


Tagatose as a Potential Nutraceutical: Production, Properties, Biological Roles, and Applications

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Abstract: Nutraceuticals are gaining importance owing to their potential applications in numerous sectors including food and feed industries. Among the emerging nutraceuticals, D-tagatose occupies a significant niche because of its low calorific value, antidiabetic property and growth promoting effects on beneficial gut bacteria. As D-tagatose is present in minute quantities in naturally occurring food substances, it is produced mainly by chemical or biological means. Recently, attempts were made for bio-production of D-tagatose using L-arabinose isomerase enzyme to overcome the challenges of chemical process of production. Applications of D-tagatose for maintaining health and wellbeing are increasing due to growing consumer awareness and apprehension against modern therapeutic agents. This review outlines the current status on D-tagatose, particularly its production, properties, biological role, applications, and the future perspectives.

Keywords: antidiabetic, D-tagatose, L-arabinose isomerase, nutraceutical

Introduction

Since ancient time, varieties of foods are being used in different forms to ensure better health. The quest for functional food has lead to the emergence of the novel concept, popularly known as nutraceutical (combination of two words: “nutrition” and “pharmaceutical”). Although, the term nutraceutical was coined by De-Felice L. Stephen in 1989, the philosophy to use food as medicine was introduced as early as 460 to 370 BC by the Greek philosopher Hippocrates through his famous quote “Let food be thy medicine and medicine be thy food.” A biomolecule is termed as nutraceutical if it influences one or more targeted physiological functions beneficially along with prevention of disease (Dobbs & Bell, 2010). Nutraceuticals are gaining importance owing to their potential applications in numerous sectors including food and feed industries. These biomolecules essentially work on the principle of “prevention is better than cure” (Samanta et al., 2015).

Currently, the preference of consumers has undergone a paradigm shift from synthetic compounds to natural bioactive molecules as these are considered to be safe without any adverse effects. In fact, the nutraceuticals are the biomolecules, which are generated through microbial fermentation or obtained from plants. There is an impartial consensus that the onset of many chronic diseases can be prevented with the regular consumption of nutraceuticals.

Following the ban over application of feed antibiotics in “food producing animals” since 2006 by European Union, the livestock

industry is on the crossroad to protect the gastrointestinal tract (Castanon, 2007). Therefore, the animal feed manufacturers are expected to depend more on the nutraceuticals to produce antibiotic-free livestock products. Among the potential nutraceuticals, D-tagatose has attracted global attention because of its low calorific value, prebiotic potentiality, and antidiabetic property in addition to its capability to prevent the lifestyle related diseases. D-Tagatose is grouped under the category of “rare sugars” because of its limited occurrence in the nature (International Society of Rare Sugars, 2011). The U.S. Food and Drug Administration (USFDA, 2010) has categorized D-tagatose in the list of Generally Recognized as Safe (GRAS) substances. The use of D-tagatose in food products is accepted in several countries including European Union, Australia, New Zealand, South Africa, and South Korea (Armstrong, Luecke, & Bell, 2009). This review outlines the current status on D-tagatose, particularly its production, properties, biological role, applications, and the future perspectives.

History of Tagatose Discovery

According to the available literatures on carbohydrate chemistry, D-tagatose was discovered by Lobry de Bruyn & Van Ekenstein in, 1897, while experimenting on the effects of mild alkali on D-galactose. They noticed that aldoses changed into epimeric aldoses in addition to one or more corresponding 2-ketoses. The former was named as D-sorbose/pseudo-tagatose and the additional molecule was named as tagatose. In 1939, Yvonne and his coworkers succeeded in alkaline conversion of D-galactose into a crystalline product and named it as α -D-tagatose (Hudson, Wolfrom, & Cantor, 1952). Later, D-tagatose was isolated from the acid hydrolysate of gum exudates of tropical tree *Sterculia setigera* (Hirst, Hough, & Jones, 1949a). The biochemical oxidation of D-talitol by *Acetobacter suboxydans* also leads to the formation of D-tagatose (Totton & Lardy, 1949). During this period, chromatography was considered as an efficient tool for isolation and identification of biomolecules of diverse nature. Paper and partition chromatography were used for identification and separation of D-tagatose (Hirst, Hough, & Jones, 1949b; Karabinos, 1952). The application of strong heat (121 °C for 8 hr) also resulted into transformation of

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milk lactose into galactose, lactulose, and tagatose (Adachi, 1958). Subsequently, a novel and improved method of chemical synthesis was developed through the formation of different derivatives of tagatose involving the reaction steps of oxidation, reduction, and removal of sulfonic groups followed by acid hydrolysis (Al-Jobore, Guthrie, & Wells, 1971). The discovery of L-arabinose isomerase from *Mycobacterium smegmatis* was documented only during the mid-seventies of twentieth century (Izumori, Yamanaka, & Elbein, 1976). D-Galactose, L-arabinose, L-arabitol, D-fucose, and dulcitol act as inducer for L-arabinose isomerase. Later, the microbial production of D-tagatose was successfully carried out from dulcitol by *Arthrobacter globiformis* (Izumori, Miyoshi, Tokuda, & Yamabe, 1984). In 1988, Spherix Inc. (formally known as Biospheric Inc.) developed D-tagatose as a low caloric sweetener. Gilbert Levin, the Chief Executive Officer of Spherix, identified D-tagatose as a low caloric value sugar during his work with other enantiomers of carbohydrates, mainly L-glucose and L-fructose. In 1992, Pfizer introduced commercial D-tagatose as a low caloric bulk sweetener through joint venture with Biospheric Inc. At the same time, an efficient chemical process of tagatose production from galactose was reported (Beadle, Saunders, & Wajda, 1992). At present, consumer awareness coupled with health consciousness turns the mindset of generation next population toward the biologically produced nutraceuticals. Therefore, current emphasis of tagatose research is primarily focused on biological production as it addresses the issues of consumers' mindset as well as food safety.

Occurrence

The natural occurrence of D-tagatose was first noticed in the gum exudates of *Sterculia setigera* (Hirst et al., 1949a). Later, its presence was detected in the oligosaccharides of lichen belonging to *Rocella* species (Lindberg, 1955). It is also naturally present in trace quantities in various foods such as sterilized powdered milk, hot cocoa, cheese, yogurts, and other dairy products (Kim, 2004). D-Tagatose has potential to replace sugar as its sweetness and taste quality is similar to sucrose. Since its natural occurrence is very low, chemical or biological production of tagatose is imminent to meet the demand.

Production of Tagatose

Tagatose can be produced both by chemical and enzymatic process. Evidently, each process of production has its own merits and demerits.

Chemical Process

The thought process for chemical synthesis of tagatose began during the fag end of 19th century (Lobry de Bruyn & Alberdavan Ekenstein, 1897). Nevertheless, the commercial production by chemical route took place only in 1992 (Beadle et al., 1992). In the chemical process of tagatose production, galactose is used as raw material in a two-step process. The first step is isomerization of D-galactose. In this step, D-galactose is allowed to react with metal hydroxide (calcium hydroxide or a mixture of calcium hydroxide and sodium hydroxide) to form an insoluble D-tagatose complex in the presence of catalyst (calcium chloride or sodium chloride). The complex is stable under alkaline medium. The second step is the neutralization with acid. During the neutralization step, acid reacts with D-tagatose in order to form insoluble salt. Thereafter, D-tagatose is separated from the insoluble salt simply by filtration. Figure 1 outlines the reaction steps for chemical synthesis of D-tagatose. Nevertheless, the chemical process of D-tagatose production suffers from several disadvantages including complex

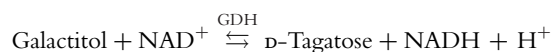
purification steps, chemical waste, and by-product formation. Hence, researchers have attempted for biological production of D-tagatose to address the issues of chemical process.

Biological Process

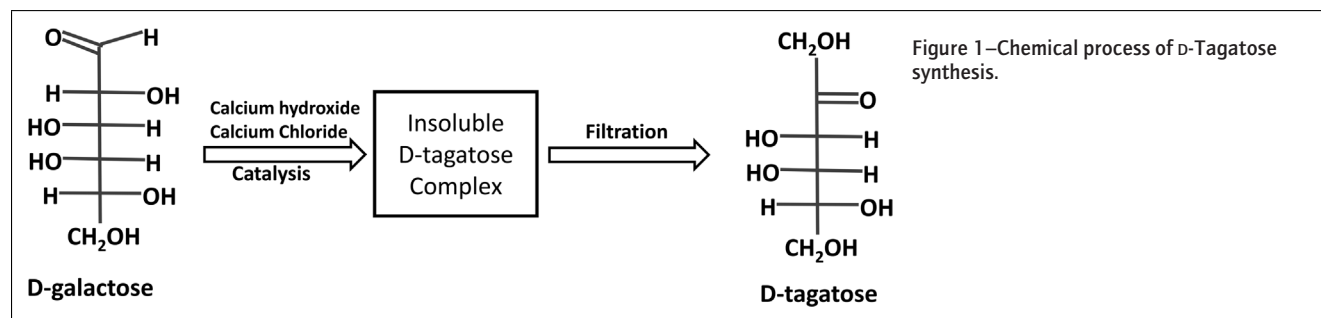
Biological production of tagatose began with the work of Izumori and his group in 1984 by using *Arthrobacter globiformis* (Izumori, Miyoshi, Tokuda, & Yamabe, 1984). In the biological process, two enzymes were explored: galactitol dehydrogenase and L-arabinose isomerase. In this endeavor, several species of fungi of Mucoraceae family were tested for their potentiality to produce tagatose in the growth medium containing psicose. Among those tested strains, highest concentration of D-tagatose (4.18 mg/mL) was recorded in the growth medium of *Rhizopus oryzae* IAM 6002 after 8 days of growth (Yoshihara, Shinohara, Hirotsu, & Izumori, 2006). The biological transformation of D-psicose to D-tagatose by the action Mucoraceae fungi takes place according to the principle of Izumoring process. It is presumed that the formation of D-tagatose involves in two biochemical steps: (i) reduction of D-psicose to D-talitol and (ii) oxidation of D-talitol to D-tagatose (Granstrom, Takata, Tokuda, & Izumori, 2004). However, enhanced yield of tagatose could be obtained by using the bacteria *Enterobacter aerogens* 230S, leading to conversion ratio over 60% from L-psicose (Rao et al., 2008).

