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-aspariginase (LA) is one of the approaches for acrylamide depletion in food stuffs. Presently, the cancer risk in the overall population has not yet had adequate 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 fabricated in edibles have set off a very significant wellbeing issue. The cut back of acrylamide in fried potatoes was determined by HPLC (High performance liquid chromatography).

Keywords: Acrylamide, Health Hazard, HPLC, Maillard Reaction, Potatoes, U.V.

Introduction

Structure of Acrylamide

Physico-Chemical Properties of Acrylamide

<table>
<thead>
<tr>
<th>NAMES</th>
<th>PROPERTIES</th>
</tr>
</thead>
<tbody>
<tr>
<td>IUPAC</td>
<td>Acrylamide</td>
</tr>
<tr>
<td>CAS Number</td>
<td>79-06-1</td>
</tr>
<tr>
<td>MolecularFormula</td>
<td>C3H4NO</td>
</tr>
<tr>
<td>Molecular weight[g/mol]</td>
<td>71.078</td>
</tr>
<tr>
<td>Properties</td>
<td>White, crystalline solid at Room temperature.</td>
</tr>
<tr>
<td>Density 30°C</td>
<td>1.127g/cm³</td>
</tr>
<tr>
<td>Melting point</td>
<td>84-84.5°C</td>
</tr>
<tr>
<td>Boiling point</td>
<td>125°C at 3.3 pa</td>
</tr>
</tbody>
</table>

Vapour pressure 0.9 pa at 25°C

log pow -0.67 to 1.67

Solubility in water 2.155 g/1 at 30°C

MAK commission classification Skin absorption

Carcinogen category

Germ cell mutagen

E.g.: - The utilize of Bacterial L-Aspariginase (LA) is one of the access for acrylamide cut-off in nourishment. As it catalyses the change (LA) to L-Aspartic acid and ammonia, The maximum Aspariginase 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 Union also 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 deriving 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 embolent the 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]

<table>
<thead>
<tr>
<th>PRODUCT</th>
<th>INVESTIGATED SAMPLES</th>
<th>MEDIUM</th>
<th>RANGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Potato chips</td>
<td>221</td>
<td>750</td>
<td>130-3680</td>
</tr>
<tr>
<td>French fries, cooked</td>
<td>54</td>
<td>250</td>
<td>20-3920</td>
</tr>
<tr>
<td>Potato sticks</td>
<td>26</td>
<td>1430</td>
<td>630-2870</td>
</tr>
<tr>
<td>Fried potatoes, cooked</td>
<td>6</td>
<td>240</td>
<td>n.n-280</td>
</tr>
<tr>
<td>Cracker bread</td>
<td>95</td>
<td>170</td>
<td>n.n-2840</td>
</tr>
<tr>
<td>Bread</td>
<td>52</td>
<td>&lt;30</td>
<td>n.n-200</td>
</tr>
<tr>
<td>Bread rolls</td>
<td>12</td>
<td>&lt;30</td>
<td>n.n-140</td>
</tr>
<tr>
<td>Breakfast cereals</td>
<td>39</td>
<td>50</td>
<td>n.n-640</td>
</tr>
<tr>
<td>Corn flakes</td>
<td>9</td>
<td>170</td>
<td>20-640</td>
</tr>
<tr>
<td>Butter cookies</td>
<td>8</td>
<td>300</td>
<td>140-1090</td>
</tr>
<tr>
<td>Ginger bread</td>
<td>17</td>
<td>350</td>
<td>130-890</td>
</tr>
<tr>
<td>Pretzel sticks</td>
<td>7</td>
<td>250</td>
<td>110-360</td>
</tr>
<tr>
<td>Powdered coffee</td>
<td>35</td>
<td>280</td>
<td>180-290</td>
</tr>
</tbody>
</table>
At moderate conditions for magnetic solid-phase extraction (MSPE) of acrylamide in potato samples proceeded by high performance liquid chromatography (HPLC). The quantification was done by HPLC with UV detection (HPLC-UV). In the current status, the cancer risk in the general population has not yet had a satisfactory result. By using a relative cancer risk model, they had an improvement of the cancer risk estimated for dietary acrylamide this can be achieved by estimation of the genotoxic contribution to the risk.

**Methodology**

**Preparation of Acrylamide**

- **Sample Preparation**: Acrylamide powder
- **Standard Preparation**: N,N’-Methylenebisacrylamide (bisacrylamide) powder
- **Materials**: Acrylamide powder, N,N’-Methylenebisacrylamide, Tris buffer, Distilled water, Ammonium persulfate (APS) solution, Tetramethylethylenediamine (TEMED), Sodium hydroxide, Methylenebisacrylamide (bisacrylamide)
- **Procedure**: Take 1g of sample, add 0.1ml carrez 1, 0.1ml carrez 2, and 9.8ml of 0.2mM acetic acid solutions.
- **Safety Precautions**: Acrylamide is toxic, and its residues or solutions can cause serious side effects. Always wear protective personal protective garments. Including gloves and safety goggles, and work in a well-ventilated area.
- **Assembly and Running**: 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.
- **Preparation of Tris Buffer**: The ratio of acrylamide to bisacrylamide may differ depending on the desired gel characteristics.

**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**

- **Add** ammonium persulfate (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).

**Optimized Conditions for Estimation Of Acrylamide by RP-HPLC**

- **Mobile phase**: Acetonitrile: Methanol (40:60 v/v)
- **Flow Rate**: 1ml/min
- **Column**: Thermo scientific model C18 column (4.6mm i.d. × 250mm: 5mm particle size) Based on 99.99% ultra-high purity silica
- **Column temperature**: 25°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**

![Standard Graph of Acrylamide](image)
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]. E.g.: Four types of edible nuts and seed were roasted at 160,180, and 200°C for 5 to 60 min 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 magnetic 12 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 8-12°C Avoidance of long frying and baking times. Use the browning of chips as an indicator of DONESS of the frying process.

Conclusion

Acrylamide is a carcinogenic and processed food and seems to be produced in the processed food due to the chemical reaction that is initiated during high temperature. Cooking the processed 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-Asparaginase (LA) is one of the possible approaches 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

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Not required

Author Contribution
All authors are contributed equally

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