



Technology Match Maker | Sustainable Ingredients for Functional Foods & Additives | Oct 2023

Novel Enzyme And Process For Biosynthesis Of D-Allulose



Lead Scientist: Dr Sudhir Singh

Organization: DBT-CIAB

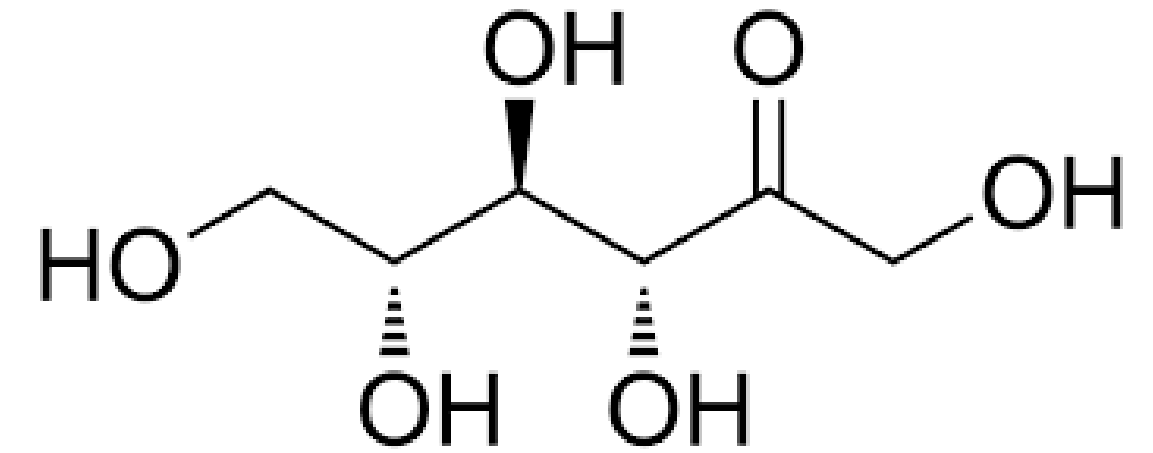
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D-Allulose

- D-Allulose or D-psicose (D-ribo-2-hexulose) is a naturally occurring rare sugar found in many fruits and grains.
- **Sensory appeal** - It is a low calorie sweetener with taste and texture comparable to table sugar.
- **Health advantage** - D-Allulose does not raise insulin levels, contains only 1/10th calories of table sugar and does not promote tooth decay,
- **Safety Aspects**- Not metabolized by the body, D-Allulose is a safe and non-carcinogenic sweetener.
- **Market demand** - With growing popularity of D-Allulose as sweetener in various food and beverages, the USFDA has reviewed and updated the nutrition label rule for D-Allulose from earlier status of carbohydrates to only 0.4 kCal/g of the sweetener.



Market Opportunity

The global market for D-Allulose exceeded USD 90M in 2022 and is anticipated to witness a steep growth of 14.8% CAGR, reaching USD 370M by 2032. [1]

Key Market Drivers are:

