# Metabolomic Technologies for Improving the Quality of Food: Practice and Promise

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Annu. Rev. Food Sci. Technol. 2016. 7:413–38

First published online as a Review in Advance on January 11, 2016

The *Annual Review of Food Science and Technology* is online at food.annualreviews.org

This article's doi: 10.1146/annurev-food-022814-015721



## Keywords

mass spectrometry, MS, nuclear magnetic resonance, NMR, central metabolism, agricultural practices, processing, storage

## Abstract

It is now well documented that the diet has a significant impact on human health and well-being. However, the complete set of small molecule metabolites present in foods that make up the human diet and the role of food production systems in altering this food metabolome are still largely unknown. Metabolomic platforms that rely on nuclear magnetic resonance (NMR) and mass spectrometry (MS) analytical technologies are being employed to study the impact of agricultural practices, processing, and storage on the global chemical composition of food; to identify novel bioactive compounds; and for authentication and region-of-origin classifications. This review provides an overview of the current terminology, analytical methods, and compounds associated with metabolomic studies, and provides insight into the application of metabolomics to generate new knowledge that enables us to produce, preserve, and distribute high-quality foods for health promotion.

## INTRODUCTION

As late as the mid-1990s, the idea of monitoring all human metabolites was firmly in the realm of science fiction, as illustrated in Greg Egan's short story "Yeyuka," in which a doctor wears a ring that constantly samples his blood, monitoring and responding to the thousands of compounds making up his metabolic profile (Egan 1998). In the fictional story, it was thus possible to identify disease at such an early stage that it could effectively be prevented. Parts of that story, such as real-time monitoring of all metabolites and real-time response to the results of that monitoring, remain science fiction, but for how long? Since the early 1980s, insulin-dependent diabetics have had the option of wearing a pump that constantly monitors one compound (blood glucose) and delivers insulin in real time to maintain appropriate blood sugar levels. Today, wearable fitness devices monitor heart rate and activity levels in real time. How long will it be until these wearable devices begin sampling a wide range of biofluids to constantly monitor health and respond to any perceived irregularities?

The emerging discipline of metabolomics seeks to accomplish what was once thought to be science fiction by providing a snapshot of animal, plant, or microbial metabolism. Previous systems biology approaches, such as the Human Genome Project (started in 1990 and completed in 2004) had hoped to provide a global understanding of human health and disease, but this promise remained unfulfilled because of the incredible complexity that lay beyond the genome, including the transcriptome and the proteome, as well as epigenetics (Monteiro et al. 2013). Genomics, transcriptomics, and proteomics, the global study of DNA, RNA, and proteins in biological systems, respectively, were thus the first three disciplines in the systems biology approach. Can the newer discipline of metabolomics deliver on the promise of individualized health care? The scientific and medical communities now understand that heart disease is far more complex than cholesterol levels alone and that diabetes cannot be controlled simply by controlling glucose levels, but are still grappling with how to achieve a truly comprehensive and individualized understanding of metabolism. The field of metabolomics aims to fill the phenotype-genotype gap that currently exists in the functional genomics era.

Metabolomic analysis of small molecules sampled from easily accessible biofluids, such as blood, saliva, and urine, builds on a long medical tradition of the study of biofluids. From the 1200s through the 1600s, the idea of balancing the four humors prevailed in medicine. The four humors, melancholic, phlegmatic, sanguine, and choleric, corresponded to four biofluids: black bile, phlegm, blood, and yellow bile (US Natl. Libr. Med. 2015). As distasteful as it may seem, the color, smell, and, yes, even the taste of urine was used for many years as a means of diagnosing disease. Ullrich Pinder's Urine Wheel, published as part of his *Epyphanie Medicorum* in 1506, illustrated urinalysis techniques that medieval physicians had already been using for hundreds of years. Twentieth century research came to recognize the importance of specific metabolites in health and disease. Today, we are familiar with blood tests and urinalysis. A full blood panel at a modern hospital may measure several dozen different analytes (sodium, glucose, creatinine, etc.) by a variety of means (Weatherby & Ferguson 2004). Likewise, modern urinalysis can be used for medical purposes or for drug testing but is similarly limited in the number of compounds it seeks to detect. In contrast, a single metabolomics experiment may identify and attempt to quantify hundreds or even thousands of compounds. Today, metabolomic studies are being conducted not only with human biofluids but with samples from all forms of life. Viewed in this way, metabolomics is simply a more comprehensive, more technologically advanced extension of both ancient and current medical practices. As a result of metabolomic research, scientists now understand, better than ever, how little we know about the importance of the presence, concentration, and flux of metabolites in health and disease.

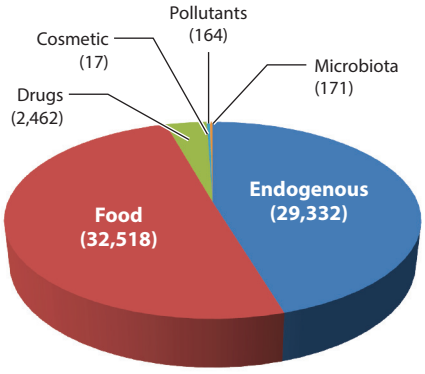


**Figure 1**

Groups of metabolites cataloged in the human metabolome database (**http://www.hmdb.ca/**; **http://foodb.ca/**).



Although originally conceived as a means of understanding human metabolism, it is now well recognized that a thorough understanding of plant, microbial, and animal metabolites is needed to appreciate how the food metabolome impacts human health. All of the small molecules that can be measured in human blood, urine, saliva, and tissue samples map onto some biochemical pathway, such as glycolysis or the citric acid cycle, but not all are strictly human in origin. Thus, human metabolism is affected by diet, microbiota, and environmental exposure. As cataloged by the Human Metabolome Database (Wishart et al. 2007), 41,933 metabolites have been identified and/or quantified in human fluids and tissues thus far. This is a tremendous increase over the 6,500 metabolites documented in 2009 (Wishart et al. 2009). Of the more than 40,000 metabolites now listed in the database, 32,518 (more than 13,000 that are uniquely diet-related) are from food sources compared to 29,332 endogenous metabolites (Wishart et al. 2013), highlighting the tremendous impact that food can have on human metabolism (**Figure 1**). A food metabolome database, referred to as FooDB, has also been released by the Wishart lab (Wishart et al. 2009) to fully document all the known chemical and biological properties of these food-related metabolites.

The expansion of the use of metabolomic technologies and terminology has resulted in a proliferation of published data. According to PubMed Medline, only two published articles used the term metabolomics in 2000, whereas a total of 2,100 peer-reviewed metabolomic studies were published in 2014. Among these publications is the promise of individualized nutrition (German et al. 2004), a better understanding of diseases and the links among them, a better understanding of drug efficacy and toxicity (Kaddurah-Daouk et al. 2008), and improved food quality and safety (Cevallos-Cevallos et al. 2009). The objectives of this review are to provide an update on the current terminology, analytical methods, and compounds associated with metabolomic studies, as well as to provide insights into the application of metabolomics for the improvement of food quality, which ultimately benefits human health and well-being.

