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Trends in Food Science & Technology 20 (2009) 557–566

TRENDS IN FOOD SCIENCE & TECHNOLOGY

Review

Metabolomic analysis in food science: a review

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Metabolomics has emerged as an important tool in many disciplines such as human diseases and nutrition, drug discovery, plant physiology and others. In food science, metabolomics has recently risen as a tool for quality, processing and safety of raw materials and final products. This article discusses the latest advances in food metabolomics from the discriminative, predictive, and informative approaches, as well as the typical methods used at each step of the metabolomic analysis.

Introduction

Metabolomics, the study of ''as-many-small-metabolites-as-possible'' in a system, has become an important tool in many research areas. Recent reviews and perspectives in the areas of human diseases (Kaddurah-Daouk & Krishnan, 2009), drug discovery (Wishart, 2008a), plant analysis (Hall, Brouwer, & Fitzgerald, 2008), human nutrition (Wishart, 2008b), and others, have shown the broad impact and rapid growth of metabolomics.

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0924-2244/$ - see front matter
doi:10.1016/j.tifs.2009.07.002

Metabolomic analyses have been generally classified as targeted or untargeted (Fig. 1). Targeted analyses focus on a specific group of intended metabolites with most cases requiring identification and quantification of as many metabolites within the group (Ramautar, Demirci, & Jong, 2006). Targeted analyses are important for assessing the behavior of a specific group of compounds in the sample under determined conditions. Targeted metabolomics typically requires higher level of purification and a selective extraction of metabolites. In contrast, untargeted (a.k.a. comprehensive) metabolomics focuses on the detection of as many groups of metabolites as possible to obtain patterns or fingerprints without necessarily identifying nor quantifying a specific compound(s) (Monton & Soga, 2007). Untargeted analyses have been used in the identification of possible fingerprints of biological phenomena such as plant diseases (Cevallos-Cevallos, Rouseff, & Reyes-De-Corcuera, 2009). Based on the specific objective of the analysis and data manipulation, most metabolomic studies can also be classified as discriminative, informative, and/or predictive (Fig. 1). Discriminative analyses have been aimed to find differences between sample populations without necessarily creating statistical models or evaluating possible pathways that may elucidate such differences. Wine has been classified by grape variety and production area by metabolomic techniques (Son *et al*., 2008). Discrimination is usually achieved by the use of multivariate data analysis (MVDA) techniques intended to maximize classification, principal components analysis (PCA) being the most used tool. PCA and other MVDA tools have been widely described in other reviews (Kemsley *et al*., 2007; van der Werf, Jellema, & Hankemeier, 2005). In contrast, informative metabolomic analyses have focused on the identification and quantification of targeted or untargeted metabolites to obtain sample intrinsic information. Informative metabolomics has been used in the development and continuous update of metabolite databases such as the human metabolome database (Wishart *et al*., 2007). Possible pathways, discovery of novel bioactive compounds, discovery of biomarkers, creation of specialized metabolite databases, and metabolites functionality studies can also be carried out by informative metabolomics. Finally, some metabolomics reports have been predictive. In this case, statistical models based on metabolite profile and abundance are created to predict a variable that is difficult to quantify by other means. Metabolite-based models

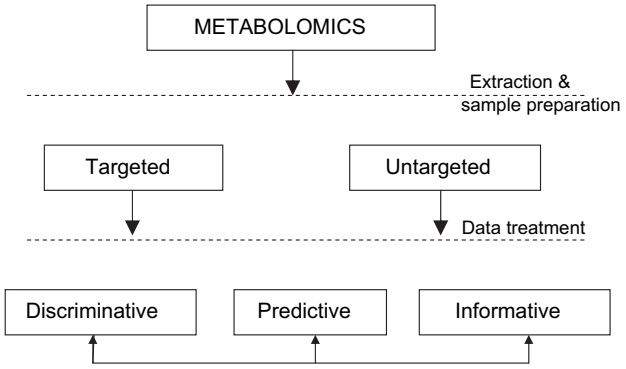

Fig. 1. General classification of metabolomics.

for prediction of green tea sensory quality have been developed (Ikeda, Kanaya, Yonetani, Kobayashi, & Fukusaki, 2007). These models are usually produced by partial least square (PLS) regression as discussed in Data treatment section of this review.

In food science, metabolomics has the potential for solving major problems worldwide as it is being applied in food research programs such as the Metabolomics for Plants, Health and OutReach (METHA-PHOR) initiative (Hall, 2007). Moreover, metabolomics is considered an efficient tool for addressing future needs in agriculture (Green, Qureshi, Long, Burfening, & Hamernik, 2007) and human nutrition (Green *et al*., 2007; Hall *et al*., 2008).

