Chemical Properties

This chapter covers the chemical reactions that are significant in the food industry and include hydrogenation, oxidation, thermal changes in double bond systems, and some reactions of the acid/ester function.

6.1 Hydrogenation

As generally practised hydrogenation involves reaction between the unsaturated centres in an oil or fat in the presence of a metallic catalyst. This is a heterogeneous reaction, involving solid, liquid, and gas, taking place on the solid catalyst surface at an appropriate temperature and pressure. After hydrogenation, the product has changed physical, chemical, and nutritional properties. Sometimes compromises have to be made between these.

Each year millions of tonnes of soybean and other unsaturated vegetable oils containing oleic, linoleic, and linolenic acids as well as diminishing levels of fish oils with more complex patterns of unsaturation are subject to hydrogenation. This reaction is practised in three major ways:

(1) Brush hydrogenation is a short reaction, applied particularly to soybean and rapeseed/canola oils, designed to reduce the level of linolenic esters (18:3) to around 4%, thereby increasing shelf life. This is not a very extensive hydrogenation process. Apart from the lowering of the level of linolenate the fatty acids are little changed and production of *trans* isomers will be minimal. The partially reduced linolenate will be converted not to linoleate but to a mixture of 18:2 isomers and

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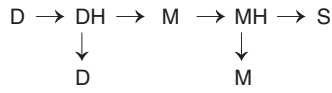
perhaps some 18:1 (not oleate). Table 2.5 contains data on the composition of various products when soybean oil of iodine value (IV) 132 is hydrogenated progressively to oil of IV 110 (brush hydrogenation), 97, 81, and 65.

(2) Partial hydrogenation is an important way of processing oils and fats to extend their range of use. The technique, first applied to such materials by the German chemist Normann, has been in operation for over 100 years and has been subject to continuous improvement during that time. The main objective is to convert a liquid oil (vegetable or fish) into a semi-solid fat that can be used as a component of a spread. Compared to brush hydrogenation this is a more extensive reaction whereby the content of polyunsaturated fatty acid is much reduced and a considerable proportion of *trans* 18:1 is formed. This results in a rise in melting point (because *trans* esters are higher melting than their *cis* isomers) that affects spreadability, oral response, and baking performance. Two other changes have also to be considered. There is an increase in oxidative stability through the complete or partial removal of the polyunsaturated fatty acids that are so easily oxidised and there is a decrease in nutritional value through the destruction of essential fatty acids and the formation of saturated acids and of unsaturated acids with *trans* configuration. During hydrogenation linoleate is reduced first to a mixture of *cis* and *trans* 18:1 isomers referred to as 'oleate' and then to stearate. The level of stearic acid may also increase (Table 2.5) but it has been argued that this is not a serious cholesterol-raising fatty acid. Along with ruminant fats, partially hydrogenated vegetable oils are the most important source of *trans* acids and, following the concern over these acids, there is now a requirement in some countries to indicate their level on the product label. As a consequence other ways to achieve the desired physical properties have been explored. These include modifications of the hydrogenation process to give less *trans* compounds and using interesterification of suitable blends as an alternative approach. Concern over *trans* acids is based on the fact that they raise LDL (low-density lipoproteins) levels and lower HDL (high-density lipoproteins) levels. This has been reported to be mainly a US problem because 75% of the fats consumed in that country are derived from soybean oil with its high

levels of polyunsaturated fatty acid. According to US legislation samples containing less than 0.5 g of non-conjugated *trans* acids per 14 g serving may be claimed as zero *trans*.

(3) Complete hydrogenation is a still more extensive process in which virtually all unsaturated acids are converted to their saturated analogues. This produces 'hardstock' which can be blended with a liquid (unsaturated) oil and subsequently interesterified. Hardstock is rich in stearic acid formed from the unsaturated C_{18} acids originally present. It usually has an IV of around 2. It contains only low levels of unsaturated acid and therefore very little *trans* acids. However, it may be necessary for the word 'hydrogenated' to appear on the label.

