India Food Safety Conclave 2025

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Acryl-off ®: A food processing aid for dietary acrylamide remediation in starchy foods

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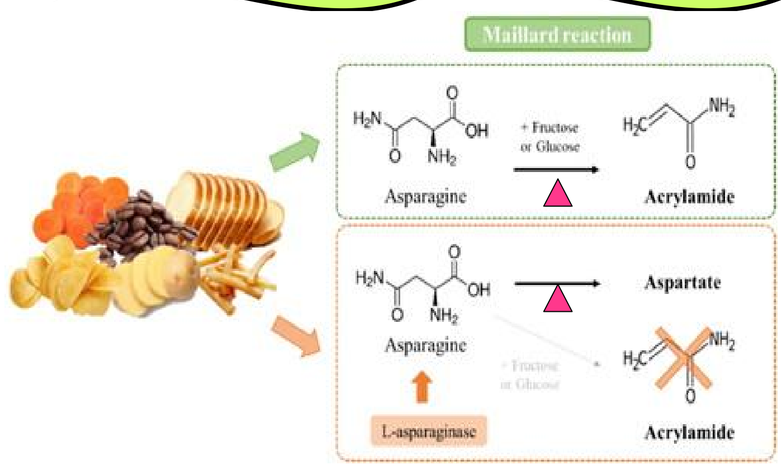
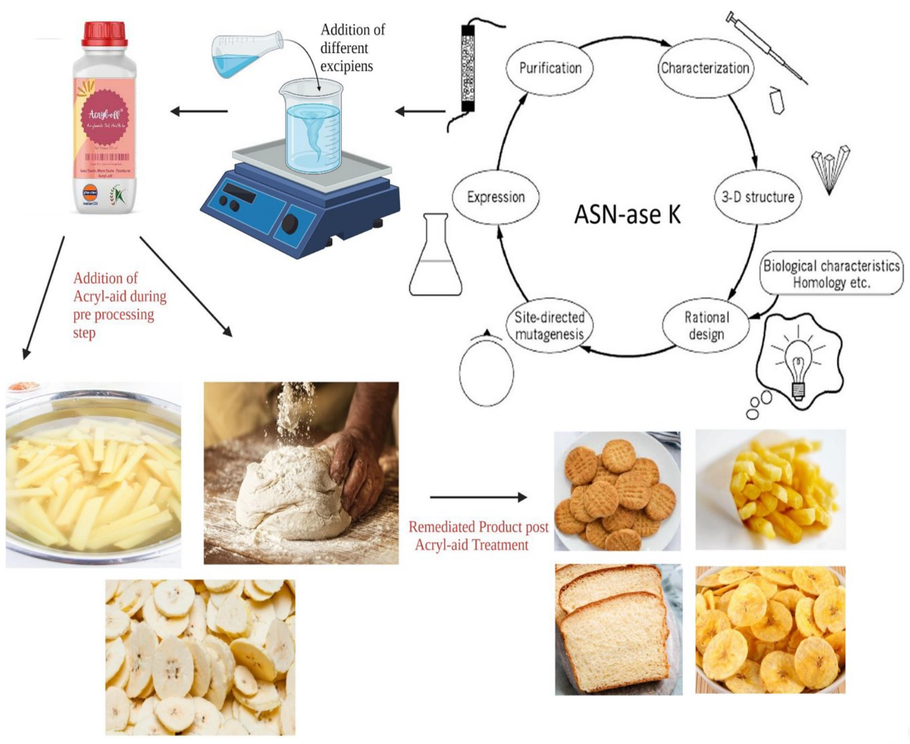
INTRODUCTION

- **Food Safety Concern: Acrylamide,** a toxic by-product of the Maillard reaction, forms during high-temperature food processing.
- **Solution:** Acryl-off — a food-grade, protein-engineered *E. coli* L-asparaginase — hydrolyzes L-asparagine into aspartic acid and ammonia, reducing acrylamide formation.
- **Protein Engineering:** Cloned, overexpressed, and purified in *E. coli*. High yield: 7000–7500 U/L with enhanced thermal and pH stability. Liquid formulation developed for practical use.
- **Application:** Tested on fries, banana chips, and bakery products. At 0.8 U/gram, achieved >75% acrylamide reduction during blanching.
- **Stability:** Stable for 90+ days at 4°C.
- **Safety:** Pre-clinical studies confirmed compliance with OECD and GHS guidelines.
- **Impact:** Safe, cost-effective, and sustainable solution for reducing acrylamide and ensuring healthier food production.