Discriminative, informative, and predictive metabolomics have been recently used in combination for quality, nutrition, and food components analysis (Wishart, 2008b) with a significant expansion to other food applications in the last two years. This paper presents an in depth review of recent metabolomics studies in food from the perspective of the extraction, separation, detection, and data treatment, as well as the application of discriminative, informative, and predictive metabolomics in the areas of food quality, safety, regulations, microbiology, and processing.

The process of metabolomic analysis

Metabolomic analyses consist of a sequence of steps including sample preparation, metabolite extraction, derivatization, metabolite separation, detection, and data treatment (Fig. 2). However, not every steps is always needed. Only detection and data analysis have been essential steps in all reported metabolomics studies. The selection of the steps depends on the type of study (untargeted *vs*. targeted), kind of sample (e.g. solids *vs*. liquids), instrumentation to be used for separation (e.g. GC *vs*. LC) and detection method (e.g. MS *vs*. NMR). Table 1 summarizes recent metabolomics studies used for food analysis.

Sample preparation

Solid samples such as apple peel (Rudell, Mattheis, & Curry, 2008) and potatoes (Dobson *et al*., 2008) are typically ground under liquid nitrogen or after freeze-drying.

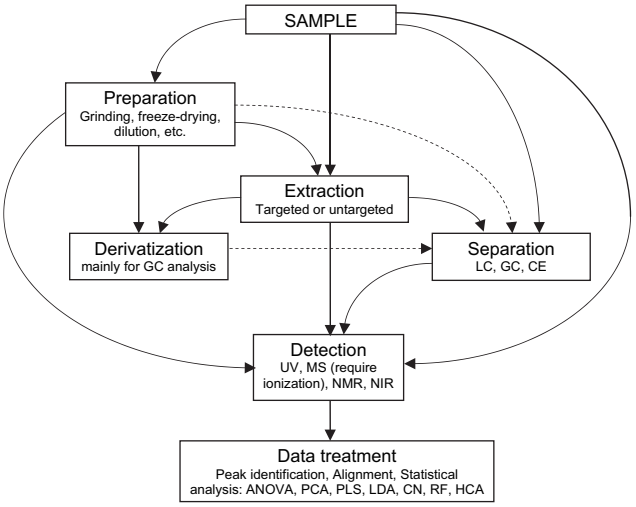

Fig. 2. Schematic representation of the process of metabolomic analysis.

Proper grinding enhances the release of metabolites during extraction. Freeze-drying acts as a concentration step and minimizes possible differences in metabolites due to dissimilarities in moisture content between groups of sample. Other concentrated liquid samples such as honey can be diluted as a preliminary step (Donarski, Jones, & Charlton, 2008). However, to maximize the amount of information to be collected, concentration steps are more suitable. For example, metabolites in wine (Son *et al*., 2008) and volatiles in olive oil (Cavaliere *et al*., 2007) have been concentrated by lyophilization and solid phase microextraction (SPME) respectively.

Extraction

The initial extraction procedure is aimed at maximizing the amount and concentration of the compounds of interest. For this reason, extraction is probably the most critical step in metabolomics. In untargeted metabolomics, the nature of compounds of interest is mostly unknown. Hence, several solvents and extraction methods should be tested and compared between the groups of samples. Most reports on untargeted food analysis do not describe preliminary comparisons among extraction solvents tested. However, the extraction methods used in foods have been similar to those found optimal in comparable research fields such as non-food plant metabolomics. For instance, the combination methanol–water–chloroform ($MeOH–H_2O–CHCl_3$) in different proportions was shown to be superior to other solvents for untargeted studies in plants such as *Arabidopsis thaliana* (Gullberg, Jonsson, Nordstrom, Sjostrom, & Moritz, 2004) because of its capacity of extracting both hydrophilic and hydrophobic compounds. Therefore, the effectiveness of $MeOH–H_2O–CHCl_3$ in green tea (Pongsuwan *et al*., 2008), potatoes (Dobson *et al*., 2008) and other foods was anticipated. For untargeted analysis, the use of sequential and selective extractions followed by metabolite analysis of each extract was previously recommended (Dixon

Table 1. Most common metabolomics processes in food analysis.

