ACRYLAMIDE QUANTITATION OF LOCALLY AVAILABLE POTATO CHIPS IN CHATTOGRAM & DEVELOPMENT OF A POTATO CHIPS MANUFACTURING PROCEDURE TO REDUCE ACRYLAMIDE LEVEL

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A thesis submitted in the partial fulfillment of the requirements for the degree of Master of Science in Food Processing and Engineering

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This is to certify that we have examined the above Master's thesis and have found that is complete and satisfactory in all respects, and that all revisions required by the thesis examination committee have been made.

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JULY 2018

Dedication

I dedicate this small piece of work to my beloved son

Muhammad Ali Zayan (Zarif)

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Author praises the "Almighty Allah" Whose divine blessings enabled the author to complete this research and writing up this thesis paper for the degree of Master of Science (MS) in Food Processing and Engineering.

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ABBREVIATIONS

$\mu g\ kg^{-1}$	Micro Gram per Kg (also known as “ppb” or “Parts Per billion”)
^0C	Degree Celsius
^0F	Degree Fahrenheit
ACN	Acetonitrile
AOAC	Association of Official Analytical Chemists
BCSIR	Bangladesh Council for Scientific and Industrial Research
$BMDL_{10}$	Benchmark Dose Lower Confidence Limit
C_3H_5NO	Acrylamide
CAC	Codex Alimentarius Commission
CF	Crude Fiber
CHO	Carbohydrate
CP	Crude Protein
$CuSO_4$	Copper Sulphate
DM	Dry Matter
DNA	Deoxyribonucleic Acid
DoE	Department of Environment
DRICM	Designated Research Institute for Chemical Measurements
EFSA	European Food Safety Authority
FAO	Food and Agriculture Organization
FDA	Food and Drug Administration
GL	Guideline
gm	Gram
GOB	Government of Bangladesh
H_2SO_4	Sulphuric Acid
H_3BO_3	Boric Acid
HCL	Hydro Chloric Acid
HPLC	High Performance Liquid Chromatography
IARC	International Agency for Research into Cancer
IUPAC	International Union for Pure and Applied Chemistry
K_2SO_4	Potassium Sulphate
kg	Kilogram
LC-MS	Liquid Chromatography – Mass Spectrometry

ml	Milliliter
MS	Mass Spectrophotometry
N	Normality
NaCl	Sodium Chloride (Natrium Chloride)
NaOH	Sodium Hydroxide (Natrium Hydroxide)
ng	Nano Gram
NH_3	Ammonia
PSA	Primary Secondary Amine
R&D	Research and Development
RF	Response Factor
RSD	Relative Standard Deviation
SAPP	Sodium Acid Pyrophosphate (Disodium Pyrophosphate)
SD	Standard Deviation
SPE	Solid Phase Extraction
TDI	Tolerable Daily Intake
TDI	Total Dietary Intake
USA	United States of America
USFDA	United States Food and Drug Administration
w/v	Weight by volume
WHO	World Health Organization

ABSTRACT

This study has been conducted to quantify the Acrylamide level in the potato chips locally available in Chattogram, Bangladesh. Several markets were surveyed to enlist the available potato chips. Among them only domestic manufactured potato chips were selected as population. Sample size was determined five (5) for the population size of this study following codex guideline and samples were selected using simple random sampling method. Three samples from different production batch of each brand were collected and analyzed for Acrylamide. The average (647.17 $\mu g\ kg^{-1}$) and highest (1523 $\mu g\ kg^{-1}$) Acrylamide content in the analyzed samples were found very high in comparison with the Quantitation in other countries. Considering most of the precautions provided by FAO and other organizations to reduce Acrylamide formation, a manufacturing method of potato chips containing reduced Acrylamide was developed. Again the potato chips was analyzed for Acrylamide. The Acrylamide level of the chips was found below detection limit. The majority of the samples of available potato chips in Chattogram are found unsafe for large quantity consumption by all age group due to presence of higher level of Acrylamide. The outcome of this study strongly recommends conducting a large scale survey and analytical study to determine the Acrylamide level in other fried and over-baked starchy foods frequently consumed in Bangladesh as well as to follow the considerations during potato chips manufacturing to mitigate the risk of Acrylamide formation in potato chips.

Keywords: Potato chips, Acrylamide analysis, Acrylamide mitigation considerations, Proximate analysis

INTRODUCTION

Acrylamide (or acrylic amide) is a chemical compound with the chemical formula C_3H_5NO. Its IUPAC name is Prop-2-Enamide. It is a white odorless crystalline solid, soluble in water, ethanol, ether, and chloroform. Presence of Acrylamide in common heated foods is considered as an important food safety problem by international authorities. Acrylamide exposure varies depending upon the population's eating habits, the way the foods are processed and prepared.

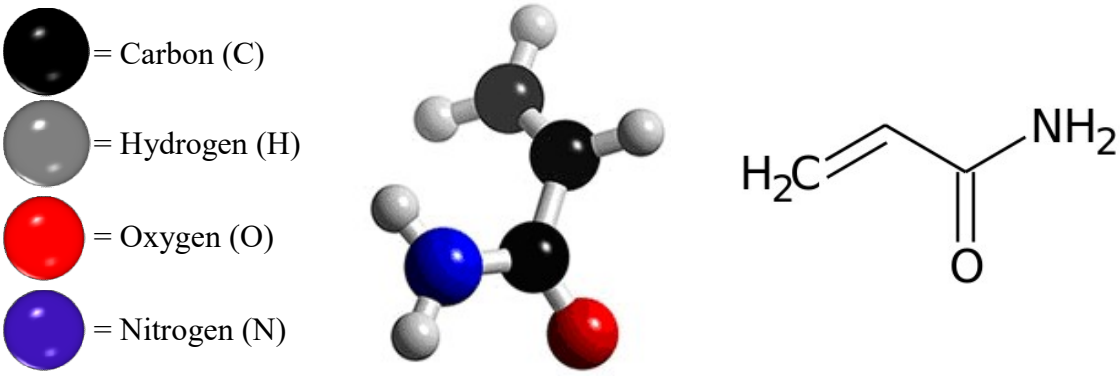

Figure 1.1: Structural Model of Acrylamide

Acrylamide has been brought in the limelight of food sector by the Swedish scientists and the Swedish National Food Authority in the year 2002. They have reported that the existence of Acrylamide in a variety of fried and baked foods, in particular in potato products such as chips. This finding has since been confirmed by many other food scientists throughout the world.

Research into Acrylamide in food shows that the chemical is formed during the frying, roasting or baking (above 120°C) of variety of foods, particularly starchy foods such as potatoes and cereal products, as a side product of the Maillard reaction. Acrylamide was discovered in foods in April 2002 by Eritrean scientist Eden Tareke in Sweden when she found the chemical in starchy foods, such as potato chips, French fries, and bread that had been heated higher than 120°C (248°F).

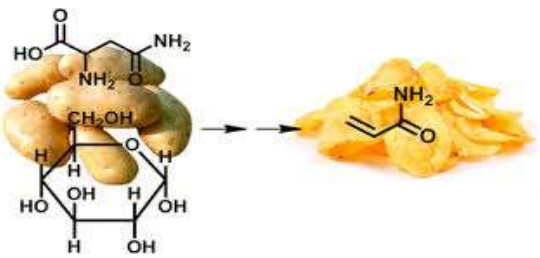

Figure 1.2: Acrylamide formation in potato chips

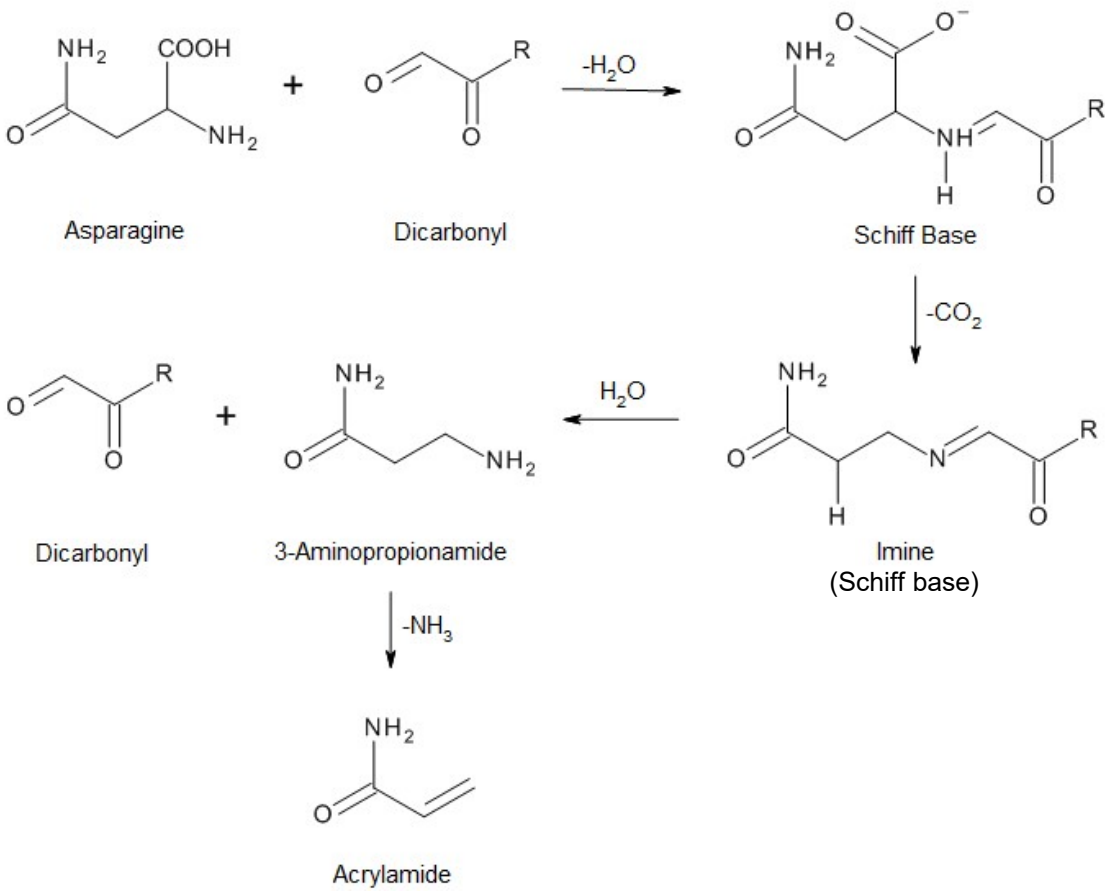

Figure 1.3: Chemical reaction involved in Acrylamide formation

The National Toxicology Program and the International Agency for Research on Cancer consider Acrylamide to be a "probable human carcinogen," based on studies in laboratory animals given Acrylamide in drinking water. Studies in rodent models have found that Acrylamide exposure poses a risk for several types of cancer.

