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Quality enhancement of potato chips through acrylamide mitigation and comparison with local potato chips in Bangladesh

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Abstract

Potato chips are popular and frequently consumed ready-to-eat food in the world. Acrylamide is a possible carcinogenic and neurotoxic compound formed in potato chips during heat processing, a public health concern. This study aimed to develop potato chips to mitigate acrylamide formation, followed by Food and Drug Administration (FDA) guidelines with some modifications. A total of 15 local chip samples were collected by a stratified random sampling method and were assessed for nutritional values, sensory attributes, microbial quality, and acrylamide levels. Acrylamide was extracted from potato chips using a novel activated charcoal method, then determined by the High- Performance Liquid Chromatography (HPLC) method and showed that only 40% of local potato chips were above the benchmark level established by the EU 2017/2158 (750 ppb) (parts per billion) and ranged from 461 ppb to 2129 ppb while in the developed chips it was not detected. The microbial results showed that the developed chips are safe according to Bangladesh Standards and Testing Institution (BSTI) standard, while the local chips contain total viable count (TVC) ($>10^4$) and yeast-mold count in a significant amount ($>10^3$). Sensory evaluation results showed that the developed potato chip samples had high ratings for all the evaluated attributes. From the nutritional point of view, fat content is lower while protein and fiber content is higher in developed potato chips. Moisture content is significantly higher in local potato chips than in developed potato chips, which is the major concern for microbial quality. As a result of the study's findings, acrylamide formation in potato chips was successfully mitigated by adhering to FDA modification guidelines without compromising quality, despite the fact that many locally produced potato chips are unfit for human consumption.

1. Introduction

The potato (*Solanum tuberosum* L.) is a major crop in Bangladesh, and it is a good source of energy, carbohydrates, and fiber (Dourado *et al*., 2019). Potato chips are the most popular ready-to-eat food in Bangladesh. According to the Food and Agriculture Organization of the United Nations and World Health Organization (2004), "ready-to-eat foods" are foods that include any food consumed in its raw, cooked, or fried stage or into a form in which it is normally consumed directly. Ready-to-eat potato chips are convenient but can contribute to less nutritional value, along with foodborne illnesses, the presence of chemical hazards, and process contaminants (Gurler *et al*., 2015). Thus, the production of safe food has become a major global concern considering consumers' health. Acrylamide, a process contaminant produced predominantly in the Maillard reaction at a high temperature (Bhuiyan *et al*., 2017; Branciari *et al*., 2020), has been brought into the limelight of the food sector by Swedish scientists when they found it in protein-rich foods in moderate levels (5-50 g/kg) and higher contents (150-4000 g/kg) in carbohydrate-rich foods, such as potato chips, french fries, beetroot, and crisp bread that had been heated above 120°C (Tareke *et al*., 2002). Recent assessments by the Joint Expert Committee on food additives confirmed that risk could not be excluded from a dietary

