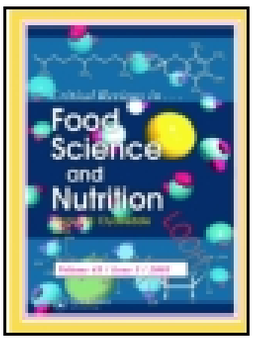

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REVIEW

A review of polyols – biotechnological production, food applications, regulation, labeling and health effects

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ABSTRACT

Food research is constantly searching for new ways to replace sugar. This is due to the negative connotations of sugar consumption on health which has driven consumer demand for healthier products and is reflected on a national level by the taxation of sugary beverages. Sugar alcohols, a class of polyols, are present in varying levels in many fruits and vegetables and are also added to foods as low calorific sweeteners. The most commonly used polyols in food include sorbitol, mannitol, xylitol, erythritol, maltitol, lactitol and isomalt. Of these, microorganisms can produce sorbitol, mannitol, xylitol and erythritol either naturally or through genetic engineering. Production of polyols by microbes has been the focus of a lot of research for its potential as an alternative to current industrial scale production by chemical synthesis but can also be used for in situ production of natural sweeteners in fermented products using microbes approved for use in foods. This review on the generation of these natural sweetening compounds by microorganisms examines the current understanding and methods of microbial production of polyols that are applicable in the food industry. The review also considers the health benefits and effects of polyol usage and discusses regulations which are applicable to polyol use.

KEYWORDS

Sweetener, food labelling, mannitol, sorbitol, xylitol, erythritol

Introduction

Polyols or sugar alcohols are derivatives of sugars formed by the reduction of the aldo or keto group to a hydroxyl group (Bieleski 1982). They are produced naturally by micro- and higher- organisms where they serve a variety of functions. In bacteria, polyol production is often a mechanism of cofactor recycling, where reduction of a sugar to polyol oxidizes $NAD(P)^+$ which is required for metabolic processes. This includes the reduction of fructose to mannitol or sorbitol and of erythrose to erythritol (Ladero et al. 2007; Veiga-Da-Cunha, Santos, and Van Schaftingen 1993; Wisselink, Weusthuis, Eggink, Hugenholtz, and Grobben 2002). Fungi also produce polyols. Osmotolerant species release polyols in response to hypo-osmotic stress (Kayingo, Kilian, and Prior 2001). Similarly, intracellular accumulation of polyols occurs in osmophilic yeast and yeast-like species to protect cells from osmotic stress (Blomberg and Adler 1992; Nevoigt and Stahl 1997). These include, erythritol, mannitol and xylitol.

While currently, most industrial production of polyols is achieved by chemical hydrogenation of sugars, significant interest in the microbiological production of polyols is apparent from the increasing number of studies concerning methods for biotechnological polyol production. Erythritol is the exception and is produced on an industrial scale using fermentation processes with osmophilic yeast such as *Moniliella* sp. and *Trichosporonoides* sp. (Varzakas, Labropoulos, and Anestis 2012). This is largely due to costs associated with catalytic hydrogenation of erythrose. On the other hand, microbial production of mannitol, sorbitol and xylitol can also offer advantages above current chemical synthesis through reduced process costs. Chemical hydrogenation of sugars typically requires harsh process parameters such as temperature and pressure along with highly pure substrate and catalysts (Castoldi, Câmara, and Aranda 2009). The milder process requirements of microbial fermentation (temperatures in the range of 20–40 °C) and the possibility to use low cost substrates present a financial advantage. Conversion of industrial wastes such as cellulosic material (xylitol production) and glycerol (erythritol production) for polyol formation by microbes has the two-fold advantage of cheap (or free) source of substrate along with converting waste materials to value added products.

Polyols have established themselves as important players in the food industry and are used as sweeteners, flavor enhancers, cooling agents, humectants, and thickeners, finding applications in bakery goods, hard candies, spreads and more. Their similarities to sugars, both in sweet flavor and physical properties have lent them to use as low-calorie sweeteners and unlike high intensity sweeteners, polyols can be used for bulk sugar replacement. Consequently, polyols and high intensity sweeteners are often used in combination in food formulations. A summary table of polyols produced by microorganisms, their physical properties and main food applications is presented (Table 1). In contrast to addition of polyols as ingredients,

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Table 1. Summary of polyols produced by microorganisms.

Polyol	Microbial production	Caloric value EU/USA (kcal/g)	Sweetness relative to sucrose (%)	Food applications
Mannitol	Homofermentative LAB Heterofermentative LAB; *Lactobacillus* spp. (group III) *Leuconostoc* spp. *Oenococcus* spp. *Weisella* spp. Yeast; *Candida* spp. Fungi; *Aspergillus candidus* *Penicillium* spp.	2.4/1.6	50-70	Hard candy coating, dusting powder for chewing gum, chewing gum
Sorbitol	*Zymomonas mobilis* Genetically engineered LAB	2.4/2.6	50-70	Hard candies, chewing gum, frozen desserts, baked goods, confectionary items
Xylitol	Yeast; *Candida* spp. *Debaromyces* spp. *Kluveromyces* spp.	2.4/2.4	100	Baked goods, hard candies, chocolate, chewing gum, ice-cream
Erythritol	Fungi; *Aureobasidium* spp. *Moniella* spp. *Penicillium* spp. *Pseudozyma tsukubaensis* *Yarrowia lipolytica* Heterofermentative LAB spp.	0.0/0.0	60-80	Baked goods, table-top sweetener, beverages

Adapted from Lenhart and Chey (2017).

fermented foods often contain natural polyols as metabolic end products of many starter cultures, whose use provides a natural means of polyol addition to foods when organisms generally recognized as safe (GRAS) or qualified presumption of safety (QPS) are used as starter cultures. Recent studies have looked at applications of polyol producing bacteria to develop novel low sugar fermented products, and these represent an emerging area in sugar replacement research (Jeske, Zannini, Lynch, Coffey, and Arendt 2018; Sahin et al. 2018).

Due to the industrial importance of polyols, discussion has also focused on the health effects of these compounds. The lower caloric value of polyols relative to sugars is partly due to their decreased breakdown in the body and also their poor absorption in the gut. While these traits have positive effects such as decreased blood sugar spike and insulin response, undigested polyols are free to be broken down by members of gut microbiota. The latter can lead to negative gastrointestinal effects such as bloating and laxation and exacerbation of symptoms in sufferers of irritable bowel syndrome (IBS; Lenhart and Chey 2017). Conversely, polyols are not metabolized by oral microbes associated with dental plaque formation and are regarded as non-cariogenic. In order to moderate the promotion of the health claims for polyols, they are included in food labeling regulations in national and international directives.

Microbial production of polyols

Industrially, polyols are largely produced by the chemical hydrogenation of their corresponding mono- and di-saccharides. Sorbitol and mannitol are made from the hydrogenation of glucose and fructose mixtures derived from invert sugar. Xylitol can be formed by the hydrogenation of xylose. The disaccharide lactose is hydrogenated to lactitol and similarly, maltose to maltitol. Isomalt is a composed of a mixture of gluco-mannitol and gluco-sorbitol. These chemical processes can be expensive and also have relatively low yields. Polyols are also extracted from natural sources, examples being xylitol, which is extracted from corn and the bark of birch trees and mannitol, which can be obtained from manna, the exudate of the manna ash tree. The prospect of biotechnological production of polyols has warranted much research and currently erythritol is the principal polyol produced on an industrial scale in this way. This section focuses on the approaches for microbial production of polyols, the strategies used to enhance productivity in various microbes, and potential low-cost materials used as substrates.

Mannitol

Mannitol production by microbes has been investigated in bacteria, yeast and fungi (Smiley, Cadmus, and Liepins 1967; Song et al. 2002; Wisselink et al. 2002). The metabolic production of mannitol differs between the organisms used. These pathways have been elucidated and their exploitation has been optimized (Figures 1A–C).

Mannitol production by homofermentative LAB

Homofermentative lactic acid bacteria (LAB) include the genera *Lactobacillus* (group I – obligately homofermentative and group II – facultatively heterofermentative), *Lactococcus*, *Enterococcus*, *Streptococcus* and *Pediococcus*. These bacteria produce mannitol through the combined actions of

