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Full Length Research Paper

Determination of acrylamide concentration in processed food products using normal phase high-performance liquid chromatography (HPLC)

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Currently, acrylamide concentration in processed food products have become a very serious health issue. The World Health Organization (WHO) and the Scientific Committee for Food (SCF) of the European Union also confirmed this concern. In laboratory scale, it was found that acrylamide causes tumors in animals. This study is aimed to determine the concentration of acrylamide in processed food products available in open market. In order to determine the acrylamide concentration, three bakery items and three fried chips from three different brands were analyzed. High-performance liquid chromatography (HPLC) technique was employed for the analysis. The overall distribution of acrylamide concentration was found to be maximum in kurkure followed by lays and minimum in banana Chips.

Key words: Processed food, high-performance liquid chromatography, acrylamide, health hazard.

INTRODUCTION

In the year 2002, the Swedish National Food administration published their comments and data on acrylamide concentration in processed foods (SNFA, 2002). In laboratory scale, acrylamide causes tumors in animals. The report of World Health Organization (WHO) (WHOPR, 2002) and the Scientific Committee for Food (SCF) of the European Union (OSCF, 2002) revealed that the concentration of acrylamide in most processed food was high e.g potato chips, fried chips, crisp bread, roast potatoes, breakfast cereals etc. It was thus confirmed that acrylamide has become a serious problem in respect to the public health (Richard et al., 1984). High concentration of acrylamide in the body usually has serious impact on health and causes various health problem issues and it has been found to be very prone to cancer (Ahmad and Gerd, 2007; Adam et al., 2003). The approach which was used for the analysis of acrylamide concentration was the high-performance liquid chromatography (HPLC) which gives proper qualitative and quantitative analysis. HPLC has been credited with qualitative and quantitative analysis of various components, biological and non-biological (Matuszewski et al 2003, Wenzl et al 2003). Biological components when analysed with HPLC require flow rates which are in separate ranges depending on the underneath composition (Eden 2002). Non-biological components are not that specific when it comes to flow rate. The flow rate plays an important role in the time taken for HPLC analysis to finish. Higher flow rate ensures faster completion of analysis process and slower flow rate makes the process slower.

Processed food items seem to be acquiring acrylamide when cooked at high temperatures especially above 120°C. Different food products get contaminated with acrylamide in domestic and commercial fronts showing that the addition or presence of preservatives, flavor enhancers, color additives do not have any role as far as acrylamide is concerned. The objective of the study is to study the presence of acrylamide in processed food items sold in bakery and super markets in India.

Selection of solvent system and the nature of the solvents play an important role in HPLC analysis of biological components (Paleologos and Kontominas, 2005). When the biological species being analysed is a processed

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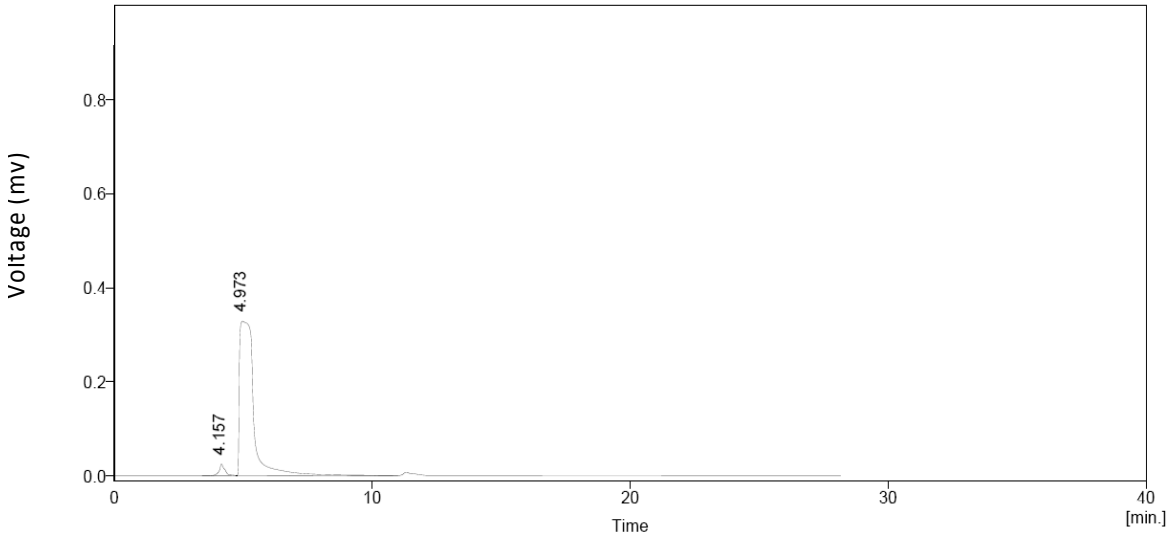