At the molecular level one or more of the following changes may occur during this reaction: hydrogenation (saturation) of unsaturated centres, stereomutation of natural *cis* olefins to their higher-melting *trans* isomers, double bond migration, and conversion of polyunsaturated fatty acids to monounsaturated and saturated acids. These are the consequences of reaction between a liquid (fatty oil) and a gas (hydrogen) occurring at a solid surface (the catalyst). In the sequence below the horizontal line shows the conversion of diene to monoene and of monoene to saturated acid/ester via the half hydrogenated states represented as DH and MH. The steps shown vertically are the reverse processes whereby DH reverts to D or a diene isomer and MH reverts to M or a monoene isomer. It is during these reverse stages that *trans* and positional isomers are formed. There are six stages altogether and it is important to understand the relative rates of these. In the conversion of D to M the first step is rate-determining and the second step is fast. Levels of DH will therefore be low and the conversion of DH back to D is slow and only important in the unusual situation that hydrogen is present in very low concentration. In the conversion of M to S the final stage is slow and rate-determining. This makes it more likely that there will be considerable recycling of M and MH leading to formation of stereochemical and positional isomers.



The catalyst used on a commercial scale is nickel on an inert support at a 17–25% level, encased in hardened fat. This preserves

the activity of the nickel in a form that is easily and safely handled. Hydrogenation is generally conducted at 180–200°C and 3 bar pressure in vessels containing up to 30 tonnes of oil. To minimise the use of catalyst it is desirable to use refined oil and the highest quality of hydrogen. Through improvements in the quality of catalyst and in equipment the requirement for catalyst has been gradually reduced. In 1960, 0.2% nickel was required but by the end of the century this was reduced 4- to 8-fold to between 0.025% and 0.05%. Reaction may proceed in a batch-wise manner with up to 8–10 batches in a 24-hour day or in a semi-continuous fashion at rate of 25–100 tonnes per day. The reaction is exothermic and appropriate cooling is required as well as stirring to distribute the heat.

Several significant variables have to be considered:

- The nature of the oil being treated.
- The extent of hydrogenation which is desired.
- The selectivity to be achieved in terms of PUFA–MUFA-saturated ratios and the ratio of *cis* to *trans* isomers.
- The quality and quantity of catalyst in terms of pore length, pore diameter, activity, and amount used.
- The reaction conditions of temperature, pressure, and degree of agitation.

The competition between hydrogenation (a change incorporating hydrogen) and isomerisation (a change not involving additional hydrogen in the product) depends on the availability of hydrogen at the catalyst surface in relation to the demand. A plentiful supply of hydrogen will promote hydrogenation, and an inadequate supply of hydrogen will allow isomerisation to become more significant. The availability of hydrogen at the catalyst surface is enhanced by increased pressure and increased agitation. The demand for hydrogen is increased with higher temperatures, higher catalyst quantity, higher catalyst activity, and more highly unsaturated oils.

The progress of the reaction can be followed in a number of ways that vary in simplicity, in speed of completion, and in the information they provide. They include: the volume of hydrogen used which will measure saturation but not isomerisation, iodine value measured by an accelerated technique that will provide similar information, refractive index, solid fat content measured by low-resolution ^{1}H-NMR, solid fat index measured by dilatometry, slip melting point, or gas chromatography of methyl esters.

Koetsier in Gunstone and 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 100–200°C of 2.60–3.36 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 180°C Koetsier gives concentrations of ~7000 and 16 mol/m^3, respectively, for the olefinic groups and the hydrogen.

6.2 Atmospheric oxidation

Unsaturated fats like other unsaturated products such as rubber and paints deteriorate as a consequence of reaction with oxygen (air) that leads, in fat-containing foods, to oxidative rancidity (another form of rancidity results from hydrolysis). Oxidative deterioration is of two kinds (autoxidation and photo-oxidation). These processes lead first to similar, but not identical, unstable allylic hydroperoxides which decompose to volatile short-chain molecules (mainly aldehydes) responsible for the undesirable odours and flavours associated with oxidative rancidity (Figure 6.1). These compounds have low but differing threshold levels so that only small quantities are necessary to produce their undesirable effects. Since the oxidation processes are influenced by heat, light, the presence of pro-oxidants (copper and iron) and antioxidants (natural or synthetic), the presence of already-oxidised material, and of air, attention must be given

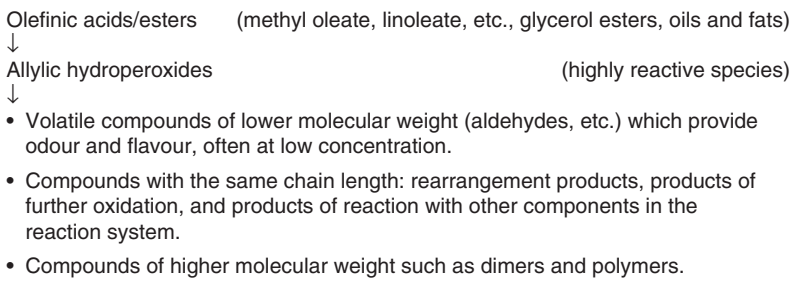


Figure 6.1 Formation and further reactions of allylic hydroperoxides.

to all these factors in the handling, transport, and storage of oils and fats and of foods containing these.

