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Sweeteners

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Abstract Polyols as sugar substitutes, intense sweeteners and some new carbohydrates are increasingly used in foods and beverages. Some sweeteners are produced by fermentation or using enzymatic conversion. Many studies for others have been published. This chapter reviews the most important sweeteners.

Keywords Aspartame · Erythritol · Fermentation · Isomalt · Maltitol · Mannitol · Production · Sorbitol · Steviol glycosides · Tagatose · Thaumatin

Contents

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1 Summary

Sweeteners, sweet substances other than sugar and related carbohydrates, are polyols or intense sweeteners. Most of these substances are produced by chemical synthesis. Among the group of polyols, erythritol and part of mannitol are produced by fermentation. Immobilized cells or enzymes are used in the production of isomalt and maltose, an intermediate for maltitol. Many papers on the production of sorbitol and xylitol by fermentation are available. Among the intense sweeteners, the building blocks of aspartame, aspartic acid and phenylalanine, are produced by fermentation, and enzymatic coupling was used in practice by one producer. Stevioside and glycyrrhizin can be modified enzymatically, and possibilities to express the genes for thaumatin were reported in several papers. Tagatose, a reduced-calorie carbohydrate, can be produced by enzymatic conversion of galactose. Important papers describing organisms, enzymes, and fermentation conditions used in practice and in studies are reviewed in this chapter.

2 Introduction

Sweet-tasting substances other than sugar have become increasingly important in food production in the course of the last decades. In certain areas such as soft drinks, the quantity of products sweetened with these substances has almost equalled the conventional, sugar-sweetened products in some countries including the United States. In others, such as in some European countries, the percentage of these beverages has increased steadily after a harmonized approval for all Member States of the European Community in 1995. In other fields of application such as sugar-free sweets and confections, polyols have been established as a noncariogenic alternative to sucrose.

Many sweet-tasting substances are known. This chapter focuses on products used in foods and beverages. Several others can be produced by fermentation, but are of no practical importance.

3 Definitions and General Aspects

The general field of sweet-tasting substances can be divided in two main sectors. One comprises sugar (sucrose) and other nutritive carbohydrates including glucose, fructose, and products obtained from hydrolyzed starch such as high-fructose corn syrup. The other sector covers products generally called sweeteners. They are noncarbohydrate alternatives such as polyols and intense sweeteners. A third group of still rather limited commercial importance comprises sweet carbohydrates of

physiological characteristics different from the standard carbohydrates normally used in food production.

3.1 Sweetness

All substances covered in this chapter are sweet. They are, however different in their sweetness intensity and characteristics of their sweetness.

Several substances show sweetness intensity in the same range as the sweetness of sucrose. These are generally polyols and also the carbohydrates described here. Others are distinguished by much a higher sweetness intensity and therefore are normally called intense or high-intensity sweeteners.

In addition to the sweetness intensity, other characteristics are important for the assessment of sweeteners, such as side-tastes, for example, bitter or licorice-like aftertastes and delayed or lingering sweetness or cooling effects. Although polyols normally have a more or less clean sweetness, most of them have a cooling effect when ingested as the dry substance. Intense sweeteners may have aftertastes, a bitter aftertaste like saccharin, a licorice-like taste like steviol glycosides, a delayed sweetness onset like thaumatin or a lasting sweetness like aspartame and sucralose. They are therefore often used in combinations balancing their taste properties.

3.2 Physiology

Most polyols are metabolized, but absorbed only slowly. Partial absorption and fermentation in the intestine result in some contribution to the calorie content of foods. The European Union uses 2.4 kcal/g or 10 kJ/g for all polyols except for erythritol which is noncaloric [10]. Other countries use other, mostly similar, but not always the same, values for polyols. Osmotic effects and microbial metabolization of polyols in the intestine can result in laxative effects causing intestinal discomfort after ingestion of larger amounts.

Most intense sweeteners are not metabolized in the human body and are therefore calorie-free. Others such as aspartame are fully metabolized but, owing to their intense sweetness, are only used in minute quantities that do not make any significant contribution to the caloric content of foods or beverages.

The caloric values of the carbohydrates covered here vary from zero calories for tagatose to the full energy value for, as an example, isomaltulose.

Polyols and intense sweeteners are suitable for diabetics within a suitable diet, whereas for the fully metabolized carbohydrates the rules for the diet should apply, although they may not be absorbed as quickly as sucrose or glucose and therefore trigger a lower blood glucose level than sucrose.

As intense sweeteners and polyols are either not or only very slowly metabolized by the bacteria of the oral cavity to acids, they are generally considered noncariogenic [89].

3.3 Applications

Polyols have a similar sweetness level to that of sugar and are therefore used in similar quantities. Important applications are sweets and confections, chewing gum, tablets, or carriers for sugar-free powders. Owing to the rather low sweetness of some polyols, they are often combined with intense sweeteners to adjust the sweetness to the customary sucrose level.

Intense sweeteners are used in too small a quantity to have any of the technological functions sugar has in many foods. Therefore their main fields of application are beverages, table-top sweeteners and dairy products, but also combinations with some polyols, for example, in confectionery products.

3.4 Regulatory Aspects

Several polyols and intense sweeteners are approved as food additives in the European Union [11]. Change of their manufacturing processes (e.g., replacement of synthetic production by fermentation) requires an additional approval [9]. The reduced-calorie and other carbohydrates are normally not food additives in the EU regulatory framework. New substances would require approval as novel food; approved substances produced by a new fermentation process would also require this approval, but could be notified as substantially equivalent to existing substances if no significant deviation from the existing product could be demonstrated [4].

