# **USE OF CHEMICAL METHODS FOR FOOD QUALITY TESTS**

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**Abstract:** In order to monitor the quality and safety of foods used in a variety of chemical methods. The main reason for the chemical analysis of today have very great significance are savings in time is often greater accuracy during operation, the ability to analyze very small samples and the ability to avoid the often very complicated chemical separation. Spectroscopic techniques constitute the most important group of techniques in instrumental chemical analysis. Atomic absorption spectroscopy (AAS) is primarily used to determine the concentration of metals in foods. UV/VIS is additional (and often high) methods for the identification of natural conjugated compounds, such as plant pigments (carotenoids), polyacetylene, porphyrins, total phenols, flavonoids, total anthocyanins, monomeric anthocyanins percentages polymer color, total antioxidant activity. Infrared spectroscopy (IR) has many advantages because it allows rapid measurement of a large number of sizes, and that when it does not destroy the sample, this technique is used for the analysis of liquids and solids such as milk, cheese and milk powder. Nuclear Magnetic Resonance (NMR) can be used to measure the fat or water soluble ingredients of dairy products. In addition to the spectroscopic analysis of the feed used and chromatographic methods: thin layer chromatography, gas chromatography, liquid chromatography high degree of separation (HPLC) and immunochemical assay (ELISA) using which they can be identified soy protein, pea protein and gluten in heat-processed products as well as the detection of different types of meat and vegetable proteins.

Keywords: spectroscopic methods, chromatographic methods, immunochemical methods, food

#### 1. INTRODUCTION

Food is any substance or product processed, partially processed or unprocessed, and intended for human consumption or can be expected to be consumed by humans (1). Nowadays, the high demands of consumers and quality standards are imposed on producers and processors of all foods the need for the most efficient production and production control. Producing and placing on the market products of uniform quality, hygienically flawless, healthy and sustainable, which do not harm consumer health, can be achieved by applying automation in production with a systematic and disciplined approach to food control, and following and applying the latest scientific knowledge (2). According to their chemical composition, food is a mixture of different compounds, which are of organic and / or inorganic origin. These compounds in food can be of natural or artificial origin. Determining the content of these compounds in food is crucial for assessing the quality and safety of food.

For food analysis, the following are used: physical, physico-chemical, chemical, microbiological and enzymatic methods of determination. This paper will discuss the most commonly used instrumental methods, which belong to the physico-chemical methods for determining the quality and safety of food. The development of an analytical strategy and methodology, which is closely related to the control during primary food production, as well as during its processing and marketing, as well as with the relevant legislation, is an imperative that provides consumers with quality and safe food. on the other hand, through the harmonization of domestic legislation with international regulations in this area, the inclusion of our country in international trade flows is ensured. Given the wide range of methods and techniques used today in the control of food safety, it is very important that the analytical result is reliable and unambiguous, ie confirmed by the confirmatory method. It is also important that analytical methods, whether those used for sample triage or confirmatory, whether multiresidual or for individual determinations, be selective, sensitive, accurate and precise (3).

Instrumental methods of qualitative and quantitative analysis of chemical substances today are very numerous and diverse, and occupy a significant place in analytical research. Instrumental analyzes measure some of the physical properties of a substance and, based on that, determine its chemical composition. Lately, they are increasingly used because almost every physical property of a compound can be the starting point for the development of an instrumental technique. The main reasons why instrumental analyzes are very important today are time savings, often greater accuracy in operation, the ability to analyze very small samples and the ability to avoid often very complicated chemical separations (4).

Many of the food analysis methods used today are based on the basics of analytics that were discovered a hundred or more years ago. Table 1 shows the scheme of analyzes used to determine the basic parameters of food quality and is based on the choice of a rapid analytical procedure for individual parameters without the need to use sophisticated equipment and chemicals (5).

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Table 1. Analyzes used to determine the basic parameters of food quality	
ORIGINAL TERMINOLOGY	ALTERNATIVE TERMINOLOGY
Moisture	Loss of moisture
Ash	Mineral matter
Raw (unprocessed) fat	Fat
	Ether extracts
Crude (unprocessed) proteins	Proteins
Nitrogen-free extracts	Carbohydrates
	Useful carbohydrates
Crude fiber	Crude fiber Useless carbohydrates
	Fibers
	Dietary fiber

In food hygiene, chemical methods play a very important role in assessing their health. In addition to assessing the freshness and sustainability of food as a basic task in food production, processing and trade, the obligation to determine the amount and type of additives in the technological process of production should be emphasized, and in that sense on the maximum permitted amounts of food additives prescribed by the World Health Organization (WHO; Codex Alimentarius) (6).

