In many foods the lipid component plays a major role in determining the overall physical characteristics, such as flavour, texture, mouthfeel and appearance. For this reason, it is difficult to develop low-fat alternatives of many foods, because once the fat is removed some of the most important physical characteristics are lost. Finally, many fats are prone to lipid oxidation, which leads to the formation of off-flavours and potentially harmful products. Some of the most important properties of concern to the food analyst are:

- Total lipid concentration
- Type of lipids present
- Physicochemical properties of lipids, e.g., crystallisation, melting point, smoke point, rheology, density and colour
- Structural organisation of lipids within a food

**Properties of Lipids**

Lipids are usually defined as those components that are soluble in organic solvents (such as ether, hexane or chloroform), but are insoluble in water. This group of substances includes triacylglycercols, diacylglycercols, monoacylglycercols, free fatty acids, phospholipids, sterols, carotenoids and vitamins A and D. The lipid fraction of a fatty food therefore contains a complex mixture of different types of molecule.
Even so, triacylglycerols are the major component of most foods, typically making up more than 95 to 99% of the total lipids present. Triacylglycerols are esters of three fatty acids and a glycerol molecule. The fatty acids normally found in foods vary in chain length, degree of unsaturation and position on the glycerol molecule. Consequently, the triacylglycerol fraction itself consists of a complex mixture of different types of molecules.

Each type of fat has a different profile of lipids present which determines the precise nature of its nutritional and physiochemical properties. The terms fat, oil and lipid are often used interchangeably by food scientists. Although sometimes the term fat is used to describe those lipids that are solid at the specified temperature, whereas the term oil is used to describe those lipids that are liquid at the specified temperature.

Sample Preparation Procedure

As with any analytical procedure, the validity of the results depends on proper sampling and preservation of the sample prior to analysis. Ideally, the composition of the sample analysed should represent as closely as possible that of the food from which it was taken. The sample preparation required in lipid analysis depends on the type of food being analysed, the nature of the lipid component and the type of analytical procedure used.

In order, to decide the most appropriate sample preparation procedure it is necessary to have a knowledge of the physical structure and location of the principal lipids present in the food. Since each food is different it is necessary to use different procedures for each one. Official methods have been developed for specific types of foods that stipulate the precise sample preparation procedure that should be followed.

In general, sample preparation should be carried out using an environment that minimises any changes in the properties of the lipid fraction. If lipid oxidation is a problem it is important to preserve the sample by using a nitrogen...
atmosphere, cold temperature, low light or adding antioxidants. If the solid fat content or crystal structure is important it may be necessary to carefully control the temperature and handling of the sample.

**DETERMINATION OF LIPID CONCENTRATION**

It is important to be able to accurately determine the total fat content of foods for a number of reasons:

- Economic (not to give away expensive ingredients)
- Legal (to conform to standards of identity and nutritional labelling laws)
- Health (development of low fat foods)
- Quality (food properties depend on the total lipid content)
- Processing (processing conditions depend on the total lipid content)

The principle physicochemical characteristics of lipids (the "analyte") used to distinguish them from the other components in foods (the "matrix") are their solubility in organic solvents, immiscibility with water, physical characteristics and spectroscopic properties. The analytical techniques based on these principles can be conveniently categorised into three different types:

(i) solvent extraction;
(ii) non-solvent extraction and
(iii) instrumental methods.

**Solvent Extraction Techniques**

The fact that lipids are soluble in organic solvents, but insoluble in water, provides the food analyst with a convenient method of separating the lipid components in foods from water soluble components, such as proteins, carbohydrates and minerals. In fact, solvent extraction techniques are one of the most commonly used methods of isolating lipids from foods and of determining the total lipid content of foods.
Sample Preparation for Solvent Extraction

The preparation of a sample for solvent extraction usually involves a number of steps:

- **Drying sample**: It is often necessary to dry samples prior to solvent extraction, because many organic solvents cannot easily penetrate into foods containing water, and therefore extraction would be inefficient.

- **Particle size reduction**: Dried samples are usually finely ground prior to solvent extraction to produce a more homogeneous sample and to increase the surface area of lipid exposed to the solvent. Grinding is often carried out at low temperatures to reduce the tendency for lipid oxidation to occur.

- **Acid hydrolysis**: Some foods contain lipids that are complexed with proteins (lipoproteins) or polysaccharides (glycolipids). To determine the concentration of these components it is necessary to break the bonds which hold the lipid and non-lipid components together prior to solvent extraction. Acid hydrolysis is commonly used to release bound lipids into easily extractable forms, e.g. a sample is digested by heating it for 1 hour in the presence of 3N HCl acid.

- **Solvent Selection**: The ideal solvent for lipid extraction would completely extract all the lipid components from a food, while leaving all the other components behind. In practice, the efficiency of solvent extraction depends on the polarity of the lipids present compared to the polarity of the solvent. Polar lipids (such as glycolipids or phospholipids) are more soluble in polar solvents (such as alcohols), than in non-polar solvents (such as hexane).

