CURRENT TRENDS IN FOOD ANALYSIS
Food analysis today is a much broader and more complex field than it was in the past. Food safety continues to be a major focus of food analysis. But in recent years, other areas of food research have blossomed, and many analytical chemists are now investigating a wide array of questions on topics ranging from authenticating the origin of foods and beverages to investigating the nutritional aspects of natural food compounds. In this new e-book, we explore some current trends in food analysis and also provide some concrete advice for preparing food samples for analysis.

We open the issue with an interview with Sastia Prama Putri of Osaka University about the role of metabolomics in food analysis. She and her colleagues have done a range of food studies, on products such as soy sauce, cheese, sake, coffee, and cocoa, using metabolomics to predict sensory attributes of food—identifying the metabolites responsible for flavor, aroma, and other characteristics—and also conducting studies on food authentication.

Next, Elena Ibañez of the Institute of Food Science Research in Madrid, Spain, talks about an extension of the ‘omics approach to the concept of “foodomics”—a framework to address the full range of challenges in food science today. She then discusses “green foodomics,” an approach to applying the concepts of green chemistry to food science. She explains what is involved in making food analysis methods green, including both the challenges and the benefits, to both the laboratory and the environment.

We close the e-book with a look at selectivity in extractions, from our regular LCGC contributor Doug Raynie. As every food analyst knows, good sample preparation is critical when dealing with the complex matrices of food and beverage samples. Raynie discusses how to take advantage of selectivity options, illustrating the case with an example of removing a fat substitute from a food product.

We hope you enjoy this new e-book and find it useful in your work in food analysis.
Selectivity in Food Extractions

The Role of Selectivity in Extractions: A Case Study

Douglas E. Raynie

Green Foodomics

Green Foodomics

An interview with Elena Ibañez

Food Metabolomics

Food Metabolomics in Practice

An interview with Sastia Prama Putri

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CURRENT TRENDS IN FOOD ANALYSIS
In recent years, the study of metabolomics has expanded into food analysis. In this interview, Sastia Prama Putri of Osaka University in Japan talks about advances in metabolomics, the need for authentication of high value food products, and the important role of gas chromatography–mass spectrometry (GC–MS) in food analysis.

Q: How did you become involved in metabolomics?
A. My research focus was initially on the discovery of novel bioactive compounds from various natural products. I joined a metabolomics laboratory in early 2011, and have since been involved in several research projects on metabolomics applications in food science and metabolic engineering fields.

Q: What is the role of your laboratory in food metabolomics?
A. The Laboratory of Bioresource Engineering, Osaka University (also known as Fukusaki laboratory) has been involved in food metabolomics research since 2007. One of the first food metabolomics demonstrations performed at the laboratory predicted the
sensory attributes contributing to the quality of food, such as green tea and watermelon.

Recently, our laboratory also conducted soy sauce research in collaboration with Kikkoman company, cheese research in collaboration with Morinaga food company, and sake research in collaboration with Gekkeikan (a renowned Japanese sake company), as well as specialty coffee research in collaboration with the Indonesian Coffee and Cocoa Research company. Through this strong partnership with food companies and food research institutes, we have demonstrated the use of metabolomics technology for assisting in product development, authentication purposes, prediction of food sensory attributes, and the identification of metabolites responsible for flavour, aroma, and other characteristics of food.

Professor Eiichiro Fukusaki is responsible for driving all of the research activities in the laboratory. He is a Full Professor at the Graduate School of Engineering, Osaka University. He has published more than 200 journal articles, book chapters, and reviews, and hold 17 domestic patents and eight international patents. His research collaborators include over 30 academic institutions and major companies from various fields, including electrical, pharmaceutical, and medical as well as the food industry. He received the Japan “Saito” Award from the Society of Biotechnology Japan in 2004.

Q: What role does metabolomics have in food and beverage analysis, and what are the practical applications?
A. Broadly speaking, the application of metabolomics technology in food science includes informative analyses to characterize and identify compounds of interest; the prediction of quantitative functional value of food by means of multivariate analysis using metabolome data as the explanatory variable (which I will define here as predictive metabolomics); and “comparative” metabolomics to determine the metabolites responsible for classification of samples by type or for discriminatory purposes.

