

Study of Cancer causing Food Product material Analysis by using UV Spectroscopy

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Abstract: Aim: To study the formation of acryl amide in banana chips by using different kind of vegetable oils as the cooking media.

Method: The Banana chips were prepared by using different vegetable oils by frying above 180-200 °C. The samples were frozen and homogenized with water in the ratio of (1:10) for 20mins. The homogenised samples were subjected to centrifugation at 10000rpm for 15mins. The supernatants liquid were collected and analysed by U.V Spectroscopy. The λ_{max} were obtained at 275nm. The Quantification of acryl amide was done by using calibration curve method. The calibration curve was plotting between concentration vs absorbance. Acrylamide was linear within the concentration range of 1 – 10 μ g/ ml at 275nm.

The slope, intercept, Standard deviation, %RSD was calculated.

Results: The sunflower oil contained (1996ng/gm) of acrylamide, coconut oil contained (1997ng/gm) of acrylamide, rice bran oil contained (2221ng/gm) of acrylamide, gingelly oil contained (2666ng/gm) of acrylamide, & palm oil contained (2220ng/gm) of acrylamide.

Conclusion: The Tolerable intake of acrylamide was reported in different articles should be at 2.6 mcg/kg of bodyweight to avoid cancer. The amount of acrylamide present in banana chips was (888-4222ng/gm). So this much amount should not produce toxic effect, but repeated intake produces toxicity.

Keywords: Acrylamide, Banana chips, Vegetable oils, UV Spectroscopy, Carcinogenicity.

1. Introduction:

Acrylamide¹ is a chemical compound and the chemical formula C₃H₅NO. Its IUPAC name is prop-2-enamide. It is a white odourless crystalline solid, soluble in water, ethanol, ether, and chloroform³. Acrylamide decomposes in the presence of acids, bases, oxidizing agents, iron, and iron salts. The discovery of acrylamide in some cooked starchy foods in 2002 prompted concerns about the carcinogenicity of those foods. Acrylamide consumption affects the people's and risk of getting cancer.

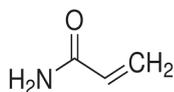
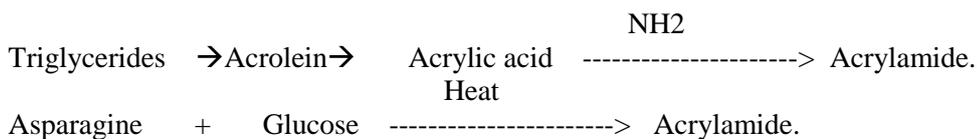


Fig:1 Structure of Acrylamide

The starch containing food is heated for longer periods of time or the fried or baked goods may be produce the acrylamide by the Maillard reaction. The reaction is taking between asparagine and reducing sugars (fructose, glucose, etc.) or reactive carbonyl temperatures above 120 °C (248 °F).

Maillard pathway² for acrylamide formation:



As per literature survey⁵⁻¹⁷ they are reported acrylamide formed in Potato chips, French fries, Noodle and soup mixes, Snack foods, chocolate products, Crackers, Nuts and nut butters etc, But nobody reported in banana chips. Hence we tried it in banana chips in different vegetable oil⁴ and acrylamide was extracted by using liquid-liquid extraction method and Quantification was done by using the UV Spectroscopy².

2. Materials and Methods:

2.1 Materials:

Banana chips fried in an 5 different oil samples, Sunflower oil (gold winner), Coconut oil (kerajam), Rice bran oil (Porna), Gingelly oil (Idhayam), Palm oil, Kadai fire bowl (small size), Glass laboratory thermometer, Distilled water, Homogeniser, Centrifugator, 0.45 um syringe filter (whatmann filter), Sodium hydroxide 1M(NaOH), Acrylamide (sigma aldrich).

