

6. Lipids

All living matter contains some fat as their membranes are made up from self-associated lipid molecules. In practical terms though, the fat content of foods varies from effectively zero (many fresh vegetables, certain fish) to 100% (cooking oils). Low fat foods are seen in this culture as being almost virtuous but also not as tasty as their high-fat alternatives. This is probably because of the nutritional and functional roles of fats in foods.

Nutrition. Fats are the densest source of calories available in the diet (7 kcal per gram) which is seen as a bad thing in the west. A high fat diet is associated with many health problems (heart disease and some cancers) as well as obesity. However not all fats are equally "bad for you" and modern lipid nutrition recommendations are increasingly complex. As well as the negative nutritive aspects of fats many lipids are essential components of the diet. Oils act as a solvent for other important molecules such as certain vitamins and flavors.

Functionality. Fats provide important texture to foods:

- Liquid fat on food is greasy (e.g., fried chicken)
- Emulsified fats give a creamy, rich mouthfeel to foods (e.g., heavy cream is smoother and richer than light cream which is "creamier" than milk)
- Semi-crystalline fats are responsible for the plastic spreadability of butter and margarine
- Largely crystalline fats are hard solids (e.g., chocolate)

1. Lipid Structure

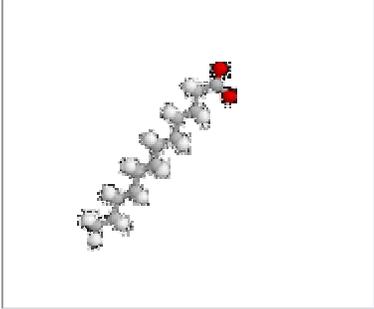
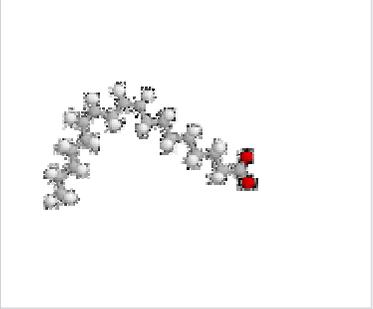
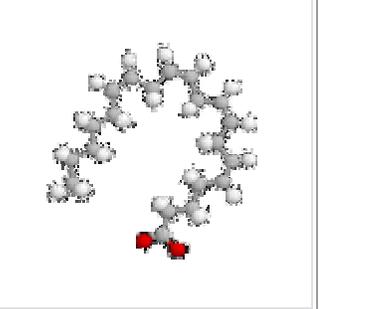
In this section we will identify the important molecules that occur in the class of compounds known as lipids.

Fatty Acids. The basic building blocks of lipids are fatty acids which are carboxylic acids with long hydrocarbon chains attached. In natural fats, the hydrocarbons are (usually):

- Linear (non-branched)
- Even numbers of carbon atoms
- Between 4 and 20 carbons long, the preponderance being of middle length (~12 carbon). Fatty acids can be classified according to their length: short chain fatty acids (4-10 carbons), medium chain fatty acids (12-14 carbons), long chain fatty acids (more than 16 carbons)
- Contain zero (unsaturated), one (monounsaturated) or more (polyunsaturated) double bonds. Triple bonds are not normally seen. Any double bonds are usually *cis* but the *trans* form is thermodynamically more stable so heating can allow the activation energy for transformation to be exceeded *cis trans* isomerization to occur. About 40% of the fat in a French fry and overall approximately 10% of western dietary fat may be *trans*. *Trans* fatty acids are thought to be harmful to

the health as they cannot be correctly metabolized by the body or incorporated into membranes.

- *cis* fatty acid force a turn in the molecular backbone so polyunsaturated fatty acids can be bent double

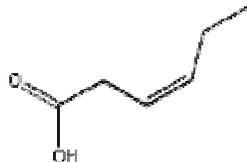
		
Lauric acid , 14 carbons - no double bonds	Linoleic acid , 16 carbons, double bonds at carbon 9 and 12 (note count carbon #1 as the carbonyl group)	Arachadonic acid , 20 carbons, double bonds at carbons 5, 8, 11, and 14

The important fatty acid structures are listed in Table 1 in the text. Common fatty acids have common names (e.g. lauric acid, stearic acid) but there are also more formal naming conventions, for example:



Hexanoic acid

- Hex - six carbons
- -anoic - an unsaturated structure
- acid - a carboxylic acid