Practical applications include prediction of quality ranking of various food products and authentication of food products, which can include products with a high economic value, such as high-quality olive oil or rare products such as Kopi Luwak. Discriminative metabolomics has been widely applied to assess food quality, food safety, and determine the origin and varietal differences of foodstuffs. In addition, metabolomics is also useful for the discrimination of a variety of important genetically modified (GM) crops and for nutrition research (nutrimetabolomics).

Q: How important is metabolomics in food analysis? In your opinion, is the need for authentication of certain food products growing?
A. The power of metabolomics lies in the acquisition of an exhaustive analytical profile by which most of the
low-molecular-weight metabolites in a cellular system are quantified as fully as possible, and the subsequent extraction of the most meaningful elements from this data using various data analysis tools (a process widely known as data mining). Metabolomics offers several advantages for the discrimination of food products because it involves the exhaustive profiling of metabolites and subsequent data processing by multivariate analysis. The use of multivariate analysis for the discrimination of food products is very important because it can capture sample complexity and extract the most meaningful elements for analysis.

The need for authentication of certain food products is growing, particularly among products with a high economic value that are prone to adulteration. Previous studies have demonstrated that metabolomics is a very useful tool for the characterization and authentication of foodstuffs because it can explore, classify, and predict geographical origin, types, varieties, and adulterations. Among the tools commonly used, partial least squares discriminant analysis (PLS-DA) (a discrimination method) has become the most popular classification method as a result of its potential and versatility.

Q: Why is gas chromatography (GC) your analytical method of choice? In your opinion, what are the reasons for the recent popularity of GC–MS analysis in food chemistry?
A. The advantages of GC–MS are high peak capacity, excellent repeatability of retention time, and readily available compound libraries, which enable compound identification without using standard compounds. In addition, GC–MS is popular in the field of food chemistry because it provides high sensitivity, reproducibility, and quantification of a large number of metabolites with a single-step extraction (1). Compound identification using GC–MS is relatively easy compared with other analytical platforms because the unique mass spectrum of each compound can be consistently obtained. This is achieved through the use of electron ionization (EI), which is currently the most commonly used ionization method in GC–MS owing to its robustness and high repeatability. Furthermore, a large number of compounds can be easily identified using the National Institute of Standards and Technology mass spectral library (2).

Q: Where will your research into food analysis take you in the future?
A. I am interested to perform research on the use of metabolomics in the improvement of post-harvest technology in various food products. I am also keen to research the effect of some food intake to improve human health (nutrimetabolomics).

References
Sastia Prama Putri is an assistant professor (Specially Appointed) at the Graduate School of Engineering at Osaka University in Japan. She received her PhD from the International Center for Biotechnology, Osaka University. She is currently working on the “JST-NSF: Metabolomics for low carbon society” project, a highly prestigious collaborative research project between Japan and the USA, focusing on the application of metabolomics technology for optimization of various higher alcohols for use as biofuels.

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Is “green foodomics” another buzzword or a new direction in food analysis? Here, Professor Elena Ibañez of the Institute of Food Science Research (CIAL), Madrid, Spain, discusses one of the lastest trends in food analysis.

Q: What is foodomics?
A. Our research group defined foodomics for the first time in 2009 as “a new discipline that studies the food and nutrition domains through the application and integration of advanced ‘omics’ technologies to improve (consumer) well-being, health, and knowledge”. Basically, we believe that foodomics can help to provide new answers to some of the important challenges (such as food safety and quality, traceability, new foods for health improvement and disease prevention, etc.) that society is facing in the 21st century.

Q: What chromatographic techniques are commonly used in foodomics?
A. The techniques typically used in foodomics are those typically used in proteomics and metabolomics, such as liquid chromatography (LC), ultrahigh-performance liquid chromatography (UHPLC), nano-LC, gas
chromatography (GC), and capillary electrophoresis (CE) hyphenated to high resolution mass spectrometry (MS). These techniques are able to provide a great deal of information at different expression levels, including protein and metabolites. Logically, an important additional step here is the use of adequate sample preparation techniques.