Galactitol 2-Dehydrogenase

Galactitol 2-dehydrogenase (GDH; EC 1.1.1.16) belongs to the protein subfamily of short chain dehydrogenase/reductase. It oxidizes the polyvalent alcohols into ketone in the presence of NAD⁺ (Jagtap, Singh, Kang, Zhao, & Lee, 2014). The significance of GDH is growing because of its enantio-selective and stereo-selective oxidative properties commonly used in pharmaceutical industries for rare sugar production. The GDH enzyme utilizes galactitol, also known as dulcitol, as substrate for production of D-tagatose as per the below reaction:



Although, the presence of D-galactitol (dulcitol) dehydrogenase in the culture supernatant of *Pseudomonas* spp. was noticed long back (Shaw, 1956), its uses in tagatose formation is being tested during eighties of the previous century. The microbial production of D-tagatose from dulcitol was first demonstrated using *Arthrobacter globiformis* (Izumori et al., 1984). Biotransformation of D-galactitol to D-tagatose takes place when the cells of *Mycobacterium smegmatis* are allowed to grow in the presence of D-galactitol (Izumori & Tsuzaki, 1988). Later, biological production of tagatose from galactitol has been demonstrated using several other microorganisms such as *Enterobacter agglomerans* (Muniruzzaman, Tokunaga, & Izumori, 1994), *Klebsiella pneumonia* (Shimonishi, Okumura, & Izumori, 1995), *Rhodobacter sphaeroides* (Huwig, Emmel, Jakel, & Giffhorn, 1997) and *Gluconobacter oxydans* (Manzoni, Rollini, & Bergomi, 2001). In due course of time, the GDH enzyme of *Rhizobium leguminosarum* was cloned and expressed in *E. coli* for bioconversion of D-galactitol into D-tagatose (Jagtap et al., 2014). The expressed GDH from *Rhizobium leguminosarum* shows highest specific activity for galactitol compared to any other GDH with a K_{cat}/K_M value of $94.9 \text{ min}^{-1} \text{ mM}^{-1}$. The industrial application of GDH for tagatose production is limited because of the higher cost of starting material D-galactitol. Moreover, the enzyme galactitol dehydrogenase also requires the presence of NAD, an expensive co-factor.

**Table 1—Sources and properties of L-arabinose isomerase.**

Microbial source	pH optima	Temperature optima (°C)	Metal ion requirement	K _{cat} /K _M (mM ⁻¹ min ⁻¹) (D-galactose)	References
Mesophilic					
<i>E. coli</i>	8.0	30	Mn ²⁺	NA	Yoon et al. (2003)
<i>Bacillus halodurans</i>	7.5 to 8.0	50	Mn ²⁺	0.4	Lee et al. (2005a)
<i>Lactobacillus plantarum</i> NC8	7.5	60	Co ²⁺ , Mn ²⁺	1.6	Chouayekh et al. (2007)
<i>Lactobacillus sakei</i> 23 K	5.0 to 7.0	30 to 40	Mg ²⁺ , Mn ²⁺	10.3	Rhimi et al. (2010)
<i>Shewanella</i> sp. ANA-3	5.5 to 6.5	15 to 35	Mn ²⁺	NA	Rhimi et al. (2011a)
<i>Lactobacillus fermentum</i> CGMCC2921	6.5	65	Mn ²⁺	9.0	Xu et al. (2011)
<i>Arthrobacter</i> sp. 22c	5.0 to 9.0	47 to 52	Not Required	NA	Wanarska and Kur (2012)
<i>Bacillus thermoglucosidasius</i>	7.0	40	Mn ²⁺	2.8	Seo (2013)
<i>Enterococcus faecium</i> DBFIQ E36	7.0	50	NA	NA	Torres et al. (2014)
<i>Pediococcus. Pentosaceus</i> PC-5	6.0	50	Co ²⁺ , Mn ²⁺	2.9	Men et al. (2014)
<i>Bacillus coagulans</i> NL01	7.5	60	Co ²⁺ , Mn ²⁺	1.0	Mei et al. (2016)
<i>Shigella flexneri</i>	8.0	40	Co ²⁺ , Mn ²⁺	0.10	Patel et al. (2017)
Thermophilic					
<i>Thermus</i> sp. IM6501	8.0	60	Mn ²⁺	NA	Kim et al. (2003a)
<i>Thermoanaerobactermathranii</i>	8.0	65	Mn ²⁺	NA	Jorgensen et al. (2004)
<i>Geobacillus thermodenitrificans</i>	7.5	60	Co ²⁺ , Mn ²⁺	0.5	Kim and Oh (2005)
<i>Geobacillus Stearothermophilus</i> T6	7.0 to 7.5	70	Mn ²⁺	4.3	Lee et al. (2005a)
<i>Alicyclobacillus acidocaldarius</i>	6.0	65	Co ²⁺ , Mn ²⁺ , Mg ²⁺	3.3	Lee et al. (2005b)
<i>Bacillus stearothermophilus</i> IAM1101	7.5	65	Mn ²⁺	NA	Cheng, Mu, and Jiang (2010a)
<i>Bacillus stearothermophilus</i> US100	7.5 to 8	80	Co ²⁺ , Mn ²⁺	8.4	Rhimi and Bejar (2006)
<i>Acidothermus cellulolyticus</i> ATCC 43068	7.5	75	Co ²⁺ , Mn ²⁺	9.3	Cheng, Mu, Zhang, and Jiang (2010b)
<i>Alicyclobacillus hesperidum</i> URH17-3-68	7.0	70	Co ²⁺	1.2	Fan et al. (2014)
<i>Thermoanaerobacterium saccharolyticum</i> NTOU1	7.0 to 7.5	70	Co ²⁺ , Mn ²⁺	2.41	Hung, Tseng, Liu, Tzou, and Fang (2014)
<i>Anoxybacillus flavithermus</i>	9.5 to 10.5	95	Ni ²⁺	5.16	Li et al. (2011)
Hyperthermophilic					
<i>Thermotoga neapolitana</i>	7.0	85	Co ²⁺ , Mn ²⁺	3.24	Kim et al. (2002)
<i>Thermotoga maritima</i>	7.0 to 7.5	90	Co ²⁺ , Mn ²⁺	8.51	Lee et al. (2004)

NA, not available.

L-Arabinose Isomerase

Lately, L-arabinose isomerase (L-AI; EC 5.3.1.4) has attracted worldwide attention among researchers because of its application in the production of rare sugars. It is an intracellular enzyme that catalyzes the isomerization of L-arabinose to L-ribulose (Heath, Horecker, Smyrniotis, & Takagi, 1958) in addition to conversion of D-galactose into D-tagatose (Izumori, Ueda, & Yamanaka, 1978). Due to numerous health benefits of D-tagatose, an extensive research is going on across the world to develop the efficient process for its production after due contemplation to food and environmental safety, human health,

and consumer awareness. The biological conversion of D-galactose into D-tagatose by application of L-AI enzyme seems to be a viable process because it relies on isomerisation of D-galactose, an industrial by-product of cheese industry (Torres, Manzo, Rubiolo, Batista-Vieraa, & Mammarella, 2014). During the past few decades, biological production of D-tagatose has been studied using L-AI from *E. coli* (Yoon, Kim, & Oh, 2003), *Lactobacillus fermentum* (Xu et al., 2011), *Bacillus coagulans* (Mei, Wang, Zang, Zheng, & Ouyang, 2016), *Geobacillus thermodenitrificans* (Kim & Oh, 2005), *Geobacillus stearothermophilus* (Lee et al., 2005a), *Thermotoga neapolitana* (Kim et al., 2002), *Thermotoga maritima* (Lee

et al., 2004), and *Anoxybacillus flavithermus* (Li, Zhu, Liu, & Sun, 2011). Different sources of L-AI and its properties are presented in Table 1.

Although, L-arabinose is the natural substrate for L-AI but it also catalyzes the isomerization of D-galactose into D-tagatose because of their similarity in configuration. The application of L-AI for D-tagatose production from D-galactose was introduced during mid-1990s of the previous century following harvesting the enzyme from lactic acid bacteria (Cheetham & Wootton, 1993). The major factors influencing the catalytic activity of L-AI are source organisms, pH, temperature and metal ion (Xu, Li, Feng, Liang, & Xu, 2014a). The catalytic efficiency of L-AI varies from 0.4 to 9.3 mM⁻¹ min⁻¹ for isomerisation of D-galactose. The temperature optima for L-AI are 30 to 50 °C for mesophilic bacteria, 60 to 80 °C for thermophilic bacteria and 85 to 90 °C for hyperthermophilic bacteria. Several metal ions play critical role on the functioning of L-AI (Table 1).

Approaches for Improvement of Tagatose Production

The production of tagatose could be enhanced by the applications of chemicals, immobilization of L-AI and protein engineering.

L-AI enzyme from *Geobacillus thermodenitrificans* has been reported to increase the productivity of D-tagatose in the presence of boric acid (Lim, Kim, & Oh, 2007) because of its higher affinity towards ketoses (De Muyndck, Beauprez, Soetaert, & Vandamme, 2006).

The immobilization helps in the improvement of catalytic activity, thermostability and temperature optima of L-AI enzyme (Xu, Li, Feng, Liang, & Xu, 2014a). Even though, alginate is the most commonly used immobilization material but, glutaraldehyde, polyethylenimine, chitopearl beads, and agarose are also used for immobilization of L-AI and recombinant cells (Jorgensen, Hansen, & Stougaard, 2004; Jung, Kim, & Oh, 2005; Kim, Yoon, Roh, & Choi, 2001a; Lim, Kim, & Oh, 2008; Ryu, Kim, Kim, Baek, & Oh, 2003). The effect of immobilization on tagatose yield is presented in Table 2.

The crystal structure of *E. coli* L-arabinose isomerase (ECAI) was first solved by Manjasetty & Chance in 2006. Later on, the crystal structure of L-AIs from *Lactobacillus fermentum* CGMCC2921 (Xu, Li, Liang, Feng, & Xu, 2015) and *Geobacillus kaustophilus* (Choi et al., 2016) were also solved. The crystal structure of all the above 3 L-AI indicates the presence of hexameric assembly. Understanding of the crystal structures of L-AI opens the door for application of protein engineering tools (directed evolution and site directed mutagenesis) to modify the enzyme properties such as substrate specificity, temperature optima, thermostability, pH optima, and enzyme activity pertaining to tagatose production (Choi et al., 2016). Different strategies of protein engineering have been employed for improving the properties of L-AI (Figure 2). Site directed mutagenesis of L-AI produced by *Geobacillus thermodenitrificans*, *Bacillus stearothermophilus*, and *Lactobacillus fermentum*, resulted into increase of the specific activity, catalytic efficiency, substrate affinity and change of pH optima (Kim, Hong, Shin, Jo, & Oh, 2014a; Li, Xu, Li, & Xu, 2012; Rhimi et al., 2009). Site-directed mutagenesis study shows that the amino acid residue Lys-269 of *Alicyclobacillus acidocaldarius* L-arabinose isomerase plays a crucial role to modify the pH optima (Lee et al., 2005b). The application of direct evolution approach in L-AI enzyme of *Geobacillus stearothermophilus* leads to higher enzyme activity (Kim, Kim, Oh, & Oh, 2006).

Use of GRAS Microorganism for L-AI Expression

Mostly, the expression of L-AI has been carried out in non-GRAS microorganism (Mei et al., 2016; Patel, Akhiani, Patel, Dedania, & Patel, 2017), which is unsuitable for food and pharmaceutical applications owing to the safety concerns. Hence, researchers have attempted to express L-AI in GRAS microorganism such as *Corynebacterium glutamicum*, *Bacillus subtilis*, and *Lactococcus lactis* (Kim et al., 2012; Salonen, Salonen, Leisola, & Nyssola, 2013). Most of these GRAS hosts express L-AI as an intracellular enzyme. This in turn increases the cost of production as it involves several additional steps including extraction and purification. Therefore, extracellular expression of L-AI can be an attractive alternative to reduce the cost of enzyme production. Recently, attempts have been made to express L-AI as an extracellular enzyme using *Lactococcus lactis* (Rhimi et al., 2015). Expression of L-AI gene in different GRAS organism is presented in Table 3.

