

A brewer's biochemistry

By **Professor Charles Bamforth**

Department of Food Science and Technology,
University of California, Davis, USA,

Part 2: Carbohydrates

This is the second in a series of articles aiming to position malting and brewing in biochemical terms for the benefit of those who have received no training in this area of science. Readers lacking a formal scientific training will find basic chemical principles described in the first article of the series.

Carbohydrates have many roles in life. Quantitatively they are the major organic materials in nature (*organic* means compounds containing carbon atoms, other than the simpler ones such as carbon dioxide).

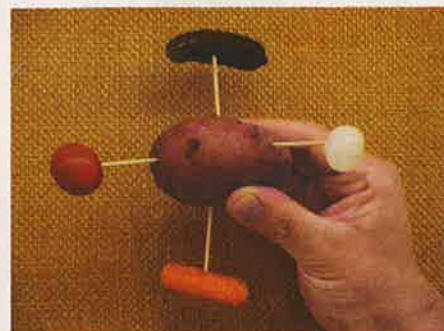
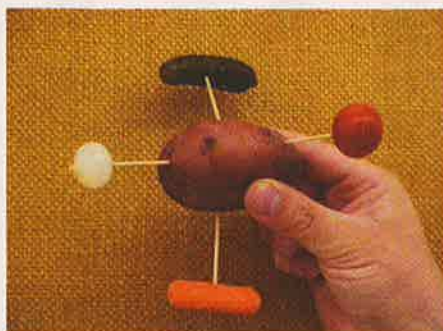
Food stores

Firstly, carbohydrates comprise the major food reserves in living systems. Two examples are the starch found in plants (for example, in the endosperm of barley) and the glycogen found in animals – and brewer's yeast. Starch and glycogen are polymers of glucose: huge numbers of separate molecules joined together to form long chains. If you completely break down starch or glycogen you would end up with glucose.

The reason an organism keeps its food reserves in this polymeric form is to avoid problems with osmotic pressure. If a concentrated solution of glucose (i.e. lots of glucose molecules per millilitre of water) is separated from a weak glucose solution by a membrane that allows passage of the molecules, then water will pass across the membrane in an attempt to equalise the strength of the solutions on both sides of the membrane.

If that membrane was the one surrounding a cell, and that cell was loaded with glucose as a store, then water from the outside of the cell would come flooding in, the cell would swell and eventually burst once the membrane had been overstretched.

However, if all those glucoses are joined together to make a molecule of starch or one of glycogen, then the concentration of *molecules* is much lower. It's the concentration of individual molecules that determines osmotic pressure, not the size of those molecules. So by maintaining glucose in a polymeric form a cell prevents osmotic stress.



A simple way to understand stereochemistry with fruit and vegetables:
Left: Fig. 1 D-glyceraldehyde and right, Fig 2 L-glyceraldehyde.

Source of energy

Why store carbohydrate any way? The answer of course is that it can be "burnt" as a fuel to generate energy. I'll talk about the pathways that do this in a later article. But suffice to say, here, that the storage materials (starch, glycogen) are broken down by enzymes to form simpler sugars, which then enter into quite complex pathways, each involving a separate enzyme-catalysed step, that progressively, in a highly controlled and efficient manner, lead to the capturing of energy.

Forming a part of important metabolic molecules

The energy is captured in the form of a high-energy molecule, called ATP (we don't need to know what the letters stand for, but trust me, it isn't the Association of Tennis Professionals). ATP can be likened to a crossbow.

When you pull back the string and secure it this is akin to the pathway by which sugar is broken down, and which leads to the accumulation of ATP – i.e. you are putting energy into the crossbow. Then when you release the catch the energy is released, hopefully in a targeted fashion. For ATP this means releasing its energy again in metabolic processes that require energy, such as making cell components, moving about and simply staying alive. The ATP is broken down.

ATP has quite a complex structure. Remarkably, at its heart is a sugar, called ribose (see later). This is an example of a sugar being part of important metabolic molecules. Ribose also finds itself in RNA (ribonucleic acid), while a ribose lacking one oxygen, ergo deoxyribose, is an important feature of the structure of DNA (deoxyribonucleic acid) which, as most folk are aware, is the genetic material of the vast majority of living cells. (RNA helps DNA translate its code into action, as we shall see in a later article.)