On the other hand, non-polar lipids (such as triacylglycerols) are more soluble in non-polar solvents than in polar ones. The fact that different lipids have different polarities means that it is impossible to select a single organic solvent to extract them all. Thus the total lipid content determined by solvent extraction depends on the nature of the organic solvent used.
to carry out the extraction: the total lipid content determined using one solvent may be different from that determined using another solvent.

In addition to the above considerations, a solvent should also be inexpensive, have a relatively low boiling point (so that it can easily be removed by evaporation), be non-toxic and be nonflammable (for safety reasons). It is difficult to find a single solvent which meets all of these requirements. Ethyl ether and petroleum ether are the most commonly used solvents, but pentane and hexane are also used for some foods.

**Batch Extraction Process**

These methods are based on mixing the sample and the solvent in a suitable container, e.g., a separatory funnel. The container is shaken vigorously and the organic solvent and aqueous phase are allowed to separate (either by gravity or centrifugation). The aqueous phase is then decanted off, and the concentration of lipid in the solvent is determined by evaporating the solvent and measuring the mass of lipid remaining: \( \% \text{Lipid} = 100 \left( \frac{M_{\text{lipid}}}{M_{\text{sample}}} \right) \). This procedure may have to be repeated a number of times to improve the efficiency of the extraction process.

In this case the aqueous phase would undergo further extractions using fresh solvent, then all the solvent fractions would be collected together and the lipid determined by weighing after evaporation of solvent. The efficiency of the extraction of a particular type of lipid by a particular type of solvent can be quantified by an equilibrium partition coefficient, \( K = \frac{c_{\text{solvent}}}{c_{\text{aqueous}}} \), where \( c_{\text{solvent}} \) and \( c_{\text{aqueous}} \) are the concentration of lipid in the solvent and aqueous phase, respectively. The higher the partition coefficient the more efficient the extraction process.

**Semi-Continuous Solvent Extraction Methods**

Semi-continuous solvent extraction methods are commonly used to increase the efficiency of lipid extraction from foods. The Soxhlet method is the most commonly used example of a semi-continuous method. In the Soxhlet method a sample is
dried, ground into small particles and placed in a porous thimble. The thimble is placed in an extraction chamber, which is suspended above a flask containing the solvent and below a condenser.

The flask is heated and the solvent evaporates and moves up into the condenser where it is converted into a liquid that trickles into the extraction chamber containing the sample. Eventually, the solvent builds up in the extraction chamber and completely surrounds the sample. The extraction chamber is designed so that when the solvent surrounding the sample exceeds a certain level it overflows and trickles back down into the boiling flask. As the solvent passes through the sample it extracts the lipids and carries them into the flask.

The lipids then remain in the flask because of their low volatility. At the end of the extraction process, which typically lasts a few hours, the flask containing the solvent and lipid is removed, the solvent is evaporated and the mass of lipid remaining is measured (M_{lipid}). The percentage of lipid in the initial sample (M_{sample}) can then be calculated: \%Lipid = 100 (M_{lipid}/M_{sample}). A number of instrument manufacturers have designed modified versions of the Soxhlet method that can be used to determine the total lipid content more easily and rapidly.

**Continuous Solvent Extraction**

The Goldfish method is similar to the Soxhlet method except that the extraction chamber is designed so that the solvent just trickles through the sample rather than building up around it. This reduces the amount of time required to carry out the extraction, but it has the disadvantage that channelling of the solvent can occur, i.e., the solvent may preferentially take certain routes through the sample and therefore the extraction is inefficient. This is not a problem in the Soxhlet method because the sample is always surrounded by solvent.

**Accelerated Solvent Extraction**

The efficiency of solvent extraction can be increased by carrying it out at a higher temperature and pressure than are
normally used. The effectiveness of a solvent at extracting lipids from a food increases as its temperature increases, but the pressure must also be increased to keep the solvent in the liquid state. This reduces the amount of solvent required to carry out the analysis, which is beneficial from a cost and environmental standpoint. Special instruments are available to carry out solvent extraction at elevated temperatures and pressures.

**Supercritical Fluid Extraction**

Solvent extraction can be carried out using special instruments that use supercritical carbon dioxide (rather than organic liquids) as the solvent. These instruments are finding greater use because of the cost and environmental problems associated with the usage and disposal of organic solvents. When pressurised $\text{CO}_2$ is heated above a certain critical temperature it becomes a supercritical fluid, which has some of the properties of a gas and some of a liquid. The fact that it behaves like a gas means that it can easily penetrate into a sample and extract the lipids, while the fact that it behaves like a fluid means that it can dissolve a large quantity of lipids (especially at higher pressures).