Sample: Purpose of analysis	Type	Extraction and preparation	Separation–detection	Data treatment	Reference
Apples: light induced changes in peel	Untargeted/discriminative	MeOH Derivatization for GC–MS	GC–MS LC–MS	PCA	Rudell *et al.*, 2008
Berries: polyphenol composition	Targeted/informative	Acetic acid + water C18 and Sephadex LH 20 columns	LC–MS DIMS	Compound identification	McDougall *et al.*, 2008
Broccoli, mustard, and brassica: glucosinolates composition	Targeted/informative	Hot water (90 °C) + sonication	$LC–MS^n$	Compound identification	Rochfort *et al.*, 2008
Broccoli: variety differentiation	Untargeted/discriminative	Freeze dried MeOH + H_2O	LC–UV–MS DIMS	PCA, ANOVA	Luthria *et al.*, 2008
Cheese: Production control	Untargeted/informative	–	IMS	Compound identification	Vautz *et al.*, 2006
E. coli: glycolisis metabolites	Targeted/informative	Indirect thermal treatment	LC–MS	Compound identification	Schaub & Reuss, 2008
Ginseng: variety differentiation	Untargeted/discriminative	Deuterated MeOH + buffered water	NMR	PCA	Kang *et al.*, 2008
Green: tea quality	Untargeted/predictive	Freeze dried MeOH + H_2O + $CHCl_3$	UPLC–TOF–MS	PCA, PLS	Pongsuwan *et al.*, 2008
Honey: origin verification	Untargeted/discriminative/ predictive	Buffered water	NMR	PLS–GP	Donarski *et al.*, 2008
Maize: GMO identification	Untargeted/discriminative	MeOH + water + ultrasonication	CE–TOF–MS	Student's t, PCA	Levandi *et al.*, 2008
Meat: quality/safety	Untargeted/discriminative	Neutral desorption	EESI–MS	PCA	Chen *et al.*, 2007
Olive oil: origin differentiation	Targeted/discriminative	SPME	GC–CI–MS	LDA Kruskal–Wallis and Wald–Wolfowitz tests	Cavaliere *et al.*, 2007
Pine mushrooms: quality differentiation	Untargeted/discriminative	MeOH + H_2O + $CHCl_3$	NMR	PCA	Cho *et al.*, 2007
Potato: GM differentiation	Untargeted/discriminative	MeOH + H_2O + $CHCl_3$ Derivatization for GC–MS	GC–MS DIMS	PCA	Catchpole *et al.*, 2005
Potato: identification of cultivars	Untargeted/discriminative/ informative	Freeze dried + MeOH + water + chloroform + derivatization	GC–TOF–MS	ANOVA, PCA	Dobson *et al.*, 2008
Potato: variety differentiation	Untargeted/discriminative/ informative	MeOH + H_2O + $CHCl_3$ Derivatization for GC–MS	GC–MS DIMS	RF	Beckmann *et al.*, 2007
Soybean: GMO differentiation	Untargeted/informative	MeOH–EtOH–H_2O	CE–TOF–MS	Compound identification	Garcia-Villalba *et al.*, 2008
Spinach: *E. coli* contamination	Untargeted/discriminative	Neutral desorption	EESI–MS	PCA	Chen *et al.*, 2007
Tomato paste: changes during production	Targeted to antioxidants/ informative Untargeted/informative	Targeted: H_2O–MeOH and MeOH–$CHCl_3$ Untargeted: Formic acid–MeOH–H_2O	LC–antioxidant detector LC–TOF–MS	ANOVA, PCA	Capanoglu *et al.*, 2008
Tomato: metabolite correlations	Untargeted/predictive	Volatiles: EDTA–NaOH–H_2O + SPME Sugars and organic acids: MeOH + derivatization	GC–MS	PCA, LDA, CN	Ursem *et al.*, 2008
Tomato: variety differentiation	Untargeted/discriminative	Lyophilization + MeOH + sonication	LC–TOF–MS NMR	PCA	Moco *et al.*, 2008
Tomato: volatiles analysis	Targeted/discriminative	EDTA–NaOH–H_2O + SPME	GC–MS	PCA, HCA	Tikunov *et al.*, 2005
Watermelon: quality evaluation	Untargeted/predictive	Buffered D_2O	NMR	PLS–LDA	Tarachiwin *et al.*, 2008
Wine: metabolite characterization	Untargeted/discriminative	Lyophilized + buffered D_2O	NMR	PCA, PLS	Son *et al.*, 2008
Yeast: aroma compounds production	Targeted/discriminative	Diethyl ether	GC–FID	PCA, PLS	Rossouw *et al.*, 2008
Yeast: strain differentiation	Untargeted/discriminative	Lyophilization + derivatization	GC–TOF–MS	PCA, HCA	MacKenzie *et al.*, 2008
Yeast: strain differentiation	Untargeted/discriminative	–	NIR	PCA LDA	Cozzolino *et al.*, 2006