Structural roles

Carbohydrates have diverse important structural roles in nature. Crab shells are carbohydrates. Closer to home, perhaps, are the β -glucans and pentosans in the starchy endosperm cell walls of barley, the cellulose in the husk, and the glucans and mannans that are present in the cell wall of

yeast. More of this later.

Carbohydrates also form a part of the structure of some proteins. Some people are adamant that the foaming polypeptides are so-called *glycoproteins*, the glyco- prefix signifying that sugars are present.

The chemistry of carbohydrates

Some basics

The word 'carbohydrate' derives from the fact that many of these compounds have the general formula $C_n(H_2O)_n$, i.e. they are 'hydrates of carbon' (hydrate as in *hydration* – inclusion of water). So, glucose, one of the simpler sugars, has the simple formula $C_6H_{12}O_6$ where $n = 6$.

The simplest carbohydrate of all is glyceraldehyde. This comprises a carbon atom to which are attached four different groups: a simple hydrogen atom (-H), a hydroxyl group (-OH), an aldehyde group (-CHO) and an alcohol group (-CH₂OH). Now these "poke out" at equal angles from the central carbon.

To get a feel for what I am talking about you may wish to avail yourself of a small potato (the carbon atom) and four cocktail sticks (Fig 1). Push the cocktail sticks into the potato such that each is equidistant from all the others. The cocktail sticks are the bonds that bind the carbon atom to each of the groups that are linked to it. Now get a cocktail onion and stick it on to one of the sticks. You will instantly realise that this is your hydrogen atom.

Similarly we can put on to the other sticks a gherkin (aldehyde group), a baby tomato (hydroxyl) and baby carrot (alcohol). (Please don't get hung up on the size issues here – for instance, the fact that the potato carbon is much bigger than the carrot which, being our alcohol, not only has a carbon but also three hydrogens and an oxygen. I am using the fruit and veg in an illustrative manner only!)

You can of course move this "fruity-veg" version of a glyceraldehyde molecule about in space and the onion, gherkin, tomato and carrot always stay in the same places relative to one another, no matter what group is uppermost.

However, let's switch the sticks holding the onion and the tomato (Fig 2). What we have now

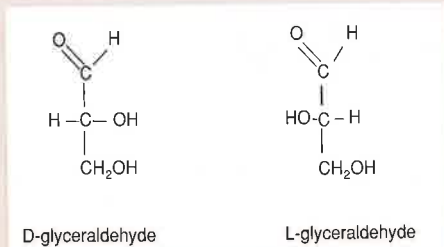


Fig 3. The real glyceraldehydes.

is the mirror image of our first molecule. And it's different! No matter what you do (short of whipping out the sticks or switching the fruit and veg) then you can't turn one around such that it looks exactly like the other.

What we have are *isomers*: molecules that differ in the orientation of the groups. There are, then, two forms of glyceraldehyde: they are known as D-glyceraldehyde and L-glyceraldehyde (Fig 3). (I always remember them by thinking of the L as meaning "left", such that the -OH points to the left when the aldehyde group is pointing upwards.)

In fact the carbohydrates that primarily concern brewers are all in the D- configuration, so we'll concentrate on that class.

Now suppose we put another carbon atom between the central carbon of glyceraldehyde and the aldehyde group. And on this new carbon we have a hydrogen (onion) and a hydroxyl (tomato). The reader will realise (I hope. If not, get some more cocktail sticks and food.) that the new addition can be introduced in two ways: in one the onions (and tomatoes) are on the same side of the molecule whereas in the other they are on opposite sides. Thus we have two new molecules, which are in fact called D-erythrose and D-threose (Fig 4a).

In this way we can keep adding more H-C-OH or HO-C-H segments (Fig 4). And each time, because we have two ways in which to make the introduction, we double the number of possible compounds.

Glyceraldehyde has three carbons – it is a *triose*. The suffix *-ose* indicates carbohydrate: most carbohydrates have this suffix (as you can see from Fig 4). Those sugars with four carbons are known as *tetroses* (of course – "tetra" meaning four). Then we have the *pentoses*, *hexoses* etc.