Biochemical Properties of D-Tagatose

D-Tagatose is a naturally occurring monosaccharide having sweetness equivalent to 90% of the sucrose. It is the stereoisomer (epimer) of D-fructose with variation at the spatial configuration of hydroxyl group at C-4 position. It is an odorless, white crystalline powder that takes part in Maillard reaction leading to browning of food. It acts as a functional sweetener with no net energy and cooling effects. The detailed biochemical properties of D-tagatose are presented in Table 4.

Metabolism of D-Tagatose

D-Tagatose is considered as a mal-absorbing sugar because of the difference in the spatial configuration at C-4 position (Buemann, Toubro, & Astrup, 1999a). Although, there is a structural similarity between fructose and tagatose, fructose carrier mediated transport system has no affinity for tagatose in the small intestine. Approximately 20% to 25% of ingested tagatose is absorbed from the small intestine and the major part of ingested tagatose reaches to the large intestine for utilization by the indigenous gut microflora (Lu, Levin, & Donner, 2008). Feeding of radio labeled D-tagatose demonstrated around 20% absorption from the small intestine in rats (Saunders, Zehner, & Levin, 1999a). In case of pigs, approximately 26% of ingested tagatose has been reported to be absorbed from the small intestine (Laerke & Jensen, 1999). The metabolic fate of tagatose is identical to that of the fructose after absorption. Nevertheless, the rate of metabolism is half of the fructose. Initially, D-tagatose is converted to D-tagatose-1-phosphate by fructokinase. Thereafter, it splits into glyceraldehyde and dihydroxyacetone phosphate by the action of aldolase. The enzyme fructokinase has lower affinity for D-tagatose as compared to D-fructose (Martinez, Carrascosa, & Nunez de Castro, 1987).

The unabsorbed fraction of D-tagatose reaches to the large intestine and is subjected to fermentation by the diverse group of microflora leading to short chain fatty acids (SCFA) production (Laerke, Jensen, & Hojsgaard, 2000). In large intestine, the D-tagatose is catabolized by the gut microflora (*Lactobacillus casei*, *Lactococcus lactis*, and *Streptococcus lactis*) through tagatose-6-phosphate pathway, a branching pathway of galactose metabolism (Yu, Hodge, & Li, 1990). D-Tagatose is metabolized into D-tagatose-6-phosphate by the action of hexokinase. Thereafter, it is metabolized into dihydroxyacetone phosphate (DHAP) and glyceraldehyde-3-phosphate (G3P) by tagatose-6-phosphate kinase and tagatose-1,6-diphosphatealdolase, respectively.

Table 2—Effect of immobilization on D-tagatose yield.

Immobilization material	Source of L-arabinose isomerase	Galactose Concentration (g/L)	Reaction condition	Tagatose yield (g/L/hr)	Conversion yield (%)	References
Alginate	<i>G. stearothermophilus</i>	300	60 °C, pH8.0	54	48	Ryu et al. (2003)
Alginate	<i>G. stearothermophilus</i>	100	65 °C, pH 8.0	13.3	50	Kim, Ryu, Kim, and Oh (2003b)
Calcium alginate	<i>T. mathranii</i>	18	75 °C, pH7.5	10	23.3	Liang et al. (2012)
Sodium alginate	<i>L. fermentum</i>	100	65 °C, pH 6.5	11.1	60	Xu et al. (2012)
Glutaraldehyde and polyethylenimine	<i>T. mathranii</i>	300	65 °C, pH 6.9	2.6	42	Jorgensen et al. (2004)
Chitopearl beads	<i>T. neapolitana</i>	300	70 °C, pH7.5	1.2	46	Lim et al. (2008)
Agarose	<i>E. coli</i>	500	30 °C, pH7.0	2.1	20	Kim et al. (2001a)
Calcium alginate	Recombinant <i>E. coli</i> cells expressing L-AI of <i>T. neapolitana</i>	180	70 °C pH-7.0	4.0	27	Hong et al. (2007)
Sodium alginate	Recombinant <i>E. coli</i> cells expressing L-AI of <i>G. stearothermophilus</i>	300	60 °C pH-7.0	2.9	19.5	Jung et al. (2005)

Table 3—GRAS microorganism used for L-AI expression.

Source of L-AI	GRAS host expression system	Specific activity of expressed L-AI (U/mg)	Bioconversion rate of D-galactose to D-tagatose (%)	Reference
<i>Geobacillus stearothermophilus</i> DSM 22	<i>Corynebacterium glutamicum</i> KCTC 13032 and <i>Bacillus subtilis</i> 168	NR	NR	Kim et al. (2012)
<i>Geobacillus thermodenitrificans</i>	<i>Bacillus subtilis</i> GSAIB-1, <i>Corynebacterium glutamicum</i> GSAIC-1 and <i>Corynebacterium glutamicum</i> mGTAIC001	NR	NR	
<i>Thermotoga neapolitana</i> DSM 5068	<i>Corynebacterium</i> sp.	NR	NR	Kim et al. (2014b)
<i>Bifidobacterium longum</i> NRRL B-41409	<i>Lactococcus lactis</i>	7.7	36	Salonen et al. (2013)
<i>Bacillus stearothermophilus</i> US100	Co-culture of <i>Lactobacillus delbrueckii</i> subsp. <i>bulgaricus</i> and <i>Streptococcus thermophiles</i>	32 ± 0.54	24	Rhimi, Chouayekh, Gouillouard, Maguin, and Bejar (2011b)
<i>Lactobacillus fermentum</i> CGMCC2921	Surface display system using the spore surface of <i>Bacillus subtilis</i> 168	2.02 × 10 ⁻¹⁰ U/spore	75 ± 3.5	Liu et al. (2014)
<i>Lactobacillus sakei</i>	<i>Lactococcus lactis</i>	22 ± 0.6	32	Rhimi et al. (2015)
<i>Geobacillus stearothermophilus</i>	<i>Bacillus subtilis</i>	2.7	NR	Cheon, Kim, Park, Han, and Kim (2009)

NR, not reported.

Biological Role of D-Tagatose

Currently, the D-tagatose is attracting attention among the researchers of different sectors because of its disease ameliorating properties (antidiabetic, obesity control, blood metabolite regulator) together with health promoting effects (anti-aging, anti-oxidant, and prebiotic).

Antidiabetic

Diabetes mellitus (type 2 diabetes) is one of the major metabolic disorders encountered by the entire world. Peripheral insulin resistance and progressive failures of pancreatic β -cell function are the two principal causes for the pathogenesis of type 2 diabetes (Kaveeshwar & Cornwall, 2014). Often, blood glucose management in type 2 diabetes remains unsatisfactory (unwanted

weight gain, hypoglycaemia, gastrointestinal distress, pancreatitis, and so on) in spite of the application of insulin therapy along with antidiabetic medicine (Ensor, Williams, Smith, Banfield, & Lodder, 2014). It clearly urges the requisite of a molecule that is not only able to control the blood sugar within the recommended levels, but also slows down the progression of diabetes through restoration of β -cell function, reduction of cardiovascular risk factors, weight management, and so on. In view of the above perspectives, extensive investigations are being carried out across the world to find out the alternative molecules to control blood glucose. D-Tagatose is a potential therapeutic molecule that can blunt the blood glucose levels in both healthy and diabetic patient (Donner, Wilber, & Ostrowski, 1996). D-Tagatose has several advantages over the conventional oral antidiabetic medicines

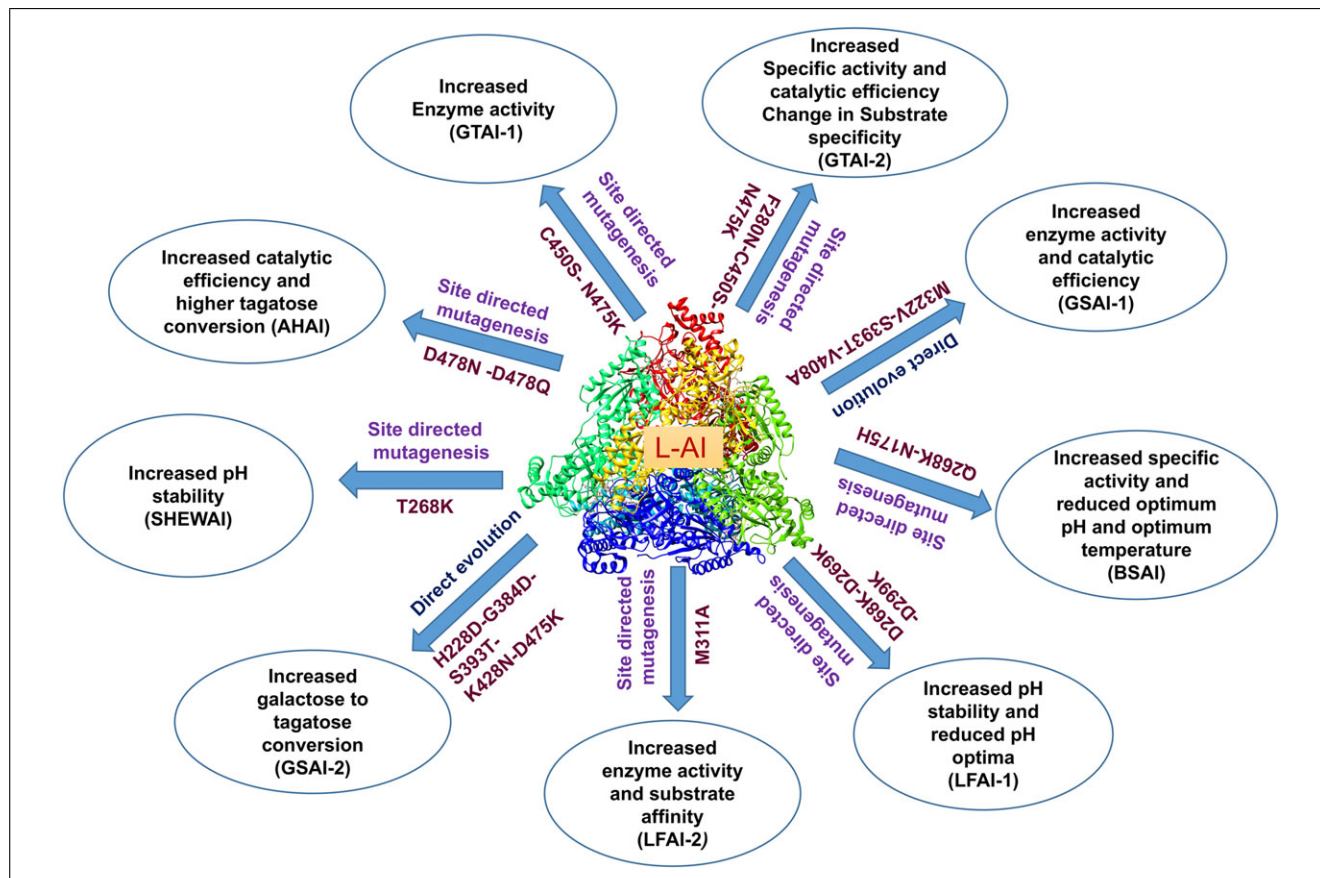


Figure 2—Protein engineering of L-arabinose isomerase (L-AI) from different sources: GTAI-1 from *Geobacillus thermodinitrificans* (Oh, Kim, & Oh, 2006); GTAI-2 from *Geobacillus thermodinitrificans* (Kim et al., 2014a); GSAI-1 from *Geobacillus stearothermophilus* (Kim, Yoon, Seo, Oh, & Choi, 2001b); GSAI-2 from *Geobacillus stearothermophilus* (Kim et al., 2006); AHA1 from *Alicyclobacillus hesperidum* (Fan et al., 2015); SHEWAI from *Shewanella* sp. (Rhimi et al., 2011a); BSAI from *Bacillus stearothermophilus* (Rhimi et al., 2009); LFAI-1 from *Lactobacillus fermentum* (Xu, Li, Feng, Zhan, & Xu, 2014b); LFAI-2 from *Lactobacillus fermentum* (Li, Xu, Li, & Xu, 2012).

including its GRAS status, hypo-glycemic properties, antioxidant activities, prevention of weight gain, and so on (Lu, Levin, & Donner, 2008). It seems that the initial findings of D-tagatose as an antidiabetic molecule are encouraging and useful.

