

SUGAR ALCOHOLS: CHEMISTRY, PRODUCTION, HEALTH CONCERNS AND NUTRITIONAL IMPORTANCE OF MANNITOL, SORBITOL, XYLITOL, AND ERYTHRITOL

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ABSTRACT

The sugar alcohols commonly found in foods are sorbitol, mannitol, xylitol, erythritol, isomalt, and hydrogenated starch hydrolysates. Sugar alcohols come from plant products such as fruits and berries. Sugar alcohols occur naturally and at one time, mannitol was obtained from natural sources. Today, they are often obtained by hydrogenation of sugars and other techniques. Sugar alcohols do not contribute to tooth decay. Consumption of sugar alcohols may affect blood sugar levels, although less than of sucrose. Sugar alcohols, with the exception of erythritol, may also cause bloating and diarrhea when consumed in excessive amounts. Mannitol and sorbitol are isomers, the only difference being the orientation of the hydroxyl group on carbon 2. Among production methods of mannitol are Industrial synthesis, Biosyntheses, Natural extraction, chemical process, microbial process. Most sorbitol is made from corn syrup, but it is also found in apples, pears, peaches, and prunes. It is converted to fructose by sorbitol-6-phosphate 2-dehydrogenase.

Xylitol is a "tooth-friendly", nonfermentable sugar alcohol. It appears to have more dental health benefits than other polyalcohols. The structure of xylitol contains a tridentate ligand, (H-C-OH)₃ that can rearrange with polyvalent cations like Ca²⁺. This interaction allows Ca²⁺ to be transported through the gut wall barrier and through. Xylitol is produced by hydrogenation of xylose, which converts the sugar (an aldehyde) into a primary alcohol. Another method of producing xylitol is through microbial processes, including fermentative and biocatalytic processes in bacteria, fungi, and yeast cells, which take advantage of the xylose-intermediate fermentations to produce high yield of xylitol.

In the body, most erythritol is absorbed into the bloodstream in the small intestine, and then for the most part excreted unchanged in the urine. About 10% enters the colon. Because 90% of erythritol is absorbed before it enters the large intestine, it does not normally cause laxative effects. Chemical and fermentative processes have been introduced for large-scale production of erythritol. Erythritol can be synthesized from dialdehyde starch by high-temperature chemical reaction in the presence of a nickel catalyst.

INTRODUCTION

Sugar alcohols (also called polyhydric alcohols, polyalcohols, alditols or glycitols) are organic compounds, typically derived from sugars, comprising a class of polyols. Contrary to what the name may suggest, a sugar alcohol is neither a sugar nor an alcoholic beverage. They are white, water-soluble solids that can occur naturally or be produced industrially from sugars. They are used widely in the food industry as thickeners and sweeteners. In commercial foodstuffs, sugar alcohols are commonly used in place of table sugar (sucrose), often in combination with high intensity artificial sweeteners to counter the low sweetness. Xylitol is perhaps the most popular sugar alcohol due to its similarity to sucrose in visual appearance and sweetness.

The sugar alcohols commonly found in foods are sorbitol, mannitol, xylitol, isomalt, and hydrogenated starch hydrolysates. Sugar alcohols come from plant products such as fruits and berries. The carbohydrate in these plant products is altered through a chemical process. The carbohydrate in these plant products is altered through a chemical process. These sugar substitutes provide somewhat fewer calories than table sugar (sucrose), mainly because they are not well absorbed and may even have a small laxative effect. Many so-called "dietetic" foods that are labeled "sugar free" or "no sugar added" in fact contain sugar alcohols. People with diabetes mistakenly think that foods labeled as "sugar free" or "no sugar added" will have no effect on their blood glucose. Foods containing these sugar alcohols need to have their calorie and carbohydrate contents accounted for in your overall meal plan, as it is carbohydrate that raises blood glucose levels. Since many people typically *overeat* "sugar free" or "no sugar added" foods, their blood glucose may be significantly elevated.

Sugar alcohols have the general formula $\text{HOCH}_2(\text{CHOH})_n\text{CH}_2\text{OH}$. In contrast, sugars have two fewer hydrogen atoms, for example $\text{HOCH}_2(\text{CHOH})_n\text{CHO}$ or $\text{HOCH}_2(\text{CHOH})_{n-1}\text{C}(\text{O})\text{CH}_2\text{OH}$. The sugar alcohols differ in chain length. Most have five- or six-carbon chains, because they are derived from pentoses (five-carbon sugars) and hexoses (six-carbon sugars), respectively. They have one OH group attached to each carbon. They are further differentiated by the relative orientation (stereochemistry) of these OH groups. Unlike sugars, which tend to exist as rings, sugar alcohols do not. They can however be dehydrated to give cyclic ethers, e.g. sorbitol can be dehydrated to isosorbide.

Sugar alcohols occur naturally and at one time, mannitol was obtained from natural sources. Today, they are often obtained by hydrogenation of sugars, using Raney nickel catalysts. The conversion of glucose and mannose to sorbitol and mannitol is given:



More than a million tons of sorbitol are produced in this way annually. Xylitol and lactitol are obtained similarly. Erythritol on the other hand is obtained by fermentation of glucose and sucrose.

Health effects

Sugar alcohols do not contribute to tooth decay (Bradshaw and Marsh, 1994). Consumption of sugar alcohols may affect blood sugar levels, although less than of sucrose. Sugar alcohols, with the exception of erythritol, may cause bloating and diarrhea when consumed in excessive amounts.

Common sugar alcohols

- Glycerol (3-carbon)
- Erythritol (4-carbon)
- Threitol (4-carbon)
- Arabitol (5-carbon)
- Xylitol (5-carbon)
- Ribitol (5-carbon)
- Mannitol (6-carbon)
- Sorbitol (6-carbon)
- Galactitol (6-carbon)
- Fucitol (6-carbon)
- Iditol (6-carbon)
- Inositol (6-carbon; a cyclic sugar alcohol)
- Volemitol (7-carbon)
- Isomalt (12-carbon)
- Maltitol (12-carbon)
- Lactitol (12-carbon)
- Maltotriitol (18-carbon)
- Maltotetraitol (24-carbon)
- Polyglycitol

Both disaccharides and monosaccharides can form sugar alcohols; however, sugar alcohols derived from disaccharides (e.g. maltitol and lactitol) are not entirely hydrogenated because only one aldehyde group is available for reduction.

The simplest sugar alcohol, ethylene glycol, is sweet but notoriously toxic. The more complex sugar alcohols are for the most part nontoxic.

Sugar alcohols as food additives

Table 1: Sugar alcohols' relative sweetness

Name	Sweetness relative to sucrose	Food energy (kcal/g)	Sweetness per food energy, relative to sucrose	Food energy for equal sweetness, relative to sucrose
Arabitol	0.7	0.2	14	7.1%
Erythritol	0.8	0.21	15	6.7%
Glycerol	0.6	4.3	0.56	180%
HSH	0.4–0.9	3.0	0.52–1.2	83–190%
Isomalt	0.5	2.0	1.0	100%
Lactitol	0.4	2.0	0.8	125%
Maltitol	0.9	2.1	1.7	59%
Mannitol	0.5	1.6	1.2	83%
Sorbitol	0.6	2.6	0.92	108%
Xylitol	1.0	2.4	1.6	62%
<i>Compare with: Sucrose</i>	<i>1.0</i>	<i>4.0</i>	<i>1.0</i>	<i>100%</i>

As a group, sugar alcohols are not as sweet as sucrose, and they have less food energy than sucrose. Their flavor is like sucrose, and they can be used to mask the unpleasant aftertastes of some high intensity sweeteners. Sugar alcohols are not metabolized by oral bacteria, and so they

do not contribute to tooth decay (Bradshaw and Marsh, 1994). They do not brown or caramelize when heated.

In addition to their sweetness, some sugar alcohols can produce a noticeable cooling sensation in the mouth when highly concentrated, for instance in sugar-free hard candy or chewing gum. This happens, for example, with the crystalline phase of sorbitol, erythritol, xylitol, mannitol, lactitol and maltitol. The cooling sensation is due to the dissolution of the sugar alcohol being an endothermic (heat-absorbing) reaction, one with a strong heat of solution (Cammenga et al., 1996).

Sugar alcohols are usually incompletely absorbed into the blood stream from the small intestines which generally results in a smaller change in blood glucose than "regular" sugar (sucrose). This property makes them popular sweeteners among diabetics and people on low-carbohydrate diets. However, like many other incompletely digestible substances, overconsumption of sugar alcohols can lead to bloating, diarrhea and flatulence because they are not absorbed in the small intestine. Some individuals experience such symptoms even in a single-serving quantity. With continued use, most people develop a degree of tolerance to sugar alcohols and no longer experience these symptoms. As an exception, erythritol is actually absorbed in the small intestine and excreted unchanged through urine, so it contributes no calories even though it is rather sweet.

The table above presents the relative sweetness and food energy of the most widely used sugar alcohols. Despite the variance in food energy content of sugar alcohols, EU labeling requirements assign a blanket value of 2.4 kcal/g to all sugar alcohols.

MANNITOL

Mannitol is a type of sugar which is also used as a medication (Wakai et al, 2013). As a sugar it is often used as a sweetener in diabetic food as it is poorly absorbed from the intestines. As a medication it is used to decrease high pressures in the eyes such as are seen in glaucoma and to lower increased intracranial pressure. Medically it is given by injection. Effects typically begin within 15 minutes and last up to 8 hours.

Common side effects from medical use include electrolyte problems and dehydration. Other serious side effects may include worsening heart failure and kidney problems. It is unclear if use is safe in pregnancy. Mannitol is in the osmotic diuretic family of medications and works by pulling fluid from the brain and eyes.

The discovery of mannitol is attributed to Joseph Louis Proust in 1806. It is on the World Health Organization's List of Essential Medicines, the most effective and safe medicines needed in a health system. The wholesale cost in the developing world is about 1.12 to 5.80 USD a dose. In the United States a course of treatment costs 25 to 50 USD. It was originally made from the flowering ash and called manna after its supposed resemblance to the Biblical food.

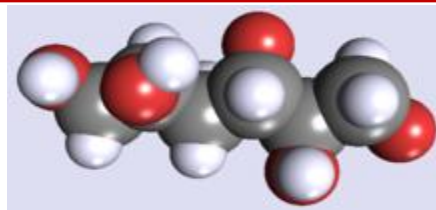
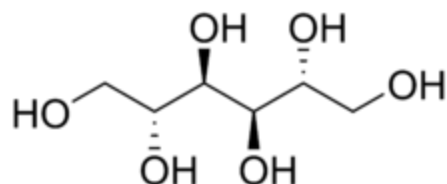


Fig. 1: Structure of D – mannitol

NUTRITIONAL AND MEDICAL USES OF MANNITOL

Mannitol is used to reduce acutely raised intracranial pressure until more definitive treatment can be applied, e.g., after head trauma.