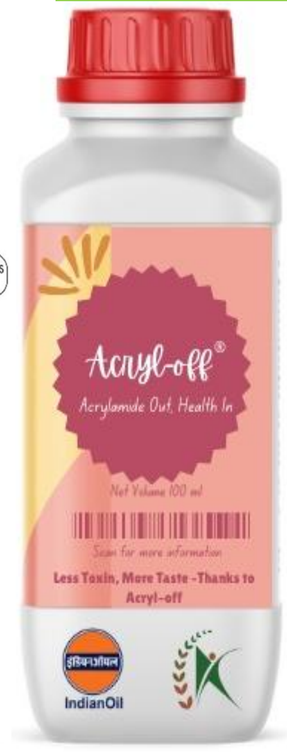
SCIENTIFIC RATIONALE



Process Overview - Scientific Rationale



Product - Acryl-off ®



What is Acryl-off ® ?

Composition
- *ASN-ase K enzyme*
- Acidity Regulators
- Anti-oxidants
- Stabilizers
- Others

Liquid Formulation Stable > 90 days at 4°C

Application: Potato Wafers, Potato chips, French Fries, Banana Chips and Potato Crisps

Novelty- Acryl-off ®

High Enzymatic activity of Acryl-off leading to lower dosage for ALARA acrylamide reduction

Parameters	EcAsa-WT	EcAsa-DM (ASN-ase K)
Km (mM)	0.5 ± 0.10	0.085 ± 0.015
Vmax	22.47 ±2.2	16.22±1.8
Min. detectable [asparagine] mM	0.4mM-0.6mM	0.07mM-0.1mM

Application	Acrylaway ® (Novozymes)	Acryl-off ®	PreventASe L ® (DSM)
Dosage	10U/ml	0.8U/gm	25 U/ml
Acrylamide Reduction %	<60%	>75%	>59%
Cost per kg of food matrix	0.32USD= Rs 27.14	0.09 USD= Rs 7.63	0.5 USD= Rs 42.41

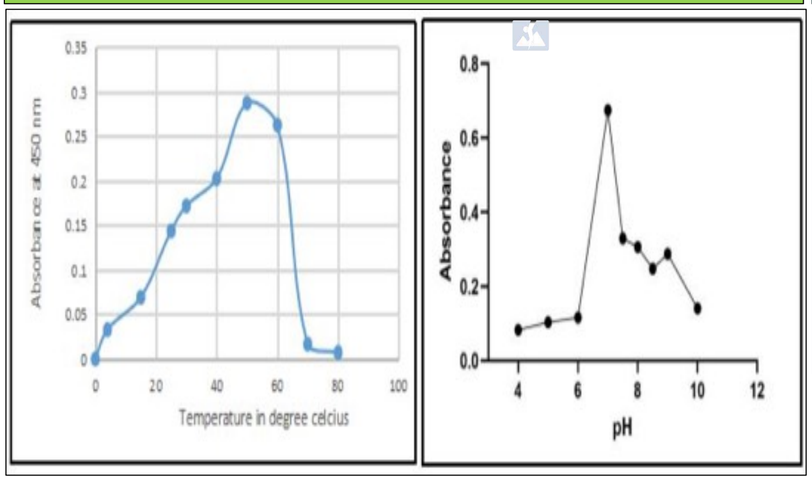
Production & Purification of ASNase K

Batch	OD at 600nm	Biomass ®
5L Chemically Defined Media	30.1	86.5 ± 2.9gm
10L Chemically Defined Media	49.9	310.8 ± 7.0 gm

Purification Scheme:
E.coli Cell Lysate ➡ Ni-NTa Affinity Chromatography ➡ Size Exclusion Chromatography ➡ Acryl-off Formulation

Category	Avg. Wet Weight (g)	Avg. Total Purified Protein (mg)	Avg. Specific Activity (Purified Protein) (U/mg)	Yield
14 L Bioreactor (ASN-ase K)	318.9	114.4	214.5	32 ± 2 mg/L
7.5 L Bioreactor (ASN-ase K)	82.9	75.0	233.9	

