Chapter 14

Determination of Food Quality by Using Spectroscopic Methods

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Additional information is available at the end of the chapter

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1. Introduction

Food is a complex system comprised predominantly of water, fat, proteins and carbohydrates together with numerous minor components. The functional properties of these components, which are governed by their molecular structure and intra- and intermolecular interactions within food system, and the amounts present define the characteristics of food products. Quality of food products refers to the minimum standards for substances to qualify as fit for human consumption or permitted to come in contact with food. Appearance, color, flavor and texture are critical aspects for the sensory quality of food. The food quality includes also chemical, biological and microbial factors, e.g. instability of food products, which limits their shelf life and is connected with irreversible chemical and enzymatic reactions [1]. Recently, public interest in food quality and production has increased, probably related to changes in eating habits, consumer behavior, and the development and increased industrialization of the food supplying chains. The demand for high quality and safety in food production obviously calls for high standards for quality and process control, which in turns requires appropriate analytical tools to investigate food.

Spectroscopic methods have been historically very successful at evaluating the quality of agricultural products, especially food. These methods are highly desirable for analysis of food components because they often require minimal or no sample preparation, provide rapid and on-line analysis, and have the potential to run multiple tests on a single sample. These advantages particularly apply to nuclear magnetic resonance (NMR), infrared (IR), and near-infrared (NIR) spectroscopy. The latter technique is routinely used as a quality assurance tool to determine compositional and functional analysis of food ingredients, process intermediates, and finished products [1]. Additionally, UV–VIS spectroscopy, fluorescence and mid-infrared (MIR) and Raman spectroscopy are used in the food quality monitoring.

The aim of this paper is to demonstrate applicability of four spectroscopic techniques, e.g. UV–VIS spectroscopy, fluorescence, infrared and Raman spectroscopy, as rapid analysis methods to determine the quality of cereals, cereals products and oils. Additionally, physical foundations of the aforementioned methods are described.

2. UV-VIS spectroscopy

Absorption spectroscopy in the UV–VIS region is based on the Lambert-Beer's law, expressed by the following equations (1, 2)

$$I = I_0 10^{-\varepsilon cl} \tag{1}$$

$$\ln \frac{I_0}{I} = \ln \frac{1}{T} = \varepsilon cl = A \tag{2}$$

where: I_0, I – intensity of light coming in and out of the sample, respectively; ε – extinction molar coefficient; c– molar concentration of substance; l– thickness of the sample (cm). The transmission of the light by the sample is shown in figure 1. Absorption of the studied sample depends on the length of the radiation wave, the thickness of the sample and the characteristic extinction coefficient at a given wavelength.

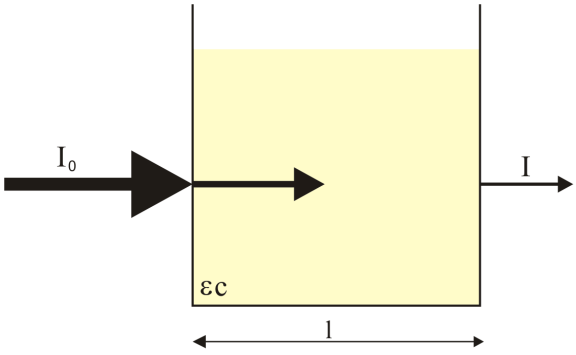

Figure 1. Illustration of Lambert-Beers's law.

The UV-VIS spectroscopy is mainly used to examine the quality of edible oils regarding a number of parameters including the anisidine value. Anisidine value is a measurement of the level of fats oxidation, and is used for the assessment of poorer quality oils. Precisely, it is the measure of aldehyde production during oxidation of fats. The anisidine value (AV) is defined as one hundred-fold value of absorbance of a solution of a fat sample containing al-

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dehydes which have reacted with p-anisidine. The aforementioned aldehydes are dienals or alka-2-enals and both are one of the final products of lipids oxidation. The highest permissible value of AV for edible oils is 8. AV is also an element of Totox (total oxidation value), another factor indicating deterioration level of total fat. The value of Totox is calculated as the sum of two-fold value of AV and peroxide value. According to european standard [2], the measurement of absorbance is performed for three solutions (p-anisidine and sample of fat (A1), acetic acid and sample of fat (A0), and, lastly, blind sample being a mixture of p-anisidine and isooctane (A2)) at the wavelength 350 nm in a 1 cmcuvette. Theanisidine value can be calculated from the formula (3)

$$AV=100QV\left[1.2\left(A_1-A_2\right)-A_0\right]m^{-1} \tag{3}$$

where: Q – content of the sample in the solution based on which the AV is expressed (g cm^{-3}); V – volume in which the fat sample is dissolved (cm^3); m – weight of the fat sample (g).

