6 Physical properties

6.1 Polymorphism, crystal structure and melting point

In the solid state, long-chain compounds frequently exist in more than one crystalline form and consequently have more than one melting point. This property of polymorphism is of both scientific and technical interest. Understanding this phenomenon is essential for the satisfactory blending and tempering of fat-containing materials such as cooking and confectionery fats, which must attain a certain physical appearance during preparation and maintain it during storage. Problems of graininess in margarine and bloom in chocolate, for example, are both related to polymorphic changes (sections 10.2 and 10.6). The experimental methods used most extensively to examine melting and crystallisation involve dilatometry, low-resolution pulsed ^{1}H NMR spectroscopy, differential scanning calorimetry, infrared spectroscopy and X-ray diffraction (Larsson, 1994).

X-ray investigations indicate that the unit cell for long-chain compounds is a prism with two short spacings and one long spacing, as indicated in Fig. 6.1. When the long spacing is less than the molecular dimension calculated from known bond lengths and bond angles, it is assumed that the molecule is tilted with respect to its end planes. Sometimes, however, the length is such as to indicate a dimeric or trimeric unit for the most stable form. The molecules assume the angle of tilt at which they are most closely packed. This will give the greatest stability and the highest melting point.

6.1.1 Alkanoic and alkenoic acids

The melting points of long-chain acids and their methyl esters are listed in Table 3.1. These values show alternation with increasing chain length, a phenomenon commonly displayed by the physical properties of long-chain compounds in the solid state and related to the arrangement of molecules in the crystals. The melting points of acids with an even number of carbon atoms in the molecule and their methyl esters plotted against chain-length fall on smooth curves lying above similar curves for the odd acids and their methyl esters. Odd acids melt lower than even acids with one less carbon atom. The two curves for saturated acids converge at 120–125ºC.

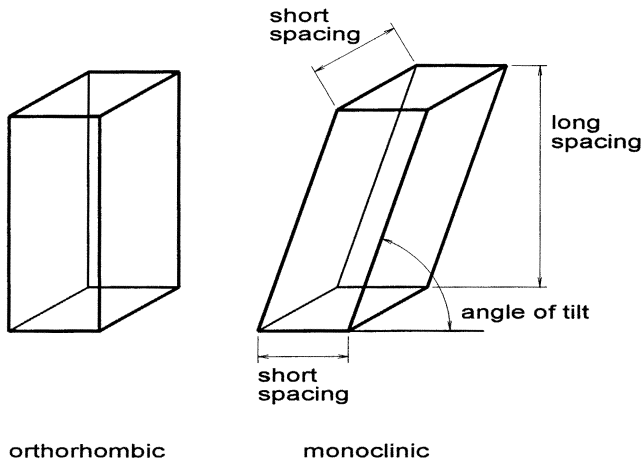

Figure 6.1 The unit cell of long-chain compounds (kindly supplied by my colleague Dr C.M. Scrimgeour).

The melting points of unsaturated acids depend on chain length and on the number, position, and configuration of the unsaturated centres. For example stearic (70ºC), oleic ($\Delta 9c$, 11ºC) elaidic ($\Delta 9t$, 45ºC) and stearolic acids ($\Delta 9a$, 46ºC) have the melting points shown. Among polyunsaturated acids those with conjugated unsaturation are higher melting than their methylene-interrupted isomers (Table 6.1).

Table 6.1 Melting points (ºC) of some mono- and poly-unsaturated acids

Monoenes	
16:1 (9*c*)	0.5
18:1 (9*c*)	16.3
20:1 (9*c*)	25
22:1 (13*c*)	33.4
Polyenes with methylene-interrupted unsaturation	
18:2 (9*c*12*c*)	−5
18:2 (9*c*12*t*)	−3
18:2 (9*t*12*t*)	29
18:3 (9*c*12*c*15*c*)	−11
18:3 (9*t*12*t*15*t*)	30
Polyenes with conjugated unsaturation	
18:2 (9*c*11*t*)	22
18:2 (9*t*11*t*)	54
18:3 (9*c*11*t*13*c*)	44
18:3 (9*c*11*t*13*t*)	49
18:3 (9*t*11*t*13*c*)	32
18:3 (9*t*11*t*13*t*)	71

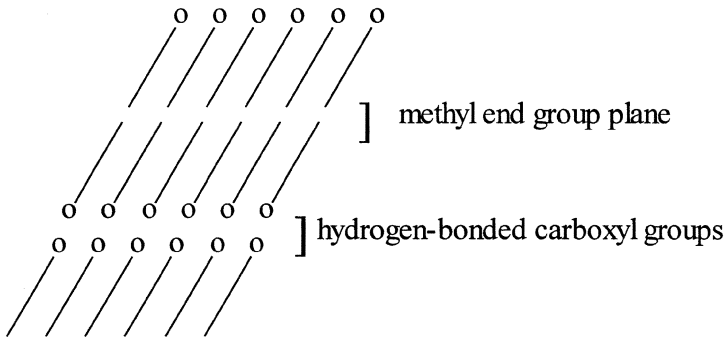

Figure 6.2 Schematic arrangement of alkanoic acid molecules in the crystalline form. o represents the polar head group (COOH) and the line represents the alkyl chain which will assume a zig-zag arrangement of successive carbon atoms.

Alkanoic acids exist in three polymorphic forms designated A, B and C for acids with an even number of carbon atoms. Form C has the highest melting point and is the most stable (physically). It is obtained by crystallisation from the melt or from polar solvents. Crystallisation from nonpolar solvents gives form A or forms B and C. The molecules crystallise in dimeric layers. Alternation of melting point for odd and even chain-length compounds results from the fact that the methyl groups in the end group plane interact differently in the odd and even series.

