Modern Analytical Techniques for Food Analysis

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Introduction

The aim of food products analysis is obtaining results, which provides information about the composition of food products or food raw material sample. This obtaining information can be carried out on different levels. These levels can be the following: elemental, molecular, and structural. The level of the chemical elements (elemental) means that answer can be given to the question that what (qualitative analysis) and how much (quantitative analysis) can be found in the given sample. Although, on the molecular level the answer can be given about what compounds and crystalline forms consist of the sample from the building elements. The examination of the structure can mean arrangement of the molecules as well (e.g.: determining the order of the amino acids in a protein). The difficulty of the analytical task differs among levels. Any technique selected for food analysis depends on what the researcher is looking for, and there is a host of food properties from which to choose. The development and application of analytical methods and techniques in food science has grown parallel to the consumers concern about what is in their food and the safety of the food they eat.

In the food industry, food safety and quality are still performed as an important issue all over the world, which are directly related to people's health and social progress. Consumers are gradually looking for quality seals and trust marks on food products, and expect manufacturers and retailers to provide products of high quality. All of these factors have underlined the need for reliable techniques to evaluate the food quality (Haiyan and Yong, 2007). Protein, Fiber and fat content are the routine biochemical food quality parameters which are employed world-wide to determine the quality of any food matrices.

Classifications of analytical methods

In case of the applied analytical methods at first one can decide whether the classical or the instrumental analytical method is better to apply. The classical analytical methods, in other words the wet-chemical methods, preceded the instrumental analytical methods by over a hundred years.

Classical analytical methods

In the first years of the analytical chemistry the majority of the analysis was done by dividing the components of the sample that should be examined. During this process precipitation, extraction or distillation was applied. Afterwards the divided components, meant to be used for qualitative analysis, were handled by other reagents with the help of which chemical reaction was used either results in coloured compound or changes of its boiling/freezing point or its solubility. Moreover, reactions which were applied leaded to variously perceptible gases (e.g.: odours) or changes in the compound's optical characteristics or optical activity. When classical analytical method is chosen for the quantitative analysis of the components (to determine its relative or absolute concentration) gravimetric or volumetric method can be used. In gravimetric measurements, the determination of the components' concentration in the given sample is led back to the changes in the mass of the examined analyte or to the mass of the precipitate that was formed with another component. In case of volumetric, also known as titrimetric methods, the component which is analysed, in form of a solution, must be reacted with the reagent, already being in the standard solution and after the reaction of all the amounts of reagent in the sample, from the loss of the amount of the standard solution (from the proportional value of the stoichiometric quantity), the concentration can be counted. All of these classical analytical methods, can be used to either for separating or defining these components, are still used in several laboratories nowadays; but the number of those, who generally use these methods is slowly decreasing due to the appearance of more developed and more conveniently applicable methods of instrumental analysis, these new methods are slowly, but surely superseding the aforementioned ones.

Modern Analytical techniques/ Instrumental analytical methods for food analysis

At the beginning of the twentieth century scientists began to take more and more advantage of the different opportunities provided by the measured components' physical correlations. With the help of them they developed better and better instrumental analytical methods which they found solution for several problems of the classical analytical methods. Such physical characteristics are for example: conductivity, electrode potential, light absorption, light emission, fluorescence and the mass-charge ratio, which were started to be used for quantitative analysis. Furthermore, highly effective chromatographic and electrophoretic techniques were also used to substitute distillation, extraction or precipitation, applied to divide the mixture of components of food or food raw material samples with unusually complex matrix before the qualitative or quantitative determination. The aforementioned new methods, used for the separation and determination of different components, are called instrumental analytical methods. The rapid development of the computer and electronics industry highly contributed to the improvement and spread of the modern instrumental analytical methods.

Performance characteristics of the analytical methods

1. Selectivity,
2. Specificity,
3. Ruggedness,
4. Measurement range,
5. Linearity,
6. Detection limit,
7. Quantitation limit,
8. Accuracy, and
9. Precision.

