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HPLC-UV Quantitative Analysis of Acrylamide in Snack Foods of India

Pal Murugan M*, Agathian G, Anil Dutt Semwal and Gopal Kumar Sharma

DRDO-Defence Food Research Laboratory, Mysore – 570 011, Karnataka, India
**E-mail: palmurugan@dfrl.drdo.in*

ABSTRACT

An investigation was carried out to determine acrylamide content in 51 popular snacks food of India by using High Pressure Liquid Chromatography (HPLC) Instrument with UV detection method. The method entails acetone extraction of acrylamide, clean up by solid extraction cartridges, isocratic elution with mobile phase of HPLC grade water, acetonitrile and formic acid followed by detection at 210 nm. The limit of detection and the limit of quantification for this method were 5.12 µg/kg and 17.08 µg/kg, respectively. The mean recoveries of acrylamide obtained by using spiked samples ranged from 91 per cent to 101.33 per cent. Acrylamide concentrations in the five group of snacks ranged from 788.99 - 4191.82 µg/kg for extruded and deep fat fried snack, 372 µg/kg to 6391 µg/kg for deep fat fried food, 435-3147 µg/kg for baked food, 434-1307 µg/kg for breakfast cereal and 471-1520 µg/kg for other snacks. Among the food products, snack foods purchased from unorganised sector showed highest concentration of acrylamide.

Keywords: HPLC-UV; Deep fat fried snacks; Extruded food; Baked food; Breakfast cereals

1. INTRODUCTION

Acrylamide, a potential carcinogen, was accidentally detected in fried and baked food matrices by Swedish scientists in the year 2002 which created alarm amongst the food scientist and consumers. The most important sources of acrylamide are deep fat fried potato products, roasted coffee beans and bakery products[1].

Acrylamide, a multipurpose organic compound used in many products in our everyday life. It exists in monomeric and a polymeric form. The monomeric form of acrylamide is toxic to the nervous system, a carcinogen in laboratory animals and a suspected carcinogen in humans. The multiple unit or polymeric form is not known to be toxic. The monomeric form of acrylamide is primarily used in research laboratories for electrophoresis. It is used extensively for the production of grout, dyes, ore & contact lenses and in the construction of dams, tunnels and sewers[2]. There are two established legal limits for Acrylamide; detectable limit of Acrylamide in drinking water and percolation of a monomeric form of acrylamide from packaging materials into food which are less than 0.5 µg/kg of uncoagulated acrylamide[3] and 10 µg/kg, respectively[4,5].

However, daily intake of some tens of micrograms of acrylamide is expected depending upon the dietary habits of the individual. Earlier studies revealed carbohydrate rich foods such as potato chips, french fries and bakery products contain higher level of acrylamide (400-7000 µg/kg) followed by protein rich foods with (5-400 µg/kg)[6]. The formation acrylamide in heat treated foods resulted from the Maillard reaction between amino acid asparagine and reducing sugars[7]. Even though, several reports revealed acrylamide (AA) forms above 120 °C, there were studies confirming that this compound can be formed at temperatures below 100°C, during drying processes between 65–130 °C[8]. Additional mechanisms of acrylamide formation involving peptides, proteins, biogenic amines and lipids have been also reported[1,9-12].

Acrylamide detection in human foods has led to extensive studies by Food and Agriculture Organisation (FAO), World Health Organisation (WHO) and European Commission to explore its formation mechanisms, levels of exposure, suitable analytical procedures and mitigation strategies in food stuffs. Since 2003, data on the occurrence of acrylamide in food commodities have been compiled by the European Food Safety Authority under European commission based on the inputs from member states and food industries[13]. The compilation showed higher levels of Acrylamide in various food products. In order reduce amount of Acrylamide in processed food, European Commission, in collaboration with the Confederation of the Food and Drink Industry (CIAA) formulated Food Drink Europe Acrylamide Toolbox 2013 with guidelines which could be effectively used by food processing industries in line with their particular needs to lower acrylamide levels in their products. Besides this, various ethnic food products from different countries[14-19] were analysed for Acrylamide content and level of exposure[20,21]. Hogervorst[22], *et al.* reported that increased risks of postmenopausal endometrial and ovarian cancer with increasing dietary acrylamide intake, among never-smokers of the Dutch population.