Q: When was the term green foodomics coined and what does it involve?
A. Foodomics can be understood as a global framework that gathers all the new challenges that the food science domain will be facing in the current post-genomic era (some of them unthinkable a few years ago) and providing new answers through the development and application of new strategies, mainly based on “omics” approaches for large-scale analysis. In this regard, one of the challenges that can impact future generations is sustainability, which is understood as a rational way of improving processes to maximize production while minimizing the environmental impact or, in the words of the Environmental Protection Agency (EPA), “sustainability creates and maintains the conditions under which humans and nature can exist in productive harmony, that permit fulfilling the social, economic, and other requirements of present and future generations”. Thus, the term green foodomics was coined as a way to highlight foodomics goals with regards to green chemistry principles, bearing in mind that sustainability can be not only a word but also a way of doing things.

Q: How easy is it to translate regular chromatography techniques to the green foodomics approach—and can it be cost-effective?
A. Application of green chemistry principles to analytical chemistry is not new, although it is true that not much attention has been given to this approach until recently. Although the analytical community has always been environmentally sensitive and the idea of improving analytical methods by reducing the consumption of solvents and reagents has always been at the forefront of the analytical chemists’ minds, the first descriptions of “green analytical chemistry” (or clean analytical methods) appeared in the mid-1990s (1). The concept and use of such an approach has evolved over the years reaching approximately 100 publications by 2011. This evolution positively affects foodomics (and green foodomics) since some of the mentioned applications deal with advanced analytical methodologies applied to food science.

The key aspects that should be considered when regarding the adverse environmental impact of analytical methods deal with reducing the amount and toxicity of solvents during sample pre-treatment, minimizing solvents and reagents during the separation and measurement steps, and developing alternative direct analytical methods.
that do not require solvents or reagents. Moreover, they should also consider developing methods able to consume fewer resources. All of this has to be done whilst maintaining or improving the analytical performance of the method. This is probably the most difficult task and is responsible for a limited translation of conventional methods to greener ones.

Undoubtedly, laboratories that follow the green analytical chemistry principles, applied or not to foodomics, can have many benefits, which include the cost in terms of waste generation and management, health risks, and resources preservation.

Q: Can you illustrate the benefits of this approach with some practical examples?
A. In a recent book chapter we published about “green foodomics” (2), we suggested the possibilities offered by tools such as life cycle analysis (LCA) to evaluate the “greenness” of a process by calculating the environmental impact of, for instance, processes and analytical techniques. In fact, a comparative LCA study was presented to quantify the green profile of some analytical techniques used for foodomics.

Specifically, six advanced analytical methods used in our laboratory for chemical characterization of supercritical rosemary antioxidant extracts were selected, namely: High performance liquid chromatography with diode-array detection (HPLC–DAD), micellar electrokinetic capillary chromatography with diode-array detection (MEKC–DAD), ultrahigh-pressure liquid chromatography with diode-array detection mass spectrometry (UHPLC–DAD–MS), capillary electrophoresis–mass spectrometry (CE–MS), supercritical fluid chromatography with flame ionization detection (SFC–FID), and gas chromatography with flame ionization detection (GC–FID). In all of them only the analytical part has been considered for LCA purposes, excluding sample preparation.

Factors considered included reagents used and amount, total analysis time, energy used, and wastes generated. By comparing the impacts produced by the different analytical methods obtained by LCA, it was possible to assess, for example, that GC–FID was the method that provided the highest impacts because of the high-energy consumption for each analysis, while MEKC–DAD analysis yielded the lowest impacts, even considering that several compounds and additives were present in the mobile phase. As for UHPLC–MS, it exhibited low impacts while providing a lot of information about the sample.

To understand the global dimension of green foodomics it is important to highlight that in our research group we have demonstrated the different effect of supercritical fluid extracts obtained from rosemary in, for example, the induction of transcription of genes that encode phase II detoxifying and antioxidant
genes in two different leukemia cell lines. This effect, together with differences in metabolic profiles, suggested that some dietary polyphenols exert differential chemopreventive effects in leukemia cells of different phenotype (3).