The anisidine value can be also measured by using Flow Injection Analysis (FIA) combined with UV–VIS spectroscopy. Thanks to the implementation of FIA, the period of time required for analysis can be significantly shortened. Additionally, the number of reagents is also maintained at very reasonable level. Sample of fat dissolved in propanol-2 is injected into continuous flow of p-anisidine with a mixture of solvents: propanol-2 and glacial acetic acid. Spectrophotometer is used as a detector, and the value of absorbance is measured at 350 nm [3].

The process of fat deterioration is also described by the peroxide value (PV). The deterioration takes place during lipids' exposition to some external factors including temperature, daylight and oxygen. It results in production of peroxides and hydroperoxides, which are regarded as products of fatty acids oxidation. The highest value of PV for oil produced through cold press extraction is 10 meq O_2 kg^{-1}, while regarding refined oil it may reach the amount of 5 meq O_2 kg^{-1}. The PV value is measured by employing UV–VIS spectrometer as detector [4, 5]. Method of PV measurement of the frying canola oil was developed by Talpur et al. [6]. The authors used stoichiometric reaction of triphenylphosphine (TPP) with the hydroperoxides to produce triphenylphosphine oxide (TPPO) which shown absorbance maximum at 240 nm. Therefore, the developed method could serve as an alternative to the titration method for the determination of PV in frying oils due to high correlation of peroxide values measured by both methods.

Another parameter of oil quality is general colour which is determined by the saturation of chlorophyll or carotenoid pigments. Unlike oil produced through cold press extraction, refined oil has low saturation intensity colour, as most pigments are removed in the oil refinement. Carotenoid pigments are included to antioxidants. For this reason, oils with high content of carotenoinds are regarded as healthier and of higher quality. The general colour is assayed spectrophotometrically for oil samples diluted in CCl_4, at two wavelengths: 460 nm for carotenoid pigments and 666 nm for chlorophyll pigments for oil samples diluted in

CCl_4 due to polish standard [7]; or 442 nm for carotenoid pigments and 668 nm for chlorophyll pigments when oil samples are dissolved in hexane [7]. Absorbance values obtained for carotenoid and chlorophyll pigments are summed up, multiplied by 1000, and given in the form of an integer or as general colour value:

$$B=\left(A_{460}+A_{666}\right)1000 \tag{4}$$

or

$$B=\left(A_{442}+A_{668}\right)1000 \tag{5}$$

The concentration of carotenoids can also be detected through the use of the UV–VIS method due to the British norm BS 684: Section 2.20:1977 [8]. The absorbance of oil diluted in cyclohexane is measured at the wavelength 445 nm and the proportion of carotenoid expressed by β-carotene content can be calculated using the following equation

$$\beta\text{-}car\left(mg\times kg^{-1}\right)=383E\left(PC\right)^{-1} \tag{6}$$

where: E – the difference in measured absorbance values for oil sample and cyclohexane; P – optical pathlength (cm); C – concentration of the sample (g $100ml^{-1}$).

Chlorophylls also influence general colour of oils, especially pheophytins, which have a prooxidative properties. They give some bitter note as well as green colouring to both cold extracted oil and olive oil from green olives. The smaller concentration of chlorophylls in oil sample, the oil has the higher quality. The chlorophyll concentration can be determined with the AOCS Official Method Cc 13d-55 [8, 9]. Following the method, the measurement of chlorophyll absorption is performed at three wavelengths, namely: 630 nm, 670 nm and 710 nm. They refer to the absorbance of the oil sample diluted in carbon tetrachloride at λ = 625.5, 665.5 and 705.5 nm for oil samples diluted in mixture of ethanol and isooctane, or λ = 630, 665 and 710 nm for oil samples diluted in mixture of ethanol and heptane. The concentration of total chlorophyll (in carbon tetrachloride) is calculated from the following equation:

$$Chl\left(mg\times kg^{-1}\right)=\left[A_{670}-0.5\left(A_{630}+A_{710}\right)\right]\left(0.901L\right)^{-1} \tag{7}$$

where: A – is the absorbance of the oil at the respective wavelength; L – the cell thickness (cm).

The main chlorophyll pigment, occurring in oils, is pheophytin α. The concentration of this dye can be calculated from the equation (8), as featured in the work of Psomiadou and Tsimidou [10]:

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$$Pheop\alpha\left(mg \times kg^{-1}\right) = 345.3\left[A_{670} - 0.5\left(A_{630} + A_{710}\right)\right]L^{-1} \quad (8)$$

where: A – is the absorbance of the oil at the respective wavelength; L - the cell thickness (mm).

3. Fluorescence

Fluorescence is the emission of light subsequent to absorption of ultraviolet or visible light of a fluorescent molecule, called a fluorophore. To typical fluorophores are included quinine, fluorescein, acridine orange, rhodamine B and pyridine 1 [11]. The general principle of the phenomenon is illustrated by Jablonski diagram, as shown in figure 2. The singlet ground, first and second electronic states are depicted by S_0, S_1, and S_2, respectively. The first step of the fluorescence is the excitation of the molecule from the ground state (S_0) to the one of excited states (S_1, S_2) by the absorption of light. This is followed by a vibrational relaxation or internal conversion, where the molecule undergoes a transition from an upper excited state (S_2) to a lower one (S_1), without any radiation. Finally, the fluorescence occurs, typically 10^{-8} s after excitation, when the electron returns to the ground state (S_0). Emitting light has energy equal to the difference between energies of ground and excited states.

