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Full Length Research Article

MICROBIAL PRODUCTION OF SWEETENERS AND THEIR INDUSTRIAL APPLICATIONS: CURRENT STATUS AND FUTURE PROSPECTS

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ABSTRACT
Sweeteners are chemical compounds with sweet taste. They are categorized into six groups, namely artificial sweeteners, modified sugars, natural calorie sweeteners, natural zero-calorie sweeteners, sugars, and sugar alcohols. Sugar alcohols, like xylitol, erythritol, sorbitol, and mannitol are primarily produced via microbial processes. The increasing demand for natural, low-calorie sweeteners is driven by health-conscious consumers and regulatory efforts to reduce excessive sugar intake. Microbial production of sweeteners has emerged as a sustainable and cost-effective alternative to traditional sweetener production. Sugar alcohols can be efficiently produced via microbial fermentation of their precursors. Applications of sweeteners range from confectionery to oral care products, cosmetics, food and beverages and pharmaceutical products; thereby demonstrating the market potential of microbial-derived sweeteners. Microbial-derived sweeteners range from less calorie to a zero-calorie sugar. More accessibility to the sugars has led to increasing demand for scalable and economical microbial production methods. Advances in biotechnology have developed microbial strains that convert renewable feedstocks into sugar alcohols with high yields and purity, boosting their commercial viability. Microbial biosynthesis can produce erythritol, xylitol, sorbitol and mannitol. Metabolic engineering continues to enhance strain performance, substrate utilization, and product yields, making microbial sweetener production more scalable and cost-effective. This review focuses on the microbial production of sweeteners, their industrial production, current status and future prospects.

Keywords: Sweeteners, Erythritol, Mannitol, Low-calorie, Precursor, Microbial.

INTRODUCTION

The growing demand for healthier and natural alternatives to traditional sugar has driven extensive research in the production of sweeteners from microbial sources. Microbial sweeteners, derived from bacteria, fungi, and yeast, offer promising solutions due to their low caloric content, potential health benefits, and sustainable production methods. These sweeteners include sugar alcohols like xylitol, mannitol and erythritol, as well as high-intensity natural sweeteners such as steviol glycosides. Microbial production systems not only provide a cost-effective means of sweetener production, but also offer greater control over product quality and consistency compared to traditional plant-based extraction methods (Hernández-Pérez *et al.,* 2020)

The biotechnological approach to sweetener production leverages microbial fermentation processes, which can convert inexpensive substrates into value-added sweetening compounds. For example, lactic acid bacteria (LAB) are employed in the production of low-calorie sugars such as tagatose and erythritol, which have attracted attention due to their favourable glycaemic properties and low caloric values (Patra *et al.,* 2009). The use of metabolic engineering has further enhanced microbial pathways, improving the efficiency and yield of these sweeteners (Philippe *et al.,* 2014). Microbial sweeteners offer a sustainable and scalable alternative to traditional sugar production, aligning with consumer preferences for low-calorie and natural products. The future of this field lies in optimizing microbial strains and fermentation processes to produce high-quality sweeteners with minimal environmental impact (Zubair *et al*., 2019).

Microbial sweeteners (sugar alcohols or polyols) as shown in Table 1 are slowly digestible carbohydrates, which are obtained by substituting an aldehyde group with a hydroxyl one (Shankar *et al.,* 2013). As most of sugar alcohols are produced from their corresponding aldose sugars, they are also called alditols (Mäkinen, 2011). Among sugar alcohols are hydrogenated monosaccharides (sorbitol, mannitol), hydrogenated disaccharides (isomalt, maltitol, lactitol) and mixtures of hydrogenated mono-di- and/or oligosaccharides (hydrogenated starch hydrolysates) (Livesey *et al.,* 2003).

Polyols are naturally present in smaller quantities in fruits as well as in certain kinds of vegetables or mushrooms, and they are also regulated as either generally recognized as safe or food additives (Fitch and Keim, 2012; Wheeler and Pi-sunyer, 2008). Food additives are substances that are added intentionally to foodstuff in order to perform certain technological functions such as to give colour, to sweeten or to help in food preservation. Although acceptable daily intake (ADI) dose has not been specified for them, they are known for their potent laxative effect and cause other gastrointestinal symptoms such as flatulence, bloating, and abdominal discomfort when eaten in excess (Grabitske and Slavin, 2008). Therefore, in order to ensure consumers with adequate information, products containing more than 10 % added polyols must include the advisory statement because excessive consumption may produce laxative effects (EFSA, 2011).

Sweetness of sugar alcohols is usually lower than the one of monosaccharide; therefore, they are used volume for-volume like sugar and are called bulk sweeteners. They are often used in combination with other sweeteners to achieve the desired level of sweetness and flavour. Similar to carbohydrates, they are not only responsible for sweet taste, they are also responsible for product texture, its preservation, filling, holding moisture and cooling

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sensation in the mouth (Health Canada, 2005). These compounds have a lower nutritional value than sugars, due to slower and incomplete absorption from the intestine, which results in indirect metabolism via fermentative degradation by the intestinal flora. Products of fermentation include short-chain fatty acids and gases (Grabitske and Slavin, 2008). Sugar alcohols such as maltitol and lactitol were found to increase mineral bioavailability in human and rats (Nakamura, 2005; Xiao *et al.,* 2013).

Table 1: Samples of Sweeteners that have been Identified and Documented (Grembecka 2015)

Sugar alcohol	Formula	Systematic name	Synonyms	Functional classes
Erythritol	$C_4H_{10}O_4$	(2R,3S)-1,2,3,4-Butanetetrol	Erythrite Meso-erythritol Tetrahydroxybutane	Flavor enhancer Humectant Sweetener
Mannitol	$C_6H_{14}O_6$	D-Mannitol	Mannite D-Mannitol	Anti-caking agent Bulking agent Humectant Stabilizer Sweetener Thickener
Sorbitol	$C_6H_{14}O_6$	D-Glucitol	D-Glucitol, D-Glucitol syrup Sorbit D-sorbitol Sorbol	Bulking agent Humectant Sequestrant Stabilizer Sweetener Thickener
Xylitol	$C_5H_{12}O_5$	D-erythro-pentitol		Emulsifier Humectant, Stabilizer Sweetener, Thickener

Major Types of Sweeteners Produced by Microorganisms

Microbial sweeteners have been produced using microorganisms such as bacteria, yeast, and fungi. Through biotechnological processes like fermentation and metabolic engineering, these microbes convert various feedstocks (e.g., sugars, starches) into low-calorie or non-caloric sweeteners (Zubair *et al.,* 2019). These sweeteners are increasingly popular as healthier alternatives to traditional sugar due to their reduced caloric content, lower glycaemic index, and sustainable production methods. Microbial sweeteners which are sugar alcohols include xylitol, erythritol, sorbitol and mannitol (Grembecka, 2015).

Xylitol

Xylitol is a naturally occurring sugar alcohol (polyol) that is widely used as a low-calorie sweetener. It is known for its sweetness, which is comparable to that of sucrose (table sugar), but with significantly fewer calories (Tiefenbacher, 2017). Xylitol is found in small quantities in fruits and vegetables, including berries, oats, mushrooms, and certain types of bark, particularly birch and beechwood (Cheng *et al.,* 2014). Its primary use is in sugar-free products such as chewing gums, candies, and oral hygiene products because of its non-cariogenic properties and its ability to prevent tooth decay. Additionally, xylitol has a very low glycaemic index, making it safe for consumption by individuals with diabetes (Rehman *et al.,* 2016).

Xylitol is a pentahydroxy sugar alcohol, structured with five hydroxyl groups that allow it to mimic the sweetness of sucrose while providing fewer calories, making it a popular low-calorie sweetener (Ahuja *et al.,* 2020). Unlike many sugars, xylitol resists fermentation by oral bacteria, which is crucial to its non-cariogenic properties; this helps inhibit cavity-causing bacteria like *Streptococcus mutans* and reduces the risk of dental caries (Peldyak and Mäkinen, 2002).

