A brewer's biochemistry

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Part 3: Lipids

This is the third 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.

Lipid is a term used to describe those chemical compounds in living organisms (such as barley, hops, yeast and you and me) that are insoluble in water. You won't be surprised to find that they comprise a diverse range of molecules, many of which are classed as fats or oils. Closely related to them are other materials that make a sizeable contribution to the lives of most of us, substances like detergents and petrols.

Unsurprisingly (because of their insolubility) the lipids are primarily found in structural components of cells, principally the membranes that surround the cells and that hold the soluble liquid components within.

Structure of lipids

Many lipids are based on combinations of *fatty acids*. These compounds have a structure comprising chains of carbon atoms joined end to end, with hydrogen atom "wings" and, at one, end, an acid group, the carboxyl group (-COOH) (Fig 1). The simplest such molecule is actually acetic acid. It has just two carbon atoms, one holding three hydrogens and the other being the carbon in the carboxyl group. Acetic acid is strictly not a lipid – for it is very water soluble (being the key ingredient of vinegar). It is only when successively more carbons and hydrogens are added that the insolubility becomes manifest, for reasons that we will discuss momentarily.

Those fatty acids with up to 14 or so carbon atoms are often referred to as "short chain fatty acids". They often have distinctive aromas, such as cheesy.

Quantitatively in living organisms the most important fatty acids have either 16 or 18 carbon atoms. (Much longer fatty acids are found in some organisms, but in progressively smaller quantities).

Of especial interest are the fatty acids containing eighteen carbon atoms, of which there are several. The first, stearic acid, has each of its carbon atoms linking through a single bond to its neighbouring carbon atoms (Fig 2). Hydrogen atoms take up the other places on the first 17 carbons, the eighteenth carbon of course being part of the carboxyl group.

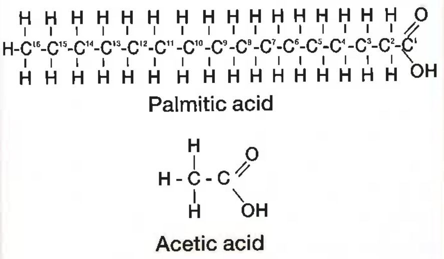

Figure 1. ***The basic structure of fatty acids.*** *Carbon 1 is the so-called "carboxyl carbon" because it forms part of the carboxyl group. As we saw in the first article of this series, this acidic group can dissociate to release a hydrogen ion (H^+), leaving $-COO^-$.*

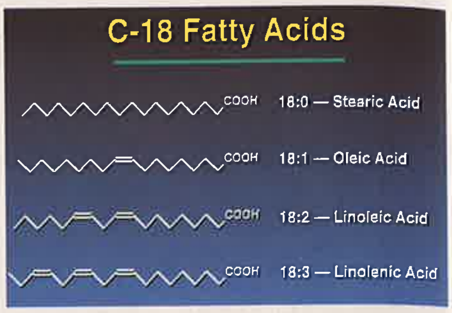

Figure 2. ***The fatty acids with eighteen carbons.*** *Some shorthand conventions are illustrated. Rather than write out all of the carbon atoms, biochemists simply draw lines, at each end of which is a carbon atom. As each carbon atom is capable of binding to four other atoms, it is taken as read that the last carbon on the left hand side has three hydrogens attached to it, and most of the other carbons have two hydrogens linked to them. The carboxyl group is, of course, different, with two bonds to one of the oxygens and one to the other, which in turn links to another hydrogen (c.f. Fig 1). The unsaturated fatty acids contain double bonds. Those carbons entering into double bond formation have only one hydrogen linked to them. The shorthand notation is to indicate the total number of carbon atoms in front of the colon, then to signify the number of double bonds after the colon. Thus 18:2 is universally recognised by biochemists as being linoleic acid.* **Thanks to Gerry Russell for this illustration.**

Oleic acid differs from stearic acid in that carbon atoms numbers 9 and 10 are held together by two bonds (Fig 1). It is said to be an *unsaturated fatty acid* whereas stearic acid is a *saturated fatty acid*.