et al., 2006). Usually, an initial hydrophilic extraction (typically with MeOH–H_2O) followed by centrifugation and hydrophobic extraction (typically with $CHCl_3$) of the pellet achieves this purpose. Sequential extraction maximized the amount of metabolites from tomato paste (Capanoglu, Beekwilder, Boyacioglu, Hall, & De Vos, 2008) finding discriminating compounds in both hydrophilic and hydrophobic fractions. Conversely, analysis of other food stuff such as potato (Dobson *et al*., 2008) and mushrooms (Cho, Kim, & Choi, 2007) showed low or no sample discrimination in the hydrophobic fractions. Similar observations made in other areas such as analysis in plant leaves (Cevallos-Cevallos *et al*., 2009) suggest a higher suitability of hydrophilic extracts for discriminative metabolomic analyses. Hydrophilic extractions in untargeted food analysis such as apple (Rudell *et al*., 2008) and broccoli (Luthria *et al*., 2008) have usually been carried out by MeOH or MeOH–H_2O. Other extractions based on deuterium oxide (D_2O) for NMR analysis are also common. Novel methods for extraction of metabolites from frozen meat, where a desorption gas hits the meat surface extracting metabolites further carried to the ionization and detection chambers, have been reported (Chen, Wortmann, & Zenobi, 2007). Extraction for targeted analysis relies on previous knowledge of the analytes nature. Polyphenols have been extracted from berries with a water–acetic acid combination (McDougall, Martinussen, & Stewart, 2008), and hot water was used for targeted analysis of glucosinolates in broccoli and mustard seeds (Rochfort, Trenerry, Imsic, Panozzo, & Jones, 2008). To maximize the number and amount of metabolites obtained and to reduce extraction time, disruption methods such as ultrasonic treatments are usually part of both untargeted and targeted extractions.

Derivatization

In food metabolomics, derivatization is commonly used prior to GC analysis in order to increase volatility of analytes. Derivatization is usually a two-step process starting with oximation (conversion of aldehydes and ketones to oximes) of the sample to reduce tautomerism (especially from monosaccharide), followed by silylation to increase volatility by reducing hydrophilicity of functional groups OH, SH or NH (Gullberg *et al*., 2004). Several oximation and silylation reagents have been tested in the past. Gullberg *et al*. (2004) reviewed previous comparisons among derivatization reagents and reported that methoxiamine hydrochloride in pyridine and *N*-methyl-*N*-trimethylsilyltrifluoroacetamide were the most appropriate reagents for oximation and silylation respectively. In food analyses, these reagents have shown to improve GC separation of metabolites from potato (Beckmann, Enot, Overy, & Draper, 2007) and other products. Derivatization times and temperatures affect each metabolite independently with major changes at the beginning of the reaction (Ma, Wang, Lu, Xu, & Liu, 2008). Therefore, preliminary experiments should be done to determine optimum derivatization times and temperatures that maximize the detection of compounds of interest. In food metabolomic analysis, several silylation reactions have been carried out at 37 °C for 90 min (Beckmann *et al*., 2007; Dobson *et al*., 2008) with good results.

Separation and detection

Separation and detection of the metabolites have been considered the key steps in metabolic profiling. Particular attention has been given to separation techniques such as liquid chromatography (LC) in its high performance (HPLC) or ultra performance (UPLC) forms, gas chromatography (GC), capillary electrophoresis (CE), as well as the coupling of these instruments to detection techniques such as mass spectrometry (MS), nuclear magnetic resonance (NMR), and near infrared spectrometry (NIR). Working principle as well as individual and hyphenated suitability of these techniques in metabolomics have been broadly discussed (Bedair & Sumner, 2008; Rochfort, 2005; Toyo'oka, 2008; Wishart, 2008b).

In food metabolomics most separation analyses have been achieved by GC, CE, and LC as seen in Table 1. Comparison and suitability of these techniques in food analysis have been discussed in other reviews (Wishart, 2008b). Among non-conventional techniques, ion mobility spectrometry (IMS), where food metabolites are carried in a flow of inert gas, ionized, and separated by a drift gas flowing in the opposite direction, has been applied to metabolomic analysis of cheese, beer, and food packaging material (Vautz *et al*., 2006). Detection methods are mostly based on UV, NIR, MS, or NMR techniques. In food metabolomics MS and NMR have been used the most (Table 1). A greater amount of data is generally obtained by using MS accompanied by high throughput separation techniques such as HPLC or UPLC as shown in Table 2. For instance, the quality of green tea has been evaluated by NMR (Tarachiwin, Ute, Kobayashi, & Fukusakii, 2007) and UPLC–MS (Pongsuwan *et al*., 2008) and statistical models from UPLC–MS yielded a higher prediction coefficient than models from NMR, probably due to the higher number of peaks detected by UPLC–MS. However, other factors

Table 2. Examples representative of common number of peaks reported in food metabolomics.[a]