When compared to developed countries, the awareness

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about the AA toxicity and level of health risk were low among the developing and under developed nations. In India, with changing lifestyles such as a steep rise in dual income level, rise in the number of women in workforce and the resultant time-paucity, preference for nuclear families, the growing acceptance of western food and the need for on-the-move cooked food, there is deep increase in consumption of ready to eat food, instant food and snack food consumption among urban population especially in Metropolitans cities. Besides Multinational Food companies, small scale industries are also in rise in snack food production with annual compounded growth rate of 15-16 percent. However, only few studies were carried out by researchers[23,24] in India, on the level of acrylamide in snack foods.

Various analytical techniques such as MS, LC-MS, LC ESI-MS, LC, HPLC DAD, HPLC, ESI-MS, LC, HPLC DAT, GC-MS, GC-HRTF, GC-MECD, GC-PCI MS have been reported for various food matrices with limit of detection (LOD) ranged from 10 ng - 10 µg level of acrylamide[25-32]. Despite good analytical performance the above methods require expensive and complicated instrumentation and long extraction procedures. In recent years, many researchers used HPLC UV detection method as reliable and rapid detection techniques to estimate acrylamide content in a food matrix coupled with different extraction procedures by using water, acetonitrile, acetone, dichloromethane, ethanol with solid phase extraction procedures to isolate acrylamide from the co-extructives[33-37]. With this brief, a comprehensive study on Acrylamide content in 51 Indian snack foods such as extruded and deep fat fried snack food products, deep fat fried, baked products, Breakfast cereals and other snacks from both organised and unorganised sectors were carried out using HPLC-UV quantification in order to create awareness about the acrylamide level among Indian snack food industries and consumers. In the present study, a modified sample extraction protocol was followed to reduce the sample preparation time.

2. MATERIALS AND METHODS

2.1. Reagents and Solutions

Acrylamide (purity > 99.9 %) from M/s Sigma Aldrich, India and other chemicals (highest purity) were obtained from Merck Millipore, India and used without further purification. A stock solution of AA (1000 mg ml^{-1}) was prepared by dissolving 0.1000 g of AA in 100 ml of de ionised water. The successive 100 mg ml^{-1} stock solutions of AA were prepared from this solution. Working standard solutions of AA (50 µg ml^{-1}, 25 µg ml^{-1}, 15 µg ml^{-1}, 10 µg ml^{-1}, 5 µg ml^{-1}, and 2.5 µg ml^{-1}) were prepared by appropriate dilution of 100 µg ml^{-1} stock solution with de ionised water. The stock solutions and working standards were stored at 4 °C maximum for 1 month.

2.2 Food samples

A total of 51 sample, namely; 7 number of extruded and deep fat fried snacks, 19 number of deep fat fried, 12 number of baked products, 3 number of breakfast cereals and 8 number of other snack foods including chips, biscuits, cakes of different companies which are popular in India were purchased from local supermarket and production site of Mysore, Karnataka, India. The samples were homogenised and the subsamples of the homogenate were stored at –20 ºC in amber colored centrifuge tube secured with plastic screw-capped lids until further analysis. The proximate compositions of food products were carried out by AOAC method.

2.3 Apparatus- HPLC UV Analysis

The liquid chromatography consisted of 24 series HPLC system and 515 pump equipped with UV-Visible variable wavelength detector (2486) set at 210 nm of M/s Waters India Pvt. Ltd was used in the present study to estimate the acrylamide content in the popular snack food of India. A Spherisorb ODS-2 column (250 x 4.6 mm 5 µm) by Waters Corporation, Massachusetts, U.S.A and thermostat at 30 ºC was used for all separation. M/s Toshniwal Process instruments ultrasonic water bath was used throughout the experiments for extraction of acrylamide from sample matrix, removal of air bubbles from mobile phase and separation of acrylamide using mobile phase. Vacuum pump used for filtration of HPLC grade water and acetonitrile was procured from M/s Associated Electrical Industries, England. Different combination of mobile phase consisted of HPLC grade water; acetonitrile and formic acid were tested for satisfactory resolution. The solvent flow rate was 0.8 mL/min with detection wavelength of 210 nm.

