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Research Article

Enzymatic Pretreatment for Acrylamide Mitigation in Baked Cookies

G. Baskar*, S. Subanjalin Joy, R. Aiswarya

Department of Biotechnology, St. Joseph's College of Engineering, Chennai – 600 119. India.

*Corresponding author's e-mail: basg2004@gmail.com

Abstract

Acrylamide is carcinogen chemical that are produced in many starchy food and in some cereal when it undergoes frying and baking. In the present work incubation time with asparaginase before frying of cookies, enzyme concentration, baking temperature and baking time are optimized for acrylamide mitigation in baked cookies fungal asparaginase. Incubation time was varied from 30-80 min and minimum acrylamide formation was observed at 60 min using 1 U of asparaginase. Cookies were baked using air fryer at optimized the conditions. The baking temperature was varied from 130-160°C and 140°C was found to be optimum. Baking time of 30 min was found to give good quality cookies with less acrylamide. The presence of acrylamide in cookies with and without asparaginase treatment was analyzed using Fourier Transform Infra-Red spectroscopy and the change in surface characteristics was studied using Scanning Electron Microscope.

Keywords: Baked Cookies; Asparaginase; Acrylamide mitigation; Pretreatment.

Introduction

Acrylamide is a carcinogen. It can trigger mutation in human, by replacing adenine with guanine and cytosine with thymine. Earlier toxicological studies suggested that acrylamide vapour irritate the eyes and skin and cause paralysis of the cerebrospinal system, and it has been demonstrated to have carcinogenic properties in animals. The International Agency for Research on Cancer (IARC) has therefore classified it as potential carcinogenic to humans [1-3].

The acrylamide formation occur in food when they directly get in contact with heat, food sample undergoes a reaction called Millard reaction were the reducing sugar and asparagine react to produce acrylamide in food [4]. Acrylamide in food is formed, if it is heated or fried in an oven or a frying pan or by microwave heating, while no acrylamide has been detected in boiled food products. Swedish findings about the high level of acrylamide in heat-treated foods were quickly confirmed by a series of government agencies. Carbohydrate-rich foods seem to generate relatively more acrylamide [5,6]. Another important aspect is that low water content seems important for the reactions, and acrylamide is nearly not detected in boiled foods containing starch. Deep frying or roasting seems to be propitious to the formation of acrylamide [7]. The major reactants leading to the formation of acrylamide by Millard reaction are reducing sugars and the amino acid asparagine which is responsible for the brown color, crust, and toasted flavor in fried and backed foods [8].

Asparaginase is an enzyme which cleaves the precursor of Millard reaction, asparagine into ammonia and aspartic acid thus reduces the formation of acrylamide during frying and baking of foods. As a food processing aid, asparaginase can effectively reduce the level of acrylamide up to 90% in a range of starchy foods without changing the taste and appearance at the end [9-11]. Thus the present work was focused on mitigation of acrylamide in baked cookies by pretreatment using fungal asparaginase from *Aspergillus terreus*.

Material and methods

Materials used

Wheat flour used in this study was purchased from local market, sugar, butter, baking powder, water Chennai, Tamilnadu. The amino acids such as L-asparagine and L-proline used in the production were purchased from HiMedia laboratories Pvt. Ltd., Mumbai, India. All other chemicals used in this study were of

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analytical grades and used without any further purification.

Production of L-asparaginase using Aspergillus terreus

The fungi *Aspergillus terreus* MTCC 1782 was obtained from Institute of Microbial Technology, Chandigarh, India. The stock culture was cultivated Czapek-Dox agar media and stored at 4°C and they are sub cultured monthly. The inoculum culture of *A. terreus* MTCC 1782 was prepared in agar slants of modified Czapek-Dox media. The prepared slants were incubated at 37°C for 4 days. The asparaginase was produced by cultivating *A. terreus* in 500 ml Erlenmeyer flask with 100 ml of modified Czapek-Dox liquid medium containing 2 g of L-proline, 1 g of L-asparagine, 0.2 g glucose, 1.0 g sodium nitrate, 0.052 g potassium chloride, 0.152 g Di-potassium hydrogen sulphate, 0.001 g zinc sulphate, 0.001 g copper sulphate, 0.001 g ferrous sulphate and 0.052 g of magnesium sulphate. The solution was maintained with pH of 6.2 and the flask was kept in orbital shaker at 32°C, 160 rpm for 4 days. The culture broth was filtered in Whatman # 2 filter paper and the filtrate was used as crude asparaginase enzyme [12].