Result Table (Uncal - D:\Asthagiri-HPLC-20A\Job Order\acrymalide-std 2)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]
1	4.157	411.166	25.379	3.1	7.2
2	4.973	12672.504	328.891	96.9	92.8
	Total	13083.670	354.270	100.0	100.0

Figure 1. HPLC analysis of standard sample.

food item, obviously the emphasis on the nature of solvent and the solvent system selection becomes more important. Polar solvents and non-polar solvents have a marked difference when it comes to biological components analysis by HPLC (Zhang et al., 2007). Usually used solvents are acetonitrile, water, methanol, ethanol, acetone etc.

MATERIALS AND METHODS

The main material for this study were three fried chips from three different companies (kurkure, lays, banana chips) and three bakeryitems (vegetable puff, chicken puff, egg puff). All these samples were procured from super markets and bakery situated inside SRM University, Chennai.

Sample preparation

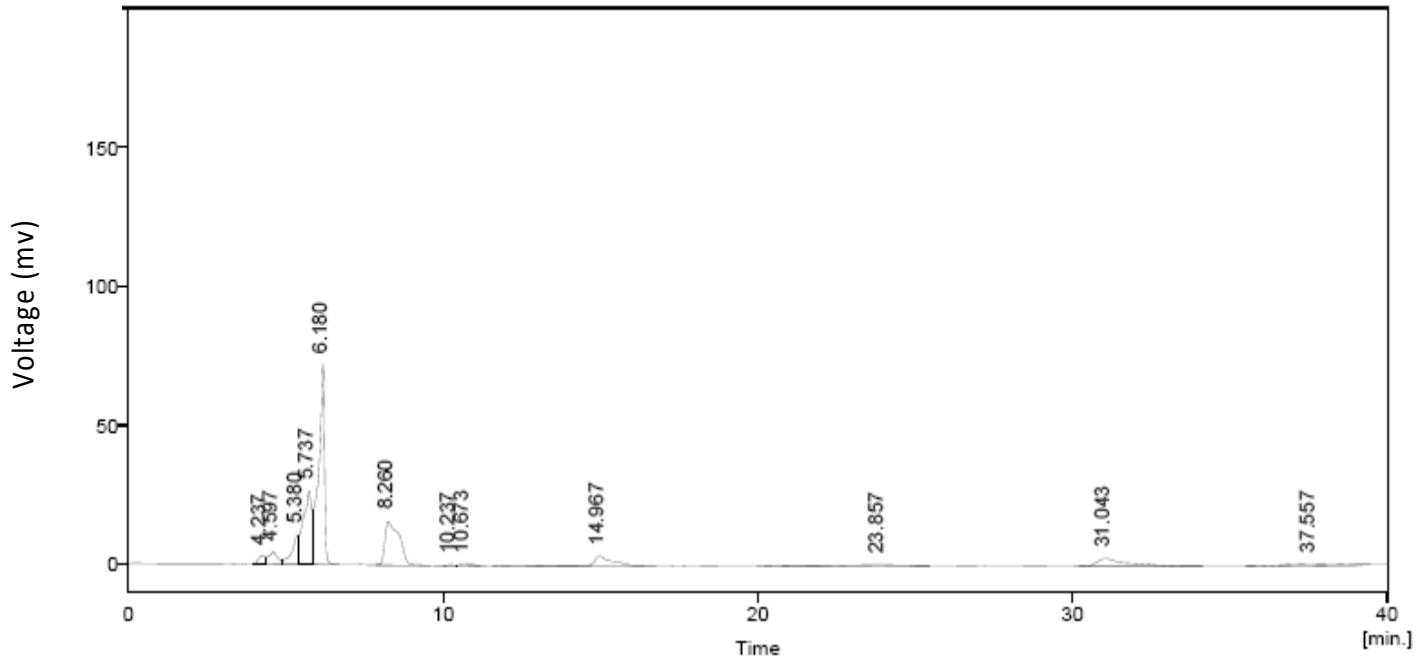
Each sample (25 g) was soaked in water (75 ml). More water was used for very dry samples. Then 500 µg/kg of D3 acrylamide in acetonitrile solution was added. The homogenate in a beaker glass covered with aluminium foil was incubated 30 min at 70°C. The beaker glass was covered by aluminium foil to prevent evaporation of water. 10 g of the homogenate was weighed into a 100 ml centrifuge glass with a screw cap and thoroughly mixed with 40 ml of 1-propanol. When the solids form lumps, mixing was supported by a blender. 10 ml (8.4 g) of the supernatant was transferred to a 25 ml pointed flask. About 200 mg of a vegetable oil were added and the water/propanol removed in a rotary evaporator at about 50 Torr and 60 - 70°C in the water bath. Evaporation was stopped as soon as no liquid was left. The residue from the evaporation, consisting of fat/added oil and often much salt, was extracted with acetonitrile and defatted with hexane. 