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intake of acrylamide because it is classified as a human carcinogen (International Agency for Research on Cancer (IARC), 1994). Currently, no regulatory limits for the presence of acrylamide in food have been established, even though many countries have formulated recommendations and guidelines to educate industries about mitigation strategies for this substance in food, such as the FDA guidance on acrylamide in foods (US Food and Drug Administration (US FDA), 2016), the Heatox project report (Hellenäs *et al*., 2005) and the acrylamide toolbox (Food Drink Europe (FDE), 2019). An average intake of acrylamide ranging from 0.2 –4 g/kg bw is considered high (Joint FAO/WHO Expert Committee on Food Additives (JECFA), 2005). Both the JECFA (2005) and the European Food Safety Authority (EFSA Panel on Contaminants in the Food Chain (CONTAM), 2015) agree that the major contributors to acrylamide intake are potato crisps and chips, coffee, bread, cakes, and biscuits. There are several approaches to reducing the levels of acrylamide in food (Albedwawi *et al.*, 2021). Since dietary exposure to acrylamide has been recognized as a major health concern, different approaches have been investigated to minimize the acrylamide level in food products. These strategies include the utilization of raw materials with low levels of precursors, controlling the process conditions (pH, temperature, time) or post-processing approaches such as evaporation and polymerization (Xu *et al*., 2016; Khorshidian *et al*., 2020; Ledbetter *et al*., 2021). Thus, the potential strategies to prevent acrylamide formation may be covered by two major approaches: removing acrylamide precursors (glucose, fructose, and asparagine) or interfering with the Maillard reaction (Capuano and Fogliano, 2011; Powers *et al*., 2013; Singh and Kaur, 2016; Genovese *et al*., 2019). Despite this, fried potato chips are highly susceptible to acrylamide formation due to asparagine and low sugar content at the high temperatures used in acrylamide formation in food processing (Parker *et al*., 2012). Due to the large consumption of fried potato chips worldwide, the reduction of undesirable acrylamide without compromising the sensory characteristics is essential (Dourado *et al*., 2019). Therefore, the major concern of food producers is to mitigate acrylamide content while keeping quality parameters unaffected. Since potato chips need to monitor the presence of acrylamide as proposed by Commission Regulation (European Union) 2019/1888, it is particularly important to conduct extensive research and quantitative analysis of the process control of acrylamide content in food products. Thus, the study aimed to develop a potato chip with low acrylamide formation during cooking and evaluate its nutritional, microbial, and sensory attributes in comparison to local potato chips available in Bangladesh.

2. Materials and methods

2.1 Sample collection

Lady Rosetta, a low acrylamide potato (Rahman *et al*., 2016), was obtained from the Bangladesh Agricultural Development Corporation (BADC) and stored at temperatures above 4°C until chip preparation. Other raw materials, such as oil, salt, and spices, were collected from the grocery stores in Chattogram, Bangladesh. Then, non-branded, locally manufactured potato chips were considered the population for the study. In total, 15 potato chip samples from 5 local brands were collected randomly for analysis and coded as LC1, LC2, LC3, LC4, and LC5, respectively. The chip developed in the Food Processing and Engineering Laboratory is coded as DC.

2.2 Media and chemicals

Mueller-Hilton agar media, buffer peptone water, plate count agar, Lauryl sulfate tryptose broth, *E. coli* broth (EC broth), Brilliant green lactose bile broth, Tetrathionate (TT) broth, Rappaport-Vassiliadis soya (RSV) broth, XLD Agar, VBM Agar Plates, Rappaport-Vassiliadis soya (RSV) broth, Muller-Kaufmann Tetrathionate Novobiocin Broth (MKTTn) enrichment medium, VBM Agar, Dichloran Rose Bengal Chloramphenicol (DRBC), and Dichloran 18% Glycerol (DG18) agar media were purchased from Hi-Media, India. Acrylamide (99.9%) was obtained from Sigma-Aldrich (Stockholm, Sweden). All other solvents, activated charcoal, and chemicals used for the analysis of acrylamide were of analytical grade.

2.3 Bacterial strains and inoculum preparation

Three bacterial strains that cause food poisoning were selected to test the potato chips. These strains were *Escherichia coli*, *Salmonella* spp. and *Shigella* spp. The bacterial strains were obtained from the culture collection of the Industrial Microbiology Section, IFST, BCSIR, Bangladesh. Each bacterial strain was subcultured overnight in Mueller-Hilton agar media at 30°C. The bacterial growth was harvested using 5 mL of sterile saline water and diluted to attain a viable cell count of 10^7CFU/mL using a spectrophotometer.