### 2. APPLICATION OF SPECTROSCOPIC METHODS IN FOOD ANALYSIS

The spectroscopic techniques used are fast, accurate and, most importantly, cheap, and they can be used to control milk, dairy products, meat, meat products and other foods. Rapid near-infrared spectroscopy in routine analytics and process control replaces common, expensive, and demanding classical analytical methods (7). These methods are most commonly used for qualitative and quantitative analysis of milk and dairy product ingredients as well as for determining the total number of bacteria in raw milk (8,9). It is of paramount importance in the analysis and research of substances. Spectroscopic techniques form the most important group of techniques in instrumental chemical analysis. According to the principle on which they are based and performed, spectroscopic techniques can be applied in the laboratory, in an industrial plant (process analysis), or in the field. The limit of identification of individual spectroscopic techniques is different and ranges in a very wide range, from a few picograms to a few grams (10). The use and application of spectroscopic techniques in the food industry is growing, and the answers they provide make it easier to solve problems in food production and distribution.

Ultraviolet / visible (UV/VIS) spectrophotometry is a spectroscopic method that includes the study of the absorption of electromagnetic radiation in the range between 200 and 800 nm. As a large number of organic compounds are not absorbed in this part of the spectrum, UV/VIS spectrophotometry, compared to other structural methods (IC, NMR, MS), has far less application for structural determinations and is mainly used as a complementary method for identifying parts of molecules. which absorb in said area, so-called chromophores. The UV/VIS spectra obtained in this way provide very useful information on the structure of the test compound. For example, it is an indispensable auxiliary (and often main) method for the identification of natural conjugate compounds, such as: plant pigments (carotenoids), polyacetylenes, porphyrins, total phenols (11), flavonoids (12), total anthocyanins, monomeric anthocyanins 13), percentage of polymer color, total antioxidant activity, etc. In addition to its application for the identification of organic compounds, UV/VIS spectrophotometry is widely used today in quantitative analysis. Its advantages over other methods are extremely high sensitivity and easy operation of the instrument (14).

Infrared radiation (IR) is electromagnetic radiation of wavelengths from 0.7 to 500 mm. Its name derives from the fact that the energies of infrared radiation are less than the energies of the visible part of the spectrum to which they continue (15). The IR method has many advantages because they allow fast measurement of a large number of sizes without destroying the sample. They can also be used for online and online process control. Near infrared radiation affects the stretching vibrations of covalent bonds C - H, N - H, and O - H in the molecule. Near infrared spectroscopy techniques use the technique of near infrared reflection (NIR) and near infrared emission (NIT) (16). These techniques can be used to analyze liquids and solids such as milk, cheeses, and milk powder (17).

Atomic absorption spectrophotometry (AAS) is an absorption method that measures the decrease in the intensity of monochromatic radiation as it passes through the atomic vapor of a sample. Hollow cathode lamps made of the element to be determined are used as the radiation source. The samples are evaporated at temperatures from 2,000 to 6,000 K, and then the absorption of electromagnetic radiation of the appropriate wavelength is measured. Due to the high sensitivity, it is possible to determine more elements from the solution even in a very low concentration range. The concentration range is of the order of 10-6 mg / dm3 (or ppm) (18). AAS is primarily used to determine the concentration of metals in foods such as metals in plant material and plant extracts (14). Samples that are already

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liquid (eg fruit juices, wine) can be easily analyzed without prior preparation, ie after dilution with water or a reagent. The basic prerequisite for analysis by atomic absorption spectrophotometry is that the sample be homogeneous and at least in a semi-liquid state. Most often, concentrations are determined from a calibration diagram obtained using a series of standard solutions of the analyzed element of known concentrations. Newer AASs are equipped with a computer device that easily programs the analysis parameters and prints the measurement results. The instrument measures each standard, ie sample, three times, conducts measurement statistics, constructs a calibration diagram and calculates the concentration of an unknown sample. The printout on the printer usually contains a display of a calibration diagram with readings for individual concentrations of the standard and the absorbances and calculated concentrations of the element in the sample (19). Important advantages of AAS are primarily the analysis of about 65 different elements in a whole range of different samples; low limits of detection (mg / cm3, mg / dm3) and possible analysis of traces of metal and small amounts of samples; high precision and accuracy; if the samples are a solution or fine suspension, the pretreatment of the samples is practically superfluous and their direct analysis is possible; AAS is more sensitive to determinations e.g. Ca, Mg, Cu, Fe, Zn, Pb, Hg, Cd in biological material. High sensitivity allows analysis of analyte traces, analysis of small amounts of smalls is possible analysis of material and dilution of solutions which reduces interference (20).