It may also be used for certain cases of kidney failure with low urine output, decreasing pressure in the eye, to increase the elimination of certain toxins, and to treat fluid build up.

Mannitol acts as an osmotic laxative in oral doses larger than 20g and is sometimes sold as a laxative for children.

The use of mannitol, when inhaled as a bronchial irritant, as an alternative method of diagnosis of exercise induced asthma has been proposed. A 2013 systematic review concluded there is insufficient evidence to support its use for this purpose at this time.

Mannitol is commonly used in the circuit prime of a heart lung machine during cardiopulmonary bypass. The presence of mannitol preserves renal function during the times of low blood flow and pressure, while the patient is on bypass. The solution prevents the swelling of endothelial cells in the kidney, which may have otherwise reduced blood flow to this area and resulted in cell damage.

Mannitol can also be used to temporarily encapsulate a sharp object (such as a helix on a lead for an artificial pacemaker) while it is passed through the venous system. Because the mannitol dissolves readily in blood, the sharp point will become exposed at its destination.

Mannitol is the primary ingredient of Mannitol Salt Agar, a bacterial growth medium, and is used in others.

Mannitol is also the first drug of choice for the treatment of acute glaucoma in veterinary medicine. It is administered as a 20% solution IV. It dehydrates the vitreous humor and, therefore, lowers the intraocular pressure. However, it requires an intact blood-ocular barrier to work.

Mannitol increases blood glucose to a lesser extent than sucrose (thus having a relatively low glycemic index) and is therefore used as a sweetener for people with diabetes, and in chewing gums. Although mannitol has a higher heat of solution than most sugar alcohols, its comparatively low solubility reduces the cooling effect usually found in mint candies and gums. However, when mannitol is completely dissolved in a product, it induces a strong cooling effect. Also, it has a very low hygroscopicity – it does not pick up water from the air until the humidity level is 98%. This makes mannitol very useful as a coating for hard candies, dried fruits, and chewing gums, and it is often included as an ingredient in candies and chewing gum. The pleasant taste and mouthfeel of mannitol also makes it a popular excipient for chewable tablets.

Contraindication on Use of Mannitol

Mannitol is contraindicated in people with anuria, congestive heart failure and active cerebral haemorrhage (except during craniotomy). The three studies that initially found that high-dose mannitol was effective in cases of severe head injury have been the subject of a recent investigation. Although several authors are listed with Dr. Julio Cruz, it is unclear whether the authors had knowledge of how the patients were recruited. Further, the Federal University of São Paulo, which Dr. Cruz gave as his affiliation, has never employed him. As a result of doubt surrounding Cruz's work, an updated version of the Cochrane review excludes all studies by Julio Cruz, leaving only 4 studies. Due to differences in selection of control groups, a conclusion about the clinical use of mannitol could not be reached.

PRODUCTION OF MANNITOL

Mannitol is classified as a sugar alcohol; that is, it is derived from a sugar (mannose) by reduction. Other sugar alcohols include xylitol and sorbitol. Mannitol and sorbitol are isomers, the only difference being the orientation of the hydroxyl group on carbon 2. Among production methods of mannitol are Industrial synthesis, Biosyntheses, Natural extraction, chemical process, microbial process, etc.

Industrial synthesis

Mannitol is commonly produced via the hydrogenation of fructose, which is formed from either starch or sucrose (common table sugar). Although starch is a cheaper source than sucrose, the transformation of starch is much more complicated. Eventually, it yields syrup containing about 42% fructose, 52% glucose, and 6% maltose. Sucrose is simply hydrolyzed into invert sugar syrup, which contains about 50% fructose. In both cases, the syrups are chromatographically purified to contain 90–95% fructose. The fructose is then hydrogenated over a nickel catalyst into a mixture of isomers sorbitol and mannitol. Yield is typically 50%:50%, although slightly alkaline reaction conditions can slightly increase mannitol yields.

Biosyntheses

Mannitol is one of the most abundant energy and carbon storage molecules in nature, produced by a plethora of organisms, including bacteria, yeasts, fungi, algae, lichens, and many plants. Fermentation by microorganisms is an alternative to the traditional industrial synthesis. A fructose to mannitol metabolic pathway, known as the mannitol cycle in fungi, has been discovered in a type of red algae (*Caloglossa leprieurii*), and it is highly possible that other microorganisms employ similar such pathways. A class of lactic acid bacteria, labeled heterofermentive because of their multiple fermentation pathways, convert either three fructose molecules or two fructose and one glucose molecule into two mannitol molecules, and one molecule each of lactic acid, acetic acid, and carbon dioxide. Feedstock syrups containing medium to large concentrations of fructose (for example, cashew apple juice, containing 55% fructose: 45% glucose) can produce yields 200 g (7.1 oz) mannitol per liter of feedstock. Further research is being conducted, studying ways to engineer even more efficient mannitol pathways in lactic acid bacteria, as well as the use of other microorganisms such as yeast and *E. coli* in mannitol production. When food grade strains of any of the aforementioned microorganisms are

used, the mannitol and the organism itself are directly applicable to food products, avoiding the need for careful separation of microorganism and mannitol crystals. Although this is a promising method, steps are needed to scale it up to industrially needed quantities.

Natural extraction

Since mannitol is found in a wide variety of natural products, including almost all plants, it can be directly extracted from natural products, rather than chemical or biological syntheses. In fact, in China, isolation from seaweed is the most common form of mannitol production. Mannitol concentrations of plant exudates can range from 20% in seaweeds to 90% in the plane tree. It is a constituent of saw palmetto (*Serenoa*). Traditionally, mannitol is extracted by the Soxhlet extraction, utilizing ethanol, water, and methanol to steam and then hydrolysis of the crude material. The mannitol is then recrystallized from the extract, generally resulting in yields of about 18% of the original natural product. Another up and coming method of extraction is by using supercritical and subcritical fluids. These fluids are at such a stage that there is no difference between the liquid and gas stages, and are therefore more diffusive than normal fluids. This is considered to make them much more effective mass transfer agents than normal liquids. The super-/sub-critical fluid is pumped through the natural product, and the mostly mannitol product is easily separated from the solvent and minute amount of byproduct. Supercritical carbon dioxide extraction of olive leaves has been shown to require less solvent per measure of leaf than a traditional extraction — 141.7 g (5.00 oz) CO₂ versus 194.4 g (6.86 oz) ethanol per 1 g (0.035 oz) olive leaf. Heated, pressurized, subcritical water is even cheaper, and is shown to have dramatically greater results than traditional extraction. It requires only 4.01 g (0.141 oz) water per 1 g (0.035 oz) of olive leaf, and gives a yield of 76.75% mannitol. Both super- and sub-critical extractions are cheaper, faster, purer, and more environmentally friendly than the traditional extraction. However, the required high operating temperatures and pressures are causes for hesitancy in the industrial use of this technique.

Chemical process for production of mannitol

Mannitol is produced industrially by high pressure hydrogenation of fructose/glucose mixtures in aqueous solution at high temperature (120–160°C) with Raney nickel as a catalyst and hydrogen gas. α -Fructose is converted to mannitol and β -fructose is converted to sorbitol. The glucose is hydrogenated exclusively to sorbitol. Due to poor selectivity of the nickel catalyst, the hydrogenation of a 50:50 fructose/glucose mixture results in an approximately 25:75 mixture of mannitol and sorbitol. It is relatively difficult to separate sorbitol and mannitol. The requirement for separation of mannitol and sorbitol results in even higher production costs and decreased yields. According to Takemura et al. (1978), the yield of crystalline mannitol in the chemical process is only 17% (w/w) based on the initial sugar substrates. If sucrose is used as starting material and the hydrogenation is performed at alkaline pH, mannitol yields up to 31% can be obtained (Schwarz 1994). The hydrogenation of pure fructose results in mannitol yields of 48%–50%.

Makkee et al. (1985) developed a process involving both bio- and chemocatalysts for the conversion of glucose/fructose mixture into mannitol. Good yields (62%–66%) were obtained by using glucose isomerase (GI) immobilized on silica in combination with a copper-on-silica catalyst (water, pH ~7.0, 70°C, 50 kgcm⁻² of hydrogen, trace amounts of buffer, Mg(II), borate, and EDTA). In another method, mannitol is produced from mannose by hydrogenation with

stoichiometric yield (100% conversion) (Devos 1995). Mannose can be obtained from glucose by chemical epimerization with a yield of 30%–36% (w/w). Thus, the mannitol yield from glucose can be as high as 36%. If the non-epimerized glucose can be enzymatically isomerized to fructose by using GI, the mannitol yields could reach 50% (w/w) (Takemura et al. 1978). However, the total cost of using the multi-steps process is not economical. Devos (1995) suggested a process in which fructose is first isomerized to mannose using mannose isomerase. However, mannose isomerase is not yet commercially available for large scale use.

Microbial production of mannitol

Lactic acid bacteria (LAB), yeast, and fungi are known to produce mannitol from fructose or glucose (Smiley et al. 1967; Song et al. 2002; Wisselink et al. 2002; Saha 2003). Both homo- and heterofermentative LAB produce mannitol (Saha 2003; Saha and Racine 2008).

Mannitol production by homofermentative LAB

Some homofermentative LAB such as *Streptococcus mutants* and *Lactobacillus leichmanii* produce small amounts of mannitol from glucose (Chalfan et al. 1975). Forain et al. (1996) reported that a strain of *L. plantarum* deficient in both L- and D-lactate dehydrogenase (LDH) produces mannitol as an end-product of glucose catabolism. LAB use several strategies for regeneration of NAD⁺ during metabolism of sugars. Hols et al. (1999) showed that disruption of the *ldh* gene in

Lactococcus lactis strain NZ20076 leads to the conversion of acetate into ethanol as a rescue pathway for NAD⁺ regeneration. Neves et al. (2000) reported that a LDH-deficient mutant of *Lc. lactis* transiently accumulates intracellular mannitol, which was formed from fructose-6-phosphate by the combined action of mannitol-1-phosphate (M-1-P) dehydrogenase and phosphatase. They showed that the formation of M-1-P by the LDH-deficient strain during glucose catabolism is a consequence of impairment in NADH oxidation caused by a greatly reduced LDH activity, the transient formation of M-1-P serving as a regeneration pathway for NAD⁺ regeneration. Gaspar et al. (2004) described the construction of *Lc. lactis* strains able to form mannitol as an end-product of glucose metabolism, using a food-grade LDH-deficient strain as genetic basis for knocking out the gene *mtlA* or *mtlF*. Resting cells of the double mutant strains ($\Delta ldh\Delta mtlA$ and $\Delta ldh\Delta mtlF$) produced mannitol from glucose, with approximately one-third of the carbon being successfully channeled to the production of mannitol.

