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Review

Liquid chromatography-high resolution mass spectrometry for the analysis of phytochemicals in vegetal-derived food and beverages

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A B S T R A C T

The recent years witnessed a change in the perception of nutrition. Diet does not only provide nutrients to meet the metabolic requirements of the body, but it also constitutes an active way for the consumption of compounds beneficial for human health. Fruit and vegetables are an excellent source of such compounds, thus the growing interest in characterizing phytochemical sources, structures and activities. Given the interest for phytochemicals in food, the development of advanced and suitable analytical techniques for their identification is fundamental for the advancement of food research. In this review, the state of the art of phytochemical research in food plants is described, starting from sample preparation, throughout extract clean-up and compound separation techniques, to the final analysis, considering both qualitative and quantitative investigations. In this regard, from an analytical point of view, fruit and vegetable extracts are complex matrices, which greatly benefit from the use of modern hyphenated techniques, in particular from the combination of high performance liquid chromatography separation and high resolution mass spectrometry, powerful tools which are being increasingly used in the recent years. Therefore, selected applications to real samples are presented and discussed, in particular for the analysis of phenols, polyphenols and phenolic acids. Finally, some hot points are discussed, such as waste characterization for high value-compounds recovery and the untargeted metabolomics approach.

1. Introduction

The increasing knowledge about the impact of diet on health is changing the opinion on the role of nutrition. Diet does not only provide adequate nutrients to meet the metabolic requirements of the body, but it can be a source of bioactive compounds able to contribute to the improvement of human health. Consequently, the identification of such compounds is crucial for both the food and the dietary integrators market, to provide customers with a healthy, balanced diet. Bioactive compounds are being studied in the prevention of cancer, heart disease, and other diseases (https://www.cancer.gov/publications/dictionaries/cancer-terms?cdrid=703278). In this context, the vegetable kingdom is a primary source of such phytochemicals with potential health benefits. At present, researchers have identified hundreds of compounds with health-promoting, disease-preventing, or curative properties, and new discoveries concerning the complex interactions between secondary metabolites in plants and health are continually made (Weidner et al., 2012). Table 1, adapted from (Sbrana, Avio, & Giovannetti, 2014), shows some of the phytochemicals occurring in edible plants with health-promoting, disease-preventing, or medicinal properties. A considerable amount of data on dietary phytochemicals accumulated over the past years has been collected and stored in electronic databases (Scalbert et al., 2011). Additionally, the advent of metabolomics, which is a powerful approach enabling the comprehensive, qualitative and quantitative analysis of all metabolites (Fiehn, 2002), prompted research in this field; high resolution mass spectrometry (HRMS) coupled to (ultra)high performance liquid chromatography ((U)HPLC) is increasingly used in metabolomics. The recent innovations in instrumentation and informatics currently allow performing a comprehensive analysis of metabolites and reliably compare samples in a semi-automated manner. However, rigorous methodologies are required. Plants have a very large metabolome: about 200,000 phytochemicals are known so far and 20,000 of them have been identified as originating from fruits, vegetables and grains; moreover, they differ in molecular weight, physico-chemical characteristics and dynamic range (Oz & Kafkas, 2017). Thus, despite the potential, a fully exhaustive characterization in plant metabolomics remains challenging.

Metabolomics is subdivided into targeted (Roberts, Souza, Gerszten, & Clish, 2012) and untargeted (Vinayavekhin & Saghatelian,

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Table 1
Health effect of some phytochemicals (adapted from (Sbrana et al., 2014) with the addition of new items and references).