Instruments based on this principle heat the food sample to be analysed in a pressurised chamber and then mix supercritical $\text{CO}_2$ fluid with it. The $\text{CO}_2$ extracts the lipid, and forms a separate solvent layer, which is separated from the aqueous components. The pressure and temperature of the solvent are then reduced which causes the $\text{CO}_2$ to turn to a gas, leaving the lipid fraction remaining. The lipid content of a food is determined by weighing the percentage of lipid extracted from the original sample.

**Nonsolvent Liquid Extraction**

A number of liquid extraction methods do not rely on organic solvents, but use other chemicals to separate the lipids from the rest of the food. The Babcock, Gerber and Detergent methods are examples of nonsolvent liquid extraction
methods for determining the lipid content of milk and some other dairy products.

*Babcock Method*

A specified amount of milk is accurately pipetted into a specially designed flask (the Babcock bottle). Sulfuric acid is mixed with the milk, which digests the protein, generates heat, and breaks down the fat globule membrane that surrounds the droplets, thereby releasing the fat. The sample is then centrifuged while it is hot (55-60°C) which causes the liquid fat to rise into the neck of the Babcock bottle. The neck is graduated to give the amount of milk fat present in wt%. The Babcock method takes about 45 minutes to carry out, and is precise to within 0.1%. It does not determine phospholipids in milk, because they are located in the aqueous phase or at the boundary between the lipid and aqueous phases.

*Gerber Method*

This method is similar to the Babcock method except that a mixture of sulfuric acid and isoamyl alcohol, and a slightly different shaped bottle, are used. It is faster and simpler to carry out than the Babcock method. The isoamyl alcohol is used to prevent charring of the sugars by heat and sulfuric acid which can be a problem in the Babcock method since it makes it difficult to read the fat content from the graduated flask. This method is used mainly in Europe, whilst the Babcock method is used mainly in the USA. As with the Babcock method, it does not determine phospholipids.

*Detergent Method*

This method was developed to overcome the inconvenience and safety concerns associated with the use of highly corrosive acids. A sample is mixed with a combination of surfactants in a Babcock bottle. The surfactants displace the fat globule membrane which surrounds the emulsion droplets in milk and causes them to coalesce and separate. The sample is centrifuged which allows the fat to move into the graduated neck of the bottle, where its concentration can then be determined.
Instrumental Methods

There are a wide variety of different instrumental methods available for determining the total lipid content of food materials. These can be divided into three different categories according to their physicochemical principles:

(i) measurement of bulk physical properties,
(ii) measurement of adsorption of radiation, and
(iii) measurement of scattering of radiation.

Each instrumental methods has its own advantages and disadvantages, and range of foods to which it can be applied.

Measurement of Bulk Physical Properties

— **Density:** The density of liquid oil is less than that of most other food components, and so there is a decrease in density of a food as its fat content increases. Thus the lipid content of foods can be determined by measuring their density.

— **Electrical conductivity:** The electrical conductivity of lipids is much smaller than that of aqueous substances, and so the conductivity of a food decreases as the lipid concentration increases. Measurements of the overall electrical conductivity of foods can therefore be used to determine fat contents.

— **Ultrasonic velocity:** The speed at which an ultrasonic wave travels through a material depends on the concentration of fat in a food. Thus the lipid content can be determined by measuring its ultrasonic velocity. This technique is capable of rapid, nondestructive online measurements of lipid content.

Measurement of Adsorption of Radiation

— **UV-visible:** The concentration of certain lipids can be determined by measuring the absorbance of ultraviolet-visible radiation. The lipid must usually be extracted and diluted in a suitable solvent prior to analysis, thus the technique can be quite time-consuming and labour intensive.
— *Infrared*: This method is based on the absorbance of IR energy at a wavelength of 5.73 m due to molecular vibrations or rotations associated with fat molecules: the greater the absorbance the more fat present. IR is particularly useful for rapid and on-line analysis of lipid content once a suitable calibration curve has been developed.

— *Nuclear Magnetic Resonance*: NMR spectroscopy is routinely used to determine the total lipid concentration of foods. The lipid content is determined by measuring the area under a peak in an NMR chemical shift spectra that corresponds to the lipid fraction. Lipid contents can often be determined in a few seconds without the need for any sample preparation using commercially available instruments.

— *X-ray absorption*: Lean meat absorbs X-rays more strongly than fat, thus the X-ray absorbance decreases as the lipid concentration increases. Commercial instruments have been developed which utilise this phenomenon to determine the lipid content of meat and meat products.

**Measurement of Scattering of Radiation**

— *Light scattering*: The concentration of oil droplets in dilute food emulsions can be determined using light scattering techniques because the turbidity of an emulsion is directly proportional to the concentration of oil droplets present.

— *Ultrasonic scattering*: The concentration of oil droplets in concentrated food emulsions can be determined using ultrasonic scattering techniques because the ultrasonic velocity and absorption of ultrasound by an emulsion is related to the concentration of oil droplets present.

