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# New Analytical Advances for Addressing Healthful Constituents in Foods

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## ABSTRACT

The tools available to analyze the chemical composition of foods and other complex samples have developed rapidly, including commercial introduction of new instruments and many examples of utility. Areas of application include identification and quantification of newly uncovered bioactive chemicals and chemical classes, adulteration and authentication of foods, determining bioavailability of both parent and metabolites of specific interest, and addressing emerging environmental contaminant issues. FT-infrared and other spectroscopic techniques have seen increasing applications particularly in the area of in situ and/or screening analyses for the presence of specific classes of chemicals. Many of these tools are based upon mass spectrometry, with LC/MS and LC/MS/MS leading the way, but new applications of MALDI-TOF, isotope mass spectrometry, and highly sensitive specific ion detection systems are finding increasing applications. Advances have also been made in both NMR and MS-based metabolomics, in sample preparation techniques, and applications of analytical chemistry to biological analytes such as microorganisms in foods, behavior in food processing, and during the digestion of foods in the body. This paper will include an overview of new/recent applications of methods and of areas where continuing method development research is needed to address current and emerging issues with food quality and safety.

Key words: Analysis, mass spectrometry, proteomics, metabolomics, genotyping, contaminants, adulteration, nutraceuticals, phytonutrients, functional foods, food safety, food healthfulness

## INTRODUCTION

Analytical advances have been made with increasing frequency, leading to new instrumentation for resolving, detecting, identifying, and measuring bioactive substances in foods. These include naturally occurring and introduced chemicals, and also microorganisms that occur as unintentional contaminants. In the area of food toxicology and safety, in addition to pathogenic microorganisms, genetically engineered foods, nanomaterials and obesity-enhancing substances are of increasing concern, along with the more long standing targets of microbial and other natural toxins, pesticide residues, persistent organic pollutants (POPs), heavy metals, mutagens formed during processing and cooking, food allergens, food additives and diseases such as hepatitis, BSE, and avian flu. In the area of diet and health, antioxidants have risen in attention for the past 15 years or so, with interest also in dietary fibers, bioavailability of nutrients, vitamins, dietary supplements, unsaturated fatty acids, and trace elements.

The evolution of analytical techniques for contaminant and constituent analysis has evolved based upon these needs (Figure 1)<sup>(1)</sup>. The modern era of analysis developed with the onset of chromatography, and particularly gas-liquid and high performance liquid chromatography in the 1970s, an area which continues to develop

with interfaces to mass spectrometry-based detectors, and other specialized detection systems. Tandem Mass Spec (MS<sup>n</sup>) represents a technique of much interest in the present era.

Microbiology meets chemistry in the evolution of modern methods for detecting and identifying foodborne pathogens, which rely increasingly on mass spectrometry. MALDI-TOF (matrix-assisted laser desorption-time of flight) mass spec can be used:

- To identify/classify pathogenic bacteria *via* biomolecules (proteins, DNA) characteristic of the pathogens
- For MS analysis of expressed proteins linked to genetic identity of bacteria
- For tandem TOF-MS (TOF-TOF) techniques which allow sequence specific fragment ion determination
- To carry out top-down proteomics to rapidly identify foodborne pathogens by comparison with MS databases.

Examples are in Fagerquist *et al.*<sup>(2)</sup> who applied MALDI-TOF-TOF MS and top-down proteomics to identify *E. coli* 0157:H7 in connection with outbreaks in leafy green vegetables in California's coastal vegetable producing areas in the 2000s.

Genome sequencing is another relatively new technique arising from modern molecular biology, and as a basis for genotyping *via* regions that vary between strains

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of microscopic organisms. Microarray technology can be built on this foundation to find which genes are present or absent in a particular isolate, and to determine strain relatedness (phylogeny) between different isolates. As an example, Parker *et al.*<sup>(3)</sup> compared genotypes of *Salmonella enterica* Serovar Enteritidis Phage Type 30 and 9c strains isolated during three outbreaks associated with raw almonds that occurred in California. Establishing that two of the outbreak strains were closely related helped in identifying risk factors as a basis for managing or preventing future food product contamination. Cluster analysis with comparative genomics indexing holds promise for minimizing ambiguity in identification as well as picking out pathogenic vs non-pathogenic strains of the causative microorganisms.

Aflatoxin detection in *Aspergillus* infected nuts, grains, and seeds provides several examples of the use of modern instrumental detection and analysis to improve the safety and quality of the food supply. LC-fluorescence and/or LC-MS are among the current methods of choice. Insect damage is often the enabling step in *Aspergillus* invasion of these commodities. The control of insects such as codling moth is thus key to control of *Aspergillus* and elimination of aflatoxins in pears, almonds, walnuts and other orchard crops. Both pheromone and non-pheromone natural attractants, identified by GC/MS and other instrumental techniques including GC-electroantennography, have been employed<sup>(4)</sup>. Electroantennography (EAG) was an early precursor to GC-olfactometry which has found multiple uses in identifying flavor and aroma agents, off flavors, adulterants, *etc.* in food products, as well as attractants for insects which can be used for pest control.

