



# Acrylamide formation in plantain (*Musa paradisiaca*) chips influenced by different ripening stages: A correlation study with respect to reducing sugars, amino acids and phenolic content



L. Shamla, P. Nisha\*

Agro Processing and Natural Products Division, CSIR-National Institute for Interdisciplinary Science and Technology, Thiruvananthapuram 695019, Kerala, India

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## ABSTRACT

The effect of ripening on the formation of acrylamide in deep fried plantain chips made from Nendran variety (*Musa paradisiaca*) was investigated. The precursors of acrylamide formation, reducing sugars (glucose and fructose) and ten major amino acids, were quantified during different stages of ripening using HPLC and correlated with acrylamide formation. The total phenolic content and total flavonoid content were also estimated and correlated with acrylamide formation. Both glucose and fructose increased during ripening and demonstrated a positive correlation on formation of acrylamide (correlation coefficient of  $r = 0.95$  and  $0.94$  respectively ( $p < 0.05$ ), whereas asparagine, was poorly correlated ( $p > 0.05$ ). The decreased levels of phenolic content during ripening of plantain were negatively correlated with acrylamide formation in the deep fried chips prepared. Thus the selection of proper ripening stage renders reduced formation of acrylamide in plantain chips to a reasonable extend.

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## 1. Introduction

Acrylamide is a process induced contaminant that is formed during high-temperature processing (e.g., frying, baking and roasting) of carbohydrate and protein rich foods. The reducing sugars and asparagine are believed to be the main precursors responsible for acrylamide formation in plant-based foods (Bent, Maragh, & Dasgupta, 2012). The Maillard reaction which involves the interaction between the free amino group of amino acids or proteins and the carbonyl group of sugars or carbohydrates has an important role in the formation of acrylamide along with time-temperature combinations. Decarboxylation and deamination of the amino acid asparagine have also been suggested as a possible pathway for the formation of acrylamide. Though different mechanisms have been postulated for the formation of acrylamide in plant-derived foods, the concentration of asparagine and reducing sugars are the major determinant factors (Friedman, 2015).

Banana/plantain is the fourth most important food crop in the world after rice, wheat, and maize followed by potato and India is the largest producer of banana in the world (Singh, Singh, Kaur, & Singh, 2016). Since plantain is a good source of carbohydrate, the formation of acrylamide during high-temperature pro-

cessing such as frying and baking is unavoidable. A study by Daniali, Jinap, Zaidul, and Hanifah (2010) on the acrylamide content of five popular Malaysian fried and baked banana based snacks revealed that the acrylamide content ranged from 74.0 to 7468.8  $\mu\text{g}/\text{kg}$  for banana fritter (pisang goreng), 28.9 to 243.7  $\mu\text{g}/\text{kg}$  for banana chips (kerepek pisang), 160.7 to 500.4  $\mu\text{g}/\text{kg}$  for sweet banana chips (kerepek pisang manis), not detected to 154.4  $\mu\text{g}/\text{kg}$  for banana cake (kek pisang) and 31.7 to 609.1  $\mu\text{g}/\text{kg}$  for banana balls (cekodok pisang). Bent et al. (2012) reported that banana chips (green & ripe) and banana fritters made from bananas were found to contain 100–430, 180 and 1090  $\mu\text{g}/\text{kg}$  of acrylamide, respectively. Effect of maturity on the formation of acrylamide in banana fritters made from *Musa paradisiaca* variety, Awak and Abu has also been reported (Daniali, Jinap, Hanifah, & Hajej, 2013). They indicated a strong correlation between the reducing sugar content and acrylamide formation as on increasing fruit maturity. But no correlation was found with asparagine and acrylamide formation in banana fritters.

Antioxidants particularly phenolic compounds have been reported to inhibit the formation of acrylamide in model and various food systems. It is reported that the phenolic compounds present in plantain extract could act as natural antioxidants which could help in mitigating the toxicity of acrylamide consumed by the body through an antioxidant protective mechanism (Jun et al., 2008). Morales, Jimenez, Garcia, Mendoza, and Beristain (2014) reported that green tea, oregano & cinnamon extracts reduced the acrylamide

\* Corresponding author.

E-mail addresses: [pnisha@niist.res.in](mailto:pnisha@niist.res.in), [bp.nisha@yahoo.com](mailto:bp.nisha@yahoo.com) (P. Nisha).

formation in fried potatoes whereas thyme and bougainvillea extracts had no effect on the formation of acrylamide. It was reported that many factors such as the nature of phenolic compounds, conditions of model reactions, and type of food matrices may contribute to the increase or decrease in levels of acrylamide formation (Jin, Wu, & Zhang, 2013). Alternatively, these additives constitute a group of phytochemicals which are highly beneficial to human health because of their antioxidant properties.