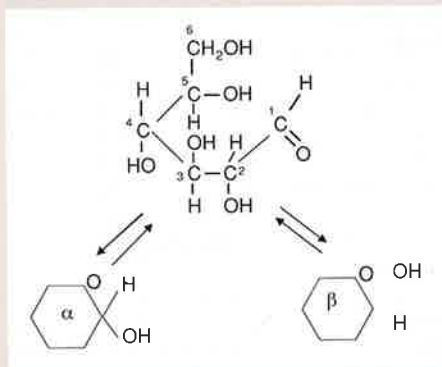


Fig 5. Glucose in linear and two ring forms.

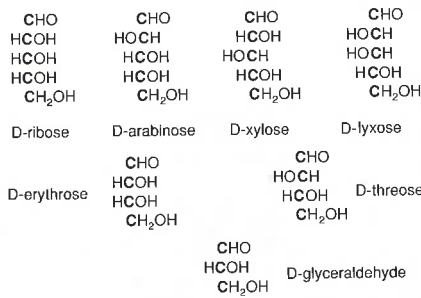


Fig 4a Every time you add a carbon you double the options;

Polymers of sugar units containing five carbons are known as *pentosans*. Polymers of glucose are known as *glucosans*, or more commonly, *glucans*.

Perusal of Fig 4b will show that all of the molecules are very similar. Small differences, though, can make all the difference in this world (as we all know). Thus mannose and glucose differ only in the way in which two of the groups are attached to one of the carbon atoms (in fact carbon atom number 2 – the convention for numbering the carbons is shown in the glucose molecule). A tiny difference – but one that makes for a different set of properties for glucose and mannose, for example their sweetness, the enzymes that act on them, etc.

Building up the complexity

In fact a sugar such as glucose has a rather more complex structure than this. For the most part it is found in a ring form, rather than a linear style. Think of one end of the molecule grabbing the other, as a snake might grip its jaws on its rear end.

In fact, there are two ring forms that are interchangeable through a linear form (Fig 5). These two forms are called α and β . In the α -form the -OH group at C-1 points downwards, for β it points upwards.

Carbon atom number 1 is also called a *reducing group* because when the molecule is in the linear form it has a free carbonyl group (-C=O), which is readily oxidised (and when something is oxidised something else is

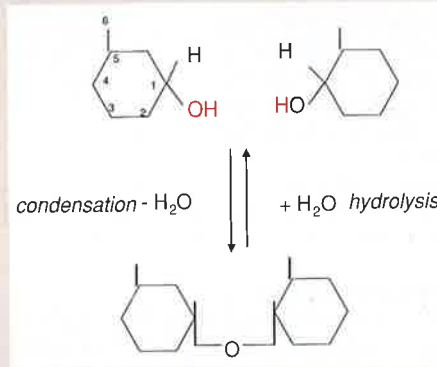


Fig 6 The formation of a disaccharide (maltose) by the splitting out of water. Reverse reaction = hydrolysis (the addition of water).

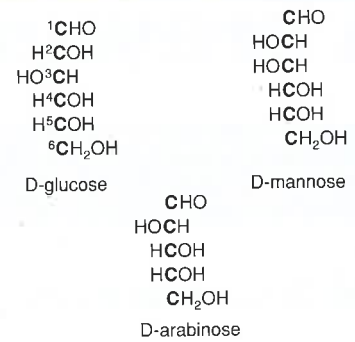


Fig 4b The two D- hexoses derived from D-arabinose.

reduced). In other words a molecule such as glucose is able to reduce another molecule because of this group. It is through this group that a sugar interacts with an amino acid (see my last article) to form coloured materials through the so-called Maillard reaction that occurs in kilning and wort boiling. If these reducing groups are eliminated (for example by polymerisation – see next section) then colour formation won't occur.

Joining sugars together

Low molecular weight carbohydrates tend to be freely soluble in water and, of course, sweet. They are called sugars. A molecule of glucose (or xylose or mannose etc) is called a *mono-saccharide*.

Individual sugar molecules can join. If their hydroxyl groups get together, then a molecule of water can be split out and they get to share the remaining oxygen atom (Fig 6). A sugar comprising two such units is called a *disaccharide*.

Add another unit and we get a *trisaccharide* – and so on. Carbohydrates containing between 2 and, say, ten units are called *oligosaccharides*. Anything bigger is a *polysaccharide*.

By now you will, of course, have realised that two glucoses (for example) could join in various ways. One of the most common ways is for the water to be split out from between carbon 1 and carbon 4. In this way we can get a 1→4 linkage (an example of a *glycosidic bond*).