## WHAT IS METABOLOMICS?

Metabolomics has been defined as the field of research that involves the characterization, including identification and quantification, of the complete collection of small molecule metabolites in a biological system. The term small molecules refers to compounds with a molecular weight

of less than 2 kilodaltons (Wishart et al. 2013). This means that large molecules, such as DNA, RNA, starch, and protein, are excluded from metabolomic data. Although the term metabolomics is often used synonymously with metabonomics, they are not the same. Metabonomics refers specifically to the changes in metabolites of a living system related to a pathophysiological state, biological stimulus, or genetic alteration (Nicholson et al. 1999). Metabolomics is a more general term. Since the inception of omics technologies, a dizzying array of new terms has appeared in the scientific literature (e.g., foodomics, lipidomics, fluxomics, mineralomics), all trying to comprehensively describe some aspect of metabolism or composition of metabolites in a biological matrix. The discovery-based approach that is employed in these studies generates new knowledge on the chemical similarities and differences between defined groups of samples. Analysis of the massive data sets produced by these experiments results in the development of new scientific questions. Therefore, metabolomics and related omics disciplines are often referred to as hypothesis-generating.

The criteria set forth for metabolomic studies state that the sample preparation, analytical method, and data analysis must include all classes of compounds; have high recovery; be robust, sensitive, reproducible, matrix independent, and universal; and have a plan for identifying unknowns (Fiehn 2001). Achieving these goals with a single analytical technology is a significant challenge given the extraordinary diversity of chemical species that make up the metabolome. In practice, metabolomic studies apply a nontargeted metabolite profiling approach to the analytical chemistry and statistical analyses used to discover changes in metabolites that occur related to some criteria of interest (e.g., a disease state, changes in a food due to processing treatments, changes in human plasma related to consumption of a food, etc.). Nontargeted or untargeted metabolomic studies aim to detect as many components as possible followed by statistical analysis to determine which components (a.k.a. differentiating metabolites) are of interest for identification and quantification. This type of nontargeted metabolite profiling is limited by the sample preparation and analytical techniques employed, the ability to handle and process large volumes of data, and the databases or other tools used for identifying unknown compounds. A nontargeted approach that aims only to observe patterns of metabolites that allow classification of samples into one or more groups of biological significance (a.k.a. a chemometric approach) is often referred to as metabolic fingerprinting. In contrast, targeted metabolomics or targeted metabolite profiling involves the quantification of a predetermined set of known compounds related to metabolic pathways of interest or a specific class of compounds. When a large number of metabolites are simultaneously quantified, this is also considered a quantitative metabolomics approach. Although most of these approaches refer to metabolite changes in a single biological material, the term metabolic footprinting has emerged in reference to the nontargeted detection of metabolite changes in a biological system due to the action of a specific microorganism.

In a nontargeted metabolomic experiment, it is common to observe hundreds to thousands of metabolite peaks per sample (**Table 1**). Data processing, although somewhat platform dependent, usually involves peak detection, assignment (with or without tentative identification), and peak-area quantification; peak alignment among samples; and prestatistical data treatment to deal with censored data and unequal variances. Given the immense quantities of data resulting from these experiments, it is necessary to create new ways to understand what often amounts to terabytes of data for single experiments. One way this is done is through the visualization of data. Principal component analysis plots, three-dimensional chromatograms, and heat maps are three common ways in which data can be visualized where simple charts and graphs cannot suffice. Smart algorithms that can study and learn from data are also being applied to ferret out meaningful results that might be overlooked by a human analyst. Other approaches map relative changes in concentrations of identified compounds onto known metabolic pathways, treating them more or less

**Table 1 Application of metabolomic technologies to improve the quality of food**

| Application | Food | Metabolomic technology | Compound coverage | Number of metabolites profiled or detected | Number of differentiating metabolites identified | Citation |
|---|---|---|---|---|---|---|
| Impact of agricultural practices and genetic lines on the chemical composition of food | Vanilla bean pods | $^1$H NMR | Organic acids, phenolic compounds, sugars | NR | 10 | Palama et al. 2009 |
| | Gilthead sea bream | $^1$H NMR | Amino acids, amines, betaines, nucleosides, nucleotides, organic acids | 38 | 6 | Savorani et al. 2010 |
| | Grape | $^1$H NMR | Amino acids, choline, organic acids, phenolic compounds, sugars | 27 | NA | Fortes et al. 2011 |
| | Mandarin oranges | $^1$H NMR | Amino acids, nucleosides, organic acids, sugars | 29 | 13 | Zhang et al. 2012 |
| | Pork | $^1$H NMR | Amino acids, amines, betaines, nucleosides, nucleotides, organic acids, sugars, sugar alcohols | 16 | 8 | Straadt et al. 2014 |
| | Salmon | $^1$H NMR | Fatty acids, nucleosides, organic acids, phospholipids, sugars | 40 | 16 | Wagner et al. 2014 |
| | Tomato | ESI-TOF-MS | Amino acids, organic acids, phenolic acids, sugars | 4,600 ion features | 17 | Overy et al. 2005 |
| | Potato | GC-MS | Amino acids, amines, organic acids, sugars (including di- and trisaccharides), sugar alcohols | 150 | NA | Roessner et al. 2000 |
| | Tomato | GC-MS | Amino acids, amines, nucleosides, nucleotides, organic acids, sugars (including di- and trisaccharides), sugar alcohols | 70 | 51 | Roessner-Tunali et al. 2003 |
| | Tomato | GC-MS | Central metabolism | NR | 889 | Schauer et al. 2006 |
| | Wheat | GC-MS | Amino acids, nucleobases, nucleotides, organic acids, sugars, sugar alcohols, sugar phosphates | 250 | 52 | Zörb et al. 2006 |
| | Sunflower | GC-MS | Central metabolism | NR | 63 | Peluffo et al. 2010 |
| | Rice | GC-MS | Amino acids, fatty acids, organic acids, polyols, sugars, sterols | 41 | NA | Lou et al. 2011 |

(*Continued*)

**Table 1 (*Continued*)**

| Application | Food | Metabolomic technology | Compound coverage | Number of metabolites profiled or detected | Number of differentiating metabolites identified | Citation |
|---|---|---|---|---|---|---|
| | Apple | GC-MS | Amino acids, amines, nucleosides, nucleotides, organic acids, phenolic compounds, sterols, sugars (including di- and trisaccharides), sugar alcohols | 248 | NA | Cuthbertson et al. 2012 |
| | Tomato | LC-MS | Amino acids, fatty acids, flavonoids, hydroxybenzoic acids, hydroxycinnamic acids, nucleosides, organic acids, phenylacetic acids, phenolic alcohols, triterpenoids | 135 | 21 | Gómez-Romero et al. 2010 |
| | Rice | LC-MS | NR | ~3,097 | 194 | Heuberger et al. 2010 |
| | Ketchup | LC-MS | Phenolic compounds, including flavanols, flavanones, and hydroxycinnamic acids | ~600 | 16 | Vallverdú-Queralt et al. 2011 |
| | Green tea | LC-MS | Alkaloids, amino acids, flavonoids, organic acids, sugars | 301 | 18 | Lee et al. 2013 |
| | Olive oil | LC-MS | Several classes of phenolic compounds | 37 | 9 | Sánchez de Medina et al. 2014 |
| | Broccoli | LC-MS | Flavonols, glucosinolates, hydroxycinnamic acids, sugars | 5,107 ion features | 12 | Sun et al. 2015 |
| | Melon | $^{1}$H NMR, FIE-MS, SPME-GC-MS | Central metabolism, phenolic compounds, and volatile alcohols, aldehydes, esters, and ketones | >1,000 | ~300 | Bernillon et al. 2013 |
| | Rice | $^{1}$H NMR, GC-MS | Amino acids, amines, fatty acids, lipids, organic acids, sugars (including di- and trisaccharides), sugar alcohols, and volatile alcohols, aldehydes, esters, hydrocarbons, ketones, and furans | NR | ~77 | Calingacion et al. 2012 |