Food analysis has exploited the characteristic advantages of fluorescence spectroscopy, i.e. high sensitivity and specificity. However, the technique alone is not usually used in this field. It is mainly linked with liquid chromatography (e.g. HPLC), and the fluorymeter is used as a detector. The combination of these techniques is still advantageous for detecting extremely low concentrations of contaminants such as toxins (mycotoxins), pathogenic microbes (bacterial species: *Salmonella*, *Escherichia coli*), antibiotics (e.g. penicilin, tetracycline, oxytetracycline), and food additives (e.g. aspartam, salicylates). Other important applications of fluorescence in food area is the analysis of structure changes in proteins, analysis of some carbohydrates and lipids in oils.[12]

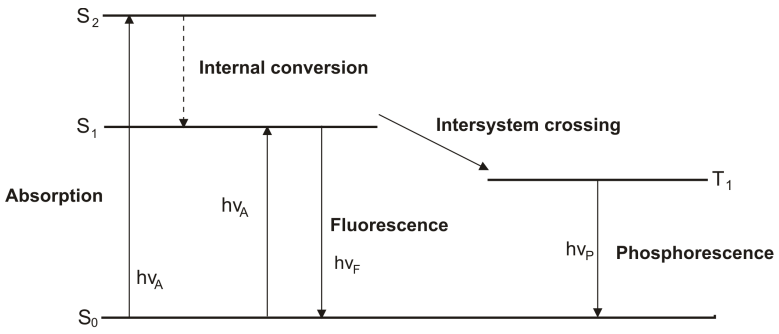

Figure 2. Jablonski diagram.

The high specificity of fluorescence is due to use of two spectra, i.e. excitation and emission spectra, while the high sensitivity of the technique is a result of measuring radiation against absolute darkness. However, the use of only excitation and emission wavelengths could limit the ability of fluorescence spectroscopy to determine the quality of food systems. To comply with this requirement, the variation in the excitation and emission wavelengths allows simultaneous determination of compounds in several food products. This could be realized by using synchronous fluorescence spectroscopy (SFS) [13].

Fluorescence spectroscopy is one of the most valuable instrumental analytical techniques for determining causes of food poisoning by analyzing concentration of toxins, especially mycotoxins. Almost all mycotoxins, apart from aflatoxins (aflatoxins B_1 and B_2 exhibit blue fluorescence, while aflatoxins G_1 and G_2 show yellow-green fluorescence [14]), do not exhibit fluorescence. For this reason, the technique is connected with other analytical techniques and spectrofluorymeter serves as a detector. Corneli and Maragos (1998) used capillary electrophoresis (CE) with a laser induced fluorescence detector to determine ochratoxin A in roasted coffee, corn and sorghum [15]. CE has also been used in corn samples to analyze fumonisin B1, which was fluorescein-labeled due to lack of a UV chromophore [16]. Maragos and Plattner (2002) developed a rapid test for deoxynivalenol (DON) in wheat using the principle of fluorescence polarization (FP) immunoassay. The assay was based on the competition between DON and a novel DON-fluorescein tracer for a DON-specific monoclonal antibody in solution. FP immunoassay utilizes the interaction of a toxin-specific antibody with a toxin-fluorophore conjugate (tracer) to effectively decrease the rate of rotation of the tracer. Binding of the antibody to the tracer increases polarization. In the presence of free toxin less of the antibody is bound to the tracer, reducing polarization [17]. The FP immunoassays have been also developed for the fumonisin mycotoxin detection [18].

Fluorescence has been also used in analysis of cereals and cereals products. Zandomeneghi [19] used fluorescence spectroscopy to differentiate between different cereal flours (e.g. rise, maize). Emission spectra of red and white wheat kernels were recorded by Ram and coworkers (2004) and a clear difference was observed between the two group of samples. This difference has been attributed to the morphological variation in the pericarp and nuclear organization of the two varieties of wheat [20]. The fluorescence spectroscopy has been also used to monitor wheat flour refinement and milling efficiency by recording emission spectra of ferulic acid and riboflavin [21].