And then we have linoleic acid, in which case we have ***two*** double bonds, between carbons 9 and 10 and carbons 12 and 13. Linoleic acid is an example of a *polyunsaturated fatty acid*, as is linolenic acid, which has a third double bond, this one between carbons 15 and 16.

For the most part in living organisms fatty acids don't exist in a free form, rather they are attached to other molecules, above all glycerol (Fig 3). Glycerol is an alcohol (in fact you might even say it's three alcohols in one, having three of the alcohol groupings, -OH). Fatty acids are, as the name indicates, acids. When you react an acid and an alcohol together (by the exclusion of water) you get something called an "ester". Esters of glycerol and fatty acids are known as *glycerides*. If just one of the –OH groups of glycerol is "esterified" by a fatty acid then we have a *monoglyceride*. Two fatty acids, and we have a *diglyceride*. Three = *triglyceride*.

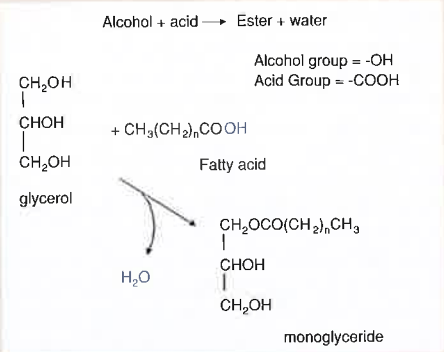

Figure 3. ***A glyceride is an ester formed between a fatty acid and glycerol.***

There are more complex forms of esterified fatty acids, which contain extra groups, such as phosphate (phospholipids), sugars (glycolipids) and sulphur (sulpholipids), but to pursue these would take us into too complex a domain. The important properties of lipids that we need to address need us to focus only on the fatty acids....oh, and the sterols.

Sterols, alongside the fatty acids, are important components of the membranes of yeast. The most important one is ergosterol (Fig 4).

Fats and oils

You'll have to take my word that the shapes of such sterols lends themselves nicely to a structural task in membranes. Fatty acids

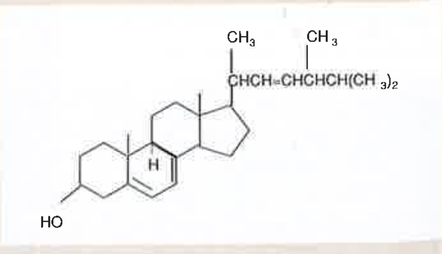

Figure 4. Ergosterol.

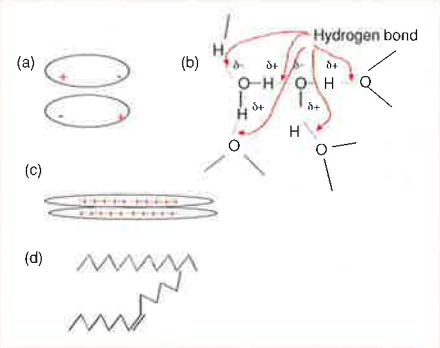

Figure 5. ***Interactions and shapes:***
a) ionic interactions
b) hydrogen bonding
c) Van der Waals forces
d) Saturated fatty acids are straight whereas unsaturated fatty acids are kinked. Imagine stacking corrugated sheets and such sheets after they have been bent.

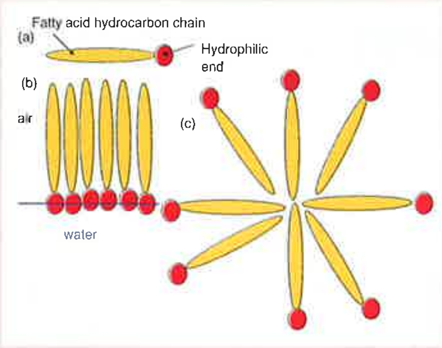

Figure 6. ***The behaviour of lipids in the presence of an aqueous solvent.***
a) schematic representation of a lipid
b) lipid behaviour at a surface
c) lipid behaviour (micelle) in the body of the aqueous system.