6.1.2 Glycerol esters

For most technical purposes the melting behaviour of triacylglycerols is more important than that of fatty acids. It has long been known that fats show multiple melting points, and as far back as 1853 glycerol tristearate was reported to have three melting points at 52, 64 and 70ºC. When the melt of a simple triacylglycerol is cooled quickly it solidifies in its lowest melting form (α) with perpendicular alkyl chains in its unit cell (the angle of tilt is 90º). When heated slowly this melts but, held just above this melting point, it will re-solidify in the β' crystalline form. In the same way a more stable β form can be obtained from the β' form. The β form has the highest melting point and is obtained directly by crystallisation from solvent. The β' and β forms have tilted alkyl chains which permit more efficient packing of the triacylglycerols in the crystal lattice. Glycerol esters with only one type of acyl chain are easy to make and have been thoroughly studied. The results have provided useful guidance but such molecules are not generally significant components of natural fats (except perhaps after complete hydrogenation). With mixed saturated triacylglycerols such as PStP (P = palmitic, St = stearic) the β form is only obtained with difficulty and such compounds usually exist in their β'

Table 6.2 Characteristics of α, β', and β forms of crystalline triacylglycerols

Form	MP	Short spacings (nm)	Infrared absorption (cm^{-1})	Hydrocarbon chain	Subcell
α	lowest	0.4	720	perpendicular	orthorhombic
β'	intermediate	0.42–0.43 and 0.37–0.40	726 719	tilted	orthorhombic
β	highest	0.46 and 0.36–0.39	717	tilted	triclinic

form. Among triacylglycerols with saturated and unsaturated acyl chains, symmetrical compounds (SUS and USU) have higher melting (more stable) β forms but the unsymmetrical compounds (USS and UUS) have stable β' forms (S = saturated and U = unsaturated acyl chains) (Table 6.2).

The stable β form generally crystallises in a double chain-length arrangement (DCL or β_2) but if one acyl group is very different from the others in either chain length or in degree of unsaturation, the crystals assume a triple chain-length arrangement (TCL or β_3) since this allows more efficient packing of alkyl chains and head groups. These crystals have the short spacing expected of a β crystalline form but the long spacing is about 50 per cent longer than usual (Fig. 6.3).

In the DCL arrangement, the molecules align themselves (like tuning forks) with two chains in extended line (to give the double-chain length) and a third parallel to these (Fig. 6.3). Some mixed glycerol esters which have a TCL form when crystallised on their own, give high-melting (well-packed) mixed crystals with a second appropriate glycerol ester. For example CPC and PCP

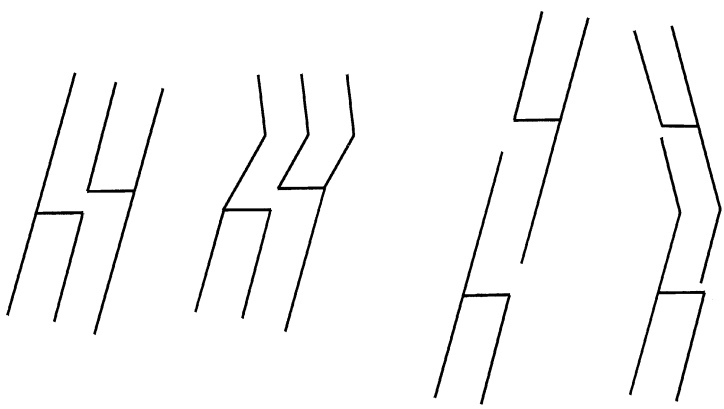

Figure 6.3 DCL and TCL structures (kindly supplied by my colleague Dr C.M. Scrimgeour).

or OPO and POP (where C = capric, P = palmitic and O = oleic). This has been described as 'compound formation'.

The methyl groups at the top and bottom of each triacylglycerol layer do not usually lie on a straight line, but form a boundary with a structure depending on the lengths of the several acyl groups. This is called the 'methyl terrace'. The molecules tilt with respect to their methyl end-planes to give the best fit of the upper methyl terrace of one row of glycerol esters with the lower methyl terrace of the next row of esters. There may be several β_2 modifications differing in the slope of the methyl terrace and in the angle of tilt.

Crystallisation occurs in two stages: nucleation and growth. A crystal nucleus is the smallest crystal that can exist in a solution and is dependent on concentration and temperature. Spontaneous (homogeneous) nucleation rarely occurs in fats. Instead heterogeneous nucleation occurs on solid particles (dust, etc.) or on the walls of the container. Once crystals are formed, fragments may drop off and either re-dissolve or act as nuclei for further crystals. The latter is not desirable in fat crystallisation so agitation should be kept to the minimum required to facilitate heat transfer. Nucleation rates for the different polymorphs are in the order $\alpha > \beta' > \beta$ so that α and β' crystals are more readily formed in the first instance, even though the β polymorph is the most stable and is favoured thermodynamically. Crystal nuclei grow by incorporation of other molecules from the adjacent liquid layer at a rate depending on the amount of supercooling and the viscosity of the melt (Mori, 1988; Timms in Gunstone & Padley, 1997; Sato, 2001a,b; Lawler & Dimick, 2002).

In the production of margarines and shortenings the β' crystalline form is preferred to the β form. β' Crystals are relatively small and can incorporate a large amount of liquid. This gives the product a glossy surface and a smooth texture. β Crystals, on the other hand, though initially small, grow into needle-like agglomerates. These are less able to incorporate liquids and produce a grainy texture. Margarines and shortenings, made from rape/canola, sunflower, or soybean oil after partial hydrogenation, tend to develop β crystals. This can be inhibited or prevented by the incorporation of some hydrogenated palm oil or palm olein which stabilise the crystals in the β' form. These changes in crystallisation pattern are linked with the larger amount of palmitic acid in the palm products. Glycerol esters with C_{16} and C_{18} acyl chains are more likely to be stable in the β' form than glycerol esters with three C_{18} chains.

Because of the importance of its melting behaviour, the polymorphism displayed by cocoa butter has been thoroughly investigated. This material is particularly rich in three 2-oleo-1,3-disaturated glycerol esters namely POP, POSt and StOSt. The solid fat has been identified in six crystalline forms designated I-VI with the melting points and double/triple chain length nature indicated in Table 6.3. Of these, form V (β_2) is the one preferred for

Table 6.3 Polymorphism in cocoa butter

	I	II	III	IV	V	VI
MP (ºC)	17.3	23.3	25.5	27.3	33.8	36.3
Chain length	D	D	D	D	T	T

D = double chain length, T = triple chain length.

chocolate. This crystalline form gives good demoulding characteristics, has a stable gloss, and shows a favourable snap at room temperature. Two procedures have been employed to promote the formation of this particular crystalline form. The most extensively used is tempering, i.e. putting molten chocolate through a series of cooling and heating processes. This optimises the production of the appropriate polymorph. An alternative procedure requires seeding of the molten chocolate with cocoa butter already prepared in form V (β_2) or VI (β_1) but this method is restricted by the difficulty of obtaining adequate supplies of these crystalline forms.