Industrial production of xylitol often involves catalytic hydrogenation of xylose, but alternative microbial production methods are being explored for their lower energy requirements and reduced environmental impact (Rafiqul and Sakinah, 2013). Additionally, xylitol's metabolism in the human body has minimal effects on blood glucose and insulin levels, a property that makes it especially beneficial for diabetic patients and those managing blood sugar levels (Ur-Rehman *et al.,* 2015). Industrial production of xylitol is carried out by a chemical hydrogenation process where Raney nickel is used as a catalyst to convert xylose from hemicellulosic hydrolysate to xylitol. It involves hydrolysis of the lignocellulosic biomass by acid to get monomeric sugars, treatment of hydrolysate to purify xylose, catalytic hydrogenation (usually carried out at a temperature of 353–413 K), xylitol purification, and xylitol crystallization (Arcaño *et al.,* 2020). By employing the catalytic hydrogenation method, about 50–60% of xylan from the hydrolysate can be converted to xylitol (Naidu *et al.,* 2018).
In food and pharmaceutical applications, stability of xylitol under heat and its moisture-retentive properties make it an ideal additive for products requiring longer shelf life and sweetness without added sugar calories (Pérez-Bibbins *et al.,* 2016).

The biotechnological conversion of xylose to xylitol is carried out by microorganisms that produce enzymes for xylose metabolism. The substrate, xylose, is obtained from the hemicellulose-rich fraction of lignocellulosic biomass such as wood, agricultural wastes, or aquatic weeds (Sindhu *et al.,* 2017; Espinoza-Acosta, 2020). Yeast is the predominant microorganism that can utilises xylose and ferments it to xylitol. Certain bacteria and filamentous fungi are also known to ferment xylose. In addition to microbial fermentation, enzymes have been used to produce xylitol as well. Furthermore, genetically engineered strains are being developed to improve xylitol yield to meet the industrial requirements (Xu *et al.,* 2019).
Microbial fermentation can be carried out under milder pressure and temperature compared to chemical methods. In the bioconversion process, organic waste can be extensively utilized and their environmental burden is reduced. Xylitol obtained through bioconversion can be used safely in food products as it does not have the risk of the presence of metal catalyst debris. The biotechnological process is safer and environmentally less polluting. Some inhibitors or impurities formed during hydrolysis of the biomass are either utilized or degraded partially by the microorganisms thus facilitating easier purification of the produced xylitol (Hernández-Pérez *et al.,* 2019). Consequently, the biotechnological method of producing xylitol has an added advantage in reducing production and purification costs and has the potential to replace chemical methods in terms of efficiency and sustainability.

Microbial Production of Xylitol

The biotechnological process of xylitol production is centered on the metabolism of xylose by microorganisms that can naturally

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utilize pentose sugars as a carbon source. Xylitol is produced by these microorganisms as an intermediate in the metabolic pathway of xylose (Narisetty *et al.,* 2022). The first step in the metabolism of D-xylose occurring involves xylose reductase which reduces D-xylose to xylitol. The xylitol thus produced is secreted out of the cell or otherwise, it is oxidized by xylitol dehydrogenase to produce D-xylulose (Yablochkova *et al.,* 2003). The secretion or oxidation of xylitol depends on the availability of cofactors. Xylose reductase requires NADPH or NADH and xylose dehydrogenase requires NADP or NAD for their activities. D-xylulose further gets phosphorylated by the action of xylulokinase and gets integrated with the non-oxidative route of the pentose phosphate pathway (Ravella *et al.,* 2012) (Figure 1). In the process of xylitol bioconversion, continuous availability of NADPH is required so that there is limited oxidation of xylitol to xylulose. Numerous research works have been done to screen microbial strains that can efficiently produce xylitol (Hernández-Pérez *et al.,* 2019; Narisetty *et al.,* 2022).

In contrast, bacteria contain the enzyme xylose isomerase that can directly convert D-xylose into D-xylulose which further enters the pentose phosphate pathway after phosphorylation. However, there are some exceptions. Some of the earlier studies done in the 1970s have shown that a few bacterial strains belonging to *Corynebacterium* and *Enterobacter* species contain the enzyme xylose reductase and have the ability to accumulate xylitol (Yoshitake *et al*., 1973a; b). In a study by Izumori and Tuzaki (1988), *Mycobacterium smegmatis* was found to produce xylitol. Several bacterial cultures belonging to the species *Cellulomonas*, *Corynebacterium*, and *Serratia* were screened for xylitol production by Rangaswamy and Agblevor (2002) and it was observed that among those, *Corynebacterium* produced maximum xylitol yield.

Recently it has been found that *Pseudomonas putida* can produce xylitol with a volumetric productivity of 0.98 g L^{-1} h^{-1} which is higher than other bacterial strains (Lugani and Sooch, 2020). Filamentous fungi such as *Penicillium chrysogenum* and *Petromyces albertensis* were first studied for xylitol production (Dahiya, 1991). However, bacteria and filamentous fungi do not favour xylitol production as much as yeast does. Yeast is widely studied due to the high xylitol productivity and assimilation of xylose. The most common yeast that produces high xylitol yield includes *Candida* and *Debaryomyces* (Guo *et al.,* 2006; Sampaio *et al.,* 2008). In a recent study by Carneiro and Almeida (2019) involving 44 isolates, it was found that *Meyerozyma species* were the best utilizers of xylose and *Wickerhamomyces anomalus* produced high xylitol yield. Candida strains such as *C. tropicalis* and *C. silvanorum* are the most promising xylitol producers as they have been found to have the highest xylose reductase activity (Yablochkova *et al.,* 2003). López-Linares *et al*. (2018) compared the production of xylitol by *Debaryomyces hansenii* and *Candida guilliermondii* and observed that *C. guilliermondii* showed high tolerance to inhibitors like furans and acids; thus possessing the advantage of not requiring additional detoxification steps. Similarly, a strain of *C. tropicalis* was found to produce xylitol with high tolerance to acetic acid in the hydrolysate (Junior *et al.,* 2019). Generally, the yield of xylitol is less if the hydrolysate is not detoxified. However, Prabhu *et al*. (2020a) obtained a high yield of xylitol using *Pichia fermentans* without detoxifying the hydrolysate. Oleaginous yeast such as *Yarrowia lipolytica* has also studied in the xylitol bioproduction (Prabhu *et al.,* 2020b).

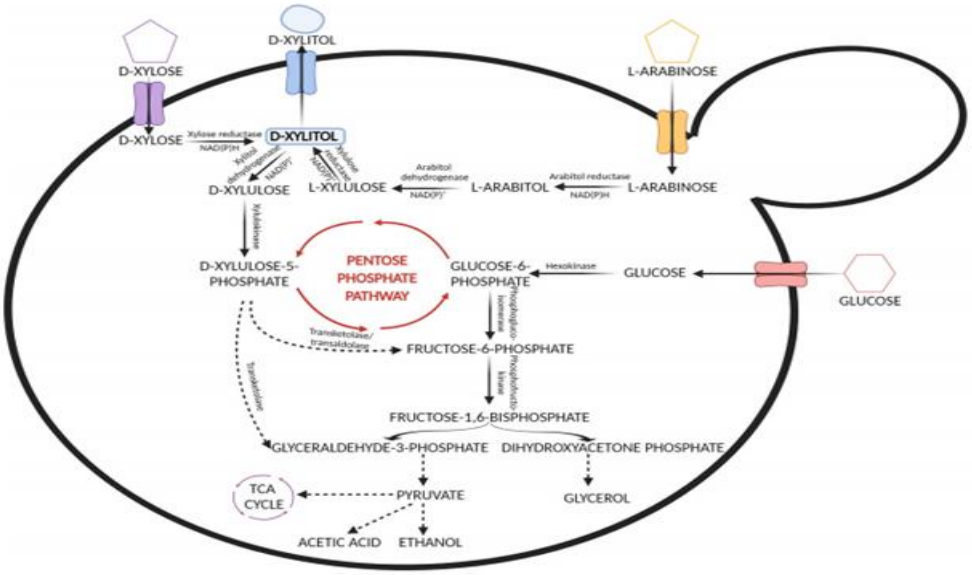

Figure 1. Metabolic pathways involved in the assimilation of xylose by yeast.

