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Review on metabolomics for food authentication

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ABSTRACT

European and global food policies require that food put on the market is authentic, which means that the label declaration matches the composition and provenance of the food item. During the last decades conventional approaches such as the determination of certain major and minor components by wet chemistry of chromatographic methods have been used for food authentication. Although many of the traditional methods are still used because they are part of product standards, new approaches that could complement existing methodologies, such as metabolomics are emerging in food authentication applications. Metabolomics aims to study multiple metabolites in a cell, a tissue or an organism. The main advantage of metabolomic applications in food authentication makes use of its untargeted nature, which can enable the detection of emerging frauds. The purpose of this review is to summarise and describe the recent metabolomic applications in the area of food authentication and to discuss its current limitations and future potential.

1. Introduction

The rights of consumers to receive truthful information about the food they buy are set out in Regulation No. 178/2002 (European Commission, 2002) (Reid, O'Donnell, & Downey, 2006). It aims at the prevention of (a) fraudulent or deceptive practices, (b) the adulteration of food and (c) any other practices which may mislead the consumer. An example of a common fraud is the substitution of one ingredient by a similar cheaper one which is difficult to recognise by the consumer and to detect by current analytical techniques. Besides, depending on the nature of the adulterants, the admixtures can also represent a health risk for the consumer and therefore the ability to trace and authenticate food products is of major concern in the food industry not only for economical but also for safety reasons.

During the last decades more traditional methods based on wet chemistry, e.g. iodine value and saponification value on edible oils, have been used for food authentication. Notwithstanding the fact that those traditional methods are still used because they are part of product standards, new approaches that could improve the expensive and time consuming methodologies, such as metabolomics are emerging in food authentication applications (Consonni, Cagliani, & Steve, 2010). These new methodologies are also being studied for their application to solve current food fraud issues where classical methods fail to detect them. For example, adulteration of extra virgin olive oil with hazelnut oil is a serious concern since it cannot be easily detected by well-established techniques because of their similarities in the triacylglycerol, sterol and fatty acid compositions (high oleic acid, low linoleic acid).

Metabolomics allows the study of multiple metabolites in a cell, a tissue or an organism. Improvements in analytical chemistry such as ultra-high performance liquid chromatography (UHPLC), high-resolution mass spectrometry (HR-MS) and software programs to process the large analytical data sets created have been responsible for the rapid development of metabolomics in the last ten years (Wolfender, Rudaz, Choi, & Kim, 2013). The general application of metabolomics in food science and nutrition has been recently reviewed (Cevallos-Cevallos, Reyes-De-Corcuera, Etxeberria, Danyluk, & Rodrick, 2009; Esslinger, Riedl, & Fauhl-Hassek, 2014; Wishart, 2008) as well as the use of metabolomics for assessing safety and quality of plant-derived food (Oms-Oliu, Odriozola-Serrano, & Martín-Belloso, 2013). In general, depending on the objective of the analysis metabolomic studies can be classified in (a) informative, where the identification and quantification of metabolites is needed to obtain information; (b) discriminative, aiming to find differences between sample populations; and (c) predictive aiming to create statistical models to predict class memberships (Cevallos-Cevallos et al., 2009). Metabolomic studies in food authentication are mainly discriminative and predictive. Understanding the reason of the classification by the identification and quantification of metabolites responsible for this classification (informative metabolomics) is not the main thrust of such studies (Cevallos-Cevallos et al., 2009).

According to Dettmer, Aronov, and Hammock (2007) and Hall (2006) two complementary approaches are used for metabolomic investigations: metabolite profiling and metabolic fingerprinting. Metabolite profiling focuses on the analysis of a group of metabolites. In most cases metabolic profiling is a hypothesis-driven approach where a set of metabolites associated to a specific pathway are studied (Oms-Oliu et al., 2013). The second approach towards metabolomics is metabolic fingerprinting. Initially in this approach the intention is not to identify each observed metabolite, but to compare patterns or "fingerprints" of

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metabolites that change in response to disease, environmental or genetic alterations. Since this approach can be simultaneously applied to a wide range of metabolites, with the only limitation of the analytical platforms used, it is closer to be a true "omics" approach. Fiehn (2002) describes a "pure" metabolomics approach as the comprehensive analysis of a metabolome comprising identification and quantification of as many metabolites as possible in a biological system.

As Madsen, Lundstedt, and Trygg (2010) highlighted, the word metabonomics is often used as synonym to metabolomics, although the original definition relates it to the metabolic response to pathophysiological stimuli or genetic modification. However, many metabolomic publications are designed as metabonomic studies and vice versa. According to these authors nowadays the two terms can be viewed more as a historical curiosity than two different disciplines and therefore we have included both types of studies in our review.

Over the past decade with new developments in analytical approaches such as MS and advanced statistical and mathematical techniques, the application of metabolomics in food authentication issues has gained increasing interest. This review will describe the main steps, technical platforms and chemometric tools used in the last five years and the application, advantages, challenges and future trends of metabolomic fingerprinting approaches for the authentication of different food commodities.

2. General workflow

To date there are no standard guidelines described for every step of the workflow in metabolomic studies. However, the Metabolomics Society coordinates an initiative (the Standards Metabolome Initiative) to facilitate data integration and to avoid duplication efforts. The aim of the initiative is not to describe how to perform metabolomic experiments but to formulate a minimum set of reporting standards that describe the experiments and associated statistical and chemometric analysis (Fiehn et al., 2007).