The synthetic glycerol ester 2-oleo-1,3-dibehenin (BOB, O = 18:1, B = 22:0) may be added to cocoa butter to prevent bloom formation by keeping it in its form V at temperatures above 30ºC (section 10.6).

Oils rich in saturated acids contain high-melting triacylglycerols which may crystallise from the oil when stored. When this is considered to be undesirable, the oil is subjected to winterization. The oil is chilled gradually and kept at around 5ºC for several hours before being filtered. The liquid fraction should then remain clear at ambient temperature. This process is applied to cottonseed oil and to partially hydrogenated soybean oil.

6.2 Spectroscopic properties

6.2.1 Ultraviolet spectroscopy

The use of ultraviolet spectroscopy in the study of lipids is confined to systems containing or generating conjugated unsaturation. It is therefore of value in the study of natural acids with conjugated unsaturation such as the dienes, trienes, tetraenes and acetylenic compounds described in section 3.7. Conjugated dienes such as CLA have a UV maximum around 230–240 nm and trienes show triple peaks around 261, 271 and 281 nm. Methylene-interrupted polyenes do not show any interesting UV absorption until double bonds migrate to form conjugated systems. This happens during autoxidation (section 7.2.2), alkali isomerisation (section 7.8) and other reactions involving doubly allylic methylene groups. UV spectroscopy is also used in the study of carotenoids with extended conjugated systems (Young & Hamilton in Hamilton & Cast, 1999; Angioni *et al*. in Dobson, 2002).

6.2.2 *Infrared spectroscopy*

Infrared spectroscopy has been applied to solid lipids to provide information about polymorphism, crystal structure, conformation and chain-length (section 6.1.2) but the commonest use of traditional IR spectroscopy has been the recognition and quantitation of *trans* unsaturation in acids and esters where unsaturation is predominantly *cis,* using neat liquids or solutions. One *trans* double bond absorbs at 968 cm^{-1}. Additional *trans* centres increase the intensity but do not change the frequency unless they are conjugated when small changes are reported.

There is no similar diagnostic absorption for a *cis* olefin but Raman spectra show strong absorption bands at 1665 ± 1 cm^{-1} (*cis* olefin), 1670 ± 1 cm^{-1} (*trans* olefin), and 2230 ± 1 cm^{-1} and 2291 ± 2 cm^{-1} (acetylenes) for the type of unsaturation indicated.

Carbonyl compounds have a strong absorption band in the region 1650–1750 cm^{-1}. The wavelength varies slightly with the nature of the carbonyl compound as in the following saturated and $\alpha\beta$-unsaturated compounds: aldehydes (1740–1720 and 1705–1680 cm^{-1}), ketones (1725–1705 and 1685–1665 cm^{-1}), acids (1725–1700 and 1715–1690 cm^{-1}) and esters (1750–1730 and 1730–1715 cm^{-1}).

Most oils with the usual mixture of saturated and unsaturated acids have similar infrared spectra. Additional bands associated with less common functional groups include hydroxyl (3448 cm^{-1}), keto (1724 cm^{-1}), cyclopropene (1852 and 1010 cm^{-1}), epoxide (848 and 826 cm^{-1}), allene (2222 and 1961 cm^{-1}), vinyl (990 and 909 cm^{-1}) and conjugated enyne systems (952 cm^{-1}).

The analytical uses of FTIR and NIR have been discussed in section 5.3.6. For more information see Chapman & Goni (1994), Ismail *et al*. in Hamilton & Cast (1999), van de Voort *et al*. in Dobson (2001) and Mossoba *et al*. in Dobson (2001).

6.2.3 *Electron spin resonance (ESR) spectroscopy*

ESR spectroscopy is used for the study of free radicals (odd electron species) but finds only limited use in lipid studies. It has been used in the study of autoxidation which occurs through a free radical mechanism (section 7.2.2) (Anderson & Skibsted in Dobson, 2002) and is also used in the study of membranes after incorporation of spin-labelled material such as 5-doxylstearic acid (shown below) (McPhail, 2003).

6.2.4 *^{1}H NMR spectroscopy*

^{1}H NMR spectroscopy is used in two ways in the study of lipids. With wide-line (low resolution) (pulsed) instruments, it is possible to determine the

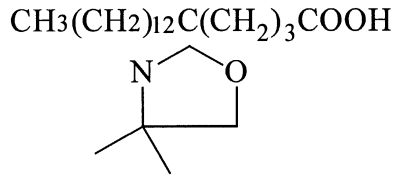

Figure 6.4 Structure of 5-doxylstearic acid.

proportion of solid and liquid in a fat and the content of oil in a seed. High-resolution spectrometers, on the other hand, are used to examine solutions and give information about the solute, which may be an individual compound or a mixture, such as a natural oil or fat.

6.2.4.1 Low resolution NMR spectroscopy

Low-resolution ^{1}H NMR or time-domain NMR is used extensively in quality control laboratories for the measurement of solid fat content, simultaneous determination of oil and moisture content, in the study of oil and water droplet size distribution and in measurements to be made through packaging. The technique has been reviewed by Meeussen in Hamilton & Cast (1999) and Todt *et al.* (2001). See also section 5.3.5.

6.2.4.2 High resolution NMR spectroscopy

A typical ^{1}H spectrum is shown in Figure 6.5. It contains signals that can be distinguished by chemical shift, coupling constant, splitting pattern and area. The last of these provides quantitative information that can be presented as mol%. The remaining parameters give structural information (Diehl, in Dobson, 2001; Knothe, 2003).

A saturated long-chain methyl ester has five signals with the following chemical shifts (ppm), number of hydrogen atoms, and splitting pattern:

• CH_3	0.90	3	triplet
• $(CH_2)_n$	1.31	2n	broad (many overlapping signals)
• -C*H*$_2$CH$_2$COOCH$_3$	1.58	2	quintet
• -CH$_2$C*H*$_2$COOCH$_3$	2.30	2	triplet
• -CH$_2$CH$_2$COOC*H*$_3$	3.65	3	singlet

Such a spectrum indicates the presence of a straight-chain saturated methyl ester but does not distinguish between homologues in a mixture. In olefinic esters there are additional signals corresponding to the:

- olefinic hydrogen atoms (-C*H*=C*H*- 5.35 ppm, 2H for oleate, 4H for linoleate, 6H for linolenate),
- allylic hydrogen atoms (C*H*$_2$CH=CHC*H*$_2$- 2.05 ppm, 4H),
- doubly allylic hydrogen atoms (=CHC*H*$_2$CH= 2.77 ppm, 2H for linoleate and 4H for linolenate).