However there are two possibilities, depending on whether the glucose providing the -OH from its C-1 atom is in the α or β configuration. Thus we can have an α 1→4 or a

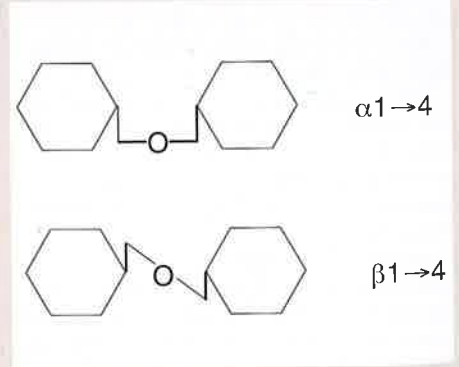


Fig 7 Two glucoses can link by either α or β bonds between carbon atoms 1 and 4.

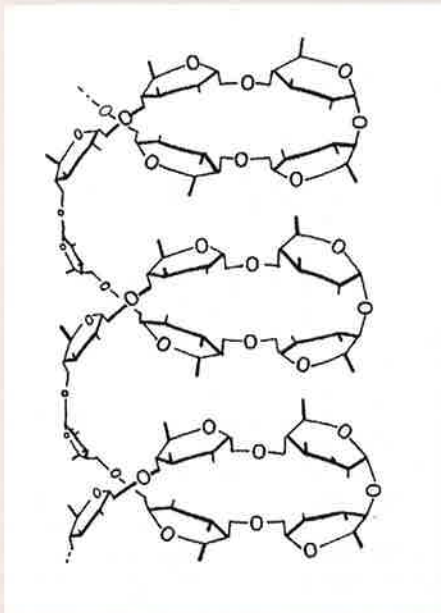


Fig 8a Amylose

$\beta 1 \rightarrow 4$ linkage (Fig 7). The two disaccharides formed are totally different. The first is maltose (the main sugar found in wort), the second is cellobiose.

When hundreds and hundreds of glucose units are linked together through $\alpha 1 \rightarrow 4$ bonds we have a molecule called amylose, which is one of the two fractions found in starch (Fig 8a). The other is called amylopectin, which differs from amylose in that it has branches that result from some of the glucoses being linked $\alpha 1 \rightarrow 6$ (Fig 8b). When loads of glucose units are linked together through $\beta 1 \rightarrow 4$ bonds then the molecule is cellulose. Just one small change in configuration (β links between the 1 and 4 carbons rather than α ones) – but what a huge difference in properties. Cellulose is found as the major component of most plant cell walls – in tree trunks for instance and ending up in paper. It is tough and difficult to chew up.

The β configuration between two glucoses allows the production of very straight chains. Not only that, there is an opportunity for hydrogen bonding to increase yet further the interaction between adjacent glucoses. (I explained hydrogen bonding in my last article). Hydrogen bonding between bundles of adjacent chains leads to fibril formation (Fig 9).

Contrast this with starch – familiar in everyone's kitchen. (You wouldn't bake your cakes from paper. Well, I wouldn't.) The α links

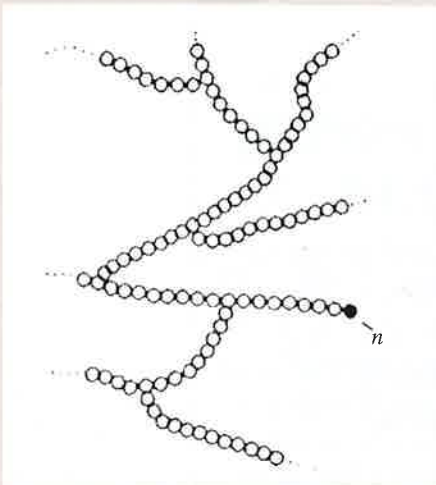


Fig 8b Amylopectin model – where the various chains join is an $\alpha 1-6$ bond. *n* indicates the sole non-reducing end.

make for a helical structure (Fig 8a) – akin to a loose spring. You will realise that we have a somewhat open, accessible structure.

The cell walls of barley endosperm are about three-quarters β -glucan. It wouldn't be sensible for this to be cellulose, because the barley embryo needs to break down its food reserve readily and it would take an eternity to get through cellulose-rich walls. Thus a third of the inter-glucose links in this case are $\beta 1 \rightarrow 3$ (Fig 10). Such links (which for the most part occur interspersed by 2 or 3 $\beta 1 \rightarrow 4$ bonds) tend to disrupt the ability of the polymer to fold in on itself and make those hydrogen bonds between side-by-side glucoses.