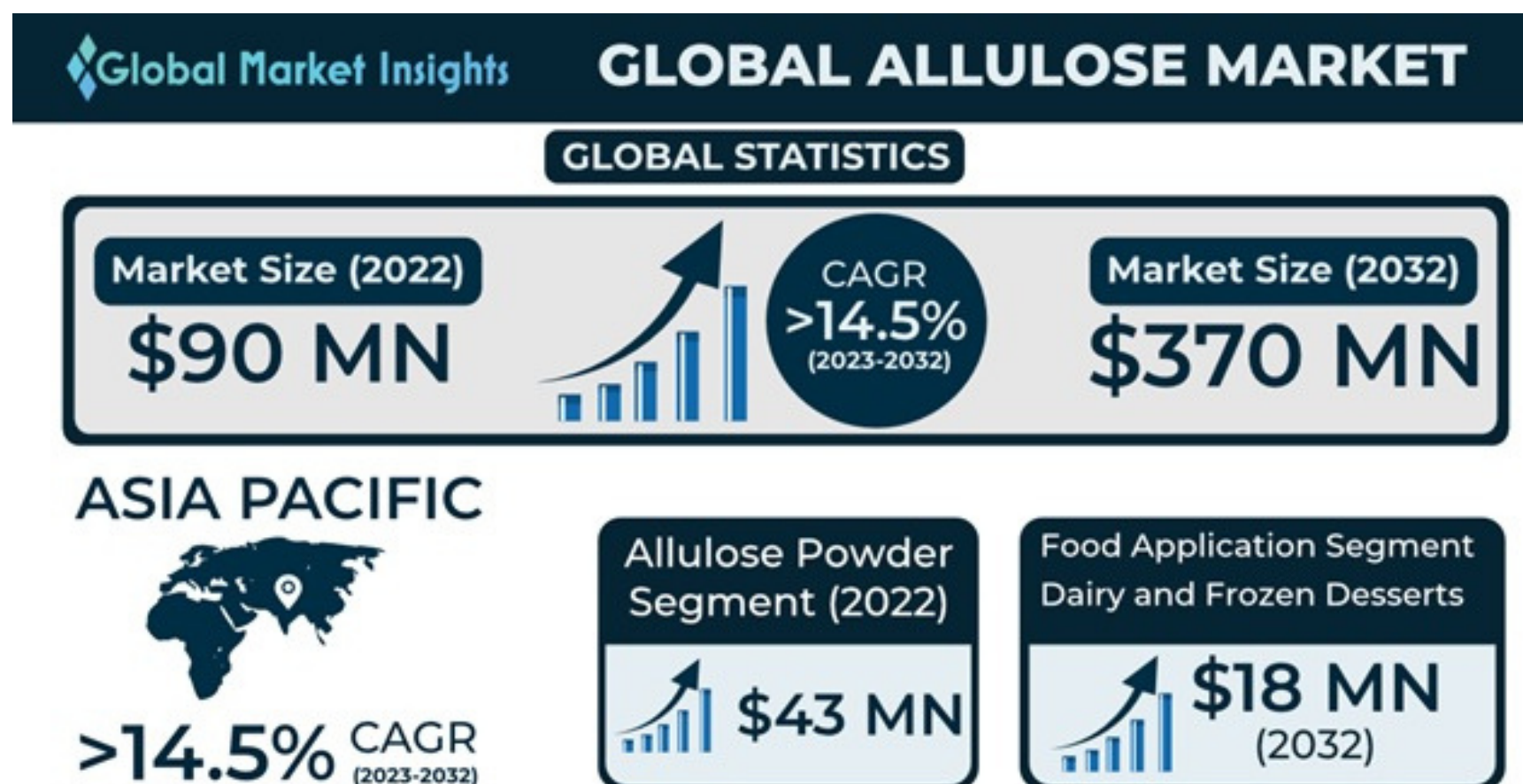
- Growing trend in use of natural sweeteners.
- Demand for healthy and low-calorie diet due to increasing health awareness and chronic diseases.
- Rising awareness for clean label products with natural ingredients.

Market leaders: Cargil, Tate & Lyle, Matsutani, Daesang, Anderson global, Ingredion etc.

Challenges in bioprocess: Optimising yield and cost of product.

Current cost: Rs. 800-1000/ kg [2].

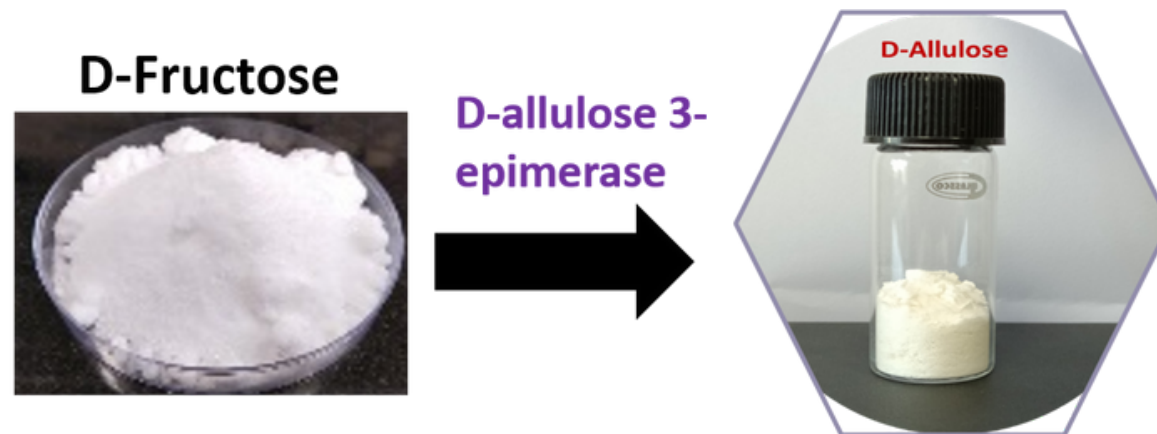
Applications: Food industry to lead the market with growing adoption in Dairy & Frozen Desserts. Other industries includes beverages such as energy drinks, natural juices etc.



