Recent Developments in Food Characterization and Adulteration Detection: Technique-Oriented Perspectives

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This review covers mainly publications that appeared in Analytical Abstracts (Royal Society of Chemistry) from January 1990 to February 2001. The number of publications on this topic continues to grow, and during the past three years (1998–2000) about 150 reviews and/or overviews have been published in the area of food. Numerous techniques and food matrices or chemical components are presented and discussed in these reviews. The present review is intentionally limited to eight techniques or classes of techniques and intends to be a “technique by technique” presentation of “what was used” or “what is used” to characterize food products and to detect their possible adulteration. The present review focuses on the following techniques: microscopic analysis; HPLC; GC, GC-(MS, FTIR); UV–visible spectrophotometry; AAS/AES, ICP-(AES, MS); IRMS, GC-IRMS, GC-C-IRMS; DSC; IR, mid-IR, and NMR (202 references). Emphasis is placed as much as possible on chemometrical treatment of analytical data, which are commonly used to achieve the final objective, either food characterization or adulteration detection. Finally, a brief description is given of the new generation of analytical systems that combine powerful analytical techniques and powerful computer software for a best extraction of the information from analytical data.

Keywords: Microscopy; HPLC; GC; MS; GC-FTIR; UV; visible spectrophotometry; AAS/AES, ICP-(AES, MS); IRMS, GC-IRMS, GC-C-IRMS; DSC; IR, mid-IR, and NMR; chemometrics; ANN; food characterization; food adulteration

INTRODUCTION

This review covers mainly publications that appeared in Analytical Abstracts (Royal Society of Chemistry) from January 1990 to February 2001. However, some older papers are also included to illustrate some historical aspects. The number of publications on this topic continues to grow, so some important contributions have certainly been omitted, for which we must apologize. For the past three years (1998–2000) about 150 reviews and/or overviews have been published in the area of food science. Numerous techniques and food matrices or chemical components are presented and discussed in these reviews. This review is therefore intentionally limited to eight techniques or classes of techniques and offers a “technique by technique” presentation of “what was used” or “what is used” to characterize food products and to detect their eventual adulteration. For a food matrix oriented presentation, see the paper published by Dennis (1). For further information related to each technique presented here and their recent developments in food science (from Jan 1998 to Dec 2000), the reader will find some additional bibliography resources at the end of this review (Tables 6 and 7).

Frauds in various consumer sectors are now commonly practiced. Raw materials the farm-produce sector are not exempt, and according to the activity report of the French General Directorate for Fair Trading, Consumer Affairs and Fraud Control (DGCCRF) (2), this practice is increasing. Authentication of food products is of primary importance for both consumers and industries, at all levels of the production process, from raw materials to finished products. From the legislative point of view, quality standards have been established through the requirement of quality labels that specify the chemical composition of each product. From the economic point of view, product authentication is essential to avoid unfair competition that can create a destabilized market and disrupt the regional economy and even the national economy. All food products targeted for adulteration are high-commercial-value products and/or produced in high tonnage around the world. Table 1 assembles products having the potential to be adulterated, adulteration modes, and techniques used for their detection. For example, Briandet et al. (3) reported that two types of adulteration could be used in coffee: adulteration with substitutes such...
as chicory, malt, cereals, caramel, maltodextrin, and glucose, and adulteration by mixing two coffee qualities (addition of a less expensive robusta coffee to an arabica coffee and sold under “pure arabica” designation). The latter practice is also applied to other common food products such as wine, fruit juice, olive oil, butter, and honey.

The detection of adulteration is a technical problem. Indeed, we must wonder about the following question: How can we detect an adulterant having approximately the same chemical composition of the food product in which it is included?

The first approach consists of determining the ratio between some chemical constituents and assumes these ratios are a constant component of the particular food. From this point of view, it seems to make sense that any addition of any substance(s) into foods will modify the value of these ratios or will highlight an anomaly in its chemical composition. This approach is frequently associated with a large set of analyses and the use of chemometrics. In this area, many pattern classification procedures (such as PCA, LDA, HCA, SIMCA, PLS, CVA, and ANN) can be applied to the dataset to compare similarities or differences of sample data with original data. Theoretically, after a multivariate analysis taking into account many analytical factors, adulterated samples form singular groups that can be easily distinguishable from authentic samples.

An alternative approach to this problem could be to search for a specific marker in the product, which could be a chemical constituent (complexes, molecules, nucleic acids) or morphological component (plant cells), that proves either the adulteration or authenticity of the food.

The third possibility for detection of adulteration in foods is the global approach of the food products. The method consists of using analytical techniques derived from physical analysis by considering the whole of the sample to show the effects of the adulteration on the physicochemical properties of the sample. Rather than measuring one chemical parameter such as pH, conductivity, water content, pt ash content, a physical analysis, such as thermal analysis (TGA + DSC), observes the thermal behavior of the food. The thermal behavior is followed over changing temperature and/or time.

To carry out these analytical controls, many techniques are used and/or usable. This review focuses on the following: microscopic analysis; HPLC; GC; GC-MS, GC-FTRIR; UV—visible spectrophotometry; AAS/AES, ICP-AES, ICP-MS; IRMS, GC-IRMS, GC-C-IRMS; DSC; and IR and mid-IR, NMR.

The final goal can be either food characterization or adulteration detection.