A number of these instrumental methods have major advantages over the extraction techniques mentioned above because they are nondestructive, require little or no sample preparation, and measurements are usually rapid, precise and
simple. A major disadvantage of the techniques which rely on measurements of the bulk physical properties of foods are that a calibration curve must be prepared between the physical property of interest and the total lipid content, and this may depend on the type of lipid present and the food matrix it is contained in.

In addition, these techniques can only be used to analyse foods with relatively simple compositions. In a food that contains many different components whose concentration may vary, it is difficult to disentangle the contribution that the fat makes to the overall measurement from that of the other components.

Comparison of Methods

Soxhlet extraction is one of the most commonly used methods for determination of total lipids in dried foods. This is mainly because it is fairly simple to use and is the officially recognised method for a wide range of fat content determinations. The main disadvantages of the technique are that a relatively dry sample is needed (to allow the solvent to penetrate), it is destructive, and it is time consuming. For high moisture content foods it is often better to use batch solvent or nonsolvent extraction techniques.

Many instrumental methods are simple to operate, rapid, reproducible, require little sample preparation and are nondestructive. Nevertheless, they are often expensive to purchase and can only be used for certain types of foods, i.e., where there is no interference from other components. In addition, calibration curves prepared for instrumental methods usually require that the fat content be measured using a standard method.

Extraction techniques tend to be more accurate and more generally applicable and are therefore the standard methods for official analysis of many food materials. Instrumental methods are most useful for rapid measurements of fat content on-line or in quality assurance laboratories of food factories where many samples must be measured rapidly.
LIPID COMPOSITION

Lipids are an extremely diverse group of compounds consisting of tri-, di- and monoacylglycerols, free fatty acids, phospholipids, sterols, carotenoids and vitamins A and D. In addition, most of these sub-groups are themselves chemically complex. All triacylglycerols are esters of glycerol and three fatty acid molecules, nevertheless, the fatty acids can have different chain lengths, branching, unsaturation, and positions on the glycerol molecule.

Thus even a lipid which consists of only triacylglycerols may contain a huge number of different chemical species. It is often important for food scientists to either know or to be able to specify the concentration of the different types of lipid molecules present, as well as the total lipid concentration. Some of the most important reasons for determining the type of lipids present in foods are listed below:

— **Legal**: Government regulations often demand that the amounts of saturated, unsaturated and polyunsaturated lipids, as well as the amount of cholesterol, be specified on food labels.

— **Food Quality**: Desirable physical characteristics of foods, such as appearance, flavour, mouthfeel and texture, depend on the type of lipids present.

— **Lipid oxidation**: Foods which contain high concentrations of unsaturated lipids are particularly susceptible to lipid oxidation, which can lead to the formation of undesirable off-flavours and aromas, as well as potentially toxic compounds e.g., cholesterol oxides.

— **Adulteration**: Adulteration of fats and oils can be detected by measuring the type of lipids present, and comparing them with the profile expected for an unadulterated sample.

— **Food Processing**: The manufacture of many foods relies on a knowledge of the type of lipids present in order to adjust the processing conditions to their optimum values, e.g. temperatures, flow rates etc.
Sample Chosen for Analysis

It is important that the sample chosen for analysis is representative of the lipids present in the original food, and that its properties are not altered prior to the analysis. Analysis of the types of lipids present in a food usually requires that the lipid be available in a fairly pure form. Thus foods which are almost entirely lipids, such as olive oil, vegetable oil or lard, can usually be analysed with little sample preparation. Nevertheless, for many other foods it is necessary to extract and purify the lipid component prior to analysis. Lipids can sometimes be extracted by simply applying pressure to a food to squeeze out the oil, e.g., some fish, nuts and seeds.

For most foods, however, more rigorous extraction methods are needed, such as the solvent or nonsolvent extraction methods described in the previous lecture. Once the lipids have been separated they are often melted and then filtered or centrifuged to remove any extraneous matter. In addition, they are often dried to remove any residual moisture which might interfere with the analysis. As with any analytical procedure it is important not to alter the properties of the component being analysed during the extraction process. Oxidation of unsaturated lipids can be minimised by adding antioxidants, or by flushed containers with nitrogen gas and avoiding exposure to heat and light.

Chromatography

Chromatography is one of the most powerful analytical procedures for separating and analysing the properties of lipids, especially when combined with techniques which can be used to identify the chemical structure of the peaks, e.g., mass spectrometry or NMR. A chromatographic analysis involves passing a mixture of the molecules to be separated through a column that contains a matrix capable of selectively retarding the flow of the molecules.

Molecules in the mixture are separated because of their differing affinities for the matrix in the column. The stronger
the affinity between a specific molecule and the matrix, the more its movement is retarded, and the slower it passes through the column. Thus different molecules can be separated on the basis of the strength of their interaction with the matrix. After being separated by the column, the concentration of each of the molecules is determined as they pass by a suitable detector.