1. Atomic absorption spectrometry

In atomic absorption spectrometry (AAS) the analysed element is transformed into free ground state atoms with energy transfer (in a flame or graphite furnace). Through this atomic vapour a light with the wavelength

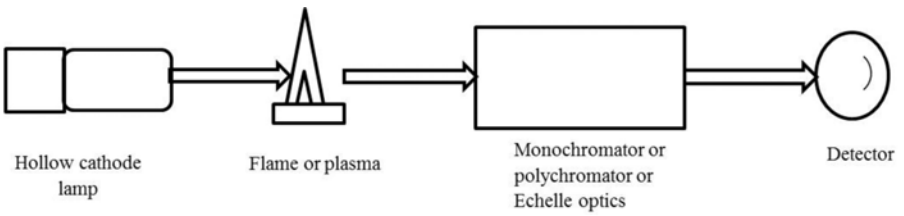

Fig. 1. Working principal of atomic absorprtion spectrometers

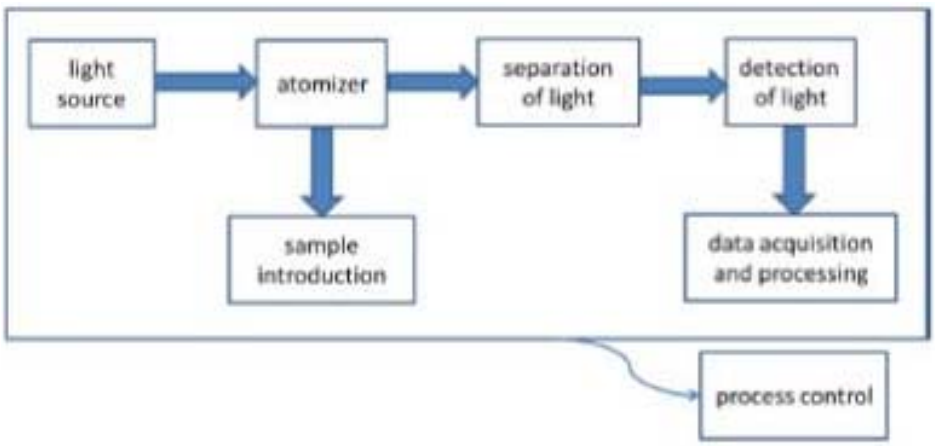

Fig. 2. Units of atomic absorprtion spectrometers

characteristic for the element that is directed through and the decrease of the intensity of light is measured. The wavelength of the used light determines the quality of the analysed material, while the relative decrease of the intensity of light determines the relative and absolute quantity of the element.

2. Inductively coupled plasma mass spectrometry (ICP-MS)

In the field of elemental analytical measurements, inductively coupled plasma mass spectrometry (ICP-MS) is one of the most sensitive method nowadays. The inductively coupled plasma mass spectrometry – issuing from its name – has 2 main parts. The first of them is the inductively coupled plasma, and the second one is the mass spectrometer which carries out the separation and the detection. In ICP-MS the ions of the measured element (isotope) are produced, and when directed into the mass spectrometer, the ions are separated in the magnetic or electrostatic field according to mass/charge (m/z). The mass-charge ratio of the isotope is typical for the quality of the element, while relative intensity of the produced ion beam is proportional to the relative or absolute quantity of the measured element.