Erythritol

Erythritol, a first-generation polyol with a glycosidic nature, is a four-carbon compound produced by hydrolysing the aldehyde or a ketone groups in carbohydrates. It can be found in nature in products like wine (130-300 mg/L), soy sauce (910mg/L), vegetables, fruits (melons–22-47 mg/kg; pears–up to 40 mg/kg), and is also present in human body in semen, lens, cerebrospinal fluid, serum and human urine (10-30mg/L) (Barbieri *et al.,* 2014; Regnat *et al.,* 2018).

Erythritol is a low-calorie sweetener produced commercially through fermentation using osmophilic yeasts and yeasts-like fungi. Some filamentous fungi and heterofermentative lactic acid bacteria have shown the production of erythritol at lower yield on a laboratory scale (Osama, 2021). It exhibits antioxidative, noncariogenic, low glycaemic, high digestive tolerance, and non-acid-forming properties (Seshadrinathan and Chakraborty, 2022). Erythritol is the only polyol that provides zero calories, as per the regulations of the Food Safety and Standards Authority of India (FSSAI), while other polyols contribute to 2kcal/g. Excessive consumption of erythritol does not have adverse effects and does not raise blood glucose levels due to its very low glycemic response which make it suitable for individuals with diabetes and obesity (Daniëlle *et al.,* 2015). Erythritol has sensory properties similar to sucrose, with a fresh taste and a quickly disappearing aftertaste that gives it a refreshing (Tiefenbacher, 2019).

Erythritol is presently utilised as an ingredient in various products, such as food coatings, baked goods, fermented milk, confectionery and chocolate (Martău *et al.,* 2020). Nearly 90 years after polyols were first introduced into the food industry; erythritol remains the most commonly ingested sweetener due to its ability to reduce salivation, non-cariogenic ability, and its lack of impact on insulin levels when used in lit foods (Chattopadhyay *et al.,* 2014; Carocho *et al.,* 2017). Sweetness of erythritol with its mouthfeel enhancing properties has led to its use as a low-calorie alternative sweetener in the food industry. Additionally, it is utilized for preventing tooth decay as the microorganisms responsible for tooth decay are unable to utilise erythritol as a carbon source (Grembecka, 2016). Erythritol is increasingly gaining a significant market share as a natural sweetener, being utilised in low-calorie foods, beverages, and as an additive in combination with high-intensity zero calorie artificial sweeteners to elevate sweetness, texture and to cover the bitter aftertaste associated with these artificial sweeteners (Peter and Claire-Lise, 2002; Marco *et al*., 2023).

Production of Erythritol in Yeast

Various fungal genera encompassing yeast and yeast-like species have adapted to thrive in environments characterized by low water activity. These osmophilic organisms possess the ability to accumulate compatible solutes such as glycerol, D-arabitol, erythritol, and mannitol when they encounter osmotic stress (Moon *et al.,* 2010). This adaptation enables them to maintain cellular integrity and function under conditions of reduced water availability, contributing to their survival in challenging environments. Erythritol production involves the pentose phosphate pathway, a metabolic route where D-erythrose-4-phosphate undergoes dephosphorylation to form D-erythrose, followed by reduction to erythritol. This pathway serves multiple functions within the cell: it generates and supplies reducing capacity in the form of NADPH for various cellular responses, provides precursor molecules for amino acid biosynthesis and nucleotide, and acts independently as a compatible solute, safeguarding and stabilizing enzymes crucial for cellular activities in response to osmotic pressure (Moon *et al.,* 2010).

The osmoregulation protective role of erythritol is particularly significant, leading to the selection of osmophilic yeasts, including *Moniliella pollinis*, *Trichosporonoides megachiliensis*, *Aureobasidium* spp., *Trigonopsis variabilis*, *Trichosporon* spp., *Torula* spp., and *Candida magnolia*, as production strains. (Lin *et al.,* 2010; Chattopadhyay *et al.,* 2014; Grembecka, 2015). These yeasts primarily produce erythritol in response to salt or osmotic stress, allowing them to thrive in environments with reduced water availability (Yang *et al.,* 2015). This dual functionality of erythritol production not only benefits the cell by providing osmotic protection but also offers industrial applications, where these yeasts are harnessed for the large-scale production of erythritol.
Unfavourable fermentation conditions can disrupt the balance of erythritol production, leading to an increased formation of glycerol, which serves as the primary osmolyte in yeasts. To address this challenge, efforts have focused on enhancing erythritol yield while minimizing glycerol production. Two main strategies have been employed: random mutagenesis and fermentation optimization (Riahna *et al*., 2025).

Random mutagenesis, involving the use of UV and chemical mutagens, has been instrumental in identifying mutants that produce higher amounts of erythritol with reduced byproducts compared to their parental strains. This improvement is attributed to enhanced enzyme activity and expression in the pentose phosphate pathway (Park *et al.,* 2016). Notably, strains like *Trichoderma megachiliensis* SNG-42, *Candida magnoliae* JH110, and *Candida magnoliae* 12-2, obtained through random mutagenesis, are currently utilized for erythritol production due to their high yield (Li *et al.,* 2016).

Although strains capable of producing erythritol can transform glucose or fructose to this compound, increased productivity and yield have been achieved through the regulation of starting glucose concentrations using fed-batch fermentation techniques, coupled with optimized media compositions and supplementation with vitamins or minerals (Park *et al.,* 2016). Moreover, fine-tuning conditions for fermentation has been shown to mitigate the undesirable glycerol accumulation rate (Li *et al.,* 2016).
Additionally, stress from salt and osmotic conditions has been identified as a crucial factor for improving erythritol yields. Research has shown that a particular DNA sequence associated with a potential stress response component of the erythrose reductase gene of *Candida magnoliae* is enhanced expressly in response to stress from salt and conditions. Such stresses can be triggered by elevated levels of sugars, potassium chloride, or sodium chloride in the fermentation medium (Park *et al.,* 2011). Understanding and leveraging these stress responses have become crucial for optimizing erythritol production processes, leading to increased yields and improved efficiency in industrial settings.

As methods for erythritol production were refined and optimized, efforts turned towards exploring alternative carbon sources beyond glucose or fructose substrates. *Yarrowia lipolytica*, a yeast species, emerged as a promising candidate capable of converting both pure and unrefined glycerol into various compounds, including sugar alcohols (Rakicka *et al.,* 2020). Glycerol, a sustainable raw material and a major byproduct of biodiesel production, presented an attractive substrate option (Mirończuk *et al.,* 2015). Moreover, metabolic engineering strategies were employed to enhance sucrose and glycerol use, leading to enhanced erythritol formation. Overexpression of the GUT1 gene, which functions as glycerol kinase in the phosphorylative glycerol catabolic pathway, facilitated glycerol assimilation, addressing complications in erythritol biosynthesis and subsequent handling (Rakicka *et al.,* 2017).

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Despite these advancements, the specific metabolic pathway for erythritol biosynthesis in *Y. lipolytica* had not been fully elucidated until a recent study and this research revealed the involvement of the pentose phosphate pathway in erythritol formation and sought to identify key genes associated with this process (Mirończuk *et al.,* 2017). Overexpression of genes involved in this pathway, such as transketolase (TKL1) and two dehydrogenases (ZWF1 and GND1), resulted in an increase in erythritol synthesis, highlighting the significance of these genes in erythritol production (Mirończuk *et al.,* 2017).

Production of Erythritol in Bacteria

The quest for more economical substrates and the exploration of alternative host organisms for erythritol production are both gaining momentum. One such organism is the heterofermentative strain of lactic acid bacterium *Oenococcus oeni*, which utilizes an alternative mechanism for NADPH reoxidation under anaerobic conditions to produce erythritol (Regnat *et al.,* 2018).

The erythritol biosynthesis pathway in *Oenococcus oeni* differs from that in yeasts. In this bacterium, glucose-6-phosphate is initially converted into fructose-6-phosphate, which then undergoes cleavage to produce erythrose-4-phosphate and acetyl-phosphate. Erythrose-4-phosphate is then reduced to erythritol-4-phosphate before being converted to erythritol through dephosphorylation (Veiga-da-Cunha *et al.,* 1993; Ortiz *et al.,* 2013).