Q: Does green foodomics benefit the consumer?
A. Green foodomics can highly benefit the consumer since it attempts to improve consumer well-being and confidence while, at the same time, decreasing contamination and health risks and preserving sustainability.

Q: What is the future for green foodomics?
A. I believe green foodomics has a brilliant future because sustainability and eco-friendliness of a process or analytical approach will not be considered just an additional advantage but a goal in itself in the future. Therefore, different approaches will have to be closely considered so that greener processes and analytical methods can be developed.

In terms of sample preparation techniques, modern pressurized extraction methods are able to provide additional advantages using significantly less solvents. On the other hand, miniaturized extraction methods are also gaining in importance. The development of integrated approaches will also help to obtain more environmentally friendly processes under the green chemistry domain.

Simultaneously, different strategies will be followed to “green” sample analysis, such as the use of novel column technologies, the revision of conventional methods to others using less solvent, miniaturization, and the use of water at high temperatures or other non-toxic solvents as chromatographic mobile phases.

References

Elena Ibáñez is a full research professor at the Institute of Food Science Research (CIAL) belonging to the National Research Council (CSIC) in Madrid, Spain. She received her PhD in Analytical Chemistry from the Autonomous University of Madrid, Spain. Her main research includes the study and development of new extraction processes based on the use of sub- and supercritical fluids to isolate bioactive compounds from natural products, and also the development of advanced analytical methods for foodomics.

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Realize one resource that provides a portfolio of products delivering connected chromatography solutions across key market workflows. A comprehensive catalog of chromatography columns and consumables showcasing some of our new product innovations including Thermo Scientific™ SMART Digest™ kits, Thermo Scientific™ Virtuoso™ Vial Indentification System, Bio LC Columns, Thermo Scientific™ Accucore™ Vanquish™ Columns, Thermo Scientific™ GC Septa and the Thermo Scientific™ LinerGOLD™ Range. These products meet the world’s changing requirements and enable our customers to make the world healthier, cleaner and safer.
Many of the extraction techniques developed over the past generation tout selectivity among their advantages. In reality, solvent selection and the use of stationary (sorbent) phases are the main mechanisms for providing selectivity. Therefore, selectivity is often limited to isolation of classes of compounds rather than individual structures. In this article, the selective removal of a fat substitute in food products is discussed to demonstrate options for obtaining selectivity during extraction.

Over the past generation or so, myriad extraction techniques were developed that have generally improved yields, lessened the amount of organic solvent used, and minimized time. Additionally, many of these techniques claim advantages concerning selectivity.

Selectivity is the ability to determine the analytes of interest in preference to other sample components (potential interferents). A recent article (1) advocated that selectivity can stem from any point in the analytical process, but as a general rule, selectivity arises from separations, selective detection schemes, and selective chemical reactions. These approaches
can balance each other. For example, if an analytical separation is not completely sufficient, the use of a selective detection method like mass spectrometry (MS) or fluorescence spectroscopy can offer the balance of the required selectivity provided that the unseparated components do not suppress the detector signal.

Majors described “just enough” sample preparation (2) in which method selectivity is matched to the qualitative or quantitative analytical requirements. For example, the QuEChERS (quick, easy, cheap, effective, rugged, and safe) method for extracting pesticides from fruits and vegetables combines salting out partitioning with dispersive solid-phase extraction (SPE) to remove matrix components, allowing effective chromatography and MS detection. As Majors points out and illustrates in Figure 1 from his original article, increasing complexity in an analytical procedure typically leads to greater selectivity.

Turning our attention back to modern extraction methods, the fundamental driving force of the technique leads to the element of selectivity. A number of sorbent-based methods, such as SPE, solid-phase microextraction, and stir-bar sorbent extraction, use chromatographic stationary phases to isolate solutes of interest from gaseous or liquid samples.