The level and nature of unsaturation is an important factor in the rate of oxidation (Table 6.1). This is the reason for the instability of (highly unsaturated) fish oils and accounts for the short shelf life of oils containing linolenic acid (soybean and rapeseed/canola oils) and the preference for oils with reduced levels of this acid available through breeding or through brush hydrogenation. The figures in Table 6.1 indicate that the polyunsaturated acids (linoleic, linolenic, etc.) oxidise much quicker than the monounsaturated oleic acid. Oxidative deterioration is strongly linked with the number of doubly activated allylic groups present in PUFA. An index of the oxidisability of vegetable and fish oils has been expressed as:

$$\text{Oxidisability} = (\text{diene}) + 2(\text{triene}) + 3(\text{tetraene}) + 4(\text{pentaene}) + 5(\text{hexaene})$$

It is not possible to prevent these oxidation reactions but they can be inhibited and the induction period (Section 4.6) should be extended as much as possible. The difficulties of inhibiting oxidation are further complicated by the fact that many foods (and biological systems) are emulsions of lipids and aqueous systems and attention has to be given to the distribution of antioxidants and pro-oxidants between these two phases and at the interface between them.

The detailed structure of the hydroperoxides that can result from oleate, linoleate, or other polyunsaturated acid is important as this controls the chemical structure of the volatile short-chain compounds, each of which has its own flavour/odour and its own threshold level. One of the problems with linolenic esters is that trienes oxidise quicker than dienes and the resulting volatile aldehydes have lower threshold values. When an oxidised fatty molecule cleaves one fragment will remain attached to glycerol (the core aldehyde) while the methyl end of the molecule will provide

Table 6.1 Relative rates of autoxidation and photo-oxidation of oleate, linoleate, and linolenate (autoxidation of methyl oleate = 1)

Reaction	Oxygen	18:1	18:2	18:3
Autoxidation	Triplet	1	27	77
Photo-oxidation	Singlet	3×10^4	4×10^4	7×10^4
Ratio of reaction rates		30,000	1500	900

the volatile component. The latter can be removed through refining but the short-chain fragment still attached to glycerol will probably remain in the oil. The sequence in Figure 6.2 shows the formation of octanal from a glycerol ester containing oleic acid via the 11-hydroperoxide. Other aldehydes from oxidised oleate, linoleate, and linolenate are listed in Table 6.2.

Autoxidation is a radical chain process. That means that the intermediates are radicals (odd electron species) and that like other chain processes there are three stages: initiation, propagation, and termination (Figure 6.2). The initiation step (not fully understood) is followed by a propagation sequence that continues, perhaps for many cycles, until stopped by one of the termination processes. The process will be accelerated by more initiation steps (involving metal ions, higher temperatures, or a poor sample containing already oxidised oil) and by less termination steps resulting in more of the propagation cycle. More important, the process will be inhibited by having fewer initiation steps or more termination steps thereby having fewer and shorter propagation cycles. This can be achieved by starting with good quality oil and by the

Table 6.2 The major hydroperoxides produced from oleate, linoleate, and linolenate during autoxidation and photo-oxidation and the volatile aldehydes resulting from these

Ester	Hydroperoxide	Double bond	Volatile aldehyde*
Oleate	8	9	11:1 (2)
	9	10	10:1 (2)
	10	8	9:0
	11	9	8:0
Linoleate	9	10,12	10:2 (2,4)
	10	8,12	9:1 (3)
	12	9,13	7:1 (2)
	13	9,11	6:0
Linolenate	9	10,12,15	10:3 (2,4,7)
	10	8,12,15	9:2 (3,6)
	12	9,13,15	7:2 (2,4)
	13	9,11,15	6:1 (3)
	15	9,12,16	4:1 (3)
	16	9,12,14	3:0

* Numbers before the colon indicate the number of carbon atoms in each aldehyde molecule and numbers after the colon indicate the number of unsaturated centres. Numbers in brackets indicate double bond position with respect to the aldehyde function.