### MICROSCOPIC ANALYSIS

Optic or electronic microscopic analyses are not very applicable techniques in the food area except for honeys, for which they are very useful. The first studies relating to the use of optical microscopic analysis as a characterization tool of the floral origin of honey appeared in the 1950s when Maurizio (4) described a quantitative study of pollens in honeys. Louveaux and Maurizio (5) used optical microscopic analysis to characterize European melliferous plants by establishing their “pollinic identity card”. The following year, Louveaux (6) applied this technique with success to 40 Canadian honeys. In 1978, Louveaux and Maurizio (7) published “Methods of Mellissopalynology”, which has since become the official method for determining honey floral origin. Since 1978, numerous honey characterization studies have been published using palynology to distinguish among honeys from various countries. Now, analysts prefer to combine microscopic analysis with physicochemical determinations to determine floral origin of honeys. This practice is used increasingly as seen in Cortopassi-Laurino et al. (8) to study bactericide effects of some Brazilian honeys and in Thrasyvoulou et al. (9) in Greek monofloral honeys. Recently, Kerkvliet et al. (10, 11) applied the Louveaux—Maurizio method to the preparation of the samples and to the detection of adulteration of honeys with cane sugar and cane sugar products using optical microscopy. This study is based on the detection of parenchyma cells, sclerous rings, and other cane sugar constitutive cells. The latest review about analytical methods to determine the geographical and botanical origin of honeys and for proving their authenticity was published by Anklam et al. (12).

With the appearance of scanning electron microscopy (SEM), the development of palynology reached a turning point in the 1980s. With certain floral species, it is impossible to highlight, on live preparations, differences in hexin structure. This problem is linked to the weak field depth of optical microscopes. Using electron microscopy, this limitation is overcome and the determination of the botanical origin of honeys having similar palynologies is now possible without ambiguity.

In this area, several works should be emphasized, particularly, the work of Dustmann et al. (13—15), where SEM was used to describe the surface pattern of pollen from honeys. In the same way, Ohe et al. (16) used SEM to study pollen from apple varieties. Despite improvements brought about by SEM, electronic microscopic analysis is not a cheap routine technique, and it requires a meticulous sample preparation. When identification of pollens from a “fresh-state” is not possible, the

### Table 1. Common Types of Food Adulteration (Courtesy of Eurofins Scientific Analytics)

<table>
<thead>
<tr>
<th>Product</th>
<th>Common Adulteration</th>
<th>Compositional Parameters</th>
<th>Common Detection Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aloe Vera Gel</td>
<td>Dilution</td>
<td>HPLC</td>
<td>*Brix, (D/H), and ¹⁸O IRMS</td>
</tr>
<tr>
<td>Berry Jam</td>
<td>Addition of cheaper berries</td>
<td></td>
<td>Gas chromatography, ¹³C IRMS, and SNIF-NMR</td>
</tr>
<tr>
<td>Fruit Juice</td>
<td>Addition of or dilution with water</td>
<td></td>
<td>¹³C IRMS</td>
</tr>
<tr>
<td>Fruit Juice</td>
<td>Addition of sugar</td>
<td></td>
<td>Molecular biology methods</td>
</tr>
<tr>
<td>Honey</td>
<td>Addition of corn or cane sugar</td>
<td></td>
<td>Chiral chromatography</td>
</tr>
<tr>
<td>Meat</td>
<td>Addition of cheaper meat</td>
<td></td>
<td>¹³C IRMS and SNIF-NMR</td>
</tr>
<tr>
<td>Milk</td>
<td>Species of origin</td>
<td></td>
<td>DNA methods</td>
</tr>
<tr>
<td>Natural Aroma</td>
<td>Addition of non-natural aroma</td>
<td></td>
<td>¹³C IRMS and SNIF-NMR</td>
</tr>
<tr>
<td>Natural Vanilla Extract</td>
<td>Addition of artificial vanillin or p-hydroxybenzaldehyde</td>
<td></td>
<td>Chromatography</td>
</tr>
<tr>
<td>Soy</td>
<td>Genetic modification (in the EU)</td>
<td></td>
<td>¹³C IRMS and SNIF-NMR</td>
</tr>
<tr>
<td>St. John’s Wort</td>
<td>Mislabeling of botanical origin</td>
<td></td>
<td>DNA methods</td>
</tr>
<tr>
<td>Vegetable Oil</td>
<td>Replacement by other herbs</td>
<td></td>
<td>Chromatography and mass spectrometry</td>
</tr>
<tr>
<td>Wine</td>
<td>Addition of cheaper seed oil</td>
<td></td>
<td>(D/H) and ¹⁸O IRMS</td>
</tr>
<tr>
<td>Wine</td>
<td>Dilution with water</td>
<td></td>
<td>¹³C IRMS and SNIF-NMR</td>
</tr>
<tr>
<td>Wine and Spirit</td>
<td>Chaptalization</td>
<td></td>
<td>¹⁴C Scintillation counting</td>
</tr>
</tbody>
</table>

*Note: *Brix, (D/H), and ¹⁸O IRMS refer to specific measurements related to sugar content and water content, respectively.
preferred technique is Erdtman’s, which consists of observing pollens after sample acetolysis (17).

**HPLC**

High-performance liquid chromatography is one of the most widely used techniques in analytical chemistry. In an industrial or laboratory setting, HPLC represents one of a series of control and research tools routinely employed.