Technique	Peaks reported	Main references
HPLC–UV	40 detected	Defernez *et al*., 2004
UPLC–MS	1560 detected	Pongsuwan *et al*., 2008
GC–MS	91–142 detected	Beckmann *et al*., 2007; MacKenzie *et al*., 2008
CE–MS	27–45 detected	Garcia-Villalba *et al*., 2008; Levandi *et al*., 2008
NMR	16–20 identified	Jahangir, Kim, Choi, & Verpoorte, 2008; Son *et al*., 2008

[a] Food matrix and extraction methods greatly influence the number of detected peaks.

such as sample variability should also be considered. Although not as sensitive as the other detection techniques, NIR has provided a fast non-destructive fingerprint in several metabolomic analyses such as strain differentiation of wine yeast (Cozzolino, Flood, Bellon, Gishen, & Lopes, 2006). Another technique, direct infusion mass spectrometry (DIMS) methods do not require a previous separation step achieving faster results as applied for broccoli (Luthria *et al*., 2008).

Data treatment

Metabolomic data have usually been submitted to compound identification and MVDA. Compound identification has been mainly achieved by database matching and comparison with pure standards ran under same conditions. Data analysis in food metabolomics is largely carried out by several chemometrics tools. Typically, metabolomics data have been aligned before comparison to correct for instrumental deviations on retention/migration times. Alignment has been shown to drastically improve the performance of MVDA techniques (Son *et al*., 2008). Examples of alignment software include MetAlign for LC–MS and GC–MS (Sumner, Urbanczyk-Wochniak, & Broeckling, 2008) or alignment tools of 32 Karat for CE. Alignment routines written in MATLAB (The MathWorks Natick, MA) have been reported. Discriminative metabolomics usually relies on multivariate methods such as PCA for sample grouping. PCA creates new variables (principal components) by linear combinations of the metabolites detected while maximizing sample variation. Grouping occurs when comparing the values of two or more principal components of each sample as used for discrimination of broccoli varieties (Luthria *et al*., 2008). On the contrary, PLS is a MVDA technique that allows sample discrimination by reduction of dimensionality while maximizing correlation between variables. PLS has been the main technique used for predictive metabolomics studies such as the creation of a metabolite-based model for sensory evaluation of watermelon (Tarachiwin, Masako, & Fukusaki, 2008). Similarly, linear discriminant analysis (LDA) with *a priori* classification hypothesis was used for discrimination of olive oil according to origin (Cavaliere *et al*., 2007). Reviews on PCA, PLS, and LD are widely found in the literature (Kemsley *et al*., 2007; van der Werf *et al*., 2005). Also, correlation techniques such as correlation network (CN) analysis have successfully lead to determining the link between metabolites and establish possible reactions in several informative metabolomics studies such as genotype differentiation of tomatoes (Ursem, Tikunov, Bovy, van Berloo, & van Eeuwijk, 2008). Genetic programming (GP) is another discriminative tool that was utilized to improve the sensitivity and selectivity of the PLS models for honey origin determination (Donarski *et al*., 2008). Most of the MVDA tools such as PCA and PLS reduce dimensionality of the data by linear combination of the original variables. In contrast, random forest (RF) analysis permits multivariate data comparison without dimensionality reduction. RF has allowed classification of potato varieties by pairwise comparisons with accuracy values greater than 92%. Also creation of a Mastermix potato model allowed discrimination of a larger number of potato varieties through RF (Beckmann *et al*., 2007). Table 1 shows that most of the MVDA in food have relied on PCA, PLS, and other linear methods. Non-linear structures associated with the data were not considered. Non-linear methods for dimensionality reduction have been shown to outperform linear MVDA tools in areas such as gene and protein expression studies (Lee, Rodriguez, & Madabhushi, 2008). However, to the best of our knowledge, application of non-linear methods for metabolomics data analysis has not been reported in foods. Non-linear PCA, Self Organizing Map (SOM), visualization induced SOM, multidimensional scaling, and other non-linear tools that have the potential to be applied to foods have been recently reviewed (Yin, 2007).

Metabolomics in food quality

Targeted metabolomics focused on volatiles has shown great potential to assess pre-harvest issues that may affect quality. Pre-harvest fungal diseases in mango (Moalemiyan, Vikram, & Kushalappa, 2007), post-harvest bacterial contamination of onions (Vikram, Hamzehzarghani, & Kushalappa, 2005) and McIntosh apples (Vikram, Prithiviraj, Hamzehzarghani, & Kushalappa, 2004), as well as diseases of stored carrots (Vikram, Lui, Hossain, & Kushalappa, 2006) have been assessed by sampling headspace metabolites followed by GC–MS analysis. In each case, the volatile profile was found to be disease-specific, and several compounds were tentatively identified by GC–MS databases. Additionally, changes in polyphenolic compounds during berries breeding (Stewart *et al*., 2007) have been characterized by informative metabolomics. Post-harvest metabolomic analysis has the potential for detection and understanding food spoilage as reviewed by Kushalappa, Vikram, and Raghavan (2008).

