4 Carbohydrates

4.1 Foreword

Carbohydrates are the most widely distributed and abundant organic compounds on earth. They have a central role in the metabolism of animals and plants. Carbohydrate biosynthesis in plants starting from carbon dioxide and water with the help of light energy, i. e., photosynthesis, is the basis for the existence of all other organisms which depend on the intake of organic substances with food.

Carbohydrates represent one of the basic nutrients and are quantitatively the most important source of energy. Their nutritional energy value amounts to 17 kJ/g or kcal/g. Even the nondigestible carbohydrates, acting as bulk material, are of importance in a balanced daily nutrition. Other important functions in food are fulfilled by carbohydrates. They act for instance as sweetening, gel- or paste-forming and thickening agents, stabilizers and are also precursors for aroma and coloring substances, especially in thermal processing.

The term carbohydrates goes back to times when it was thought that all compounds of this class were hydrates of carbon, on the basis of their empirical formula, e. g. glucose, $C_6H_{12}O_6$ ($6C+6H_2O$). Later, many compounds were identified which deviated from this general formula, but retained common reactions and, hence, were also classed as carbohydrates. These are exemplified by deoxysugars, amino sugars and sugar carboxylic acids. Carbohydrates are commonly divided into monosaccharides, oligosaccharides and polysaccharides. Monosaccharides are polyhydroxy-aldehydes or -ketones, generally with an unbranched C-chain. Well known representatives are glucose, fructose and galactose. Oligosaccharides are carbohydrates which are obtained from <10 carbohydrate units, which formally polymerize from monosaccharides with the elimination of water to give full acetals, e. g. by the reaction:

$$n\ C_6H_{12}O_6 \xrightarrow{-(n-1)\ H_2O} C_{6n}H_{10n+2}O_{5n+1} \qquad (4.1)$$

Well known representatives are the disaccharides saccharose (sucrose), maltose and lactose, and the trisaccharide raffinose, and the tetrasaccharide stachyose.

In polysaccharides, consisting of n monosaccharides, the number n is as a rule >10. Hence, the properties of these high molecular weight polymers differ greatly from other carbohydrates. Thus, polysaccharides are often considerably less soluble in water than mono- and oligosaccharides. They do not have a sweet taste and are essentially inert. Well known representatives are starch, cellulose and pectin.

4.2 Monosaccharides

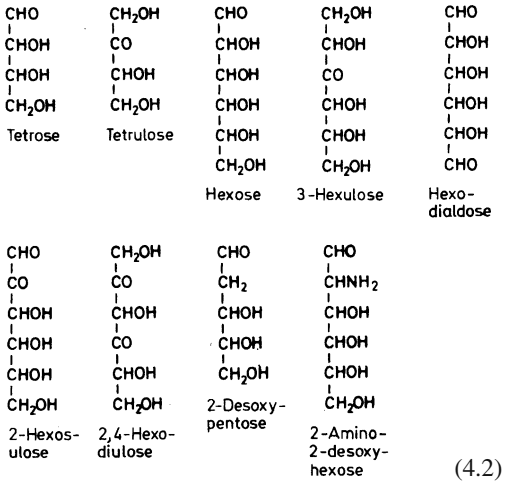
4.2.1 Structure and Nomenclature

4.2.1.1 Nomenclature

Monosaccharides are polyhydroxy-aldehydes (aldoses), formally considered to be derived from glyceraldehyde, or polyhydroxyketones (ketoses), derived from dihydroxyacetone by inserting CHOH units into the carbon chains. The resultant compounds in the series of aldoses are denoted by the total number of carbons as trioses, for the starting glyceraldehyde, and tetroses, pentoses, hexoses, etc. The ketose series begins with the simplest ketose, dihydroxyacetone, a triulose, followed by tetruloses, pentuloses, hexuloses, etc. The position of the keto group is designated by a numerical prefix, e. g. 2-pentulose, 3-hexulose.

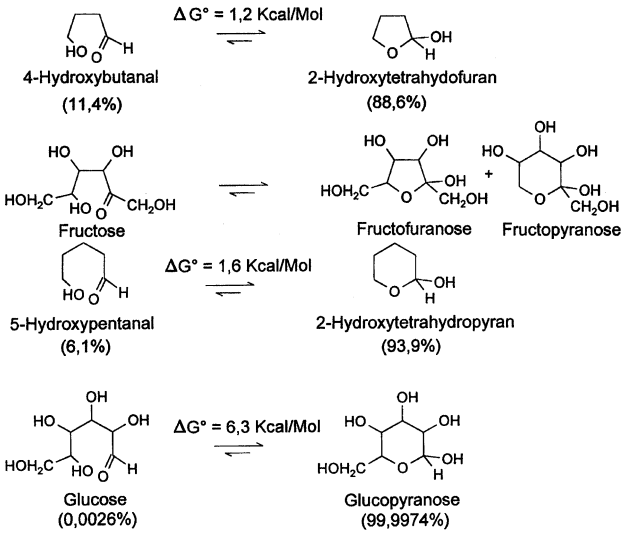
When a monosaccharide carries a second carbonyl group, it is denoted as a -dialdose (2 aldehyde groups), -osulose (aldehyde and keto groups) or -diulose (2 keto groups). Substitution of an HO-group by an H-atom gives rise to a deoxy sugar, and by an H_2N-group to aminodeoxy compounds (cf. Formula 4.2). Analogous to 4- or 5-hydroxypentanal, aldoses (starting from

tetroses) and ketoses (starting from 2-pentuloses) undergo intramolecular cyclization with hemiacetal formation to form lactols (Formula 4.3). With the exception of erythrose, monosaccharides are crystallized in these cyclic forms and, even in solution, there is an equilibrium between the open chain carbonyl form and cyclic hemiacetals, with the latter predominating. The tendency to cyclize is, compared to hydroxyaldehydes, even more pronounced in monosaccharides, as shown by ΔG°-values and equilibrium concentrations in 75% aqueous ethanol (cf. Formula 4.3).



(4.2)

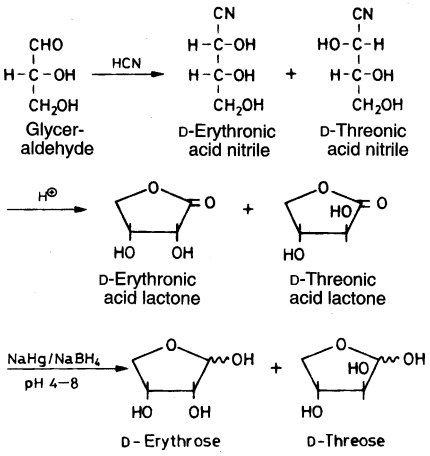
Lactols can be considered as tetrahydrofuran or tetrahydropyran derivatives, hence, they are also denoted as furanoses or pyranoses.



(4.3)

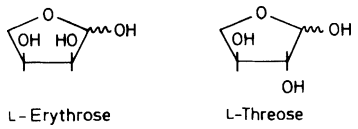
4.2.1.2 Configuration

Glyceraldehyde, a triose, has one chiral center, so it exists as an enantiomer pair, i.e. in D- and L-forms. According to the definition, the secondary hydroxyl group is on the right in D-glyceraldehyde and on the left in L-glyceraldehyde. Although this assignment was at first arbitrarily made, it later proved to be correct. It is possible by cyanhydrin synthesis to obtain from each enantiomer a pair of diastereomeric tetroses (*Kiliani–Fischer synthesis*):



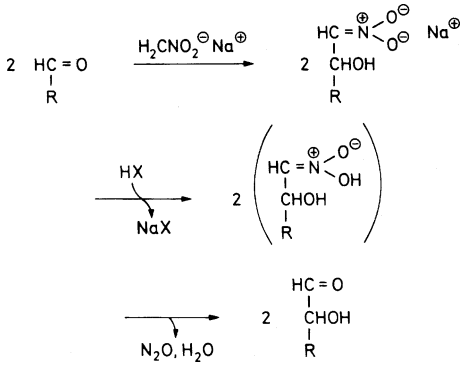
(4.4)

Correspondingly, L-erythrose and L-threose are obtained from L-glyceraldehyde:



(4.5)

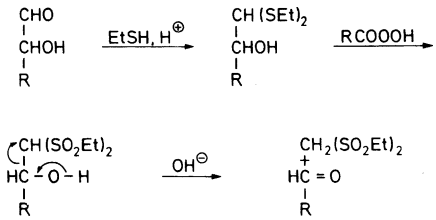
The nitriles can also be directly reduced to diastereomeric aldoses with $PdO/BaSO_4$, by passing the lactone intermediate stage. Another reaction for the formation of monosaccharides is the nitroalkane synthesis. The epimeric nitro compounds, obtained by the reaction of an aldose with nitromethane as anions, are separated and converted to the corresponding aldoses by an acinitroalkane cleavage (*Nef*-reaction):



(4.6)

After repeated cyanhydrin reactions, four tetroses will provide a total of eight pentoses (each tetrose provides a pair of new diastereomers with one more chiral center), which can then yield sixteen stereoisomeric hexoses. The compounds derived from D-glyceraldehyde are designated as D-aldoses and those from L-glyceraldehyde as L-aldoses.

An important degradation reaction of aldoses proceeds via the disulfone of the dithioacetal:



(4.7)

Figure 4.1 shows the formulas and names for D-aldoses using simplified *Fischer* projections. The occurrence of aldoses of importance in food is compiled in Table 4.1. Epimers are monosaccharides which differ in configuration at only one chiral C-atom. D-Glucose and D-mannose are 2-epimers. D-glucose and D-galactose are 4-epimers.

The enantiomers D- and L-tetrulose, by formally inserting additional CHOH-groups between the keto and existing CHOH-groups, form a series of D- and L-2-ketoses. Figure 4.2 gives D-2-ketoses in their simplified *Fischer* projections.

Data are provided in Table 4.2 on the occurrence of ketoses of interest in food.

For the simplified presentation of structures, abbreviations are used which usually consist of the first letters of the name of the monosaccharide. Figure 4.1 gives the configuration prefix derived from the trivial names, representing a specified configuration applied in monosaccharide classification. Thus, systematic names for D-glucose and D-fructose are D-gluco-hexose

Table 4.1. Occurrence of aldoses

Name, structure	Occurrence
Pentoses	
D-Apiose (3-C-Hydroxymethyl-D-glycerotetrose)	Parsley, celery seed
L-Arabinose	Plant gums, hemicelluloses, pectins, glycosides
2-Deoxy-D-ribose	Deoxyribonucleic acid
D-Lyxose	Yeast-nucleic acid
2-O-Methyl-D-xylose	Hemicelluloses
D-Ribose	Ribonucleic acid
D-Xylose	Xylanes, hemicelluloses plant gums, glycosides
Hexoses	
L-Fucose (6-Deoxy-L-galactose)	Human milk, seaweed (algae), plant gums and mucilage
D-Galactose	Widespread in oligo- and polysaccharides
D-Glucose	Widespread in plants and animals
D-Mannose	Widespread as polysaccharide building blocks
L-Rhamnose (6-Deoxy-L-mannose)	Plant gums and mucilage, glycosides

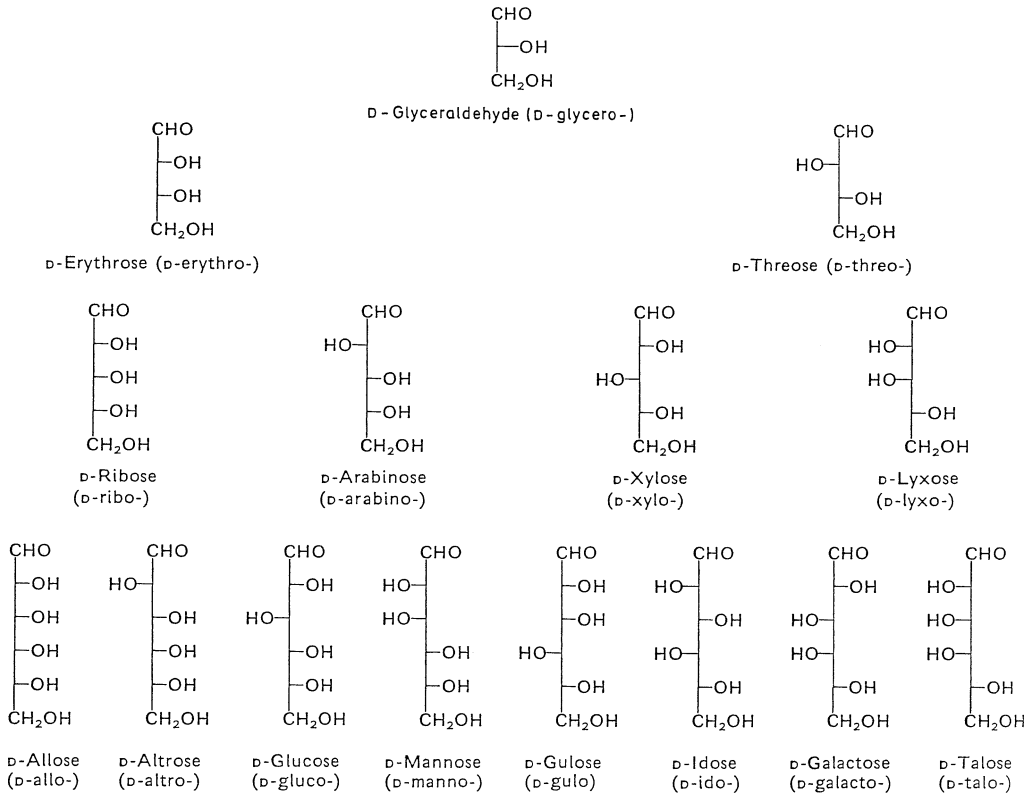


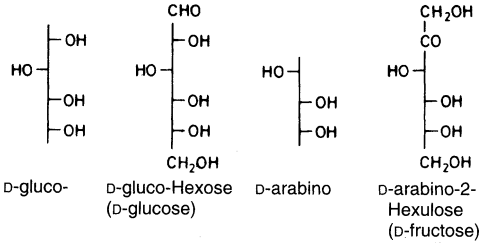
Fig. 4.1. D-Aldoses in *Fischer* projection

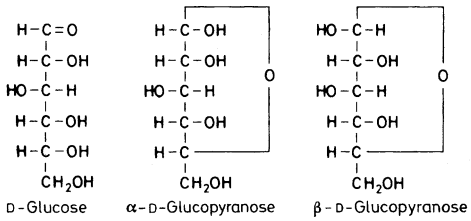
and D-arabino-2-hexulose. Such nomenclature makes it possible to systematically denote all monosaccharides that contain more than four chiral centers. According to this procedure the portion of the molecule adjacent to the carbonyl group is given the maximal possible prefix, while the portion furthest from the carbonyl group is denoted first. In the case of ketoses, the two portions of the molecule separated by the keto group are given. In a combined prefix designation, as with aldoses, the portion which has the C-atom furthest from the keto group is mentioned first. However, when a monosaccharide does not have more than four chiral centers, a designation in the ketose series may omit the two units separated by the keto group. The examples in Formula 4.8 illustrate the rule.

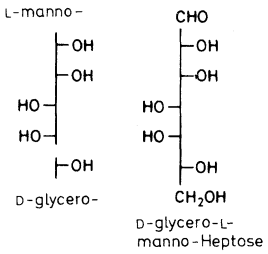
Lactol formation provides a new chiral center. Thus, there are two additional diastereomers for each pyranose and furanose. These isomers are called anomers and are denoted as α and β-forms. Formula 4.9 illustrates the two anomeric D-glucose molecules in *Tollens* ring formulas and *Haworth* projections.

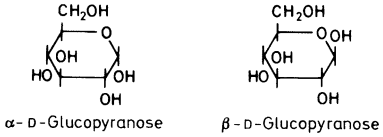
Table 4.2. Occurrence of ketoses

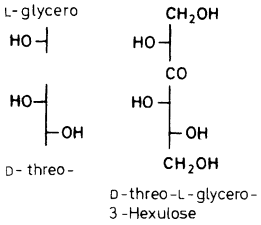
Name, structure	Occurrence
Hexulose	
D-Fructose	Present in plants and honey
D-Psicose	Found in residue of fermented molasses
Heptulose	
D-manno-2-Heptulose	Avocado fruit
Octulose	
D-glycero-D-manno-2-Octulose	Avocado fruit
Nonulose	
D-erythro-L-gluco-2-Nonulose	Avocado fruit

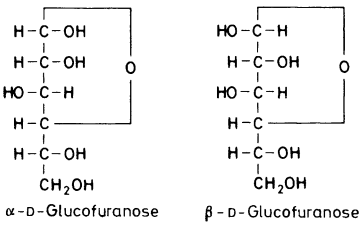




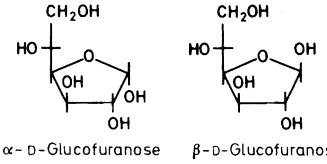




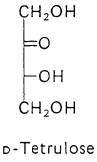




(4.8)



(4.9)



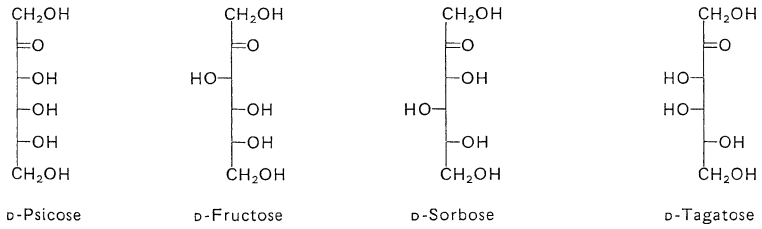
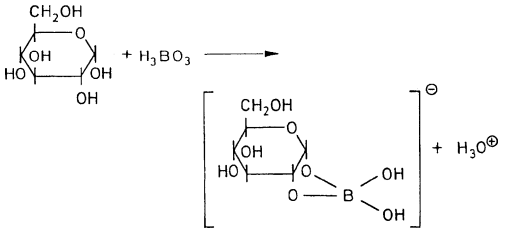


Fig. 4.2. D-Ketoses in *Fischer* projection

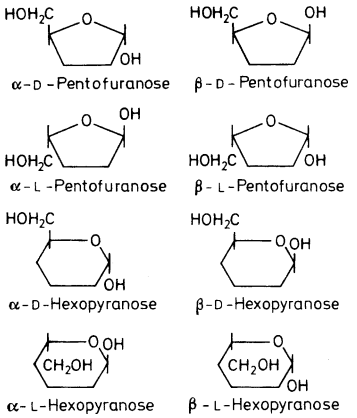
The cis-arrangement of the two adjacent HO-groups in positions C-1 and C-2 of α-D-glucopyranose, unlike its β-anomer, increases the conductivity of boric acid. A borate complex is formed which is a stronger acid than boric acid itself (cf. Formula 4.10).

In *Tollens* ring formula, in the D-series, the α-anomer has the hydroxyl group at C-1 on the right and the β-anomer has this OH-group on the left.

In *Haworth* projections the HO-group of α/β-anomers of the D-series usually occurs below/above the pyranose or furanose ring planes, while in the L-series the reverse is true (cf. Formula 4.11).



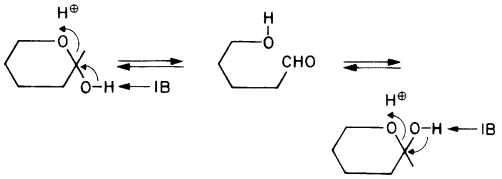
(4.10)



(4.11)

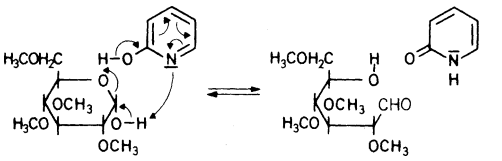
Each monosaccharide can exist in solution together with its open chain molecule in a total of five forms. Due to the strong tendency towards cyclization, the amount of the open chain form is greatly reduced. The contribution of the different cyclic forms to the equilibrium state in a solution depends on the conformation. An aqueous D-glucose solution is nearly exclusively the two pyranoses, with 36% α- and 64% β-anomer, while the furanose ring form is less than 1%. The equilibrium state varies greatly among sugars (cf. Table 4.6).

The transition into the different hemiacetal forms, called mutarotation, proceeds via the open-chain carbonyl compound. The acid- and base-catalyzed ring opening is the rate limiting step of the reaction:



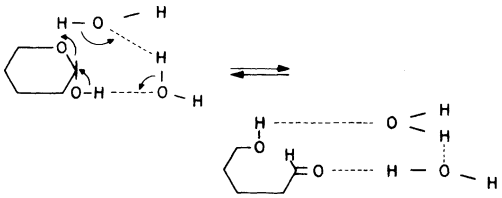
(4.12)

2,3,4,6-Tetramethyl-D-glucose reaches equilibrium in benzene rapidly through the concerted action of cresol and pyridine as acid–base catalysts (Table 4.3). Bifunctional reagents, like 2-pyridone and benzoic acid, are especially efficient acid–base catalysts in both polar and nonpolar solvents:



(4.13)

Water can also be a bifunctional catalyst:



(4.14)

Table 4.3. Mutarotation rate of 2,3,4,6-tetramethyl-D-glucose (0.09 mol/l) in benzene

Catalyst	($k\,min^{-1}$)	k_{rel}
–	7.8×10^{-5}	1.0
Pyridine (0.1 mol/l)	3.7×10^{-4}	4.7
p-Cresol (0.1 mol/l)	4.2×10^{-4}	5.4
Pyridine + p-cresol (0.1 mol/l)	7.9×10^{-3}	101
2-Pyridone (0.1 mol/l)	1.8×10^{-1}	2307
Benzoic acid (0.1 mol/l)	2.2	28,205

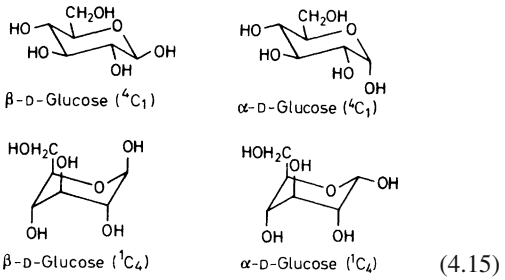
The reaction rate for the conversion of the α-and β-forms has a wide minimum in an aqueous medium in a pH range of 2–7, as illustrated in section 10.1.2.2 with lactose, and the rate increases rapidly beyond this pH range.

4.2.1.3 Conformation

A series of physicochemical properties of monosaccharides can be explained only by the conformation formulas (*Reeves* formulas).

The preferred conformation for a pyranose is the so-called chair conformation and not the twisted-boat conformation, since the former has the highest thermodynamic stability. The two chair C-conformations are $^4C_1$ (the superscript corresponds to the number of the C-atom in the upper position of the chair and the subscript to that in the lower position; often designated as C1 or "O-outside") and $^1C_4$ (often designated as 1C, the mirror image of C1, and C-1 in upper and C-4 in lower positions, or simply the "O-inside" conformer). The $^4C_1$-conformation is preferred in the series of D-pyranoses, with most of the bulky groups, e. g., HO and, especially, CH_2OH, occupying the roomy equatorial positions. The interaction of the bulky groups is low in such a conformation, hence the conformational stability is high. This differs from the C_4-conformation, in which most of the bulky groups are crowded into axial positions, thus imparting a thermodynamic instability to the molecule (Table 4.4).

β-D-Glucopyranose in the $^4C_1$-conformation is an exception. All substituents are arranged equatorially, while in the $^1C_4$ all are axial (Formula 4.15). α-D-Glucopyranose in the $^4C_1$-conformation has one axial group at C-1 and is also lower in energy by far (cf. Table 4.5).



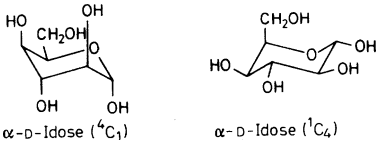
(4.15)

Table 4.4. Free energies of unfavorable interactions between substituents on the tetrahydropyran ring

Interaction	Energy kJ/mole [a]
$H_{ax} - O_{ax}$	1.88
$H_{ax} - C_{ax}$	3.76
$O_{ax} - O_{ax}$	6.27
$O_{ax} - C_{ax}$	10.45
$O_{eq} - O_{eq}/O_{ax} - O_{eq}$	1.46
$O_{eq} - C_{eq}/O_{ax} - C_{eq}$	1.88
Anomeric effect[b]	
for O^{c2}_{eq}	2.30
for O^{c2}_{ax}	4.18

[a] Aqueous solution, room temperature.
[b] To be considered only for an equatorial position of the anomeric HO-group.

The arrangement of substituents differs e. g., in α-D-idopyranose. Here, all the substituents are in axial positions in the $^4C_1$-conformation (axial HO-groups at 1, 2, 3, 4), except for the CH_2OH-group, which is equatorial. However, the $^1C_4$-conformation is thermodynamically more favorable despite the fact that the CH_2OH-group is axial (cf. Table 4.5):



(4.16)

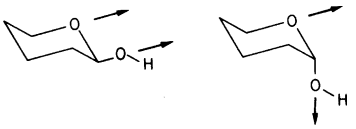
A second exception (or rather an extreme case) is α-D-altropyranose. Both conformations (O-outside and O-inside) have practically the same stability in this sugar (cf. Table 4.5).

The free energy of the conformers in the pyranose series can be calculated from partial interaction energies (derived from empirical data). Only the 1,3-diaxial interactions (with exception of the interactions between H-atoms), 1,2-gauche or staggered (60°) interactions of two HO-groups and that between HO-groups and the CH_2OH-group will be considered. The partial interaction energies are compiled in Table 4.4, the relative free enthalpies ΔG° calculated from these data for various conformers are presented in Table 4.5. In addition to the interaction energies an effect is considered

Table 4.5. Relative free enthalpies for hexopyranoses

Hexopyranose	Conformation	ΔG° (kJ/mole)
α-D-Glucose	$^4C_1$	10.03
	$^1C_4$	27.38
β-D-Glucose	$^4C_1$	8.57
	$^1C_4$	33.44
α-D-Mannose	$^4C_1$	10.45
β-D-Mannose	$^4C_1$	12.33
α-D-Galactose	$^4C_1$	11.91
β-D-Galactose	$^4C_1$	10.45
α-D-Idose	$^4C_1$	18.18
	$^1C_4$	16.09
β-D-Idose	$^4C_1$	16.93
	$^1C_4$	22.36
α-D-Altrose	$^4C_1$	15.26
	$^1C_4$	16.09

which destabilizes the anomeric HO-group in the equatorial position, while it stabilizes this group in the axial position. This is called the *anomeric effect* and is attributed to the repulsion between the parallel dipoles. If the 1-OH group (β-anomer) is in the equatorial position (cf. Formula 4.17), repulsion results from the polarized bonds carbon atom 5 – ring oxygen and carbon atom 1 – oxygen of the anomeric OH-group. The repulsion forces the anomeric HO-group to take up the more stable axial or α-position:



(4.17)

The other substituents also influence the anomeric effect, particularly the HO-group in C-2 position. Here, due to an antiparallel dipole formation, the axial position enhances stabilization better than the equatorial position. Correspondingly, in an equilibrium state in water, D-mannose is 67% in its α-form, while α-D-glucose or α-D-galactose are only 36% and 32%, respectively (Table 4.6). The anomeric effect (dipole–dipole interaction) increases as the dielectric constant of the solvent system decreases e. g., when water is replaced by methanol.

Alkylation of the lactol HO-group also enhances the anomeric effect (Table 4.7). A reduction of the dielectric constant of the solvent (e. g., transition from water to methanol), resulting in an increase in the dipole–dipole interaction, also enhances the anomeric effect. Conformational isomers of furanose also occur since its ring is not planar. There are two basic conformations, the envelope (E) and the twist (T), which are the most stable; in solu-

Table 4.7. Methylglucoside isomers in methanol (1% HCl) at equilibrium state[a]

Compound	α-Pyranoside	β-Pyranoside	α-Furanoside	β-Furanoside
Methyl-D-glucoside	66	32.5	0.6	0.9
Methyl-D-mannoside	94	5.3	0.7	0
Methyl-D-galactoside	58	20	6	16
Methyl-D-xyloside	65	30	2	3

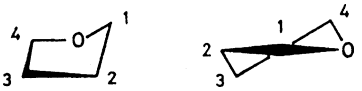
[a] Values in %.

Table 4.6. Equilibrium composition[a] of aldoses and ketoses in aqueous solution

Compound	T (°C)	α-Pyranose	β-Pyranose	α-Furanose	β-Furanose
D-Glucose	20	36	64	–	–
D-Mannose	20	67	33	–	–
D-Galactose	20	32	64	1	3
D-Idose	60	31	37	16	16
D-Ribose	40	20	56	6	18
D-Xylose	20	35	65	–	–
D-Fructose	20	–	76	4	20

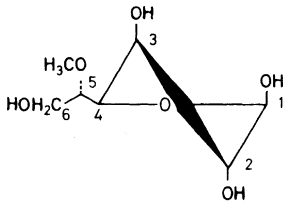
[a] Values in %.

tion a mixture exists of conformers similar in energy (cf. Formula 4.18).



^1E $^4T_3$ (4.18)

An anomeric effect preferentially forces the anomeric HO-group into the axial position. This is especially the case when the HO-group attached to C-2 is also axial. When pyranose ring formation is prevented or blocked, as in 5-O-methyl-D-glucose, the twisted $^3T_2$-conformer becomes the dominant form:



5-O-Methyl-β-D-glucose ($^3T_2$) (4.19)

A pyranose is generally more stable than a furanose, hence, the former and not the latter conformation is predominant in most monosaccharides (Table 4.6).

The composition of isomers in aqueous solution, after equilibrium is reached, is compiled for a number of monosaccharides in Table 4.6. Evidence for such compositions is obtained by polarimetry, by oxidation with bromine, which occurs at a much higher reaction rate with β- than α-pyranose and, above all, by nuclear magnetic resonance spectroscopy (^{1}H-NMR).

In proton magnetic resonance spectroscopy of sugars, the protons bound to oxygen, which complicate the spectrum, are replaced by derivatization (O-acyl derivatives) or are exchanged for deuterium when the sugar is in D_2O solution. The chemical shift of the retained protons bound covalently to carbon varies. Due to less shielding by the two oxygens in α position, the proton on the anomeric carbon atom appears at a lower magnetic field than other protons, the chemical shift increasing in the order pyranoses $<$ furanoses in the range of $\delta = 4.5-5.5$ (free monosaccharides). As a result of the coupling with the H-atom at C-2, the anomeric proton appears as a doublet. Furthermore, an axial proton (β-form of D-series) appears at higher field than an equatorial proton (α-form of D-series). The sugar conformation is elucidated from the coupling constant of neighboring protons: equatorial–equatorial, equatorial–axial (small coupling constants) or axial-axial (larger coupling constants).

The proton resonance spectrum of D-glucose ($^1C_4$-conformation) in D_2O is shown in Fig. 4.3. The figure first shows the signals of the protons at C-2 to C-6 in the range of 3.2–3.9 ppm. The large coupling constant of the doublet at δ 4.62 (7.96 Hz) shows a diaxial position of the H-atoms at C-1/C-2 and, thus, the equatorial position of the hydroxy group at C-1. This indicates the β-D-glucopyranose conformation. The equatorial proton in α-D-glucopyranose (5.2 ppm) appears at lower field (higher ppm). The smaller coupling constant of the doublet at δ 5.2 ($J = 3.53$ Hz) confirms the axial/equatorial arrangement of the H-atoms at C-1/C-2 of α-D-glucopyranose.

The content of both anomers in aqueous solution can be calculated from the signal areas. α- and β-Glucofuranoses are not present in aqueous solution (Table 4.6).

Elucidation of sugar conformation can also be achieved by ^{13}C-nuclear magnetic resonance spectroscopy. Like ^{1}H-NMR, the chemical shifts differ for different C-atoms and are affected by the spatial arrangement of ring substituents.

4.2.2 Physical Properties

4.2.2.1 Hygroscopicity and Solubility

The moisture uptake of sugars in crystallized form is variable and depends, for example, on the sugar structure, isomers present and sugar purity. Solubility decreases as the sugars cake together, as often happens in sugar powders or granulates. On the other hand, the retention of food moisture by concentrated sugar solutions, e. g., glucose syrup, is utilized in the baking industry.

The solubility of mono- and oligosaccharides in water is good. However, anomers may differ substantially in their solubility, as exemplified by α- and β- lactose (cf. 10.1.2.2). Monosaccharides

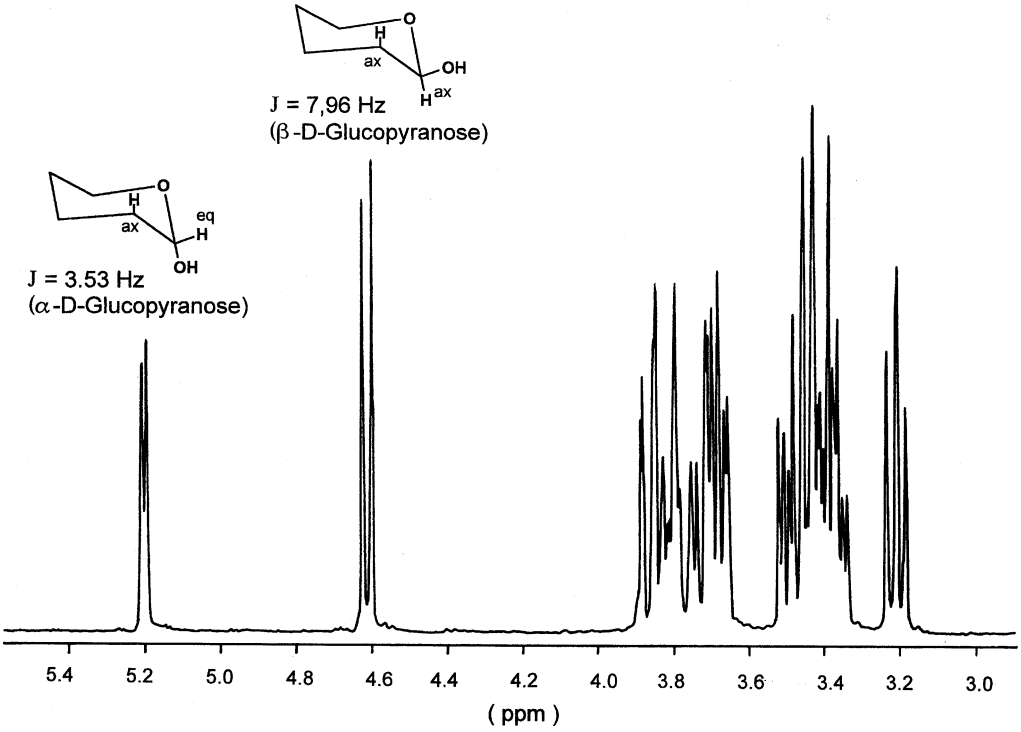


Fig. 4.3. Proton resonance spectrum of D-glucose (in D_2O)

are soluble to a small extent in ethanol and are insoluble in organic solvents such as ether, chloroform or benzene.

4.2.2.2 Optical Rotation, Mutarotation

Specific rotation constants, designated as [α] for sodium D-line light at 20–25°C, are listed in Table 4.8 for some important mono- and oligosaccharides. The specific rotation constant $[\alpha]_\lambda^t$ at a selected wavelength and temperature is calculated from the angle of rotation, α, by the equation:

$$[\alpha]_\lambda^t = \frac{\alpha}{l \cdot c} \tag{4.20}$$

where l is the polarimeter tube length in decimeters and c the number of grams of the optically active sugar in 100 ml of solution. The molecular rotation, [M], is suitable for comparison of the rotational values of compounds with differing molecular weights:

$$[M]_\lambda^t = M[\alpha]_\lambda^t \tag{4.21}$$

where M represents the compound's molecular weight. Since the rotational value differs for anomers and also for pyranose and furanose conformations, the angle of rotation for a freshly prepared solution of an isomer changes until an equilibrium is established. This phenomenon is known as mutarotation. When an equilibrium exists only between two isomers, as with glucose (α- and β-pyranose forms), the reaction rate follows first order kinetics:

$$-\frac{dc_\alpha}{dt} = k \cdot c_\alpha - k' \cdot c_\beta \tag{4.22}$$

A simple mutarotation exists in this example, unlike complex mutarotations of other sugars, e. g., idose which, in addition to pyranose, is also largely in the furanose form. Hence, the order of its mutarotation kinetics is more complex.

Table 4.8. Specific rotation of various mono- and oligosaccharides

Compound	$[\alpha]_D$[a]	Compound	$[\alpha]_D$[a]
Monosaccharides		*Oligosaccharides* (continued)	
L-Arabinose	+105	Kestose	+28
α-	+55.4	Lactose	+53.6
β-	+190.6	β-	+34.2
D-Fructose	−92	Maltose	+130
β-	−133.5	α-	+173
D-Galactose	+80.2	β-	+112
α-	+150.7	Maltotriose	+160
β-	+52.8	Maltotetraose	+166
D-Glucose	+52.7	Maltopentaose	+178
α-	+112	Maltulose	+64
β-	+18.7	Manninotriose	+167
D-manno-2-Heptulose	+29.4	Melezitose	+88.2
D-Mannose	+14.5	Melibiose	+ 143
α-	+29.3	β-	+ 123
β-	−17	Palatinose	+ 97.2
D-Rhamnose	−7.0	Panose	+ 154
D-Ribose	−23.7	Raffinose	+ 101
D-Xylose	+18.8	Saccharose	+ 66.5
α-	+93.6	α-*Schardinger*-Dextrin	+151
Oligosaccharides (including disaccharides)		β-*Schardinger*-Dextrin	+ 162
Cellobiose	+34.6	γ-*Schardinger*-Dextrin	+180
β-	+14.2	Stachyose	+146
Gentianose	+33.4		
Gentiobiose	+10		
α-	+31		
β-	−3		

[a] Temperature: 20–25 °C.

4.2.3 Sensory Properties

Mono- and oligosaccharides and their corresponding sugar alcohols, with a few exceptions, are sweet. β-D-Mannose has a sweet-bitter taste, and some oligosaccharides are bitter, e. g. gentiobiose.

The most important sweeteners are saccharose (sucrose), starch syrup (a mixture of glucose, maltose and malto-oligosaccharides) and glucose. Invert sugar, fructose-containing glucose syrups (high fructose corn syrup), fructose, lactose and sugar alcohols, such as sorbitol, mannitol and xylitol, are also of importance. The sugars differ in quality of sweetness and taste intensity. Saccharose is distinguished from other sugars by its pleasant taste even at high concentrations. The taste intensity of oligosaccharides drops regularly as the chain length increases.

The taste intensity can be measured by determining the recognition threshold of the sugar (the lowest concentration at which sweetness is still perceived) or by comparison with a reference substance (isosweet concentrations). The threshold value is related to the affinity of sweet-taste chemoreceptors for the sweet substance and is of importance in elucidation of reltionships between the chemical structure of a compound and its taste. For practical purpose, the use of a reference substance is of greater importance: taste intensity is dependent on concentration and varies greatly among sweet compounds.

Saccharose is the reference substance usually chosen. Tables 4.9, 4.10 and 4.11 list some sugar sweetness threshold values and relative sweet-

Table 4.9. Taste threshold values of sugars in water

Sugar	Recognition threshold		Detection threshold	
	mol/l	%	mol/l	%
Fructose	0.052	0.94	0.02	0.24
Glucose	0.090	1.63	0.065	1.17
Lactose	0.116	4.19	0.072	2.60
Maltose	0.080	2.89	0.038	1.36
Saccharose	0.024	0.81	0.011	0.36

ness values. Only mean values are given with deviations omitted. The recognition threshold values for saccharose cited in the literature vary from 0.01 to 0.037 mol/l.

Taste quality and intensity are dependent not only on a compound's structure but on other taste reception parameters: temperature, pH and the presence of additional sweet or non-sweet compounds.

The temperature dependence of the taste intensity is especially pronounced in the case of D-fructose (Fig. 4.4). It is based on the varying intensity of sweetness of the different isomers:

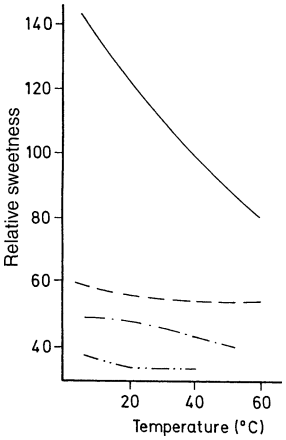


Fig. 4.4. Temperature dependence of the relative sweetness of some sugars (based on saccharose ≙ 100 at each temperature; — D-fructose, – – – D- glucose, –·–·– D-galactose, –··–··– maltose) (according to *Shallenberger* and *Birch*, 1975)

β-D-fructopyranose is the sweetest isomer, and its concentration decreases as the temperature increases (Fig. 4.5).

Table 4.10. Relative sweetness of sugars and sugar alcohols to sucrose[a]

Sugar/ sugar alcohol	Relative sweetness	Sugar/ sugar alcohol	Relative sweetness
Saccharose	100	D-Mannitol	69
Galactitol	41	D-Mannose	59
D-Fructose	114	Raffinose	22
D-Galactose	63	D-Rhamnose	33
D-Glucose	69	D-Sorbitol	51
Invert sugar	95	Xylitol	102
Lactose	39	D-Xylose	67
Maltose	46		

[a] 10% aqueous solution.

Table 4.11. Concentration (%) of iso-sweet aqueous solutions of sugars and sugar alcohols

D-Fructose	D-Glucose	Lactose	Saccharose	D-Sorbitol	Xylitol
0.8	1.8	3.5	1.0		
1.7	3.6	6.5	2.0		
4.2	8.3	15.7	5.0	9.3	8.5
8.6	13.9	25.9	10.0	17.2	9.8
13.0	20.0	34.6	15.0		
16.1	27.8		20.0	28.2	18.5
	39.0		30.0		25.4
	48.2		40.0	48.8	

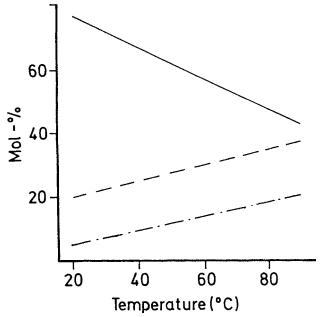


Fig. 4.5. Temperature dependence of the mutarotation equilibrium of D-fructose, (— β-D-fructo-pyranose, – – – β-D-fructofuranose, –·–·– α-D-fructofuranose) (according to *Shallenberger* and *Birch*, 1975)

Furthermore, an interrelationship exists between the sugar content of a solution and the sensory assessment of the volatile aroma compounds present. Even the color of the solution might affect taste evaluation. Figures 4.6–4.9 clarify these phenomena, with fruit juice and canned fruits as selected food samples.