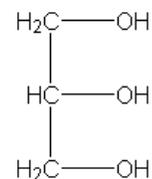
Hexenoic acid

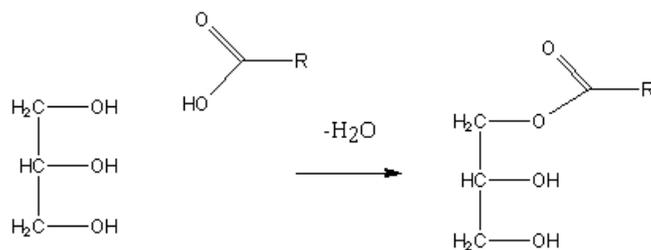
- Hex - six carbons
- -enoic - an saturated structure
- acid - a carboxylic acid

Glycerol esters

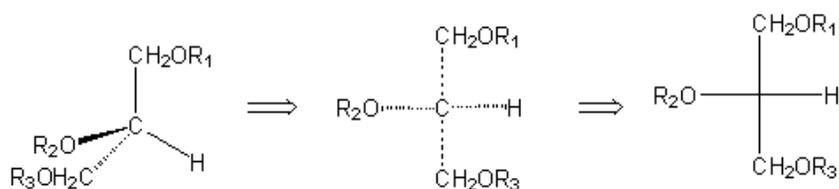
Fatty acids do occur in foods but the vast majority (~99%) of them are bound as esters of glycerol. **Glycerol** is a three carbon polyol. It is a clear, viscous highly water soluble liquid with a mild sweet taste sometimes used as a humectant in foods (e.g., added to dried food to keep them moist and pliable with very low water activities)

The three alcohol groups can esterify with carboxylic acids (a dehydration reaction) to form mono-, di-, and tri-glycerides. (More correctly these are known as mono- (or di- or tri-) acyl glycerols.) Mono- and di-glycerides are amphiphilic and triglycerides are hydrophobic and water insoluble.





Glycerol is a symmetrical molecule but the central carbon becomes chiral with certain substituents. Like sugars, the Fisher projection is used to differentiate between the chiral forms of glycerol derivatives (note – in the structures shown below the subscript numbers with the R groups serve merely to differentiate the fatty acids present rather than suggest there are multiple R-groups at different bonding sites).



The convention for glycerols is to arrange the central hydroxyl left (i.e up from page). This is the stereospecific numbering (*sn*) convention and assigns a fixed number to the two ends (carbon one at the top, three at the bottom). So *sn*-StPO would have stearic acid in position 1 (at the top) and palmitic and oleic in positions 2 and 3 respectively. The triglyceride *sn*-OPSt although containing the same atoms, is a distinct molecule with different properties. In this convention you can use *rac*- (racemic) to designate a mixture of stereoisomers, or if the composition is unknown or unimportant.

Phospholipids. Glycerol can esterify with molecules other than fatty acids, for example phospholipids are glycerol esterified with at least one mono- or di-ester of phosphoric acid. Very often the phospho-substituent on the glycerol contains another unusual substituent (a carbohydrate or charged molecule) whilst the other two positions contain normal fatty acids. Phospholipids are amphiphilic molecules and many spontaneously form membranes.

2. Lipid Oxidation

In this section we are mainly concerned with lipid autoxidation but we will initially deal briefly with lipolysis. Lipolysis is the enzymatic (lipase) or thermal hydrolytic cleavage of ester links holding fatty acids to glycerol. Free fatty acids, particularly the small ones, are quite volatile and have some aroma so their formation can lead to off flavors. There are very few free fatty acids in "normal" food oils.

Avoiding enzymatic lipolysis requires the oil must be separated from the protein (enzyme) fraction of the food as soon as possible in the extraction process, or the enzyme should be rapidly thermally inactivated. Once formed, free fatty acids can be converted to soaps at high pH (i.e., sodium salts) and extracted to remove the flavor.

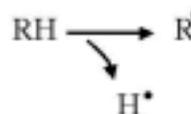
Autoxidation. Lipid autoxidation is the catalyzed radical reaction between unsaturated fatty acids (and triglycerides) and molecular oxygen. Lipid oxidation leads to food spoilage as there is extensive molecular fragmentation leading to the formation of volatile (and extremely smelly) products. The formation of flavor from oxidized lipids is known as rancidity. In certain cases (some fried foods and cheeses) some rancid flavor is desirable but usually rancid flavors are extremely unpleasant. Once radical oxidation has started in a food or biological system it may spread to non-lipid components, in food this may be as simple as vitamin loss but can also cause protein damage. There are also some health concerns as any oxidation induced *in vivo* can lead to genetic damage and may be carcinogenic. Extensive lipid oxidation can lead to some lipid polymerization with consequent increases in viscosity and browning.

