Part 4. Carbohydrates

Carbohydrates are essential in foods as an energy source (starch is the main source of human calories), a flavoring (simple sugars are usually sweet) and as a functional ingredient (sucrose allows ice cream to be soft in the freezer; xanthan gum thickens a low-fat salad dressing). As with all our approaches to food ingredients/constituents we will first examine the structure of carbohydrates and then try to elucidate how their structures allow them to function as they do.

1. Carbohydrate Structure

As their name suggests, carbohydrates basically made up from sugar and water, i.e., C\(x\)(H\(_2\)O)\(_y\), although this ratio is often not strictly true and occasionally other atoms may be present. The carbons are arrange in a chain (most often 5-6 atoms) functionalized with alcohol groups. The terminal carbon either carries either an aldehyde or a ketone functional group.

Fructose is a ketose sugar (i.e., with a ketone functional group). It has five alcohol groups (i.e., fructose is a polyol).

Glucose has the same atoms as fructose but it is an aldose sugar (i.e., with an aldehyde functional group).

Note that in both of these diagrams hydrogens and carbons are unlabelled

Each of these sugars contains several chiral carbons so there are many mirror image versions of the same functional groups. Fructose has three chiral carbons so 2\(^3\) different version. Glucose has four so 2\(^4\) different versions. All of the different sugars have distinct properties.

The bond angles around each carbon atom are tetrahedral so each carbon in a sugar has a fixed three dimensional configuration of groups bonded two it. If all four of the bonding groups are different, then there are two distinct arrangements that cannot be superimposed, i.e., the carbon is chiral. The two different versions have the same chemical formula but can have very different properties. Chiral carbons are three dimensional structures that are hard to represent on paper so various conventions have been developed to convey the shape information. The most common of these is the Fisher projection where the carbon is arranged as the center point of a cross with the up (out of plane) groups left and right and the down (out of plane) groups top and bottom. A further simplification of the Fisher projection commonly used in sugar chemistry is the Rosanoff projection where the cross shape is retained along with its implications of configuration, but only alcohol groups are drawn in (as a straight line) and
aldheyde/ketone groups as a circle. These conventions are illustrated below with
glyceraldehydes, a three-carbon molecule that is the basis of aldose sugars.

\[
\text{CHOCH(OH)CH}_2\text{OH}
\]

\[
\begin{align*}
\text{D-glyceraldehyde} & : & \text{CHO} \\
& & \text{CHO} \\
& & \text{CH}_2\text{OH}
\end{align*}
\]

\[
\begin{align*}
\text{L-glyceraldehyde} & : & \text{CHO} \\
& & \text{HO} \\
& & \text{CH}_2\text{OH}
\end{align*}
\]

