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ROLE OF HPLC IN ESTIMATION OF ACRYLAMIDE AND MICROBIOLOGICAL APPROACHES FOR DEPLETION OF ACRYLAMIDE IN FOOD STUFFS.

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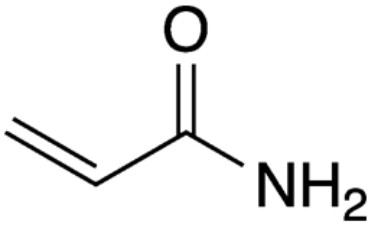
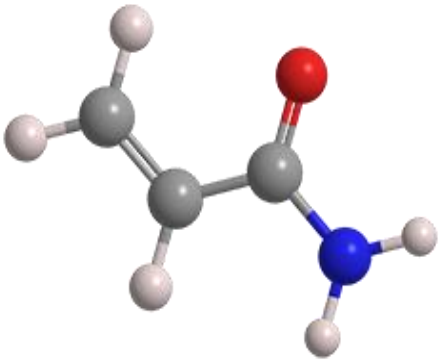
Abstract

Acrylamide is known to be a mutagenic, nephrotoxic, and carcinogenic compound. Acrylamide is a snug processed food via, the nonenzymatic browning reaction and through the reaction connecting Asparginine and a carbonyl compound. The utilize of bacterial L-asparginase (LA) is one of the approaches for acrylamide depletion in food stuffs. Presently, the cancer risk in the overall population has not yet had anadequate response. But the acrylamide cancer tests are one of the basis tests of in vivo doses of Glycidamide (GA) in rats and exhibit in the cancer tests. In the actual stage, acrylamide concentration is fabricatedin edibles have set off a very significant wellbeingissue. The cut back of acrylamide in fried potatoes was determined by HPLC (High performance liquid chromatography).

Introduction

Structure of Acrylamide





Physico-Chemical Properties of Acrylamide
Table: 1 [1]

NAMES	PROPERTIES
IUPAC	Acrylamide
CAS Number	79-06-1
MolecularFormula	C_3H_5NO
Molecular weight[g/mol]	71.078
Properties	White, crystalline solid at Room temperature.
Density 30^{0}c	1.127g/cm^3
Melting point	84-84.5^{0}c
Boiling point	125c at 3.3 pa
Vapour pressure	0.9 pa at 25^{0}c
log pow	-0.67 to 1.67
Solubility in water	2.155 g/1 at 30^{0}c
MAK commission classification	Skin absorption Carcinogen category Germ cell mutagen

E.g.: - Theutilize of Bacterial L-Asparginase (LA) is one of theaccess foracrylamide cut-off in nourishment. As it catalyses the change (LA) to L-Aspartic acid and ammonia.The maximum Asparginase activity (47IU/ML) was arrived in the middle having orange peel. Reduction of acrylamide in fried potatoes was determined by HPLC [6].

At present, Acrylamide concentration is processed in frozen food products have developed into a very deliberate health issue. The WHO and the scientific board for food of the European Unionalso proved the concern. In laboratory scale, it was initiate that acrylamide causes cancerous in animals [7].

E.g.:-If we examine a hotel the oil they use for cooking is used for many times, so that the structure of acrylamide is broken and that oil is being consumed by animals and they are suffering diseases such as cancer, due to that humans are getting cancer.

Figure: 1

In order to detect the Acrylamide attentiveness three bakery items and three fried chips from three different brands were analysed.HPLC technique was engaged for the analysis. The universal distribution of acrylamide concentration was start up to be maximum in kurkure followed by lays and minimum in banana chips.Acrylamide is a poisonous compound and a thermal marker of food derriving from non enzymatic browning [8].

Maillard Reaction [8]

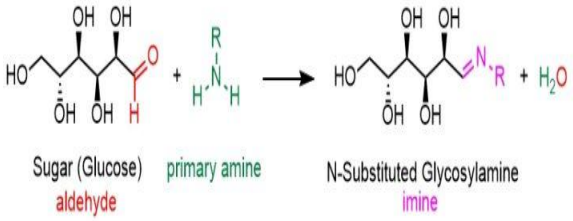
The process start with the formation of glycosylamine from the chemical reaction between an amine and The Maillard reaction is an organic named reaction which is named after the French chemist Louis Camille Maillard. It is sometimes referred to as non-enzymatic browning. The Maillard reaction is a relatively complex process that involves heat-induced chemical reactions between reducing sugar.

Some common examples in which the Maillard reaction is responsible for the change in colours and flavours of food items include:

- The appearance of caramel from sugar and milk.
- The browning of bread during the preparation of toast.
- The change in colour and flavour when meat is heated at higher temperature.
- The change in colour during the processes of condensed milk.

  In the cooking of nearly all forms of foods, the Maillard reaction takes place, while the basic sugars and amino acids present create different aromas. The Maillard reaction is speeded up by high-temperature cooking Because heat increases the rate of chemical reactions and accelerates water evaporation.