Mannitol production by heterofermentative LAB

A number of heterofermentative LAB of the genera *Lactobacillus*, *Leuconostoc*, and *Oenococcus* can produce mannitol directly from fructose (Saha 2003). In addition to mannitol, these bacteria may produce lactic acid, acetic acid, carbon dioxide, and ethanol. The process is based on the ability of the LAB to use fructose as an electron acceptor and reducing it to mannitol using the enzyme mannitol 2-dehydrogenase (MDH). Saha and Nakamura (2003) reported that nine strains of heterofermentative LAB (*L. brevis* NRRL B-1836, *L. buchneri* NRRL B-1860, *L. cellobiosus* NRRL B-1840, *L. fermentum* NRRL B-1915, *L. intermedius* NRRL B-3693, *Leu. amelilobiosum* NRRL B-742, *Leu. citrovorum* NRRL B-1147, *Leu. mesenteroides subsp. dextranicum* NRRL B-1120, and *Leu. paramesenteroides* NRRL B-3471) produce mannitol from fructose. The strain *L. intermedius* NRRL B-3693 produced 198 g mannitol from 300 g fructose l⁻¹ in pH-controlled (pH 5.0) fermentation at 37°C. The time of maximum mannitol production varied greatly from

15 h at 150 g fructose to 136 h at 300 g fructose l^{-1} . The bacterium converted fructose to mannitol from the early growth stage. One-third of fructose can be replaced with other substrates such as glucose, maltose, starch plus glucoamylase (simultaneous saccharification and fermentation, SSF), mannose, and galactose. Two-thirds of fructose can also be replaced by sucrose. The bacterium co-utilized fructose and glucose (2:1) simultaneously and produced very similar quantities of mannitol, lactic acid, and acetic acid in comparison with fructose only. The glucose was converted to lactic acid and acetic acid, and fructose was converted into mannitol.

Application of fed-batch fermentation by feeding equal amounts of substrate and medium four times decreased the maximum mannitol production time of fructose (300 gl^{-1}) from 136 to 92 h. The yields of mannitol, lactic acid, and acetic acid were 202, 53, and 39 gl^{-1} , respectively. With glucose (150 gl^{-1}) alone, the bacterium produced D- and L-lactic acids in equal ratios (total, 70 gl^{-1}) and ethanol (38 gl^{-1}) but no acetic acid.

A competitive production process for mannitol by fermentation would require inexpensive raw materials. Saha (2006) studied the production of mannitol by *L.*

intermedius NRRL B-3693 using molasses as a carbon source. The bacterium produced mannitol (104 gl^{-1}) from a mixture of molasses and fructose syrup (1:1; total sugars, 150 gl^{-1} ; fructose/glucose, 4:1) in 16 h. Several kinds of inexpensive organic and inorganic nitrogen sources and corn steep liquor (CSL) were evaluated for their potential to replace more expensive nitrogen sources derived from Bactopeptone and Bacto-yeast extract. Soy peptone (5 gl^{-1}) and CSL (50 gl^{-1}) were found to be suitable substitutes for Bacto-peptone (5 gl^{-1}) and Bacto-yeast extract (5 gl^{-1}), respectively. The bacterium produced 105 g mannitol from 150 g molasses and fructose syrup (1:1) in 22 h using 5 g soy peptone and 50 g CSL l^{-1} . The effects of four salt nutrients (ammonium citrate, sodium phosphate, $MgSO_4$, and $MnSO_4$) on the production of mannitol by *L. intermedius* NRRL B-3693 in a simplified medium containing 300 g fructose, 5 g soy peptone, and 50 g CSL l^{-1} in pH controlled fermentation at 5.0 at 37°C were evaluated using a fractional factorial design (Saha 2006). Only $MnSO_4$ was found to be essential for mannitol production and 33 $mg l^{-1}$ was found to support maximum mannitol production. The bacterium produced 200 g mannitol, 62 g lactic acid, and 40 g acetic acid from 300 g fructose l^{-1} in 67 h. Saha and Racine (2007) improved the fermentation process further for the production of mannitol by *L. intermedius* NRRL B-3693. A fed-batch protocol overcame limitations caused by high substrate concentrations. The fed-batch process resulted in the accumulation of 176 g mannitol from 184 g fructose and 92 g glucose l^{-1} of final fermentation broth in 30 h with a volumetric productivity of 5.9 $gl^{-1}h^{-1}$. Further increases in volumetric productivity of mannitol were obtained in a continuous cell-recycle fermentation process that reached more than 40 $gl^{-1}h^{-1}$.

Mannitol production by filamentous fungi

Several filamentous fungi produce mannitol from glucose. Yamada et al. (1961) showed that glucose is first converted to F-6-P, which is then reduced to M-1-P in the presence of NADH, and M-1-P is hydrolyzed to mannitol by a specific phosphatase in *Pircularia oryzae*. Smiley et al. (1967) studied the biosynthesis of mannitol from glucose by *Aspergillus candidus*. The fungal strain converted glucose to mannitol with 50% yield based on glucose consumed in 10–16 days by feeding glucose daily with a volumetric productivity of 0.15 $gl^{-1}h^{-1}$ and a yield of 31.0 mol%. The presence of glucose in the medium was essential to prevent metabolism of mannitol. Nelson et al. (1971) reported the production of mannitol from glucose and other sugars by conidia of *A.*

candidus. Low pH (~3.0) favored the percentage yield but decreased the fermentation rate. *A. candidus* produced mannitol from 2% glucose in 75% yield (based on sugar consumed) in 7 days at 28 °C. The fungus converts glucose to mannitol via F-6-P and M-1-P (Strandberg 1969). Lee (1967) determined the carbon balance for fermentation of glucose to mannitol by *Aspergillus sp.* The products found were: cells (17% of carbon input), CO₂ (26%), mannitol (35%), glycerol (10%), erythritol (2.5%), glycogen (1%), and unidentified compounds (8%). Cell-free enzyme studies indicated that mannitol was produced via the reduction of fructose-6-phosphate.

Hendriksen et al. (1988) screened 11 different *Penicillium* species for production of mannitol. All strains produced mannitol and glycerol from sucrose. The highest amount of mannitol (43 g l⁻¹) was produced by *P. scabrosum* IBT JTER4 and the highest combined yield of mannitol and glycerol (65 g l⁻¹) was obtained with *P. pH aethiopicum* IBT MILA 4 when grown on sucrose (150 g l⁻¹) and yeast extract (20 g l⁻¹) at 6.2 and 25 °C for 12 days. However, the volumetric productivity of mannitol from sucrose by the high mannitol producer *P. scabrosum* was only 0.14 g l⁻¹ h⁻¹. *Penicillium sp.* uses the same metabolic route for conversion of glucose to mannitol as *A. candidus* (Boosaeng et al. 1976). El-Kady et al. (1995) screened 500 filamentous fungal isolates belonging to ten genera and 74 species and identified *Aspergillus*, *Eurotium*, and *Fennellia* species as high (>1.82 g l⁻¹) producers of mannitol cultivated on liquid glucose–Czapek’s medium fortified with 15% NaCl and incubated at 28 °C as static cultures for 15 days.

Domelsmith et al. (1988) demonstrated that four fungal cultures—*Alternaria alternata*, *Cladosporium herbarum*, *Epicoccum purpurascens*, and *Fusarium pallidoroseum* isolated from cotton leaf dust produced mannitol and are a probable source of mannitol found in cotton dust.

Mannitol production by recombinant microorganisms

Although a few homofermentative LAB produce mannitol from glucose; production level is very low. Several heterofermentative LAB produce excellent quantities of mannitol (~66%) from fructose. They also produce lactic acid and acetic acid as coproducts. This is why a number of recombinant microorganisms have been developed to either overproduce mannitol or limit or eliminate the production of co-products (lactic acid and acetic acid). In this section, we review the literature dealing with the construction of recombinant organisms for mannitol production. Kaup et al. (2004) constructed an efficient *Escherichia coli* strain for mannitol production from fructose in a whole cell biotransformation. The strain expressed NAD⁺-dependent MDH from *Leu. pseudomesenteroides* ATCC 12291 (Hahn et al. 2003) for the reduction of fructose to mannitol, NAD⁺-dependent formate dehydrogenase (FDH) from *Mycobacterium vaccae* N10 (Galkin et al. 1995) for NADH regeneration, and the glucose facilitator from *Zymomonas mobilis* (Weisser et al. 1995; Parker et al. 1995) for the uptake of fructose without concomitant phosphorylation. The strain produced about 66 g mannitol from 90 g fructose l⁻¹ within 8 h with a yield of 73% and a specific mannitol productivity of >4 g per g cell dry weight (cdw) h⁻¹. Kaup et al. (2005) reported that supplementation of this recombinant strain with extracellular GI resulted in the formation of 145.6 g mannitol from 180 g glucose l⁻¹. They have co-expressed the *xylA* gene of *E. coli* in this recombinant *E. coli* strain which formed 83.7 g mannitol from 180 g glucose l⁻¹. Sasaki et al. (2005) cloned a gene encoding MDH from *L. reuteri* and expressed in *E. coli*. The purified recombinant enzyme works optimally at 37°C and pH 5.4 for conversion of fructose to mannitol. Aarnikunnas et al. (2003) used metabolic engineering of *L. fermentum* for production of mannitol and pure L-lactic acid or pyruvate. The authors first developed genetic

tools to modify *L. fermentum* and then proceeded to inactivate first *ldhD* gene and then *ldhL* gene in order to create a bacterium that could produce mannitol and either pure L-lactic acid or pyruvic acid in a single process. In bioreactor cultivations, the single mutant strain constructed by inactivation of the *ldhD* gene produced mannitol and L-lactic acid.