In the United States, intense sweeteners with the exception of steviol glycosides are regulated as food additives; polyols are either Generally Recognized As Safe (GRAS) or approved as food additives (Anonymous). Substances occurring in nature are GRAS eligible. For these substances, submission of a GRAS notice to the US Food and Drug Administration (FDA) is possible. They are considered acceptable unless the FDA objects or asks questions within 90 days after submission [5].

Generally, a high purity is required for food uses. The specifications laid down in legislation, are, however, slightly different among the EU, USA, and international proposals.

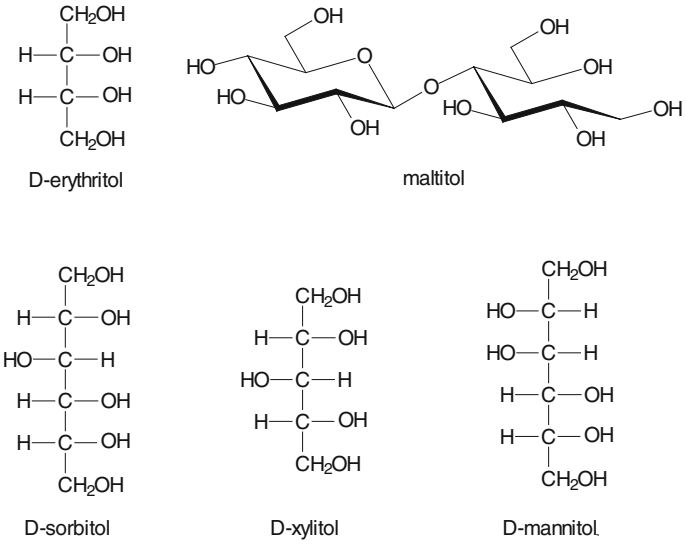

Fig. 1 Structures of commercially produced polyols

4 Polyols

4.1 Erythritol

4.1.1 General Aspects and Properties

Erythritol (*meso*-erythritol, *meso*-1,2,3,4-Tetrahydroxybutan; Fig. 1) has been known for a long time. Its potential use as a bulk sweetener was, however, recognized rather late.

Erythritol is a natural constituent of several foods and beverages in levels sometimes exceeding 1 g/kg. Its solubility in water is approximately 370 g/L at room temperature and increases with increasing temperature. Erythritol melts at 121 C and is stable up to more than 160 C and in a pH range from 2 to 10.

Depending on the concentration used, erythritol is approximately 60 % as sweet as sucrose. It is noncariogenic and not metabolized in the human body which means that it is more or less calorie-free [26].

In the European Union, erythritol is approved as E 968 for a large number of food applications [11]. It is GRAS in the United States [6, 8, 12] and also approved in many other countries.

4.1.2 Microorganisms Producing Erythritol

Microorganisms producing erythritol have been known for many years [140]. Papers describing microorganisms producing yields of 35–40 % of the sugar used in the medium were published as early as 1960 and 1964, and the need carefully to control nitrogen and phosphorus levels in the medium were also highlighted [39, 139]. Further research resulted in the discovery of a variety of organisms. Among these are *Aspergillus niger* [102], *Aurobasidium sp*. [49], *Beauveria bassiana* [145], *Candida magnoliae* [158], *Moniliella sp*. [87], especially *Moniliella pollinis* [29], *Penicillium sp.* [80], *Pseudozyma tsukubaensis* [55], *Torula corallina* [77], *Trigonopsis variabilis* [65], *Trichosporonoides sp*. [90], and especially *Trichosporonoides megachiliensis* [131], *Ustilagomycetes sp*. [44], and *Yarrowia lipolytica* [122]. Patent applications specify a number of different species.

4.1.3 Biochemical Pathways

Different types of microorganisms use different pathways for the biosynthesis of erythritol.

For *C. magnoliae*, transaldolases and transketolases are involved [139]. For mutant strains of *C. magnoliae*, up-regulated enzymes of the citric acid cycle with resulting higher NADH and ATP formation, down-regulated enolase, and up-regulated fumarase with improved conversion of erythritol-4-phosphate to erythritol were held responsible for the higher yields of erythritol [73]. The enolase, erythrose reductase, is an NAD(P)H-dependent homodimeric aldose reductase [78, 79]. Reduction of fumarate production resulted in higher yields of erythritol inasmuch as fumarate is a strong inhibitor of erythrose reductase, the enzyme converting this substance to erythritol [77].

Trichosporonoides megachiliensis mainly uses the pentose phosphate way for the production of erythritol. Transketolase activity was correlated with erythritol yields under various production conditions. It is therefore concluded that transketolase appears to be a key enzyme for formation of erythritol in this organism [131].

In *Y. lipolytica*, glucose is supposed to be converted to erythrose-4-phosphate via the pentose phosphate pathway and reduced by erythrose reductase to erythritol-4-phosphate with subsequent hydrolysis of the ester bond [121].

4.1.4 Production

The synthesis of erythritol is rather difficult. One of the possibilities is the catalytic reduction of tartaric acid with Raney nickel, which does, however, also produce threitol, a diastereomere of erythritol that requires separation of both. Threitol may be isomerized which increases the yields of erythritol. Another chemical synthesis starts from butane-2-diol-1.4 which is reacted with chlorine in aqueous alkali to yield erythritol-2-chlorohydrin and can be hydrolyzed with sodium carbonate

solution. Synthesis from dialdehyde starch in the presence of a nickel catalyst at high temperatures is also possible [16].

Owing to the special physiological properties of erythritol, commercial interest increased with the discovery of an increasing number of microorganisms able to produce this substance. Today, the commercial production of erythritol is apparently only based on fermentation.