Figure:-



A current, European supervision established mitigation calculate and benchmark levels for its depletion in many products emboldenthe utilize of colorimetric scales providing a statistical interaction between colour intensity and acrylamide content.This was immersed an Acrylamide assurance by liquid chromatography paired to mass spectrometry in baked potato samples prepared at different time, temperature and damp conditions.Therefore, the portions of prepared product characterized by distinctive colours were sampled to fabricate a colour scale. These are same in colours, even attained under different cooking conditions were characterized by same Acrylamide levels [10].

Acrylamide concentrations in various food Categories

Table: 2 [11]

PRODUCT	INVESTIGATED SAMPLES	MEDIUM	RANGE
Potato chips	221	750	130-3680
French fries,cooked	54	250	20-3920
Potato sticks	26	1430	630-2870
Fried potatoes, cooked	6	240	n.n-280
Cracker bread	95	170	n.n-2840
Bread	52	<30	n.n-200
Bread rolls	12	<30	n.n-140
Breakfast cereals	39	50	n.n-640
Corn flakes	9	170	20-640
Butter cookies	8	300	140-1090
Ginger bread	17	350	130-890
Pretzel sticks	7	250	110-360
Powdered coffee	35	280	180-290

At moderate conditions for magnetic solid-phase extraction(MSPE)of Acrylamide in potato samples proceeded by High performance liquid chromatography (HPLC). [13]The quantification was done by HPLC with U.V detection (HPLC-UV).[14]In the current status, the cancer risk in the general population has not yet had a satisfactory resut.By using a relative cancer risk mode they had an improvement of the cancer risk estimated for dietary acrylamide this can be acheived by estimation of the Genotoxic contribution to the risk.

Methodology

Sample Preparation of Acrylamide

- Take 1g of sample
- Add 0.1ml carrez 1, 0.1ml carrez2and 9.8ml 0f 0.2mM acetic acid solutions.
- The mixture was mixed for about 2min by the help of vortex
- The suspension was centrifuged at 5000rpm for 10min.at -5^{0}c
- The supernatant was filtered through 0.45mg syringe filter
- Measured by HPLC-MS.

Standard Preparation of Acrylamide

Materials

1. Acrylamide powder
2. N,N'-Methylenebisacrylamide (bisacrylamide) powder
3. Tris buffer
4. Distilled water
5. Ammonium persulfate (APS) solution
6. Tetramethylethylenediamine (TEMED)
7. Weighing balances
8. Stirring rod
9. Graduated cylinders
10. Beakers

Procedure

Safety Precautions

- Acrylamide is toxic, and its residues or solutions can cause serious side effects.
- Always wear an appropriate personal protective garments.
- Including glovesand safety goggles, and work in a well-ventilated area.

Preparation of Tris Buffer

- Dissolve Tris buffer in distilled water to the required concentration.
- Adjust the pH using concentrated hydrochloric acid or sodium hydroxide as required.
- The typical pH range for polyacrylamide gel solutions is around 8.8 to 9.0.

Weigh Acrylamide and Bisacrylamide

- Weigh the appropriate quantities of acrylamide and bisacrylamide using a weighing balance.
- The ratio of acrylamide to bisacrylamide may differ depending on the desired gel characteristics.
- A common ratio is 29:1 for separating small proteins and 37.5:1 for larger proteins.

Preparation of Monomer Solution:

- Mix well the weighed acrylamide and bisacrylamide in a beaker.
- Add distilled water to make a concentrated monomer solution.
- Stir the mixture until the powders are completely dissolved.

Add APS and TEMED

- Addammoniumpersulfate (APS) solution and tetramethylethylenediamine (TEMED) to the monomer solution.
- APS acts as the initiator, and TEMED is the catalyst.
- The typical concentrations are 0.1-0.2% for APS and 0.05-0.1% for TEMED

Polymerization

- Immediately transfer the monomer solution into the gel casting apparatus, ensuring that no air bubbles are trapped.
- Insert a comb to create wells for sample loading. Allow the gel to polymerize.

Gel Running Buffer:

- Prepare a running buffer using Tris and glycine or Tris and SDS, based on the type of electrophoresis (native or SDS-PAGE).

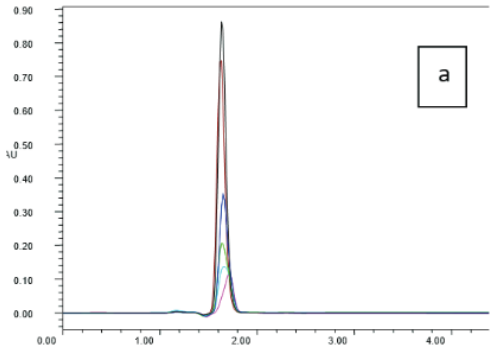
Assembly and Running

- Assemble the gel in the electrophoresis apparatus, fill the chamber with running buffer, and load your samples into the wells.
- Run the gel at the appropriate voltage and time form specific experiment.

