Biotechnical modification of agro-bulk carbohydrates into specialty mono-, oligo- or polysaccharides

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1. Introduction

A number of mono- and disaccharides are available in bulk quantities at high purity and at low prices (1). Sucrose, obtained as such and glucose, obtained by depolymerisation of starch are the cheapest pure bulk carbohydrates and available in very large quantities. Sucrose, with a world production of over 100 million tons per year, is the world's most ubiquitously produced pure chemical on earth. Worldwide glucose production is estimated at 10 million tons per year. Slightly more expensive carbohydrates such as maltose, lactose and fructose are also readily available. Although these carbohydrates combine the desirable characteristics of cheapness, availability and not to forget sustainability, they are up to now not extensively used as organic bulk chemicals. However, they constitute a potential green source of a wide range of desired chemicals, either derived chemically or biotechnologically.

Biotechnical carbohydrate modifications are usually performed in aqueous media under relatively mild conditions. Pressure is rarely of importance and temperature can go as high as 100°C (α-amylase starch liquefaction). Contrary to a common belief, concentrations in biotechnical carbohydrate modifications are often very high. Batch fermentations are performed at dry matter contents of up to 450 g/l (erythritol production) and enzymatic conversions go up to 650 g/l (leucrose synthesis). A fed batch fermentation for the production of L-sorbose goes even higher up to 800 g/l, in which part of the L-sorbose spontaneously crystallizes during the fermentation (3). Substrate conversion

efficiencies can be quite low but very often the commercially applied conversions have nearly quantitative conversion efficiencies of over 95%. Selectivity can be extremely high or conveniently low depending on the enzyme involved. Stability of the biocatalyst can be very good or disappointingly poor. Industrial enzymatic conversions based on immobilized enzymes have half life times measured in several months, glucose isomerase serving as a typical example.

Current biotechnical carbohydrate modifications are mostly based on microbial technology. Cheap carbohydrates such as sucrose (molasses), glucose and starch are almost universally used as carbon sources in industrial fermentation processes. The worldwide total usage of carbohydrate fermentation feedstocks is about 30 million tons per year. They are converted into a wide range of compounds, ranging from industrial enzymes over vitamins, antibiotics, organic acids, bio-pharmaceuticals, peptides, vaccines, amino acids,... Only in a number of cases is the desired end product also a carbohydrate. This review paper will be restricted on purpose to those fermentation/bioconversion processes in which the carbohydrate substrate is converted to another carbohydrate.

2. Carbohydrate syntheses involving complex fermentation pathways

Although the actual workhorses in biotechnical carbohydrate modifications are the enzymes, one must differentiate between carbohydrate modifications that employ a complex biochemical route or a single enzyme. Some carbohydrates can only be produced through involvement of a complex biosynthesis pathway. Such processes are usually performed via a fermentation process with a suitable microbial strain that possesses the concerned pathway and which is often metabolically blocked and/or deregulated, thereby overproducing the desired carbohydrate.

2.1. D-Ribose

D-Ribose is an aldopentose, which is mainly used for the synthesis of riboflavin (vitamin B_2). It is recently also used to synthesize flavour enhancers (inosine monophosphate and guanosine monophosphate) and for treating heart diseases and other pathological conditions. It is also used as a starting material for producing antiviral nucleoside analogs such as ribavirine (anti-herpes) and zidovudine (AZT) used in the treatment of AIDS.

Ribose was previously produced by chemical synthesis, mainly through epimerisation of D-arabinose, derived from glucose. These production techniques have now been virtually completely abandoned in favour of a fermentative production method, mainly developed and performed in Japan by Takeda Chemical Industries.

Ribose is produced from glucose by submerse aerobic fermentation using special blocked and deregulated strains of *Bacillus* sp. Ribose concentrations of >90 g/l are obtained in a fermentation time of 72 hours (4). Ribose can then be isolated from the culture broth by ion exchange (salts removal) and subsequent precipitation in ethanol.

The (over)production of ribose by the microbial strains is mainly due to the transketolase negative genotype of the used mutants. The strains are blocked in their pentose phosphate pathway, thus leading to an accumulation of ribose. The so-called tkt^- mutants accumulate over 40 g/l ribose in the culture broth. Successive mutations that stimulate the activity of the enzyme glucose dehydrogenase have led to a further increase in ribose concentration up to 90 g/l.