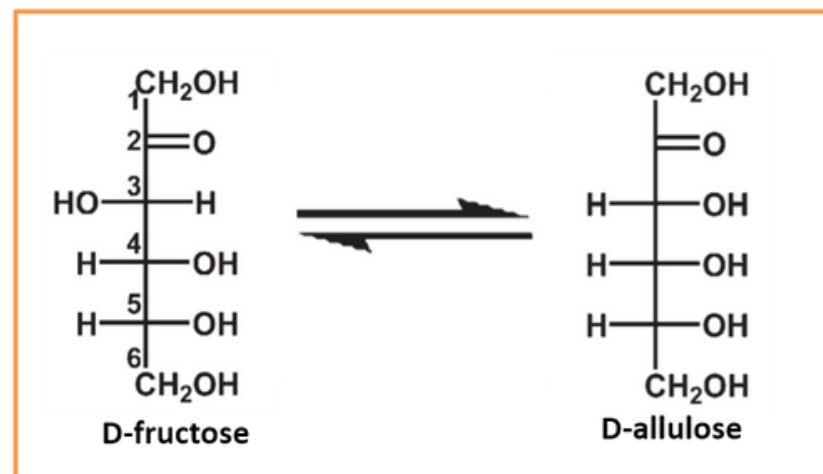
Who Should Be Interested?

Who?	Why?
Functional foods and nutraceuticals companies	<ul style="list-style-type: none">• New range of products• Novel products for niche markets
Manufacturers of food ingredients	<ul style="list-style-type: none">• New product addition in portfolio
Sugar and Rare sugars manufacturers	<ul style="list-style-type: none">• Higher value products from sugar building blocks• New product in portfolio
Manufacturers of bio-synthesized value added chemicals	<ul style="list-style-type: none">• New products and forays into new markets• Opportunity to disrupt markets & displace incumbents
Industrial enzyme companies	<ul style="list-style-type: none">• New products addition in portfolio

About the Technology



The first report on D-allulose from India



Enzyme variants of CIAB

Enzyme sources	Optimum pH range	Optimum temperature range (°C)	Half- life (minutes)
Smt3-d-allulose 3-epimerase (SAE)	6-9	55-65	943 (50 °C) 260 (55 °C)
DaeM	5-10	70-85	13860 (60°C), 2700 (70°C), 150 (80°C)
Bacillus sp.(DaeB)	6-10	45-65	36000 (50°C), 1320 (55°C)

Process features:

- **Novel bioprocess for D-allulose using novel enzymes**
 - **Novel Enzymes (D-allulose 3 epimerase) - thermally stable with excessive half-life (160 hrs at 60 deg C) and longer shelf life.**
 - **Process optimised for high yield:**
 - **30% conversion of D-fructose to D-Allulose.**
 - **Working temp range (50-70 deg C) ☒ For faster reaction and avoid contamination.**
 - **Working pH range (slightly acidic) ☒ reduce browning and minimal by-products.**

Product features:

- **Quality: High quality, pure white coloured D-Allulose powder with no browning and minimal by-products.**
- **Conversion Yield: 30%**
- **Estimated Cost: Techno-economical analysis to be performed.**

D-Allulose As Low Calorie Sweetener

- **D-Allulose enhances storage stability of food products.**
 - Increases water-holding capacity of gelling food materials.
 - Improves texture and mouthfeel.
- **The daily recommended intake level of D-allulose is 0.9 g/kg of body weight.**
 - For an average indian adult, daily sugar consumption was calculated to be 49 g [1].
 - Calories from 49 g of sugar as sweetener ~ 196 kCal.
 - Replacing this sugar with same quantity of D-Allulose, adds only 17.64 kCal.
- **D-Allulose has many physiological benefits including:**
 - Synergistic activity with probiotic microorganisms in decreasing adiposity.
 - D-Allulose has good gastrointestinal tolerance.