Raw materials with low precursor concentration would generate less Acrylamide in any form of cooking, industrial or domestic, and would reduce the need to adapt processes (Halford *et al.*, 2012). Hence, potato varieties that are low in Acrylamide precursors but give desirable sensory attributes when fried or oven baked are keenly sought. Krause *et al.* (2006) stated that Verdi was an optimum variety for the manufacture of chips because even after storage at 4^0C for 4 months, tuber reducing sugar levels were low.

Thus the study is aimed to detect the level of Acrylamide in available potato chips and to develop a new method of potato chips preparation which will result in lower Acrylamide level in the finished product.

The objectives of the study are followed by;

1. To determine the Acrylamide level in Potato Chips available in Chittagong;
2. To develop a manufacturing method of Potato Chips considering all factors related to Acrylamide level in finished product;
3. Proximate analysis of the potato chips prepared in laboratory;
4. To determine the Acrylamide level in the prepared Potato Chips.

REVIEW OF LITERATURE

Although no significant studies were found conducted in Bangladesh, sufficient recent research and studies are available throughout the world regarding carcinogenicity of Acrylamide, formation of Acrylamide, Food risk group, Acrylamide quantity in Potato chips and Acrylamide reduction considerations in potato chips.

International Agency for Research into Cancer (IARC, 1994) has classified Acrylamide as a probable human carcinogen. It has also been shown to be neurotoxic in humans and may affect reproductive processes.

2.1 Acrylamide in Foods

In June 2002 the World Health Organization (WHO) and the United Nations Food and Agriculture Organization (FAO) convened a meeting of 23 scientific experts. The experts recognized the presence of Acrylamide in food as major concern in humans based on ability to induce cancer and heritable mutations in laboratory animals and the urged investigation of the possibilities for reducing the levels of Acrylamide in food by changes in formulation, processing and other practices.

On April 24, 2002, scientists at the Swedish National Food Administration and Stockholm University reported the discovery of Acrylamide in variety of fried and oven-baked foods. The initial Swedish research suggested that Acrylamide formation is particularly associated with traditional high-temperature cooking processes for certain carbohydrate-rich foods. Subsequent studies in Norway, Switzerland, the United Kingdom and the United States confirmed the Swedish discovery of Acrylamide in certain foods.

Acrylamide was found in nearly all food items analyzed so far, which raises the possibility that it might be present in other food items not yet analyzed. The highest average levels of Acrylamide were found in crisps and chips, there was however a wide range, from not detectable to 3.5 mg/kg product (FAO/WHO, 2002).

In a study of Tawfik *et al.* (2008), the Acrylamide level in different food groups were found in order, mashed-roasted potato (8974 $\mu g\ kg^{-1}$) > fried pasta (1600 $\mu g\ kg^{-1}$) > soluble coffee (816 $\mu g\ kg^{-1}$) > biscuits (810 $\mu g\ kg^{-1}$) > potato chips (620 $\mu g\ kg^{-1}$) >

cocoa powder (256 μg kg^{-1}) > crisp bread (439 μg kg^{-1}) > fried rice (430 μg kg^{-1}) > roasted Turkish coffee (282 μg kg^{-1}) > cereal breakfast (215 μg kg^{-1}) > butter cookies (151 μg kg^{-1}).

According to EFSA (2012), it is evident that the main sources of human dietary exposure to Acrylamide are those of fried potato (~272–570 μg kg^{-1}), bakery products (~75–1044 μg kg^{-1}), breakfast cereals (~149 μg kg^{-1}) and coffee (~229–890 μg kg^{-1}).

2.2 Carcinogenicity of Acrylamide

According to European Food Safety Authority (EFSA, 2015), Acrylamide and its metabolite glycidamide are genotoxic and carcinogenic. Since any level of exposure to a genotoxic substance could potentially damage DNA and lead to cancer, EFSA's scientists conclude that they cannot set a tolerable daily intake (TDI) of acrylamide in food. Instead, EFSA's experts estimated the dose range within which acrylamide is likely to cause a small but measurable tumour incidence (called "neoplastic" effects) or other potential adverse effects (neurological, pre- and post-natal development and male reproduction). The lower limit of this range is called the Benchmark Dose Lower Confidence Limit ($BMDL_{10}$). For tumours, experts selected a $BMDL_{10}$ of 0.17 mg/kg-bw/day. For other effects, neurological changes were seen as the most relevant with a $BMDL_{10}$ of 0.43 mg/kg-bw/day.

Dybing *et al.*(1992) reported that, both Acrylamide and Glycidamide bind to hemoglobin in red blood cells, and the determination of the resulting adducts provides an estimation of the internal dose that accounts for both absorption and metabolism of these compounds over the life of the red blood cells (120 days).

Animal studies of Friedman (2003) documented that high doses of Acrylamide (>203 mg kg^{-1}) caused adverse developmental and reproductive effects in neonatal rodents.

Chen *et al.* (2012) modeled that if the Acrylamide content in French fries is higher than 168 μg kg^{-1} the estimated cancer risk for adolescents aged 13–18 years in Taiwan would be higher than the target excess lifetime cancer risk for high consumers (95^{th} percentile), the excess cancer risk being 3.8×10^{-6} to 1.9×10^{-5} for boys and 3.0×10^{-6} to 1.5×10^{-5} for girls.

2.3 Acrylamide Formation & Mitigation

Tareke *et al.* (2002) proved in their study that Acrylamide is formed during the cooking of carbohydrate-rich food at elevated temperatures. When the frying temperatures were very high (180^0C – 190^0C), the Acrylamide levels increased exponentially at the end of frying. This was most likely due to the fact that Acrylamide formation occurs mainly at the surface of potatoes when the temperature is likely to rise to >120^0C, when Acrylamide formation is believed to form.

In a study of Zyzak *et al.* (2003) Asparagine, through its participation in the Maillard reaction, has been identified as the major precursor of Acrylamide, and heat-treated products containing relatively high amounts of asparagine have been shown to yield correspondingly high Acrylamide concentrations.

According to Pedreschi *et al.* (2014), Acrylamide mitigation techniques can be separated into three different types. Firstly, starting materials low in Acrylamide precursors can be used to reduce the Acrylamide in the final product. Secondly, process conditions may be modified, in order to decrease the amount of Acrylamide formation. Thirdly, post-process intervention could be used to reduce Acrylamide.

Zyzak *et al.* (2003) conducted a research focused on the formation mechanism of Acrylamide. He used commercial asparaginase from Aldrich (A2925 from *Erwinia chrysanthemi*), 50 U added to 60gm of mashed potato slurry (15 g potato, 45 g water), to hydrolyze the asparagine, in order to verify that asparagine is indeed the precursor of Acrylamide. The asparaginase achieved an 88% asparagine reduction that led to 99% Acrylamide reduction in a micro-waved mashed potato snack, heated at full power until brown.

According to Jackson and Al-Taher, (2005) although Acrylamide is not present in raw potato (before cooking or processing), both asparagine and reducing sugar content varied significantly among different cultivars emphasizing the importance of the starting raw material on the Acrylamide forming potential in later processing stages.

Koutsidis *et al.*, (2008 & 2009) stated that, Acrylamide is formed in heated mainly starchy foods through the process of the Maillard reaction in which sugars react with the amino acid asparagine the role of which is well established. However the relative

importance of different sugars and / or carbonyls as reactive species the type of model system as well as the conditions employed may play a crucial role in its formation.

Ciesarová *et al.* (2006) set up a model system to examine the importance of all the Acrylamide formation related factors, such as temperature, dosage and application time. However, there were insufficient time and temperature points studied to determine optimum activity. Applying asparaginase to dried potato powder led to a 90% Acrylamide reduction in cooked product.

Another study by Pedreschi *at al.* (2011) focused on the combination of asparaginase (Acrylaway®) and conventional blanching, alongside their individual usage. Blanching using hot water at 85^0C to treat the potato tuber samples for 3.5min was compared with enzymatic mitigation using an asparaginase solution (10000 ASNU/L) at 50^0C for 20min. One ASNU is defined as the amount of asparaginase that produces one micromole of ammonia per minute under standard conditions (pH 7; 37^0C). Experimental results showed that blanching and enzyme treatments have a similar effect on Acrylamide reduction (17%). By combining the two methods, almost 90% of Acrylamide was mitigated.

MATERIALS AND METHODS

3.1 Location and study period

The study was conducted in the Laboratory of Department of Environment (Chattogram), Food Processing laboratory of Department of Food processing and Engineering, Chittagong Veterinary and Animal Sciences University and Chemical analysis laboratory of Bangladesh Council for Scientific and Industrial Research (BCSIR, Chattogram). The study period was 6 (six) months from January 2018 to June 2018.

3.2 Acrylamide Quantitation

3.2.1 Sampling method and Sample Collection

Sampling method is determined according to General Guidelines on Sampling (CAC/GL 50-2004). According to CAC guidelines appropriate sample size is 5 (five) for the population of this study. Samples are collected from local grocery and food shops situated at Chattogram, Bangladesh. Three (3) packets (replications) of different batch of production of same branded chips were collected. In total 15 samples were collected from the local grocery shops and super-shops of Chattogram, Bangladesh. Samples were coded as shown in the table 3.1.