(*Continued*)

**Table 1** (*Continued*)

| Application | Food | Metabolomic technology | Compound coverage | Number of metabolites profiled or detected | Number of differentiating metabolites identified | Citation |
|---|---|---|---|---|---|---|
| | Melon | $^1$H NMR, GC-MS | Amino acids and volatile alcohols, aldehydes, esters, hydrocarbons, ketones, and sulfur compounds | 70 | NA | Allwood et al. 2014 |
| | Green tea | LC-MS, GC-MS | Polyphenols and central metabolism | 595 | 20 | Ku et al. 2010 |
| | Pepper | LC-MS, GC-MS | Acyclic diterpenoids, alkaloids, branched-chain amino acid derivatives, fatty acid derivatives, flavonoids, phenolic compounds, phenylpropanoids, and volatile esters, ketones, fatty acids, monoterpenes, and sesquiterpenes | 1,290 | 678 | Wahyuni et al. 2013 |
| Identification of novel bioactive compounds in foods | Tomato | LC-MS | Amino acids, fatty acids, flavonoids, hydroxybenzoic acids, hydroxycinnamic acids, nucleosides, organic acids, phenylacetic acids, phenolic alcohols, triterpenoids | 135 | 21 | Gómez-Romero et al. 2010 |
| | Potato | LC-MS | Amino acids, anthocyanins, flavonols, glycoalkaloids, hydroxycinnamic acids and amides, organic acids | 31 | NA | Chong et al. 2013 |
| Changes in food composition during postharvest handling, processing, and storage | Soy sauce | $^1$H NMR | Amino acids, amines, organic acids, polyols, sugars (including oligosaccharides) | 37 | 25 | Ko et al. 2009 |
| | Beef | $^1$H NMR | Amino acids, amines, nucleotides, organic acids and derivatives, purine bases and derivatives, sugars | 27 | 12 | Graham et al. 2010 |
| | Gilthead sea bream | $^1$H NMR | Amino acids, amines, betaines, nucleosides, nucleotides, organic acids | 38 | 6 | Savorani et al. 2010 |
| | Edamame | CE-MS | Central metabolism | 126 | 76 | Sugimoto et al. 2010 |
| | Pear | GC-MS | Amino acids, amines, chlorogenic acids, fatty acids, nucleobases, organic acids, sugars, sugar alcohols, triterpenes | 64 | 18 | Pedreschi et al. 2009 |

(*Continued*)

**Table 1** (*Continued*)

| Application | Food | Metabolomic technology | Compound coverage | Number of metabolites profiled or detected | Number of differentiating metabolites identified | Citation |
|---|---|---|---|---|---|---|
| | Fermented soybean paste | GC-MS | Amino acids, organic acids, fatty acids | 41 | 9 | Park et al. 2010 |
| | Barley | GC-MS | Amino acids, amines, fatty acid methyl esters, fatty alcohols, hydrocarbons, organic acids, sterols, sugars | 587 | 173 | Frank et al. 2011 |
| | Peach | GC-MS | Amino acids, amines, fatty acids, organic acids, phenylpropanoids, polyols, sugars, sugar alcohols | 47 | 38 | Lauxmann et al. 2014 |
| | Port wine | GC-MS | Volatile compounds | NR | 6 | Castro et al. 2014 |
| | Tomato paste | LC-MS | Alkaloids, amino acids, glycosylated alkaloids, hydroxycinnamic acids and derivatives, flavonoids, organic acids, saponins | 3,177 ion features | 41 | Capanoglu et al. 2008 |
| | Beer | LC-MS | Flavonoids, organic acids, peptides, purines | NR | 16 | Heuberger et al. 2012 |
| | Green tea | LC-MS | NR | 561 | 12 | Kim et al. 2013 |
| | Wine grapes | $^{1}$H NMR, GC-MS | Amino acids, fatty acids, organic acids, peptides, sugars, volatile organic compounds | ~500 | 40 | Pinu et al. 2014 |
| | Broccoli, tomato, and carrot | $^{1}$H NMR, LC-MS, LC-MRM, GC-MS | Polar primary metabolites, antioxidant vitamins, carotenoids, glucosinolates, flavonoids, oxylipins, phenylpropanoids, sugar nucleotides, tocopherols, volatile organic compounds | NR | >128 | Lopez-Sanchez et al. 2015 |
| | Sake | CE-MS, LC-MS | Amino acids, organic acids, short peptides, sugars | 195 | 35 | Sugimoto et al. 2012 |
| | Semolina pasta | LC-MS, GC-MS | Amino acids, B vitamins, carotenoids, fatty acids, fatty alcohols, organic acids, sterols, sugars, sugar alcohols, tocopherols | 69 | NA | Beleggia et al. 2011 |
| | Tomato | LC-MS, GC-MS, SPME-GC-MS | NR | NR | 30 | Thissen et al. 2011 |

(*Continued*)

**Table 1** (*Continued*)

| Application | Food | Metabolomic technology | Compound coverage | Number of metabolites profiled or detected | Number of differentiating metabolites identified | Citation |
|---|---|---|---|---|---|---|
| Authentication and traceability of foods based on characteristic metabolite profiles | Mozzarella cheese | $^1$H NMR | Fatty acids, fatty alcohols, organic acids, sugars | 37 | 5 | Mazzei & Piccolo 2012 |
| | Wine | $^1$H NMR | Alcohols, aromatics, sugars, organic acids | 46 | 8 | López-Rituerto et al. 2012 |
| | Hazelnut | $^1$H NMR | Amino acids, choline, nucleobases, nucleosides, organic acids, phenolic compounds, polyols, sterols, sugars | 71 | 47 | Caligiani et al. 2014 |
| | Olive oil | ESI-TOF-MS | Triglycerides and fatty acids | NR | NR | Goodacre et al. 2002 |
| | Fruit juices | LC-MS | NR | NR | 21 | Jandrić et al. 2014 |
| | Coffee | LC-MS, GC-FID | NR | NR | NR | Choi et al. 2010 |

Abbreviations: $^1$H NMR, hydrogen-1 nuclear magnetic resonance; CE-MS, capillary electrophoresis–mass spectrometry; ESI-TOF-MS, electrospray ionization–time-of-flight–mass spectrometry; FIE-MS, flow injection electrospray–mass spectrometry; GC-FID, gas chomatography–flame ionization detector; GC-MS, gas chromatography–mass spectrometry; LC-MRM, liquid chromatography–multiple reaction monitoring; LC-MS, liquid chromatography–mass spectrometry; NA, not applicable; NR, not reported; SPME-GC-MS, solid phase microextraction–gas chromatography–mass spectrometry.



as biological circuits to determine which pathways may be stimulated or inhibited as well as the biological significance of those findings. More recently, tools for mapping metabolic networks independently of known biochemical pathways aim to overcome the limited knowledge in current pathway databases (Grapov et al. 2015). Numerous data treatments, statistical approaches, databases, and pathway mapping tools have been developed to enable discovery of differentiating metabolites and link these changes to metabolic fluxes and their biological significance. The details of these bioinformatics workflows have been the subject of recent books, book chapters, and review articles, and are changing rapidly to address the challenges and demands of metabolomic studies (Johnson et al. 2015).