The overall conclusion is that the composition and concentration of a sweetening agent has to be adjusted for each food formulation to provide optimum sensory perception.

A prerequisite for a compound to be sweet is the presence in its structure of a proton donor/acceptor system (AH/B-system), which may be supplemented by a hyrophobic site X. This AH/B/X-system interacts with a complementary system of the taste receptor site located on the taste buds. Based on studies of the taste quality of sugar derivatives and deoxy sugars, the following AH/B/X-systems have been proposed

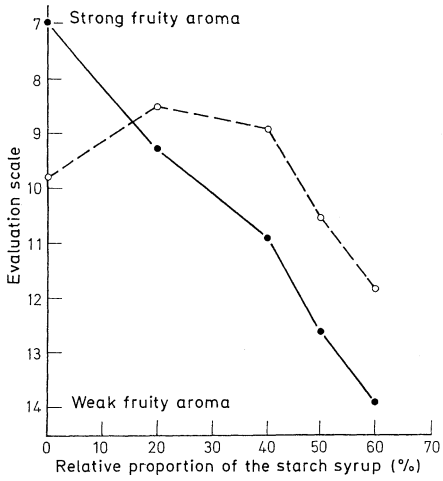


Fig. 4.6. Sensory evaluation of the "fruity flavor" of canned peaches at different ratios of saccharose/starch syrup (●—●60°, ○—○50° Brix) (according to *Pangborn*, 1959)

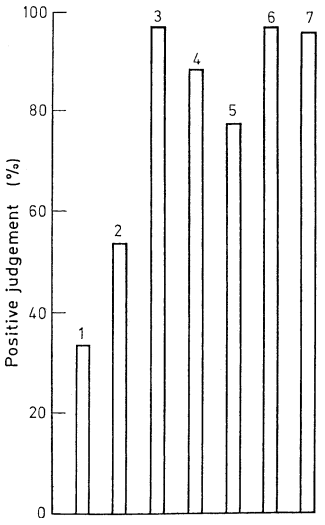


Fig. 4.7. Sensory evaluation of canned cherries prepared with different sweeteners 1, 2, 3: 60, 50, 40% saccharose, 4: 0.15% cyclamate, 5: 0.05% saccharin, 6: 10% saccharose + 0.10% cyclamate, 7: 10% saccharose + 0.02% saccharin (according to *Salunkhe*, 1963)

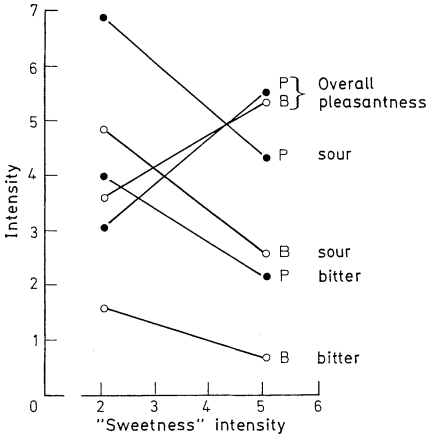
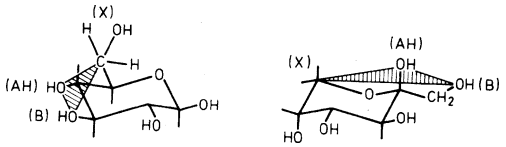


Fig. 4.8. Sensory evaluation of the categories "overall pleasantness", "sour" and "bitter" versus sweetness intensity. B bilberry (○—○) and P (●—●) cranberry juice (according to *Sydow*, 1974)

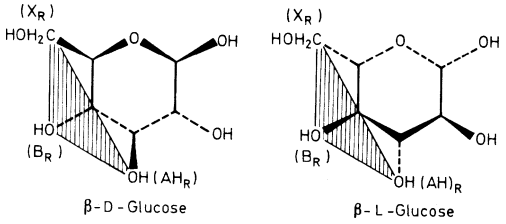
Fig. 4.9. Bitter taste threshold values of limonin (○—○) and naringin ($\times 10^{-1}$ ●—●) in aqueous saccharose solution (according to *Guadagni*, 1973)

for β-D-glucopyranose and β-D-fructopyranose respectively:



(4.23)

β-D-glucopyranose and β-L-glucopyranose are sweet. Molecular models show that the AH/B/X-system of both sugar components fits equally well with the complementary receptor system $AH_R/B_R/X_R$ (Formula 4.24):



(4.24)

With asparagine enantiomers, the D-form is sweet, while the L-form is tasteless. Here, unlike D- and L-glucose, only the D-form interacts with the complementary $AH_R/B_R/X_R$-system:

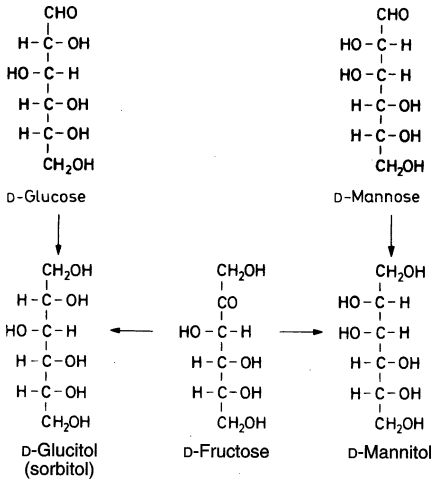
(4.25)

As described in 8.8.1.1, the AH/B/X-system has been extended to explain the large differences in structure and sweetening strength which can exist in compounds of different substance classes.

4.2.4 Chemical Reactions and Derivatives

4.2.4.1 Reduction to Sugar Alcohols

Monosaccharides can be reduced to the corresponding alcohols by $NaBH_4$, electrolytically or via catalytic hydrogenation. Two new alcohols are obtained from ketoses, e. g., fructose, since a new chiral center is formed:



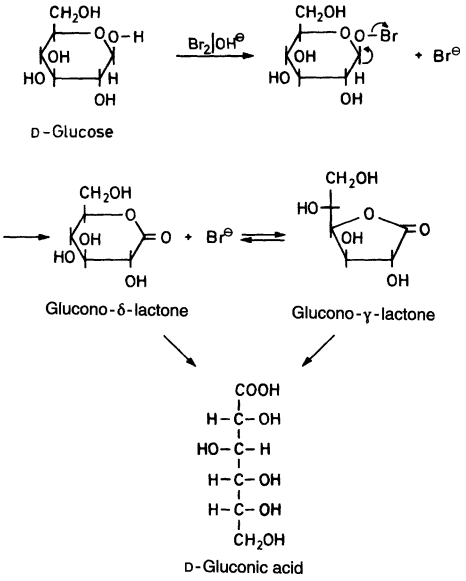
(4.26)

The alcohol name is derived from the sugar name in each case by replacing the suffix -ose or -ulose with the suffix -itol. The sugar alcohols of importance in food processing are xylitol, the only one of the four pentitols (mesoribitol, D,L-arabitol, mesoxylitol) used, and only D-glucitol (sorbitol) and D-mannitol of the ten stereoisomeric forms of hexitols [meso-allitol, meso-galactitol (dulcitol), D,L-glucitol (sorbitol), D,L-iditol, D,L-mannitol and D,L-altritol]. They are used as sugar substitutes in dietetic food formulations to decrease water activity in "intermediate moisture foods", as softeners, as crystallization inhibitors and for improving the rehydration characteristics of dehydrated food. Sorbitol is found in nature in many fruits, e. g., pears, apples and plums. Palatinitol (a mixture of glucopyranosyl glucitol and glu-

copyranosyl mannitol) is a sugar alcohol. It is produced using biotechnological methods by the rearrangement of sucrose (1 → 2 to 1 → 6), followed by reduction. Maltitol, the reduction product of the disaccharide maltose, is being considered for wider use in food formulations.

4.2.4.2 Oxidation to Aldonic, Dicarboxylic and Uronic Acids

Under mild conditions, e. g., with bromine water in buffered neutral or alkaline media, aldoses are oxidized to aldonic acids. Oxidation involves the lactol group exclusively. β-Pyranose is oxidized more rapidly than the α-form. Since the β-form is more acidic (cf. 4.2.1.3), it can be considered that the pyranose anion is the reactive form. The oxidation product is the δ-lactone which is in equilibrium with the γ-lactone and the free form of aldonic acid. The latter form prevails at pH > 3.



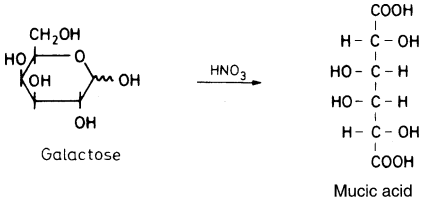
(4.27)

The transition of lactones from δ- to γ-form and vice versa probably proceeds through an intermediary bicyclic form.

The acid name is obtained by adding the suffix–onic acid (e. g. aldose → aldonic acid).

Glucono-δ-lactone is utilized in food when a slow acid release is required, as in baking powders, raw fermented sausages or dairy products.

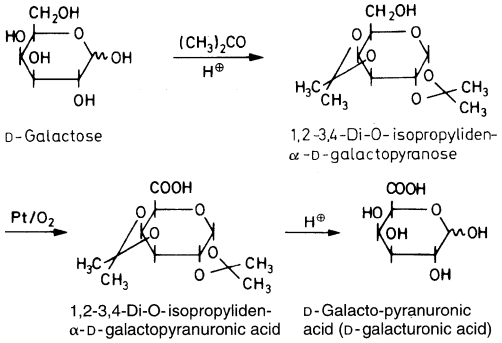
Treatment of aldose with more vigorous oxidizing agents, such as nitric acid, brings about oxidation of the C-1 aldehyde group and the CH_2OH-group, resulting in formation of a dicarboxylic acid (nomenclature: stem name of the parent sugar + the suffix -aric acid, e. g. aldose → aldaric acid). Thus, galactaric acid (common or trivial name: mucic acid) is obtained from galactose:



(4.28)

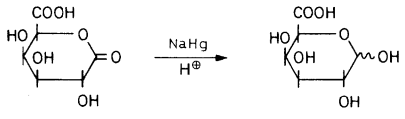
The dicarboxylic acid can, depending on its configuration, form mono- or dilactones.

Oxidation of the CH_2OH-group by retaining the carbonyl function at C-1, with the aim of obtaining uronic acids (aldehydocarboxylic acids), is possible only by protecting the carbonyl group during oxidation. A suitable way is to temporarily block the vicinal HO-groups by ketal formation which, after the oxidation at C-6 is completed, are deblocked under mild acidic conditions:



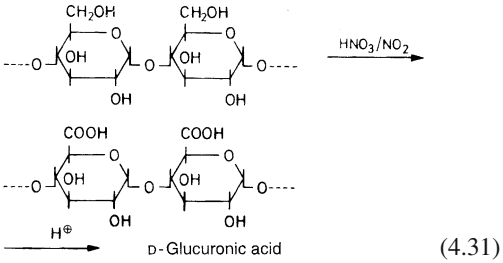
(4.29)

An additional possibility for uronic acid synthesis is the reduction of monolactones of the corresponding aldaric acids:



(4.30)

Another industrially applied glucuronic acid synthesis involves first oxidation then hydrolysis of starch:



(4.31)

Depending on their configuration, the uronic acids can form lactone rings in pyranose or furanose forms.

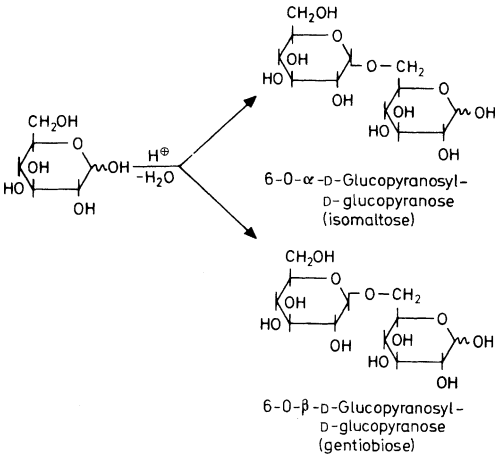
A number of uronic acids occur fairly abundantly in nature. Some are constituents of polysaccharides of importance in food processing, such as gel-forming and thickening agents, e. g. pectin (D-galacturonic acid) and sea weed-derived alginic acid (D-mannuronic acid, L-guluronic acid).

4.2.4.3 Reactions in the Presence of Acids and Alkalis

In the absence of amine components, monosaccharides are relatively stable in the pH range 3–7. Beyond these pH limits, more or less extensive conversions occur, depending on the conditions. Enolizations and subsequent elimination of water with retention of the C-chain predominate in an acidic medium. In a basic medium, enolizations with subsequent fragmentation (*retro*-aldol reactions) and secondary reactions of the fragments (aldol additions) predominate.

4.2.4.3.1 Reactions in Strongly Acidic Media

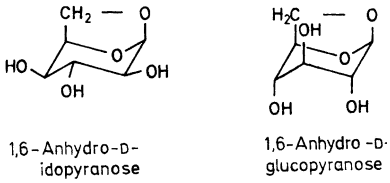
The reverse of glycoside hydrolysis (cf. 4.2.4.5) i.e. re-formation of glycosides, occurs in dilute mineral acids. All the possible disaccharides and higher oligosaccharides, but preferentially isomaltose and gentiobiose, are obtained from glucose:



(4.32)

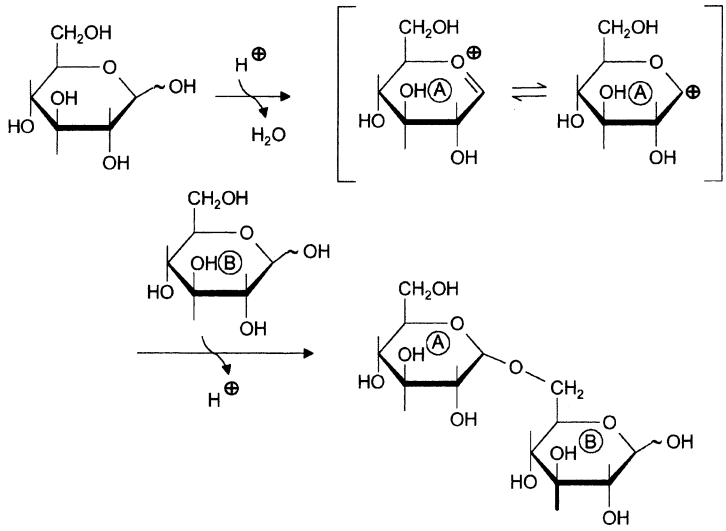
Such reversion-type reactions are observed, e. g., in the acidic hydrolysis of starch.

In addition to the formation of intermolecular glycosides, intramolecular glycosidic bonds can be readily established when the sugar conformation is suitable. β-Idopyranose, which occurs in the $^1C_4$-conformation, is readily changed to 1,6-anhydroidopyranose, while the same reaction with β-D-glucopyranose ($^4C_1$-conformation) occurs only under more drastic conditions, e. g., during pyrolysis of glucose, starch or cellulose. Heating glucose syrup above 100 °C can form 1,6-anhydrogluco-pyranose, but only in traces:



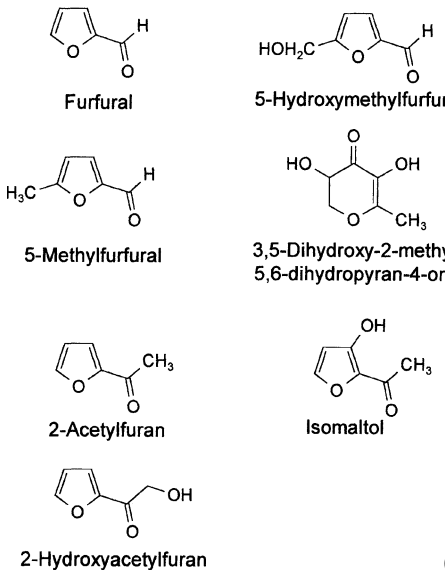
(4.33)

In the formation of reversion products, it is assumed that in the presence of strong acids an oxonium cation is formed which, as an alkylating agent, reacts with the nucleophilic hydroxy groups with the cleavage of H^+ (Formula 4.34).



(4.34)

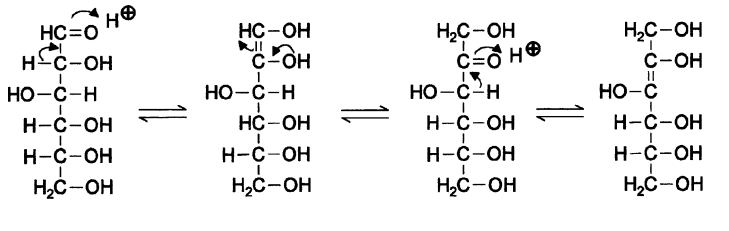
Dehydrating Reactions. The heating of monosaccharides under acidic conditions, e. g., during pasteurization of fruit juices and baking of rye bread, gives rise to a large number of furan and pyran compounds (examples in Formula 4.35). The formation of these compounds can be explained by enolizations and dehydrating reactions of the carbohydrates. It is noticeable that in some compounds, the aldehyde group of an aldose is formally retained at C-1 (furfural, 5-hydroxymethyl furfural, 5-methylfurfural) and in other components, the aldehyde group is reduced to a methyl group. As explained later, this indicates the course of formation in each case.



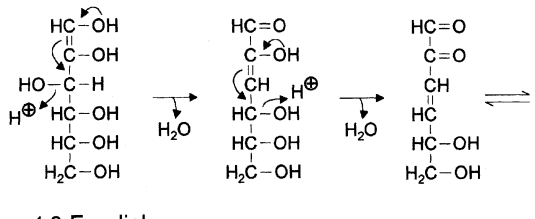
(4.35)

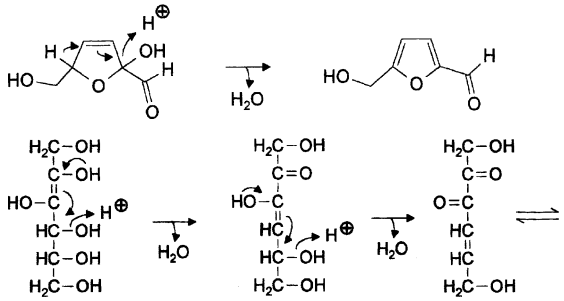
The reaction pathway in acid starts slowly with enolization to important intermediates called enediols. Glucose gives rise to 1,2-enediol, and fructose to 2,3-enediol as well (Formula 4.36). Starting with the enediols, the further course of the reaction is shown below.

The steps in the formation of 5-hydroxymethyl furfural (HMF) from 1,2-enediol is shown in Formula 4.37. HMF is also used as an indicator for the heating of carbohydrate containing food, e. g., honey. The (*retro-Michael* addition) water elimination at C-3 and subsequently at C-4 leads to a 1,2-diulose (3,4-dideoxyosone), which after cyclization to a hemiacetal, a dihy-drofuran, releases another molecule of water, producing HMF. In the same way, e. g., furfural can be made from pentoses and 5-methylfurfural from the 6-methylpentose rhamnose. 2-Hydroxyacetylfuran, which is preferentially formed from fructose, can be obtained starting from the corresponding 2,3-enediol by water elimination at C-4, followed by C-5 (Formula 4.38).

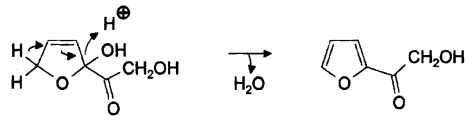


(4.36)

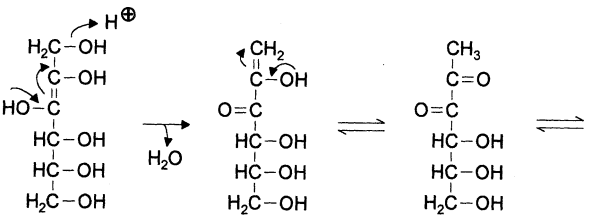


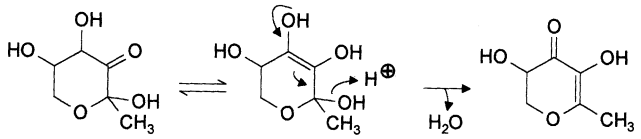


(4.37)



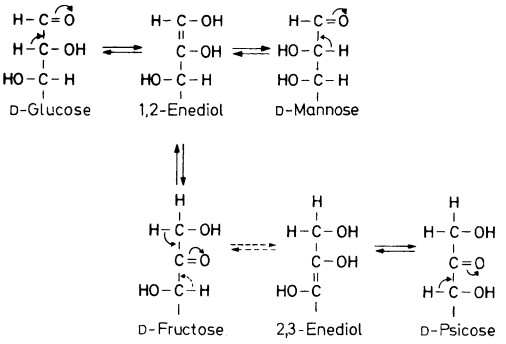
(4.38)





(4.39)

With the 2,3-enediol, not only water elimination at C-4, but also the elimination of the hydroxyl group at C-1 is possible (Formula 4.39). This reaction pathway gives, among other compounds, 3,5-dihydroxy-2-methyl-5,6-dihydropyran-4-one, which is also used as an indicator for the heating of food. The formation of two different enediols is the reason for the wider product spectrum from ketoses, like fructose, than from aldoses. In the presence of amino compounds, all the reactions mentioned here proceed very easily also in the pH range 3–7. Since free amino acids are present in many foods, the reactions shown here also occur in connection with the pathways discussed in 4.2.4.4.

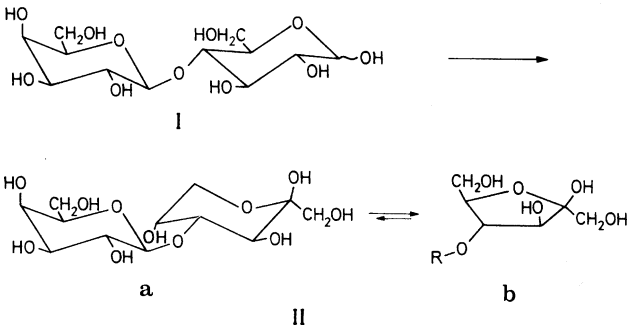


(4.40)

4.2.4.3.2 Reactions in Strongly Alkaline Solution

Alkaline reaction conditions occur in food, e. g., in the isolation of sucrose from sugar beet and in the production of alkali-baked goods. Apart from enolization reactions, which can also occur under acidic conditions but proceed much faster under alkaline conditions, the degradation of the carbohydrate skeleton is an important characteristic of base-catalyzed degradation reactions. Glucose, mannose and fructose are in equilibrium through the common 1,2-enediol. Also present is a small amount of psicose, which is derived from fructose by 2,3-enolization:

In this isomerization reaction, known as the *Lobry de Bruyn–van Ekenstein* rearrangement, one type of sugar can be transformed to another sugar in widely differing yields. The reaction plays a role in transforming an aldose to a ketose. For example, in the presence of sodium aluminate as a catalyst, lactose (4-O-β-D-galactopyranosido-D-glucopyranose, I) is rearranged into lactulose (4-O-β-D-galacto-pyranosido-D-fructose):

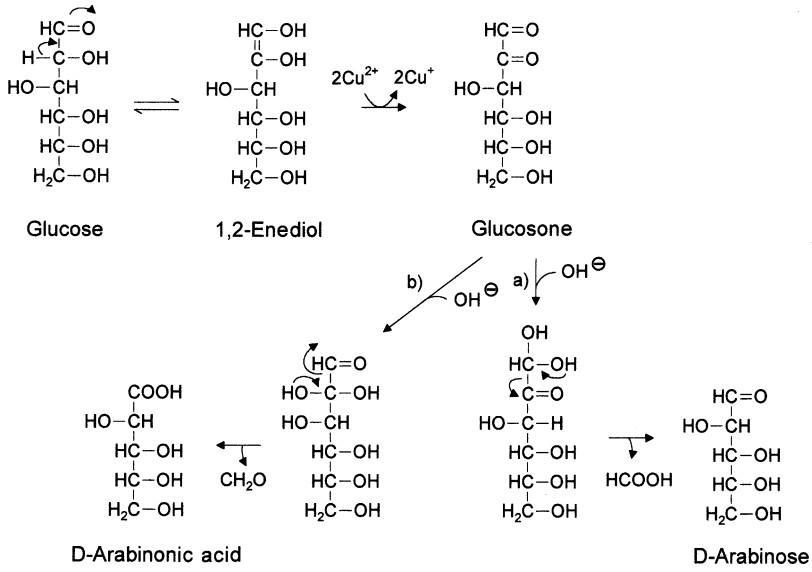


(4.41)

In this disaccharide, fructose is present mainly as the pyranose (IIa) and, to a small extent, as the furanose (IIb).

Lactulose utilization in infant nutrition is under consideration since it acts as a bifidus factor and prevents obstipation.

Oxidation of the enediol occurs in the presence of oxygen or other oxidizing agents, e. g., Cu^{2+}, resulting in carboxylic acids. In such a reaction with glucose, the main products are D-arabinonic and



(4.42)

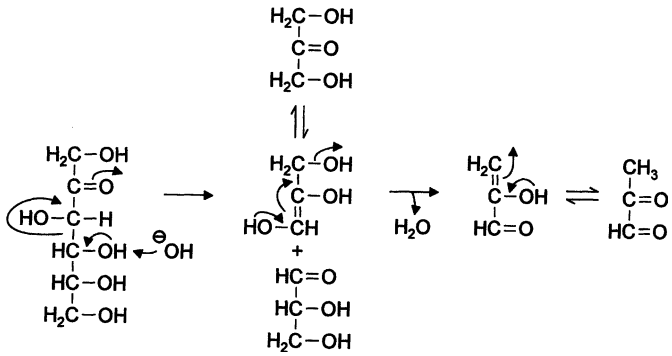
formic acids in addition to formaldehyde and D-arabinose (Formula 4.42). Depending on reaction conditions, particularly the type of alkali present, further hydroxyacids are also formed due to enolization occurring along the molecule.

The nonstoichiometric sugar oxidation process in the presence of alkali is used for both qualitative and quantitative determination of reducing sugars (*Fehling's* reaction with alkaline cupric tartrate; *Nylander's* reaction with alkaline trivalent bismuth tartrate; or using *Benedict's* solution, in which cupric ion complexes with citrate ion).

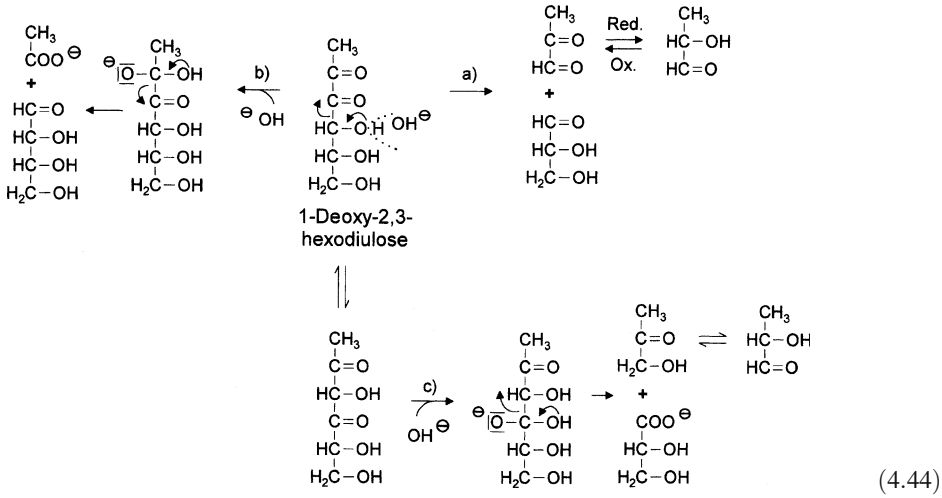
Hydroxyaldehydes and hydroxyketones are formed by chain cleavage due to retroaldol reaction under nonoxidative conditions using dilute alkali at elevated temperatures or concentrated alkali even in the cold.

For example, fructose can yield glyceraldehyde and dihydroxyacetone (Formula 4.43), and the latter easily undergoes water elimination to give 2-oxopropanal (methylglyoxal). Starting from 1-deoxy-2,3-hexodiulose, several degradation pathways leading to short-chain compounds are possible (Formula 4.44). Among other compounds, 2-oxopropanal, monohydroxyacetone, acetic acid, glyceraldehyde or glyceric acid can be formed by *retro*-aldol reactions (a), α-dicarbonyl cleavages (b) and β-dicarbonyl cleavages (c).

Since enolization is not restricted to any part of the molecule and since water elimination and redox reactions are not restricted in amount, even the spectrum of primary cleavage products is great. These primary products are highly



(4.43)



(4.44)

reactive and provide a great number of secondary products by aldol condensations (*retro*-reactions) and intramolecular *Cannizzaro* reactions.

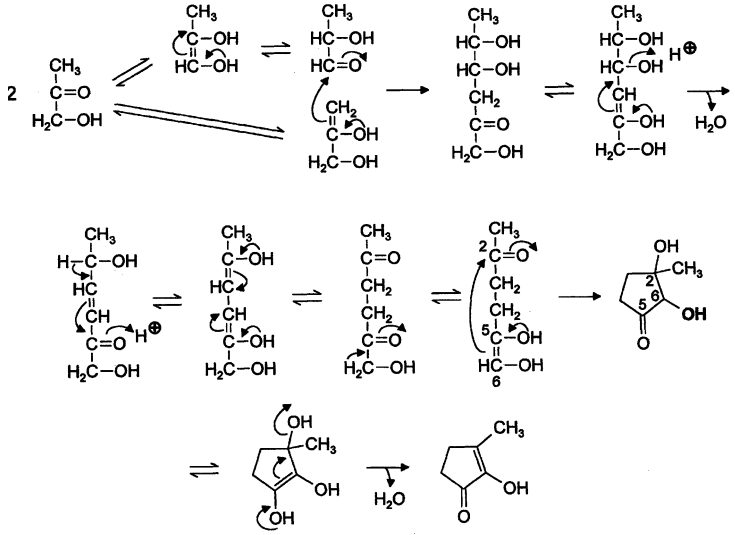
The compounds formed in fructose syrup of pH 8–10 heated for 3 h are listed in Table 4.12. The cyclopentenolones are typical caramel-like aroma substances. The formation of 2-hydroxy-3-methyl-2-cyclopenten-1-one as an example is shown in Formula 4.46. The compound 1,3,5-trideoxy-2,5-hexulose is formed as an intermediate via the aldol condensation of 2 molecules of monohydroxyacetone (or the isomer 2-hydroxypropanone). The linking of the C-atoms 6 and 2 then leads to a carbocycle which yields the target compound on water elimination. Analogously, the substitution of l-hydroxy-2-butanone or 3-hydroxy-2-butanone for one molecule of hydroxyacetone can give rise to 2-hydroxy-3-ethyl- or 2-hydroxy-3,4-dimethyl-2-cyclopenten-1-one.

Among other compounds, C-3 fragments are also precursors for the formation of 4-hydroxy-2,5-dimethyl-3(2H)-furanone (II, furaneol), which can be formed, e. g., from 2-hydroxypropanal and its oxidation product, 2-oxopropanal (I in Formula 4.47). The substitution of 2-oxobutanal for 2-oxopropanal yields the homologous ethylfuraneol. In a similar way, 3-hydroxy-4,5-dimethyl-2(5H)-furanone (sotolon) can be formed from 2,3-butanedione (diacetyl) and glycolaldehyde (Formula 4.48).

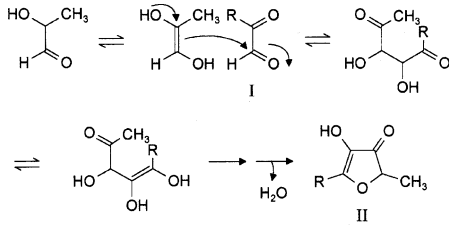
Saccharinic acids are specific reaction products of monosaccharides in strong alkalies, particularly of alkaline-earth metals. They are obtained in each case as diastereomeric pairs by benzilic acid rearrangements from deoxy-hexodiuloses according to Formula 4.48a. In fact, 1-deoxy-2,3-hexodiulose yields saccharinic acid, 3-deoxy-1,2-hexodiulose yields metasaccharinic acid and 4-deoxy-2,3-hexodiulose yields isosaccharinic acid (Formula 4.48b).

Table 4.12. Volatile reaction products obtained from fructose by an alkali degradation (pH 8–10)

Acetic acid
Hydroxyacetone
1-Hydroxy-2-butanone
3-Hydroxy-2-butanone
4-Hydroxy-2-butanone
Furfuryl alcohol
5-Methyl-2-furfuryl alcohol
2,5-Dimethyl-4-hydroxy-3-(2H)-furanone
2-Hydroxy-3-methyl-2-cyclopenten-1-one
3,4-Dimethyl-2-hydroxy-2-cyclopenten-1-one
3,5-Dimethyl-2-hydroxy-2-cyclopenten-1-one
3-Ethyl-2-hydroxy-2-cyclopenten-1-one
γ-Butyrolactone

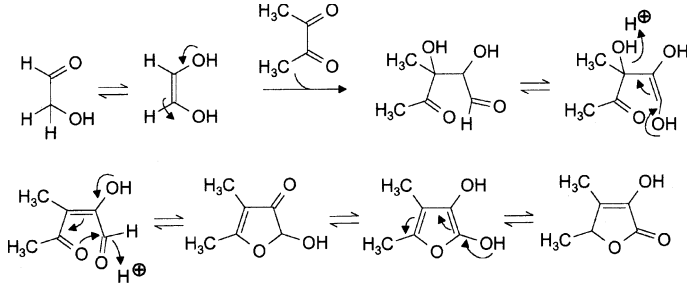


(4.46)

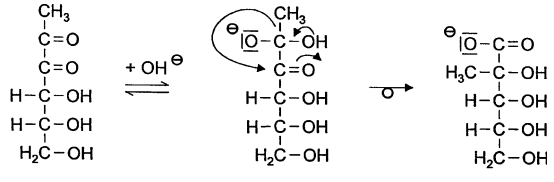


R = Methyl (I : 2-Oxopropanal; II : Furaneol)
R = Ethyl (I : 2-Oxobutanal; II : Ethylfuraneol)

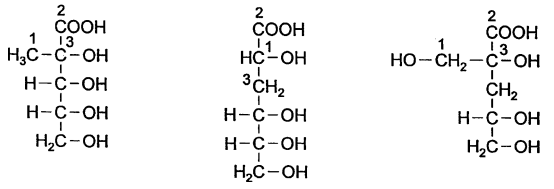
(4.47)



(4.48)



(4.48 a)

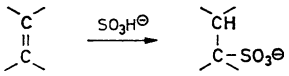


(4.48 b)

Ammonia catalyzes the same reactions as alkali and alkaline earths. Reactive intermediary products can polymerize further into brown pigments ("sucre couleur") or form a number of imidazole, pyrazine and pyridine derivatives.

4.2.4.3.3 Caramelization

Brown-colored products with a typical caramel aroma are obtained by melting sugar or by heating sugar syrup in the presence of acidic and/or alkaline catalysts. The reactions involved were covered in the previous two sections. The process can be directed more towards aroma formation or more towards brown pigment accumulation. Heating of saccharose syrup in a buffered solution enhances molecular fragmentation and, thereby, formation of aroma substances. Primarily dihydrofuran-ones, cyclopentenolones, cyclohexenolones and pyrones are formed (cf. 4.2.4.3.2). On the other hand, heating glucose syrup with sulfuric acid in the presence of ammonia provides intensively colored polymers ("*sucre couleur*"). The stability and solubility of these polymers are enhanced by bisulfite anion addition to double bonds:



(4.50)

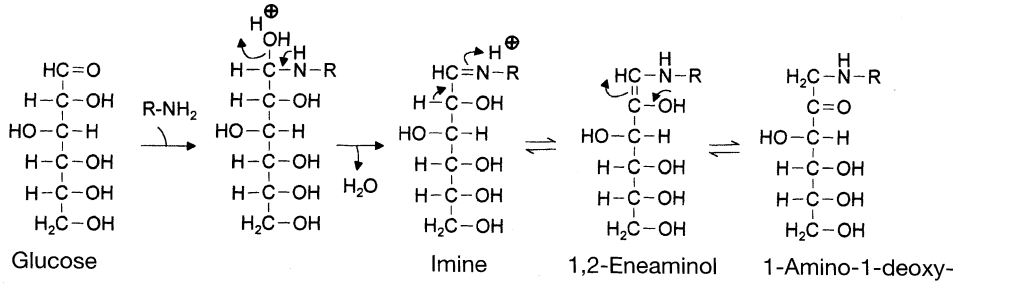
4.2.4.4 Reactions with Amino Compounds (*Maillard* Reaction)

In this section, the formation of N-glycosides as well as the numerous consecutive reactions classed under the term *Maillard* reaction or nonenzymatic browning will be discussed.

N-Glycosides are widely distributed in nature (nucleic acids, NAD, coenzyme A). They are formed in food whenever reducing sugars occur together with proteins, peptides, amino acids or amines. They are obtained more readily at a higher temperature, low water activity and on longer storage.

On the sugar side, the reactants are mainly glucose, fructose, maltose, lactose and, to a smaller extent, reducing pentoses, e. g., ribose. On the side of the amino component, amino acids with a primary amino group are more important than those with a secondary because their concentration in foods is usually higher. Exceptions are, e. g., malt and corn products which have a high proline content. In the case of proteins, the ε-amino groups of lysine react predominantly. However, secondary products from reactions with the guanidino group of arginine are also known. In fact, imidazolin-ones and pyrimidines, which are formed from arginine and reactive α- and β-dicarbonyl compounds obtained from sugar degradation, have been detected.

The consecutive reactions of N-glycosides partially correspond to those already outlined for acid/base catalyzed conversions of monosaccharides. However, starting with N-containing intermediates, which with the nitrogen function possess a catalyst within the molecule, these reactions proceed at a high rate under substantially milder conditions, which are present in many foods.



(4.50)

These reactions result in:

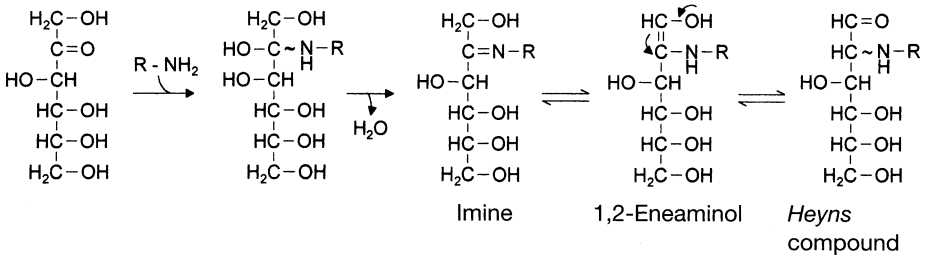
- Brown pigments, known as melanoidins, which contain variable amounts of nitrogen and have varying molecular weights and solubilities in water. Little is known about the structure of these compounds. Studies have been conducted on fragments obtained after *Curie* point pyrolysis or after oxidation with ozone or sodium periodate. Browning is desired in baking and roasting, but not in foods which have a typical weak or other color of their own (condensed milk, white dried soups, tomato soup).
- Volatile compounds which are often potent aroma substances. The *Maillard* reaction is important for the desired aroma formation accompanying cooking, baking, roasting or frying. It is equally significant for the generation of off-flavors in food during storage, especially in the dehydrated state, or on heat treatment for the purpose of pasteurization, sterilization and roasting.
- Flavoring matter, especially bitter substances, which are partially desired (coffee) but can also cause an off-taste, e. g., in grilled meat or fish (roasting bitter substances).
- Compounds with highly reductive properties (reductones) which can contribute to the stabilization of food against oxidative deterioration.
- Losses of essential amino acids (lysine, arginine, cysteine, methionine).
- Compounds with potential mutagenic properties.
- Compounds that can cause cross-linkage of proteins. Reactions of this type apparently also play a role *in vivo* (diabetes).

4.2.4.4.1 Initial Phase of the Maillard Reaction

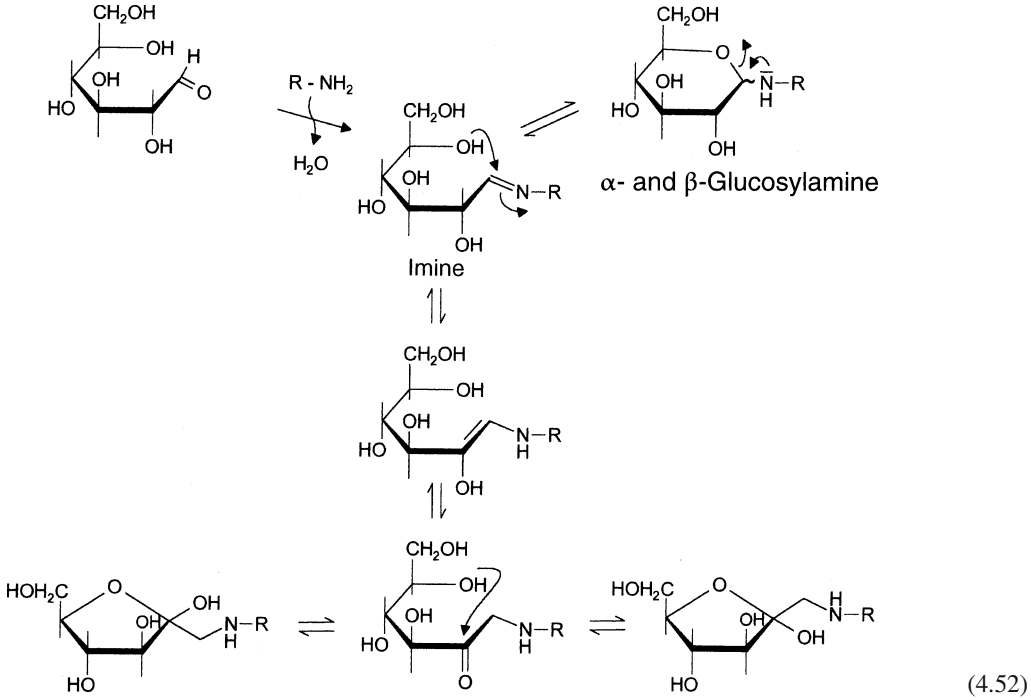
D-Glucose will be used as an example to illustrate the course of reactions occurring in the early phase of the *Maillard* reaction. The open-chain structures will be used for simplification although the hemiacetal forms are predominantly present in solution.