Table 3.1: Sample Codes

Sample No. / Brand No.	1	2	3
1	B1S1	B1S2	B1S3
2	B2S1	B2S2	B2S3
3	B3S1	B3S2	B3S3
4	B4S1	B4S2	B4S3
5	B5S1	B5S2	B5S3

B = Brand, S= Sample

3.2.2 Sample Preparation

25 (twenty five) grams of each potato chips sample was homogenized and 02 (two) grams of each sample was weighed (using HR-250AZ, A&D company) into a 50 (fifty) ml polypropylene centrifuge tube (Falcon tube). Aqueous extraction of Acrylamide was initiated by the addition of 05 (five) ml of Hexane (95% Anhydrous,

Sigma-Aldrich), 10 (ten) ml Ultra pure Water and 10 (ten) ml Acetonitrile (99.8% , Sigma-Aldrich). The sample was vortexed (using Scilogex vortex mixer) and shaken well for 60 (sixty) seconds. 8 (eight) gm $MgSO_4$ (Magnesium Sulphate Heptahydrate, Merck) and 0.5 gm NaCl (Sodium Chloride, Himedia) was added to the tube. The tube was again vortexed and shaken well for 05 (five) minutes. Then the solution was allowed to settle down until the layer immediate next to hexane layer becomes transparent. Then the Hexane layer was discarded to facilitate collection of extract from the next layer. After that the middle transparent layer (the layer immediate next to hexane layer) was collected. 100 mg Primary Secondary Amine (PSA, Sigma-Aldrich) was added to 2 ml extract and vortexed for 1 (one) minute. Then it was centrifuged (using Hermle Z360 Centrifuge machine) at 11,000 rpm for 10 (ten) minutes. 01 (one) ml of the supernatant was taken in a vial and evaporated to dryness.

3.2.3 Acrylamide Analysis

Acrylamide quantitation of the samples and the developed potato chips were conducted with the Thermo Scientific Surveyor HPLC system. A thermo Scientific Hypercarb 2.1 × 50 mm column was utilized as the analytical LC column. Separation of Acrylamide was achieved under iso-electric conditions using 100% water as the mobile phase at a flow rate of 0.4mL/min. the injection volume for all LC experiments was 10 µL.

To eliminate the need for solid phase extraction (SPE) purification prior to the analysis of the food sample extracts, a column switching LC method was employed.

Briefly, the sample extract was loaded onto a 2.1 × 50nm Thermo Scientific Aquasil C18 column, which was positioned before a 6-port switching valve. The eluant from the C18 column was diverted to waste except for the period when Acrylamide was eluted from the C18 column, whereby the valve was switched to the Hypercarb column for MS/MS detection. This column switching method required a second Thermo Scientific Surveyor MS pump, which also delivered 100% water at 0.4mL/min. Both surveyor MS pumps and the 6-port switching valve were controlled using Thermo Scientific Xcalibur Software.

3.3 Potato Chips Manufacturing Method development

3.3.1 Considerations for method development

The study was also aimed at developing a specific potato chips manufacturing method considering most of the issues mentioned in different studies which lead to lower Acrylamide level producing method for potato chips. Considerations maintained in this study from FDA guidelines, codex guidelines and other research studies are as mentioned hereby;

a) Storage of raw potato above 4^0C after harvesting
b) Cutting potatoes into thinner slices
c) Prolonged soaking of potato flakes in Calcium or Sodium salt solution at temperature higher than ambient temperature
d) Blanching potato flakes with Sodium Acid Pyrophosphate (SAPP) or Disodium Pyrophosphate and avoiding over baking
e) Using lower frying temperature (below 175^0C or 347^0F) when lower moisture content is achieved

3.3.2 Potato chips manufacturing method

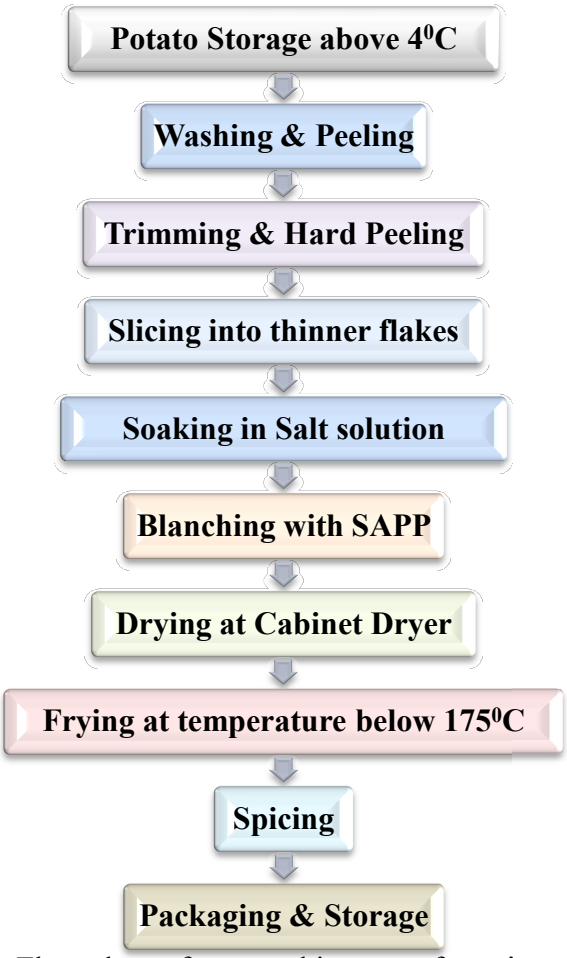

Figure 3.1: Flow chart of potato chips manufacturing method

Step 1: Storage of Potato above 4^0C after Harvesting

Potatoes undergo "Cold sweetening" if stored below 4^0C after harvesting. Thus reducing sugar content increases and promotes Acrylamide level during frying and in finished product. It is necessary to store potatoes above 4^0C to keep reducing sugar content low. It was ensured that the potatoes purchased for the study were kept at temperature above 4^0C after harvesting.

Step 2: Washing & Peeling

Properly stored potatoes are needed to be washed before peeling to remove the mud and other extraneous matters. Thorough washing after peeling also reduces reducing sugar content of potato. Collected potatoes were washed thoroughly in running water and peeled using hand peeler. Peeling was done carefully to reduce unnecessary waste.

Step 3: Trimming, Peeling & Slicing

Hard trimming after peeling is necessary to remove any residual skin, discoloration, disease, insect injury, black spot and green parts. After trimming, peeling is again necessary to remove leftover skins. Then peeled and trimmed potatoes are needed to be sliced to thinner slices to reduce Acrylamide production due to less thermal requirements. Thus peeled potatoes were trimmed and again peeled to remove leftover skins sliced to thinner slice possible.

Step 4: Soaking in Salt Solution

Addition of Calcium or Sodium salt before blanching reduces Acrylamide formation upto 60%. But excess calcium salt compromises frying quality and excess sodium salt compromises the nutritive value. Since calcium is not compatible with SAPP, flakes were soaked in .5 to 1.0 percent sodium chloride solution for one (1) minute prior to blanching with SAPP solution.

Step 5: Blanching with SAPP

To prevent darkening of potato slices they are needed to be blanched along with SAPP (Sodium Acid Pyrophosphate or Disodium Pyrophosohate). Potato flakes were blanched at 50^0C for 3 to 5 minutes with 0.5 to 1.0 percent SAPP Solution.

Step 6: Drying

Drying is required to reduce moisture content. Lower moisture content of potato flakes lead to less time and lower temperature requirement for frying. Lower frying temperature and reduced frying time combination ultimately reduces Acrylamide content in the final chips.

Step 7: Frying below 175^0C or 347^0F

Higher frying temperature leads to higher Acrylamide formation and darkening. On the contrary lower frying temperature leads to higher frying time requirement and unpleasant texture, color and appearance of the chips. This also compromises the crispness of the chips. To avoid Acrylamide formation maintaining the color, texture and appearance of final product, potato flakes were fried at higher temperature until the moisture content came to 1.3 to 1.5 percent and then the flakes were fried below 175^0C or 447^0F until the desired moisture content and color achieved.

Step 8: Spicing

Prepared Potato chips were mixed thoroughly with different spices for taste and palatability.

Step 9: Cooling

After spicing, potato chips were cooled to ambient temperature to facilitate packaging and storage.

Step 10: Acrylamide Quantitation & Proximate Analysis

Three Samples were taken from the prepared chips for Acrylamide Quantitation and proximate analysis.

Step 10: Packaging and storage

Finished potato chips were packed in air tight impermeable packet to retain the compositional attributes. Packed potato chips were stored in cool and dry place to maintain the quality until further sensory evaluation and attributive tests.

3.4 Proximate Analysis & Acrylamide Quantitation of Developed Potato Chips

Proximate analysis of the prepared potato chips was carried out in the Food Processing Laboratory of Food processing and Engineering Department under the Faculty of Foods Science and Technology of Chittagong Veterinary and Animal Sciences University.

3.4.1 Moisture Content

Determination of moisture content of the prepared potato chips was done following AOAC (2000) method.

Method

1) An empty dish was dried I the oven at 105^0C for 3 hours and transferred to desiccator to cool. The empty dish and the lid were weighed.

2) 3gm of sample was weighed to the dish and spread to uniformity

3) The dish with sample was placed in the oven. It was dried for 3 hours at 105^0C

4) After drying, the dish was transferred with partially covered lid to the desiccator to cool.

5) The dish and its dried sample were reweighed.

Calculation

$$\text{Moisture (\%)} = \frac{(W_1 - W_2)}{W_1} \times 100$$

Here, W_1 = weight (g) of sample before drying

W_2 = weight (g) of sample after drying

3.4.2 Crude Protein

Protein content of the prepared potato chips was determined using AOAC (2000) method.

Reagents required

- Kjeldahl catalyst: Mix of 9 part of Potassium Sulphate (K_2SO_4) with 1 part of Copper Sulphate ($CuSO_4$)
- Sulphuric Acid (H_2SO_4)

- 40% NaOH solution
- 0.2 N HCL Solution
- 4% H_3BO_3
- Indicator solution: Mix 100 ml of 0.1% methyl red (in 95% ethanol) with 200 ml of 0.2% Bromocresol Green (in 95% ethanol)

Method

1) Sample (0.5 – 1.0 gm) was placed in digestion flask.

2) 5 gm of Kjeldahl catalyst and 200 ml of concentrated H_2SO_4 was added to the digestion flask.

3) A blank was prepared containing the above chemicals except sample. The flask was placed inclined position and heated gently until frothing ceases. The solution was boiled briskly until it became clear.

4) It was cooled and 60 mL of distilled water was added cautiously.

5) The flask was immediately connected to digestion bulb on condenser and with tip of condenser immersed in standard acid and 5 – 7 drops of mix indicator in receiver. The flask was rotated to mix content thoroughly; then was heated until NH_3 distilled.

6) The receiver was removed, tip of the condenser was washed and the excess standard acid distilled with standard NaOH solution was titrated.