Lactobacillus sanfranciscencis, a LAB strain derived from sourdough, presents another noteworthy example. Under stress conditions, this bacterium exhibits the ability to produce erythritol as an additional byproduct of metabolism (Ortiz *et al.,* 2013). This discovery opens up intriguing possibilities for utilizing functional lactic starter cultures capable of synthesizing polyols such as erythritol. One compelling application of this capability is the development of newly developed fermented foods naturally sweetened with these low-calorie sugars. By incorporating erythritol-producing lactic acid bacteria into fermentation processes, food producers can create healthier product options without compromising on taste or sensory quality. This innovative approach aligns with the growing consumer demand for nutritious and flavorful food choices, offering exciting avenues for the future of food production and consumption (Ortiz *et al.,* 2013).

Production of Erythritol in Filamentous Fungi

Erythrose reductase plays a pivotal role in erythritol production, and extensive research has focused on characterizing and purifying various forms of this enzyme in yeasts (Deng *et al.,* 2012). In addition to yeasts, fungi like *Trichoderma reesei* have garnered attention for their industrial utility, particularly for their capacity to secrete large quantities of cellulases and hemicellulases. Notably, erythrose reductase (ER) is found naturally in *Trichoderma reesei* and has been thoroughly defined (Jovanovic *et al.,* 2013). This enzyme characterization provides valuable insights into the enzymatic machinery involved in erythritol biosynthesis, offering opportunities for further optimization and enhancement of erythritol production processes using fungal systems.

The production of erythritol in *Trichoderma reesei* follows a pathway similar to that in yeast, specifically the pentose phosphate pathway. The process begins with erythrose-4-phosphate, which is first subjected to dephosphorylation. After dephosphorylation, the resulting compound is then reduced to form erythritol. This reduction reaction depends on NADPH and is reversible.

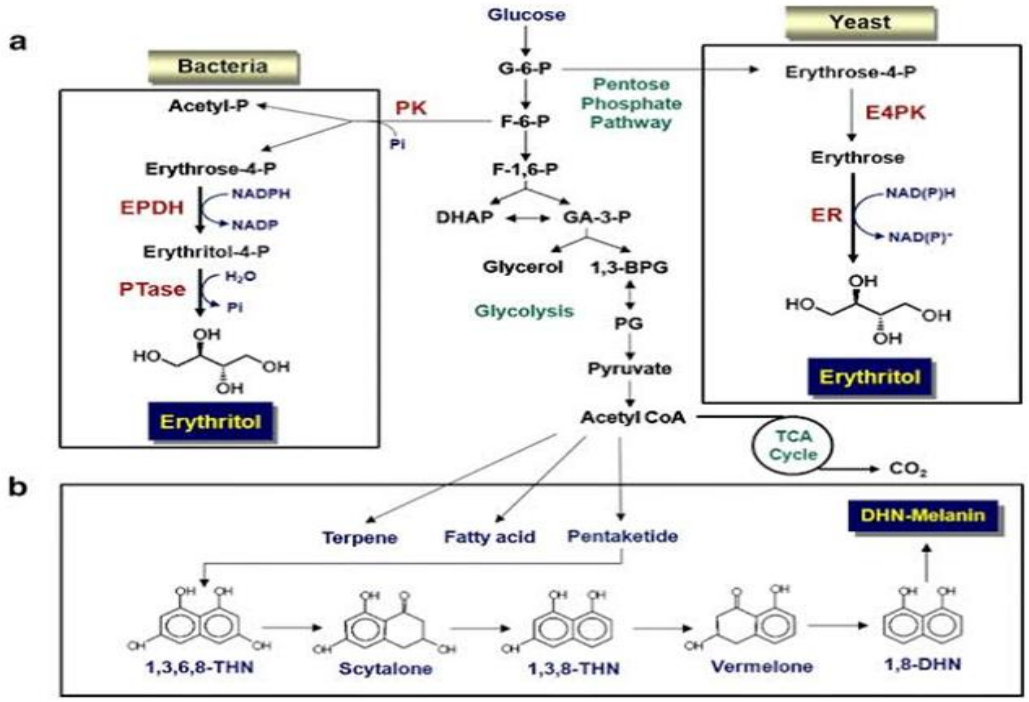

Figure 2: Pathway of Erythritol Biosynthesis (Moon *et al.,* 2010)

Even though the natural fermentation levels of erythritol in this organism are not as high as those found in yeasts, the organism offers other significant benefits. Notably, it has the ability to grow on lignocellulosic materials. This capability means it has the ability to use inexpensive biowaste materials as its ideal carbon source, which makes it a cost-effective option for erythritol production. By using biowaste, the production process can be made more sustainable and economical, leveraging low-cost raw materials for generating erythritol. The strain is highly capable of growing and producing erythritol when using wheat straw as a substrate. According to Jovanovic *et al.* (2014), a strain with overexpressed erythrose reductase demonstrates significantly increased erythritol production when grown on pretreated wheat straw. This suggests that by optimizing the substrate, improving the strain, engineering the metabolic pathways, and refining the fermentation process, this method could offer a promising alternative to the use of costly fermentation substrates. Such advancements could make the production of erythritol more efficient and economically viable by utilizing low-cost, readily available materials like wheat straw.

Mannitol

Mannitol, a six-carbon sugar alcohol, is widely used across multiple industries due to its favorable physicochemical and physiological properties. Its applications span the food, pharmaceutical, and medical fields. Mannitol is especially valued for its functional roles as a sweetener, osmotic agent, and therapeutic excipient (Kazuki *et al*., 2021).

Mannitol is about half as sweet as sucrose, but what makes it particularly appealing is the cooling sensation it imparts when consumed, which can help to mask bitterness in pharmaceutical formulations. This sweetening effect enhances its use in various medications and lozenges, improving taste profiles and patient acceptability. Mannitol has a melting point of approximately 168°C and demonstrates notable chemical stability. Due to its low glycaemic index and slow metabolism, mannitol is ideal for diabetic and low-calorie products, offering a safer alternative to traditional sugars for people with blood sugar sensitivities (Shawkat *et al.,* 2012; Chen *et al.,* 2020).

Traditionally, mannitol is produced through the hydrogenation of fructose in a chemical synthesis process. However, this method is limited by relatively low yield rates and high production costs. In recent years, interest has shifted toward microbial and enzymatic methods of mannitol production, which offer more sustainable, cost-effective alternatives (Min *et al*., 2018).

Production of Mannitol

Microbial production of mannitol typically involves fermentation by specific microorganisms, including LAB, fungi, and certain yeast species, which convert fructose into mannitol under controlled conditions. Lactic acid bacteria, such as *Leuconostoc mesenteroides*, have shown particularly high efficiency in this conversion process, utilizing fructose as an electron acceptor and producing mannitol as a primary metabolite. Enzymes like mannitol dehydrogenase (MDH) play a crucial role, catalyzing the conversion of fructose to mannitol in the presence of the coenzyme NADPH or NADH (Saha and Racine, 2011; Dai *et al.,* 2017). In microbial production systems, high concentrations of mannitol (up to 180 g/L) have been achieved, especially under optimized fermentation conditions. In such systems, glucose is typically converted to lactic acid and acetic acid; while fructose is reduced to mannitol, allowing for efficient co-fermentation and improved mannitol yields. This microbial pathway does not only reduce energy consumption, but also minimizes the need for high-temperature processing and complex purification steps required in chemical synthesis. Moreover, by using agricultural and food waste products as substrates, microbial production can be both economically and environmentally advantageous (Chen *et al.,* 2020).

Advances in biotechnology, particularly in metabolic and protein engineering, have significantly improved microbial production of mannitol. By genetically modifying strains of LAB and optimizing their metabolic pathways, researchers have enhanced the efficiency of mannitol production. For example, certain metabolic engineering strategies involve the overexpression of MDH and the knockout of competing pathways, allowing for higher mannitol yields and better resource utilization. These engineered strains can use renewable substrates, like fructose derived from lignocellulosic biomass, further supporting sustainable production (Dai *et al.,* 2017).