Assay of asparaginase activity

The activity of asparaginase was estimated by Nesslerization method. The enzymatic reaction mixture contains 0.1 ml of the asparaginase was added with 0.9 ml of 0.1 M phosphate buffer along with 1 ml of 0.04 M of L-Asparagine. This mixture was incubated at 37°C for 10 min and the reaction was stopped by adding 0.5 ml of 15% Trichloroacetic acid. The solution was mixed thoroughly and centrifuged at 6000 rpm for 10 min at 4°C. Supernatant 0.1 ml was taken in a separate tube and diluted to 8 ml with distilled water and mixed with 1 ml of 2 M NaOH and 1 ml of Nesslers reagent. The mixture was incubated for 10 min at room temperature and absorbance was noted at 480 nm [13].

Cookies sample preparation, pretreatment and baking

Dough was prepared using 100 g cup of wheat flour, 200 g of powder sugar, 50 g of butter, 5 g of baking powder and asparaginase solution with concentration. The dough was incubated with asparaginase and made in to small pieces and baked. The dough incubation time was varied from 30 to 80 min. The asparaginase a concentration was varied from 1 to 4 U. The baking temperature was varied from 130 to 160 and baking time was varied from 10 to 30 min. The baked cookies at different conditions were analyzed for acrylamide content. The surface characteristics and functional group changes of baked cookies were studied using Scanning Electron Microscope (SEM) and (Fourier Transform Infra-Red spectroscopy (FT-IR) respectively.

Quantitative analysis of acrylamide content

Estimation of acrylamide in fried sample was carried out by volumetric method using Potassium bromide 10 ml of sample extract was added with 10 ml of 10% potassium bromide, 1 M potassium bromate, and 1 M of sulfuric acid. The mixture was incubated at 10 min in dark and then 10 ml of potassium iodide was added. This solution was titrated against sodium thiosulphide, for the disappearance of dark purple color to light yellow. Then 5 drop of starch was added and again titrated with sodium thiosulphide. The amount of acrylamide reduced was then calculated [14].

Results and discussion

Effect of incubation time of dough with asparaginase on acrylamide mitigation in cookies

The effect of incubation time of dough in asparaginase solution on acrylamide mitigation was studied. The dough incubation time was varied from 30 to 80 min with an interval of 10 min. The baking temperature and time was maintained at 140°C in 30 min respectively. Asparaginase concentration of 1U was used. As dough was incubated for 30 min the acrylamide concentration was 1200 µg/kg (Fig. 1). Increase in incubation time increased up to 50 min, the acrylamide concentration in fried cookies. The lowest acrylamide concentration of 1100 µg/kg was obtained at 60 min of incubation of dough with asparaginase solution.

Effect of asparaginase concentration and baking time on acrylamide mitigation in cookies at baking temperature of 130°C

The effect of baking temperature and time studied on acrylamide mitigation in asparaginase pretreated cookies. The effect of baking time (10-30 min) and asparaginase

concentration (1-4 U) on acrylamide mitigation in cookies at 130°C baking temperature was observed as shown in Fig. 2. Acrylamide concentration for 1 U asparaginase at 10 min baking time was 2500 µg/kg. Acrylamide concentration was found decreased as baking time increased. Acrylamide concentration decreased to 2000 µg/kg for 30 min baking time. The acrylamide concentration increased as the concentration of asparaginase increased, but decreased with increase in between baking time. The acrylamide concentration of 2700 µg/kg was reported in 10 min and gradually decreased 2300 µg/kg in 30 min for 2 U asparaginase concentration. The acrylamide concentration decreased as baking time was increased for 2 U asparaginase concentration. The formation of acrylamide was 2700 µg/kg in 10 min baking time and it was 2500 µg/kg in at 30 min. The acrylamide concentration was increased to 3300 µg/kg in 10 min for 4 U asparaginase concentration and decreased to 2600 µg/kg at 30 min.