3 ml acetonitrile and 20 ml hexane were added and mixed with the sample with the help of an ultrasonic bath. The acetonitrile phase was transferred into a 10 ml reagent glass with screw cap by means of a Pasteur pipette, losing acetonitrile rather than carrying along hexane. The acetonitrile phase was extracted by another 5 ml hexane, 1.5 ml of the acetonitrile phase was then transferred into a 1.5 ml autosampler vial. Butyramide solution (internal standard, IS) was added. For the common 25 g sample swollen with 75 ml water this meant 5 µl of a 25 mg/l solution in acetonitrile (Peter and Hugo, 2002).

HPLC data analysis

HPLC instrument used was Phenomenex – 5u (Luna) C18 column, 4.6 × 250 mm with mobile phase ACN: H_2O (70:30) that is, acrylamide: water. The flow rate was fixed to 0.5 ml/min and the detection wavelength was set as 202 nm. Mode of the HPLC instrument was isocratic and injection volume was 20 µl.

RESULTS AND DISCUSSION

The acrylamide concentration was analysed using HPLC and estimation was done. Standard acrylamide was determined with 2 characteristic peaks observed at 4.157 and 4.973 (Figure 1). Then the analysis of banana chips



Result Table (Uncal - D:\Asthagiri-HPLC-20A\Job Order\B1)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]
1	4.237	45.374	3.035	1.6	2.1
2	4.597	84.129	4.372	3.0	3.1
3	5.380	161.587	10.588	5.7	7.5
4	5.737	473.523	26.331	16.7	18.6
5	6.180	960.417	71.916	33.9	50.9
6	8.260	492.175	15.805	17.4	11.2
7	10.237	4.129	0.267	0.1	0.2
8	10.673	23.075	0.971	0.8	0.7
9	14.967	162.516	3.534	5.7	2.5
10	23.857	58.496	0.345	2.1	0.2
11	31.043	198.672	2.859	7.0	2.0
12	37.557	76.381	0.666	2.7	0.5
13	47.757	88.928	0.510	3.1	0.4
	Total	2829.401	141.201	100.0	100.0