Chromatography can be used to determine the complete profile of molecules present in a lipid. This information can be used to: calculate the amounts of saturated, unsaturated, polyunsaturated fat and cholesterol; the degree of lipid oxidation; the extent of heat or radiation damage; detect adulteration; determine the presence of antioxidants. Various forms of chromatography are available to analyse the lipids in foods, e.g. thin layer chromatography (TLC), gas chromatography (GC), and high pressure liquid chromatography (HPLC).

*Lipid Fractions by TLC*

TLC is used mainly to separate and determine the concentration of different types of lipid groups in foods, e.g. triacylglycerols, diacylglycerols, monoacylglycerols, cholesterol, cholesterol oxides and phospholipids. A TLC plate is coated with a suitable absorbing material and placed into an appropriate solvent. A small amount of the lipid sample to be analysed is spotted onto the TLC plate. With time the solvent moves up the plate due to capillary forces and separates different lipid fractions on the basis of their affinity for the absorbing material.

At the end of the separation the plate is sprayed with a dye so as to make the spots visible. By comparing the distance that the spots move with standards of known composition it is possible to identify the lipids present. Spots can be scraped off and analysed further using techniques, such as GC, NMR or mass spectrometry. This procedure is inexpensive and allows rapid analysis of lipids in fatty foods.
**Fatty Acid Methyl Esters by GC**

Intact triacylglycerols and free fatty acids are not very volatile and are therefore difficult to analyse using GC (which requires that the lipids be capable of being volatised in the instrument). For this reason lipids are usually derivitised prior to analysis to increase their volatility. Triacylglycerols are first saponified which breaks them down to glycerol and free fatty acids, and are then methylated.

\[
\text{Triacylglycerol} \xrightarrow{\text{OH}_2\text{N}x\text{OH}} \text{Fatty acid methyl esters (FAMEs)} + \text{methylated glycerol}
\]

Saponification reduces the molecular weight and methylation reduces the polarity, both of which increase the volatility of the lipids. The concentration of different volatile fatty acid methyl esters (FAMEs) present in the sample is then analysed using GC. The FAMES are dissolved in a suitable organic solvent that is then injected into a GC injection chamber.

The sample is heated in the injection chamber to volatilise the FAMES and then carried into the separating column by a heated carrier gas. As the FAMES pass through the column they are separated into a number of peaks based on differences in their molecular weights and polarities, which are quantified using a suitable detector. Determination of the total fatty acid profile allows one to calculate the type and concentration of fatty acids present in the original lipid sample.

**Chemical Techniques**

A number of chemical methods have been developed to provide information about the type of lipids present in edible fats and oils. These techniques are much cruder than chromatography techniques, because they only give information about the average properties of the lipid components present, e.g. the average molecular weight, degree of unsaturation or amount of acids present. Nevertheless, they are simple to perform and do not require
expensive apparatus, and so they are widely used in industry and research.

**Iodine Value (IV)**

The iodine value (IV) gives a measure of the average degree of unsaturation of a lipid: the higher the iodine value, the greater the number of C=C double bonds. By definition the iodine value is expressed as the grams of iodine absorbed per 100g of lipid. One of the most commonly used methods for determining the iodine value of lipids is "Wijs method". The lipid to be analysed is weighed and dissolved in a suitable organic solvent, to which a known excess of iodine chloride is added. Some of the ICl reacts with the double bonds in the unsaturated lipids, while the rest remains:

\[
R-\text{CH}=\text{CH}-R + \text{ICl}_\text{excess} \rightarrow R-\text{CHI}-\text{CHCl}-R + \text{ICl}_\text{remaining}
\]

The amount of ICl that has reacted is determined by measuring the amount of \( \text{ICl}_\text{remaining} \) after the reaction has gone to completion (\( \text{ICl}_\text{reacted} = \text{ICl}_\text{excess} - \text{ICl}_\text{remaining} \)). The amount of ICl remaining is determined by adding excess potassium iodide to the solution to liberate iodine, and then titrating with a sodium thiosulfate (\( \text{Na}_2\text{S}_2\text{O}_3 \)) solution in the presence of starch to determine the concentration of iodine released:

\[
\text{ICl}_\text{remaining} + 2\text{KI} \rightarrow \text{KCl} + \text{KI} + I_2
\]

\( I_2 + \text{starch} + 2\text{Na}_2\text{S}_2\text{O}_3 \) (blue) \( \rightarrow 2\text{NaI} + \text{starch} + \text{Na}_2\text{S}_2\text{O}_6 \) (colourless)

Iodine itself has a reddish brown colour, but this is often not intense enough to be used as a good indication of the end-point of the reaction. For this reason, starch is usually used as an indicator because it forms a molecular complex with the iodine that has a deep blue colour. Initially, starch is added to the solution that contains the iodine and the solution goes a dark blue. Then, the solution is titrated with a sodium thiosulfate solution of known molarity.