Erythrytitol fermentations mostly use osmophilic yeasts. Based on regulatory submissions for commercial production, *T. megachiliensis*, *M. pollinis* [7], and *Y. lipolytica* [12] are used. It is also claimed that *P. tsukubaensis* and *Aureobasidium sp.* are used for commercial production [95].

Erythritol-producing microorganisms often produce other polyols such as ribitol. Nevertheless, some strains had a rather high yield of erythritol. A two-step fermentation of *C. magnoliae* on 400 g/L glucose resulted in a 41 % conversion rate and a productivity of 2.8 g/Lh [124]. *M. pollinis* cultivated on glucose and several nitrogen sources yielded erythritol concentrations up to 175 g/L with a conversion rate of 43 %. Oxygen limitation resulted in ethanol formation, and nitrogen limitation in strong foaming. A mutant gave even better yields [17].

Aerobically on glucose cultured *P. tsukubaensis* KN 75 produced 245 g/L of erythritol with an especially high yield of 61 %. The productivity was 2.86 g/Lh. Scale-up from 7-L laboratory fermenter to 50,000-L industrial scale resulted in productivities similar to the laboratory value [55].

Several factors influence productivity and conversion rates. Investigated were, among others, supplementation of the medium with Mn^{2+} and Cu^{2+} for *Torula sp*. Supplementation with Mn^{2+} resulted in lower intracellular concentrations of erythritol, whereas Cu^{2+} increased the activity of erythrose reductase [75]. Phytic acid, inositol, and phosphate also had a positive effect on the yields in *Torula sp.* by increasing the cell growth and increasing the activity of erythrose reductase [76].

A further increase in productivity was obtained by using mutant strains. Examples are an osmophilic mutant strain of *C. magnoliae* with a yield of 200 g/L, a conversion rate of glucose of 43 %, and a productivity of 1.2 g/Lh [70]. Among several mutants of *Moniliella sp*. 440 fermented in 40 % glucose and 1 % yeast extract, the highest yields were 237.8 g/L [88].

Many aspects of fermentation of an osmophilic fungus are described in a thesis by [16]. A survey covers the most important aspects of fermentation [58].

Owing to the commercial importance of erythritol, much information on production conditions is laid down in patent applications. They describe new strains or species producing erythritol and new mutants that have no commercial importance or none as yet. Also specific compositions of the media, methods to reduce viscosity of the media and specific processing, purification, and crystallization conditions are claimed.

Strains not producing polysaccharides eliminate problems caused by increasing viscosity of the medium such as reduced oxygen transfer rates with increasing formation of ethanol and difficulties in filtration during processing of the medium [147].

The use of inorganic nitrogen sources, especially nitrates, as the main nitrogen source for fermentation of *M. pollinis* was claimed to facilitate the adjustment of the pH, the purification, and also to increase the erythritol yields [30].

Common isolation and purification steps are filtration or centrifugation to remove the microorganisms, demineralization with anion exchangers, other types of chromatographic separation, decolorization with activated carbon, and crystallization and recrystallization [125].

4.2 Isomalt

4.2.1 General Aspects and Properties

Isomalt is a more or less equimolar mixture of 1-*O*-α-D-glucopyranosy-D-mannitol-dihydrate and 6-*O*-α-D-glucopyranosyl-D-sorbitol. Different production conditions, however, allow variations in the ratio of the two products. The solubility in water is about 24.5 % (w/w) at room temperature, but varies with the composition and increases with increasing temperature. In addition to the dry isomalt, a syrup is available.

Isomalt is, depending on the concentration, approximately 45–60 % as sweet as sucrose, stable under normal processing conditions of foods, and noncariogenic [132].

In the European Union, isomalt is approved as E 953 for a large number of food applications [11]. It is GRAS in the United States and also approved in many other countries.

Owing to its low glycemic index, isomaltulose, an intermediate of the production, has found increasing interest as a food ingredient in recent years.

4.2.2 Microorganisms Transforming Sucrose into Isomaltulose

For commercial production of isomalt, the sucrose starting material has to be transformed into isomaltulose. The enzyme for this transformation is a glycosyltransferase (sucrosemutase). An organism producing this enzyme suitable for commercial use is commonly named *Protaminobacter rubrum*. It is, however, claimed that it should be *Serratia plymuthica* [36]. Several other organisms have a similar enzymatic activity. Among these are *Erwinia sp* D 12 [59], *E. rhapontici* [155], and *Klebsiella terrigena* JCM 1687 [143].

A variety of enzymes from other sources and cloning into other organisms has been described in the literature. However, they seem to have no commercial importance or none as yet.

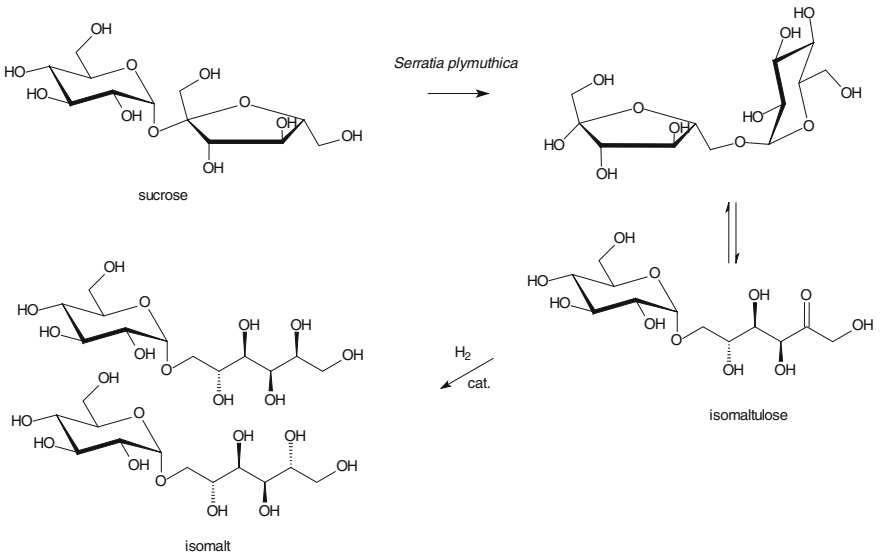

Fig. 2 Production of isomalt from sucrose

4.2.3 Production

For the production of isomalt sucrose is converted to isomaltulose which is then hydrogenated to yield a mixture of the two components of isomalt (Fig. 2). Although the production of isomalt itself from isomaltulose is a chemical hydrogenation, transformation of sucrose into isomaltulose requires enzymatic transformation.