2.2. Meso-erythritol

Ferruzzi has developed a fermentation process to produce meso-erythritol from glucose by an aerobic fermentation process, employing an osmophilic yeast strain, *Moniliella tomentosa* (5). The fermentation process is performed with glucose as a substrate in a high concentration of 450 g/l. Glucose is converted to a polyol mixture of erythritol, ribitol and glycerol from which the erythritol can be crystallized in high yield and purity. Erythritol is an interesting C_4 polyol, as it combines desirable properties such as toothfriendliness, good tolerance and moderate sweetness. The polyol is not taken up by the body and does not undergo fermentation in the colon; consequently it has a low caloric value (<0.4 kcal/g). The combination of these properties makes erythritol an interesting low caloric bulk sweetener. It may also be used for bulk applications such as for the production of polyurethanes and alkyd resins.

2.3. α, α-Trehalose

Ajinimoto Co. has recently started production of α, α-trehalose by fermentation with a *Corynebacterium* or *Brevibacterium* strain (6). The selected strain extracellularly excretes trehalose upon induction by osmotic pressure variation. The osmo-inducer can either be a salt or a sugaralcohol. The fermentation is performed aerobically and lasts 20-40 hours, after which the trehalose content is 50 g/l.

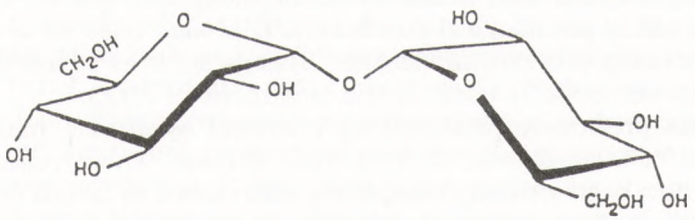


Fig. 1. Structure of α, α-trehalose.

Trehalose is a glucose α-1, α1-glucose non-reducing disaccharide, having special interesting properties (Fig. 1). The addition of trehalose to biological material largely increases its stability towards drying, heating and freezing processes. These desirable properties are thought to be due to the stable glass physical state that is formed by trehalose. Also there is evidence that the carbohydrate molecule replaces structural surface-bound water of proteins. Up to now trehalose is produced by extraction from yeasts, making this a very expensive carbohydrate. If the price of trehalose decreases interesting applications may be envisaged in foods and pharmaceuticals.

3. Carbohydrates formed by dehydrogenase enzymes

Hydrogenation or dehydrogenation of carbohydrates implies the conversion of a keto group into an alcohol group or vice versa. The enzymes performing this task are the dehydrogenases. They usually require a cofactor NAD(P)H transporting the hydrogens as reducing equivalents. Regio/enantiospecific (de)hydrogenations are notoriously difficult to perform by organic chemistry and require complex protection procedures. Enzymes however usually perform the task of picking out one keto or alcohol group and converting it to an alcohol or keto group with very high precision and yield. These enzymes have one main drawback, which is their cofactor dependance. The cofactor NAD(P)H is very expensive and difficult to retain in a reactor. A number of strategies have been developed to circumvent these problems.

The usual way to use a dehydrogenase lies in the continuous regeneration of the cofactor in a continuous reactor, usually a membrane reactor. The enzyme and the cofactor are then retained by the membrane, while the substrate and product can freely diffuse through. Two ways are posssible to retain the rather small cofactor in the reactor. One way is by increasing its molecular weight by coupling it covalently to a polymer e.g. polyethylene or dextran. However, this often results in serious inhibition of the enzyme. Another way is by using special negatively charged ultrafiltration membranes, repulsing the cofactor. The best retention coefficients are obtained when using NADP, as this is charged twice.

The regeneration of the cofactor can be performed by using for instance formate dehydrogenase, supplying reducing equivalents from the conversion of formate into carbon dioxide (Fig. 2) (10).

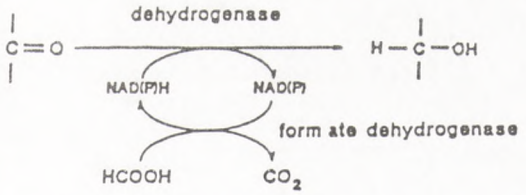


Fig. 2. Continuous regeneration of cofactor NAD(H) with formate as a hydrogen donor.

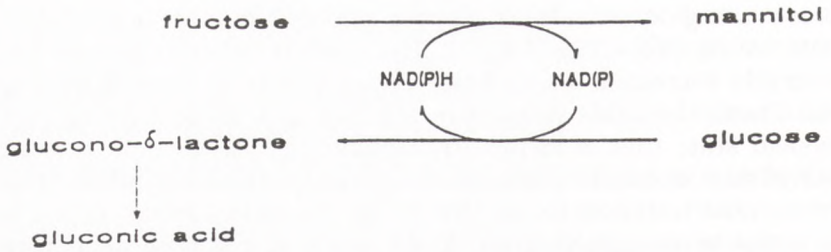


Fig. 3. Simultaneous synthesis of gluconate and mannitol from a glucose/fructose mixture.

