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Study of extraction procedures, and analytical methods using nanoLC-MS for the identification and monitoring of polyphenols and their metabolites in biological samples

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Abstract

With the growing interest in the use of metabolomic technologies for a widerange of biological targets, food applications related to nutrition and quality are rapidly emerging. Metabolomics offers us the opportunity to gain deeper insights into, and have better control of, the fundamental biochemical basis of the things we eat. So doing will help us to design modified breeding programmes aimed at better quality produce; optimised food processing strategies and ultimately, improved (micro)nutrient bioavailability and bioefficacy.

A better understanding of the pathways responsible for the biosynthesis of nutritionally relevant metabolites is key to gaining more effective control of the absence/level of presence of such components in our food. Applications of metabolomic technologies in both applied and fundamental science strategies are therefore growing rapidly in developing countries in popularity.

Keywords: Phenolic, metabolomics, pharmokokinetics, bioactive, esterification etc.

INTRODUCTION

Metabolomics is defined as the unbiased identification and quantification of all metabolites presents in a specific biological sample (from an organism) [1]. Since metabolomics cannot be carried out in its totally, the use of different analytical approaches or categories can help answer specific types of questions. Nowadays, one of the main strategies applied in this field is metabolomic profiling that consist in the elucidation of the function of a whole pathway or intersecting pathways and does not require the characterization of the entire metabolome. For this purpose, it is required a specific analytical methodology to identify and quantify a group of chosen class of metabolites, with known (target analysis) or unknown identity (non-target analysis). In this field, phenolic compounds and their metabolites have received considerable attention since can act as potentially protective factors, against

degenerative diseases (cataracts, macular degeneration, neurodegenerative diseases, and diabetes mellitus), cancer and cardiovascular diseases [2]. This is supported by *in vitro* and *in vivo* studies, but also by clinical and epidemiologic studies [3-5]. However, to understand the role of phenolic compounds in disease prevention, it is essential to have a thorough knowledge of the mechanisms underlying it, and provide information about the metabolomic pathways, and the pharmacokinetics of their absorption and bioavailability using *in vitro* and *in vivo* assays.

So far, numerous studies have demonstrated the absorption of phenolic compounds after consumption of foods rich in them, by indirect measure of the antioxidant activity in plasma [6,7], or by estimation of the recovery of phenolic compounds excreted in urine, with respect to the ingested dose [8-10]. Besides, some simple flavonoids and phenolic acids, such as quercetin, chlorogenic acid and caffeic acid, and their metabolites have been detected in plasma [11-14]. However, few data on more complex phenolic compounds are available. Another problem is that complex polyphenols are not commercially available and studies using individual compounds are not possible. In addition, the comprehensive study of those bioactive phenolic compounds is necessary, since structural parameters, such as molecular weight, glycosylation and esterification, largely influences their absorption and bioavailability [15]. Information on the effect of food matrix on the bioavailability of phenolic compounds is increasing in recent years. Thus, further studies are necessary in order to evaluate the influence of the food matrices on the metabolization of these compounds.

A great number of studies have evaluated the metabolization of phenolic compounds of olive (*Olea europea*) fruits and oil, being mainly focused on oleuropein, hydroxytyrosol and tyrosol. It has been described that hydroxytyrosol was absorbed through Caco-2 monolayer, a cell line derived from a human colorectal carcinoma, which has become established as a model of the small intestinal epithelium. Bidirectional passive diffusion is likely the main mechanism of transport of hyroxytyrosol, and it has been postulated 100% absorption in humans [16]. In addition, homovanilic acid was the main metabolite of hydroxytyrosol. Some studies have described the bioavailability of hydroxytyrosol and tyrosol in humans, being detected in urine [9,17,18]. It was also concluding that the absorption of these compounds depends on the initial dose, and they were mainly excreted in urine as glucuronide conjugates.

On the other hand, absorption of dietary flavonoid glycosides could be mediated by sugar transporter [19]. In addition, since hydroxytyrosol and tyrosol are metabolites of glycosides compounds in urine, a mechanism of hydrolysis could occur in gastrointestinal tract, before absorption or inside intestinal cells, in blood or, even, in liver after absorption [20].

There is relatively little information about the absorption and metabolization of phenolic compounds of *Hibiscus sabdariffa* (hibiscus) and *Lippia citriodora* (lemon verbena). Concerning to this point, a pharmacokinetics study of anthocyanidin glycosides from hibiscus leaves extract has shown a little oral bioavailability of several anthocyanins in humans [21]. However, it is necessary to perform further studies on both the intact glycosides and their *in vivo* metabolites or conjugates in human plasma and urine. Concerning lemon verbena, It has been shown that verbascoside, which represent one of the most abundant compounds of this plant, presented a low oral bioavailability [22-24].

Research methodology

This research focussed on the development of new analytical methodologies using nanoLC-MS (TOF/QTOF) in order to study the metabolomic of polyphenolic compounds derived from plant extracts that have proven bioactivity.

Objectives of the research paper

The main objective can be divided into the following partial objectives:

- Characterization of the phenolic compounds profile of crude extracts from *Olea europea*, *Hibiscus sabdariffa* and *Lippia citriodora*.
- Evaluation of the bioactive properties of the latter extracts in breast cancer cell lines (*Olea europea extracts)*, in mouse model of obesity (hibiscus extracts) and in humans (lemon verbena extracts).
- Development of extraction procedures, and analytical methods using nanoLC-MS (TOF/QTOF) for the identification and monitoring of polyphenols and their metabolites in biological samples.