Nucleophilic compounds like amino acids or amines easily add to the carbonyl function of reducing carbohydrates with the formation of imines (*Schiff* bases). As a result of the hydroxyl group present in the α-position (Formula 4.50), the imines can rearrange via the 1,2-eneaminols corresponding to the 1,2-enediol (cf. Formula 4.36). This rearrangement leads to an aminoketose called an *Amadori* compound (1-amino-1-deoxyketose) after its discoverer. If fructose reacts in a corresponding way with an amine or an amino acid (Formula 4.51), an aminoaldose, called a *Heyns* compound (2-amino-2-deoxyaldose), is formed. Since the addition of the amine to fructose or the addition of the H-atom to the intermediate aminoenol can proceed from two sides, an enantiomeric pair is obtained in each case.

Amadori compounds with different amino acid residues have been detected in many heated and stored foods, e. g., in dried fruit and vegetables, milk products, cocoa beans or soy sauce. *Amadori* compounds are also found in the blood serum, especially of patients suffering from Diabetes mellitus. As secondary amino acids, *Amadori* and *Heyns* compounds can be analytically detected by amino acid analysis (cf. protein section).



(4.51)

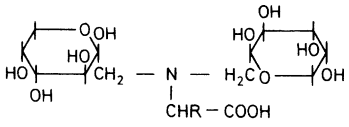


(4.52)

The reasons for the partial stability of such *Amadori* compounds in comparison with imines can be explained by the cyclic molecular structures.

The imine (Formula 4.52) formed by the reaction of glucose with the amine is easily converted to the cyclic hemiaminal, α- and β-glucosylamine. However, N-glycosides of this type are relatively instable because they very easily mutarotate, i. e., they are easily hydrolyzed via the open-chain imine or are converted to the respective α- and β-anomer. However, the *Amadori* rearrangement leads to furanose, which as a hemiacetal, has a stability to mutarotation comparable with that of carbohydrates.

The *Amadori* compounds can react further with a second sugar molecule, resulting in glycosylamine formation and subsequent conversion to di-D-ketosylamino acids ("diketose amino acids") by an *Amadori* rearrangement:



Di-D-fructosylamino acid (4.54)

4.2.4.4.2 Formation of Deoxyosones

Amadori products are only intermediates formed in the course of the *Maillard* reaction. In spite of their limited stability, these products can be used under certain conditions as an analytical indicator of the extent of the heat treatment of food. Unlike the acidic (pH <3) and alkaline (pH >8) sugar degradation reactions, the *Amadori* compounds are degraded to 1-, 3-, and 4-deoxydicarbonyl compounds (deoxyosones) in the pH range 4–7. As reactive α-dicarbonyl compounds, they yield many secondary products. Formulas 4.54–4.57 summarize the degradation reactions starting with the *Amadori* compound.

Analogous to fructose (cf. Formula 4.37), amino-l-deoxyketose can be converted to 2,3-eneaminol as well as 1,2-eneaminol (Formula 4.54) by enolization. Analogous to the corresponding 1,2-enediol, water elimination and hydrolysis of the imine cation gives 3-deoxy-1,2-diulose, also called 3-deoxyosone (Formula 4.55).

Like the corresponding 2,3-enediol, the 2,3-eneaminol has two different β-elimination options. Formula 4.56 shows the elimination of the amino acid by a *retro-Michael* reaction with

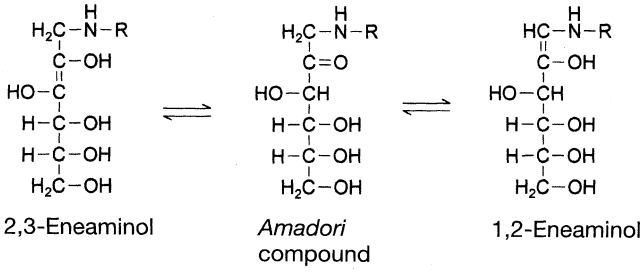
formation of 1-deoxy-2,3-diulose, also called 1-deoxyosone. In addition, 4-deoxy-2,3-diulose, also called 4-deoxyosone, can be formed by water elimination at C-4 of 2,3-eneaminol (Formula 4.57). In contrast to the previously mentioned pathways, the amino acid residue remains bound to the carbohydrate in this reaction path. As shown in the reaction scheme, all three deoxyosones occur in different cyclic hemiacetal forms.

As in the case of the deoxyosones, the concentrations of *Amadori* and *Heyns* compounds vary, depending on the reaction conditions (pH value, temperature, time, type and concentration of the educts). As a result, there is a change in the product spectrum and, consequently, in the color, taste, odor, and other properties of the food in each case.

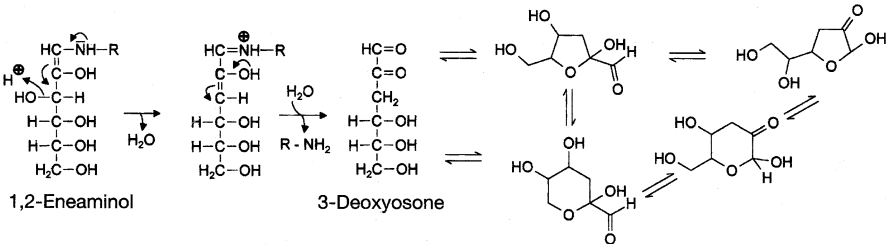
Like all α-dicarbonyl compounds, deoxyosones can be stabilized as quinoxalines by a trapping reaction with o-phenylenediamine (Formula 4.58) and subsequently quantitatively determined using liquid chromatographic techniques. In this way, deoxyosones were detected for the first time as intermediates in carbohydrate degradation.

The stable secondary products of the *Maillard* reaction, that are isolated from many different reaction mixtures and have known structures, can be generally assigned to a definite deoxyosone by a series of plausible reaction steps (enolization, elimination of water, retroaldol cleavage, substitution of an amino function for a hydroxy function etc.).

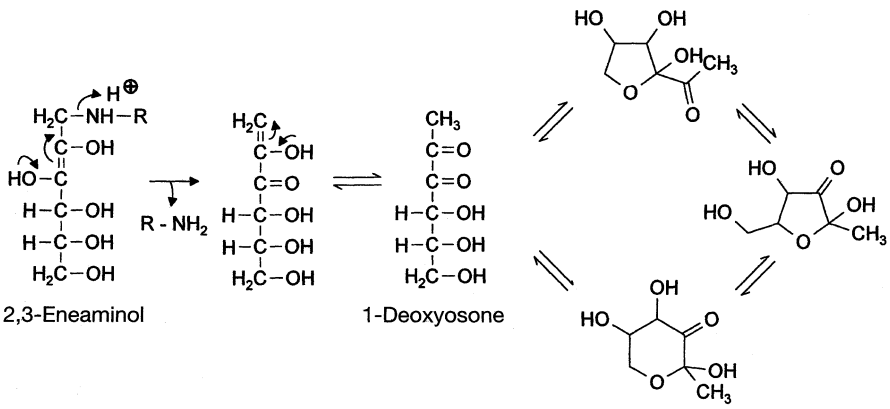
Of the large number of secondary products known today, a few typical examples will be dealt with here for each deoxyosone.



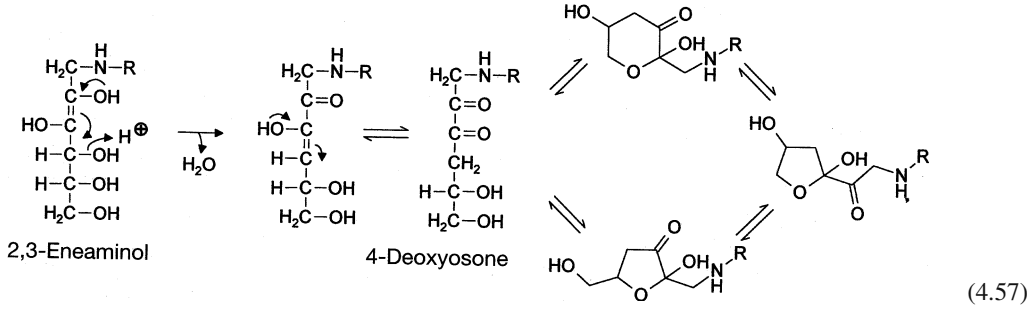
(4.54)



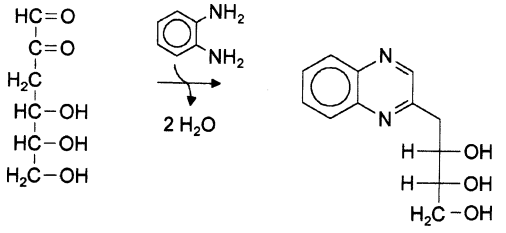
(4.55)



(4.56)



(4.57)



(4.58)

4.2.4.4.3 Secondary Products of 3-Deoxyosones

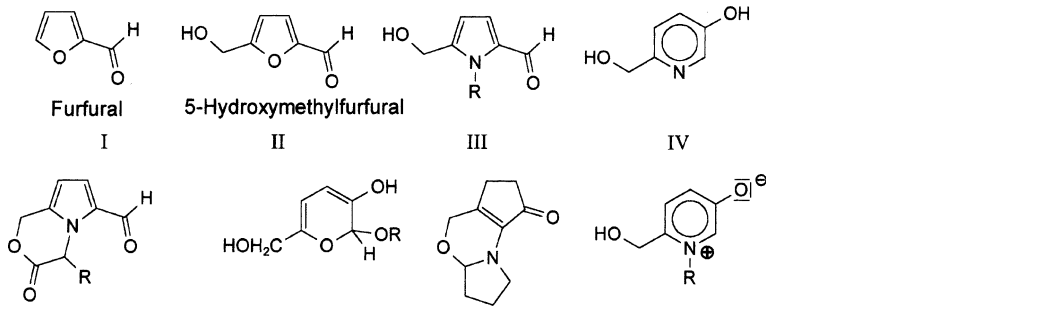
Formula 4.59 shows examples of products obtained on the decomposition of 3-deoxyosones. The best known compounds are 5-hydroxymethylfurfural from hexoses (HMF, II in Formula 4.59) and furfural from pentoses (I in Formula 4.59). Taking the furanoid structures of 3-deoxyosone as a basis (Formula 4.55), 3,4-dideoxyosone is obtained after ring opening, enolization, and water elimination (Formula 4.60). Water elimination from the hemiacetal form of 3,4-dideoxyosone directly yields HMF. Taking into account the water elimination required to form 3-deoxyosone (cf. Formula 4.55), 5-hydroxymethylfurfural is formed from hexose by the stoichiometric elimination of 3 mols of water.

In the presence of higher concentrations of ammonia, primary amines or amino acids, 3-deoxyosone preferentially gives rise to 2-formyl-5-hydroxymethylpyrrole (III in Formula 4.59) or the corresponding N-alkylated derivatives rather than to HMF. The most important reaction intermediate is 3,4-dideoxyosone (cf. Formula 4.60), which can react with amino compounds with the elimination of water to give the corresponding pyrrole (Formula 4.61) or pyridine derivatives (Formula 4.62). The reaction with ammonia plays a role, especially in the production of sugar couleur.

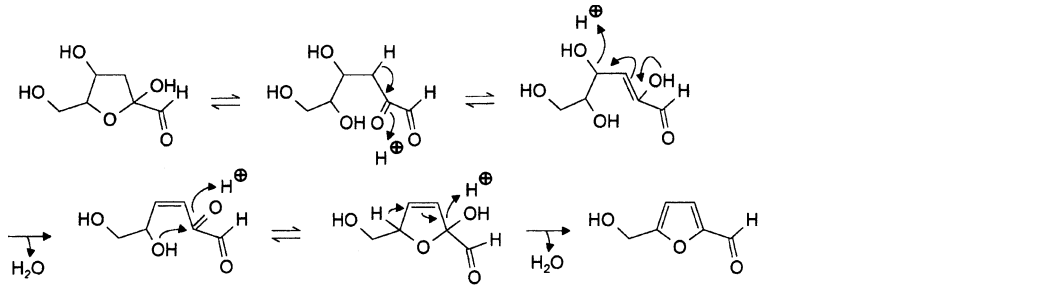
If pyrrole formation occurs with an amino acid, this product can react further (Formula 4.63) to yield a bicyclic lactone (V in Formula 4.59). Other secondary products of 3-deoxyosone are compounds with a pyranone structure. In fact, β-pyranone (VI in Formula 4.59) is under discussion as the most important intermediate. It can be formed from the pyranose hemiacetal form of 3-deoxyosone (Formula 4.64). This compound has been identified only in the full acetal form (e. g., with carbohydrates on drying) because only this structure makes a relatively stable end product possible. The compounds mentioned have acidic hydrogen atoms in position 4, easily allowing condensation reactions with aldehydes and polymerization or the formation of brown dyes.

Another compound obtained from 3-deoxyosone via a relatively complex reaction is maltoxazine (VII in Formula 4.59), which has been identified in malt and beer. This compound could be formed from 3,4-dideoxyosone, which first undergoes a *Strecker* reaction with the secondary amino acid proline with decarboxylation to give the 1-pyrroline derivative (Formula 4.65). Enolization, formation of a five-membered carbocyclic compound and nucleophilic addition of the hydroxymethyl group to the pyrroline cation yields the tricyclic maltoxazine. In general, the formation of such carbocyclic compounds is favored in the presence of secondary amino acids like proline.

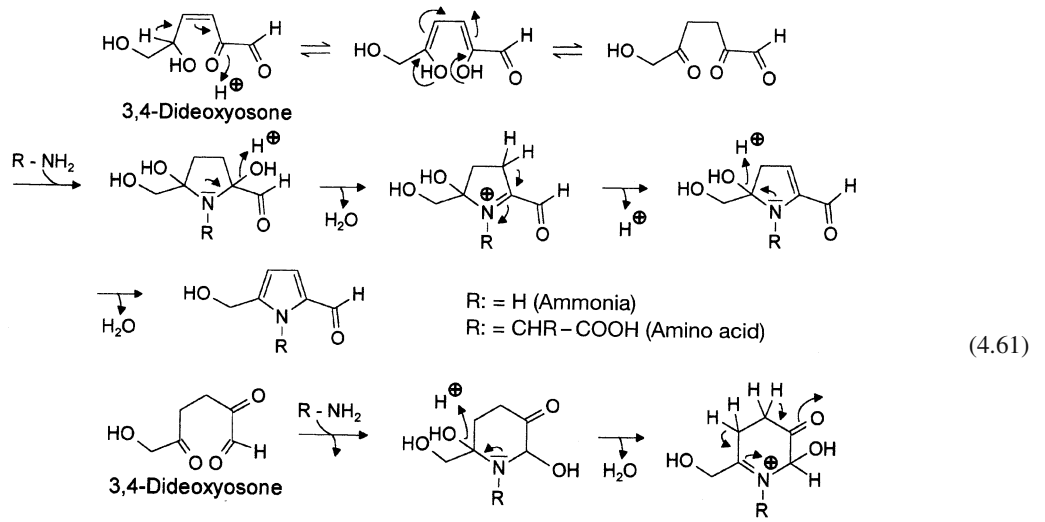
3-Deoxyosones predominantly form pyrazines and imidazoles with ammonia. The following compounds were isolated from sugar coloring (cf. Formula 4.66).



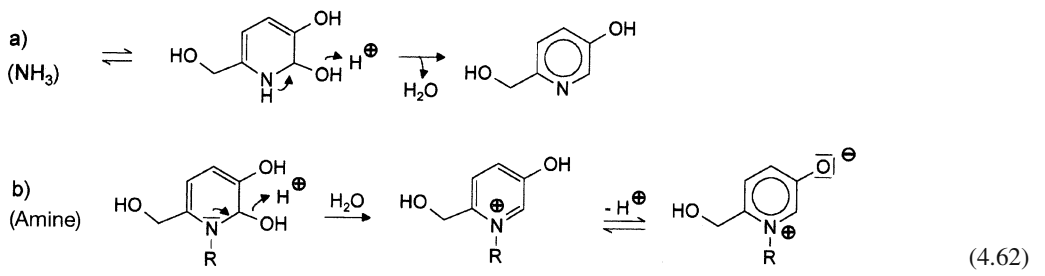
(4.59)



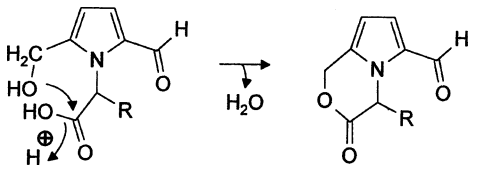
(4.60)



(4.61)

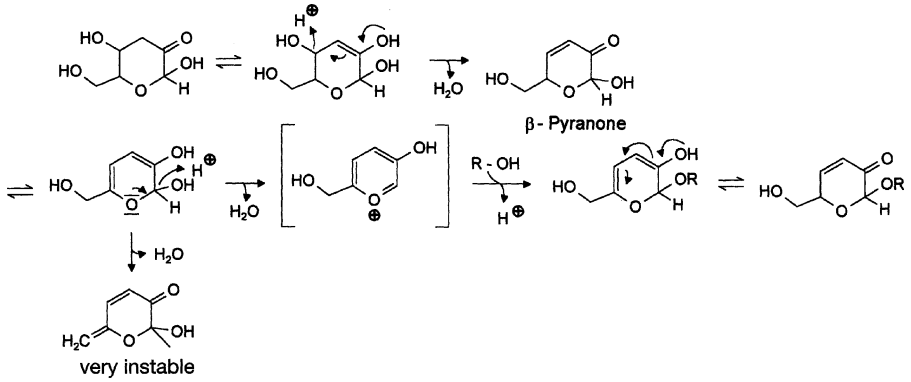


(4.62)

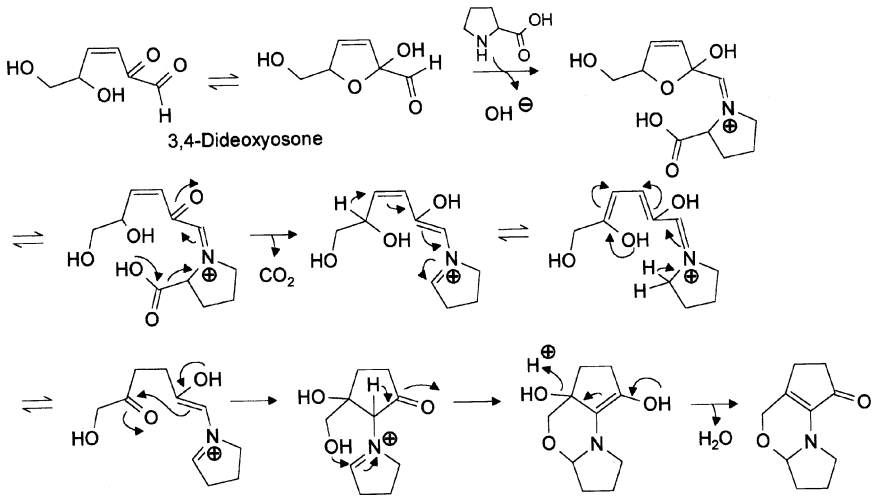


(4.63)

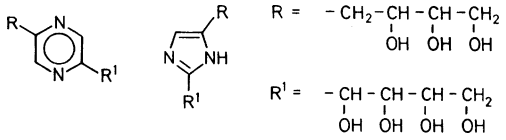
by reduction at C-1 of the carbohydrate (cf. Formula 4.56), all these compounds contain a methyl or acetyl group at position 2 of the furan or pyran derivatives. The product structures show that apart from the water elimination at C-1 leading to 1-deoxyosone, other dehydrations occurred at C-2, C-5 and/or C-6.



(4.64)



(4.65)

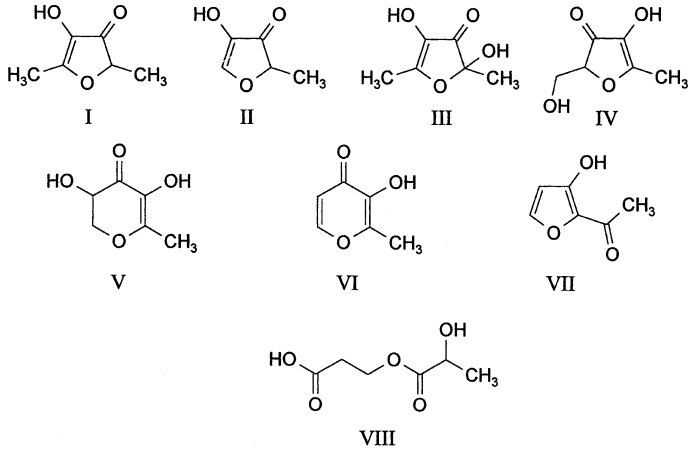


(4.66)

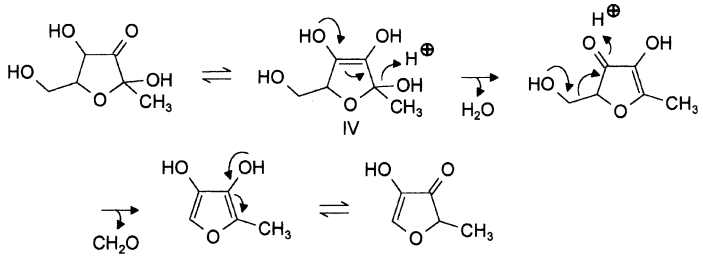
4.2.4.4.4 Secondary Products of 1-Deoxyosones

Unlike the 3-deoxyosones which have been studied for a long time, the 1-deoxyosones were detected only a few years ago. Formula 4.67 shows known compounds derived from 1-deoxyosone. Since 1-deoxyosones formally occur

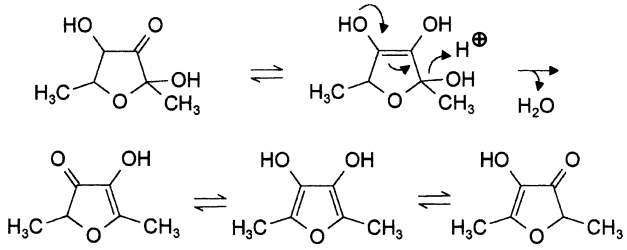
In the reaction yielding 3-hydroxy-5-hydroxymethyl-2-methyl-(5H)-furan-4-one (IV in Formula 4.67), this compound can be directly formed by water elimination from the furanoid hemiacetal of 1-deoxyosone (Formula 4.68). It was found that isomerization to the 4-hydroxy-3-oxo compound does not occur under the conditions relevant to food. On the other hand, it is interesting that significant degradation to norfuraneol occurs (Formula 4.68). Norfuraneol is also formed as the main reaction product on the degradtion of the 1-deoxyosones of pentoses.



(4.67)



(4.68)

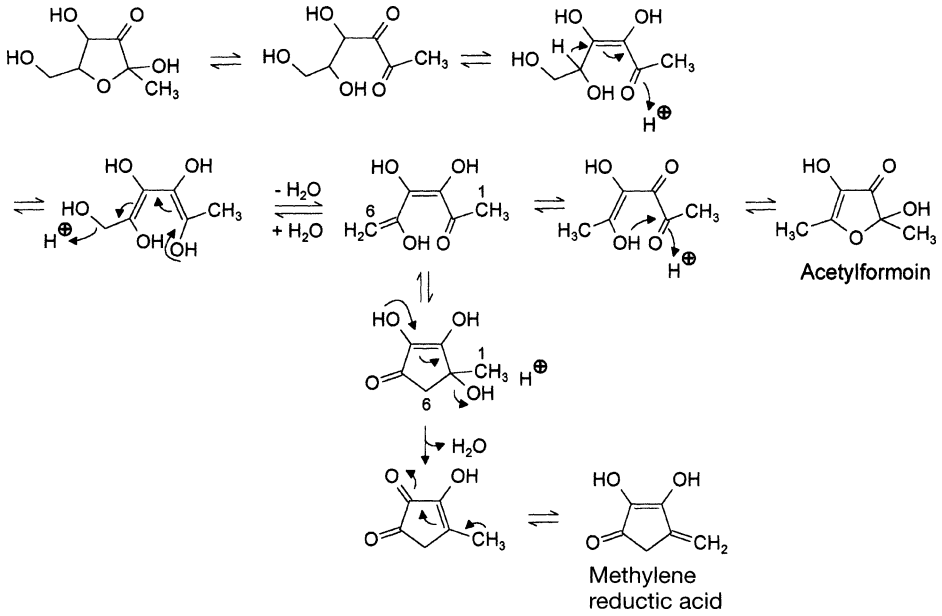


(4.69)

The compound 4-hydroxy-2,5-dimethyl-3(2H)-furanone (furaneol, I in Formula 4.67) is the corresponding degradation product from the 6-deoxy-L-mannose (rhamnose) (Formula 4.69). Furaneol can also be formed from hexose phosphates under reducing conditions (cf. 4.2.4.4.6) and from C-3 fragments (cf. Formula 4.47). With a relatively low odor threshold value, furaneol has an intensive caramel-like odor. It is interesting that furaneol is also biosynthesized in plants, e. g., in strawberries (cf. 18.1.2.6.9) and pineapples (cf. 18.1.2.6.10).

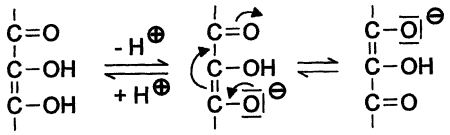
The formation of another degradation product of 1-deoxyosone, acetylformoin (III in Formula 4.67) is shown in Formula 4.70. In comparison with the formation of furanone in the synthesis of acetylformoin (cf. Formula 4.68), the water elimination at C-6 of the carbohydrate skeleton occurs *before* cyclization to the furan derivative. Although further water elimination is no longer easy, it is suggested to explain the formation of methylene reductic acid (Formula 4.70).

As a result of the presence of an enediol structure element in the α-position to the oxo function in the open-chain structures of acetylformoin, this compound belongs to the group of substances called reductones. Substances of this type, e. g., also vitamin C (ascorbic acid), are weakly acidic (Formula 4.71), reductive (Formula 4.72) and exhibit antioxidative properties. The latter are attributed to the possible formation of resonance-stabilized radicals (Formula 4.73) and also to the disproportionation of two radicals with

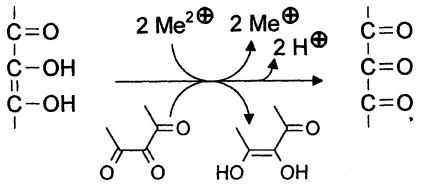


(4.70)

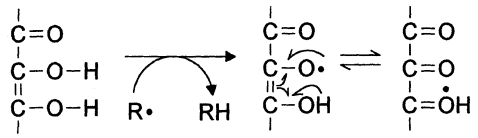
re-formation of the reductone structure (Formula 4.74). Reductones reduce $Ag^{\oplus}$, $Au^{3\oplus}$, $Pt^{4\oplus}$ to the metals, $Cu^{2\oplus}$ to $Cu^{\oplus}$, $Fe^{3\oplus}$ to $Fe^{2\oplus}$ and Br_2 or I_2 to $Br^{\ominus}$ or $I^{\ominus}$ respectively. Reductones are present as mono-anions at pH values <6. The di-anion occurring under alkaline conditions is easily oxidized in the presence of O_2.



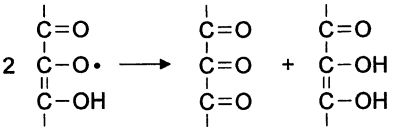
(4.71)



(4.72)



(4.73)

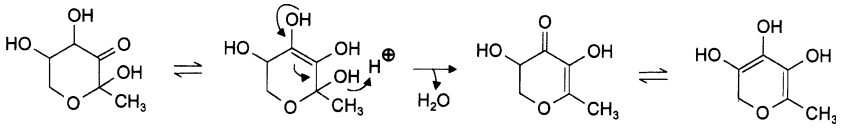


(4.74)

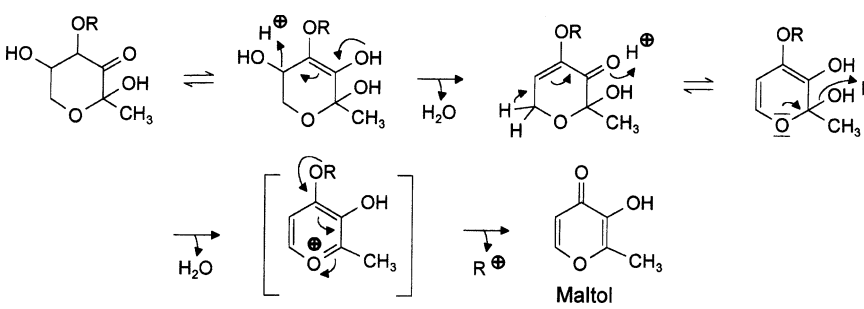
The compound 3,5-dihydroxy-2-methyl-5,6-dihydropyran-4-one (V in Formula 4.67) is also formed from the pyranoid hemiacetals of 1-deoxy-2,3-hexodiulose (Formula 4.75). In comparison, maltol is preferentially formed from disaccharides like maltose or lactose (Formula 4.76) and not from dihydroxypyranone by water elimination. The formation of maltol from monosaccharides is negligible. A comparison of the decomposition of 1-deoxyosones from the corresponding cyclic pyranone structure clearly shows (cf. Formula 4.75 and 4.76) that the glycosidically bound carbohydrate in the disaccharide directs the course of water elimination in another direction (Formula 4.76). It is the stabilization of the intermediates to quasi-aromatic maltol which makes possible the cleavage of the glycosidic bond with the formation of maltol. Parallel to the formation of maltol, isomaltol derivatives which still contain the second carbohydrate molecule are also formed from disaccharides (Formula 4.77). Indeed, the formation of free isomaltol is possible by the hydrolysis of the

glycosidic bond. β-Galactosyl-isomaltol was detected as the main product in heated milk. However, glucosyl-isomaltol is formed from maltose in much lower amounts. In this case, the formation of maltol dominates. The galactosyl residue clearly favors the formation of furanoid 1-deoxyosone from lactose, whereas the pyranoid 1-deoxyosone is preferentially formed from maltose (cf. Formula 4.76 and 4.77).

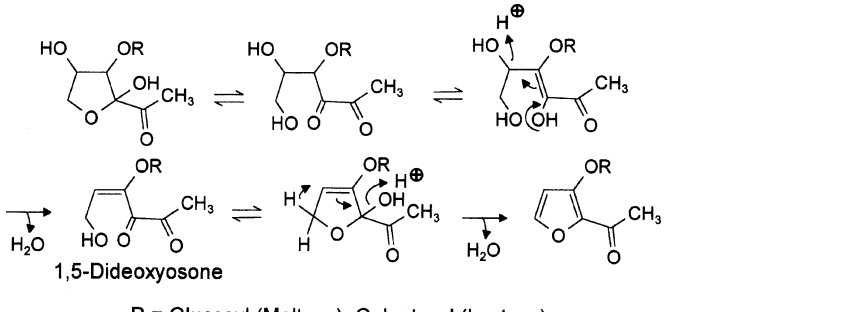
An open-chain compound, the lactic acid ester of β-hydroxypropionic acid (VIII in Formula 4.67) can also be formulated from 1-deoxyosone via 1,5-dideoxyosone. Hydration of this β-dicarbonyl compound and subsequent cleavage of the bond between C-2 and C-3 directly yield the lactic acid ester (Formula 4.78). Among the compounds mentioned, acetylformoin is one of the comparatively



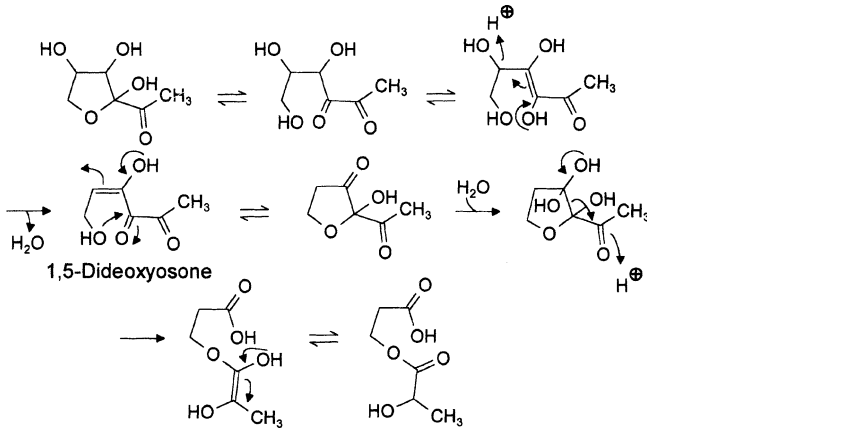
(4.75)



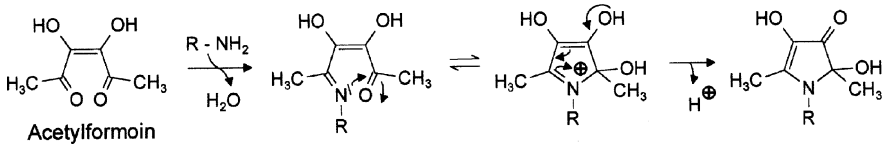
(4.76)



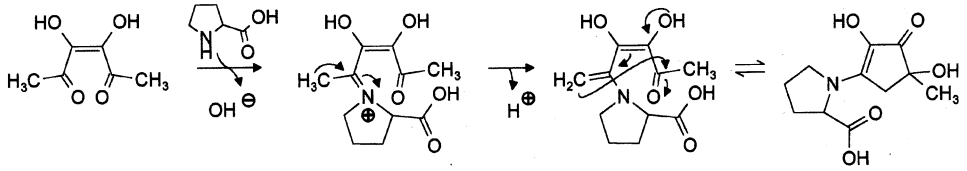
(4.77)



(4.78)



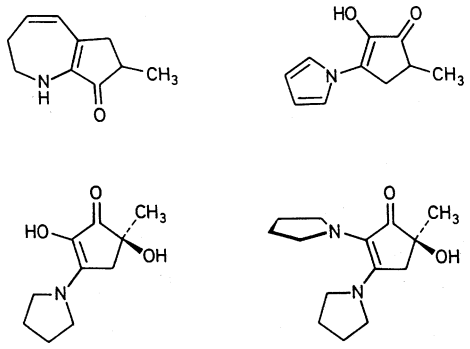
(4.79)



(4.80)

instable compounds which undergo reactions with other amino components. In the presence of mainly primary amines (amino acids), aminoreductones are formed (pyrrolinones, Formula 4.79) and in the presence of secondary amino acids, relatively stable carbocyclic compounds (Formula 4.80).

The following compounds were detected in reaction mixtures containing proline and hydroxyproline. Their formation must proceed via the 1-deoxyosones as well:

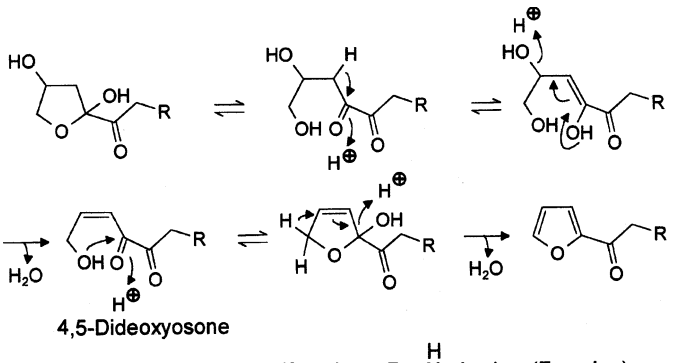


(4.81)

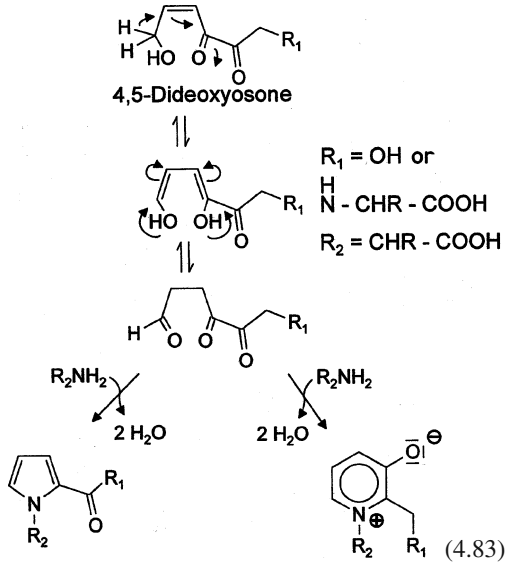
The pyrrolidino- and dipyrrolidinohexose reductones were characterized as bitter substances obtained from heated proline/saccharose mixtures (190 °C, 30 min, molar ratio 3:1; c_{sbi}: 0.8 and 0.03 mmol/l).

4.2.4.4.5 Secondary Products of 4-Deoxyosones

As shown in Formula 4.38, 2-hydroxyacetylfuran is one the reaction products of 4-deoxyosone. However, this compound is preferentially formed in carbohydrate degradation in the absence of amine components. If it is formed from the *Amadori* product in accordance with Formula 4.57, the amino acid remains in the reaction product, producing furosine (Formula 4.82). In the presence of higher concentrations of primary amines, the formation of 2-hydroxyacetylfuran (and also furosine) is significantly suppressed in favor of the corresponding pyrrole and pyridiniumbetaine (Formula 4.84). The reason for this is that the triketo structures formed from 4,5-dideoxyosone by enolization react with primary amines, amino acids or ammonia to give pyrrole and pyridine derivatives (Formula 4.83).

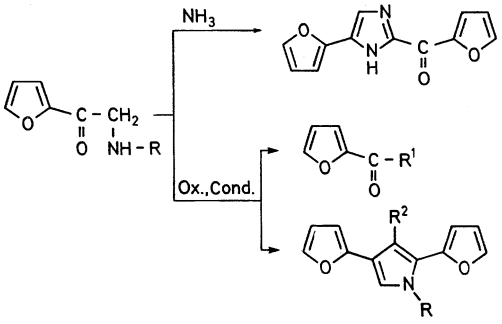


(4.82)

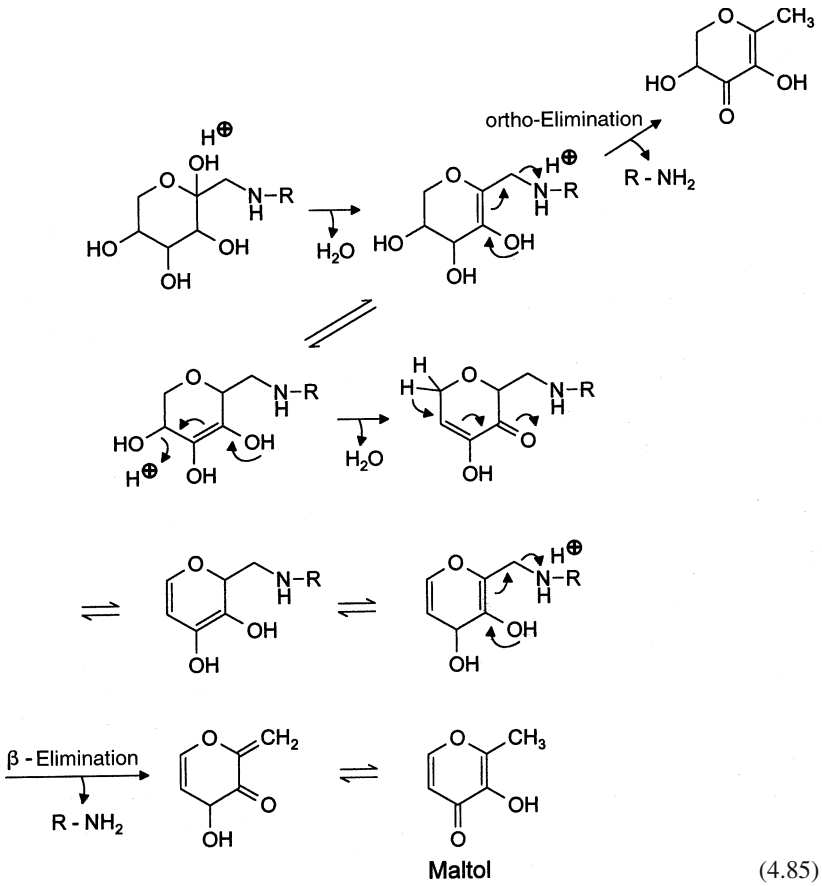


(4.83)

With ammonia, aminoacetylfuran is very easily converted to 2-(2-furoyl)-5-(2-furyl)-1 Himidazole, known as FFI, which was previously isolated from acid hydrolysates from protein/glucose reaction mixtures:



(4.84)



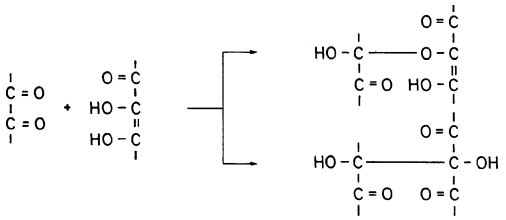
(4.85)

Various oxidation and condensation products (R^1 = OH, CONHR; R^2 = OH, NHR) were isolated from a heated, neutral solution. It can be assumed from the structures shown that protein cross-linkages are possible if, e. g., R_1 and R_2 represent the ε-amino group of lysine (Formula 4.83).

In conclusion, it should be mentioned here that more recent studies also assume the direct elimination of water from the cyclic hemiacetals. This is shown in Formula 4.85 for the direct formation of maltol from the *Amadori* product.

4.2.4.4.6 Redox Reactions

In the course of the *Maillard* reaction, deoxyosones and reductones, e. g., acetylformoin (cf. III, Formula 4.67), are formed. They can react to give enol and triketo compounds via an addition with disproportionation (Formula 4.86). Redox reactions of this type can explain the formation of products which are not possible according to the reactions described till now. In fact, it has recently been found that, for example, glucose 6-phosphate and fructose-1,6-diphosphate, which occur in baker's yeast and muscle, form 4-hydroxy-2,5-dimethyl-3(2H)-furanone to a large extent. Since the formation from hexoses (or hexose phosphates) is not explainable, reduction of the intermediate acetylformoin (Formula 4.87) must have occurred. As shown, this reduction can proceed through acetylformoin itself or other reductones, e. g., ascorbic acid. Such redox reactions also play an important role in the formation of the aroma substances 2-acetyl-1-pyrroline and 2-acetyltetrahydropyridine, which have been shown to exhibit slight oxidizability of α-eneaminols (cf. 5.3.1.6). Furthermore, such processes also play a part in the formation of carboxymethyllysine (cf. 4.2.4.4.9).