\[
\begin{align*}
\text{D-glyceraldehyde is the starting point for all of the D-series sugars (the most important). Three carbon sugars are not common in our diet but we can imagine them as the starting point of a family of molecules created by adding a CH(OH) between the aldehyde and the first chiral carbon. Each new carbon added is also chiral so there are two alternative configurations. The following diagram shows the D-series of sugars with D-glyceraldehyde in the center. The outer ring contains the hexosuloses; they have four chiral carbons so 16 enantiomers but only 8 of these are in the D-series. Some important D-sugars are marked on the diagram.}
\end{align*}
\]

```plaintext
D-xylose

D-galactose

D-glyceraldehyde

D-glucose

D-mannose
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The chain forms of sugars shown above are not prevalent in nature. The carbonyl carbon (part of the aldehyde or ketone functional group) can react with an alcohol to form an ether link. In sugars the bond angles conspire to make the alcohol on carbon 4 or 5 of the same molecule particularly reactive in this respect yielding a 5 (i.e., furanose) or 6 (i.e., pyranose) membered ring respectively. The combined alcohol-carbon-ether structure produced is a hemiacetal.

As shown in the diagram, the alignment of the hemiacetal alcohol (formed from the carbonyl oxygen) can either lie axially (α-form) or equatorially (β-form) to the ring depending on the way the alcohol initially attacked the carbonyl. The “new” alcohol has made carbon number one chiral so there are two new forms known as the α and β anomers.

Monomeric sugars can therefore from five possible structures – linear (open), α-pyranose, β-pyranose, α-furanose, and β-furanose. The ring forms are by far the most prevalent with the linear form typically present at about 0.02% at any instant. However the system is very dynamic and individual molecules are constantly transforming from ring-form to ring-form through the linear intermediate. The linear form is the only one with a free carbonyl that allows the sugar to take part in important reactions. An excellent demonstration of sugar structure is available from the University of Hertfordshire (http://www.herts.ac.uk/natsci/Bio/schools/glucose/glucose.htm).

**Oxidation and Reduction.** An aldehyde can readily be oxidized to a mixture of a carboxylic acid and other products while ketones cannot. The oxidation must be coupled with the reduction of another group and this is commonly exploited in a chemical test for reducing sugars where Cu(II) is reduced to Cu(I) and forms a brick-red precipitate. Ketose sugars can interconvert with aldose sugars and give a positive reducing sugar test and so are also referred to as reducing sugars. The interconversion (isomerisation) is catalyzed by enzyme (e.g., glucose isomerase) or high pH.

Aldehyde groups can be reduced (typically by catalytic hydrogenation) to their corresponding alcohols, i.e., glucose to glycitol or xylose to xylitol. Sugar alcohols are
sometimes used as reduced calorie sweeteners, particularly in chewing gums and diabetic foods. They often give a mild cooling sensation in the mouth.

**Disaccharides.** A hemiacetal can be attacked by another alcohol to lose water and form a full acetal. The attacking alcohol can come from any of the alcohols on a second sugar to form a disaccharide. This type of link, a glycosidic bond, is described as an α or β (depending on the orientation of the hemiacetal alcohol) \(\#\) (where \# is the number of the carbon on the second bonding sugar. Some examples include:

- **Maltose or malt sugar,** is formed from starch by enzymatic digestion. It is used for its mild sweetening taste and characteristic flavor. Structurally, maltose is two glucose pyranose rings joined by an α 1-4 glycosidic linkage. The 1 carbon on the second ring is unreacted so can open, isomerize and close to form α and β versions of maltose. It can also be oxidized, so maltose behaves as a reducing sugar.

- **Lactose** is a major component of the non-fat solids of milk (about 5% by weight in fluid milk). It is a dimer of two different monosaccharides, glucose and galactose, joined by a 1-4 β glycosidic link. The galactose can still open to form an oxidizable aldehyde therefore lactose is a reducing sugar. Lactose is vulnerable to acid hydrolysis and the enzyme lactase is required to split the glycosidic link for digestion. Lactase is absent in many adults so the sugar can accumulate in the gut where it is fermented by bacteria to form acid and gas.

  Lactose can crystallize in two types of crystal, the α-hydrate and the β-anhydrous. (The α form is much less soluble than the β form.) Commercial lactose is extracted from whey and crystallized as α-lactose. Crystals larger than about 10 mm can be perceived and as α-lactose does not melt well its crystals can lead to "sandiness" defect in certain dairy products, importantly those with a high solids content and/or cold storage (condensed milk, ice cream). To avoid the formation of large crystals it is often useful to add seed crystals to prevent too much supercooling.