The development of novel metabolomic techniques such as IMS has permitted monitoring of quality attributes during food processing. Because IMS allows *in situ* automatic sampling, it can be used for determining the completion of certain processes assuring standard quality based in a group of metabolites. This type of analysis fits the needs of biotechnological food processes in which metabolites are changing with time. Targeted informative (concentration aimed) IMS has been applied for the detection of diacetyl and 2,3 pentadione compounds in beer to determine the endpoint of the fermentation (Vautz *et al*., 2006). Discriminative metabolomics has allowed classification of health supplements based on their quality and origin (Kooy, Verpoorte, & Meyer, 2008; Liu, Si, Wan, Lin, & Xu, 2008).

Future trends will involve the use of discriminative and predictive metabolomics as the ultimate tool for quality control. The metabolite profile of products meeting minimum standards can be used as a baseline for quality

acceptance. Individual samples obtained during processing can be analyzed and compared to the baseline through MVDA techniques to determine acceptability of the batch produced. Moreover, accidental adulteration of food (e.g. allergenic inclusion or microbial contamination) can be detected by appearance of uncommon peaks in the sample's metabolic profile. Informative metabolomics can elucidate the nature of the peaks of interest. In addition, combinations of predictive and informative metabolomics have the potential to become the single all-parameter analysis tool. Quality parameters are usually individually measured complicated and costly protocols. Many of these parameters can be quantified in a single run of informative metabolomics whereas others (e.g. sensory attributes) can be estimated by predictive models based on sample metabolite profile, providing a cost-effective alternative to quality analyses. Predictive models have been developed to estimate sensory attributes of green tea (Ikeda *et al*., 2007; Pongsuwan *et al*., 2008; Tarachiwin *et al*., 2007), watermelon (Tarachiwin *et al*., 2008) and mushrooms (Cho *et al*., 2007). Similarly, metabolomics has the potential of identifying compounds that dictate consumer taste preferences. Consumers' preferences can be obtained by taste panels while discriminating compounds can be identified by metabolomics techniques. Sensory evaluations with various concentrations of the chosen compounds will confirm their impact on consumer preferences (Fig. 3).

Metabolomics in food safety

Many untargeted discriminative tools have been applied in food safety. Amongst the many techniques, neutral desorption extractive electrospray-ionization MS (EESI-MS) was able to discriminate *Escherichia coli*-contaminated spinach through the presence of unidentified high molecular weight peaks (Chen *et al*., 2007). Even though no attempt to determine the limit of detection of *E. coli* was made, the technique clearly shows potential for rapid pathogen detection in food. Additionally, the same technique discriminated spoiled fish through the presence of putrescine, cadaverine, and histamine, showing a great potential of this type of analysis in food safety. Informative and predictive metabolomics in fresh raw fish have been recommended as tools to provide evidence of water contamination, temperature stress, and the fish health conditions at the moment of the catch (Samuelsson & Larsson, 2008).

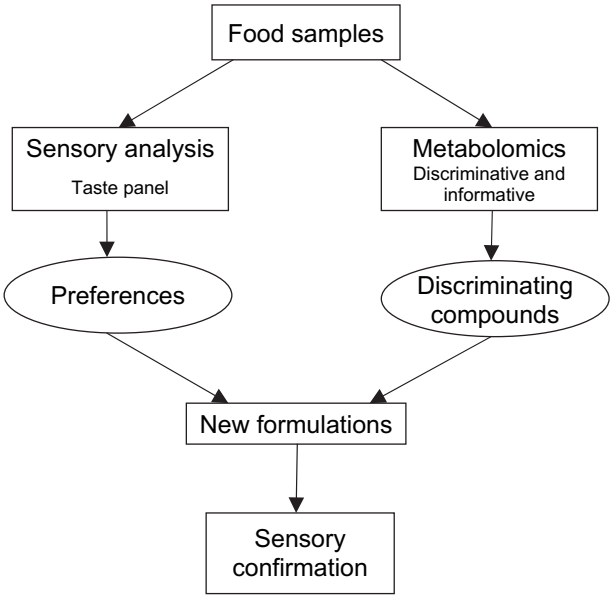

Fig. 3. Potential application of metabolomics for understanding consumer preferences.