Another way is by coupling a hydrogenation to a dehydrogenation with a common cofactor. For instance an equimolar mixture of glucose and fructose can be converted into respectively gluconic acid and mannitol (Fig. 3) (11).

These enzymatic conversions often suffer from serious drawbacks such as incomplete conversions, excessive loss of expensive cofactor, and slow conversion rates when enzymes with high K_m values are used. The cofactor is usually the main cost factor.

A very interesting alternative has recently been discovered (12,13). An enzyme complex from Zymomonas mobilis has the ability to convert an equimolar mixture of glucose and fructose into gluconic acid and sorbitol, without the need for an added cofactor. The enzyme complex is thought to consist of two enzymes, one converting glucose to gluconic acid, the other converting fructose to sorbitol. A tightly bound non-diffusable cofactor transfers the reducing equivalents. Thus all problems with cofactor production and retention are excellently circumvented by this approach. Although this is by now the only known example of such an enzyme complex, its high potential will hopefully lead to more of these discoveries.

The most commonly used industrial way to circumvent the cofactor problems is by using fermentation (Fig. 4). In this way the cell metabolism takes care of the synthesis of both the cofactor and the dehydrogenase. Moreover the cofactor is constantly regenerated by the general metabolism and is nicely retained in the cell by the cell membrane. Conversion efficiencies are usually

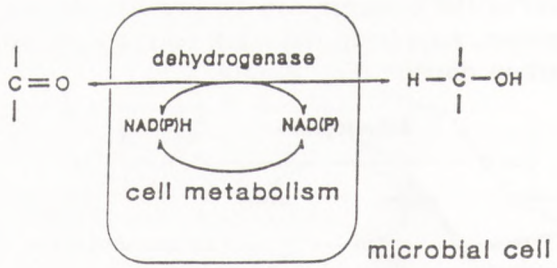


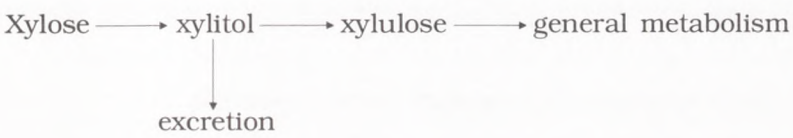
Fig. 4. Continuous regeneration of the cofactor NAD(H) by the general cell metabolism.

very high, fast and selective. Both hydrogenations and dehydrogenations can be performed using a wide variety of microorganisms. The most important examples will be given here.

3.1. Hydrogenations performed by viable cells

Xylitol is a carbohydrate that has been successfully introduced into the market. It has a high sweetening power and is non-cariogenic, which makes it very suitable for application in chewing gum. It is now primarily produced by chemical hydrogenation of xylose, obtained from natural sources such as acid hydrolysed corn cobs, nut shells, or wood hydrolysates such as sulphite waste liquor. The xylose from these sources must first undergo substantial purification before being hydrogenated with hydrogen gas under high temperature and pressure using a nickel catalyst.

There is increasing interest for producing xylitol from xylose by xylose utilizing yeasts. The pathway of xylose utilisation is as follows: first xylose is reduced to xylitol with the enzyme xylose reductase. Xylitol is consequently dehydrogenated to xylulose with the enzyme xylitol dehydrogenase. Xylulose is then further metabolized.



Thus, under suitable conditions xylitol is accumulated instead of being further metabolized. These conditions usually imply a controlled supply of oxygen to promote growth, but limiting further metabolism of xylitol. Yeast species such as *Pachysolen tannophilus*, *Pichia stipitis* and *Candida guilliermondii* are most cited as useful microorganisms. Xylitol conversion yields of 50-76% are obtained (14).

Recently a new approach was followed. The genetic code for xylose reductase enzyme from *Pichia stipitis* was cloned into a host, *S. cerevisiae*, a yeast not naturally utilizing xylose. The recombinant strain converts xylose to xylitol in a higher yield (95%) than the natural xylitol producers, but the conversion rate is markedly lower (15).