- **Sucrose** is a disaccharide formed by the reaction of α-glucopyranose and β-fructofuranose. Because the acetal formed is a 1-1 linkage, neither ring can open and expose reducing groups. Sucrose is table sugar.
2. The Maillard Reaction

Maillard reactions occur between reducing sugars and amino groups to form brown pigments and flavor compounds. In foods this occurs mainly between simple sugars and amino groups of proteins. The reaction does not need oxygen to proceed. The reaction proceeds faster at high pH. Ascorbic acid (vitamin C) can also brown in a Maillard-like manner both alone and in the presence of amino compounds.

There is no one simple reaction responsible for the many effects of the Maillard reaction. In fact the process has been defined as “the sequence of events that begins with reaction of the amino group of amino acids with a glycosidic hydroxyl group of sugars; the sequence terminates with the formation of brown nitrogenous polymers or melanoidins” (from John deMan "Principles of Food Chemistry"). The reaction is believed to occur as follows:

1. **Formation of an N-glucosamine from an aldose or ketose reacting with and amino group.** Glucose (or any other sugar) reacts with an amine. The amine can be ammonia, a free amino acid, a peptide, or a protein. It is frequently lysine as the ε-amino group is the most reactive of all amino acids.

2. **Rearrangement of the glycosamine via the Amadori mechanism.** The product is dehydrated and then can either cyclize to form a glucosamine, or isomerize to form an amino-fructose derivative sometimes called the Amadori product.

3. **Degradation of Amadori product.** The Amadori product is quite unstable and will isomerize through several intermediates to form deoxyhexosulose. The DH can react further with another amino group or can cyclize to form hydroxy methyl furfural (HMF). HMF has a mildly sweet caramel flavor.

4. **Condensation and polymerization.** The HMF and other intermediates can react with each other and more amino compounds to form a complex mix of high molecular weight polymers. The actual reaction mechanism is complicated and the structure of the products only understood up to a point.

The reactants are largely colorless but the larger and greater number of polymers formed the darker brown the solution (food) becomes. There is frequently a lag time in the browning reaction before any color is seen during which the intermediates accumulate and the polymers grow to produce color. Different starting ingredients can be used to produce different color pigments and this can be exploited to some extent in the controlled formation of process colors.

**Reaction of Maillard Products with Proteins.** Several Maillard products contain the highly reactive α-dicarbonyl structure (e.g., 3-deoxy hexosulose) which can react with an amino group, in particular the ε-amino of lysine. The adduct breaks down to form an aldehyde amongst other products. Aldehydes have strong, distinctive aromas and their
formation is important in many "cooked" flavors (e.g., meaty, nutty, toasted). The reaction shown is the Strecker degradation. Cross linking of proteins as part of Maillard product formation can lead to some insolubilization and loss of protein functionality.

Amino acids, most importantly lysine, are destroyed as a result of the Maillard reaction. Several of the heterocyclic compounds formed have been shown to be carcinogenic and mutagenic. Some Maillard products are antioxidants and have been claimed to have some health benefits.

**Control of the Maillard Reaction.** The Maillard reaction proceeds much faster at high temperatures (doubling or tripling in rate about every 10°C) so keeping a reaction mixture (food) cool can limit the browning. In fact, Maillard browning is usually only associated with cooked foods or very long term storage.

Reactions sometime show a maximum rate at reduced water activities before dropping to zero at or near the monolayer moisture value. This effect is particularly strong for Maillard chemistry and intermediate moisture foods brown many times faster than their fully hydrated counterparts. The drying process itself is particularly difficult, as the foods have to pass through the intermediate moisture danger-zone at elevated temperatures.

The reaction is slower under acid conditions and is inhibited by the additive sulfur dioxide (under acid conditions, amine groups may be protonated and less reactive).

4. **Polysaccharides**

As saccharide chains grow longer they start to exhibit more “polymer like” properties (e.g., viscous, tendency to gel) rather than “small sugar like” properties (e.g., sweet, tend to crystallize). There are a huge range of polysaccharide ingredients and here we will examine a few of the more important starting with starch