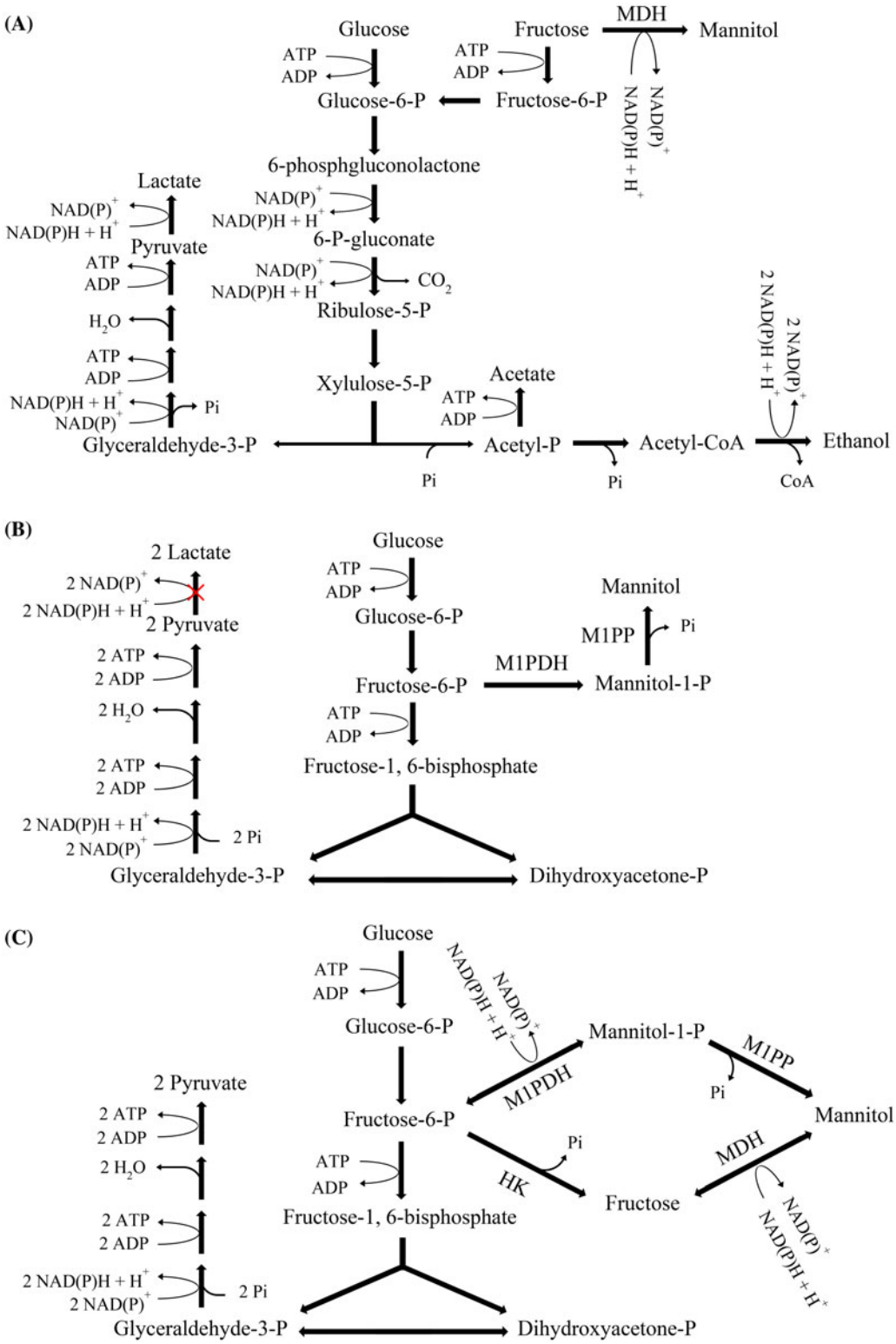

Figure 1. Pathways for microbial production of mannitol by heterofermentative LAB (1 A), homofermentative LAB (1B) and fungi and yeast (1 C). HK – hexokinase, M1PDH – mannitol-1-phosphate dehydrogenase, M1PP – mannitol-1-phosphate phosphatase, MDH – mannitol dehydrogenase. A crossed out step in a pathway denotes a target for genetic modification to increase mannitol production.

mannitol-1-phosphate (M-1-P) dehydrogenase and its phosphatase which respectively convert the glycolytic intermediate fructose-6-phosphate (F-6-P) to M-1-P and remove the phosphate, yielding mannitol (Neves et al. 2000). F-6-P production requires one ATP and is an intermediate of the Embden-Meyerhof pathway which generates energy (ATP) from glucose in homofermentative bacteria. The production of mannitol therefore, is energy inefficient for the cell and occurs when cells are stressed due to an inability to regenerate NAD^+. This has been exploited through genetic engineering in multiple studies by knock-out of the lactate dehydrogenase gene, *ldh*. Studies on metabolite production

in *ldh* deficient strains of *L. lactis* (Neves et al. 2000; Neves et al. 2002) and *L. plantarum* (Ferain, Schanck, and Delcour 1996) have reported mannitol production. In Gaspar et al. (2004) engineered a double knock out strain deficient in *ldh* and *mtlF*, *L. lactis* FI10089 (Δ*ldh*Δ*mtlF*). *mtlF* encodes the EIIA protein of the phosphoenolpyruvate phosphotransferase system (PTS) mannitol transporter (PTS-mannitol), the knock-out strain FI10089 is unable to consume formed mannitol after alternative carbon sources are exhausted, which improves mannitol yields. Resting cells of strain FI10089 produced 0.33 g/g mannitol from glucose. In later work by the same group, the identification of alternative lactose dehydrogenase encoding genes *ldhB* and *ldhX* led to the deletion of *ldhB* in FI10089 to create strain FI10089Δ*ldhB*. Over expression of the mannitol biosynthetic pathway was achieved in this strain by introduction of the lactococcal *mtlD* gene (encoding M-1-P dehydrogenase) and the *mtlP* gene (encoding M-1-P phosphorylase) from *Eimeria tenella* on the nisin-inducible expression vector pNZ-*mtlDmtlP* in strain FI10089Δ*ldhB*. Induction of the expression of these genes in the strain resulted in a mannitol yield of 0.42 g/g during growth on glucose (Gaspar, Neves, Gasson, Shearman, and Santos 2011). The above work shows that genetic modification of homofermentative LAB can result in mannitol yields above 40% from glucose. Nevertheless, wild type heterofermentative LAB have been made to elicit higher yields than this from fructose as discussed below.

Mannitol production by heterofermentative LAB

Heterofermentative LAB genera include *Lactobacillus* (group III- obligate heterofermentative), *Leuconostoc*, *Oenococcus* and *Weissella*. These bacteria do not possess the aldolase cleavage enzyme of the homofermentative pathway, but instead cleave the 5-carbon xylulose-5-phosphapate with a phosphoketolase enzyme and produce carbon dioxide, lactic acid, acetic acid and/or ethanol as fermentation end products (Gänzle 2015). Heterofermentative LAB use alternative electron acceptors as a means of NAD^+ regeneration a relevant example being fructose, which when used as an electron acceptor is converted to mannitol by the activity of mannitol 2-dehydrogenase (MDH) (Wisselink et al. 2002). A variety of heterofermentative LAB strains have been studied for their capacity to produce mannitol from fructose under differing conditions. Work with the strain *L. intermedius* NRRL B-3693 by (Saha and Nakamura 2006) reported production of 198.3 g/L (0.66 g/g yield) when grown in batch fermentation with 300 g/L fructose after 136 h of growth. Improved fructose based productivity was achieved using a fed-batch format; after 92 h of fermentation 202.5 g/L mannitol (0.67 g/g yield) was generated. These studies represent the maximum yield of mannitol when fructose is the sole carbon source. If only fructose is present as a carbon source it is used for fermentation and as an electron acceptor. With the preferential production of acetate over ethanol the cell can produce a net 2 ATP per sugar molecule fermented. However, as the intermediate acetyl-phosphate is not reduced to ethanol, a deficit of 2 NAD^+ results from this pathway. Therefore, per fructose molecule that is fermented to lactate and acetate 2 fructose molecules are reduced to mannitol to maintain the redox balance of the cell. The theoretical maximum yield is 2 moles of mannitol per 3 moles of fructose consumed or 0.66 g/g. Alternatively, when glucose is available, complete reduction of fructose can occur (100% mannitol yield) if a 1:2 ratio of glucose to fructose is applied (Wisselink et al. 2002). Batch fermentations using this format with 100 g/L fructose and 50 g/L glucose in the media produced 83 g/L mannitol (0.89 g/g) with *L. fermentum* (Weymarn, Von Hujanen, and Leisola 2002). In later work by Racine and Saha (2007) on *L. intermedius* strain B-3693 in a pH controlled, continuous cell recycle fermentation system 94.7 g/L mannitol was produced in media containing a mixture of glucose (50 g/L) and fructose (100 g/L) with largely improved volumetric productivity of mannitol (28.40 g/L/h). Notably, this work was performed using a low cost medium applicable for biotechnological production of mannitol, where the costly ingredients of de Man-Rogosa-Sharpe (MRS) media such as bacto-peptone and bacto-yeast extract were substituted with soy peptone, corn steep liquor and manganese sulfate. Cashew apple juice has been investigated as an alternative nutrient source for mannitol production by heterofermentative LAB (Fontes, Honorato, Rabelo, and Rodrigues 2009; Honorato, Rabelo, Gonçalves, Pinto, and Rodrigues 2007). Cashew apples are a by-product of cashew nut cultivation, and the apple has a high sugar content (approx. 100 g/L). In media containing 50 g/L reducing sugar from cashew apple juice (28 g/L fructose, 22 g/L glucose), 20 g/L yeast extract and 20 g/L phosphate as K_2HPO_4, 18 g/L of mannitol was produced by *Lc. mesenteroides* B-512F. Sugarcane molasses (42% sucrose, 4% fructose, 3% glucose) were used as substrates for mannitol production in MRS supplemented medium by *L. reuteri* CRL 1101. When 7.5% molasses was used in supplemented MRS under aeration at 37 °C, 90% of fructose consumed (35.7 g/L) was converted to mannitol, producing 32.4 g/L mannitol (Ortiz, Fornaguera, Raya, and Mozzi 2012).

