Water content, one of the most important properties of food

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Abstract

Water is present in practically every foodstuff. The water content has significant importance for a number of reasons. The determination of water content is therefore the most frequent general analysis performed on foodstuffs. A survey of the most widely used methods is given. They are classified into direct and indirect methods. Direct methods aim at the determination of the water as such. The physical techniques among them are based on a separation of the water. Heating techniques which measure a mass loss under certain conditions are principally problematic as they cannot distinguish between water and other volatile matter. Chemical direct methods are based on a chemical reaction of the water molecules. The most important technique is the Karl Fischer titration. Indirect methods can either measure a property of the sample that depends on the water content, or they observe the response of the water molecules to a physical influence. The latter group comprises the use of low-resolution nuclear magnetic resonance, near infrared spectroscopy and microwave techniques. These extremely rapid methods need a very product specific calibration against a reference method, because water molecules in different bonding situations may behave differently. © 2001 Elsevier Science Ltd. All rights reserved.

1. Different bonding situations of water

Water occurs in different bonding situations. This has an influence on the separability of the water and the possibility to detect it. Bulk or “free” water is easy to determine. This comprises water in gaseous or liquid mixtures and also water at the outer surface, in big cavities and large pores of solid products. Separation of this water fraction is reversible and detection presents no problem. The situation becomes more complicated for water in capillary interstices and in oligomolecular layers adsorbed at the surface. Whereas chemical detection of this water fraction is usually still well feasible, the necessary heat to separate this water fraction from the matrix increases, making it less accessible to physical separation methods. For the last and very tightly bound so-called monolayer water the separation energy becomes very high and will lead to decomposition reactions.

2. Classification of methods for determining water content

Several methods exist to determine the water content of foodstuffs (Isengard, 1995). They may be classified into different groups. Direct methods are those that aim at a quantitative determination of the water itself. The physical techniques among these measure the amount of water obtained or the mass loss observed after separation of the water from the other components in a product. Chemical methods are based on a selective reaction of the water in the sample. Both techniques may be combined by chemically determining the water obtained by separation. Indirect methods may either determine a macroscopic property of the sample which depends on its water content, or measure the response of the water molecules in the sample to a physical influence.

3. Direct methods

3.1. Direct methods based on physical separation of the water

3.1.1. Desiccation by water transfer

One possibility to separate the water from the other components in the product is to place the sample in a desiccator close to a very hygroscopic substance like diphosphorus pentoxide or molecular sieve. The difference in mass before and after the transfer of the water is measured. This process leads, of course, only to a...
distribution of the water in an equilibrium which depends on the difference in hygroscopicity of the two products competing for the water. A part of the water will therefore remain in the sample.

3.1.3. Process

3.1.2. Distillation

Water can also be separated by distillation. Compounds forming an azeotropic mixture with water, like toluene or xylene, that separates again after condensation are often used (ISO, 1980). The water obtained is usually measured by volume. Incomplete separation of the water in the measuring tube and imprecise reading may affect the accuracy and the reproducibility of this technique (Rückold & Isengard, 1999; Suparton, Rückold, & Isengard, 1998).

3.1.3. Oven drying

The most frequently applied method is based on the mass loss that the product undergoes by a heating process. These drying techniques with convective heating principle comprise ordinary oven drying and vacuum oven drying.

It is important to be aware that drying techniques do not measure the water content as such. The result is a mass loss under the conditions applied. These conditions can principally be freely chosen and the results are, consequently, variable. Even the results of official methods with a certain parameter set are only a convention by definition and do not necessarily reflect the true water content. Drying to a constant mass is often required, but a real constancy is only achieved in rare cases. Tightly bound water escapes detection, but a distinction between ‘free’ and ‘bound’ water is nevertheless hardly possible (Rückold, Grobecker, & Isengard, 2000). The mass loss is not only caused by water, but also by all the substances volatile under the drying conditions, either already contained in the original sample or produced by the heating process. The application of low pressure in vacuum ovens reduces the danger of producing volatile decomposition compounds but does not allow a distinction to be made between water and other volatile substances already present in the product. The results of drying methods should therefore not be termed as “water content”; ‘moisture’ is often used instead, but “mass loss” would be the most correct expression.