2.2 Determination of Maximum Wavelength

As per literature survey²⁰ the acrylamide soluble in water and it shows UV absorbance in alkali condition .The 10gm of standard acrylamide transfer to 100ml standard flask then make up to 100ml with distilled water. Pipette out 1ml of above solution diluted to 100ml with 1M sodium hydroxide (10ug /ml).The standard stock solution was scanned between 200 and 400 nm using the same solvent as blank. The spectrum was observed in that range and the λ_{max} was found to be 275nm and it's used as a wavelength. The graph are shown in figure:2

2.3 Calibration Curve:

The standard stock solution of Acrylamide (1.0 – 10 ml) was transferred into series of 10 ml volumetric flasks and made up to the volume with water. The absorbance of 1,2, 3.....10 μg / ml solutions were measured at 275 nm. The calibration curve was plotting between concentration vs absorbance. Acrylamide was linear within the concentration range of 1 – 10 μg / ml at 275nm.

2.4 Preparation of Sample

Test Samples (banana chips) was prepared in the laboratory using 5 different oils (sunflower oil, coconut oil, rice bran oil, gingelly oil, palm oil). The outer layer of banana was peeled off with the help of knife. Then the inner portion of fruit was sliced in to thin slices using vegetable Slicer. At first it has to be fried in one type of oil. When the chips that turns crispy, they were taken out from the oil. The same procedure was used to preparing the chips using other subsequent oils. Here the temperature should be maintained constant for all the five types of oils (180°C-200°C). The prepared samples were homogenised using a homogeniser. Each samples were homogenised separately in a specialised homogenised tubes with the addition of water, for proper mixing of samples in the water for 20mins.The homogenised samples were centrifuged by filling it in a centrifuge tubes at 10000rpm for 15mins. The supernatants liquid of all the samples have been collected.

2.4.1 Identification Test by UV Spectroscopy:

The supernatant liquid was scanned between 200-400 nm. The sample graphs have been compared with the standard graphs. The peak determination is seemed at 275nm in both the sample and standard. So the presence of acrylamide is confirmed in the samples.

2.4.2 Identification Test by Biuret test^[18&19]

The biuret test used for detecting the presence of peptide bonds. The presence of peptides, a copper(II)

ion forms violet– coloured co-ordination complexes in alkaline solution. An aqueous sample was treated with an equal volume of 1% strong base (sodium or potassium hydroxide most often) followed by a few drops of aqueous copper(II) sulfate. If the solution turns purple, protein is present. A peptide of a chain length of at least 3 amino acids is necessary for a significant, measurable color shift with these reagents.

2.4.3 Quantification of acrylamide by UV spectroscopy:

The 1ml of supernatant solution from sample was transferred in to 100ml standard flask, and make up to 100ml with 1M sodium hydroxide. Then the samples were measured the absorbance at 275 nm using sodium hydroxide as blank. The process will be repeated for three times for all the samples. The graphs are shown in figure:3

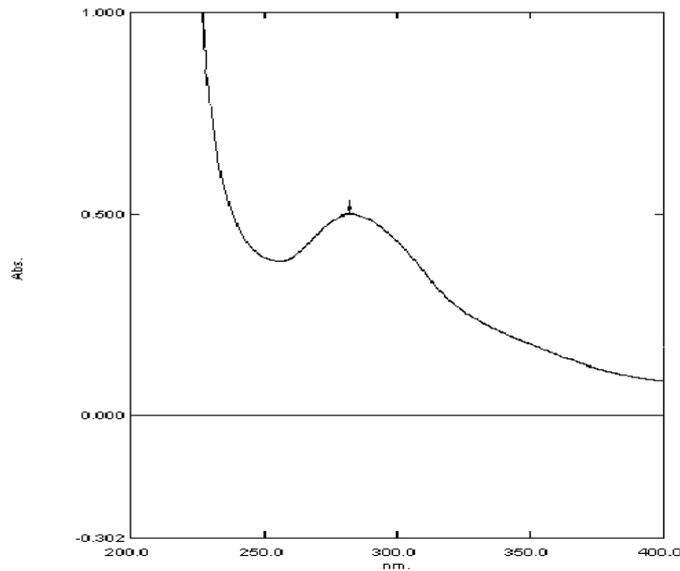


Fig:2 Absorption Spectrum of Standard Acrylamide In Water(10mcg/ml)

