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Characterising fats and oils

Fats and oils are essential components of our food and their effective characterisation is integral to ensuring quality. This guide outlines the recognised methods to assess fat and oil properties

Fats and oils are lipid materials that are generally soluble in organic solvents, but insoluble in water. While these lipids are chemically similar – in that both are composed of triglycerides – the common distinction is that fats are solid at room temperature and oils are liquid.

Fats and oils have various characteristics – such as melting behaviour, water content and free fatty acid levels – which determine their quality and behaviour. These characteristics can be measured with methods such as titration and UV/VIS spectroscopy.

Melting behaviour

While pure substances have a sharp melting point, complex mixtures such as oils and fats – which consist of mixed triglycerides – gradually soften as the temperature rises and melt over a relatively large temperature interval. Therefore, better characterisation is achieved with the dropping point.

The **dropping point** of an edible fat or oil is the specific temperature at which a sample becomes sufficiently fluid to form a drop that falls from a standardised cup with an orifice, under defined test conditions. The sample preparation as well as the measuring method are described in the AOCS Cc 18-80 method. Table 1 (*following page*) indicates the dropping points of a selection of edible fats.

During dropping point determination, the first drop leaving the standardised cup is detected using a video-based image analysis process. Simultaneous analysis of two samples yields the mean value and the standard deviation of the single results.

The entire measurement is recorded in colour, allowing the process to be followed live and subsequently reproduced as many times as desired.

The **slip melting point** (SMP) can be used to characterise fat and oil mixtures that do not have a sharp melting point.

USP <741>, ISO 6321 and AOCS Cc 3-25 are examples of methods using SMP as a quality control measure to identify and check the purity of substances in foods and drugs.

Palm oil, for example, is used in a wide variety of food products and its constitutents are solid (stearin) and liquid (olein). The main components of stearin melt at higher temperatures than those of olein. By blending different amounts of stearin and olein, the SMP of palm oil can be used to differentiate between palm oil blends used in various food production processes to improve texture and taste or maintain quality and shelf life.

The SMP is the temperature at which a column of solid begins to rise in a capillary tube with both ends open due to a combination of buoyancy and the molten outside surface of the solidified fat.

To determine the SMP, an inner slip melting point capillary tube containing a column of fat (10mm in length) crystallised under controlled conditions is immersed in water, which is then heated at a specific ▶

Type of oil	Mean value (°C)	Standard deviation (°C)	n
Canola oil	-5.95	0.30	4
Red palm oil	23.13	0.44	4
Cocoa butter	29.75	0.17	4
Palm fat	36.55	0.17	4
Margarine	37.20	0.33	12
Pasteurised butter	37.45	0.12	12
Pure ghee	37.92	0.12	12
Hydrogenated vegetable oil	41.82	0.19	12

Source: Mettler Toledo

Table 1: Dropping point of edible fats and oils

Type of oil	Density (g/cm³)	Temperature
Castor oil	0.9500-0.9740	15°C
Cocoa butter	0.9450-0.9760	15°C
Coconut oil	0.9190-0.9370	15°C
Grapeseed oil	0.9190-0.9360	15°C
Mustardseed oil	0.9210-0.9470	15°C
Palm oil	0.9210-0.9470	15°C
Rapeseed (canola) oil	0.9100-0.9170	15°C
Sesame oil	0.9210-0.9420	15°C
Sunflower oil	0.9200-0.9270	15°C

Source: Mettler Toledo

Table 2: Density ranges for edible oils at specified temperature

rate. The temperature at which the column of fat is observed to start rising in the inner capillary tube is recorded as the SMP.

When measured manually via visualisation with the human eye, the SMP can often be difficult to detect due to the transparency of certain substances.

SMP instruments offer fully automated detection based on camera image analysis.

Moisture and water content

A low water content in fats and oils prevents their spoilage and water determination is therefore essential to ensure quality and safety. According to the Asia and Pacific Coconut Community (APCC), water content should not exceed 0.1–0.5%.