Calculation

$$\text{Protein (\%)} = \frac{(A-B)\times N \times 1.4007 \times 6.25}{W}$$

Here, A = Volume (mL) of 0.2 N HCL used sample titration

B = Volume (mL) of 0.2 N HCL used in blank titration

N = Normality of HCL

W = Weight (gm) of sample

14.007 = Atomic weight of Nitrogen

6.25 = The Protein-Nitrogen conversion factor for fish and its by-products

3.4.3 Ash Content

Ash content of the prepared potato chips was determined using AOAC (2000) method.

Method

1) The crucible and the lid were placed in the furnace at 550^0C overnight to ensure that impurities in the surface of crucible were burned off.

2) The crucible was cooled in the desiccator for 30 minutes.

3) The crucible and the lid were weighed to 3 decimal places.

4) 5 gm of sample was weighed into the crucible and was heated over low Bunsen flame with lid half covered. The crucible and the lid were placed in the furnace when fumes were no longer produced.

5) The sample was heated at 550^0C overnight. During heating the lid was uncovered. After complete heating, the lid was placed to prevent loss of fluffy ash. Then it was cooled down in the desiccator.

6) The crucible and the lid were weighed when the sample turned into gray.

Calculation

$$\text{Ash (\%)} = \frac{\text{Weight of Ash}}{\text{Weight of sample}} \times 100$$

3.4.4 Fat content

Fat content of the prepared potato chips was determined using AOAC (2000) method.

Reagent: Petroleum ether

Method

1) The bottle and the lid were placed in the oven at 150^0C overnight to ensure that weight of bottle is stable.

2) 3 – 5 gm of sample was weighed to paper filter and wrapped.

3) Then the sample was taken into extraction thimble and has been transferred into Soxhlet.

4) 250 mL petroleum ether was filled into the bottle and the bottle was taken to heating mantle.

5) The Soxhlet apparatus was connected and the water was turned on to cool them and then the heating mantle was switched on.

6) The sample was heated for 14 hours.

7) The solvent was evaporated using vacuum condenser.

8) The bottle was incubated at 80^0C – 90^0C until complete evaporation of the solvent and the bottle is completely dried.

9) After drying, the bottle was transferred with partially covered lid to the desiccator to cool. The bottle and its dried content were weighed.

Calculation

$$\text{Fat (\%)} = \frac{\text{Weight of Fat}}{\text{Weight of sample}} \times 100$$

3.4.5 Crude Fibre

Crude Fibre content of the prepared potato chips was determined using AOAC (2000) method.

Reagents

1. H_2SO_4 Solution 1.25% (w/v)
2. NaOH Solution 1.25% (w/v)
3. N-Octanol as antifoam
4. HCL Solution 1% (w/v)

Method

1) 2.0 gm of sample was weighed accurately

2) 125ml 1.25% H_2SO_4 solution was added to the beaker.

3) 3-5 drops of N-Octanol was added as antifoam agent.

4) Sample solution was boiled for 30 minutes.

5) Then the solution was washed three times to make it acid free.

6) After draining the last wash, 125ml 1.25% NaOH and 3-5 drops of antifoam was added.

7) Then again the solution was boiled for 30 minutes.

8) Then it was filtered and the residue was washed as above.

9) Second wash was performed with1% HCL solution to make it acid free.

10) The residue was dried in the hot air oven at 105^0C up to constant weight.

11) The residue was cooled in the desiccator and weighed.

12) The residue was burnt up to no smoke.

13) Then the residue was ignited in the muffle furnace up to white ash (550^0C – 600^0C, 4 – 6 hours).

14) The ash was weighed and the value was deducted to get fiber weight.

Calculation

$$\text{Crude fibre (\%)} = \frac{W - W_1}{W_2} \times 100$$

Here, W = Weight of crucible, Crude fibre and ash

W_1 = Weight of crucible and ash

W_2 = Weight of sample

3.4.6 Total Carbohydrate

Total carbohydrate content of the sample was determined as total carbohydrate by difference, that is by subtracting the measured protein, fat, ash and moisture from 100 (Pearson, 1970).

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

3.5 Statistical Analysis

Statistical analysis (Mean, Standard Deviation) was done in MS Excel 2013.

RESULTS

4.1 Acrylamide quantitation of samples

Acrylamide Samples were prepared in the Chemical Laboratory of Chittagong Divisional Laboratory under the Department of Environment (DoE), Government of Bangladesh (GOB). Acrylamide quantitation was performed in the Designated Reference Institute for Chemical Measurements (DRICM) of Bangladesh Council for Scientific and Industrial Research (BCSIR), Dhaka.

Function: f(x) = 3.64879*x + 0.0610482

Rr1 = 0.9998715

Rr2 = 0.9997430

MeanRF = 4.560924e - 001

RF SD = 1.067812e – 001

RF% RSD: 23.412192

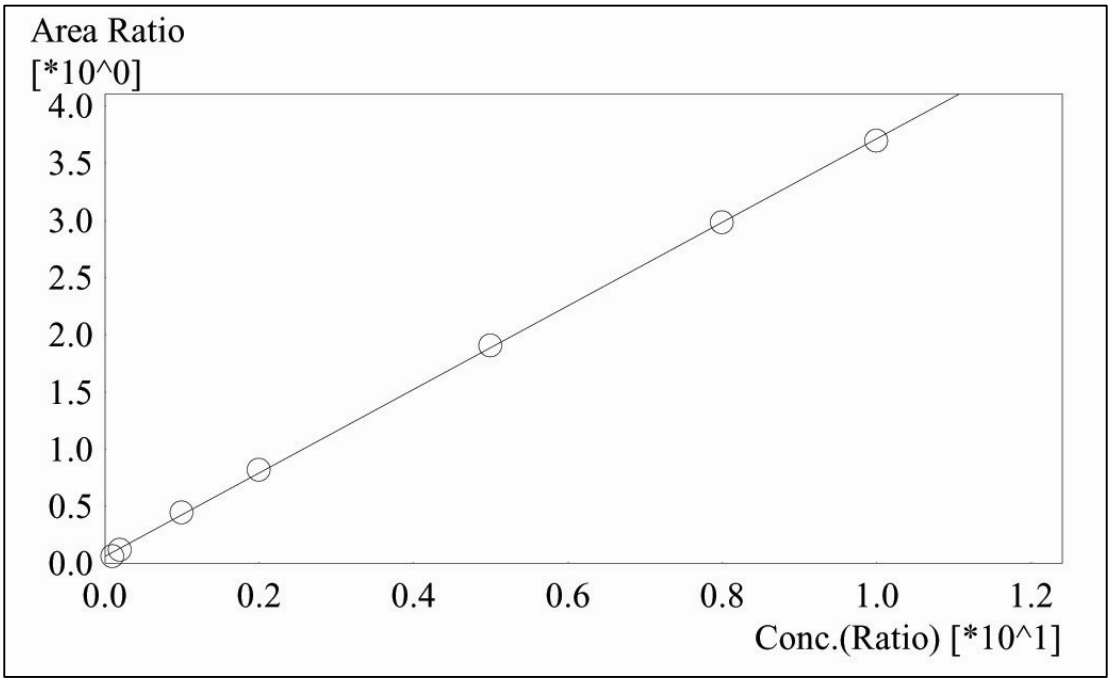

Figure 4.1: Calibration curve for Acrylamide standard solutions

Table 4.1: Detected Acrylamide level in collected samples

Brand no. \ Replication	Acrylamide Level (µg kg^{-1}or ppb)				
	S1	S2	S3	Standard Deviation (SD)	Mean
B1	< dl	< dl	< dl	-	-
B2	< dl	< dl	< dl	-	-
B3	875	987	812	88.64	891.33
B4	1523	1180	1372	7.51	525.67
B5	119	684	214	302.53	339

dl = detection limit, B=Brand, S=Sample, ppb = parts per billion

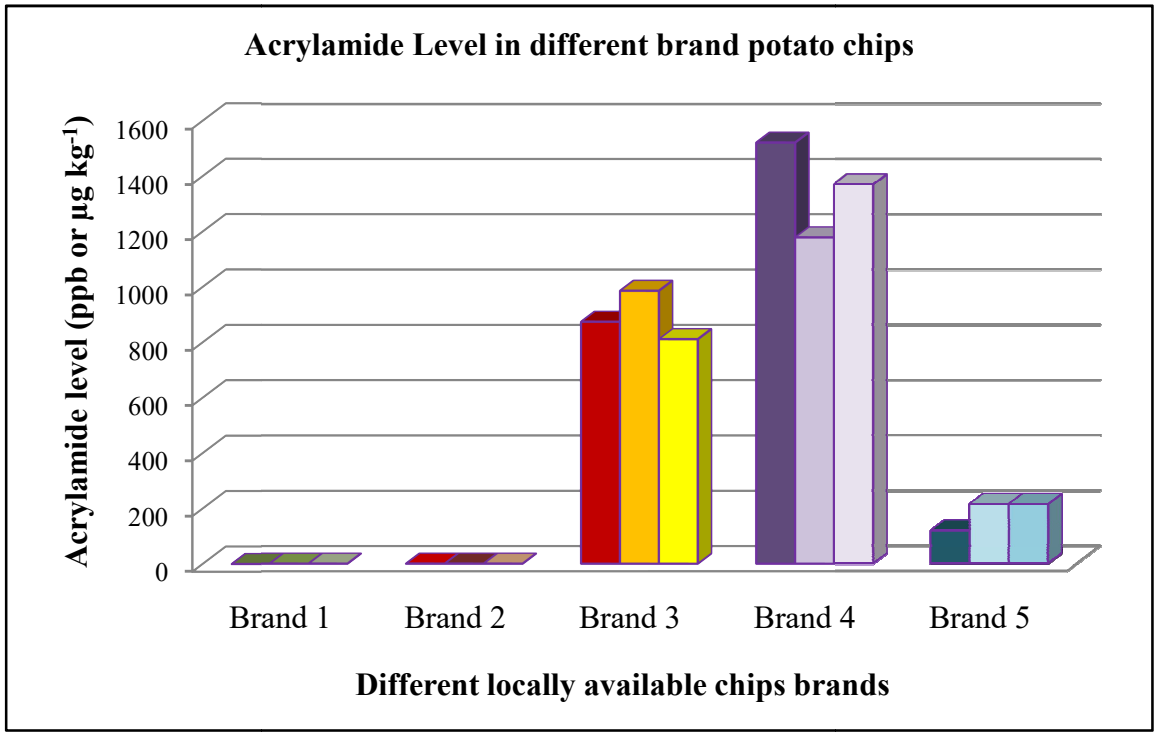

Fig 4.2: Acrylamide level in samples of different potato chips brands

From the tabular and graphical presentation it is seen that highest (1523 µg kg^{-1}) level of Acrylamide was found in the samples of brand 4. And also the samples of brand 3 contains higher amount of Acrylamide. Acrylamide level was found below detection limit (5 ppb) in the samples of brand 1 and brand 2.

