices. Slices were soaked in distilled water and subjected to pretreatment along with asparaginase. During soaking of slices in asparaginase solution will cleave amine group from asparagine. The tapioca slices were soaked in asparaginase solution (2 U/g) for 10 min at 60°C Pretreated slices were fried at 180°C for 15 min in a home pro air fryer (Model no: GLA-601)[14].

Characterization of fried Tapioca chips

The changes in surface morphology and physical characteristics of asparaginase pretreated and without pretreated fried tapioca chips were studied using Scanning Electron Microscopy (QUANTA 200). The qualitative analysis of acrylamide was studied by Liquid Chromatography with Mass Spectrometry. Changes in functional groups and relative presence of acrylamide in asparaginase pretreated and without pretreated fried tapioca chips were studied using Fourier Transform Infra-Red spectroscopy (BRUKER α-T FT-IR) and Liquid Chromatography with Mass Spectrometry.

Results and discussions

Characterization of fried tapioca chips by Scanning Electron Microscopy

The surface morphology and the absorption of oil over the surface of fried tapioca chips were studied by Scanning Electron Microscopy. Fig. 1 and 2 indicates the surface of asparaginase treated and untreated fried tapioca chips. Fig. 1 indicates that the structure and topology of treated sample was more concise and smooth in nature. The treated chips were more microgranular in nature which made the fried chips are more porous and crispy for eating. Due to the uniform dispersion nature of tapioca sample it absorbs more amount of oil over the surface. Treated samples absorbed less oil because of its smooth surface and microgranular nature. In case of untreated fried chips as shown in Fig. 2, the surface was observed with certain aggregations which might have resulted due to the exposure of food samples at high frying temperature due Maillard reaction in the presence of asparagine [15,16].

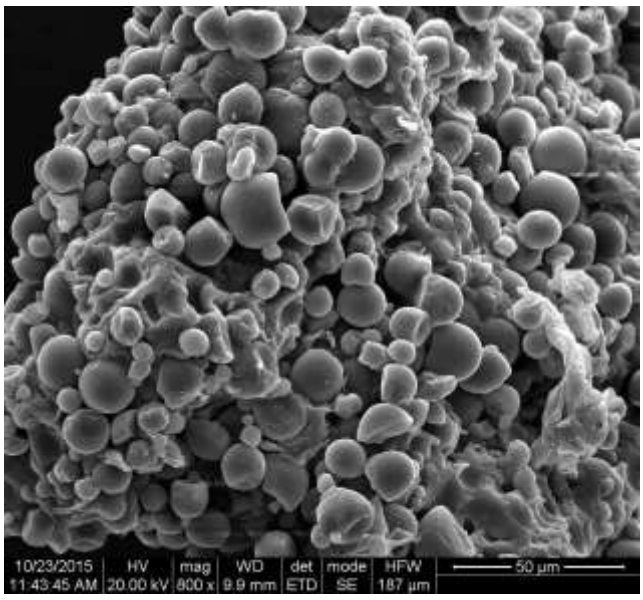

Fig. 1. SEM analysis of asparaginase treated fried tapioca chips

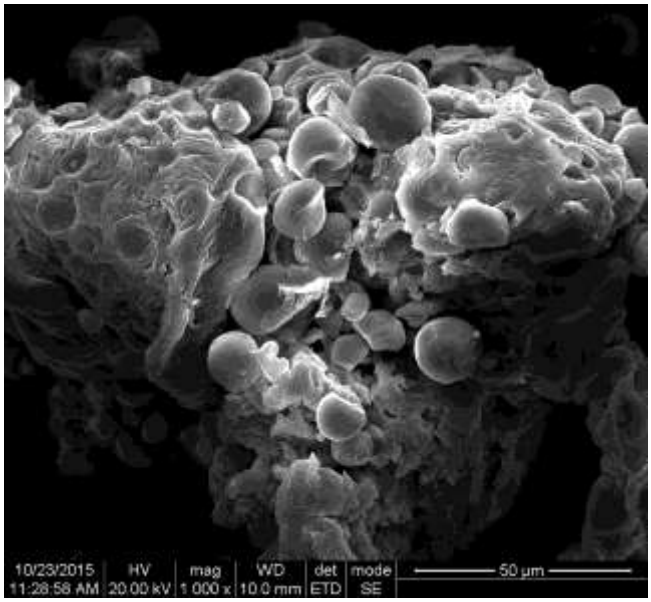

Fig. 2. SEM analysis of untreated fried tapioca chips

Characterization of fried tapioca chips by Fourier Transform Infrared Spectroscopy