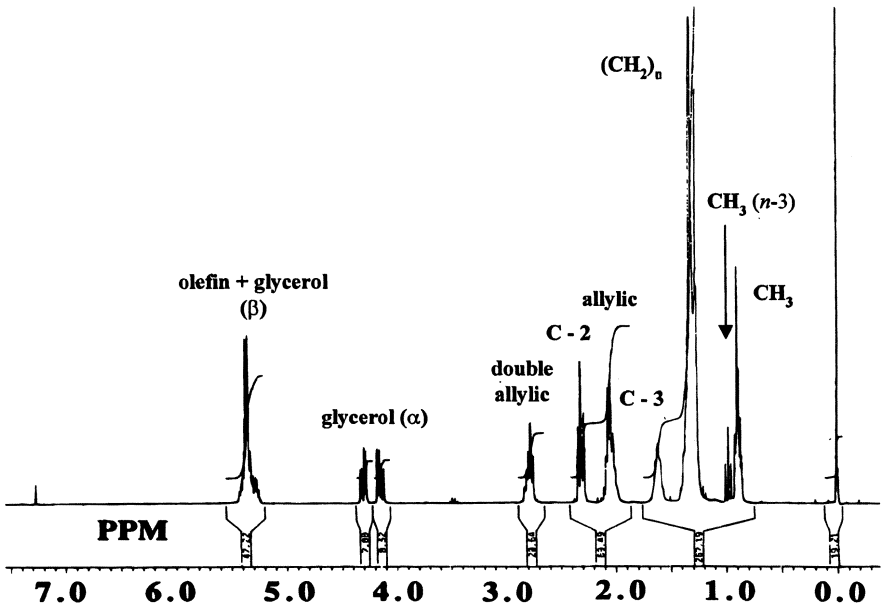

Figure 6.5 Typical ^{1}H NMR spectrum of a vegetable oil – see text for discussion (kindly supplied by my colleague Dr C.M. Scrimgeour).

For linolenate and other n-3 esters the proximity of a double bond affects the C*H*$_3$ signal which then appears at 0.98 ppm. This makes it possible to distinguish the n-3 esters from other common fatty esters with their C*H*$_3$ signal at 0.90 ppm. Glycerol esters have five hydrogen atoms associated with the glycerol unit. There is a one-proton signal at 5.25 ppm (C*H*OCOR) overlapping with olefinic signals and a four-proton signal split between 4.12 and 4.28 ppm (C*H*$_2$OCOR).

There is a growing interest in using ^{1}H NMR spectroscopy to obtain information about the composition of triacylglycerol mixtures occurring naturally. The recognition and measurement of total n-3 acids and of Δ-4 acids (DHA) in fish oils has been discussed in section 5.3.5.

For vegetable oils containing the usual mixture of saturated acids and C_{18} unsaturated acids useful information can be obtained by ^{1}H NMR procedures that are non-destructive and require no chemical reactions. The signal at 2.30 ppm (α-methylene function) provides a measure of all the acyl groups. The signals at 0.89 and 0.98 ppm distinguish linolenate (n-3) from all other esters. Signals at 2.77 ppm are a combined measure of triene (linolenate) and diene (linoleate) and those at 2.05 ppm relate to all of linolenate, linoleate and oleate. The intensity of these signals can be used to calculate the composition (%mol) in terms of oleic, linoleic, linolenic and total saturated acids.

6.2.5 *^{13}C NMR spectroscopy*

^{13}C NMR spectra are based on natural ^{13}C atoms present at a level of 1.1 per cent in organic compounds. The spectra provide two kinds of information: the chemical shift of each signal (usually around 50 signals in a natural mixture) and their relative intensities. The former is of qualitative value and permits identification of important structural features. The latter, with appropriate safeguards, provides quantitative information of analytical value. Chemical shifts may vary slightly with concentration of the solution under study and (rather more) with the solvent employed. Most measurements are made with solutions of about 1M concentration and $CDCl_3$ is the solvent most commonly used. Other solvents include mixtures of CD_3OD and $CDCl_3$, $(CD_3)_2SO$, D_2O and C_6D_6. Figure 6.6 shows a typical spectrum of vegetable oils.

The chemical shift of a carbon atom depends on its total environment to a distance of six or more atomic centres. For example, in glycerol trioleate, the signals for the olefinic carbon atoms (C_9 and C_{10}) differ from one another and also to a small extent depending on whether the oleate is an α or β chain (attached to primary or secondary glycerol hydroxyl groups). The C-1 signal is also slightly different for saturated and $\Delta 9$ unsaturated chains. In these examples, the difference is produced by structural changes up to 11 atomic centres away. This makes the spectrum more complex, but also more informative when all the chemical shifts have been assigned.

In another example, the methyl groups at the end of the acyl chains in glycerol tripalmitate give one signal at around 14.1 ppm which is well separated from other signals and hence easily recognised. The difference

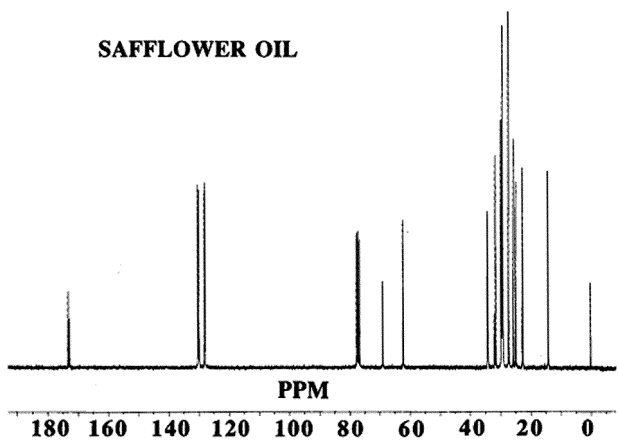

Figure 6.6 ^{13}C NMR spectrum of safflower oil – for assignment of signals see text. The signals around 80 ppm come from the solvent. (Taken with permission from <www.lipid.co.uk>.)

between α and β chains for this signal in this molecule is too small to be observed but in a vegetable oil containing saturated and unsaturated chains, the resonance at 14.1 ppm appears as a cluster of two or more signals. Each is indicative of a different environment for the methyl group and may result from *n*-3, *n*-6 or n-9 acids where the closest double bond affects the chemical shift of the methyl signal.