The double mutant strain created by inactivating the *ldhL* gene produced mannitol and pyruvate. In addition, the mutant produced 2, 3-butanediol and the volumetric productivity of mannitol was decreased. Helanto et al. (2005) described the construction and characterization of a random mutant of *Leu. pseudomesenteroides* that is unable to grow on fructose and the positive effects of the mutation on mannitol production. They have performed the inactivation of its fructokinase activity with random mutagenesis and screening the mutants unable to grow on fructose. The fructose uptake of the mutant was unaltered and the mutant converted fructose to mannitol when grown in a medium containing both glucose and fructose. The yield of mannitol from fructose was improved from 74 to 86 mol%. A fructokinase-negative mutant could enable higher pH to be used in the mannitol production process without lowering the yield. Wisselink et al. (2004) cloned and over-expressed *mtlD* from *L. plantarum* in *Lc. lactis* in different genetic backgrounds (a wild-type strain, an LDH-deficient strain, and a strain with reduced phosphofructokinase activity). Small amounts (<1%) of mannitol were formed by growing cells of *mtlD*-over-expressing LDH-deficient and phosphofructokinase reduced strains. The resting cells of the LDH deficient transformant converted 25% of glucose (3.6 g l^{-1}) to mannitol. They concluded that the *mtlD* over-expressing LDH-deficient *Lc. lactis* strain seemed to be the most promising strain for mannitol production. Liu et al. (2005) have cloned and expressed the *mtlK* gene encoding MDH from *L. brevis* in *E. coli*. The genetically engineered *E. coli* strain was able to catalyze the reduction of fructose to mannitol. Costenoble et al. (2003) demonstrated that mannitol is produced under anaerobic conditions by a glyceroldefective mutant of *Saccharomyces cerevisiae* expressing the *mtlD* gene from *E. coli* coding for NADH-dependent M-1-P dehydrogenase. Improving efflux of the formed mannitol seems to be necessary for obtaining anaerobic growth and a sustained mannitol production. Baumchen and Bringer-Meyer (2007) over-expressed MDH gene (*mdh*) from *Leu. pseudomesenteroides* and co-expressed FDH gene (*fdh*) in *Corynebacterium glutamicum* ATCC 13032. The recombinant *C. glutamicum* cells produced mannitol at a constant production rate of $0.22 \text{ g (g cdw)}^{-1}\text{h}^{-1}$. Expression of the glucose/fructose facilitator gene *glf* from *Z. mobilis* in *C. glutamicum* led to a 5-fold increased productivity of $1.25 \text{ g (g cdw)}^{-1}\text{h}^{-1}$, yielding 87 g mannitol from 93.7 g fructose. In repetitive fed-batch biotransformation, 285 g mannitol was formed over a period of 96 h with an average productivity of $1.0 \text{ g (g cdw)}^{-1}\text{h}^{-1}$.

Enzymatic production of mannitol

Mannitol can be enzymatically produced from fructose in a one pot synthesis by using NADH-dependent MDH or NADPH-dependent MDH. Saha (2004) purified MDH from *L. intermedius* NRRL B-3693 and showed that the purified enzyme can convert fructose to mannitol completely in the presence of NADPH. The cofactor dependency of the enzyme is a major limitation. A number of strategies such as enzymatic, electrochemical, chemical and photochemical, and biological methods are available for cofactor regeneration (Chenault and Whitesides 1987). A two-enzyme system can be used for cofactor regeneration with simultaneous conversion of two substrates into two products of interest (Wichmann et al. 1981). One example is the simultaneous conversion of fructose and formate using the enzymes MDH and FDH (Parmentier et al. 2005).

FDH converts formate to CO₂ and reduces NAD to NADH. MDH uses NADH to convert fructose to mannitol and regenerates NAD.

Synthesizing reaction: Fructose + NADH + H⁺ = Mannitol + NAD⁺

Regenerating reaction: Formic acid + NAD⁺ = CO₂ + NADH

Slatner et al. (1998) achieved a volumetric mannitol productivity of 2.25 g l⁻¹ h⁻¹ using a recombinant MDH from *P. fluorescens* overexpressed in *E. coli* and FDH from *C. boidinii* with a final product concentration of 72 g l⁻¹ and a fructose conversion of 80% in the system. The other product CO₂ is easily separated from mannitol. Mannitol was crystallized from the ultrafiltered product solution in 97% purity and 85% recovery, thus allowing reuse of enzymes for repeated batch production of mannitol. Another example of the two enzyme system for regeneration of cofactor is MDH and glucose dehydrogenase (GDH) system using glucose/fructose mixture (1:1) and simultaneous synthesis of mannitol and gluconic acid (Howaldt et al. 1988). NAD requiring GDH converts glucose to gluconic acid and generates NADH. MDH uses NADH to convert fructose to mannitol and regenerates NAD.

Synthesizing reaction: Fructose + NADH + H⁺ = Mannitol + NAD⁺

Regenerating reaction: Glucose + NAD⁺ + H₂O = Gluconic acid + NADH

Gluconic acid (market size, 35,000 tons year⁻¹), a multifunctional carbonic acid, is a bulk chemical used in the chemical, pharmaceutical, food, beverage, textile and other industries. It can be used for cleaning purposes and in the construction industry, where it can be used as a cement additive to increase cement resistance and stability under extreme climatic conditions, e.g., frost and floods. The MDH used was from *P. fluorescens*, *Torulaspora delbruckii*, and *Schizophyllum commune*, and the FDH was from *Bacillus megaterium*.

Downstream processing consists of the separation of enzymes from the product solution with the aid of reactor integrated membranes and of the isolation of the two products—mannitol and gluconic acid—by electrodialysis, ion-exchange chromatography, and fractional crystallization. Both NADH- and NADPH-dependent MDHs have been purified from a number of microorganisms. As for example, NADH-dependent MDH has been purified from *Lb. brevis*, *Leu. mesenteroides*, *P. fluorescens*, *Rhodobacter sphaeroides*, *Saccharomyces cerevisiae*, and *T. delbruckii* (Brunker et al. 1997). NADPH-dependent MDH has been purified from *A. parasitius*, *C. magnoliae*, *Z. mobilis*, and *Gluconobacter suboxydans* (Lee et al. 2003). Schneider and Giffhom (1989) reported an increase of 8.3-fold of MDH activity by constructing a strain (pAK82) from *R. sphaeroides* Si4 and by producing high cell concentrations via fed-batch cultivation in a bioreactor in comparison to batch cultivation of the wild-type strain. Mannose can be reduced to mannitol enzymatically. However, the reversible reaction favors mannitol oxidation and is thus not suitable (Stoop et al. 1998). Baumchen et al. (2007) developed an in vivo system for the biotransformation of fructose to mannitol by the expression of *mdh* gene encoding MDH from *Leu. pseudomesenteroides* in *B. megaterium*. The NADH reduction necessary for MDH activity was regenerated via the oxidation of formate to CO₂ by co-expression of *fdh* gene encoding *Mycobacterium vaccae* N10 FDH. Recombinant *B. megaterium*

produced up to 10.6 g mannitol per l at the shaking flask scale. Whole cell biotransformation in a fedbatch bioreactor increased the mannitol production to 22 g l^{-1} with a specific productivity of 0.32 $\text{g (g cdw)}^{-1}\text{h}^{-1}$ and a mannitol yield of 0.91 mol mol^{-1} . However, the substrate uptake was the limiting factor of the overall biotransformation. Song et al. (2008) also demonstrated that mannitol can be produced from glucose in a two step enzymatic process using a *Thermotoga neapolitana* xylose isomerase (GI) and *T. martina* MDH. However, in the absence of a cofactor regeneration system, the final mannitol concentration only reached 19 mM.

Medical, Food and Nutrition Applications of mannitol

Mannitol is used as a sweet-tasting bodying and texturing agent. It reduces the crystallization tendency of sugars and is used as such to increase the shelf life of foodstuffs. Crystalline mannitol exhibits a very low hygroscopicity, making it useful in products that are stable at high humidity. It is only about half as sweet as sucrose. Mannitol exhibits reduced physiological calorie value (1.6 kcalg^{-1}) compared to sucrose (4 kcalg^{-1}). It has a low solubility in water of only 18% (w/v) at 25 °C and 13% (w/v) at 14°C (Perry et al. 1997). In comparison, the solubility limit of sorbitol in water is about 70% (w/v) at 25°C. Mannitol is sparingly soluble in organic solvents such as ethanol and practically insoluble in ether, ketones, and hydrocarbons (Schwarz 1994). It forms orthorhombic crystals and the crystals have a melting point at 165–168 °C (Schwarz 1994). Mannitol is extensively used in chewing gum. It is chemically inert and is commonly used in the pharmaceutical formulation of chewable tablets and granulated powders. Mannitol prevents moisture absorption from the air, exhibits excellent mechanical compressing properties, does not interact with the active components, and has a sweet cool taste owing to its high negative heat of solution ($\sim 121 \text{ kJkg}^{-1}$) that masks the unpleasant taste of many drugs. The complex of boric acid with mannitol is used in the production of dry electrolytic capacitors. It is an extensively used polyol for production of resins and surfactants.

Mannitol is used in medicine as a powerful osmotic diuretic (to increase the formation of urine in order to prevent and treat acute renal failure and also in the removal of toxic substances from the body) and in many types of surgery for the prevention of kidney failure (to alter the osmolarity of the glomerular filtrate) and to reduce dye and brain oedema (increased brain water content). Hypertonic mannitol can enhance the transport of drugs across the blood–brain barrier for the treatment of life-threatening brain diseases (Rapoport 2001; Miller 2002). Inhaled mannitol improves the hydration and surface properties of sputum in patients with cystic fibrosis (Daviskas et al. 2010). Mannitol hexanitrate is a well-known vasodilator, used in the treatment of hypertension (Johnson 1976). Mannitol is also a scavenger of hydroxyl radicals (Shen et al. 1997).

XYLITOL

Xylitol is a sugar alcohol used as a sweetener. Xylitol is categorized as a polyalcohol or sugar alcohol (alditol). It has the formula $\text{CH}_2\text{OH}(\text{CHOH})_3\text{CH}_2\text{OH}$ and is an achiral isomer of pentane-1,2,3,4,5-pentol. Unlike other natural or synthetic sweeteners, xylitol is actively beneficial for dental health by reducing caries (cavities) to a third in regular use and helpful to remineralization. Multiple studies utilizing electron microscopy have indicated that xylitol is effective in inducing remineralization of deeper layers of demineralized enamel. Fair evidence was found that xylitol (as chewing gum, lozenges, nasal spray, etc.) reduced the incidence of acute middle ear infection in healthy children.

Xylitol is naturally found in low concentrations in the fibers of many fruits and vegetables, and can be extracted from various berries, oats, and mushrooms, as well as fibrous material such as corn husks and sugar cane bagasse. However, industrial production starts from xylan (a hemicellulose) extracted from hardwoods or corncobs, which is hydrolyzed into xylose and catalytically hydrogenated into xylitol. A study in laboratory rats that compared xylitol to other artificial sweeteners found that xylitol had fewer or no side effects, had fewer calories, and was less likely to cause cavities (that is, had lower cariogenicity) than sucrose (table sugar). Xylitol contains asymmetric carbon atoms, but it is not *chiral* because the molecule as a whole is symmetrical.