3.1.4. Infrared, halogen and microwave drying

To shorten the long determination times in drying ovens with convective heating principle, more efficient heating sources have been introduced. In such dryers the samples are exposed, on the pan of a built-in balance to infrared (or ‘halogen’) or microwave radiation and the loss in mass is registered. Different heating modes and end-point criteria are available. The more intensive way of heating in these dryers as compared with usual drying ovens makes samples even more susceptible to decomposition reactions, resulting in the production of volatile matter and, thus, pretending a higher water content of the sample. The results can vary in a very broad range depending on the drying parameters applied.

Mass-loss results can, however, be matched with the results of another method, particularly a reference method, by adjusting the parameters in an appropriate way (Brack & Isengard, 1995; Chin, Kimball, Hung, & Allen, 1985; Handwerk & Maaz, 1987; Heinz & Isengard, 2000; Isengard & Färber, 1998; Isengard & Prager, 1998; Isengard & Walter, 1998). In these cases the two errors by leaving a part of the water undetected and by determining other volatile substances as water compensate each other. Such calibrations are particularly relevant for the rapid techniques mentioned and must be established for every type of product in a specific way. All the parameters like drying mode or programme including temperature and time, stop criterion, sample size and sample distribution on the pan of the balance and, in some situations, even the time interval between consecutive measurements must be considered (Isengard & Färber, 1999).

3.2. Direct methods based on a chemical reaction

3.2.1. Calcium carbide and calcium hydride methods

Water reacts with calcium hydride to give hydrogen (Eq. (1)) and with calcium carbide to give ethyne (Eq. (2)). The amount of these gases can be measured by volume taking temperature and pressure into account (Lück, 1964)

\[ 2\text{H}_2\text{O} + \text{CaH}_2 \rightarrow \text{Ca(OH)}_2 + 2\text{H}_2 \]  
\[ 2\text{H}_2\text{O} + \text{CaC}_2 \rightarrow \text{Ca(OH)}_2 + \text{C}_2\text{H}_2 \]  

(1)  
(2)

The sample needs to be mixed thoroughly with the reagent to bring it in direct contact with the water. Water in the interior of the product particles will not be detected.

3.2.2. Karl Fischer titration

The by far most important chemical determination method is the Karl Fischer titration (Fischer, 1935; Scholz, 1984). It is based on a two-step reaction. In the first step an alcohol ROH (normally methanol) is esterified with sulphur dioxide; to obtain a quantitative reaction, the ester is neutralised by a base Z to yield alkyl sulphite (Eq. (3a)). The ‘classical’ pyridine has been replaced by other bases like imidazole in modern reagents (Scholz, 1984). In the second step alkyl sulphite is oxidised by iodine to give alkyl sulphate in a reaction that requires water; the base, again, provides for a quantitative reaction (Eq. (3b))
ROH + SO₂ + Z → ZH⁺ + ROΣO₂⁻ (3a)
ZH⁺ + ROΣO₂⁻ + I₂ + H₂O + 2Z
   → 3ZH⁺ + ROΣO₂⁻ + 2I⁻ (3b)

Overall reaction:
3Z + ROH + I₂ + H₂O → 3ZH⁺ + ROΣO₂⁻ + 2I⁻ (4)