Moisture content can include both water and volatiles and halogen moisture analysers are a fast and accurate way to determine moisture content.

Karl Fischer or water-specific titration is an effective means to determine water content in fats and oils. The coulometric technique is preferred given the typical lower water content of fats and oils. However, sample dissolution may pose challenges and chloroform (or chloroform-containing solvent mixtures), may be required for the complete dissolution of fats and oils in KF reagents.

An alternative that avoids such solvents is the warming of the KF titration cell prior to dissolution of the sample. This requires jacketed KF titration cells which can be connected to circulating thermostats.

Tips and hints:

- Sample handling is the most frequent source of errors in KF titration and meticulous sample preparation and administration is a pre-requisite for reliable results. It is recommended, for example, to rinse the syringe with a small amount of sample before the sample is administered.
- Small deviations of slightly drifting balance readings can already be of significant influence. Thus, the balance reading must be perfectly stable when the weight is recorded.
- A balance with a resolution of 0.01mg is needed for precise results.
- 1-decanol or fat-specific solvents sold by specialty reagent manufacturers are recommended. Chloroform is an excellent solvent but its use is restricted due to its toxicity.
- The dissolution capacity of the solvent should be considered in order to ensure that that accuracy and reproducibility (RSD) are high.
- Hard fats are best titrated at elevated temperature. They dissolve quickly at 50°C in a methanol-chloroform solvent (1:1).
- Since edible oils typically contain less than 0.1% water, a larger sample size of up to 10g is required to bring the amount of water to the optimal range for volumetric KF titration. For coulometric titration, 1g is optimal.

Free fatty acids (FFAs)

Free fatty acids (FFAs) in oils and fats are prone to oxidation, leading to rancidity and degradation over time. They can form when bruised fruits or seeds are used for oil production, when harvest is delayed, or fruits and seeds are stored before processing. FFAs can reduce the quality of an oil and are a quality indicator. The FFA content in an oil can be determined by non-aqueous titration.

In this method, 2-4g of sample are dissolved in an ethanol-ether solvent mixture and titrated with potassium hydroxide (0.02 M KOH in ethanol). The indication for non-aqueous applications is pH, and units are given as mg KOH per g sample.

Tips and hints:

- Since ethanol and ether solvents may contain titratable acids, a blank determination of the solvent mixture should be made and factored into the calculation of results.
- In the past, phenolphthalein was used to indicate the endpoint of the titration (when it turned pale pink). Now, however, a pH electrode is used and titration is carried out to the inflection point of the neutralisation curve. The end of titration is automatically detected with an auto-titrator.
- After each titration, the electrode should be rinsed and cleaned with a solvent mixture to avoid cross-contaminating the next sample.
- To ensure hydration of the electrode's glass membrane, the electrode is stored in water overnight, or when not in use. Before titration, the electrode should be rinsed with the solvent mixture.

Analytical methods

Titration is a classic analytical technique that allows the quantitative determination of a specific substance dissolved in a suitable solvent. The substance can be a pure compound or a component of a sample. However, several groups of samples or substances – typically, fats and oils or food samples containing fats and oils – require organic solvents to dissolve.

Titration has undergone a great deal of development, from manual operation of glass burettes and colour indicators to automated instruments that perform a complete analysis, while electrodes or other sensors indicate the progress of the titration. Instead of manual calculations by chemists and technicians, modern auto-titrators automatically evaluate results and prepare the documentation required.

Differential scanning calorimetry (DSC) is a thermal analysis technique and one of the most widely used tests in food processing. It can be used to quantify and determine:

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UV/VIS spectroscopy is used to distinguish different grades of olive oils

- The liquid fraction in edible fats.
- The thermal (oxidation) stability of oils and fats.
- Polymorphic transitions in solid fats.
- Melting behaviour in fats.
- Crystallisation behaviour.
- The influence of hydrogenation on melting behaviour.