2.4 Microbiological analysis

Microbiological analysis was performed using the procedures of the Bacteriological Analytical Manual (Maturin and Peeler, 2001; Tournas *et al.,* 2001). A sample (25 g) was aseptically weighed and properly enriched with 225 mL of buffer peptone water (Hi-Media, India). For total viable count (TVC), an aliquot (1

https://doi.org/10.26656/fr.2017.8(6).528

mL) of potato chip was diluted in 9 mL buffer peptone water, thus placed on plate count agar (Hi-Media, India) following the serial dilution method, and incubated at 37°C for 24 hrs. After the incubation, the number of colonies was observed and reported as a log of colony forming units (CFU/g). Total Coliforms and *Escherichia coli* counts were performed on a screw cap tube using Lauryl sulfate tryptose broth (Hi-Media, India), *E. coli* broth (EC broth), and Brilliant green lactose bile broth (Hi-Media, India). The most probable number (MPN) of Coliforms per gram of potato chip was determined by confirming gas formation in the number of tubes. Following the streak plate technique with an enriched sample, the sample was enriched with Tetrathionate (TT) broth and Rappaport-Vassiliadis soya (RVS) broth for 18 hrs at 37°C. The detection of *Salmonella* spp. was performed according to the International Organization for Standardization 6579-1 (2017). From each product, 25 g was taken into a sterile BagFilter bag with 225 mL of buffered peptone water. The sample was homogenized in a paddle blender at 100 rpm for 20 s before being incubated at 37°C for 24 hrs. After that, 0.1 mL aliquots were added to 0.9 mL of Muller-Kaufmann Tetrathionate Novobiocin Broth (MKTTn) enrichment medium, and portions of 1 mL were added to 10 mL of Rappaport enrichment medium-Vasiliadis Soya (RVS). Tubes were incubated for 24-48 hrs at 37°C and 42°C, respectively. After 24-48 hrs, aliquots of 0.1 mL were removed from each enrichment medium, and the loop was inoculated onto XLD agar and VBM agar plates. Incubation was executed at 37°C for 30 hrs for the detection of characteristic colonies. *Salmonella-Shigella* (SS) agar and Hektoen enteric agar were used and incubated for 24 h at 37°C for detection of *Shigella* spp. Yeast and mold counts were determined by the spread plate method with Dichloran 18% glycerol (DG18) agar media and Dichloran Rose Bengal Chloramphenicol (DRBC) media. The plates were incubated at 37°C for 5 days. The number of CFU was counted and reported as log CFU/g. All the microbial experiments were conducted in triplicate.

2.5 Preparation of potato chips

The potato chip preparation method was developed following FDA guidelines (FDA, 2016) with slight modifications to mitigate the acrylamide level (Figure 1). Firstly, the selected potato variety low in acrylamide precursor was collected and stored above 4°C until chip preparation. It was ensured that the potatoes purchased for the study were kept at a temperature above 4°C after harvesting. The collected potatoes were washed thoroughly in running water and peeled using a hand peeler. Then peeled and trimmed potatoes were sliced into thinner slices (1 mm) to reduce acrylamide due to lower thermal requirements. Then the potato slices were soaked in a 0.5% sodium chloride solution for 1 min before blanching at 70°C for 1 min. Then these chips were fried at 160°C for 3 mins. Post-drying was done to reduce moisture content (1.3% to 1.5%) at 105°C for 75 mins (Kita *et al*., 2004). After spicing, potato chips were cooled to an ambient temperature to facilitate packaging and storage. To preserve the compositional properties of the finished potato chips, they were packed in an airtight, impermeable packet. To maintain quality until further sensory evaluation and attributive tests, packed potato chips were stored in a cool, dry place.