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Figure 1: Just-enough sample preparation represents a continuum of methodologies.
Analytes are retained by their attraction to a stationary phase of similar polarity and are selectively eluted via choice of an appropriate solvent. The techniques aimed at solid samples, including supercritical fluid extraction (SFE), pressurized fluid extraction, microwave extraction, and ultrasound extraction, rely on the application of energy (often heat) to drive the analyte into an appropriate solvent. In all of these techniques, both sorbent- and solvent-based, the key to selectivity is the match between analyte polarity and polarity of the extracting phase. In other words, “like dissolves like.” Thus, extractions are usually considered crude separation techniques, providing compound class selectivity and less utility for the selective isolation of specific, individual compounds. Of course, volatility is the major contributor to selectivity for gas-phase techniques.

If the primary selectivity mechanism in extractions is solute polarity (that is, matching solute polarity with the solvent or sorbent following the “like dissolves like” principle), is selectivity possible during chemical extraction? Is selectivity beyond compound class selectivity possible? Do extractions need to be selective or is selectivity solely a function of subsequent chromatography and detection?

To look at an example of extraction selectivity within the “like dissolves like” polarity context, let’s consider the example of fat analysis in food products and, more specifically, the example of sucrose ester fat substitutes.

Fatty Acid Methyl Ester Analysis

The United States Nutrition Labeling and Education Act (NLEA) of 1990 requires the labeling of selected nutrients on prepackaged food products. One issue with this requirement deals with the concept of “total fat.” What is a “fat”? Are lipoproteins considered lipid or protein? The next concern is their analysis. If “fats” are based on the fatty acid moiety, how can they be measured? Fatty acids are not volatile enough for gas chromatography (GC) analysis. They do not contain any chromophores necessary for ultraviolet detection in liquid chromatography (LC). (Remember, at the time, LC–MS was not as widely accepted as it is currently.) The polarity of the acidic group can irreversibly adsorb to active sites on chromatographic stationary phases via hydrogen bonding, depending on the type of chromatography. Consequently, the total fat listed on nutritional labels is based on the acid hydrolysis and formation of methyl esters of an organic extract, using a nonpolar solvent, of the food product. The “total fat” listed on the nutritional label is that of a triglyceride based on the resulting fatty acid methyl ester (FAME) composition (3,4). An overview of the formation of methyl esters from triglycerides is presented in Figure 2.

In the FAME method, samples are dissolved in a nonpolar solvent and a catalyst like BF₃ dissolved in methanol is added. Sometimes methanolic acid or base is used. After mild heating,
back-extraction with water removes the polar components. The FAME sample is dried and characterized by GC with flame ionization detection (FID). The esterification is facilitated with an alkylation derivatizing agent to condense the carboxyl group of the fatty acid with the methanol hydroxyl. The catalyst aids the reaction by protonating the acid group to promote the formation of the ester and water. The stability of the methyl ester, or FAME, allows GC separation by boiling point or unsaturation.

The Procter and Gamble Company began marketing sucrose esters, called olestra or the tradename Olean, as fat substitutes in the mid-1990s. The sucrose ester structure is shown in Figure 3. In this figure, the R group is either hydrogen or any fatty acid. By varying the number of fatty acids connected to the sucrose molecule by ester linkages or by changing the carbon chain length of the fatty acids, the properties of the olestra molecule can be altered. Under appropriate conditions, the olestra molecule can have boiling points, viscosity, mouth feel, and other properties similar to common vegetable oils. Because they are not naturally occurring lipids (though they are made from naturally occurring compounds), the sucrose esters are not subject to enzymatic digestive action. Hence, they can be substituted for vegetable oils in selected applications, such as the frying of potato chips and similar salted snacks.

If we review the acid hydrolysis and esterification reactions for the FAME analysis, olestra in food products would
be hydrolyzed along with triglycerides and other fats. The resulting FAMEs would be indistinguishable regarding their source, olestra or triglyceride. Thus, a selective analysis to determine NLEA “total fats” in the presence of olestra is needed. Here we will present three possibilities to garner the necessary selectivity during the sample preparation process.