Production of mannitol by yeasts

Yeasts typically require longer growth times than LAB which translates to lower volumetric productivities of mannitol during fermentations, although some yeast species have demonstrated comparable yields of mannitol from fructose and can also produce mannitol from glucose and glycerol (Onishi and Suzuki 1968; Saha and Racine 2011; Song et al. 2002; Yoshikawa et al. 2014). *Candida magnolia* has been the focus of mannitol production studies for many years. A novel strain of *C. magnoliae* was isolated from fermentation sludge and demonstrated production of 67 g of mannitol from 150 g of fructose following a 168 h batch fermentation (Song et al. 2002). A fed-batch fermentation process for mannitol production from fructose by *C. magnolia* was optimized by investigating the optimal fructose and glucose feed-rate and maximum fructose concentration on mannitol yield and volumetric productivity. Optimal results were observed with a glucose/fructose ratio of 1:20, with 250 g/L fructose and 12.5 g/L glucose in the medium generating a mannitol yield of 84% from fructose and a productivity of 1.94 g/L/h (J. K. Lee, Song, and Kim 2003). Similar results were achieved with a

mutant *C. magnolia* strain R9. In fed-batch fermentation following a growth phase with 100 g/L glucose, 300 g/L fructose was added and resulted in the formation of 240 g/L mannitol (0.81 g/g yield) with a productivity of 4.0 g/L/h (Savergave, Gadre, Vaidya, and Narayanan 2011). Studies have also examined mannitol production from alternative carbon sources. Resting cells of *Candida magnoliae* NCIM 3470 were cultivated on various carbon sources at 100 g/L and produced high quantities of mannitol when fermenting fructose (44.5 g/L mannitol) and glycerol (51 g/L mannitol) over 96 h (Khan, Bhide, and Gadre 2009). *Candida azyma* NBRC10406 was able to produce 50.8 g/L mannitol when grown in raw glycerol (~25% v/v) supplemented with $CaCl_2$ (0.2%) in a batch fermentation (Yoshikawa et al. 2014). The fermentation conditions were optimized for mannitol formation by *Candida parapsilosis* strain SK26.001 isolated from sugarcane juice. A maximum mannitol concentration of 97.1 g/L mannitol was recorded after the consumption of 284 g/L glucose during fed batch fermentation of the strain (Meng, Zhang, Wei, Mu, and Miao 2017).

Fungal production of mannitol

Metabolic pathways involving mannitol production have been identified in a number of fungal genera including *Aspergillus, Botyrtis, Penicillium* and *Trichothecium*. In these cases, a cyclic pathway involves the enzymes hexokinase, M-1-P dehydrogenase, M-1-P phosphatase and mannitol dehydrogenase (Hult, Veide, and Gatenbeck 1980). Published research focusing on the biotechnological production of mannitol by fungi is quite limited. An early study optimized the process for mannitol production by *Aspergillus candidus* resulting in a 50% yield of mannitol from glucose (22 g/L mannitol produced). Glucose feeding was essential to prevent consumption of accumulated mannitol (Smiley et al. 1967). A more recent study of mannitol production by various *Penicillium* strains reported the accumulation of 40 g/L of mannitol in fermentate following growth for 10 days in media containing 150 g/L sucrose by the strain *Penicillium scabrosum* IBT JTER 4 (Hendriksen et al. 1988).

Sorbitol

Production of sorbitol by microorganisms has exploited the bacterium *Zymomonas mobilis* which can convert fructose to sorbitol. Optimization of the process including genetic modification in *Z. mobilis* has been extensively studied. Genetic engineering strategies have also been used to construct lactic acid bacteria with the ability to produce sorbitol (Figures 2A and B).

Production of sorbitol by Zymomonas mobilis

Zymomonas mobilis is a Gram-negative, facultatively anaerobic, bacillus with unique metabolic characteristics. It metabolizes glucose by the Entner-Doudoroff (ED) pathway and has been shown to convert over 95% of utilized glucose into equimolar quantities of CO_2 and ethanol (Kersters and De Ley 1968; Swings and De Ley 1977). *Z. mobilis* has been the focus of considerable interest following the isolation of a glucose-fructose oxidoreductase (GFOR) which oxidizes glucose to glucono-δ-lactone and consequently reduces fructose to sorbitol by a classic ping-pong mechanism (Hardman and Scopes 1988; Zachariou and Scopes 1986). Resultant glucono-δ-lactone is rapidly converted to gluconic acid by gluconolactonase. This enzyme responsible for sorbitol production was found to have tightly bound NADP cofactor which acts as a hydrogen carrier for the redox reaction. Early studies of sorbitol and gluconic acid production by *Z. mobilis* during fermentation of fructose and glucose gave poor yields as ethanol was primarily produced gluconic acid was consumed (Strohdeicher, Schmitz, Bringer-Meyer, and Sahm 1988; Viikari 1984). A new approach to improve sorbitol yields was introduced by Chun and Rogers (1988) in which *Z. mobilis* cells were permeabilised by treatment with 10% (v/v) toluene to remove soluble cofactors and high energy compounds necessary for the conversion of gluconic acid to ethanol. This approach resulted in yields of 290 g/L (>0.97 g/g) for sorbitol in batch fermentations with 300 g/L of both fructose and glucose. Also as part of this work, immobilization of the toluene treated cells in calcium–alginate beads was assessed and while it was observed to result in slightly lower yields, was successful for repeated semi-batch cultures with slight decreases in enzyme activity between cycles. A number of other approaches for permeabilisation and immobilization of *Z. mobilis* for sorbitol and gluconic acid production have been described and patented (Bringer-Meyer and Sahm 1989; Ferraz, Borges, and Alves 2000; Rehr and Sahm 1991). Invertase treatment of sucrose was employed as a cheaper source of fructose and glucose for continuous sorbitol production. When combined with immobilized toluene-treated cells in a recycle packed-bed reactor, 20% sucrose gave a productivity in the region of 5.20 g/L/h sorbitol (Ro and Kim 1991). Generation of sorbitol by *Z. mobilis* using the low-cost substrate sugar cane molasses was optimized with a systematic approach which examined four variables: molasses (total reducing sugar) concentration, temperature, agitation and culture time assessed using a 2^{4-1} factorial scheme. Cell permeabilisation was not performed however and low sorbitol production (13.87 g/L) from 300 g/L reducing sugar, was broadly similar to sorbitol yields in prior studies without permeabilisation (Cazetta, Celligoi, Buzato, Scarmino, and Da Silva 2005).

Bioengineering approaches for sorbitol production

Foreseeably, *Z. mobilis* has been subjected to genetic engineering to improve sorbitol production. Liu et al. (2010) constructed a recombinant strain of Z. *mobilis* harboring the plasmid pHW20a-*gfo* for increased expression of GFOR. In a batch fermentation, a 1.7 fold increase of GFOR activity was found in the recombinant strain versus wild type. The research group also assessed the effectiveness of several divalent cations at inhibiting the ED pathway to reduce ethanol production and reported that Zn^{2+} gave the best results with a 100% yield of sorbitol from fructose consumed in batch fermentation with 160 g/L of both fructose and glucose combined with 2 g/L $ZnSO_4.7H_2O$. Sorbitol production is also possible through the genetic engineering of

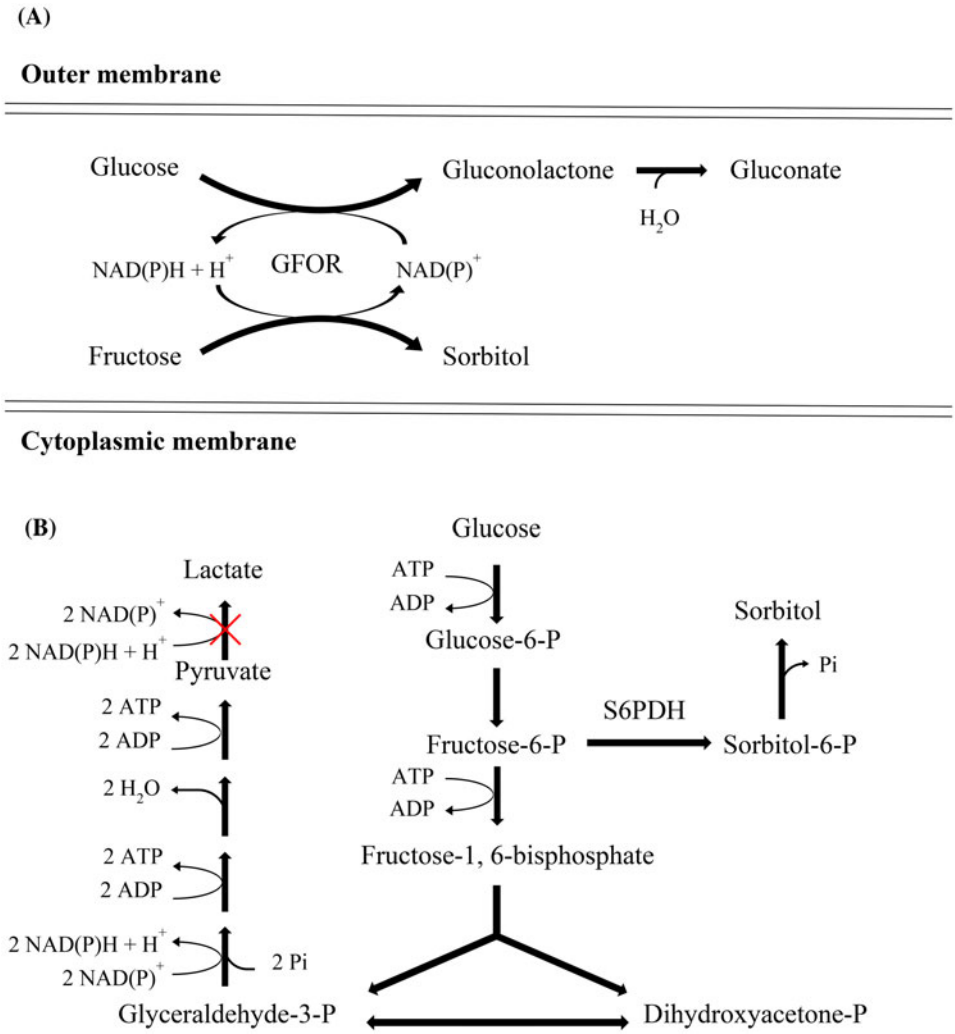

Figure 2. Pathways for microbial production of sorbitol. Sorbitol production by Zymomonas mobilis (2 A), and through genetic engineering of lactic acid bacteria (2B). GFOR – glucose fructose oxidoreductase, S6PDH – sorbitol-6-phosphate dehydrogenase.