2.4. Sample Extraction Procedure

The sample preparation procedure included defatting of sample with dichloromethane and Ethanol, extraction of acrylamide with acetone and cleaning by solid phase extraction cartridges using the modified method of Wang[38], *et al*.

Finely ground and homogenised snack food sample (10.0 g) was defatted twice by adding 50 mL dichloromethane and 5 mL ethanol and followed by 50 mL dichloromethane alone and shaken vigorously in butterfly shaker for 2 hour at room temperature. The mixture was centrifuged (M/s Kubota Corporation Tokoyo, Japan) at 3500 rpm for 20 min at 15 ºC. The supernatant was discarded and the precipitate was dried under vacuum oven at 40 ºC. For the extraction of Acrylamide, 20 mL of acetone was added to the defatted sample. The samples were shaken vigorously with butterfly shaker (M/S Scientific and Allied products, Bangalore, India) for 30 minute. The samples were placed in an ultrasonic water bath at 40°C for about 20 minute which followed by centrifugation at 3000 rpm for 25 minute at 15 ºC. The solvents were filtered through a filter paper (Whattman No.1). The filtrate was evaporated under flash evaporator (M/s Superfit, India) to dryness. 2 ml of mobile phase, HPLC grade water: acetonitrile (40:60) was added and shaken thoroughly to dissolve the residue. The aqueous solution was purified using Solid Phase Extraction tubes (ODS, 500 mg, and 3 mL capacity) and injected to the column using a 20 µl injection loop.

3. STATISTICAL ANALYSIS

The experimental results were expressed as mean ± SD of triplicate measurements. The data were subjected to regression analysis by using SPSS for Windows standard version 11.5 and significance was accepted at $P \leq 0.05$.