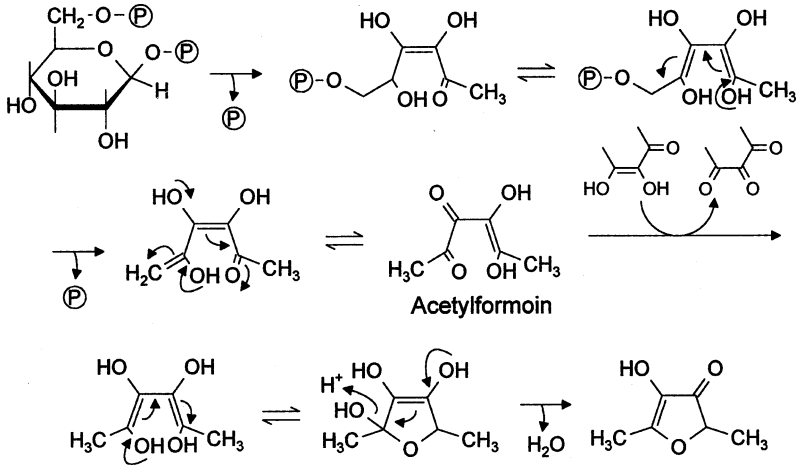


(4.86)

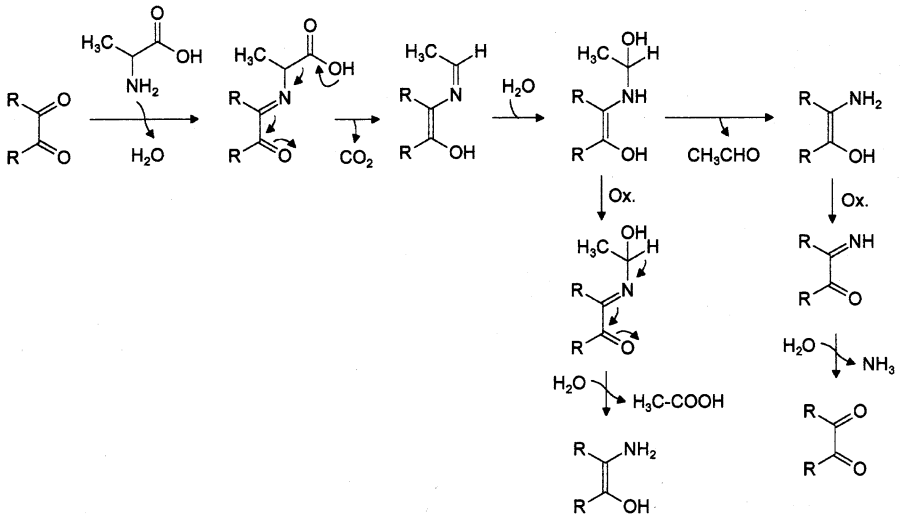
4.2.4.4.7 *Strecker* Reaction

The reactions between α-dicarbonyl compounds, like the deoxyosones obtained in the *Maillard* reaction, and amino acids are classed under the term *Strecker* reaction. This reaction leads to the formation of aldehydes (*Strecker* aldehydes), CO_2 and α-aminoketones on oxidative decarboxylation of the α-amino acids (Formula 4.88). It occurs in foods at higher concentrations of free amino acids and under more drastic reaction conditions, e. g., at higher temperatures or under pressure.

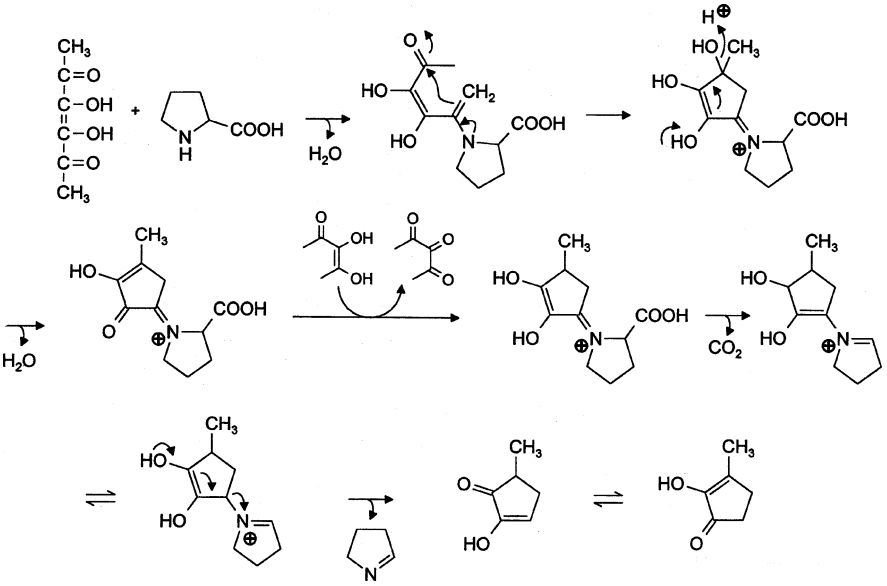
The aldehydes, which have one C-atom less than the amino acids, possess a considerable aroma potential, depending on the amino acid degraded.



(4.87)



(4.88)



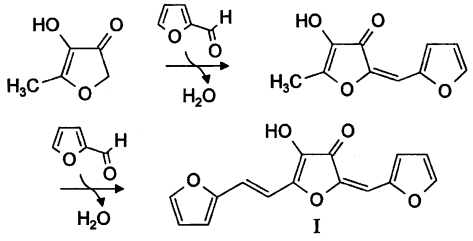
(4.89)

Strecker aldehydes which are important for their aroma are methional, phenylacetaldehyde, 3- and 2-methylbutanal and methylpropanal (cf. 5.3.1.1). Other compounds which are formed via the *Strecker* degradation and influence the aroma of food are H_2S, NH_3, 1-pyrroline (cf. 5.3.1.6) and cysteamine (cf. 5.3.1.4). Recently, the corresponding *Strecker* acids have also been found, especially in the presence of oxygen. They can be formed via the oxidation of the intermediate eneaminol (Formula 4.88). All the α-dicarbonyl compounds obtained on carbohydrate degradation as well as the reductones can undergo *Strecker* reactions. The product spectrum is significantly increased due to the redox reactions of the resulting intermediates. The complex course of a *Strecker* reaction is represented in Formula 4.89 with the formation of 2-hydroxy-3-methyl-cyclopent-3-enone. Apart from the pathway shown in Formula 4.46, this reaction is also of importance. When amino acids with functional groups in the side chain are involved, even more complex reactions are possible (cf. 5.3.1.4–5.3.1.8).

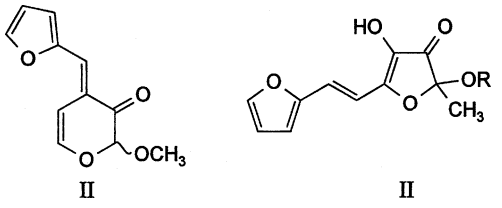
4.2.4.4.8 Formation of Colored Compounds

As a result of the mostly brown colors (bread crust, meat) formed by the heating of reducing carbohydrates with amine components, the *Maillard* reaction is also called nonenzymatic browning. Clinical biochemical studies have recently shown that these browning products partly exhibit antimutagenic and anticarcinogenic properties. As a result of the complex course of the reaction, however, it has only rarely been possible to identify colored compounds till now. One of the first colored compounds identified in model reactions of xylose/amines and furfural/norfuraneol is compound I in Formula 4.90. It is under discussion that this compound is formed via condensation reactions of the CH-acidic compound norfuraneol with the aldehyde group of furfural. Similar condensation reactions of 3-deoxyosone with furfural and of acetylformoin with furfural in model reactions led to the formation of the yellow products II and III (Formula 4.91).

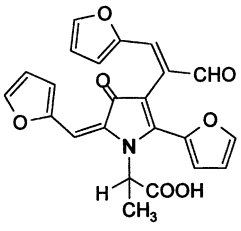
However, both compounds could be stabilized only as the full acetal, e. g., in alcoholic solutions. In general, it is assumed today that condensation reactions between nucleophilic/electrophilic intermediates of the *Maillard* reaction result in the formation of the colored components, which are also called *melanoidins*.



(4.90)

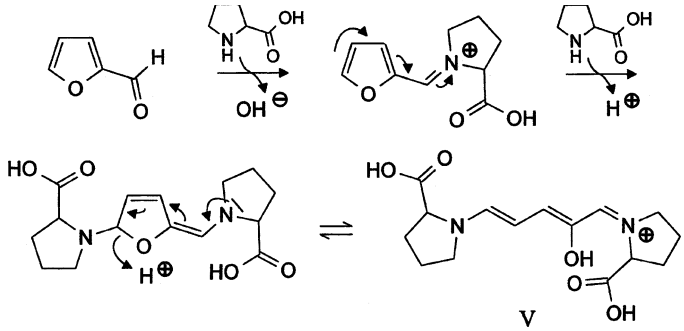


(4.91)

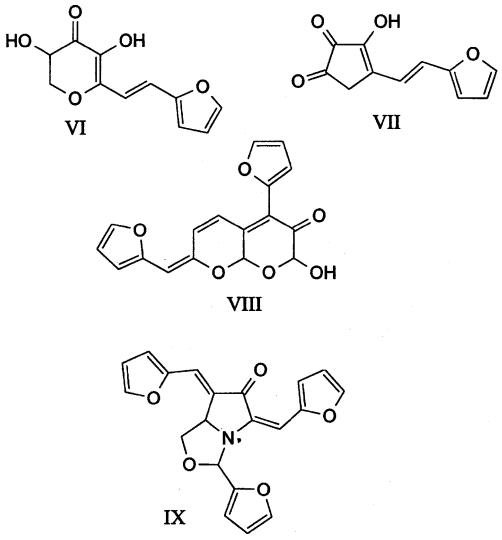


(4.92)

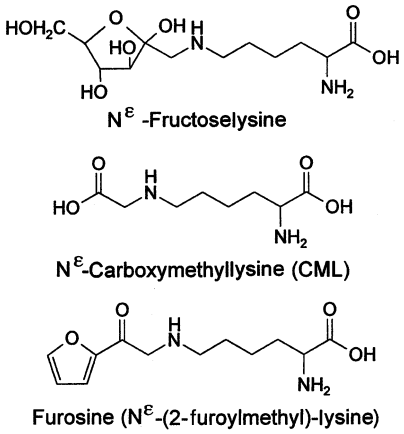
A red colored pyrroline dye (IV, Formula 4.92) could be identified in a model reaction of furfural and alanine. This dye is formed from 4 molecules of furfural and 1 molecule of alanine. Labeling experiments with ^{13}C showed that one open-chain molecule of furfural is inserted into the pyrrolinone structure. The proline/furfural reaction system indicated further that ring opening proceeds via a cyanine dye with the structure illustrated in V, Formula 4.93. Other colored compounds could be obtained by the condensation of 3,5-dihydroxy-2-methyl-5,6-dihydropyran-4-one with furfural (VI, Formula 4.94) and of 3-hydroxy-4-methyl-3-cyclopenten-1,2-dione (methylene reductic acid) with furfural (VII, Formula 4.94). Both dyes were also obtained by heating the *Amadori* product of proline and glucose in the presence of furfural. The orange colored compound VIII and the red compound IX were identified in a reaction system containing xylose/alanine/furfural (Formula 4.94).

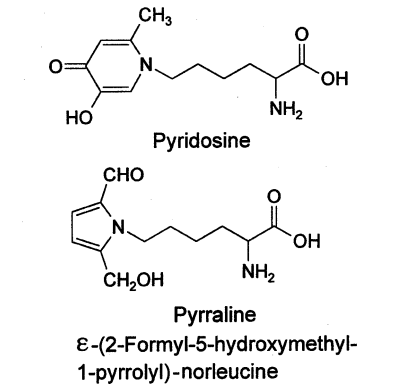


(4.93)



(4.94)





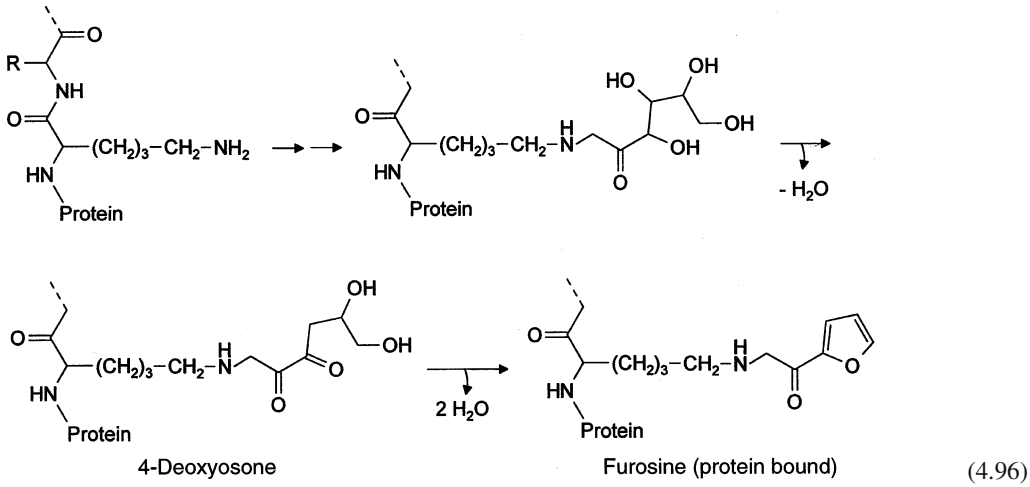
(4.95)

4.2.4.4.9 Protein Modifications

The side chains of proteins can undergo post-translational modification in the course of thermal processes. The reaction can also result in the formation of protein cross-links. A known reaction which mainly proceeds in the absence of carbohydrates, for example, is the formation of dehydroalanine from serine, cysteine or serine phosphate by the elimination of H_2O, H_2S or phosphate. The dehydroalanine can then lead to protein cross-links with the nucleophilic side chains of lysine or cysteine (cf. 1.4.4.11). In the presence of carbohydrates or their degradation products, especially the side chains of lysine and arginine are subject to modification, which is accompanied by a reduction in the nutritional value of the proteins. The structures of important lysine modifications are summarized in Formula 4.95. The best known compounds are the *Amadori* product N^{ε}-fructoselysine and furosine, which can be formed from the former compound via the intermediate 4-deoxyosone (Formula 4.96). To detect of the extent of heat treatment, e. g., in the case of heat treated milk products, furosine is released by acid hydrolysis of the proteins and quantitatively determined by amino acid analysis. In this process, all the intermediates which lead to furosine are degraded and an unknown portion of already existing furosine is destroyed. Therefore, the hydrolysis must occur under standardized conditions or preferably by using enzymes. Examples showing the concentrations of furosine in food are presented in Table 4.13.

Table 4.13. Concentration of furosine in heated milk products

Product	Furosine (mg/kg protein)
Raw milk	35–55
Milk (pasteurized)	48–75
Milk (ultrahigh heated)	500–1800
Sterile milk	5000–12,000
Milk powder	1800–12,000
Baby food (powder)	9300–18,900
Noodles	400–8500
Bakery products	200–6000



(4.96)

Pyridosine (Formula 4.95) is also formed by the degradation of N^{ε}-fructoselysine, but in lower amounts than furosine (ratio ca. 3:1). It is assumed that 1-deoxyosone is the precursor.

Carboxymethyllysine is also formed from N^{ε}-fructoselysine and is also used as an indicator of the degree of thermal treatment of protein containing foods. This compound can be produced in different ways starting with oxidized N^{ε}-fructoselysine or the reaction of glyoxal with the lysine side chain. The reaction path shown in Formula 4.97 takes into account general mechanisms of carbohydrate degradation with cleavage of β-dicarbonyl compounds.

Pyrraline (Formula 4.95) is also formed as a modification of the amino acid lysine in proteins. The reaction partner is, according to Formula 4.61, 3,4- dideoxyosone obtained from 4-deoxyosone. Pyrraline is found in high concentrations especially in foods that have been subjected to strong thermal treatment, e. g., biscuits and pastries (Table 4.14). The concentrations in milk are clearly less than those of furosine (Table 4.13). The cross-linkage of proteins is also possible via the pyrrole residues of 2 molecules of pyrraline. The corresponding dimers have already been detected in model reactions. (cf. Formula 4.100).

The amino acid arginine can also be modified at the guanidino group, e. g., by reaction with α-dicarbonyl compounds from carbohydrate degradation. The compounds characterized were, among others, those formed from the reaction with methylglyoxal (I, Formula 4.98), 3-deoxyosone (II, Formula 4.98), a pentandione (III, Formula 4.98) and glyoxal (IV, Formula 4.98). The synthesis of GLARG is displayed in Formula 4.99. It is interesting that glyoxal reacts with the N-atoms 1 and 2 of the guanidino group (Formula 4.99), whereas methylglyoxal bridges N-2 and N-3.

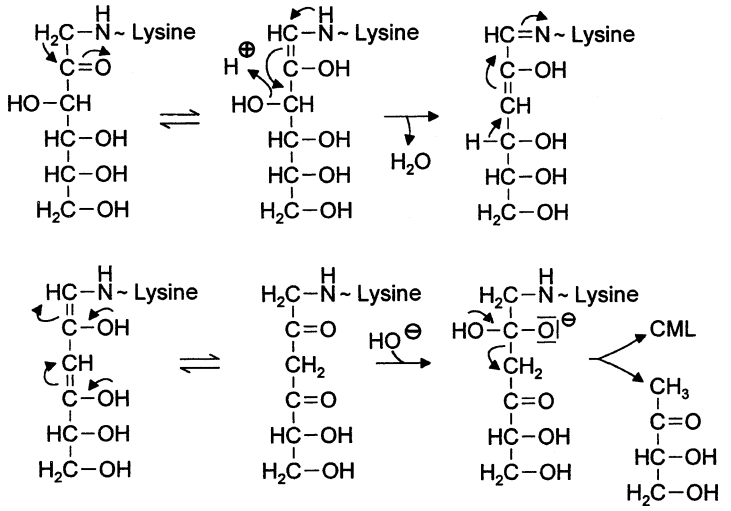
Among the identified compounds, only ornithino-imidazolinone (I, Formula 4.98) has been quanti-

Table 4.14. Concentrations of pyrraline in foods

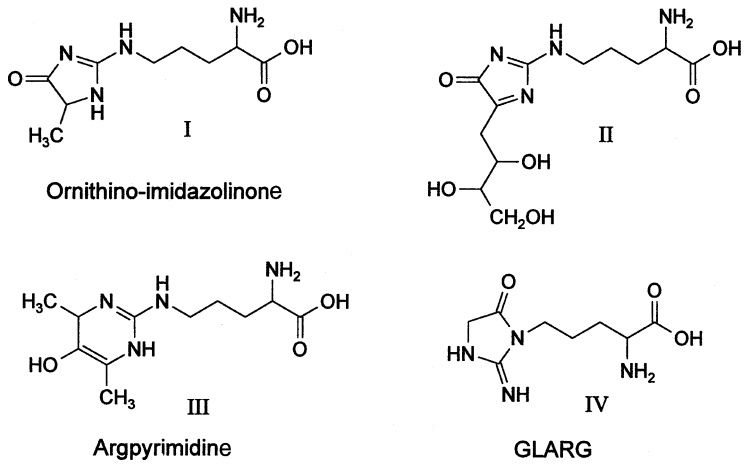
Foods	Pyrraline (mg/kg protein)
UHT milk	<2–5
Sterile milk	60–80
Condensed milk	30–135
Pretzels	220–230
White bread crust	540–3680
White bread crumb	25–110
Nibbling biscuits	970–1320

Table 4.15. Concentrations of ornithino-imidazolinone (OIZ) in foods

Foods	OIZ (mg/kg protein)	Arginine loss (%)
Alkali-baked products	9000–13,000	20–30
Pretzel crust	25,000–28,000	60–70
Coffee beans (roasted)	7000–9000	20–25
Nibbling biscuits	6000–20,000	15–40

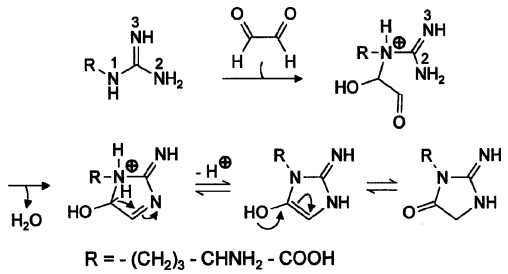


(4.97)



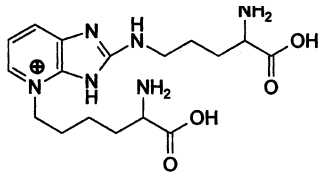
(4.98)

tatively determined in different foods. The data (Table 4.15) show that especially in alkali-baked products, about 60–70% of the arginine present in the flour reacts to imidazolinone.

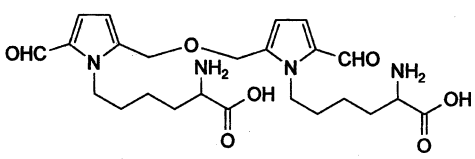


(4.99)

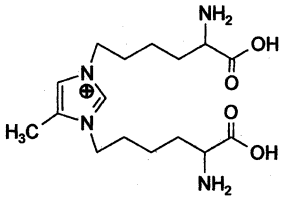
Apart from the modification of amino acid side chains in individual protein strands, cross-linkage of two protein chains can also occur. Some of the structures are shown in Formula 4.100. Pentosidine was first found in physiological protein. It strongly fluoresces and is formed by bridging an arginine residue with a lysine residue via a pentose. The concentrations of pentosidine in food are comparatively low (Table 4.16). The formation of pentosidine is assumed to be as shown in Formula 4.101. Formation of the *Amadori* product with the ε-amino group of lysine is followed by water elimination at C-2 and C-3 of pentose with the formation of the 4,5-diulose, which condenses with the



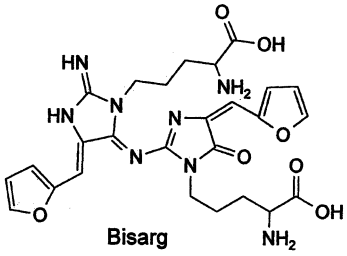
Pentosidine



Bis-pyrraline

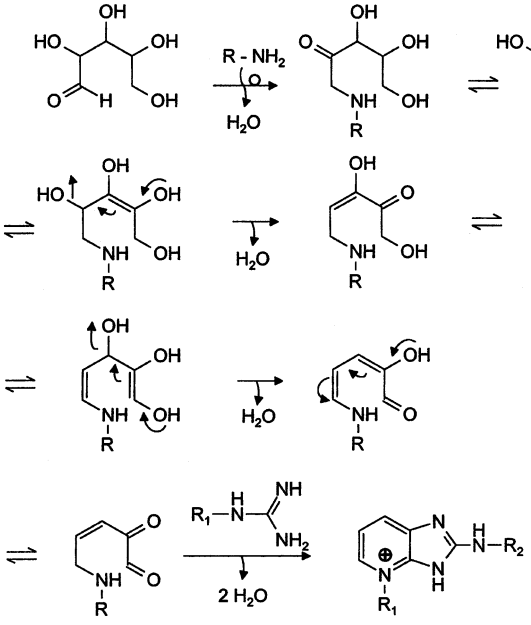


MOLD (Methylglyoxal-derived lysine dimer)



Bisarg

(4.100)



R : Lysine
R_1 : Arginine

(4.101)

Table 4.16. Concentrations of pentosidine in foods

Foods	Concentration (mg/kg protein)
Sterile milk	0.1–2.6
Condensed milk	0.3–0.6
Bread crust	0.4–2.6
Salt pretzel	9.3–22.8
Roasted coffee	10.8–39.9

guanidino group of arginine. The recently identified compound Bisarg (Formula 4.100) is a condensation product of 2 molecules each of arginine, glyoxal and furfural. Apart from the protein cross-linking properties, the intensive brown-orange color of Bisarg should be mentioned.

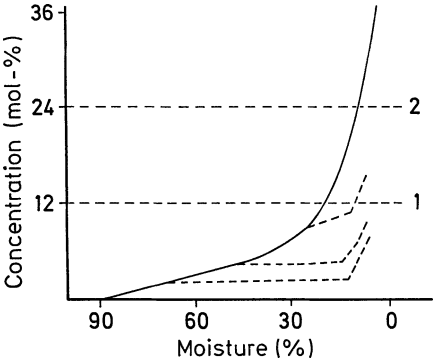


Fig. 4.10. Increase of *Amadori* compounds in two stage air drying of carrots as influenced by carrot moisture content. — 10, 20, 30 min at 110 °C; – – – 60 °C; sensory assessment: 1) detection threshold 2) quality limit (according to *Eichner* and *Wolf*, in *Waller* and *Feather*, 1983)

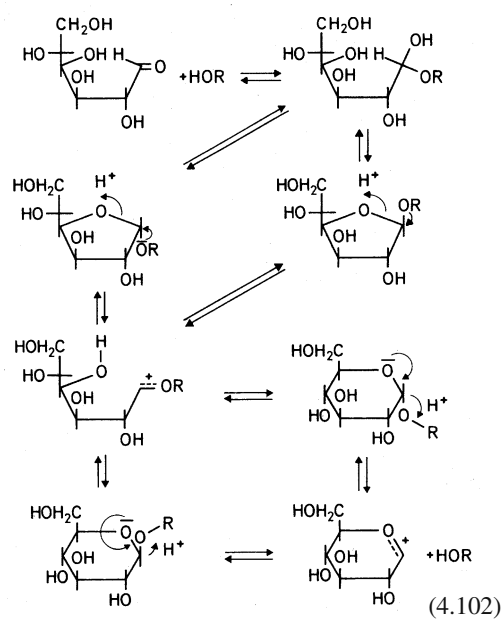
4.2.4.4.10 Inhibition of the Maillard Reaction

Measures to inhibit the *Maillard* reaction in cases where it is undesirable involve lowering of the pH value, maintenance of lowest possible temperatures and avoidance of critical water contents (cf. 0.3.2) during processing and storage, use of nonreducing sugars, and addition of sulfite. Figure 4.10 demonstrates by the example of carrot dehydration the advantages of running a two-stage process to curtail the *Maillard* reaction.

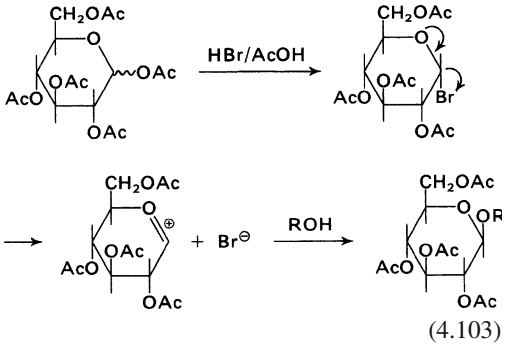
4.2.4.5 Reactions with Hydroxy Compounds (O-Glycosides)

The lactol group of monosaccharides heated in alcohol in the presence of an acid catalyst is substituted by an alkoxy or aryloxy group, denoted as an aglycone (*Fischer* synthesis), to produce alkyl- and arylglycosides. It is assumed that the initial reaction involves the open form. With the majority of sugars, the furanosides are formed in the first stage of reaction. They then equilibrate with the pyranosides. The transition from furanoside to pyranoside occurs most probably through an open carboxonium ion, whereas pyranoside isomerization is through a cyclic one (cf. Reaction 4.102). Furanosides are obtainable by stopping the reaction at a suitable time. The equilibrium state in alcohol is, as in water, dependent on conformational factors. The alcohol as solvent and its R-moiety both increase the anomeric effect and thus α-pyranoside becomes a more favorable form than was α-pyranose in aqueous free sugar solutions (Table 4.7). In the system D-glucose/methanol in the presence of 1% HCl, 66% of the methylglucoside is present as α-pyranoside, 32.5% as β-pyranoside, and only 0.6% and 0.9% are in α- and β-furanoside forms. Under the same conditions, D-mannose and D-galactose are 94% and 58% respectively in α-pyranoside forms.

A highly stereospecific access to glycosides is possible by C-1 bromination of acetylated sugars.



(4.102)

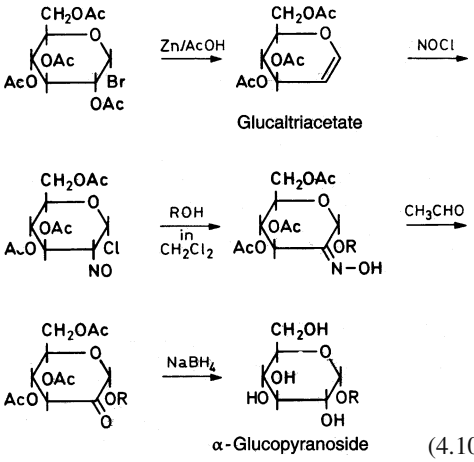


(4.103)

In the reaction of peracetylated sugar with HBr, due to the strong anomeric effect, α-halogenide is formed almost exclusively (cf. Formula 4.103).

This then reacts, probably through its glycosyl cation form. Due to the steric influence of the acetylated group on C-2, the 1,2-trans-glycoside is preferentially obtained, e. g., in the case of D-glucose, β-glucoside results.

Acetylglycosyl halogenides are also used for a highly stereoselective synthesis of α-glycosides. The compound is first dehalogenated into a glycal. Then, addition of nitrosylchloride follows, giving rise to 2-deoxy-2-nitroso-glycosylchloride. The latter, in the presence of alcohol, eliminates HCl and provides a 2-deoxy-2-oximino-α-glycoside. Reaction with ethanal yields the 2-oxo compound, which is then reduced to α-glycoside:

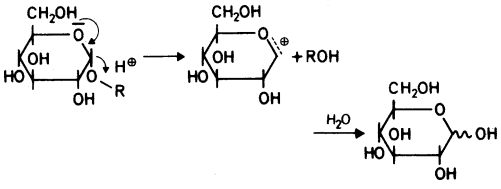


(4.104)

O-Glycosides are widely distributed in nature and are the constituents, such as glycolipids, glycoproteins, flavanoid glycosides or saponins, of many foods.

O-Glycosides are readily hydrolyzed by acids. Hydrolysis by alkalies is achieved only under drastic conditions which simultaneously decompose monosaccharides.

The acid hydrolysis is initiated by glycoside protonation. Alcohol elimination is followed by addition of water:



(4.105)

Table 4.17. Relative rate of hydrolysis of glycosides (a: 2 mol/l HC1, 60 °C; b: 0.5 mol/l HCl, 75 °C)

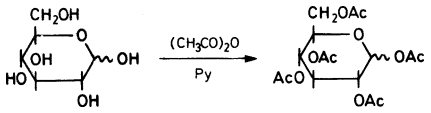
Compound	Hydrolysis condition	K_{rel}
Methyl-α-D-glucopyranoside	a	1.0
Metyhl-β-D-glucopyranoside	a	1.8
Phenyl-α-D-glucopyranoside	a	53.7
Phenyl-β-D-glucopyranoside	a	13.2
Methyl-α-D-glucopyranoside	b	1.0
Methyl-β-D-glucopyranoside	b	1.9
Methyl-α-D-mannopyranoside	b	2.4
Methyl-β-D-mannopyranoside	b	5.7
Methyl-α-D-galactopyranoside	b	5.2
Methyl-β-D-galactopyranoside	b	9.2

The hydrolysis rate is dependent on the aglycone and the monosaccharide itself. The most favored form of alkylglycoside, α-pyranoside, usually is the isomer most resistant to hydrolysis. This is also true for arylglycosides, however, due to steric effects, the β-pyranoside isomer is synthesized preferentially and so the β-isomer better resists hydrolysis.

The influence of the sugar moiety on the rate of hydrolysis is related to the conformational stability. Glucosides with high conformational stability are hydrolyzed more slowly (cf. data compiled in Table 4.17).

4.2.4.6 Esters

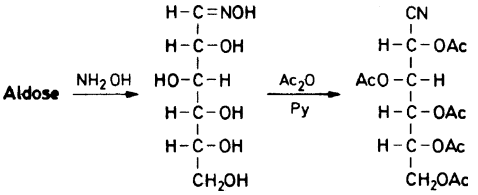
Esterification of monosaccharides is achieved by reaction of the sugar with an acyl halide or an acid anhydride. Acetylation, for instance with acetic anhydride, is carried out in pyridine solution:



(4.106)

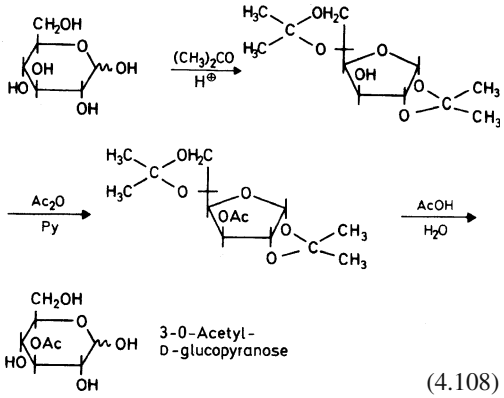
Acyl groups have a protective role in some synthetic reactions. Gluconic acid nitrile acetates (aldonitrile acetates) are analytically suitable sugar derivatives for gas chromatographic separation and identification of sugars. An advantage

of these compounds is that they simplify a chromatogram since there are no anomeric peaks:



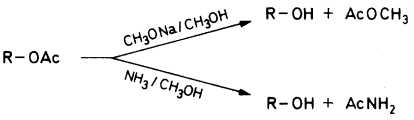
(4.107)

Selective esterification of a given HO-group is also possible. For example, glucose can be selectively acetylated in position 3 by reacting 1,2,5,6-di-O-isopropylidene-α-D-glucofuranose with acetic acid anhydride, followed by hydrolysis of the diketal:



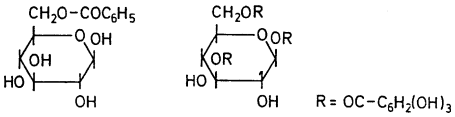
(4.108)

Hydrolysis of acyl groups can be achieved by interesterification or by an ammonolysis reaction:



(4.109)

Sugar esters are also found widely in nature. Phosphoric acid esters are important intermediary products of metabolism, while sulfuric acid esters are constituents of some polysaccharides. Examples of organic acid esters are vacciniin in blueberry (6-benzoyl-D-glucose) and the tannintype compound, corilagin (1,3,6-trigalloyl-D-glucose):



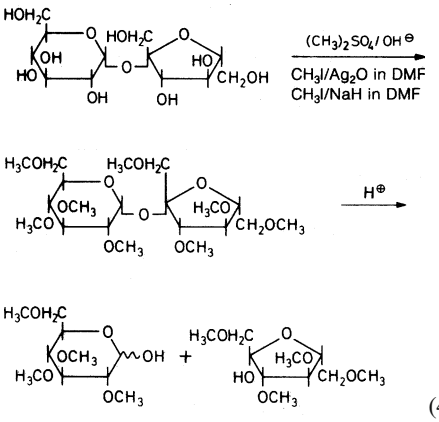
(4.110)

Sugar esters or sugar alcohol esters with long chain fatty acids (lauric, palmitic, stearic and oleic) are produced industrially and are very important as surface-active agents. These include sorbitan fatty acid esters (cf. 8.15.3.3) and those of saccharose (cf. 8.15.3.2), which have diversified uses in food processing.

4.2.4.7 Ethers

Methylation of sugar HO-groups is possible using dimethylsulfate or methyliodide as the methylating agent. Methyl ethers are of importance in analysis of sugar structure since they provide data about ring size and linkage positions.

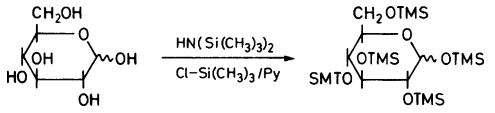
Permethylated saccharose, for example, after acid hydrolysis provides 2,3,4,6-tetra-O-methyl-D-glucose and 1,3,4,6-tetra-O-methyl-D-fructose. This suggests the presence of a $1,2'$-linkage between the two sugars and the pyranose and furanose structures for glucose and fructose, respectively:



(4.111)

Trimethylsilyl ethers (TMS-ethers) are unstable against hydrolysis and alcoholysis, but have remarkable thermal stability and so are suitable for gas chromatographic sugar analysis. Treatment of a sugar with hexamethyldisilazane and

trimethylchlorosilane, in pyridine as solvent, provides a sugar derivative with all HO-groups silylated:



(4.112)

4.2.4.8 Cleavage of Glycols

Oxidative cleavage of vicinal dihydroxy groups or hydroxy-amino groups of a sugar with lead tetraacetate or periodate is of importance for structural elucidation. Fructose, in a 5-membered furanose form, consumes 3 moles of periodate (splitting of each α-glycol group requires 1 mole of oxidant) while, in a pyranose ring form, it consumes 4 moles of periodate.

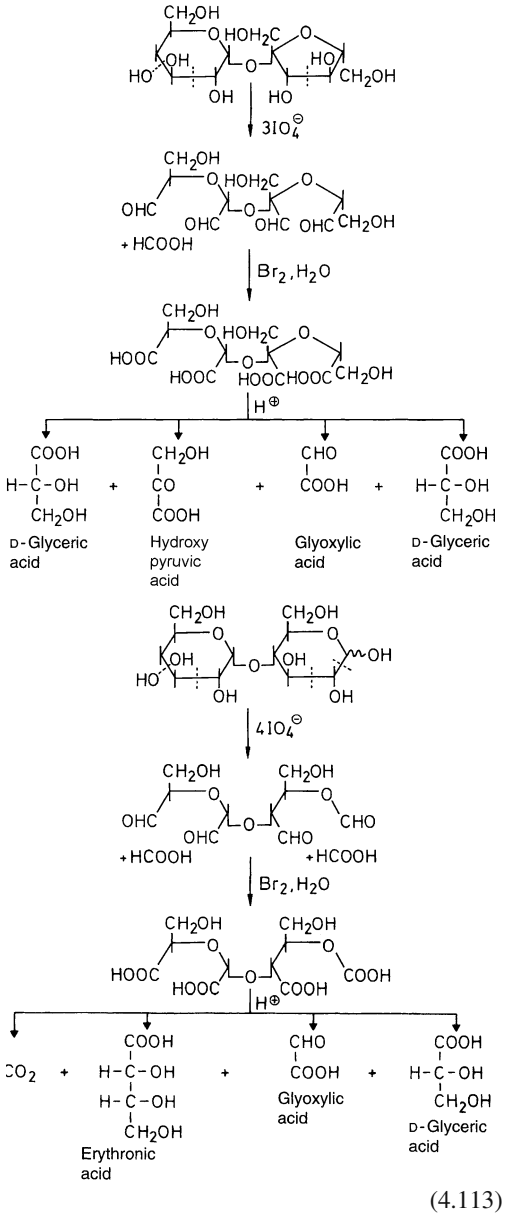
Saccharose consumes 3 moles (cf. Reaction 4.113) and maltose 4 moles of periodate. The final conclusion as to sugar linkage positions and ring structure is drawn from the periodate consumption, the amount of formic acid produced (in the case of saccharose, 1 mole; maltose, 2 moles) and the other carbonyl fragments which are oxidized additionally by bromine to stable carboxylic acids and then released by hydrolysis. The glycol splitting reaction should be considered an optional or complementary method to the permethylation reaction applied in structural elucidation of carbohydrates.

4.3 Oligosaccharides

4.3.1 Structure and Nomenclature

Monosaccharides form glycosides (cf. 4.2.4.5). When this occurs between the lactol group of one monosaccharide and any HO-group of a second monosaccharide, a disaccharide results.

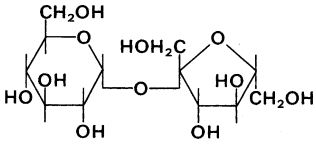
Compounds with up to about 10 monosaccharide residues are designated as oligosaccharides. When a glycosidic linkage is established only between the lactol groups of two monosaccharides, then a *nonreducing disaccharide* is formed, and when one lactol group and one alcoholic HO-group are involved, a *reducing disaccharide* results. The former is denoted as a glycosylglycoside, the latter as a glycosylglycose, with additional data for linkage direction and positions. Examples are saccharose and maltose:



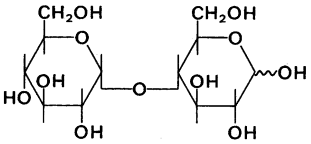
(4.113)

An abbreviated method of nomenclature is to use a three letter designation or symbol for a monosaccharide and suffix *f* or *p* for furanose or pyranose. For example, saccharose and maltose

can be written as O-β-D-Fru$f(2 \rightarrow 1)$α-D-Glc$p$ and O-α-D-Glc$p(1 \rightarrow 4)$D-Glc$p$, respectively.

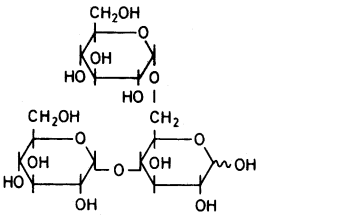


β-D-Fructofuranosyl-α-D-glucopyranoside (saccharose)



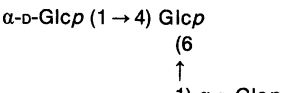
O-α-D-Glucopyranosyl-(1 → 4)-D-glucopyranose (maltose) (4.114)

Branching also occurs in oligosaccharides. It results when one monosaccharide is bound to two glycosyl residues. The name of the second glycosyl residue is inserted into square brackets. A trisaccharide which represents a building block of the branched chain polysaccharides amylopectin and glycogen is given as an example:



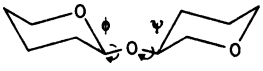
O-α-D-Glucopyranosyl-(1→4)-O-[α-D-glucopyranosyl-(1→6)]-D-glucopyranose (4.115)

The abbreviated formula for this trisaccharide is as follows:



(4.116)

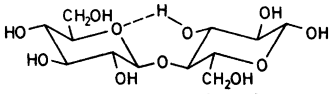
The conformations of oligo- and polysaccharides, like peptides, can be described by providing the angles Φ and ψ:



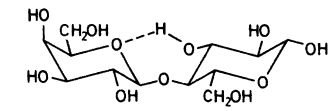
(4.117)

A calculation of conformational energy for all conformers with allowed Φ, ψ pairs provides a Φ, ψ diagram with lines corresponding to iso-conformational energies. The low-energy conformations calculated in this way agree with data obtained experimentally (X-ray diffraction, NMR, ORD) for oligo- and polysaccharides.