homofermentative lactic acid bacteria to express the enzyme sorbitol-6-phosphate dehydrogenase (S6PDH) (Ladero et al. 2007; Nissen, Pérez-Martínez, and Yebra 2005). *L. casei* was genetically engineered by Nissen, Pérez-Martínez, and Yebra (2005) to introduce the S6PDH gene, *gutF*, into the *lac* operon. It was observed that sorbitol production was increased by inactivation of the L-lactose dehydrogenase gene, *ldhL*. Sorbitol production was also achieved in *L. plantarum* by overexpression of the native S6PDH genes, *srlD1* and *srlD2* in strains constructed by Ladero et al. (2007) which were also deficient in the D- and L-lactate dehydrogenase genes. In the absence of the reduction of pyruvate to lactate as a means of NAD^+ formation, cell metabolism shifted to sorbitol production to oxidize NADH. It was observed that in both engineered constructs, aeration had a negative impact on sorbitol formation. This was postulated to be due to the oxidation of NADH by NADH oxidase in the presence of oxygen.

Erythritol

Erythritol differs from the other polyols which are predominantly produced by chemical processes in that its industrial production is achieved through biological processes. The largest commercial erythritol producers are Cargill (USA), Mitsubishi Chemical Corporation (Japan) and Bolak Corporation (Korea) and it is generally produced by bioconversion of glucose rich substrates by yeasts or yeast-like fungi (Figure 3A and B; Moon, Jeya, Kim, and Lee 2010).

Fungal production of erythritol

Yeast and yeast-like species from a number of fungal genera are able to grow in conditions of low water activity. These osmophilic species accumulate compatible solutes including glycerol, D-arabitol, erythritol and mannitol when exposed to osmotic stress (Moon et al. 2010). In several erythritol producing yeasts, erythrose-4-phosphate (E-4-P) formed in the pentose phosphate pathway can be converted to erythrose by dephosphorylation and subsequently reduced to erythritol with concomitant oxidation of NAD(P)H to $NAD(P)^+$ (Rzechonek, Dobrowolski, Rymowicz, and Mirończuk 2018). The erythrose reductase enzyme has been identified in multiple erythritol-producing yeasts including *Torula* species, *Moniliella megachiliensis*, *Moniliella pollinis*, *Candida magnolia* and *Yarrowia lipolytica* (Janek,

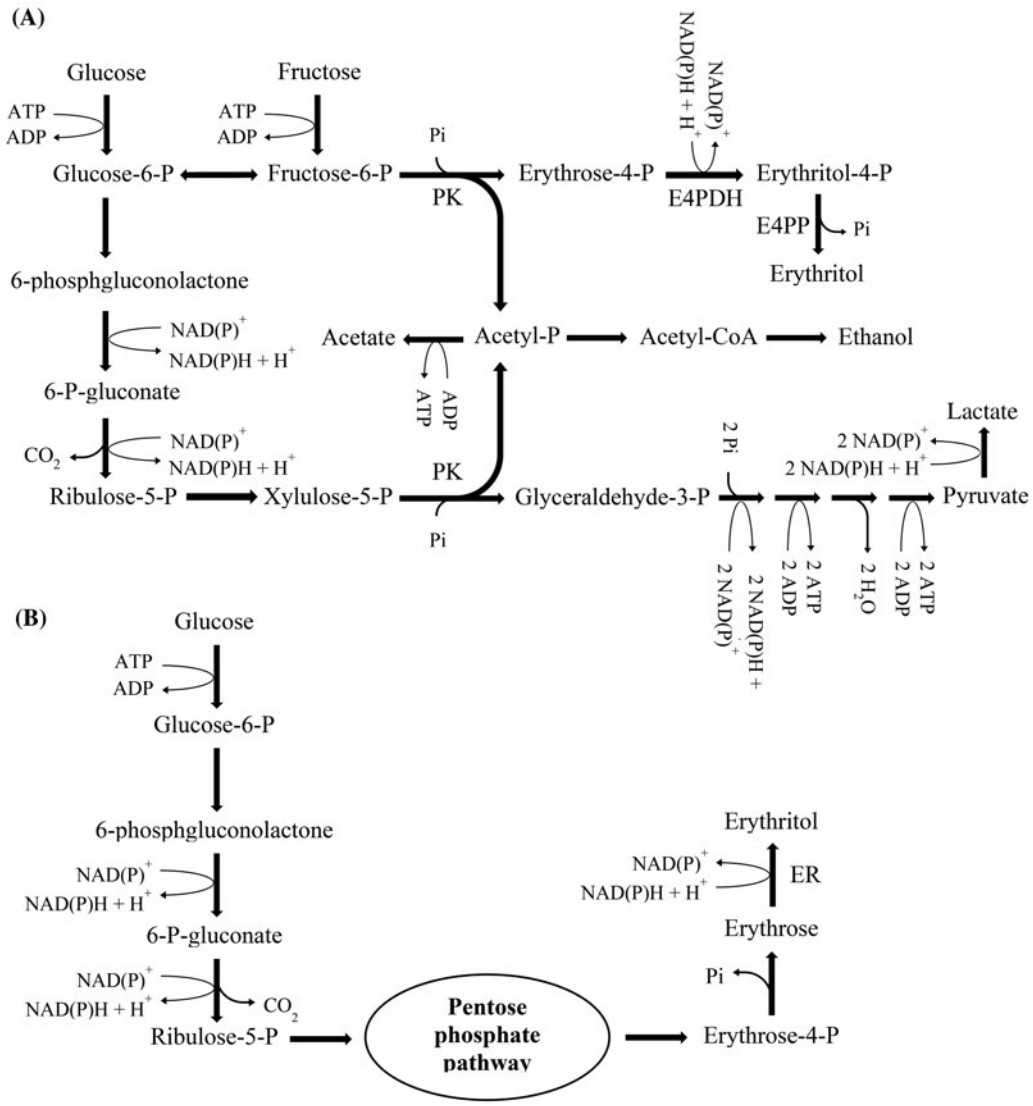

Figure 3. Pathways for microbial production of erythritol by heterofermentative lactic acid bacteria (3 A) and fungi (3B). E4PDH – erythritol-4-phosphate dehydrogenase, E4PP – erythritol-4-phosphate phosphatase, ER – erythrose reductase, PK – phosphoketolase.

Dobrowolski, Biegalska, and Mirończuk 2017; D. H. Lee, Lee, Ryu, and Seo 2010; J. K. Lee, Koo, and Kim 2002; J. Lee, Kim, Ryu, Seo, and Kim 2003) although, a gene encoding the enzyme that dephosphorylates E-4-P has not yet been identified, suggesting the activity of a nonspecific kinase for this purpose. To improve the natural production of erythritol in yeast, mutation by ultra violet (UV) irradiation and chemical mutagen exposure has been reported resulting in the generation of highly active erythritol producing mutant strains (Ishizuka et al. 1989; Lin et al. 2010). Successive mutation of *Aureobasidium* sp. SN-124A by UV irradiation and N-methyl-N'-nitro-N-nitrosoguanidine (NTG) formed the mutant SN-G42 with superior properties. The strain was negative for foam formation during fermentation and exhibited increased yields of erythritol from glucose even at increased substrate concentrations up to 470 g/L glucose, which had been observed to result in decreased erythritol yields in the wild type strains. In batch fermentation, 400 g/L glucose was converted to 175 g/L erythritol (Ishizuka et al. 1989). In other work, a mutant strain, *Penicillium* sp. KJ-UV29 derived from the wild type *Penicillium* sp. KJ81 using conventional UV and NTG treatments. Strain KJ-UV29 produced higher erythritol yields (15 g/L by KJ-UV29, 11.7 g/L by KJ81) along with less of the by-product glycerol (6.1 g/L by KJ-UV29, 19.4 g/L by KJ81) during fermentation. Under optimized condition the mutant strain produced 45.2 g/L erythritol during batch fermentation with 300 g/L sucrose, almost twice that of the wild type strain (Lee and Lim 2003). Iterative rounds of mutation and strain selection of *Moniliella* sp. 440 resulted in the mutant strain N61188-12. The mutant showed superior erythritol yields in flask culture which translated to the formation of 152.4 g/L of erythritol when grown in 350 g/L glucose at 2000 L scale (Lin et al. 2010). The wild type osmophilic yeast strain *Pseudozyma tsukubaensis* KN75 exhibited the highest reported erythritol yields at industrial scale. In a 50,000 L batch fermentation of glucose, 241 g/L erythritol (61% erythritol yield) was achieved following optimization of pH, temperature and dissolvable oxygen content (Jeya et al. 2009). The unconventional yeast *Yarrowia lipolytica* has received interest for its ability to produce erythritol from glycerol, a waste product of many industrial processes (Rymowicz,