One of the most important edible oils is olive oil, which market price depends on its quality. The most expensive is the extra-virgin olive oil (EVOO) owing to its high quality. For economic reasons, it may be adulterated by the addition of cheaper oils such as refined olive oil, residue oil, synthetic olive oil-glycerol products, seed oils and nut oils. For this reason, a rapid method to detect such a practice is important for quality control and labeling purposes [13]. Sayago et al. [22] applied fluorescence spectroscopy for detecting hazelnut oil adulteration in virgin olive oils. Kyriakidis and Skarkalis [23] used excitation wavelength of 360 nm to differentiate between common vegetable oils, including olive oil, olive residual oil, refined olive oil, corn oil, soybean oil, sunflower oil and cotton oil. All the oils studied showed a strong fluorescence band at 430 – 450 nm, except for virgin olive oil, which exhibited a low

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intensity at both 440 and 455 nm, a medium band around 681 nm and a strong one at 525 nm. The latter two bands have been ascribed to chlorophyll and vitamin E compounds, respectively. All refined oils showed only one intense peak at 445 nm, which is due to fatty acid oxidation products formed as a result of the large percentage of polyunsaturated fatty acids present in these oils. Fluorescence is regarded as the technique that provided the best models for anisidine and iodine values, oligomers and vitamin E content in deteriorated oil after repeated frying cycles [24].

4. Infrared spectroscopy

Infrared (IR) radiation was discovered by F.W Herschel in 1800. This is an electromagnetic radiation extending from 780 nm to 1 mm. The IR range is divided into the following three bands: near-infrared (NIR; 780 nm – 5 μm), mid-infrared (MIR; 5 – 30 μm) and far-infrared (FIR; 30 – 1000 μm).

Infrared (IR) spectroscopy is a technique based on the vibrations of the atoms of a molecule. An infrared spectrum is commonly obtained by passing infrared radiation through a sample and determining what fraction of the incident radiation is absorbed at a particular energy. The energy at which any peak in an absorption spectrum appears corresponds to the frequency of a vibration of a part of a sample molecule. There are different kinds of vibrations observed in the infrared as well as Raman spectra. Vibrations observed in diatomic molecules are shown in fig.3. IR spectroscopy gives information on molecular structure through the frequencies of the normal modes of vibration of the molecule. A normal mode is one in which each atom executes a simple harmonic oscillation about its equilibrium position. All atoms move in phase with the same frequency, while the center of gravity of the molecule does not move. There are 3N-6 normal modes of vibrations (known as fundamentals) of a molecule (3N-5 for linear molecules), where N is the number of atoms. For a molecule with no symmetry, all 3N-6 fundamental modes are active in the IR and may give rise to absorptions. There are also observed overtones in the IR spectra. Overtones has frequencies corresponding approximately to twice, three times etc. that of the fundamental. The frequencies of many overtone bands are in the NIR region [25].

Vibrations of certain functional groups such as –OH, $–NH_2$, $–CH_3$, C=O, $C_6H_5–$, and so on always give rise to bands in the IR spectrum within well-defined frequency ranges regardless of the molecule containing the functional group. The IR spectrum of any compound that contains a C=O group has a strong band between 1800 and 1650 cm^{-1}. Compounds containing $–NH_2$ groups have two IR bands between 3400 and 3300 cm^{-1}. The spectrum of a compound containing the $C_6H_5–$ group has sharp peaks near 1600 and 1500 cm^{-1} due to stretching modes of the benzene ring. The explanation of these characteristic diatomic group frequencies lies in the approximately constant values of the stretching force constant of a bond in different molecules. Thus, the IR spectrum can be regarded as a 'fingerprint' of the molecule [26].

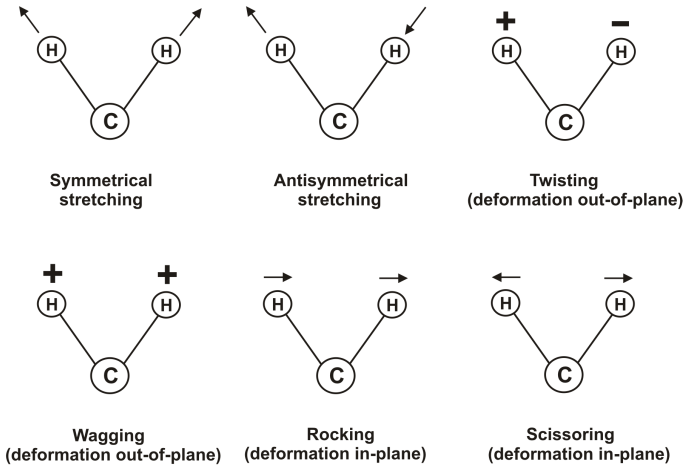

Figure 3. Oscillations observed in diatomic molecules.

4.1. Near-infrared spectroscopy

Near-infrared (NIR) spectroscopy has been primarily employed in the quantitative analysis of foods. The spectroscopy has been applied to measure moisture, fat, protein and carbohydrate content in wide variety of foods. The most significant advantage is its ability to determine simultaneously several components in a food sample within a short time. The precision of NIR analysis for a wide range of applications is comparable to or better than that of the chemical techniques it replaces. On the other hand, the main disadvantage of NIR quantitative analysis is that it requires calibration using samples of known composition. This has seriously limited the use of NIR spectroscopy because of the large amount of time and expense required for the development of calibrations. This disadvantage is compounded by the problem of calibration instability resulting from changes in sample or instrument characteristics over time, which can make frequent recalibration necessary, and the lack of transferability of calibrations owing to optical differences between instruments. Other disadvantages of NIR analysis include the need for high-precision spectroscopic instruments, the complexity of data treatment, and the lack of sensitivity for minor constituents [27].