4.2 Potato chips manufacturing method development

A method was successfully developed in the laboratory of Department of Food Processing and Engineering under Faculty of Foods Science and Technology, Chittagong Veterinary and Animal Sciences University to reduce Acrylamide level in finished potato chips. The considerations were found easy to implement during processing of potato and proceedings to final product (Potato chips).

4.3 Proximate analysis of Developed Potato Chips

Proximate analysis of the laboratory prepared potato chips was performed in the laboratory of the Department of Animal Science and Nutrition under Faculty of Veterinary Medicine, Chittagong Veterinary and Animal Sciences University.

Table 4.2: Proximate Analysis of developed potato chips samples

S/N	Parameter	S1	S2	S3	Mean
1	**Dry Matter (%)**	96.32	98.15	98.63	97.70
2	**Moisture (%)**	3.68	1.85	1.37	2.30
3	**Ash (%)**	3.11	2.97	3.01	3.03
4	**Crude Protein (%)**	9.89	10.16	9.95	10.00
5	**Ether Extract/Fat (%)**	12.91	12.94	12.76	12.87
6	**Crude Fiber (%)**	8.32	8.77	8.23	8.44
7	**Carbohydrates (%)**	71.65	72.06	71.69	71.8

S=Sample

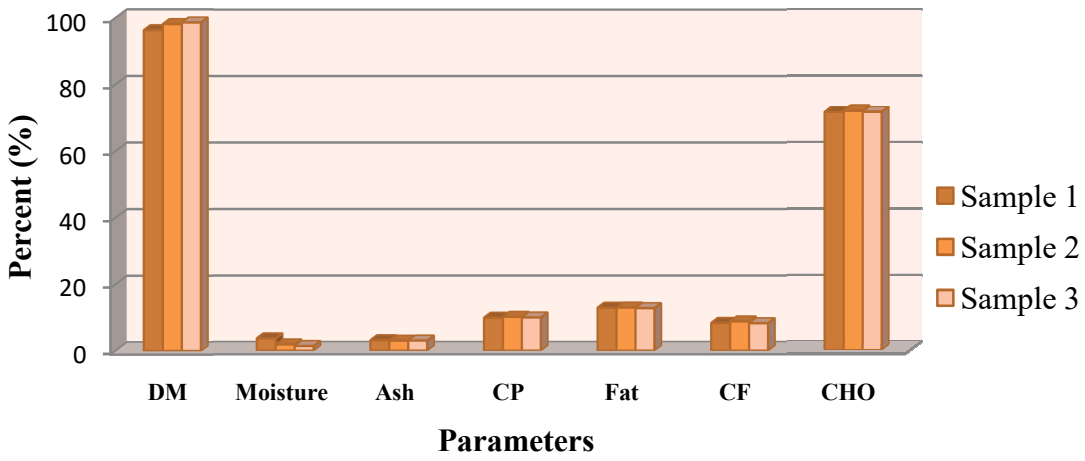

Figure 4.3: Comparative percentage of different parameters of prepared potato chips samples

4.4 Acrylamide quantitation of Developed Potato Chips

The developed potato chips samples were further analyzed for Acrylamide level in the Chemical Laboratory of Chittagong Divisional Laboratory under the Department of Environment (DoE), Government of Bangladesh (GOB). The Acrylamide levels were found below detection limit.

Table 4.3: Acrylamide level in developed potato chips

Samples	**S1**	**S2**	**S3**
Acrylamide Level (µg kg^{-1}or ppb)	< dl	<dl	<dl

S= Sample, ppb= parts per billion, dl = detection limit

DISCUSSION

In this study, Acrylamide level was found in the range from below detection limit (5 ppb) to 1,523 ppb. Many countries have performed analysis of different kinds of foods for Acrylamide level. The concentration in the potato chips samples were found 620 ppb at Kingdom of Saudi Arabia (Tawfik *et al.*, 2008), 693 ppb to 2,050 ppb at USA (USFDA, 2008), 433 ppb at Turkey (Senyuva *et al.*, 2005), 82 ppb to 4,245 ppb at India (Shamla *et al.*, 2014) and so on. Since Acrylamide and its oxidized derivative glycidamide both are carcinogenic and can damage DNA and induce tumours and cancer upon dietary exposure, it is a burning issue regarding the heat processed starchy foods throughout the world. The findings indicate the danger regarding exposure to Acrylamide for the population of Bangladesh.

Codex Alimentarius Commission, WHO and FAO provided many guidelines to check Acrylamide formation in the processed starchy foods maintaining the sensory and nutritive attributes. In the present study, the considerations regarding post harvest storage temperature, peeling depth, slice thickness, repetitive washing, soaking in salt, use of SAPP, frying time and temperature combination were maintained to develop a potato chips manufacturing method. Deep peeling was done to remove most of the reducing sugar from surface. Potatoes were sliced in thinner flakes to shorten the time requirement for frying. Potato flakes were washed thoroughly to washout reducing sugar content. Flakes were soaked in salt solution and were blanched in SAPP solution to reduce Acrylamide formation through Maillard reaction. Potato flakes were fried below 175^0C temperature for short time so that the rest of the reducing sugar content cannot produce Acrylamide. The developed potato chips exerted somehow satisfactory result and needs some improvement to be made to the method.

Tangkanakul *et al.* (1999) analyzed some potato chips sample collected from retail markets of Thailand. The proximate analysis ranges were moisture 1.91% to 4.54%, protein 2.81% to 8.5%, fat 11.09% to 36.25%, carbohydrate 48.75% to 74.73%, ash 2.11% to 4.17% and crude fibre 0.81% to 2.23%. Wipawee *et al.* (2012) found moisture 4.80%, protein 4.78%, crude lipid 21.78%, crude fiber 3.93%, ash 1.90% and carbohydrate 67.61% in proximate analysis of potato chips fried at 180^0C. Potato flakes were fried below 175^0C in the current study. The proximate analysis result was

found as moisture 2.30%, ash 3.03%, crude protein 10%, ether extract/fat 12.87%, crude fibre 8.44% and carbohydrates 71.8%.

Food Drink Europe (2013) suggested that reducing slice thickness can reduce Acrylamide formation. Hendriksen *et al.* (2009) showed in their study that washing in ambient temperature water and blanching prior to frying results in reduce Acrylamide formation during frying. Morales *et al.* (2008) reported in their study that frying temperature below 175^0C can reduce the Acrylamide formation in potato chips during frying. According to codex guidelines, using SAPP during blanching can reduce the Acrylamide concentration in a large extent. The Acrylamide level of the developed potato chips samples were found below detection limit (5ppb) which indicates the considerations were able to reduce Acrylamide formation in potato chips during processing.

CONCLUSIONS

Acrylamide has been listed as “Probable human carcinogen” by the IARC. Many food groups have been analyzed for Acrylamide. Starchy and potato products undergone high temperature processing were found most vulnerable food groups. Among those food groups, potato chips is notable. In Bangladesh potato chips is frequently consumed by all age groups of people. This study has successfully quantified Acrylamide level in some samples of locally available potato chips at Chattogram which point out that the Acrylamide level in the potato are high enough. This also indicates the unawareness of the processors about Acrylamide formation in their manufactured foods. This study showed some considerations can mitigate Acrylamide formation without affecting qualitative and nutritive parameters to a significant extent. These considerations can be helpful for the food manufacturers to reduce the Acrylamide level in their products.

RECOMMENDATIONS & FUTURE PERSPECTIVES

7.1 Recommendations for the potato chips manufacturers

It is recommended for the Potato chips manufacturers to follow the considerations maintained in the R&D part of this study to mitigate formation of Acrylamide in their manufactured potato chips. Many countries of Europe and America have already established Acrylamide policy for starchy foods which undergo heat processing and vulnerable for Acrylamide formation. For this policy the vulnerable food groups are strictly checked before imported to those countries. Bangladeshi potato chips as well as other foods may fall under restriction while exported to those countries because of Acrylamide level. So it is wise to adopt the considerations to mitigate Acrylamide formation and reduce the risk to export restriction.

7.2 Recommendations for the Policy makers

Since no Acrylamide policy has been developed or published in Bangladesh, there is a huge scope for the policy makers to take some measures and play vital role in the sector of food safety. Therefore policy makers from different regulatory authorities of Bangladesh can;

- Fund different research projects to find out the lethal dose and maximum allowable limit of dietary Acrylamide for the population of Bangladesh;
- Set the limit of Acrylamide in different heat processed (Baked / Cooked / Fried / Roasted / Smoked) starchy foods which are vulnerable to formation of Acrylamide;
- Put restriction in the domestic market to prohibit trading and marketing of Acrylamide rich foods.

7.3 Recommendations for researchers

Very few studies have been conducted in Bangladesh regarding Acrylamide quantitation in different food groups, exposure level of general people to dietary Acrylamide. The researchers can

- Quantify Acrylamide in different food groups in Bangladesh;
- Determine exposure level to dietary Acrylamide;
- Determine the sources of Acrylamide in our daily consumed foods;

- Determine cost effective Acrylamide formation mitigation technology in the perspective of Bangladesh.