## FOOD METABOLITES

The complete collection of small molecules present in foods, now commonly referred to as the food metabolome, mainly comprises metabolites from animals, plants, and microorganisms, which may be further altered by microorganisms, processing, storage, and, to a small degree, unintentional chemical contamination (**Figure 2**). Although certain metabolites are uniquely contributed by each of these sources (examples shown in **Figure 2**), there are many common molecules contributed by the various sources and a complex range of interactions that significantly impact the makeup of the food metabolome. Molecules associated with central carbon metabolism are common to the three food metabolite sources: animals, plants, and microorganisms. These primary

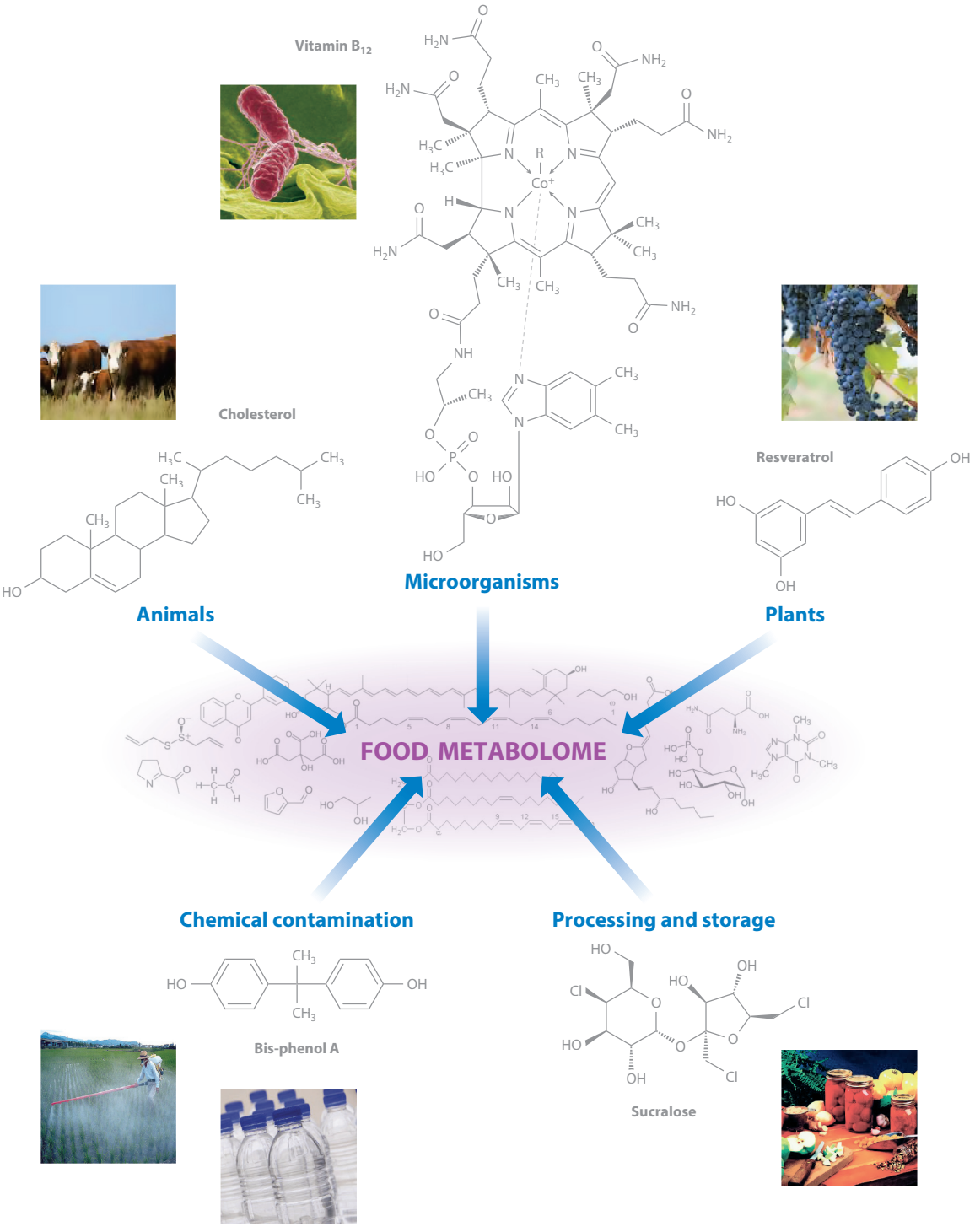


**Figure 2**

Sources of metabolites that constitute the food metabolome.

metabolites are directly involved in the growth and development of the organisms and include small molecules such as sugars, sugar phosphates, amino acids, fatty acids, and organic acids. Although these classes of compounds are shared among sources, the occurrence and abundance of specific compounds or specific combinations of compounds are often characteristic of an individual food. Therefore, the ability to simultaneously detect and quantify a large number of primary metabolites in a single analysis (Fiehn et al. 2000) is a significant advancement that is being widely applied for improving the quality of food (**Table 1**).

## Plant Secondary Metabolites

Plant-derived foods are a source of diverse metabolites. In addition to the primary metabolites shared by animals and microorganisms, the secondary plant metabolites, that are not directly involved with growth and development, are implicated in a range of activities within the plant and a host of benefits for human health. Secondary metabolites, such as flavonoids, phenolic acids, terpenes, alkaloids, and sulfur-containing compounds, can be further divided into several compound classes based upon their chemical composition. As described in more detail below, plant secondary metabolites comprise a diverse and varied collection of molecules with a wide range of chemical properties.

The flavonoid phenolic compounds are built on a diphenylpropane ($C_6$-$C_3$-$C_6$) skeleton, and they are one of the largest groups of naturally occurring phenolic compounds (Robards & Antolovich 1997, Spanos & Wrolstad 1992). Flavonoids are often found as glycosides with the flavonoid molecule linked to a glucose molecule. Classes of flavonoids are differentiated based on the number of substituent hydroxyl groups and the degree of unsaturation or oxidation of the three-carbon segment of the structure. Flavonoid phenolic compounds can be subdivided into multiple classes; flavanones, flavonols and flavones, anthocyanins, catechins, and biflavans. Flavonol and flavone glycosides are nearly ubiquitous among plants, with flavonols more prevalent in vegetables than flavones, typically occurring as glycosides (Herrmann 1988, Spanos & Wrolstad 1992). Formation of flavonol glycosides is light dependent; thus, these compounds are typically observed in leaf tissues or the skins of fruits (Herrmann 1976). The most abundant flavonol glycosides in commonly consumed fruits and vegetables are quercetin and kaempferol. Anthocyanins are molecules that consist of an aglycone with a varying number of sugar residues attached to the hydroxyl group in the 3 position (Jennings & Carmichael 1980). Cyanidins are the most prevalent anthocyanins in fruits (Ishikura & Sugahara 1979). Catechins are some of the most widely occurring flavonoids and are unique in that they have two asymmetric carbons leading to four possible isomers (Spanos & Wrolstad 1992).