Optimized Conditions for Estimation Ofacrylamide by RP-HPLC

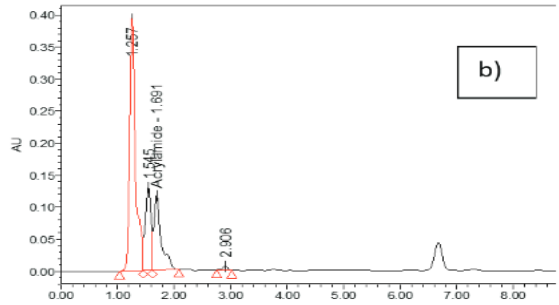
Mobile phase-Aceto nitrile: Methanol (40-60v/v)
Flow-Rate-1ml/min
Column-Thermo scientific model C_{18}column (4.6mm.i.d.x250mm:5mm particle size) Based on 99.99%ultra-high purity silica
Column temperature: 25^{0}c
Run time: 12min.
Column Type: Rp column C18 bonded phase.
Mobile Phase: Water: Acetonitrile 50:50
Detection Method: UV detection

Standard Graph of Acrylamide

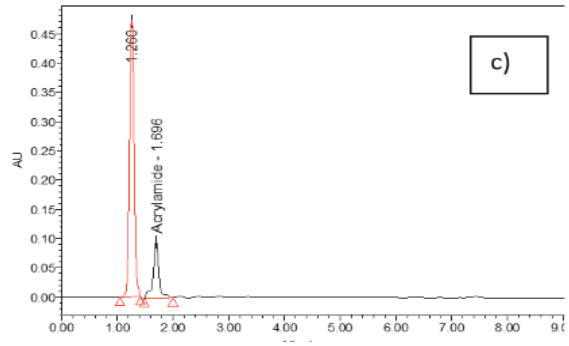


a) Acrylamide standards

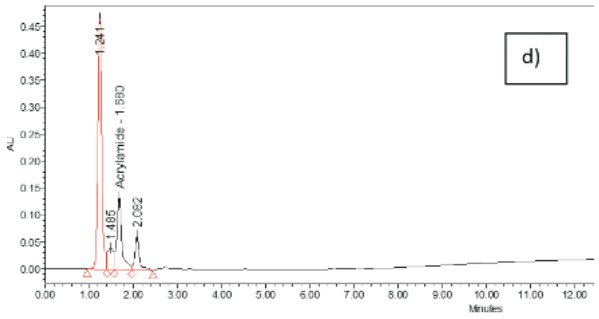
Sample Graphs:



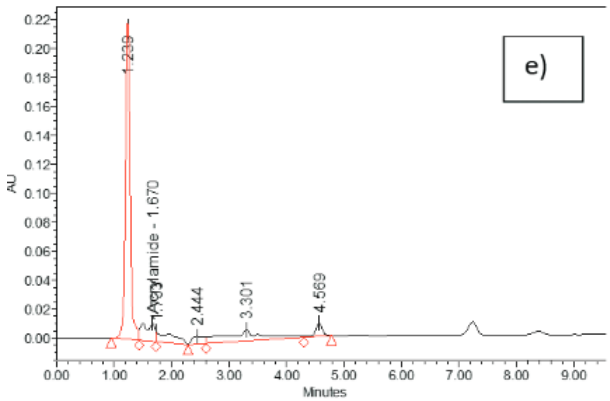
b) Kurkure Hyderabadi



c) Potato chips lays



d) Kajubadam biscuits



e) Honey loops (Kellogg's)

Future Directions

- To reduce the acrylamide in foods they must maintain the neutral pH with a restricted amount of reducing sugar content but rich in sucrose and free amino acids should be maintained [16]. **Eg:-**Four types of edible nuts and seed were roasted at 160,180,and 200^{0}c for 5 to 60min to represent these dry systems.The changes in the concentration of reactants and products of acrylamide formation were noticed during roasting.
- At present scenario, some of the people are using the magnetic12 dummy molecularly imprinted nanoparticles for the pre-concentration of acrylamide from potato chips and it could be the future alternative for the depletion of acrylamide in food stuffs.
- To reduce the exposure of acrylamide avoid eating a lot of carbohydrate-rich foods **E.g.:-** French fries
- For keeping the acrylamide levels to a minimum filter, change oils and clean cooking equipment as often as needed suppliers.
- Immediately stop the reusing old, dirty oil and cooking equipment that will increase the levels of acrylamide in deep-fried foods.
- While baking bread and sweet and savoury bakery products should be cook to a golden yellow or lighter colour.
- To reduce the acrylamide in potatoes store the potato tubers at temperature not less than 8-12c.Avoidance of long frying and baking times. Use the browning of chips as an indicator of DONESS of the frying process.

Conclusion

Acrylamide[15]is a carcinogenic and processed foodand seems to be produced in the processed food due to the chemical reaction that is initiated during high temperature. Cooking theprocessed food items in the absence of acrylamide will be extremely beneficial in the health aspects of the public by and large. The use of Bacterial L-Asparginase (LA) is one of the possibleapproaches for acrylamide depletion in food stuffs. As it catalyses the transformation (LA) to L-Aspartic acid and ammonia.

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Conflict of Interest

There is no conflict of interest

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Ethical Considerations

Not required

Infamed Consideration

Not required

Author Contribution

All authors are contributed equally

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