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Research Article

Optimization of Enzymatic Pretreatment and Frying Conditions for Acrylamide Mitigation in Fried Tapioca Chips

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Abstract

Asparaginase is the enzyme which will cleave amino group from asparagine and prevent the formation of acrylamide in fried foods. The mitigation of acrylamide formation in fried tapioca chips by using fungal asparaginase 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 optimum conditions for maximum mitigation of acrylamide in fried tapioca chips were found to be 10min of soaking time, 2 U/g of asparaginase concentration in soaking solution, soaking at 60°C followed by frying at 180°C for 15 min. The fungal asparaginase was found to be effective in mitigation of acrylamide in tapioca fried chips.

Keywords: Asparaginase; Fried foods; Tapioca Chips; Acrylamide; Mitigation.

Introduction

Acrylamide is produced in food when it is directly contacted with heat at high temperature [1]. Swedish National Food Administration (SNFA) and University of Stockholm together in early 2002 announced that some food produce acrylamide when it is cooked in high temperature, the acrylamide produced is relatively high level [2]. Acrylamide formation is seen in fried animal food when it is fried and when it is demonstrated in rat [3]. As it was found in animal food when it is heated in high temperature, they also found that acrylamide formation in common human foods when it cooked in high temperature which leads to an interesting study of neurotoxicity of dietary exposure to acrylamide. Human gets direct expose to acrylamide thought breakdown from high carbohydrate foods like potato chips, roasted cereals and bread. Some food packing cover also contains monomer of acrylamide in which we get expose in indirect way [4].

Acrylamide formation is not traced in boiled foods it lead to the hypothesis that high heat produce acrylamide formation in food even it was heated in oven [5,6]. Cooked starchy food produce acrylamide in them when they undergo an reaction called Millard reaction which cause brown colour with crispiness in baked and fried food. During heating asparagine amino acid and reducing sugar undergoes Millard reaction that leads to the production of acrylamide in starch food. Fungal asparaginase is an enzyme [7] that will reduce the formation of acrylamide in food up to 90% without changing or reducing the taste of food. As acrylamide is a carcinogen toxicology study were done early, they suggested that acrylamide vapour cause irritation in skin and eye and paralysis of the cerebrospinal system [8].

As we know that were used in many industry's employee used to expose themselves to acrylamide, long time expose lead to damage in nervous system [9,10], it is also considered as genetic and reproductive toxin [11-13], with mutagenic that will all the cytosine to adenine it was experienced in both in vitro and in vivo studies [14]. These things lead the scientist to know that it is an inheritance toxin, so low level of expose to acrylamide is safe [4,15]. As acrylamide is known as carcinogen which would cause cancer, mitigation of acrylamide should be done in food in order to reduce the level of expose towards the chemical [16,17].

Low level of water in food is important in acrylamide formation in food, that happen when food is roasted or fried however acrylamide formation are not seen in boiled foods. Some starchy food can boiled they don't produce acrylamide formation in them during boiling

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[18]. In early 2002 Swedish announced that acrylamide formation during Millard reaction is due to reducing sugar like glucose and by an amino acid called asparagine, that cause huge interest towards this area [19-21].

This conventional frying process increases the intensity of drying with decreased moisture content in cereals and enhances the acrylamide formation [22,23]. The various factors such as species type, roast degree and brew length reported to influence acrylamide formation in espresso coffee [24]. Vacuum treatment of potatos reported to reduce acrylamide formation [25]. Mitigation of acrylamide can be done in three different ways like physical, chemical, biological method. Few examples of physical method includes soaking and blanching, chemical methods include soaking in organic solvents or in base solution and in case of biological methods samples are treated in enzyme solution or amino acid solution.

In the present study fungal asparaginase enzyme was used to reduce the acrylamide formation in fried food, which is known to have carcinogenic properties. Different parameters like soaking and frying were evaluated to study the reduction in acrylamide formation in treated and untreated fried tapioca chips.

Material and methods

Materials used

Tapioca (*Manihot esculenta)* used in this study was purchased from local vegetable market, 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. The fungi *Aspergillus terrus* 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. All other chemicals used in this study were of analytical grades and used without any further purification.