H-bonds fulfill a significant role in conformer stabilization. Cellobiose and lactose conformations are well stabilized by an H-bond formed between the HO-group of C-3 in the glucose residue and the ring oxygen of the glycosyl residue. Conformations in aqueous solutions appear to be similar to those in the crystalline state:

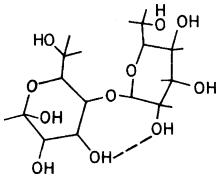


O-β-D-Glucopyranosyl-(1→4)-D-glucopyranose (cellobiose)

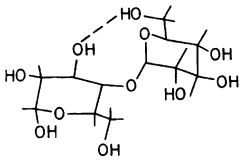


O-β-D-Galactopyranosyl-(1→4)-D-glucopyranose (lactose) (4.118)

In crystalline maltose and in nonaqueous solutions of this sugar, a hydrogen bond is established between the HO-groups on C-2 of the glucosyl and on C-3 of the glucose residues (4.119). However, in aqueous solution, a conformer partially present is stabilized by H-bonds established between the CH_2OH-group of the glucosyl residue and the HO-group of C-3 on the glucose residue (4.120). Both conformers correspond to the two energy minima in the Φ, ψ diagram.



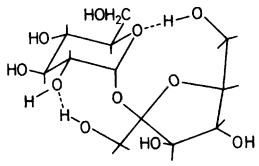
(4.119)



(4.120)

Two H-bonds are possible in saccharose, the first between the HO-groups on the C-1 of the fruc-

tose and the C-2 of the glucose residues, and the second between the HO-group on the C-6 of the fructose residue and the ring oxygen of the glucose residue:



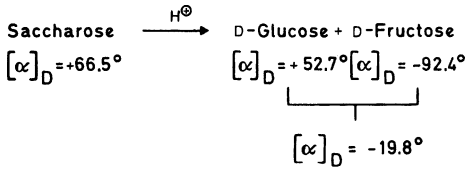
β -D-Fructofuranosyl -α-D-glucopyranoside (saccharose)

(4.121)

4.3.2 Properties and Reactions

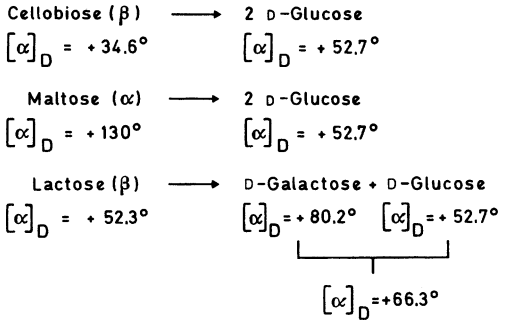
The oligosaccharides of importance to food, together with data on their occurrence, are compiled in Table 4.18. The physical and sensory properties were covered with monosaccharides, as were reaction properties, though the difference between reducing and nonreducing oligosaccharides should be mentioned. The latter do not have a free lactol group and so lack reducing properties, mutarotation and the ability to react with alcohols and amines.

As glycosides, oligosaccharides are readily hydrolyzed by acids, while they are relatively stable against alkalies. Saccharose hydrolysis is denoted as an inversion and the resultant equimolar mixture of glucose and fructose is called invert sugar. The term is based on a change of specific rotation during hydrolysis. In saccharose the rotation is positive, while it is negative in the hydrolysate, since D-glucose rotation to the right (hence its name dextrose) is surpassed by the value of the left-rotating fructose (levulose):



(4.122)

Conclusions can be drawn from mutarotation, which follows hydrolysis of reducing disaccharides, about the configuration on the anomeric C-atom. Since the α-anomer has a higher specific rotation in the D-series than the β-anomer, cleavage of β-glycosides increases the specific rotation while cleavage of α-glycosides decreases it:

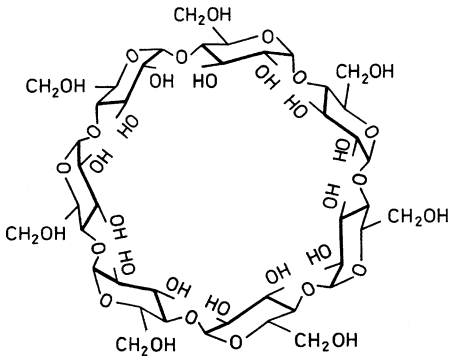


(4.123)

Enzymatic cleavage of the glycosidic linkage is specified by the configuration on anomeric C-1 and also by the whole glycosyl moiety, while the aglycone residue may vary within limits.

The methods used to elucidate the linkage positions in an oligosaccharide (methylation, oxidative cleavage of glycols) were outlined under monosaccharides.

The cyclodextrins listed in Table 4.18 are prepared by the action of cyclomaltodextrin glucanotransferase (E. C. 2.4.1.19), obtained from *Bacillus macerans*, on maltodextrins. Maltodextrins are, in turn, made by the degradation of starch with α-amylase. This glucanotransferase splits the α-1,4-bond, transferring glucosyl groups to the nonreducing end of maltodextrins and forming cyclic glucosides with 6-12 glucopyranose units. The main product, β-cyclodextrin, consists of seven glucose units and is a non-hygroscopic, slightly sweet compound:



(4.124)

Table 4.18. Structure and occurrence of oligosaccharides

Name	Structure	Occurrence
Disaccharides		
Cellobiose	O-β-D-Glc*p*-(1 → 4)-D-Glc*p*	Building block of cellulose
Gentiobiose	O-β-D-Glc*p*-(1 → 6)-D-Glc*p*	Glycosides (amygdalin)
Isomaltose	O-α-D-Glc*p*-(1 → 6)-D-Glc*p*	Found in mother liquor during glucose production from starch
Lactose	O-β-D-Gal*p*-(1 → 4)-D-Glc*p*	Milk
Lactulose	O-β-D-Gal*p*-(1 → 4)-D-Fru*p*	Conversion product of lactose
Maltose	O-α-D-Glc*p*-(1 → 4)-D-Glc*p*	Building block of starch, sugar beet, honey
Maltulose	O-α-D-Glc*p*-(1 → 4)-D-Fru*f*	Conversion product of maltose, honey, beer
Melibiose	O-α-D-Gal*p*-(1 → 6)-D-Glc*p*	Cacao beans
Neohesperidose	O-α-L-Rha*p*-(1 → 2)-D-Glc*p*	Glycosides (naringin, neohesperidin)
Neotrehalose	O-α-D-Glc*p*-(1 → 1)-β-D-Glc*p*	Koji extract
Nigerose	O-α-D-Glc*p*-(1 → 3)-D-Glc*p*	Honey, beer
Palatinose	O-α-D-Glc*p*-(1 → 6)-D-Fru*f*	Microbial product of saccharose
Rutinose	O-α-L-Rha*p*-(1 → 6)-D-Glc*p*	Glycosides (hesperidin)
Saccharose	O-β-D-Fru*f*-(2 → 1)-α-D-Glc*p*	Sugar beet, sugar cane, spread widely in plants
Sophorose	O-β-D-Glc*p*-(1 → 2)-D-Glc*p*	Legumes
Trehalose	O-α-D-Glc*p*-(1 → 1)-α-D-Glc*p*	Ergot (*Claviceps purpurea*), young mushrooms
Trisaccharides		
Fucosidolactose	O-α-D-Fuc*p*-(1 → 2)-O-β-α-Gal*p*-(1 → 4)-D-Gal*p*	Human milk
Gentianose	O-β-D-Glc*p*-(1 → 6)-O-α-D-Glc*p*-(1 → 2)-β-D-Fru*f*	Gentian rhizome
Isokestose (1-Kestose)	O-α-D-Glc*p*-(1 → 2)-O-β-D-Fru*f*-(1 → 2)-β-D-Fru*f*	Product of saccharase action on saccharose as a substrate
Kestose (6-Kestose)	O-α-D-Glc*p*-(1 → 2)-O-β-D-Fru*f*-(6 → 2)-β-D-Fru*f*	Saccharose subjected to yeast saccharase activity, honey
Maltotriose	O-α-D-Glc*p*-(1 → 4)-O-α-D-Glc*p*-(1 → 4)-D-Glc*p*	Degradation product of starch, starch syrup
Manninotriose	O-α-D-Gal*p*-(1 → 6)-O-α-D-Gal*p*-(1 → 6)-D-Glc*p*	Manna
Melezitose	O-α-D-Glc*p*-(1 → 3)-O-β-D-Fru*f*-(2 → 1)-α-D-Glc*p*	Manna, nectar
Neokestose	O-β-D-Fru*f*-(2 → 6)-O-α-D-Glc*p*-(1 → 2)-β-D-Fru*f*	Product of saccharase action on saccharose as a substrate
Panose	O-α-D-Glc*p*-(1 → 6)-O-α-D-Glc*p*-(1 → 4)-D-Glc*p*	Degradation product of amylopectin, honey
Raffinose	O-α-D-Gal*p*-(1 → 6)-O-α-D-Glc*p*-(1 → 2)-β-D-Fru*f*	Sugar beet, sugar cane, widely distributed in plants
Umbelliferose	O-α-D-Gal*p*-(1 → 2)-O-α-D-Glc*p*-(1 → 2)-β-D-Fru*f*	Umbelliferae roots
Tetrasaccharides		
Maltotetraose	O-α-D-Glc*p*-(1 → 4)-O-α-D-Glc*p*-(1 → 4)-O-α-D-Glc*p*-(1 → 4)-D-Glc*p*	Starch syrup
Stachyose	O-α-D-Gal*p*-(1 → 6)-O-α-D-Gal*p*-(1 → 6)-O-α-D-Glc*p*-(1 → 2)-β-D-Fru*f*	Widespread in plants (artichoke, soybean)
Higher oligosaccharides		
Maltopentaose	[O-α-D-Glc*p*-(1 → 4)]$_4$-D-Glc*p*	Starch syrup
α-*Schardinger*-Dextrin, Cyclohexaglucan (α, 1 → 4)		Growth of
β-*Schardinger*-Dextrin, Cycloheptaglucan (α, 1 → 4)		*Bacillus macerans*
γ-*Schardinger*-Dextrin, Cyclooctaglucan (α, 1 → 4)		on starch syrup

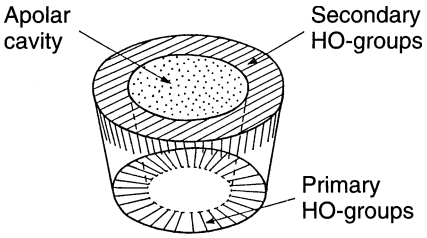


Fig. 4.11. Schematic representation of the hollow cylinder formed by β-cyclodextrin

The β-cyclodextrin molecule is a cylinder (Fig. 4.11) which has a primary hydroxyl (C6) rim on one side and a secondary hydroxyl (C2, C3) rim on the other. The surfaces made of pyranose rings are hydrophobic. Indeed, the water of hydration is very easily displaced from this hydrophobic cavity by sterically suitable apolar compounds, which are masked in this way. In food processing, β-cyclodextrin is therefore a suitable agent for stabilizing lipophilic vitamins and aroma substances and for neutralizing the taste of bitter substances

4.4 Polysaccharides

4.4.1 Classification, Structure

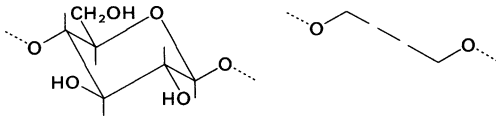
Polysaccharides, like oligosaccharides, consist of monosaccharides bound to each other by glycosidic linkages. Their acidic hydrolysis yields monosaccharides. Partial chemical and enzymatic hydrolysis, in addition to total hydrolysis, are of importance for structural elucidation. Enzymatic hydrolysis provides oligosaccharides, the analysis of which elucidates monosaccharide sequences and the positions and types of linkages.
Polysaccharides (glycans) can consist of one type of sugar structural unit (homoglycans) or of several types of sugar units (heteroglycans). The monosaccharides may be joined in a linear pattern (as in cellulose and amylose) or in a branched fashion (amylopectin, glycogen, guaran). The frequency of branching sites and the length of side chains can vary greatly (glycogen, guaran). The monosaccharide residue sequence may be periodic, one period containing one or several alternating structural units (cellulose, amylose or hyaluronic acid), the sequence may contain shorter or longer segments with periodically arranged residues separated by nonperiodic segments (alginate, carrageenans, pectin), or the sequence may be nonperiodic all along the chain (as in the case of carbohydrate components in glycoproteins).

4.4.2 Conformation

The monosaccharide structural unit conformation and the positions and types of linkages in the chain determine the chain conformation of a polysaccharide. In addition to irregular conformations, regular conformations are known which reflect the presence of at least a partial periodic sequence in the chain. Some typical conformations will be explained in the following discussion, with examples of glucans and some other polysaccharides.

4.4.2.1 Extended or Stretched, Ribbon-Type Conformation

This conformation is typical for 1,4-linked β-D-glucopyranosyl residues (Fig. 4.12 a), as occur, for instance, in cellulose fibers:



(4.125)

This formula shows that the stretched chain conformation is due to a *zigzag* geometry of monomer linkages involving oxygen bridging. The chain may be somewhat shortened or compressed to enable formation of H-bonds between neighboring residues and thus contribute to conformational stabilization. In the ribbon-type, stretched conformation, with the number of monomers in turn denoted as n and the pitch (advancement) in the axial direction per monomer unit as h, the range of n is from 2 to ± 4, while h is the length of a monomer unit. Thus, the chain given in Fig. 4.12 a has $n = -2.55$ and $h = 5.13$ Å.
A strongly pleated, ribbon-type conformation might also occur, as shown by a segment of

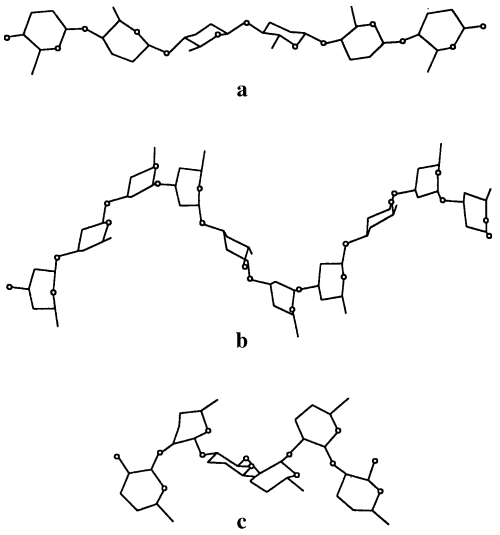
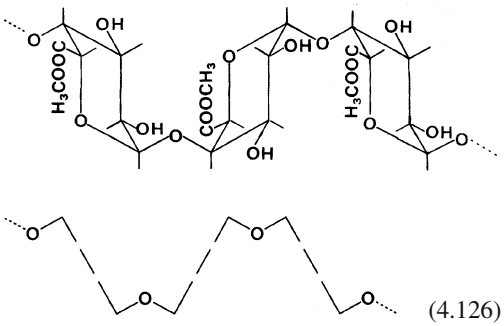


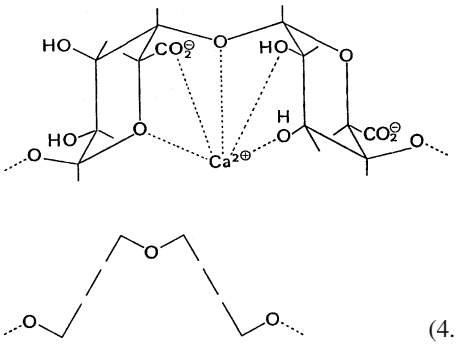
Fig. 4.12. Conformations of some β-D-glucans. Linkages: **a** $1 \rightarrow 4$, **b** $1 \rightarrow 3$, **c** $1 \rightarrow 2$ (according to *Rees*, 1977)

a pectin chain (1,4-linked α-D-galactopyranosyluronate units):



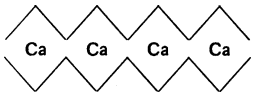
(4.126)

and the same pleated conformation is shown by an alginate chain (1,4-linked α-L-gulopyranosyluronate units):



(4.127)

Ca^{2+} ions can be involved to stabilize the conformation. In this case, two alginate chains are assembled in a conformation which resembles an egg box (*egg box type of conformation*):

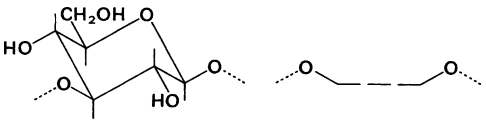


(4.128)

It should be emphasized that in all examples the linear, ribbon-type conformation has a zigzag geometry as a common feature.

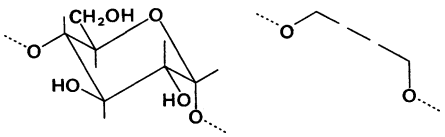
4.4.2.2 Hollow Helix-Type Conformation

This conformation is typical for 1,3-linked β-D-glucopyranose units (Fig. 4.12, b), as occur in the polysaccharide lichenin, found in moss-like plants (lichens):



(4.129)

The formula shows that the helical conformation of the chain is imposed by a U-form geometry of the monomer linkages. Amylose (1,4-linked α-D-glucopyranosyl residues) also has such a geometry, and hence a helical conformation:



(4.130)

The number of monomers per turn (n) and the pitch in the axial direction per residue (h) is highly variable in a hollow helical conformation. The value of n is between 2 and ±10, whereas h can be near its limit value of 0. The conformation of a $\beta(1 \rightarrow 3)$-glucan, with $n = 5.64$ and $h = 3.16$ Å, is shown in Fig. 4.12, b. The helial conformation can be stabilized in various ways. When the helix diameter is large, inclusion (clathrate) compounds can be formed (Fig. 4.13, a; cf. 4.4.4.14.3). More extended or stretched chains, with smaller helix diameter,

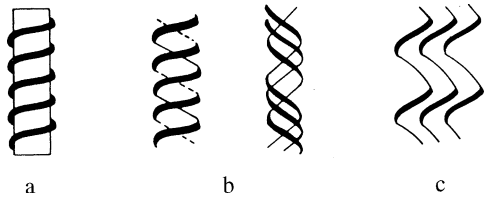
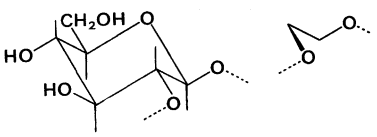


Fig. 4.13. Stabilizatioin of helical conformations. **a** Clathrate compounds, **b** coiled double or triple helices, **c** "nesting" (according to *Rees*, 1977)

can form double or triple stranded helices (Fig. 4.13, b; cf. 4.4.4.3.2 and 4.4.4.14.3), while strongly-stretched chains, in order to stabilize the conformation, have a zigzag, pleated association and are not stranded (Fig. 4.13, c).

4.4.2.3 Crumpled-Type Conformation

This conformation occurs with, for example, 1,2-linked β-D-glucopyranosyl residues (Fig. 4.12, c). This is due to the wrinkled geometry of the monomer O-bridge linkages:

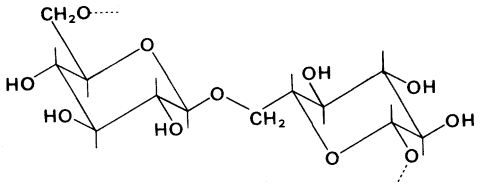


(4.131)

Here, the n value varies from 4 up to −2 and h is 2–3 Å. The conformation reproduced in Fig. 4.12, c has $n = 2.62$ and $h = 2.79$ Å. The likelihood of such a disorderly form associating into more orderly conformations is low. Polysaccharides of this conformational type play only a negligible role in nature.

4.4.2.4 Loosely-Jointed Conformation

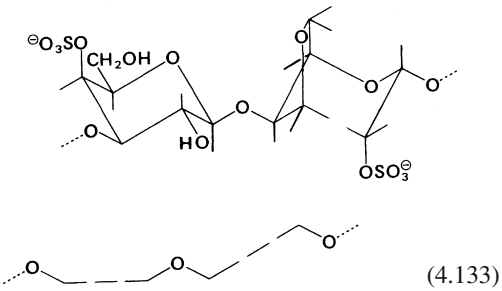
This is typical for glycans with 1,6-linked β-D-glucopyranosyl units, because they exhibit a particularly great variability in conformation.
The great flexibility of this glycan-type conformation is based on the nature of the connecting bridge between the monomers. The bridge has three free rotational bonds and, furthermore, the sugar residues are further apart:



(4.132)

4.4.2.5 Conformations of Heteroglycans

The examples considered so far have demonstrated that a prediction is possible for a homoglycan conformation based on the geometry of the bonds of the monomer units which maintain the oxygen bridges. It is more difficult to predict the conformation of a heteroglycan with a periodic sequence of several monomers, which implies different types of conformations. Such a case is shown by ι-carrageenan, in which the β-D-galactopyranosyl-4-sulfate units have a U-form geometry, while the 3,6-anhydro-α-D-galactopyranosyl-2-sulfate residues have a zigzag geometry:



(4.133)

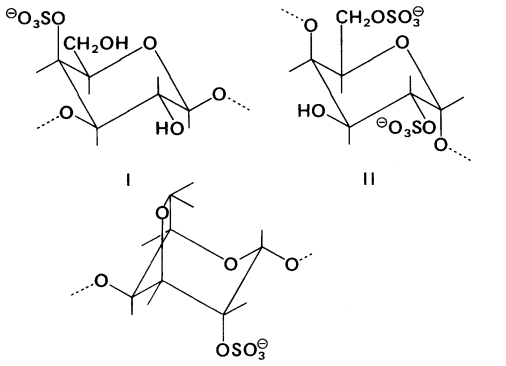
Calculations have shown that conformational possibilities vary from a shortened, compressed ribbon band type to a stretched helix type. X-ray diffraction analyses have proved that a stretched helix exists, but as a double stranded helix in order to stabilize the conformation (cf. 4.4.4.3.2 and Fig. 4.19).

4.4.2.6 Interchain Interactions

It was outlined in the introductory section (cf. 4.4.1) that the periodically arranged monosaccharide sequence in a polysaccharide can be interrupted by nonperiodic segments. Such

sequence interferences result in conformational disorders. This will be explained in more detail with ι-carrageenan, mentioned above, since it will shed light on the gel-setting mechanism of macromolecules in general.

Initially, a periodic sequence of altering units of β-D-galactopyranose- 4-sulfate (I, conformation $^4C_1$) and α-D-galactopyranose-2,6-disulfate (II, conformation $^4C_1$) is built up in carrageenan biosynthesis:



I II III (4.134)

When the biosynthesis of the chain is complete, an enzyme-catalyzed reaction eliminates sulfate from most of α-D-galactopyranose-2,6-disulfate (II), transforming the unit to 3,6-anhydro-α-D-galactopyranose-2-sulfate (III, conformation $^1C_4$). This transformation is associated with a change in linkage geometry. Some II-residues remain in the sequence, acting as interference sites. While the undisturbed, ordered segment of one chain can associate with the same segment of another chain, forming a double helix, the nonperiodic or disordered segments can not participate in such associations (Fig. 4.14).

In this way, a gel is formed with a three-dimensional network in which the solvent is immobilized. The gel properties, e. g., its strength, are influenced by the number and distribution of α-D-galactopyranosyl-2,6-disulfate residues, i.e. by a structural property regulated during polysaccharide biosynthesis.

The example of the τ-carrageenan gel-building mechanism, involving a chain–chain interaction of sequence segments of orderly conformation, interrupted by randomly-coiled segments corresponding to a disorderly chain sequence, can be applied generally to gels of other macromolecules. Besides a sufficient chain length, the

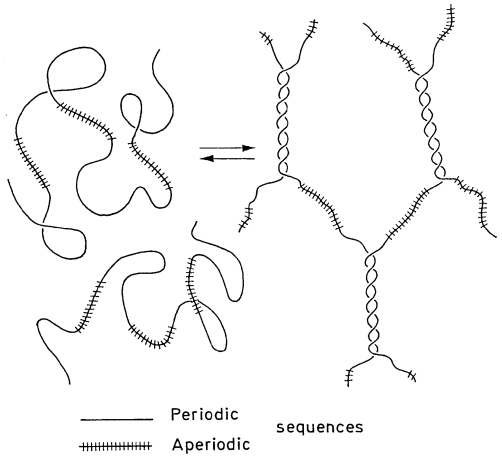


Fig. 4.14. Schematic representation of a gel setting process (according to *Rees*, 1977)

structural prerequisite for gel-setting ability is interruption of a periodic sequence and its orderly conformation. The interruption is achieved by insertion into the chain of a sugar residue of a different linkage geometry (carrageenans, alginates, pectin), by a suitable distribution of free and esterified carboxyl groups (glycuronans) or by insertion of side chains. The interchain associations during gelling (network formation), which involve segments of orderly conformation, can then occur in the form of a double helix (Fig. 4.15,a); a multiple bundle of double helices (Fig. 4.15,b); an association between stretched ribbon-type conformations, such as an egg box model (Fig. 4.15,c); some other similar associations (Fig. 4.15,d); or, lastly, forms consisting of double helix and ribbon-type combinations (Fig. 4.15,e).

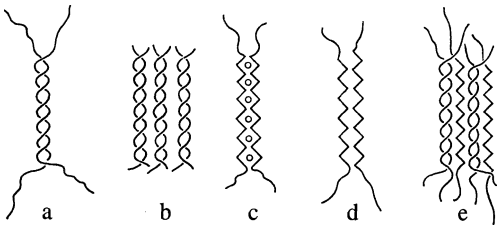


Fig. 4.15. Interchain aggregation between regular conformations. **a** Double helix, **b** double helix bundle, **c** egg-box, **d** ribbon–ribbon, and **e** double helix, ribbon interaction

4.4.3 Properties

4.4.3.1 General Remarks

Polysaccharides are widely and abundantly distributed in nature, fulfilling roles as:

- Structure-forming skeletal substances (cellulose, hemicellulose and pectin in plants; chitin, mucopolysaccharides in animals).
- Assimilative reserve substances (starch, dextrins, inulin in plants; glycogen in animals).
- Water-binding substances (agar, pectin and alginate in plants; mucopolysaccharides in animals).

As a consequence, polysaccharides occur in many food products and even then they often retain their natural role as skeletal substances (fruits and vegetables) or assimilative nutritive substances (cereals, potatoes, legumes). Isolated polysaccharides are utilized to a great extent in food processing, either in native or modified form, as: thickening or gel-setting agents (starch, alginate, pectin, guaran gum); stabilizers for emulsions and dispersions; film-forming, coating substances to protect sensitive food from undesired change; and inert fillers to increase the proportion of indigestible ballast substances in a diet (cf. 15.2.4.2). Table 4.19 gives an overview of uses in food technology.

The outlined functions of polysaccharides are based on their highly variable properties. They vary from insoluble forms (cellulose) to those with good swelling power and solubility in hot and cold water (starch, guaran gum). The solutions may exhibit low viscosities even at very high concentrations (gum arabic), or may have exceptionally high viscosities even at low concentrations (guaran gum). Some polysaccharides, even at a low concentration, set into a thermoreversible gel (alginates, pectin). While most of the gels melt at elevated temperatures, some cellulose derivatives set into a gel.

These properties and their utilization in food products are described in more detail in section 4.4.4, where individual polysaccharides are covered. Here, only a brief account will be given to relate their properties to their structures in a general way.

4.4.3.2 Perfectly Linear Polysaccharides

Compounds with a *single* neutral monosaccharide structural unit and with *one* type of linkage (as occurs in cellulose or amylose) are denoted as perfectly linear polysaccharides. They are usually insoluble in water and can be solubilized only under drastic conditions, e. g. at high temperature, or by cleaving H-bonds with alkalies or other suitable reagents. They readily precipitate from solution (example: starch retrogradation). The reason for these properties is the existence of an optimum structural prerequisite for the formation of an orderly conformation within the chain and also for chain–chain interaction. Often, the conformation is so orderly that a partial crystallinity state develops. Large differences in properties are found within these groups of polysaccharides when there is a change in structural unit, linkage type or molecular weight. This is shown by properties of cellulose, amylose or β-1,3-glucan macromolecules.

4.4.3.3 Branched Polysaccharides

Branched polysaccharides (amylopectin, glycogen) are more soluble in water than their perfectly linear counterparts since the chain–chain interaction is less pronounced and there is a greater extent of solvation of the molecules. Solutions of branched polysaccharides, once dried, are readily rehydrated. Compared to their linear counterparts of equal molecular weights and equal concentrations, solutions of branched polysaccharides have a lower viscosity. It is assumed that the viscosity reflects the “effective volume” of the macromolecule. The “effective volume” is the volume of a sphere with diameter determined by the longest linear extension of the molecule. These volumes are generally larger for linear than for branched molecules (Fig. 4.16). Exceptions are found with highly pleated linear chains. The tendency of branched polysaccharides to precipitate is low. They form a sticky paste at higher concentrations, probably due to side chain–side chain interactions (interpenetration, entanglement). Thus, branched polysaccharides are suitable as binders or adhesives.

Table 4.19. Examples of uses of polysaccharides in foods

Area of application/food	Suitable polysaccharides
Stabilization of emulsions/suspensions in condensed milk and chocolate milk	Carrageenan, algin, pectin, carboxymethylcellulose
Stabilization of emulsions in coffee whiteners, low-fat margarines	Carrageenan
Stabilization of ice cream against ice crystal formation, melting, phase separation; improvement of consistency (smoothness)	Algin, carrageenan, agar, gum arabic, gum tragacanth, xanthan gum, guaran gum, locust bean flour, modified starches, carboxymethylcellulose, methylcellulose
Water binding, improvement of consistency, yield increase of soft cheese, cream cheese, cheese preparations	Carrageenan, agar, gum tragacanth, karaya gum, guaran gum, locust bean flour, algin, carboxymethylcellulose
Thickening and gelation of milk in puddings made with and without heating, creams; improvement of consistency	Pectin, algin, carrageenan, guaran gum, locust bean flour, carboxymethylcellulose, modified starches
Water binding, stabilization of emulsions in meat products (corned beef, sausage)	Agar, karaya gum, guaran gum, locust bean flour
Jellies for meat, fish, and vegetable products	Algin, carrageenan, agar
Stabilization and thickening, prevention of synaeresis, freeze-thaw stability of soups, sauces, salad dressing, mayonnaise, ketchup; obtaining "body" in low-fat and low-starch products	Gum tragacanth, algin, karaya gum, xanthan gum, guaran gum, locust bean flour, carboxymethyl-cellulose, propylene glycol alginate, modified starches
Stabilization of protein foam in beer, whipped cream, meringues, chocolate marshmallows	Algin, carrageenan, agar, gum arabic, karaya gum, xanthan gum
Prevention of starch retrogradation in bread and cakes, water binding in dough	Agar, guaran gum, locust bean flour, carrageenan, xanthan gum
Thickening and gelation of fruit pulp (confiture, jams, jellies, fruit pulp for ice cream and yoghurt)	Pectin, algin
Gelation of jelly candies, jelly beans, glaze, icing, water-dessert jellies	Pectin, algin, carrageenan, agar, gum arabic, modified starches
Sediment stabilization in fruit juices, obtaining "body" in beverage powders	Algin, pectin, propylene glycol alginate, gum arabic, xanthan gum, guaran gum, methylcellulose
Stabilization of powdery aroma emulsions, encapsulation of aroma substances	Gum arabic, gum ghatti, xanthan gum

4.4.3.4 Linearly Branched Polysaccharides

Linearly branched polysaccharides, i.e. polymers with a long "backbone" chain and with many short side chains, such as guaran or alkyl cellulose, have properties which are a combination of those of perfectly linear and of branched molecules. The long "backbone" chain is responsible for high solution viscosity. The presence of numerous short side chains greatly weakens interactions between the molecules, as shown by the good solubility and rehydration rates of the molecules and by the stability even of highly concentrated solutions.

4.4.3.5 Polysaccharides with Carboxyl Groups

Polysaccharides with carboxyl groups (pectin, alginate, carboxymethyl cellulose) are very soluble as alkali salts in the neutral or alkaline pH range. The molecules are negatively charged due to carboxylate anions and, due to their repulsive charge forces, the molecules are relatively stretched and resist intermolecular associations. The solution viscosity is high and is pH-dependent. Gel setting or precipitation occurs at $pH \leq 3$ since electrostatic repulsion ceases to exist. In addition, undissociated carboxyl groups dimerize through

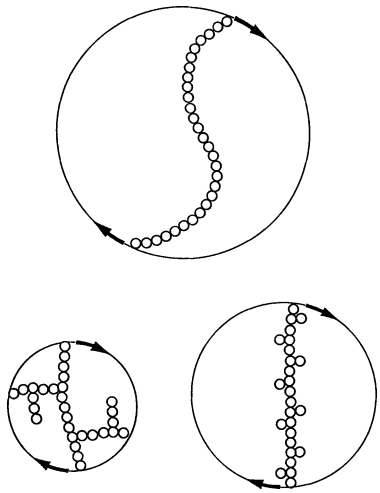


Fig. 4.16. Schematic representation of the "effective volumes" of linear, branched and linearly branched types of polysaccharides

H-bridges. However, a divalent cation is needed to achieve gel setting in a neutral solution.

4.4.3.6 Polysaccharides with Strongly Acidic Groups

Polysaccharides with strongly acidic residues, present as esters along the polymer chains (sulfuric, phosphoric acids, as in furcellaran, carrageenan or modified starch), are also very soluble in water and form highly viscous solutions. Unlike polysaccharides with carboxyl groups, in strongly acidic media these solutions are distinctly stable.

4.4.3.7 Modified Polysaccharides

Modification of polysaccharides, even to a low substitution degree, brings about substantial changes in their properties.

4.4.3.7.1 Derivatization with Neutral Substituents

The solubility in water, viscosity and stability of solutions are all increased by binding neutral substituents to linear polysaccharide chains. Thus the properties shown by methyl, ethyl and hydroxypropyl cellulose correspond to those of guaran and locust bean gum. The effect is explained by interference of the alkyl substituents in chain interactions, which then facilitates hydration of the molecule. An increased degree of substitution increases the hydrophobicity of the molecules and, thereby, increases their solubility in organic solvents.

4.4.3.7.2 Derivatization with Acidic Substituents

Binding acid groups to a polysaccharide (carboxymethyl, sulfate or phosphate groups) also results in increased solubility and viscosity for reasons already outlined. Some derivatized polysaccharides, when moistened, have a pasty consistence.

4.4.4 Individual Polysaccharides

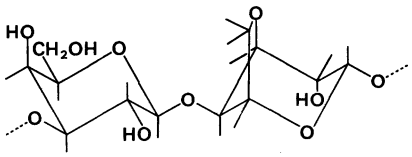
4.4.4.1 Agar

4.4.4.1.1 Occurrence, Isolation

Agar is a gelatinous product isolated from seaweed (red algae class, *Rhodophyceae*), e. g., *Gelidium spp., Pterocladia spp.* and *Gracilaria spp.*, by a hot water extraction process. Purification is possible by congealing the gel.

4.4.4.1.2 Structure, Properties

Agar is a heterogenous complex mixture of related polysaccharides having the same backbone chain structure. The main components of the chain are β-D-galactopyranose and 3,6-anhydro-α-L-galactopyranose, which alternate through $1 \rightarrow 4$ and $1 \rightarrow 3$ linkages:



(4.135)

The chains are esterified to a low extent with sulfuric acid. The sulfate content differentiates between the agarose fraction (the main gelling component of agar), in which close to every tenth galactose unit of the chain is esterified, and the agaropectin fraction, which has a higher sulfate esterification degree and, in addition, has pyruvic acid bound in ketal form [4,6-(l-carboxyethylidene)-D-galactose]. The ratio of the two polymers can vary greatly. Uronic acid, when present, does not exceed 1%. Agar is insoluble in cold water, slightly soluble in ethanolamine and soluble in formamide. Agar precipitated by ethanol from a warm aqueous dispersion is, in its moist state, soluble in water at 25 °C, while in the dried state it is soluble only in hot water. Gel setting occurs upon cooling. Agar is a most potent gelling agent as gelation is perceptible even at 0.04%. Gel setting and stability are affected by agar concentration and its average molecular weight. A 1.5% solution sets to a gel at 32–39 °C, but does not melt below 60–97 °C. The great difference between gelling and melting temperatures, due to hysteresis, is a distinct and unique feature of agar.

4.4.4.1.3 Utilization

Agar is widely used, for instance in preparing nutritive media in microbiology. Its application in the food industry is based on its main properties: it is essentially indigestible, forms heat resistant gels, and has emulsifying and stabilizing activity. Agar is added to sherbets (frozen desserts of fruit juice, sugar, water or milk) and ice creams (at about 0.1%), often in combination with gum tragacanth or locust (carob) bean gum or gelatin. An amount of 0.1–1% stabilizes yoghurt, some cheeses and candy and bakery products (pastry fillings). Furthermore, agar retards bread staling and provides the desired gel texture in poultry and meat canning. Lastly, agar has a role in vegetarian diets (meat substitute products) and in desserts and pretreated instant cereal products.

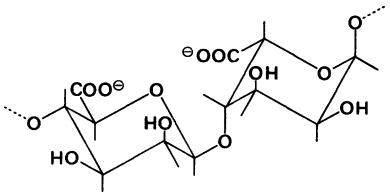
4.4.4.2 Alginates

4.4.4.2.1 Occurrence, Isolation

Alginates occur in all brown algae (*Phaeophyceae*) as a skeletal component of their cell walls. The major source of industrial production is the giant kelp, *Macrocystis pyrifera*. Some species of *Laminaria, Ascophyllum* and *Sargassum* are also used. Algae are extracted with alkalies. The polysaccharide is usually precipitated from the extract by acids or calcium salts.

4.4.4.2.2 Structure, Properties

Alginate building blocks are β-D-mannuronic and α-L-guluronic acids, joined by 1 → 4 linkages:



(4.136)

The ratio of the two sugars (mannuronic/guluronic acids) is generally 1.5, with some deviation depending on the source. Alginates extracted from *Laminaria hyperborea* have ratios of 0.4–1.0. Partial hydrolysis of alginate yields chain fragments which consist predominantly of mannuronic or guluronic acid, and also fragments where the two uronic acid residues alternate in a 1:1 ratio. Alginates are linear copolymers consisting of the following structural units:

$$[\rightarrow 4)\text{-}\beta\text{-D-Man}p\text{A}(1 \rightarrow 4)\text{-}\beta\text{-D-Man}p\text{A}(1 \rightarrow]_n$$
$$[\rightarrow 4)\text{-}\alpha\text{-L-Gul}p\text{A}(1 \rightarrow 4)\text{-}\alpha\text{-L-Gul}p\text{A}(1 \rightarrow]_m$$
$$[\rightarrow 4)\text{-}\beta\text{-D-Man}p\text{A}(1 \rightarrow 4)\text{-}\alpha\text{-L-Gul}p\text{A}(1 \rightarrow]_p \quad (4.137)$$

The molecular weights of alginates are 32–200 kdal. This corresponds to a degree of polymerization of 180–930. The carboxyl group pK-values are 3.4–4.4. Alginates are water soluble in the form of alkali, magnesium, ammonia or amine salts. The viscosity of alginate solutions is influenced by molecular weight and the counter ion of the salt. In the absence of di- and trivalent cations or in the presence of a chelating agent, the viscosity is low ("long flow" property). However, with a rise in multivalent cation levels (e. g., calcium) there is a parallel rise in viscosity ("short flow"). Thus, the viscosity can

be adjusted as desired. Freezing and thawing of a Na-alginate solution containing Ca^{2+} ions can result in a further rise in viscosity. The curves in Fig. 4.17 show the effect on viscosity of the concentrations of three alginate preparations: low, moderate and high viscosity types. These data reveal that a 1% solution, depending on the type of alginate, can have a viscosity range of 20–2000 cps. The viscosity is unaffected in a pH range of 4.5–10. It rises at a pH below 4.5, reaching a maximum at pH 3–3.5.

Gels, fibers or films are formed by adding Ca^{2+} or acids to Na-alginate solutions. A slow reaction is needed for uniform gel formation. It is achieved by a mixture of Na-alginate, calcium phosphate and glucono-δ-lactone, or by a mixture of Na-alginate and calcium sulfate.

Depending on the concentration of calcium ions, the gels are either thermoreversible (low concentration) or not (high concentration). Figure 4.18 shows a schematic section of a calcium alginate gel.

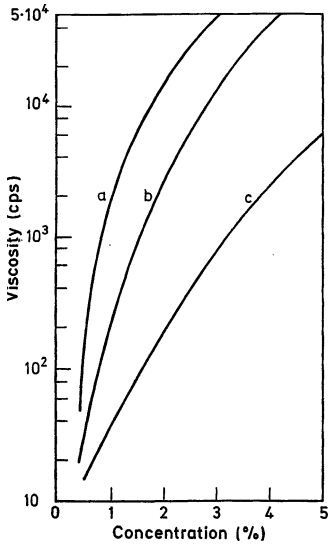


Fig. 4.17. Viscosity of aqueous alginate solutions. Alginate with (*a*) high, (*b*) medium, and (*c*) low viscosity

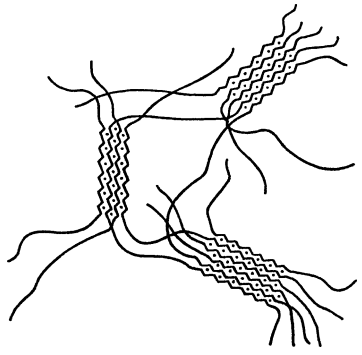


Fig. 4.18. Schematic representation of a calcium alginate gel (cross-linkage by egg box formation, cf. Formula 4.120; according to *Franz*, 1991)

4.4.4.2.3 Derivatives

Propylene glycol alginate is a derivative of economic importance. This ester is obtained by the reaction of propylene oxide with partially neutralized alginic acid. It is soluble down to pH 2 and, in the presence of Ca^{2+} ions, forms soft, elastic, less brittle and syneresisfree gels.

4.4.4.2.4 Utilization

Alginate is a powerful thickening, stabilizing and gel-forming agent. At a level of 0.25–0.5% it improves and stabilizes the consistency of fillings for baked products (cakes, pies), salad dressings and milk chocolates, and prevents formation of larger ice crystals in ice creams during storage. Furthermore, alginates are used in a variety of gel products (cold instant puddings, fruit gels, dessert gels, onion rings, imitation caviar) and are applied to stabilize fresh fruit juice and beer foam.