Rywińska, and Marcinkiewicz 2009). In a study of functional overexpression of genes involved in glycerol metabolism by Mirończuk, Biegalska, and Dobrowolski (2017), transketolase was shown to play a crucial role in erythritol production from glycerol. Overexpression of transketolase resulted in twofold improvement in erythritol synthesis in shake-flasks experiments. The proposed pathway followed the conversion of glycerol to glyceraldehyde-3-phosphate and addition of a two-carbon fragment by transketolase from fructose-6-phosphate to form xylulose-5-phosphate and erythrose-4-phosphate. Introduction of a heterologous invertase gene from *S. cerevisiae* to *Y. lipolytica* produced a strain with the capacity to synthesize erythritol from a combination of raw glycerol and molasses with good production levels of 119 g/L.

Production of erythritol by lactic acid bacteria

Erythritol production by heterofermentative LAB is possible through the presence of a nonspecific phosphoketolase or a separate fructose 6-phosphpate phosphoketolase enzyme with the ability to convert fructose-6-phosphate to erythrose-4-phosphate and acetyl phosphate. These variants of the pentose phosphate phosphoketolase which is pivotal to glucose metabolism in heterofermenters, have been described in *Leuconostoc*, *Lactobacillus* and *Oenococcus* (Goldberg and Racker 1962; Holzer and Schroeter 1962; Veiga-Da-Cunha, Firme, San Romao, and Santos 1992). *Leuconostoc oenos* (reclassified as *Oenococcus oenos* (Dicks, Dellaglio, and Collins 1995)), a wine associated LAB, was studied to elucidate the enzymes involved in erythritol formation by Veiga-Da-Cunha, Santos, and Van Schaftingen (1993). Xylulose-5-phosphate phosphoketolase from cell extracts showed activity on fructose-6-phosphate, suggesting that the phosphoketolase was not pentose specific. An erythritol-4-phosphate dehydrogenase was proposed to reduce the erythrose-4-phosphate, followed by the action of a phosphatase to give erythritol. However, erythritol production was observed to only occur under anaerobic conditions when acetyl Co-A precursors were limited, suggesting high yields of erythritol in LAB to be unlikely. Low level production of erythritol was reported in the plant isolate *L. florum* 2 F (Tyler et al. 2016). While NAD(P)H recycling plays an important role in stimulating erythritol production, the existence of a variety of possible pathways for this function in heterofermentative LAB, as described by Zaunmüller et al. (2006), renders erythritol production in wild type LAB incompatible with industrial production.

Xylitol

Research into the biotechnological production of xylitol has been focused on the use of agro-industrial waste as substrate (De Albuquerque, Da Silva, De MacEdo, and Rocha 2014). A number of species can convert the nutrients present in detoxified, hydrolyzed lignocellulosic waste to produce this valuable polyol (Figure 4). Processing of lignocellulosic waste has been optimized for treatment of the wastes and for microbial growth and xylitol productivities.

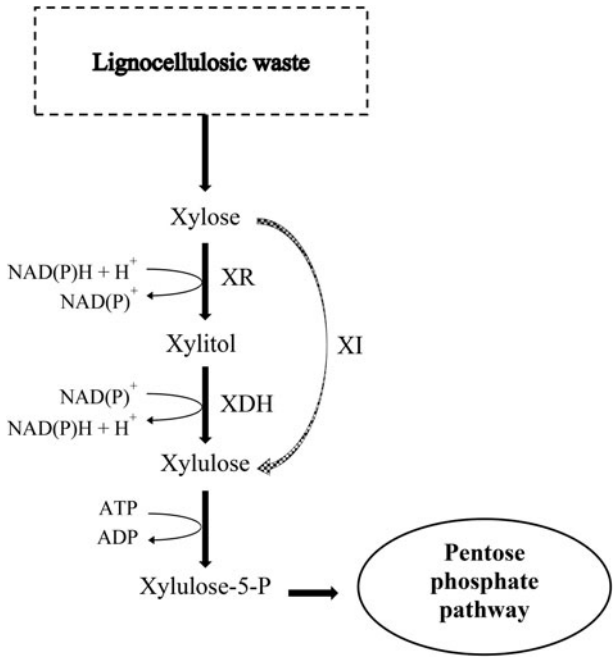

Figure 4. Pathway for xylitol production by yeast. XDH – xylitol dehydrogenase, XI – xylitol isomerase, XR – xylitol reductase. Lined arrow represents a reaction present in some species.

Xylitol production by yeast

The pathway for xylitol production in yeast and fungi involves the interconversion of xylose, xylitol and xylulose. Xylose is first reduced to xylitol by an NAD(P)H dependant xylose reductase (EC 1.1.1.21); resulting xylitol can accumulate and be secreted from the cell or further oxidized to xylulose by the NAD(P) dependant xylitol dehydrogenase (EC 1.1.1.9) (Chen, Jiang, Chen, and Qin 2010). Oxygen limitation plays an important role in limiting the conversion of xylitol to xylulose. In many yeasts, it was reported that different co-factor specificities of the reducing and oxidizing reaction lead to the accumulation of NADH in low oxygen conditions, which inhibit the reduction of xylitol (Silva et al. 1994). Glucose can also negatively impact xylitol yield from xylose through its repression of xylose metabolism (Silva, Roberto, Felipe, and Mancilha 1996). In some yeasts and molds, a xylulose isomerase is present which converts xylose to xylulose in a single step (Banerjee, Archana, and Satyanarayana 1994; Vongsuvanlert and Tani 1988). Xylulose is phosphorylated by xylulose hexokinase and enters the pentose phosphate pathway for energy metabolism. Several species of the genera *Candida*, *Debaryomyces* and *Kluyveromyces* have been studied for their ability to produce xylitol from lignocellulosic waste. In work by de Arruda et al. (2011), *Candida gutilliermondii* FTI 20037 yielded 50.5 g/L xylitol from xylose (0.81 g/g) with a maximum productivity of 0.60 g/L/h during batch fermentation of detoxified sugarcane bagasse, when pre-cultured in medium containing xylose to induce enzyme activity. In another study, hemicellulosic hydrolysate of horticultural waste was used as a xylose source for *C. athensensis* SB18 fermentation by Zhang et al. (2012). Pre-culture was also performed in xylose containing media, and batch fermentation achieved the same yield (0.81 g/g), with 100.1 g/L xylitol

formed at a rate of 0.98 g/L/h. In addition, Miura et al. (2013) investigated the use of bamboo hemicellulosic hydrolysate for fermentation by *C. magnoliae*. With a low xylose content of 19 g/L in the bamboo hydrolysate, 10.5 g/L xylitol was produced (0.59 g/g) with a volumetric productivity of 0.42 g/L/h. Other low xylose substrates have been assessed in other laboratories: for example fermentation of urea supplemented cashew apple bagasse hydrolysate by *Kluyveromyces marxtanus* was reported to produce 6.01 g/L xylitol (0.50 g/g) (Tiago Lima De Albuquerque, Gomes, Marques, Da Silva, and Rocha 2015). Immobilization of cells has been studied to improve xylitol yields and enable recycling of cells. In a study using calcium alginate immobilized *Debaryomyces hansenii* cells, 71.2 g/L xylitol (0.82 g/g) was formed in sugar cane bagasse (Prakash, Varma, Prabhune, Shouche, and Rao 2011). The immobilized cells demonstrated both sustained bioconversion rates and yields of xylitol over five batches of reuse. An important process in the use of lignocellulosic waste hydrolysates is the removal of inhibitory compounds formed during carbohydrate degradation such as furan aldehydes and phenolic compounds (Jönsson, Alriksson, and Nilvebrant 2013). Xylitol production in non-detoxified hemicellulosic hydrolysate has also been studied. Yewale et al. (2016) optimized the immobilization of *C. tropicalis* for xylitol production from non-detoxified corn cob hemicellulosic hydrolysate. These authors reported that calcium alginate bead immobilized cells produced 41 g/L xylitol (0.73 g/g) with a volumetric productivity of 0.43 g/L/h in shake flask experiments. Comparable to the results of Prakash et al. (2011), the immobilized cells showed no decline in bioconversion rates when recycled for five batches in repeated batch studies. A complete review of the challenges involved in xylitol production with whole cells was recently published by Dasgupta et al. (2017).