Additionally, research has identified several factors that enhance the fermentation process, including optimal pH, temperature, and nutrient availability. Studies have shown that heterofermentative LAB strains, such as *Lactobacillus reuteri* and *Leuconostoc mesenteroides*, are capable of producing mannitol with nearly 100% conversion efficiency when co-fermented with glucose and fructose under controlled conditions. This approach reduces by-products and maximizes mannitol yield, making microbial production highly competitive with chemical methods (Saha and Racine, 2011).

Sorbitol

Sorbitol is a sugar alcohol (polyol) that is commonly used as a low-calorie sweetener in various food, pharmaceutical, and cosmetic products. Naturally occurring in many fruits such as apples, pears, peaches, and prunes, sorbitol is widely known for its sweet taste and hygroscopic properties (ability to retain moisture) (Marques *et al*., 2016). It is less sweet than sucrose, with about 50-60% of its sweetness, but offers a lower caloric content of approximately 2.6 calories per gram compared to 4 calories per gram for table sugar (Silveira and Jonas, 2002). Its low caloric content, non-cariogenic properties, and low glycemic index have made it popular in diabetic-friendly foods, sugar-free products, and oral care products. It is one of the most widely used sugar alcohols in the global food industry (Grembecka, 2015).

Sorbitol is a hexitol, meaning it has six hydroxyl (-OH) groups attached to six carbon atoms. It is a stereoisomer of mannitol, meaning it shares the same chemical formula but differs in the arrangement of atoms. Sorbitol is a white, odorless crystalline powder or syrup that is highly soluble in water due to its numerous hydroxyl groups. This property makes it highly effective as a humectant and stabilizer, giving it wide-ranging applications in the food and pharmaceutical industries (Nezzal *et al.,* 2009).

Production of Sorbitol

Microbial production of sorbitol is an innovative and sustainable alternative to conventional chemical synthesis, offering potential for efficient, eco-friendly production using various microorganisms. Sorbitol, a polyol commonly used as a low-calorie sweetener, humectant, and stabilizer, can be biotechnologically produced via

the fermentation of glucose and fructose by specific bacteria and engineered microbial strains. Recent studies have focused on optimizing microbial processes for sorbitol production, enhancing yield, and improving industrial feasibility (Yupaporn *et al*., 2024).
Microbial production of sorbitol primarily involves the bacterium *Zymomonas mobilis*, which utilizes glucose-fructose oxidoreductase (GFOR) in a one-step reaction to produce sorbitol from fructose and glucose. This enzymatic pathway is unique to *Z. mobilis* and enables it to efficiently produce sorbitol alongside gluconic acid in a co-metabolic process (Silveira and Jonas, 2002). This method has shown significant industrial promise, as the one-step reaction is both time-efficient and reduces the need for multiple processing steps, lowering production costs compared to chemical synthesis (Silveira and Jonas and, 2002).

To improve sorbitol yields and production efficiency, genetic engineering techniques have been employed to enhance the metabolic pathways in certain bacteria. *Lactobacillus casei* and *Lactobacillus plantarum* have been engineered to produce sorbitol by integrating the sorbitol-6-phosphate dehydrogenase (gutF) gene, allowing these strains to convert glucose into sorbitol with high efficiency. Notably, *Lactobacillus plantarum* mutants with deficient lactate dehydrogenase activities exhibited enhanced NADH availability, thus improving sorbitol synthesis while also reducing the formation of unwanted byproducts (Ladero *et al.,* 2007). This genetic modification aligns the strain's metabolic output toward sorbitol, improving productivity and offering a robust solution for large-scale production.

A recent research has explored the use of photosynthetic organisms such as cyanobacteria, specifically *Synechocystis* sp PCC 6803, for sorbitol production. By introducing sorbitol-6-phosphate dehydrogenase (S6PDH) from apple and optimizing photosynthetic conditions, researchers achieved significant sorbitol yields, which are promising for environmentally sustainable production. The incorporation of a theophylline-inducible riboswitch allowed controlled expression of S6PDH, alleviating toxicity issues and boosting sorbitol productivity under light-driven conditions (Chin *et al.,* 2018).

Industrial-scale microbial production of sorbitol requires the optimization of fermentation conditions. One approach includes the use of immobilized cell systems to enable repeated batch fermentation, thus improving cell viability and stability. This technique, applied with *Lactobacillus plantarum* in sodium alginate beads, has demonstrated stable sorbitol yields across multiple fermentation cycles, presenting a sustainable solution for long-term sorbitol production (Zuriana and Sakinah, 2017).

The biotechnological production of sorbitol is expected to expand with advances in metabolic engineering and fermentation technology. Engineered microbial strains can facilitate high-yield, low-cost production processes that align with growing demands for natural and sustainable sweeteners in food and pharmaceutical industries. Further exploration of photosynthetic sorbitol production and genetic modification strategies may pave the way for environmentally friendly sorbitol synthesis at an industrial scale.

Future Prospects of microbial sweeteners

The future of microbial production for sweeteners like xylitol, erythritol, mannitol, and sorbitol shows great promise, driven by advances in metabolic engineering, cost-effective fermentation, and sustainable practices. These sugar alcohols are not only valuable as low-caloric, low-glycemic Sweeteners, but also as compounds with important therapeutic benefits. Here is an overview of the future prospects and innovations shaping the field.

Enhanced Microbial Strains through Genetic Engineering

One of the most impactful advancements in the microbial production of sugar alcohols has been the development of genetically engineered microbial strains with optimized pathways for producing xylitol, erythritol, mannitol, and sorbitol. For instance, LAB have been engineered to produce higher yields of polyols due to their unique fermentative metabolism, which can be manipulated to optimize redox balance and produce sugar alcohols with high efficiency. Since LAB are already used in food fermentation, they are ideally suited for producing functional foods where the polyols can be generated directly in situ (Monedero *et al.,* 2010). This capacity to ferment sugars efficiently and under safe conditions for human consumption is an asset that could expand their use in both the food and pharmaceutical industries.

Utilisation of Renewable Biomass for Cost-Effective Production

Another promising trend is the use of renewable biomass, such as lignocellulosic waste, as a raw material for microbial production of xylitol and other sugar alcohols. Biomass materials, including agricultural residues and byproducts, contain sugars that can be converted into polyols through microbial processes. For instance, xylose, a sugar derived from biomass, can be fermented into xylitol. By using waste as a feedstock, the production process becomes more sustainable and reduces reliance on chemically intensive methods. Biomass-derived xylitol has been shown to be an economically viable alternative, especially when enzymatic hydrolysis is employed to break down the biomass into sugars efficiently (Prakasham *et al.,* 2009). This shift toward renewable sources is anticipated to make sugar alcohol production more environmentally friendly and cost-effective.

Advances in Synthetic Biology and Metabolic Engineering

Synthetic biology offers another avenue for improving microbial production of these sugar alcohols. Using techniques from synthetic biology, researchers have been able to create "super strains" of microbes specifically designed for high-yield production of xylitol and other polyols from inexpensive substrates like glucose. Metabolic engineering techniques, such as manipulating the redox balance and reducing unwanted byproduct formation, have further enhanced production efficiency. For example, engineering yeasts to produce xylitol directly from glucose by optimizing NADPH availability has shown considerable promise in reducing production costs and increasing yield. These engineered strains are now closer to achieving industrial-scale production standards (Xu *et al.,* 2019). The future holds the potential for further advancements through synthetic biology to create strains that perform well even under industrial conditions, making microbial xylitol production a competitive alternative to chemical synthesis.

Increased Efficiency through Immobilised Bioreactor Systems

Using immobilized microbial cultures in bioreactors is another promising approach to improving production efficiency. Immobilization allows microbes to be used for longer periods without degradation, making the process more stable and reducing

the cost of replenishing biocatalysts. Research has shown that immobilized cultures are effective in producing high yields of xylitol and mannitol, and they offer better control over the fermentation process. This technique has been tested in bioreactors and has shown to increase microbial reusability, thus making it a viable solution for scaling up production to meet industry demands (Pérez-Bibbins *et al.,* 2016). This development could be particularly impactful for producing xylitol in large quantities as it allows for more continuous and consistent production.

