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-asparaginase 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 acrylamide 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.

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. Deep fat frying is extensively used in food processing both industrially and at home and fried potato products are one of its largest applications. 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.

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. The confirmation of this method was accomplished through selective

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To link to this article: http://dx.doi.org/10.12944/CRNFSJ6.1.25
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. The main pathway of acrylamide formation in fried potato products is the reaction of free asparagine and reducing sugars (asparagines route). Therefore the contents of these precursors in fried products are important and have to be controlled.

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-asparaginase 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. Blanced 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.

Application of L-asparaginase 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 NH₃ 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) 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 10 g of acrylamide in 100 ml of distilled water. Then one ml of acrylamide solution was diluted to 100 ml of 1M sodium hydroxide. This sample was considered as standard stock solution of acrylamide. The standard stock solution of acrylamide (1 – 10 μl) was transferred into series of 10 ml test tubes and made up to the volume with distilled water. The absorbances of known concentrations 1, 2, 3…10 μg/ml solutions were measured at 275 nm 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 – 10 μg/ml at 275 nm.
Preparation of Sample
The potato chips of different treatments with L-asparaginase 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.

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.

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.

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 maitiard reaction. The results obtained with respect to proximate composition are in conformity with “Nutrient data laboratory” United States Department of Agriculture.

| 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.
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 T₀ treated with 2.0U enzyme concentration scored maximum i.e. 9, whereas chips treated with 1.5U enzyme concentration (T₃) scored 8, however chips treated with 1.0U enzyme concentration (T₂) and chips treated with 0.2U enzyme concentration (T₁) scored same i.e. 7 while T₄ scored least i.e. 6. It can be seen from the score that for color, chips treated with 2.0U enzyme concentration (T₀) was good as compare to other samples because of high dose of L-asparaginase enzyme and low acrylamide content there was no brown color formation whereas the control sample (T₀) had brown color because of the high acrylamide content and it was not treated with enzyme. It was found that taste of T₀ and T₃ scored maximum i.e. 9 than other samples while T₄ and T₃ scored less i.e. 7,due to typical note of potato chips in control sample and low dose of enzyme concentration in T₄ 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 (T₃) scored maximum i.e. 9 as compared to all samples however chips treated with 0.2U enzyme concentration (T₁) scored less i.e. 7. The crispiness of T₃ and T₅ scored maximum i.e. 9 than other samples whereas chips treated with 2.0U enzyme concentration (T₄) sample scored least i.e. 7. In case of mouth feel control sample (T₀) 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 (T₃) and chips treated with 2.0U enzyme concentration (T₄) were better than chips treated with 0.2U enzyme concentration (T₁) and chips treated with 1.0U enzyme concentration (T₂).
Table 4: Effect of enzyme treatments on sensory characteristics of fried potato chips

| Treatments | Color | Taste | Texture | Crispiness | Mouth feel | Overall acceptability |
|------------|-------|-------|---------|------------|------------|-----------------------|
| T<sub>0</sub> | 6±0.13 | 9±0.13 | 8±0.19 | 9±0.16 | 8±0.21 | 8±0.12 |
| T<sub>1</sub> | 7±0.15 | 8±0.11 | 7±0.16 | 8±0.13 | 6±0.13 | 9±0.11 |
| T<sub>2</sub> | 7±0.19 | 7±0.18 | 8±0.28 | 8±0.24 | 6±0.15 | 7±0.12 |
| T<sub>3</sub> | 8±0.11 | 9±0.27 | 9±0.21 | 9±0.31 | 7±0.23 | 9±0.13 |
| T<sub>4</sub> | 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<sub>0</sub> – Control, T<sub>1</sub>-0.2U, T<sub>2</sub>- 1.0U, T<sub>3</sub>-1.5U, T<sub>4</sub>-2.0U Enzyme concentration

**Conclusion**
It was concluded that the application of L-asparaginase enzyme to the potato slices before 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-asparaginase 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.

**Acknowledgements**
Facilities provided by Department of Chemical Technology, Dr.Babasaheb Ambedkar Marathwada University, Aurangabad, Maharashtra are gratefully acknowledged.

**Conflict of Interest**
There is no conflict of interest.

**Funding source**
Dr.Babasaheb Ambedkar Marathwada University, Aurangabad, Maharashtra.

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