Nuclear magnetic resonance imaging (NMR) can be used to measure the fat or soluble constituents of dairy products. Due to the sensitivity of the measurements to mechanical manipulations of the structure, this technique gave very good results when examining the microstructure of the pear (21). During the squeezing of the cheese, the amount of water in the whey was monitored, but also in the pear by this technique as information of the interaction of water and pears (22). Nuclear magnetic resonance imaging has been applied to study changes in the structure of  $\beta$ -lactoglobulin when heated at pH 2 and 7.4 units at 55°C (23), but also the formation of the structure of ice cream (24).

## 3. APPLICATION OF CHROMATOGRAPHIC METHODS IN FOOD ANALYSIS

Of the chromatographic techniques in food analysis, thin layer chromatography, gas chromatography, high performance liquid chromatography (HPLC) are the most widely used. In this case, too, the procedures for preparing food samples for chromatographic analyzes of food are extremely important (25).

High performance chromatography is a type of liquid chromatography (LiCid Chromatography, LC) that has been significantly improved in terms of selectivity and resolution of mixture components. This was achieved by applying a column filled with a stationary phase composed of spherical micro-particles with a diameter of  $2-5 \mu m$ , or porous monolithic materials that significantly lead to a drop in pressure in the column (26). HPLC uses the selected structural property of the substances and does not change their chemical nature during the analysis. Depending on the chemical structure, the ingredients of the mixture spend different time in the column because they have different retention affinities on the stationary phase. After separating the constituents of the mixture, they are determined by an electrical signal using an appropriate detector, which gives a chromatogram-curve of the signal strength versus time. The area of dependence is directly determined by the concentration of the compound, and the physicochemical properties of the compound determine its position on the chromatogram, retention time (retention time, tR). This parameter is measured from the moment the sample and standard are inserted into the column until they exit the column. The chromatogram provides information on the number of components contained in the analyzed mixture based on the number of signals, the concentrations of individual components based on the relevant signal surfaces (quantitative analysis) and the compound present (based on retention times - qualitative analysis) (27).

HPLC is a method used to purify and identify anthocyanins which are the most important group of water-soluble pigments in plants and comprise a group of over 500 different compounds responsible for the colors of many plants (28,13). Anthocyanins can be separated according to polarity, so that they can be seen on the chromatogram at different retention times. Anthocyanins can be quantified via any purified standard. Cyanidin-3-glycoside is usually used as a standard, and quantification of anthocyanins by HPLC is performed at a wavelength of 520 nm (28,29).

The separation of the components from the mixture by gas chromatography (GC) is based on the difference in the partition coefficients between the stationary liquid and mobile gas phases. Gas chromatography (GC) is a technique most commonly used in combination with mass spectrometry (MS). Complex mixtures can be very easily separated by gas chromatography, and MS is used to identify individual components, because the mass spectrum provides information about their structure. The individual components of the mixture appear on the gas chromatogram in the form of separate peaks. Retention time can serve as a quantity for qualitative definition, but this is not a reliable way, so it should not be used to determine the composition of unknown and previously unidentified compounds (30,31).

### 4. APPLICATION OF IMMUNOCHEMICAL METHODS IN FOOD ANALYSIS

As one of the tests for the detection of pathogens in food based on immunological characteristics, the enzyme-linked immunosorbent assay (ELISA) is widely used. The method is designed to replace detection or isolation on a solid substrate, is relatively easy to perform, can be applied to a large number of pathogens, can be semi-automatic and gives a quick result. Depending on the needs, the goal of this test may be to detect the genus, species or serotype of the microorganism. However, a positive result obtained by an ELISA test must be confirmed by a conventional test. The biggest advantage of this method is the performance of negative screening, ie the possibility of including a significantly larger number of samples that can be tested for the presence or absence of a certain pathogen, assuming that there is an acceptable level of false negative results. Positive results obtained by ELISA techniques must be confirmed by analytical techniques such as aqueous chromatography or gas chromatography and by fluorescent, ultra violet or concentrated spectrophotometric detection techniques (25).

The usefulness of the ELISA method has been confirmed by many authors (32) in proving the types of meat and various plant proteins that are often used as additional ingredients in meat production, for the detection of enrofloxacin residues in chicken muscle and liver (33). Using appropriate antibodies and standards, it is possible to quantify soy proteins, pea proteins and gluten in heat-treated products by ELISA. The ELISA method has been tested several times for the purpose of qualitative and quantitative determination of denatured soy proteins (34).

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