Metabolomics has the potential to assess the safety of novel pre- and post-harvest technologies. Unintended effects of genetic modification of foods can been assessed by untargeted discriminative analyses (Chao & Krewski, 2008; Zdunczyk, 2006). Catchpole *et al*. (2005) utilized untargeted discriminative metabolomics to differentiate genetically modified (GM) potatoes from non-treated ones. Sample differentiation occurred based on the intended variation of fructans in GM samples. After removal of fructans derivatives from the model, no discrimination was observed, suggesting that GM potatoes are similar in composition to the original ones. Similarly, intended increase in flavonoid concentration in GM tomatoes have been confirmed through targeted informative metabolomics (Le Gall, DuPont, *et al*., 2003) whereas small non-intended variations were detected by untargeted analysis (Le Gall, Colquhoun, Davis, Collins, & Verhoeyen, 2003), concluding that no major unintended changes occurred after genetic modification. Future trends would involve the use of informative metabolomics to assess the safety of new or controversial processing technologies such as irradiation.

Metabolomics for compliance with food regulations

Differences in food metabolite profile can be due among other things to genotype and growing conditions (e.g. climate, soil composition, fertilization, and irrigation). Establishing baseline regional and varietal variability in metabolite profiles provides reliable information about the origin and authenticity of the food. Country of origin regulations was verified by discriminative and predictive metabolomics. Origins of honey (Donarski *et al*., 2008), olive oil (Cavaliere *et al*., 2007) and wine (Son *et al*., 2008), have been determined by discriminative and predictive metabolomics. Regulations in many countries do not allow the use of GM foods, and GM verification analyses are complicated and expensive. Discriminative and predictive analysis have been used to differentiate genetic modification in maize (Levandi, Leon, Kaljurand, Garcia-Canas, & Cifuentes, 2008), soybean (Garcia-Villalba *et al*., 2008), potatoes (Catchpole *et al*., 2005; Le Gall, Colquhoun, *et al*., 2003), and wheat (Shewry, Baudo, Lovegrove, & Powers, 2007). Additionally, metabolomics can be used for compliance verification of labeled ingredients. These analyses have relied in the use of discriminative metabolomics to differentiate among varieties of several fruits and

vegetables. For instance, MVDA techniques were used to differentiate cherry tomato from other varieties such as beef and round tomatoes by SPME–GC–MS (Tikunov *et al*., 2005), LC–MS and NMR (Moco, Forshed, De Vos, Bino, & Vervoort, 2008). Variety differentiation has also been applied to broccoli (Luthria *et al*., 2008), wines (Pereira *et al*., 2007; Son *et al*., 2008), ginseng (Kang *et al*., 2008; Shin *et al*., 2007), and potatoes (Dobson *et al*., 2008; Parr, Mellon, Colquhoun, & Davies, 2005).

Metabolomics in food microbiology

Research aimed at identifying bacterial contamination of foods has benefited from the use of metabolomics. Bacteria identification and confirmation is traditionally done by complex numerous biochemical tests. In contrast, discriminative and predictive analyses have the potential for identifying and confirming bacterial contamination. These analyses are mostly MS-based (Ecker *et al*., 2008). Microorganisms are initially grown in culture media, then concentrated (typically by centrifugation) and internal metabolites are extracted through cell disruption processes such as ultrasound or bead beating processes before separation or detection occurs. By following this method and the use of a matrix assisted laser desorption/ionization time of flight mass spectrometry (MALDI–TOF–MS) to detect high molecular weight compounds, 12 species of *Aspergillus* and 5 strains of *Aspergillus flavus* have been classified with 95–100% accuracy (Hettick *et al*., 2008). Similar methods have been used to classify *E. coli* and *Yersinia* according to growing culture media, species, and strain (Parisi *et al*., 2008). Metabolomics can also be used for understanding microbial metabolism. Dynamics of glycolysis in *E. coli* have been assessed under systemic variation of growth rate and different glucose availability (Schaub & Reuss, 2008) generating information on how glycolysis is affected under these conditions.

Wine and baking yeasts have been differentiated from medical strains by using DIMS and GC–TOF–MS (MacKenzie *et al*., 2008). In addition, exo-metabolites of several wine yeast strains were analyzed by HPLC and GC–FID to compare aroma relevant compounds to gene expression (Rossouw, Nas, & Bauer, 2008) showing the potential of metabolomics for assessing gene expressions.