In the farm-produce industry, HPLC is used as a quality control tool because it can separate various chemical constituents of mixtures, but it is also often used for characterizing food products or to detect adulteration. Blanch et al. (18) proposed various approaches to evaluate the authenticity of olive oil and hazelnut oil by HPLC and HPLC-GC. This work of characterization is based on the hazelnut oil marker [filbertone: (E)-5-methylhept-2-en-4-one], being absent in olive oil, which allows the detection of olive oil adulteration (19). Similarly, Silva et al. (20) uses the specificphenolic compound profile present in commercial quince jams to detect adulteration of quince jams by pear or apple purée. After determining that the phenolic profiles are similar, they used reversed-phase HPIC-PAD method to find a specific marker of pear purée (arbutin), proving the fraudulent adulteration of analyzed samples. Moully et al. (21) proposed a simultaneous method to determine flavone glucosides and polymethoxylated flavones in citrus juices such as orange and lemon juices. Guyot et al. (22) analyzed phenolic compounds in various French ciders, whereas Bengoechea et al. (23) made the same analyses on manufactured purées and concentrates from peach and apple fruits. The previous examples illustrate the second approach consisting of the identification of a specific marker of the natural food product or the marker belonging to the adulterant. The third approach can be illustrated in the application of HPLC data in the classification of cheese varieties by multivariate analysis of HPLC profiles as shown by Mohler-Smith et al. (24). They applied both principal component analysis and linear discriminant analysis to 55 peak areas from each cheese chromatogram, with >90% correctly classified. The utilization of linear discriminant analysis after principal component analysis improves the classification of the dataset as opposed to the direct utilization of the discriminant analysis.

Numerous chemical compounds are extensively analyzed by HPLC either for species characterization or for authenticity

**Table 2. Main Analyses Carried out on Honeys for 25 Years by HPLC**

<table>
<thead>
<tr>
<th>analyzed compounds</th>
<th>aim of study</th>
<th>year (ref)</th>
</tr>
</thead>
<tbody>
<tr>
<td>amino acids and sugars</td>
<td>storage effects on honey chemical composition and properties</td>
<td>1976 (120)</td>
</tr>
<tr>
<td>sugars</td>
<td>determination of oligosaccharides origin</td>
<td>1988 (131)</td>
</tr>
<tr>
<td>amino acids</td>
<td>floral origin and/or geographical determinations</td>
<td>1975 (132, 133)</td>
</tr>
<tr>
<td>flavonoids</td>
<td></td>
<td>1989 (134)</td>
</tr>
<tr>
<td>sugars</td>
<td></td>
<td>1990 (136)</td>
</tr>
<tr>
<td>phenolic compounds</td>
<td></td>
<td>1997 (137)</td>
</tr>
<tr>
<td>amino acids enantiomers</td>
<td></td>
<td>2000 (138)</td>
</tr>
<tr>
<td>sugars and conductivity</td>
<td></td>
<td>1994 (139, 140)</td>
</tr>
<tr>
<td>organic acids</td>
<td></td>
<td>1996 (141)</td>
</tr>
<tr>
<td>sugars, amino acids, HMF, glucosidases, yeast, free acids, diastase, lactone, ash organic acids</td>
<td>chemical characterization and chemical composition</td>
<td>1992 (143)</td>
</tr>
<tr>
<td>sugars</td>
<td>chemical composition</td>
<td>1994 (144)</td>
</tr>
<tr>
<td>HMF</td>
<td>HMF determination methodology</td>
<td>1996 (145)</td>
</tr>
<tr>
<td>abscissic acid</td>
<td>marker research into a floral species</td>
<td>1996 (146)</td>
</tr>
<tr>
<td>pyrrolizidine-derived compounds</td>
<td>sensibility improvements for alkaloid compound determination in honeys</td>
<td>1997 (147)</td>
</tr>
<tr>
<td>flavonoids</td>
<td></td>
<td>1997 (148)</td>
</tr>
<tr>
<td>kaempferol</td>
<td></td>
<td>1998 (149)</td>
</tr>
</tbody>
</table>

research: flavonoids in fruit juices (25, 26), organic acids in apple juices (22, 27), phenolic pigments in black tea liquors (28, 29), proline isomers and amino acids in wines (30–32), and anthocyanins in jams (33), juices, etc. HPLC has been very widely used for 25 years for the determination of the chemical constituents of food. Most of the chemical compounds cited previously were also found, analyzed, and studied in honeys either for characterization purposes or for adulteration detection objectives (cf. Table 2). The diversity of the compounds mentioned above shows the high complexity of certain food matrices such as honey and other nontransformed natural products.

**GC, GC-FTIR, AND GC-MS**

Gas chromatography is used for separating volatile organic compounds. Numerous detectors for this kind of chromatography have been developed since the 1950s, and several of them have high sensitivity and/or selectivity. (For further information about various kinds of chemical detectors such as NPD, ECD, AED, FID, MS, and FTIR, see ref 34). The attraction of hyphenated techniques, such as GC-MS or GC-FTIR, comes from the necessity to determine the identity of separated chemical compounds. The principles of these techniques as well as the working process are described elsewhere (34), but here we will discuss some applications of GC, GC-MS, and GC-FTIR to food products.