**Supercritical Fluid Extraction**

Perhaps the easiest method, conceptually, to address the isolation of FAME from total fats from those originating from olestra would be at the level of the extraction, meaning we would selectively extract olestra from the total fats. (That is, we’re assuming that we must perform FAME analysis of total fats to comply with the requirements of the NLEA.) This brings us back to the issue of solvent polarity or “like dissolves like.” Because olestra is designed to have properties substantially similar to vegetable oils, which are composed primarily of triglycerides, the solvents used in the dissolution and extraction of either olestra or triglycerides would likely be very similar. This brings us to the solvent extraction method where we have the most variation in solubility conditions with a single solvent: SFE. SFE almost always uses carbon dioxide, perhaps mixed with small amounts of organic cosolvents, near or above its critical point of 31.1 °C and 72.9 atm. Lipids and lipophilic materials are highly soluble in supercritical carbon dioxide, and the use of this solvent for the extraction and fractionation of lipids is well-reviewed (5–7). By making subtle changes in the operating temperature or pressure, somewhat dramatic changes in solvating ability can occur. These changes may allow the fractionation of members of a single compound class on the basis of either polarity or molecular weight. Because the molecular weights of olestra molecules are at least double that of triglycerides, it is conceivable that SFE could be used to either selectively extract olestra and triglycerides from each other, or to selectively precipitate one from the other. This has not been reported in the peer-reviewed literature, so will remain theoretical for now. No other solvent extraction methods will be able to achieve this level of selectivity as easily.

**Solid-Phase Extraction**

The next step up in complexity toward gaining the requisite selectivity during the sample preparation of olestra-containing food products would be to use a sorbent-based method to separate olestra from total fats postextraction. SPE is the most basic of these techniques and perhaps the most directly applicable to our hypothetical scenario. SPE can, in many ways, be regarded as an elementary form of LC. A stationary phase is placed onto a support material and put into a cartridge, disk, or other vehicle. Liquid samples are placed onto the SPE sorbent where total retention is achieved. Then analytes and interferents are isolated from each other.
by the judicious elution with selective solvents.

Tallmadge and Lin (8) used reversed-phase LC to determine the percent olestra in lipid samples. They found an octadecylsilane column (Zorbax, Agilent Technologies) appropriate to separate olestra from other lipophilic sample components in samples of soybean-oil olestra and heated or unheated cottonseed-oil olestra in soybean oil. The percentage of olestra in these samples varied from 5% to 90% and relative recoveries of 99.2% to 106.0% were reported. Thus, it seems possible that with minimal additional method development a protocol could be developed that involves an extraction of total fats and olestra from the sample food product, SPE separation of olestra from the total fats, and FAME analysis of the total fats.

**Lipase Hydrolysis**

Simultaneously, perhaps the most obvious and the most direct means of addressing the proposed situation is to explore the fundamental chemistry behind the problem. Again, olestra is created by esterification of sucrose with fatty acids, but because it lacks the glycerol backbone, it is not subject to enzymatic digestion as are triglycerides. Can this resistance to digestion be exploited in the conversion of total fats to FAMEs to the exclusion of olestra? This is the approach taken in a method validated under the Association of Official Analytical Chemists (AOAC) Peer-Verified Methods Program (9). A modified version of AOAC Method 983.2.3 was used, in which a chloroform–methanol extraction of olestra-containing snacks was performed. This extract contained both the total fat and olestra. The hydrolysis portion of the FAME analysis used a lipase to hydrolyze the total fats, leaving the unaltered olestra. The fatty acids resulting from the lipase hydrolysis were precipitated as calcium salts and the olestra was extracted with hexane. The fatty acid salts were redissolved and esterified before GC analysis. Recoveries of 101% (6% relative standard deviation [RSD]) for total fat and 104% (6% RSD) for saturated fat were reported. Repeatability and reproducibility were also studied and the method was standardized for fatty acid carbon chains of 6–24. This represents the official method for the determination of total fats in packaged food products containing olestra fat substitute.

**Summary**

Analytical selectivity can occur during any step of an analytical method, but typically it occurs during the separation or detection steps rather than during sample preparation. Selectivity during chemical (analytical) extractions is almost exclusively limited to solute polarity. Consequently, selective extractions beyond compound class separations will be difficult. Although selective sample preparation is not the typical case, increasing complexity of the procedures
can lead to selective analysis. This column installment presented a scenario in which selectivity during the characterization procedure was not possible, but selectivity during solvent extraction (SFE) and sorbent-based extraction (SPE) or via selective reactions was shown.

References

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