The consumption of iodine is measured. In the coulometric variation of the Karl Fischer titration, iodine is formed from iodide in the titration cell by anodic oxidation. In the volumetric variation, which is more relevant in food analysis, iodine is added in a solution. The sample is placed in the titration cell which contains the working medium which has been titrated to dryness before the addition of the sample. This working medium consists of methanol in the so-called one-component technique; the titration solution then contains all the other chemical components, iodine, sulphur dioxide and the base, dissolved in an appropriate solvent. In the two-component technique, the working medium contains sulphur dioxide and the base dissolved in methanol; the titration agent is a methanolic solution of iodine. The water equivalent of the respective titrating solution is determined by titrating standards with a known water content. The end-point indication, both in the coulometric and the volumetric varieties, is based on an electrochemical effect. Two platinum electrodes, submerged in the working medium in the titration cell, are polarised either by a constant current (bipotentiometric or voltametric technique) or a constant voltage (biamperimetric technique) and the voltage, respectively, the current to maintain this situation is monitored. After the water of the sample is consumed, iodine can no longer react and the redox couple iodine/iodide is then present and renders the respective oxidation and reduction possible. This makes the voltage necessary to maintain the constant current drop abruptly (voltametric technique) or makes the current resulting from the constant voltage rise abruptly (biamperimetric technique). This sudden change is used to indicate the end point. When the voltage remains below (respectively, the current above) a certain chosen value for a certain time, the determination is completed. This so-called stop delay time is important in order to allow for the detection of water that may not be immediately available, particularly when samples are analysed which are not or not completely soluble in the working medium. In these cases water reaches the working medium by diffusion and extraction processes only with a certain delay.

A major aspect of the Karl Fischer titration is that water must get in direct contact with the reagents, a fact that may cause problems with insoluble samples. Several measures exist, however, to provide for a practically complete water detection (Isengard, 1998). They comprise a long stop delay time, the external extraction of the water and titration of an aliquot of this solution, the internal extraction in the titration vessel prior to the start of the titration, the reducing of the particle size by external sample preparation or by using a homogeniser in the titration cell, the working at elevated temperatures, the addition of solvents to the working medium or the replacement of methanol by other alcohols (Schöfski, 1998; Wünsch & Grünke, 1998) in order to change the polarity.

3.3. Combined direct methods

3.3.1. Evaporation and Karl Fischer titration
The water obtained by a separation technique can also be determined chemically. For this purpose the sample may be heated in a vial of an automatic sample changer (Faas, 1999) or in a weighing boat which is placed in a tubular oven (Supartono & Isengard, 1997). The volatile compounds are then led into the titration cell of a Karl Fischer apparatus where the water is determined selectively. Both the coulometric and the volumetric techniques may be used for this application.

3.3.2. Evaporation and diphosphorus pentoxide method

Another technique (Grobecker, Conneely, Pertsch, & Wagner, 1994) consists in heating the product in a chamber and conducting the volatile matter (water and possibly other compounds) into a detector cell containing diphosphorus pentoxide which absorbs the water by forming different forms of phosphoric acid (Eqs. (5a) and (5b)) give the idealised example of orthophosphoric acid. This reaction product is then electrolysed to yield hydrogen and oxygen and at the same time regenerating diphosphorus pentoxide (Eqs. (6a) and (6b)). Summarily, water is electrolysed (Eq. (7)).

Absorption: 2P₂O₅ + 6H₂O → 4H₃PO₄ (5a)

Dissociation:

4H₃PO₄ + 4H₂O → 4H₅O⁺ + 4H₂PO₄⁻ (5b)

Cathodic reduction:

4H₅O⁺ + 4e⁻ → 4H₂O + 2H₂ (6a)

Anodic oxidation:

4H₂PO₄⁻ → 2P₂O₅ + 4H₂O + O₂ + 4e⁻ (6b)

Overall reaction: 2H₂O → 2H₂ + O₂ (7)

Other substances that react with diphosphorus pentoxide and thus interfering with the reaction scheme must not evaporate from the sample. The water concentration in the moist air flow must not be too high to assure a complete absorption in the detector cell. The original water content must therefore be relatively low or the sample size must be very small. This latter condition can
be problematic for the representativeness of the results for heterogeneous samples.

4. Indirect methods

4.1. Indirect methods based on the measurement of a macroscopic sample property that depends on its water content

4.1.1. Densimetry, polarimetry, refractometry, electrical measurements

The water content affects various properties of the sample. This concerns for instance density. If only approximate results are demanded and if the composition of the product is simple and differs only in water content, a calibration of the water content against density can be established. In a similar way polarimetry and refractometry may also serve as a method for solutions that differ only in water content. A wide field is the application of electric properties of samples to measure their water content (Kupfer, 1997a). These techniques comprise the determination of conductivity, resistance, capacitance or permittivity. As these properties do not, however, depend exclusively on the water content of the product, a calibration is inevitable.