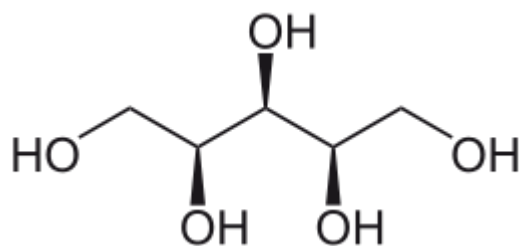


Fig. 2: Structure of Xylitol

Properties

One gram of xylitol contains 2.43 kilocalories (kcal), as compared to one gram of sugar, which has 3.87 kcal. Xylitol has virtually no aftertaste, and is advertised as "safe for diabetics and individuals with hyperglycemia." This tolerance is attributed to the lower effect of xylitol on a person's blood sugar, compared to that of regular sugars as it has an extremely low glycemic index of 7 (glucose has a GI of 100).

Nutrition and Health benefits of Xylitol

Dental care: Xylitol is a "tooth-friendly", nonfermentable sugar alcohol. It appears to have more dental health benefits than other polyalcohols. The structure of xylitol contains a tridentate ligand, (H-C-OH)₃ that can rearrange with polyvalent cations like Ca²⁺. This interaction allows Ca²⁺ to be transported through the gut wall barrier and through saliva which may allow enamel to remineralize before dental cavities form.

Early studies, from Finland in the 1970s, found, compared with chewing sucrose-sweetened gum, xylitol resulted in nearly two fewer cavities or missing teeth. Cavity-causing bacteria prefer six-carbon sugars or disaccharides, while xylitol is non-fermentable and cannot be used as an energy source - while still being taken up into the cell (due to similar shape) and interfering with bacterial growth and reproduction. The harmful micro-organisms are starved in the presence of xylitol, allowing the mouth to remineralize damaged teeth with less interruption. This same property renders it unsuitable for making bread as it interferes with the ability of yeast to digest sugars. At least six grams of xylitol per day, in three to five chewing episodes, is thought to be needed for dental efficacy. A source of xylitol that releases it slowly, and a one- to three-minute initial pulse are thought to improve the dental effect.

Xylitol also inhibits the growth of *Streptococcus pneumoniae*, as well as the attachment of *Haemophilus influenzae* on the nasopharyngeal cells.

The perception of sweetness obtained from consuming xylitol causes the secretion of saliva which acts as a buffer against the acidic environment created by the microorganisms in dental plaque. Increase in salivation can raise the falling pH to a neutral range within few minutes of xylitol consumption.

However, despite these promising conjectures two systematic reviews of clinical trials could not find conclusive evidence that xylitol was indeed superior to other polyols such as sorbitol or equal to that of topical fluoride in its anti-caries effect.

In the 33-month Xylitol for Adult Caries Trial, participants were given lozenges of either five grams of xylitol or a sucralose-sweetened placebo. While this study initially found no statistically significant reduction in 33-month caries increment among adults at an elevated risk of developing caries, a further examination of data from this study revealed a significant reduction in the incidence of root caries in the group that received xylitol.

In March, 2015, Cochrane published a review of the entire body of evidence surrounding xylitol's effects on dental caries. Their conclusion was that, while low-quality evidence suggests that over 2.5 to 3 years of use, a fluoride toothpaste containing xylitol may reduce caries when compared to a fluoride-only toothpaste, the remaining body of evidence is of low to very low quality and is insufficient to determine whether any other xylitol-containing products can prevent caries in infants, older children, or adults.

Xylitol is categorized by the U.S. Food and Drug Administration as a food additive. Like other sugar alcohol-sweetened products, xylitol-sweetened products are allowed to be labeled with the claim that they do not promote dental cavities.

Diabetes: Possessing approximately 33% fewer calories, xylitol is a lower-calorie alternative to table sugar. Absorbed more slowly than sugar, it does not contribute to high blood sugar levels or the resulting hyperglycemia caused by insufficient insulin response. This characteristic has also proven beneficial for people suffering from metabolic syndrome, a common disorder that includes insulin resistance, hypertension, hypercholesterolemia, and an increased risk for blood clots. Xylitol is used as a sweetener in medicines, chewing gum and pastilles.

Source of energy: In the human gut xylitol is not absorbed as well as glucose or fructose; the unabsorbed xylitol acts as a dietary soluble fiber in helping to maintain certain aspects of gut function. Bacterial fermentation, mainly in the large gut, partly converts xylitol to short-chain fatty acids that the gut can absorb as fuel for energy production in oxidative metabolic pathways. Xylitol also is useful in recovery after heavy exercise because the human body converts absorbed xylitol to glucose 6-phosphate and glycogen. The conversion is however slow, so that the xylitol amounts to a low-GI source of energy.

Ear infection: Xylitol chewing gum appears to decrease rates of acute otitis (inflammation of the ear) media in children going to daycare by 25%. Xylitol nasal sprays have also been shown to decrease incidence of acute otitis media as well as being a very effective way of both assisting and stimulating the body's own natural nasopharyngeal washing, and reducing both bacterial colonization and allergenic pollution, with their accompanying problems.

Osteoporosis: A feed containing Xylitol increased bone volume in rat studies conducted in 2001 and 2011, these results have generated interest in the sugar that would examine if it could be a human treatment for osteoporosis.

Toxicity of Xylitol

In humans: Xylitol has no known toxicity in human. However, some report heart palpitations after consuming it. In one study, participants consumed a monthly average of 1.5 kg of xylitol with a maximum daily intake of 430 g with no apparent ill effects. Like most sugar alcohols, xylitol has a laxative effect because sugar alcohols are not fully broken down during digestion; however, the effect varies from person to person. In one study of 13 children, four experienced diarrhea from xylitol's laxative effect when they ate more than 65 grams per day. Studies have reported that adaptation occurs after several weeks of consumption.

As with other sugar alcohols, with the exception of erythritol, consumption of xylitol in excess of one's "laxation threshold" (the amount of sweetener that can be consumed before abdominal discomfort occurs) can result in temporary gastrointestinal side effects, such as bloating, flatulence, and diarrhea. Adaptation (that is, an increase of the laxation threshold) occurs with regular intake. Xylitol has a lower laxation threshold than some sugar alcohols, but is more easily tolerated than mannitol and sorbitol.

In dogs: Xylitol is often fatal to dogs. According to the ASPCA Animal Poison Control Center, the number of cases of xylitol toxicosis in dogs has significantly increased since the first reports in 2002. Dogs that have eaten foods containing xylitol (greater than 100 milligrams of xylitol consumed per kilogram of bodyweight) have presented with low blood sugar (hypoglycemia), which can be life-threatening. Low blood sugar can result in a loss of coordination, depression, collapse and seizures in as little as 30 minutes. Intake of doses of xylitol (greater than 500 – 1000 mg/kg bwt) has been implicated in liver failure in dogs, which can be fatal. The possible cause of hypoglycemia experienced by dogs is that xylitol in chewing gum is released more slowly and absorbed over longer period than when it is consumed as a pure form.

In wild birds: Thirty Cape sugarbirds died within 30 minutes of drinking a solution made with xylitol, from a feeder in a garden in Hermanus, South Africa. It is suspected that it triggered a massive insulin release, causing an irreversible drop in blood sugar.

PRODUCTION OF XYLITOL

Xylitol is produced by hydrogenation of xylose, which converts the sugar (an aldehyde) into a primary alcohol. Another method of producing xylitol is through microbial processes, including fermentative and biocatalytic processes in bacteria, fungi, and yeast cells, which take advantage of the xylose-intermediate fermentations to produce high yield of xylitol. Common yeast cells used in effectly fermenting and producing xylitol are *Candida tropicalis* and *Candida guilliermondii*. Some of the production – chemical and biotechnological – processes for Xylitol are discussed below.

Chemical process

Xylitol is manufactured industrially by reducing pure xylose, obtained from hardwood or hemicellulosic hydrolysate in the presence of a Raney nickel catalyst. The chemical synthesis of xylitol starts with the extraction of xylose from hemicellulose by acid-catalyzed hydrolysis. After color removal and purification, xylose-rich hemicellulosic hydrolysate can be employed for xylitol production through hydrogenation of xylose at 80–140°C and hydrogen pressures up to 50 atmospheres in the presence of metal catalysts (Raney nickel). The xylitol solution formed requires further purification by chromatography, and then concentration and crystallization of the

product to obtain pure xylitol. The xylitol yield is only about 50–60% of the xylan fraction and thus the xylitol production process is expensive due to the extensive separation and purification stages.

Biotechnological processes

Fermentation process:

The fermentation process uses bacteria, fungi, and yeast for xylitol production from commercial pure xylose or hemicellulosic hydrolysate. The production of xylitol using bacteria and fungi has been studied to a lesser extent compared to that using yeast strains. A few bacteria, such as *Enterobacter liquefaciens*, *Corynebacterium* sp., and *Gluconobacter oxydans*, have been reported to produce xylitol. There are very few studies regarding xylitol production from D-xylose using filamentous fungi. Yeasts are considered as the best xylitol producers among the microorganisms. As a result, yeasts have been studied extensively in the last few decades by several researchers. Forty-four yeast strains from the five genera were screened by Barbosa *et al.* (1988) for their ability to convert D-xylose to xylitol. *Candida guilliermondii* and *C. tropicalis* were found to be the best xylitol producers and these yeasts produced 77.2 g l⁻¹ xylitol from 104 g l⁻¹ xylose using high cell densities and a defined medium under aerobic conditions. The fermentation conditions were optimized by da Silva and Afschar (1994) during continuous cultivation of *Candida tropicalis* for xylitol production. *C. tropicalis* produced xylitol at a yield of 77–80% of theoretical value (0.91 g g⁻¹) in a medium containing 100 g l⁻¹ D-xylose.

The screening of different xylose-assimilating yeast has confirmed that the best xylitol producers belong to the genus *Candida*. In the fermentation process using yeast, the yield of xylitol obtainable from D-xylose is in a range of 65–85% of the theoretical value. The production of xylitol through the fermentation process is limited by certain factors, such as precise control of culture conditions, expensive nutrients, huge water consumption, and the type of process. Thus, the application of the fermentation process on an industrial level is time consuming, being associated with some preparatory activities such as sterilization and regular inoculums development involving input of energy, labor, and time, leading to decreased productivity. The advantage of the fermentation process over chemical procedures is its lower cost due to the non-necessity of extensive xylose purification. The fermentative xylitol production has been studied as an alternative, but its viability is dependent on the optimization of the various fermentation variables such as nutritional composition (substrate, nitrogen source, and micronutrients), the culture and process conditions, as well as the genetic nature of the microorganisms.