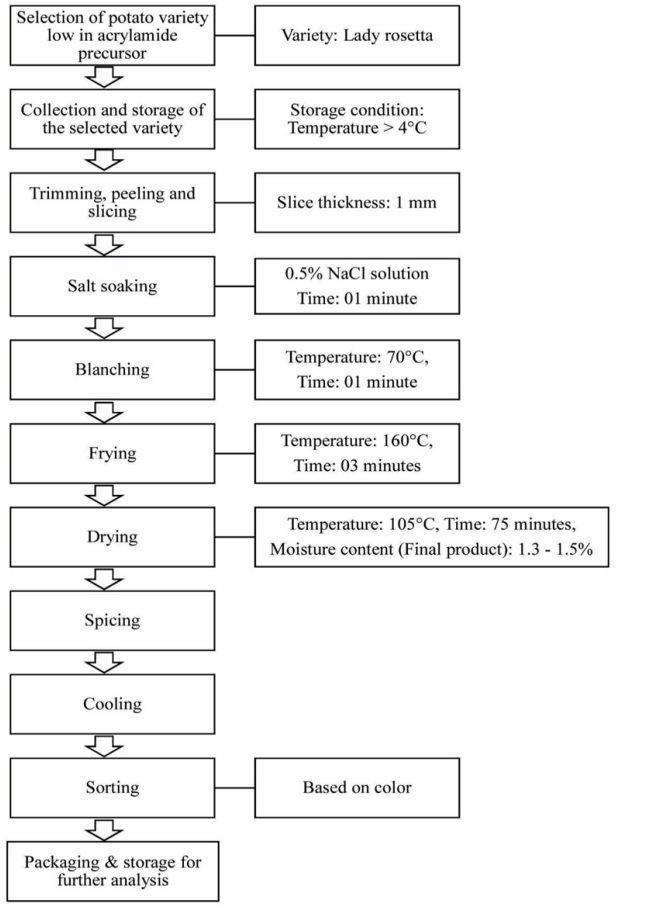

Figure 1. Process flow chart of developing potato chips preparation method for less acrylamide production.

2.6 Proximate analysis

Proximate analysis (moisture, protein, fat, crude fiber, and ash content) of the developed and local potato chips was done using the Association of Official Analytical Collaboration (AOAC) International (2005) method. Briefly, each sample was finely ground, weighed, and placed (10 g) into a 50 mL test tube. The total carbohydrate content of the sample was determined by the difference value.

Carbohydrate (%) = 100 – (Moisture % + Ash % + Protein % + Fat %)

The energy was calculated with Atwater's

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conversion factors, 9 kcal/g for fat and 4 kcal/g for carbohydrate and protein (Sánchez-Peña *et al*., 2017). The following equation was used to calculate energy.

Energy (kcal) = (9 × fat) + (4 × carbohydrate) + (4 × protein)

2.7 Sensory evaluation of developed potato chips

A panel of 10 assessors with experience in the descriptive evaluation of crispy potato products was used to evaluate the developed potato chips. The developed potato chips were subjected to sensory evaluation. Using the sensory attributes (appearance, taste, crispness, color, and overall acceptability), sensory evaluation was carried out by ten trained members of CVASU. The scores were based on a hedonic scale (9-point method). To score crispness, the instruction was to evaluate all aspects and qualities of the sound produced including deformability and brittleness. The chips were served in random order, each on its own glass plate, and each was labeled with a two-digit random code. Between sample evaluations, panelists were instructed to rinse their mouths with water.

2.8 Acrylamide quantitation of developed and local chips

2.8.1 Acrylamide extraction by charcoal treatments

A novel method was used to extract acrylamide from both local and developed potato chips. Ten grams of each potato chip were crushed in a vessel and dissolved in 100 mL of methanol (MeOH) for 3 hrs. After that, all of the methanolic extracts were filtered through a 45-mm filter paper and transferred to 50 g of activated charcoal. This process was repeated thrice. The application of activated charcoal plays a major role in the decolorization of methanolic extracts. Finally, the decolorized methanolic extract from the activated charcoal treatment was dried in a rotary evaporator, weighed, and refrigerated at 4°C for further analysis.