2.1. Experimental design, sample handling and preparation

Before starting any metabolomic study the experimental design has to be established. If possible, a power analysis should be carried out to ensure that the number of samples is adequate and to reduce the technical variability in order to obtain valid data (Dettmer et al., 2007). To have access to authentic samples is very important. In the case of plant material, the harvesting method requires special attention because rapid metabolic changes can occur from the moment at which plants are harvested (Kim & Verpoorte, 2010). Also to obtain reliable data, representative quality control samples should be included in the workflow (Dettmer et al., 2007; Dunn et al., 2011). Proper storage conditions of the samples are also crucial as metabolic activity may be high. This activity has to be stopped or quenched to stop changes in concentration or structure of the metabolites (Dunn et al., 2008). Therefore, reduced temperatures during sample preparation (4 °C) and storage (−80 °C) are usually employed (Kim & Verpoorte, 2010).

Sample preparation is an important step in the workflow. It involves the extraction of the compounds into a format compatible with the analytical platform to be used. In cases of low-abundant metabolites it can include pre-concentration steps. Headspace extraction is one of the most common sample preparation techniques for the analysis of volatile compounds from different matrices. It is a fast method as clean-up steps are not necessary. However the method is only suitable for detection of highly volatile components of the matrix. It has been used recently for the characterisation of coconut oil (Mansor, Che Man, & Rohman, 2011). Another example of a sample preparation technique is solid phase microextraction (SPME) for the characterisation of the volatile fraction of food matrices. This methodology is simple to use, relatively fast, does not require solvent extraction and allows characterisation of the headspace composition in contact with the sample. Although SPME pre-concentration is the most common technique used for volatile compounds there are other alternatives such as Stir Bar Sorptive Extraction (SBSE) and Purge and Trap (PT). The SBSE procedure has been used in wine characterisation (Tredoux et al., 2008), and its advantage against SPME is that higher sensitivity can be achieved due to a larger sorbent phase volume. However, SBSE cannot be fully automated as is the case for SPME and SBSE does not offer the possibility of selecting a stationary phase based on the chemical properties of the compounds of interest (alternative phases for SBSE are not commercially available as yet). PT has been used recently for the extraction of volatile compounds in honey (Tananaki, Thrasyvoulou, Giraudel, & Montury, 2007), but this technique is not very common because of long extraction times and low sensitivities. If the aim of the study is the characterisation not only of volatile compounds present in the samples and the analytical platform is a gas chromatograph (GC), an extraction step followed by a derivatisation procedure has to be used. It is important to highlight that reaction conditions used to prepare derivatives of individual metabolites suitable for analysis by GC must be carefully optimised to avoid artefact formation.

For other applications, extraction protocols vary depending on whether the matrix is solid or liquid. For liquid matrixes, sample preparation can be very simple such as sonication for degassing purposes in beer (Mattarucchi et al., 2010) or centrifugation for removal of solid particles in fruit juices (Vaclavik, Schreiber, Lacina, Cajka, & Hajslova, 2012) and the sample extraction procedure is sometimes omitted (direct injection of the sample) (Cajka, Riddellova, Tomaniova, & Hajslova, 2011; Luthria et al., 2008). For solid matrixes the sample, which is often freeze-dried or grinded to a fine powder (Choi, Choi, Park, Lim, & Kwon, 2010; Luthria et al., 2008), is extracted with methanol (Gómez-Romero, Segura-Carretero, & Fernández-Gutiérrez, 2010; Moco, Forshed, de Vos, Bino, & Vervoort, 2008; Vaclavik, Lacina, Hajslova, & Zweigenbaum, 2011), methanol/water (Choi et al., 2010; Luthria et al., 2008) or ethanol/water (Vallverdú-Queralt, Medina-Remón, Casals-Ribes, Amat, & Lamuela-Raventós, 2011). The extraction step is often repeated two or three times combining the extracts for analysis. Another sample preparation and pre-concentration technique is solid phase extraction (SPE) (Beretta, Caneva, Regazzoni, Bakhtyari, & Maffei Facino, 2008; Tarantilis, Troianou, Pappas, Kotseridis, & Polissiou, 2008). SPE is a separation process by which compounds dissolved in the sample (mobile phase) are separated in a solid through which the samples are passed (stationary phase). These compounds of interest can be removed from the stationary phase by rinsing it with the appropriate eluent. However, this procedure can be lengthy and only specific compounds are recovered.

2.2. Detection technologies

Although metabolomics aims to create a profile of all the metabolites present in a tissue, no single analytical method has been capable of extracting and detecting all the different molecules at once. The challenges of detecting simultaneously the whole "metabolome" arise in the variety of chemical structures, the large range of concentrations at which metabolites are present in a matrix and the capability of the analytical platforms. Fingerprinting detection methods used in food science include vibrational spectroscopic techniques (such as infrared (IR) and Raman techniques), nuclear magnetic resonance (NMR) spectroscopy, a range of MS-based techniques and others such as flame ionisation detectors (FID) or sensor arrays. Looking at the research published in the area of food authentication in the last five years, NMR and MS-based studies have gained more importance (Fig. 1). This section intends to give a very general overview of the detection possibilities available in the area and to encourage the reader to further explore them.

2.2.1. Vibrational spectroscopy

Vibrational spectroscopy is a non-invasive fingerprinting method that allows rapid and non-destructive analysis. In the last years, vibrational spectroscopic techniques such as Fourier Transform-IR spectroscopy