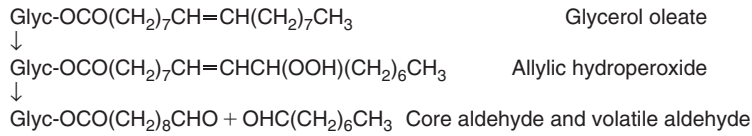


Figure 6.2 Formation of a typical core aldehyde and a short-chain volatile aldehyde (octanal) from a glycerol ester through the appropriate allylic hydroperoxide. Other aldehydes result from other hydroperoxides (Table 6.2).

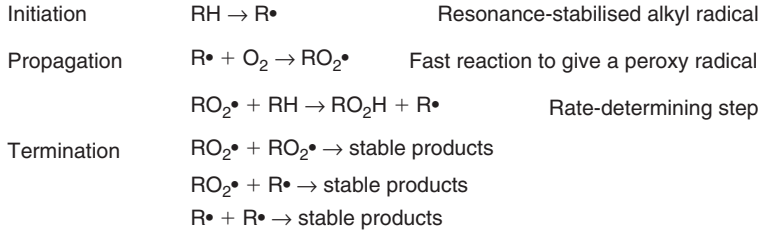


Figure 6.3 Olefin autoxidation. RH represents an olefinic compound in which H is attached to an allylic carbon atom. RO_2H is a hydroperoxide.

presence of appropriate antioxidants (see below). The initiation and propagation steps involve breaking a C−H in the glycerol ester. The energy required to remove hydrogen from a saturated methylene group, an allylic methylene group, and a doubly allylic methylene group (as at C11 in linoleate) is 100, 75, and 50 kcal, respectively. These values relate to the relative ease of oxidation of saturated, oleate, and linoleate (Table 6.1). The allyl radical first produced is resonance stabilised so that the radical centre is spread over different carbon atoms giving rise to the various hydroperoxides listed in Table 6.2. In the oxidation of olefinic lipids there is normally an induction period during which reaction is very slow and deterioration is not significant, followed by a quicker stage of undesirable oxidation. One purpose of antioxidants is to extend this induction period.

Sterols are also subject to oxidation. Cholesterol (Section 1.5) contains a cyclic double bond ($\Delta 5$) and two tertiary carbon atoms in its side chain (C-20 and C-25), all sites where oxidation may occur. Some cholesterol oxides are produced as part of the normal metabolism of cholesterol to bile acids. At higher levels these affect human health by contributing to the development of atherosclerosis. Oxidised animal-based foods represent a primary source

$$\textit{cis}\text{-RCH=CHCH}_2\text{R}' + {}^1\text{O}_2 \rightarrow \textit{trans}\text{-RCH(OOH)CH=CHR}'$$

Figure 6.4 Reaction of olefin with singlet oxygen to give allylic hydroperoxides with double bonds in a different position and of changed configuration.

of oxidised cholesterol. Such products are not present in fresh foods but are formed during handling prior to consumption, mainly through autoxidation. Cholesterol esters are predominantly of linoleic acid while free cholesterol is associated with polyunsaturated fatty acids in phospholipids in cell membranes. In both cases oxidation can be initiated in the polyunsaturated fatty acids and then involve the cholesterol molecule. This holds in the animal (before being prepared as food) and in the human, and in both oxidation can be retarded by appropriate dietary antioxidants. Processing conditions should also be adapted to minimise oxidation as, for example, in the preparation of spray-dried eggs. Between 0.5% and 1.0% of dietary cholesterol may be oxidised and the primary oxidation products include 7-α-hydroxy-, 7-β-hydroxy, and 7-keto-cholesterol, cholesterol α- and β-epoxides, 3,5,6-trihydroxycholesterol, and 20- and 25-hydroxycholesterol (Cuppett, 2003).

Photo-oxidation is a quicker reaction between olefin and a light-activated form of oxygen. The activation process requires a sensitiser such as chlorophyll, riboflavin, myoglobin, erythrosine, rose bengal, or methylene blue. The sensitiser absorbs energy from a photon and this energy is eventually passed to oxygen, raising it from the triplet to the more reactive singlet state. Singlet oxygen reacts rapidly with double bonds by an ene reaction to give an allylic hydroperoxide. Photo-oxidation differs from autoxidation in that it is faster (Table 6.1) and its rate is related to the number of double bonds rather than to the number of doubly allylic functions. It is inhibited by appropriate quencher molecules like carotene rather than by the range of compounds that inhibit autoxidation.

