OPTIMIZATION OF THE ENZYMATIC PRODUCTION OF THE LOW-CALORIE BULK SWEETENER D-TAGATOSE

EFBT/LFoRCe

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INTRODUCTION

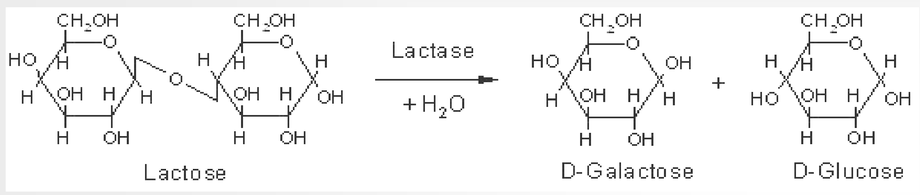
D-tagatose is a sweet-tasting monosaccharide with interesting nutritional and physiological properties. Due to the complexity, low yields and environmental implications, the chemical production of D-tagatose was stopped in 2006. This research is aimed at evaluating the economic feasibility of the enzymatic production of D-tagatose from whey permeate (lactose). In a first step, lactose in whey permeate is hydrolyzed to a mixture of D-glucose and D-galactose. Secondly, D-galactose is isomerized to D-tagatose by means of an L-arabinose isomerase from the thermophillic organism *Geobacillus stearothermophilus*. The idea of producing commercially tagatose through galactose isomerization is based on commercial production of high fructose corn syrup (HFCS).

BENEFITS

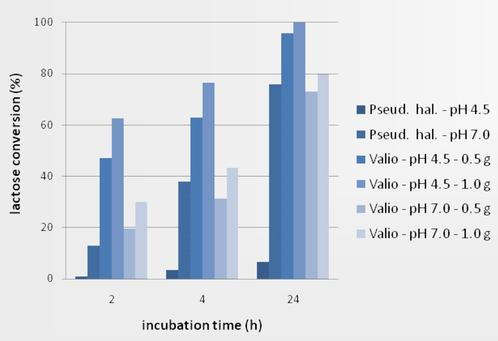
- → *Prebiotic activity*
- → *Low glycaemic impact*
- → *Reduced energy value*
- → *Non-cariogenicity*

ENZYMATIC HYDROLYSIS OF LACTOSE

In order to produce tagatose from whey permeate, lactose needs to be enzymatically hydrolyzed with a β-galactosidase to a mixture of D-glucose and D-galactose. Afterwards, the produced galactose is converted to tagatose. For galactose production from lactose, the β-galactosidase from the psychrophillic organism *Pseudoalteromonas haloplanktis* (CIP, Liège) is studied and compared to the immobilized Valio IML enzyme (Valio, Finland).

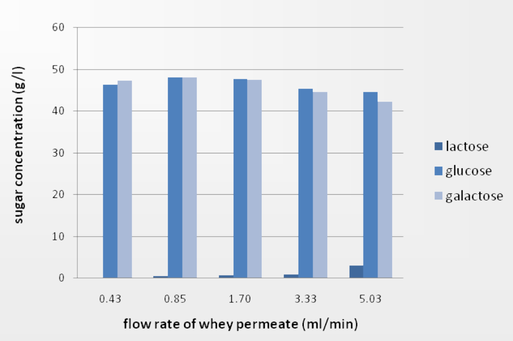


Lactose conversion in whey permeate is visualized for both enzymes at pH 4.5 and pH 7.0 as a function of incubation time at 25°C.



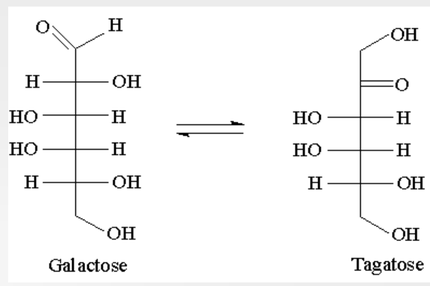
With the lactase from *Pseudoalteromonas haloplanktis*, a higher hydrolysis degree is observed at pH 7.0 compared to pH 4.5; in contrast to Valio IML, which shows a better hydrolysis at pH 4.5. With 1.0 g of Valio IML enzyme, complete lactose conversion is obtained within 24 hours. In addition, optimal incubation temperature is determined for Valio IML.