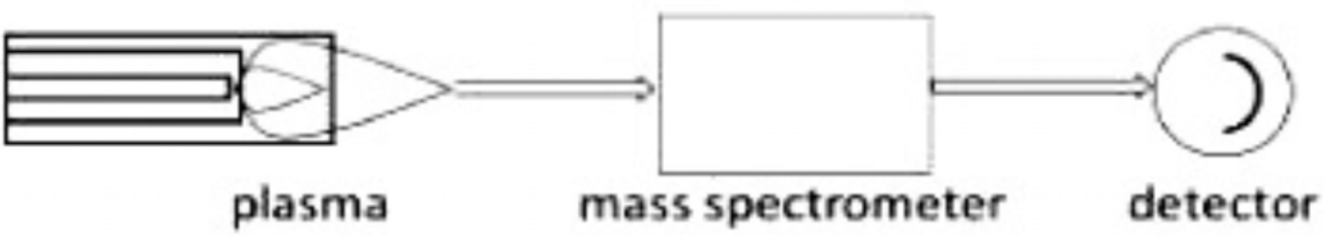

Fig. 3. Working principal of inductively coupled plasma mass spectrometers

3. Chromatography techniques

Chromatography is a useful separation method in the field of food analysis, and has a great impact in analytical chemistry.

3.1 Gas chromatography

Gas chromatography is a column chromatography technique, where the mobile phase is gas and the stationary phase is either an immobilized liquid or a solid packed in a closed tube. GC is useful for separation of thermally stable volatile components of a mixture (for example fatty acid methyl esters). During the gas–liquid GC the sample is vaporized and injected into the head of the column. By using a controlled temperature gradient, the sample is transported through the column by the mobile phase, which usually is an inert gas. The volatile components then are separated based on boiling point, molecular size, and polarity.

GC has been used for the determination of fatty acids, triglycerides, cholesterol and other sterols, gases, solvent analysis, water, alcohols, and simple sugars, as well as oligosaccharides, amino acids and peptides, vitamins, pesticides, herbicides, food additives, antioxidants, nitrosamines, polychlorinated biphenyls, drugs, flavor compounds, and many more.

3.2 Supercritical fluid chromatography

Supercritical fluid chromatography (SFC) refers to chromatography that is performed above the critical pressure (Pc) and critical temperature (Tc) of the mobile phase. A supercritical fluid (or compressed gas) is neither a liquid nor a typical gas. The combination of Pc and Tc is known as the critical point. A supercritical fluid can be formed from a conventional gas by increasing the pressure or from a conventional liquid by raising the temperature.

Carbon dioxide frequently is used as a mobile phase for SFC, because it is not a good solvent for polar and high molecular-weight compounds. Other supercritical fluids are nitrous oxide, trifluoromethane, sulphur hexafluoride, pentane and ammonia. The high diffusivity and low viscosity of supercritical fluids mean decreased analysis times and improved resolution compared to LC.

SFC offers a wide ranges of selectivity adjustment, by changes in pressure and temperature as well as changes in mobile phase composition and the stationary phase. SFC makes possible separation of nonvolatile, thermally labile compounds that are not amenable to GC. SFC can be performed by using either packed columns or capillaries, and has used primarily for nonpolar compounds. Fats, oils, and other lipids are compounds which SFC is increasingly applied.

3.3 High-performance liquid chromatography

Originally, high-performance liquid chromatography (HPLC) was the acronym for high pressure liquid chromatography, reflecting the high operating pressures generated by early columns. By the late 1970s, high-performance liquid chromatography had become the preferred term, emphasizing the effective separations achieved. HPLC can be applied to the analysis of any compound with solubility in a liquid that can be used as the mobile phase. Although most frequently employed as an analytical technique, HPLC also may be used in the preparative mode. There are many advantages of HPLC over traditional low-pressure column liquid chromatography: Speed, because many analyses can be accomplished in 30 min or less, a wide variety of stationary phases, improved resolution and greater sensitivity, because various detectors can be employed, and easy sample recovery, because of less eluent volume to remove. A basic HPLC system consists of a pump, injector, column, detector, and data system. HPLC is widely used for the analysis of small molecules and ions, such as sugars, vitamins, and amino acids, and is applied to the separationand purification of macromolecules, such as proteins and polysaccharides.