The enzyme sucrosemutase is sensitive to glutaraldehyde, therefore cross-linking is not possible. For industrial use it is, however, not necessary to isolate the enzyme, as immobilized cells of the organism can be used. Addition of sodium alginate to the cultivated cells and subsequent addition of calcium acetate immobilizes the cells. This allows for the use of the cells in a bed reactor, and also facilitates the separation of the product from the reaction mixture.

The long-term stability of the immobilized organism is high and can exceed 5,000 h, even if high sucrose concentrations of 550 g/L are used. The yields are about 80–85 % with 9–11 % of trehalulose and small quantities of other saccharides as by-products.

Prior to hydrogenation, free sucrose has to be removed. This is carried out by nonviable cells of *Saccharomyces cerevisiae*. Remaining by-products of the reaction are converted to the respective sugar alcohols.

Although the hydrogenation of isomaltulose theoretically should yield an equimolar mixture of the two constituents of isomalt, the share of each component may vary between 43–57 % depending on the conditions of hydrogenation [120].

An alternative possibility is the direct cultivation of suitable microorganisms such as *P. rubrum* on sucrose-containing juices obtained during the production of

beet and cane sugar. It is claimed that glucose and fructose produced during the transformation are consumed by the microorganisms which results in lower amounts of by-products [24].

4.3 Maltitol

4.3.1 General Aspects and Properties

Maltitol is α-D-glucopyranosyl-1.4-glucitol. The solubility in water is approximately 1,750 g/L at room temperature. Maltitol is stable under the common processing conditions of foods. In addition to dry maltitol several types of syrups are available.

Maltitol is, depending on the concentration, approximately 90 % as sweet as sucrose and noncariogenic [60].

In the European Union, maltitol is approved as E 965 for a large number of food applications. It is GRAS in the United States and also approved in many other countries.

4.3.2 Production

Maltitol is produced by chemical hydrogenation of maltose, which can be obtained by enzymatic degradation of starch under conditions similar to those used for other starch hydrolysates such as glucose. The Starting material can be the different commercially available starches including corn, potato, and others. A partially degraded starch, which can be obtained by treatment with diluted hydrochloric or sulphuric acid and subsequent neutralization or with heat-stable α-amylase, is then subjected to enzyme treatment for further degradation to maltose-rich products.

Enzymes used for maltose production are β-amylases, fungal α-amylases, α-1.6-glucosidases, maltogenic amylases, and debranching enzymes, preferably with high temperature optimum.

Examples can be found in patent applications for processes for production of maltose and maltitol [33, 34, 41, 97, 109, 141].

4.4 Mannitol

4.4.1 General Aspects and Properties

D-mannitol (D-mannohexan-1.2.3.4.5.6-hexaol) is a constituent of several plants including the Manna ash, several edible plants, and seaweed. Parts of the latter contain up to 10 % mannitol by weight. The solubility in water is approximately

230 g/L at room temperature and it increases with increasing temperature. Mannitol is stable under the common processing conditions of foods.

Mannitol is approximately 50 % as sweet as sucrose and non-cariogenic [52].

In the European Union, maltitol is approved as E 421 for a large number of food applications. In the United States, mannitol produced by hydrogenation of glucose or fructose solutions or by fermentation by *Zygosaccharomyces rouxii* or *Lactobacillus intermedius* is approved for several food applications. It is also approved in many other countries.

4.4.2 Microorganisms Producing Mannitol

Several microorganisms are able to produce mannitol, some of which have been known for a long time [105]. Among these are several species of *Aspergillus* [135], *C. magnoliae* [137], several species of *Lactobacillus* [153], especially *L. intermedius*, [128], *Leuconostoc* [20], *Penicillium* [148], or *Torulopsis* [104] and *Z. rouxii* [101].

4.4.3 Biochemical Pathways

Several heterofermentative lactic acid bacteria produce mannitol in large amounts, using fructose as an electron acceptor. Under anaerobic conditions, acetylphosphate produced in the metabolization of glucose would normally be converted to ethanol. In the presence of fructose it is used as an electron acceptor and converted to mannitol by mannitol dehydrogenase. The enzyme requires $NADH_2$ or $NADPH_2$, which is regenerated during hydrogenation of fructose. The now possible conversion of acetylphosphate to acetic acid is energetically advantageous for the organism [136]. *C. magnoliae* also uses mannitol dehydrogenase [13]. *Aspergillus sp*. uses glucose as the starting material and reduces to fructose-6-phosphate instead of fructose [81].

4.4.4 Production

The by far largest quantity of mannitol is produced by chemical hydrogenation of fructose which yields a mixture of mannitol and sorbitol. The mixture is subjected to fractionated crystallization. As direct sorbitol production is less costly, the processing costs have mostly to be borne by mannitol which makes it more expensive than sorbitol. Production from seaweed seems to be of limited importance.