The literature seems to show the GC-MS coupling as the most widely used hyphenated technique (>50%). GC comes in second, with ~20% of cases, and third is GC-FTIR (<10%). However, the use of GC-FTIR is increasing because it is a nondestructive technique with respect to the sample. Moreover, GC-FTIR gives some structural information on the chemical functional groups of the molecules (acid, alcohol, amine, aromatic groups, etc.). Gas chromatography is generally used to discriminate among different varieties of the same product, adulteration detection, and organic compound authentication and identification (cf. Table 3). GC or GC-MS is frequently used in association with a large set of analyses and with chemometric data treatments. This is an illustration of the second approach (able to detect adulteration and appropriate for characterization) presented in the Introduction of this review. Sivertsen et al. (35) presented a study in which PCA and CVA were applied to evaluate similarities among wines from the same regions and
the main differences among wines from the different regions. Twenty-one sensory attributes (i.e., color intensity, suppleness, astringency, fruit acidity, etc.) and 35 chemical compounds (i.e., ethanol, ethyl acetate, malic acid, lactic acid, glycerol, isobutanol, γ-butyrolactone, etc.) have been measured to classify 22 red wines. About 82% of samples were correctly classified by the chemical analysis, whereas the classification obtained from the sensory data set was ∼64%. Nogueira et al. (36) used PCA and HCA for characterization of commercially available Madeira wines according to different types and ages. Volatile compounds such as 1-propanol, 2-methyl-1-propanol, 2-propen-1-ol, and 3-methyl-1-butanol were measured and quantified by GC or GC-MS, yielding 30 physicochemical parameters usable for pattern classification. After PCA, 14 variables seem to describe adequately the samples according to age, and the first two PCs explain 53% of the variability among all samples studied. To improve the results, the authors employed discriminant analysis, particularly, CVA, to determine which physicochemical variables better discriminate between the type and age groups. Two discriminant functions were obtained explaining 95.5% of the variability, clearly showing a good separation of the overall groups of samples involved. In the same way, König and Schreier (37) describe a PCA/HCA application allowing the selection of aroma constituents of apple juice as key components in the authenticity verification of this product.

A complementary and very powerful tool increasingly used for the characterization of foods is that of artificial neural networks (ANNs), for which the reader may find a vast quantity of information in the scientific literature, the detailed principle of which will not be described here. The main advantage of the utilization of ANNs is that certain classes of ANNs using “supervised learning” tools can learn from sets of chemical data for which the sensory output is known. This is the case of ANNs using an algorithm based on the back-propagation of error. However, ANNs need a large set of data to work, which represents their main disadvantage. Angerosa et al. (38) applied ANN (using back-propagation algorithm) to the olive oil headspace results (input) with the aim of predicting panel test scores (output). A high level of accuracy was found, with ∼96% of answers being correct. This result suggests, according to the authors, that sensory evaluation from the panel test could be successfully replaced by the dynamic headspace analysis—ANN coupled approach.

**UV–VISIBLE SPECTROPHOTOMETRY**

Numerous techniques based on protein structure, peptides, and/or amino acids have been developed to determine the nitrogenous content of food products such as cheese, milk, or honey. They have also been developed to characterize food products containing various kinds of substances such as fatty acids, sugars, vitamins, or mineral elements. Most of these techniques are spectrophotometric or fluorometric methods. Two types of chemical reactions used by these methods can be distinguished:

1. First are reactions allowing protein or peptide quantification as the Kjeldahl method (IDF standard, 1964) based on the determination of the nitrogen content after a sample mineralization step. Another way is Hull’s method (1947) using a complexation reaction of tyrosine with the Folin–Ciocalteu reagent. The complex formed is blue. This method was used again and improved later by Citti et al. (1963) for the determination of nitrogenous compounds in milk. Lowry’s method (1951), improved by Creighton (1993), belongs to the most widely used colorimetric methods in analytical chemistry and biochemistry. Based on the biuret reaction, Lowry’s method (1951) is based on the reaction between Cu²⁺ ions and the Lowry reagent (pH >8). Finally, Soedjak’s (39) method has to be highlighted because it is based on the formation of a pink complex, stable for pH <6, between proteins and erythrosine B.

2. Nowdays, the evaluation of the protein content is mainly based on the Kjeldhal method (seen above) and the Bradford method (40). The method is based on the reaction between Bradford’s reagent (Coomassie Brilliant Blue G-250, phosphoric acid, and ethanol) and proteins leading to the formation of a protein–dye complex. A blue coloration is developed in the