2.8.2 Acrylamide quantification by HPLC

Approximately 10 mg of each dried refrigerator sample were dissolved in HPLC grade MeOH at a concentration of 10 mg/mL. The methanolic solution was filtered through a 0.22 µm syringe filter (Pall Corporation, Port Washington, USA). The standard acrylamide solution was prepared in HPLC grade MeOH at a concentration of 10 mg/mL. The DionexUltimateTM 3000 HPLC system (ThermoFisher Scientific, Germany) was used to quantify acrylamide in both local and developed potato chips. Acrylamide calibration standards and, separately, both sample extracts were injected into the HPLC using a 20 µL injection syringe onto a C18 RP (4.6 × 250 mm, 5µm) column. The confirmatory analysis of the standard acrylamide was performed on the same instrument, coupled with a UV detector. Separation of acrylamide was achieved under isocratic conditions using a mixture of methanol: water (15:85, v/v) as the mobile phase flow rate at 0.8 mL/min, and the detection wavelength was observed at 210 nm (Longhua *et al*., 2012). A calibration curve was made to define the level of acrylamide in both samples; a linear calibration curve was achieved using an acrylamide standard solution at a concentration of 10 mg/mL. Chromeleon 6 software was used to acquire and process chromatographic data. For the reproducibility of the results for the determination of acrylamide, all samples were injected with HPLC three times.

2.9 Statistical analysis

The R programming software (R Core Team, 2022) was used for the analysis of variance (ANOVA) and Turkey's HSD test. In the "Agricolae" R package for computational analysis of data in this study, a P-value<0.05 was considered statistically significant for comparing the different groups (Mendiburu and Yaseen, 2020).

3. Results and discussion

3.1 The nutrient composition of developed and local potato chips

The results of the nutritional composition (moisture, ash, protein, and fat) of developed potato chips and local potato chips are presented in Figure 2. The moisture content of all local potato chip samples ranged from 2.54 to 4.43%, where the moisture of local chip LC2 (4.43%) was significantly higher than LC1 (3.79%), LC3 (3.97%), LC4 (3.82%), and LC5 (2.54%), respectively. Compared with the local potato chips, the moisture content of the developed potato chips was significantly lower (1.50%), which is an indicator of food quality by enhancing the product shelf-life during storage by inhibiting microbial growth and chemical reactions in food (Purwani *et al*., 2006). The ash, fat, and protein content of developed chips were 3.03%, 22.20% and 5.94%, respectively. The ash, fat, and protein contents of all the local potato chip samples ranged from 1.68 to 3.79%, 27.95 to 42.72% and 2.77 to 7.62%, respectively. Lower fat content was found in developed potato chips compared with all local potato chips, which may reduce the risk factors for coronary heart disease. The dry matter, carbohydrate, crude fiber, and energy values of developed and local potato chip samples are shown in Figure 3. The amounts of dry matter, carbohydrates, and crude fiber in local potato chips range from 95.50 to 97.460%, 40.49 to 62.04% and 0.07 to 5.30%, respectively. The developed potato chip sample showed the highest dry matter (98.5%) and crude fiber (8.44%)

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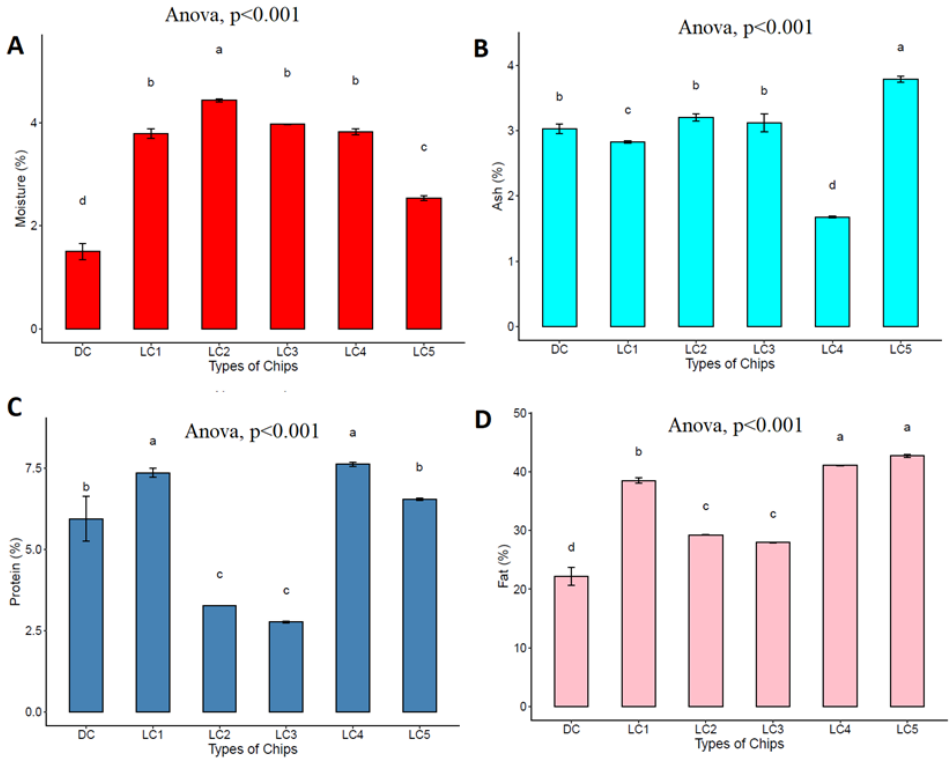