Phytochemicals	Plant	Health promoting activities	References
Activity on metabolic diseases			
Curcumin, capsaicin, catechins	Curcuma, chili peppers, cocoa	Antidiabetic	(Leiherer, Mündlein, & Drexel, 2013)
Amorfrutins	*Glycyrrhiza foetida* root	Antidiabetic, lipid-lowering	(Weidner et al., 2012)
Proanthocyanidins	Seed shells	Antiobesity	(Yokota, Kimura, Ogawa, & Akihiro, 2013)
Luteolin, phytosterols, isoflavones	Artichoke, soy	Hypocholesterolemic activity	(Gebhardt, 1997; Mannarino, Ministrini, & Pirro, 2014)
Anti-carcinogenic activity			
Theaflavins, thearubigins	Black teas	Antiproliferative activity	(Bhattacharya, Halder, Mukhopadhyay, & Giri, 2009)
Baicalein, baicalin, curcumin, quercetin, lutein	Garlic, lemon, carrot, basil, grape	Antiproliferative action on leukemia, hepatoma, melanoma, breast, bladder, pancreas, ovary, brain, kidney, lung, colon, and stomach carcinoma cells	(Cherng, Shieh, Chiang, Chang, & Chiang, 2007; Rai, Kaur, Jacobs, & Singh, 2010; Russo, Nigro, Rosiello, D'Arienzo, & Russo, 2007; Shehzad, Lee, & Lee, 2013)
Erythrodiol, uvaol, oleanolic, maslinic acid	Olive fruits	Cytotoxic effects on human breast cancer cells	(Allouche et al., 2011)
Isothiocyanates, dithiolethiones, sulforaphanes	Brassicaceae	Inhibition of carcinogenesis by modulating carcinogens metabolism and detoxification	(Ares, Nozal, & Bernal, 2013; Dillard & Bruce German, 2000; Tang et al., 2010)
Gamma-carotene, lycopene, lutein	Cruciferous vegetables	Protection against uterine, prostate, breast, colorectal, lung, and digestive tract cancers	(Jian, Du, Lee, & Binns, 2005; Toniolo et al., 2001)
Other activities			
S-allyl cysteine, allicin	Garlic	Antihypertensive	(Shouk, Abdou, Shetty, Sarkar, & Eid, 2014)
Luteolin, kaempferol, apigenin, myricetin	Teas, onions, apples	Anti-inflammatory and antibacterial activities	(Dillard & Bruce German, 2000)
Beta-sitosterol and its glycosides	Legumes, cereals, corn oil, nuts	Antioxidant activity, hypocholesterolemic activity, anti-inflammatory, antineoplastic, antipyretic, and immune system-modulating activity	(Loizou, Lekakis, Chrousos, & Moutsatsou, 2010; Woyengo, Ramprasath, & Jones, 2009)
Tocotrienols, tocopherols	Palm oil	Antioxidant, antiproliferative and apoptotic activities, preventing or reducing the risk of breast cancer	(McIntyre, Briski, Gapor, & Sylvester, 2000)
Phenolics in general, especially polyphenolics	Figs and grape seeds	Antioxidant, amyloid disease prevention and therapy, apoptotic on cancer cells	(Antunes-Ricardo et al., 2014; Ares et al., 2013; Lourenço, Gago, Barbosa, De Freitas, & Laranjinha, 2008; Stefani & Rigacci, 2014)
Epicatechin, epigallocatechin, epicatechin-gallate, epigallocatechin-gallate	Grapes, berries, cocoa, green tea, nuts	Reduction of dysregulations and degenerative phenomena, anticarcinogenic effects	(Lambert & Elias, 2010; Yang, Lambert, & Sang, 2009)
Carotenoids	Carrots, tomatoes	Antioxidants that might aid in the prevention of several human chronic degenerative diseases, such as cancer, cardiovascular diseases and age-related eye diseases	(Bijttebier, D'Hondt et al., 2014)

2010) approaches. In the targeted approach a set of selected, known metabolites are identified and quantified by using authentic standards or available databases (in the latter case a specific list of compounds is screened but no reference compounds are available for confirmation). The resulting data can be used as input for statistical analysis to distinguish sample classes with accuracy. In contrast, untargeted metabolomics aims to obtain information about all detectable compounds (no hypothesis is made on which compounds are present in the sample and the aim is to analyze everything, to achieve the widest metabolic coverage). Although this aim has not been reached yet, clear progress in this direction is ongoing. Suspect screening is an intermediate approach, which relies on specific information on some compounds, such as molecular formula and structure, suspected to be present in the sample (Flamini et al., 2013). This approach is relatively much more employed in the environmental analysis (Krauss, Singer, & Hollender, 2010; Scheibner, Squibb, Greco, & Steiner, 2016) than in phytochemical research.

Sometimes only metabolites related to either specific metabolic pathways or a class of compounds are investigated (Theodoridis, Gika, Want, & Wilson, 2012; Wolfender, Marti, Thomas, & Bertrand, 2015).

Until recently, most of the plant metabolite studies relied on analysis/quantitation of selected bioactive compounds. The development of powerful analytical techniques is essential for the advancement in phytochemical research. Due to the complexity of natural matrices, hyphenated HPLC-HRMS techniques appear a powerful tool and have been increasingly used in the recent years. Despite the very large number of reviews dealing with phytochemical analysis by chromatography-MS (Ignat, Volf, & Popa, 2011; Khoddami, Wilkes, & Roberts, 2013; Valls, Millán, Martí, Borràs, & Arola, 2009; Wang, Zhang, Yan, Han, & Sun, 2014; Wu et al., 2013), none of them was devoted to phytochemicals in food, neither were they focused on methodological aspects peculiar to HRMS, nor on issues related to the chemical classes. Considering all these aspects, the purpose of this review is to present and discuss the modern analytical approaches, based on HPLC and UHPLC coupled with HRMS and tandem mass spectrometry (MS/MS), employed to characterize and/or identify phytochemicals in food of plant origin. Decoctions, infusions and medicinal herbs in general will not be included in this review. The advantages, as well as the limitations of the various methods, are reported in order to provide an overview of the most suitable approaches for class-specific or multiclass analysis.

2. Sample preparation

2.1. General remarks

Sample preparation is a critical step, which determines the compounds isolated and the accuracy of both qualitative and quantitative results; therefore, all the aspects of the procedures should be considered from the beginning. In principle, sample preparation methods for HRMS do not differ significantly from traditional methods for low resolution MS analysis (Kim & Verpoorte, 2010). However, in HRMS experiments large sets of analytes are usually investigated, then the extraction should be a general one able to extract them all. Due to the presence of a wide variety of phytochemicals in plants, with very different polarities, no single solvent or solvent mixture is suitable for this purpose. Several extractions with solvents having different polarity (at least a polar one and a non-polar one) are needed to cover the entire range of