

# Shedding light on food fraud: spectrophotometric and spectroscopic methods as a tool against economically motivated adulteration of food

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**Abstract.** Intentional modification of food or substitution of food ingredients with the aim of gaining profit is food fraud or economically motivated adulteration (EMA). EMA appeared in the food supply chain, and following the global expansion of the food market, has become a world-scale problem for the global economy. Food frauds have involved oils, milk and meat products, infant formula, honey, juices, spices, etc. New legislation was enacted in the last decade in order to fight EMA. Effective analytical methods for food fraud detection are few and still in development. The majority of the methods in common use today for EMA detection are time consuming and inappropriate for use on the production line or out of the laboratory. The next step in the evolution of analytical techniques to combat food fraud is development of fast, accurate methods applicable using portable or handheld devices. Spectrophotometric and spectroscopic methods combined with chemometric analysis, and perhaps in combination with other rapid physico-chemical techniques, could be the answer. This review discusses some analytical techniques based on spectrophotometry and spectroscopy, which are used to reveal food fraud and EMA.

## 1. Introduction

Cases of food adulteration in order to increase profit margins are dated back to the earlier ages of civilization. Intentional modification of food or substitution of food ingredients with the aim of gaining profit is food fraud or economically motivated adulteration (EMA) [1]. Unfortunately, EMA occurs today and, following advances in food technology, has become more sophisticated. EMA appears in food supply chains and is conducted by food producers, processors, distributors and/or retailers [2]. Following the global expansion of the food market, the consequences of EMA have emerged into a world-scale problem and have great impact on the global economy.

EMA inevitably leads to production of food with lowered nutritional value. This type of fraud not only has financial impacts on consumers, and society in general, but, more importantly, has adverse effects on health and food safety. Prolonged diet with such food could have devastating consequences to public health [3]. Recent reports exposed that food frauds and adulterations include a vast variety of food, such as oils, milk and meat products, infant formula, honey, juices and spices. In order to protect consumers and eliminate EMA, or to lessen its possibility, new legislation relating to the adulteration of food and food labeling was enacted in last decade [4,5]. Legislation for containing food fraud is still in development but, in order to be effective, needs strong logistic support. In addition to government agencies and inspectorates, essential support in revealing EMA includes development of analytical



methods to detect food frauds. As it is difficult to formulate any effective legal basis for suppressing EMA, it is also a burdensome task to develop and apply appropriate analytical methods for its detection [6].

Knowledge of the specificity and characteristics of analytical techniques is essential for detecting EMA of food. Such knowledge should be helpful in choosing appropriate analytical techniques for detection of adulterants of interest in a specified food matrix by controlling laboratories. Also, this could be a guideline for research laboratories to focus their exploration into development of new or improvement of existing methods. In a reciprocal way, it could help regulators to establish legal frameworks for revealing food frauds and EMA.

Various methods and techniques of chemical analysis are used to reveal adulterants in food [7]. They can be divided into several groups based on: absorption of electromagnetic radiation – colorimetric and spectroscopic methods and nuclear magnetic resonance (NMR); chromatographic; molecular and immunological methods; elemental, isotopic and mass spectrometry techniques; and chemometric methods based on statistical analysis of data obtained by some of these previously mentioned techniques. Especially in recent years, the need for rapid methods for food authentication and food fraud has arisen, as well as the need for methods that can be performed on-site. Therefore, the aim of this review is not to list all the methods for the detection of EMA, but to indicate approaches that can lead us to, or at least closer to, achieving that goal. The most promising methods are spectroscopic methods. They are quick, simple, require small quantities of the food/sample, and are usually non-destructive and non-invasive. Miniaturization of spectrophotometers and the manufacture of portable or hand-held instruments means *in-situ* measurements have become a reality. There are many portable spectrophotometers on market today. They can measure reflectance or transmittance of food/samples and can be used for direct measurement without sample pre-treatment. This makes them suitable for control on production lines, in storage areas and shipping departments, or indeed, everywhere where it is necessary to perform fast measurements. Furthermore, an overview of recent methods based on the absorption of electromagnetic radiation in the infrared (IR), visible or ultraviolet ranges used to detect EMA of food is presented.

## 2. Spectrophotometric methods

Methods such as sensitive spectrophotometry, differential spectrophotometry, derivative spectrophotometry, and spectrophotometry using chemometrics [8] can be used to reveal EMA in beverages, drinks, confectionery, spices, milk, olive oil, jams and sweets. Spectrophotometric measurement of specific extinction increase at 270 nm can be used to detect adulteration of extra-virgin olive oil by the addition of refined olive oil or other vegetable oils. Stigmasta-3,5-diene, a characteristic compound of refined oils, has an absorption peak at 270 nm [9]. The method is simple, rapid, low-cost, and eminently suitable for *in-situ* measurement.