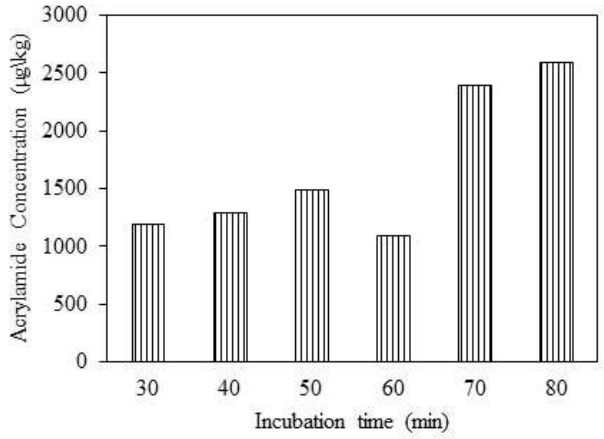

Fig. 1. Effect of incubation time of dough with asparaginase on acrylamide mitigation in cookies

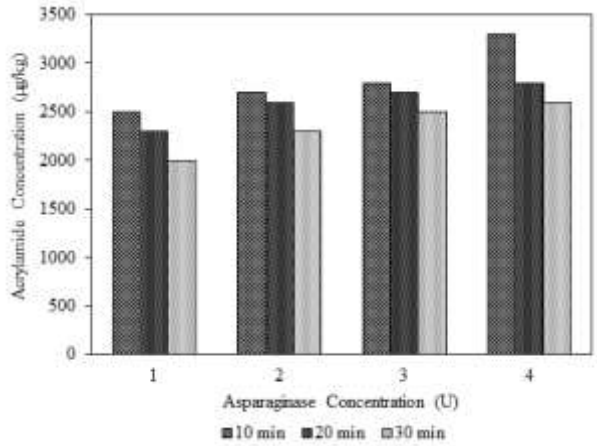

Fig. 2. Effect of asparaginase concentration and baking time on acrylamide mitigation in cookies at baking temperature of 130°C

Effect of asparaginase concentration and baking time on acrylamide mitigation in cookies at baking temperature of 140°C

The cookies was baked well in both outside and inside at 140°C. The acrylamide concentration was low for 1 U asparaginase concentration then other concentrations and it was decreased with increase in baking time. The acrylamide concentration of 1700 µg/kg was obtained at 10 min baking time and it was reduced to 1100 µg/kg at 30 min (Fig. 3). At 2 U asparaginase concentration the acrylamide concentration was increased when comparing 1 U. The acrylamide concentration of 2100 µg/kg in 10 min was decreased to 1900 µg/kg in 30 min for 2 U asparaginase concentration. The acrylamide concentration was 2300 µg/kg in 10 min and it was 1400 µg/kg in 30 min for 3U asparaginase concentration. The acrylamide concentration of 2400 µg/kg at 10 min and 1500 µg/kg and 30 min for asparaginase concentration of 4U. Thus there was a significant decrease in acrylamide concentration with increase in baking time but no significant decrease in acrylamide concentration due to increase in asparaginase concentration at baking temperature of 140°C.

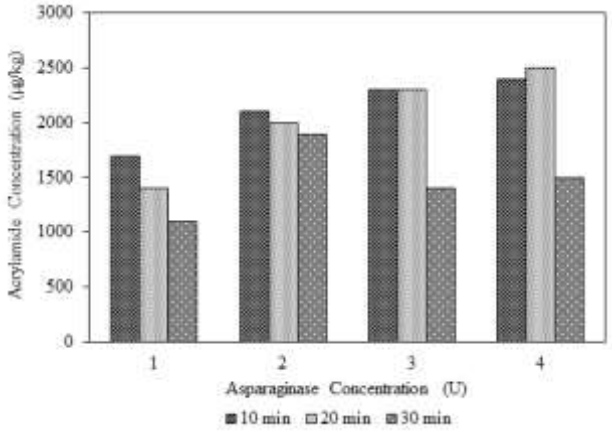

Fig. 3. Effect of asparaginase concentration and baking time on acrylamide mitigation in cookies at baking temperature of 140°C

Effect of asparaginase concentration and baking time on acrylamide mitigation in cookies at baking temperature of 150°C

At baking temperature of 150°C, the acrylamide concentration was 1500 µg/kg in 10 min and 1300 µg/kg in 30 min for 1 U asparaginase concentration (Fig. 4). The acrylamide concentration was 1600 µg/kg in 10 min and 1500 µg/kg in 30 min for 2 U asparaginase concentration. The acrylamide concentration was 1700 µg/kg in 10 min and 2000 µg/kg in 30 min for 3 U asparaginase

concentration. The acrylamide concentration was 1800 µg/kg in 10 min and 2100 µg/kg in 30 min for 4U asparaginase concentration. At 150°C the cookies have charred at 30 min which might be the reason for increase in acrylamide concentration.