Figure 2. The bar graphs compare the moisture (A), ash (B), protein (C), and fat (D) content of developed (DC) and local (LC) potato chips, respectively. Data are presented as mean±SD, n = 3. Bars with different notations are statistically significantly different (p-value <0.05) using Turkey's HSD test.

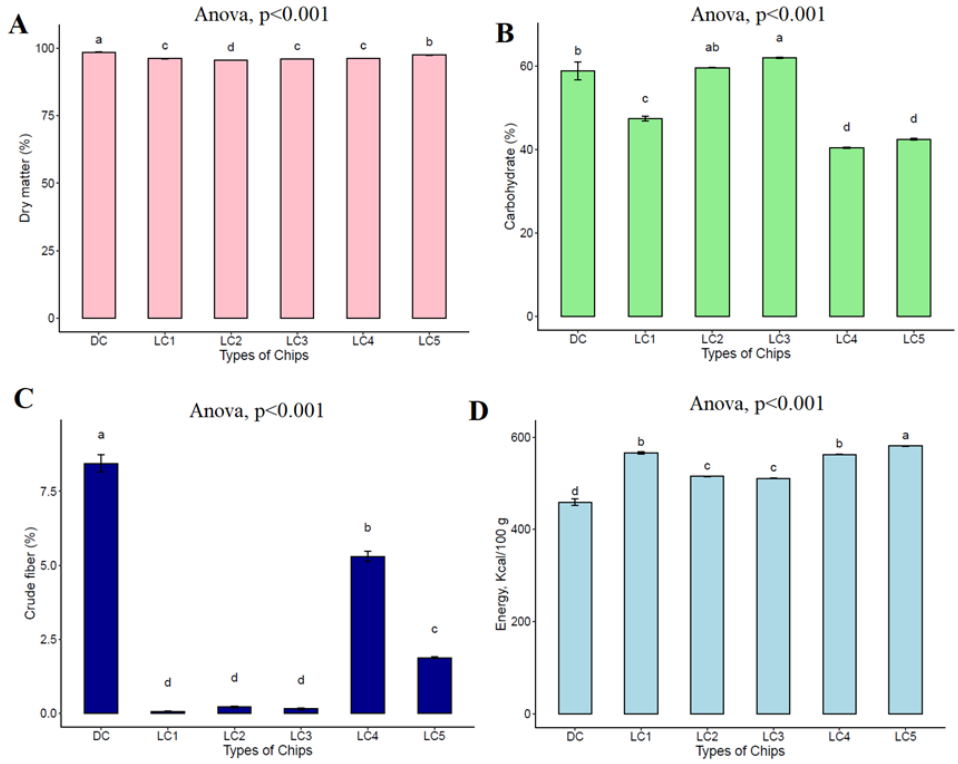

Figure 3. In the bar graphs, the comparative dry matter (A), carbohydrate (B), crude fiber (C) and energy (D) content of developed (DC) and local (LC) potato chips are illustrated. Data are presented as mean±SD, n = 3. Bars with different notations are statistically significantly different (p-value <0.05) using Turkey's HSD test.

content. According to BSTI standards for potato chips (BDS 1927: 2017), the maximum values for moisture %, fat%, and ash% are ≤ 3%, ≤ 37% and ≤ 4%, respectively. The developed potato chips were compliant compared with the local potato chip sample. Among the local potato chip samples, LC5 shows the highest energy value (580.76 kcal/100 g) compared with others.