While there is any \( I_2 \) remaining in the solution it stays blue, but once all of the \( I_2 \) has been converted to \( I \) it turns colourless.
Thus, a change in solution appearance from blue to colourless can be used as the end-point of the titration. The concentration of C=C in the original sample can therefore be calculated by measuring the amount of sodium thiosulfate needed to complete the titration. The higher the degree of unsaturation, the more iodine absorbed, and the higher the iodine value. The iodine value is used to obtain a measure of the average degree of unsaturation of oils, and to follow processes such as hydrogenation and oxidation that involve changes in the degree of unsaturation.

**Saponification Number**

The saponification number is a measure of the average molecular weight of the triacylglycerols in a sample. Saponification is the process of breaking down a neutral fat into glycerol and fatty acids by treatment with alkali:

\[
\text{Triacylglycerol} + 3 \text{KOH} \rightarrow \text{Glycerol} + 3 \text{Fatty acid salts of potassium}
\]

The saponification number is defined as the mg of KOH required to saponify one gram of fat. The lipid is first extracted and then dissolved in an ethanol solution which contains a known excess of KOH. This solution is then heated so that the reaction goes to completion. The unreacted KOH is then determined by adding an indicator and titrating the sample with HCl. The saponification number is then calculated from a knowledge of the weight of sample and the amount of KOH which reacted. The smaller the saponification number the larger the average molecular weight of the triacylglycerols present.

**Acid Value**

The acid value is a measure of the amount of free acids present in a given amount of fat. The lipids are extracted from the food sample and then dissolved in an ethanol solution containing an indicator. This solution is then titrated with alkali (KOH) until a pinkish colour appears. The acid value is defined as the mg of KOH necessary to neutralise the fatty acids present
in 1g of lipid. The acid value may be overestimated if other acid components are present in the system, e.g. amino acids or acid phosphates. The acid value is often a good measure of the break down of the triacylglycerols into free fatty acids, which has an adverse effect on the quality of many lipids.

**Instrumental Methods**

A variety of instrumental methods can also be used to provide information about lipid composition. The most powerful of these is nuclear magnetic resonance (NMR) spectroscopy. By measuring the chemical shift spectra it is possible to determine the concentration of specific types of chemical groups present, which can be used to estimate the concentration of different types of lipids. Indirect information about the average molecular weight and degree of unsaturation of the oils can be obtained by measuring physical properties, such as density or refractive index.

The refractive index increases with increasing chain length and increasing unsaturation, whereas the density decreases with increasing chain length and decreasing unsaturation. Measurements of the refractive index or density can therefore be used to monitor processes that involve a change in the composition of oils, e.g. hydrogenation, which decreases the degree of unsaturation.

**ANALYSING LIPID OXIDATION IN FOODS**

Foods which contain high concentrations of unsaturated lipids are particularly susceptible to lipid oxidation. Lipid oxidation is one of the major forms of spoilage in foods, because it leads to the formation of off-flavours and potentially toxic compounds. Lipid oxidation is an extremely complex process involving numerous reactions that give rise to a variety of chemical and physical changes in lipids:

\[
\text{reactants} \rightarrow \text{primary products} \rightarrow \text{secondary products}
\]

(unsaturated lipids and \(O_2\)) \(\rightarrow\) (peroxides and conjugated dienes) \(\rightarrow\)

(ketones, aldehydes, alcohols, hydrocarbons)
Food scientists have developed a number of methods to characterise the extent of lipid oxidation in foods, and to determine whether or not a particular lipid is susceptible to oxidation.

**Chromatography**

Chromatography is the most powerful method of monitoring lipid oxidation because it provides a detailed profile of the fatty acids and other molecules present in lipids. Valuable information about the lipid oxidation process is obtained by measuring changes in this profile with time, especially when peaks are identified using mass spectrometry or NMR. It is possible to monitor the loss of reactants and the formation of specific reaction products using chromatography. These measurements may be made on non-polar lipids extracted from the food, water-soluble reaction products present in the aqueous phase of a food or volatile components in the head-space of a food.

**Lipid Oxidation**

Lipid oxidation depends on the reaction between unsaturated fatty acids and oxygen. Thus it is possible to monitor the rate at which it occurs by measuring the uptake of oxygen by the sample as the reaction proceeds. Usually, the lipid is placed in a sealed container and the amount of oxygen that must be input into the container to keep the oxygen concentration in the head-space above the sample constant is measured. The more oxygen that has to be fed into the container, the faster the rate of lipid oxidation. This technique is therefore an example of a measurement of the reduction in the concentration of reactants.