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APPENDIX A: PHOTO GALLERY

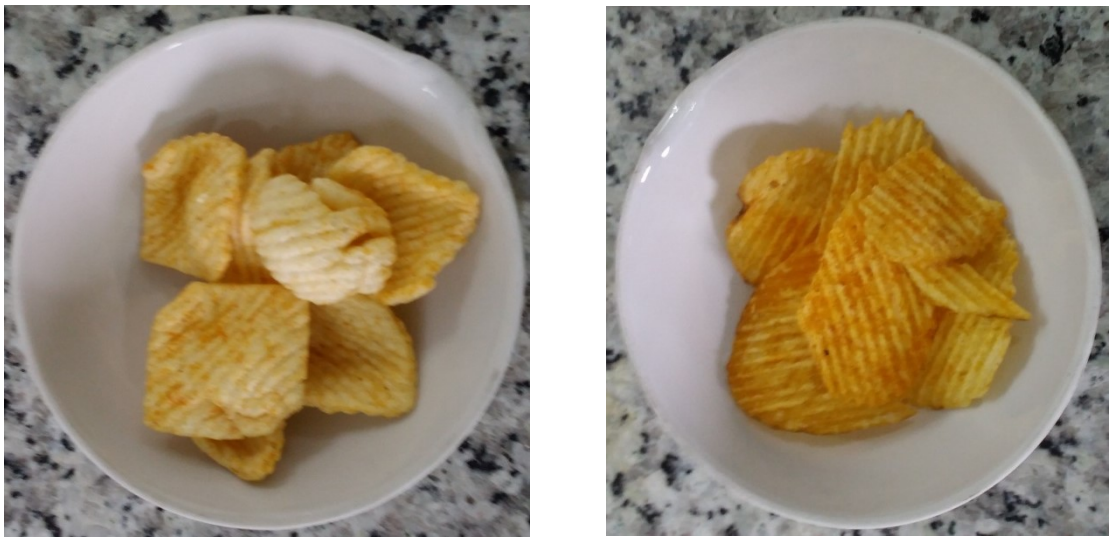


Figure A1: Potato chips samples

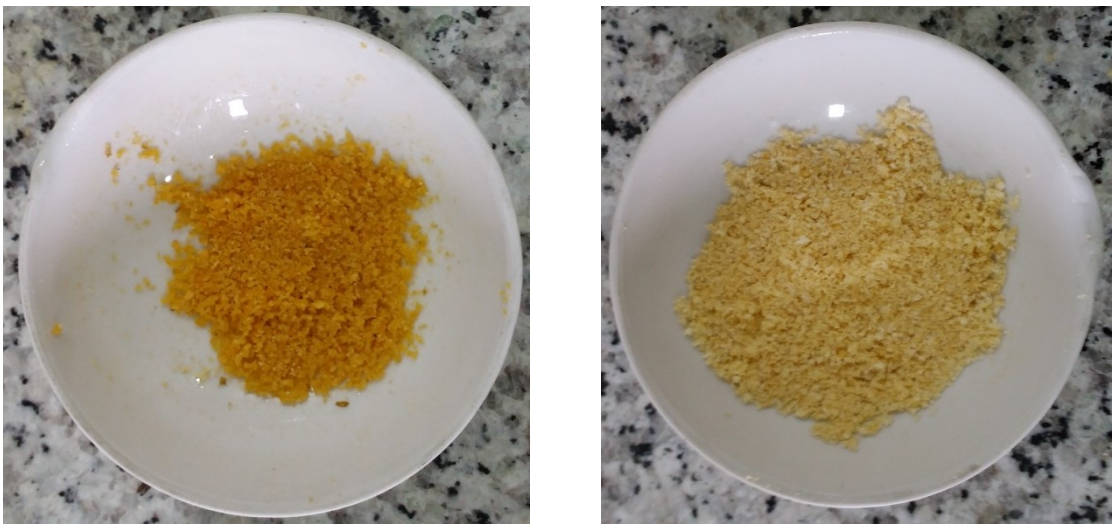


Figure A2: Ground Potato chips samples

Figure A3: Ground Potato chips samples

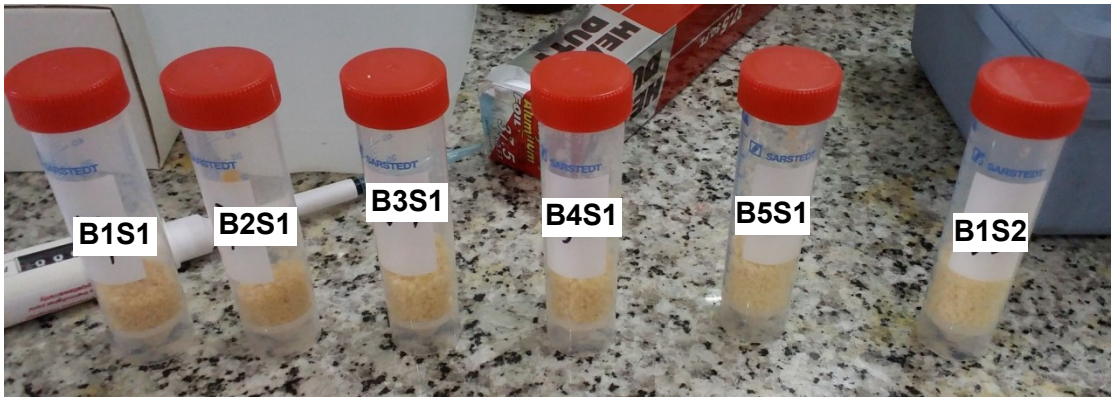


Figure A4: Sample taken in falcon tube

Figure A5: Weighing in analytical balance

Figure A6: Vortexing

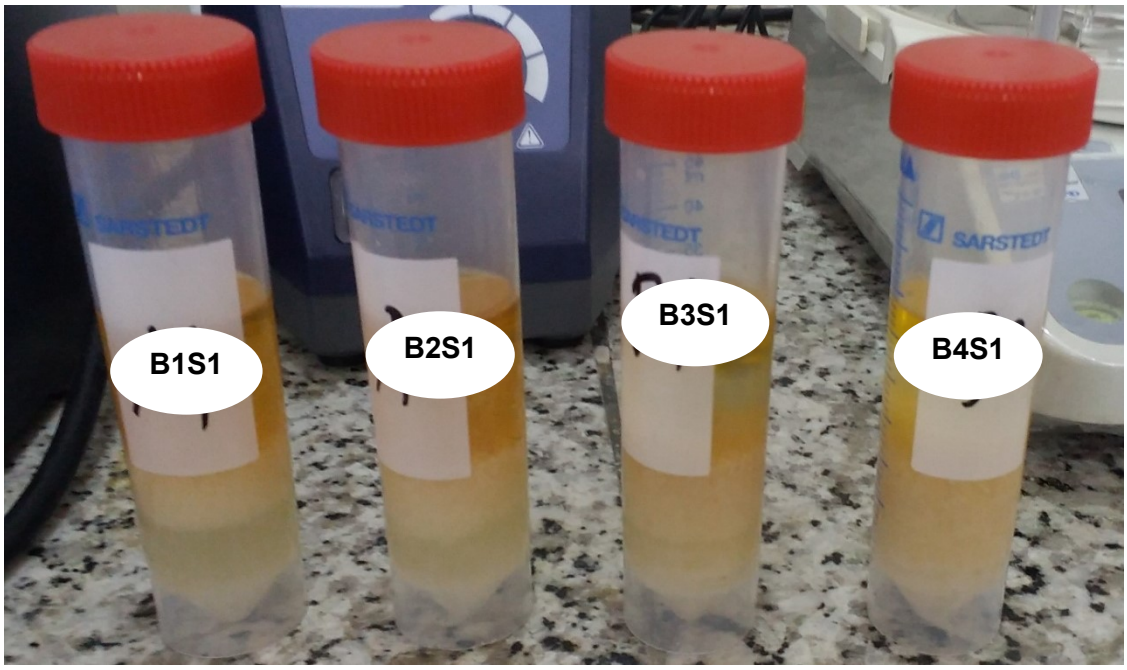


Figure A7: Mixture of Sample, Acetonitrile, hexane and pure water (before vortex)

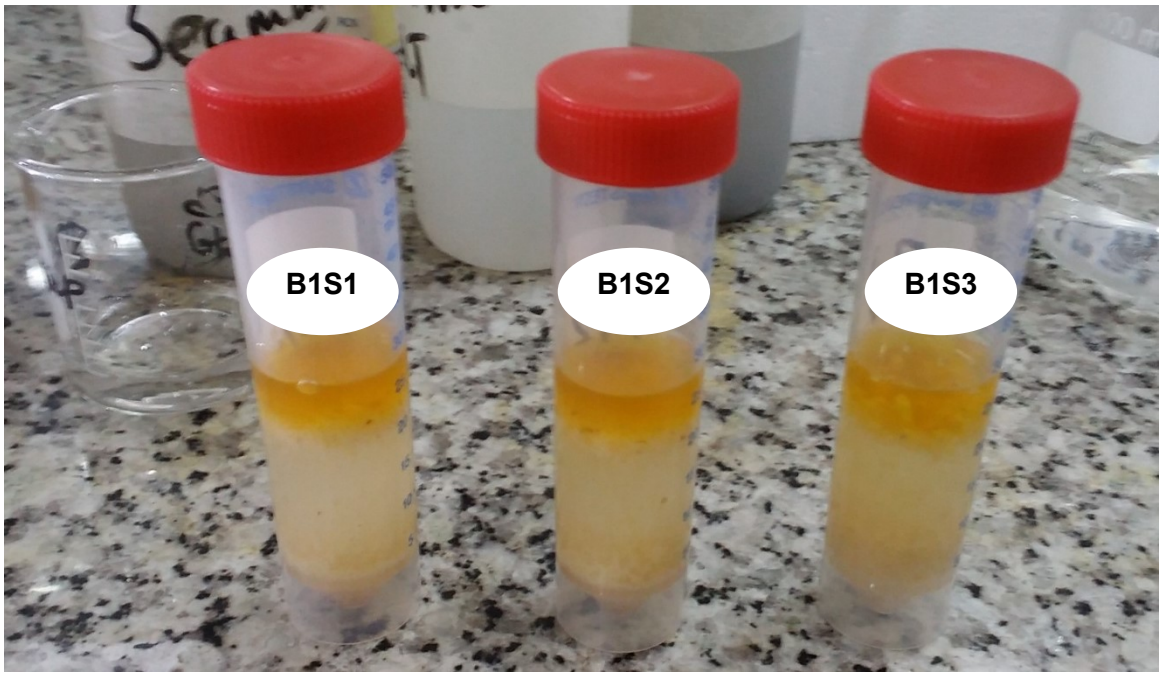


Figure A8: Mixture of Sample solution (after vortex)