Antioxidants are materials present in oils and fats to protect them against autoxidation. They may be natural compounds already present in the oils such as tocols (Section 1.6) and ferulic acid esters (structure below) or they may be natural or synthetic compounds added by the technologist. There is not enough natural antioxidant to meet demand so synthetic compounds must be used in some cases. Some of the natural antioxidants may be lost

during refining so that refined oils are generally less stable than crude oils. The eight different tocols have differing antioxidant activity so that total tocol content is not an adequate measure of antioxidant activity. (Table 6.3 and Section 1.5) Antioxidants may also be lost during food preparation so prepared foods may be less stable than the ingredients from which they are made. Autoxidation can be inhibited (induction period extended and shelf life lengthened) but it cannot be reversed so antioxidants should be added as early as possible after the refining process. The amount of antioxidant employed must be the optimum. There are legal limits for the synthetic compounds and tocopherols may act as pro-oxidants at higher concentrations. In the case of emulsions, as opposed to bulk oils, it is important to consider the distribution of antioxidants and pro-oxidants between the oil and water phases. To avoid the reaction of existing hydroperoxides with metal ions which generates more radical species, it is important to keep hydroperoxides and metal ions apart and this may be assisted by controlling the pH of the emulsion and by selection of appropriate emulsifiers.

Some plants have other natural antioxidants in their leaves or seeds. Familiar examples include oat oil (with α-tocopherol,

Table 6.3 Vitamin E content (mg/100 g) of some vegetable oils and of butter and lard

	Tocopherols					Tocotrienols						
Oil	α	β	γ	δ	Total	α	β	γ	δ	Total	Grand total	IU
Soybean	10	–	59	26	96					0	96	24
Corn	11	5	60	2	78					0	78	20
Rapeseed	17		35	1	53					0	53	30
Sunflower	49		5	1	55					0	55	73
Groundnut	13		22	2	37					0	37	23
Cottonseed	39		39		78					0	78	64
Safflower	37		17	24	80					0	80	61
Palm	26		32	7	65	14	3	29	7	53	118	49
Coconut	Trace		Trace		1	Trace		2	Trace	3	4	1
Olive	20	1	1		22					0	22	30
Wheat germ	121	65	24	25	235	2	17			19	254	233
Rice	12	4	5		21	18	2	57		77	98	30
Butter	2				2						2	3
Lard	1				1	1				1	2	2

IU represents total vitamin E content calculated on a weighted basis for the effect of each tocol.

Source: Adapted from Stone and Papas in Gunstone (2003). See also Table 1.3.

α-tocotrienol, and avenathramides), sesame oil (with sesamin, sesamolin, and sesaminol, all of which are derivatives of sesamol – 3,4-methylenedioxyphenol), and ricebran oil (with tocotrienols, avenasterols, and oryzanols which are sterol esters of ferulic acid).

4-OH-3-OMeC_6H_3CH=CHCOOH ferulic acid

Antioxidants are also present in herbs and spices and while these can sometimes be used as extracts their food use is limited by strong flavours that may or may not be acceptable in other foods. Tea leaves are a rich source of antioxidants (catechins) as are many fruits and vegetables containing flavonoids. Dietary consumption of these as whole foods provides a good source of the antioxidants required by the body to counter oxidative damage to protein and to DNA caused by radicals produced through lipid oxidation. This applies also to vegetables containing carotenes. Rosemary leaves contain powerful antioxidants such as carnosic acid, carnosol, and rosmarinic acid and rosemary extracts are available for use as antioxidants. Vitamin C (ascorbic acid) acts as an oxygen scavenger, removing traces of residual oxygen in a packed and sealed product. It is water-soluble but can be used in a lipid-soluble form as ascorbyl palmitate. Phospholipids show ill-defined antioxidant activity possibly through activity as a chelating agent and/or emulsifier.

The supply of natural antioxidants is insufficient to meet demand so some use of synthetic antioxidants is obligatory. Even so-called natural vitamin E may have been submitted to a chemical reaction (permethylation) in which tocols with only one or two methyl groups have been converted to their trimethyl derivative (α-tocopherol, Figure 1.5).

The synthetic compounds that can be used as antioxidants in food are strictly controlled, as is the level at which they may be used. The matter is complicated in that not all countries have agreed to the same list of acceptable compounds. This becomes important for materials that are traded between countries having different permitted antioxidants. Obviously the antioxidants must be non-toxic and that must apply also to the products produced from them as a result of their antioxidant activity.