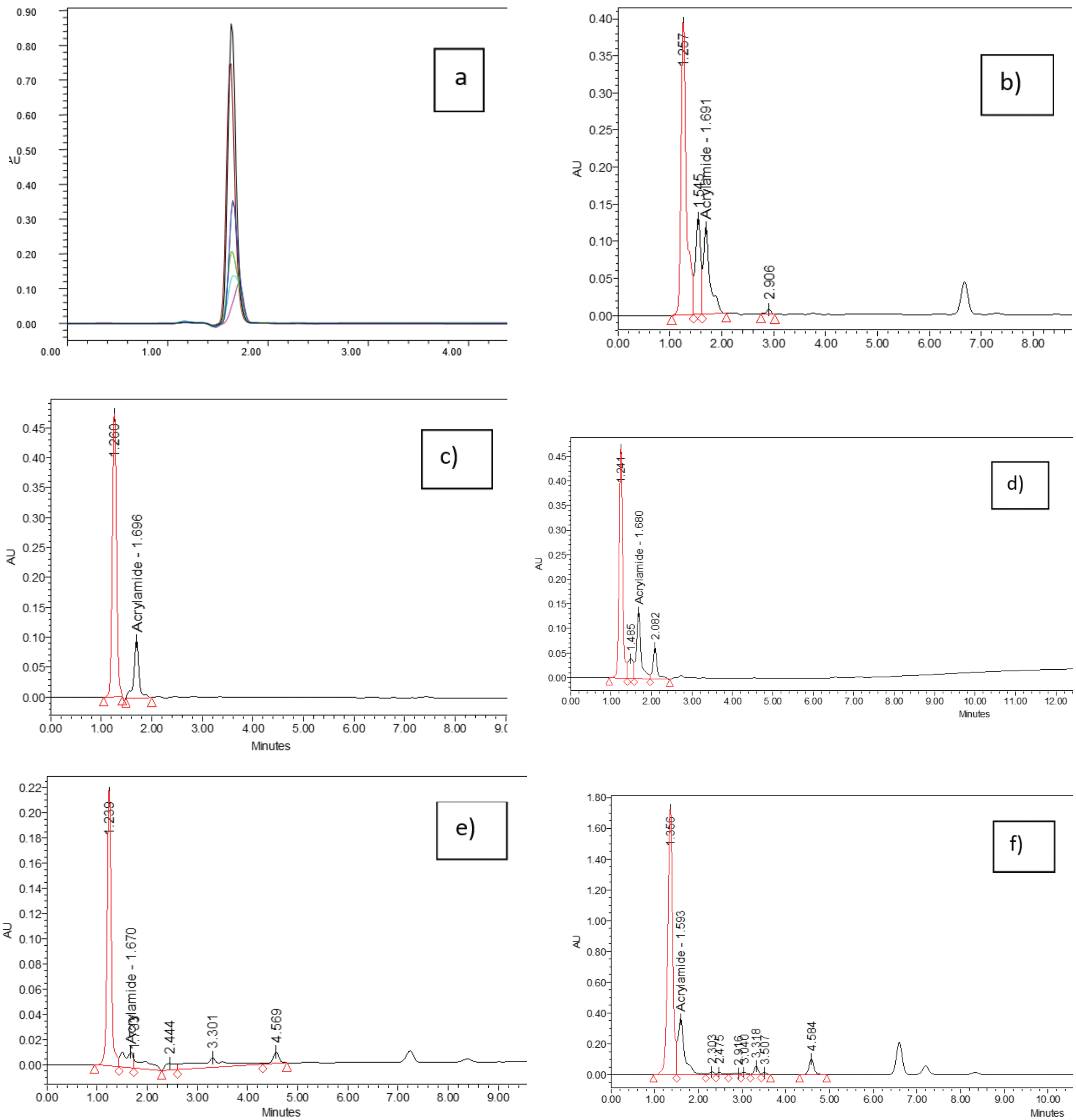

Figure 1. HPLC chromatograms of a) Acrylamide standards b) Kurkure Hyderabadi, c) Potato chips lays d) Kajubadam biscuits e) Honey loops (Kellogg's) f) Rite bite.

4. RESULTS AND DISCUSSION

4.1 Optimisation of the Analysis Conditions

Different eluent compositions; namely pure water or mixed with different portions of acetonitrile and formic acid and different column temperature were evaluated to obtain satisfactory resolution. It is observed from the study that, when pure water was used as eluent, the peak of AA was overlapped with other peaks. The study also revealed a negative relationship between retention time and column temperature between 35-45 °C. So the column temperature was kept at 30 °C to get stable retention time. It was determined that an isocratic elution pattern using 40 per cent (v/v) HPLC grade water, 60 per cent (v/v) acetonitrile and (0.10 % v/v formic acid can separate acrylamide baseline from interferents at a reasonable retention time of 1.6 minutes. The eluent i.e., acetonitrile, water, formic acid gave highly resolved chromatograms. The use of 0.1 per cent formic acid helped in reduction of noise and negative peaks. The flow rate was maintained at 0.8 ml min. Detection was performed at 210 nm with the injection volume of 20 µl. Each calibration solution was analysed in triplicate and average value of the results was used as representative for each points. All the extracts gave clear chromatograms at the desired retention time. Compared to the existing reports[34,35], the present isocratic elution condition presented the shortest analysis time for separation. A representative chromatograms showing in standard and representative samples of each food group is shown in Fig. 1.

Table 1. Proximate composition of commercial snacks

Sample name	Total Carbohydrate %	Sugar %	Crude Fat %	Crude Protein %
Extruded & Deep Fat Fried snacks				
Kurkure Hyderabadi Hungama	53.6	2.9	35.7	6.4
Kurkure masala Munch	53.6	3	35.7	6.4
Bingo Galatamasti	54.3	0.2	36.4	6.7
Bingo Tangles	51.5	5.6	32.8	10.9
Full toss Masala sticks (Parleys)	56.6	1.4	36	5.4
Instant rice kheer	78.13	46.23	10.12	7.3
Vegetablised Noodles	63.61	0	21.68	7.98
Deep Fat Fried Foods				
Potato Chips – magic masala (Lays)	51.5	4.1	35.5	7
Potato chips classic salt (Lays)	51.7	2.6	35.5	7
Potato Chips (Parles)	56.7		35.2	6.1
Potato chips (Parles)	56.7	1.4	35.2	6.1
Potato chips (unorganised sector)	52.7	2.2	34.5	6.5
Potato chips (unorganised sector)	49.7	2.5	37.5	6.7
Potato chips (unorganised sector)	50	3.5	38.2	6.1
Tapioca chips (unorganised sector)	52	1.6	33.1	5.1
Banana chips (unorganised sector)	60.84	1.2	32.04	1.6
Banana chips (Relish)	62	1.2	32.01	1.8
Cruchy rings	80	23	11	9
Moong Dhal (Bikanerwala)	50.35		20.94	19.51
Moong dhal (unorganised sector)	53.35		19	18.5
Khara boondi	39	1	40	14
AlooBoojiya (Bikanerwala)	42.43	0.23	42.31	7.16
Masala Kabuli Channa	55.3	0	23	9
Chow chow mix	44.4	1.1	34.6	13.4
Kachori	55	10	32	7.1
Samosa	53.14	1.5	31.17	6.54
Butter Murukku	49.6	0	38.3	5.3
Baked food				
Multigrain biscuit (unorganised)	65	23	24	4
Multigrain (Britania)	66	21	19.5	7.5
Kajubadam Biscuits (Sunfeast)	67.6	25	22	7.5
Cashew Badam (unorganised)	65.2	28.7	23	6.1
Malted ragi cookies	64	27	24	7
Bread Rusk (Modern)	80	23	11	9
Bread Rusk (Britannia)	80	24	10.8	8.8

