

# Technical Brief

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## Bio-manufacturing of low-calorie sweetener D-allulose using novel enzymes

### Technology Summary

An enzymatic process for production of D-allulose, a zero calorie functional sugar, with anti-diabetic and anti-obesity potential has been developed. Novel enzymes with high thermal stability and good turnover number have been discovered for the biosynthesis of D-allulose.

### Background

d-Allulose, a C-3 epimer of d-fructose, is a noncaloric sugar substitute with antidiabetes and antiobesity potential. It has 70% of the relative sweetness of sucrose, with almost zero caloric value. With several characteristics of physiological significance, d-allulose has wide-ranging applications in the food and pharmacology industries. However, it is found in rare abundance in nature. Although the chemical synthesis of d-allulose is possible, low yields, byproduct formation, and poor thermostability of the enzyme limit its industrial suitability. In recent years, there have been efforts to establish a sustainable bioprocess based on the enzymatic and microbiological synthesis of d-allulose from d-fructose as feedstock.

### Technology Description

A novel d-allulose 3-epimerase gene (daeM) was identified from the metagenomic resource of a hot-water reservoir. The protein was expressed and purified using standard protocols. The enzyme was then analyzed further for its kinetics, stability, substrate specificity, and its catalytic activity for the production of d-allulose from d-fructose. The scientists also carried out whole cell catalysis with d-fructose, which resulted in a bioconversion yield of 30%. A variety of d-fructose-containing samples, such as apple juice, mixed fruit juice, and honey, were also subjected to enzymatic treatment, resulting in a d-allulose conversion yield in the range of 26% to 32%.

### Market Potential

The global allulose market value is estimated to reach ~US\$ 210 Mn by the end of 2020. According to the report, the allulose market is anticipated to reach ~US\$ 450 Mn by 2030, at a CAGR of ~8%.

### Value Proposition

- Enormous potential of the biocatalyst for the cost-effective large-scale production of D-allulose
- Highly stable novel enzyme with high half life (160 h at 60°C compared to 6 h at 60°C for currently reported maximum thermostability).
- Working temperature in the range of 75°C to 80°C, which confers several advantages, such as minimal contamination, enhanced substrate and product solubility, and higher reactivity with higher process yield
- Working pH range of 6-11 which causes reduced browning
- Whole cell catalysis with 30% bioconversion eliminates the need for protein extraction and purification. whole recombinant cells were able to biosynthesize d-allulose in apple juice, mixed fruit juice, and honey.

### Applications

Dairy Industry (ice-creams, yoghurts), Food industry: Protein bars, baked goods, candies, cereals, salad dressings, Beverages: Sports supplements, juices, aerated drinks, Therapeutic food

### Technology Status

- Demonstrated at lab scale using a 5 L fermenter
- Patent protected
- Seeking interested industry partners

### References

<https://www.marketwatch.com/press-release/allulose-market-global-industry-analysis-size-share-growth-trends-and-forecast-2021-07-13>

<https://journals.asm.org/doi/10.1128/AEM.02605-19>