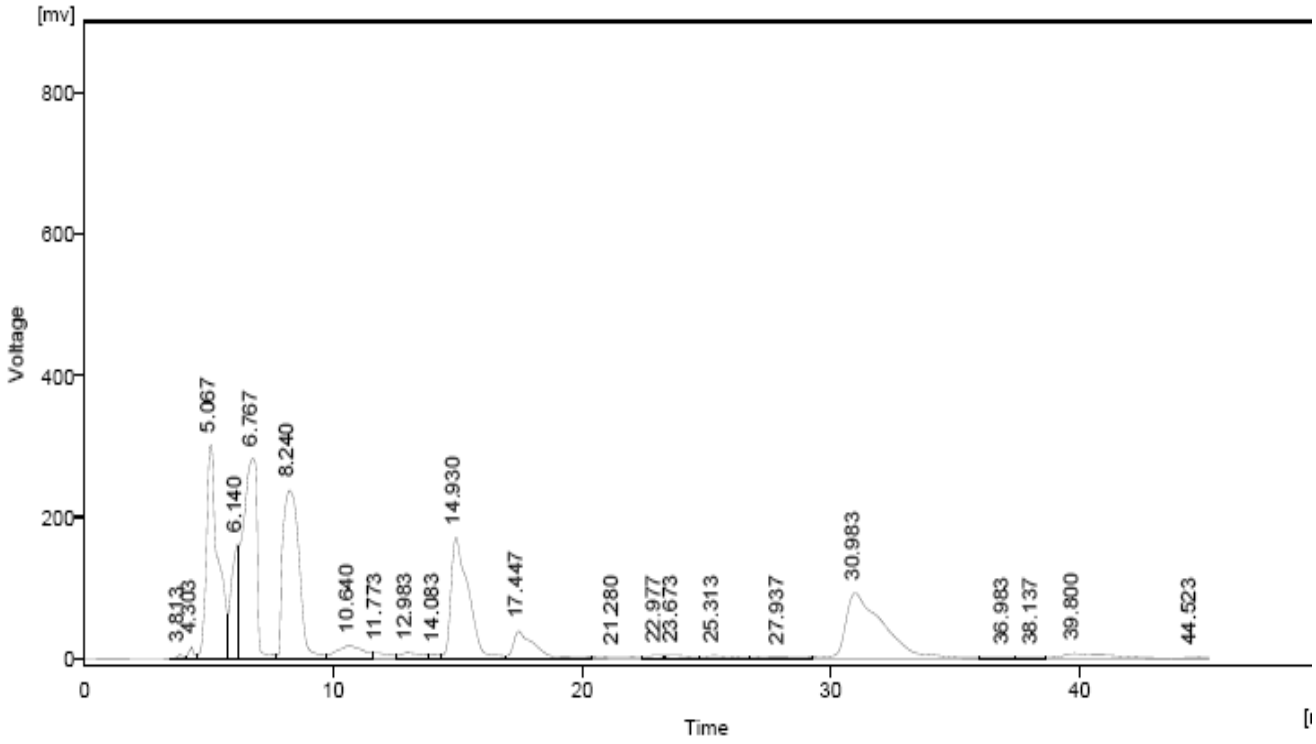
Figure 2. HPLC analysis of banana chips sample.

was done. It had 13 characteristic peaks and characteristic peak of 6.180 had the highest area percentage (Figure 2). Banana chips were found to have the lowest concentration of acrylamide among the 6 samples chosen for the study.

HPLC analysis of chicken puff revealed the acrylamide concentration to be moderate with 15 characteristic peaks and 7.913 having the highest area percentage. The acrylamide concentration was in between vegetable puff and egg puff (Figure 3). In analysis of egg puff sample, 11 characteristic peaks were observed with 7.947 having the highest area percentage (Figure 4). The acrylamide concentration was found to be quite high in the egg puff sample when compared with chicken puff but the concentration of acrylamide was lower than the vegetable puff.