NIR region of the IR spectrum are due to overtones and combinations of the fundamental vibrations observed in the MIR region. Overtones has frequencies corresponding approximately to twice, three times etc. that of the fundamental, while combination bands arise by interaction of two or more vibrations taking place simultaneously and the frequency of a combination band is the sum of multiples of the relevant fundamental frequencies. Vibrations involving C–H, O–H, N–H and possibly S–H and C=O bonds are responsible for the majority of the observed absorption bands in the NIR region. Table 1 presents principal absorption bands of water, oil, protein and starch, which are observed in the NIR region.

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Wavelength [nm]	Assignment
	Water
1454	1st overtone O–H stretching
1932	O–H combinations
	Proteins
1208	2nd overtone C–H stretching
1465	1st overtone H–N and O–H stretching
1734	1st overtone C–H stretching
1932 2058 2180	combinations N–H and O–H stretching
2302 2342	combination C–H stretching
	Oil
1210	2nd overtone C–H stretching
1406	1st overtone H–N and O–H stretching
1718 1760	1st overtone C–H stretching
2114	combinations N–H and O–H stretching
2308 2346	combination C–H stretching
	Starch
1204	2nd overtone C–H stretching
1464	1st overtone H–N and O–H stretching
1932 2100	combinations N–H and O–H stretching
2290 2324	combination C–H stretching

Table 1. Principal absorption bands of water, oil, protein and starch observed in the NIR region.

The application of NIR analysis in wheat and wheat products has included flour yield, damaged starch, water absorption, dough development time, extensibility and loaf volume measurements. The use of NIR technology to determine the protein and moisture contents of both wheat and flour is now routine practice in flour mills worldwide. It is used for testing each delivery of wheat in order to make decisions about acceptance, price and binning; for determination of conditioning time from measurement of hardness; and for analyzing flour to check that it complies with specifications before shipment to the customer [28].

NIR was used in Australia to predict optimum fertilizer requirements of cereal crops by analysis of total nitrogen and carbohydrate (fructan) in plant tissue samples [29]. Wheat hardness had been measured in both meal and whole grain by using NIR [30]. NIR spectroscopy has also been shown to be useful in the study of changes in starches during processing and storage [31]. This utility is primarily due to the sensitivity of the O–H stretching mode overtone absorptions of starch and of the water bound to starch to changes in hydrogen bonding that accompany changes in starch structure. The spectroscopy combined with chemometrics has been applied to discriminate wheat varieties [32]. It was developed a discriminant equation, which gave 94% correctly identified varieties.

Recently, NIR spectroscopy is often connected with hyperspectral imaging system. Canadian wheat classes has been determined by using near-infrared hyperspectral imaging (NIR-HSI) system [33]. Seventy-five relative reflectance intensities were extracted from the scanned images and used for the differentiation of wheat classes using a statistical classifier and an artificial neural network (ANN) classifier. Classification accuracies were 100%. This imaging system has also been used to detect spectral differences between healthy and damaged products, which are connected with different chemical composition of these products. Singh et al. [34, 35] studied insect-damaged kernels. It was observed that insect-damaged kernels had less starch compared to healthy ones, due to consumption of starch by insects during their development.

The study of food authenticity involves establishing whether a sample is genuine in terms of its description, including geographical origin. The application of NIR for authenticity testing of coffee, fruit pulps, milk powders, orange juice, pig carcasses, rice, sausages, sugars, vegetable oils, wheat grain and wheat flour have been reviewed by Downey [36]. This spectroscopy is required to classify within a series of possible classes, to identify a particular kind of adulteration or to quantify adulteration.

4.2. Mid-infrared spectroscopy

Mid-infrared (MIR) spectroscopy can both provide information on structure-functionality relationships and serve as a quantitative analysis tool. For this reason, it is regarded as a highly valuable technique for both food-related research and quality control purposes in the food industry. The Fourier-transform infrared spectroscopy (FTIR) is used the most often.

The mid-infrared spectrum (4000 – 400 cm^{-1}) includes four regions: the X–H stretching region (4000 – 2500 cm^{-1}), the triple-bond region (2500 – 2000 cm^{-1}), the double-bond region (2000 – 1500 cm^{-1}) and the fingerprint region (1500 – 600 cm^{-1}). The fundamental vibrations of the X–H stretching region are generally due to O–H, C–H and N–H stretching. Vibrations of C≡C and C≡N bonds are mainly observed in the triple-bond stretching region. Whereas absorption bands corresponding to C=C, C=O and C=N occurs in the double-bond region. The “fingerprint” bands are connected mostly with bending and skeletal vibrations The important mid-infrared bands associated with major food components (water, proteins, fats and carbohydrates) are summarized in table 2 [25].