UV/VIS spectroscopy is used to distinguish different olive oil grades. Cold-pressed extra virgin olive oil is the highest quality olive oil while olive oils that lack a precise quality declaration are usually blends of refined and virgin or extra virgin olive oils, and are of lower quality.

Lower quality olive oils contain conjugated dienes and trienes. These and other compounds are formed as a result of oxidative degradation processes in the oil. The conjugated carbon-carbon double bonds of the dienes and trienes absorb UV light in the 200-300nm wavelength range.

In contrast, substances with non-conjugated double bonds that are also present in extra virgin oil (such as unsaturated fatty acids) do not absorb light in this spectral range. This provides the basis for a simple method to check the quality of olive oil: low absorption in the 200-300nm spectral range points to high quality extra virgin oil, while high absorption indicates an olive oil of lower quality.

The International Olive Council has defined three criteria that must be fulfilled for an olive oil to be given the extra virgin label when UV/VIS spectroscopy is used for quality control.

Quality indicators

Density: Oil refineries process oils and fats from different origins and before crude oils are unloaded and transferred to storage facilities, a fast quality check is needed to assess parameters such as free fatty acids (FFA) and colour.

Density and refractive indexes allow quick confirmation of the identity of the product in question. Density may also be used to identify mixture properties, as well as for the design of process piping and storage tanks.

Digital density meters and refractomers can measure the density or associated values of liquids in a short time (typically a minute) and require only a small quantity of sample (1ml). A built-in Peltier thermostat enables the adjustment of the sample to the appropriate measurement temperature.

In digital density meters, the density measurement is based on the electromagnetically-induced oscillation of a U-form tube made of glass. A magnet is fixed to the U-tube and a transmitter induces the oscillation. Table 2 (*see page 26*) lists density ranges for edible oils at a specified temperature.

In digital refractometers, a light passes through a prism and hits the sample, and is partly refracted and partly reflected. The reflected light is measured via an optical sensor and the boundary between the dark and light areas corresponds to the critical angle required to calculate the refractive index.

Iodine value: Fats and oils are mixtures of triglycerides, which are made up of three fatty acids linked to glycerol by fatty acyl esters. Fatty acids are long chain hydrocarbons with carboxyl groups (COOH groups), and can be classified as saturated or unsaturated based on the number of double bonds present.

The iodine value (IV) can be used to determine the level of unsaturation in fatty acids, as their double/triple bonds will react with iodine. The higher the IV, the more unsaturated fatty acids are present, which are susceptible to oxidation and rancidification.

IV is defined as the weight of iodine absorbed by 100g of sample (fats, oils), and is an indication of the number of double bonds: the iodine in the Wijs reagent reacts with double bonds in the lipids. The reaction time is one hour and samples are kept in the dark.

Tips and hints:

- The addition of mercuric acetate solution increases the reaction rate of the iodine and can reduce the reaction time to five minutes.
- The excess of Wijs reagent should be 50-60% to ensure complete reaction of the sample with iodine. The sample size should therefore be adjusted depending on the expected iodine value (the degree of unsaturation). Only 40–50% of the iodine in the Wijs reagent should undergo reaction with the sample's double bonds.
- It is recommended to carry out a blank determination more than once to obtain a reliable value. Use the average (mean value) in calculations.

Peroxide value: The peroxide value (PV) of an oil or fat is a primary indication of its deterioration by oxidation, which leads to unpleasant tastes and odours due to the formation of hydroperoxides. These hydroperoxides are measured quantitatively on the basis of their ability to liberate iodine from acidic solutions of potassium iodide.

The sample is dissolved in an acetic acid-chloroform solvent and a saturated solution of potassium iodide (KI) is added, initiating reaction of the peroxides to form iodine.

This article is based on a guide written by Mettler Toledo, a global manufacturer and marketer of precision instruments for use in laboratory, industrial and food retailing applications