49 g Sugar => 196 kCal



49 g D-Allulose => 17.64 kCal

Current Status

Technology Status:

- Demonstrated at lab-scale 5L fermenter.

IP Status: Patent filed and granted in India.

- Priority date: 21st June 2018
- Coverage: IN
- Granted Patent No. IN353054
- Patent filed: 202311012386; 202011018495

Publications:

- Patel et al, Improved operational stability of D-psicose 3-epimerase by a novel protein engineering strategy and D-psicose production from fruit and vegetable residues, *Bioresour. Technol.* 216 (2016), 121–127.
- Patel et al, Development of a thermo-stable and recyclable magnetic nanobiocatalyst for bioprocessing of fruit processing residues and D-allulose synthesis, *Bioresour. Technol.* 247 (2018), 633–639.
- Patel et al, A novel D-Allulose 3-epimerase gene from the metagenome of a thermal aquatic habitat and D-Allulose production by *Bacillus subtilis* whole-cell catalysis, *Applied and Environmental Microbiology* 86 (05), e02605–19. (IF 4.016) (2020).
- Patel et al, D-allulose 3-epimerase of *Bacillus* sp. origin manifests profuse heat-stability and noteworthy potential of D-fructose epimerization, *Microbial Cell Factories* 20(60), 1–16 (IF 4.187) (2021).



Team & Organisation



Lead Scientist:

Dr Sudhir P Singh, FNAAS

- **Scientist D at DBT – CIAB.**
- **Honors and Awards:** Fellow of the National Academy of Agricultural Sciences (FNAAS); Member of NASI; HIRALAL Chakravarty Award (DST); Young Scientist Award, IBA (France); SBS–Madurai Kamraj Univ Genomics Award.
- **Expertise:** Gene mining, biocatalyst engineering, bioprocessing, bioproduct generation and enzyme characterization

CIAB – Center of Innovative and Applied Bioprocessing, Mohali, is an autonomous institute of DBT, India, mainly focused in research work related to secondary Agriculture and development of value added products from different types of bio resources.

Key assets and strengths of the team:

- 10 total patents filed, 5 granted in India; 50+ publications in bioprocess, enzyme characterization and functional biomolecules.
- **Team Strength:** 6
- **Well equipped labs and analytical facilities:**
 - 5 L fermenter
 - Enzyme characterization assays
 - Protein Purification system
 - Membrane separation unit
 - HPLC and Gas chromatography
- **Industry projects/ Tech transfer:** Good track record of technology transfer and working with industries.

Next Steps

- Team has developed the background science, invented new enzyme and demonstrated proof-of-concept at lab-scale.
- Next phase includes further process development:
 - Scale-up for synthesis of enzyme
 - Scale-up for synthesis of D-allulose and downstream processing
- Optimize for quality and cost of final product as per industry requirements

Seeking:

- **Industrial partners interested in technology licensing.**
- **Industrial partners interested in sponsoring further technology advancement and scale up.**
- **Industrial partners interested in raising 3rd party funds for a collaborative project.**
- **Industry interested in tapping scientist capabilities as an expert/consultant.**



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References

- Slide 1 - <https://www.12taste.com/product/anderson-advanced-ingredients-allsweet-allulose-crystalline-powder/>
- Slide 2 - <https://www.fda.gov/media/123342/download?attachment>
- Slide 2 - <https://foodinsight.org/allulose-labeling>
- Slide 3 - [1] <https://www.gminsights.com/industry-analysis/allulose-market>
- Slide 3 - [2] www.indiamart.com
- Slide 6 - [1] <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4389505/>