Possibilities to produce mannitol by fermentation were studied using several organisms. They mostly use fructose as an acceptor for hydrogen and glucose as a source of carbon. In a fed-batch culture of *C. magnoliae* with 50 g/L of glucose as the initial carbon source and increasing levels of fructose up to 300 g/L in 120 h,

248 g/L of mannitol were obtained from 300 g/L of fructose equivalent to a conversion rate of 83 % and a productivity of 2.07 g/Lh [138].

High yields were obtained from *Lactobacillus fermentum* grown in a batch reactor. The conversion rates increased from 25 to 35 C to 93.6 % with average and high productivities of 7.6 and 16.0 g/Lh [153]. A fast mannitol production of 104 g/L within 16 h was obtained from *L. intermedius* on molasses and fructose syrups in a concentration of 150 g/L with a fructose-to-glucose rate of 4:1 [126]. High productivity (26.2 g/Lh) and conversion rates (97 mol%) were obtained in a high cell density membrane cell recycle bioreactor. Increase of the fructose concentration above 100 g/L reduced the productivity [154]. A fed-batch process with *L. intermedius* yielded 176 g/L of mannitol from 184 g/L fructose and 94 g/L glucose within 30 h. The productivity of 5.6 g/Lh could be increased to more than 40 g/Lh at the expense of reduced mannitol yield and increased residual substrate concentrations [112].

As mannitol is more expensive than sorbitol, production by fermentation may become an alternative to hydrogenation of fructose.

4.5 Sorbitol

4.5.1 General Aspects and Properties

The solubility of D-sorbitol (D-glucitol, is D-glucohexan-1.2.3.4.5.6-hexaol) in water is up to approximately 2,350 g/L at room temperature. Sorbitol is stable under the common processing conditions of foods. In addition to the dry sorbitol, syrups are available.

Sorbitol is, depending on the concentration, approximately 60 % as sweet as sucrose and noncariogenic [52].

In the European Union, sorbitol is approved as E 420 for a large number of food applications, in the United States as GRAS, and is also approved in many other countries.

Sorbitol is generally produced by chemical hydrogenation of glucose or, together with mannitol, by chemical hydrogenation of fructose.

4.5.2 Fermentation

Several microorganisms are known to produce significant amounts of sorbitol, especially after genetic engineering.

Zymomonas mobilis grown on glucose, fructose, or sucrose produced sorbitol in addition to the main product, ethanol. Strain ZM31 gave the highest concentrations of 43 g/L when grown on 250 g/L of sucrose. As the mechanism, inhibition of fructokinase by free glucose and reduction of fructose by a dehydrogenase is assumed [14]. In a hollow fiber membrane reactor, a productivity of 10–20 g/Lh

was found for *Z. mobilis* on 100 g/L each of glucose and fructose. Gluconic acid was produced simultaneously with similar productivities [107]. Immobilized cells of *Z. mobilis* in combination with immobilized invertase produced sorbitol with a productivity of 5.11 g/Lh and gluconic acid with a productivity of 5.1 g/Lh on 20 % sucrose in a recycle packed-bed reactor [117]. Immobilized and permeabilized cells of *Z. mobilis* reached more than 98 % conversion of equimolar concentrations of glucose and fructose to sorbitol and gluconic acid and maximum concentrations of 295 g/L each [115].

A high conversion rate of 61–65 % was found in a *Lactobacillus plantarum* strain with a high expression of two sorbitol-6-phosphate dehydrogenase genes grown on glucose. Small amounts of mannitol were also detected [72].

A high conversion of fructose with 19.1 g/L of sorbitol from 20 g/L of fructose with methanol as the energy source was reported for small-scale fermentation of *Candida boidinii* No. 2201 [144].

Inasmuch as glucose as the starting material and hydrogenation leads to a low-cost production process it seems unlikely that production of sorbitol by fermentation will play a significant role, at least in the near future.

4.6 Xylitol

4.6.1 General Aspects and Properties

The solubility of D-xylitol (D-xylopentan-1.2.3.4.5-pentaol) in water is approximately 1,690 g/L at room temperature. Xylitol is stable under the common processing conditions of foods.

Xylitol is, depending on the concentration, similarly or slightly sweeter than sucrose and noncariogenic [159].

In the European Union, xylitol is approved as E 967 for a large number of food applications. In the United States, it is approved for use in foods following Good Manufacturing Practice and it is also approved in many other countries.

4.6.2 Microorganisms Producing Xylitol

Xylitol can be formed through reduction of xylose by a xylose reductase, in many organisms a NADPH-dependent enzyme [2].

Microorganisms producing xylitol have been studied extensively. Many organisms are able to produce xylitol. Among these are *C. boidinii* [150], *Candida guilliermondii* [103], *C. magnoliae* [69], *Candida maltosa* [37], *Candida mogii* [146], *Candida parapsilosis* [99], *Candida peltata* [127] *Candida tropicalis* [133], *Corynebacterium sp.* [113], especially *Corynebacterium glutamicum* [130], *Debaryomyces hansenii* [106], *Hansenula polymorpha* [129], *Mycobacterium*

smegmatis [50], *Pichia sp.,* especially *Pichia caribbica*, *Issatchenkia sp.,* and *Clavispora sp.* [142].

Not only mutants of *C. tropicalis* [35, 54, 56, 114] and *C. magnoliae* [69], but also genetic engineering was used in several organisms to improve xylitol production. Genetic engineering was used to replace the xylose reductase in some organisms in which this enzyme is significantly repressed in the presence of glucose [53].