Administration of 75 g of D-tagatose 30 min prior to glucose tolerance test in diabetic patient results into significant blunts in the rise of blood glucose levels without influencing the blood insulin concentration (Donner, Wilber, & Ostrowski, 1999). The dose ranging trial in human volunteers with type 2 diabetes indicates that the minimum dose requirement of D-tagatose for controlling the acetylated hemoglobin is 5.0 g, three times in a day (Ensor et al., 2014). However, further studies in human volunteers originating from both the United States and India reveals that tagatose dose of 15 g, three times in a day is highly effective to reduce the acetylated hemoglobin levels (Ensor, Banfield, Smith, Williams, & Lodder, 2015). Nevertheless, the effect of D-tagatose on lowering of glycosylated hemoglobin was more distinct in American as compared to the Indian populations.

Though, the understanding of the mechanism of action on D-tagatose as an antidiabetic agent is incomplete; the plausible mechanistic pathway has been framed based on the understanding of several studies involving both fructose and tagatose. The hepatic metabolism of D-tagatose is similar to D-fructose. In liver, D-tagatose is metabolized into D-tagatose-1-phosphate by the fructokinase. D-Tagatose-1-phosphate is further metabolized into glyceraldehyde (GA) and dihydroxyacetonephosphste (DHAP).

The transient accumulation of tagatose-1-phosphate induces the translocation of glucokinase enzyme, which in turn enhances the conversion of glucose into glucose-6-phosphate (Agius, 1998). It stimulates the glycogen synthase enzyme for conversion of glucose into glycogen (Seoane et al., 1996). Ercan-Fang et al. (2002) reported that D-tagatose-1-phosphate inhibits the glycogen phosphorylase, an enzyme involved in hydrolysis of glycogen into glucose. The net effect of the projected mechanistic theory suggests an increase of glycogen synthesis coupled with decrease of glycogen hydrolysis (Figure 3). Additionally, D-tagatose also inhibits carbohydrate digesting enzymes such as sucrase and maltase. These are the major intestinal enzymes influencing the intestinal carbohydrate metabolism. Therefore, by inhibiting the activity of sucrase and maltase in the small intestine, tagatose also lowers the blood sugar level.

Prebiotic

Prebiotics are the non-digestible short chain oligosaccharides that confer benefits to the host through selective growth stimulation of one or limited number of beneficial gut microflora (Gibson & Roberfroid, 1995; Samanta et al., 2015). Based on the understanding of multifaceted role of gut microflora on human health and function, current emphasis of nutraceutical researchers is focused on manipulation of gut microflora through application of bioactive carbohydrates for gut health and wellbeing, value addition of dairy and confectionary products, feed additive, and so

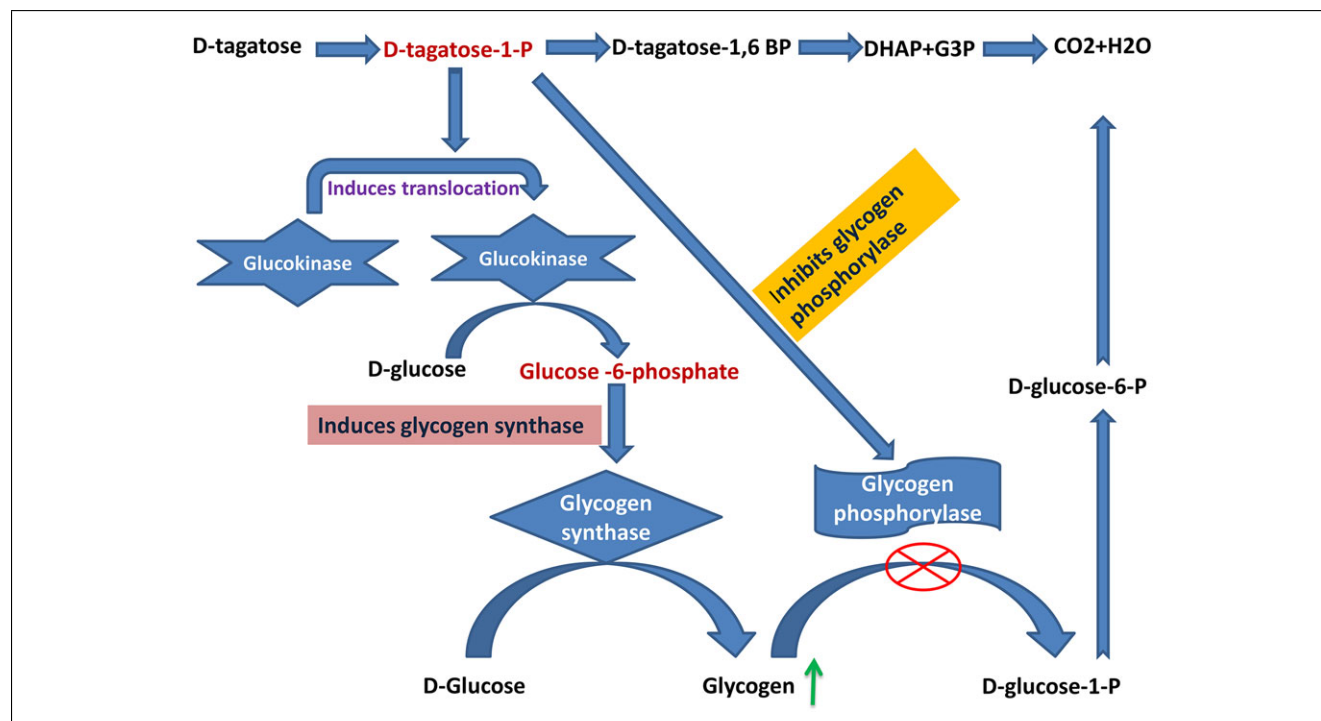


Figure 3—Plausible mechanism of action of D-tagatose.

on. Therefore, it gives enough opportunities to consider tagatose as a prebiotic. A major fraction (75% to 80%) of ingested tagatose reaches to the large intestine for utilization by the indigenous microflora leading to the production of SCFA (Lu, Levin, & Donner, 2008). In pig model, no traces of D-tagatose are detected in the feces receiving 10% tagatose in their diet (Laerke & Jensen, 1999). Under the *in vitro* fermentation system, the rate of production of SCFA was found four times higher if fecal inoculums were obtained from the volunteers receiving 10 g tagatose three times in a day for a period of 2 weeks (Bertelsen, Jensen, & Buemann, 1999). The same volunteers also exhibited increased population of beneficial bacteria (lactobacilli) and reduced population of pathogenic bacteria in the gastrointestinal tract. *In vitro* study with fecal inoculums of pig reflected production of acetate, formate, propionate, butyrate, valerate, caproate, and some heptanoate from D-tagatose fermentation (Laerke et al., 2000). Butyrate is the major fuel for colonocytes and it helps in their development inside the large intestine. It acts as a powerful anti-inflammatory agent and plays crucial role in preventing colon cancer (Canani et al., 2011).

Obesity Control

Obesity is one of the important risk factor for diabetes, cardiovascular disease, hypertension, dys-lipidemia, and coronary heart disease. Weight management plays an important role to reduce the risk of obesity related disorders. For many diabetic patients, weight loss is the key factor to control the blood glucose level and it may eliminate the need for medication. D-Tagatose is a promising agent to control obesity because of its low caloric value. Long-term therapy of diabetes with D-tagatose causes progressive weight loss in human volunteers (Donner, 2006). It is also reduces food intake in normal healthy men (Buemann, Toubro, Raben, Blundell, & Astrup, 2000). One of the common problems associated with the pregnancy is overeating by an individual. It leads to excess weight

gain, which in turn increases the health risks to both mother and fetus. In a study with rats, D-tagatose reduces food intake without any body weight loss (Levin, 2001).

Anti-Aging Property

It is postulated that the control of energy intake is one of the major steps for reducing the risk of serious illness as well as lengthening the quality of life (Masoro, 1992). Therefore, low energy sugar such as D-tagatose is capable to manage energy intake without compromising the taste of sugars. The cross linking of protein with carbohydrates (glycosylation) in muscle and brain tissue is the major cause of aging (Bunn & Higgins, 1981). Diet restriction maintains consistently lower plasma glucose and insulin level, which in turn retards the process of age linked disease and thereby lengthen the life span (Masoro, 1993). Preliminary investigation demonstrates that D-tagatose has the ability to lower the plasma glucose concentration (Ensor et al., 2015). Therefore, it could act as an effective anti-aging molecule for better human health and wellbeing.

Antioxidant Property

Reactive oxygen species (ROS) are produced as a result of numerous metabolic reactions within the cells. Although, low levels of ROS is required for specific physiological activities, but an intricate balance should be maintained between ROS generation and its clearance for healthy status. Frequently, the entire ROS are not detoxified by the defense mechanism of the body, resulting cell damage or activation of apoptosis related genes (Nakazawa, Genka, & Fujishima, 1996). D-Tagatose has the potential to attenuate cell damages caused by the intracellular free radicals. In a study with cultured murine hepatocytes, D-tagatose (20 mM) was shown to prevent oxidative cell damage caused by the redox cycling drug nitrofurantoin (NFT) as compared to the equimolar amount of glucose, mannitol, or xylose (Paterna, Boess, Staubli,

Table 4—Biochemical properties of D-tagatose.

Property	Nature/value
Common name	D-tagatose
Synonyms	1. D-tagatopyranose, D-lyxo-hexulose, D-tagatopyranoside
Category	Rare sugar
Physical form	White crystalline solid
Empirical formula	C ₆ H ₁₂ O ₆
Molecular weight	180.16
Solubility in water	160 g/100 mL at 20 °C
Melting temperature	133–137 °C
Heat of solution	−42.3 kJ/kg at 20 °C
pH stability	2 to 7
Relative sweetness	92% of sucrose
Browning effect	Turns brown like sucrose at high temperature
Caloric value	1.5 kcal/g
Regulatory status	Generally recognized as safe (GRAS)
Hygroscopic	Lower, similar to sucrose

& Boelsterli, 1998). D-Tagatose protects against the iron-induced cytotoxicity by suppressing the formation of iron-catalyzed free radicals production from membrane lipid peroxidation and protein carbonyl formation (Paterna et al., 1998). It is suggested that the weak iron chelating property of D-tagatose is responsible for its antioxidant property. D-Tagatose also gives protection against the damage of liver cells caused by pro-oxidant drugs (Valeri, Boess, Wolf, Goldlin, & Boelsterli, 1997).