Mannitol is a common polyol derived from mannose and is extensively used. It is now produced by catalytic hydrogenation of fructose using a nickel catalyst and hydrogen gas. This hydrogenation yields mannitol, as well as its isomer sorbitol in about equal amounts, due to the poor selectivity of the nickel catalyst used. This leads to a less efficient production process as sorbitol can be produced cheaper by hydrogenation of glucose. Numerous process improvements to increase the ratio of mannitol/sorbitol formation have been suggested and patented (16). Mannitol is a common reserve prod-

uct of many fungi and yeasts and its production by fermentation has often been tempted, but the yields and productivities were too low to compete with the chemical hydrogenation process.

Recently, a new fermentation process capable of converting fructose quantitatively to mannitol has been developed (17). The process makes use of a heterofermentative lactic acid bacterium, *Leuconostoc mesenteroides*. This heterofermentative lactic acid bacterium is capable of using fructose as an alternative electron acceptor, thereby reducing it to mannitol, with the enzyme mannitol dehydrogenase. In the process the reducing equivalents are delivered by the conversion of glucose into D-lactic acid and acetic acid. Based on the hydrogen balance the following (theoretical) fermentation equation can be derived.

2 fructose + 1 glucose ⟶ 2 mannitol + D-lactic acid + acetic acid + CO_2

By careful control of process conditions, high conversion efficiencies over 90% and final mannitol concentrations of 150 g/l could be obtained in less than a day of fermentation. By using a special mutant strain, quantitative conversion and a further concentration increase up to 185 g/l mannitol, very close to the solubility limit could be obtained.

3.2. Dehydrogenations performed by viable cells

Regiospecific dehydrogenations of carbohydrates are a well established biotechnical modification. Some of these processes are performed industrially on a very large scale. These conversions excellently demonstrate how a biotechnical conversion performs the (chemically very difficult) task of dehydrogenating a specific hydroxyl group with high precision and yield. The hydrogenations are important as they provide a specific site in the molecule for chemical synthesis, e.g. reductive amination of the resulting keto group. Most of these conversions are performed by acetic acid bacteria, usually *Gluconobacter oxydans* is used (18). Recently another organism has been described, *Agrobacterium tumefaciens*, performing similar dehydrogenations on other substrates (19).

Gluconobacter oxydans is an acetic acid bacterium, showing strong ketogenesis towards carbohydrates. The dehydrogenations are very regiospecific and obey to an empirical rule, known as the Bertrand-Hudson rule. The secundary alcohol involved in the oxidation, and the cis-vicinal secondary alcohol group, must have a D-configuration with respect to the primary alcohol adjacent to the site of oxidation (Fig. 5).

Although a few exceptions to the rule exist, it nevertheless has a high validity in predicting the susceptibility of a substrate. Following this rule many substrates can be dehydrogenated, e.g. D-sorbitol to L-sorbose, per-

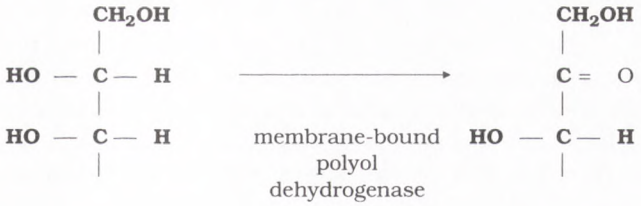


Fig. 5. Schematic representation of the substrate specificity for dehydrogenations according to the Bertrand-Hudson rule.

seitol to perseulose, erythritol to erythrulose, mannitol to fructose, fructose to 5-keto-fructose, glycerol to dihydroxyaceton, ribitol to ribulose, gluconic accid to 5-ketogluconic acid, to name just a few. The fermentations are generally referred to as ketogenic fermentations. Only a few of these conversions are performed on an industrial scale. Other conversions are performed on a lab-scale to synthesize a compound of interest for further study or use in research.

A number of procedures are put forward for converting a polyol into its keto-derivative (18). If the cells are capable of using the substrate that is to be dehydrogenated as a suitable carbon source it is possible to grow the organism with the substrate as the sole carbon source; only a small part of the substrate will be used for the bacterial metabolism. If the substrate is not suitable for maintaining growth the problem may be solved by adding a metabolizable carbon source to the medium. If the substrate is inhibitory to growth of the cells the conversion may be performed by first growing the cells on another carbon source, after which the conversion can be performed with washed viable cells.