**Peroxides**

Peroxides (R-OOH) are primary reaction products formed in the initial stages of oxidation, and therefore give an indication of the progress of lipid oxidation. One of the most commonly used methods to determine peroxide value utilises the ability of peroxides to liberate iodine from potassium iodide. The
lipid is dissolved in a suitable organic solvent and an excess of KI is added:

\[ \text{ROOH} + \text{Kl}_{\text{excess}} \rightarrow \text{ROH} + \text{KOH} + \text{I}_2 \]

Once the reaction has gone to completion, the amount of ROOH that has reacted can be determined by measuring the amount of iodine formed. This is done by titration with sodium thiosulfate and a starch indicator:

\[ \text{I}_2 + \text{starch} + 2\text{Na}_2\text{S}_2\text{O}_3 \text{(blue)} \rightarrow 2\text{NaI} + \text{starch} + \text{Na}_2\text{S}_4\text{O}_6 \text{(colourless)} \]

The amount of sodium thiosulfate required to titrate the reaction is related to the concentration of peroxides in the original sample. There are a number of problems with the use of peroxide value as an indication of lipid oxidation. Firstly, peroxides are primary products that are broken down in the latter stages of lipid oxidation. Thus, a low value of PV may represent either the initial or final stages of oxidation. Secondly, the results of the procedure are highly sensitive to the conditions used to carry out the experiment, and so the test must always be standardised. This technique is an example of a measurement of the increase in concentration of primary reaction products.

**Conjugated Dienes**

Almost immediately after peroxides are formed, the non-conjugated double bonds (C=C-C=C) that are present in natural unsaturated lipids are converted to conjugated double bonds (C=C-C=C). Conjugated dienes absorb ultraviolet radiation strongly at 233nm, whereas conjugated trienes absorb at 268nm. Thus oxidation can be followed by dissolving the lipid in a suitable organic solvent and measuring the change in its absorbance with time using a UV-visible spectrophotometer.

In the later stages of lipid oxidation the conjugated dienes (which are primary products) are broken down into secondary products (which do not absorb UV-visible light strongly).
which leads to a decrease in absorbance. This method is therefore only useful for monitoring the early stages of lipid oxidation. This technique is an example of a measurement of the increase in concentration of primary reaction products.

**Thiobarbituric Acid (TBA)**

This is one of the most widely used tests for determining the extent of lipid oxidation. It measures the concentration of relatively polar secondary reaction products, i.e., aldehydes. The lipid to be analysed is dissolved in a suitable non-polar solvent which is contained within a flask. An aqueous solution of TBA reagent is added to the flask and the sample is shaken, which causes the polar secondary products to be dissolved in it. After shaking the aqueous phase is separated from the non-polar solvent, placed in a test-tube, and heated for 20 minutes in boiling water, which produces a pink colour.

The intensity of this pink colour is directly related to the concentration of TBA-reactive substances in the original sample, and is determined by measuring its absorbance at 540 nm using a UV-visible spectrophotometer. The principle source of colour is the formation of a complex between TBA and malanaldehyde, although some other secondary reaction products can also react with the TBA reagent. For this reason, this test is now usually referred to as the thiobarbituric acid reactive substances (TBARS) method. TBARS is an example of a measurement of the increase in concentration of secondary reaction products.

**Lipid Oxidation Tests**

Rather than determining the extent of lipid oxidation in a particular food, it is often more important to know its susceptibility to oxidation. Normally, oxidation can take a long time to occur, e.g., a few days to a few months, which is impractical for routine analysis. For this reason, a number of accelerated oxidation tests have been developed to speed up this process. These methods artificially increase the rate of lipid oxidation by exposing the lipid to heat, oxygen, metal catalysts, light or enzymes. Even so there is always some
concern that the results of accelerated tests do not adequately model lipid oxidation in real systems.

A typical accelerated oxidation test is the active oxygen method (AOM). A liquid sample is held at 98°C while air is constantly bubbled through it. Stability is expressed as hours of heating until rancidity occurs, which may be determined by detection of a rancid odour or by measuring the peroxide value. Another widely used accelerated oxidation test is the Schaal Oven Test. A known weight of oil is placed in an oven at a specified temperature (about 65°C) and the time until rancidity is detected is recorded by sensory evaluation or measuring the peroxide value.

**Physicochemical Properties**

In addition to their nutritional importance lipids are also used in foods because of their characteristic physicochemical properties, such as mouthfeel, flavour, texture and appearance. They are also used as heat transfer agents during the preparation of other foods, e.g. for frying. It is therefore important for food scientists to have analytical techniques that can be used to characterise the physicochemical properties of lipids.

**Solid Fat Content (SFC)**

The solid fat content (SFC) of a lipid influences many of its sensory and physical properties, such as spreadability, firmness, mouthfeel, processing and stability. Food manufacturers often measure the variation of SFC with temperature when characterising lipids that are used in certain foods, e.g., margarine and butter. The solid fat content is defined as the percentage of the total lipid that is solid at a particular temperature, i.e. \( \text{SFC} = 100 \frac{M_{\text{solid}}}{M_{\text{total}}} \) where \( M_{\text{solid}} \) is the mass of the lipid that is solid and \( M_{\text{total}} \) is the total mass of the lipid in the food.