Blood Factor Regulation

Plasma fibrinogen, prothrombin time (PT) and activated partial thromboplastin time (APTT) are important factors for the clinical assessment of blood coagulation. Maintaining the optimum level of these parameters is imminent for reducing the onset of diseases. D-Tagatose plays a significant role in regulating the blood factors such as red blood cell (RBC) count, PT, APTT, and fibrinogen. In a study with rats, feeding of D-tagatose (15% to 20% by weight) for a period of 8 weeks increased the RBC counts and plasma fibrinogen within the desirable limit (Levin, 2006). The same study also reported a reduction in PT and APTT within the prescribed limit. These results indicate that D-tagatose can be used for the improvement of blood coagulation parameters. In case of anemic patient, administration of D-tagatose (30 g/day) ameliorated the anemic condition by increasing the RBC count (Levin, 2006). It is also reported that the administration of D-tagatose (15 g/day) increased the fibrinogen level with simultaneous decrease of PT and APTT in hemophilic patient diagnosed with prolonged PT and APTT (Levin, 2006).

Pregnancy and Fetal Development

D-Tagatose has a potential role on improvement of fertility, promotion of healthy fetal development, enhancing probability of delivering live fetus in addition to control of food intake during pregnancy. There is higher percentage of live births in rats supplemented with D-tagatose (Kruger, Whittaker, Frankos, & Schroeder, 1999a). In a study with rats, D-tagatose increased fertility, probability of live birth, and higher birth weight of new born (Levin, 2001).

Applications

Among the list of rare sugars, D-tagatose occupies an important place because of its application potentiality in several sectors including food, pharmaceuticals, packaging, cosmetics, and so on (Levin, Zehner, Saunders, & Beadle, 1995). Besides its sweetness

Table 5—Application potentialities of D-tagatose.

Product type	Expected proportion of D-tagatose
Biscuits, cookies, cake, and pies	10%
Breakfast cereals	3 g/serving
Diet/sugar free soft drinks	2%
Non-diet soft drinks	3%
Frostings	15%
Confectionary	25%
Frozen yogurt, ice-cream	7.5%
Soft candies	10%
Sugar free chewing gum	60%
Hard candies	10% to 15%
Formula diets	3%
Baked food such as short breads and muffins	2%
Chocolates	15%

properties, it has several beneficial properties such as low glycemic index, reduced energy value, prebiotic, antioxidant, antiplaque, and so on (Patel et al., 2017). It can be used as a low caloric bulk sweetener in a wide variety foods, health products, beverages, and dietary supplements. The flavor enhancing properties of D-tagatose makes it as a perfect and probable agent to mask the unpleasant taste of medicines. Application potentiality of D-tagatose is presented in Table 5.

Safety and Toxicity

The safety and toxicity aspects of tagatose have been investigated in both animal model and human subjects. When the tagatose consumption increases beyond 10%, adverse effects (increased liver weight and hypertrophy) are noticed in rats (Kruger et al., 1999a). Hence, 5% level of tagatose is considered as safe dose without any side effects. Reproductive performance of rat is not affected even though tagatose consumption reaches up to 20 g/kg body weight/day (Kruger, Whittaker, Frankos, & Trimmer, 1999b). Human clinical trials with D-tagatose consumption are mostly focused on its urecemic and gastrointestinal effects. The elevated level of plasma uric acid is associated with purine metabolism disorder and development of gout. There is a transient increase of plasma uric acid concentration in both healthy and non-insulin dependent diabetes mellitus population following single oral dose of 75 g of D-tagatose (Saunders, Donner, Sadler, Levin, & Makris, 1999b). A lower dose of D-tagatose (45 g/day; 15 g TID) is considered to be safe in healthy human subjects because it does not show any adverse effects on plasma uric acid, glycogen levels as well as functioning of liver (Boesch et al., 2001). Similarly, consumption of 45 g D-tagatose/day (15 g TID) for a period of 1 year does not cause any adverse effects on plasma uric acid levels in non-insulin dependent diabetes mellitus patient (Donner, 2006). The above dose of D-tagatose also helps in reduction of postprandial plasma glucose levels. Nevertheless, few reports indicate gastrointestinal disturbances (nausea, diarrhea and light to moderate flatulence) following the consumption of 30 g of D-tagatose in a single dose (Buemann, Toubro, Raben, & Astrup, 1999b). Keeping in view the above considerations, the “No Observed Adverse Effect Level” (NOAEL) for tagatose is set at 45 g/day or 0.75 g/kg body weight/day (World Health Organization, 2004).

Conclusions

D-Tagatose, C-4 epimer of D-fructose, is a rare sugar. Recently, it has attracted greater interest among several industries such as food, pharmaceuticals, packaging, and cosmetics. The major

impediments that restrict its large scale applications are limited availability, lack of commercial process and inadequate knowledge on beneficial roles. The chemical process was considered to be an ideal method for large scale D-tagatose production during the previous century. Nevertheless, consumer awareness coupled with safety issues forced the researchers to develop biological process of D-tagatose production. Biological methods offer advantages over chemical methods, because enzyme-catalyzed reactions are often highly enantio-selective and regioselective. In this endeavor, L-AI emerged as a most useful enzyme for biological production of D-tagatose. Despite untiring efforts, limited success has been achieved towards biological production of D-tagatose because of lower bio-conversion efficiency of L-AI, metal ion requirement, poor thermostability and low affinity of the enzyme for D-galactose. A better understanding about structure-function relationship of the existing L-AI will help to increase its affinity and selectivity towards D-galactose. The application of protein engineering together with genomic tools could enhance the bioconversion efficiency for D-tagatose production by modifying the functional properties of L-AI. Tetrameric structure of thermophilic and hyperthermophilic L-AI is still unresolved. These structures need to be solved to get a better understanding about the ligand (D-galactose) and enzyme (L-AI) interaction. Application of high throughput screening or selection method will help to evaluate individual protein variants. It will increase the possibility to screen specific mutants with higher catalytic activity. More research needs to be carried out to explore new sources of biocatalysts, especially from different GRAS microorganism in addition to the enzyme expression and secretion in a food grade microbial host. Application of GRAS expression system will be an exciting alternative to satisfy the food safety issues.

More information needs to be gathered on minute changes happened due to regular intake of D-tagatose. As further research findings on the applications of D-tagatose will continue to accumulate, its potential efficacy for clinical application will be clear. A better understanding of structure-function relationship of D-tagatose along with the identification of the metabolic profile of target microorganism will help to determine the specific health attribute of D-tagatose as a prebiotic. More *in vivo* data, particularly in animals and human intervention studies are required at this point of time to find out the effective daily dose of D-tagatose as an antidiabetic agent. New development in metabolic engineering and molecular technologies will help to answer the unsolved questions related to the mechanistic pathway of D-tagatose.

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Author Contribution

Sohini Roy wrote the manuscript with critical inputs and corrections by J. Chikkerur, S.C. Roy, A. Dhali, A.P. Kolte, Manpal Sridhar, and A.K. Samanta. Corresponding author did the final editing. All the authors considerably contributed in conceptualization and framing of the manuscript.

References

Adachi, S. (1958). Formation of lactulose and tagatose from lactose in strongly heated milk. *Nature*, 181(4612), 840–841. <https://doi.org/10.1038/181840a0>