Nonflavonoid phenolic compounds mainly consist of phenolic acids. Phenolic acids are involved in nutrient uptake, protein synthesis, enzyme activity, and photosynthesis in plants (Robbins 2003). Phenolic acids comprise cinnamic and benzoic acids (Spanos & Wrolstad 1992). Gallic acid, a precursor of tannins, is one of the principal phenolic acids (Crozier et al. 2006b). Cinnamic acid is a $C_6$-$C_3$ compound that can be converted to a range of hydroxycinnamates referred to collectively as phenylpropanoids. Of the hydroxycinnamates, ρ-coumaric, caffeic, and ferulic acids are the most common (Crozier et al. 2006b). Cinnamic acids attached to other compounds in the form of esters are naturally occurring in apple, pear, and grape. Benzoic acids typically occur as free acids in these fruits. Large amounts of cinnamic acids and catechins are formed early in apple fruit development, but the levels decrease significantly during the rapid growth of the fruit, with the concentration of these compounds stabilizing as the fruit matures (Spanos & Wrolstad 1992). Similar trends are observed with tannin levels as hazelnuts mature (Crozier et al. 2006b). Stilbenes are polyphenolic compounds with a $C_6$-$C_2$-$C_6$ structure. Resveratrol is the most commonly occurring stilbene and

is present in plant tissues mainly in the *trans* isomer form. Grapes, wine, soy, and peanuts are the major dietary sources of stilbenes (Crozier et al. 2006b).

Terpenes are a highly diverse set of metabolites. Carotenoids found in crop plants common in the human diet are present in the roots, leaves, shoots, seeds, fruit, and flowers (Fraser & Bramley 2004). Structurally, carotenoids are isoprenoids that generally have eight isoprene units joined such that the linking of units is reversed at the center of the molecule (Fraser & Bramley 2004). Carotenoids have a long polyene chain ranging from three to fifteen conjugated double bonds (Fraser & Bramley 2004). The conjugated double-bond structure provides carotenoids with their characteristic color. Carotenoids are important in the human diet in two common forms, α-carotene and β-carotene, which are vitamin A precursors (Humphrey & Beale 2006). Other important dietary carotenoids include lycopene, zeaxanthin, and lutein. Carotenoids are found at high levels in parsley, spinach, watercress, and carrots. The main dietary source of lycopene is tomato, with watermelon, guava, and papaya as alternative sources (Fraser & Bramley 2004). Triterpenes include the subgroup of the steroid family. The steroids consist of a flat cyclopenta[*a*]phenanthrene skeleton and an aliphatic side chain (Humphrey & Beale 2006). The phytosterols are the predominant steroids found in plants, and they have been linked to health-promoting effects, including the offsetting of cholesterol buildup in the human bloodstream (Piironen et al. 2000).

Alkaloids are nitrogen-containing, low-molecular-weight compounds that are typically derived from amino acids and are found in approximately 20% of plants. Three classes of alkaloids are typically consumed in food plants: purine alkaloids, steroidal glycoalkaloids, and betalains. The most abundant purine alkaloids are caffeine and theobromine. Caffeine is found in coffee, tea, and maté (Ashihara & Crozier 2001). Theobromine is commonly found in cacao seeds as well as in coffee. The purine alkaloids are synthesized from xanthosine (Zulak et al. 2006). Steroidal glycoalkaloids are found in many commonly consumed plants of the Solanaceae family, including potatoes, eggplants, and tomatoes (Zulak et al. 2006). Steroidal glycoalkaloids and aglycones have been linked to cancer prevention and reduced cholesterol levels. Despite their predominately health-promoting effects, the particular steroidal alkaloids found in green potatoes are toxic to humans (Zulak et al. 2006). Betalains are nitrogen-containing, water-soluble pigments that replace anthocyanins in flowers and fruits of most families in the Caryophyllales (Strack et al. 2003). Betalains can be further subdivided into betacyanins, which provide a red-violet color, and betaxanthins, which provide a yellow color (Strack et al. 2003). Betacyanins are immonium conjugates of betalamic acid and *cyclo*-dopa, and betaxanthins are immonium conjugates of betalamic acid and amino acids or amines (Strack et al. 2003, Zulak et al. 2006). Betaxanthins can also exist in various glycosidic and acylglycosidic forms (Zulak et al. 2006). The predominant food sources of betalains are red beets and cactus fruits (Zulak et al. 2006).

Sulfur-containing compounds come from two plant sources in the diet: the glucosinolate-myrosinase system and the alliin-alliinase system. The glucosinolate-myrosinase system is found in cruciferous plants such as cabbage and broccoli, whereas the alliin-alliinase system occurs in crops such as garlic, onion, and leeks (Mithen 2006). In cruciferous crops, glucosinolates are the most abundant secondary metabolites. Glucosinolates comprise β-thioglucoside *N*-hydroxysulfates with a side chain and a sulfur-linked β-D-glucopyranose moiety (Fahey et al. 2001). Approximately one-third of glucosinolates contains a sulfur atom in various states of oxidation (Fahey et al. 2001). The most frequently occurring glucosinolates are those that contain either straight or branched carbon chains. In cruciferous crops, the most abundant are indolylmethyl and *N*-methoxyindolylmethyl glucosinolates, both of which are derived from tryptophan (Fahey et al. 2001, Mithen 2006). Cruciferous crops also contain a small amount of methionine-derived or phenylalanine-derived glucosinolates. The sulfur-containing compounds

resulting from the alliin-alliinase system are the result of hydrolysis of nonvolatile alkyl and alkenyl-substituted L-cysteine sulfoxides as a result of the action of the enzyme alliinase following tissue disruption (Kubec et al. 2013, Mithen 2006). Tissue disruption and the resulting enzymatic activity produce a large number of sensory-active alliaceous compounds. More comprehensive knowledge of the composition of the chemically diverse set of plant metabolites in foods will enhance our ability to produce and preserve foods for optimal human health.

### Microbial Secondary Metabolites

Microorganisms contribute a vast array of metabolites to the food metabolome. The action of microbial enzymes on macromolecules (carbohydrates, proteins, and lipids) releases a variety of different sugars, oligosaccharides, amino acids, peptides, and fatty acids. Microbiota associated with foods also possess a number of other enzyme activities that can alter the food metabolome. The enzymatic action of microbes to change the metabolite composition of foods is either exploited (food fermentations) or controlled (preservation processes), depending on the nature of the food and desired finished product. In addition, microorganisms also produce a large number of secondary metabolites that differ in chemical structure from those in plants or animals. Some 22,500 biologically active, secondary microbial metabolites have been documented in the scientific literature or databases (Bérdy 2005). Furthermore, gut microbiota–produced metabolites that influence animal physiology and health (Lee & Hase 2014) may also influence the composition of animal-based foods.

## TECHNOLOGICAL PLATFORMS

A great challenge facing metabolomics researchers is the incredible chemical diversity of the compounds in the metabolome. These include sugars, fatty acids, peptides, vitamins, hormones, and flavonoids, to name a few, that may result from of an organism's metabolism or come from diet or environmental exposures. This means that there is no one size fits all approach to effectively analyze all of these compounds. Because of the diversity of compounds present, metabolomic analyses have historically included antibody and RNA trapping methods, nuclear magnetic resonance (NMR), and liquid and gas chromatography. Today, mass spectrometry–based platforms predominate because of both their ability to identify a wide range of compounds and their high-throughput capacity.