Hydrolyses are performed at different temperatures at pH 4.0. With 1.0 g enzyme, lactose conversion is already 95.6 % and 97.5 % at respectively 9°C and 15°C. 25°C results in the highest degree of lactose conversion (approximately 100 %) with the immobilized Valio enzyme as well for 0.5 g as for 1.0 g enzyme. Finally, experiments are performed in a packed bed reactor at 25°C as visualized below. Optimal flow rate of whey permeate is determined with 400 ml enzyme. Above a flow rate of 1.7 ml/min, lactose conversion starts to decline.

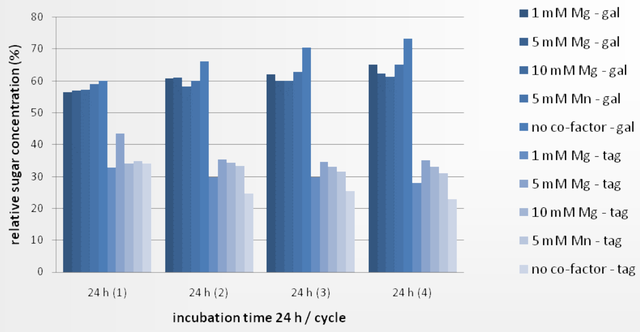


ENZYMATIC ISOMERIZATION OF D-GALACTOSE TO D-TAGATOSE

After lactose hydrolysis, an equimolar mixture of D-glucose and D-galactose is obtained. During subsequent enzymatic isomerization with an L-arabinose isomerase, D-galactose is converted to D-tagatose. L-arabinose isomerases fall within the general class of intramolecular oxidoreductases and more specifically, the group of aldose isomerases, which are capable of interconverting aldoses in their corresponding ketoses. L-arabinose isomerases in nature catalyze the isomerization of L-arabinose to L-ribulose. Galactose to tagatose conversion is considered to be a side activity of most arabinose isomerase enzymes.

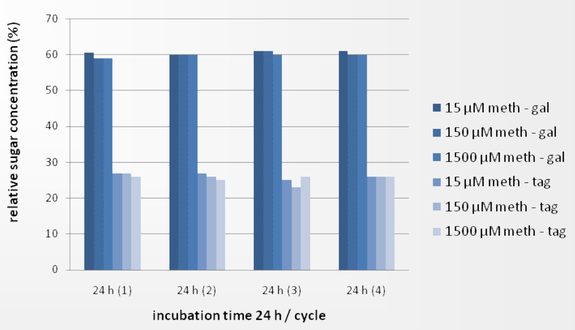


The influence of cofactors Mg^{2+} and Mn^{2+} on stability of the L-arabinose isomerase enzyme from *Geobacillus stearothermophilus* is evaluated. Isomerization tests are performed in a synthetic galactose solution of 200 g/l with/without cofactors as Mg^{2+} or Mn^{2+}. 5.0 g immobilized enzyme is added to 25.0 ml galactose solution at 60°C.



4 cycles of 24 hours are carried out. There is a clear positive influence of cofactors Mg^{2+} and Mn^{2+} on the stability of the L-arabinose isomerase. The best stability is observed in the presence of 5 mM Mg^{2+}. Approximately 34% tagatose is formed after 24 hours.

The loss of enzymatic activity of the immobilized L-arabinose isomerase can be caused by oxidation reactions. The influence of methionine on L-arabinose isomerase stability is investigated. First incubations with methionine are performed at 53°C in synthetic galactose solutions of 200 g/l with 5 mM Mn^{2+}.



No distinct differences are observed between the presence of 15, 150 and 1500 µM methionine in the sugar solutions at 53°C (5.0 g enzyme + 25 ml galactose solution). Similar results are obtained at a temperature of 60°C.

CONCLUSION

As first natural sugar replacer, tagatose is an innovation in its category. It has been approved by the FDA in the USA as Generally Recognized as Safe (GRAS) in 2001. Today, D-tagatose is already present in a lot of healthy food products, beverages and diet additives. As sugar substitute, tagatose will help to control weight, tooth decay and will be a major help in comfort of diabetics, especially type 2 patients. The results show that D-tagatose can be produced from lactose in a two-steps enzymatic process. Complete lactose hydrolysis is feasible (*Pseudoalteromonas haloplanktis* versus Valio IML). In a second step, galactose is enzymatically isomerized with the thermophillic L-arabinose isomerase from *Geobacillus stearothermophilus.* 34% tagatose is formed during repeated incubation cycles at 60°C in the presence of 5 mM Mg^{2+}.