Sustainability and Lower Energy Costs in Production

The microbial production of sugar alcohols has several advantages over traditional chemical methods. Microbial methods typically require lower temperatures and avoid the use of harsh chemicals, making the process more eco-friendly. By producing erythritol, mannitol, and other polyols through microbial processes, industries can lower energy costs and reduce their environmental impact. Studies underscore that these sustainable practices are essential in making microbial sugar alcohols more appealing to both consumers and industries focused on reducing their carbon footprint (Rice *et al.,* 2020).

Therapeutic and Functional Food Potential

The unique health properties of xylitol, erythritol, mannitol, and sorbitol, such as their low-caloric content and minimal glycemic impact, position them as ideal ingredients in functional foods. Products formulated with these sugar alcohols are valuable for health-conscious consumers, including those managing diabetes or seeking weight control. Future production of these sugar alcohols is likely to focus on meeting the growing demand for functional foods and low-calorie sweeteners. As research continues to highlight the metabolic benefits of these sugar alcohols, such as their prebiotic effects and potential role in dental health, demand is expected to increase (Paulino *et al.,* 2021).

In summary, the future of microbial sweetener production appears bright. Continued innovations in metabolic engineering, renewable biomass utilisation, and sustainable practices are making the production of xylitol, erythritol, mannitol, and sorbitol more economically viable and environmentally sustainable. These advancements will likely expand the use of these sweeteners in health products and functional foods, supporting an increased focus on natural, low-calorie sweeteners.

Conclusion

The microbial production of sweeteners, specifically xylitol, erythritol, mannitol, and sorbitol, is a rapidly advancing field with significant implications for both the food industries and health sector. Currently, microbial production presents a viable and sustainable alternative to chemical synthesis, offering lower energy costs, reduced environmental impact, and the ability to utilize renewable biomass. Recent advancements in metabolic engineering and synthetic biology have enabled the development of specialized microbial strains that can produce these sugar alcohols with higher yields and efficiency, even from low-cost substrates like agricultural wastes. Furthermore, innovations in bioreactor designs, including the use of immobilized microbial cultures, have improved the scalability and economic feasibility of microbial processes, making industrial-scale production more attainable.

In terms of applications, these microbial sweeteners serve the growing demand for low-caloric, low-glycaemic, and functional ingredients in food, beverages, and health products. As natural, calorie-reducing alternatives, they offer considerable benefits for diabetes management, weight control, and oral health, fitting well into the trend towards functional and health-promoting foods. Looking forward, the industry is poised to benefit from further genetic and metabolic optimization of microbial strains, enhanced bioprocessing technologies, and expanded use of renewable feedstocks. These developments promise to reduce production costs and increase product accessibility, making microbial sweeteners a central component in the push for sustainable, health-focused food production.

REFERENCES

Ahuja, V., Macho, M., Ewe, D., Singh, M., Saha, S., and Saurav, K. (2020). Biological and pharmacological potential of xylitol: a molecular insight of unique metabolism. *Foods*, 9.

Arcaño, Y. D., García, O. D., Mandelli, D., Carvalho, W. A., and Pontes, L. A. (2020). Xylitol: a review on the progress and challenges of its production by chemical route. *Catal. Today*. 344, 2-14.

Barbieri, G., Barone, C., Bhagat, A., Caruso, G., Conley, Z.R., and Parisi, S., (2014). Sweet compounds in foods: sugar alcohols. In: The Influence of Chemistry on New Foods and Traditional Products; Springer: Berlin/Heidelberg, Germany, pp. 51-59.

Carneiro, C. V., and Almeida, J. R. (2019). Xylitol production: identification and comparison of new producing yeasts. *Microorganisms*. 7, 484.

Carocho, M., Morales, P., and Ferreira, I., (2017). Sweeteners as food additives in the XXI century: a review of what is known, and what is to come. *Food Chemistry and Toxicology*. 107:302-317.

Chattopadhyay, S., Raychaudhuri, U., and Chakraborty, R. (2014). Artificial sweeteners–a review. *Journal of Food Science and Technology*. 51:611-621.

Chen, M., Zhang, W., Wu, H., Guang, C., and Mu, W. (2020). Mannitol: physiological functionalities, determination methods, biotechnological production, and applications. *Applied Microbiology and Biotechnology,* 104 (15): 6941-6951.

Cheng, H., Lv, J., Wang, H., Wang, B., Li, Z., and Deng, Z. (2014). Genetically engineered Pichia pastoris yeast for conversion of glucose to xylitol by a single-fermentation process. Appl. *Microbiol. Biotechnol*. 98, 3539-3552

Chin, T., Okuda, Y., and Ikeuchi, M. (2018). Sorbitol production and optimization of photosynthetic supply in the cyanobacterium *Synechocystis* sp. PCC 6803. *Journal of Biotechnology*, 25-33

Dahiya, J. S. (1991). Xylitol production by Petromyces albertensis grown on medium containing D-xylose. *Can. J. Microbiol*. 37, 14-18.

Dai, Y., Meng, Q., Mu, W., and Zhang, T. (2017). Recent advances in the applications and biotechnological production of mannitol. *Journal of Functional Foods, 36*, 404–409.

Daniëlle, M., Gertjan, J., Peter de Cock, Douwina, B., Angela, B., and Aalt, B., (2015). Health effects of erythritol. *Nutrafoods*.14: 3-9.

Deng, H., Han, Y., Liu, Y., Jia, W., and Zhou, Z., (2012). Identification of a newly isolated erythritol-producing yeast and cloning of its erythrose [corrected] reductase genes. *J Ind Microbiol Biotechnol*. 39 (11):1663-1672.

EFSA (2011) Scientific opinion on the substantiation of health

claims related to the sugar replacers xylitol, D-tagatose, xylitol, sorbitol, mannitol, maltitol, lactitol, isomalt,erythritol, D-tagatose, isomaltulose, sucralose and polydextrose and maintenance of tooth mineralisation by decreasing tooth demineralisation. EFSA Journal 9 (4): 2076. http://www.efsa.europa.eu/en/efsajournal/doc/2076.pdf. Accessed 10 October 2024

Espinoza-Acosta, J. L. (2020). Biotechnological production of xylitol from agricultural waste. *Biotecnia*. 22, 126–134.

Fitch, C. and Keim, K., (2012) Position of the Academy of Nutrition and Dietetics: use of nutritive and nonnutritive sweeteners. *J Acad Nutr Diet*. 112:739–758

Grabitske, H.A. and Slavin, J.L., (2008) Perspectives in practice low-digestible carbohydrates in practice. *J Am Diet Assoc*. 108:1677–1681

Grembecka, M., (2015). Sugar alcohols-their role in the modern world of sweeteners: A review. *Eur. Food Res. Technol*. **241**(1): 15–16.

Grembecka, M., (2016) Sugar Alcohols as Sugar substitutes in Food Industry. In: Merillon J-M, Ramawat KG, eds. Sweeteners: Pharmacology, Biotechnology, and Applications. Cham: Springer International Publishing; 2016:1-27.

Guo, C., Zhao, C., He, P., Lu, D., Shen, A., and Jiang, N. (2006). Screening and characterization of yeasts for xylitol production. *J. Appl. Microbiol*. 101, 1096–1104.

Health Canada (2005) http://www.hc-sc.gc.ca/fn-an/securit/addit/sweeten-edulcor/polyols_polydextose_factsheet-polyols_polydextose_fiche-eng.php. Accessed 10 May 2024

Hernández-Pérez, A. F., de Arruda, P. V., Sene, L., da Silva, S. S., Kumar Chandel, A., and de Almeida Felipe, M. D. (2019). Xylitol bioproduction: state-of-the-art, industrial paradigm shift, and opportunities for integrated biorefineries. *Crit. Rev. Biotechnol*. 39, 924–943.