The conversions are in general quantitative, and occur at high concentrations. The L-sorbose fed bath fermentation can go as high as 800 g/l L-sorbose, in which part of the L-sorbose spontaneously crystallises during fermentation (3). The enzymes responsible for the conversion are membrane-bound polyoldehydrogenases and directly transfer the hydrogens from the substrate over an electron transport to oxygen, the terminal electron acceptor in the conversion. Consequently, the fermentations are highly aerobic. Remarkably, little or no energy (ATP) is generated from this electron transport so that low biomass levels (< 5 g/l) are generally obtained.

The production of L-sorbose from sorbitol is by far the most important ketogenic fermentation process. Also dihydroxyacetone, 2-ketogluconate, 5-ketogluconate, 2,5-diketogluconate and glucono-δ-lacton are produced on an industrial scale by fermentation with *G. oxydans*. 2-Ketogluconate is used as a precursor for the synthesis of D-arabino-ascorbic acid (iso-vitamin C, erythorbic acid). It has a low vitamin activity but can be used in foods as an antioxidant. Dihydroxyaceton finds its main application as a sun-less

tanning agent, used in cosmetics. It reacts with the proteins in the upper layers of the skin, forming coloured (Maillard) reaction products. L-Sorbose is a key intermediate in the Reichstein-Grüssner synhesis of L-ascorbic acid (vitamin C), until now the only commercially applied synthetic route to L-ascorbic acid.

The Reichstein-Grüssner synthesis route is a combination of biosynthetic and chemical steps. Recently quite a number of alternative routes have been put forward, replacing nearly all chemical steps by biosynthetic steps (20). These alternatives offer a nice display of the efforts that are being put into goal-oriented development of carbohydrate modifications to synthesize an economically important carbohydrate derivative such as 2-keto-L-gulonic acid (2-KLG), the direct precursor of ascorbic acid. 2-KLG can be readily converted to L-ascorbic acid in a simple and efficient way. Several possible routes lie open to the production of 2-KLG. One alternative route staying close to the Reichstein-Grüssner route converts sorbose into 2-KLG by fermentation via the so-called sorbosone pathway. In this pathway sorbose is further dehydrogenated over sorbosone to 2-KLG. *Gluconobacter melanogenus* was found to be the best producer of 2-KLG from screening experiments (21). Another way for converting sorbose into 2-KLG passes over L-idose and L-idonic acid, as this pathway is found in *Pseudomonas* strains and others (22).

A completely different route starts from glucose, first converting it to 2,5-diketo-gluconic acid (2,5-DGA), which is subsequently hydrogenated at the 5-keto position to 2-KLG. This route has been applied successfully in high yield and concentration using a two-stage ("tandem") fermentation process (23). In this process glucose is first converted to 2,5-DGA in high yield and concentration (300 g/l) using *Erwinia* sp. In a next fermentation step 2,5-DGA is stereospecifically reduced to 2-KLG with *Corynebacterium* sp., using additional glucose to serve as a hydrogen donor in the process. The final fermentation broth of the two-stage process contained 106 g/l 2-KLG. The overall yield was 85% from the initial glucose.

An even further step was to combine the action of the two organisms by cloning the gene for 2,5-diketogluconic acid reductase from *Corynebacterium* into *Erwinia* sp. (24). The recombinant organism thus produced 2-KLG directly from glucose in a single fermentation step. The fermentation yields were improved by using mutants defective in the use of 2,5-DGA and 2-KLG as a sole carbon source for growth. The obtained yield was 49% in a final concentration of 19.8 g/l after 72 hours of fermentation. Further optimization may increase the yield and concentration of the product.

An enzymatic procedure to produce L-ascorbic acid starting from D-galacturonic acid has recently been proposed. Its consists of coupling an enzymatic hydrogenation to a dehydrogenation with a common cofactor in a membrane reactor. In the process D-galacturonic aid is converted to 2-keto-L-galactonic acid, which can be readily converted chemically to L-ascorbic acid. D-galacturonic acid can be easily obtained by hydrolysis of pectin, an abundant plant polysaccharide.

Although no alternative chemical or biosynthetic route has reached the economical importance of the Reichstein-Grüssner synthesis yet, current developments in the microbial biosynthesis of 2-keto-L-gulonic acid may provide an important base for future industrial production of L-ascorbic acid.