**Table 3. Main Studies in Food Area Using Gas Chromatography and Its Couplings for the Past 10 Years**

<table>
<thead>
<tr>
<th>food product</th>
<th>analyzed compounds</th>
<th>technique(s)</th>
<th>aim of study</th>
<th>year (ref)</th>
</tr>
</thead>
<tbody>
<tr>
<td>apricots purée</td>
<td>volatile compounds</td>
<td>GC-MS</td>
<td>characterization and/or classification</td>
<td>1990 (150)</td>
</tr>
<tr>
<td>wines</td>
<td>aroma</td>
<td>GC (FID)</td>
<td></td>
<td>1996 (151)</td>
</tr>
<tr>
<td>tea</td>
<td>fatty acids and volatile compounds</td>
<td>GC + GC-MS</td>
<td></td>
<td>1998 (152)</td>
</tr>
<tr>
<td>orange essential oils</td>
<td>polymethoxylavones</td>
<td>GC-MS</td>
<td></td>
<td>1998 (153)</td>
</tr>
<tr>
<td>wines</td>
<td>volatile compounds</td>
<td>GC + GC-MS (ITD)</td>
<td></td>
<td>1995 (154)</td>
</tr>
<tr>
<td>raspberry</td>
<td>aroma</td>
<td>GC-FTIR + GC-MS</td>
<td></td>
<td>1997 (155)</td>
</tr>
<tr>
<td>orange juices</td>
<td>lipidic fraction</td>
<td>GC-MS</td>
<td></td>
<td>1998 (156)</td>
</tr>
<tr>
<td>honeys</td>
<td>amino acids</td>
<td>CG</td>
<td>geographic origin determination</td>
<td>1981 (157)</td>
</tr>
<tr>
<td></td>
<td>sugars</td>
<td>CG (FID)</td>
<td>floral or botanical origin determination</td>
<td>1995 (158)</td>
</tr>
<tr>
<td></td>
<td>carboxylic acids</td>
<td>CG (FID) + CG-SM</td>
<td></td>
<td>1995 (159)</td>
</tr>
<tr>
<td></td>
<td>norsoprenoids, monoterpenes, and other volatile compounds</td>
<td>CG (capillary) + GC-MS</td>
<td></td>
<td>1997 (160)</td>
</tr>
<tr>
<td></td>
<td>sugars</td>
<td>CG + TLM</td>
<td></td>
<td>1987 (161)</td>
</tr>
<tr>
<td></td>
<td>oligosaccharides</td>
<td>GC + GC-MS</td>
<td></td>
<td>1998 (162)</td>
</tr>
<tr>
<td></td>
<td>amino acids</td>
<td>CG</td>
<td></td>
<td>1992 (163)</td>
</tr>
<tr>
<td></td>
<td>hesperetin and methylvitanrolnitate</td>
<td>CG + HPLC</td>
<td>search of a marker of floral origin</td>
<td>1994 (164)</td>
</tr>
<tr>
<td>white wine</td>
<td>volatile compounds</td>
<td>GC-MS</td>
<td>geographic origin</td>
<td>1995 (165)</td>
</tr>
<tr>
<td>essential oils</td>
<td>terpenes</td>
<td>GC-MS</td>
<td></td>
<td>1994 (166)</td>
</tr>
<tr>
<td>essential oils</td>
<td>linalool, limonene, acetate of linalyl, and related compounds</td>
<td>GC (chiral)</td>
<td>adulteration detection and/or authentication</td>
<td>1997 (167)</td>
</tr>
<tr>
<td>European virgin olive oil</td>
<td>volatile and nonvolatile compounds</td>
<td>GC</td>
<td></td>
<td>1997 (168)</td>
</tr>
<tr>
<td>green coffee</td>
<td>sterul-derived compounds</td>
<td>GC</td>
<td></td>
<td>1998 (169)</td>
</tr>
<tr>
<td>compounded beverages</td>
<td>sterol compounds</td>
<td>GC-MS</td>
<td></td>
<td>1998 (170)</td>
</tr>
<tr>
<td>honeys</td>
<td>sugars</td>
<td>CG (capillary)</td>
<td></td>
<td>1995 (171)</td>
</tr>
<tr>
<td>blackcurrant</td>
<td>terpenes</td>
<td>HPLC + GC-MS</td>
<td>impact of volatile compounds on taste</td>
<td>1995 (172)</td>
</tr>
<tr>
<td>auk</td>
<td>volatile sulfur compounds</td>
<td>FDP-GC + GC-MS</td>
<td>identification</td>
<td>1998 (173)</td>
</tr>
<tr>
<td>cherry</td>
<td>volatile, nonvolatile compounds, sugars, and organic acids</td>
<td>GC-MS</td>
<td>physicochemical properties</td>
<td>1998 (174)</td>
</tr>
<tr>
<td>citrus essential oils</td>
<td>fructo-oligosaccharides</td>
<td>GC-MS</td>
<td>detection of chemical contaminants</td>
<td>1999 (175)</td>
</tr>
</tbody>
</table>
spectrophotometric cuvettes in ~2 min and is stable for 1 h. The measurement is made at 595 nm. The reliability and the robustness of this method are its main advantages, and this is the principal reason for which Bradford’s method is now preferred over other methods. Moreover, this method can be easily automated, and the Bio-Rad firm has developed a version for microtiter plates well adapted to a routine utilization (41). The sensitivity of this variant is suitable for this type of measurements. In addition, this method removes most of the problems involved in the procedures described above (i.e., Lowry’s method), which are subject to interference by compounds such as potassium ions, magnesium ions, EDTA, carbohydrates, thiol reagents, and Tris buffer.

(2) On the other hand, specific chemical reactions exist permitting amino acid determinations or amino groups only, such as ninhydrin reagent, used for the first time by Ruhemann in 1910 but modified and improved many times. One of the last applications of this method is published by Wilkinson et al. (42), who utilized the chemical reaction to derivatize amino acid samples in order to inject them in GC determinations.

Ninhydrin normally forms blue complexes with most amino acids, except with those having a tertiary nitrogen, such as proline and hydroxyproline, with which it forms pink complex (43).

The last three methods that are classed in this category are the TNSB (trinitrobenzenesulfonic acid) method, the method using o-phthalaldehyde, or, better, that using fluorescamine. The first forms yellow complexes with primary amines, whereas the second, which reacts with α-amino group of amino acids and short peptides, give a fluorescent compound. The third, using 4-phenylspiro[furan-2(3H)-1-phthalan]-3,3′-dione] (fluorescamine), reacts quasi-instantaneously with primary amines to give also a fluorescent compound at room temperature. Further details on these various approaches are given by Wallace and Fox (44) for specific cheese and cheese fraction applications.