Preparation of inoculum

The inoculum culture of *Aspergillus terrus* MTCC 1782 was cultivated on agar slants of modified Czapek-Dox media. For 100 ml of modified Czapek-Dox media, 5 ml of solution-A, 5 ml of solution-B, 0.1 ml of solution C, 89.9 ml of distilled water, 3g of glucose and 2g of agar was added. The solution-A was prepared by adding 0.1 g of L-asparagine, 0.4 g of sodium nitrate, 0.1 g of potassium chloride 0.1 g of magnesium sulphate and 0.002 g of ferrous sulphate in 10 ml of distilled water and stored in refrigerator. The solution-B was prepared by adding 0.2 g of Di-potassium hydrogen phosphate in 10 ml of distilled water and solution C was prepared by 0.1 g of Zinc sulphate and 0.05 g of Copper sulphate in 10 ml of distilled water such that both solution B and C were stored in refrigerator. The prepared slant was incubated at 37°C for 4 days [26].

Production of L-asparaginase using A. terrus

The production of asparaginase enzyme was carried out by inoculating *Aspergillus terrus* in 500 ml Erlenmeyer flask with modified Czapek-Dox liquid medium containing 2 g of L-Proline, 1g of L-asparagine, 0.2g Glucose, 1.0 g Sodium nitrate, 0.052g Pottasium chloride, 0.152g Di-potassium hydrogen sulphate, 0.001g zinc sulphate, 0.001g copper sulphate, 0.001g ferrous sulphate and 0.052g 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. After the incubation period for growth they are filtered in Whatman #2 filter paper [26].

Assay of asparaginase activity

The activity of asparaginase was estimated by Nesslerization method. The enzymatic reaction mixture contains 0.1 ml of the crude enzyme was added with 0.9ml of 0.1M phosphate buffer along with 1ml of 0.04M 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 1ml of 2M NaOH and 1ml of Nesslers reagent. The mixture was incubated for 10 min at room temperature and absorbance was noted at 480 nm [27].

Sample preparation and pretreatment

The food sample Tapioca was collected and made into slices of same size. The slices were then subjected to pretreatment such as soaking it

in distilled water along with asparaginase. The slices were immediately rinsed after cutting to eliminate little amount of starch material that is adhered to the surface of the food samples. During soaking the enzyme will cleave amine group from asparagine. Both soaking temperature and time were optimized and then subjected for frying in air fryer (Home pro air fryer model no: GLA-601).

Optimization of soaking and frying parameter

Soaking is one of the pretreatment methods to reduce the acrylamide formation in fried food product. Soaking in distilled water tends to reduce the glucose and asparagine level which are main sources for acrylamide formation during Millard reaction. Optimization of soaking parameters reduces the acrylamide content prior to the frying process. In this study the samples were soaked in distilled water along with enzyme such that soaking temperature and time was optimized simultaneously. The sliced samples were soaked in water and the temperature was varied from 50-65°C for 10 min.

During soaking, asparaginase enzyme is used to treat the tapioca sample. Asparaginase concentration was varied from 1 U to 4 U. Frying is the process were heat will be directly applied in food, by which the food under goes Millard reaction and produce acrylamide in food products. The tapioca sample was fried in different oils by keeping the frying temperature as constant to denote the amount of acrylamide formation, in the sample using different oils. The different oil used was sunflower oil, gingelly oil, groundnut oil. The food samples after treatment and without treatment are fried in various frying temperature and time. Frying temperature varied from 160°C-200°C with interval of 20°C.

Quantitative analysis of acrylamide content in fired Tapioca chips

Estimation of acrylamide in fried sample was carried out by volumetric method using Potassium bromide 10ml 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 10ml 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 [28].

Results and discussions

Effect of frying oil on acrylamide mitigation

The type of frying oil plays an important role in the formation of acrylamide and as they are involved in acrylamide formation at higher temperatures. The treated sample and non-treated sample was deep fried using different oils known as ginger, groundnut oil and sunflower oil at a constant temperature and time. In this study, the sample was fried in deep-fryer and the food samples were subjected to air fryers in order to identify the lowest acrylamide formation during frying process. It was found the concentration of acrylamide was low (1400 µg\kg) in treated samples fried under air fryer whereas the un-treated samples was found with high concentration acrylamide (2200 µg\kg). In case of frying under gingerly oil produced low acrylamide concentration of 1500 µg\kg (Fig. 1). Hence, for further optimization process air fryer was used for frying as it produces low acrylamide concentration.