- Agius, L. (1998). The physiological role of glucokinase binding and translocation in hepatocytes. *Advances in Enzyme Regulation*, 38, 303–331. [https://doi.org/10.1016/S0065-2571\(97\)00001-0](https://doi.org/10.1016/S0065-2571(97)00001-0)
- Al-Jobore, A. A. H., Guthrie, R. D., & Wells, R. D. (1971). A new route for the synthesis of D-tagatose. *Carbohydrate Research*, 16(2), 474–476. [https://doi.org/10.1016/S0008-6215\(00\)81187-8](https://doi.org/10.1016/S0008-6215(00)81187-8)
- Armstrong, L. M., Luecke, K. J., & Bell, L. N. (2009). Consumer evaluation of bakery product flavor as affected by incorporating the prebiotic tagatose. *International Journal of Food Science*, 44(4), 815–819. <https://doi.org/10.1111/j.1365-2621.2009.01909.x>
- Beadle, J. R., Saunders, J. P., & Wajda, T. J., inventors (1992). Process of manufacturing tagatose. U.S. Patent 5078796A. Retrieved from <https://www.google.ch/patents/US5078796>
- Bertelsen, H., Jensen, B. B., & Buemann, B. (1999). D-tagatose: A novel low caloric sweetener with prebiotic properties. *World Review of Nutrition Dietetics*, 85, 98–109. Retrieved from <https://doi.org/10.1159/000059685>
- Boesch, C., Ith, M., Jung, B., Bruegger, K., Erban, S., Diamantis, I., ... Bar, A. (2001). Effect of oral D-tagatose on liver volume and hepatic glycogen accumulation in healthy male volunteers. *Regulatory Toxicology and Pharmacology*, 33(2), 257–267. <https://doi.org/10.1006/rtp.2001.1470>
- Buemann, B., Toubro, S., & Astrup, A. (1999a). Human gastrointestinal tolerance to D-tagatose. *Regulatory Toxicology and Pharmacology*, 29(2 Pt 2), S71–S77. <https://doi.org/10.1006/rtp.1998.1265>
- Buemann, B., Toubro, S., Raben, A., & Astrup, A. (1999b). Human tolerance to single, high dose of D-tagatose. *Regulatory Toxicology and Pharmacology*, 29(2), S66–S70. <https://doi.org/10.1006/rtp.1998.1252>
- Buemann, B., Toubro, S., Raben, A., Blundell, J., & Astrup, A. (2000). The acute effect of D-tagatose on food intake in human subjects. *British Journal of Nutrition*, 84(2), 227–231. <https://doi.org/10.1017/S000711450000146X>
- Bunn, H. F., & Higgins, P. J. (1981). Reactions of monosaccharides with proteins: Possible evolutionary significance. *Science*, 213(4504), 222–224. <https://doi.org/10.1126/science.12192669>
- Canani, R. B., Costanzo, M. D., Leone, L., Pedata, M., Meli, R., & Calignano, A. (2011). Potential beneficial effects of butyrate in intestinal and extra intestinal diseases. *World Journal of Gastroenterology*, 17(12), 1519–1528. <https://doi.org/10.3748/wjg.v17.i12.1519>
- Castanon, J. I. R. (2007). History of the use of antibiotic as growth promoters in European poultry feeds. *Poultry Science*, 86(11), 2466–2471. <https://doi.org/10.3382/ps.2007-00249>
- Cheetham, P. S. J., & Wootton, A. N. (1993). Bioconversion of D-galactose into D-tagatose. *Enzyme and Microbial Technology*, 15(2), 105–108. [https://doi.org/10.1016/0141-0229\(93\)90032-W](https://doi.org/10.1016/0141-0229(93)90032-W)
- Cheng, L., Mu, W., & Jiang, B. (2010a). Thermostable L-arabinose isomerase from *Bacillus stearothermophilus* IAM11001 for D-tagatose production: Gene cloning, purification and characterization. *Journal of the Science of Food and Agriculture*, 90(8), 1327–1333. <https://doi.org/10.1002/jsfa.3938>
- Cheng, L., Mu, W., Zhang, T., & Jiang, B. (2010b). An L-arabinose isomerase from *Acidothermus cellulolyticus* ATCC 43068: Cloning, expression, purification, and characterization. *Applied Microbiology and Biotechnology*, 86(4), 1089–1097. <https://doi.org/10.1007/s00253-009-2322-z>
- Cheon, J., Kim, S. B., Park, S. W., Han, J. K., & Kim, P. (2009). Characterization of L-arabinose isomerase in *Bacillus subtilis*, A GRAS host, for the production of edible tagatose. *Food Biotechnology*, 23(1), 8–16. <https://doi.org/10.1080/08905430802671873>
- Choi, J. M., Lee, Y. J., Cao, T. P., Shin, S. M., Park, M. K., Lee, H. S., ... Lee, D. W. (2016). Structure of the thermophilic L-arabinose isomerase from *Geobacillus kaustophilus* reveals metal-mediated inter subunit interactions for activity and thermostability. *Archives of Biochemistry and Biophysics*, 596, 51–62. <https://doi.org/10.1016/j.abb.2016.02.033>
- Chouayekh, H., Bejar, W., Rhimi, M., Jelleli, K., Mseddi, M., & Bejar, S. (2007). Characterization of an L-arabinose isomerase from the *Lactobacillus plantarum* NC8 strain showing pronounced stability at acidic pH. *FEMS Microbiology Letters*, 277(2), 260–267. <https://doi.org/10.1111/j.1574-6968.2007.00961.x>
- De Muynck, C., Beauprez, J., Soetaert, W., & Vandamme, E. J. (2006). Boric acid as a mobile phase additive for high performance liquid chromatography separation of ribose, arabinose and ribulose. *Journal of Chromatography A*, 1101(1–2), 115–121. <https://doi.org/10.1016/j.chroma.2005.09.068>
- Dobbs, C. M., & Bell, L. N. (2010). Storage stability of Tagatose in buffer solutions of various compositions. *Food Research International*, 43(1), 383–386. <https://doi.org/10.1016/j.foodres.2009.10.010>
- Donner, T. W. (2006). The metabolic effects of dietary supplementation with D-tagatose in patients with type 2 diabetes. *Diabetes*, 55(Suppl.1), 461.
- Donner, T., Wilber, J., & Ostrowski, D. (1996). D-tagatose: A novel therapeutic adjunct for non-insulin-dependent diabetes. *Diabetes*, 45(Suppl. 2), 125A
- Donner, T. W., Wilber, J. F., & Ostrowski, D. (1999). D-tagatose, a novel hexose: Acute effects on carbohydrate tolerance in subjects with and without type 2 diabetes. *Diabetes, Obesity and Metabolism*, 1(5), 285–291. <https://doi.org/10.1046/j.1463-1326.1999.00039.x>
- Ensor, M., Williams, J., Smith, R., Banfield, A., & Lodder, R. A. (2014). Effects of three low-doses of D-tagatose on glycemic control over six months in subjects with mild type 2 diabetes mellitus under control with diet and exercise. *Journal of Endocrinology, Diabetes & Obesity*, 2(4), 1057.
- Ensor, M., Banfield, A. B., Smith, R. R., Williams, J., & Lodder, R. A. (2015). Safety and efficacy of D-tagatose in glycemic control in subjects with type 2 diabetes. *Journal of Endocrinology, Diabetes & Obesity*, 3(1), 1065.
- Ercan-Fang, N., Gannon, M. C., Rath, V. L., Treadway, J. L., Taylor, M. R., & Nuttall, F. Q. (2002). Integrated effects of multiple modulators on human liver glycogen phosphorylase α . *American Journal of Physiology-Endocrinology and Metabolism*, 283(1), E29–E37. <https://doi.org/10.1152/ajpendo.00425.2001>
- Fan, C., Liu, K., Zhang, T., Zhou, L., Xue, D., Jiang, B., & Mu, W. (2014). Biochemical characterization of a thermostable L-arabinose isomerase from a thermophilic bacterium, *Alicyclobacillus hesperidum* URH17–3–68. *Journal of Molecular Catalysis B: Enzymatic*, 102, 120–126. <https://doi.org/10.1016/j.molcatb.2014.02.001>
- Fan, C., Xu, W., Zhang, T., Zhou, L., Jiang, B., & Mu, W. (2015). Engineering of *Alicyclobacillus hesperidum* L-arabinose isomerase for improved catalytic activity and reduced pH optimum