Hernández-Pérez, A.F., Jofre, F.M. and Gómez-López, A. (2020). Microbial production of artificial sweeteners. In *Advances in Bioactive Food Ingredients*. pp. 179-204).

Izumori, K., and Tuzaki, K. (1988). Production of xylitol from Dxylulose by Mycobacterium smegmatis. *J. Ferment. Technol*. 66, 33–36.

Jovanovic, B., Mach, L., and Mach-Aigner, R., (2013). Characterization of erythrose reductases from filamentous fungi. *AMB Express.* 3 (1): 43.

Jovanovic, B., Mach, L., and Mach-Aigner, R., (2014). Erythritol production on wheat straw using *Trichoderma reesei*. *AMB Express*. 4(1):34.

Junior, W. G. M., Pacheco, T. F., Trichez, D., Almeida, J. R., and Gonçalves, S. B. (2019). Xylitol production on sugarcane biomass hydrolysate by newly identified Candida tropicalis JA2 strain. Yeast. 36, 349–361.

Kazuki Matsui , Tomoya Nakagawa , Tomonori Okumura , Miki Ya mane , Yuji Tokunaga and Shoji Yokota.(2021). Potential pharmacokinetic interaction between orally administered drug and osmotically active excipients in pediatric polypharmacy. *European Journal of Pharmaceutical Sciences.* 165**,** 105934.

Ladero, V., Ramos, A., Wiersma, A., Goffin, P., Schanck, A., Kleerebezem, M., Hugenholtz, J., and Hols, P. (2007). High-Level Production of the Low-Calorie Sugar Sorbitol by *Lactobacillus plantarum* through Metabolic Engineering. *Applied and Environmental Microbiology,* 73, 1864-1872

Li, L., Yang, T., Guo, W., Ju, X., Hu, C., Tang, B., Fu, J., Gu, J., and Zhang, H., (2016). Construction of an efficient mutant strain of *Trichosporonoides oedocephalis* with HOG1 gene deletion for production of erythritol. *J Microbiology and Biotechnology*. 26 (4):700–709.

Lin, S., Wen, C., Wang, P., Huang, J., Wei, C., Chang, J., and Chu, W., (2010). High-level production of erythritol by mutants of osmophilic *Moniliella* sp. *Process Biochemistry*.45 (6): 973-979.

Livesey, G. (2003) Health potential of polyols as sugar replacers, with emphasis on low-glycaemic properties. *Nutr Res Rev*. 16:163–191

López-Linares, J. C., Romero, I., Cara, C., Castro, E., and Mussatto, S. I. (2018). Xylitol production by *Debaryomyces hansenii* and *Candida guilliermondii* from rapeseed straw hemicellulosic hydrolysate. *Bioresource Technology*. 247, 736-743.

Lugani, Y., and Sooch, B. S. (2020). Fermentative production of xylitol from a newly isolated xylose reductase producing Pseudomonas putida BSX-46. LWT. 134, 109988.

Mäkinen KK (2011) Sugar alcohol sweeteners as alternatives to sugar with special consideration of xylitol. *Med Princ Pract*. 20:303-320.

Marco Witkowski , Ina Nemet, Hassan Alamri, Jennifer Wilcox, Nilaksh Gupta, Nisreen Nimer, Arash Haghikia, Xinmin S. Li, Yuping Wu, Prasenjit Prasad Saha, Ilja Demuth, Maximilian König, Elisabeth Steinhagen-Thiessen, Tomas Cajka, Oliver Fiehn, Ulf Landmesser, W.H.Wilson Tang, Stanley L. Hazen. (2023). The artificial sweetener erythritol and cardiovascular event risk. *Nat Med*. 29 (3): 710-718. doi:10.1038/s41591-023-02223-9.

Marques, C., Tarek, R., Šára, M., and Brar, S. K. (2016). Sorbitol production from biomass and its global market. In *Biomass Resources and Biorefining* (pp. 217-227).

Martău, A., Coman, V., and Vodnar, C., (2020). Recent advances in the biotechnological Production of erythritol and mannitol. *Critical Review Biotechnology*. 40, 608-622.

Min Z, Lei G, Chao C, Jiangfeng M, Fengxue X, Junli L, Hao W & Min J. (2018). Recent advances in microbial production of mannitol: utilisation of low-cost substrates, strain development and regulation strategies. *World Journal of Microbiology and Biotechnology*. 34: (41) https://doi.org/10.1007/s11274-018-2425-8

Mirończuk, M., Biegalska, A., and Dobrowolski, A., (2017) Functional overexpression of genes involved in erythritol synthesis in the yeast *Yarrowia lipolytica*. *Biotechnology Biofuels*. 10 (1):77.

Mirończuk, M., Rakicka, M., Biegalska, A., Rymowicz, W., and Dobrowolski, A., (2015). A two-stage fermentation process of erythritol production by yeast *Y. lipolytica* from molasses and glycerol. *Bioresource Technology.* 198:445-455.

Monedero, V., Pérez-Martínez, G., and Yebra, M. J. (2010). Perspectives of engineering lactic acid bacteria for biotechnological polyol production. *Applied Microbiology and Biotechnology,* 86 (4): 1003-1015.

Moon, J., Jeya, M., Kim, W., and Lee, K., (2010). Biotechnological production of erythritol and its applications. *Appl Microbiology and Biotechnology*. 86 (4):1017-1025.

Naidu, D. S., Hlangothi, S. P., and John, M. J. (2018). Bio-based products from xylan: A review. Carbohydr. *Polym*. 179, 28-41

Nakamura, S., (2005) Bioavailability of cellobiose and other nondigestible and/or nonabsorbable sugar substitutes and related topics. *Nutrition*. 21:1158-1159.

Narisetty, V., Cox, R., Bommareddy, R. R., Agrawal, D., Ahmad, E., Pant, K. K., et al. (2022). Valorisation of xylose to renewable fuels and chemicals, an essential step in augmenting the commercial viability of lignocellulosic biorefineries. *Sustain. Energy Fuels*. 6, 29–65.

Nezzal, A., Aerts, L., Verspaille, M., Henderickx, G., and Redl, A. (2009). Polymorphism of sorbitol. *Journal of Crystal Growth,* 311, 3863-3870

Ortiz, M.E., Bleckwedel, J., Raya, R.R., and Mozzi, F., (2013). Biotechnological and in situ food production of polyols by lactic acid bacteria. *Applied Microbiology and Biotechnology*. 97 (11):4713-4726.

Osama Ibrahim, (2021). Erythritol Chemical Structure, Biosynthesis Pathways, Properties, Applications, and Production. *International Journal of Microbiology and Biotechnology*. 6 (3): 59-70.

Park, C., Oh, J., Jo, H., Jin, S., and Seo, H., (2016). Recent advances in biological production of sugar alcohols. *Current Opinion Biotechnology*. 37: 105-113.

Park, H., Lee, Y., Ryu, W., Seo, H., and Kim, D., (2011). Role of osmotic and salt stress in the expression of erythrose reductase in *Candida magnoliae*. *J Microbiology and Biotechnology*. 21(10):1064-1068.

Patra, F., Tomar, S.K., and Arora, S. (2009). Technological and functional applications of low calorie sweeteners from lactic acid bacteria. *Journal of Food Science*, 74(1), 16-22.

Paulino, B. N., Molina, G., Pastore, G., and Bicas, J. (2021). Current perspectives in the biotechnological production of sweetening syrups and polyols. *Current Opinion in Food Science,* 41, 36-43.

Peldyak, J., and Mäkinen, K. (2002). Xylitol for caries prevention. *Journal of dental hygiene*, 76 (4): 276-85.

Peter de Cock‡ and Claire-Lise Bechert. (2002). Erythritol. Functionality in noncaloric functional beverages. *Pure and Applied Chemistry*, 74 (7): 1281-1289.

Pérez-Bibbins, B., Torrado-Agrasar, A., Salgado, J. M., Mussatto, S. I., and Domínguez, J. M. (2016). Xylitol production in immobilized cultures: A recent review. *Critical Reviews in Biotechnology,* 36 (4), 691-704.