3.4 Gas Chromatography-Mass Spectromatry (GC-MS)

Gas Chromatography–Mass Spectrometry (GC-MS) is a hyphenated analytical technique that combines the separation properties of gas-liquid chromatography with the detection feature of mass spectrometry to identify different substances within a test sample. GC is used to separate the volatile and thermally stable substitutes in a sample whereas GC-MS fragments the analyte to be identified on the basis of its mass. The further addition of mass spectrometer in it leads to GC-MS/MS. Superior performance is achieved by single and triple quadrupole modes.

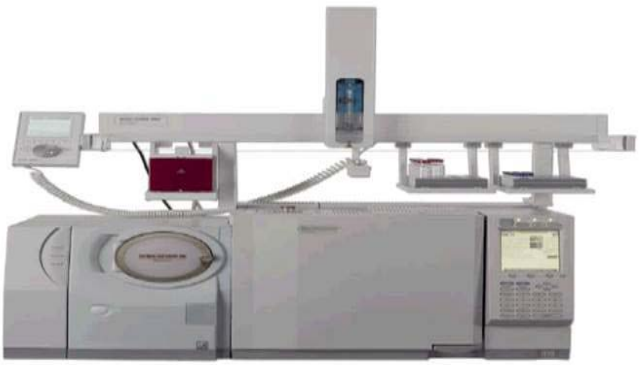

Fig. 4. A typical GC-MS with head space of Shimadzu company

Foods and beverages have several aromatic compounds existing naturally in native state or formed while processing. GC-MS is exclusively used for the analysis of esters, fatty acids, alcohols, aldehydes, terpenes etc. GC-MS is also used to detect and measure contaminants, spoilage and adulteration of food, oil, butter, ghee that could be harmful and should to be controlled and checked as regulated by governmental agencies. It is used in the analysis of piperine, spearmint oil, lavender oil, essential oil, fragrance reference standards, perfumes, chiral compounds in essential oils, fragrances, menthol, allergens, olive oil, lemon oil, peppermint oil, yiang oil, straw berry syrup, butter triglycerides, residual pesticides in food and wine.

4. Infra-red (IR) spectroscopy

Infra-red spectroscopy is used to measure IR radiation absorbed by or reflected from a sample. The absorption of IR radiation is related to the changes of vibrational or rotational energy states of molecules. Its applications for analysis of gaseous, liquid or solid samples, identification of compounds and their quantitative analysis *etc.* The IR spectrum obtained for functional groups of molecules, constitution of molecules and interaction among molecules provides information about the samples.

Main components of an instrument

1. radiation source

2. measuring (and reference) cell
3. wavelength selector
4. detector (transducer)

Types of instruments

1. Simple instruments with a filter
2. Classical instruments with a monochromator
3. Instruments based on an interferometer (FTIR)