Fried chips brand kurkure was found to have the highest concentration of acrylamide when compared with other bakery items and puff items. 22 characteristic peaks were observed with the 6.767 having the highest peak percentage (Figure 5). Kurkure's acrylamide concentration was established to be the highest due to various factors such as degree of frying, compositional difference with other processed food items.

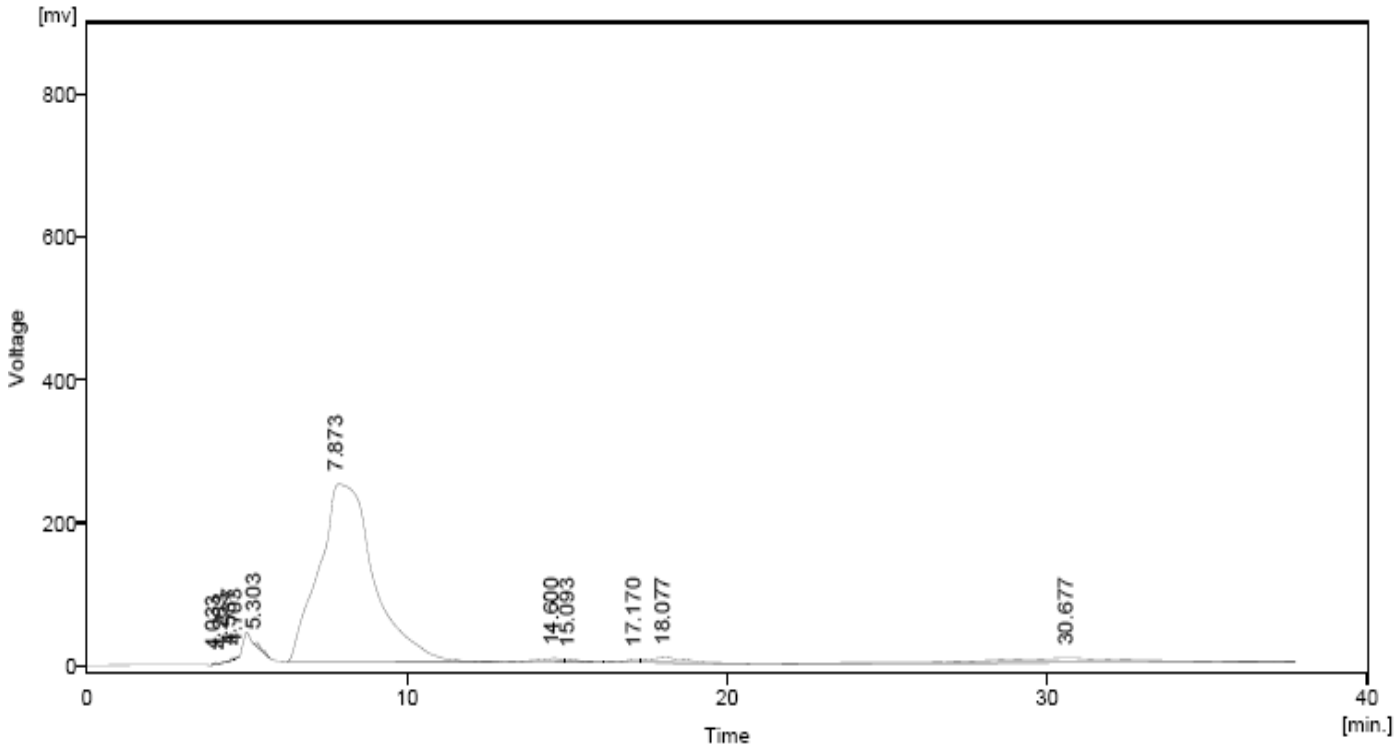
Figure 6 represents HPLC analysis of fried chips lays which had the second highest concentration as far as