Genetic engineering strategies for xylitol production

Genetic modification strategies to increase yields of xylitol from yeasts have included improved transport, expression of xylose reductase, deletion of xylitol dehydrogenase and improved co-factor recycling. A recent review by Dasgupta et al. (2017) described in depth the methods taken to apply these genetic strategies. Due to the established role of *Saccharomyces cerevisiae* as a model organism for recombinant protein expression, it is commonly used as a host to engineer xylitol production. Runquist, Hahn-Hägerdal, and Rådström (2010) compared the effect of the expression of three heterologous xylose transporters: Gxf1 (*Candida intermedia*), Sut1 (*Pichia stipites*) and At5g59250 (*Arabidopsis thaliana*) and showed significantly increased transport rates of xylose in construct containing the two former genes. To enable xylitol production from xylose in *S. cerevisiae*, the XR of *P. stipitis* was expressed by Oh et al. (2013). Glucose has an inhibitory effect on xylose uptake. To overcome this effect, Oh and colleagues successfully engineered *S. cerevisiae* D-10-BT expressing a cellodextrin transporter and intracellular β-glucosidase for simultaneous utilization of xylose for xylitol production and cellobiose for cell growth. Furthermore, to improve the resupply of NAD(P)H, constructs of DB-10-BT overexpressing different cytosolic $NADP^+$ dependent dehydrogenases were also generated. A cellobiose metabolizing strain with increased dehydrogenase activity had peak xylitol productivity at 0.62 g/L/h and achieved xylitol yields of 1 g/g from xylose consumed. Genetic engineering has also been applied to the natural xylose assimilating yeast (mentioned above) and typically involves knockout of xylitol dehydrogenase. This has been achieved by chemical and UV mutagenesis in *C. tropicalis* OVC5 (Ko et al. 2011) and gene knockout by homologous recombination in *K. marxianus YLUA005* (Zhang et al. 2012). Both strains exhibited improved xylitol production compared to controls.

In situ production of polyols in foods

The prospect of *in situ* polyol production by microbes through food fermentation is an area of recent and increasing interest. Food grade yeast and bacteria have been used in fermentation processes for food and beverage production for centuries. Recent research has characterized the microbial communities and metabolite profile of many of these fermentations such as sourdough (De Vuyst and Vancanneyt 2007; Van Der Meulen et al. 2007), kimchi (Jung et al. 2011), fermented vegetable products (Wouters et al. 2013; D. Wouters et al. 2013), the fermented soybean food *Doenjang* (Namgung et al. 2010) and cocoa beans (Papalexandratou et al. 2011). The microbial communities of such fermentations generally include heterofermentative *Lactobacilli, Leuconostoc* and *Weissella* spp. (Papalexandratou et al. 2011; Dorrit Wouters et al. 2013). Metabolite analysis reveals typical fermentative end-products including lactic acid, acetic acid, ethanol and mannitol. The levels of mannitol produced in these fermentations is relative to the quantity of fructose available in the fermentation substrate material. When analyzed in a study on spelt and wheat sourdough fermentations it was found that initial fructose content was no more than 0.2 g/Kg (Van Der Meulen et al. 2007). Vegetative matter contained more fructose; kimchi had an initial fructose content ranging from 50 – 60 mM and leak material contained 15 – 35 mM (Jung et al. 2011; D. Wouters et al. 2013). The resulting mannitol concentration during fermentation of these substrates were 50 – 60 mM, kimchi; 30 – 80 mM (leak); and less than 6 mM in sourdough, although erythritol was also detected during sourdough fermentations at concentrations below 5 mM.

A recent study on the application of sourdough to burger buns involved the addition of fructose to sourdough as substrate for increased mannitol production by a specific *Leuconostoc citreum* starter culture (Sahin et al. 2018). In this case, the sourdough formulation involved a 10% replacement of wheat flour with fructose (initial concentration of 115.4 g/Kg). Mannitol formation was maximal at 42 h fermentation with 96.9 g/Kg produced. Non-fructose supplemented sourdough had a mannitol concentration of 9.2 g/Kg at this time. Application of the high mannitol sourdough to burger buns in which sugar content was reduced by 50%,

led to a burger bun with the same flavor intensity as the full sugar burger bun according to sensory analysis. In another study by Jeske et al. (2018) on the fermentation of quinoa-based milk substitutes, a combination of enzymatic conversion of glucose to fructose with glucose isomerase and bacterial fermentation with *Leuconostoc citreum* or *Lactobacillus brevis* was used to enhance mannitol production during sugar consumption with the aim of developing a novel milk-like substitute with a low glycaemic load. Initial glucose content of the milk-like substrate was reduced from 90.9 g/L to 72.1 g/L with the converted glucose forming 15.5 g/L fructose. Following the 24 h fermentation with *Lc. citreum* or *L. brevis*, 15.6 g/L and 13.1 g/L mannitol were formed, respectively. The *Lc. citreum* fermented milk was observed to have a 40% reduction in glucose content and 35% reduction in glycaemic load.

Studies like these demonstrating *in situ* natural production of polyols in fermented products represents an emerging field in sugar replacement. Thus, in the appropriate fermentation matrix, polyol producing starter strains such as those mentioned above, can be applied in the preparation of natural, sugar reduced, fermented products, assuming they meet regulatory guidelines for use in food.

Regulations pertaining to polyols and their health effects

Polyol containing products can express health claims such as non-cariogenic and reduced energy but also come with some warnings. The substantiation of health effects caused by polyols by researchers has led to the approval of some health claims on polyol containing products by governing bodies in the USA and Europe along with mandatory warnings on products with high polyol content. Changes in food labeling standards have also changed the way polyols are labeled. These studies, regulations, claims and standards are discussed below.

Reduced risk of dental caries

One of the most significant applications of polyols in the food industry is in the chewing gum sector. Here, xylitol has been the dominant polyol, although sorbitol and mannitol are also used, with each being incorporated alone or in blends with other polyols. In a study by Holgerson et al. (2007), sugar free (polyol containing) chewing gums were reported to help to reduce dental plaque formation and caries. When school children were administered with xylitol or sorbitol and maltitol chewing gum pellets three times daily over four weeks, both groups were reported to have significantly less visible plaque and lower sucrose induced lactic acid formation. However, only the xylitol group showed a significant reduction in the cariogenic mutans streptococci. A systematic review Deshpande and Jadad (2008) compiling data from original randomized controlled trials and observational studies on reduction of dental caries by polyol-containing chewing gums found that sorbitol and xylitol are the most common polyols in sugar free chewing gum formulations; and although there was a lack of comparison with the efficacy of other polyols there was sufficient evidence to support the use of xylitol and sorbitol gums to prevent dental caries as part of normal oral hygiene. This sentiment has been reflected in the USA by the US Food and Drug Administration (FDA) under Final rule "*Food Labelling: Health claims: Dietary Sugar Alcohols and Dental Caries*". Here, it is stated that foods containing polyols and containing less than 0.5 g of sugars per labeled serving (eligible for sugar free labeling), may be labeled with the health claim "may reduce the risk of dental caries" and numerous other variations thereof (FDA 1996). This is also reflected in Europe by the European Food Safety Authority (EFSA) who in the EFSA Journal article "*Scientific Opinion on the substantiation of a health claim related to sugar-free chewing gum and reduction of tooth demineralisation which reduces the risk of dental caries pursuant to Article 14 of Regulation (EC) No 1924/2006*", stated that "A cause and effect relationship has been established between the consumption of sugar-free chewing gum and reduction of tooth demineralisation and a reduction in incidence of caries. Tooth demineralisation may contribute to increased risk of caries" (EFSA 2010).

Low caloric, glycaemic and insulinaemic characteristics

The inclusion of polyols in the food and other industries has been driven by their lower caloric, glycaemic and insulinaemic properties in comparison to sugars. This along with having similar physical properties to sugar has led to their application in many products on the market (Table 1). When consumed in the same amounts as sugar based products, polyol containing products provide a lower calorie content per serving. In Europe, according to Regulation (EU) No 1169/2011 of the European Parliament and of the Council (2011) on the provision of food information to consumers, the energy value for polyols is 2.4 kcal/g, with the exception of erythritol, which contains 0 kcal/g, while carbohydrates (including sugars) are given the energy conversion factor of 4 kcal/g. In the USA, polyols have been allocated individual energy values by the US FDA in the Code of Federal Regulations under title 21 CFR 101.9 Nutritional labeling of food. Energy values range from 1.6 – 2.4 kcal/g for polyols, with the exception of erythritol with an energy value of 0 kcal/g (FDA). In accordance with these regulations, erythritol presents the best option for sugar replacement with polyols in the manufacture of low calorie food products. Other claims relevant to polyol use are seen in the European regulation No 1924/2006 regarding the labeling of energy-reduced foods which states, "A claim that a food is energy-reduced, and any claim likely to have the same meaning for the consumer, may only be made where the energy value is reduced by at least 30%, with an indication of the characteristic(s) which make(s) the food reduced in its total energy value." (European Parliament and the Council of the European Union 2007).

The glycaemic index (GI) is an indication of the ability of a food or carbohydrate to increase blood glucose levels

(Jenkins et al. 1981). The GIs of polyols are much lower than those of sugars (glucose, GI = 100; sucrose, GI = 65) owing to their low digestibility (Livesey 2003). As such, polyols also elicit a lower insulinaemic response as defined by Livesey (2003) who measured the insulin index (II) under the same condition as GI but included a blood insulin measurement to determine the insulin response to consumption (Livesey 2003). Low GI and II diets have been shown to benefit those diagnosed with type II diabetes mellitus (Wolever et al. 1992a; Wolever et al. 1992b). Using polyols as sugar replacers in foods presents low GI options not only for diabetics, but also consumers wanting to follow low GI diets for other health reasons.