Enzymatic process

The production of xylitol from xylose by using enzyme technology is an alternative and promising approach. The enzymatic conversion of D-xylose into xylitol using xylose reductase (XR) of *Candida pelliculosa* coupled with the oxidoreductase system of *Methanobacterium* sp. has been reported by Kitpreechavanich *et al.* (1984). The authors observed that the xylose was stoichiometrically converted to xylitol with an equivalent consumption of NADPH and that an almost quantitative conversion of xylose to xylitol was achieved using a NADP⁺-toxylose ratio of over 1:30, whereas the coenzyme was successfully regenerated and retained using a membrane reactor. About 90% conversion of xylose to xylitol could be achieved at 35°C and pH 7.5 after a 24 h reaction period.

Nidetzky *et al.* (1996) optimized the production of xylitol from xylose by XR from *Candida tenuis* coupled with glucose dehydrogenase from *Bacillus cereus* for regenerating the NADH in an enzyme reactor. In this system, the substrate was converted at concentrations of 300 g l^{-1} xylose, with a 96% yield and xylitol productivity of $3.33 \text{ g l}^{-1} \text{ h}^{-1}$. Neuhauser *et al.* (1998) reported on the *C. tenuis* XR-mediated NADH-dependent xylose reduction coupled with formate dehydrogenase (FDH) from *C. boidinii* for the byproduct-free recycling of NADH used in a pH-controlled enzyme reactor. In this process, a fed-batch conversion of 0.5 M xylose to xylitol using yeast XR yielded productivities of $2.8 \text{ g l}^{-1} \text{ h}^{-1}$. To optimize the performance of the XR catalyzed reactions for xylitol synthesis, the effect of several process variables on productivity needs to be studied: pH, temperature, initial substrate, and coenzyme concentration.

SORBITOL

Sorbitol, less commonly known as glucitol, is a sugar alcohol with a sweet taste which the human body metabolizes slowly. It can be obtained by reduction of glucose, changing the aldehyde group to a hydroxyl group. Most sorbitol is made from corn syrup, but it is also found in apples, pears, peaches, and prunes. It is converted to fructose by sorbitol-6-phosphate 2-dehydrogenase. Sorbitol is an isomer of mannitol, another sugar alcohol; the two differ only in the orientation of the hydroxyl group on carbon 2. While similar, the two sugar alcohols have very different sources in nature, melting points, and uses.



Fig. 3: Structure of Sorbitol

Uses of Sorbitol

Sweetener: Sorbitol is a sugar substitute. It may be listed under the inactive ingredients listed for some foods and products. Its INS number and E number is 420. Sorbitol has approximately 60% the sweetness of sucrose (table sugar). Sorbitol is referred to as a nutritive sweetener because it provides dietary energy: 2.6 kilocalories (11 kilojoules) per gram versus the average 4 kilocalories (17 kilojoules) for carbohydrates. It is often used in diet foods (including diet drinks and ice cream), mints, cough syrups, and sugar-free chewing gum. It also occurs naturally in many stone fruits and berries from trees of the genus *Sorbus*.

Laxative: Sorbitol can be used as a laxative via an oral suspension or enema. As with other sugar alcohols, gastrointestinal distress may result when food products that contain sorbitol are consumed. Sorbitol exerts its laxative effect by drawing water into the large intestine, thereby stimulating bowel movements. Sorbitol has been determined safe for use by the elderly, although it is not recommended without consultation with a clinician. Sorbitol is found in some dried fruits and may contribute to the laxative effects of prunes. Sorbitol was discovered initially in the

fresh juice of mountain ash (*Sorbus aucuparia*) berries in 1872. It is found in the fruits of apples, plums, pears, cherries, dates, peaches, and apricots.

Medical applications: Sorbitol is used in bacterial culture media to distinguish the pathogenic *Escherichia coli* O157:H7 from most other strains of *E. coli*, as it is usually incapable of fermenting sorbitol, but 93% of known *E. coli* strains are capable of doing so.

A treatment using sorbitol and ion-exchange resin sodium polystyrene sulfonate (tradename Kayexalate), helps remove excess potassium ions when in a hyperkalemic state. The resin exchanges sodium ions for potassium ions in the bowel, while sorbitol helps to eliminate it. In 2010 the U.S. FDA issued a warning of increased risk for GI necrosis with this combination. Sorbitol is also used in the manufacture of soft gels to store single doses of liquid medicines.

Health care, food, and cosmetic uses: Sorbitol often is used in modern cosmetics as a humectant and thickener. Sorbitol often is used in mouthwash and toothpaste. Some transparent gels can be made only with sorbitol, as it has a refractive index sufficiently high for transparent formulations. Sorbitol is used as a cryoprotectant additive (mixed with sucrose and sodium polyphosphates) in the manufacture of surimi, a processed fish paste. It is also used as a humectant in some cigarettes. Sorbitol sometimes is used as a sweetener and humectant in cookies and other foods that are not identified as "dietary" items.

Medical importance

Aldose reductase is the first enzyme in the sorbitol-aldose reductase pathway responsible for the reduction of glucose to sorbitol, as well as the reduction of galactose to galactitol. Too much sorbitol trapped in retinal cells, the cells of the lens, and the Schwann cells that myelinate peripheral nerves can damage these cells, leading to retinopathy, cataracts and peripheral neuropathy, respectively. Aldose reductase inhibitors, which are substances that prevent or slow the action of aldose reductase, are currently being investigated as a way to prevent or delay these complications, which frequently occur in the setting of long-term hyperglycemia that accompanies poorly controlled diabetes. It is thought that these agents may help to prevent the accumulation of intracellular sorbitol that leads to cellular damage in diabetics.

Adverse medical effects

People with untreated celiac disease often present sorbitol malabsorption, as a result of the small bowel damage. Sorbitol malabsorption is an important cause for persisting symptoms in patients already on a gluten-free diet. It has been suggested that sorbitol hydrogen breath test is a useful tool to detect celiac disease because of a strict correlation between cut-off value and intestinal lesions. Nevertheless, its use to diagnosis in clinical practice is not recommended by the Rome Consensus Conference on "Methodology and indications of H₂-breath testing in gastrointestinal diseases" and may be indicated for research purpose.

It has been noted that the sorbitol added to sodium polystyrene sulfonate (SPS, used in the treatment of hyperkalemia) can cause complications in the GI tract, including bleeding, perforated colonic ulcers, ischemic colitis and colonic necrosis, particularly in patients with uremia. The authors of the paper in question cite a study on rats (both non-uremic and uremic) in which all uremic rats died on a sorbitol enema regimen, whilst uremic rats on non-sorbitol regimens – even with SPS included – showed no signs of colonic damage. In humans, it is

suggested that the risk factors for sorbitol-induced damage include "... immunosuppression, hypovolemia, postoperative setting, hypotension after hemodialysis, and peripheral vascular disease." They conclude that SPS-sorbitol should be used with caution, and that "Physicians need to be aware of SPS-sorbitol GI side effects while managing hyperkalemia."

Overdose effects

Ingesting large amounts of sorbitol can lead to abdominal pain, flatulence, and mild to severe diarrhea. Sorbitol ingestion of 20 grams (0.7 oz) per day as sugar-free gum has led to severe diarrhea leading to unintended weight loss of 11 kg (24 lb; 1 st 10 lb) in eight months, in a woman originally weighing 52 kg (115 lb; 8 st 3 lb); another patient required hospitalization after habitually consuming 30 grams (1 oz) per day.

PRODUCTION OF SORBITOL FROM MAIZE STARCH

Sorbitol can be produced from maize starch by simultaneous hydrolysis and hydrogenation using a nickel catalyst and pressures of approximately 100 atmospheres. The presence of certain metallic salts e.g. magnesium, nickel or calcium chloride improves the efficiency of the conversion and can be accomplished by two steps.

1. Conversion of Maize starch into liquid glucose/Dextrose on hydrolysis.
2. Conversion of Dextrose/liquid glucose into sorbitol on hydrogenation under high pressure.

Conversion of Maize Starch into liquid glucose:-

The raw materials required for the manufacture of glucose are starch and mineral acid; Amylolytic enzymes may also be use for starch hydrolysis. The conversion of starch to glucose takes place through a series of steps in which carbohydrates of progressively decreasing complexity-soluble starch, dextrans like amylo dextrin, erythro dextrin and achro-dextrin, maltose and dextrose are formed. The composition of the hydrolysate is determined largely by the concentration and temperature of the acid used for catalysing the hydrolysis.

For the manufacture of glucose a starch suspensions in water containing 35-40% starch is mixed with sufficient hydrochloric acid to give a concentration of 0.012 to 0.02N acid in the final mixture and heated in an autoclave to a temperature of 140-160. The product is held at this temperature for 15-20 minutes. The reaction mixture is tested with iodine and if not starch is present, as indicated by the colour test the pressure is released and the liquid transferred to a neutralizing tank. The acid is neutralized with soda ash. Proteins fats, fatty acids, and colloidal material are coagulated by adjusting the PH to 4-3. The mixture is passed through a filter press, the filtrate is decolourised by activated carbon and the clear filtrate clear filtrate concentrated in a triple effect evaporator. Treatment with activated carbon is repeated and the liquid further concentrated in a vacuum pan. The concentrated syrup (40-45~ Be) is quickly cooled and tranferred to storage silas. The product obtained contains 43% dextose on dry basis. Glucose is prepared also by enzymic conversion of starch or by a combination of acid conversion, neutralisation and enzymic conversions (U.S. Patent 2,201,609 of 1940).

Raw Materials

The principal raw materials required for the manufacture of glucose is starch. Excepting glucose and foods Ltd. who purchase their requirement of maize starch, other factories utilize upto 20% of their any productions of maize starch. Maize was being imported from U.S.A. for making starch but the price of maize was reported to be somewhat higher than that of imported starch. Efforts have been made to use indogenous tapioca starch as the raw material by some manufacturers. In fact Kamla Sugar Mills Ltd, India, has attempted to utilize only tapioca starch for the manufacture of dextrose. The manufacturing process employed in India is the same as that in use in other countries. Maize starch is suspended in water and cooked after adding hydrochloric acid, in a converter under a pressure of 30 lbs/inch. For 15 minutes the hydrolysis is interrupted when a test sample of the hydrolysate fails to give a blue coloration with iodine. The pressure is released and the charge run into a wooden vat, where it is neutralised with a solution of soda ash and the reaction adjusted to the Iso electric point to coagulate colloidal impurities.

The liquor is filtered decolorized with activated carbon and filtered again. The filtrate (200Be) is concentrated to 30-32 Be under vacuum, when all inorganic salts other than sodium chloride precipitate out. The syrup is treated with activated carbon, filtered, and concentrated to 45 Be.