4.1 Near-infrared (NIR) Spectroscopy

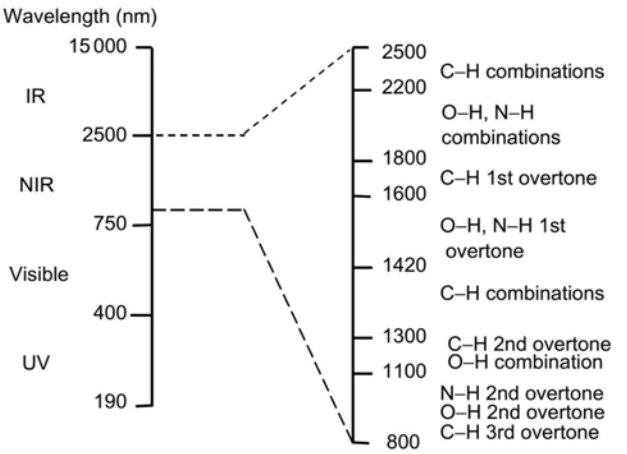

Fig. 5. Principal types of NIR absorption bands and their locations

Near-infrared spectroscopy (NIRS) provides an alternative, non-destructive technology for measuring constituents of biological materials with little sample preparation and is able to provide reliable and accurate results of larger range of samples of multiple properties at one time (Stuth *et al.*, 2003). NIRS is widely used for the quantitative determination of quality attributes such as moisture, protein, fat, and kernel hardness in agriculture and food products (Williams and Norris, 2001). NIRS is broadly accepted in quality assessment of foods, beverages and various other matrices in contemporary scientific fraternity. NIRS is an accepted method to predict forage fiber traits of barley straw (Mathison *et al.*, 1999), rice (Jin, 2007), green cereal crops (Bruno-Soares *et al.*, 1998), leguminous shrubs (Garcia *et al.*, 2004), and oat hulls (Redaelli, 2007).

4.2 Fourier Transform Infrared (FTIR) Spectroscopy

FT-IR is a spectroscopic technique that makes use of the naturally occurring electromagnetic spectrum defined by the wavelengths between 2,500 nm and 25,000 nm. This is the 'mid-infrared' region so the method referred to as 'mid infrared'. Generally, though, it is the name of a technique used to convert measurement data into a usable result (Fourier Transform) that is popular, hence, Fourier Transform Infrared, or FTIR for short. Fourier transform infrared spectroscopy (FTIR) has been available to researchers since the early 1970s (Griffiths & de Haseth, 1986).

FTIR advantages

The overall advantages of using FTIR analysis are that it provides rapid analysis data for better decision making in food and agriculture production processes. It is particularly useful for testing liquid samples such as milk and wine. Compared to traditional analysis methods it requires little or no sample preparation and no chemicals or consumables. It is non-destructive, operator friendly, fast, reliable and precise.

How FTIR works

- Light from a broad-band light source containing the full spectrum of wavelengths to be measured is through a device called an interferometer.
- The interferometer modifies the light in a special way to allow for subsequent processing of the data
- The beam is passed through the sample where a sample-dependent absorption takes place.
- The light is detected and passed to a computer.
- The computer processes all the data to infer what the absorption is at each wavelength and generates a spectrum corresponding to the data using the Fourier Transform technique.

Proximate analysis of foods is one area which can benefit from FTIR, as food systems are mainly composed of fats, proteins, carbohydrates, and moisture, all of which contribute to the gross spectrum obtained. Characteristic absorption bands are associated with these components, e.g. the carbonyl ester and CH signals associated with fat, the amide signals for protein, the COH bands for carbohydrate and the HOH bending absorption of water. Although water absorbs strongly across the IR spectrum, it can readily be ratioed or subtracted out of the spectrum to reveal

the residual absorptions due to other components. In principle, simple standardized, quantitative preparation procedures can be developed to dissolve and disperse most food components in water, or another solvent if selective extraction is required, so that samples are suitable for FTIR analysis.

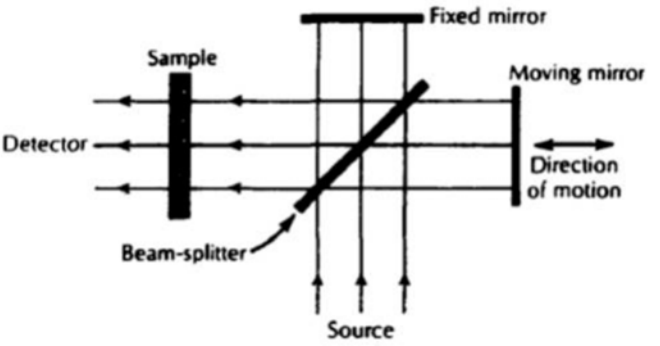

Fig. 7. Schematic diagram of the essential components of an interferometer

Conclusion

Traditional analytical methods *viz.* Folin-Lowry (Protein), Gravimetric (fiber) and Soxhlet method (oil content) are time tested but are tedious and time consuming. These methods are suitable for laboratory level analysis where representative samples can be analyzed. But at industrial level, these methods are not fitted in the scheme and could not serve the purpose of screening or monitoring of quality parameters of each product.

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