Sample name	Total Carbohydrate %	Sugar %	Crude Fat %	Crude Protein %
Rusk (unorganised sector)	79	27	12	7.1
Cake (Unorganised sector)	52	31	22	4
Cake (Unorganised sector)	55	28	21	3.5
Milk Cake (Britannia)	50	30	20	6
Khara biscuit	65.6	10	22	7.5
Breakfast Cereals				
Honey loops (Kellogg's)	86.2	31.7	1.3	6.6
Choco Flakes (Kellogg's)	82.96	34.81	2.59	8.88
Choco crunchy rings (Kellogg's)	85.7	26.29	2.96	7.4
Star moon (Kellogg's)	82.59	34.44	2.96	8.51
Others				
Snack bar (Snickers)	58.5	49.1	25.4	8.7
Snack bar (Rite bites)	65.71	38.1	6.57	9.1
Honey Crunch	64.45	38.6	18.05	9.9
Wheat Poustic	77.10	4.8	15.50	1.8
Milky chocolate (Cadbury)	64.5	57.1	23.2	9.5
Vacuum fried apple chips	68.1	12.4	24.66	6.4
Vacuum fried Pear chips	67.27	13.2	24.24	5.36
Vacuum fried Papaya chips	65.4	12.1	25.14	5.25

4.2 Validation of the Analysis Method

A linear calibration curve using the five concentration AA levels was plotted with regression equation, y = 0.318 + 0.0000078x, where y is the concentration of acrylamide (µg/kg) and x is the peak area. The peak area is linearly related to the concentration of acrylamide. The limit of detection (LOD) and the limit of quantification were calculated by LOD= (3.3XSD/S) and LOQ (10XSD/S) where SD is standard deviation of response and S is the slope of calibration curve. The LOD and LOQ were 5.12 µg/kg and 17.08 µg/kg, respectively. Precision was determined in terms intraday repeatability and inter day reproducibility as relative standard deviation (RSD). The relative standard deviation (n = 8) for determination of 40 µg g^{-1} of acrylamide is 1.3. In addition, recovery test was also carried out by adding known amount of acrylamide (500 µg/kg) to the homogenised representative food matrixes of each group, i.e., extruded and deep fat fried, deep fat fried, baked, breakfast cereals and other snacks (Table 4). The recoveries ranged from 91-101.33 per cent with less than 5 per cent co-efficient of variation. Our results indicated better LOD and LOQ values and similar recoveries when compared to few reports available on quantification of acrylamide in food products with HPLC-UV detection. The method proposed in the present study also showed good intra-inter day precision and was able to determine required level of acrylamide. Paleobogos and Kontomiros (2005) developed normal phase HPLC with UV detection for the analysis of acrylamide and methacrylamide in baked foods with LOD of 10 µg L^{-1} and recovery in the range of 95-103 per cent[34]. Wang[38], *et al.* reported LOD and LOQ values of 8 µg/kg and 25 µg/kg and 89-103 per cent recovery during analysis of acrylamide in baked and deep fat fried Chinese food using HPLC UV detection with ODS-C18 column.