4.1.2. Water activity

Water content and water activity are correlated via sorption isotherms which are very product-specific. If the isotherm should be known for the product in question and if the water activity is measured, water content can be read from the isotherm.

4.2. Indirect methods based on the measurement of the response of the water molecules to a physical influence

These extremely rapid methods that can even be adapted to serve as in-line or at-line techniques comprise low-resolution nuclear magnetic resonance (LR-NMR), near infrared (NIR) and microwave (MW) spectroscopy. The response of each water molecule may however be different depending on the bonding state within a given product. As the distribution of these bonding states is again different from one product to another, a very product-specific calibration against a reference method is necessary.

4.2.1. Low-resolution NMR spectroscopy

The hydrogen nuclei of the compounds contained in a sample have a nuclear spin. Placed in a magnetic field they precess around the field axis with the so-called Larmor frequency which depends on the nature of the nucleus and which is proportional to the magnetic field strength. These spins can be excited and synchronised by using a short, strong radiofrequency pulse, resulting in an oscillating magnetic field that induces an alternating voltage, the NMR signal. The magnitude of these oscillations, which is proportional to the number of hydrogen atoms in the sample, can be measured. After switching off the aligning radio frequency pulse, a relaxation is observed as the nuclear spins fall back into their original state, resulting in a decay of the NMR signal. The rate of this decay depends very much on the surroundings of the hydrogen atoms. In a solid environment the oscillations are heavily damped, and the decay is very rapid. After about 70 μs the NMR signal has disappeared. In a liquid environment, however, the amplitude of the NMR signal may still have 99% of its original value at that time. This allows a distinction to be made between solids, oils and water based on the different decay times, as well as to measure the amount of these different components in the sample (Barker & Koch, 1990; Field, 1989; Günther, 1983; Harz & Weisser, 1986).

Water is not determined as such, but rather hydrogen atoms in a certain environment. Free water is easily detected, while strongly bound water does not fall into this category. All the transitory states may occur. This makes a product-specific calibration necessary to correlate the NMR results with those of a reference method. For practical reasons the water content should be below at about 20% because of the long relaxation time of hydrogen nuclei in free water.

4.2.2. NIR spectroscopy

In NIR spectroscopy, the light absorbance in the range of about 800-2500 nm is measured either in a transmission mode for solutions or in a reflectance mode for solids or even in a combination of both (“transreflectance”) for dispersions. While “classical” infrared spectroscopy is based on the first harmonic oscillations of chemical bonds in molecules, NIR spectroscopy registers overtones of these and combination oscillations. These are much weaker and therefore do not necessitate very thin layers or high dilution rates as required in IR spectroscopy. The disadvantage is the great number of bands in this wavelength area, which cannot be separated into single peaks. Practically every substance in a sample contributes to the NIR spectrum, which becomes more or less a continuum consisting of an overlay of an enormous number of peaks that cannot be attributed precisely to a certain component, although water gives signals at 1450 and 1940 nm. The measurements are also influenced by temperature and by the colour and the particle size of the sample. Therefore analyses need a very product-specific calibration against a reference method based on a great number of single measurements in the whole range of expected water contents.

Originally, NIR spectrometers worked with several wavelength filters, still a widely used technique for many
applications, particularly for the determination of water. The values obtained for each wavelength are each given a mathematical factor with a statistical weight, which is calculated by empirical mathematical approximation to match the result (the sum of these values, plus a constant factor) with the result obtained by a reference method. The quality of the evaluation increases with the number of filters used. An important step forward was the exploitation of the entire NIR wavelength range by the application of Fourier-transform NIR and the use of chemometric evaluation methods (Burns & Ciurczak, 1992; Hirschfeld & Stark, 1984; Molt, 1992; Osborne & Fearn, 1988).