The technologies that are primarily being used for metabolomic investigations include NMR, liquid chromatography–mass spectrometry (LC-MS), and gas chromatography–mass spectrometry (GC-MS). Each of these techniques has advantages and limitations (**Figure 3**), and a single analytical technique to comprehensively study the metabolome is not yet readily available (Wishart 2008). Nonetheless, rapid developments in analytical instrumentation and data-handling technologies have dramatically increased the ability to perform extensive metabolite profiling.

### Nuclear Magnetic Resonance

NMR techniques, specifically $^{1}$H NMR (hydrogen-1 nuclear magnetic resonance), are a popular choice for metabolomic profiling because they are fast and simple (Kim et al. 2011). The development of methodologies utilizing NMR has been attractive for many reasons. NMR allows for high-throughput analysis and requires minimal sample preparation (Krishnan et al. 2005, Kruger et al. 2008). Although NMR-based techniques lack sensitivity compared to mass spectrometry–based approaches, they are the most uniform detection techniques (De Vos et al.

| NMR | LC-MS | GC-MS |
|---|---|---|
| **Advantages** | **Advantages** | **Advantages** |
| • Rapid and robust analysis<br>• No separation or derivatization<br>• Detects all organic classes<br>• Large pool of software and databases for metabolite identification | • Excellent sensitivity<br>• Flexible technology<br>• Potential for detecting largest portion of metabolome | • Sensitive<br>• Excellent separation capability<br>• Large pool of databases for metabolite identification |
| **Limitations** | **Limitations** | **Limitations** |
| • Limited sensitivity<br>• Large instrument footprint | • Less robust than GC or NMR<br>• Poorer chromatographic resolution than GC | • Requires compounds that are volatile or made volatile via chemical derivatization |

**Figure 3**

Advantages and limitations of the most widely used metabolomic technologies for improving the quality of food. Abbreviations: GC-MS, gas chromatography–mass spectrometry; LC-MS, liquid chromatography–mass spectrometry; NMR, nuclear magnetic resonance.

2007). Signals on NMR spectra are proportional to the molar concentration of the compound, allowing for the direct comparison of concentrations of each compound without requiring calibration curves for each (Kim et al. 2010). By comparing the peak intensity with an internal standard, the absolute concentration of all the metabolites in the sample can be calculated. Additionally, use of NMR allows for the simultaneous detection of primary and secondary metabolites, and NMR-based techniques are generally able to identify 30–150 metabolites in plant samples (Kim et al. 2011). For these reasons, NMR has been successfully used in a number of studies to detect the major compounds that change in foods in response to agricultural practices, genetic lines, processing, and storage (**Table 1**). The main limitation of NMR is its low sensitivity. Because of lower sensitivity, larger sample amounts are required. More recently introduced reduced-detection-volume NMR probes allow for analysis of smaller sample volumes, and advances in hardware, specifically low temperature probes, have increased the signal-to-noise ratio up to 16-fold per scan (Kim et al. 2010). Nonetheless, the lower sensitivity compared to mass spectrometry methods makes NMR an ideal choice for quality control applications in which high sensitivity is not required (Kim et al. 2011). This is illustrated by several recent studies that used $^{1}$H NMR metabolite profiles to authenticate and/or classify foods by origin or the processing method to which they were subjected

(Caligiani et al. 2014, López-Rituerto et al. 2012, Mazzei & Piccolo 2012). NMR is also limited by the large amounts of signal overlap on the spectra. Overlapping signals make compound identification more difficult and decrease the accuracy of peak integration. However, signal overlap is significantly reduced in two-dimensional NMR compared to traditional $^{1}$H NMR (Kim et al. 2010).

## Liquid Chromatography–Mass Spectrometry

LC-MS–based methodologies are typically used to detect secondary plant metabolites, including alkaloids, saponins, phenolic acids, phenylpropanoids, flavonoids, glucosinolates, polyamines, and their derivatives (De Vos et al. 2007). An LC-MS metabolomic platform was first applied in foods to develop a metabolome database for tomato (Moco et al. 2006). It is the preferred method for analyzing semipolar metabolites when a soft ionization technique (e.g., electrospray ionization, atmospheric pressure chemical ionization) is employed. More polar solvents are often employed for the extraction of metabolites from samples to be analyzed with LC-MS (Kim et al. 2011). An advantage of LC-MS compared to GC-MS is that samples can more easily be recovered after fractionation and that sample derivatization is not required. However, LC-MS metabolite libraries for identification are not as easily transferrable as they are in GC-MS (Bedair & Sumner 2008). To achieve better identification of metabolites during profiling, many research groups have developed in-house libraries to be used for automatic identification. The first of these libraries, called the Metabolome Tomato Database (MoTo DB), focused on providing information on metabolites present in the tomato fruit based on literature and experimentally derived data (Moco et al. 2006). Development and implementation of metabolite databases have provided a baseline for further research. For tomatoes, the MoTo DB has been utilized to inform research on how the antioxidant and metabolite profiles change during the production of tomato paste (Capanoglu et al. 2008). LC-MS–based techniques have been further expanded to include LC-MS-MS methodologies (Alvarez et al. 2008). This technological platform is most well suited for the identification of novel bioactive compounds in plant foods because of the compatibility of LC separations with the diverse classes of secondary metabolites in plants. For example, 21 and 31 novel compounds were identified from tomato and potato, respectively, including phytochemicals from various classes encompassing amino acids, anthocyanins, fatty acids, flavonoids, glycoalkaloids, hydroxybenzoic acids, hydroxycinnamic acids and amides, nucleosides, organic acids, phenylacetic acids, phenolic alcohols, and triterpenoids (Chong et al. 2013, Gómez-Romero et al. 2010).

## Gas Chromatography–Mass Spectrometry

Despite the limitation of requiring a volatile metabolite or a volatile metabolite derivative, GC-MS–based metabolomic platforms have been developed and widely applied for metabolite profiling in plants (Fiehn et al. 2000, Gullberg et al. 2004, Lisec et al. 2006, Roessner et al. 2000, Rudell et al. 2008, Weckworth et al. 2004, Zörb et al. 2006), microorganisms (Barsch et al. 2004, Bölling & Fiehn 2005, Koek et al. 2006, O'Hagan et al. 2005, Strelkov et al. 2004, van der Werf et al. 2008), and human fluids and tissues (Begley et al. 2009, Denkert et al. 2008, Jiye et al. 2005, Mal et al. 2009, O'Hagan et al. 2005, Pasikanti et al. 2008). GC-MS has been broadly applied to the analysis of food volatiles for decades and is a powerful tool for obtaining metabolite information. GC-MS may also be applied to the study of nonvolatile components in foods that are first chemically derivatized with one or more trimethylsilyl (TMS) group(s), allowing the analysis of a number of chemical classes such as mono- and disaccharides, sugar alcohols, organic acids, amino acids, and long-chain fatty acids (Cuthbertson et al. 2012, Lou et al. 2011, Peluffo et al. 2010,