- using random and site-directed mutagenesis. *Applied Biochemistry and Biotechnology*, 177(7), 1480–92. <https://doi.org/10.1007/s12010-015-1828-3>
- Gibson, G. R., & Roberfroid, M. B. (1995). Dietary modulation of the human colonic microbiota: Introducing the concept of prebiotics. *Journal of Nutrition*, 125(6), 1401–1412.
- Granstrom, T. B., Takata, G., Tokuda, M., & Izumori, K. (2004). Izumoring: A novel and complete strategy for bio-production of rare sugars. *Journal of Bioscience and Bioengineering*, 97(2), 89–94. [https://doi.org/10.1016/S1389-1723\(04\)70173-5](https://doi.org/10.1016/S1389-1723(04)70173-5)
- Heath, E., Horecker, B., Smymiotis, P., & Takagi, Y. (1958). Pentose fermentation by *Lactobacillus plantarum* II L-arabinose isomerase. *Journal of Biological Chemistry*, 231(2), 1031–1037.
- Hirst, E. L., Hough, L., & Jones, J. K. N. (1949a). Composition of the gum of *Sterculiasitena*: Occurrence of D-tagatose in nature. *Nature*, 163(4135), 177. <https://doi.org/10.1038/163177b0>
- Hirst, E. L., Hough, L., & Jones, J. K. N. (1949b). The structure of *Sterculia setigera* gum. Part I. An investigation by the method of paper partition chromatography of the products of hydrolysis of the gum. *Journal of the Chemical Society*, 3145–3151. <https://doi.org/10.1039/JR9490003145>
- Hong, Y. H., Lee, D. W., Lee, S. J., Choe, E. A., Kim, S. B., Lee, Y. H., ... Pyun, Y. R. (2007). Production of D-tagatose at high temperatures using immobilized *Escherichia coli* cells expressing L-arabinose isomerase from *Thermotoga neapolitana*. *Biotechnology Letters*, 29(4), 569–574. <https://doi.org/10.1007/s10529-006-9277-2>
- Hudson, C. S., Wolfrom, M. L., & Cantor, S. M. (1952). *Advances in carbohydrate chemistry*. New York, USA: Academic Press, Inc.
- Hung, X. G., Tseng, W. C., Liu, S. M., Tzou, W. S., & Fang, T. Y. (2014). Characterization of a thermophilic L-arabinose isomerase from *Thermoanaerobacterium saccharolyticum* NT0U1. *Biochemical Engineering Journal*, 83, 121–128. <https://doi.org/10.1016/j.bej.2013.04.026>
- Huwig, A., Emmel, S., Jakel, G., & Giffhorn, F. (1997). Enzymatic synthesis of L-tagatose from galactitol with galactitol dehydrogenase from *Rhodobacter sphaeroides* D. *Carbohydrate Research*, 305(3–4), 337–339. [https://doi.org/10.1016/S0008-6215\(97\)00265-6](https://doi.org/10.1016/S0008-6215(97)00265-6)
- International Society of Rare Sugars. (2011). Kagawa University, Miki, Japan. Retrieved from <https://sites.google.com/site/rare Sugars/activity>
- Izumori, K., Yamanaka, K., & Elbein, D. (1976). Pentose metabolism in *Mycobacterium smegmatis*: Specificity of induction of pentose isomerases. *Journal of Bacteriology*, 128(2), 587–591.
- Izumori, K., Ueda, Y., & Yamanaka, K. (1978). Pentose metabolism in *Mycobacterium smegmatis*: Comparison of L-arabinose isomerases induced by L-arabinose and D-galactose. *Journal of Bacteriology*, 133(1), 413–414.
- Izumori, K., Miyoshi, T., Tokuda, S., & Yamabe, K. (1984). Production of D-tagatose from dulcitol by *Arthrobacter globiformis*. *Applied and Environmental Microbiology*, 48(5), 1055–1057.
- Izumori, K., & Tsuzaki, K. (1988). Production of D-tagatose from D-galactitol by *Mycobacterium smegmatis*. *Journal of Fermentation Technology*, 66(2), 225–227. [https://doi.org/10.1016/0385-6380\(88\)90052-0](https://doi.org/10.1016/0385-6380(88)90052-0)
- Jagtap, S. S., Singh, R., Kang, Y. C., Zhao, H., & Lee, J. K. (2014). Cloning and characterization of a galactitol 2-dehydrogenase from *Rhizobium leguminosarum* and its application in D-tagatose production. *Enzyme and Microbial Technology*, 58–59, 44–51. <https://doi.org/10.1016/j.enzmictec.2014.02.012>
- Jorgensen, E., Hansen, O. C., & Stougaard, P. (2004). Enzymatic conversion of D-galactose to D-tagatose: Heterologous expression and characterization of a thermostable L-arabinose isomerase from *Thermoanaerobacter mathranii*. *Applied Microbiology and Biotechnology*, 64(6), 816–822. <https://doi.org/10.1007/s00253-004-1578-6>
- Jung, E. S., Kim, H. J., & Oh, D. K. (2005). Tagatose production by immobilized recombinant *Escherichia coli* cells containing *Geobacillus stearothermophilus* L-arabinose isomerase mutant in a packed-bed bioreactor. *Biotechnology Progress*, 21(4), 1335–1340. <https://doi.org/10.1021/bp050078p>
- Karabinos, J. V. (1952). Psicose, sorbose and tagatose. *Advances in Carbohydrate Chemistry*, 7, 99–136. [https://doi.org/10.1016/S0096-5332\(08\)60083-1](https://doi.org/10.1016/S0096-5332(08)60083-1)
- Kaveeshwar, S. A., & Cornwall, J. (2014). The current state of diabetes mellitus in India. *Australasian Medical Journal*, 7(1), 45–48. <https://doi.org/10.4066/AMJ.2013.1979>
- Kim, P. (2004). Current studies on biological tagatose production using L-arabinose isomerase: A review and future perspective. *Applied Microbiology and Biotechnology*, 65(3), 243–249. <https://doi.org/10.1007/s00253-004-1665-8>
- Kim, P., Yoon, S. H., Roh, H. J., & Choi, J. H. (2001a). High production of D-tagatose, a potential sugar substitute, using immobilized L-arabinose isomerase. *Biotechnology Progress*, 17(1), 208–210. <https://doi.org/10.1021/bp000147u>
- Kim, P., Yoon, S. H., Seo, M. J., Oh, D. K., & Choi, J. H. (2001b). Improvement of tagatose conversion rate by genetic evolution of thermostable galactose isomerase. *Biotechnology and Applied Biochemistry*, 34(Pt2), 99–102. <https://doi.org/10.1042/BA20010025>
- Kim, B. C., Lee, Y. H., Lee, H. S., Lee, D. W., Choe, E. A., & Pyun, Y. R. (2002). Cloning, expression and characterization of L-arabinose isomerase from *Thermotoga neapolitana*: Bio-conversion of D-galactose to D-tagatose using the enzyme. *FEMS Microbiology Letters*, 212(1), 121–126. <https://doi.org/10.1111/j.1574-6968.2002.tb11254.x>
- Kim, J. W., Kim, Y. W., Roh, H. J., Kim, H. Y., Cha, J. H., Park, K. H., & Park, C. S. (2003a). Production of tagatose by a recombinant thermostable L-arabinose isomerase from *Thermus sp.* IM6501. *Biotechnology Letters*, 25(12), 963–967. <https://doi.org/10.1023/A:1024069813839>
- Kim, H. J., Ryu, S. A., Kim, P., & Oh, D. K. (2003b). A feasible enzymatic process for D-tagatose production by an immobilized thermostable L-arabinose isomerase in a packed-bed bioreactor. *Biotechnology Progress*, 19(2), 400–404. <https://doi.org/10.1021/bp025675f>
- Kim, H. J., & Oh, D. K. (2005). Purification and characterization of an L-arabinose isomerase from an isolated strain of *Geobacillus thermodenitrificans* producing D-tagatose. *Journal of Biotechnology*, 120(2), 162–73. <https://doi.org/10.1016/j.jbiotec.2005.06.004>
- Kim, H. J., Kim, J. H., Oh, H. J., & Oh, D. K. (2006). Characterization of a mutated *Geobacillus stearothermophilus* L-arabinose isomerase that increases the production rate of D-tagatose. *Journal of Applied Microbiology*, 101(1), 213–221. <https://doi.org/10.1111/j.1365-2672.2006.02975.x>
- Kim, S. B., Lee, Y. M., Park, S. W., Kim, J. H., Song, S. H., & Lee, K. P., inventors (2012). Recombinant GRAS strains expressing thermophilic arabinose isomerase as an active form and method of preparing food grade tagatose by using the same. U.S. Patent 8137946B2. Retrieved from <https://www.google.com/patents/US8137946>
- Kim, B. J., Hong, S. H., Shin, K. C., Jo, Y. S., & Oh, D. K. (2014a). Characterization of a F280N variant of L-arabinose isomerase from *Geobacillus thermodenitrificans* identified as a D-galactose isomerase. *Applied Microbiology and Biotechnology*, 98(22), 9271–9281. <https://doi.org/10.1007/s00253-014-5827-z>
- Kim, S. B., Lee, Y. M., Park, S. W., Kim, J. H., Song, S. H., Lee, K. P., ... Choi, H., inventors (2014b). Arabinose isomerase expressed from *Corynebacterium* genus and tagatose manufacturing method by using it. U.S. Patent 8802393B2
- Kruger, C. L., Whittaker, M. H., Franks, V. H., & Schroeder, R. E. (1999a). Developmental toxicity study of D-tagatose in rats. *Regulatory Toxicology and Pharmacology*, 29(2 Pt 2), S29–S35. <https://doi.org/10.1006/rtp.1998.1261>
- Kruger, C. L., Whittaker, M. H., Franks, V. H., & Trimmer, G. W. (1999b). 90-Day oral toxicity study of D-tagatose in rats. *Regulatory Toxicology and Pharmacology*, 29(2 Pt 2), S1–S10. <https://doi.org/10.1006/rtp.1998.1262>
- Lærke, H. N., & Jensen, B. B. (1999). D-Tagatose has low small intestinal digestibility but high large intestinal fermentability in pigs. *Journal of Nutrition*, 129(5), 1002–1009.
- Lærke, H. N., Jensen, B. B., & Hojsgaard, S. (2000). In vitro fermentation pattern of D-tagatose is affected by adaptation of microbiota from gastrointestinal tract of pigs. *Journal of Nutrition*, 130(7), 1772–1779.
- Lee, D. W., Jang, H. J., Choe, E. A., Kim, B. C., Lee, S. J., Kim, S. B., ... Pyun, Y. R. (2004). Characterization of a thermostable L-arabinose (D-galactose) isomerase from the hyperthermophilic bacterium *Thermotoga maritima*. *Applied and Environmental Microbiology*, 70(3), 1397–1404. <https://doi.org/10.1128/AEM.70.3.1397-1404.2004>
- Lee, D. W., Choe, E. A., Kim, S. B., Eom, S. H., Hong, Y. H., Lee, S. J., ... Pyun, Y. R. (2005a). Distinct metal dependence for catalytic and structural functions in the L-arabinose isomerases from the mesophilic *Bacillus halodurans* and the thermophilic *Geobacillus stearothermophilus*. *Archives of Biochemistry and Biophysics*, 434(2), 333–343. <https://doi.org/10.1016/j.abb.2004.11.004>
- Lee, S. J., Lee, D. W., Choe, E. A., Hong, Y. H., Kim, S. B., Kim, B. C., & Pyun, Y. R. (2005b). Characterization of a thermoacidophilic L-arabinose isomerase from *Alcalyobacillus acidocaldarius*: Role of Lys-269 in pH optimum. *Applied and Environmental Microbiology*, 71(12), 7888–7896. <https://doi.org/10.1128/AEM.71.12.7888-7896.2005>
- Levin, G. V., Zehner, L. R., Saunders, J. P., & Beadle, J. R. (1995). Sugar substitutes: Their energy values, bulk characteristics, and potential health benefits. *The American Journal of Clinical Nutrition*, 62(5 Suppl), 1161S–1168S.
- Levin, G. V., inventor (2001). Increased fertility and improved fetal development drug. U.S. Patent 6225452 B1. Retrieved from <https://www.google.co.in/patents/US6225452>
- Levin, G. V., inventor (2006). Use of tagatose to enhance key blood factors. European Patent 1173 186B1. Retrieved from <https://data.epo.org/publication-server/rest/v1.0/publication-dates/20060614/patents/EP1173186NWB1/document.pdf>
- Li, Y., Zhu, Y., Liu, A., & Sun, Y. (2011). Identification and characterization of a novel L-arabinose isomerase from *Anoxybacillus flavithermus* useful in D-tagatose production. *Extremophiles*, 15(3), 441–450. <https://doi.org/10.1007/s00792-011-0375-2>
- Li, G., Xu, Z., Li, S., & Xu, H. (2012). Probing the essential catalytic residues and substrate affinity in thermophilic L-arabinose isomerase by homology modeling and site-directed mutagenesis. *Chinese Journal of Catalysis*, 33(10), 1717–1723. <https://doi.org/10.3724/SPJ.1088.2012.20606>
- Liang, M., Chen, M., Liu, X., Zhai, Y., Liu, X., Zhang, H., ... Wang, P. (2012). Bioconversion of D-galactose to D-tagatose: Continuous packed bed reaction with an immobilized thermostable L-arabinose isomerase and efficient purification by selective microbial degradation. *Applied Microbiology and Biotechnology*, 93(4), 1469–1474. <https://doi.org/10.1007/s00253-011-3638-z>
- Lim, B. C., Kim, H. J., & Oh, D. K. (2007). High production of D-tagatose by the addition of boric acid. *Biotechnology Progress*, 23(4), 824–828. <https://doi.org/10.1021/bp070056y>
- Lim, B. C., Kim, H. J., & Oh, D. K. (2008). Tagatose production with pH control in a stirred tank reactor containing immobilized L-arabinose isomerase from *Thermotoga neapolitana*. *Applied Biochemistry and Biotechnology*, 149(3), 245–253. <https://doi.org/10.1007/s12010-007-8095-x>
- Lindberg, B. (1955). Studies on the chemistry of lichens. VIII. Investigation of a Dermatocarpon and some *Rocella* species. *Acta Chemica Scandinavica*, 9(6), 917–919.
- Liu, Y., Li, S., Xu, H., Wu, L., Xu, Z., Liu, J., & Feng, X. (2014). Efficient Production of D-tagatose using a food-grade surface display system. *Journal of Agriculture and Food Chemistry*, 62(28), 6756–6762. <https://doi.org/10.1021/jf501937j>
- Lobry de Bruyn, C. A., & Alberda van Ekenstein, W. (1897). Action des alcalis sur les sucres. I: Transformation de la galactose. Les tagatoses, et la galatose. *Recueil des Travaux Chimiques des Pays-Bas*, 16(9), 262–273. <https://doi.org/10.1002/recl.18970160902>
- Lu, Y., Levin, G. V., & Donner, T. W. (2008). Tagatose, a new antidiabetic and obesity control drug. *Diabetes Obesity and Metabolism*, 10(2), 109–134. <https://doi.org/10.1111/j.1463-1326.2007.00799.x>
- Manjasetty, B. A., & Chance, M. R. (2006). Crystal structure of *Escherichia coli* L-arabinose isomerase (ECAI), the putative target of biological tagatose production. *Journal of Molecular Biology*, 360, 297–309.
- Manzoni, M., Rollini, M., & Bergomi, S. (2001). Biotransformation of D-galactitol to tagatose by acetic acid bacteria. *Process Biochemistry*, 36(10), 971–977. [https://doi.org/10.1016/S0032-9592\(01\)00137-6](https://doi.org/10.1016/S0032-9592(01)00137-6)
- Martinez, P., Carrascosa, J. M., & Nunez de Castro, I. (1987). Ketose induced respiratory inhibition in isolated hepatocytes. *Revista Espanola De Fisiologia*, 43(2), 163–171.
- Masoro, E. J. (1992). Aging and proliferative homeostasis: Modulation by food restriction in rodents. *Laboratory Animal Science*, 42(2), 132–137.
- Masoro, E. J. (1993). Dietary restriction and aging. *Journal of the American Geriatrics Society*, 41(9), 994–999. <https://doi.org/10.1111/j.1532-5415.1993.tb06767.x>
- Mei, W., Wang, L., Zang, Y., Zheng, Z., & Ouyang, J. (2016). Characterization of an L-arabinose isomerase from *Bacillus coagulans* NL01 and its application for D-tagatose production. *BMC Biotechnology*, 16(1), 55. <https://doi.org/10.1186/s12896-016-0286-5>
- Men, Y., Zhu, Y., Zhang, L., Kang, Z., Izumori, K., Sun, Y., & Ma, Y. (2014). Enzymatic conversion of D-galactose to D-tagatose: Cloning, over expression and characterization of L-arabinose isomerase from *Pediococcus pentosaceus* PC-5. *Microbiological Research*, 169(2–3), 171–178. <https://doi.org/10.1016/j.micres.2013.07.001>
- Muniruzzaman, S., Tokunaga, H., & Izumori, K. (1994). Isolation of *Enterobacter agglomerans* strain 221e from soil, a potent D-tagatose producer from galactitol. *Journal of Fermentation and Bioengineering*, 78(2), 145–148. [https://doi.org/10.1016/0922-338X\(94\)90253-4](https://doi.org/10.1016/0922-338X(94)90253-4)
- Nakazawa, H., Genka, C., & Fujishima, M. (1996). Pathological aspects of active oxygen-free radicals. *The Japanese Journal of Physiology*, 46(1), 15–32. <https://doi.org/10.2170/jjphysiol.46.15>
- Oh, H. J., Kim, H. J., & Oh, D. K. (2006). Increase in D-tagatose production rate by site-directed mutagenesis of L-arabinose isomerase from *Geobacillus thermodenitrificans*. *Biotechnology Letters*, 28(3), 145–149. <https://doi.org/10.1007/s10529-005-5327-4>