The Radical Reaction. All radical reactions proceed via initiation, propagation, and terminations steps:

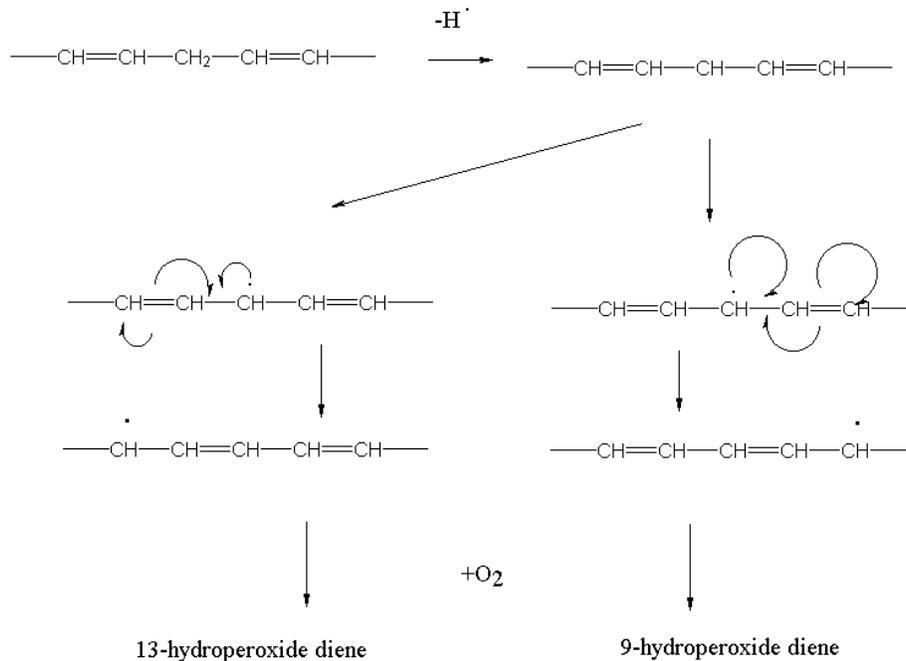
- **Initiation.** The homolytic cleavage of a covalent bond to form two free radicals. There are no radical groups on the left hand side of the reaction and two on the right.
- **Propagation.** The transfer of the radical character from one molecule to another. Propagation always has one radical species on the left hand side of the reaction and one on the right.
- **Termination.** Two radicals come together to form a covalent bond. There are two radical groups on the left hand side of the reaction and none on the right.

In lipids the typical reaction scheme is:

Initiation: A hydrogen atom is abstracted from a methylene group adjacent to a C=C bond, usually one between two, i.e., -CH=CH-CH₂-CH=CH-. The structure is a 1,4-pentadiene.

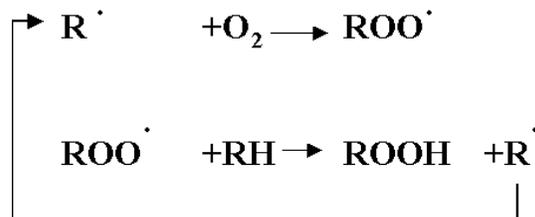


Sometimes the radical center formed can migrate along an unsaturation to localize on another carbon before further reacting.



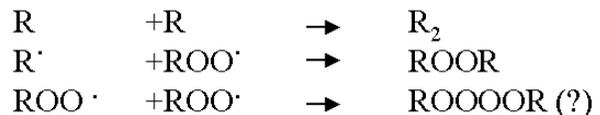
A consequence of this is there are often several starting materials for the next phase of the reaction and a mixture of breakdown products. The products of resonance stabilization of free radicals in unsaturated fatty acids are conjugated dienes (to adjacent C=C).

Propagation: The lipid radical rapidly reacts with molecular oxygen to yield a peroxy radical. The peroxy radical reacts with fresh lipid (often the same type of 1,4 pentadiene structure) taking a hydrogen atom to form a lipid hydroperoxide and leaving a new lipid radical which can react with molecular oxygen and continue the cycle.