In the honey area, fluorometric methods were not very widely applied (45–47). On the other hand, spectrophotometric methods have been widely used for about 20 years to make specific analyses of some chemical compounds of honeys. White et al. (48) used UV–visible spectrophotometry to determine the proline content in American honeys, and Davies et al. (49) applied this method for geographical origin determination to distinguish between English and Welsh honeys. In these two cases, the method is based on the ninhydrin reaction to produce colored samples. The most recent spectrophotometric application in the honey area makes use of enzymatic processes for determining various parameters such as glycerol content (50), which can be considered to be an indicator of the fermentation state of honey. α-Gluconic acid (51), galactose, lactose (52), and l-malic acid (53) contents are other honey constituents that can be evaluated by enzymatic methods. All of these methods work as honey characterization tools and together provide a good indication of honey quality.

**AAS/AES/ICP-AES**

Atomic absorption (AAS) or atomic emission (AES) are very old analytical techniques, older than GC or HPLC. AAS/AES use the physical properties of any chemical element that composes the matter, particularly the common possibility for all mineral elements listed in the Mendeleev classification, to absorb or to emit a monochromatic beam with a wavelength specific of each of them. This absorption or emission is considered to be a fingerprint of the particular element being analyzed.

Determination of the mineral content in food products started in the 1930s. Shuette et al. were interested by minerals in honeys and highlighted the relationship between the pigmentation degree and the mineral content. Later, McLelan et al. (54) used AAS to measure calcium, magnesium, potassium, and sodium contents in honeys, followed by Petrovic et al. (55), who determined the selenium content from only Eastern Croatia honeys. AAS/AES and ICP-AES are very useful techniques for the mineral content determination of food products (56). More and more applications of these techniques are now available in the literature, particularly applied to wines (57), cheeses (58), sugars (59), and honeys (60–62).

Some studies have been published in recent years concerning detection of the adulteration in food products by AAS/AES, theses techniques having been mainly used to make characterizations, as, for example, done by Moussa (63) and Lapierre (64), in determining the geographical origin of honeys. However, recently Simpkins et al. (65) suggested the use of AAS and ICP-AES to detect adulteration of Australian orange juices by showing that many elements are present in various concentrations depending on the origin of the orange juice. By using principal component analysis, the mixing of two qualities of orange juice was verified. Here again, the application of this type of technique in food analysis can require the treatment of a large quantity of data that implies the utilization of chemometric methods. Sun et al. (66) applied both discrimination analytical methods (ACP, HCA, Bayes discrimination method, and Fischer discrimination method) and ANN (back-propagation architecture) methods to ICP-OES (optical emission spectrometry) data to classify wine samples from six different regions. The results obtained by ANN compared with those obtained by conventional Bayes discrimination analysis and Fischer discrimination analysis methods show a satisfactory prediction coefficient (100%), whereas the Bayes method gives a discrimination rate of 98.8% for the prediction set and the Fischer method provides a discrimination rate of 91.8% for the prediction set. However, the ANN approach takes the longest computing time among all of the methods.

**IRMS**

For many years, isotope ratio mass spectrometry (IRMS) has been the official technique to detect adulteration in honey and other food products. The first major work in this area was presented by White and Doner, who proposed a method to detect a potential industrial syrup addition (from C-4 plants) in food products and particularly in honeys (67).

The principle of IRMS is easy to understand. The procedure consists of measuring the isotope ratio of an analyte converted into a simple gas, isotopically representative of the original sample before entering the ion source of an IRMS system. The system performs isotope ratio measurements (1H/2H, 15N/14N, 13C/12C, 18O/16O, and 34S/32S) continuously on gases of H2, N2, CO2, CO, and SO2, respectively. For example, in the case of carbon, the 13C/12C ratio is naturally constant in all biological products but can fluctuate from one C source to another (atmospheric CO2, C4 plants, C3 plants, animal carbon, etc.), so it is easy to detect whether a these different C sources were mixed because the 13C/12C ratio of the product will have shifted compared to that of the natural product.

For more theoretical and practical details see the review by Meier-Augenstein (68), who presents all aspects of the IRMS system utilization detailing the sample preparation, derivatization, isotopic calibration, etc., and gives examples of many applications on flavors and fragrances, wine, fruit juice, vegetable oils, and honey with more than 160 references.
Concerning honeys, the reference works in this area are those of White, Winters, and Martin (69, 70) for both the initial method development (δ13C measurements on honeys) and the setting of the improved method (internal standard method: measure of δ13C on honey proteins). These analytical developments were followed by a study on the detection of adulteration of honey by continuous-flow isotope ratio mass spectrometry (71). The method consisted of passing the sample through a GC combustion system and elemental analyzer inlets to determine the δ13C value in honey. The author claimed 1.6% of high-fructose corn syrup adulteration could be detected.

DSC

Sometimes, chemical analytical methods need to be complemented by experiments using new indicators derived from physical analysis such as thermal analysis. The most widely used technique in this area is differential scanning calorimetry (DSC), which originated from the pharmaceutical (72, 73) and polymer material industries (74–80). The application of temperature-programmed scans causes some structural modifications (phase transitions, glass transition, etc.) or decompositions. Thermal techniques such as DSC and others can monitor these transformations by measuring their consequences: thermal effects (variation of temperature or energy), variation of physical properties of food products (heat capacity, Young’s modulus, conductivity, variation of the volume, etc.), gas production, variation of mass, etc... These phenomena allow the determination of the type of transformation occurring in the studied products and determine their thermodynamic and kinetic properties.

The common transformations observable by DSC are the denaturation of proteins and their thermal stabilities (81–83); fusion or crystallization of carbohydrates, phase transitions of polysaccharides; thermal properties of frozen foods; fusion or crystallization of lipids and interactions with other chemical compounds (84, 85); gelatization of native starch or starch in foods (86–91); and thermal history.