To obtain quantitative data attention has to be given to the protocol for obtaining the spectrum. In particular, the problem of relaxation has to be overcome either by adding a relaxation agent such as $Cr(acac)_3$ (chromium acetonylacetonoate) and/or by including a delay time between successive scans of the spectrum. This will add to the time required to collect the spectrum. Spectrometers now available operate at a frequency for ^{13}C of 68MHz or more and spectra are generally obtained using an NOE (nuclear Overhauser effect) suppressed, inverse-gated, proton-decoupled technique.

Exciting pulses have a 45–90º pulse angle and acquisition times (including delay times) are 1–20 sec per scan. The number of scans is usually 1000 or more. The sample size for a routine ^{13}C NMR spectrum is normally 50–100 mg and the spectrum is obtained in 20–30 minutes. With smaller samples, high quality spectra can be obtained with as little as 1 mg but with a correspondingly longer acquisition time.

In using ^{13}C NMR data (chemical shifts and intensities), the first step is to assign as many of the chemical shifts as possible. If the substance under study is a mixture, many individual signals will appear as clusters. This makes interpretation more difficult, but eventually provides additional information. It is wise to ignore signals in the methylene envelope (29.4–29.9 ppm) resulting from mid-chain carbon atoms that are not greatly influenced by nearby functional groups. Instead, examine the easily-recognised shifts associated with the following carbon atoms: ω1 (around 14.1 ppm), ω2 (22.8), ω3 (32.1), C-1 (174.1), C-2 (34.2), C-3 (25.1), glycerol (68.9 and 62.1), olefinic (127–132) and allylic (27.3 and 25.6).

Assignments of chemical shift are often made on the basis of available knowledge. Existing information has been built up over the past 30 years assisted by the study of ^{2}H-containing compounds and the use of chemical shift reagents (Gunstone, website). Where the necessary information is not available more advanced spectroscopic procedures will assist assignment. These can also be made on the basis of line-width and relaxation measurements. Easily recognised carbon atoms present in most triacylglycerols have been cited above. This provides enough information to make a preliminary assignment to the signals in a spectrum such as that of sunflower seed oil (Fig. 6.6).

From the peak areas of appropriate signals, the average number of double bonds per triacylglycerol molecule and the average molecular weight can be calculated and hence the iodine value (excluding unsaponifiable material). These are based on signals at 24.85 (C-3, a measure of total acyl chains),

25.62 (L11, a measure of linoleic acid), 27.15 (O8, O11, L8, L14, monoenes and dienes) and the multiplet at 29.45 ppm (mid-chain methylene) (Ng & Gee, 2001).

In magic-angle spinning mode it is possible to examine solid samples and to determine the fatty-acid composition of seeds individually or in small batches. For example, the composition of a transformed canola seed was calculated from the five signals at 21.1 (Ln 17), 23.3 (C-17 for O, L, and sat), 25.6 (C-3 all acyl groups), 26.2 and 26.05 (L11, Ln11 and 14), 28.0 ppm (O8 and 11, L8 and 14, Ln8) (Hutton *et al*., 1999). ^{31}P NMR spectroscopy is discussed in section 5.4.3.

6.2.6 Mass spectrometry

Mass spectrometry is a procedure used to determine the structure of individual molecules. Originally, these had to be isolated by standard procedures, but it is now more usual to combine the mass spectrometer with GC or HPLC so that individual components of a mixture can be separated by chromatography and identified by mass spectrometry. If the compound is already known, then its mass spectrum can be compared with that already reported and contained in a data bank (Christie, website). If the compound is novel it may be possible to identify it by application of the basic principles of mass spectrometry. When a chromatographic separation precedes mass spectrometry then it is also desirable to quantify the data so that the proportion of each molecular species is also known. This is usually achieved by measurement of the total ion current, but accurate quantification requires calibration with standards or the use of isotopic internal standards. In the combined GC-MS procedures, it is also necessary to select derivatives that combine good chromatographic properties (satisfactory separation under simple GC or HPLC conditions) with good spectroscopic properties (molecular and/or fragment ions that lead to easy recognition of the molecule). This last may require a selection of the appropriate spectroscopic procedure.

When a molecule is ionised (electronically or chemically) it forms a molecular ion (M^+). This may fragment to give one charged (A^+) and one uncharged (B) particle and a mass spectrometer is a device for producing and examining the charged particles. These are separated according to their mass to charge ratio (*m/z,* where *z* is usually 1). With high-resolution instruments, this value can be measured with such accuracy as to indicate the molecular formula of each ion. The intensity of each peak is related to that of the base peak (largest) which is given a value of 100.

$$M \rightarrow M^+ \rightarrow A^+ + B$$

Electron ionisation (EI) is the most widely used ionisation technique. This occurs through an exchange of energy between electrons emitted by a glowing

filament (usually at 70 eV) and vaporised sample molecules. Under these conditions the molecular ion usually fragments in one or more ways.

Chemical ionisation (CI) results from gas phase reactions between a small amount of sample and a large amount of reactant gas (such as methane, ammonia, or isobutene) itself ionised by EI-producing reactant gas ions (CH_5^+, NH_4^+, $C_4H_9^+$). CI is usually softer than EI, with the result that more of the molecular ion is available for detection and fragmentation is less extensive. This usually makes interpretation simpler. The following CI techniques are used by lipid analysts and have been discussed in section 5.3.7:

- atmospheric pressure chemical ionisation (APCI),
- fast atom bombardment (FAB),
- collision induced dissociation (tandem mass spectrometry, MS/MS).

For the structural identification of fatty acids, MS procedures linked to GC or HPLC have replaced the older classical methods. Mass spectrometry was first carried out on methyl esters but these are not very satisfactory because under EI the double bonds migrate along the chain and cannot be located unequivocally. Several methods of 'fixing' the double bond were devised but only one of these, applied mainly to monoene esters, remains in use. For both mono and polyunsaturated acids, the methyl esters have been replaced by other acid derivatives that give useful structural information. Appropriate fatty-acid derivatives are generally examined by EI and triacylglycerols by one of the CI methods.