A variety of methods have been developed to measure the temperature dependence of the solid fat content. The density of solid fat is higher than the density of liquid oil, and so there
is an increase in density when a fat crystallises and a decrease when it melts. By measuring the density over a range of temperatures it is possible to determine the solid fat content—temperature profile:

$$SFC = \frac{\rho - \rho_L}{\rho_s - \rho_L} \times 100$$

where \( \rho \) is the density of the lipid at a particular temperature, and \( \rho_L \) and \( \rho_s \) are the densities of the lipid if it were completely liquid or completely solid at the same temperature. The density is usually measured by density bottles or dilatometry. More recently, instrumental methods based on nuclear magnetic resonance (NMR) have largely replaced density measurements, because measurements are quicker and simpler to carry out (although the instrumentation is considerably more expensive).

Basically, the sample is placed into an NMR instrument and a radio frequency pulse is applied to it. This induces a NMR signal in the sample, whose decay rate depends on whether the lipid is solid or liquid. The signal from the solid fat decays much more rapidly than the signal from the liquid oil and therefore it is possible to distinguish between these two components.

Techniques based on differential scanning calorimetry are also commonly used to monitor changes in SFC. These techniques measure the heat evolved or absorbed by a lipid when it crystallises or melts. By making these measurements over a range of temperatures it is possible to determine the melting point, the total amount of lipid involved in the transition and the SFC-temperature profile.

**Melting Point**

In many situations, it is not necessary to know the SFC over the whole temperature range, instead, only information about the temperature at which melting starts or ends is required. A pure triacylglycerol has a single melting point that occurs at a specific temperature. Nevertheless, foods lipids contain
a wide variety of different triacylglycerols, each with their own unique melting point, and so they melt over a wide range of temperatures. Thus the "melting point" of a food lipid can be defined in a number of different ways, each corresponding to a different amount of solid fat remaining. Some of the most commonly used "melting points" are:

- **Clear point**: A small amount of fat is placed in a capillary tube and heated at a controlled rate. The temperature at which the fat completely melts and becomes transparent is called the "clear point".

- **Slip point**: A small amount of fat is placed in a capillary tube and heated at a controlled rate. The temperature at which the fat just starts to move downwards due to its weight is called the "slip point".

- **Wiley melting point**: A disc of fat is suspended in an alcohol-water mixture of similar density and is then heated at a controlled rate. The temperature at which the disc changes shape to a sphere is called the "Wiley melting point".

**Cloud Point**

This gives a measure of the temperature at which crystallisation begins in a liquid oil. A fat sample is heated to a temperature where all the crystals are known to have melted. The sample is then cooled at a controlled rate and the temperature at which the liquid just goes cloudy is determined. This temperature is known as the cloud point, and is the temperature where crystals begin to form and scatter light. It is often of practical importance to have an oil which does not crystallise when stored at 0°C for prolonged periods. A simple test to determine the ability of lipids to withstand cold temperatures without forming crystals, is to ascertain whether or not a sample goes cloudy when stored for 5 hours at 0°C.

**Smoke, Flash and Fire Points**

These tests give a measure of the effect of heating on the
physicochemical properties of lipids. They are particularly important for selecting lipids that are going to be used at high temperatures, e.g., during baking or frying. The tests reflect the amount of volatile organic material in oils and fats such as free fatty acids.

— The smoke point is the temperature at which the sample begins to smoke when tested under specified conditions. A fat is poured into a metal container and heated at a controlled rate in an oven. The smoke point is the temperature at which a thin continuous stream of bluish smoke is first observed.

— The flash point is the temperature at which a flash appears at any point on the surface of the sample due to the ignition of volatile gaseous products. The fat is poured into a metal container and heated at a controlled rate, with a flame being passed over the surface of the sample at regular intervals.

— The fire point is the temperature at which evolution of volatiles due to the thermal decomposition of the lipids proceeds so quickly that continuous combustion occurs (a fire).

Rheology of Lipids

The rheology of lipids is important in many food applications. Rheology is the science concerned with the deformation and flow of matter. Most rheological tests involve applying a force to a material and measuring its flow or change in shape. Many of the textural properties that people perceive when they consume foods are largely rheological in nature, e.g., creaminess, juiciness, smoothness, brittleness, tenderness, hardness, etc. The stability and appearance of foods often depends on the rheological characteristics of their components. The flow of foods through pipes or the ease at which they can be packed into containers are also determined by their rheology.

Liquid oils are usually characterised in terms of their flow properties (viscosity), whereas viscoelastic or plastic "solids"
are characterised in terms of both their elastic (elastic modulus) and flow properties. A wide variety of experimental techniques are available to characterise the rheological properties of food materials. One of the most important rheological characteristics of lipids is their “plasticity”, because this determines their “spreadability”.

The plasticity of a lipid is due to the fact that fat crystals can form a three-dimensional network that gives the product some solid-like characteristics. Below a certain stress (known as the “yield stress”) the product behaves like a solid with an elastic modulus because the crystal network is not disrupted, but above this stress it flows like a liquid because the crystal network is continually disrupted. Rheological techniques are therefore needed to measure the change in deformation of a lipid when stresses are applied.

REFERENCES