Temperature & pH Stability study



Temperature & pH range optimization study of ASN-ase K

The data shows a high range of temperature and pH of ASNase K for optimal usage in a wide range of food processing applications

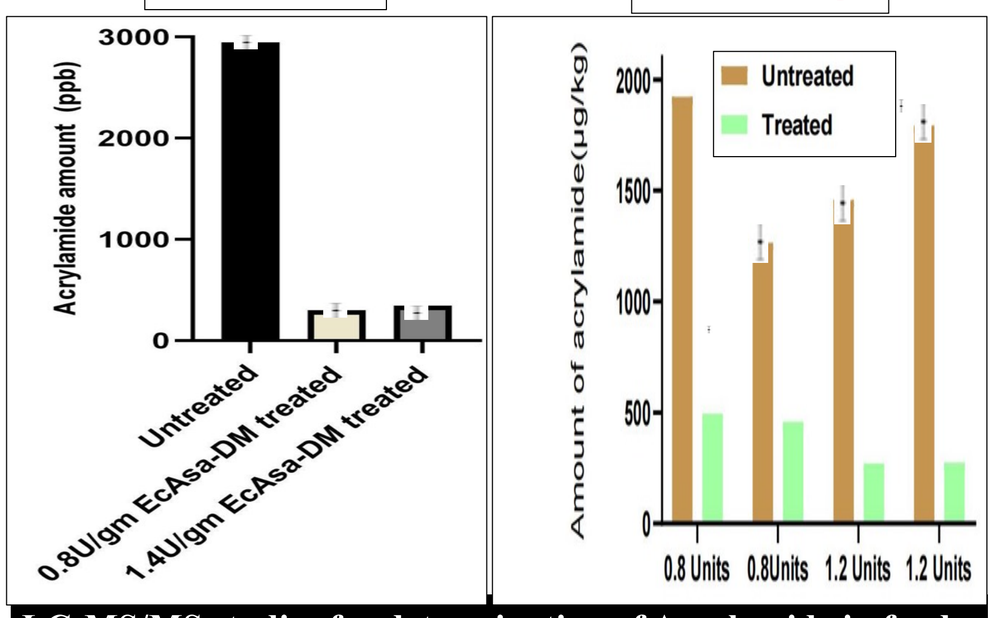
Safety and Efficacy : Acryl-off ®

- **Residual Enzyme:** ***Inactive*** and **digested like dietary protein**.
- **Production Organism**: **GRAS** category; **absent** in the final enzyme preparation.
- **Toxicological Findings**: **No toxicity or mortality**; no increase in **IgE/IgG antibody** titres at 125 IU/mice.
- **Oral Toxicity**: NOAEL at **1000 mg/kg body** weight/day after **28 days** of **repeated oral administration** in Sprague Dawley rats.
- **Pathological Findings**: No gross or **histopathological effects** related to Acryl-off.
- **Conclusion**: Acryl-off is **safe** with no measurable toxicological impact, validated as per **OECD, AAALAC,** and **CPSEA guidelines**

Acrylamide Reduction using Acryl-off ®

Untreated (+) Acryl-off | Untreated (+) Acryl-off

Potato Fries | Banana Chips



LC-MS/MS studies for determination of Acrylamide in foods: Acryl-off treatment resulting in >75% acrylamide reduction without change in its organoleptic properties (Texture and flavor).

Summary

1. **Ease of production**, **affordable** one stop solution with **high specific activity**
2. **Minimal usage (U/kg)** during food processing to ensure acrylamide mitigation content (ALARA- As low as reasonably achievable)
3. Patented processing aid product which ***eliminates the use of harmful additives*** in processed food.
4. **No change** in existing industrial usage step and organoleptic properties of food post remediation.
5. **No existing commercial product** available in India & best **cost per kg** economics for industrial application

Intellectual Property

Patent - "*An enzyme and formulation thereof for reducing formation of acrylamide in food processing*" with application no filed 202231025075 at Indian Patent Office, Kolkata

Trademarks :**Trademarks Filed "Acryl-off" & "Acryl-kill"** :5487997 & 5487998

Acknowledgments

KIIT Technology Business Incubator – KIIT-TBI; Department of BioTechnology, Government of India; IndianOil; birac Ignite Innovate Incubate; MSME Ministry of Micro, Small & Medium Enterprises