Strains of *C. tropicalis* with a disrupted gene for xylitol dehydrogenase which catalyzes the oxidation of xylitol to xylose were studied [68]. In one strain, genes were co-expressed that respectively encode glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase, under the control of glyceraldehyde-3-phosphate dehydrogenase promoter [2]. In another strain, a highly efficient xylose reductase from *Neurospora crassa*, which is not expressed as such in *C. tropicalis*, was modified and placed in a strain under control of a constitutive glyceraldehyde-3-phosphate dehydrogenase of *C. tropicalis*. This allowed for the use of glucose as a co-substrate with xylose [53]. A gene for an NADH-dependent xylose reductase from *C. parapsilosis* was transferred to *C. tropicalis* which resulted in dual co-enzyme specificity [79].

Higher productivities in *C. glutamicum* were especially achieved when the possible formation of toxic intracellular xylitol phosphate was avoided by elimination of genes encoding xylulokinase (XylB) and phosphoenolpyruvate-dependent fructose phosphotransferase (PTSfru) to yield the strain CtXR7 [130].

Further examples comprise the modification of *Escherichia coli* W3110 to produce xylitol from a mixture of glucose and xylose [61] and *E. coli* containing xylose reductase genes from several sources [23]. Xylitol-phosphate dehydrogenase genes were isolated from *Lactobacillus rhamnosus* and *Clostridium difficile* and expressed in *Bacillus subtilis* [108]. D-xylose reductase from *Pichia stipitis* CBS 5773 and the xylose transporter from *Lactobacillus brevis* ATCC 8287 were expressed in active form in *Lactococcus lactis* NZ9800 [98], and *S. cerevisiae* was supplemented with a xylose reductase gene from *P. stipitis* [82].

4.6.3 Production

Xylitol is mostly produced by chemical hydrogenation of xylose which is obtained by hydrolysis of xylans of plants such as birch and beech trees, corn cobs, bagasse, or straw, but also by fermentation of xylose, for example, using *Candida* species.

Xylose, especially for hydrogenation, requires a high purity. It may be obtained from wood extracts or pulp sulfite liquor, a waste product of cellulose production, by fermentation with a yeast that does not metabolize pentoses. Some strains of *S. cerevisiae*, *Saccharomyces fragilis*, *Saccharomyces carlsbergensis*, *Saccharomyces pastoanus,* and *Saccharomyces marxianus* are suitable for this purpose [51]. Hydrolysates of xylan-rich material are often treated with charcoal and ion-exchangers to remove by-products causing problems in hydrogenation or fermentation.

Many studies of xylitol production by fermentation have been published. Different organisms, substrates, and conditions were investigated. As the starting material, xylose or xylose in combination with glucose was used. Fermentation was carried out in batch reactors as well as continuously [134].

Among the variations studied was cell recycling in a submerged membrane bioreactor for *C. tropicalis* with a high productivity of 12 g/Lh, a conversion rate of 85 % and a concentration of 180 g/L [71]. Many studies addressed the immobilization of cells such as *S. cerevisiae* [119], *C. guilliermondii* [19], or *D. hansenii* [28], especially with calcium alginate.

In some studies, high xylitol concentrations, conversion rates and productivities were achieved. For *C. tropicalis,* concentrations of 290 g/L, a conversion rate of 97 %, and a productivity of more than 6 g/Lh [66], and 180 g/L, 85 % conversion, and 12 g/Lh were reported [71]. For *C. guilliermondii,* a concentration of 221 g/L (conversion rate of 82.6 %; [92]), for *C. glutamicum,* a concentration of 166 g/L at 7.9 g/Lh [130], and for *D. hansenii*, a concentration of 221 g/L and a conversion rate of 79 % [27] were reported. With *S. cerevisiae*, productivities of up to 5.8 g/Lh were observed [119].

4.7 Others

Polyols can generally be produced by hydrogenation of sugars and some also by fermentation. Most of the other polyols are, however, of no commercial interest for the food industry. The only other polyol of some importance is lactitol (E 966), produced by chemical hydrogenation of lactose, a constituent of milk. It seems that no possibilities for production of lactitol by fermentation have been investigated.

5 Intense Sweeteners

5.1 Aspartame

5.1.1 General Aspects and Properties

Aspartame (*N*-L-aspartyl-L-phenylalanine-1-methyl ester, 3-amino-*N*-(α-carbomethoxy-phenethyl)-succinamic acid-*N*-methyl ester) is an intense sweetener widely used in foods and beverages. Its solubility in water is approximately 10 g/L at room temperature. Aspartame is not fully stable under common processing and storage conditions of foods and beverages with the highest stability around pH 4.3 [1].

Aspartame is about 200 times sweeter than sucrose with a clean, but slightly lingering sweetness. It is used as the single sweetener, but often also in blends with

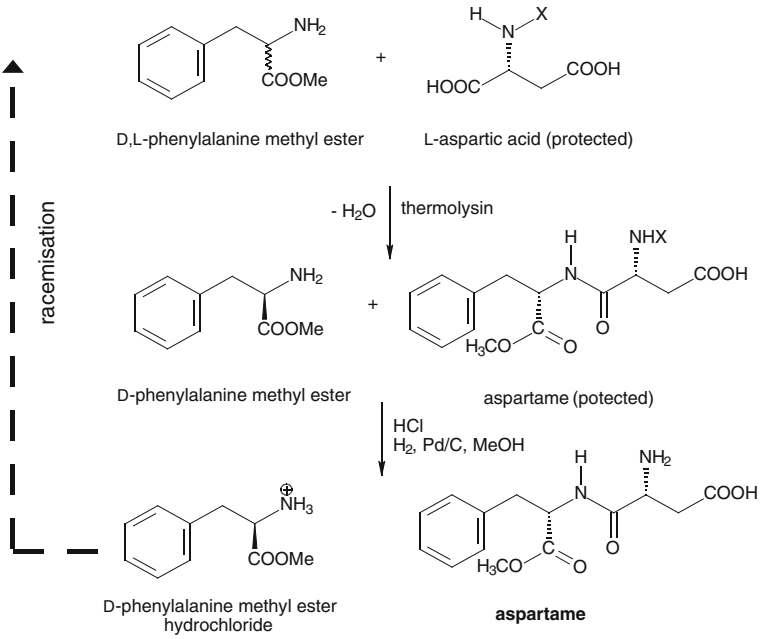

Fig. 3 Production scheme of aspartame

other intense sweeteners owing to synergistic taste enhancement and taste quality improvement often seen in such blends.