Olefinic esters react with dimethyldisulfide (MeSSMe) and iodine to give a bis(methylthio) derivative the mass spectrum of which shows a molecular ion and two or three large fragment ions that together clearly indicate the position of the SMe groups and hence, the double bond.

$$-CH{=}CH- \rightarrow -CH(SMe)CH(SMe)-$$

For example, methyl oleate gives a molecular ion at 390 ($C_{21}H_{42}O_2S_2$) and two large fragment ions at 173 ($C_9H_{18}SMe$) and 217 ($C_{10}H_{18}O_2SMe$). A third fragment ion at 185 corresponds to the loss of methanol (32 mass units) from the peak at 217. These clearly show that methyl oleate is Δ9-18:1 but do not indicate the configuration of the double bond. However *cis* and *trans* monoenes form *threo* and *erythro* adducts respectively and although these have similar mass spectra they are separated by gas chromatography. The procedure is less satisfactory with polyunsaturated acids but there are other ways of examining these. Nevertheless, the method was used to identify a 21:5 acid as the Δ6,9,12,15,18 isomer. The acid was subjected to partial reduction with hydrazine and hydrogen peroxide (section 7.1.2) and the monoene fraction was isolated by silver ion chromatography. Mass spectrometric examination of the bis(methylthio) adducts then showed key fragments at *m/z*

257 and 175 for the Δ6 acid, 215 and 217 (Δ9), 173 and 259 (Δ12), 131 and 301 (Δ15) and 89 and 343 (Δ18).

Polyunsaturated acids are better examined as picolinyl esters or as 2-alkyl-4,4-dimethyloxazoles (DMOX).When these molecules are ionised, the charge is carried on the nitrogen atom and double bond ionisation and isomerisation are minimised. Radical-induced cleavage occurs evenly along the chain and gives a series of relatively abundant ions of high mass resulting from the cleavage of each C-C bond. When a double bond or other functional group is reached then diagnostic ions appear (section 5.3.7).

The picolinyl esters are made from the free acids and picolinyl alcohol either via the acid chloride (reaction with oxalyl chloride) or through interaction with 1,1′-carbonyldiimidazole in dichloromethane in the presence of 4-pyrrolidinopyridine as catalyst. Another method involves interesterification of triacylglycerols or phospholipids with potassium *tert*-butoxide and 3-hydroxymethylpyridine for two minutes at room temperature.

The spectrum shows some fragments of low mass characteristic of picolinates resulting from $ArCH_2^+$ (93), $ArCH_2O^+$ (108), $ArCH_2OC(OH)=CH_2^+$ (151) and $ArCH_2OC(O)=CH_2^+$ (164) where Ar is C_5H_4N or C_5H_5N. In addition there is a molecular ion peak and a series of other high mass fragments which, correctly interpreted, will indicate a structure for the picolinate. For example, the ester from α-linolenic acid has peaks at 369 (M+, the 18:3 picolinate which is a C_{24} compound), 354 (M^+-15), 340, 326, 312, 298, 272, 258, 232, etc. Most of these ions differ by 14 mass units from their neighbour, representing loss of CH_2, but something different happens between 298 and 272 and between 258 and 232 where there is a loss of 26 mass units (C_2H_2 representing a –CH=CH– unit). These fragments indicate the presence of double bonds at Δ9 and Δ12. The double bond nearest to the carboxyl group is not always easily spotted, but with experience it is not too difficult to define (Fig. 6.7).

Comparison of the fragment ions for stearic and oleic picolinates (Table 6.4) show how it is possible to determine the double-bond position. The features of note are: the C_{10} to C_{17} fragments are two mass units lower for oleate than for stearate while those up to C_8 have the same mass; there are enhanced signals for the C_{11} and C_{12} fragment ions related to allylic groups at C_8 and C_{11}; and, most significantly, the C_9 fragment is missing and the C_8 and C_{10} fragments differ by 26 mass units. These concepts can be extended to linoleate and linolenate. With yet more double bonds, the interpretation becomes more complex, but it is usually possible to recognise the unsaturated centre closest to the methyl group. It may then be assumed that the remaining unsaturated centres have the usual methylene-interrupted pattern.

DMOX derivatives are made by heating the lipid with 2-amino-2-methyl-1-propanol in a nitrogen atmosphere at 180ºC (2 hours for free acids, 18 hours for methyl or glycerol esters). Their spectra show peaks at 113 and 126

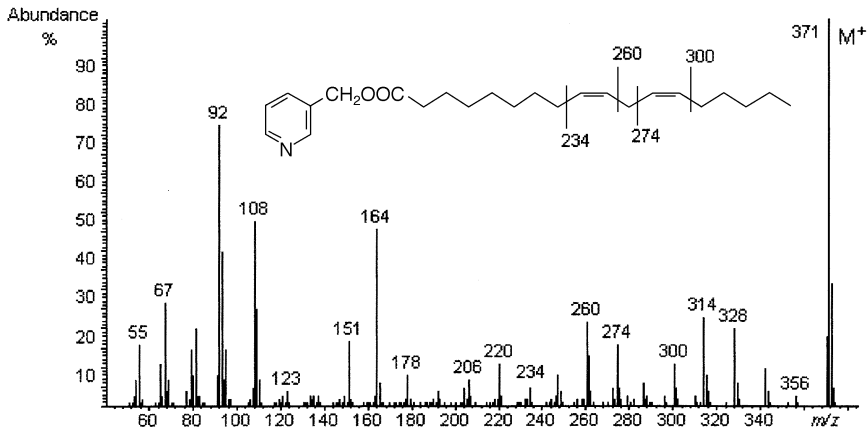

Figure 6.7 Mass spectrum of linoleic acid as the picolinate. (Taken with permission from <www.lipid.co.uk>.)

common to all DMOX derivatives, along with a molecular ion and a series of fragments differing by 14 mass units except that some pairs differ by only 12 mass units. These are indicative of olefinic centres and are interpreted according to Christie (2003, p. 289): 'if there is an interval of 12 mass units between the most intense peaks of clusters of ions containing n and n-1 carbon atoms then there is a double bond between carbon n+1 and n in the acyl chain'. Spectra of DMOX derivatives of many acids are available on Christie's website.