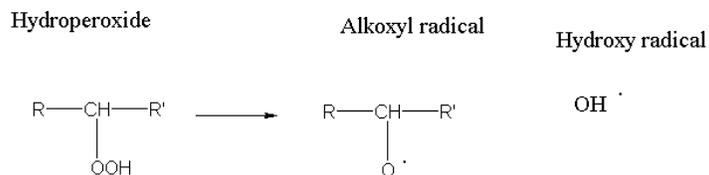
The net effect of this reaction is to consume oxygen and produce lipid hydroperoxides.

Termination: Any two radicals can combine to form a non-radical product. The rate of these reactions is initially limited by the relatively small numbers of radicals present in the system.

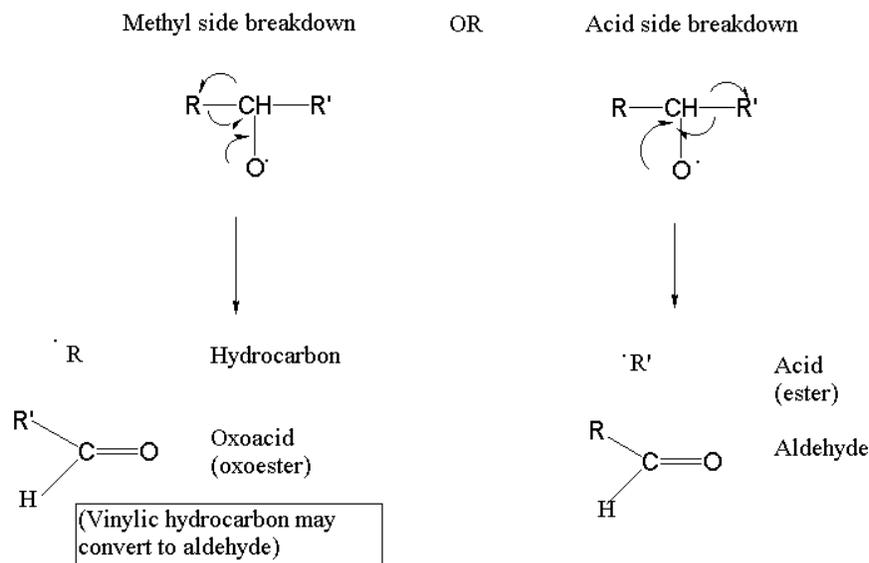


So a single radical generation event can lead to the production of large quantities of lipid hydroperoxides. Lipid hydroperoxides are relatively innocuous, having no bad smell or color, but they can and do spontaneously breakdown via another radical mechanism.

Hydroperoxide breakdown. The first step of hydroperoxide breakdown is the homolytic cleavage of the oxygen-oxygen bond to produce an alkoxy and hydroxy radical.

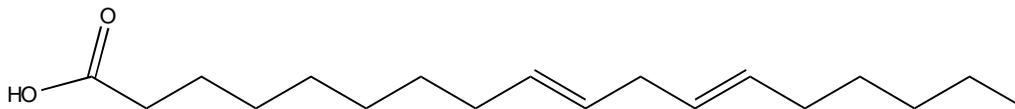


The next step is for the radical electron to "fold" into the C-O bond along with a single electron from either the C-R or C-R' bond to form C=O. The other electron from C-R (or R') moves to the R (or R') and cleaves the lipid molecule. The bond that breaks can either be left or right (methyl or carbonyl end) of the central carbon forming one of two possible pairs of products.



The most important products are the aldehydes which are volatile and have characteristic smells. Each unsaturated fatty acid can produce several types of aldehydes and as there are many fatty acids so rancidity tends to have a "broad" range of perceived flavor and aroma. Many of the reaction products (acids, hydrocarbons, and aldehydes in the figure above) are unsaturated and can oxidize further. The large number of highly reactive radicals present means the products of lipid oxidation are very highly complex.

Question 1: Predict the likely autoxidation products of this fatty acid:



(note the double bonds are drawn as trans for convenience - in a real fat they are **cis**)

Factors affecting autoxidation rate

- **Fatty acid composition.** In general the rate of lipid oxidation increases very rapidly with the number of unsaturations present in a fatty acid.

This is particularly important for polyunsaturated fatty acids essential in the diet, esp. the ω -6 and ω -3 fatty acids in fish oils. The rapid oxidation of fish oils is believed to contribute to the short shelf life of fish.