4.4.4.3 Carrageenans

4.4.4.3.1 Occurrence, Isolation

Red sea weeds (*Rhodophyceae*) produce two types of galactans: agar and agar-like polysaccharides, composed of D-galactose and 3,6-anhydro-L-galactose residues, and carrageenans and related polysaccharides, composed of D-galactose and 3,6-anhydro-D-galactose which are partially sulfated as 2-, 4- and 6-sulfates and 2,6-disulfates. Galactose residues are alternatively linked by $1 \rightarrow 3$ and $1 \rightarrow 4$ linkages. Carrageenans are isolated from *Chondrus* (*Chondrus crispus*, the Irish moss), *Eucheuma, Gigartina, Gloiopeltis* and *Iridaea* species by hot

water extraction under mild alkaline conditions, followed by drying or isolate precipitation.

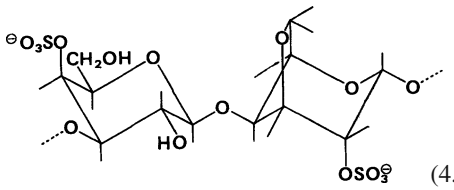
4.4.4.3.2 Structure, Properties

Carrageenans are a complex mixture of various polysaccharides. They can be separated by fractional precipitation with potassium ions. Table 4.20 compiles data on these fractions and their monosaccharide constituents. Two major fractions are $\varkappa$ (gelling and K^+-insoluble fraction) and λ (nongelling, K^+-soluble).

Table 4.20. Building blocks of carrageenans

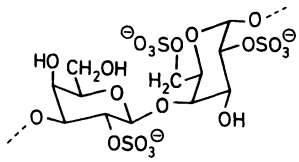
Carrageenan	Monosaccharide building block
$\varkappa$-Carrageenan	D-Galactose-4-sulfate, 3,6-anhydro-D-galactose-2-sulfate
$\varkappa$-Carrageenan	D-Galactose-4-sulfate, 3,6-anhydro-D-galactose
λ-Carrageenan	D-Galactose-2-sulfate, D-galactose-2,6-disulfate
μ-Carrageenan	D-Galactose-4-sulfate, D-galactose-6-sulfate, 3,6-anhydro-D-galactose
ν-Carrageenan	D-Galactose-4-sulfate, D-galactose-2,6-disulfate, 3,6-anhydro-D-galactose
Furcellaran	D-Galactose-D-galactose-2-sulfate, D-galactose-4-sulfate, D-galactose-6-sulfate, 3,6-anhydro-D-galactose

$\varkappa$-Carrageenan is composed of D-galactose, 3,6-anhydro-D-galactose and ester-bound sulfate in a molar ratio of 6:5:7. The galactose residues are essentially fully sulfated in position 4, whereas the anhydrogalactose residues can be sulfated in position 2 or substituted by α-D-galactose-6-sulfate or -2,6-disulfate. A typical sequence of $\varkappa$- (or ι-)carrgeenan is:



(4.138)

The sequence favors the formation of a doublestranded helix (Fig. 4.19). λ-Carrageenan contains as the basic building block β-D-Gal*p*-(1 → 4)-α-D-Gal*p* (cf. Formula 4.139), which is joined through a 1,3-glycosidic linkage to the polymer. Position 6 of the second galactose residue is esterified with sulfuric acid as is ca. 70% of position 2 of both residues. The high sulfate content favors the formation of a zigzag ribbon-shaped conformation.



(4.139)

The molecular weights of $\varkappa$- and λ-carrageenans are 200–800 kdal. The water solubility increases

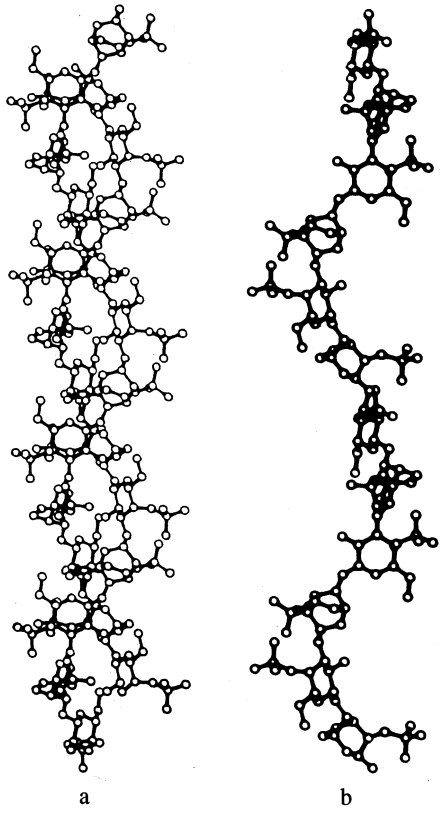


Fig. 4.19. ι-Carrageenan conformation. **a** Double helix, **b** single coil is presented to clarify the conformation (according to *Rees*, 1977)

as the carrageenan sulfate content increases and as the content of anhydrosugar residue decreases. The viscosity of the solution depends on the carrageenan type, molecular weight, temperature, ions present and carrageenan concentration.

As observed in all linear macromolecules with charges along the chain, the viscosity increases exponentially with the concentration (Fig. 4.20). Aqueous $\varkappa$-carrageenan solutions, in the presence of ammonium, potassium, rubidium or caesium ions, form thermally reversibly gels. This does not occur with lithium and sodium ions.

This strongly suggests that gel-setting ability is highly dependent on the radius of the hydrated counter ion. The latter is about 0.23 nm for the former group of cations, while hydrated lithium (0.34 nm) and sodium ions (0.28 nm) exceed the limit. The action of cations is visualized as a zipper arrangement between aligned segments of linear polymer sulfates, with low ionic radius cations locked between alternating sulfate residues. Gel-setting ability is probably also due to a mechanism based on formation of partial double helix structures between various chains. The extent of intermolecular double helix formation, and thus the gel strength, is greater, the more uniform the chain sequences are. Each substitution of a 3,6-anhydrogalactose residue by another residue, e. g., galactose-6-sulfate, results in a kink within the helix and, thereby, a decrease in gelling strength. The helical conformation is also affected by the position of sulfate groups. The effect is more pronounced with sulfate in the 6-position, than in 2- or 4-positions. Hence, the gel strength of $\varkappa$-carrageenan is dependent primarily on the content of esterified sulfate groups in the 6-position.

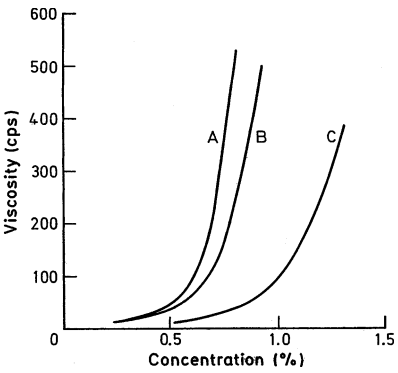


Fig. 4.20. Viscosity curves of carrageenan aqueous solutions. A: *Eucheuma spinosum, C: Chondrus crispus*, B: A and C in a ratio of 2:1, 40 °C, 20 rpm (according to *Whistler*, 1973)

The addition of carubin, which is itself non-gelling, to $\varkappa$-carrageenan produces more rigid, more elastic gels that have a lower tendency towards synaeresis. Carubin apparently prevents the aggregation of $\varkappa$-carrageenan helices.

The 6-sulfate group can be removed by heating carrageenans with alkali, yielding 3,6-anhydrogalactose residues. This elimination results in a significantly increased gel strength.

Carrageenans and other acidic polysaccharides coagulate proteins when the pH of the solution is lower than the proteins' isoelectric points. This can be utilized for separating protein mixtures.

4.4.4.3.3 Utilization

Carrageenan utilization in food processing is based on the ability of the polymer to gel, to increase solution viscosity and to stabilize emulsions and various dispersions. A level as low as 0.03% in chocolate milk prevents fat droplet separation and stabilizes the suspension of cocoa particles. Carrageenans prevent syneresis in fresh cheese and improve dough properties and enable a higher amount of milk powder incorporation in baking. The gelling property in the presence of K^+ salt is utilized in desserts and canned meat. Protein fiber texture is also improved. Protein sedimentation in condensed milk is prevented by carrageenans which, like κ-casein, prevent milk protein coagulation by calcium ions. Carrageenans are also used to stabilize ice cream and clarify beverages.

4.4.4.4 Furcellaran

4.4.4.4.1 Occurrence, Isolation

Furcellaran (Danish agar) is produced from red sea weed (algae *Furcellaria fastigiata*). Production began in 1943 when Europe was cut off from its agar suppliers. After alkali pretreatment of algae, the polysaccharide is isolated using hot water. The extract is then concentrated under vacuum and seeded with 1–1.5% KCl solution. The

separated gel threads are concentrated further by freezing, the excess water is removed by centrifugation or pressing and, lastly, the polysaccharide is dried. The product is a K-salt and contains, in addition, 8–15% occluded KCl.

4.4.4.4.2 Structure, Properties

Furcellaran is composed of D-galactose (46–53%), 3,6-anhydro-D-galactose (30–33%) and sulfated portions of both sugars (16–20%).
The structure of furcellaran is similar to χ-carrageenan. The essential difference is that χ-carrageenan has one sulfate ester per two sugar residues, while furcellaran has one sulfate ester residue per three to four sugar residues. Sugar sulfates identified are: D-galactose-2-sulfate, -4-sulfate and -6-sulfate, and 3,6-anhydro-D-galactose-2-sulfate. Branching of the polysaccharide chain can not be excluded. Furcellaran forms thermally reversible aqueous gels by a mechanism involving double helix formation, similar to χ-carrageenan.
The gelling ability is affected by the polysaccharide polymerization degree, amount of 3,6-anhydro-D-galactose, and by the radius of the cations present. K^+, NH_4^+, Rb^+ and Cs^+ from very stable, strong gels. Ca^{2+} has a lower effect, while Na^+ prevents gel setting. Addition of sugar affects the gel texture, which goes from a brittle to a more elastic texture.

4.4.4.4.3 Utilization

Furcellaran, with milk, provides good gels and therefore it is used as an additive in puddings. It is also suitable for cake fillings and icings. In the presence of sucrose, it gels rapidly and retains good stability, even against food grade acids. Furcellaran has the advantage over pectin in marmalades since it allows stable gel setting at a sugar concentration even below 50–60%. The required amount of polysaccharide is 0.2–0.5%, depending on the marmalade's sugar content and the desired gel strength. To keep the hydrolysis extent low, a cold aqueous 2–3% solution of furcellaran is mixed into a hot, cooked slurry of fruits and sugar.
Furcellaran is also utilized in processed meat products, such as spreadable meat pastes and pastry fillings. It facilitates protein precipitation during brewing of beer and thus improves the final clarification of the beer.

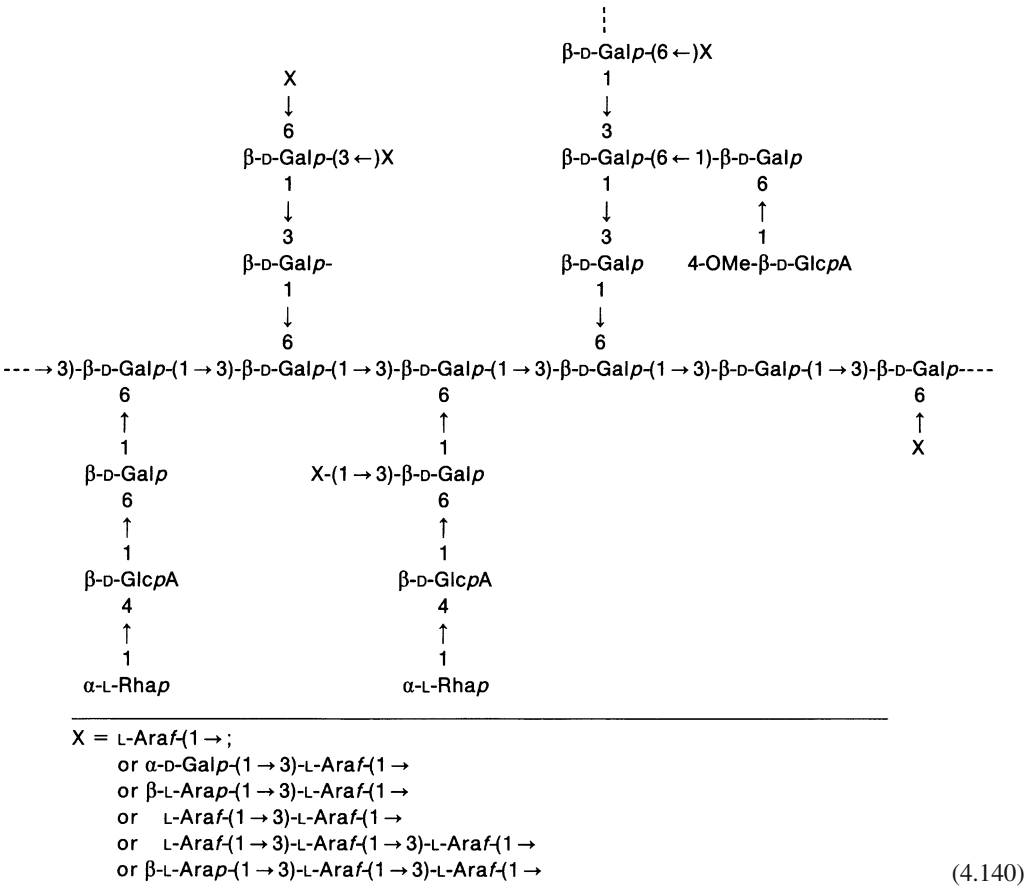
4.4.4.5 Gum Arabic

4.4.4.5.1 Occurrence, Isolation

Gum arabic is a tree exudate of various *Acacia* species, primarily *Acacia Senegal*, and is obtained as a result of tree bark injury. It is collected as air-dried droplets with diameters from 2–7 cm. The annual yield per tree averages 0.9–2.0 kg. The major producer is Sudan, with 50–60,000 t/annum, followed by several other African countries. Gum arabic has been known since ancient Egypt as "kami", an adhesive for pigmented paints.

4.4.4.5.2 Structure, Properties

Gum arabic is a mixture of closely related polysaccharides, with an average molecular weight range of 260–1160 kdal. The main structural units, with molar proportions for the gum exudate *A. senegal* given in brackets, are L-arabinose (3.5), L-rhamnose (1.1), D-galactose (2.9) and D-glucuronic acid (1.6). The proportion varies significantly depending on the Acacia species. Gum arabic has a major core chain built of β-D-galactopyranosyl residues linked by $1 \rightarrow 3$ bonds, in part carrying side chains attached at position 6 (cf. Formula 4.140).
Gum arabic occurs neutral or as a weakly acidic salt. Counter ions are Ca^{2+}, Mg^{2+} and K^+. Solubilization in 0.1 mol/l HCl and subsequent precipitation with ethanol yields the free acid. Gum arabic exhibits marked emulsifying and film-forming properties, which are caused not only by its structure, but also by the slight admixture (ca. 2%) of a protein. The serine and threonine residues of this protein are thought to be covalently bound to the carbohydrate.
The interfacial activity of gum arabic is low compared to that of proteins. The proportion of gum arabic to oil used in formulations has to be ap-



(4.140)

proximately 1:1. In contrast, a protein oil ratio of about 1:10 is used in an emulsion stabilized by milk proteins.

Gum arabic is very soluble in water and solutions of up to 50% gum can be prepared. The solution viscosity starts to rise steeply only at high concentrations (Fig. 4.21). This property is unlike that of many other polysaccharides, which provide highly viscous solutions even at low concentrations (Table 4.21).

Table 4.21. Viscosity (mPas) of polysaccharides in aqueous solution as affected by concentration (25 °C)

Concentration (%)	Gum arabic	Tragacanth	Carrageenan	Sodium alginate	Methyl cellulose	Locust bean gum	Guaran gum
1		54	57	214	39	59	3025
2		906	397	3760	512	1114	25,060
3		10,605	4411	29,400	3850	8260	111,150
4		44,275	25,356		12,750	39,660	302,500
5	7	111,000	51,425		67,575	121,000	510,000
6		183,500					
10	17						
20	41						
30	200						
40	936						
50	4163						

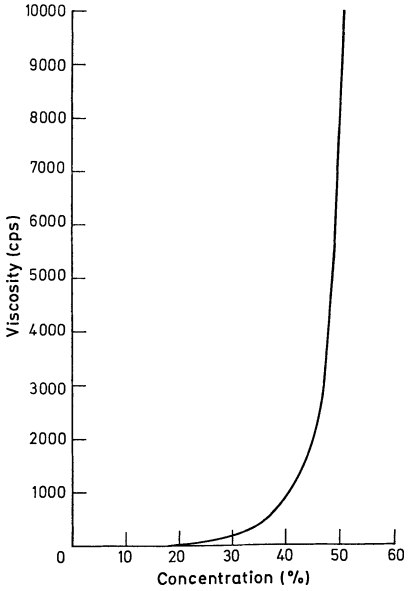


Fig. 4.21. Viscosity curve of an aqueous gum arabic solution (according to *Whistler*, 1973) (25.5 °C, Brookfield viscometer)

4.4.4.5.3 Utilization

Gum arabic is used as an emulsifier and stabilizer, e. g., in baked products. It retards sugar crystallization and fat separation in confectionery products and large ice crystal formation in ice creams, and can be used as a foam stabilizer in bever ages. Gum arabic is also applied as a flavor fixative in the production of encapsulated, powdered aroma concentrates. For example, essential oils are emulsified with gum arabic solution and then spray-dried. In this process, the polysaccharide forms a film surrounding the oil droplet, which then protects the oil against oxidation and other changes.

4.4.4.6 Gum Ghatti

4.4.4.6.1 Occurrence

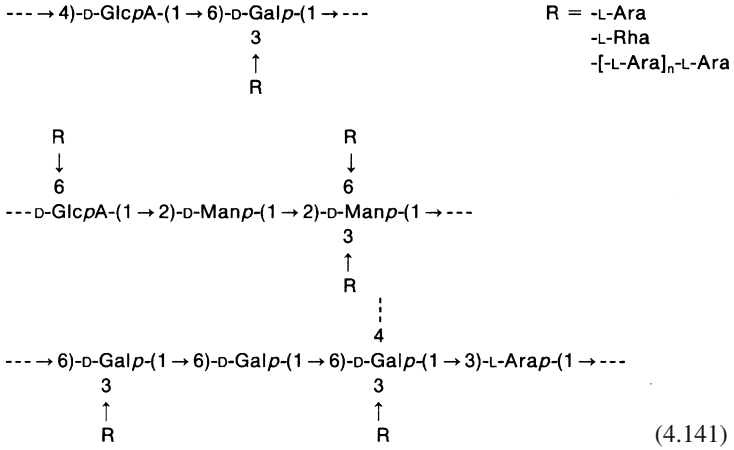
Gum ghatti is an exudate from the tree *Anogeissus latifolia* found in India and Ceylon.

4.4.4.6.2 Structure, Properties

The building blocks are L-arabinose, D-galactose, D-mannose, D-xylose, and D-glucuronic acid. L-Rhamnose has also been detected. The sugars are partially acetylated (5.5% acetyl groups based on dry weight). Three characteristic structural elements have been detected (cf. Formula 4.141). This acidic polysaccharide occurs as a Ca/Mg salt. Gum ghatti is soluble in water to the extent of ca. 90% and dispersible. Although it produces solutions that are more viscous than gum arabic, it is less soluble.

4.4.4.6.3 Utilization

Like gum arabic, gum ghatti can be used for the stabilization of suspensions and emulsions.



(4.141)

4.4.4.7 Gum Tragacanth

4.4.4.7.1 Occurrence

Gum tragacanth is a plant exudate collected from *Astragalus* species shrubs grown in the Middle East (Iran, Syria, Turkey).

4.4.4.7.2 Structure, Properties

Gum tragacanth consists of a water-soluble fraction, the so-called tragacanthic acid, and the insoluble swelling component, bassorin. Tragacanthic acid contains 43% of D-galacturonic acid, 40% of D-xylose, 10% of L-fucose, and 4% of D-galactose. Like pectin, it is composed of a main polygalacturonic acid chain which bears side chains made of the remaining sugar residues (Formula 4.142). Bassorin consists of 75% of L-arabinose, 12% of D-galactose, 3% of D-galacturonic acid methyl ester, and L-rhamnose.

Its molecular weight is about 840 kdal. The molecules are highyl elongated (450 × 1.9 nm) in aqueous solution and are responsible for the high viscosity of the solution (Table 4.21). As shown in Fig. 4.22, the viscosity is highly dependent on shear rate.

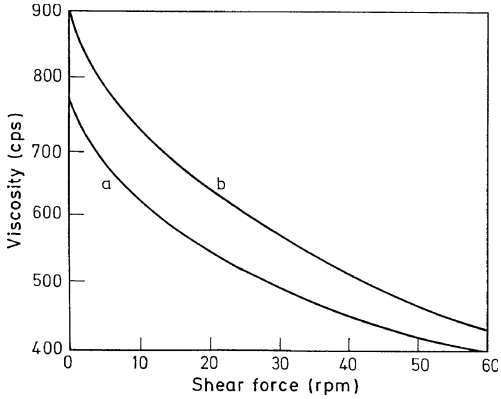


Fig. 4.22. The effect of shear rate on viscosity of aqueous tragacanth solutions. **a** Flake form tragacanth, 1%; **b** tragacanth, ribbon form, 0.5% (according to *Whistler*, 1973)

4.4.4.7.3 Utilization

Gum tragacanth is used as a thickening agent and a stabilizer in salad dressings (0.4–1.2%) and in fillings and icings in baked goods. As an additive in ice creams (0.5%), it provides a soft texture.

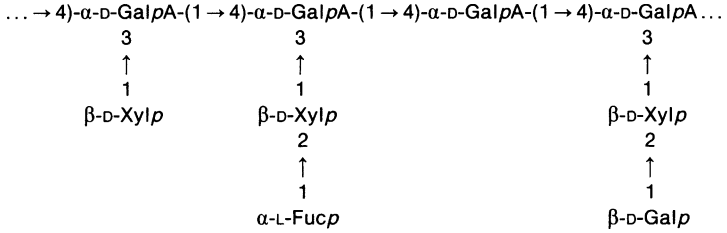
4.4.4.8 Karaya Gum

4.4.4.8.1 Occurrence

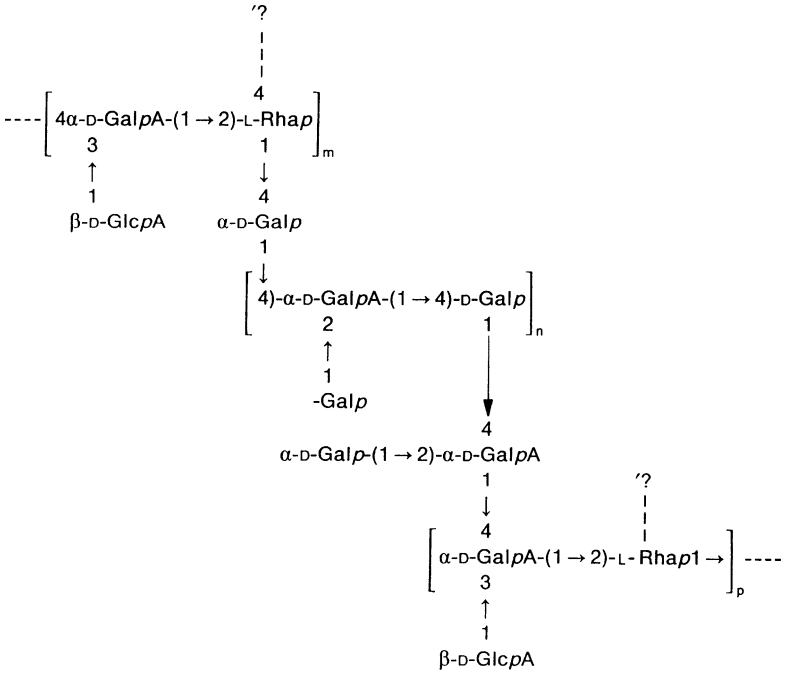
Karaya gum, also called Indian tragacanth, is an exudate from an Indian tree of the *Sterculia ureus* and other *Sterculia* species.

4.4.4.8.2 Structure, Properties

The building blocks are D-galactose, L-rhamnose, D-galacturonic acid, and L-glucuronic acid. The sugars are partially acetylated (13% acetyl groups based on dry weight). The molecule consists of three main chains which are polymers of different disaccharide units (cf. Formula 4.143). The main chains carry side chains and are also covalently linked via the side chains.



(4.142)



(4.143)

As a result of the strong cross-linkage, this polysaccharide is insoluble in water and resistant to enzymes and microorganisms. However, it swells greatly even in cold water. Suspensions have a pasty consistency at concentrations of more than 3%.

4.4.4.8.3 Utilization

Karaya gum is used as a water binder (soft cheese), a binding agent (meat products like corned beef, sausages), a stabilizer of protein foams (beer, whipped cream, meringues), and as a thickener (soups, sauces, salad dressings, mayonnaise, ketchup). It increases the freeze-thaw stability of products, prevents synaeresis of gels, and provides "body".

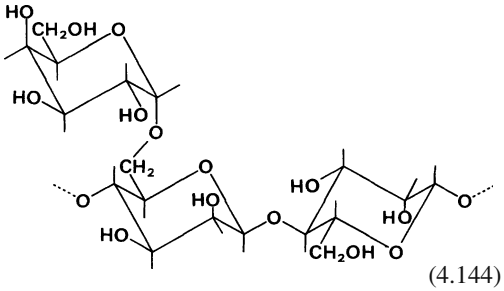
4.4.4.9 Guaran Gum

4.4.4.9.1 Occurrence, Isolation

Guar flour is obtained from the seed endosperm of the leguminous plant *Cyamopsis tetragonoloba.* The seed is decoated and the germ removed. In addition to the polysaccharide guaran, guar flour contains 10–15% moisture, 5–6% protein, 2.5% crude fiber and 0.5–0.8 ash. The plant is cultivated for forage in India, Pakistan and the United States (Texas).

4.4.4.9.2 Structure, Properties

Guaran gum consists of a chain of β-D-mannopyranosyl units joined by 1 → 4 linkages. Every second residue has a side chain, a D-galactopyranosyl residue that is bound to the main chain by an α(1 → 6) linkage (cf. Formula 4.126).



(4.144)

Guaran gum forms highly viscous solutions (Table 4.21), the viscosity of which is shear rate dependent (Fig. 4.23).

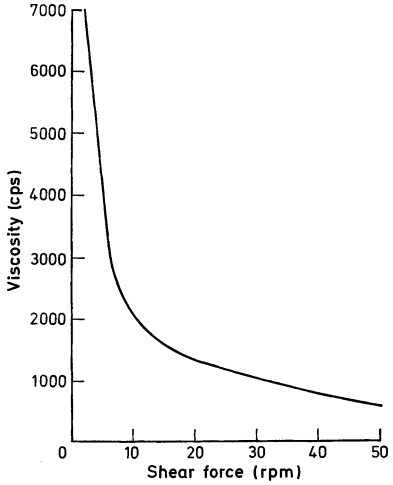


Fig. 4.23. Viscosity of 1% aqueous guar solution at 25 °C versus shear rate (rpm.). Viscometer: Haake rotovisco (according to *Whistler*, 1973)

4.4.4.9.3 Utilization

Guaran gum is used as a thickening agent and a stabilizer in salad dressings and ice creams (application level 0.3%). In addition to the food industry, it is widely used in paper, cosmetic and pharmaceutical industries.

4.4.4.10 Locust Bean Gum

4.4.4.10.1 Occurrence, Isolation

The locust bean (carob bean; St. John's bread) is from an evergreen cultivated in the Mediterranean area since ancient times. Its long, edible, fleshy seed pod is also used as fodder. The dried seeds were called "carat" by Arabs and served as a unit of weight (approx. 200 mg). Even today, the carat is used as a unit of weight for precious stones, diamonds and pearls, and as a measure of gold purity (1 carat $= 1/24$ part of pure gold). The locust bean seeds consist of 30–33% hull material, 23–25% germ and 42–46% endosperm. The seeds are milled and the endosperm is separated and utilized like the guar flour described above. The commercial flour contains 88% galactomannoglycan, 5% other polysaccharides, 6% protein and 1% ash.

4.4.4.10.2 Structure, Properties

The main locust bean polysaccharide is similar to that of guaran gum: a linear chain of $1 \rightarrow 4$ linked β-D-mannopyranosyl units, with α-D-galactopyranosyl residues $1 \rightarrow 6$ joined as side chains. The ratio mannose/galactose is 3 to 6; this indicates that, instead of every second mannose residue, as in guaran gum, only every 4th to 5th is substituted at the C-6 position with a galactose molecule.
The molecular weight of the galactomannan is close to 310 kdal. Physical properties correspond to those of guar gum, except the viscosity of the solution is not as high (cf. Table 4.21).

4.4.4.10.3 Utilization

Locust bean flour is used as a thickener, binder and stabilizer in meat canning, salad dressings, sausages, soft cheeses and ice creams. It also improves the water binding capacity of dough, especially when flour of low gluten content is used.

4.4.4.11 Tamarind Flour

4.4.4.11.1 Occurrence, Isolation

Tamarind is one of the most important and widely grown trees of India (*Tamarindus indica*; date of India). Its brown pods contain seeds which are rich in a polysaccharide that is readily extracted with hot water and, after drying, recovered in a powdered form.

4.4.4.11.2 Structure, Properties

The polysaccharide consists of D-galactose (1), D-xylose (2) and D-glucose (3), with respective molar ratios given in brackets. L-Arabinose is also present. The suggested structure is presented in Formula 4.145.
The polysaccharide forms a stable gel over a wide pH range. Less sugar is needed to achieve a desired gel strength than in corresponding pectin gels (Fig. 4.24). The gels exhibit only a low syneresis phenomenon.

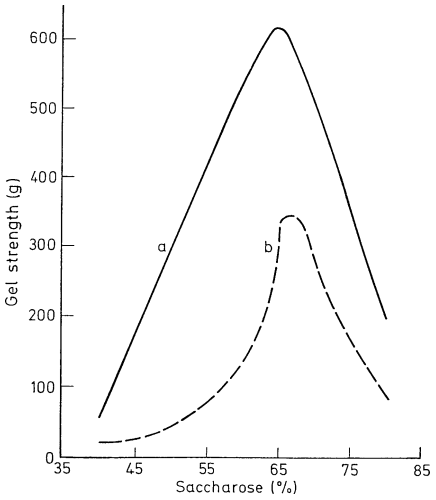


Fig. 4.24. Gel strength of (*a*) tamarind flour and (*b*) pectin from lemons versus saccharose concentration (according to *Whistler*, 1973)

4.4.4.11.3 Utilization

The tamarind seed polysaccharide is a suitable substitute for pectin in the production of marmalades and jellies. It can be used as a thickening agent and stabilizer in ice cream and mayonnaise production.

4.4.4.12 Arabinogalactan from Larch

4.4.4.12.1 Occurrence, Isolation

Coniferous larch-related woods (*Larix* species; similar to pine, but shed their needle-like leaves) contain a water-soluble arabinogalactan of 5–35% of the dry weight of the wood. It can be isolated from chipped wood by a counter-current extraction process, using water or dilute acids. The extract is then usually drum dried.

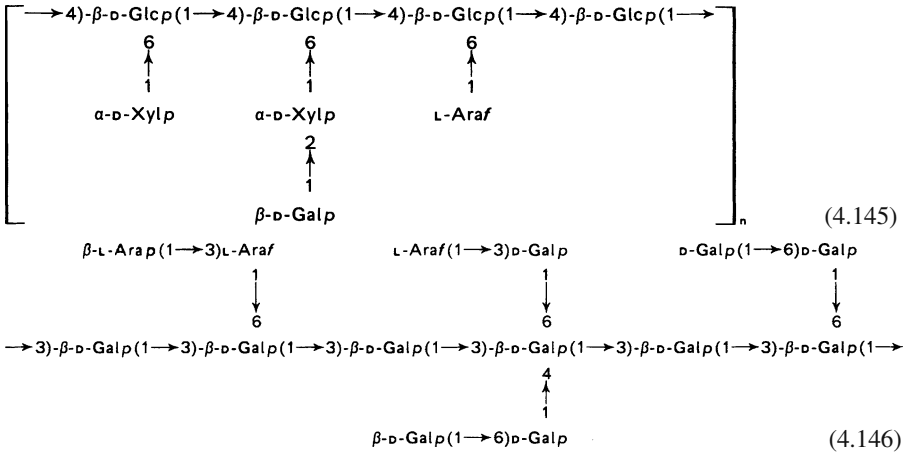
4.4.4.12.2 Structure, Properties

The polysaccharide consists of straight chain β-D-galactopyranosyl units joined by 1 → 3 linkages and, in part, has side chains of galactose and arabinose residues bound to positions 4 and 6. The suggested structure is given in Formula 4.146.

In general, the polysaccharide is highly branched. The molecular weight is 50–70 kdal. The molecule is nearly spherical in shape, so its aqueous solution behaves like a *Newton*ian fluid. The viscosity is exceptionally low. At a temperature of 20 °C, the viscosity of a 10% solution is 1.74 cps, a 30% solution 7.8 cps at pH 4 or 8.15 cps at pH 11, and a 40% solution 23.5 cps. These data show that the viscosity is practically unaffected by pH. The solution acquires a thick paste consistency only at concentrations exceeding 60%.

4.4.4.12.3 Utilization

Arabinogalactan, due to its good solubility and low viscosity, is used as an emulsifier and stabilizer, and as a carrier substance in essential oils, aroma formulations, and sweeteners.



(4.145)

(4.146)

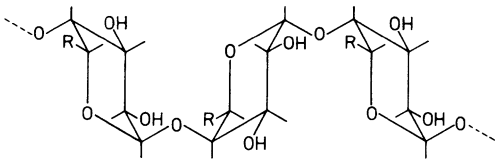
4.4.4.13 Pectin

4.4.4.13.1 Occurrence, Isolation

Pectin is widely distributed in plants. It is produced commercially from peels of citrus fruits and from apple pomace (crushed and pressed residue). It is 20–40% of the dry matter content in citrus fruit peel and 10–20% in apple pomace. Extraction is achieved at pH 1.5–3 at 60–100 °C. The process is carefully controlled to avoid hydrolysis of glycosidic and ester linkages. The extract is concentrated to a liquid pectin product or is dried by spray- or drum-drying into a powered product. Purified preparations are obtained by precipitation of pectin with ions which form insoluble pectin salts (e. g. Al^{3+}), followed by washing with acidified alcohol to remove the added ions, or by alcoholic precipitation using isopropanol and ethanol.

4.4.4.13.2 Structure, Properties

Pectin is a polysaccharide mixture with a complicated structure containing at least 65% of galacturonic acid (GalA). Three structural elements are involved in the make-up of a pectin molecule: a homogalacturonan (cf. Formula 4.147) consisting of (1 → 4) linked α-D-GalA, a galacturonan with differently arranged side chains (building blocks: apiose, fucose, arabinose, xylose), and a rhamnogalacturonan with a backbone consisting of the disaccharide units [→ 4)-α-D-GalA-(1 → 2)-α-L-Rha-(1 →] and with its rhamnose residues linked by arabinan and galactan chains. In pectins, the GalA residues



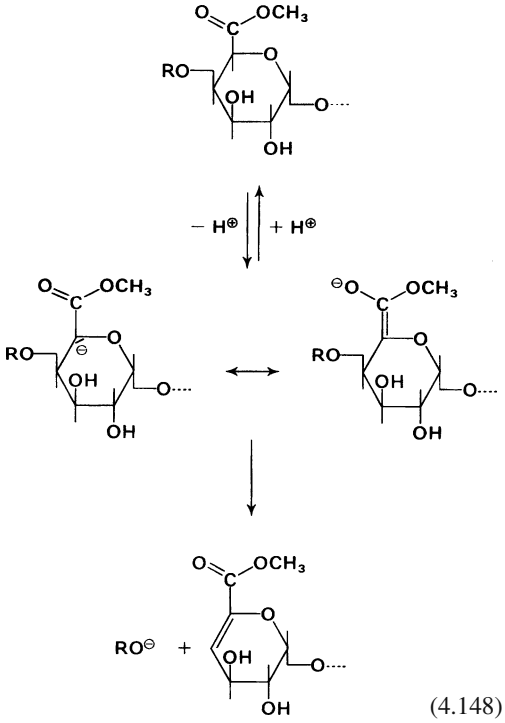
(4.147)

are esterified to a variable extent with methanol, while the HO-groups in 2- and 3-positions may be acetylated to a small extent. Pectin stability is highest at pH 3–4. The glycosidic linkage hydrolyzes in a stronger acidic medium. In an alkaline medium, both linkages, ester and glycosidic, are split to the same extent, the latter by an elimination reaction (cf. Formula 4.148).

The elimination reaction occurs more readily with galacturonic acid units having an esterified carboxyl group, since the H-atom on C-5 is more acidic than with residues having free carboxyl groups.

At a pH of about 3, and in the presence of Ca^{2+} ions also at higher pH's, pectin forms a thermally reversible gel. The gel-forming ability, under comparable conditions, is directly proportional to the molecular weight and inversely proportional to the esterification degree. For gel formation, low-ester pectins require very low pH values and/or calcium ions, but they gelatinize in the presence of a relatively low sugar content. High-ester pectins require an increasing amount of sugar with rising esterification degree. The gelsetting time for high ester pectins is longer than that for pectin products of low esterification degree (Table 4.22).

Apart from the degree of esterification, gel formation is also influenced by the distribution of the ester groups in the pectin molecule.



(4.148)

Table 4.22. Gelling time of pectins with differing degrees of esterification

Pectin type	Esterification degree (%)	Gelling time[a] (s)
Fast gelling	72–75	20–70
Normal	68–71	100–135
Slow gelling	62–66	180–250

[a]Difference between the time when all the prerequisites for gelling are fulfilled and the time of actual gel setting.

4.4.4.13.3 Utilization

Since pectin can set into a gel, it is widely used in marmalade and jelly production. Standard conditions to form a stable gel are, for instance: pectin content <1%, sucrose 58–75% and pH 2.8–3.5. In low-sugar products, low-ester pectin is used in the presence of Ca^{2+} ions. Pectin is also used to stabilize soured milk beverages, yoghurts and ice creams.

4.4.4.14 Starch

4.4.4.14.1 Occurrence, Isolation

Starch is widely distributed in various plant organs as a storage carbohydrate. As an ingredient of many foods, it is also the most important carbohydrate source in human nutrition. In addition, starch and its derivatives are important industrially, for example, in the paper and textile industries.

Starch is isolated mainly from the sources listed in Table 4.23. Starch obtained from corn, potatoes, cassava, and wheat in the native and modified form accounted for 99% of the world production in 1980. Some other starches are also available commercially. Recently, starches obtained from legumes (peas, lentils) have become more interesting because they have properties which appear to make them a suitable substitute for chemically modified starches in a series of products.

Starches of various origin have individual, characteristic properties which go back to the shape, size, size distribution, composition, and crystallinity of the granules. The existing connections are not yet completely understood on a molecular basis.

Table 4.23. Raw materials for starch

Raw material	Starch production 1980[a]
Raw materials of industrial importance	
Corn	77
Waxy corn	
Potato	10
Cassava	8
Wheat	4
Rice	
Waxy rice	
Other raw materials	
Sago palm	Kouzu
Sweet potato	Water chestnut
Arrowroot	Edible canna
Negro corn	Mungo bean
Lotus root	
Taro	Lentil

[a] % of the world production.

In some cases, e. g., potato tubers, starch granules occur free, deposited in cell vacuoles; hence, their isolation is relatively simple. The plant material is disintegrated, the starch granules are washed out with water, and then sedimented from the "starch milk" suspension and dried. In other cases, such as cereals, the starch is embedded in the endosperm protein matrix, hence granule isolation is a more demanding process. Thus, a counter-current process with water at 50 °C for 36–48 h is required to soften corn (steeping step of processing). The steeping water contains 0.2% SO_2 in order to loosen the protein matrix and, thereby, to accelerate the granule release and increase the starch yield. The corn grain is then disintegrated. The germ, due to its high oil content, has a low density and is readily separated by flotation. It is the source for corn oil isolation (cf. 14.3.2.2.4). The protein and starch are then

separated in hydrocyclones. The separation is based on density difference (protein < starch).
The protein by-product is marketed as animal feed or used for production of a protein hydrolysate (seasoning). The recovered starch is washed and dried.
In the case of wheat flour, a dough is made first, from which a raw starch suspension is washed out. After separation of fiber particles from this suspension, the starch is fractionated by centrifugation. In addition to the relatively pure primary starch, a finer grained secondary starch is obtained which contains pentosans. The starch is then dried and further classified. The residue, gluten (cf. 15.1.5), serves, e. g., as a raw material in the production of food seasoning (cf. 12.7.3.5) and in the isolation of glutamic acid. If dried gently, it retains its baking quality and is used as a flour improver. In the case of rye, the isolation of starch is impeded by the relatively high content of swelling agents. Starch isolated from the tubers of various plants in tropical countries is available on the market under a variety of names (e. g., canna, maranta, and tacca starch). The real sago is the product obtained from the pith of the sago palm.
Starch is a mixture of two glucans, amylose and amylopectin (cf. 4.4.4.14.3 and 4.4.4.14.4).
Most starches contain 20–30% amylose (Table 4.24). New corn cultivars (amylomaize) have been developed which contain 50–80% amylose. The amylose can be isolated from starch, e. g., by crystallization of a starch dispersion, usually in the presence of salts ($MgSO_4$) or by precipitation with a polar organic compound (alcohols, such as n-butanol, or lower fatty acids, such as caprylic or capric), which forms a complex with amylose and thus enhance its precipitation.
Normal starch granules contain 70–80% amylopectin, while some corn cultivars and millet, denoted as waxy maize or waxy millet, contain almost 100% amylopectin.