The four important synthetic antioxidants discussed here are solid compounds and may be conveniently used as solutions in propylene glycol, monoacylglycerols, or vegetable oils. They are mono

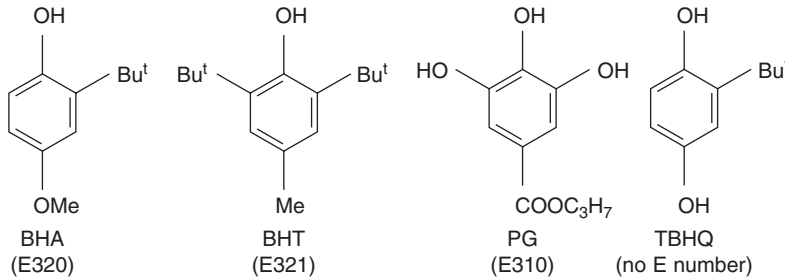


Figure 6.5 The structures and E numbers of synthetic antioxidants. TBHQ has no E number because it is not a permitted antioxidant in the EU.

or dihydric phenols (represented as ArOH) and react readily with a peroxy radical to give a phenoxy radical (ArO·) stabilised by extensive delocalisation of the odd electron over the aromatic system. The E numbers indicate that they may be used in Europe within prescribed limits (Figure 6.5).

Butylated hydroxyanisole (BHA, E320) shows good solubility in fat and reasonable stability in fried and baked products. It is very effective with animal fats and less so with vegetable oils. It shows marked synergism with BHT (butylated hydroxytoluene) and PG (propyl gallate) and can be used at a maximum level of 200 ppm.

BHT, E321 is less soluble than BHA and is not soluble in the propylene glycol frequently used as a solvent for antioxidants. It is synergistic with BHA but not with PG and can be used to a maximum level of 200 ppm.

Synergism is the term used to describe the observation where the efficacy of two or more components is greater than the sum of the effects for individual components and indicates some co-operative activity.

PG, E310 is less soluble than BHA or BHT. It does not generally survive cooking as it decomposes at 148°C. Nevertheless it is effective when used with BHA and may be used up to 100 ppm.

Tertbutyl hydroquinone (TBHQ) is acceptable in USA and many other countries but not in EU-27 and hence does not have an E number. It is very effective with vegetable oils, has good solubility, and is stable at high temperatures. It is frequently used during oil transport and storage and can be subsequently removed during deodorisation.

6.3 Thermal changes

Unsaturated centres may undergo undesirable changes when heated. This is particularly important when the fatty acyl chains contain three or more methylene interrupted olefinic centres and when the temperature exceeds 180°C. This problem may arise during deodorisation of soybean and rapeseed oils containing up to 10% of linolenic acid (18:3) or of fish oils with eicosapentaenoic acid (EPA) (20:5) and docosahexaenoic acid (DHA) (22:6). At elevated temperatures the risk of oxidative change is enhanced but even in the absence of air undesirable changes occur at elevated temperatures. Studies on the refining of vegetable oils have shown that stereoisomerism of linolenic acid with three double bonds occurs from 220°C upwards and is quicker than for linoleate with only two double bonds. The changes that may occur during exposure of polyunsaturated fatty acids to higher temperatures include:

- cyclisation (formation of five- and six-membered carbocyclic ring systems);
- geometrical isomerism (conversion of the natural all-*cis* polyunsaturated fatty acid to isomers with both *cis* and *trans* double bonds);
- polymerisation (producing dimers, trimers, and oligomers of enhanced molecular weight).

All these changes are undesirable on nutritional grounds since the nutritional value of the polyunsaturated fatty acid depends on them retaining their all-*cis* pattern of unsaturation. Special analytical methods may be required to detect these changes.

6.4 Reactions of the carboxyl/ester function

Oils and fats are glycerol esters and the ester functions are reactive centres which can be modified in various ways. For example, they can be hydrolysed. The suffix -lysis means splitting so that hydrolysis implies splitting with water and the final products are fatty acids and glycerol. This reaction generally requires a catalyst which may be acidic, basic, or enzymatic. When catalysed by

a lipase the reaction may be described as lipolysis. Hydrolysis is an important reaction in the oleochemical industry but in the food industry the conversion of glycerol esters to other esters is more important.