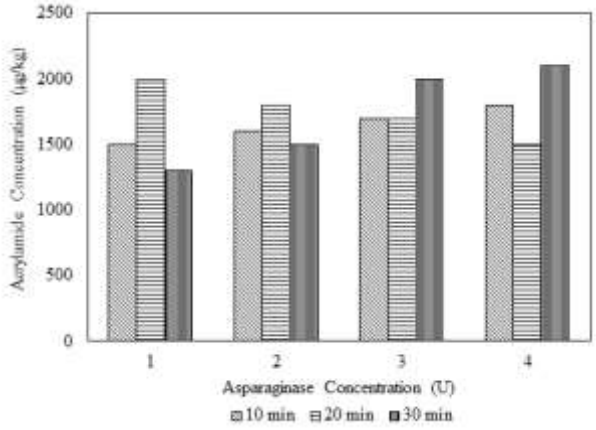

Fig. 4. Effect of asparaginase concentration and baking time on acrylamide mitigation in cookies at baking temperature of 150°C

Effect of asparaginase concentration and baking time on acrylamide mitigation in cookies at baking temperature of 160°C

The erratic change in acrylamide concentration in cookies was observed at 160°C baking temperature (Fig. 5). At 1U asparaginase concentration, the acrylamide concentration was 1700 µg/kg in 10 min and increased to 1800 µg/kg at the baking time 30 min. At 2U asparaginase concentration, the acrylamide concentration was 1600 µg/kg in 10 min and decreased to 1400 µg/kg in 30 min of baking time. At 3U asparaginase concentration, the acrylamide concentration was erratic in manner, at baking time of 10 min the acrylamide concentration was 1500 µg/kg and increased to 2100 µg/kg at 20 min then decreased to 1500 µg/kg in 30 min. At 4 U asparaginase concentrations the acrylamide concentration was high when compared with other asparaginase concentration. At 10 min the acrylamide concentration was 1800 µg/kg and at 20 min it was increased to 2200 µg/kg in 20 min.

The effect on baking temperature and time on acrylamide mitigation in untreated cookies was studied at 140°C for different baking time namely 10, 20 and 30 min (Fig. 6). Acrylamide concentration in baked cookies was increased with increase in baking time. At 10^{th} min the Acrylamide concentration of 2650, 2750 and 3050 µg/kg was found in untreated cookies in 10, 20, and 30 min of baking time respectively (Fig. 6). It was observed that change in baking temperature and time has significant effect on acrylamide mitigation in asparaginase treated baked of cookies. Acrylamide concentration was reduced to 1100 µg/kg at baking temperature of 140 C in 30 min using 1 U asparaginase concentration. Thus significant reduction (64%) of acrylamide in baked cookies was achieved by asparaginase pretreatment of dough before baking.

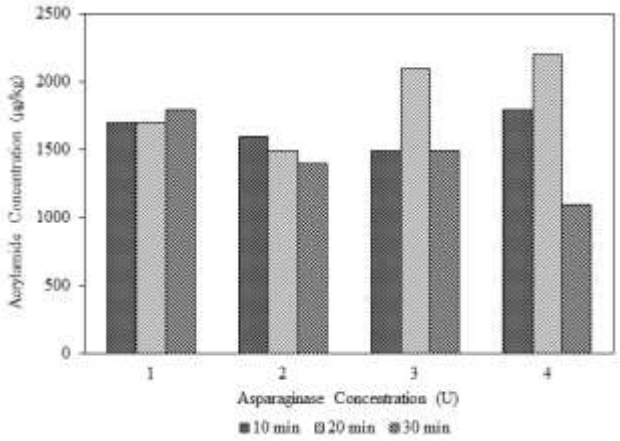

Fig. 5. Effect of asparaginase concentration and baking time on acrylamide mitigation in cookies at baking temperature of 160°C