4.4.4.14.2 Structure and Properties of Starch Granules

Starch granules are formed in the amyloplasts. These granules are simple or compound and consist of concentric or eccentric layers of varying density. They are of varying size (2–150 μm), size distribution, and shape (Table 4.24). In addition to amylose and amylopectin, they usually contain small amounts of proteins and lipids. They are examined by using various physical methods, including light microscopy, small-angle light scattering, electron microscopy, X-ray diffraction, small-angle neutron scattering, and small-angle X-ray scattering. On the basis of X-ray diffraction experiments, starch granules are said to have a semicrystalline character, which indicates a high degree of orientation of the glucan molecules. About 70% of the mass of a starch granule is regarded as amorphous and ca. 30% as crystalline (Table 4.24). The amorphous regions contain the main amount of amylose, but also a considerable part of the amylopectin. The crystalline regions consist primarily of amylopectin. Although this finding was surprising at first because of the branched structure of amylopectin (cf. 4.4.4.14.4), it was deduced from the fact that amylose can be dissolved out of the granule without disturbing the crystalline character and that even amylose-free starches, like waxy corn starch, are semicrystalline. The degree of crystallinity depends on the water content. It is 24% for air-dried potato starch (19.8% of water), 29–35% for the wetted product (45–55% of water), and only 17% for starch dried via P_2O_5 and subsequently rehydrated.
On the basis of results obtained from different physical methods, the model shown in Fig. 4.25 is under discussion for the crystalline regions of the starch granule. It contains double helices of amylopectin (cf. 4.4.4.14.4), mixed amylose/amylopectin double helices, V helices of amylose with enclosed lipid (cf. 4.4.4.14.3), free amylose, and free lipid.
With the aid of X-ray diffraction diagrams, native starches can be divided into types A, B, and C. An additional form, called the V-type, occurs in swollen granules (Fig. 4.26). While types A and B are real crystalline modifications, the C-type is a mixed form. The A-type is largely present in cereal starches, and the B-type in potatoes, amylomaize, and in retrograded starches (resistant starch, cf. 4.4.4.16.3). The C-type is not only observed in mixtures of corn and potato starches, but it is also found in various legume starches.
When suspended in cold water, air-dried starch granules swell with an increase in diameter of 30–40%. If this suspension is heated, irreversible

Table 4.24. Shape, composition, and properties of different starch granules

Source	Shape[a]	Diameter (μm)	Crystallinity (%)	Gelatinization temperature (°C)	Swelling at 95°C[b]	Amylose		Amylopectin	
						Percentage (%)[c]	Polymerisation degree	Iodine binding constant[d]	Polymerisation degree[e]
Cereal									
Wheat	l,p	2–38	36	53–65	21	22–28	2100	0.21	19–20
Rye	l	12–40		57–70		28		0.74	26
Barley	l	2–5		56–62		22–29	1850		26
Corn	p	5–25		62–70	24	28	940	0.91	25–26
Amylomaize			20–25	67–87		52–80	1300	0.11	23
Waxy corn	p		39	63–72	64	0–1			20–22
Oats		5–15		56–62		27	1300		20
Rice	p	3–8	38	61–78	19	14–32		0.59	
Waxy rice				55–65	56	1			
Millet	p,s	4–12		} 69–75[g]	} 22[g]				
Sorghum	p,s	4–24				21–34			
Waxy sorghum				68–74	49				
Legumes									
Horsebean	s,o	17–31		64–67		32–34	1800	1.03	23
Smooth pea	n(si)	5–10		} 57–70[h]		33–35	1300	1.66	26
Wrinkled pea	n(c)	30–40				63–75	1100	0.91	27
Roots and tubers									
Potato	e	15–100	25	58–66		23	3200	0.58	24
Cassava	semi-s,s	5–35	38[f]	52–64		17		1.06	

[a] l = lenticular, p = polyhedral, s = spherical, o = oval, n = kidney-shaped, el = elliptical, si = simple, c = compound.
[b] Weight of swollen starch, based on its dry weight; loss of soluble polysaccharides is considered.
[c] Based on the cum of amylose and amylopectin.
[d] mg iodine/100 mg starch.
[e] Cleavage degree of polymerization, determined by degradation of branches with pullulanase or isoamylase.
[f] Tapioca.
[g] Millet.
[h] Pea.

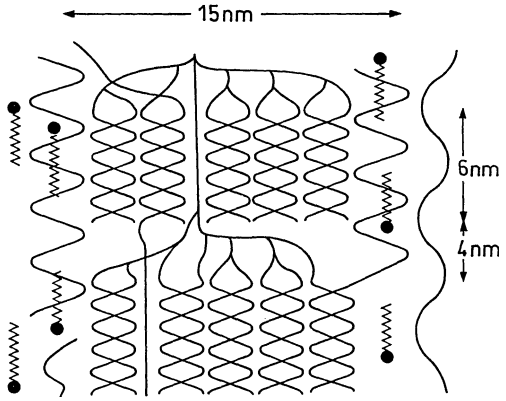


Fig. 4.25. Model of a crystalline region in a starch granule (according to *Galliard*, 1987). Amylopectin double helix; mixed double helix of amylose and amylopectin; V-helix of amylose and enclosed lipid; free lipid; free amylose

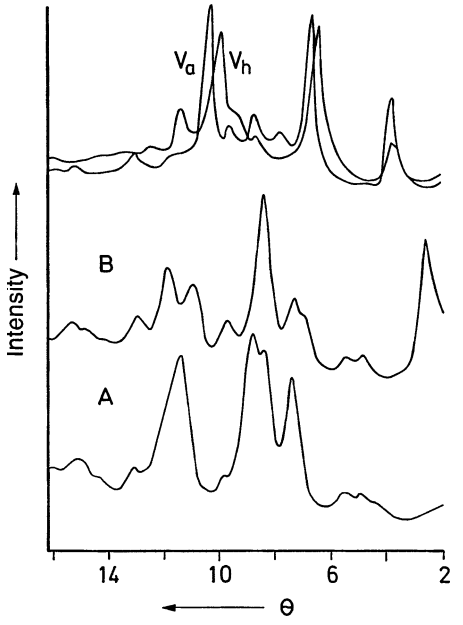


Fig. 4.26. X-ray diffraction diagrams of starches: A-type (cereals), B-type (legumes) and V-type (swollen starch, V_a: water free, V_h: hydrated) (according to *Galliard*, 1987)

changes occur starting at a certain temperature, which is characteristic of each type of starch (50–70 °C, cf. Table 4.24), called the gelatinization temperature. The starch granules absorb 20–40 g of water/g of starch, the viscosity of the suspension rising steeply. At the same time, a part of the amylose diffuses out of the granule and goes into solution. Finally, the granule bursts. In the first step of gelatinization, the starch crystallites melt and form a polymer network. This network breaks up at higher temperatures (ca. 100 °C), resulting in a solution of amylose and amylopectin. In gelatinization, water first diffuses into the granule, crystalline regions then melt with the help of hydration, and, finally, swelling gives rise to a solution through further diffusion of water. In this process, hydrogen bridges between glucose chains in the crystallites are primarily disrupted, and perhaps some of those in the amorphous regions as well. It is probable that the swelling of the amorphous regions facilitates the dissolving out of amylose from the crystallites, which are thereby destabilized. As with heating in water, the same effect occurs when starch is suspended in other solvents, e. g., liquid ammonia or dimethyl sulfoxide, or mechanically damaged, e. g., by dry grinding.

The course of gelatinization depends not only on the botanical origin of the starch and the temperature used, but also on the water content of the suspension (Fig. 4.27). Thus, dried starch with 1–3% of water undergoes only slight changes up to a temperature of 180 °C, whereas starch with 60% of water completely gelatinizes at temperatures as low as 70 °C.

If an aqueous starch suspension is maintained for some time at temperatures below the gelatinization temperature, a process known as tempering, the gelatinization temperature is increased, apparently due to the reorganization of the structure of the granule. Treatment of starch at low water contents and higher temperatures results in the stabilization of the crystallites and, consequently, a decrease in the swelling capacity. Figure 4.28 shows the resulting change in the X-ray diffraction spectrum from type B to type A, using potato starch as an example.

The changes in the physical properties caused by treating processes of this type can, however, vary considerably, depending on the botanical origin of the starches. This is shown in Table 4.25 for potato and wheat starch. On wet heating, the swelling capacity of both starches decreases, although to different extents. On the other hand, there is a decrease in solubility only of

Table 4.25. Physicochemical properties of starches before (1) and after (2) heat treatment in the wet state ($T = 100\,^{\circ}C$, $t = 16\,h$, $H_2O = 27\%$)

Property	Wheat starch		Potato starch	
	1	2	1	2
Start of gelatinization (°C)	56.5	61	60	60.5
End of gelatinization (°C)	62	74	68	79
Swelling capacity at 80 °C (ratio)	7.15	5.94	62.30	19.05
Solubility at 80 °C (%)	2.59	5.93	31.00	10.10
Water binding capacity (%)	89.1	182.6	102.00	108.7
Enzymatic vulnerability (% dissolved)	0.44	48.55	0.57	40.35

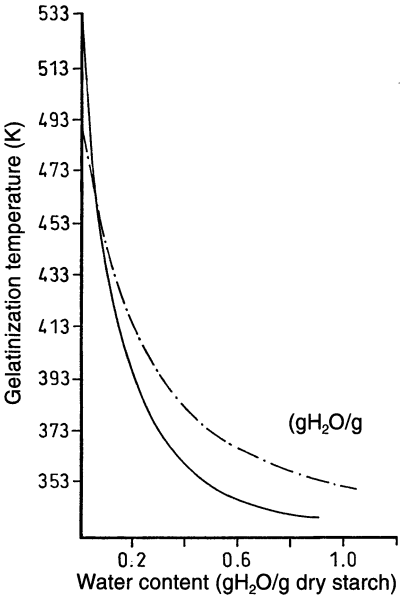


Fig. 4.27. Gelatinization temperature of differently hydrated starches (— potato starch, – · – wheat starch, determined by differential thermal analysis, differential calorimetry, and double refraction) (according to *Galliard*, 1987)

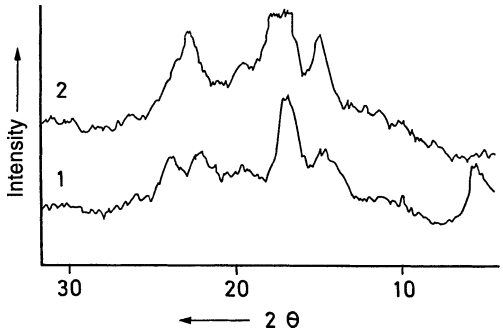


Fig. 4.28. X-ray diffraction diagrams of potato starch before (1) and after (2) thermal treatment (102 °C/16 h) at a water content of 40%. The pattern of native starch (18.3% of water) corresponds to the B-type and that of treated starch (24.2% of water) to the A-type (according to *Galliard*, 1987)

potato starch, while that of wheat starch clearly increases. The explanation put forward to explain these findings is that the amorphous amylose of potato starch is converted to an ordered, less soluble state, while the amylose of cereal starch, present partially in the form of helices with enclosed lipids, changes into a more easily leachable state.

A gelatinization curve for potato starch is presented in Fig. 4.29. The number of gelatinized starch granules was determined by microscopy. Another way to monitor gelatinization as a function of temperature is to measure the viscosity of a starch suspension. The viscosity curves in Fig. 4.30 show that, as mentioned above, the viscosity initially increases due to starch granule swelling. The subsequent disintegration of the swollen granule is accompanied by a drop in viscosity. The shape of the curve varies greatly for different starches.

Potato starch shows a very high maximum (~4000 *Brabender* units), followed by a steep drop. Waxy corn starch exhibits similar behavior, except that the maximum is not as high. In normal corn starch, the maximum is still lower, but the following drop is slight, i. e., the granules are more stable. Under these conditions, amylomaize starch does not swell, even though ca. 35% of

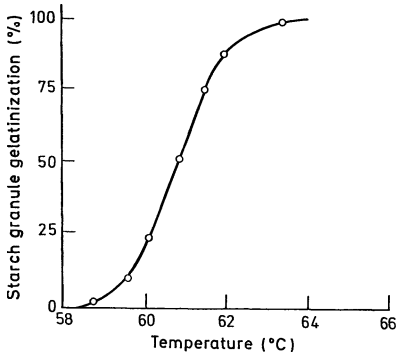


Fig. 4.29. Potato starch gelatinization curve (according to *Banks* and *Muir*, 1980)

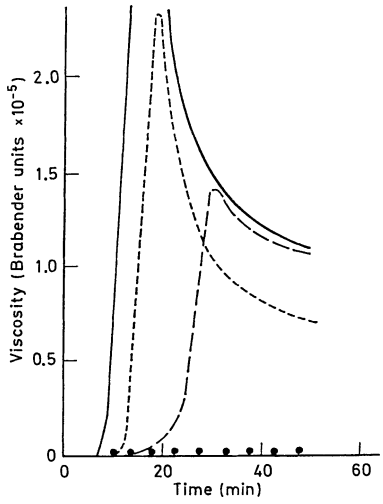


Fig. 4.30. Gelatinization properties of various starches (according to *Banks* and *Muir*, 1980). Brabender viscoamylograph. 40 g starch/460 ml water, temperature programming: start at 50 °C, heated to 95 °C at a rate of 1.5° C/min. Held at 95 °C for 30 min — potato, - - - waxy corn, – – – normal corn, and ● ● ● amylomaize starch

the amylose goes into solution. The viscosity of a starch paste generally increases on rapid cooling with mixing, while a starch gel is formed on rapid cooling without mixing.

Amylose gels tend to retrograde. This term denotes the largely irreversible transition from the solubilized or highly swollen state to an insoluble, shrunken, microcrystalline state (Fig. 4.31). This state can also be directly achieved by slowly cooling a starch paste. The tendency towards retrogradation is enhanced at low temperatures, es-

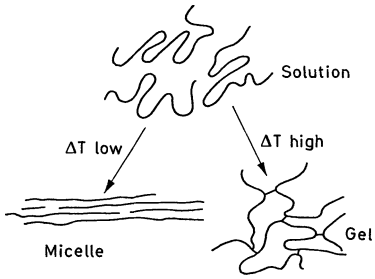


Fig. 4.31. Behavior of amylose molecules during cooling of a concentrated aqueous solution

pecially near 0 °C, neutral pH values, high concentration, and by the absence of surface active agents. It also depends on the molecular weight and on the type of starch, e. g., it increases in the series potato < corn < wheat. The transitions described from very water-deficient starting states via very highly swollen states or solutions to more or less shrunken states are linked to changes in the interactions between the glucans and to conformational changes. At present, these changes cannot be fully described because they greatly depend on the conditions in each case, e. g., even on the presence of low molecular compounds.

It is known that the gelatinization temperature is increased by polyhydroxy compounds (glycerol, sugar) and decreased by salts (NaCl, $CaCl_2$), as presented in Fig. 4.32 (top) as a function of water activity, which is lowered by the dissolved substances (a_w, cf. 0.3.1). Apart from the activity of the solvent water, if its volume fraction (v_1), which changes in reverse order to the volume fraction of the solute, is considered and if the gelatinization temperature is plotted against $\ln a_w/V_1$, instead of a_w, the effect of the different dissolved substances is unified (Fig. 4.32, bottom). The reason is that polyhydroxy compounds cause a large change in v_e and a small change in a_w, while a small change in v_e is linked to a large change in a_w in the case of the salts.

Lipids also influence the properties of starch. Like free amino acids, monoglycerides or fatty acid esters of hydroxy acids, lipids form inclusion compounds with amylose (cf. 4.4.4.14.3). Like di- and triglycerides, they also reduce the swelling capacity and solubility by inhibiting water diffusion. Therefore, both degreasing as well as lipid addition are of importance as physical modification methods of starches.

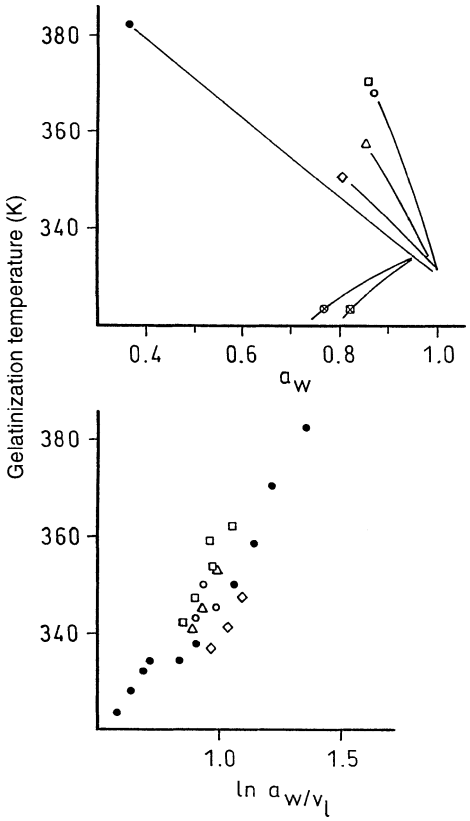
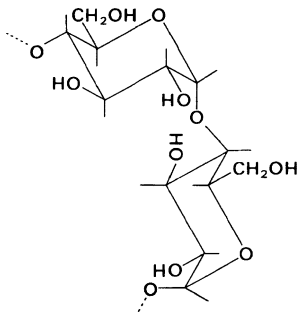


Fig. 4.32. Gelatinization temperature of potato starch as a function of water activity a_w (*top*) and of the natural logarithm of the quotient of activity a_w to volume fraction v_l of water (*bottom*); • glycerol, ○ maltose, □ saccharose, △ glucose, ◇ ribose, ⊗ NaCl, ⊠ $CaCl_2$ (according to *Galliard*, 1987)

4.4.4.14.3 Structure and Properties of Amylose

Amylose is a chain polymer of α-D-glucopyranosyl residues linked $1 \rightarrow 4$:



(4.149)

Enzymatic hydrolysis of the chain is achieved by α-amylase, β-amylase and glucoamylase. Often, β-amylase does not degrade the molecule completely into maltose, since a very low branching is found along the chain with $\alpha(1 \rightarrow 6)$ linkages. The molecular size of amylose is variable. The polymerization degree in wheat starch lies between 500 and 6000, while in potatoes it can rise up to 4500. This corresponds to a molecular weight of 150–750 kdal. X-ray diffraction experiments conducted on oriented amylose fibers make possible the assignment of the types of starch mentioned above to definite molecular structural elements. Oriented fibers of the A-type were obtained by cutting and stretching thin films of acetylamylose at 150 °C, deacetylation in alcoholic alkali, and conditioning at 80% relative air humidity and 85 °C. Type B fibers were obtained in a corresponding manner by conditioning the deacetylated material at room temperature for three days at 80% and for another three days at 100% relative air humidity, followed by aftertreatment in water at 90 °C for 1 h. The diffraction patterns obtained with these oriented fibers corresponded to those of types A and B given by native starch powders, allowing the development of structural models.

The structural elements of type B are left-hand double helices (Fig. 4.34a), which are packed in a parallel arrangement (Fig. 4.33). One turn of the double helix is 2.1 nm long, which corresponds to 6 glucose residues, i. e., three residues from each glucan chain. Hydrogen bridges between the amylose molecules stabilize the double helix. The central channel surrounded by six double helices is filled with water (36 H_2O/unit cell). The A-type is very similar to the B-type, except that the central channel is occupied by another double helix, making the packing more close. In this type, only eight molecules of water per unit cell are inserted between the double helices. The transition from type B to type A achieved by wet heating has been described already (4.4.4.14.2, Fig. 4.28). It is difficult to bring the postulated antiparallel arrangement of the double helices into line with the requirements of biosynthesis, where a parallel arrangement can be expected. It is possible that the present experimental data do not exclude such an arrangement.

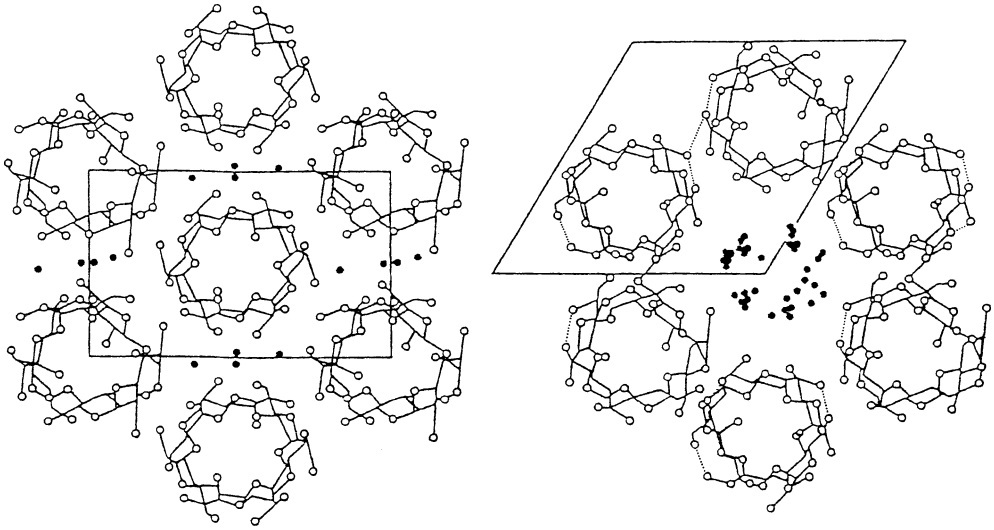


Fig. 4.33. Unit cells and arrangement of double helices (*cross section*) in A-amylose (*left*) and B-amylose (*right*) (according to *Galliard*, 1987)

The double helix mentioned above and shown in Fig. 4.34 can, depending on conditions, change into other helical conformations.

In the presence of KOH, for instance, a more extended helix results with 6 glucose residues per helical turn (Fig. 4.34, b) while, in the presence of KBr, the helix is even more stretched to 4 residues per turn (Fig. 4.34, c). Inclusion (clathrate) compounds are formed in the presence of small molecules and stabilize the V starch conformation (Fig. 4.34, d); it also has 6 glucose residues per helical turn. Stabilization may be achieved by H-bridges between O-2 and O-3 of neighboring residues within the same chain and between O-2 and O-6 of the residues i and $i+6$ neighbored on the helix surface. Many molecules, such as iodine, fatty acids, fatty acid esters of hydroxycarboxylic acids (e. g., stearyllactate), monoglycerides, phenols, arylhalogenides, n-butanol, t-butanol, and cyclohexane are capable of forming clathrate compounds with amylose molecules. The helix diameter, to a certain extent, conforms to the size of the enclosed guest molecule; it varies from 13.7 Å to 16.2 Å. While the iodine complex and that of n-butanol have 6 glucose residues per turn in a V conformation, in a complex with t-butanol the helix turn is enlarged to 7 glucose residues/turn (Fig. 4.34, e). It is shown by an α-naphthol clathrate that up to 8 residues are allowed (Fig. 4.34, f). Since the helix is internally hydrophobic, the enclosed "guest" has also to be lipophilic in nature. The enclosed molecule contributes significantly to the stability of a given conformation. For example, it is observed that the V conformation, after "guest" compound removal, slowly changes in a humid atmosphere to a more extended B conformation. Such a conformational transition also occurs during staling of bread or other bakery products. Freshly baked bread shows a V spectrum of gelatinized starch, but aged bread typically has the retrograded starch B spectrum. Figure 4.35 illustrates both conformations in the form of cylinder projections. While in V amylose, as already outlined, O-2 of residues i and O-6 of residues $i+6$ come into close contact through H-bridges, in the B pattern the inserted water molecules increase the double-strand distance along the axis of progression (h) from 0.8 nm for the V helix to 1 nm for the B helix.

Cereal starches are stabilized by the enclosed lipid molecules, so their swelling power is low. The swelling is improved in the presence of alcohols (ethanol, amyl alcohol, tert-amyl alcohol). Obviously, these alcohols are dislodging and removing the "guest" lipids from the helices.

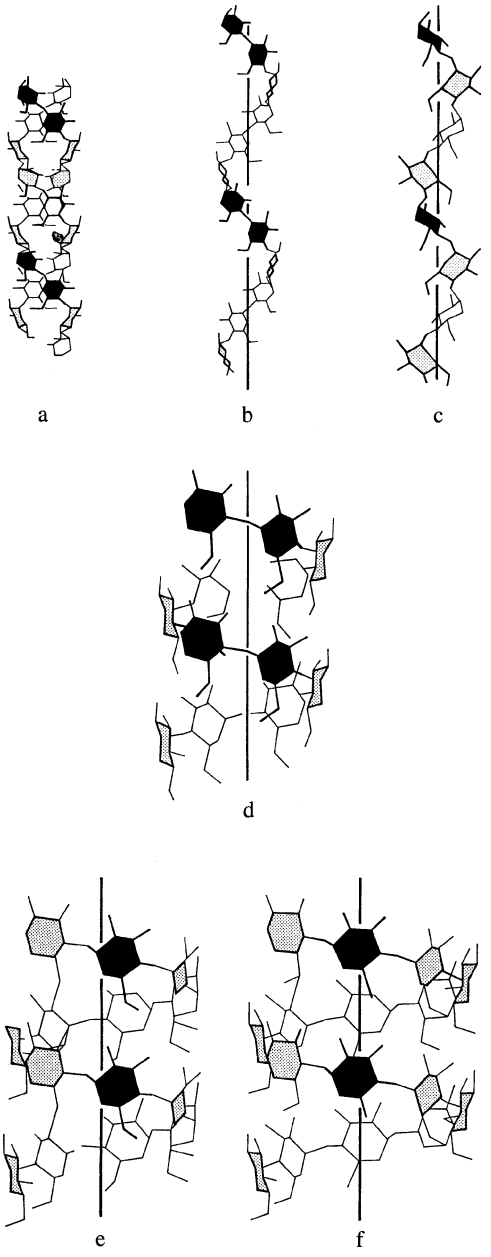


Fig. 4.34. Amylose conformation (for explanation see text) (according to *Rees*, 1977)

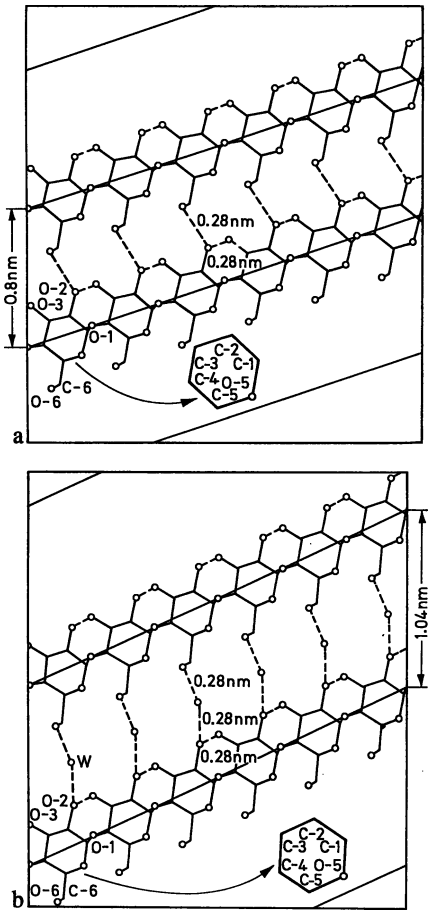
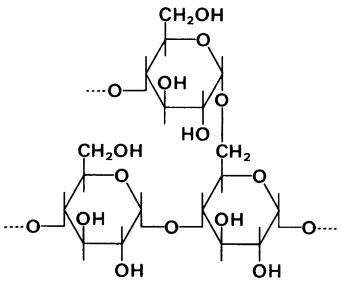


Fig. 4.35. Amylose: V-conformation (**a**) and B-conformation (**b**) in a cylinder projection (according to *Ebert*, 1980)

4.4.4.14.4 Structure and Properties of Amylopectin

Amylopectin is a branched glucan with side chains attached in the 6-position of the glucose residues of the principal chain:



(4.150)

An average of 20–60 glucose residues are present in short chain branches and each of these

branch chains is joined by linkage of C-1 to C-6 of the next chain. The proposed structural models (Fig. 4.36) suggest that amylopectin also has double helices organized in parallel. As mentioned above, the main portion of a starch granule's crystalline structure is apparently derived from amylopectin. The structural modell II in Fig. 4.36 clearly shows from left to right the sequence of more compact (crystalline) and less compact (amorphous) sections. In this model, a distinction is made between shorter A-chains that are free of side chains and longer B chains that bear side chains. In the B chains, sections with compact successive side chains (cluster) alternate with branch-free sections.

The degree of polymerization of amylopectin (wheat) lies in the range of 3×10^5–3×10^6 glucose units, which corresponds to a molecular mass of 5×10^7–5×10^8. One phosphoric acid residue is found for an average of 400 glucose residues.

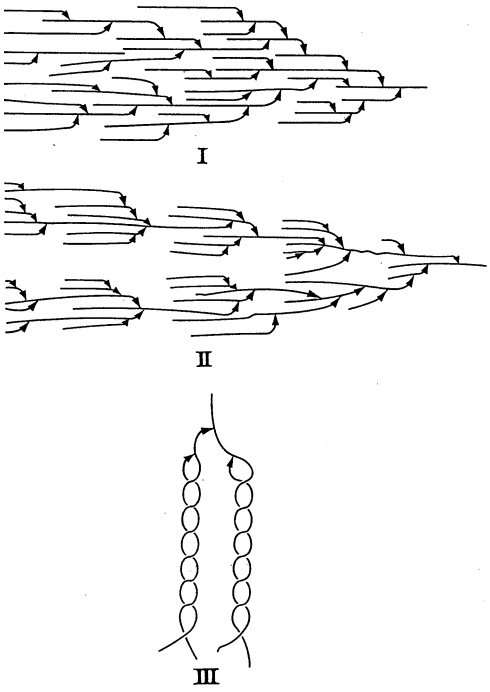


Fig. 4.36. Structural models (I, II) for amylopectin with parallel double helices. III is an enlarged segment of I or II (according to *Banks* and *Muir*, 1980)

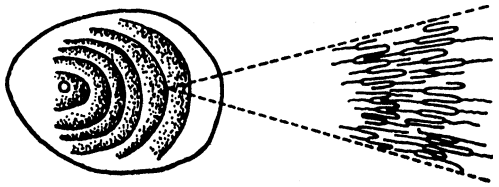


Fig. 4.37. Arrangement of amylopectin molecules in a starch granule

The organization of amylopectin molecules in starch granules is shown in Fig. 4.37: it is radial, the reducing end being directed outwards.

Enzymatic degradation of amylopectin is similar to that of amylose. The enzyme β-amylase degrades the molecule up to the branching points. The remaining resistant core is designated as "limit-dextrin".

Amylopectin, when heated in water, forms a transparent, highly viscous solution, which is ropy, sticky and coherent. Unlike with amylose, there is no tendency toward retrogradation. There are no staling or aging phenomena and no gelling, except at very high concentrations. However, there is a rapid viscosity drop in acidic media and on autoclaving or applying stronger mechanical shear force.

4.4.4.14.5 Utilization

Starch is an important thickening and binding agent and is used extensively in the production of puddings, soups, sauces, salad dressings, diet food preparations for infants, pastry filling, mayonnaise, etc. Corn starch is the main food starch and an important raw material for the isolation of starch syrup and glucose (cf. 19.1.4.3).

A layer of amylose can be used as a protecting cover for fruits (dates or figs) and dehydrated and candied fruits, preventing their sticking together. Amylose treatment of French fries decreases their susceptibility to oxidation. The good gelling property of a dispersable amylose makes it a suitable ingredient in instant puddings or sauces. Amylose films can be used for food packaging, as edible wrapping or tubing, as exemplified by a variety of instant coffee or tea products. Amylopectin utilization is also diversified. It is used to a large extent as a thickener or stabilizer

Table 4.26. Utilization of amylopectin and its derivatives

Starch	Utilization
Unmodified waxy starch (also in blend with normal starch and flours)	Salad dressing, sterilized canned and frozen food, soups, broth, puffed cereals, and snack food
Pregelatinized waxy starch or isolated amylopectin	Baked products, paste (pâté) fillings, sterilized bread, salad dressing, pudding mixtures
Thin boiling waxy starch	Protective food coatings
Cross-linked waxy starch	Paste fillings, white and brown sauces, broth, sterilized or frozen canned fruit, puddings, salad dressing, soups, spreadable cream products for sandwiches, infant food
Waxy starch, hydroxypropyl ether	Sterilized and frozen canned food
Waxy starch, carboxymethyl ether	Emulsion stabilizer
Waxy starch acetic acid ester	Sterilized and frozen canned food, infant food
Waxy starch succinic- and adipic acid esters	Sterilized and frozen canned food, aroma encapsulation
Waxy starch sulfuric acid ester	Thickenig agent, emulsion stabilizer, ulcer treatment (pepsin inhibitor)

and as an adhesive or binding agent. Table 4.26 lists the range of its applications.

4.4.4.14.6 Resistant Starch

Starch and its degradation products which are not absorbed in the small intestine are called resistant starch (RS). RS can, however, be metabolized by the bacteria of the colon. Acetic acid, propionic acid and butyric acid are formed, stimulating the growth of the cells of the intestinal epithelium. Especially butyric acid has been found to positively affect health. A distinction is made between 4 forms of RS: Type I, starch enclosed in cells (e. g., coarse-ground grain or legumes), Type II, native starch granules (e. g., in bananas, potatoes), Type III, starch fractions produced on retrogradation (e. g., in boiled potatoes, bread crumb), and Type IV, starch modified by the *Maillard* reaction or caramelization (formation of glycosidic bonds which are not hydrolyzed by α-amylase).

Only amylose, and not amylopectin, is involved in Type III RS. The formation of RS depends on the temperature and on the water and lipid content. Indeed, 20% of RS is obtained on autoclaving corn starch. The yield can be raised to about 40% by heating under pressure and cooling (ca. 20 cycles). The optimal amylose/water ratio is 1:3.5 (g/g). Lipids complexed by amylose inhibit RS formation (cf. 15.2.4.1).

Type III RS consists of 60–70% of double helical α(1–4)polyglucan aggregates and only 25–30% of crystalline structures. It is assumed that the high content of the double helical conformation, which is similar to that of amylose type B, limits the activity of α-amylases.

Various methods have been proposed for the determination of RS, e. g., RS equals total starch minus digestible starch. The results are only comparable if the incubation conditions and the α-amylases used correspond.

4.4.4.15 Modified Starches

Starch properties and those of amylose and amylopectin can be improved or "tailored" by physical and chemical methods to fit or adjust the properties to a particular application or food product.

4.4.4.15.1 Mechanically Damaged Starches

When starch granules are damaged by grinding or by application of pressure at various water contents, the amorphous portion is increased, resulting in improved dispersibility and swellability in cold water, a decrease in the gelatinization temperature by 5–10 °C, and an increase in enzymatic vulnerability. In bread dough made from flour containing damaged starch, for instance, the uptake of water is faster and higher and amylose degradation greater.

4.4.4.15.2 Extruded Starches

The X-ray diffraction diagram changes on extrusion of starch. The V-type appears first, followed

by its conversion to an E-type at higher temperatures (>185 °C), and reformation of the V-type on cooling. The E-type apparently differs from the V-type only in the spacing of the V helices of amylose.
Extruded starches are easily dispersible, better soluble, and have a lower viscosity. The partial degradation of appropriately heated amylose shows that chemical changes also occur at temperatures of 185–200 °C. Apart from maltose, isomaltose, gentiobiose, sophorose, and 1,6-anhydroglucopyranose appeared.

4.4.4.15.3 Dextrins

Heating of starch (<15% of water) to 100–200 °C with small amounts of acidic or basic catalysts causes more or less extensive degradation. White and yellow powders are obtained which deliver clear or turbid, highly sticky solutions of varying viscosity. These products are used as adhesives in sweets and as fat substitutes.

4.4.4.15.4 Pregelatinized Starch

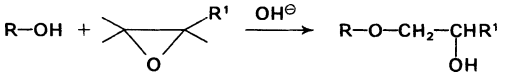
Heating of starch suspensions, followed by drying, provides products that are swellable in cold water and form pastes or gels on heating. These products are used in instant foods, e. g., pudding, and as baking aids (cf. Table 4.26).

4.4.4.15.5 Thin-Boiling Starch

Partial acidic hydrolysis yields a starch product which is not very soluble in cold water but is readily soluble in boiling water. The solution has a lower viscosity than the untreated starch, and remains fluid after cooling. Retrogradation is slow. These starches are utilized as thickeners and as protective films (cf. Table 4.26).

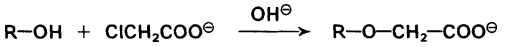
4.4.4.15.6 Starch Ethers

When a 30–40% starch suspension is reacted with ethylene oxide or propylene oxide in the presence of hydroxides of alkali and/or alkali earth metals (pH 11–13), hydroxyethyl- or hydroxypropyl-derivates are obtained ($R' = H, CH_3$):



(4.151)

The derivatives are also obtained in reaction with the corresponding epichlorohydrins. The substitution degree can be controlled over a wide range by adjusting process parameters. Low substitution products contain up to 0.1 mole alkyl group/mole glucose, while those with high substitution degree have 0.8–1 mole/mole glucose. Introduction of hydroxyalkyl groups, often in combination with a small extent of cross-linking (see below) greatly improves starch swelling power and solubility, lowers the gelatinization temperature and substantially increases the freeze–thaw stability and the paste clarity of highly-viscous solutions. Therefore, these products are utilized as thickeners for refrigerated foods (apple and cherry pie fillings, etc), and heat-sterilized canned food (cf. Table 4.26).
Reaction of starch with monochloroacetic acid in an alkaline solution yields carboxymethyl starch:

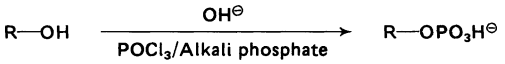


(4.152)

These products swell instantly, even in cold water and in ethanol. Dispersions of 1–3% carboxymethyl starch have an ointment-like (pomade) consistency, whereas 3–4% dispersions provide a gel-like consistency. These products are of interest as thickeners and gelforming agents.

4.4.4.15.7 Starch Esters

Starch monophosphate ester is produced by dry heating of starch with alkaline orthophosphate or alkaline tripolyphosphate at 120–175 °C:



(4.153)

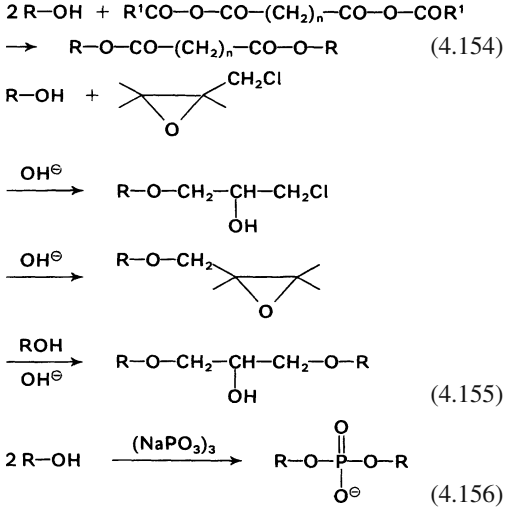
Starch organic acid esters, such as those of acetic acid, longer chain fatty acids (C_6–C_{26}), succinic,

adipic or citric acids, are obtained in reactions with the reactive derivatives (e. g., vinyl acetate) or by heating the starch with free acids or their salts. The thickening and paste clarity properties of the esterified starch are better than in the corresponding native starch.

In addition, esterified starch has an improved freeze–thaw stability. These starches are utilized as thickeners and stabilizers in bakery products, soup powders, sauces, puddings, refrigerated food, heat-sterilized canned food and in margarines. The starch esters are also suitable as protective coatings, e. g., for dehydrated fruits or for aroma trapping or encapsulation (cf. Table 4.26).

4.4.4.15.8 Cross-Linked Starches

Cross-linked starches are obtained by the reaction of starch (R—OH) with bi- or polyfunctional reagents, such as sodium trimetaphosphate, phosphorus oxychloride, epichlorohydrin or mixed anhydrides of acetic and dicarboxylic acids (e. g., adipic acid):



The starch granule gelatinization temperature increases in proportion to the extent of cross-linking, while the swelling power decreases (Fig. 4.38). Starch stability remains high at extreme pH values (as in the presence of food acids) and under conditions of shear force. Cross-linked starch derivatives are generally used when high starch stability is demanded.

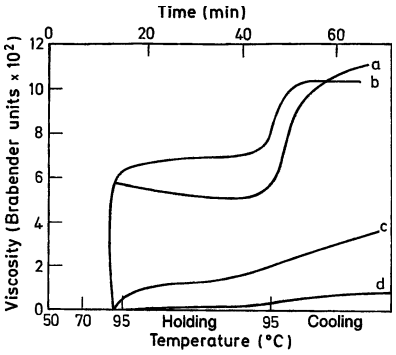
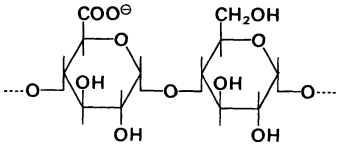


Fig. 4.38. Corn starch viscosity curves as affected by crosslinking degree. Instruments: Brabender amylograph; *a* control, *b* crosslinked with 0.05%, *c* 0.10%, *d* 0.15% epichlorohydrin (according to *Pigman*, 1970)

4.4.4.15.9 Oxidized Starches

Starch hydrolysis and oxidation occur when aqueous starch suspensions are treated with sodium hypochlorite at a temperature below the starch gelatinization temperature range. The products obtained have an average of one carboxyl group per 25–50 glucose residues:



Oxidized starch is used as a lower-viscosity filler for salad dressings and mayonnaise. Unlike thin-boiling starch, oxidized starch does not retrograde nor does it set to an opaque gel.

4.4.4.16 Cellulose

4.4.4.16.1 Occurrence, Isolation

Cellulose is the main constituent of plant cell walls, where it usually occurs together with hemicelluloses, pectin and lignin. Since cellulase enzymes are absent in the human digestive tract, cellulose, together with some other inert polysaccharides, constitute the indigestible carbohydrate of plant food (vegetables, fruits or cereals), referred to as dietary fiber. Cellulases are also absent in the digestive tract of animals, but herbivorous an-

imals can utilize cellulose because of the rumen microflora (which hydrolyze the cellulose). The importance of dietary fiber in human nutrition appears mostly to be the maintenance of intestinal motility (peristalsis).