Philippe, R.N., De Mey, M., Anderson, J., & Caimi, P. (2014). Biotechnological production of natural zero-calorie sweeteners. *Current Opinion in Biotechnology*, 26, 155-161.

Prabhu, A. A., Bosakornranut, E., Amraoui, Y., Agrawal, D., Coulon, F., Vivekanand, V., et al. (2020a). Enhanced xylitol production using non-detoxified xylose rich pre-hydrolysate from sugarcane bagasse by newly isolated *Pichia fermentans*. *Biotechnol. Biofuels*. 13, 1-5.

Prabhu, A. A., Thomas, D. J., Ledesma-Amaro, R., Leeke, G. A., Medina, A., Verheecke-Vaessen, C., et al. (2020b). Biovalorisation of crude glycerol and xylose into xylitol by oleaginous yeast *Yarrowia lipolytica. Microb*. *Cell Factories*. 19, 1-8.

Prakasham, R., Rao, R. S., and Hobbs, P. (2009). Current trends in biotechnological production of xylitol and future prospects. *Current Trends in Biotechnology and Pharmacy,* 3 (1), 8-36.

Rafiqul, I., and Sakinah, M. (2013). Processes for the production of xylitol—A review. *Food Reviews International,* 29 (2), 127-156.

Rakicka, M., Biegalska, A., Rymowicz, W., Dobrowolski*, A.,* and Mironczuk, M., (2017). Polyol production from waste materials by genetically modified *Yarrowia lipolytica*. *Bioresource Technology*. 243:393-399.

Rakicka, M., Mironczuk, M., Celinska, E., Białas, W., and Rymowicz, W., (2020). Scale-up of the erythritol production technologyeProcess simulation and techno-economic analysis. *Journal of Cleaner Production*. 257, 120533

Rangaswamy, S., and Agblevor, F. A. (2002). Screening of facultative anaerobic bacteria utilizing D-xylose for xylitol production. *Applied Microbiology and Biotechnology*. 60, 88-93.

Ravella, S. R., Gallagher, J., Fish, S., and Prakasham, R. S. (2012). "Overview on commercial production of xylitol, economic analysis and market trends," in D-Xylitol, eds. S. S. Da Silva and A. K. Chandel (Berlin, Springer), 291-306.

Regnat, K., Mach, R.L., and Mach-Aigner, A.R. (2018). Erythritol as sweetener - wherefrom and whereto? *Appl. Microbiol. Biotechnol*. 102, 587-595.

Rehman, S., Murtaza, M. A., and Mushtaq, Z. (2016). "Xylitol as sweetener,"in Sweeteners, Reference Series in Phytochemistry, eds J. M. Merillon and K. Ramawat (Cham: Springer), 1-21.

Riahna Kembaren*, Arli Aditya Parikesit, Jocelyn Nataniel, Nethania Angeline Dharmawan, Charlivo Mikaichi Dungus, Priscilla Angelique and Solmaz Aslanzadeh. (2025). Optimization of erythritol production through fermentation using molasses as carbon source. *Acta Biochimica Polonica*. 1-14.

Rice, T., Zannini, E., Arendt, E. K., and Coffey, A. (2020). A review of polyols – biotechnological production, food applications, regulation, labeling and health effects. *Critical Reviews in Food Science and Nutrition, 60*(12), 2034-2051.

Saha, B., and Racine, F. M. (2011). Biotechnological production of mannitol and its applications. *Applied Microbiology and Biotechnology,* 89 (4), 879-891.

Sampaio, F. C., Chaves-Alves, V. M., Converti, A., Passos, F. M., and Coelho, J. L. (2008). Influence of cultivation conditions on xylose-to-xylitol bioconversion by a new isolate of *Debaryomyces hansenii*. *Bioresource Technology*. 99, 502-508.

Seshadrinathan, S., and Chakraborty, S., (2022). Fermentative Production of Erythritol from Cane Molasses Using *Candida magnoliae*: Media Optimization, Purification, and Characterization. *Sustainability*.**14**, 10342.

Shankar, P., Ahuja, S., and Sriram, K. (2013) Non-nutritive sweeteners: review and update. *Nutrition*. 29:1293-1299

Shawkat, H., Westwood, M., and Mortimer, A. (2012). Mannitol: A review of its clinical uses. *Continuing Education in Anaesthesia Critical Care & Pain, 12*(2), 82-85.

Silveira, M., and Jonas, R. (2002). The biotechnological production of sorbitol. *Applied Microbiology and Biotechnology,* 59, 400-408.

Sindhu, R., Binod, P., Pandey, A., Madhavan, A., Alphonsa, J. A., Vivek, N., Gnansounou E. , Castro E. [7], Faraco V. (2017). Water hyacinth a potential source for value addition: an overview. *Bioresour. technol*. 230, 152-162.

Tiefenbacher, F. (ed.). (2017). "Technology of Main Ingredients—Sweeteners and Lipids," in The Technology of Wafers and Waffles: Operational Aspects, (Academic Press), 123-225.

Tiefenbacher, K.F., (2019). Glossary of terms in wafers, waffles

and adjuncts. In: Tiefenbacher KF, editor. The technology of wafers and waffles II. London: Academic Press; 325-411.

Ur-Rehman, S., Mushtaq, Z., Zahoor, T., Jamil, A., and Murtaza, M. (2015). Xylitol: a review on bioproduction, application, health benefits, and related safety issues. *Critical Review in Food Science and Nutrition*, 55, 1514 - 1528.

Veiga-da-Cunha, M., Santos, H., and Van Schaftingen, E., (1993). Pathway and regulation of erythritol formation in *Leuconostoc oenos*. *J Bacteriology*. 175 (13):3941-3948.

Wheeler, M.L. and Pi-Sunyer, X. (2008) Carbohydrate issues. Type and amount. *J Am Diet Assoc*. 108: S34-39

Xiao, J., Li, X., Min, X., and Sakaguchi, E., (2013) Mannitol improves absorption and retention of calcium and magnesium in growing rats. *Nutrition*. 29:325-331

Xu, Y., Chi, P., Bilal, M., and Cheng, H. (2019). Biosynthetic strategies to produce xylitol: an economical venture. *Appl. Microbiol. Biotechnol*. 103, 5143-5160.

Yablochkova, E. N., Bolotnikova, O. I., Mikhailova, N. P., Nemova, N. N., and Ginak, A. I. (2003). The activity of xylose reductase and xylitol dehydrogenase in yeasts. *Microbiology*. 72, 414-417.

Yang, B., Dai, M., Zheng, Y., Zhu, L., Zhan, B., and Lin, C., (2015). Proteomic analysis of erythritol-producing *Yarrowia lipolytica* from glycerol in response to osmotic pressure. *J Microbiol Biotechnol*. **25**(7):1056-1069.

Yoshitake, J., Ishizaki, H., Shimamura, M., and Imai, T. (1973a). Xylitol production by an *Enterobacter* species. *Agr Biol Chem*. 37, 2261-2267

Yoshitake, J., Shimamura, M., and Imai, T. (1973b). Xylitol production by a *Corynebacterium* species. *Agr Biol Chem*. 37, 2251-2259.

Yupaporn P, Bunyapon J, Sudarat T, Preekamol K, Mamoru Y and Pornthap T. (2024). Sorbitol production from mixtures of molasses and sugarcane bagasse hydrolysate using the thermally adapted *Zymomonas mobilis* ZM AD41. *Scientific Reports*. **14**, 5563 https://doi.org/10.1038/s41598-024-56307-8

Zubair H., Ibrahim M I., Dawar K, Syed H. A., Syed O A., Mir M. B., Tanzeel U., Muhammad S H. & Nadeem H. S. (2019). Production of Microorganism-Based-Low-Calorie Sugars. Application of Bio-Additives for the Food Industry. Pp 89-129.

Zuriana, S. A., and Sakinah, A. (2017). Production of sorbitol by repeated batch fermentation using immobilized *Lactobacillus plantarum* strain (BAA-793) via solid-state fermentation. *Journal of Food Science,* 1*,* 176-182.