Free fatty acids are more prone to oxidation than those attached to glycerol so lipolysis can lead to autoxidation.

- **Temperature.** The rate of lipid oxidation increases rapidly with temperature.
- **Water activity.** As with most food chemical reactions, the rate of lipid oxidation decreases as the water activity is lowered from one towards the monolayer value (~ 0.2). However many lipid oxidation reactions increase in rate under very low water activities ($a_w < \text{monolayer}$) and rancidity can be a major problem in dehydrated foods. The rate of lipid oxidation is believed to increase because the lipid hydroperoxides are dehydrated and break down more readily and because non-hydrated metal ions are more effective catalysts.
- **Metal ions.** Although unsaturated lipids can spontaneously react with oxygen the rate of the uncatalyzed reaction is many times slower than the catalyzed reaction. If the amount of free metal is restricted the rate will be slower. Particularly effective metal catalysts are soluble salts of iron and copper, and the use of these metals in food processing equipment may cause problems - particularly due to corrosion or poor quality welding. Control of metal ions is often achieved by the use of a class of antioxidants known as metal chelators which tightly bind free catalytic ions and render them unavailable. A good example is EDTA which binds iron.
- **Oxygen.** If oxygen could be completely eliminated from a product it could not oxidize. In practice this is very hard to achieve absolutely as only a very small amount of oxidation (and therefore oxygen) is required to make a food inedible. Oxygen is very soluble in both water and oil and there is usually enough present to allow oxygen to proceed to an unacceptable level.

Even if this oxygen was eliminated most natural fats contain a considerable amount of pre-existing hydroperoxides that can break down and lead to oxidation products in the absence of additional oxygen.

- **Light.** Light is a source of energy that can lead to the formation of radical initiators. Ultraviolet light is particularly harmful as it is higher energy than visible light. It would be better to store most fatty foods in the dark but this is not practical in retail.

Antioxidants. Fat naturally present in animal and plant tissues is relatively resistant to oxidation due to the presence of natural antioxidants (e.g., tocopherols, certain pigments, some enzymes) but, when the fat is extracted the purification process remove a lot of the antioxidants and the oil is less stable. Natural and synthetic antioxidants frequently added back to fatty foods - particularly those prone to oxidation.

There are two main classes of antioxidant: molecules which prevent the action of catalysts (e.g., metal chelators) and molecules which are oxidized preferentially to the lipid. It is the second class we will consider here. Sacrificial antioxidants are oxidized more easily than the lipids they are designed to protect. The idea is that when an oxidation-causing event occurs (e.g., the generation of the radical), the antioxidant will rapidly react and itself take on the radical character. The antioxidant radical formed is more stable than a lipid radical so tends not to react further and cause damage to the valuable components of the food. In forming a radical, the antioxidant is "destroyed" and has no further antioxidant activity so will only delay not completely prevent lipid oxidation.

In the diagram the process is shown as opening up another pathway to get rid of the radical avoiding the unstable lipid radical that would lead to flavor.

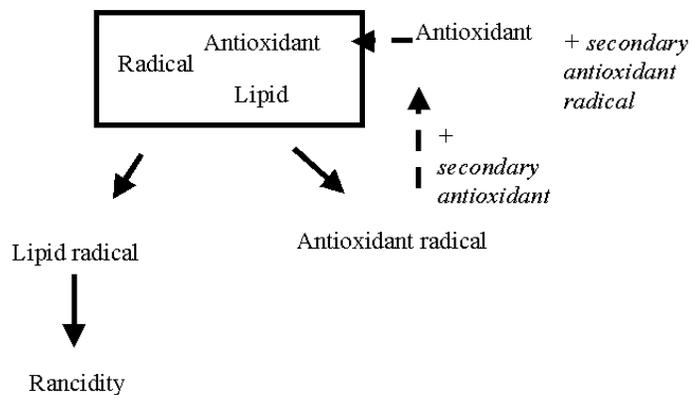
Sacrificial antioxidants are often divided into primary and secondary. Primary antioxidants are those which remove the radical. Secondary antioxidants remove the radical character from the primary antioxidant radical returning it to a "working" form.

Secondary antioxidants extend the lag before oxidation is seen. Good examples of primary and secondary antioxidants are vitamins E and C in living cells.