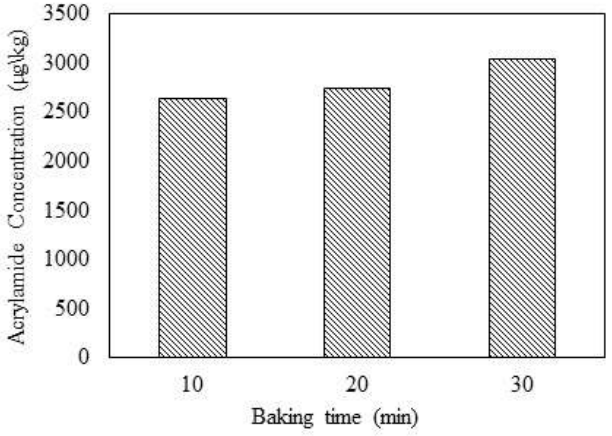

Fig. 6. Effect of baking time on acrylamide formation in cookies without treatment at optimal baking temperature and time

Characterization of baked cookies by Fourier Transform Infrared Spectroscopy and Scanning Electron Microscope

The changes in functional groups and chemical bonding in baked cookies was analyzed using Fourier Transform Infrared Spectroscopy Fourier Transform Infra-Red spectroscopy (FT-IR) (BRUKER α-T FT-IR). The asparaginase treated cookies sample (Fig. 7(a)) showed with trace amount of acrylamide in the spectrum region of 1201-1699 cm^{-1} whereas the untreated cookies samples (Fig. 7(b)) showed strong

bending vibrational within the molecules in the fingerprint region indicating high amount of acrylamide content. The % of transmittance was increased in untreated samples whereas; it was 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 untreated cookies confirmed by the increase in transmittance region. The surface of the baked cookies was characterized by Scanning Electron Microscopy (SEM, QUANTA 200). The surface of the asparaginase treated cookies was homogeneous, and smooth in nature (Fig. 8(a). The untreated cookies were observed with irregular and rough surface (Fig. 8(b). Thus asparaginase pretreatment not only helps to decrease the asparaginase but also increase the surface quality of baked cookies [15].