The functional group and chemical bonding was analyzed using Fourier Transform Infrared Spectroscopy (FT-IR). The capping element between the enzyme and surface of tapioca chips were confirmed by mode of vibration spectrum as shown in Fig. 3 and 4. The asparaginase treated tapioca sample (Fig. 3) showed with trace amount of acrylamide in the spectrum region of 1201-1699 cm^{-1} whereas the untreated tapioca samples (Fig. 4) showed strong bending vibrational within the molecules in the fingerprint region indicating huge amount of acrylamide content. The % of transmittance was increased in untreated samples whereas they decreased in case of treated samples. This might be due to the presence of various bonds and the energy that is corresponding to various regions. The presence of C-H, C=C was noted in the range of 3251.196 cm^{-1} and 1643.87 cm^{-1} respectively in both the samples. The C-N stretching mode was observed in both samples at 1242.6 cm^{-1} such that more heat was applied to un-treat sample was confirmed by the increase in transmittance region.

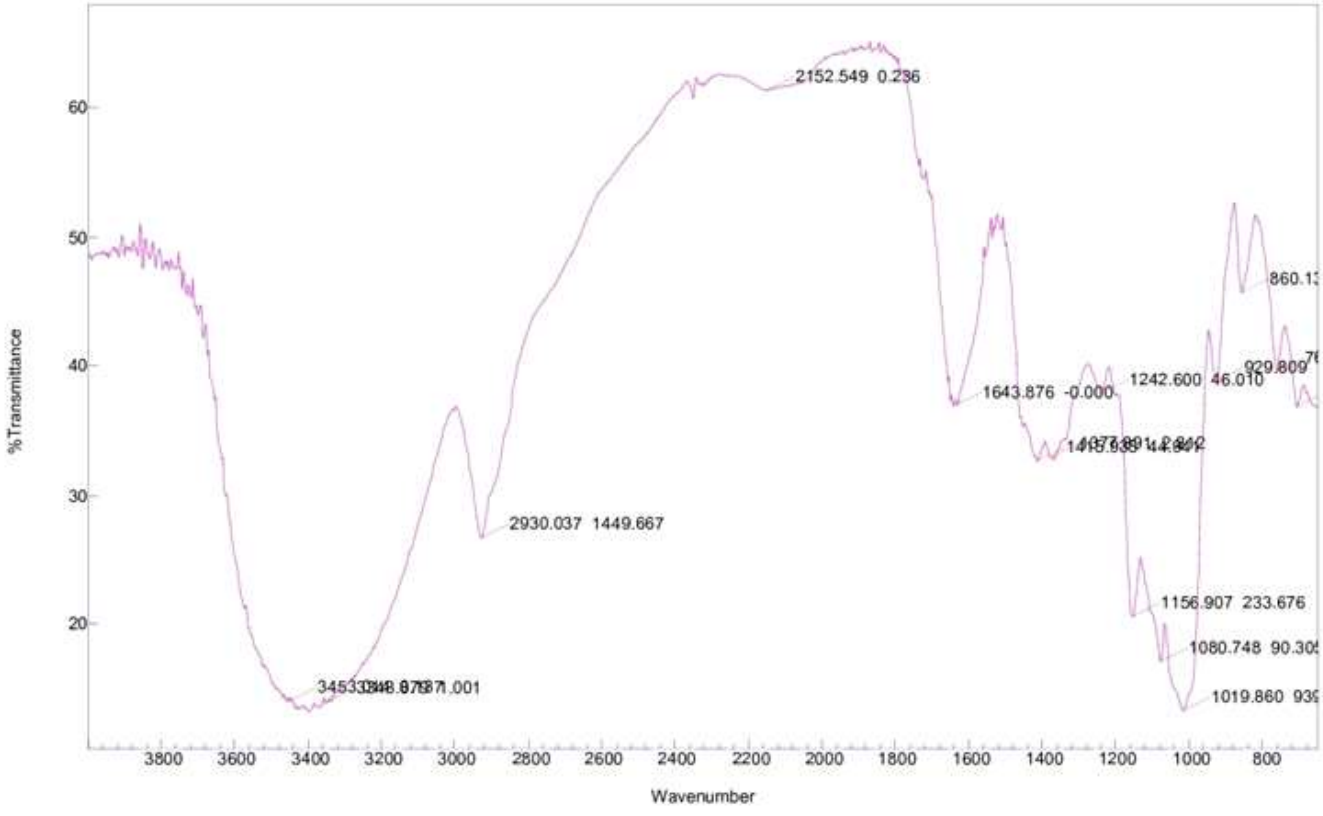

Fig. 3. FTIR analysis of asparaginase treated fried tapioca chips

Qualitative analysis of acrylamide in fried tapioca chips using liquid chromatography mass spectroscopy

The qualitative analysis of acrylamide *in fried tapioca chips* was carried out by Liquid chromatography-Mass Spectrometry. The presence of acrylamide was analyzed using electrospray ionization in positive mode. The characteristic fragmented peak transition was acquired by Multiple Reaction Monitoring. The Fig. 5 and 6 shows the chromatogram of acrylamide present in food. The positive ions were noted at 72 m/z (Fig. 5) and the retention

time of acrylamide was noted at 13.32 min (Fig. 6). The chromatogram shows various small fragmented transition peaks acquired by the multiple reaction mode at 55 m/z. During the time of elution, the compound was found to have peak area percentage of 0.155%.