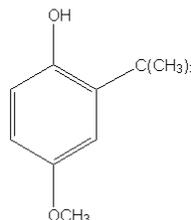
In order to work, a primary antioxidant must (1) React rapidly with radicals, (2) Produce a stable radical on reaction (i.e., not react further). Examples of natural and synthetic antioxidants permitted as food additives include:

- **BHA/BHT**

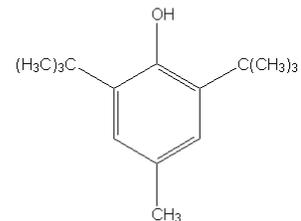
Butylated hydroxyanisole and butylated hydroxytoluene are similar, synthetic molecules used as primary antioxidants in many foods. The phenol group in both BHA and BHT is vulnerable to having a hydrogen atom removed, leaving a radical. The



BHA
Butylated hydroxyanisole

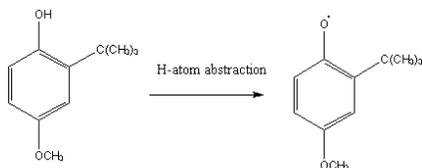


BHT
Butylated hydroxytoluene

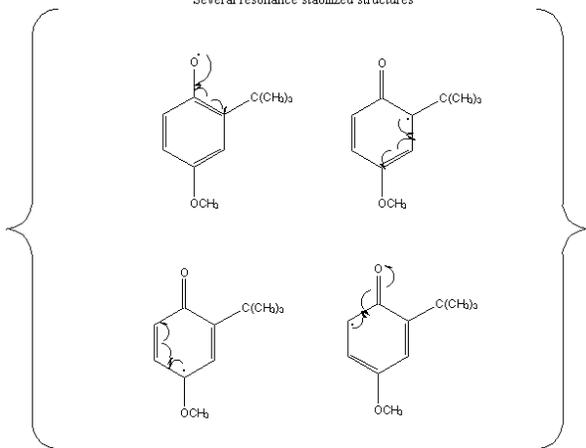


radical formed is relatively stable because it can efficiently delocalize around the benzene ring (shown below for BHA):

Butylated hydroxyanisole



Several resonance stabilized structures



In general a structure is stabilized by extensive resonance delocalization of electrons but BHA is even more stable because:

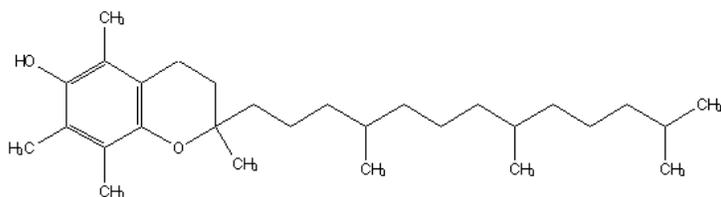
- The single (highly reactive) electron spends a lot of its "time" on the benzene carbon adjacent to the tertiary butyl group. The tertiary butyl group is electropositive and draws the electron a little away from the benzene carbon further stabilizing that position.
- The tertiary butyl is extremely bulky and prevents the physical approach of other molecules that might react with the radical if they could get close enough.

(BHT reacts very similarly to BHA).

• Tocopherols

Tocopherols are natural primary antioxidants. There are a whole group of very similar molecules all with similar structures. Many of them have Vitamin E activity, for example α -tocopherol:

Alpha Tocopherol



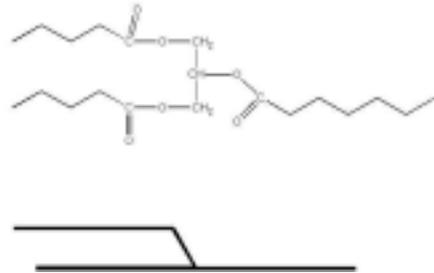
Tocopherols are lipid soluble molecules but the concentration of hydroxyl groups on one end makes them slightly amphiphilic. The hydrogen atoms can be abstracted from the hydroxyl groups and the resulting radical delocalizes extensively around the heterocyclic structure (figure 39 in the Fennema text).

3. Lipid Crystallization

Lipid crystallization is an example of a phase transition and some parallels can be drawn between this and the formations of gas bubbles in a can of soda or avoiding gritiness during lactose crystallization - in particular the supersaturation of a solution before the phase transition occurs.