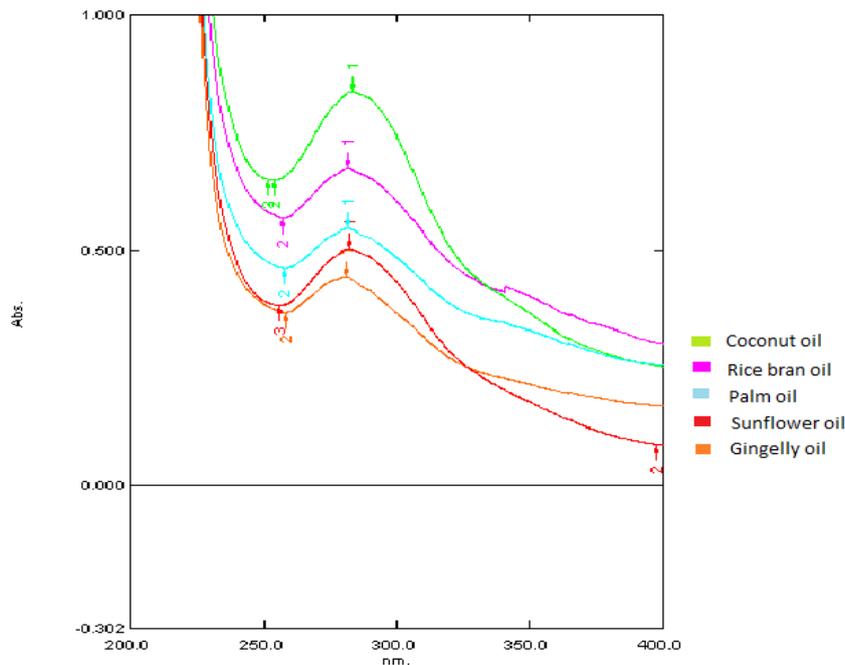


Fig : 3 Absorption Spectrum of Banana Chips Extract by Using Water

3. Results:

Table :1 Linearity studies of Acrylamide

S.no	Standard samples of acrylamide (ng/ml)	Absorbance
1	1000	0.102
2	2000	0.210
3	3000	0.304
4	4000	0.408
5	5000	0.512
6	6000	0.599
7	7000	0.702
8	8000	0.807
9	9000	0.903
10	10000	1.012

Table : 2 Optical characteristics of Standard Acrylamide

Parameters	Values*
λ_{\max} (nm)	275
Beer's law limit (ng/ ml)	1000 – 10000
Slope(m)	0.100152
Intercept(c)	0.005067
Sandell's sensitivity ($\mu\text{g}/\text{cm}^2/0.001 \text{ A.U}$)	0.009985
Molar absorptivity ($\text{L mol}^{-1} \text{ cm}^{-1}$)	7154.784
Correlation coefficient (r)	0.999888
Standard error	0.004809