In the European Union, aspartame is approved as E 951 for a large number of food applications. In the United States, it is approved as a multipurpose sweetener for food and beverage uses and it is also approved in many other countries.

5.1.2 Production

Aspartame is produced from L-aspartic acid and L-phenylalanine and methanol or alternatively L-phenylalanine methyl ester. The standard process uses common chemical methods of peptide synthesis. Enzymatic coupling of the two amino acids is also possible. *N*-formyl-L-aspartic acid and L- or D.L-phenylalanine methyl ester can be condensed to aspartame by thermolysin-like proteases [43] The formylated aspartame can be deformylated chemically or with a formylmethionyl peptide deformylase to yield the sweetener [111].The enzymatic coupling does not require L-phenylalanine but can start from the racemic product obtained in chemical synthesis, and the remaining D-phenylalanine can be racemized again [151] (Fig. 3).

Production processes based on fermentation are available for the two main components, aspartic acid and phenylalanine [40, 83]

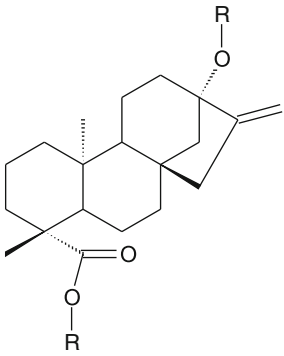

Fig. 4 Structure of steviol glycosides; R = mono- or disaccharide residues

5.2 Steviol Glycosides

5.2.1 General Aspects and Properties

Steviol glycosides (Fig. 4) are a family of related substances occurring in *Stevia rebaudiana*, a plant originating in South America, but now also cultivated in Asian countries especially. Main components are typically stevioside and rebaudioside A. The ratio of the different components varies, depending on the product. It may be changed by breeding, which aimed especially at an increase in Rebaudioside A, the product with the best sensory properties. Depending on the composition, steviol glycosides are 200–300 times as sweet as sugar but leave a more or less pronounced bitter and licorice aftertaste. They are stable under normal processing conditions of foods and beverages, but only poorly soluble in water [18].

In the European Union, steviol glycosides are approved as E 960 for a large number of food applications. In the United States, several preparations are GRAS. Steviol glycosides are also approved in many other countries, especially in Asia and South America.

Steviol glycosides are extracted from the leaves of the Stevia plant. The extracts are purified further by flocculation and treatment with ion exchangers before crystallization of the steviol glycosides.

5.2.2 Enzymatic Modifications

To overcome the taste disadvantages of steviol glycosides and their limited solubility, enzymatic modifications were studied. Transglycosylations were used to improve taste quality and solubility. Among the different products obtained, α-glucosyl stevioside seems to be the most interesting. Glucosylated steviosides can be obtained from stevioside and α-glucosyl oligosaccharides including maltose, maltooligosaccharides, or sucrose in the presence of glucosyltransferases [93]. Effective transglycosylation was also achieved with dextrin dextranase of *Acetobacter capsulatus* in a mixture of stevioside and a starch hydrolysate with

isoamylase [157]. Glucosyl stevioside has a less pronounced aftertaste than stevioside, is better soluble, of similar sweetness as stevioside, and approved in Japan but neither in Europe nor in the United States.

Transglycosylations of the other steviol glycosides are also possible but apparently of lower, if any, practical importance.

5.3 Thaumatin

5.3.1 General Aspects and Properties

Thaumatin is a mixture of sweet proteins occurring in the arils of the fruits of the African plant *Thaumatococcus daniellii*. Thaumatins I and II are the main components, but four more thaumatin molecules are known [67]. The proteins may be extracted with water. Thaumatin is about 2,000–2,500 times sweeter than sucrose but has a lingering sweetness. In addition to its sweet taste, it has flavor-enhancing properties. It is freely soluble in water and of fairly good stability [42].

In Europe, thaumatin is approved as E 957 for use as a sweetener. It is also approved in a variety of other countries, but in the United States, GRAS as a flavor enhancer only.

5.3.2 Fermentation

Genes encoding thaumatin, mostly thaumatin II, were expressed in several organisms. Among the organisms heterologously producing thaumatin are *Aspergillus awamori* [32, 96], *A. oryzae* [38], *E. coli* [25], *Penicillium roqueforti* [31], *Pichia pastoris* [91], and *Streptomyces lividans* [48]. Thaumatin I was produced in *P. pastoris*, too [47]. The thaumatins A and B, but not Thaumatin I were secreted by engineered *S. cerevisiae* [74]. Proteolytic activities of the production organism may impair the yields, as steps to eliminate this activity significantly improved the yields [96]. When specifically investigated, the secreted products were sweet [25, 32, 47, 91].

The recombinant expression in plant cells was studied, too. The secretion of small levels of thaumatin by recombinant hairy root cells of tobacco could be achieved. However, the yields decreased with increasing amounts of proteases in the medium [110].

5.4 Others

Most intense sweeteners are synthetic products [151]. Approved for food use are acesulfame K (E 950), cyclamate (E 952), neohesperidin dihydrochalcone (E 959),

saccharin (E 954), sucralose (E955), and neotame (E 961). Aspartame–acesulfame salt (E 962) is produced by a reaction of acesulfame acid with aspartame.