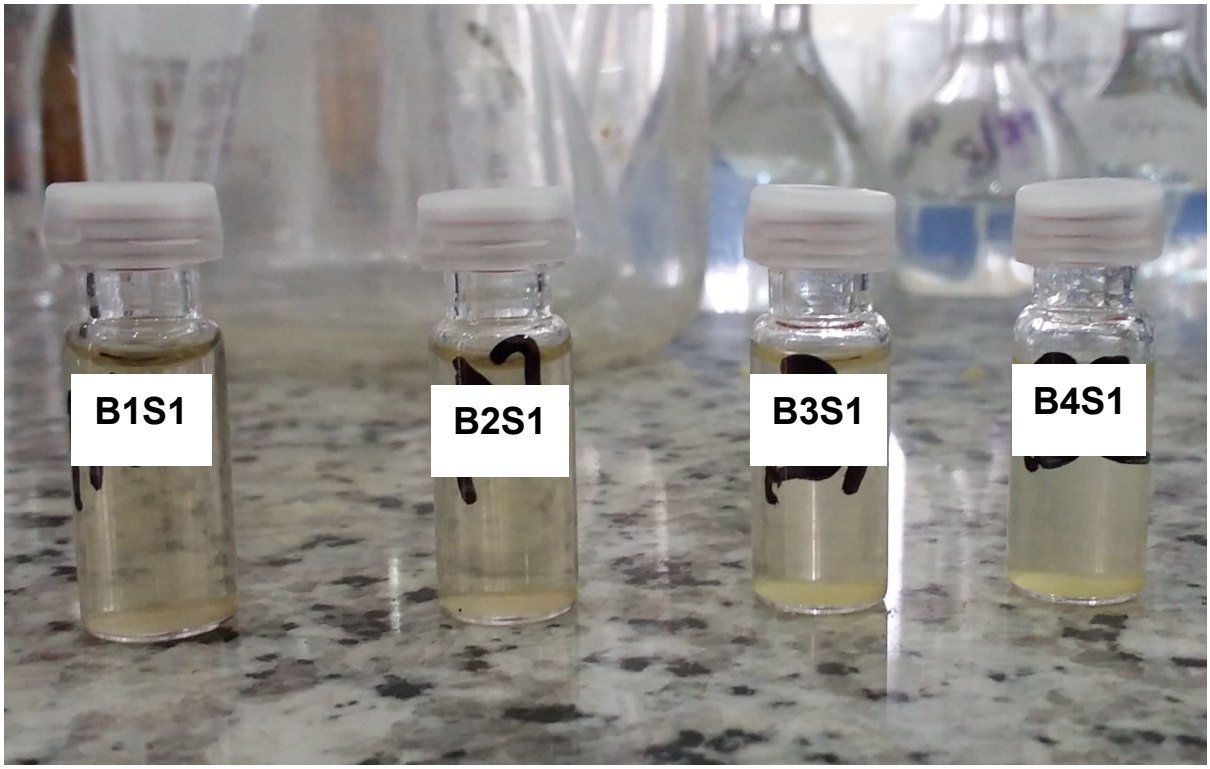


Figure A9: Acrylamide extract separated from sample solution after centrifugation