Chips made by deep frying of raw matured plantain are a popular snack product in many parts of the world. The chips prepared by deep frying the core of unripe plantain, variety- Nendran (*Musa paradisiaca*) at a stage of maturity II, peel still completely green, is one of the most favorite snack items among children as well as adults, especially in South Asian countries. The plantain/banana ripening is usually divided into seven ripening stages depending on its maturity (Soltani, Alimardani, & Omid, 2011) and the colors were reproduced and translated to a numerical scale where stage I-entirely green; II-green with a trace of yellow; III-more green than yellow; IV-more yellow than green; V-yellow with a trace of green; VI-entirely yellow; VII-entirely yellow with brown speckles. Ripe plantain (Nendran variety) at maturity III & IV are also used for making chips that will be slightly sweeter in taste. A preliminary survey on the market samples carried out by the authors revealed reasonable levels of acrylamide in plantain chips (unripe & ripe) which were high in ripe plantain chips (Shamla & Nisha, 2014). The survey warranted a detailed investigation on the influence of different ripening stages on acrylamide formation in plantain chips as there were no reports on how the maturity affects the sugar & amino acid profile and the phenolic content of plantain. Recent studies on the mitigation of acrylamide formation in foods revealed that when polyphenols are added as antioxidants to food systems; it will influence the Maillard reaction affecting formation of acrylamide in food systems (Cheng, Chen, Zhao, & Zhang, 2015; Xu et al., 2014). A good documentation of the precursors such as reducing sugars and asparagine in plantain along with the total phenolic & flavonoid content could be helpful in understanding the formation of acrylamide in plantain chips. Therefore, the present study was undertaken with an objective to investigate the changes in the composition of plantain fruit during different stages of ripening and its correlation with the formation of acrylamide in deep fried chips. Thus, the proper information regarding maturity will help to understand the level of precursors, total phenolic & flavonoid content thereby we can reduce the levels of acrylamide in the final product to a greater extent.

## 2. Materials and methods

### 2.1. Reagents and chemicals

Standard acrylamide (>99%), D-(+) glucose, D-(–) fructose, sodium phosphate, sodium carbonate, aluminium chloride hexahydrate, potassium acetate, sodium hydroxide, gallic acid, quercetin, aluminium chloride, Folin-Ciocalteu reagent, amino acid standards and *o*-phthalaldehyde (OPA) were purchased from Sigma-Aldrich (St. Louis MO, USA). HPLC water was purified on a Milli-Q system (Millipore India Pvt Ltd, Bangalore, India). Solvents used were methanol, acetic acid & acetonitrile of high-performance liquid chromatography (HPLC) grade. Oasis HLB (30 mg, 1 ml) solid phase extraction (SPE) cartridges were obtained from Waters Corp. (Milliford, Massachusetts USA). Minigen syringe filters (0.22 µm diameter) were obtained from Genetix Biotech Asia Pvt. Ltd, New Delhi, India.

### 2.2. Sample preparation

A bunch of plantain with around twenty tiers, variety Nendran (*Musa Paradisiaca*) at stage I maturity (green), was harvested from

an authenticated agricultural farm in Trivandrum, Kerala, India and was kept at ambient conditions. Chips were made from plantain using maturity stages I–V. Two days of subsequent intervals were given for each ripening stages two, three, four and five respectively, after harvesting. Plantains were peeled manually, and the remaining fruit was used for making chips. The proximate composition, reducing sugars, amino acids, and phenolic compounds of the peeled fruit were estimated. Fig. 1 represents maturity stages I–V of plantain, plantain fruit after peeling and the chips prepared from it.

### 2.3. Preparation of plantain chips

As chips cannot be made from fully ripened plantain, only stages of I–V were selected for preparing plantain chips in the present study. After peeling the plantain manually, the remaining fruit was sliced into thin rounds with thickness of approximately 1 mm. The sliced samples (500 g) were deep fried in coconut oil (3 L) using a stainless steel electrical deep fat fryer (NOVA, Flomatic Industries PTE Ltd, Singapore) at 165 °C for 7 min. The temperature and time of frying adopted for the study was optimized earlier based on product quality in terms of texture (by rupture test of banana chips using texture analyser, TA-HDi, Stable Microsystems, UK, using a ball probe) and color (*L*, *a* and *b* values using ColourFlex EZ, HunterLab Instruments, Virginia, USA) and sensory analysis in comparison with the commercial samples. The temperature was monitored by the use of a thermometer TTX 110 type T temperature prob (Ebro, Germany). For each stage, chips were prepared in triplicates, using the fruit from different tiers. Freshly prepared chips from each ripening stage were analyzed for the acrylamide content using the procedure given under 2.9.

### 2.4. Chemical compositional analysis

The standard procedures of AOAC (2005) were used for the determination of moisture, ash, crude fat and protein contents of all stages of raw plantain. Triplicate samples of all the five stages of plantain were oven-dried at 100 °C transferred to a desiccator, and allowed to cool at room temperature for moisture content. The sample weights were recorded before and after heat treatment in a muffle furnace (550 °C for 12 h) for the ash content determination. Micro-Kjeldahl method was used for the protein estimation with nitrogen to protein conversion factor of 6.25 and fat content was determined using Soxhlet extraction. Total carbohydrate was calculated using the difference method.

### 2.5. Determination of reducing sugars by HPLC

The reducing sugars, glucose and fructose of plantain fruit (fresh samples after peeling as mentioned under 2.2), were measured using Shimadzu HPLC (Kyoto, Japan) technique using a reversed phase Supelcosil LC-NH<sub>2</sub> column (25 cm × 4.6 mm, 5 µm) equipped with a Refractive Index detector. The standards of glucose and fructose in the concentration range 5–15 mg/mL were used for quantification. The sample extraction was carried out according to Vivanti, Finotti, and Friedman (2006) with slight modifications. For the sugar analysis, the sample preparation was as follows. 1 g of plantain fruit (after peeling) paste was mixed with 10 ml acetonitrile/water (8.5:1.5 v/v) and stirred for 5–15 min. The suspension was centrifuged at 1700g for 10 min, and the supernatant was passed through 0.22 µm syringe filter. The mode of elution used was isocratic with the mobile phase consisting of (85:15 v/v) acetonitrile/water at a flow rate of 1 mL/min.

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