Enzyme Application for Reduction of Acrylamide Formation in Fried Potato Chips

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Abstract:

Acrylamide is a carcinogenic compound formed in starchy food during heat processing. The application of L-asparaginase was found effective method to prevent acrylamide formation in fried potato chips. This enzyme efficiently catalyzes the conversion of amino acid L-asparagine into L-aspartic acid, which is not an acrylamide precursor. The acrylamide formation is considerably limited by the application of this enzyme in fried food products. The potato chips were prepared and treated with different concentrations of L-asparginase enzyme viz. 0.2 IU, 1.0 IU, 1.5 IU and 2.0 IU respectively. The treated chips along with the control sample were subjected to various physicochemical and sensory analysis in general and acryalmide content in particular. It was observed that the acrylamide formation was drastically reduced to 0.019 ppm in the chips treated with 2.0 IU enzyme concentration with better sensory quality characteristics as compared to untreated control chips in which acrylamide formation was 15.65 ppm.

Keywords:

Acrylamide; Fried potato Chips; L-asparaginase; L-asparagine.

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Introduction

Frying is a cooking method that creates unique textures and flavors in foods. Potato (*Solanum tuberosum*) is one of the world's important agricultural crops consumed by millions of people over worldwide. Potatoes are mostly cooked by frying and consumer preferred to have deep fried potato products.[1] Deep fat frying is extensively used in food processing both industrially and at home and fried potato products are one of its largest applications.[2] Acrylamide is a chemical compound formed from food components during heat treatment like frying, baking, roasting and extrusion as a result of the maillard's reaction between asparagine and reducing sugars.[3]

Recent findings of acrylamide in foods have focused research on the possible mechanisms of formation. Researchers found a method for the formation of acrylamide from the reaction of the amino acid asparagine and a carbonyl-containing compound at typical cooking temperatures.[4] The confirmation of this method was accomplished through selective removal of asparagine using asparaginase which resulted in a reduced level of acrylamide in a heated starchy food. The potential ability of different potato varieties to form acrylamide during heat treatment depends on the concentration of reducing sugars (especially glucose and fructose) and

asparagines. The potato cultivars show large differences in their potential to form acrylamide which was mainly linked to their sugar contents.[5] The main pathway of acrylamide formation in fried potato products is the reaction of free aspargine and reducing sugars (asparagines route). Therefore the contents of these precursors in fried products are important and have to be controlled.[6]

Acrylamide having adverse carcinogenic effects on human health. It is known to be neurotoxic (causing peripheral neuropathy) in humans and a reproductive toxic agent in rodents. Acrylamide is positive in a number of tests for genotoxicity, inducing chromosomal aberrations, micronuclei, sister chromatid exchange, polyploidy, aneuploidy and other mitotic disturbances in mammalian cells in the absence of metabolic activation.

The present investigation was taken to study the effect of L-asparaginase for acrylamide content reduction in fried potato chips.

Materials and Methods

Potatoes (Cv *Kufri Chipsona)*, soybean oil, salt was purchased from local market of Aurangabad city. L-asparginase enzyme was purchased from HIMEDIA Laboratories, Mumbai.

Proximate analysis of Potato

The proximate analysis of potato was carried out before frying. Protein, carbohydrate, fat, ash, moisture content, etc. were determined in the food laboratory of Department of Chemical Technology, Dr. Babasaheb Ambedkar Marathwada University, Aurangabad.

The large sized potatoes were selected for preparation of chips. The selected potatoes were washed and peeled. The peeled potatoes were cut into round slices by slicer having 4.5 cm diameter and 2 mm thickness. Potato slices were blanched at 65°C for one minute followed by cooling in ice water at 15°C for 5 min. Blanched potato slices were soaked in 2.5 % salt solution for 10 minute. The potato slices were dried in cabinet tray drier at 60° C for 20 minute. The potato chips were made by frying in electric fryer at 190°C ± 5°C for 6 minute using soybean oil. The cooled fried potato chips were packed in LDPE bags, stored at room temperature till further analysis.[3]

Table 1: Proximate analysis of potato per 100g

Parameters	Values
Energy (KJ)	322±1.26
Protein (g)	2.0±0.06
Total Carbohydrate (g)	17.47±0.13
Total Fat (g)	0.1±0.02
Ash Content (g)	0.8±0.01
Moisture (g)	79±1.54