From 1992 to 1999, five important reviews on the applications of thermal analysis were published. Several analytical areas are involved, such as instrumentation, thermodynamic measurements, reaction kinetics, inorganic compounds, organic and polymeric materials, biological, and medical and pharmaceutical studies. (92, 93). Many aspects of the applications in the drug development process, such as calibration, purity determination, quantification of crystallinity, polymorphism, degradation, decomposition, and glass transition, have been thoroughly treated (72). In the area of food, DSC is also widely used, but its utilization is more recent than in other industrial domains. Foods are multicomponent systems containing proteins, carbohydrates, lipids, starch, and water. Therefore, multiple interactions can arise between food components and lead to some modifications of the thermal behavior of foods (72, 94). A good understanding of their thermal properties is essential for a good definition of product quality (95–103) and for detecting alteration (104) or adulteration (105–107). Moreover, a thermal analytical approach in food has great value in the quality assurance of food (108).

Table 4 presents some additional calorimetric studies on foods with both the aims of each study and the parameter(s) measured to achieve the objective.

**IR, 1H, AND 2H NMR SPECTROSCOPES**

This review cannot be complete without taking into account the applications of spectroscopic techniques to food characterization and authentication.

**IR Spectroscopy.** One of the spectroscopic techniques most commonly used in the food product adulteration area or quality control of manufactured products is infrared spectroscopy (IR and mid-IR). The wavelength range is classically <2500 cm⁻¹.

A major advantage of IR spectroscopy versus other previously used techniques lies in its nondestructive nature, like NMR applications, which will be described later in this review.

From a general point of view, spectroscopic techniques yield structural information that constitutes a molecular fingerprint.
of the sample. It makes sense to think that kind of technique could be used either as a characterization tool or as a technical means to detect food adulteration. Downey et al. (109) suggest various possible approaches using IR spectroscopy coupled with chemometric tools to perform food authentication.

Table 5 presents the most interesting work published in the past seven years using IR spectroscopy for food characterization and food authentication.

With respect to honey, only one IR contribution (110) was reported from 1990 to 2000, probably because of the chemical complexity of this matrix, which is essentially constituted by various sugars (mono-, di-, tri-, and oligosaccharides) but also contains numerous aromatic substances, proteins, water, microsopic figurative elements such as pollens, vegetable cells, etc. The authors proposed a regression model (obtained by PLS methods) allowing the determination of the major constituents of honey (moisture, fructose, glucose, sucrose, and maltose) based on the IR measurements and classical physicochemical determinations (official methods of AOAC).

NMR Spectroscopy. For a few years, nuclear magnetic resonance (NMR) techniques such as $^1$H NMR, $^2$H NMR monodimensional or bidimensional (2D-NMR), and SNIF-NMR complemented the analysis possibilities offered by IR spectroscopy. The late 1980s and the early 1990s were particularly productive in reviews reporting the use of NMR applied to food materials with the aim of food characterization as well as adulteration detection (111–115). Vogel et al. (119) propose, in a study on fruit juices, a new screening method using $^1$H NMR combined with chemometrics. Their approach consists of applying principal component analysis (PCA) to reduce the data dimensionality from $^1$H NMR spectra. Effectively, when the analyzed substance is not pure (constituted by different types of molecules) as with a mixture, the generated $^1$H NMR spectrum is too complex to be interpreted visually. By applying PCA to these spectra, it is possible to combine correlated variables (peaks in spectra) to form a new smaller group of independent variables, which are called principal components. These components are oriented and constructed in order to explain the maximum variance contained in the original data. Results obtained by this method show this approach is very pertinent and adapted to detect adulteration because it allows a good discrimination between nonadulterated samples and samples adulterated by various compounds (sucrose, inverted beet sugar 50%, sodium benzoate, etc.).

More recently, Sacchi et al. (120) presented the high-field $^1$H NMR (600 MHz) contribution in olive oil characterization and geographical origin determination. This study is oriented toward minor component analysis of olive oil such as sterols, saturated and unsaturated aliphatic aldehydes, and various volatile compounds. Results obtained indicate that samples are correctly classified in 96% of cases and that the discrimination between classes is sufficient to obtain a good characterization of geographical origin of olive oils. In the past year, Spyros et al. (121) presented a new facile method using $^{31}$P NMR to characterize olive oils to determine their geographical origin. This method is based on the phosphorylation of the free hydroxyls of the mono- and diglycerides with a specific reagent and the integration of the appropriate peaks in the $^{31}$P NMR spectrum. The method was tested with certain model compounds bearing labile protons with the purpose of quantitative measurements of 1,2-diglycerides/1,3-diglycerides ratios of virgin olive oils from different geographical origins.

In a characterization study of Citrus honeys, Lindner et al. (122) measured the D/H ratio and $^13$C values on honey sugars using SMRI.