3.2 Microbial analysis of local and newly developed potato chips

As a dry food item, potato chips are a popular ready-to-eat food when it comes to food safety issues. But there is a possibility of contamination if good manufacturing practices are not maintained. And consumers,

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particularly children, may suffer serious health consequences as a result of consuming contaminated chips. According to BSTI Standards (BDS 1927:2017), the maximum permissible limit in potato chips for the total viable bacterial count is 5.0×10^4 CFU/g, and total Coliforms 10 MPN/g, *E. coli*, *Salmonella* spp., and *Shigella* spp., are absent. In this study, the total viable count (TVC) in the developed potato chips was within the acceptable limit (Table 1), whereas total Coliforms, *E. coli*, *Salmonella* spp., *Shigella* spp., yeast, and mold counts were also absent. However, yeast and mold counts were observed to be unsatisfactory ($>10^3$) according to East African Standards (EAS, 2010) in all local chips. No other pathogens were found, although all local chips had a significant number of total viable count bacteria ($>10^4$) that are present at unsatisfactory levels due to the likelihood of microbial contamination brought on by residual water activity, environmental factors, production processing faults, humidity, and temperature. However, in the Bangladesh Standard for potato chips (BDS 1927:2017), there is no specified limit for yeast and mold. According to the research, the developed potato chips are comparable to all local potato chips in terms of quality from a microbiological standpoint.

3.3 Acrylamide quantitation in developed and local potato chips

In this study, the standard acrylamide average (tR) and the sample mean tR were 4.390 and 4.386 mins, respectively (Figure 4). According to these values, it was confirmed that both of the samples were almost similar. Therefore, a conclusion can be drawn that the analyzed samples contained acrylamide. In this study, the acrylamide content in the developed potato chips was below the detection limit. For local potato chips, acrylamide content was detected in the range of 461-2129 ppb, where the average acrylamide content in LC3, LC4, and LC5 were 2030.67, 1017.67, and 466 ppb, respectively. In Ethiopia, acrylamide levels were obtained ranging from 211 µg/kg to 3515 µg/kg in potato chips (Deribew and Woldegiorgis, 2021). A Chinese study reported higher acrylamide concentrations (3016 µg/kg) in potato crisps (Yuan *et al*., 2007). In Romania, 50 potato chip samples showed acrylamide concentrations ranging up to 1782 µg/kg (Oroian *et al*., 2015). The differences in acrylamide levels and the wide range of levels can be attributed to several factors. In addition to raw materials (Amrein *et al*., 2004) and storage conditions (Powers *et al*., 2017), higher frying temperatures and extended cooking times can result in increased acrylamide formation in potato chips (Matthäus *et al*., 2004; FDA, 2016) and could have led to variations in acrylamide levels. For the limit of human exposure to acrylamide, a benchmark level of 750 ppb for potato crisp made from fresh potatoes and potato dough, potato-based crackers, and other potato products like chips, has been established by the Commission Regulation (EU) 2017/2158. In this study, among the local potato chips, only 40% of the samples had