Polyol consumption and gastrointestinal symptoms

In the context of reported gastrointestinal response to polyols, dietary intake restrictions of these substances may be discussed as part of the fermentable oligo-, di-, mono-saccharides and polyols (FODMAP) group. These molecules are understood to be poorly absorbed in the intestines and instead become substrates for fermentation by specific intestinal bacteria. Polyol fermentation can result in gas formation leading in some instances to bloating, flatus, borborygmus or more severe symptoms, nausea and diarrhea (Oku and Nakamura 2007). These symptoms are also likely to be associated with the osmotic effect of non-absorbed polyols on the intestinal lumen causing increased water retention (Hyams 1983; Langkilde, Andersson, Schweizer, and Würsch 1994; Lenhart and Chey 2017). Polyols are believed to be absorbed by passive diffusion and the amount of absorption varies between polyol types (Mansueto, Seidita, D'alcamo, and Carroccio 2015). The gastrointestinal tolerance of polyols also varies, a number of studies have assessed dose dependant tolerance and symptomology of polyol consumption in different matrices either as independent or comparative studies (Lee and Storey 1999; Storey, Lee, Bornet, and Brouns 2007; Tetzloff, Dauchy, Medimagh, Carr, and Bär 1996; Zumbe and Brinkworth 1992). Considerable focus has been given to the effects of polyols on patients exhibiting IBS. These include gut transit, the gut microbiome and gastrointestinal symptoms; the topic is reviewed by Lenhart and Chey (2017). The relief of gastrointestinal symptoms achieved by implementing low FODMAP diets has also been the subject of recent work. In the study of Halmos et al. (2014) thirty IBS patients undertook 21 day eating periods of a reduced FODMAP diet followed by a non-reduced diet with a 21 day washout period between each. Results showed a significant reduction in gastrointestinal symptoms while on the low FODMAP diet for the IBS patient group. However, in a control group of healthy individuals symptoms were minimal and if present, nonspecific to either diet. Similar results were found in a randomized controlled study by McIntosh et al. (2017). However, an adaptive tolerance to polyols is believed to develop following regular consumption. Adaptation has been reported to vary between individual human subjects, specific polyols and maximum tolerated doses (Culbert et al. 1986). The Turku sugar studies performed in Finland in the 1970s involved a two year feeding trial with xylitol. An overview of the study stated that participants experiencing diarrhea after large doses of xylitol began to show no symptoms as adaptation took place (Scheinin and Mäkinen 1976). Polyols nevertheless continue to be used in a wide range of products and their success in the market may be an indication of general tolerance to the typically consumed doses. To inform consumers of potential gastrointestinal effects caused by polyol consumption, European Union regulation No 1169/2011 on the provision of food information to consumers states that products containing over 10% polyol content must include the comment, "excessive consumption may produce laxative effects" (European Parliament and the Council of the European Union 2011).

Food labeling: Aspects relating to polyols, sugar replacement and sweeteners

Polyols are regularly exploited both as bulk replacers and as sweetness substitutes for sugar. Their use has been driven by an acknowledgement of the health damaging effects of over consumption of sugar. The World Health Organization (WHO) has recently made recommendations for a reduction in added sugar in the diet. A daily intake of sugar amounting to no more than 10% of the daily energy intake is advised in order to help reduce the risk of overweight and tooth decay, while a sugar intake of less than 5% of daily calories is stated to further reduce health risks (World Health Organization 2015). This opinion has been reflected by the introduction of sugar taxes in a number of countries. In Europe, France was one of the early regulators with a tax on sugary drinks being enforced in 2013. The United Kingdom and Republic of Ireland introduced sugar taxes subsequently along with Portugal, illustrating a trend in policy change across Europe. Similarly, several Latin American countries have introduced taxes on sugary beverages, with Brazil set to follow suit this year. A number of cities in the USA have also applied sugar taxes; and Canada is also set to introduce a tax in the 2018/2019 budget year.

In the European directive EC No 1169-2011, the layout and information requirements of food labels were redefined to make the appropriate information accessible to the consumer. Polyol content is to be included under total carbohydrates on food labels given that the definition of 'sugars' only includes all mono- and di- saccharides but excludes polyols. Regulation (EU) 1169/2011 on food information to consumers requires any food containing a sweetener(s) to carry 'with sweetener(s)' as a statement accompanying the name of the food. Foods containing both an added sugar or sugars and a sweetener or sweeteners must carry the statement 'with sugar(s) and sweetener(s)' as a statement accompanying the name of the food. In the USA, the FDA published their final rule on changes to nutritional labels in 2016. The document states that the "sugars" section of a nutritional label is now to state "total sugar" along with a section detailing how much of this is "added sugar". Importantly, polyols have not been characterized as added

sugars for this purpose. Therefore, in the USA, products using polyols as sugar replacers can include health claims such as "sugar free" and "reduced sugar" provided the requirements of these claims are met. In instances when a product displays a claim regarding sugar content or polyol content, polyols must be included on the nutritional label (Food and Drug Administration 2016). As food additives, polyols have been subject to assessment for their safe use in foods. Both FDA and EFSA approve polyols for use in food. Regardless, both bodies state a requirement for labels to acknowledge potential laxative effects. While the latter clause is applicable to all polyols in Europe, it only includes mannitol and sorbitol in the USA.

Polyol properties and food applications

Polyols have been applied to foods for decades owing to their functional properties including sweetness, solubility, hygroscopicity, crystallisation and heat stability. Polyols approved for use in the EU include mannitol, sorbitol, xylitol, erythritol, isomalt, maltitol, lactitol. Differing properties between specific polyols lend them to applications in particular products for sucrose replacement. The purpose of replacement of added sugars in foods with lower calorie sweet alternatives is self-explanatory. However, it is well known that the role of sugar in foods is not limited to providing sweetness. It also plays a role in important characteristics such as texture, hardness and other physicochemical properties in individual food products. Thus, research has focused on the outcomes of sugar replacement with polyols from the point of view of maintaining these characteristics where relevant. Each polyol will be discussed under its applications, advised dosage and health effects.

In confectionary polyols have been applied as sugar replacers in a number of products including, jellies, hard candies, chewing gum, ice-cream and chocolate coatings. These conventionally high sugar products are a prime target for the reduction of sugar in the diet; and several studies have evaluated how polyol replacement of sugar can reproduce the physical properties inherent to sugar in these cases.

Mannitol

Mannitol, also known as mannite, is a hexitol. Its sweetness is 50-60% of that of sucrose. It is non-hygroscopic leading to its use in sugar free coatings and in manufacturing as a dusting agent to prevent stickiness on the surface of products. It is typically used in chewing gum both as a bulking and dusting agent and similarly in mint candy. O'Donnell and Kearsley (2012) reported that the negative heat associated with mannitol solution resulting in a cooling effect in the mouth makes it favorable for these applications. Mannitol is also used as a sucrose alternative in in sugar free chocolate coatings and in cake frostings and studies have found it to have a varied performance as a sugar substitute in bakery goods. In cookies for example, mannitol retarded dough spreading, forming a unsatisfactory product that scored poorly in sensory analyses (Zoulias, Piknis, and Oreopoulou 2000). However, in another study concerning sucrose replacement in burger buns, up to 50% replacement with mannitol produced a satisfactory product. Physical property differences were attributed to an inability of bakers' yeast to ferment polyols, which in turn affected specific volume and crumb structure (CO_2 production) while a decrease in Maillard reaction associated with sucrose in the control buns, resulted in increased lightness in the mannitol-containing product (Sahin et al. 2018).

Sorbitol

Sorbitol, also a hexitol, is an isomer of mannitol. The molecules differ in the planar orientation of the hydroxyl group on the second carbon resulting in differing properties for each molecule (Kearsley 2001). Sorbitol has a relative sweetness (to sucrose) of 60% and is 20-fold more soluble in water than mannitol leading to its preferential use in many applications (Ortiz, Bleckwedel, Raya, and Mozzi 2013; Silveira and Jonas 2002). Unlike mannitol, sorbitol is hygroscopic leading it to use in the prevention of drying in confectionary and baked products. An important application of sorbitol is as a bulking agent in chewing gum. Crystalline sorbitol does not cause dental caries, and combined with its sweet taste and pleasant cooling effect, it is very practical for chewing gum and hard candies (Kearsley 2001). O'Donnell and Kearsley (2012) reported that sorbitol can also be used in ice-cream formulation to replace sucrose; and in this application, it is generally combined with other sweeteners such as polydextrose and high intensity sweeteners. In another study, Vilela et al. (2015) described the use of sorbitol to replace sucrose in jams and reported rheological properties, texture profile and sensory profile of strawberry, raspberry and cherry jams. Cherry jams prepared with sorbitol exhibited lower water activity associated with better prevention of microbial growth. In addition, sorbitol jams also had good spreading characteristics.