SNIF-NMR. Site-specific natural isotope fractionation nuclear magnetic resonance is probably the best answer to the question of adulterations. Stemming from the work of G. J. Martin and colleagues at l’Université de Nantes, France, SNIF-NMR is the most sophisticated and most specific method available for the determination of food product authenticity. Introduced in the early 1980s (123), this method is based on the measurement of deuterium/hydrogen (D/H) ratios at specific sites in a molecule. For example, to detect clandestine addition of sugar or modified sugar syrups in a juice or concentrate, the sample is fermented under controlled conditions and the resulting alcohol is distilled off. Alcohol recovery is monitored by determining the alcoholic grade of both the fermented juice and the distillate. D/H ratios are measured at the methyl (D/H)$_3$ and methylene (D/H)$_n$ sites of the ethanol molecule. The sample is declared to be adulterated if the (D/H)$_3$ value in ethanol derived from the sample falls below a certain level. It has been shown that the (D/H)$_3$ value is significantly lower in ethanol derived from beet sugar than in ethanol originating from grape or citrus fruits. One of the first applications of SNIF-NMR was to detect the adulteration of wines (124). Since then, numerous contributions of SNIF-NMR have been reported. Day et al. (125) showed the value of SNIF-NMR measurements coupled with AAS trace element analysis in the determination of the geographical origin of wines. Eleven trace elements were analyzed (K, Ca, Mg, Mn, Fe, Zn, Cu, Rh, Al, Sr, and Ba) by AAS, and five isotopic parameters ([D/H)$_{\text{H}_2}$, $^\text{13}$O$_{\text{H}_2}$, (D/H)$_{\text{H}_2}$, $^\text{13}$C$_{\text{H}_2}$), including two site-specific ratios, were determined using SNIF-NMR and IRMS. Martin et al. (126) applied SNIF-NMR alone or SNIF-NMR and stable isotope ratio analysis mass spectrometry (SIRA-MS) (127) to detect added sugar and to assess authentication of fruit juices. The influence of the deuterium content of the fermentation water on the isotopic parameters was shown, and a way for normalizing the results was proposed (adulteration triangle, interpretation rules for SNIF-NMR and SIRA-MS analysis applied to detection of beet sugar and/or cane sugar in fruit juice). The strength of this technique comes from its capacity to be applied to any raw material or any food product (128, 129). The only weakness of this method is the need for a large knowledge base or database for comparison purposes, but this limitation is diminishing as the SNIF-NMR method is more and more frequently used for food characterization or adulteration detection.

CONCLUSION

With the objective of characterization of the food product, whether one adopts a global approach or a chemical compound approach, all of the techniques presented here are well suited...
and provide interesting information. The ideal is to, as much as possible, arrange several techniques to gather a set of additional data allowing a better characterization of the food from the physicochemical point of view. In such a context and in an approach by chemical compounds, it seems evident that chromatographic techniques such as HPLC and GC (and their coupled variants) are particularly well adapted to the characterization of the food from a molecular point of view without there being a real necessity of identifying the totality of detected compounds (chemometric treatment of the chromatograms). However, it is also evident that the characterization of products may not be attainable with a purely molecular or macro-molecular vision and that the mineral constituent often turns out to be interesting and even necessary for the characterization. In this area, only spectroscopic techniques such as atomic absorption (AAS, AES, ICP-AES, etc.) can bring the expected answer. As for UV–visible spectrophotometry, it joins a molecular approach of the characterization but with the necessity of knowing the colorimetric properties of the measured compounds. If the global approach of the characterization is chosen, the best results will be obtained from techniques stemming from the domain of the thermal analysis such as DSC. More and more, IR spectroscopy and NMR spectroscopy are used in association with chemometric treatment to characterize mixtures of chemical compounds and foods. Finally, IRMS turns out to be successful both for a global characterization of the sample and for a compound by compound characterization (GC-IRMS and GC-C-IRMS), too. For the purpose of detection of adulteration, two approaches are also possible: the global analysis and the search for tracer(s) of adulteration. In the first case, IRMS and the spectroscopic techniques will doubtless give the best results, whereas in the second case a better approach will be to use chromatographic techniques, optical microscopy, and GC-IRMS. Besides, the significant development of data processing software (Statistica, S-Plus, Statgraphics, Unscrambler, etc.) provides considerable help in the resolution of the problems of charac-

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terization as well as in the search for and the detection of nonconformities and adulterations. Supplemetning the classical chemometric tools, such as PCA, LDA, HCA, SIMCA and PLS methods, we see interesting developments of various solutions based on artificial neural networks applied to food problems. The main advantages of these "neuromimetic" structures are their robustness faced with fuzzy data and their capacity to resolve problems containing nonlinear solutions. The future of the analysis of foodstuffs seems to be linked to the increasing development of the analytical solutions marrying powerful analytical devices and data processing software such as the headspace GC-MS coupling, known as "Chemsensor" (from Agilent Technology) or "Prometheus" (from Alpha MOS), which is a headspace MS system.

**ABBREVIATIONS USED**

- PCA, principal component analysis
- LDA, linear discriminant analysis
- HCA, hierarchical cluster analysis
- SIMCA, soft independent modeling of class analogy
- PLS, partial least squares
- CVA, canonical variate analysis
- ANN, artificial neural network
- HPLC, high-performance liquid chromatography
- GC, gas chromatography
- MS, mass spectrometry
- GC-FTIR, GC–Fourier transform infrared spectroscopy
- AAS, atomic absorption spectroscopy
- AES, atomic emission spectroscopy
- ICP, inductively coupled plasma
- IRMS, isotopic ratio mass spectrometry
- TGA, thermogravimetric analysis
- DSC, differential scanning calorimetry
- IR, infrared spectroscopy
- NMR, nuclear magnetic resonance spectroscopy
- SNIF-NMR, site-specific natural fractionation nuclear magnetic resonance spectroscopy
- HPIC-PAD, high-performance ionic chromatography–pulsed amperometric detection

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LITERATURE CITED


(21) Silva, B. M.; Andrade, P. B.; Mendes, G. C.; Valen...
Reviews