MS procedures, combined with a chromatographic separation system, also give valuable insight into the structure and composition of triacylglycerol mixtures such as milk fats (Currie & Kallio, 1993), vegetable oils (Byrdwell, 1998, 2001; Neff *et al*., 2001) and fish oils. In general, identification depends on molecular ions that define the number of both carbon atoms and double

Table 6.4 Significant fragment ions in the mass spectra of stearic, oleic, linoleic and linolenic picolinates

		Number of carbon atoms in the acyl chain of the fragment ions													
Ester	[M]+	17	16	15	14	13	12	11	10	9	8	7	6	5	4
18:0	375	360	346	332	318	304	290	276	262	248	234	220	206	192	178
18:1	373[a]	358	344	330	316	302	288[a]	274[a]	260	*	234	220	206	192	178
18:2	371[a]	356	342	328	314	300	*	274	260	*	234	220	206	192	178
18:3	369[a]	354	340	*	314	300	*	274	260	*	234	220	206	192	178

[a] Enhanced signal.
* Missing fragment, gap of 26 mass units between adjacent signals, gap of 40 mass units between somewhat enhanced signals.

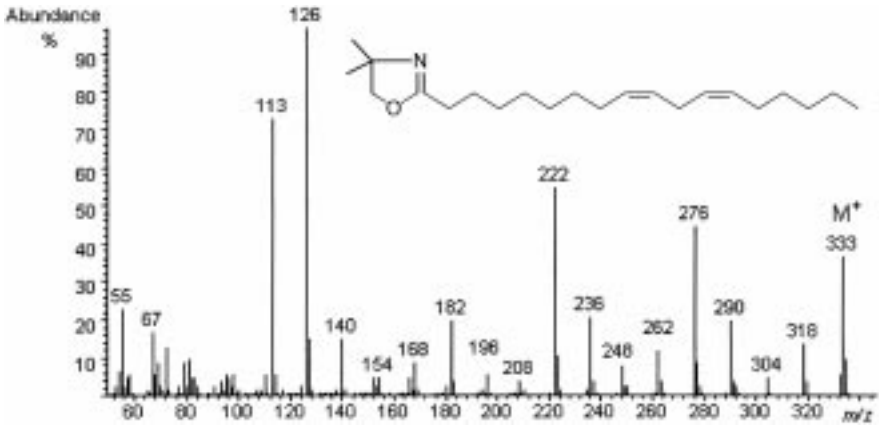

Figure 6.8 Mass spectrum of linoleic acid as the DMOX derivative. (Taken with permission from <www.lipid.co.uk>.)

bonds in each triacylglycerol molecule. In addition fragment ions indicate the nature of each acyl group in terms of its number of carbon atoms and unsaturated centres and in some cases will define the distribution of fatty-acyl residues between the primary (*sn*1/3) and secondary (*sn*-2) glycerol positions. Quantitative determination of mixtures is still a problem because the MS responses of triacylglycerols vary with the molecular structure. This topic is intensively reviewed by Laakso & Manninen in Hamilton & Cast (1999) and Laakso in Dobson (2002).

In measuring the molecular distribution of triacylglycerols using MALDI-TOF, a mixture of analyte and matrix such as α-cyano-4-hydroxycinnamic acid [4-$HOC_6H_4CH{=}C(CN)COOH$] is deposited on a stainless steel plate and placed in the ion source of the MS. Sodium ions are often present in the matrix or are added to the sample as sodium acetate. When a laser beam is directed at the sample, ions are formed by cationisation of triacylglycerol molecules $(M+Na)^+$. With soybean oil, for example, a series of peaks corresponding to 54:n (n = 2–9) and 52:n (n = 2–5) are apparent in the spectrum. Most of these can be identified on the basis of the known fatty-acid composition (Ayorinde, 2000).

Reverse phase HPLC followed by APCI MS gives a molecular ion $(M+H)^+$ and fragment ions corresponding to M-RCOOH. For example, the StLO fraction gives peaks at 885.6 $(M+H)^+$, 605.4 $(StO)^+$, 603.5 $(StL)^+$ and 601.4 $(OL)^+$. Among the DAG the 1,3 isomer is less intense than the 1,2 and 2,3 isomers and this makes it possible to identify the fatty acid at the 2 position (Byrdwell 1998, 2001; Neff *et al*., 2001).

Mass spectrometry of lipids has also been reviewed by Christie (1998), Laakso & Manninen (1999); Roach *et al*. in Hamilton & Cast (1999); Dobson & Christie (2002); Laakso (2002) and Korachi *et al*. in Dobson (2002).

6.3 Other physical properties

6.3.1 Density

Density may not seem an exciting physical property to many technologists, but it is very important in the trading of oils since shipments are sold on a weight basis but measured on a volume basis. These two values are related by density, so it is important to have correct and agreed values for this unit. This is not the same for all oils. It depends on fatty-acid composition and minor components as well as on the temperature. An equation taking these variables into account is based on iodine value, saponification value and temperature (Pantzaris, 1985).

$$d = 0.8543 + 0.000308(SV) + 0.000157(IV) - 0.00068t$$

where d = apparent density (g/ml or kg/L), SV = saponification value, IV = iodine value, and t = temperature (ºC).

Density can be defined in various ways and the correct form must be used when relating volume to weight.

- Density (absolute density or density in vacuum) is: Mass in vacuum of a volume of oil at t°*C* ÷ volume of the oil at the same temperature expressed in g/mL or kg/L.
- Apparent density (density in air, weight-by-volume, or litre-mass) is: Mass in air of a volume of oil at t°*C* ÷ volume of the oil at the same temperature expressed in g/mL or kg/L.
- Relative density (specific gravity, density in relation to water) is: Mass in air of a given volume of oil at t_1ºC ÷ mass in air of same volume of water at t_2ºC. This is a ratio without units. It is important to note that two temperatures are involved and the value is meaningless unless both figures are cited. This is the value most usually employed and equations exist to connect these three expressions.

Further information is given by Gunstone (2000).

6.3.2 Viscosity

Viscosity can be reported as kinematic viscosity or dynamic viscosity with the two values being related through density. The viscosity of a vegetable oil depends on its chemical composition (summarised in the iodine value and saponification value) and the temperature of measurement. Equations have been derived which permit calculation of viscosity from knowledge of the other three parameters. These have been developed empirically from observation with a range of oils at different temperatures (Duff & Prasad, 1989; Toro-Vazquez & Infante-Guerro, 1993). Coupland & McClements (1997) and

Fisher (1998) have related viscosity with density, refraction, surface tension and other physical properties.