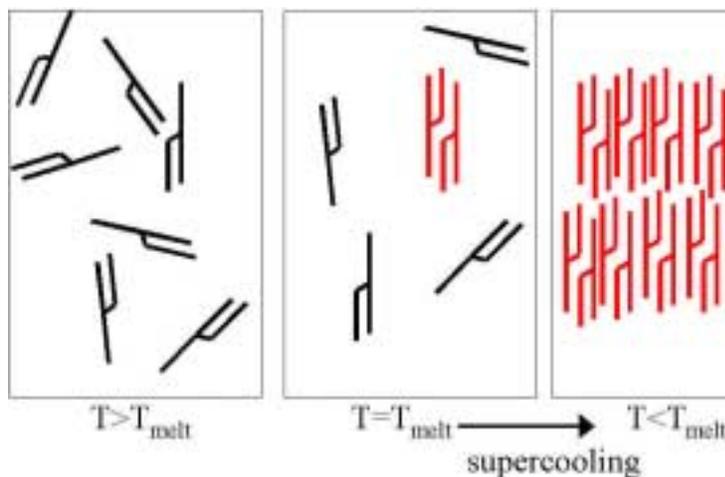
Triglyceride molecules take on a "tuning fork" conformation. (Note unsaturation in any of the fatty acids can make the shape very twisted).

In a liquid oil the triglyceride molecules are orientated randomly to one another and constantly in motion. In a crystalline fat they are tightly packed in a regular repeating pattern. To undergo a liquid to solid transition the oil must first be cooled to reduce the average thermal motion of each molecule enough that they will start to interact with each other. Once the thermal energy is low enough a solid can form. The interactions between oil molecules in a crystal are largely Van der Waals.



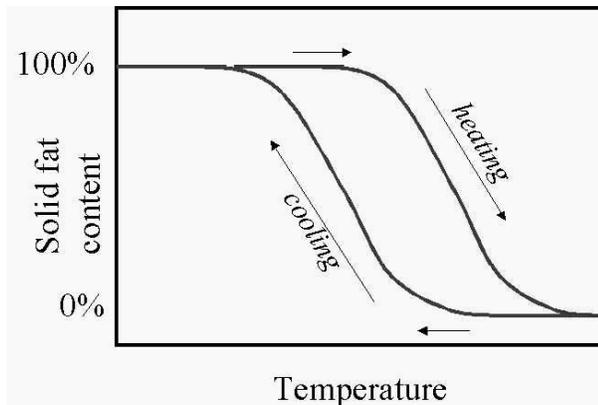
Crystallization is a liquid to solid phase transition divided into two distinct processes: nucleation and growth.

Nucleation. Nucleation is the formation of a solid in a liquid matrix. The very smallest crystals have a very small radius of curvature and so the lipid molecules are more soluble. The consequence is the smallest crystal embryos tend to redissolve before they grow - unless the temperature is considerably below the melting point of the fat when the thermodynamic pressure to crystallize becomes too great and the nuclei begin to grow. Therefore a liquid oil may remain liquid below its melting point for a considerable time - supercooling. Crystals melt at their thermodynamic melting point - there is no superheating so there is often *hysteresis* between solids content on cooling and heating.



Growth. The second phase of crystallization is growth. During the growth phase liquid triglycerides align themselves at the surface of the crystal and are incorporated. The nuclei will grow until they overlap and fill the container with solid fat.

Oils contain a mixture of fatty acids with very many different triglyceride molecules present. Any pure compound has a characteristic melting point so each triglyceride present will crystallize at a unique temperature slightly different from each different triglyceride present. As a consequence real food oils have a *melting range* rather than a melting point. The solid fat content of a food can be measured as a function of temperature by nuclear magnetic resonance (NMR) or differential scanning calorimetry (DSC) and will reduce from 100% to 0% liquid oil over a range of as few as 2 or 3 and as many as 20 or more degrees Kelvin.



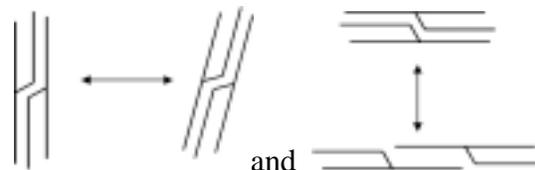
The solids content of common food fats changes from 0-100% over the range of temperatures found in the manufacture, storage, and use of foods. The solids content is an important determinant of food texture - particular in high fat foods. Liquid cooking oil is completely liquid. Butter is soft at room temperature but will harden in the refrigerator. Chocolate is hard at room temperature but will melt in the mouth. Partly crystalline fats can be seen as a dispersed system with solid crystals in a liquid oil continuous phase. The individual crystals are 2-3 μm long and needle-shaped but they can aggregate to form a viscoelastic network (e.g., margarine). Texture changes caused by temperature are because more fat crystallizes and reinforces the network (causing a shift from liquid-like to solid-like behavior).