4.3. Estimation of Acrylamide Content in Commercial Snack Food of India

Acrylamide, a polar and low weight molecule is readily incorporated and distributed in animals and humans and is known to exhibit carcinogenicity and neurotoxicity. Neurotoxicity of acrylamide characterised by ataxia and skeletal muscle weakness was reported in workers occupationally engaged in polymer and paper industries due to high levels of exposure through inhalation or dermal absorption where acrylamide was used as raw material[5]. In rat and mice models, No Observable Effect Level (NOEL) for neuro-toxic effect of acrylamide is in the range of 0.2 to 10 mg/kg body wt/day and is far above dietary exposure. The exposure to AA through food intake varies from country to country and depends upon food habits. For example, in Germany, high consumption of 240 g/day of bread and bread rolls account for 25 per cent of acrylamide intake. LoPachin[39] that neurotoxicity of acrylamide might be cumulative and dietary exposure might not be negligible. Several studies conducted worldwide to estimate acrylamide content of potato based products such as potato chips and french fries indicated high acrylamide contents ranging from 1270-5312 µg/kg while coffee based products reported up to 7300 µg/kg of acrylamide[40]. Chinese deep fat fried ethnic foods namely *paicha, youtiao, yougao crisp matua* and *mahua* contained acrylamide in the range of 212-248 µg/kg[37]. Indian traditional extruded snacks prepared using blends of potato flour and semolina showed acrylamide concentration of 704 to 1560 µg/kg[41]. Shamla & Nisha[23] reported high acrylamide content in deep fat fried foods of India such as potato chips (1456.5 µg/kg) and jack fruit and plantain chips (426. 2 µg/kg).

In the present study, proximate composition analysis was carried out for all selected snack foods to estimate their

Table 2. Acrylamide content in commercial snacks & permitted level of consumption

Sample name	PPB (µg /Kg) of Sample	Weight can be consumed per day (g)
Extruded & deep fat fried snacks		
Kurkure hyderabadi hungama	4191.82	13.76
Kurkure masala munch	4072.33	14.17
Bingo galatamasti	2337.26	24.69
Bingo tangles	1795.41	32.14
Full toss masala sticks (parleys)	3516.55	16.41
Instant rice kheer	788.99	73.13
Vegetablised noodles	Not Detected	Not applicable
Deep fat fried foods		
Potato chips – magic masala (lays)	2593.35	22.25
Potato chips classic salt (lays)	1064.30	54.21
Potato chips (parles)	2902.99	19.88
Potato chips (parles)	1661.74	34.72
Potato chips (unorganised sector)	5754.32	10.03
Potato chips (unorganised sector)	6391.73	9.03
Potato chips(unorganised sector)	5228.26	11.04
Tapioca chips (unorganised sector)	683.44	84.43
Banana chips (unorganised sector)	1003.13	57.52
Banana chips (relish)	1003.12	57.52
Cruchy rings	3786.03	15.24
Moong dhal (bikanerwala)	371.56	155.29
Moong dhal (unorganised sector)	425.14	135.72
Khara boondi	2474.72	23.32
Alooboojiya (bikanerwala)	3033.85	19.02
Masala kabuli channa	2209.59	26.11
Chow chow mix	2522.98	22.87
Kachori	745.02	77.45
Samosa	1473.73	39.15
Butter murukku	Not Detected	Not applicable
Baked food		
Multigrain biscuit (unorganised)	4449.67	12.97
Multigrain (britania)	2223.87	25.95
Kajubadam biscuits (sunfeast)	3099.79	18.61
Cashew badam (unorganised)	4167.78	13.84
Spicy malted ragi cookies	3667.35	15.73
Bread rusk (modern)	3147.88	18.33
Bread rusk (britannia)	2502.52	23.06
Rusk (unorganised sector)	3282.52	17.58
Cake (unorganised sector)	1033.89	55.81
Cake (unorganised sector)	614.64	93.88
Milk cake (britannia)	1369.05	42.15
Khara biscuit	Not Detected	Not applicable
Breakfast cereals		
Honey loops (kellogg's)	434.37	132.84
Choco flakes (kellogg's)	1307.98	44.11
Choco crunchy rings(kellogg's)	1050.44	54.93
Star moon (kellogg's)	Not Detected	Not applicable
Others		
Snack bar (snickers)	1520.74	37.94
Snack bar (rite bites)	1475.32	39.11
Honey crunch	471.37	122.41
Wheat poustic	803.79	71.78
Milky chocolate (cadbury)	1505.58	38.32
Vacuum fried apple chips	Not Detected	Not applicable
Vacuum fried pear chips	Not Detected	Not applicable
Vacuum fried papaya chips	Not Detected	Not applicable