Note: ± is standard deviation from the mean value

Application of L-asparginase enzyme

L-asparaginase lyophilized enzyme powder was dissolved in deionized water to obtain a solution with enzyme concentration of 100 $U.ml^{-1}$ (1 unit is the amount of enzyme which releases 1 µmol NH_3 from L-asparagine per minute at pH 8.6 and 37°C). The prepared solution was used for pre-treatment of potato slices in concentrations of 0.2 U (T1), 1.0 U (T2), 1.5 U (T3) and 2.0 U (T4) per g of sample, respectively before frying. The samples were incubated for 30 min, respectively at room temperature on a shaker (exact conditions are given for each experiment)[7] and compared with control (T0).

Detection of Acrylamide content in potato chips

Standard curve of Acrylamide by UV- Spectroscopy

The standard acrylamide solution was prepared by dissolving 10g of acrylamide in 100ml of distilled water. Then one ml of acrylamide solution was diluted to 100ml of 1M sodium hydroxide. This sample was considered as standard stock solution of acrylamide. The standard stock solution of acrylamide (1 – 10ml) was transferred into series of 10ml test tubes and made up to the volume with distilled water. The absorbances of known concentrations 1, 2, 3…10µg/ml solutions were measured at 275nm wavelength. The calibration curve was plotted between concentration vs absorbance. It is represented in Fig.1. Acrylamide was liner within the concentration range of 1 – 10µg/ml at 275nm.[8]

Preparation of sample

The potato chips of different treatments with L-asparginase enzyme were converted into fine powder followed by homogenization using a homogenizer. Each samples were homogenized with the addition of water (the ratio of powder to water was taken as 1:10), for proper mixing it kept for 20 minutes in water. The homogenized samples were allowed to centrifuge at 10000 rpm for 15 minutes. The supernatants liquid of all the samples were collected. The one ml of supernatant solution from each sample was added up to 100 ml with 1M sodium hydroxide. The absorbance was measured at 275 nm wavelength using water as a blank. The process was repeated for three times for all the samples. The concentration of acrylamide was measured from the different samples.[8]

Table 2: The effects of concentration of L-asparginase enzyme on acrylamide level in fried potato chips (ppm)

Treatments	Acrylamide content of potato chips (ppm)*
T_0 (Control)	15.650±1.69
T_1 (0.2U)	0.022±0.014
T_2 (1.0U)	0.020±0.011
T_3 (1.5U)	0.019±0.009
T_4(2.0U)	0.018±0.009

*Each value is the average of three determinations

Physicochemical Analysis

The prepared potato chips were subjected to physicochemical analysis for various parameters such as protein, carbohydrate, fat, ash, moisture content, etc. as per the standard methods.[9]

Sensory analysis

The prepared potato chips were subjected to sensory analysis for various quality parameters such as appearance, color, taste, texture, crispiness, mouth feel and overall acceptability by semi trained panel of judges with the help of 9 point hedonic scale.[9]

Table 3: Physicochemical analysis of fried potato chips per 100g

Parameters	Values
Energy (KJ)	547±1.62
Protein (g)	6.56±0.21
Total Carbohydrate (g)	49.77±1.34
Total Fat (g)	37.48±1.26
Ash Content (g)	3.96±0.19
Moisture (g)	2.29±0.17

Results and Discussion

The proximate composition of potato slices before frying was estimated with respect to energy content, protein, carbohydrate, fat, ash content, moisture, etc. (Table 1). It was observed that the potato is a rich source of nutrients in general and carbohydrates in particular. The protein content was 2 g which might be having number of amino acids that plays important role in the maillard reaction. The results obtained with respect to proximate composition are in conformity with “Nutrient data laboratory” United States Department of Agriculture.

The effects of different concentration of L-asparginase enzyme on acrylamide level in fried potato chips presented in Table 2. It was observed that in the control sample (T_0) acryalamide level was 15.65 ppm which was very high. Whereas the chips treated with 0.2U enzyme concentration (T_1) was significantly reduced from 15.65 ppm to 0.022 ppm. However the chips treated with 1U enzyme concentration (T_2) was slightly decreased with 0.020 ppm with

decreasing trend for the next treatment of 1.5U enzyme concentration (T_3) and 2.0U enzyme concentration (T_4) with 0.019 ppm and 0.018 ppm respectively. Sumithra *et al.*, 2015[8] also studied the effect of enzyme on level of acrylamide. It was concluded that the potato chips treated with 2.0U enzyme concentration (T_4) had shown the significant reduction in acrylamide content.