Another aspect of the *Aspergillus* and aflatoxin contamination of tree nuts which involved analytical chemistry was in tracking down why tree nut species varied so much in their ability to support aflatoxin production by invading codling moth. The mix of identified polyphenol antioxidants formed during the feeding and spoilage of the infested nut tissue inhibited aflatoxin production quite efficiently in almonds, due to high contents of ellagic acid—a particularly potent polyphenolic antioxidant<sup>(5)</sup>.

In the area of adulteration, a significant problem along with the threat of bioterrorism, LC/tandem MS proves to be quite useful because of its speed and access to information in databases of chemicals with potential for illegal use in foods. Melamine, for example, was identified in kidney tissue of poisoned pets by this technique<sup>(6)</sup>. In another area of toxicological significance, acrylamide was identified first as an agent of occupational illness and then, using serendipity in part, as a contaminant in French fried potatoes and other high temperature, primarily fried foods<sup>(7)</sup>. A current challenge being addressed with instrumental analytical techniques is represented by the migration of plastics additives from plastic food utensils and containers into foods, including baby foods<sup>(8)</sup>.

There are many examples of the identification and

quantitation of health beneficial chemicals in foods. For example, Huang and Mazza presented a review of the application of LC and LC-MS for the analysis of melatonin and serotonin in edible plants<sup>(9)</sup>. These indolamines possess a broad spectrum of neurological bioactivity, but only recently were found in plants at significant concentrations, up to 220 micrograms per gram in tomatoes<sup>(10)</sup>. They function as antioxidants in people consuming these chemicals in components of the diet. HPLC is now routinely used for their analysis, with a variety of detectors including MS.

Metabolomics is a newer approach to characterizing chemicals responsible for biological functions and activities in cells, tissues, body fluids, and whole organisms. GC-MS, LC-MS, and NMR are most often used to address the daunting task of analyzing for all small molecules in a sample, both for identification but also for observing changes in metabolites when the organism is exposed to drugs, toxicants, nutritional agents, stress, or other substances or states that can affect the health status of the organism in question. Both global approaches, addressing all of the small molecules in a sample, and targeted metabolomics, addressing individual groups of metabolites, can be used. Metabolomics has several applications in food and nutrition sciences<sup>(11)</sup>:

- For food composition and characterization
- For food adulteration and deterioration
- For diet monitoring and metabolism
- For physiological response monitoring

Erdman *et al.*<sup>(12)</sup> identified and summarized flavanoid and polyphenol consumption in adults using a metabolomics approach. Froehlich *et al.*<sup>(13)</sup> used a metabolomics approach to target oligosaccharides in human milk, which was found to contain more oligosaccharides than proteins and that less than half of those present had been identified. Dallas *et al.*<sup>(14)</sup> performed N-linked glycan profiling of human milk by high-performance microfluidic chip LC/TOF/tandem MS. Oligosaccharides play prominent roles in promoting a healthy gut microbiota, including the transformation of ingested polyphenols to more bioavailable and effective forms in safeguarding human health, particularly critical in the early period of life of neonates as they move from sole reliance on human milk to a more varied diet of milk and other foods<sup>(15)</sup>.

Although not 'metabolomics', multiresidue approaches to evaluating pesticides and other contaminants in foods have much in common with metabolomics-broad application for screening all contaminants of concern in food samples, relatively rapidly. Filagenzi *et al* used the procedure dubbed QuEChers<sup>(16)</sup> to screen for chemical contaminants of concern in food-related matrices using QuEChERS extraction, UHPLC and high resolution mass spectrometry. This multiclass, multiresidue analytical approach is rapidly catching on worldwide as a watchdog technique to ensure the safety of the food supply.

The sheer amount of data produced using new tech-

niques of analysis will challenge the ingenuity of analysts and instrument companies to come up with formats for data storage, data processing, and data retrieval. Such areas as fingerprinting samples based upon their chemical content, in situ methods, principal component analysis, and transformation of data will be particularly important to a future of data-rich characterization of samples, using both existing techniques as well as new ones which are certain to be developed.

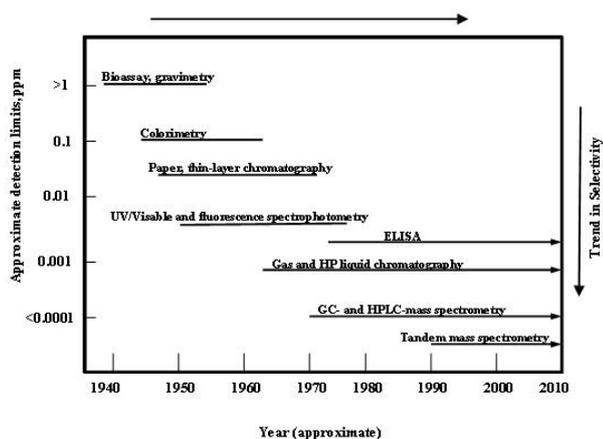


Figure 1. Development of chemical analytical methods over time. Adapted from Seiber<sup>(1)</sup>.

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