Table 3. Acrylamide in snack food samples from Indian market

Sample	Range (µg /Kg)
Extruded snacks	788.99 - 4191.82
Deep fat fried snacks	371.56- 6391.73
Baked foods	435.52 - 4449.67
Breakfast cereals	434.37 - 1307.98
Others	1.37 - 1520.74

nutritional content (Table 1). The estimation of acrylamide content in popular Indian snack foods revealed acrylamide content of extruded and deep fat fried snack ranged from 788.99 µg/kg - 4191.82 µg/kg, deep fat fried snack foods from 371.56 µg/kg - 6391.73 µg/kg, baked foods from 614.64 µg/kg - 4449.67 µg/kg, breakfast cereals from 434.37 µg/kg - 1307.98

Table 4. Recovery test of the HPLC-UV method (n=3)

Sample	Acrylamide added (µg /Kg)	Recovery (%)	CV (%)
Potato chips	500	101.33	1.13
Kurkure Hyderabadi Hungama	500	94.33	2.20
Khara boondi	500	91.26	1.55
Aloo Boojiya	500	93.53	3.03
Choco crunchy rings	500	94.0	3.25

µg/kg and other snack foods from 471 µg/kg to 1520.74 µg/kg as shown in Table 3. The extruded and deep fat fried food matrices from organised food processing industries Kurkure Hyderabadi Hungama showed highest level of acrylamide i.e 4191.82 µg/kg followed by Kurkure masala Munch 4072.33 µg/kg which is similar to the findings of Singh[24]. The high level of acrylamide in kurkure may be attributed dual thermal treatment i.e. extrusion and deep fat frying. The acrylamide content was found to be nil detected in vegetablised noodle.

In deep fat fried food matrices, 7 sample of potato chips procured from both large-scale food processing industries such as Pepsico and Parley (4 Sample) and unorganised processing sector (3 sample) were analysed for acrylamide content. The results showed in Table 2 revealed that potato chips procured from an unorganised cottage industries contained high amount of acrylamide ranging from 5228.26 µg/kg - 6391.73 µg/kg which is 2-3 time higher than chips procured from organised food production units (1064.30 µg/kg - 2902.99 µg/kg). The high acrylamide content of potato chips from unorganised sector may due to unscientific processing condition such as high temperature and extended frying time, frequent frying and repeated use of frying oil. The plantain (banana) chips showed acrylamide content ranging from 1033 µg/kg which is similar to earlier reports by Shamla & Nisha[23]. Among the savories studied Aloo (Potato) Boojia showed highest level of acrylamide i.e 3033.85 µg/kg followed by chow chow mix (2522.98 µg/kg) and Khara Boondi (2474.72 µg/kg). The addition of fried potato in aloo boojiya savory may be the reason for highest level of acrylamide. Fried Moong Dhal a deep fat fried snack prepared from Green gram showed less amount of acrylamide due to presence lower level of reducing sugar in the raw material. The acrylamide content was found nil in butter murukku a traditional south Indian dish which may be due to low level of reducing sugar in the product.

The baked food matrices from organised and unorganised sectors were also subjected to acrylamide content estimation. The baked foods from unorganised sectors such as cashew badam biscuits and malted *ragi* (Finger millet) cookies showed higher acrylamide content followed by rusks and cakes (Table 2). The high level of acrylamide contents in cashew badam biscuits may be due to the presence roasted cashew, badam, nuts, molasses and flavoring agents which are cooked several times which leads to formation of acrylamide[42]. The high amount of acrylamide in malted *ragi* cookies is due to use of sprouted *ragi* grains for making cookies. Claus[43], *et al.* revealed sprouting of cereals grains leads to increased protease activity which results in high amount of asparagine a potential precursor for acrylamide formation. In the spiced based baked foods such as *Khara* (Spice) biscuit which has no sugar showed no acrylamide content.