Polymorphism

"Polymorphic forms are crystalline phases of the same chemical composition that differ amongst themselves in structure but yield the same liquid phase on melting."

Triglyceride molecules are capable of packing into a number of distinct crystal lattice forms known as polymorphic forms (polymorphs). Their crystal packing can vary in angle and/or spacing of the tuning forks.

Note in the right hand figure the structure at the top is double chain packing and at the bottom triple chain packing.



In many fats there is only one reasonable stable polymorph so there is no need to distinguish between solid forms (you practically only ever see one) but in cocoa butter (the basic fat in chocolate) there are several variably stable polymorphs and good quality chocolate requires finding the right one.

		Melting point	Chain Packing
I	β'_2	17	double
II	α	21	double
III	mixed	25.5	double
IV	β'_1	28	double
V	β_2	34.5	triple
VI	β_1	36	triple

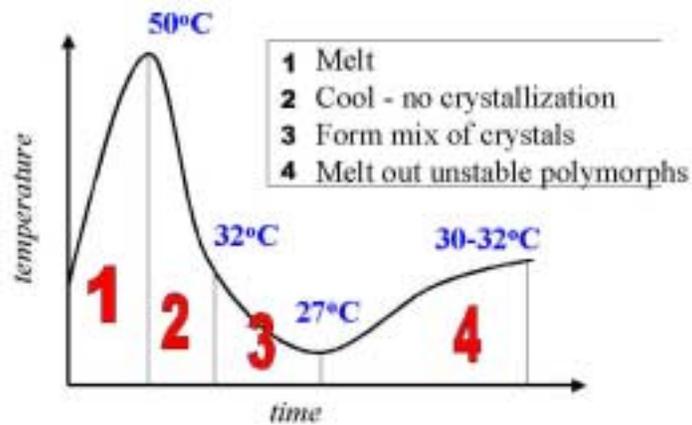
Selected properties of the different polymorphic forms of cocoa butter are listed in the table and can be summarized as follows:

- Desirable forms have a high melting point.
- The higher melting point, the more stable.
- More stable fats are denser.
- More stable forms are slow to form.
- Like crystals will grow from like.
- Cocoa butter can convert from a *less* to *more* stable form.

These points are exploited in the tempering process.

Tempering. The last step before liquid chocolate is poured into a mold to solidify into a bar is tempering. Tempering is required to ensure the fat crystallizes in the right polymorphic form. In tempering, liquid chocolate is poured into a gently mixed container and the temperature altered according to a fixed program:

1. The fat is heated to about 50°C. At this temperature all the solids melt so there are no residual nuclei
2. The fat is cooled, at about 32°C crystallization of the stable β' crystals will start
3. but cooling is continues to create a large mixed crop of different crystal polymorphs.
4. Re-heating the mixed crystals melts out the less stable polymorphs leaving only the stable ones.



When the fat is cooled again the stable crystals (~1-2% of the fat) will act as nuclei for the rest of the lipid and the whole bulk will crystallize into the right form.

After solidification, well tempered chocolate is hard - has good "snap", it has a glossy surface, and it is readily detached from molds. It is also resistant to bloom.

Bloom. Fat bloom is the formation of a white mould like surface coating on solid chocolate bars. Bloom can form slowly over hours, days or months of storage. Under the electron microscope, bloom looks like jagged, broken crystals sticking up from the surface. Bloom is not toxic, harmful (or mould) but it looks unpleasant and is an important reason for the rejection of chocolate.

Bloom is the formation of large (polymorph VI) fat crystals on the surface of the fat. It is caused by:

- Poorly tempered chocolate which can slowly stabilize itself into a more dense, stable polymorph.
- Well tempered chocolate that is melted then resets.
- Temperature abuse, particularly storing product hot or temperature cycling.

Heating melts some fat which can more readily diffuse through the solid fraction (to the surface) where cooling allows it to crystallize.

Very often the causes of bloom are beyond the manufacturer's control (often retail and consumer storage) but, as with all branded goods, the manufacturer will be held responsible. Aside from educating retailers it is possible to modify the lipid to retard bloom formation - often by adding butter fat or an emulsifier (e.g., sorbitan monostearate).