The synthesis of 3-ketoderivatives from various disaccharides by bioconversion with *Agrobacterium tumefaciens* has recently been optimized (25). A specific dehydrogenase from *A. tumefaciens* catalyzes the oxidation at C3 position of a variety of saccharides, preferentially disaccharides. For example sucrose is dehydrogenated to 3-ribo-hexopyranos-3-ulosyl-α(1-2)β-D-fructofuranoside (or 3-keto-sucrose). Biochemically the conversion is similar to those with *G. oxydans*, the dehydrogenase is coupled to an electron transport chain, with oxygen as the terminal electron acceptor. In the optimized process, the bioconversion is separated from the fermentation as the two processes have different optimal conditions. First the cells are cultivated in an aerobic fermentation. Then the cells are separated from the broth and added to the substrate. The conversion takes place in an aerobic environment, preferably at very high oxygen saturation. The obtained yield of 3-keto-sucrose is 60%. The process has potential in supplying a specific site for chemical synthesis into the polyol molecule which sucrose actually is. Also other disaccharides such as palatinose are oxidized, equally at the C3 position of the glucose moiety of the disaccharide.

4. Carbohydrates formed by oxidase enzymes

Carbohydrate oxidases are a group of enzymes with interesting biotechnological perspectives. The enzymes generally lead to dehydrogenation of the substrate carbohydrate, either converting a hydroxyl group in a keto group, the free aldehyde group of an aldose in a carboxyl group, or the terminal hydroxyl group into an aldehyde. Oxygen is the cosubstrate which is concomitantly reduced to hydrogen peroxide. The hydrogen peroxide is usually decomposed into water and oxygen by catalase action.

$$\text{substrate} + O_2 \xrightarrow{\text{oxidase}} \text{oxidized substrate} + H_2O_2$$

$$2\ H_2O_2 \xrightarrow{\text{catalase}} 2\ H_2O + O_2$$

The only commercially applied enzyme of this group is glucose oxidase. It finds extensive application in the biotechnological production of gluconic acid (26). Other oxidases have been discovered but still await application.

Many potential applications for oxidases exist but the enzyme either has not yet been discovered or is too expensive to produce. Once these bottle-necks are solved oxidases may become another valuable tool in carbohydrate technology. Goal-oriented screening methods for specific enzymes have recently been developed (27). A limited overview of some already used or known oxidases will be presented here.

Glucose oxidase is a common enzyme, usually produced by fermentation with *A. niger*. The enzyme catalyses the oxidation of glucose to glucono-δ-lactone, which subsequently hydrolyses rapidly to gluconic acid.

$$\text{glucose} + O_2 \longrightarrow \text{glucono-}\delta\text{-lactone} + H_2O_2$$

$$\text{glucono-}\delta\text{-lactone} \longrightarrow \text{gluconic acid}$$

The production of gluconic acid is commonly performed by fermentation with *A. niger*. About 45.000 tons per year are produced worldwide. Gluconic acid can also be produced by fermentation with *G. oxydans* (see above), but this approach is less utilized since side-products (2-ketogluconate and 5-ketogluconate) can be formed. The conversion is nearly quantitative, conversion yields of 97% are mentioned. Sodium gluconate is the most important product, finding use as a chelating agent in alkalic environments. Glucono-δ-lactone is produced to a lesser extent for use as an acidulant in food processing.

The enzyme glucose oxidase is used in purified form for diagnostic purposes, i.e. the selective determination of glucose in blood, foods,... The enzyme also has a potential for the selective removal of glucose in food stuffs such as e.g. eggs. Liquid egg treated with glucose oxidase can be heated and dried using conventional methods where glucose would otherwise give rise to Maillard reactions. Glucose can be selectively oxidized by a pyranose oxidase at its C2 position to 2-keto-glucose (or glucosone).

$$\text{glucose} + O_2 \longrightarrow \text{2-keto-glucose} + H_2O_2$$

The enzyme can be obtained from various sources. *Aspergillus oryzae* and white rot fungi such as *Polyporus obtusus* and *Peniophora gigantea* have been described as good extracellular producers of the enzyme (28,29). In white rot fungi the oxidases are thought to supply the lignolytic enzyme system with hydrogen peroxide. The enzyme from *P. gigantea* acts best on D-glucose as a substrate, but equally on L-sorbose and D-xylose. Also glucose derivatives such as methyl-ß-D-glucoside are oxidized.

High yields of glucosone are obtained with the enzyme from *Polyporus* sp. with low by-product formation. On the basis of this enzyme, various processes

have been developed and patented, although not yet applied. One process which has been suggested is the production of fructose from glucose (30). Glucose is first enzymatically dehydrogenated to glucosone, which can be subsequently chemically hydrogenated using a nickel catalyst and hydrogen gas to fructose. Thus glucose can be quantitatively converted to fructose.

$$\text{glucose} \xrightarrow{\text{oxidase}} \text{glucosone} \xrightarrow{H_2 / Ni} \text{fructose}$$

Another patented process aims at mannitol production in which the glucosone is hydrogenated to a mannitol/sorbitol mixture in a ratio of 3.2 (30).