Table 4: Effect of enzyme treatments on sensory characteristics of fried potato chips

Treatments	Color	Taste	Texture	Crispiness	Mouth feel	Overall acceptability
T_0	6±0.13	9±0.13	8±0.19	9±0.16	8±0.21	8±0.12
T_1	7±0.15	8±0.11	7±0.16	8±0.13	6±0.13	9±0.11
T_2	7±0.19	7±0.18	8±0.28	8±0.24	6±0.15	7±0.12
T_3	8±0.11	9±0.27	9±0.21	9±0.31	7±0.23	9±0.13
T_4	9±0.28	7±0.27	8±0.24	7±0.27	7±0.17	6±0.12

*Each value is the average of 10 determinations Where T_0 -Control, T1-0.2U, T2 – 1.0U, T3-1.5U, T_4-2.0U Enzyme concentration

The physicochemical parameters of potato chips after frying were studied. The fried potato chips contained protein 6.56%, carbohydrate 49.77%, fat 37.48% whereas ash 3.96% and moisture 2.29% (Table 3). While estimating the results of physicochemical analysis for all the treatments it was observed that the nutritional value of fried potato chips does not change with the addition of enzyme treatment of L-asparginase. The nutritional information was same for all the treatments. The results obtained with respect to physicochemical analysis are in conformity with "Nutrient data laboratory" United States Department of Agriculture.

The sensory analysis of potato chips were carried out for various sensory parameters like color, taste, texture, crispiness, mouth feel, overall acceptability by semi trained panel of 10 judges by using 9 point hedonic scale (Table 4). The color of sample T_4 treated with 2.0U enzyme concentration scored maximum i.e. 9, whereas chips treated with 1.5U enzyme concentration (T_3) scored 8, however chips treated with 1.0U enzyme concentration (T_2) and chips treated with 0.2U enzyme concentration (T_1) scored same i.e. 7 while T_0 scored least i.e. 6. It can be seen from the score that for color, chips treated with 2.0U enzyme concentration (T_4) was good as compare to other samples because of high dose of L-asparginase enzyme and low acrylamide content there was no brown color formation whereas the control sample (T_0) had brown color because of the high acrylamide content and it was not treated with enzyme. It was found that taste of T_0 and T_3 scored maximum i.e. 9 than other samples while T_2 and T_4 scored less i.e.7 due to typical note of potato chips in control sample and low dose of enzyme concentration in T_3 sample it was highly accepted for taste whereas because of mild synthetic note of enzyme in other samples, they were least accepted. In case of texture chips treated with 1.5U enzyme concentration (T_3) scored maximum i.e. 9 as compared to all samples however chips treated with 0.2U enzyme concentration (T_1) scored less i.e. 7. The crispiness of T_0 and T_3 scored maximum i.e. 9 than other samples whereas chips treated with 2.0U enzyme concentration (T_4) sample scored least i.e.7 In case of mouth feel control sample (T_0) scored high i.e. 9 it may be because that desired typical taste of potato chips seen in it, while the chips treated with 1.5U enzyme concentration (T_3) and chips treated with 2.0U enzyme concentration (T_4) were better than chips treated with 0.2U enzyme concentration (T_1) and chips treated with 1.0U enzyme concentration (T_2).

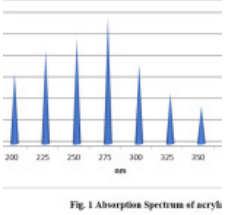

Fig. 1 Absorption Spectrum of acryli

Figure 1: Absorption Spectrum of acrylamide

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Conclusion

It was concluded that the application of L-asparginase enzyme to the potato slices befire frying shows significantly lower level of acrylamide content as compare to control sample. The

nutritional composition and sensory properties of chips were not affected by the treatment with L-asparginase enzyme. The acrylamide formation was drastically reduced to 0.019 ppm in the chips treated with 2.0 IU enzyme concentration as compared to control chips in which acrylamide formation was 15.65 ppm.

There is no conflict of interest.

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