Result Table (Uncal - D:\Asthagiri-HPLC-20A\Job Order\K)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]
1	3.813	86.765	5.100	0.1	0.4
2	4.303	229.387	16.546	0.4	1.2
3	5.067	9778.891	301.121	16.3	22.0
4	6.140	3250.984	161.004	5.4	11.7
5	6.767	11000.588	282.542	18.4	20.6
6	8.240	10866.842	237.345	18.1	17.3
7	10.640	1340.096	18.844	2.2	1.4
8	11.773	347.907	7.596	0.6	0.6
9	12.983	465.251	7.856	0.8	0.6
10	14.083	183.496	6.292	0.3	0.5
11	14.930	8315.627	170.875	13.9	12.5
12	17.447	2263.906	38.362	3.8	2.8
13	21.280	264.609	2.666	0.4	0.2
14	22.977	176.679	4.244	0.3	0.3
15	23.673	287.914	4.159	0.5	0.3
16	25.313	287.641	3.675	0.5	0.3
17	27.937	256.135	2.114	0.4	0.2
18	30.983	9514.422	92.143	15.9	6.7
19	36.983	62.163	0.897	0.1	0.1
20	38.137	73.626	1.038	0.1	0.1
21	39.800	811.819	6.559	1.4	0.5
22	44.523	14.167	0.310	2.366e-02	2.263e-02
	Total	59878.916	1371.288	100.0	100.0

Figure 5. HPLC analysis of Kurkure sample.



Result Table (Uncal - D:\Asthagiri-HPLC-20A\Job Order\L)

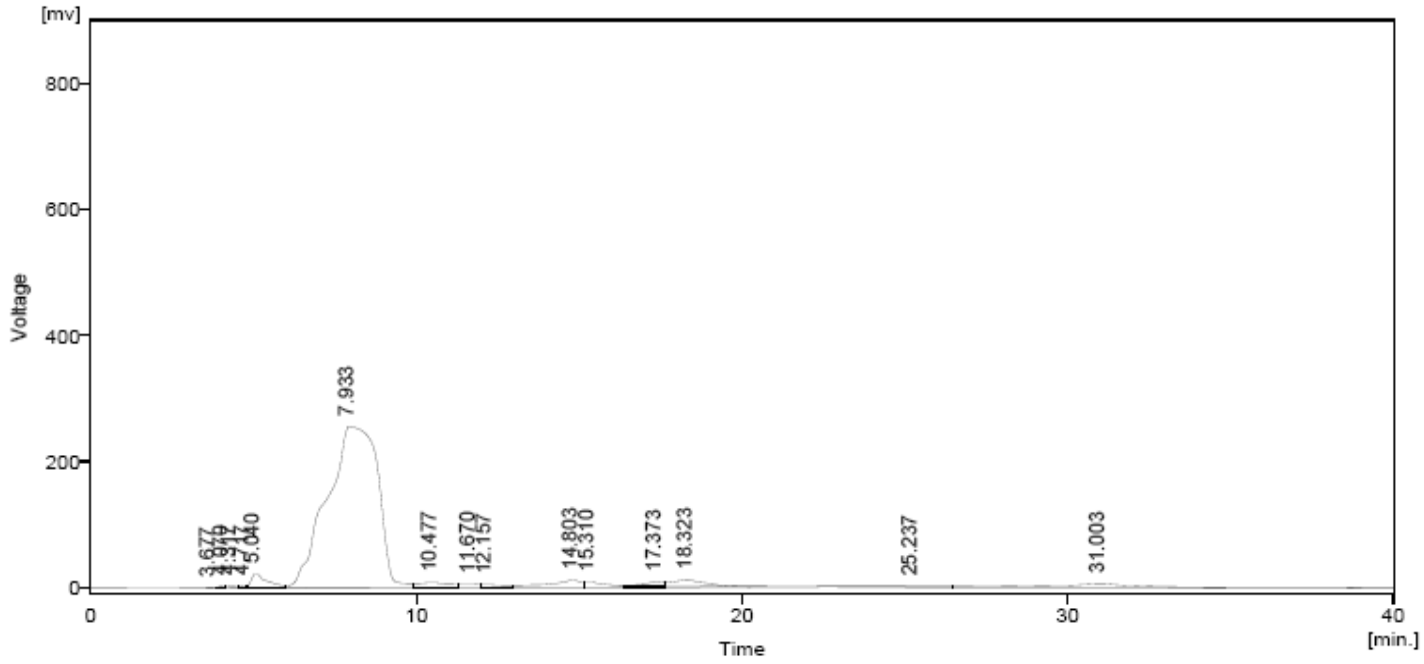
	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]
1	4.033	13.374	1.645	4.017e-02	0.6
2	4.263	9.396	1.249	2.822e-02	0.4
3	4.537	25.660	3.133	0.1	1.1
4	4.703	7.217	0.946	2.168e-02	0.3
5	5.303	93.513	5.238	0.3	1.8
6	7.873	29817.415	248.095	89.6	86.3
7	14.600	282.589	5.416	0.8	1.9
8	15.093	142.800	3.752	0.4	1.3
9	17.170	163.871	4.189	0.5	1.5
10	18.077	584.283	7.294	1.8	2.5
11	30.677	2151.239	6.575	6.5	2.3
	Total	33291.358	287.531	100.0	100.0