Starch is tremendously important to plants as their main fuel store and to the animals that eat them. From the plant’s point of view the goal is to store as much reduced carbon in a small amount of space as possible without disrupting cell function. The plant must be able to access the store when needed. Given these objectives, what design options are open to the plant?

- Small molecule sugars would create a huge osmotic pressure if stored in sufficient quantities to be useful. It is necessary to polymerize the sugar to reduce the number of molecules present and hence the osmotic effects.
- A large concentration of free polymers would be too viscous to allow the cell to function probably. It is necessary to pack the polymers together in some dense function.

These needs are met by storing the reduced carbon in starch granules.
Starch is comprised of two types of polymeric glucose. Amylose is linked via $\alpha$-1,4 bonds. It typically has a degree of polymerization (DP) of approximately 180-320 units and a molecular weight of about 1,000,000. About 1 in 200 links there may be a branch but it is basically a linear polymer. The axial-equatorial nature of the link forces the polymer into a helical structure. The core of the helix is slightly hydrophobic so amylose can form inclusion complexes with certain lipids and iodine. Amylopectin is a much larger molecule (molecular weight $\sim$100,000,000). It is also has about one an $\alpha$1,4 chain but approximately every 20 residues there is an $\alpha$1,6 link branching off the chain. Amylopectin has one reducing end (a glucose that can unfold and expose the aldehyde functional group). The $\alpha$1,4 chain that flows from that is the C-chain. Branches from the C-chain are B-chains and branches from that are A-chains. The overall structure of the molecules is believed to look like a branching tree. The dense helices of the C-chains are close to each other and can form a double helix.

In a typical starch amylose and amylopectin are present at a ratio of about 1:4. However this ratio will vary according to the biological source of the starch and certain genetic mutants are commercially available with artificially modified rations. Waxy starches are effectively pure amylopectin (with a few percent amylose) and sugary mutants have elevated levels of amylose (e.g., su-maize).

A starch granule is a lens-shaped object a few micrometers to a few tens of micrometers in diameter. Under polarized light it appears as a bright Maltese cross. Polarizing light microscopy shows only the crystalline structures as bright but the presence of a bright Maltese cross does not mean just those regions of the granule are crystalline. Instead the cross shape is characteristic of radial symmetry about the intersection point of the cross. There must be bands of alternating crystalline and non-crystalline (i.e., amorphous) materials present in a granule. Further evidence for this model comes from electron microscopy studies of granules that have been sliced open. If the granule is sliced than etched with acid to digest out the non-crystalline portions, a series of raise (i.e., not etched therefore crystalline) bands are seen.

The crystalline regions are believed to be due to the formation of double helices between A-chains of the amylopectin molecule. The molecule’s C-chain (and single free aldehyde group) is orientated towards the center of the granule and radiates out towards the edge. The B- and A-chains radiate from that but only the spacing of the branch points allows the relatively short A-chains to naturally lie close together and form their double helices. The double helices pack together like stacks of pipes to form the crystalline lamellae seen in electron microscopy and inferred from the polarized light microscopy. The location of
amylose is not clear in this model but is believed to be non crystalline and lie in the amorphous portions of the granule alongside the B-chains of the amylopectin.

Starch granules are insoluble, although they will swell as they take on a little water. However as they are heated in the presence of water at a critical temperature they will suddenly loose their Maltese crosses and swell to many times their original diameter in a process known as gelatinization. In fact complete swelling of granules is often limited due to a lack of available water. The swollen granules readily leak amylose out of their structure and can be disrupted by shear forces acting.