Xylitol

Xylitol has a long history of use as a sweetener, primarily owing to its relative sweetness to sucrose of 100%, making it the sweetest polyol described in the context of food. Xylitol occurs naturally at low levels in a variety of fruits and vegetables. Due to its acceptance as reducing the risk of dental caries, the main application for xylitol has been in chewing gum. Studies by Mäkinen (2011), have shown that regular consumption reduces the risk of dental caries and can help to mineralize the teeth. A study of complete sucrose replacement with polyols in sponge cakes showed xylitol to be the best candidate polyol, largely due to its ability to match the sweetness and flavor. Cakes with xylitol in the place of sucrose also compared well in their physical properties such as specific volume and light intensity (Ronda, Gómez, Blanco, and Caballero 2005). Xylitol has also been used effectively in the formulation of hard candies, chocolate, ice-cream and baked goods, where it has been concluded that it is an adequate sugar replacer (Aidoo, Depypere, Afoakwa,

and Dewettinck 2013; Mushtaq, Rehman, Zahoor, and Jamil 2010; Soukoulis, Rontogianni, and Tzia 2010).

Erythritol

Erythritol is unique in that it is the only 4-carbon polyol commonly used as a sweetener. It also occurs naturally in fruit and vegetables (melons, peaches, mushrooms) and is naturally present in fermented products such as wine, sake and soy sauce. Erythritol has a relative sweetness of 60%, it is temperature stable along with being stable at both acidic and alkali pH. It does not contribute to Maillard browning and is also hygroscopic (Grembecka 2015; O'Donnell and Kearsley 2012). Erythritol is used extensively in the food industry due to its favorable properties. A recent study by de Cock et al. (2016) demonstrated superior outcomes of erythritol over xylitol and sorbitol for dental plaque suppression and risk reduction highlighting its potential in oral health applications. Studies on the replacement of sucrose in baked goods with this polyol have shown it to function well for partial sucrose replacement, while total replacement can cause adverse effects on product properties. In a study on Danish cookies, 50% of sucrose could be replaced with erythritol while retaining good physical and sensory properties. In addition, there was no difference in hedonic score between the control and the 50% erythritol replaced cookies (Sheng Dun Lin, Lee, Mau, Lin, and Chiou 2010). In a similar study on chiffon cakes, 50% erythritol replacement of sucrose showed no significant difference in physical properties. Cakes with 75% erythritol replacement were acceptable but showed differences in crust color and sweetness from the 100% sucrose control (S. D. Lin, Hwang, and Yeh 2003). In a study on muffins with complete sucrose replacement by several polyols erythritol scored worst as a candidate for 100% sucrose replacement. This was largely due to its effect on starch gelatinization in the dough batter leading to the production of short and hard muffins (Martínez-Cervera, Salvador, and Sanz 2014).

Disaccharide polyols commonly used in foods

Maltitol is a disaccharide composed of glucose and sorbitol and has the chemical name 4-O-α-glucopyranosyl-D-sorbitol. It is formed from maltose and consists of a glucose and sorbitol unit. Maltitols sweetness is up to 90% that of sucrose, and due to the disaccharide structure they are similar in properties (Grembecka 2015; Nabors 2012).

Maltitol is regularly used in sugar replaced chocolate spreads and chocolate flavored coatings. In ice-cream sugar replacement maltitol produced the most functionally similar ice-cream to a sucrose control (Soukoulis et al. 2010). It is extensively used for this application in sugar free dairy products.

Lactitol, also a disaccharide polyol is composed of galactose and sorbitol. As the name suggests, lactitol is derived from the hydrogenation of lactose. Although lactitol only has 40% of the sweetening power of sucrose it has a good solubility in comparison. This makes bulk substitution of sucrose with lactitol widely applicable with minimal change to processing while intense sweeteners are used to bridge the sweetness deficit (O'Donnell and Kearsley 2012). This polyol is useful in the preparation of no added sugar baked goods in which it gives similar product characteristics to sucrose equivalents. It is also used in sugar free chocolate production, again due to the similarities to sucrose in its properties such as molecular weight. These favorable properties also extend to ice-cream formulations where its comparable effect to sucrose on freezing point depression and high solubility are functional (O'Donnell and Kearsley 2012).

Isomalt is a mixture of two disaccharide polyols a glucose and mannitol disaccharide (GPM) and a glucose and sorbitol disaccharide (GPS) given the generic name isomalt (isomers of hydrogenated maltulose). The product is sold as different ratios of GPM and GPS suited for specific application requirements. The sweetening power of isomalt is 45–60% compared to sucrose. Applications of isomalt products include hard candies, chocolate and baked goods (O'Donnell and Kearsley 2012).

Conclusion

Sugar alcohols are used widely in the food industry as sweeteners and thickeners. As such, economically advantageous methods for their production are constantly under investigation. Biotechnological production of the polyols mannitol, sorbitol, xylitol and erythritol is possible using a variety of different organisms and the processes can yield relatively high amounts often from low cost substrate materials. Mannitol can be formed from glucose by homofermentative LAB under conditions of redox imbalance which can be induced by knock-out of *ldh* and compromise of the NAD^+ regeneration capacity of cells. Heterofermentative LAB reduce fructose to mannitol by the action of a mannitol-2-dehydrogenase as a means of NAD^+ regeneration. In processes applying a 2:1 ration of fructose to glucose the theoretical yield is 100% and indeed using continuous cell recycling fermentation, a yield of 0.94 g/g was achieved. Alternative, low cost sources of nutrients such as corn steep liquor and cashew apple juice when supplemented appropriately can be effectively used for mannitol production. Sorbitol production is largely achieved using the Gram negative *Z. mobilis* which possesses a glucose-fructose oxidoreductase. Processes applying the immobilization and permeabilisation of *Z. mobilis* cells have achieved yields of sorbitol approaching 100% at high substrate concentrations. Sorbitol production by *Z. mobilis* using the low-cost substrate sugar cane molasses as carbon source has been reported but yields were lower. Nevertheless, investigation of the use of low cost media along with optimized processes of cell permeabilisation and encapsulation is likely to provide an avenue for more economic industrial scale production of sorbitol. Xylitol production is possible with a variety of yeast and yeast-like fungal species. The substrate for xylitol formation, xylose, can be extracted from hemicellulosic wastes of agricultural industries. While appreciably high yields (up to 80%) can be achieved form these extracts, slow cell growth

and relatively low substrate concentrations result in low volumetric productivities. Further research to optimize the growth rate and productivity of yeasts will improve the commercial potential of such processes. Erythritol can be produced in large quantities by osmophilic yeast, and wild type strains are often successfully mutated by UV irradiation and/or chemical mutagenesis to develop more active isolates. Biotechnological production of erythritol is the predominant means of industrial scale production with multiple species capable of producing high yields from glucose and sucrose on large scale. Interestingly, the species *Y. lipolytica* can produce erythritol efficiently from glycerol an industrial waste material. Genetic engineering to overexpress genes encoding enzymes involved in erythritol synthesis can improve erythritol production in *Y. lipolytica*. While erythritol production is described in some LAB species, productivities are generally low, this however may be of use for *in situ* erythritol formation in fermented foods in which large quantities are less desirable. Overall, the use of industrial waste material for fermentative production of polyols is an important example of the potential for biological processes in the development of circular economic practices, an area currently undergoing substantial growth.

Polyols have been recognized to have a number of health benefits which have encouraged their use in foods. These claims have been substantiated by research, and food policy makers have approved the use of claims pertaining to the reduced dental caries risk and the reduced energy intake of sugar replaced foods containing polyols. In addition, policy makers have laid out rules for the communication of these benefits to consumers on food labels. Conversely, the potential for gastrointestinal side effects caused by consumption of polyols is recognized and similar labeling regulations are applied in cases where high amounts of polyols may be consumed. Food labeling now requires polyols to be included in ingredient listings and as an individual component of the nutritional label under carbohydrates. While polyols are not recognized as added sugars, products with polyols added as sweeteners are required to state the presence of sweeteners. This recognition in terms of food labeling regulations are indicative of the prominence of polyols in the food market. Continued research into the health effects of polyol consumption will improve our understanding of both the beneficial and undesired results of polyols in the diet, and also help to inform consumers how to approach polyol consumption on an individualized basis. Nonetheless, negative connotations of consumers towards FODMAPs will continue to play a role in acceptance.

The differing physical properties of polyols including varying sweetness intensities, solubility and hygroscopicity among others, open them to alternate applications in food. While all of the monosaccharide polyols have seen use in chewing gum formulations, their application to baked goods, candies and other confectionary differs. Hygroscopic mannitol is suitable as a dusting agent for gum and candies. The high solubility of sorbitol lends it to use in liquid systems such as chocolate and ice-cream. Xylitol, the sweetest polyol, is a model for quantitative sugar replacement. The low caloric value of erythritol make it of particular use in the formulation of low-calorie sugar free products. Disaccharide polyols are also widely used. Assessment of the effects of sugar replacement with polyols on the physical and sensory characteristics of unstudied food formulations, along with continued comparison of different polyols for their optimal applications will present new avenues for their application.

Polyols are an important food additive whose application has seen an increase in a climate where consumer demand is driven by health awareness and sugar over consumption is at the forefront of government policy. Research continues to focus on processes for microbial production of polyols and the promising field of applying fermentation processes for in situ polyol production in foods is emerging. It is important now more than ever for consumers to be aware of the health effects of polyol consumption, both positive and negative. Current food labeling regulations aim to give consumers appropriate information in an understandable format, and as the range of products containing polyols is increasing they are becoming a common feature on food labels.

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