4.4.4.16.2 Structure, Properties

Cellulose consists of β-glucopyranosyl residues joined by 1 → 4 linkages (cf. Formula 4.158).
Cellulose crystallizes as monoclinic, rod-like crystals. The chains are oriented parallel to the fiber direction and form the long b-axis of the unit cell (Fig. 4.39). The chains are probably somewhat pleated to allow intrachain hydrogen bridge formation between O-4 and O-6, and between O-3 and O-5 (cf. Formula 4.159).
Intermolecular hydrogen bridges (stabilizing the parallel chains) are present in the direction of the a-axis while hydrophobic interactions exist in the c-axis direction. The crystalline sections comprise an average of 60% of native cellulose. These sections are interrupted by amorphous gel regions, which can become crystalline when moisture is removed. The acid- or alkali-labile bonds also apparently occur in these regions. Microcrystalline cellulose is formed when these bonds are hydrolyzed. This partially depolymerized cellulose product with a molecular weight of 30–50 kdal, is still water insoluble, but does not have a fibrose structure.
Cellulose has a variable degree of polymerization (denoted as DP; number of glucose residues per chain) depending on its origin. The DP can range from 1000 to 14,000 (with corresponding molecular weights of 162 to 2268 kdal). Because of its high molecular weight and crystalline structure, cellulose is insoluble in water. Also, its swelling power or ability to absorb water, which depends partly on the cellulose source, is poor or negligible.

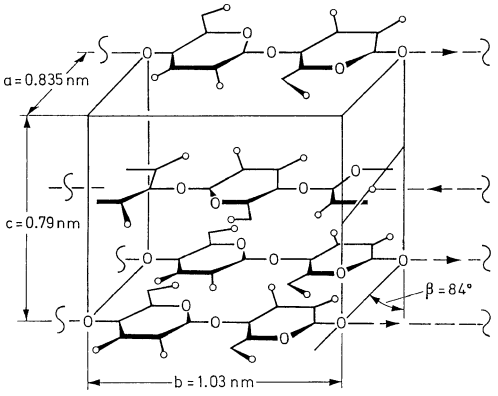


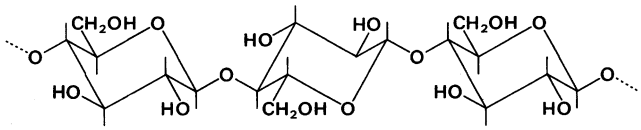
Fig. 4.39. Unit cell of cellulose (according to *Meyer* and *Misch*)

4.4.4.16.3 Utilization

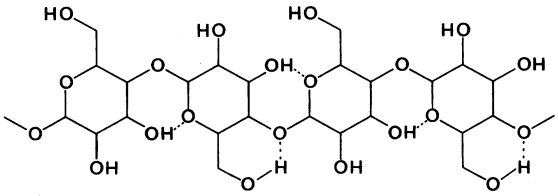
Microcrystalline cellulose is used in low-calorie food products and in salad dressings, desserts and ice creams. Its hydration capacity and dispersibility are substantially enhanced by adding it in combination with small amounts of carboxymethyl cellulose.

4.4.4.17 Cellulose Derivatives

Cellulose can be alkylated into a number of derivatives with good swelling properties and improved solubility. Such derivatives have a wide field of application.



(4.158)



(4.159)

4.4.4.17.1 Alkyl Cellulose, Hydroxyalkyl Cellulose

The reaction of cellulose with methylchloride or propylene oxide in the presence of a strong alkali introduces methyl or hydroxypropyl groups into cellulose (cf. Reaction 4.160). The degree of substitution (DS) is dependent on reaction conditions.

Mixed substituted products are also produced, e. g., methylhydroxypropyl cellulose or methylethyl cellulose. The substituents interfere with the normal crystalline packing of the cellulose chains, thus facilitating chain solvation. Depending on the nature of the substituent (methyl, ethyl, hydroxymethyl, hydroxyethyl or hydroxypropyl) and the substitution degree, products are obtained with variable swelling powers and water solubilities. A characteristic property for methyl cellulose and double-derivatized methylhydroxypropyl cellulose is their initial viscosity drop with rising temperature, setting to a gel at a specific temperature. Gel setting is reversible. Gelling temperature is dependent on substitution type and degree. Figure 4.40 shows the dependence of gelling temperature on the type of substitution and the concentration of the derivatives in water. Hydroxyalkyl substituents stabilize the hydration layer around the macromolecule and, thereby, increase the gelling temperature. Changing the proportion of methyl to hydroxypropyl substituents can vary the jelling temperature within a wide range.

The above properties of cellulose derivatives permit their diversified application (Table 4.27). In baked products obtained from gluten-poor or gluten-free flours, such as those of rice, corn or rye, the presence of methyl and methylhydroxypropyl celluloses decreases the crumbliness and friability of the product, enables a larger volume of water to be worked into the dough and, thus, improves the extent of starch swelling during oven baking. Since differently substituted celluloses offer a large choice of gelling temperatures, each application can be met by using the most suitable derivative. Their addition to batter or a coating mix for meats (panure) decreases oil uptake in frying. Their addition to dehydrated fruits and vegetables improves rehydration characteristics and texture upon reconstitution. Sensitive foods can be preserved by applying alkyl cellulose as a protective coating or film. Cellulose derivatives can also be used as thickening agents in low calorie diet foods. Hydroxypropyl cellulose is a powerful emulsion stabilizer, while methylethyl cellulose has the property of a whipping cream: it can be whipped into a stable foam consistency.

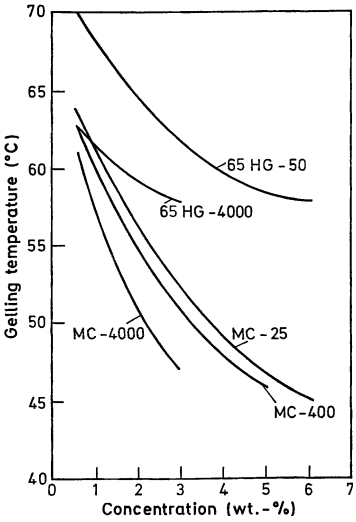
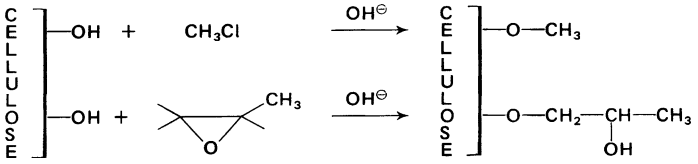


Fig. 4.40. Gelling behavior of alkyl celluloses (according to *Balser*, 1975). MC: methyl cellulose, HG: hydroxypropylmethyl cellulose with a hydroxypropyl content of about 6.5%. The numerical suffix is the viscosity (cps) of a 2% solution

4.4.4.17.2 Carboxymethyl Cellulose

Carboxymethyl cellulose is obtained by treating alkaline cellulose with chloroacetic acid.



(4.160)

Table 4.27. Utilization of cellulose derivatives (in amounts of 0.01 to 0.8%)

Food product	Cellulose derivative[a]			Effect								
	1	2	3	A[b]	B	C	D	E	F	G	H	I
Baked products	+		+		+		+		+			
Potato products	+	+			+		+					+
Meat and fish	+		+	+		+						+
Mayonnaise, dressings	+		+	+	+			+				
Fruit jellies	+			+	+	+						
Fruit juices	+			+								
Brewery	+	+							+	+		
Wine	+	+							+	+		
Ice cream, cookies	+			+	+			+				
Diet food	+	+	+		+							

[a] 1: Carboxymethyl cellulose, Na-salt; 2: methyl cellulose; 3: hydroxypropyl methyl cellulose.
[b] A: Thickening effect; B: water binding/holding; C: cold gel setting; D: gel setting at higher temperatures; E: emulsifier; F: suspending effect; G: surface activity; H: adsorption; and I: film-forming property.

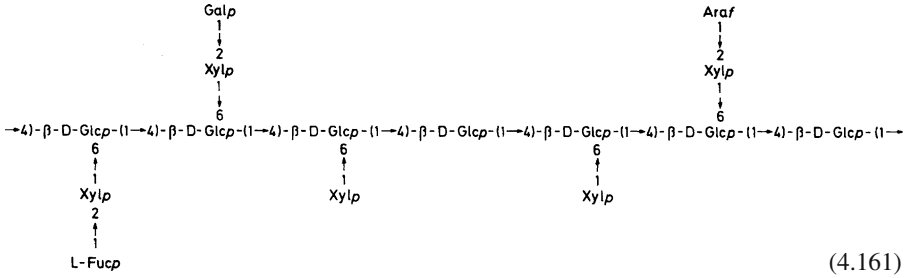
The properties of the product depend on the degree of substitution (DS; 0.3–0.9) and of polymerization (DP; 500–2000). Low substitution types (DS $\leq$0.3) are insoluble in water but soluble in alkali, whereas higher DS types (>0.4) are water soluble. Solubility and viscosity are dependent on pH.

Carboxymethyl cellulose is an inert binding and thickening agent used to adjust or improve the texture of many food products, such as jellies, paste fillings, spreadable process cheeses, salad dressings and cake fillings and icings (Table 4.27). It retards ice crystal formation in ice cream, stabilizing the smooth and soft texture. It retards undesired saccharose crystallization in candy manufacturing and inhibits starch retrogradation or the undesired staling in baked goods. Lastly, Carboxymethyl cellulose improves the stability and rehydration characteristics of many dehydrated food products.

4.4.4.18 Hemicelluloses

The term hemicelluloses refers to substances which occupy the spaces between the cellulose fibrils within the cell walls of plants. Various studies, e. g., on apples, potatoes, and beans, show that xyloglucans dominate in the class *Dicotyledoneae*. A section of the structure of a xyloglucan from runner beans is presented in Formula 4.161.

In the class *Monocotyledoneae*, the composition of the hemicelluloses in the endosperm tissue varies greatly, e. g., wheat and rye contain mainly arabinoxylans (pentosans, cf. 15.2.4.2.1), while β-glucans (cf. 15.2.4.2.2) predominate in barley and oats.



(4.161)

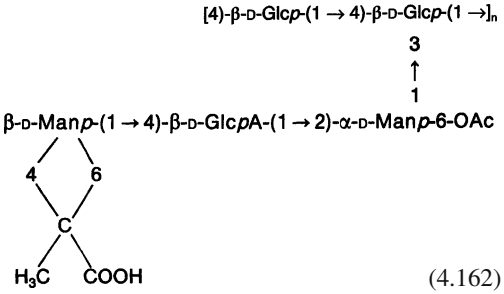
4.4.4.19 Xanthan Gum

4.4.4.19.1 Occurrence, Isolation

Xanthan gum, the extracellular polysaccharide from *Xanthomonas campestris* and some related microorganisms, is produced on a nutritive medium containing glucose, NH_4Cl, a mixture of amino acids, and minerals. The polysaccharide is recovered from the medium by isopropanol precipitation in the presence of KCl.

4.4.4.19.2 Structure, Properties

Xanthan gum can be regarded as a cellulose derivative. The main chain consists of 1,4 linked β-glucopyranose residues. On an average, every second glucose residue bears in the 3-position a trisaccharide of the structure β-D-Man*p*-(1 → 4)-β-D-Glc*p*A(1 → 2)-α-D-Man*p* as the side chain. The mannose bound to the main chain is acetylated in position 6 and ca. 50% of the terminal mannose residues occur ketalized with pyruvate as 4,6-O-(1-carboxyethylidene)-D-mannopyranose (cf. Formula 4.162; Glc*p*A: glucuronic acid).



(4.162)

The molecular weight of xanthan gum is $>10^6$ dal. In spite of this weight, it is quite soluble in water. The highly viscous solution exhibits a pseudoplastic behavior (Fig. 4.41). The viscosity is to a great extent, independent of temperature. Solutions, emulsions and gels, in the presence of xanthan gums, acquire a high freeze–thaw stability.

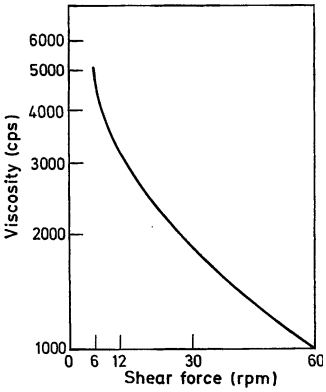


Fig. 4.41. Viscosity of aqueous xanthan gum solution as affected by shear rate (according to *Whistler*, 1973). Viscometer: Brookfield model LVF

4.4.4.19.3 Utilization

The practical importance of xanthan gum is based on its emulsion-stabilizing and particle-suspending abilities (turbidity problems, essential oil emulsions in beverages). Due to its high thermal stability, it is useful as a thickening agent in food canning. Xanthan gum addition to starch gels substantially improves their freeze-thaw stability.

Xanthan gum properties might also be utilized in instant puddings: a mixture of locust bean flour, Na-pyrophosphate and milk powder with xanthan gum as an additive provides instant jelly after reconstitution in water. The pseudoplastic thixotropic properties, due to intermolecular association of single-stranded xanthan gum molecules, are of interest in the production of salad dressings, i. e. a high viscosity in the absence of a shear force and a drop in viscosity to a fluid state under a shear force.

4.4.4.20 Scleroglucan

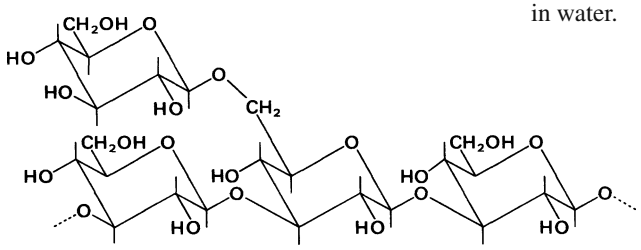
4.4.4.20.1 Occurrence, Isolation

Sclerotium species, e. g., *S. glucanicum*, produce scleroglucan on a nutritive medium of glucose, nitrate as N-source and minerals. The polysaccharide is recovered from the nutritive medium by alcoholic precipitation.

4.4.4.20.2 Structure, Properties

The "backbone" of scleroglucan is a β-1,3-glucan chain that, on an average, has an attached glucose

as a side chain on every third sugar residue (cf. Formula 4.163).



(4.163)

The polysaccharide has a molecular weight of about 130 kdal and is very soluble in water. Solutions have high viscosities and exhibit pseudoplastic thixotropic properties.

4.4.4.20.3 Utilization

Scleroglucan is used as a food thickener and, on the basis of its good film-forming property, is applied as a protective coating to dried foods.

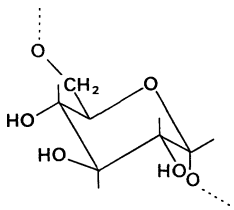
4.4.4.21 Dextran

4.4.4.21.1 Occurrence

Leuconostoc mesenteroides, Streptobacterium dextranicum, Streptococcus mutans and some other bacteria produce extracellular dextran from saccharose with the help of α-1,6-glucan: D-fructose-2-glucosyl transferase (dextran sucrase, EC 2.4.1.5).

4.4.4.21.2 Structure, Properties

Dextran is an α-1,6-glucan (Formula 4.164; molecular weight $M_r = 4-5 \times 10^7$ dal) with several glucose side chains, which are bound to the main chain of the macromolecule primarily through 1,3-linkages but, in part, also by 1,4- and 1,2-linkages.



(4.164)

On an average, 95% of the glucose residues are present in the main chain. Dextran is very soluble in water.

4.4.4.21.3 Utilization

Dextran is used mostly in medicine as a blood substitute. In the food industry it is used as a thickening and stabilizing agent, as exemplified by its use in baking products, confections, beverages and in the production of ice creams.

4.4.4.22 Inulin and Oligofructose

4.4.4.22.1 Occurrence

Inulin occurs as a reserve carbohydrate in many plant families, e. g., scorzonera, topinambur, chicory, rye, onion and dahlia bulb.

4.4.4.22.2 Structure

Inulin contains about 30 furanoid D-fructose units in a β-1,2-linkage. This linear polysaccharide has α-glucose residues in 2,1-bonding at its ends. Individual α-glucose residues in 1,3-bonding have also been detected in the interior of the polysaccharide. Inulin (M, 5000–6000) is soluble in warm water and resistant to alkali.

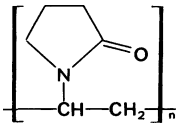
4.4.4.22.3 Utilization

Inulin is nondigestible in the small intestine, but is degraded by the bacteria in the large intestine. It can be used in many foods as a sugar and fat substitute (cf. 8.16.1.2), e. g., biscuits, yoghurt, desserts and sweets. Inulin yields D-fructose on acid or enzymatic hydrolysis. Oligofructans have a slightly sweet taste due to the lower degree of polymerization.

4.4.4.23 Polyvinyl Pyrrolidone (PVP)

4.4.4.23.1 Structure, Properties

This compound is used as if it were a polysaccharide-type additive. Therefore, it is described here. The molecular weight of PVP can range from 10–360 kdal.



(4.165)

It is quite soluble in water and organic solvents. The viscosity of the solution is related to the molecular weight.

4.4.4.23.2 Utilization

PVP forms insoluble complexes with phenolic compounds and, therefore, is applied as a clarifying agent in the beverage industry (beer, wine, fruit juice). Furthermore, it serves as a binding and thickening agent, and as a stabilizer, e. g., of vitamin preparations. Its tendency to form films is utilized in protective food films (particle solubility enhancement and aroma fixation in instant tea and coffee production).

4.4.5 Enzymatic Degradation of Polysaccharides

Enzymes that cleave polysaccharides are of interest for plant foods. Examples are processes that occur in the ripening of fruit (cf. 18.1.3.3.2), in the processing of flour to cakes and pastries (cf. 15.2.2.1), and in the degradation of cereals in preparation for alcoholic fermentation (cf. 20.1.4). In addition, enzymes of this type are used in food technology (cf. 2.7.2.2) and in carbohydrate analysis (cf. Table 2.16 and 4.4.6). The following hydrolases are of special importance.

4.4.5.1 Amylases

Amylases hydrolyze the polysaccharides of starch.

4.4.5.1.1 α-Amylase

α-Amylase hydrolyzes starch, glycogen, and other 1,4-α-glucans. The attack occurs inside the molecule, i. e., this enzyme is comparable to endopeptidases. Oligosaccharides of 6–7 glucose units are released from amylose. The enzyme apparently attacks the molecule at the amylose helix (cf. 4.4.4.14.3) and hydrolyzes "neighboring" glycoside bonds that are one turn removed. Amylopectin is cleaved at random; the branch points (cf. 4.4.4.14.4) are overjumped. α-Amylase is activated by Ca^{2+} ions (cf. 2.3.3.1 and 2.7.2.2.2).

The viscosity of a starch solution rapidly decreases on hydrolysis by α-amylase (starch liquefaction) and the iodine color disappears. The dextrins formed at first are further degraded on longer incubation, reducing sugars appear and, finally, α- maltose is formed. The activity of the enzyme decreases rapidly with decreasing degree of polymerization of the substrate.

Catalysis is accelerated by the gelatinization of starch (cf. 4.4.4.14.2). For example, the swollen substrate is degraded 300 times faster by a bacterial amylase and 10^5 times faster by a fungal amylase than is native starch.

4.4.5.1.2 β-Amylase

This enzyme catalyzes the hydrolysis of 1,4-α-D-glucosidic bonds in polysaccharides (mechanism, 2.4.2.5), effecting successive removals of maltose units from the nonreducing end. Hydrolysis is linked to a Walden inversion at C-1, giving rise to β-maltose. This inversion, which can be detected polarimetrically, represents a definite characteristic of an exoglycanase.

In contrast to amylose, amylopectin is not completely hydrolyzed. All reaction stops even before branch points are reached.

4.4.5.1.3 Glucan-1,4-α-D-glucosidase (Glucoamylase)

This glucoamylase starts at the nonreducing end of 1,4-α-D-glucans and successively liberates β-D-glucose units. In amylopectin, α-1,6-branches are cleaved ca. 30 times slower than α-1,4-bonds.

4.4.5.1.4 α-Dextrin Endo-1,6-α-glucosidase (Pullulanase)

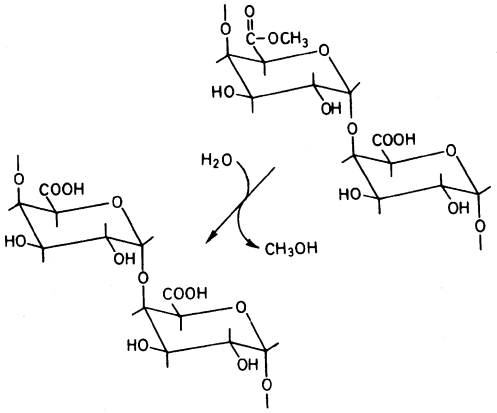
This enzyme hydrolyzes 1,6-α-D-glucosidic bonds in polysaccharides, e. g., in amylopectin, glycogen, and pullulan. Linear amylose fragments are formed from amylopectin.

4.4.5.2 Pectinolytic Enzymes

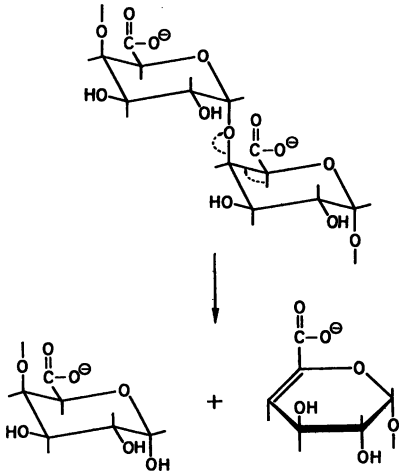
Pectins (cf. 4.4.4.13) in plant foods are attacked by a series of enzymes. A distinction is made between:

- Pectin esterases which occur widely in plants and microorganisms and demethylate pectin to pectic acid (Formula 4.166).
- Enzymes which attack the glycosidic bond in polygalacturonides (Table 4.28). These include hydrolases and lyases which catalyze a transelimination reaction (see Formula 4.167). The double bond formed in the product of the last mentioned reaction results in an increase in the absorption at 235 nm.

The second group can be further subdivided according to the substrate (pectin or pectic acid) and to the site of attack (endo-/exo-enzyme), as shown in Table 4.28. The endo-enzymes strongly depolymerize and rapidly reduce the viscosity of a pectin solution.



(4.166)



(4.167)

Table 4.28. Enzymes that cleave pectin and pectic acid

Enzyme	EC No.	Substrate
Polygalacturonase	3.2.1.15	
Endo-polymethyl galacturonase		Pectin
Endo-polygalacturonase		Pectic acid
Exo-polygalacturonase	3.2.1.67	
Exo-polymethyl galacturonase		Pectin
Exo-polygalacturonase		Pectic acid
Pectin lyase	4.2.2.10	
Endo-polymethyl galacturonlyase		Pectin
Pectate lyase	4.2.2.2	
Endo-polygalacturonate lyase		Pectic acid
Exo-polygalacturonate lyase	4.2.2.9	Pectic acid

Polygalacturonases occur in plants and microorganisms. They are activated by NaCl and some by Ca^{2+} ions as well.

Pectin and pectate lyases are only produced by microorganisms. They are activated by Ca^{2+} ions and differ in the pH optimum (pH 8.5–9.5) from the polygalacturonases (pH 5–6.5).

4.4.5.3 Cellulases

Hydrolysis of completely insoluble, microcrystalline cellulose is a complicated process. For this purpose, certain microorganisms produce particles called cellusomes (particle weight ca. 10^6). During isolation, these particles readily disintegrate into enzymes, which synergistically perform cellulose degradation, and components, which, among other things, support substrate binding. At least three enzymes are involved in the degradation of cellulose to cellobiose and glucose:

$$\text{Cellulose} \xrightarrow[C_1]{C_x} \text{Cellobiose} \xrightarrow{\text{Cellobiase}} \text{Glucose} \qquad (4.168)$$

As shown in Table 4.29, the C_1 and C_x factors, which were found to be endo- and exo-1,4-β-glucanases respectively, hydrolyze cellulose to cellobiose. Since the C_1 factor is increasingly inhibited by its product, a cellobiase is needed so that cellulose breakdown is not rapidly brought to a standstill. However, cellobiase is also subject to product inhibition. Therefore, complete cellulose degradation is possible only if cellobiase is present in large excess or the glucose formed is quickly eliminated.

4.4.5.4 Endo-1,3(4)-β-glucanase

This hydrolase is also called laminarinase and hydrolyzes 1,3(4)-β-glucans. This enzyme occurs together with cellulases, e. g., in barley malt, and is involved in the degradation of β-glucans (cf. 15.2.4.2.2) in the production of beer.

4.4.5.5 Hemicellulases

The degradation of hemicelluloses also proceeds via endo- and exohydrolases. The substrate specificity depends on the monosaccharide building blocks and on the type of binding, e. g., endo-1,4-β-D-xylanase, endo-1,5-α-L-arabinase. These enzymes occur in plants and microorganisms, frequently together with cellulases.

4.4.6 Analysis of Polysaccharides

The identification and quantitative determination of polysaccharides plays a role in the examination of thickening agents, balast material etc.

4.4.6.1 Thickening Agents

First, thickening agents must be concentrated. The process used for this purpose is to be modified depending on the composition of the food. In general, thickening agents are extracted from the defatted sample with hot water. Extracted starch is digested by enzymatic hydrolysis (α-amylase, glucoamylase), and proteins are separated by precipitation (e. g., with sulfosalicylic acid). The polysaccharides remaining in the solution are separated with ethanol. An electropherogram of the polysaccharides dissolved in a borate buffer provides an initial survey of the thickening agents present. It is sometimes difficult to identify and, consequently, differentiate between the added polysaccharides and those that are endogenously present in many foods. In simple cases, it is sufficient if the electropherogram is supported by structural analysis. Here, the polysaccharides are permethylated (cf. 4.2.4.7), then subjected to acid hydroysis, reduced with sodium borohydride (cf. 4.2.4.1) and converted to partially methylated alditol acetates by acetylation of the OH-groups (cf. 4.2.4.6).

These derivatives of the monosaccharide structural units are then qualitatively and quantitatively analyzed by gas chromatography on capillary columns. In more difficult cases, a preliminary separation of acidic and neutral polysaccharides on an ion exchanger is recommended. Methanolysis or hydrolysis of polysaccharides containing uronic acids and anhydro sugars are critical due to losses of these labile building blocks.

Reductive cleavage of the permethylated polysaccharide is recommended as a gentle alternative to hydrolysis. In this process, partially methylated anhydroalditolacetates are formed as shown in Fig. 4.42, using a galactomannan as an example. Conclusions about the structure of the polysaccharide can be drawn from the result of the qualitative and quantitative analysis, which is carried out by gas chromatography/mass

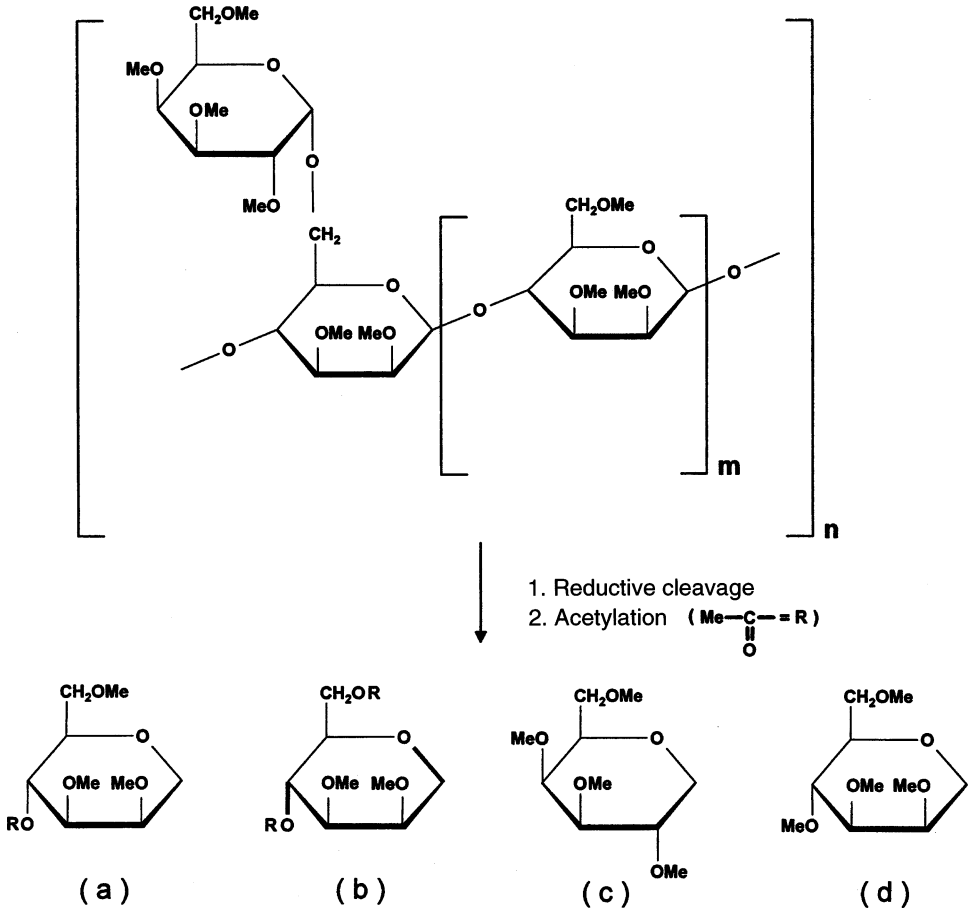


Fig. 4.42. Reductive depolymerization of a permethylated galactomannan (according to *Kiwitt-Haschemie* et al., 1996) 1. Reductive cleavage with triethylsilane and trimethylsilylmethanesulfonate/boron trifluoride 2. Acetylation with acetic anhydride and N-methylimidazole

spectrometry. In the example presented here, the derivative 4-O-acetyl-1,5-anhydro-2,3,6-tri-O-methyl-D-mannitol (*a* in Fig. 4.42) results from the 1,4-linked D-mannose, the structural unit of the main chain. The derivative 4,6-di-O-acetyl-1,5-anhydro-2,3-di-O-methyl-D-mannitol (*b*) indicates the structural unit which forms the branch and the derivative 1,5-anhydro-2,3,4,6-tetra-O-methyl-D-galactitol (*c*) indicates the terminal D-galactopyranose of the side chain. The derivative 1,5-anhydro-2,3,4,6-tetra-O-methyl-D-mannitol (*d*) produced in small amounts shows the end of the main chain formed by D-mannopyranose. The appearance of glucose in the structural analysis indicates glucans or modified glucans, e. g., modified starches or celluloses. The identification of thickening agents of this type is achieved by the specific detection of the hetero-components, e. g., acetate or phosphate.

4.4.6.2 Dietary Fibers

Gravimetric methods are the methods of choice for the determination of dietary fibers (cf. 15.2.4.2). In the defatted sample, the digestible components (1,4-α-glucans, proteins) are enzymatically hydrolyzed (heat-stable α- amylase, glucoamylase, proteinase). After centrifugation,

Table 4.29. Cellulases

EC No.	Name	Synonym	Reaction
3.2.1.4	Cellulase	C_x factor CMCase[a], endo-1,4-β-glucanase	Endohydrolysis of 1,4-β-D-glucosidic bonds
3.2.1.91	Cellulose 1,4-β-cellobiosidase	C_1 factor avicellase	Exohydrolysis of 1,4-β-D-glucosidic bonds with formation of cellobiose from cellulose or 1,4-β-glucooligosaccharides. The attack proceeds from the nonreducing end.
3.2.1.21	β-Glucosidase	Cellobiase amygdalase	Hydrolysis of terminal β-D-glucose residues in β-glucans

[a] CMC: carboxymethylcellulose; the enzyme activity can be measured via the decrease in viscosity of a CMC solution.

the insoluble fibers remain in the residue. The water soluble fibers in the supernatant are isolated by precipitation with ethanol, ultrafiltration or dialysis. The protein and mineral matter still remaining with the soluble and insoluble dietary fibers is deducted with the help of correction factors.

4.5 References

Angyal, S.J.: Zusammensetzung und Konformation von Zuckern in Lösung. Angew. Chem. *81*, 172 (1969)

Balser, K.: Celluloseäther. In: Ullmanns Encyklopädie der technischen Chemie, 4. edn., Vol. *9*, S. 192. Verlag Chemie: Weinheim. 1975

Banks, W., Muir, D.D.: Structure and chemistry of the starch granule. In: The biochemistry of plants (Eds.: Stumpf, P.K., Conn, E.E.), Vol. *3*, p. 321, Academic Press: New York. 1980

Brouns, F., Kettlitz, B., Arrigoni, E.: Resistanz starch and "the butyrate revolution". Trends Food Sci. Technol. *13*, 251 (2002)

Birch, G.G. (Ed.): Developments in food carbohydrate-1 ff, Applied Science Publ.: London. 1977 ff.

Birch, G.G., Parker, K.J. (Eds.): Nutritive sweeteners. Applied Science Publ.: London. 1982

Birch, G.G., Parker, K.J. (Eds.): Dietary fibre. Applied Science Publ.: London. 1983

Birch, G.G. (Ed.): Analysis of food carbohydrate. Elsevier Applied Science Publ.: London. 1985

Blanshard, J.M.V., Mitchell, J.R. (Eds.): Polysaccharides in food. Butterworths: London. 1979

Büser, W., Erbersdobler, H.F.: Carboxymethyllysine, a new compound of heat damage in milk products. Milchwissenschaft *41*, 780 (1986)

Davidson, R.L. (Ed.): Handbook of water-soluble gums and resins. McGraw-Hill Book Co.: New York. 1980

Ebert, G.: Biopolymere. Dr. Dietrich Steinkopff Verlag: Darmstadt. 1980

Erlingen, R.C., Delcour, J.A.: Formation, analysis, structure and properties of type III enzyme resistant starch. J. Cereal Sci. *22*, 129 (1995)

Friedman, M.: Food browning and its prevention: An Overview. J. Agric. Food. Chem. *44*, 631 (1996)

Galliard, T. (Ed.): Starch: Properties and Potential. John Wiley and Sons: Chichester. 1987

Gidley, M.J., Cooke, D., Darke, A.H., Hoffmann, R.A., Russell, A.L., Greenwell, P.: Molecular order and structure in enzyme-resistant retrograded starch. Carbohydrate Polymers *28*, 23 (1995)

Glomb, M.A., Monnier, V.M.: Mechanism of protein modification by glyoxal and glycolaldehyde, reactive intermediates of the Maillard reaction. J. Biol. Chem. *270*, 10017 (1995)

Guadagni, D.G., Maier, V.P., Turnbaugh, J.G.: Effect of some citrus juice constituents on taste thresholds for limonine and naringin bitterness. J. Sci. Food Agric. *24*, 1277 (1973)

Henle, T., Zehetner, G., Klostermeyer, H.: Fast and sensitive determination of furosine. Z. Lebensm. Unters. Forsch. *200*, 235 (1995)

Henle, T., Schwarzenbolz, U., Klostermeyer, H.: Detection and quantification of pentosidine in foods. Z. Lebensm. Unters. Forsch. *204*, 95 (1997)

Hill, R.D., Munck, L. (Eds.): New approaches to research on cereal carbohydrates. Elsevier Science Publ.: Amsterdam. 1985

Hofmann, T.: Characterization of the chemical structure of novel colored Maillard reaction products from furan-2-carboxaldehyde and amino acids. J. Agric. Food Chem. *46*, 932 (1998)

Hofmann, T.: 4-Alkylidene-2-imino-5-[4-alkylidene-5-oxo-1,3-imidazol-2-inyl]azamethylidene-1,3-imidazolidine. A novel colored substructure in melanoidins formed by Maillard reactions of bound arginine with glyoxal and furan-2-carboxaldehyde. J. Agric. Food Chem. *46*, 3896 (1998)

Hofmann, P., Münch, P., Schieberle, P.: Quantitative model studies on the formation of aroma-active aldehydes and acids by Strecker type reactions. J. Agric. Food Chem. *48*, 434 (2000)

Hough, L., Phadnis, S.P.: Enhancement in the sweetness of sucrose. Nature *263*, 800 (1976)

Jenner, M.R.: Sucralose. How to make sugar sweeter. ACS Symposium Series *450*, p. 68 (1991)

Kiwitt-Haschemie, K., Renger, A., Steinhart, H.: A comparison between reductive-cleavage and standard methylation analysis for determining structural features of galactomannans. Carbohydrate Polymers *30*, 31 (1996)

Ledl, F., Severin, T.: Untersuchungen zur *Maillard*-Reaktion. XIII. Bräunungsreaktion von Pentosen mit Aminen. Z. Lebensm. Unters. Forsch. *167*, 410 (1978)

Ledl, F., Severin, T.: Untersuchungen zur *Maillard*-Reaktion. XVI. Bildung farbiger Produkte aus Hexosen. Z. Lebensm. Unters. Forsch. *175*, 262 (1982)

Ledl, F., Krönig, U., Severin, T., Lotter, H.: Untersuchungen zur *Maillard*-Reaktion. XVIII. Isolierung N-haltiger farbiger Verbindungen. Z. Lebensm. Unters. Forsch. *177*, 267 (1983)

Ledl, F.: Low molecular products, intermediates and reaction mechanisms. In: Amino-carbonyl reactions in food and biological systems (Eds.: Fujimaki, M., Namiki, M., Kato, H.), p. 569, Elsevier: Amsterdam. 1986

Ledl, F., Fritul G., Hiebl, H., Pachmayr, O., Severin, T.: Degradation of *Maillard* products. In: Aminocarbonyl reactions in food and biological systems (Eds.: Fujimaki, M., Namiki, M., Kato, H.). p. 173, Elsevier: Amsterdam. 1986

Ledl, F.: Chemical Pathways of the *Maillard* Reaction. In: The *Maillard* Reaction in Food Processing, Human Nutrition and Physiology (Eds.: Finot, P.A. et al.) p. 19, Birkhäuser Verlag: Basel. 1990

Ledl, F., Schleicher, E.: Die *Maillard*-Reaktion in Lebensmitteln und im menschlichen Körper – neue Ergebnisse zu Chemie, Biochemie und Medizin. Angewandte Chemie *102*, 597 (1990)

Ledl, F., Glomb, M., Lederer, M.: Nachweis reaktiver *Maillard*-Produkte. Lebensmittelchemie *45*, 119 (1991)

Lehmann, J.: Chemie der Kohlenhydrate. Georg Thieme Verlag: Stuttgart. 1976

Loewus, F.A., Tanner, W. (Eds.): Plant carbohydrates I, II. Springer-Verlag: Berlin. 1981/82

Nedvidek, W., Noll, P., Ledl, F.: Der Einfluß des *Strecker*abbaus auf die *Maillard*-Reaktion. Lebensmittelchemie *45*, 119 (1991)

Pagington, J.S.: β-Cyclodextrin and its uses in the flavour industry. In: Developments in food flavours (Eds.: Birch, G.G., Kindley, M.G.), p. 131, Elsevier Applied Science: London. 1986

Pigman, W., Horton, D. (Eds.): The Carbohydrates. 2nd edn., Academic Press: New York. 1970–1980

Pilnik, W., Voragen, F., Neukom, H., Nittner, E.: Polysaccharide. In: Ullmanns Encyklopädie der technischen Chemie, 4. edn., Vol. *19*, S. 233, Verlag Chemie: Weinheim. 1980

Preuß, A., Thier, H.-P.: Isolierung natürlicher Dickungsmittel aus Lebensmitteln zur capillargaschromatographischen Bestimmung. Z. Lebensm. Unters. Forsch. *176*, 5 (1983)

Radley, J.A. (Ed.): Starch production technology. Applied Science Publ.: London. 1976

Rees, D.A.: Polysaccharide shapes. Chapman and Hall: London. 1977

Reilly, P.J.: Xylanases: structure and function. Basic Life Sci. *18*, 111 (1981)

Rodriguez, R., Jimenez, A., Fernandez-Bolanos, J., Guillen, R., Heredia, A.: Dietary fibre from vegetable products as source of functional ingredients. Trends Food Sci. Technol. *17*, 3 (2006)

Salunkhe, D.K., McLaughlin, R.L., Day, S.L., Merkley, M.B.: Preparation and quality evaluation of processed fruits and fruit products with sucrose and synthetic sweeteners. Food Technol. *17*, 203 (1963)

Scherz, H., Mergenthaler, E.: Analytik der als Lebensmittelzusatzstoffe verwendeten Polysaccharide. Z. Lebensm. Unters. Forsch. *170*, 280 (1980)

Scherz, H.: Verwendung der Polysaccharide in der Lebensmittelverarbeitung. In: Polysaccharide. Eigenschaften und Nutzung (Hrsg.: Burchard, W.), S. 142, Springer-Verlag: Berlin. 1985

Scherz, H., Bonn, G.: Analytical Chemistry of Carbohydrates, Georg Thieme Verlag, Stuttgart, 1998

Schweizer, T.F.: Fortschritte in der Bestimmung von Nahrungsfasern. Mitt. Geb. Lebensmittelunters. Hyg. *75*, 469 (1984)

Shallenberger, R.S., Birch, G.G.: Sugar chemistry. AVI Publ. Co.: Westport, Conn. 1975

Shallenberger, R.S.: Advanced sugar chemistry, principles of sugar stereochemistry. Ellis Horwood Publ.: Chichester. 1982

Sutherland, I.W.: Extracellular polysaccharides. In: Biotechnology (Eds.: Rehm, H.-J., Reed, G.), Vol. 3, p. 531, Verlag Chemie: Weinheim. 1983

Szente, L., Szeijtli, J.: Cyclodestrins as food ingredients. Trends Food Sci Technol. *15*, 137 (2004)

Von Sydow, E., Moskowitz, H., Jacobs, H., Meiselman, H.: Odor – Taste interaction in fruit juices. Lebensm. Wiss. Technol. *7*, 18 (1974)

Voragen, A.G.J.: Technological aspects of functional food-related carbohydrates. Trends Food Sci. Technol. *9*, 328 (1998)

Waller, G.R., Feather, M.S. (Eds.): The *Maillard* reaction in foods and nutrition. ACS Symposium Series 215, American Chemical Society: Washington, D.C. 1983

Wells-Knecht, K.J., Brinkmann, E., Baynes, J.W.: Characterization of an imidazolium salt formed from glyoxal and N-hippuryllysine: a model for Maillard reaction crosslinks in proteins. J. Org. Chem. *60*, 6246 (1995)

Whistler, R.L. (Ed.): Industrial gums. 2nd edn., Academic Press: New York. 1973

Willats, W.G.T., Knox, J.P., Mikkelsen, J.D.: Pectin: new insights into an old polymer are starting to gel. Trends Food Sci. Technol. *17*, 97 (2006)

Yaylayan, V.: In search of alternative mechanisms for the *Maillard* reaction, Trends Food Sci Technol *1*, 20 (1990)