NIR spectroscopy has the advantage that different components and properties of the sample can be measured (after respective calibration) simultaneously.

4.2.3. MW spectroscopy

The velocity of microwaves depends on the dielectric properties of the material they cross. As the dielectric constant increases, the propagation rate becomes smaller and with it the wavelength. The small dipoles of water molecules can easily be oriented in the rapidly oscillating electromagnetic field. This results in an extraordinarily high dielectric constant and explains the great effect of water on the microwave wavelength. The water molecules cannot however follow the oscillations of the field exactly; a small time lag occurs, resulting in partial conversion of the field energy into translational energy, and thus in a gradual damping of the amplitude of the microwaves.

The sample is placed between the emitter and the receiver of the microwaves. For physical reasons no metallic or other highly conductive substances must be present between these microwave antennae. Measurement of the shift of the wavelength and the attenuation of the amplitude of the waves (and thus the microwave energy) after passing through the sample can be used to determine the amount of water present. Several wavelengths may be used in quick succession for the same sample. The mean value of these measurements is more reliable than the result obtained with a single wavelength. The effect depends on the interaction of the waves with the water molecules and thus their number. This means that the measurement is dependent on the concentration of water as well as on the thickness and density of the product layer. These other properties must therefore either be kept constant (as parameters of the calibration) or be measured and be accounted for (Kraszewski, 1980; Kraszewski, 1998; Kupfer, 1997b).

Only freely movable water can be measured accurately, as crystallised or tightly bound water molecules cannot be oriented in the field in the same way. Different intermediate states exist, an aspect that must be covered by the calibration which must therefore be product-specific.

The principle of the microwave resonator method is mainly the same as described for MW spectroscopy (Kent & Meyer, 1982; Kupfer, 1997c; Meyer & Schilz, 1980). Here, too, the wavelength shift and the attenuation of the microwave energy are monitored. The difference is that the measurement is carried out in a resonator chamber, in which standing microwaves are produced. Their frequency equals the resonance frequency of the chamber. The plot of the measured diode signal against the frequency has the form of a slim peak with the maximum at the resonance frequency. This frequency changes as soon as a sample is brought into the resonator chamber, the shift being dependent on the amount of water in the product. At the same time the peak becomes lower and broader. These quantities are evaluated mathematically, allowing calculation of the water content and the packing density independently of each other. A product-specific calibration is also necessary.

5. Determination of water content – a challenge for the analysts

Water is present in every foodstuff, the range of possible water contents reaching from extremely low values in dried products to extremely high values in beverages. Water content is of utmost significance in many respects. Physical properties like conductivity for heat and electrical current, density and particularly rheological behaviour depend on the water content of a product. This has an influence on the design of technological processes. Corrosiveness is dependent on the presence of water. Water may also present an impurity of substances. In reference materials the water content is important in as far as specifications are given on the basis of either dry matter or of the initial water content which, however, may change. Water is necessary for microbiological life and for most of the enzymatic activities and, thus, influences the stability and the shelf-life of foodstuffs. Storage volume and mass depend on the amount of water in the good and so do transport costs. As water is relatively cheap, its amount, particularly in expensive products, is interesting from the commercial point of view and for this and other reasons legal regulations exist.

Water determination is, for practical reasons, therefore a challenge for the analysts as it is certainly the most frequent analysis performed on foodstuffs. But it is a challenge for the analysts also for a second reason. As different methods for water determination exist, the question arises which approach yields the correct result. The problem becomes more difficult due to the fact that water in food is distributed in different bonding states. In particular situations only one of these water fractions may be interesting, for instance only
surface water which provokes caking of a powder, or ‘available’ water which is decisive for microbiological stability, or total water to calculate the contents of components of a product on the basis of dry matter. Where are the limits of such fractions? Can a certain technique distinguish between such fractions? What is an appropriate criterion to judge the correctness of a result? Is this, after all, principally possible, as nobody really knows the true value?

The field of water determination in food (and other materials) will certainly remain a challenge for the analysts for quite some time.

References