Conversion of Liquid Glucose/Dextrose into Sorbitol:-

The catalytic hydrogenation of dextrose yield sorbitol Dextrose is dissolved in worm distilled water until, 50% solution is obtained. The final solution contains small amounts of inorganic salts which could poison the catalyst during hydrogenation. These impurities are removed by passing, the solution through a cationic-anionic exchange system.

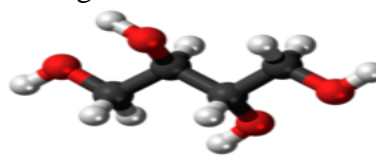
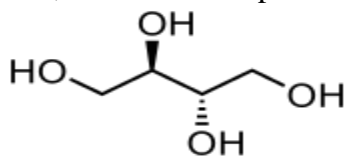
In the batch process, catalyst is prepared prior to hydrogenation by treatment of Raney nickel, which is an aluminum-nickel alloy containing about 50% nickel by wt. The catalyst is activated by dissolving aluminium from the matrix via treatment with warm (60°C) 25% caustic soda. Aluminium is dissolved from the matrix and treatment with deionized water removes the sodium aluminate which is formed. Catalyst is fed in slurry form to a hydrogenation reactor by the use of nitrogen under pressure. A typical concentration of catalyst is 2% nickel (based on glucose. After catalyst and deionized dextrose solution are charged into the pressure auto clave/reactor the hydrogen flow is started and hydrogenation takes place at about 1000 ps ig and upto 3 hr of time During the reaction the process temperature is controlled below 150°C.

Upon completion of reaction auto clave/pressure is reduced by venting hydrogen to a gas holder, but sufficient pressure is maintained to move reaction products out of the auto clave/reactor crude sorbitol solution is then passed through two filter system for removal of catalyst. Since the catalyst is pyrophoric it is recycled to the aut claves without coming in contact with air.

The freshly made sorbitol solution is purified by passage through a three stage deionization system that contain a cationic, anionic and mixed bed column Metallic cations (Such as nickel) are removed in the first stage, and gluconate anions are absorbed in the second. Further treatment may be given with activated carbon to remove trace organic impurities. Deionized solution contains about 50% sorbitol. Commercial sorbitol of 70% concentration is the result of evaporation at 45- 50°C under a low pressure of 50 – 80 mm Hg. Crystalline sorbitol is obtained by further concentration and crystallization and is sold in both pellet and powder form.

ERYTHRITOL

Erythritol ((2*R*,3*S*)-butane-1,2,3,4-tetraol) is a sugar alcohol (or polyol) that has been approved for use as a food additive in most countries. It was discovered in 1848 by Scottish chemist John Stenhouse. It occurs naturally in some fruit and fermented foods. At the industrial level, it is produced from glucose by fermentation with a yeast, *Moniliella pollinis*. Erythritol is 60–70% as sweet as sucrose (table sugar) yet it is almost noncaloric, does not affect blood sugar, does not cause tooth decay, and is partially absorbed by the body, excreted in urine and feces. Under U.S. Food and Drug Administration (FDA) labeling requirements, it has a caloric value of 0.2 calories per gram (95% less than sugar and other carbohydrates), though nutritional labeling varies from country to country. Some countries, such as Japan and the United States, label it as zero-calorie, while the European Union currently labels it at 0 cal/g.



Erythritol

Erythritol and human digestion

In the body, most erythritol is absorbed into the bloodstream in the small intestine, and then for the most part excreted unchanged in the urine. About 10% enters the colon. Because 90% of erythritol is absorbed before it enters the large intestine, it does not normally cause laxative effects, as are often experienced after consumption of other sugar alcohols (such as xylitol and maltitol), although extremely large doses can cause nausea and borborygmi (stomach rumbling).

Side effects of erythritol

In general, erythritol is free of side effects in regular use. Doses over 50 grams (1.8 oz) can cause a significant increase in nausea and stomach rumbling. Rarely, erythritol can cause allergic hives (urticaria). When compared with other sugar alcohols, it is also much more difficult for intestinal bacteria to digest, so it is less likely to cause gas or bloating than other polyols, such as maltitol, sorbitol, or lactitol.

According to a study conducted in 2014, erythritol functions as an insecticide toxic to the fruit fly *Drosophila melanogaster*.

Physical properties of erythritol

Heat of solution

Erythritol has a strong cooling effect (endothermic, or positive heat of solution) when it dissolves in water, which is often combined with the cooling effect of mint flavors. The cooling effect is present only when erythritol is not already dissolved in water, a situation that might be experienced in an erythritol-sweetened frosting, chocolate bar, chewing gum, or hard candy. The cooling effect of erythritol is very similar to that of xylitol and among the strongest cooling effects of all sugar alcohols.

Blending for sugar-like properties

Erythritol is commonly used as a medium in which to deliver high-intensity sweeteners, especially stevia derivatives, serving the dual function of providing both bulk and a flavor similar to that of table sugar. Diet beverages made with this blend, thus contain erythritol in addition to the main sweetener. Beyond high-intensity sweeteners, erythritol is often paired with other bulky ingredients that exhibit sugar-like characteristics to better mimic the texture and mouth feel of sucrose. The cooling effect of erythritol is rarely desired, hence other ingredients are chosen to dilute or negate that effect. Erythritol also has a propensity to crystallize and is not as soluble as sucrose, so ingredients may also be chosen to help negate this disadvantage. Furthermore, erythritol is not hygroscopic, meaning it does not attract moisture, which can lead to the drying out of products, in particular baked goods, if another hygroscopic ingredient is not used in the formulation.

Inulin is often combined with erythritol because of inulin's offering a complementary negative heat of solution (exothermic, or warming effect when dissolved, which helps cancel erythritol's cooling effect) and noncrystallizing properties. However, inulin has a propensity to cause gas and bloating in those having consumed it in moderate to large quantities, in particular in individuals unaccustomed to it. Other sugar alcohols are sometimes used with erythritol, in particular isomalt, because of its minimally positive heat of solution, and glycerin, which has a negative heat of solution, moderate hygroscopicity, and noncrystallizing liquid form.

Erythritol and dental bacteria

Erythritol is tooth-friendly; it cannot be metabolized by oral bacteria, so it does not contribute to tooth decay. Erythritol is preferentially utilized by the *Brucella* bacteria spp. The presence of erythritol in the placentas of goats, cows, and pigs has been proposed as an explanation for the accumulation of *Brucella* bacteria found at these sites.

Can Erythritol Cause Digestive Upset?

In small amounts, erythritol is not supposed to cause digestive upset and diarrhea that other sugar alcohols like sorbitol and xylitol are known to cause, because erythritol is a smaller molecule and 90 percent of erythritol is absorbed in the small intestine and excreted for the most part unchanged in urine.

There are some people who report side effects such as diarrhea, stomach upset, and headache after consuming regular amounts of erythritol in food or beverages. The amount needed to cause symptoms varies greatly based on your individual tolerance. Some find that even small amounts of sugar alcohols upset their stomach, while others can tolerate higher amounts before they experience gastrointestinal symptoms. Consuming over 50 grams of erythritol may result in nausea or stomach rumbling.

Physiochemical properties and functions of erythritol

Erythritol, a tetra-carbon sugar alcohol [1,2,3,4-butanetetrol, molecular weight (MW) 122.12], is a symmetrical molecule, and therefore, it exists only in one form, the mesoform. It forms anhydrous crystals with a moderately sweet taste without off-taste or odors. Crystals melt at 122°C to form a colorless and brilliant nonviscous melt. Erythritol's chemical properties are similar to those of other polyols in that it has no reducing end-groups and thus has excellent heat and acid stability. It differs in having a low solubility, and its heat of solution is very low.

However, compared with the group of polyols presently used as sugar replacers, erythritol has the lowest molecular weight—which gives it different properties, such as higher osmotic pressure and lower water activity in solution. The most important and special nutritional properties that differentiate erythritol from other polyols are due to its small molecular size.

Not only animal toxicological but also clinical studies have consistently demonstrated the safety of erythritol even when consumed on a daily basis in high amounts. More than 90% of ingested erythritol is not metabolized by the human body and is excreted unchanged in the urine without changing blood glucose and insulin levels. A little amount of erythritol can be metabolized in some reversible metabolic reactions such as dehydrogenation to D- or L-erythrulose by NAD-dependent cytoplasmic polyol dehydrogenase or phosphorylation by glycerol kinase to erythritol-1-phosphate followed by dehydrogenation to D-erythrulose 1-phosphate via α -glycerophosphate dehydrogenase in humans (Chu and Ballou 1961; Maret and Auld 1988).

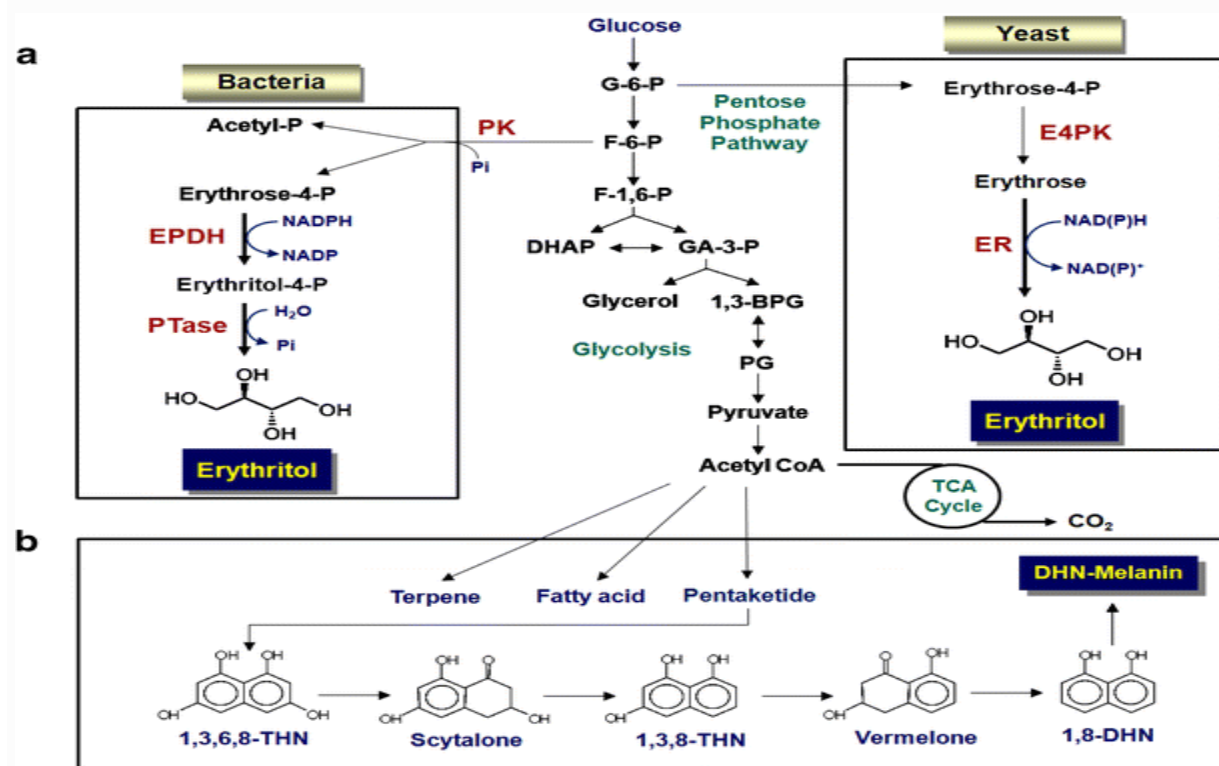
Background of erythritol production

Erythritol is produced industrially beginning with enzymatic hydrolysis of the starch from corn to generate glucose. Glucose is then fermented with yeast or another fungus to produce erythritol. Other methods such as electrochemical synthesis are in development. Chemical and fermentative processes have been introduced for large-scale production of erythritol. Erythritol can be synthesized from dialdehyde starch by high-temperature chemical reaction in the presence of a nickel catalyst. This process has not been industrialized because of its low efficiency. Large-scale production of erythritol uses fermentative processes with pure glucose, sucrose, and glucose from chemically and enzymatically hydrolyzed wheat and corn starches. Erythritol can be produced by microbial methods that utilize osmophilic yeasts and some bacteria.