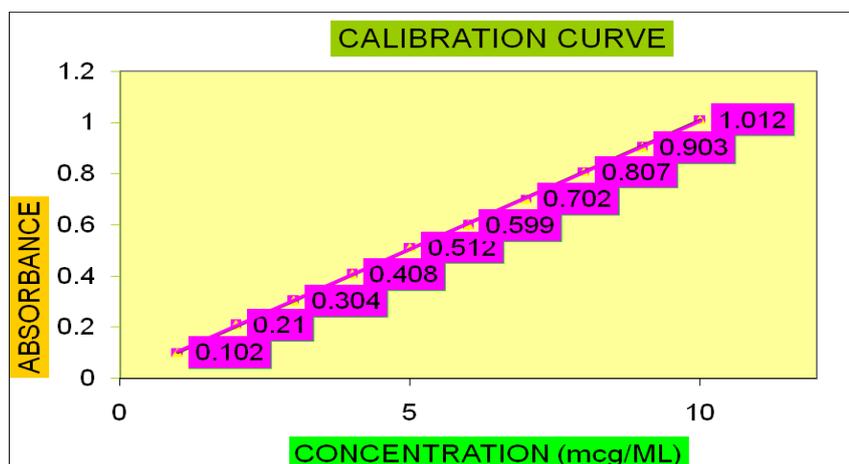


Fig : 4 Calibration curve of standard Acrylamide (1- 10mcg/ml)

3.1 Quantification of Acrylamide in Banana chips:

The Quantification of acrylamide from banana chips by using different oils was carried out by UV spectroscopy. The acrylamide was extracted by using liquid –liquid extraction. The absorbance are measured at 275 nm using water as blank. The amount was calculated by $Y=mX+C$ formula. The process will be repeated for three samples and the values obtained are as follows: sunflower oil (889-2880ng/gm), coconut oil (1111-3110ng/gm), rice bran oil (888-3555ng/gm), gingelly oil (888-4222ng/gm), & palm oil (1111-3111ng/gm). The values are shown in Table :3.

Table: 3 Quantification of acrylamide in banana chips in five oil samples:

Types of Oils in Which Has Been Fried.	Sunflower Oil		Coconut Oil		Rice Bran Oil		Gingelly Oil		Palm Oil	
	Abs	Conc ng/gm	Abs	Conc ng/gm	Abs	Conc ng/gm	Abs	Conc ng/gm	Abs	Conc ng/gm
1)	0.504	889	0.836	1111	0.675	888	0.444	888	0.547	1111
2)	0.508	2220	0.837	1770	0.677	2222	0.446	2888	0.548	2440
3)	0.510	2880	0.839	3110	0.678	3555	0.447	4222	0.550	3111
Standard deviation	1014.169775		1018.649596		1333.500031		1678.050059		1017.880314	
% rsd	50.80162508		51.0089933		60.02250703		62.94261286		45.83669981	

4. Discussion:

The samples of banana chips were prepared by using five different oils (sunflower oil, coconut oil, rice bran oil, gingelly oil & palm oil), and these samples were analysed using U.V spectroscopy. The acrylamide from banana chips was extracted by using water and it confirms by using Biuret chemical test and UV spectroscopy. The supernatant liquid were scanned between 200-400nm. The maximum absorbance is formed at 275nm for both the standard and test samples. As per reference the UV absorbance for acrylamide is between 245nm to 280nm. Hence it confirms the presence of acrylamide in banana chips.

The quantification of acrylamide in banana chips was calculated by $Y=mX+C$ formula. The values obtained are as follows: sunflower oil (889-2880ng/gm), coconut oil (1111-3110ng/gm), rice bran oil (888-3555ng/gm), gingelly oil (888-4222ng/gm), & palm oil (1111-3111ng/gm). The maximum and minimum amount acrylamide formed in banana chips are as follows: (MAX:4222ng/gm – MIN:888-ng/gm).

The Tolerable intake of acrylamide was reported in different articles should be at 2.6 mcg/kg of bodyweight to avoid cancer [19&25]. The amount of acrylamide present in banana chips was (888-4222ng/gm). So this much amount will not produce toxic effect, but repeated intake will produce toxicity. As per our process, the Gingelly oil produced maximum amount of acrylamide in preparation of banana chips whereas sunflower oil has minimum amount of acrylamide.

Table: 4 Mean concentration of Acrylamide in banana chips using five different oils.

Samples used	Quantification of acrylamide in banana chips (mean values) in (ng/gm)
Gingelly oil	2666
Rice bran oil	2221
Palm oil	2220
Coconut oil	1997
Sunflower oil	1996

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