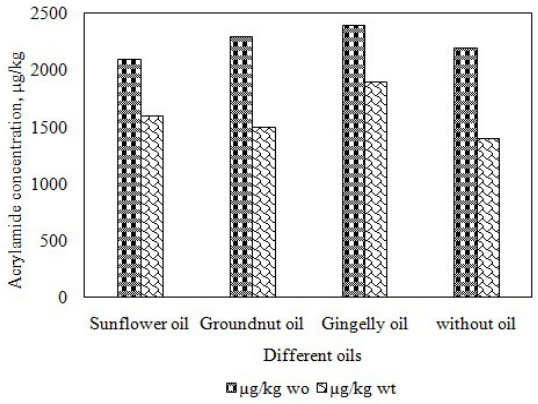

Fig. 1. Effect of frying oil on acrylamide mitigation in fried tapioca chips

Effect of asparaginase concentration on acrylamide mitigation

Asparaginase tends to cleave amide group from asparagines into aspartic acid, which reduces the formation of acrylamide in fried food products. The tapioca sample was soaked in the distilled water along with the enzyme as it reduces the level of glucose and asparagine in tapioca sample. In this study, the concentration of enzyme was varied from 1-4 U were the

optimum concentration was found to 2 U with the acrylamide concentration of 1400 µg/kg for treated samples (Fig. 2). Hence the optimal asparaginase concentration for enzyme concentration was found as 2 U.

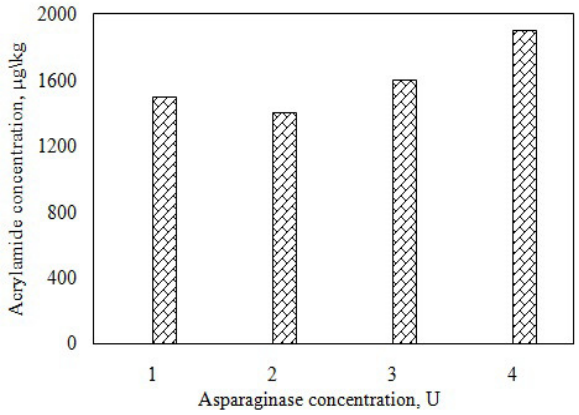

Fig. 2. Effect of asparaginase concentration on acrylamide mitigation in fried tapioca chips

Effect of soaking time and temperature on acrylamide mitigation

Soaking temperature and time plays a vital role in the reduction of acrylamide as it leaches the presence of compound. The Fig. 3 indicates the effect of soaking time and the time was varied from 5-20 min were the acrylamide formation was found to be low when compared to other soaking time. In case of soaking temperature as shown in Fig. 4 the increase in temperature increases the formation and the optimum temperature was found to be 60°C due to the increased extraction of glucose The acrylamide concentration at optimized soaking condition was reduced to 1400 µg/kg for treated samples.

Effect of frying temperature and time on acrylamide mitigation

Frying temperature is one of the important parameters to be monitored as they are directly involved in the formation of acrylamide respectively [29]. In order to investigate the optimum temperature, the temperature was varied from 160-200°C. The effect of frying temperature and time on acrylamide mitigation in asparaginase treated fried tapioca chip is shown in Fig. 5. The effect of frying temperature and time on acrylamide mitigation in untreated fried tapioca chips is shown in Fig. 6.

The acrylamide formation at various temperatures such as 160°C, 180°C and 200°C was 2000 µg/kg, 2300 µg/kg and 1600 µg/kg. It was observed that the food samples were not suitable for consumption at 200°C and were charred whereas at 160°C food samples were not fried properly, but at 180°C proper cooking was done, hence the optimized condition was considered as 180°C. The frying time was varied from 5-20 min with interval of 5 min and the optimized time was found to be at 15 min.