Current methods for quantification of bacteria in food still rely on lengthy techniques such as plate count and most probable number. Metabolomic analysis coupled to sensor development can provide critical information for the detection and quantification of bacteria. Metabolomics has been successfully used for the discovery of specific biomarkers in areas such as plant physiology (Glauser *et al*., 2008). The discovery of bacteria biomarkers and their monitoring throughout growth phases has the potential to become a quantitative tool if related to the final bacteria colony forming units (CFU). Sensors may be developed to monitor the formation of the biomarker in the culture broth and the rate of biomarker production can be incorporated into an algorithm that predicts the CFU. Metabolomics studies during *E. coli* growth have shown the time-related progression of several metabolites (Koek, Muilwijk, & van der Werf, 2006).

Moreover, metabolomics has the potential to find new antimicrobial compounds as well as to determine the analytes responsible for the antimicrobial characteristics of certain plants and foods. Zhi, Yu, and Yi (2008) utilized discriminative metabolomics based on HPLC to identify dihydrocucurbitacin F-25-O-acetate as the major antimicrobial component of the herb *Hemsleya pengxianensis*. PCA data showed that *Staphylococcus aureus* treated with dihydrocucurbitacin F-25-O-acetate clustered with those treated with the herb extract.

Metabolomics in food processing

Food processing involves the combination of physical and chemical events that may cause important changes in food components. These changes can be detected by metabolomics. The production of cheonggukjang (a soybean and rice straw fermented drink) has been monitored by informative and discriminative untargeted analysis using NMR (Choi, Yoon, Kim, & Kwon, 2007). The method showed the expected time-related reduction of sugars (e.g. sucrose and fructose) and increase of acetic acid, tyrosine, phenylalanine and others. Final products were differentiated as a function of fermentation time by PCA. In addition, Capanoglu *et al*. (2008) utilized both targeted and untargeted informative metabolomic analyses to show that several flavonoids such as rutin, naringenin and derivates, as well as some alkaloids increased significantly after the breaking step (fruit chopping). The appearance of new flavonoids was explained by the possible activation of pertinent enzymes after wounding. In addition, reduction of these compounds after the pulping step was observed because of the high presence of these analytes in the removed skin and seeds. Metabolomics can also be used to understand the suitability of certain varieties for processing. For instance, several potato varieties are preferred for frying whereas other for baking. To assess differences, potato varieties have been analyzed by flow infusion electrospray-ionization MS (FI-ESI-MS) and compound identification was aided by GC–MS (Beckmann *et al*., 2007). Cultivars Salara and Agria were low in tyrosine (major substrate for polyphenol oxidases) making them suitable for slicing and frying. Tyrosine is also a precursor of aroma and flavor compounds in boiled potatoes by Strecker degradation. Cultivars found to be high in tyrosine (Désirée and Granola) are more suitable for baking (Beckmann *et al*., 2007). This type of analysis has shown the potential for providing valuable information to food product and process development industry.

Informative metabolomics has the potential to assess unintended effects during processing and pre-processing such as changes in nutrient composition, degradation of health-related compounds, and formation of new compounds like toxins. In addition informative and discriminative metabolomics have the potential to study other pre-processing

scenarios such as organic food production, and denominations such as "cage free" or "grade A".

Conclusions

Metabolomics has shown to be an important tool for the progress of the main food science areas such as compliance of regulations, processing, quality, safety, and microbiology. Recent studies suggest that the potential of metabolomics in food science can be expanded to the area of food product development by determining the compounds responsible for consumers' taste preferences.

The development of rapid technologies such as DIMS, IMS, and EESI has helped the growth of metabolomics in food science. However, further improvement on these techniques is necessary to overcome sensitivity and compound identification issues.

Most of the data analyses in food have relied in linear MVDA tools, not considering possible non-linear aspects of the samples. Future trends should involve the use of non-linear tools for dimensionality reduction in food metabolomics.

Even though metabolomic analyses in food have been much diversified, most studies can be considered as discriminative (Table 1) with very few compounds identified. Therefore, the development of a food metabolome database, as suggested by Wishart (2008b) is needed in order to facilitate compound identification and the development of informative metabolomics. In addition, most reports have focused on fruit and vegetables (Table 1) leaving the meat, seafood, and related areas still underexplored. Because of some metabolic similarities, identification of many compounds in red meat can be carried out by using available human metabolome databases (Wishart, 2008b).

Metabolomics' successful association to other analytical areas such as genomics has been demonstrated, showing the potential of metabolite profiling to be linked to other areas as well. Metabolomics as a first step for sensor development has the potential to introduce a series of new rapid methods for food analysis. In this area, bacteria biomarkers can be discovered by metabolomics techniques and sensors can be developed for rapid detection of the selected biomarkers. To achieve this purpose, studies on microbial biomarkers identification involving different levels of bacterial contamination, accompanying flora, and biomarker response in food are needed.

The rapid growth of metabolomics in the food science area suggests the potential of discriminative, predictive, and informative analyses to solve the most important problems and provide important information to the food industry.

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