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The MIR spectroscopy, similar to NIR spectroscopy, is applying to analysis of moisture, protein, carbohydrate and fat content in food products. Owing to the intense absorption of MIR radiation by water, FTIR was used to determination of moisture of food emulsions e.g. butter [37] and mayonnaise [38]. FTIR technique is also well established as a powerful tool for the study of protein secondary structure, based primarily on examination of the amide I region (1600 – 1700 cm^{-1}) [39]. Proteins are widely used as ingredients in the food processing industry because of their useful properties such as emulsification, gelation and thickening. These properties are highly related to the secondary structure of protein, which can change during processing and storage of food products [27]. Examination of amide I region are made by mathematical process called deconvolution. Deconvoluted amide I band of wheat gluten are presented in figure 4 [40].

Wavenumber [cm^{-1}]	Assignment
	Water
3200 – 3600	O–H stretching
1650	H–OH stretching
	Proteins
1600 – 1690	Amide I (C=O stretching)
1480 – 1575	Amide II (C–N stretching and N–H bending)
1230 – 1300	Amide III (C–N stretching and N–H bending)
	Fats
2800 – 3000	C–H stretching
1725 – 1745	C=O stretchnig
970	C=C–H bending
	Carbohydrates
2800 – 3000	C–H stretching
800 – 1400	Skeletal stretching and bending

Table 2. The major bands of food components localized in MIR region.

MIR spectroscopy play a crucial role in research on the chemistry of fats and oils. An important result of early investigations of the IR spectra of fats and oils was the identification of an absorption band at 996 cm^{-1} which is characteristic for isolated trans double bonds. Although the double bonds in naturally occurring fats and oils predominantly have the cis configuration, extensive cis-trans isomerization occurs during industrial catalytic hydrogenation processes, which are widely employed to convert oils to fats and to increase the oxidative stability of polyunsaturated oils. Van de Voort et al. [41] developed a method for the simultaneous determination of trans unsaturation, cis unsaturation and total unsaturation,

which is traditionally expressed as the iodine value (IV), as well as saponification number (SN) (a measure of weight-average molecular weight).

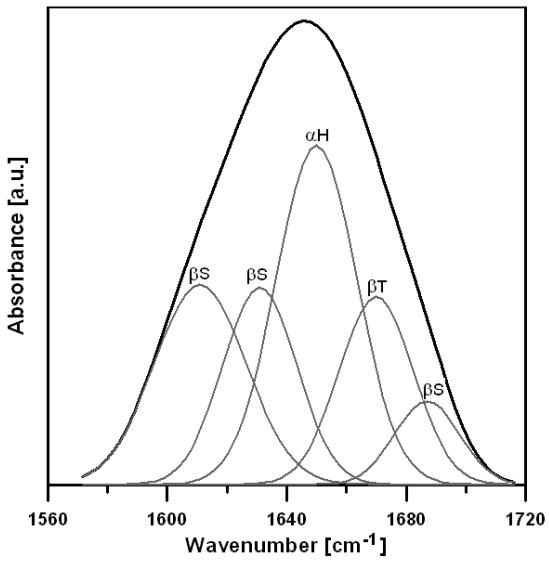

Figure 4. Deconvoluted amide I band of wheat gluten (αH - α-helix, βS - β-sheet, βT - β turns).

Another area of application of FTIR spectroscopy that has been investigated is its use in the assessment of oil quality and stability. The reaction between unsaturated lipids and atmospheric oxygen under ambient conditions, termed lipid autooxidation, is a leading cause of deterioration of fats and oils, as well as of any lipid-containing food, as it gives rise to the off-flavors and unpalatable odors associated with oxidative rancidity. FT-IR spectroscopy proved to be the most direct and accurate method of monitoring gross changes in the frying oil over time [24, 42]. A quantitative FTIR method was used for monitoring the oxidative state of frying oils, based on the determination of anisidine value (AV), a measure of aldehydes that are major secondary oxidation products in polyunsaturated oils, has also been reported [43]. FTIR methods have also been developed to serve as alternatives to the peroxide value (PV) test, which is widely employed by the fats and oils industry to assess the oxidative status and stability of refined oils. This method entails measurement of the hydroperoxide OO–H stretching absorption, which is observed at 3444 cm^{-1} in the spectra of neat oils [44]. FTIR spectroscopy was also used to understand in more detail the mechanisms of thermally induced oxidative processes (thermal oil degradation) in extra-virgin olive oils [45].

FTIR methods for the determination of other minor components present in oils, including free fatty acids in refined [46] and crude oils [47], β-carotene in palm oil [48], and phospholipids in vegetable oils [49], have also been reported.