The need for both heart and moisture to gelatinize starch granules provides a strong suggestion that a glass transition must be involved in starch gelatinization and indeed the first step seems to involve the non-crystalline portion of the granule. As the temperature is increased the crystalline regions will begin to melt or dissolve in the water but in this case they are physically locked into place by the concentrated, fairly dry polymer in the amorphous regions. Heat and moisture eventually softens the amorphous region and induces a glass to rubbery transition. The softer rubber cannot contain the crystalline regions and they rapidly melt and the granule swells and soaks up a huge amount of water as the structures holding it together are disrupted. The amylose is not covalently linked into the granule structure so can diffuse out into the water. The network of mainly amylopectin ion the swollen granule is still linked together but can be disrupted under shear to leave granule fragments known as ghosts. (Indeed some people have suggested that starch granules contain only a single huge amylopectin molecule!)

Starch gelatinization is associated with a sudden increase in solution viscosity because the hydrodynamic radius of the swollen granule is so many times larger than the native granule. However if the swollen granules are intensively mixed or allowed to heat for a prolonged period, particularly under acid conditions, the viscosity will decrease as the swollen granules are ruptured. If a suspension of swollen granules is allowed to cool it will gel. As we have seen in our general discussion of macromolecular functionality, gelation requires the formation of some form of interchain linkage to support solid-like behavior. In starch the links are initially the formation of double helices between amylose chains (i.e., retrogradation). The amylose molecules are smaller and are therefore able to diffuse faster but over the next days and weeks the larger amylopectin molecules may also begin to retrograde and increase the gel strength.

The natural gelatinization and retrogradation behavior of starch are important in controlling the texture of many foods but natural granules are not sufficiently robust, or are otherwise inconvenient for many food processing operations. Various chemically

1 Frequently confused with gelation. These are different processes!
2 Frequently confused with gelatinization. These are different processes!
and physically modified starch ingredients are available to improve on nature. Some specific defects and their solutions include:

- **Starch must be cooked to act as a thickening agent.** Pre-gelatinized starch is made by quickly cooking a starch paste between two hot rollers and drying the product. Pre-gelatinized starch rapidly rehydrates without further cooking and is a useful thickening agent in dried sauces and salad dressings that require no further cooking.

- **Starch suspensions are not stable to heating.** Swollen granules rapidly break down in hot, stirred and acid conditions and lose their viscosity. A classic example of this problem is when cans of fruit pie filling mix being retorted. The heat, acid and very high temperatures rapidly depolymerize the swollen granules and the finished product would be fruit pieces sitting at the bottom of can of colored sugar syrup. Chemical cross links can be added to the alcohol groups of the starch chain (i.e., cross linked starch) to stabilize the molecule and prevent breakdown. Cross links also make a granule slightly more resistant to gelatinization. Typical cross links include acetic anhydride esters and they are used at levels of DS$^3=0.002-0.2$. Cross linked starch must be labeled as “modified starch”.

- **Starch gels change their properties during storage.** The slow retrogradation of amylopectin means the texture of a starch gel will change and frequently show some syneresis. Monofunctional reagents (commonly acetate or phosphate, DS$\approx0.1$) can be chemically added to the starch molecules. These modifications do not fit well into the double helical arrangement and thus inhibit retrogradation and allow the formation of a stable gel. The stabilized starches formed must also be labeled as “modified starch”.

As well as an ingredient in its own right, starch is a useful feedstock to make other reagents by partially or completely depolymerizing them. Depolymerization can involve either acid or enzymatic hydrolysis of the polymers. The more extensive the hydrolysis, the lower the molecular weight of the product. The size of glucose oligimers made from starch hydrolysis is usually expressed as a dextrose (i.e., glucose) equivalents (=reducing power of the sample relative to that of glucose). In general the lower the molecular weight of the product (or higher the DE), the lower the viscosity of the product, the sweeter the flavor, the greater the tendency towards nonenzymatic browning, and the easier it is for the products to crystallize. Some examples of starch gelatinization products include: dextrins, daltodextrin, corn syrup (DE=20-60, a few glucose units).

The great advantage of starch is the tremendous efficiency of midwestern corn farmers means it is incredibly cheap. However limitations to the functionality of even modified starch in combination with the costs and label declarations associated with modification mean other polysaccharides have useful roles as food ingredients, typically as gelling and thickening agents. (See also http://www.lsbu.ac.uk/water/hydro.html)