Miniaturization has emerges as one of the critical trends in modern analytical chemistry, being of special attention for the development of miniaturized separation methods. Since its introduction by Karlsson y Novotny en 1988 [25], the nano-LC is challenging and complementary to conventional LC. It has been applied as powerful tool in proteomics [26,27], pharmaceutical industry [28], environmental analysis [29,30] and for enantiomer separations [31]. In food analysis, nanoLC has also been used [32,33], whereas its application in metabolomics is more limited. The nanoLC technique allows us to work with very low flows of mobile phases and also to inject small quantities of samples. Thus, by coupling the nano LC system with high-resolution mass spectrometry, we can produce an extremely powerful analytic tool that offers the high speed and efficiency of nano LC with the high resolving power, generation of mass accuracy, selectivity, sensitivity and structural information provided by high-resolution mass spectrometry, such as time-of-flight (TOF) and hybrids such as quadrupole-time-of-flight (Q-TOF) analyzers. This latter allows both MS and MS/MS analysis, obtaining more comprehensive structural information of the compounds.

The review article involves the study of analytical methods to study the metabolomics of polyphenolic compounds from plant extracts and functional foods that have proven bioactivity. Within this context, different crude extracts from plants such as Olea europea, Hibiscus sabdariffa and Lippia citriodora, which have demonstrated bioactivity in breast cancer, obesity and inflammatory processes respectively, will be used in cell models, animal models and human studies. The biological samples obtained, such as cytoplasm and rat and human plasma, will be deproteinized under controlled conditions (as many polyphenols may interact with albumin or other proteins) and preconcentrated using solid-phase-extraction cartridges and coupled columns. These treated samples will be analysed by nano LC coupled to high-resolution mass spectrometry using time-of-flight (TOF) and hybrid quadruple-time-of-flight (Q-TOF) analysers. Thus, the application of these analytical techniques in the identification and the quantification of metabolites of phenolic compounds, currently represents an important and growing field of research, due to the complex and diverse structures that may exists in biological samples and at very low levels. The methods developed for samples of the different models (cell, animal and human) will provide us with information about the metabolic pathways of these polyphenolic compounds from bioactive extracts and their active mechanisms as well as information about the pharmacokinetics of their absorption in the different models both “in vitro” and “in vivo”.

Timeless and relevance of review article

The new methodology will be expected to presents better sensitivity, with less consumption of mobile phases, especially inter-day repeatability and provides results with highly accurate quantification. The outcomes of this proposal will be expected to open up the application field of this technique to cover a large variety of compounds and its advantages will make it especially useful for the analysis of samples containing low concentration of phenolic compounds, as for instance, in biological samples. Further investigations should be targeted on its Industrial applications.

Both the European Union and India are countries with a wide plant biodiversity which has tremendous medicinal therapy application. In addition there are many food products supplied in India by incorporating plant product additives which has been reported to reduce various risks raised by the synthetic food preservatives/chemicals. Metabolomics are classified into primary and secondary, through the boundaries between these groups can be blurred. Primary metabolites, such as organic acids, fatty acids, nucleotides and amino acids play essential roles in growth and development, respiration and photosynthesis and hormone and photosynthesis.This project certainly would end up with fruitful natural plant extracts or constituents in particular from to identify and quantify all the metabolites in a biological

systems and so to contribute to researcher understanding of the complex molecular interactions with biological systems which will make a platform/relation to the host country and the country where the candidate came from. EU and India have already collaborated in a number of areas where both parties have mutual interests. If a efficient biodirectional passive diffusion which is the main mechanism of transport of polyphenols is obtained from the outcome, then it will connect both countries to have long term relation in developing still effective and efficient new analytical methodologies using nano LC-MS (TOF/QTOF) which could answer the future plant extracts and metabolomics related issues.

Still, relatively little is known about the nano applications of metabolomics in medical science and pharmaceutical industries development in most developing countries. The development and marketing of technologies related to metabolomics require significant research efforts because most markets require scientific evidence and proof of functionality. This involves identifying functional compounds and assessing their biological effect, taking into account bioavailability in humans and potential changes during processing and clinical trials on product efficacy in order to gain approval for health-enhancing marketing claims. This research requires time, financing, and skilled labour, especially for products destined for export markets. Lastly, innovation and research capacity is required to screen local biodiversity to uncover potential new sources. This is also a management culture challenge for researchers because the best results can be obtained through partnerships between formal science institutions and indigenous communities.

Conclusion

Concluding, developing countries such as India can enjoy the benefits of the applications of nano to metabolomics to expand options for food and pharmaceutical industries and to promote growth in the sector through partnerships between research centres, private entrepreneurs, and indigenous communities. However, the success requires sufficient proof to establish the health claim and capacity to accurately market the new technologies to consumers in high-end markets. Countries that are interested in this sector should also assess the opportunities at the national level because these technologies cover such a broad group of food and pharmaceutical industries that some can find demand in the domestic market, while others can be targeted for export. Identification of specific export markets, certification and other regulations, and consumer demand are product and/or ingredient specific, and largely dictate the possibilities for development. Further studies could establish the most critical bottlenecks in production systems and identify opportunities with the greatest potential for rural employment creation and competitive advantage for small-scale pharmaceutical industries.

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