Esters can be made from fatty acids and alcohols but it is often more convenient to transform existing esters (such as triacylglycerols) to other esters by reaction with an alcohol (alcoholysis), an acid (acidolysis), or another ester (interesterification) using a catalyst that may be chemical or a lipase.

Two important alcoholysis procedures are methanolysis and glycerolysis. The former is used in the conversion of glycerol esters to methyl esters and is used on a mg scale for analytical purposes (gas chromatography) and on a multi-tonne scale to produce esters for biofuel or as an intermediate in the production of fatty alcohols. Glycerolysis involves reaction of triacylglycerols with glycerol to produce monoacylglycerols and diacylglycerols. The former, as such or after further modification, are much used in the food industry as emulsifying agents (Section 8.11).

Acidolysis is less commonly employed but it may be used to incorporate (say) lauric acid (12:0) into a $C_{16/18}$ oil.

Interesterification can be carried out on a single oil, itself a non-random mixture of triacylglycerols, or on a blend of oils. An old example of the first of these is the interesterification (randomisation) of lard. This fat is unusual in having a high level of palmitic acid in the *sn*-2 position. During reaction with sodium methoxide the lard fatty acids become randomly distributed. Overall there is no change in fatty acid composition but a change in triacylglycerol composition. As a consequence of this modification the randomised lard is a better shortening. More commonly, interesterification is applied to a blend of oils. The oils that are mixed can differ in their level of unsaturation (an unsaturated oil and a hydrogenated oil), in their range of chain length (such as a lauric oil and a $C_{16/18}$ oil), or in the presence of a less common fatty acid such as γ-linolenic acid, EPA, or DHA in one of the components.

Mixtures of esters may be interesterified with an appropriate basic catalyst. When this happens the natural non-random mixture is ultimately converted to a randomised mixture. The change will be even more marked with a mixture of two different types of oils such as a lauric oil and a non-lauric oil. Alteration is particularly apparent in the fatty acids present in the *sn*-2 position. Before interesterification of a vegetable oil these will be mainly unsaturated acids

but after complete randomisation the fatty acids at all three positions will be the same. These changes have important effects on the physical (particularly melting behaviour) and nutritional properties of the modified fat.

Interesterification is used in newer methods of producing spreads with a reduced content of *trans* acids. Hydrogenation producing large amounts of *trans* acids, can be replaced by interesterification of a soft fat with a hard fat. Most spreads produced in Europe now have a very low level of *trans* acid though this may be less true for cooking fats and industrial spreads. When randomisation is complete triacylglycerol composition can be calculated from the fatty acid composition since the amount of an individual triacylglycerol (ABC) will depend only on the proportions of each of these acids (a%, b%, c%, respectively). The level of this single glycerol ester will be 100 [a/100 $\times$ b/100 $\times$ c/100]% and the level of all isomers having one A, one B, and one C acyl chain will be six times this figure since there are six stereoisomers meeting this requirement.

Ester–ester interchange can be achieved without a catalyst at temperatures above 200°C but is usually carried out at 20–100°C with a basic catalyst such as sodium hydroxide, sodium methoxide, or sodium potassium alloys. At ~80°C the reaction takes 30–60 min and may be carried out on a multi-tonne scale. The oil should be free of water, carboxylic acid, and hydroperoxide as these compounds will destroy the catalyst. The true catalyst is thought to be either a diacylglycerol anion [$ROCOCH_2CH(OCOR)CH_2O^-$] formed by interaction of a triacylglycerol molecule with sodium methoxide or the enolate [$ROCOCH_2CH(OCOR)CH_2OCO^-{=}CHR'$] resulting from removal of a proton from the α-methylene function. The product will contain some free acid if NaOH is used as catalyst or methyl ester, if the catalyst is NaOMe and must be refined to remove these. The amount of catalyst must be as low as possible to minimise these losses.

Directed interesterification is a modification of the normal process when the reaction is conducted at a lower temperature (25–35°C). Under these conditions the less soluble triacylglycerols crystallise from the solution. This disturbs the equilibrium in the liquid phase and this will be continually re-established. The consequence is to raise the levels of SSS and of UUU triacylglycerols in the final interesterified product.

With enzymatic catalysts, interesterification leads to structured lipids of the types described below.

Nutritional and other physiological properties of fats (triacylglycerols) depend on their detailed structure. Until now, use has been made of materials provided by agriculture modified in minor ways such as fractionation, partial hydrogenation, and interesterification as described in earlier sections of this chapter. Individual triacylglycerols can be synthesised in the laboratory in modest quantities but these will not usually be appropriate for large-scale human consumption. By exploiting the specificity of lipases it is now possible to produce large quantities of oils and fats approximating to a specification designed to optimise some important physical and/or nutritional property.