Erythritol production by yeasts

Yeast species of genera, e.g., *Zygosaccharomyces*, *Debaryomyces*, *Hansenula*, and *Pichia* are able to grow in environments with low water activity (in the presence of high sugar or salt concentrations). These osmotolerant yeasts accumulate compatible solutes when encountering salt or osmotic stress. Compatible solutes protect and stabilize enzymes, enabling the cellular functions in osmotic conditions. Glycerol is the most common osmolyte in yeasts, but sugar alcohols such as D-arabitol, erythritol, and mannitol may also serve as osmolytes. The sugar alcohols produced may also have a role in redox balancing or as storage compounds. The role of the pentose phosphate pathway in yeasts and other eukaryotic organisms is to produce reducing power in the form of NADPH for the cellular reactions and also to produce precursors such as D-ribose 5-phosphate and D-erythrose 4-phosphate for nucleotide and amino acid biosynthesis. The erythritol found in yeast and fungus species is synthesized via the pentose phosphate pathway. Erythritol, possibly acting as an osmosolute, is synthesized from D-erythrose 4-phosphate after dephosphorylation and reduction reactions. Several yeast species such as *Torula corallina*, *Candida magnoliae*, and *Ustilaginomycetes* are known to produce erythritol, and the strains or their “mutagenised” derivatives reach over 40% yields of erythritol on D-glucose ($w w^{-1}$). As shown in Fig. 1, the production of erythritol results from the reduction and dephosphorylation of a C-4 moiety by an erythritol dehydrogenase and a

phosphotransferase-like enzyme, the C-4 compound, resulting itself from the splitting of a C-6 intermediate metabolite by a phosphoketolase. This C-6 compound is fructose 6-phosphate originating from the isomerization of glucose 6-phosphate, and the phosphoketolase is either the same or a different enzyme from the one catalyzing the splitting of xylulose 5-phosphate into glyceraldehyde 3-phosphate and acetyl phosphate. The two phosphoketolase activities are borne by a single enzyme with a much higher affinity for pentulose phosphate than for hexulose phosphate.



a. Pathway of erythritol biosynthesis in yeast and bacteria. EPDH erythritol-4-phosphate dehydrogenase, PK phosphoketolase, E4PK erythrose-4-phosphate kinase, Pi inorganic phosphate, PTase, phosphatase, ER erythrose reductase, G-6-P glucose 6-phosphate, F-6-P fructose 6-phosphate, F-1,6-P fructose 1,6-diphosphate; GA-3-P, glyceraldehyde 3-phosphate, 1,3-BPG 1,3-diphoglycerate, PG phosphoglycerate.

b. Pentaketide pathway for melanin biosynthesis in fungi. CoA coenzyme A, TCA tricarboxylic acid, 4HNR, tetrahydroxynaphthalene reductase, 2-HJ, S-hydroxyjuglone; THN, trihydroxynaphthalene; DHN, dihydroxynaphthalene

Eukaryotes contain erythrose reductase to catalyze the hydrogenation of erythrose. Characterization of erythrose reductase in *C. magnoliae* indicated that it was one of the aldose reductase with a high preference for NADH in contrast to the typical aldose reductase acting with NADPH (Lee et al. 2003c). During the biosynthesis of erythritol in yeasts such as *C. magnoliae*, 1 mol of glucose is converted to 1 mol of erythrose-4-phosphate followed by its dephosphorylation into erythrose, which is reduced into erythritol by erythrose reductase. The

synthesis of erythritol via the pentose phosphate pathway is favored by growth under glycolytic rather than gluconeogenic condition in *Aspergillus nidulans*. Under these conditions, the high carbon fluxes through both the pentose phosphate pathway and glycolysis generate sufficient reduction capacity to lead to the overflow of the various polyols.

Erythritol production by lactic acid bacteria

The enzymatic pathway involved in the formation of erythritol in *Leuconostoc oenos*, and the effects of oxygen on the regulation of erythritol production from glucose have been reported. The metabolism of glucose in heterolactic acid bacteria is initiated by the oxidation of glucose 6-phosphate to gluconate 6-phosphate. Following oxidative decarboxylation of the latter, ribulose 5-phosphate is converted to xylulose 5-phosphate, which is then split into acetyl phosphate and glyceraldehyde 3-phosphate by a pentose phosphate phosphoketolase present in all heterolactic acid bacteria. The existence of a nonspecific phosphoketolase has been described for *Leuconostoc mesenteroides*, *Lactobacillus plantarum*, *Acetobacter xylinum*, *Bifidobacterium bifidum*, and *Bifidobacterium globosum*, whereas separate xylulose 5-phosphate and fructose 6-phosphate phosphoketolase have been described for *L. mesenteroides*, *Bifidobacterium dentium*, *L. plantarum*, *Thiobacillus novellus*. The pathway of formation of erythritol from glucose in *L. oenos* was shown to involve the isomerization of glucose 6-phosphate to fructose 6-phosphate by a phosphoglucose isomerase, the cleavage of fructose 6-phosphate by a phosphoketolase, the reduction of erythrose 4-phosphate by an erythritol 4-phosphate dehydrogenase and, finally, the hydrolysis of erythritol 4-phosphate to erythritol by a phosphatase. The erythritol-producing bacteria were the only ones in which the concentration of hexose 6-phosphate increased in the absence of O₂. It is shown that, under anaerobic conditions, the NADPH/NADP ratio is high, which inhibits glucose 6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase. This inhibition, in turn, results in the accumulation of glucose 6-phosphate and fructose 6-phosphate. The increase in the concentration of fructose 6-phosphate and the accompanying decrease in that of xylulose 5-phosphate allow phosphoketolase to cleave the fructose-6-phosphate, leading to the formation of erythrose 4-phosphate and ultimately to erythritol biosynthesis.

Industrial production of erythritol

Erythritol can be produced by a chemical process where dialdehyde starch is converted into erythritol by a high-temperature chemical reaction in the presence of a nickel catalyst. Due to low yields, the chemical process did not reach to industrialization. Erythritol is commercially produced by Bolak Corporation (Whasung, Kyungki-do, Korea), Cargill Food & Pharm Specialties (Blair, Nebraska, USA), and Mitsubishi Chemical Corporation (Tokyo, Japan). Glucose from chemically and enzymatically hydrolyzed wheat and corn starches is used as a major carbon source to produce erythritol by the fermentation of yeast-like fungi such as *Torula* sp. and *Moniliella pollinis*. Erythritol can be purified by ion exchange chromatography to remove charged impurities, followed by crystallization of the pure polyol. Ion exchange chromatography using cation and anion exchange resins is performed immediately following membrane filtration of fermentation broth. The solution is then concentrated and allowed to crystallize. The crystalline erythritol product has a final purity of more than 99.5%. Other microorganisms that have been reported to produce erythritol

include *Pichia*, *Zygoichia*, *Candida*, *Torulopsis*, *Trigonopsis*, and *Moniliella tomentosa* var. *pollis*. Erythritol production using these strains could not be applied on an industrial scale due to byproducts such as glycerol and ribitol.

In industrial production, the yield and formation rate of erythritol by *Aureobasidium* sp. SN-G42 have been found to reach 47%, $2.0 \text{ g l}^{-1} \text{ h}^{-1}$, and 96 h, respectively, in a 100,000-l fermentor with medium components and oxygen-transfer rate improved to decrease cells and byproducts. The size of a fermentation tank for erythritol production was scaled up to 200,000 l for commercial production (Kasumi et al. 1998). Hirata et al. (1999) isolated *Ustilaginomyces* sp. 618A-01 from pollen that did not produce glycerol as a byproduct; however, the culture time was 500–1,000 h. With regard to fermentation methods, Park et al. (1998) reported that a fed-batch culture with a 5-l fermentor feeding glucose increased the formation rate to $1.86 \text{ g l}^{-1} \text{ h}^{-1}$, which was 23% greater than that with batch fermentation using *Trichosporon* sp. Sawada et al. studied cell-recycled continuous culture using a 1,000-l fermentor and a continuous centrifuge in which the yield was 55.0% and the formation rate was $3.0 \text{ g l}^{-1} \text{ h}^{-1}$ for 55 days (Sawada et al. 2002). Scale-up studies for erythritol production by *Pseudozyma tsukubaensis* from a laboratory scale (7-l fermenter) to pilot (300 l) and plant (50,000 l) scales using the dissolved oxygen as a scale-up parameter were reported. Erythritol production at the pilot and plant scales was similar to that at the laboratory scale (245 g l^{-1}).

CONCLUSION

Sugar alcohols are organic compounds, typically derived from sugars: contrary to what the name may suggest, a sugar alcohol is neither a sugar nor an alcoholic beverage. They are white, water-soluble solids that can occur naturally or be produced industrially from sugars. They are used widely in the food industry as thickeners and sweeteners. Sugar alcohols are usually incompletely absorbed into the blood stream from the small intestines which generally results in a smaller change in blood glucose than "regular" sugar (sucrose). This property makes them popular sweeteners among diabetics and people on low-carbohydrate diets. However, like many other incompletely digestible substances, overconsumption of sugar alcohols can lead to bloating, diarrhea and flatulence because they are not absorbed in the small intestine.

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