Combining spectrophotometric methods with other analytical techniques enhances their possible application in detecting food EMA. Spectrophotometric methods and thin layer chromatography (TLC) [10] were applied to detect sweets and jams adulteration with synthetic colorants. Eight synthetic food dyes were used in the reported research [10]. The Kjeldahl method and spectrophotometric methods were employed to evaluate detection of milk adulteration by nitrogen-rich compounds such as melamine, ammonium sulfate or urea [11]. Standard (Kjeldahl) methods fail to identify adulteration by nitrogen compounds, and classical spectrophotometric methods for protein are not sensitive to these compounds. In combination, the proposed methods were able to recognize nitrogen added to milk and/or whey. It is suggested that this combination of Kjeldahl and spectrophotometric methods should be used to screen for milk adulteration by these compounds [11].

However, spectrophotometric techniques have limited application and often, they must necessarily be combined with other analytical techniques.

## 3. Color measurement

Measurement of color in HunterLab and CIE L\*a\*b\* systems were used as supplementary techniques, mainly to describe visual properties of food [12]. Color of food can sometimes be improved by adding prohibited dyes [13]. Adulteration by adding coloring stuffs in food is maybe the oldest and most common means of food fraud. Colorings are added (fraudulently and legally) to various foods from beverages to meat products and from confectionery to spices and oils. Reasons for the widespread usage of dyes in EMA is twofold: first, synthetic dyes are cheap and easily available, and, second, staining of lower quality food or food manufactured from low-grade ingredients provides higher profit. Therefore, the measurement of the surface color of food in HunterLab and CIE L\*a\*b\* systems is usually used in assessment of food quality and safety [14]. However, the potential of such methods for detecting EMA is not yet fully recognized. Some efforts were made to employ food color measurements for detecting EMA and compliance with regulatory requirements [15]. The rapid method based on CIE L\*a\*b\* measurements combined with chemometrics was used to estimate food coloring contents in meat products [15]. The method was developed to determine the presence and quantification of one permitted and two prohibited food dyes in frankfurters and fine-chopped boiled sausages, and it might also be useful during sensory evaluation of meat products for the assessment of the added food colorants.

#### **4. Fluorescence spectroscopy**

Fluorescence spectroscopy has the lowest limits of detection of all spectroscopic analytical techniques, but it is limited to polyaromatic hydrocarbons (PAHs) and heterocyclic compounds with rigid skeletons. It is a sensitive, selective and nondestructive technique. Development of new generation fluorescence spectrometers with powerful software for acquisition and interpretation of spectra resulted in application of this technique for EMA detection. Therefore, total synchronous fluorescence (TsyF) with the partial least square (PLS) model was used for the differentiation of virgin olive oil from olive-pomace, corn, sunflower, soybean, rapeseed and walnut oils [16]. Other applications of fluorescence spectroscopy combined with extensive use of chemometric tools were in food quality assessment of animal (dairy, meat, fish and egg) and vegetable (oils, cereal, sugar, fruit and vegetable) products [17-19].

The combination of fluorescence spectroscopy with other techniques such as Fourier transformed infrared spectroscopy (FTIR) was reported for authentication of extra-virgin olive oil. PLS method was used for both fluorescence and FTIR spectra analysis. The results suggest that FTIR and fluorescence could be a useful tool for analysis and detecting adulteration of extra-virgin olive oil with pomace oil.

#### **5. Infrared spectroscopy**

Infrared spectroscopy (IR), based on absorption of heat rays by molecular bonds with dipole moment, has been used for food quality and food authenticity assessments. In accordance with the applied spectral range, IR can be divided into two techniques – mid infrared (MIR) and near infrared (NIR). In MIR (fingerprint range), IR radiation is absorbed as a consequence of fundamental vibrations of atoms in the molecules. NIR spectra are the result of superposition of composite, overtone and high frequency absorption bands at shorter wavelengths.

Methods based on IR spectroscopy are among the most widely used methods for revealing EMA of food. Both MIR and NIR were used for detection of food frauds and authenticity of honey, meat, meat products, cheeses, milk, fish, spices, olive oil, and so forth.

The application of attenuated total reflectance (ATR) MIR microspectroscopy was evaluated as a rapid method for detection and quantification of milk adulteration [20]. Milk samples were spiked with different concentrations of whey, hydrogen peroxide, synthetic urine, urea and synthetic milk. Soft Independent Modeling of Class Analogy (SIMCA) pattern recognition analysis with Partial Least Squares Regression (PLSR) were used for estimation of adulterant levels. Results showed that MIR-microspectroscopy can provide an alternative methodology to the dairy industry for screening potential fraudulent practice for economic adulteration of cow's milk [20].