The breakfast cereals selected for the study was from organised sector and the acrylamide level was highest in choco flakes up to 1307 µg/kg and found to be lowest in honey loops of Kelloggs brand (Table 2). The addition of cocoa beans, roasted nuts and flavor compounds may be attributed to high acrylamide content. It also observed in other snack food category, snack bars and milky chocolate contained high amount of acrylamide when compared to wheat poustic, honey crunch and vacuum fried chips. It is inferred from the present study that the variation in the acrylamide content in different food matrices may be due to different concentration of precursors such as level of asparagine, sugar, different processing condition as frying, time duration, temperature and baking condition. Detailed study has to be carried out on Indian processing conditions which will help in reduction of acrylamide in popular Indian snack foods. Among the five categories of traditional Indian snack studied the deep fat fried snack foods showed high amount of acrylamide followed by extruded and deep fat fried foods. The acrylamide content was found to be low among the breakfast cereals which may be due to varying method of food processing and low thermal treatment.

In the present study, the average per day consumption of snacks with safer levels of acrylamide was calculated based on the average body weight (57.7 kg) of Asian man with permissible limit of acrylamide intake as recommended by WHO. It can be used ready reckoner to avoid over consumption snack food with high level of acrylamide which leads to several health hazards. The per day permitted level of snack food ranged from 13.76 g to 71.13 g for extruded and deep fat fried snack food, 9.03 g to 155.29 g for deep fat fried snack food, 12.97 g to 93.88 g for baked food, 54.93 g to 132.84 g for breakfast cereals and 37.94 g to 122.41 g for other snack food matrices.

5. CONCLUSIONS

A precise, accurate and rapid HPLC-UV detection method was developed to determine acrylamide content of snack food products. The proposed method has better limit of detection and RSD values in comparison to previously reported studies on acrylamide detection. The present method was successfully applied for estimation of acrylamide content of 51 number of commercially available packed snack foods of different categories from organised and unorganised sectors in India. The acrylamide content ranged from 371.56 µg/kg - 6391.73 µg/kg of snack foods. The highest content of acrylamide was detected in potato chips from unorganised food processing units. Acrylamide content in Indian foods were comparatively higher than European countries, so care should be taken in material selection and processing methods by food processing industries to have lower level of acrylamide. The data on the per day consumption level of snack food will guide consumers to reduce lower level of acrylamide intake.

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CONTRIBUTORS

Dr Pal Murugan M, did his PhD in Agricultural Extension from Indian Agricultural Research Institute (IARI), Pusa, and New Delhi. Presently working as Scientist 'D' in DRDO-Defence Food Research Laboratory, Mysuru and contributed significantly in the development of Jiffy Food mixes. He has more than 20 research paper and 3 patent to his credit. He has received LIMCA Book of Record Award-2012, National technology day Award, Laboratory scientist of the year.
Contribution in the present study: He has planned and executed the study

Mr Agathian G, did his Masters in Chemistry from University of Madras, Chennai. He is presently working as Technical Officer 'A' in DRDO-Defence Food Research Laboratory, Mysuru and working on the development of light weight Jiffy Food products for the armed forces.
Contribution in the present study: He has performed HPLC analytical study

Dr Anil Dutt Semwal did his PhD in Food Chemistry from University of Mysore, Karnataka. He is working as Director of DRDO-Defence Food Research Laboratory, Mysuru. He has significantly contributed in the development of convenience foods, extruded technology and extensively worked on the factors affecting the stability of various precooked dehydrated foods. He also actively engaged in the commercialisation of DFRL product and technologies and transferred more than 250 technologies to Industries. He has more than 110 research paper and 20 patent to his credit.

Contribution in the present study: He provided support and Guidance and Edited manuscript

Dr Gopal Kumar Sharma obtained his PhD in Chemistry from Lucknow University. Currently, he is working as an Associate Director in DRDO-Defence Food Research Laboratory, Mysuru. He has significantly contributed in separation and identification of various flavours and off flavour compounds in processed foods, development convenience food and indigenisation of low cost processing equipment. He has more than 120 research papers and 25 patent to his credit. He has transferred 103 food products and technologies for commercialisation.
Contribution in the present study: He provided support, guidance and overall supervision of work.