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Likewise fluorescence, MIR spectroscopy is used to study adulteration of olive oil by other edible oils. The edible oils widely employed in virgin olive oil adulteration can be lower quality olive oil (refined or pomace olive oil) or other vegetable or seed oils such as corn, peanut, cottonseed, sunflower, soybean and poppy seed oils [50]. Gurdeniz and Ozen [51] wanted to demonstrate that MIR spectroscopy connected with chemometrics is a rapid method to detect and quantify adulteration of extra-virgin olive oil (EVOO) with vegetable oils (rapeseed, cottonseed, and corn-sunflower binary mixture). The adulteration of EVOO by different concentrations of palm oil was studied by using FTIR technique [52]. Not only EVOO is adulterated by a low-quality edible oils but also virgin coconut oil (VCO), which possesses several biological activities such as antiviral and antimicrobial. Rohman and Che Man used FTIR spectroscopy combined with chemometrics to determine the level of adulteration of VCO with corn and sunflower oils [53].

4.3. Far-infrared spectroscopy

The region below 400 cm^{-1} down to 10 cm^{-1} is defined as the far-infrared. The region below 200 cm^{-1} is not readily accessible and there are not many useful spectra-structure correlations in this region. However, compounds containing halogen atoms, organometallic compounds and inorganic compounds absorb in the far-infrared and torsional vibrations and hydrogen bond stretching modes are found in this region [26].

5. Raman spectroscopy

Raman effect arises from the interaction of incident photons with electrons of the matter under investigation (inelastic scattering of the incident light). During this interaction the photon can lose (Stokes' process; $h(\nu-\nu_R)$) or gain (anti-Stokes' process; $h(\nu+\nu_R)$) energy equal to the vibrational energy of the atoms (see fig.5). Consequently the vibrational energy of the atoms increases or decreases. Such communication is possible for the motions of atoms, which modulate the polarizability of the molecule. Intense Raman bands will be observed from non-polar groups, particularly aromatic rings, the vibrations of which produce considerable modulation of polarizability. The resulting Raman spectrum, presented in wave numbers (cm^{-1}) as the difference between the excited and emitted photon energy, is the vibrational spectrum of the molecule. The effect is very weak, because the energy exchange probability is low [54].

Raman spectroscopy (RS) is a vibrational spectroscopy technique, based on the Raman effect. It is an irreplaceable tool for the study of biological events at the molecular level and for identification of molecules. There are several reasons for this. First of all, RS measures the vibrations of atoms. This implies that the positions of the bands, widths and intensities are sensitive to the molecular structure. Vibrations of some molecular groups are very characteristic and therefore can be used for the identification of certain groups or even whole molecules. Second, water causes weak Raman scattering, and consequently molecules can be studied in their natural environment without solvent interference. The third reason for the growing interest in RS is re-

lated to the considerable intensity enhancement (up to 10^6) observable in resonance Raman spectroscopy (RRS) and surface-enhanced Raman spectroscopy (SERS). Fourthly, because the Raman effect is instantaneous (timescale in order of 10^{-14} s), and due to the recent advances in laser technology, the time-resolved action of molecules down to picosecond resolution can be followed, retaining the value of structural information [54].

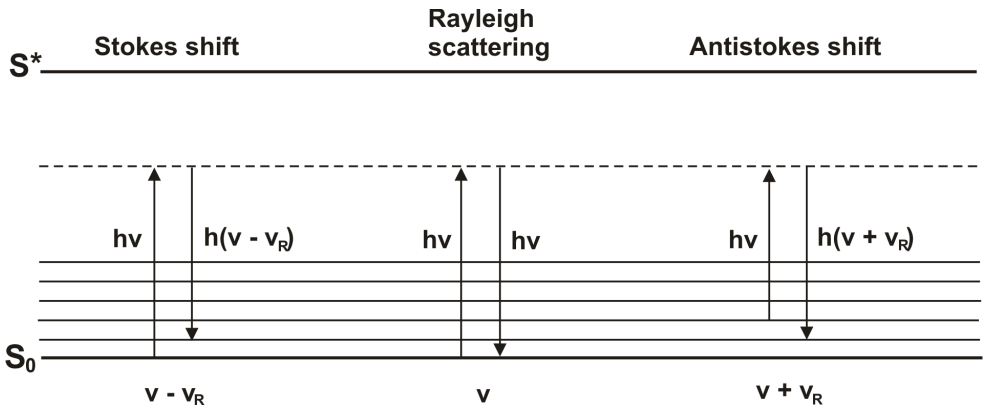

Figure 5. Scheme of scattering of the light.

The limitations of the technique must also be considered. First of all, the probability of Raman scattering is very low, so the effect is weak and high concentrations of samples are required. Secondly, in Raman experiments excitation within the electronic absorption band often causes photodegradation of the molecule. To avoid such problems, low laser powers, moving samples and independent inspection of the sample integrity are often employed. Finally, many molecules, or impurities in the sample, exhibit intense fluorescence, obscuring the Raman spectra [54].