Galactose oxidase is an enzyme produced by several fungi. It specifically oxidizes D-galactose at its C6 position to give rise to galacto-hexodialdose with concomittant generation of hydrogen peroxide. An extracellular galactose oxidase is produced by the fungus *Giberella fujikuroi* (31). The enzyme was found to be a true galactose oxidase, as it acts on galactose and not on glucose or glycerol. The enzyme was characterized and found to be a copper requiring metalloprotein.

5. Carbohydrates formed by transferases

Transferases are a class of enzymes that have recently been extensively explored. The enzymes break up a glycosidic bond, and transfer the released monosaccharide unit from the donor molecule to an acceptor molecule, thereby forming another specific glycosidic bond. The acceptor molecule is usually another carbohydrate, but can be as simple as a phosphate group or as complex as UTP. Various di- and oligosaccharides can thus be conveniently synthesized. A few examples are given below.

5.1. Leucrose

Leucrose is a keto-disaccharide that consists like sucrose of a glucose and fructose moiety which are differently bound (5-O-(α-D-glucopyranosyl)-D-fructopyranose). Leucrose is formed from sucrose by the action of dextransucrase, a glucosyltransferase that normally catalyses the formation of dextran from sucrose. In the normal reaction glucose is transferred by the enzyme from sucrose to the non-reducing end of the growing dextran chain.

$$\begin{aligned} &\text{Glu-Fru} + \text{Glu} \longrightarrow \text{Glu-Glu} + \text{Fru} \\ &\text{Glu-Fru} + \text{Glu-Glu} \longrightarrow \text{Glu-Glu-Glu} + \text{Fru} \\ n\ &\text{Glu-Fru} + \text{Glu-Glu} \longrightarrow \text{Glu-(Glu)}_n\text{-Glu} + n\ \text{Fru} \end{aligned}$$

sucrose → dextran

In the presence of high concentrations of fructose however, fructose acts as the acceptor and thus a new glucose-fructose disaccharide is formed, its bond being different from that of the starting sucrose molecule.

$$\begin{array}{lcl} \text{Glu-Fru}_1 + \text{Fru}_0 & \longrightarrow & \text{Glu-Fru}_0 + \text{Fru}_1 \\ \text{Glu-Fru}_2 + \text{Fru}_1 & \longrightarrow & \text{Glu-Fru}_1 + \text{Fru}_2 \\ \text{sucrose} & & \text{leucrose} \end{array}$$

In the process no net fructose is converted. In essence sucrose is converted to leucrose. Pfeifer and Langen (Germany) has developed a process for industrial production of leucrose from saccharose (32). The enzymatic reaction is performed in a concentrated solution of 65% consisting of 1/3 sucrose and 2/3 fructose at 25°C. The enzyme is obtained by fermentation with a *Leuconostoc mesenteroides* strain, by which the enzyme is extracellularly excreted. The conversion efficiency is about 90%. After the conversion is finished the leucrose is separated from fructose by chromatography. Leucrose is then obtained in pure form by crystallisation. Leucrose displays desirable characteristics as a bulk sweetener. It is essentially non-cariogenic and is resorbed easily without any incompatibility problems, as it is broken down to its glucose and fructose units as for sucrose. It has a sweetness of about 50% compared to sucrose. It is a reducing sugar with a high stability towards acid of the glucose-fructose bond, contrary to the acid-labile sucrose.

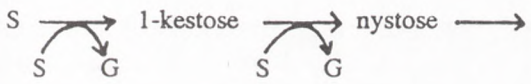
5.2. Isomaltulose

Isomaltulose (palatinose) consists as sucrose of a glucose and fructose moiety which are differently bound (6-O-(α-D-glucopyranosyl)-D-fructopyranose). It is produced from sucrose by an enzymatic conversion performed by sucrose mutase, found in a number of microorganisms such as *Protaminobacter rubrum*, *Serratia* sp. and *Erwinia* sp. The enzyme is found in the periplasm of the bacteria. Südzucker (Germany) has developed an industrial process for the production of this disaccharide (33). In the production process, cells of *Protaminobacter rubrum* are first cultivated by fermentation, after which they are immobilized as whole cells in alginate gel. The substrate consists of a pure sucrose solution which is pumped through a column with the immobilised cells. Careful sterility measures have to be taken to ensure that the immobilized cells in the column do not become infected. The obtained conversion efficiency is about 80%. The main side product found is trehalulose (about 10% of the initial amount of sucrose). Also glucose, fructose, isomaltose and oligosaccharides are found in the mixture in smaller amounts. The columns are run so that the sucrose content is still 1-2% of the initial content to minimize by-product formation. The converted sucrose solution is then evaporated and the isomaltulose can be obtained by crystallisation. Isomaltulose displays desirable characteristics as a bulk sweetener. It is non-cariogenic,