However there are still plenty of $-OH$ groups poking out and they can interact with their look-alikes on adjacent chains. In this way are built up aggregates which increase the viscosity of solutions and reduce the solubility of the molecules (ergo the brewer encounters problems with β -glucans)

The other polysaccharide in the cell walls of the starchy endosperm of barley is the pentosan arabinoxylan (Fig 11). By now you should have got the drift: in this case we have long chains of xyloses joined through $\beta 1 \rightarrow 4$ bonds. Arabinoses are joined on to some of the xyloses by $\alpha 1 \rightarrow 2$ and $\alpha 1 \rightarrow 3$ links.

The glucans and the pentosans in the barley walls are robust enough to provide structural integrity whilst being sufficiently amenable to enzymic digestion. Which reminds me to

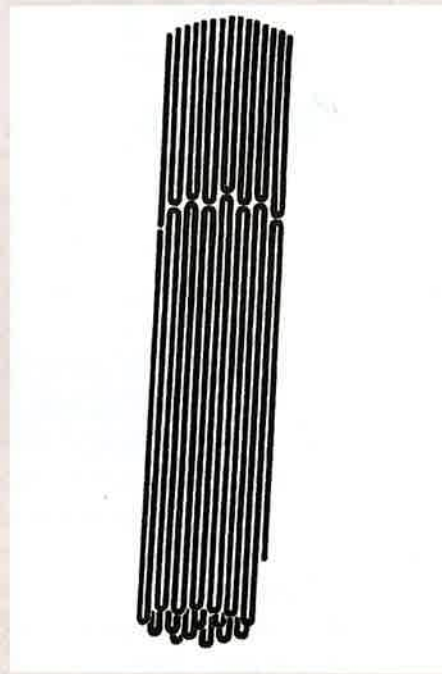


Fig 9 Cellulose fibrils.

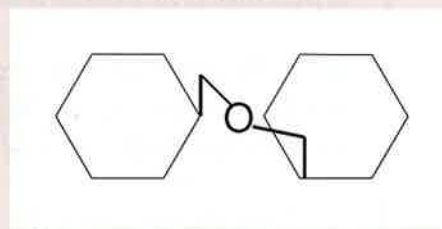


Fig 10 Laminaribiose.

highlight the fact that an organism such as barley needs to develop at least one different enzyme for every type of glycosidic linkage that needs to be broken down (Table 1).

In this way the organism can control the breakdown of its food reserve: if one enzyme broke down everything we'd have chaos. The hydrolysis involves the addition of water (i.e. the restoration of the water that is split out when two monosaccharides condense together to form a disaccharide, and so on).

Some of these enzymes are said to be *endo* acting in that they attack a polysaccharide molecule in the middle to produce smaller oligosaccharides. Others are *exo* enzymes, which attack from the end of a molecule, chopping off one or two sugar units. These will tend to travel

Table 1 Enzymes breaking down carbohydrates in brewing

Substrate	Enzyme	Bond broken	Endo or Exo-acting	Major Product
Starch (amylose & amylopectin)	α -amylase	$\alpha 1 \rightarrow 4$	endo	dextrins
Starch (amylose & amylopectin)	β -amylase	$\alpha 1 \rightarrow 4$	exo	maltose
Starch (amylopectin)	Limit dextrinase	$\alpha 1 \rightarrow 6$	endo	linear dextrins
Starch dextrins	α -glucosidase	$\alpha 1 \rightarrow 4$	exo	glucose
β -Glucans	β -glucanase	$\beta 1 \rightarrow 4$	endo	β -oligosaccharides
β -oligosaccharides	β -glucosidase	$\beta 1 \rightarrow 4$ and $\beta 1 \rightarrow 3$	exo	glucose
Xylanase	Arabinoxylan (xylan backbone)	$\beta 1 \rightarrow 4$	endo	Xylo-oligosaccharides
Arabinase	Arabinoxylan	$\alpha 1 \rightarrow 2$, $\alpha 1 \rightarrow 3$		Xylan backbone
Xylosidase	Xylan oligosaccharides	$\beta 1 \rightarrow 4$	exo	Xylose