Figure 6. HPLC analysis of lays sample.

acrylamide concentration is concerned. It had acrylamide concentration second only to kurkure with all other bakery items, puff items being lower than it. Lays had 11 characteristic peaks with 7.873 having the highest area percentage.

Vegetable puff had 16 characteristic peaks with 7.933 having the area percentage (Figure 7). The concentration of acrylamide concentration was lower than kurkure and lays. The acrylamide concentration was higher than chicken puff, egg puff and banana chips.

HPLC has been credited with qualitative and quanti-tative analysis of various components, biological and non-biological. Biological components when analysed with HPLC require flow rates which are in separate ranges depending on the underneath composition. Non-biological components are not that specific when it comes to flow rate. The flow rate plays an important role in the time taken for HPLC analysis to finish. Higher flow rate ensures faster completion of analysis process and slower flow rate makes the process slower.



Result Table (Uncal - D:\Asthagiri-HPLC-20A\Job Order\V.O)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]
1	3.677	2.170	0.245	6.665e-03	0.1
2	4.070	34.511	2.878	0.1	0.8
3	4.317	65.958	3.549	0.2	1.0
4	4.717	45.001	3.668	0.1	1.1
5	5.040	717.652	20.982	2.2	6.0
6	7.933	27552.419	255.461	84.6	73.6
7	10.477	503.623	7.317	1.5	2.1
8	11.670	186.890	4.987	0.6	1.4
9	12.157	200.922	4.242	0.6	1.2
10	14.803	736.349	10.928	2.3	3.1
11	15.310	362.667	7.823	1.1	2.3
12	17.373	380.290	8.056	1.2	2.3
13	18.323	832.059	11.069	2.6	3.2
14	25.237	84.172	0.532	0.3	0.2
15	31.003	846.820	5.321	2.6	1.5
16	39.817	8.055	0.163	2.474e-02	4.708e-02
	Total	32559.557	347.219	100.0	100.0

Figure 7. HPLC analysis of vegetable puff sample.

Conclusion

Acrylamide is a carcinogenic and processed foods available at super markets seem to be having considerable amount of it. Acrylamide seems to be produced in the processed food due to the chemical reaction that is initiated during high temperature cooking. Although differences are present in the amount of acrylamide in the 6 samples taken for the study, there is no denying the fact that all these samples contained acrylamide. The processed food items in the absence of acrylamide will be extremely beneficial in the health aspects of the public by and large.

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