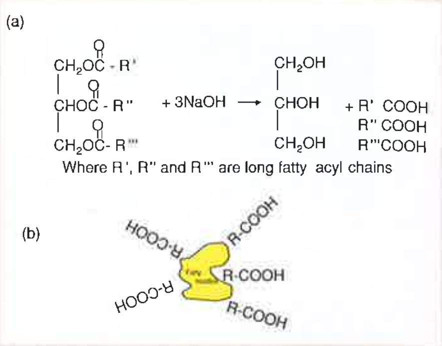

Figure 7. ***Soaps and detergents:***
a) Soaps are formed by alkaline hydrolysis of glycerides
b) Soap (or detergent) interacts with the hydrophobic chains associating with the fatty particle and the hydrophilic ends associating with the water.

(particularly when in the form of phospholipids) are also major membrane components. In particular fluidity and flexibility is important.

The structure of a lipid impacts greatly on its properties. Moving into the kitchen momentarily, we find that butter is a solid (at room temperature) and it is referred to as a *fat*. The stuff that comes in bottles and that we use to cook our French-Fries is referred to as an *oil*, because it is a liquid at room temperature. Examples are sunflower oil, corn oil and olive oil.

Clearly olive oil is much more fluid than butter (unless you heat the butter up). Olive oil contains a high proportion of unsaturated fatty acids, which have a lower melting point (the temperature at which they go from being a solid to a liquid) than do the saturated fatty acids, which are found in butter.

The insolubility of lipids

I started this discourse by saying that lipids are united as a group of substances by the common feature of water-insolubility. To explain, let's talk a bit about the forces that hold separate molecules together (*See panel below*).

The strongest of these forces are between molecules that have fully fledged charges, positive and negative. Opposite charges attract, so if we have a couple of adjacent molecules, each of which has a full positive and a full negative charge, then the charges on each will attract the opposite charge on the other and the two molecules will associate (Fig 5a). An example is common salt (NaCl, which we can also write Na^+Cl^-). To break the units apart demands a lot of energy to overcome the strong inter-molecular forces – you try heating salt and you'll neither melt it nor boil it.

In some molecules there are "incomplete" charges. The best example is water. For reasons I explained in the first article of the series, oxygen tends to hog the electrons and pull them away from hydrogen in the water (rather like Mrs Bamforth and the duvet), so it has a partial negative charge, whereas hydrogen has a partial positive charge.

These partial charges allow adjacent water molecules to associate, through something called "hydrogen bonding" (Fig 5b). The opportunity is for vast networks of water molecules to associate together to form well organised structures. At room temperature water is of course a liquid. The hydrogen bonding needs quite a lot of energy to overcome it, so water only boils at 100°C.

But what of compounds that don't have any charge whatsoever? An example of such a compound is a fatty acid, such as the palmitic acid shown in Fig 1. (For the purpose of this discussion, let's ignore the –COOH group, which adds complexity to the argument. It is the rest of the fatty acid molecule that largely determines its solubility properties.) Another example would be a sterol, such as ergosterol (Fig 4).

Such molecules are held together by something called *van der Waals* forces (Fig 5c). As we found in the first article in the series, atoms and molecules contain positive and negative charges. In a molecule such as palmitic acid they balance one another out. However the individual negatively charged electrons are moving about the whole time, creating infinitesimal localised regions with net positive or negative charges. Association of the tiny opposite charges on adjacent molecules holds them together, but the interactions are very weak.

Thus some lipids (e.g. sunflower oil) are liquid at room temperature, whereas others (e.g. lard) don't need much input of energy to liquefy them. Whereas the saturated fatty acids have a regular zig-zag structure that lends itself to packing together, the double bonds in unsaturated fatty acids put kinks into the molecules, rendering them less easy to pack together (Fig 5d).

Fats, with their higher proportion of saturated fatty acids, therefore are solid whereas the unsaturated fatty acid-rich oils are liquid.

Another way to overcome the interactions between molecules of the same type is to intersperse other molecules between them: that is, to try to dissolve them. Sodium chloride will dissolve in water because the strong interactions between the positively and negatively charged ions can be overcome and replaced by many smaller positive-negative interactions between the sodium and chloride and the water molecules.