NIR methods with latent variable models applied to the spectral data were developed and used to estimate proximate composition, fatty acid profile, fillet yield and cooking loss, and to classify the available dataset by the rearing farm and genetic strain of raw and cooked freeze-dried rainbow trout (*Oncorhynchus mykiss*) fillets [21]. No major differences were observed between the results obtained from raw freeze-dried fillets and those obtained from cooked freeze-dried fillets, except for the estimation of some chemical constituents of interest, such as C22:6n-3 and polyunsaturated fatty acid content, both of which were better estimated in cooked freeze-dried fillets [21].

In a recent study, methodology based on the combination of MIR and NIR measurements and chemometric data processing was reported [22]. Such approach could overcome the disadvantages of both techniques and offer more accurate and reliable results in detecting the EMA.

## 6. Raman spectroscopy

Raman spectroscopy (RS), similar to IR, gives spectral information on fundamental vibrations of functional groups in a molecule. It is based on the inelastic scattering of incident radiation through its interaction with vibrating molecules [23]. RS and MIR are complementary techniques, but RS has some advantages over MIR. Water has weak absorption in RS and does not cause interference in Raman spectra. Taking into account the fact that most foods contain more or less water, the lack of interference due to water is essential for the reliability of methods for determining food authenticity. Absorption bands in Raman spectra are narrower than IR spectral bands. This is of significant importance for RS application, because narrow bands mean less overlapping, especially in complex matrices such as foods. RS also can be applied to analysis of foods directly through plastic or glass bottles and wrappers, bags and other transparent packaging materials [24].

RS scattering is weak, and new, improved techniques like Surface Enhanced Raman Spectroscopy (SERS), in contrast to classic Raman spectroscopy, provide lower detection limits, allowing applications in food adulterant determination [7]. For example, three Raman modalities, normal Raman, FT-Raman and SERS, combined with principal component analysis (PCA), were evaluated as a tool for detecting Sudan I dye in culinary spices. The results show that SERS is the most appropriate modality capable of providing a proper Raman signal when a complex matrix is analyzed [25]. RS combined with chemometrics was also applied to examine adulterations of honey by syrup and sugar solutions, olive oil by vegetable oils or pomace olive oils, and meat and fish [26].

## 7. Chemometrics

Almost all recent spectroscopic methods for food authentication use the chemometric approach for development and validation processes. This brief review would not be complete without a section dedicated to the chemometric application for food authenticity analysis. The reason for employing chemometrics in spectrophotometric and spectroscopic techniques is that spectra obtained by these aforementioned techniques are large datasets which cannot be resolved by conventional analysis. Chemometric tools, such as data mining, data fusion etc., are crucial for handling datasets generated by analytical methods for EMA detection.

A sample spectrum is, in fact, an “image” of the sample and can be treated in the same way as any digital image analysis. Data pre-processing, enhancement, segmentation, feature extraction and data classification for generation of applicable methods to spectroscopic data are based on similar mathematical models to those used in digital image processing for facial recognition or satellite image analysis [27]. Chemometrics resembles digital extraction and clean-up of the sample instead of using physical manipulation for clean-up; this is a major obstacle for common application of chemometric methods, because their calibration and validation necessitates a large number of samples. They are matrix-dependent too, and every change in matrix composition means a new calibration and validation process. Despite these disadvantages, the development of analytical methods based on spectroscopy combined with chemometrics is becoming more important, and the further efforts are being directed toward improvement of chemometric tools to become more robust and less sensitive to the influence of the matrix.

## 8. Conclusion

General features of spectrophotometric and spectroscopic methods are their simplicity, rapidity, and ability to be directly applied to foods/ingredients in different aggregate states, with fast, minimal or without any preparation. Production of new, cheaper and miniature, portable or handheld instruments for spectroscopy made them applicable on production lines, outdoors, in storage areas, etc. Their potential dual measurement modality, transmittance and reflectance, make spectroscopic methods useful in the resolution of various analytical tasks.

The combination of spectroscopic techniques with chemometrics has made them applicable for revelation of EMA and authentication of food. The majority of new spectroscopic methods for detecting food frauds are based on chemometric analysis. Although they are still under development, some of these methods have already been incorporated into benchtop and portable instruments. Further improvement of spectroscopy combined with chemometrics will require development of methods which are more robust and less sensitive to the influence of the matrix.

The next aspect to be addressed is the combination of spectroscopy with other analytical techniques, such as measurement of thermal, electrical or electrochemical properties of the food. This combination could improve reliability of results, and accuracy of determination and classification without loss of rapidity, simplicity or other advantages. Any additional data on the characteristic of the food, or by extension, other sample types, will contribute to a better and more accurate answer as to whether the food/sample is authentic or not. As the coupling of chromatographic techniques with mass spectrometry has made a significant breakthrough in analytical practice in the past, the integration of spectroscopic methods, either with one another or with other physico-chemical techniques, could make significant progress in detecting EMA of food in the future. That future requires rapid and reliable methodology for revealing EMA, which is fundamental for the production of safe and healthy food.

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