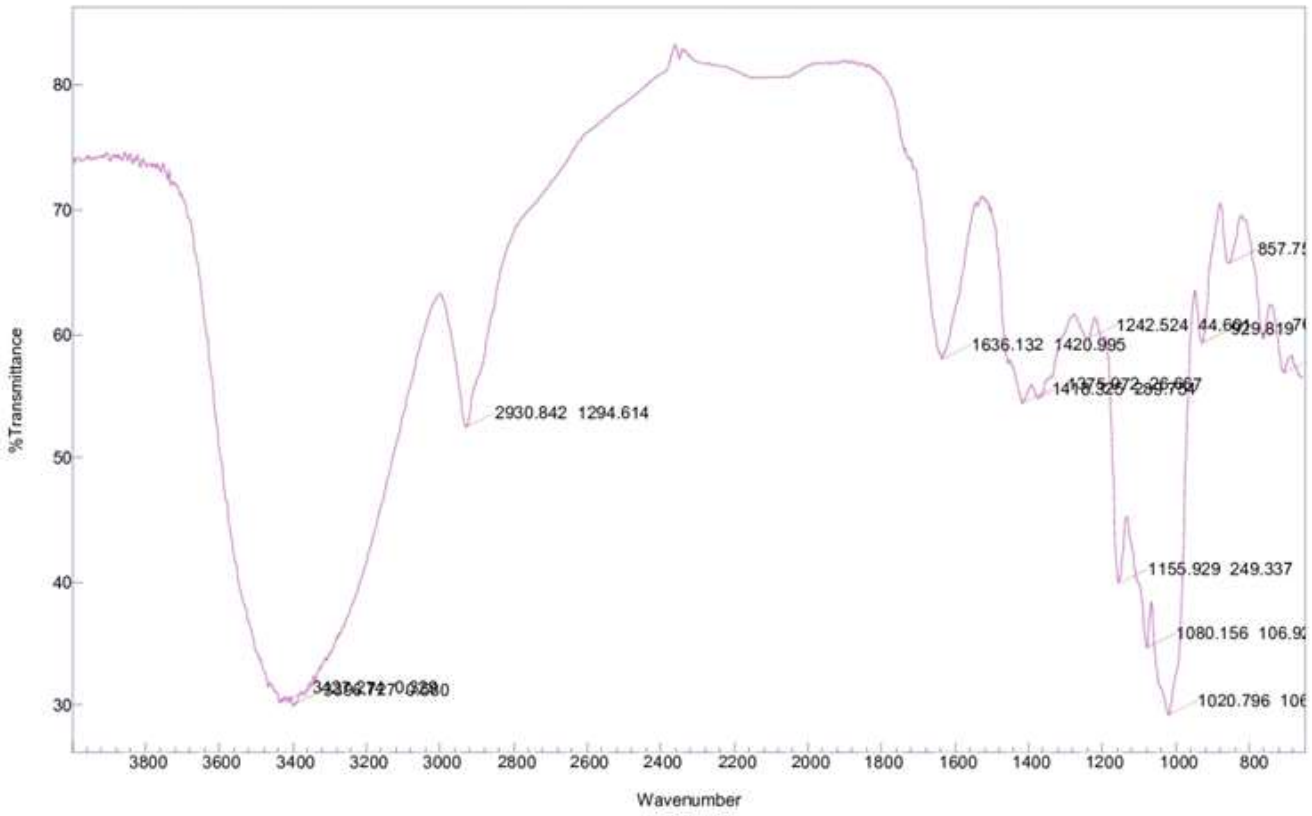

Fig. 4. FTIR analysis of untreated fried tapioca chips

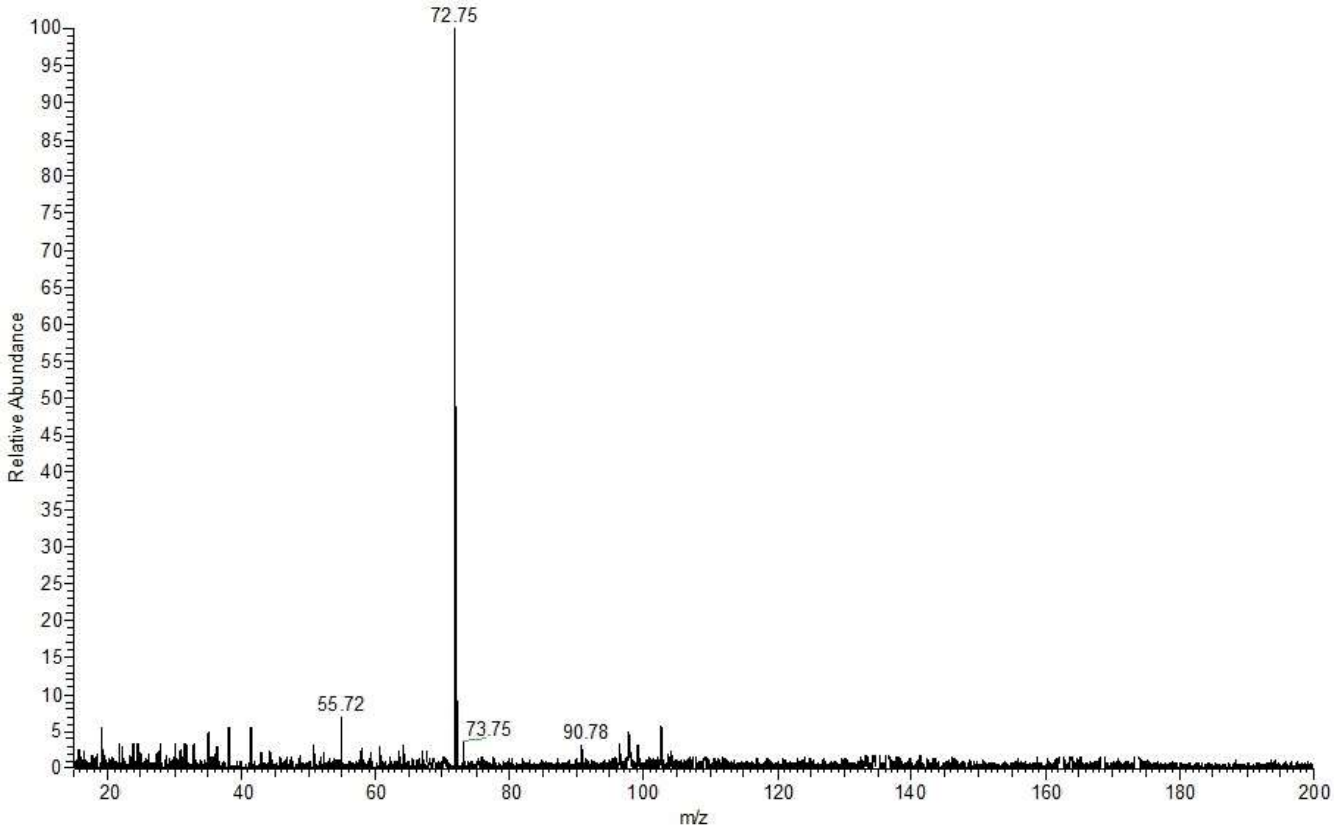

Fig. 5. ESI-MS spectrum of acrylamide in fried tapioca chips by LC-MS

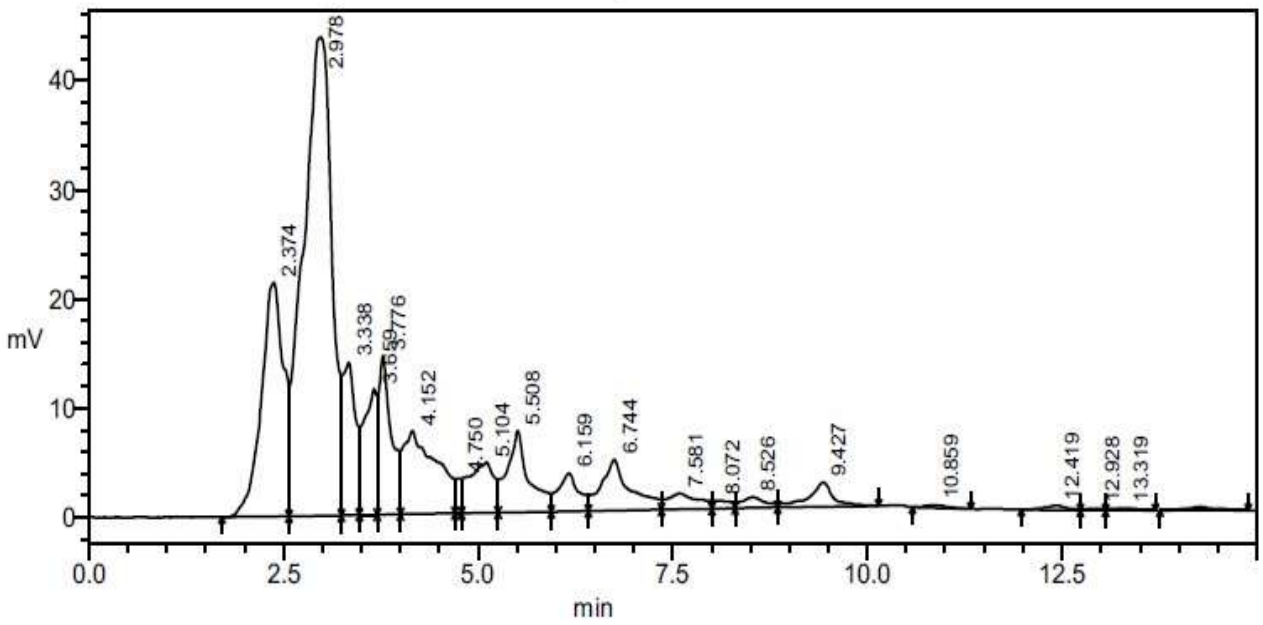

Fig. 7. Retention time of acrylamide in fried tapioca chips by LC-MS

Conclusions

Asparaginase treated chips reported for less surface aggregation, smooth and microgranular surface structure. Thus it makes the fried tapioca chips are more porous and crispy. The characterization of fried tapioca chips using FT-IR spectrum analysis proved that the asparaginase treated samples were found to have low acrylamide content. The retention time of 13.32 min in LC-MS chromatogram has confirmed the level acrylamide in fried tapioca chips. Thus it is confirmed that the frying of tapioca slices after pretreatment using asparaginase for reduction of acrylamide has changed the physicochemical characteristics of fried chips.

Acknowledgement

This work was financially supported under Scheme of Start-Up Research Grant (Young Scientists, Engineering Sciences, File No. YSS/2014/000170) by Science and Engineering Research Board, Department of Science and Technology, Government of India.

Conflicts of Interest

Authors declare no conflict of interest.

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