- Patel, M. J., Akhiani, R. C., Patel, A. T., Dedania, S. R., & Patel, D. H. (2017). A single and two step isomerization process for D-tagatose and L-ribose bio-production using L-arabinose isomerase and D-lyxose isomerase. *Enzyme and Microbial Technology*, 97, 27–33. <https://doi.org/10.1016/j.enzmictec.2016.11.001>
- Paterna, J. C., Boess, F., Staubli, A., & Boelsterli, U. A. (1998). Antioxidant and cytoprotective properties of D-tagatose in cultured murine hepatocytes. *Toxicology and Applied Pharmacology*, 148(1), 117–125. <https://doi.org/10.1006/taap.1997.8315>
- Rao, D., Gullapalli, P., Yoshihara, A., Jenkinson, S. F., Morimoto, K., Takata, G., ... Izumori, K. (2008). Direct production of L-tagatose from L-psicose by *Enterobacter aerogenes* 230S. *Journal of Bioscience and Bioengineering*, 106(5), 473–480. <https://doi.org/10.1263/jbb.106.473>
- Rhimi, M., & Bejar, S. (2006). Cloning, purification and biochemical characterization of metallic-ions independent and thermoactive L-arabinose isomerase from the *Bacillus stearothermophilus* US100 strain. *Biochimica et Biophysica Acta*, 1760(2), 191–199. <https://doi.org/10.1016/j.bbagen.2005.11.007>
- Rhimi, M., Aghajari, N., Juy, M., Chouayekh, H., Maguin, E., Haser, R., & Bejar, S. (2009). Rational design of *Bacillus stearothermophilus* US100 L-arabinose isomerase: Potential applications for D-tagatose production. *Biochimie*, 91(5), 650–653. <https://doi.org/10.1016/j.biochi.2009.01.014>
- Rhimi, M., Ihammami, R., Bajic, G., Boudebouze, S., Maguin, E., Haser, R., & Aghajari, N. (2010). The acid tolerant L-arabinose isomerase from the food grade *Lactobacillus sakei* 23K is an attractive D-tagatose producer. *Bioresource Technology*, 101(23), 9171–9177. <https://doi.org/10.1016/j.biortech.2010.07.036>
- Rhimi, M., Bajic, G., Ihammami, R., Boudebouze, S., Maguin, E., Haser, R., & Aghajari, N. (2011a). The acid-tolerant L-arabinose isomerase from the mesophilic *Shewanella* sp. ANA-3 is highly active at low temperatures. *Microbial Cell Factories*, 10, 96. <https://doi.org/10.1186/1475-2859-10-96>
- Rhimi, M., Chouayekh, H., Gouillouard, L., Maguin, E., & Bejar, S. (2011b). Production of D-tagatose, a low caloric sweetener during milk fermentation using L-arabinose isomerase. *Bioresource Technology*, 102(3), 3309–3315. <https://doi.org/10.1016/j.biortech.2010.10.078>
- Rhimi, M., Bermudez-Humaran, L. G., Huang, Y., Boudebouze, S., Gaci, N., Garnier, A., ... Maguin, E. (2015). The secreted L-arabinose isomerase displays anti-hyperglycemic effects in mice. *Microbial Cell Factories*, 14, 204. <https://doi.org/10.1186/s12934-015-0391-5>
- Ryu, S. A., Kim, C. S., Kim, H. J., Baek, D. H., & Oh, D. K. (2003). Continuous D-tagatose production by immobilized thermostable L-arabinose isomerase in a packed-bed bioreactor. *Biotechnology Progress*, 19(6), 1643–1647. <https://doi.org/10.1021/bp0340739>
- Salonen, N., Salonen, K., Leisola, M., & Nyssola, A. (2013). D-Tagatose production in the presence of borate by resting *Lactococcus lactis* cells harboring *Bifidobacterium longum* L-arabinose isomerase. *Bioprocess and Biosystems Engineering*, 36(4), 489–497. <https://doi.org/10.1007/s00449-012-0805-2>
- Samanta, A. K., Jayapal, N., Jayaram, C., Roy, S., Kolte, A. P., Senani, S., & Sridhar, M. (2015). Xylooligosaccharides as prebiotics from agricultural by-products: Production and applications. *Bioactive Carbohydrate and Dietary Fibre*, 5(1), 62–71. <https://doi.org/10.1016/j.bcdf.2014.12.003>
- Saunders, J. P., Zehner, L. R., & Levin, G. V. (1999a). Disposition of D-[U-14C]-tagatose in the rat. *Regulatory Toxicology and Pharmacology*, 29(2), S46–S56. <https://doi.org/10.1006/rtp.1998.1251>
- Saunders, J. P., Donner, T. W., Sadler, J. H., Levin, G. V., & Makris, N. G. (1999b). Effects of acute and repeated oral doses of D-tagatose on plasma uric acid in normal and diabetic humans. *Regulatory Toxicology and Pharmacology*, 29(2 Pt 2), S57–S65. <https://doi.org/10.1006/rtp.1998.1264>
- Seo, M. J. (2013). Characterization of an L-arabinose isomerase from *Bacillus thermoglucosidarius* for D-tagatose production. *Bioscience, Biotechnology, and Biochemistry*, 77(2), 385–388. <https://doi.org/10.1271/bbb.120723>
- Seoane, J., Gomez-Foix, A. M., O'Doherty, R. M., Gomez-Ara, C., Newgard, C. B., & Guinovart, J. J. (1996). Glucose 6-phosphate produced by glucokinase, but not hexokinase I, promotes the activation of hepatic glycogen synthase. *Journal of Biological Chemistry*, 271(39), 23756–23760. <https://doi.org/10.1074/jbc.271.39.23756>
- Shaw, D. R. D. (1956). Polyol dehydrogenases. 3. Galactitol dehydrogenase and D-iditoldehydrogenase. *Biochemical Journal*, 64(3), 316–322.
- Shimonishi, T., Okumura, Y., & Izumori, K. (1995). Production of L-tagatose from galactitol by *Klebsiella pneumoniae* strain 40b. *Journal of Fermentation and Bioengineering*, 79(6), 620–622. [https://doi.org/10.1016/0922-338X\(95\)94759-K](https://doi.org/10.1016/0922-338X(95)94759-K)
- Torres, P. R., Manzo, R. M., Rubiolo, A. C., Batista-Viera, F. D., & Mammarella, E. J. (2014). Purification of an L-arabinose isomerase from *Enterococcus faecium* DBFIQ E36 employing a biospecific affinity strategy. *Journal of Molecular Catalysis B: Enzymatic*, 102, 99–105. <https://doi.org/10.1016/j.molcatb.2014.01.023>
- Totton, E. L., & Lardy, H. A. (1949). The synthesis of D-tagatose by biochemical oxidation and by improved chemical method. *Journal of the American Chemical Society*, 71(9), 3076–3078. <https://doi.org/10.1021/ja01177a037>
- USFDA. (2010). GRAS exemption claim for D-tagatose as an ingredient in foods. U.S. Food and Drug Administration. Retrieved from <https://www.fda.gov/downloads/Food/IngredientsPackagingLabeling/GRAS/NoticeInventory/ucm269560.pdf> (Accessed on 7th February, 2018)
- Valeri, F., Boess, F., Wolf, A., Goldlin, C., & Boelsterli, U. A. (1997). Fructose and tagatose protect against oxidative cell injury by iron chelation. *Free Radical Biology and Medicine*, 22(1–2), 257–268. [https://doi.org/10.1016/S0891-5849\(96\)00331-0](https://doi.org/10.1016/S0891-5849(96)00331-0)
- Wanarska, M., & Kur, J. (2012). A method for the production of D-tagatose using a recombinant *Pichia pastoris* strain secreting β -D-galactosidase from *Arthrobacter chlorophenolicus* and a recombinant L-arabinose isomerase from *Arthrobacter* sp 22c. *Microbial Cell Factories*, 11, 113. <https://doi.org/10.1186/1475-2859-11-113>
- World Health Organization. (2004). Evaluation of certain food additives and contaminants. Sixty first report of the Joint FAO/WHO expert committee on food additives. WHO Technical Report Series 922. Retrieved from https://apps.who.int/iris/bitstream/10665/42849/1/WHO_TRS_922
- Xu, Z., Qing, Y. J., Li, S., Feng, X. H., Xu, H., & Ouyang, P. K. (2011). A novel L-arabinose isomerase from *Lactobacillus fermentum* CGMCC2921 for D-tagatose production: Gene cloning, purification and characterization. *Journal of Molecular Catalysis B: Enzymatic*, 70(1–2), 1–7. <https://doi.org/10.1016/j.molcatb.2011.01.010>
- Xu, Z., Li, S., Fu, F. G., Li, G. X., Feng, X., Xu, H., & Ouyang, P. K. (2012). Production of D-tagatose, a functional sweetener, utilizing alginate immobilized *Lactobacillus fermentum* CGMCC2921 cells. *Applied Biochemistry and Biotechnology*, 166(4), 961–973. <https://doi.org/10.1007/s12010-011-9484-8>
- Xu, Z., Li, S., Feng, X., Liang, J., & Xu, H. (2014a). L-Arabinose isomerase and its use for biotechnological production of rare sugars. *Applied Microbiology and Biotechnology*, 98(21), 8869–8878. <https://doi.org/10.1007/s00253-014-6073-0>
- Xu, Z., Li, S., Feng, X., Zhan, Y., & Xu, H. (2014b). Function of aspartic acid residues in optimum pH control of L-arabinose isomerase from *Lactobacillus fermentum*. *Applied Microbiology and Biotechnology*, 98, 3987–3996. <https://doi.org/10.1007/s00253-013-5342-7>
- Xu, Z., Li, S., Liang, J., Feng, X., & Xu, H. (2015). Protein purification, crystallization and preliminary X-ray diffraction analysis of L-arabinose isomerase from *Lactobacillus fermentum* CGMCC2921. *Acta Crystallographica Section F*, 71, 28–33. <https://doi.org/10.1107/S2053230X14025321>
- Yoon, S. H., Kim, P., & Oh, D. K. (2003). Properties of L-arabinose isomerase from *Escherichia coli* as biocatalyst for tagatose production. *World Journal of Microbiology and Biotechnology*, 19(1), 47–51. <https://doi.org/10.1023/A:1022575601492>
- Yoshihara, K., Shinohara, Y., Hirotsu, T., & Izumori, K. (2006). Bioconversion of D-psicose to D-tagatose and D-talitol by *Mucoraceae* Fungi. *Journal of Bioscience and Bioengineering*, 101(3), 219–222. <https://doi.org/10.1263/jbb.101.219>
- Yu, P. L., Hodge, R. A., & Li, X. P. (1990). *In vitro* expression of Lac-PTS and tagatose 1,6-bisphosphate aldolase genes from *Lactococcus lactis subsp cremoris* plasmid pDI-21. *Applied Microbiology and Biotechnology*, 33(6), 677–679. <https://doi.org/10.1007/BF00604936>