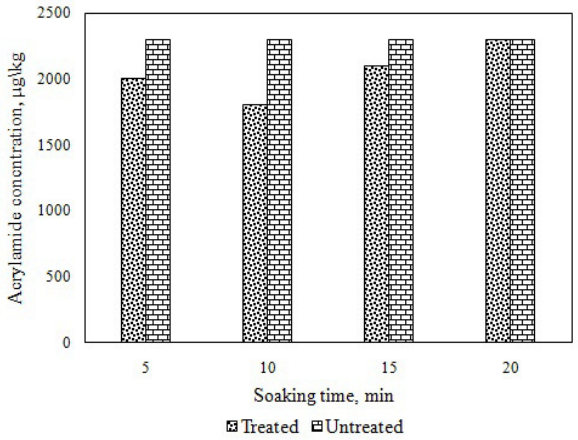

Fig. 3. Effect of soaking time on acrylamide mitigation in fried tapioca chips

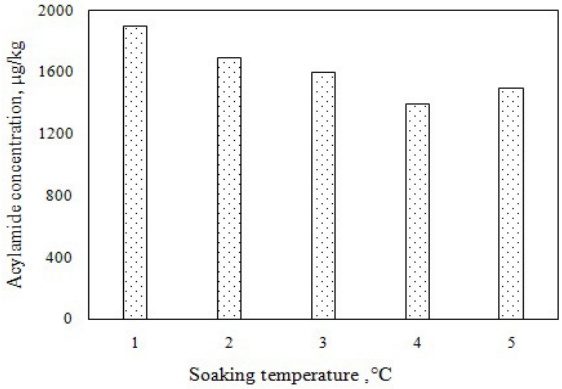

Fig. 4. Effect of soaking temperature on acrylamide mitigation in fried tapioca chips

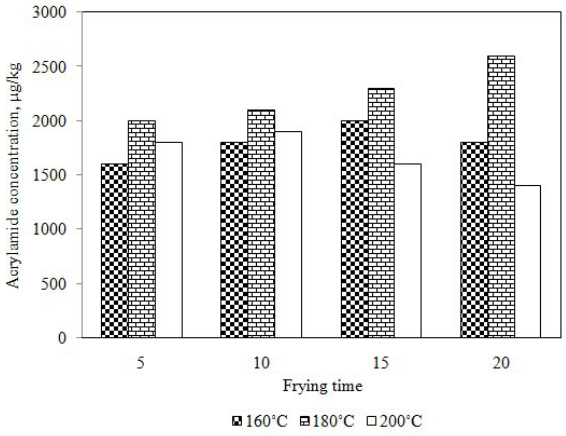

Fig. 5. Effect of frying temperature and time on acrylamide mitigation in asparaginase treated fried tapioca chips

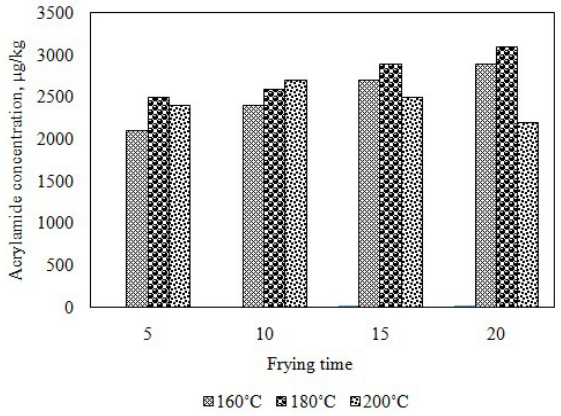

Fig. 6. Effect of frying temperature and time on acrylamide mitigation in untreated fried tapioca chips

Conclusions

The reduced acrylamide content in fried tapioca chips was achieved by asparaginase pretreatment. It showed positive outcome on optimization of asparaginase on various parameters. The acrylamide formation was found low at asparaginase concentration of 2 U/g. The pretreatment studies were done for food samples indicating the effectiveness of method prior to frying process. Studies on frying also showed that increase in temperature increases the acrylamide concentration such that 200°C was found optimum for frying of tapioca chips at 10 min.

Conflict of interest

Authors declare there are no conflicts of interest.

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