Roessner et al. 2000, Roessner-Tunali et al. 2003, Schauer et al. 2006, Zörb et al. 2006). A review by Kanani et al. (2008) highlighted the areas of the GC-MS metabolomic platform that require standardization to yield unbiased results. These included extraction, derivatization, adjustment for multiple derivatives per metabolite, proper equipment parameters to ensure operation in the linear range, and improvements in peak alignment software tools. The development of two-dimensional gas chromatography–time-of-flight mass spectrometry (GC × GC-TOFMS) provides the potential to carry out separations of volatile chemical components using two separation mechanisms by connecting columns of different bonded phases in series (Adahchour et al. 2008, Marriott & Shellie 2002). The increased separation efficiency of GC × GC-TOFMS as compared to one-dimensional GC-MS results in an increase in the number of compounds that can be efficiently separated and detected and higher quality mass spectra for facilitating identifications for both volatile organic compounds (Adahchour et al. 2005, Rocha et al. 2007, Shellie et al. 2001) and trimethyl silylated, nonvolatile compounds (Guo & Lidstrom 2008, Hope et al. 2005, Kusano et al. 2007, Li et al. 2009, Mohler et al. 2006, Welthagan et al. 2005). Optimization of instrumental analytical parameters for a nonpolar/polar column combination made it possible to detect more than 1,800 metabolite peaks in human serum (O'Hagan et al. 2007). Furthermore, Koek et al. (2008) found that a polar/nonpolar column combination resulted in better resolution of components and greater use of the separation space with similar separation efficiency. This technology has been successfully adapted for the nontargeted profiling of volatile compounds in fermented cucumber (Johanningsmeier & McFeeters 2011) and metabolic footprinting of *Lactobacillus buchneri* during fermented cucumber spoilage (Johanningsmeier & McFeeters 2015), resulting in the identification of 92 volatile and nonvolatile compounds related to spoilage from several thousand metabolite peaks detected.

## Capillary Electrophoresis–Mass Spectrometry

Capillary electrophoresis coupled to mass spectrometry (CE-MS) is rapidly gaining attention as a powerful metabolomic platform for ionic, weakly ionic, and highly polar metabolites (Ramautar et al. 2015). Soga and colleagues were among the first to demonstrate the suitability of CE-MS for metabolomics, using this platform for the quantitative analysis of 352 metabolites related to central metabolism and the detection of more than 1,600 metabolites in a microbial culture during sporulation (Soga et al. 2003). In recent years, this technique has been successfully applied to study the changes in edamame composition under differing storage conditions in relation to sensory properties (Sugimoto et al. 2010) and to monitor the effects of pasteurization and storage on sake composition (Sugimoto et al. 2012). One additional advantage of CE-MS is the efficient separation of molecules that allows multiplexing for high-throughput analysis of samples (Kuehnbaum et al. 2013).

## Multiple Platform Methods

Given the diversity of chemical species that make up the food metabolome, a single analytical method platform is unlikely to yield a comprehensive metabolite profile. Recent developments in metabolomic platforms rely on multiple analytical instruments and methods to achieve the broadest coverage of metabolites possible. This approach was recently applied to study the effect of processing treatments on the metabolite composition of broccoli, tomato, and carrot purees. The metabolomic platform comprising $^{1}$H NMR, LC-MS, and GC-MS methods gave broad coverage of polar primary metabolites, antioxidant vitamins, carotenoids, glucosinolates, flavonoids,

oxylipins, phenylpropanoids, sugar nucleotides, tocopherols, and volatile organic compounds, resulting in the identification of 128 differentiating metabolites that varied among vegetables and were impacted by the order of processing operations (Lopez-Sanchez et al. 2015). At a recent Metabolomics Society meeting, a combination of reverse-phase LC-MS in positive and negative ion modes, hydrophilic interaction chromatography (HILAC) coupled to MS (Spagou et al. 2010), and GC-MS (for both volatile compound analysis and TMS derivatives for coverage of central metabolism) was demonstrated to achieve the greatest coverage of currently known metabolic maps (Siuzdak et al. 2015).

## METABOLOMIC TECHNOLOGIES FOR IMPROVING FOOD QUALITY

Whether fresh or processed, newly purchased or nearing the expiration date, the foods we eat are never in stasis. Foods routinely undergo chemical, biochemical, and physical changes pre- and postharvest, throughout processing, and over the course of their shelf life. Some of these changes, such as enzymatic browning, may be inherent to the food itself. Other changes may be due to the addition of other ingredients, freezing/thawing, thermal processes, or fermentation by microbes. Regardless of processing or storage, chemical and biochemical reactions in grains, fruits, vegetables, dairy products, and meat continue throughout the shelf life of a food, right up until the time it is eaten by a consumer. These changes in food metabolites directly affect food quality, which has implications for human health and well-being. Thus, a metabolomic approach can be quite powerful for furthering our understanding of the links between food composition and nutritional and sensory quality. The associated technological platforms are being used in several research areas that will lead to production of foods that enhance health and well-being. Metabolomic studies in food science aim to evaluate changes in food metabolites as related to agricultural practices, processing, and storage; identify novel bioactive compounds; and determine authenticity and designation of origin for verifying the quality of finished products (**Table 1**). **Table 1** shows representative studies for the types of approaches and findings but is not a comprehensive list, as new studies are being published every week in these areas.

### Impact of Agricultural Practices and Genetic Lines on Food Quality

The impact of agricultural practices and genetic lines on the chemical composition of animals and plants used as foods is a major area that is benefiting from the development of metabolomic technologies. Traditionally, cultivars and field conditions have been selected based primarily on agronomic targets such as disease resistance and yield. Combining these criteria with comprehensive metabolite profiling allows for more strategic selections that include quality-associated chemical composition and desirable production traits. These studies have revealed the effects of varying agricultural practices on plant metabolism, which translated into elevated glucosinolate content in broccoli (Sun et al. 2015); higher vanillin content in *Vanilla* pods (Palama et al. 2009); changes in the sugar, organic acid, and amino acid composition in mandarin oranges (Zhang et al. 2012); green tea with higher phytochemical and sensory values when shade grown (Ku et al. 2010, Lee et al. 2013); and a better understanding of ripening processes in tomato (Roessner-Tunali et al. 2003) and grapes (Fortes et al. 2011). $^{1}$H NMR techniques applied in pork breeding and aquaculture revealed differences in amino acids, amines, and nucleosides (Savorani et al. 2010, Straadt et al. 2014, Wagner et al. 2014). A metabolomics approach is also being employed to settle the organic versus conventional agriculture debate. A profiling of wheat grains using a GC-MS platform showed similarities in 44 of 52 metabolites detected in

organic and conventionally grown wheat (Zörb et al. 2006). However, ketchup produced from organically grown tomatoes had higher contents of antioxidant phytochemicals compared to the conventionally grown counterpart (Vallverdú-Queralt et al. 2011), emphasizing the importance of platform selection and scope of inference in conducting metabolomic studies.

Many studies in this realm used metabolite profiling in combination with cultivar selections and sometimes genetic information to link quality traits to both metabolic markers and single nucleotide polymorphisms (SNPs) or quantitative trait loci (QTLs). Examples in tomato (Overy et al. 2005, Schauer et al. 2006) and rice (Calingacion et al. 2012, Heuberger et al. 2010, Lou et al. 2011) were successful in linking metabolites to genetic information to facilitate strategic breeding efforts. Metabolomic studies have also been used to explore the phenotypic diversity in melon (Allwood et al. 2014, Bernillon et al. 2013), apple (Cuthbertson et al. 2012), pepper (Wahyuni et al. 2013), and olives (Sánchez de Medina et al. 2014), demonstrating the ability to separate varieties by their metabolite profiles and identify differentiating phytochemicals.