$^3$ Defined as Degree of Substitution the number of free alcohols per residue containing a modification. The maximum DS is therefore 3.
**Cellulose and Cellulose Derivatives.** Cellulose is the main polymeric content of plants and is mainly located as a structural material in cell walls. Chemically cellulose molecules are like very large amylose molecules but the glycosidic links are β1-4 (as opposed to α1-4 in amylase) which are indigestible by human enzymes and so cellulose behaves as dietary fiber. The β1-4 links in cellulose allow the molecule to lie as a flat ribbon while β1-4 links put a step into the chain conformation that facilitated helix formation in amylose. In the cell wall the cellulose chains lie parallel to one another and form crystalline regions supported by strong interchain hydrogen bonds. However, the very long chains are hard to line up to form a perfect crystal over their entire length and the crystalline regions are interspersed with non-crystalline amorphous regions. Cellulose fibers form into bundles connected at intervals along their length by the crystalline regions. They are very rigid and strong and are completely water insoluble. They are resistant to our digestive enzymes and make up the bulk of the dietary fiber we consume.

Cellulose can be modified to make a number of useful products. Most simply the amorphous regions can be hydrolyzed out to form short rods of microcrystalline cellulose. This can be dried to form an insoluble powder useful both as a fiber additive and as a dusting form shredded cheese to prevent it sticking. The microcrystalline cellulose bundles can be disrupted by intense shear and acid. The fibers formed are then derivatized to form carboxymethyl cellulose, methyl cellulose or hydroxypropyl methyl cellulose. Cellulose gums tend to be highly viscous and can be used to form gels.

**Xanthan Gum.** Xanthan gum is an extracellular polysaccharide produced by the bacterium *xanthomonis campestris*. The xanthan gum backbone is very similar to cellulose (β1-4 polyglucose) but does not crystallize because alternate residues have an α1-6 linked 3-sugar residue attached (specifically -(3,1)-α-linked D-mannopyranose-(2,1)-β-D-glucuronic acid-(4,1)-β-D-mannopyranose). The side chains contain carboxylic acid residues (glucuronic acid) which are negatively charged at all but the lowest pH and their intrachain electrostatic repulsion helps keep the molecule relatively linear in solutions. The extended conformation of a xanthan molecule contributes to its high viscosity. The molecules also form weak, transient cross links as double helices between two chains. Xanthan gum is a non-gelling polysaccharide but forms very viscous, temperature-stable solutions.

**Alginate.** Alginate is extracted from seaweed. It is a linear polysaccharide containing two types of residue (i.e., a co-polymer): β-D-
mannopyranosyluronic acid and (M) α-L-gulopyrasonic acid (G). The structure tends to be either sequences of MMMMMM…., GGGGGG…., or MGMGMGMGM…. The M/G ratio is important in determining the overall quality of the product. The polyguluronic acid regions (G-block) can bind calcium with the negative charge on the carboxylic acid residues to form the “egg-box structure” shown. Alginate solutions are viscous and will gel in the presence of calcium or acid to form soft, temperature-stable gels. Propylene glycol can be reacted with the carboxylic acid residues to form propylene glycol alginate (PGA) that has a reduced sensitivity to calcium.

**Pectin.** Pectin is extracted from fruit – particularly citrus pulp. Chemically it is largely a linear polymer of polygalacturonic acid with varying degrees of methyl esterification. If more than 50% of the acid groups are present as methyl esters (i.e., DE>50%) it is classified as a high-methoxy pectin otherwise it is a low-methoxy pectin. It also contains “hairy regions” with considerable side chains of the main backbone. High methoxy pectin will gel in the presence of acid and high sugar concentrations and low methoxy pectin will gel in the presence of calcium but there is a continuum of properties between these two extremes.

Gelation of pectin requires the formation of cross-links between two different polymer chains. The charged groups on the galacturonic acid residues repel one another and this can only be overcome for high-methoxy pectin by acidifying (to protonate and remove charge) and adding sugar (to compete for the hydration water). Low methoxy pectin carries too much charge to aggregate under these conditions and instead calcium is. The calcium ions complex with the negative charges from the galacturinic acid to form local polymer-polymer bonds. The hairy regions never cross link and allow the pectin to form a well-hydrated gel.