The relation between temperature and viscosity for selected oils has been described by several authors (Timms, 1985; Ibemesi & Igwe, 1991; Lang *et al*., 1992; Noureddini *et al*., 1992; Tasioula-Margari & Demetropoulos, 1992).

6.3.3 Refractive index

The refractive index is easily measured on small amounts of material. Refractive index increases with chain length (though not in a linear fashion) and with increasing unsaturation. Geometric isomers differ from one another and methylene-interrupted polyenes differ from those with conjugated unsaturation. Triacylglycerols have higher values than free acids. Values for commercial oils are cited in Table 6.5.

6.3.4 Solubility of gases in oils

A recent discussion (Hilder in Gunstone & Padley, 1997) on the solubility of gases in oils includes the data presented in Tables 6.6 and 6.7 for oxygen, nitrogen and air. When an oil is in contact with air, the dissolved gases will depend on their individual solubility as well as their concentration in air. The high solubility of the monatomic argon enhances its concentration so that one per cent in air becomes three per cent of the gases in the oil.

Koetsier in Gunstone & Padley (1997) has summarised data on the solubility of hydrogen in vegetable oil. This information is obviously important for hydrogenation. He cites solubility values (maximum concentration in oil at a given temperature and pressure) from two sources at 1 bar and 100–200ºC of 2.60–3.36 mol/m^3 and 2.76–3.40 mol/m^3. The concentration of hydrogen is thus much lower than the concentration of unsaturated centres and for a fish oil of iodine value hydrogenated at 5 bar and 180º Koetsier gives concentrations of around 7000 mol/m^3 and 16 mol/m^3 respectively for the olefinic groups and the hydrogen.

6.3.5 Other physical properties

Gross heats of combustion (HG) for saturated and unsaturated triacylglycerols can be related to the number of valence electrons (EN). The following equations have been derived.

$$HG = -109.20 + 26.39\ EN \qquad \text{saturated triacylglycerols}$$
$$HG = 115.87 + 25.88\ EN \qquad \text{unsaturated triacylglycerols}$$
$$HG = 1\ 896\ 000/SN - 0.6\ IV - 1600$$

Coupland & McClements (1997) reported several physical properties

Table 6.5 Physical and chemical properties of selected commodity oils and fats

	Specific gravity (temperature ºC)	Ref index (40ºC)	Ref index (25ºC)	Iodine value	Saponi–fication value	Titre (ºC)	Unsaponifiable (%)	Mp (ºC)
Cocoa butter	0.973–0.980 (25/25)	1.456–1.458	–	32–40	192–200	45–50	0.2–1.0	31–35
Coconut	0.908–0.921 (40/20)	1.448–1.450	–	6–11	248–265	–	<1.5	23–26
Corn	0.917–0.925 (20/20)	1.465–1.468	1.470–1.473	107–128	187–195	–	1–3	–
Cottonseed	0.918–0.926 (20/20)	1.458–1.466		100–115	189–198	–	<2	–
Linseed	0.930–0.936 (15.5/15.5)[d]	1.472.1.475	1.477–1.482	170–203	188–196	19–21	0.1–2.0	–
Olive	0.910–0.916 (20/20)	–	1.468–1.471	75–94	184–196	–	1.5	–3–0
Palmkernel	0.899–0.914 (40/20)	1.452–1.488	–	14–21	230–254	–	<1.1	24–26
Palm	0.891–0.899 (50/20)	1.449–1.455[e]	–	50–55	190–209	–	<1.4	33–40
Palm olein	0.899–0.920 (40/20)	1.459–1.459	–	>55	194–202	–	<1.4	–
Palm stearin	0.881–0.891 (60/20)	1.447–1.451	–	<49	193–205	–	<1.0	–
Peanut	0.914–.0.917 (20/20)	1.460–1.465	–	86–107	187–196	–	<1.1	–
Rape[a]	0.910–0.920 (20/20)	1.465–1.469	–	94–120	168–181	–	<0.21[f]	–
Rape[b]	0.914–0.920 (20/20)	1.465–1.467	–	110–126	182–193	–	<0.21[f]	–
Sesame	0.915–0.923 (20/20)	1.465–1.469	–	104–120	187–195	–	<2.1	–
Soybean	0.919–0.925 (20/20)	1.466–1.470	–	124–139	189–195	–	<1.6	–
Sunflower	0.918–0.923 (20/20)	1.467–1.469	1.472–1.476	118–145	188–194	–	<1.6 (max 2.0)	–
Sunflower[c]	0.915–0.920 (20/20)	–	1.467–1.469	75–90			0.8–1.0 (max 2.0)	–

[a] High-erucic rape seed oil.
[b] Low-erucic rape seed oil.
[c] High-oleic sunflower seed oil.
[d] Also 0.924–0.930 (25/25).
[e] 50ºC.
[f] These values are correctly copied from the source but they are in error. Better values are 0.5–1.2 per cent.
Source: AOCS (1997).

Table 6.6 Solubility of oxygen and nitrogen in oils

Temp (ºC)	Oxygen (ppm, 1 bar)	Nitrogen (ppm, 1 bar)
0	170	80
25	180	85
50	185	90
75	190	95
100	200	105
125	*	110
150	*	115

Source: Hilder in Gunstone & Padley (1997).
* Oxygen solubilities at higher temperatures are not reliable because oxidation occurs.

Table 6.7 Gas content of oil saturated with air

	Solubility (ppm)	Air dissolved in oil (ppm)
Oxygen	180	38
Nitrogen	85	66
Argon	270	3

Source: Hilder in Gunstone & Padley (1997).

(density, viscosity, adiabatic expansion coefficient, thermal conductivity, specific heat, ultrasonic velocity and ultrasonic attenuation coefficient) for a number of liquid oils. Timms (1978) reviewed and significantly extended information on the heats of fusion of glycerides. He derived an equation for the heat of fusion of mono acid glycerides in the β polymorph form and showed how this could be adapted to calculate the heat of fusion of most glycerides of commercial interest. Chumpitaz *et al*. (1999) recently reported the surface tension of lauric, myristic, palmitic and oleic acids and of tricaprylin and tripalmitin, over a range of temperatures. These data are important for processes involving gas-liquid contact such as distillation and stripping columns, deodorisers, reactors and equipment for physical refining.

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