## Food Processing and Storage

One of the major factors affecting economic outcomes for food producers is food spoilage. Although there is significant disagreement as to exactly what percentage of the food produced in the United States and around the world is lost due to waste and or spoilage (estimates range widely from 10% to 50%), there is general agreement that food spoilage represents a significant loss to producers, which is passed on to the consumer in the form of higher food prices (Parfitt et al. 2010). Therefore, food producers are continually seeking ways to predict, monitor, and extend the shelf life of their products. Metabolomic technologies provide a means to predict the end of shelf life before spoilage is readily apparent and to determine the effects of treatments to extend shelf life. This approach has been applied to a wide variety of food products, including fruits, vegetables, beer, and meats. A few notable examples follow. Broccoli microgreens are known for their potential health-promoting properties, particularly those related to glucosinolate content, which can be enzymatically converted to anticancer compounds. Preharvest calcium chloride application increased levels of potentially bioactive glucosinolates in broccoli microgreens (Sun et al. 2015). Apples are often kept in cold storage for periods of up to six months prior to being brought to market. Treatment of Granny Smith apples with the antioxidant diphenylamine lessened some of the physical damage associated with cold storage. This observed protective effect was correlated with oxidation-related markers in the apples (Leisso 2013). The decline in the desirable sensory properties of edamame over several storage times and temperatures was correlated with metabolomic profiles. Higher levels of phenolic compounds, phospholipids, and gamma-amino butyric acid were associated with higher storage temperatures, whereas variations in amino acid levels were most closely associated with sensory changes in edamame (Sugimoto et al. 2010).

Although fermentation is an ancient food preservation process, it is well recognized that beer, wine, and other products age or mature, indicating a change in chemical composition over time. A metabolomic study comparing freshly brewed beer to beer stored for 16 weeks at low and ambient temperatures indicated significant oxidation in the beer stored at ambient temperatures. A sensory panel corroborated the presence of stale and oxidized flavor (Heuberger et al. 2012). Because fermented foods represent a collaboration of sorts between a food and a fermenting microbe, it is no surprise that the metabolomic analyses of these foods reveal a wide range of compounds. A combined LC-MS-MS and GC-MS analysis of soy sauces detected 237 and 366 nonvolatile and volatile metabolites, respectively. Comparing these metabolites to sensory panel data revealed that dipeptides were important to perceived sweetness in soy sauce (Yamamoto et al. 2014).

In addition to its usefulness for understanding the shelf life of plant products, metabolomics has also been applied to the study of changes in beef and fish products over their respective shelf lives to understand what compounds are produced during the microbial spoilage of meats. Changes in metabolites may result from the degradation of meat by its own enzymes or from the metabolism of bacteria, yeasts, and molds. Because the presence of spoilage microbes may not be readily apparent to consumers until meat is obviously spoiled, metabolomic technologies have been used to predict the shelf life of meat at various storage temperatures, with or without modified atmosphere. These studies identified an array of compounds that correlated with shelf life, with microbial growth, and with sensory attributes (Argyri et al. 2015, Nychas 2008). Although frozen storage might seem to render foods inert, a study of frozen fish roe indicated that this is not the case. Although no major changes were observed in the fish roe during the first six months of storage, a year of frozen storage resulted in changes in amino acids, sugars, and other compounds, showing that both proteolysis and lipolysis continued (Piras 2014). Whether in fruits, vegetables, meat, or fish, the ability to predict changes in food quality over the course of a product's shelf life using metabolomic technologies could allow the food industry to provide more realistic expiration or use-by dates. A more comprehensive understanding of the food metabolite composition during processing and storage will lead to improved preservation methods that optimize health-promoting compound content and minimize undesirable changes that cause sensory defects.

Many food metabolites serve as flavors or flavor precursors. Although macronutrients, such as proteins and starches, also play important roles in the sensory properties of foods, many of the aroma, taste, and color compounds important to food systems are small molecules. Some of these compounds may be naturally occurring, whereas others may result from processing or be created during food storage. There are also interactions between molecules that can significantly change the flavor impact of volatile compounds. For these reasons, researchers in flavor chemistry and sensory analysis are making increasing use of metabolomic techniques. A typical study design might involve conducting a sensory panel and simultaneously examining the volatile and nonvolatile flavor components in the samples, with the goal of obtaining a more complete understanding of the small molecule metabolites affecting the flavor profile of the food products. Since 2010, a number of studies have applied a metabolomic approach to better understand the links between the chemical composition and the sensory properties of a variety of foods, including strawberries, carrots, rice, coffee, pork, soy sauce, beer, and wine. For example, thirty-one volatile flavor compounds, including esters, terpenes, and furans, were identified as being important to strawberry flavor (Schweiterman et al. 2014). Five carrot varieties could be distinguished based on the variations in the levels of thirty nonvolatile compounds detected using $^{1}$H NMR, most of which were hydrophilic (Clausen et al. 2014). An NMR-based metabolomic assessment of roasted coffee was able to rapidly and accurately predict sensory panel scores (Wei et al. 2014), and more than 250 volatile compounds were detected using a nontargeted GC-MS platform and analyzed via partial least squares regression, which allowed the identification of key volatile compounds associated with the characteristic flavors of Hunter Valley Semillon wine (Schmidtke et al. 2013). One of the most interesting findings across a range of studies and food products is the central importance of amino acids in flavor profiles.

## LIMITATIONS AND CRITICISMS

In some cases, metabolomic experiments may reveal changes or differences in composition that have already been documented in the scientific literature or could have been found using a hypothesis-driven approach rather than a hypothesis-generating metabolomic study. This leads to criticisms of the work during the publication process. Employing a metabolomic

platform that is sensitive and has broad metabolite coverage in combination with a carefully designed experiment will protect against such a scenario by making it more likely that novel information can be generated. Another serious limitation is that it is common to detect 3–20-fold more compounds than can be readily identified in the highly sensitive MS-based metabolomic methods (LC-MS, GC-MS, and CE-MS). High-resolution mass spectrometry that employs expensive instrumentation is essential for narrowing the list of possible identifications of unknown compounds to a manageable number of leads. Even when high-resolution mass spectra are collected, the process of identification is slow. Thus, methods for more efficient identification of unknown compounds and the expansion of databases for metabolomic workflows are areas of active engagement in the metabolomics community (Dunn et al. 2013). However, even with this current limitation, the information gained in metabolomic experiments routinely exceeds that of traditional compositional analysis of foods because of the nontargeted approach, which enables the detection of changes in metabolites that are not already known or expected.

## RAMIFICATIONS AND FUTURE DIRECTIONS

Although significant advancements have been made that enable the profiling of hundreds to thousands of molecules in a single experiment, it is projected that there are hundreds of thousands of possible chemical species in the food metabolome, most of which are yet to be identified. To achieve the promise of metabolomics for improved human health, we need a global understanding of how an environmental perturbation (i.e., consumption of a specific food or combination of foods) impacts the metabolism of an organism (i.e., an individual human with a specific physiological state). We must also be able to fully characterize the chemical composition of foods by generating specific knowledge regarding the effects of agricultural practices, genetic lines, postharvest physiology, processing, and storage. This grand goal can be achieved through expansion and coordination of shared data efforts such as the Human Metabolome Database and the FooDB food component database, among others; further development of metabolomic workflows that enable the sensitive, rapid detection and identification of food metabolites; and continued collaboration between food scientists, nutritionists, and biomedical researchers to determine which food components (and appropriate intake levels) are contributing to chronic disease prevention and optimal health and well-being.

## DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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of Food Science
and Technology
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