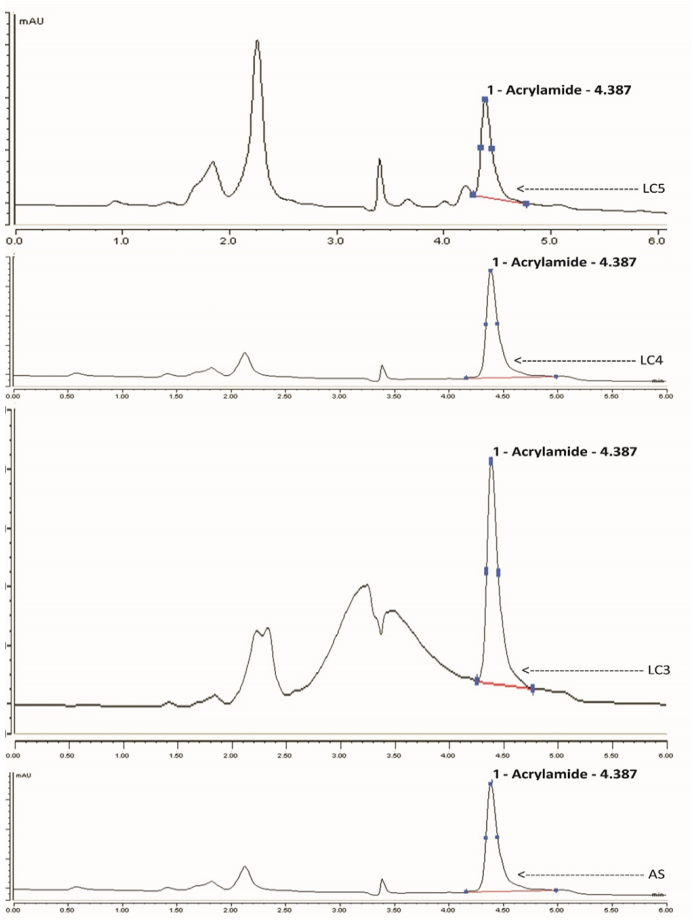

Figure 4. HPLC chromatogram of local potato chips sample LC3, LC4, and LC5 versus acrylamide standard (AS).

Table 1. Microbial quality evaluation of developed and local potato chips.

Microorganisms	DC	LC1	LC 2	LC 3	LC 4	LC 5
Total viable count, CFU/g	4.21×10^4	2.72×10^5	5.45×10^5	2.90×10^5	6.63×10^5	7.27×10^5
Total Coliforms, MPN/g	<0.3***	<0.3***	<0.3***	<0.3***	<0.3***	<0.3***
E. coli	<0.3***	<0.3***	<0.3***	<0.3***	<0.3***	<0.3***
Salmonella spp.	Absent	Absent	Absent	Absent	Absent	Absent
Shigella spp.	Absent	Absent	Absent	Absent	Absent	Absent
Yeast and mold, CFU/g	<10*	$>10^3$ **	$>10^3$ **	$>10^3$ **	$>10^3$ **	$>10^3$ **

*<10 indicates the absence of test organisms in 1g of sample, ** $>10^3$ indicates the presence of test organisms at an unsatisfactory level in 1 g of sample, ***MPN<0.3 indicates the absence of test organisms in 1 g as per MPN chart.

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acrylamide, higher than the baseline level set by the European Commission (EC), but the developed chip is free from acrylamide formation.

3.4 Sensory assessment of the developed potato chips

The results of the sensory evaluation revealed that the developed chip was generally accepted for all attributes evaluated, as none scored below the minimum acceptable rating of 5 on the 9-point hedonic scale. The average score for appearance, color, crispness, taste, and overall acceptability was 8.7±0.48, 8.6±0.51, 8.8±0.63, 8.2±0.63, and 8.5±0.5, respectively (Table 2).

Table 2. Sensory attributes of developed potato chips.

Sensory attributes	Score
Appearance	8.7±0.48
Color	8.6±0.51
Crispness	8.8±0.63
Taste	8.2±0.63
Overall acceptability	8.5±0.52

Values are presented as mean±SD, n = 10.

4. Conclusion

This study revealed the mitigation of acrylamide in potato chips without disturbing nutritional, microbial, and sensory quality attributes through the statistical analysis of data by following the FDA guidelines with slight modifications. However, when compared to a developed chip, the nutritional and microbial quality, as well as the acrylamide level, of most of the locally produced potato chips are unfit for human consumption.

Conflict of interest

The authors declare no conflicts of interest.

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