Raman spectroscopy, which is regarded as a complementary technique to IR spectroscopy, is similarly applied to study water, carbohydrate, protein and fat structure in food samples. Raman bands of major food components are shown in table 3. As it was mentioned above, weak Raman scattering of water is the advantage, but it also makes difficult to observe changes in water structure due to weak Raman signals. However, changes in water structure have been observed as decrease of intensity of the O–H stretching band at 3250 cm^{-1} relative to the C–H band at 2938 – 2942 cm^{-1}. It was a result of interaction between water molecules and food proteins [55]. Structure of food proteins may also be analyzed by using RS. The –CO–NH– amide or peptide bond has several distinct and conformationally sensitive vibrational modes, with the amide I and III bands being the most commonly used for secondary structure characterization. [56]. Changes in food carbohydrate structure inducing by processing or storage can be monitored by this technique. Using RS technique interactions of carbohydrates with other food components, particularly with water have also been studied [27].

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Wavenumber [cm^{-1}]	Assignment
	Water
3200 – 3600	O–H stretching
	Proteins
510 525 545	S–S stretching
630 – 670 700 – 745	C–S stretching
1235 – 1245	Amide III (C–N stretching and N–H bending)
1600 – 1700	Amide I (C=O stretching and N–H bending)
2550 – 2580	S–H stretching
2800 – 3000	C–H stretchnig
	Fats
1441	CH_2 bending
1457	$CH_3 – CH_2$ bending
1656	C=C stretching
2855 – 2960	C – H stretching
	Carbohydrates
836	C – C stretching
1064	C – O stretching
2912 2944	C – H stretching
3451	O – H stretching

Table 3. Raman bands of major food component.

Raman spectroscopy, likewise IR spectroscopy, has been used to quantify and characterized the lipid components of food systems, including quantitative analysis of the degree of unsaturation and the content of cis and trans isomers, identification or detection of adulteration of various oils, characterization of polymorphism and chain packing, and monitoring of interactions with other food components or changes induced by processing or storage, such as autooxidation or isomerization. Rapid quantitative analysis of unsaturation and cis and trans isomers content has been reported by using dispersive laser Raman spectroscopy and FT-Raman spectroscopy [57, 58, 59]. Low-resolution Raman spectroscopy was used to monitor oxidation status of olive oil. Primary and secondary oxidation parameters (e.g. peroxide

value) were obtained in a rapid, non-destructive and direct way [60]. FT-Raman spectroscopy combined with multivariate analysis procedures was applied to determine the level of adulteration of virgin olive oil by some vegetable oils (soybean, corn and raw olive residue oils) [61].

Nowadays, Raman spectroscopy is often combined with microscopy (Raman microspectroscopy). The combination results in an analytical method that allows spatially resolved investigation of the chemical composition of heterogeneous food and food ingredients. Both qualitative and quantitative information can be obtained using microspectroscopy. A number of organic compounds and functional groups can be identified by their unique pattern of absorption, and the intensity of the absorption may be used for the calculation of the relative concentration in the sampled entity. Furthermore, samples of microscopic size can be analyzed directly, in air, at ambient temperature and pressure, wet or dry, and in many cases without destroying the sample [62]. Confocal Raman microspectroscopy has been applied to obtain information about microstructure and chemical composition of wheat grain [63]. The work was focused on the protein content and composition of the starchy endosperm and on the aleurone cell walls in arabinoxylan and ferulic acid derivatives. Confocal Raman microscopy was also used to follow the evolution of protein content and structure during grain development of various wheat varieties selected on the basis of hardness level and aptitude to separation of peripheral layers during milling. Raman microspectroscopy is not only a powerful technique to identify cereal components, but it also gives information about secondary structure and configuration of these proteins. For instance, the technique permits to determine the conformation of a non-specific wheat phospholipid transfer protein, and to study the role of disulphide bridges in the stabilization of the α-helical structure. In addition, Raman microspectroscopy was used to determine the secondary structure and conformation of puroindolines, lipid-binding protein of wheat [64].

6. Conclusions

Spectroscopic methods seem to be very successful at evaluating the food quality. They are used to qualitative as well as quantitative analysis of food products. Furthermore, they provide information on structure-functionality relationships (e.g. secondary structure of proteins). These techniques can be used independently or combined with other analytical techniques such as chromatography and served as detector. Some of them (IR and Raman spectroscopy) are regarded as rapid, no sample preparation techniques which additionally can be used on-line.

The above described methods are mainly used to determine technological parameters (anisidine value, peroxide value and general color) and authenticity (kind and level of adulteration) of edible oils. They can also be used to differentiate cereal classes (wheat classes), determine quality of flours, and level of the insects and fungal damage in food products. But first of all, the spectroscopic methods are applied to determine content of the major food components (water, proteins, lipids and carbohydrates). Although some researches claimed that spectroscopy is unsuitable tool for studying food products, nowadays it is used commonly and with a great success.

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