Neotame (E 961), (*N*-(*N*-(3,3-dimethylbutyl)-L-α-aspartyl-L-phenylalanine 1-methyl ester, is obtained by reacting aspartame with 3,3-dimethylbutyraldehyde.

Advantame, *N*-[*N*-[3-(3-hydroxy-4-methoxyphenyl) propyl-α-aspartyl]-L-phenylalanine 1-methyl ester, monohydrate, is synthesized from aspartame and 3-(3-hydroxy-4-methoxyphenyl)-propionaldehyde. It is not yet approved in the European Union and the United States.

A variety of other sweet-tasting compounds was discovered in plants [67]. Most of these have no commercial importance. Dried aqueous extracts of *Siraitia grosvenori* (formerly *Momordica grosvenori*) are called Luo Han Guo and are regarded GRAS in the United States, but not approved in Europe.

Glycrrhizin, triterpene glycoside salts occurring in the roots of licorice, is not approved as a sweetener, but as a flavoring. Extracts from the roots are up to 100 times sweeter than sucrose. The *3-O-β*-D-monoglucuronide can be prepared using an enzyme from *Cryptococcus magnus*. It is more than 900 times sweeter than sucrose [94], but not approved in Europe and the United States.

6 Carbohydrates

6.1 Isomaltulose

Isomaltulose, 6-*O*-α-D-Glucopyranosyl-D-fructofuranose, is a carbohydrate that has found interest owing to its low glycemic index and noncariogenicity [152]. It is approved as a novel food in the European Union and GRAS in the United States. Production details are given above under isomalt.

6.2 Tagatose

6.2.1 General Aspects and Properties

D-tagatose is a carbohydrate occurring in small amounts in several foods. The solubility in water is approximately 580 g/L at room temperature. As a ketohexose, tagatose reacts in foods in browning reactions like other ketohexoses, for example, fructose (Fig. 5).

Tagatose is, depending on the concentration, approximately 92 % as sweet as sucrose and noncariogenic. The caloric value of tagatose is generally set to 1.5 kcal/g [149].

In the European Union, tagatose is approved as a novel food. In the United States, tagatose has GRAS status and it is also approved in many other countries.

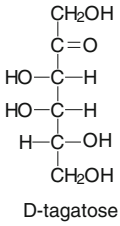

Fig. 5 Fischer projection of the keto hexose tagatose

6.2.2 Microorganisms Producing Tagatose

Enzymatic transformation of galactose into tagatose is possible with L-arabinose isomerase which is found in many microorganisms. Enzymes stable at high temperatures were found in *Acidothermus cellulolytics* [21], *Anoxybacillus flavithermus* [84], *Geobacillus thermodenitrificans* [100], *Thermoanaerobacter mathranii* [85], *Thermotoga maritime*, *Geobacillus stearothermophilus* [46], *Thermotoga neapolitana* [86], and *Thermus sp*. [63]. A thermostable galactose isomerase was isolated from bacteria [64].

Mutations were induced to increase the production rates of tagatose, for example, in *G. thermodenitrificans* [100] or *G. stearothermophilus* [62].

Genetic engineering to improve the performance of fermentation and to use common organisms was reported in several studies. The overexpression of genes of *T. mathranii* [57], *Bacillus stearothermophilus* [21], *T. neapolitana* [45], or *A. cellulolytics* [22] in *E. coli* was described.

6.2.3 Production

Tagatose is produced from galactose, which can be obtained by enzymatic hydrolysis of lactose, the main carbohydrate of milk. Galactose is separated from glucose by chromatography and either isomerized by treatment with calcium hydroxide, subsequent precipitation of calcium carbonate with carbon dioxide, filtration, demineralization with ion exchangers and crystallization [15], or converted enzymatically.

Especially high conversion rates of 96.4 % were obtained with an enzyme extract of an engineered *E. coli* [118], and of 60 % at 95 C for *A. flavithermus* in the presence of borate [84]. Conversion rates of 58 % were reported for an enzyme obtained from a mutant of *G. thermodenitrificans* [100], of 54 % at 60 C for a recombinant enzyme of *Thermus sp*. expressed in *E. coli* [63], and of more than 50 % at 75 C for *E. coli* containing an enzyme of *A. cellulolytics* [21, 22].

Immobilized enzymes or whole cells were used for practical applications. In some studies, high yields and productivities were achieved.

Immobilized L-arabinose isomerase in calcium alginate produced 145 g/L of tagatose with 48 % conversion of galactose and a productivity of 54 g/Lh in a packed-bed reactor [123]. An enzyme of *T. mathranii* immobilized in calcium

alginate had its optimum at 75 C with a conversion rate of 43.9 % and a productivity up to 10 g/Lh with, however, lower conversion. After incubation of the resulting syrup with *S. cerevisiae,* purities above 95 % were achieved [85]. The enzyme of *T. neapolitana* immobilized on chitopearl beds gave a tagatose concentration of 138 g/L at 70 C [86].

Lactobacillus fermentum immobilized in calcium alginate had a temperature optimum of 65 C. A conversion rate of 60 % and a productivity of 11.1 g/Lh were obtained in a packed-bed reactor after addition of borate [156].

Direct production of tagatose in yogurt was possible by expressing the enzyme of *B. stearothermophilus* in *Lactobacillus bulgaricus* and *Streptococcus thermophilus* [116].

6.3 Others

A variety of other reduced-calorie or caloric sweeteners was studied in the course of the last years. Properties, production cost, or lack of advantages over established sweet-tasting carbohydrates resulted in no market success [152].

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