has a sweetness of 42% compared to sucrose and has about the same energetic value as sucrose. It is considerably less soluble than sucrose and upon intake of doses higher than 50 g per day gastrinal upset caused by fermentation in the colon may occur. As isomaltulose is a reducing sugar it undergoes browning reactions in foods. To overcome this, isomaltulose (palatinose) can be hydrogenated with a nickel catalyst to palatinit, an equimolecular mixture of 1-O-(α-D-glucopyranosyl)-D-mannitol and 1-O-(α-D-glucopyranosyl)-D-sorbitol. Palatinit equally is applied as a bulk sweetener with similar properties as isomaltulose.

5.3. Levans and neosugar

Microbial levans consist of ß-2,6 polyfructan molecules. They are formed from sucrose by a fructosyltransferase produced by a number of organisms such as *Bacillus* sp., *Streptococcus* sp., *Aureobasidium* sp. and *Aspergillus* sp. The enzyme is produced by fermentation during which the enzyme is secreted extracellularly. Thus high and low molecular levans can be produced by fermentation starting from a fermentation medium containing sucrose, beet juice, cane or beet molasses. Yields are 20-60%. The high molecular weight levan may find application in foods as a thickener. With β-fructosyltransferases from *A. niger* a mixture of oligo-levans is formed from sucrose (34).



The resulting fructo-oligosaccharide mixture, so-called neosugar can be added to foods to promote Bifidus growth in the colon.

5.4. Glucose-1-phosphate

Sucrose phosphorylase is an enzyme obtained from a number of microorganisms such as *Pseudomonas saccharophila* and *Leuconostoc mesenteroides* (35). It is a glucosyltransferase transferring glucose from sucrose to a number of acceptor molecules, phosphate being the most effective acceptor, forming α-glucose-1-phosphate.

$$\text{sucrose} + P_i \longrightarrow \alpha\text{-glucose-1-phosphate} + \text{fructose}$$

The reaction is reversible so that an enzymatic way to produce sucrose form starch can be based on it (36). It uses starch phosphorylase to produce α-glucose-1-phosphate from starch, which is subsequently coupled to fructose (derived from starch) with sucrose phosphorylase. The approach however

is too complex for practical use. Moreover, the equilibrium favours phosphorolysis of sucrose instead of synthesis. Fructose and other similar carbohydrates such as L-sorbose and xylitol are also good acceptors (37). The enzyme for instance catalyses the following reaction:

$$\alpha\text{-G-1-P} + \text{D-xylose} \longrightarrow \text{4-O-}\alpha\text{-D-glucopyranosyl-xylitol} + P_i$$

In this way such complex disaccharides can be conveniently synthesized. The enzyme has an optimum pH of 7.3 and has a good stability. It is currently industrially applied in immobilized form to produce α-glucose-1-phosphate from sucrose. The process has turned a previously very expensive product as α-glucose-1-phosphate into a commodity product. It can be used as a phosphate donor in enzymatic syntheses. It may also find use as a source of glucose, protected at the glycosidic carbon atom.

6. Conclusion

The biotechnology to produce and modify mono/disaccharides is already largely developed. However, if one has a closer look at the class of enzymes that are mainly used, it is clear that the hydrolases are the most important, followed by the transferases that recently have been extensively explored. However, all enzymes that are cofactor dependent are applied to a limited extent. The difficulty arising with the involved cofactor is no doubt the main reason for this. However, the surprising new enzyme activities that have been discovered recently show that the potential to find completely new ways of modifying carbohydrates is by no means exhausted. Screening for new enzymes should therefore attract more attention to further enrich the toolbox for carbohydrate modifications.

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Biotechnical modification of agro-bulk carbohydrates into specialty mono-, oligo- or polysaccharides

Summary

An overview is given of various biotechnical carbohydrate modifications. Fermentations involving complex fermention patways for the production of specialty carbohydrates are mentioned. Bioconversions involving a single enzyme are discusssed. Enzymatic conversions involving dehydrogenases, oxidases and tranferases are discussed in detail.

Key words:
carbohydrate, fermentation, bioconversion.

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