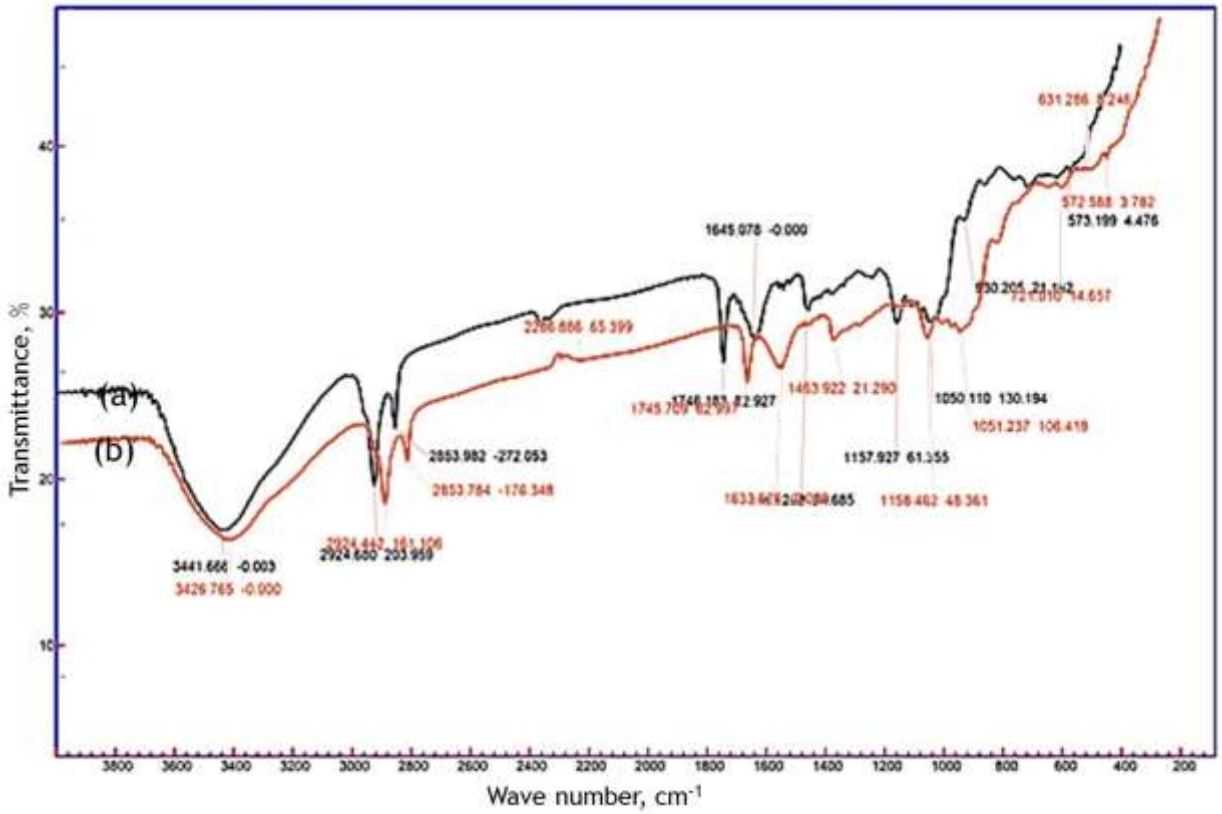

Fig. 7. FT-IR spectrum baked cookies (a) asparaginase treated (b) without asparaginase treatment

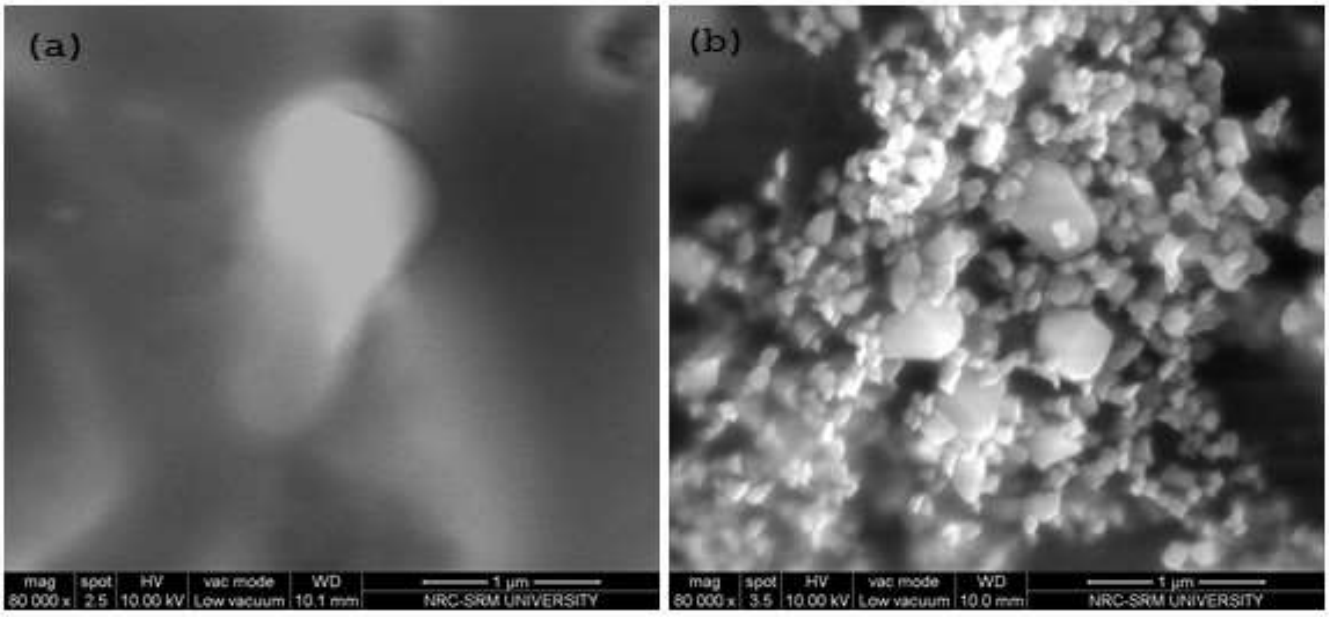

Fig. 8. SEM image of baked cookies (a) asparaginase pretreated (b) without asparaginase treatment

Conclusions

The baking temperature and time has significant effect on acrylamide mitigation in asparaginase treated baked of cookies. Asparaginase pretreatment of dough before baking has significantly reduced the acrylamide content in baked cookies. Acrylamide concentration was reduced to 1100 µg/kg at baking temperature of 140°C in 30 min using 1 U asparaginase concentration. The characterization of baked cookies proved that asparaginase treated cookies were found to have low acrylamide content with homogeneous and smooth surface. Thus asparaginase pretreatment not only helps to decrease the asparaginase and increase the surface quality of baked cookies.

Conflicts of Interest

Authors declare no conflict of interest.

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