This is not possible with something like a

States of matter

MATTER can basically divided into solids, liquids and gases. They principally differ in the intensity of the forces between the component molecules and the distance between those molecules.

In a *solid* the molecules are close together and so there is an opportunity for close range interactions between molecules. The stronger those interactions, the greater the resistance of those molecules to be driven apart.

It demands energy to drive those molecules apart. The most obvious source of this energy is heat, which invigorates the molecules and increases their tendency to break apart from one another. When they gain this energy and achieve mobility they turn into *liquids*. There is still sufficient inter-molecular interaction to loosely hold the molecules together. The temperature at which this solid to liquid transition occurs is, of course, the *melting point*. The stronger the links between molecules, the higher the melting point.

Put in more energy and the intermolecular interactions are overcome, the molecules can move about largely independently – and we have a *gas*. The temperature at which the liquid-gas transition occurs is the *boiling point*. The stronger the links between molecules, the higher the boiling point.

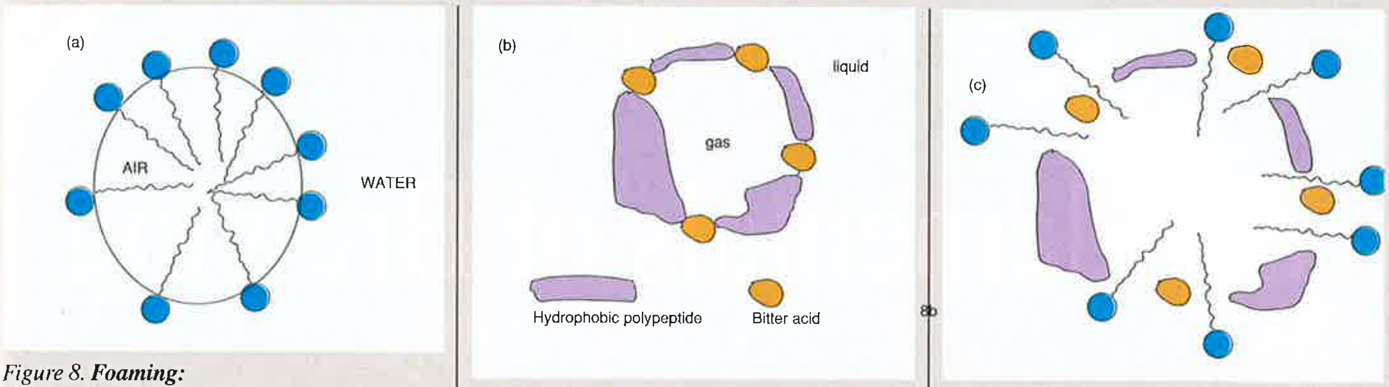

Figure 8. ***Foaming:***
a) detergent-stabilised foam
b) protein-bitter acid stabilised foam
c) mutual collapse of foam by the simultaneous presence of protein, bitter acid and lipid.

fatty acid. So a fatty acid does not dissolve in water: it is *hydrophobic* ("water-hating"). It will dissolve in something that is held together by similar forces. So things like chloroform and petroleum products will dissolve fats (as anyone needing to remove a greasy stain from clothing knows). The rule of thumb is "like dissolves like".

Amphipathicity

A little earlier I said we'd ignore the carboxyl group. But we can't for long. The carboxyl group is of itself *hydrophilic* ("water-loving"). In a glyceride, the glycerol portion is the hydrophilic bit. So part of the molecule is hydrophilic and part is hydrophobic. These molecules are said to be *amphipathic* (Fig 6a).

Now if you add an oil (say sunflower oil) to water, what do you see? The oil will collect atop the water (being less dense). You can't see it, but what is happening is that the hydrophilic parts of the lipids have orientated so as to be dipping into the water where they are quite comfortable, with the hydrophobic portions pointing away from the water (Fig 6b).

Within the body of the water lipid may exist in the form of so-called *micelles* (Fig 6c). Again it is the hydrophilic groups that gather on the outside, able to interact with water. The hydrophobic chains hide away inside.