The cost of the enzyme is generally so high that their use is only economic for high-value products but these difficulties are being overcome as enzyme producers develop immobilised enzymes of greater stability with a longer useful life. At the same time our understanding of enzyme structure allows changes to be made leading to enhanced selectivity. Beyond this there is growing willingness to pay more for fats for which approved health claims can be made. Enzymatic reactions are considered to be more 'natural' or 'greener'.

Lipases show several different kinds of specificity which can be exploited. The most common is 1,3-regiospecificity in which reaction is confined to the *sn*-1 and 3 positions of a triacylglycerol with no change at the *sn*-2 position. Lipases may also show specificity for selected fatty acids with which they associate. This specificity may depend on the unsaturated centres and especially the position of the double bond closest to the carboxyl group or may be related to chain length.

There are many reports of preparations of structured triacylglycerols which can be achieved in one-step or two-step processes. In the former, an oil or fat has some of its *sn*-1/3 acyl chains replaced by different fatty acids. The acyl donor may be an ester such as an alkyl ester or triacylglycerol mixture or an acid (acidolysis). The reaction is illustrated in very simple form in the following equation. In reality the reaction is more complicated since neither reactant will be the individual species indicated in the equation.

$$\text{Gl-ABC} + \text{D (as acid or ester)} \rightleftharpoons \text{Gl-DBC} + \text{Gl-ABD} + \text{Gl-DBD}$$

The products represent racemic triacylglycerols with acyl chains A–D. The *sn*-2 position is unaffected by this process.

Good results have been obtained with lipases such as those from *Rhizomucor miehei* (Lipozyme), *Rhizopus delemar*, and *Candida antarctica*. Incorporation of reactant is usually in the range 40–65%. This procedure is simpler than the two-step reaction but the products are less pure.

In the two-step process triacylglycerols are selectively deacylated at the *sn*-1 and 3 positions by enzyme-catalysed ethanolysis and pure 2-monoacylglycerol is isolated. The monoacylglycerol is then acylated at the free hydroxyl positions using a 1,3 specific lipase and an appropriate acyl donor which may be free acid, alkyl ester, or vinyl ester.

$$\text{Gl-ABC} \rightarrow \text{Gl-(OH)B(OH)} \rightarrow \text{Gl-DBD}$$

Two-step synthesis of a structured triacylglycerol proceeding through a 2-monoacylglycerol which is isolated and purified before the second step. Gl stands for the glycerol residue; OH represents the free OH groups in a monoacylglycerol; and A, B, C, and D are acyl groups.

There is a greater control of the reaction when this is conducted in a carefully selected solvent but this involves additional handling and cost and the aim is to produce bulk products having the desired properties as simply and cheaply as possible.

Typically a plug-in reactor (1 m^3), containing Lipozyme TL IM prepared from *Thermomyces lanuginose* lipase is supplied by the enzyme producer. Two oils such as palm oil (or palm stearin) and palm kernel (or coconut) oil are passed through the reactor and emerge 1 h later as interesterified oil. The reaction occurs at 70°C which is 30°C lower than for chemical interesterification, no downstream processing is required, and the product has no acids with *trans* unsaturation. This approach is being used to produce spreads with a low level of *trans* acids.

Many laboratory experiments are concerned with attempts to produce triacylglycerols of the type MLM where M is an easily metabolised fatty acid of medium-chain length (frequently C_8) and L is a long-chain acid including nutritionally important fatty acids such as EPA or DHA.

The nature of the fatty acid in the *sn*-2 position is controlled by the selection of starting material. Vegetable oils will provide sources of oleic and linoleic acid in this position and fish oils will be used for the long-chain polyunsaturated fatty acids. In a preliminary stage

the levels of these important acids may be enhanced prior to interesterification (Section 2.7).

Human milk fat is unusual in that it is rich in triacylglycerols containing a saturated acid (palmitic) in the *sn*-2 position. This is unusual among natural fats so a product with this structural feature called 'Betapol' has been developed for addition to infant formula. In theory, tripalmitin is reacted with unsaturated acids in the presence of a 1,3-stereospecific lipase (from *Rhizomucor miehei*). In practice the reactants are a palm stearin rich in tripalmitin and a mixture of canola and sunflower oils rich in oleic acid.