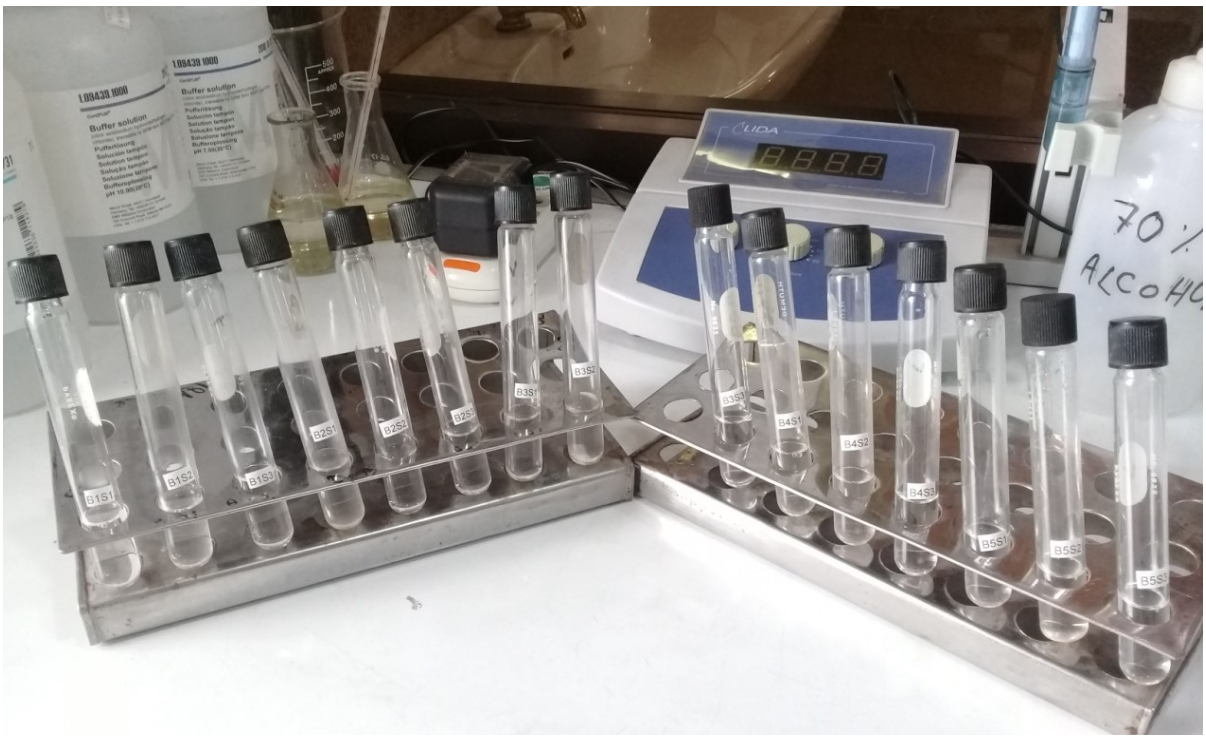


Figure A10: Acrylamide extract prepared for quantitation

Figure A11: Potatoes before washing

Figure A12: Peeled potatoes

Figure A13: Thin slices of Potato

Figure A14: Soaking in Salt solution

Figure A15: Blanching with SAPP

Figure A16: Drying in cabinet dryer after blanching

Figure A17: Spicing after frying

Figure A18: Packed Finished Potato chips

Figure A19: Grinding

Figure A20: Ground chips for Proximate Analysis

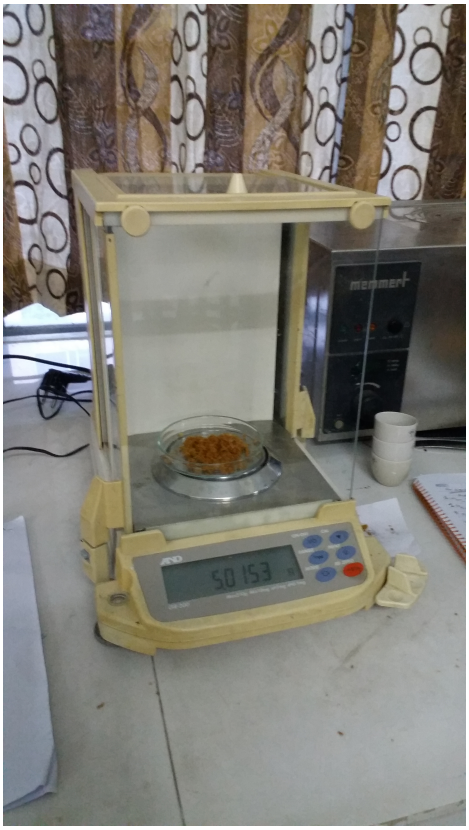


Figure A21: Weighing Sample

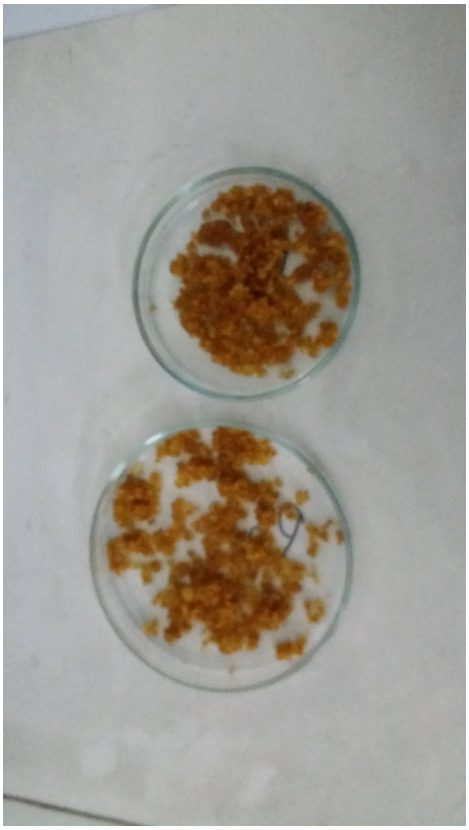


Figure A22: Ground chips taken in Petri-dish for moisture content determination

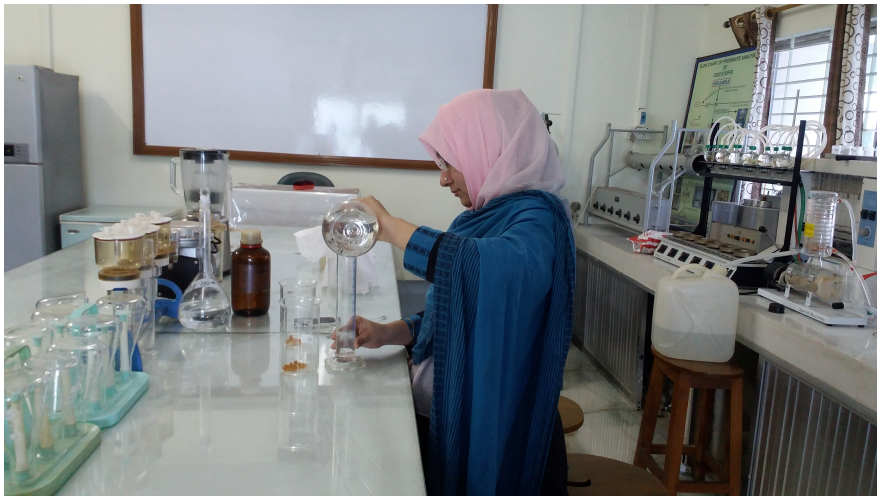


Figure A22: Sample preparation for Crude Fiber determination

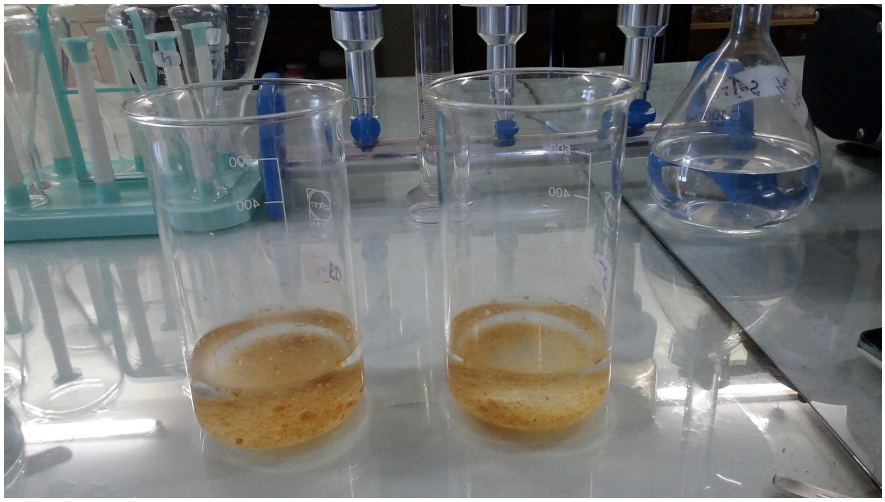


Figure A23: Sample Solution for Crude Fiber determination

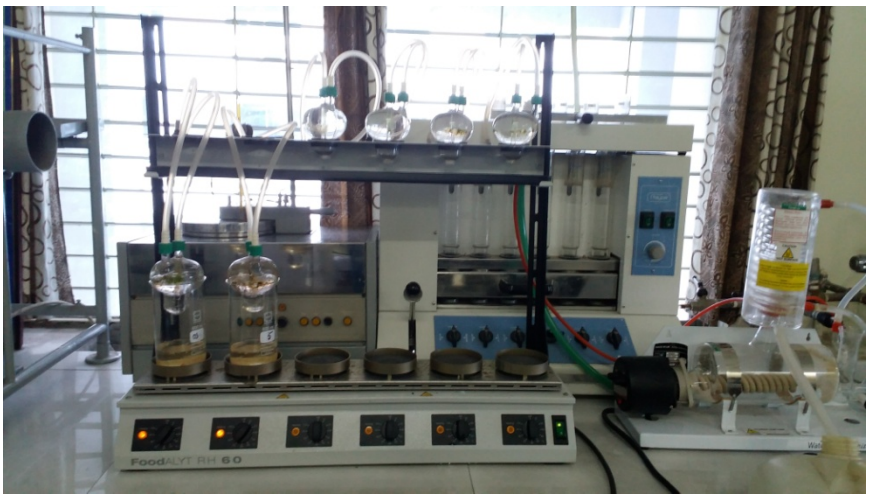


Figure A24: Boiling with alkali and acid

Figure A25: Filtration to separate water insoluble fiber

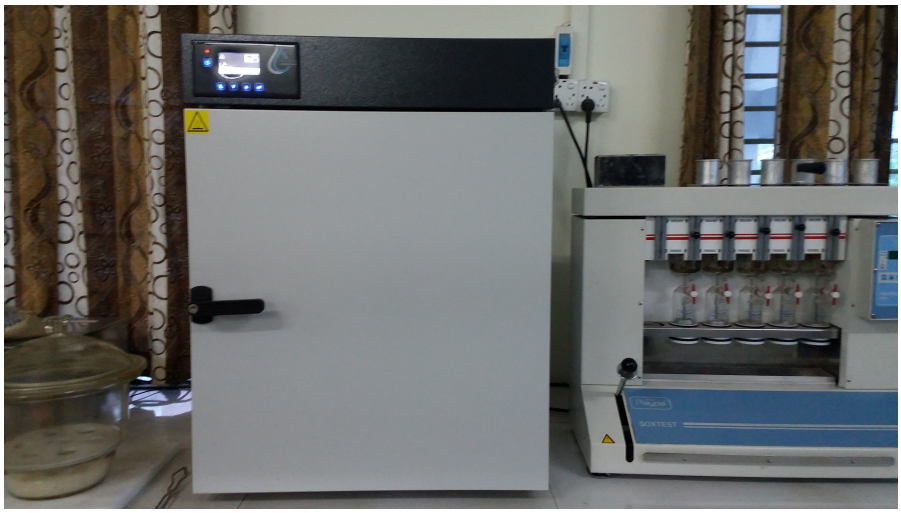


Figure A26: Hot air oven

Figure A27: Dried crucibles

Figure A28: Taking dried crucible for ash determination

Figure A29: Sample kept in muffle furnace for ash determination

Figure A30: Muffle Furnace

Figure A31: Sample in Soxhlet apparatus for fat determination

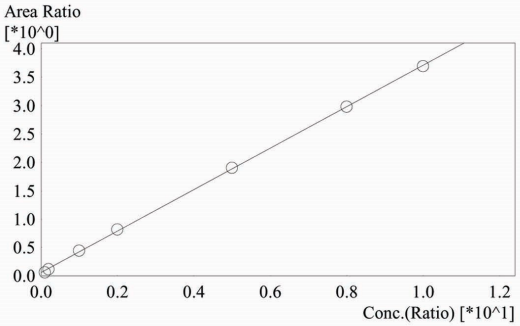
APPENDIX B: REPORTS

Designated Reference Institute for Chemical Measurements (DRiCM)
Bangladesh Council of Scientific & Industrial Reaserch
Dr. Qudrat-i-Khuda Road, Dhanmondi, Dhaka-1205, Bangladesh
Tel:+880-2-9671830, Fax:+880-2-58613819

ANALYTICAL REPORT
(Calibration Curve)

Calibration Curve

ID# : MSS18073002 and MSS18073003 m/z : STD 72>55, 27 and IS 75>58, 30
Name : Acrylamide
Quantitative Method : Internal Standard
Function : f(x)=0.364879*x+0.0610482
Rr1=0.9998715 Rr2=0.9997430
MeanRF: 4.560924e-001 RF SD: 1.067812e-001 RF %RSD: 23.412192
FitType : Linear
ZeroThrough : Not Through
Method File Name: C:\LCMS Data\Acrylamide\Linearity\Cal 2\Acrylamide&IS_Cal_method02.lcm



MS Summary(Compound)

ID#1 Compound Name: ISTD m/z: 75.10>58.00

Title	Sample ID	Std. Conc.	Acquired Conc	Unit	Area	Ret. Time
Acry_STD_5ppb_02.lcd	Acry_STD_5ppb_02	50	1.000	ppb	353122	1.858
Acry_STD_10ppb_02.lcd	Acry_STD_10ppb_02	50	1.000	ppb	332656	1.851
Acry_STD_50ppb_02.lcd	Acry_STD_50ppb_02	50	1.000	ppb	311965	1.854
Acry_STD_100ppb_02.lcd	Acry_STD_100ppb_02	50	1.000	ppb	324291	1.852
Acry_STD_250ppb_02.lcd	Acry_STD_250ppb_02	50	1.000	ppb	308124	1.848
Acry_STD_400ppb_02.lcd	Acry_STD_400ppb_02	50	1.000	ppb	307129	1.865
Acry_STD_500ppb_02.lcd	Acry_STD_500ppb_02	50	1.000	ppb	302232	1.872
Average			1.000		319931	1.857
%RSD			0.000		5.653	0.462
Standard Deviation			0.000		18086	0.009

ID#2 Compound Name: Acrylamide m/z: 72.10>55.00

Title	Sample ID	Std. Conc.	Acquired Conc	Unit	Area	Ret. Time
Acry_STD_5ppb_02.lcd	Acry_STD_5ppb_02	5	0.100	ppb	21595	1.866
Acry_STD_10ppb_02.lcd	Acry_STD_10ppb_02	10	0.200	ppb	40156	1.855
Acry_STD_50ppb_02.lcd	Acry_STD_50ppb_02	50	0.998	ppb	139078	1.868
Acry_STD_100ppb_02.lcd	Acry_STD_100ppb_02	100	1.981	ppb	265271	1.864
Acry_STD_250ppb_02.lcd	Acry_STD_250ppb_02	250	4.967	ppb	586608	1.862
Acry_STD_400ppb_02.lcd	Acry_STD_400ppb_02	400	7.960	ppb	915615	1.884
Acry_STD_500ppb_02.lcd	Acry_STD_500ppb_02	500	9.954	ppb	1116127	1.887
Average			3.737		440636	1.869
%RSD			106.094		99.824	0.628
Standard Deviation			3.965		439862	0.012

Chittagong Veterinary and Animal Sciences University

Department of Animal Science and Nutrition

Khulshi, Chittagong-4225

Proximate Analysis Report

Name of the owner: MS Student, FST, CVASU Date: **08/08/2018**

Sample Name: **Potato Chips**

Serial No.	Name of the Nutrient or Proximate component	Result Percentage (%)
1.	**Dry matter**	**97.70%**
2.	**Moisture**	**2.30%**
3.	**Crude protein (CP)**	**10.00%**
4.	**Crude Fiber (CF)**	**8.44%**
5.	**Ash**	**3.03%**
6.	**Ether Extract/Fat**	**12.87%**
7.	Calcium (Ca)	-
8.	Phosphorus (P)	-
9.	ME Kcal/Kg	-
10.	Sand/Silica	-

Islam 08.08.2018

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BIOGRAPHY

Meher Nahid, author of this study is MS fellow in the Department of Food Processing and Engineering under Faculty of Food Science and Technology at the Chittagong Veterinary and Animal Sciences University. Author achieved her Bachelor of Science in Food Science and Technology from the Chittagong Veterinary and Animal Sciences University with a distinction 1st class 1st in the year 2016. Author received University Gold Medal Award on February 11, 2018 for her distinction in graduation. She also had been awarded the Prime Minister Gold Medal – 2016 at Shapla Hall of Prime Minister's Office on February 25, 2018. Author keeps interest in research regarding food processing, bio-preservation, dietary replacement of drugs, identification of potential threats in frequently consumed foods in Bangladesh and others.