Structures of the type shown in Fig 6b are exactly analogous to those found in the membranes of living cells, save that the latter tend to comprise a bilayer of lipid, with the hydrophobic chains on the two layers pointing towards each other in the middle, with the respective hydrophilic groups outside.

The action of soaps (and other detergents) in dissolving grease and other hydrophobic "dirt" is analogous to this micelle formation. Soaps are essentially the salts of fatty acids, made by reversing the esterification of glycerides (Fig 7a). The hydrophobic chains on the soaps can associate with "lumps" of grease and dirt, whilst presenting their charged, hydrophilic ends to the water (Fig 7b). In this way the unwanted material is solubilized.

Foam

Those of us who have ventured as far as the kitchen (and who avoid the use of mechanical dishwashers – energy crises in certain places demand it!) are familiar with foams in the sink. These are due, of course, to "washing up liquid" (detergent).

On filling the bowl, air is whipped in to the system and the detergent stabilises the resultant bubbles by forming an interactive surface "skin" that counters the force of surface tension that is seeking to collapse the bubble (Fig 8a).

Of course, as some of us are wont to say ad nauseam, lipids and detergents are ***bad*** news for beer foam. How can this be? The answer lies in the different ways in which beer foams and detergent foams are stabilised. As we saw in the first article of this series, beer foams are stabilised by interactions between polypeptides that are rich in hydrophobic character (which drives them into the head) and the very hydrophobic bitter acids (Fig 8b).

If we have a mixture of protein and detergent or lipid, we have mutual interference. The proteins can't associate together to form a barrier against bubble collapse, and neither can the detergent (Fig 8c). Result: foam collapse.

Lipids and oxygen

The unsaturated fatty acids are very susceptible to oxidation. It's those pesky double bonds that oxygen goes for, starting a cascade reaction leading to the development of materials that can give rancid and cardboard characters to products, including beer.

There was a time in the manufacture of cooking fats when such materials were industrially hydrogenated, to eliminate the double bonds and therefore lengthen the shelf life of the product. Not really a solution for brewers worried about staling (or is it?).

Ironically, oxygen is involved in the metabolic pathways leading to the synthesis of unsaturated fatty acids (and sterols). This is why yeast needs some oxygen to allow it to kick off fermentations efficiently.

Lipids and energy

There is some lipid inserted within the amylose component of starch (see second article in the series), however most of the lipid in a cereal such as barley is found in the germ. The embryo uses the lipid as a high concentration energy reserve.

As we shall see when we talk about metabolism, living organisms break down fuels using oxygen to release energy. It is exactly analogous to burning coal or wood on the fire. In this combustion process carbon dioxide and water are produced. Now if you compare the structure of a fatty acid with that of, say, glucose, you will realise immediately that there is already a lot more oxygen pro rata in the glucose molecule than in the fatty acid. In other words, the glucose is already reasonably oxidised.

This means that there is more potential energy available from the oxidation of a fatty acid than from a carbohydrate – nutritionists assign a calorific value of 9 thousand calories for each gram of fat, but 4 thousand calories per gram for carbohydrate and protein.

This is why it makes sense for bodies to store food as fat rather than carbohydrate: you need to carry less weight around. In fact a 120 pound person would be 150 pounds if there was no such things as a fat reserve. That would really get Mrs B. on my case. ■

CORRIGENDUM

In the last Biochemistry article in the May edition, there was an error in the last line of the caption to Figure 8b. It should have read as follows:

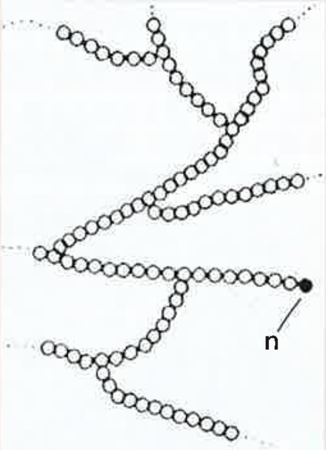

Fig 8b. Amylopectin model – where the various chains join is an α*1-6 bond.*
n indicates the sole ***reducing*** *end.*