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Development of personalised functional foods needs metabolic profiling

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Abstract

Purpose of review

There is growing interest in applying metabolic profiling technologies to food science as this approach is now embedded into the foodomics toolbox. This review aims at exploring how metabolic profiling can be applied to the development of functional foods.

Recent findings

One of the biggest challenges of modern nutrition is to propose a healthy diet to populations worldwide that must suit high inter-individual variability driven by complex gene-nutrient-environment interactions. Although a number of functional foods are now proposed in support of a healthy diet, a one-size-fits-all approach to nutrition is inappropriate and new personalised functional foods are necessary. Metabolic profiling technologies can assist at various levels of the development of functional foods, from screening for food composition to identification of new biomarkers of food intake to support diet intervention and epidemiological studies.

Summary

Modern 'omics' technologies, including metabolic profiling, will support the development of new personalised functional foods of high relevance to twenty-first-century medical challenges such as controlling the worldwide spread of metabolic disorders and ensuring healthy ageing.

Keywords: functional foods, nutrition, metabolic profiling, metabolomics

Introduction

The term “functional food” can be defined as a food product that confers some beneficial effects on health beyond the basic nutritional values of food [1]. As such, it is sometimes difficult to determine whether a food product should be classified or not as “functional”. Functional food can therefore be either naturally rich or artificially enriched in substances (living organisms or nutrients) that are known for their beneficial effects such as probiotics, prebiotics, essential fatty acids, flavonoids, vitamins etc. In this regard, many natural products can be considered “functional” such as milk, numerous fruits and vegetables and oily fishes. To determine whether a food product has any specific health beneficial effect, one must measure the health impact of a complex food matrix following digestion (Figure 1). This can be achieved by measuring a number of physiological and biochemical parameters following diet intervention studies or epidemiologic surveys in relation with health outcome (e.g. HDL-LDL ratio in relation with cardiovascular disease). However, testing individuals with various genetic backgrounds results in high inter-individual variability and makes it difficult to detect subtle metabolic variations induced by a specific food intake. In addition, the gut microbiota is a highly variable environmental factor that must be considered as it inevitably interacts with the digestive process and may influence the overall outcome of ingested food.

These complex gene-nutrient-environment interactions are the same challenges that Linus Pauling faced in 1971 when he developed a new technique to enable “the thorough quantitative analysis of body fluids” that “might permit differential diagnosis of many diseases in a more effective way than is possible at the present time” [2]. In this pioneering publication, Pauling laid the foundations of a new analytical field that would lead to the development of modern metabolic profiling technologies [3]. Indeed, using a hypothesis-generating approach based on the untargeted measurement of the ensemble of metabolites present in a biological sample, also called metabolomics or metabolic fingerprinting, has proved extremely

useful over the last decade in identifying potential disease markers [4] and is now embedded into the “foodomics” toolbox [5]. Hence, beyond its applications in the medical field, metabolic profiling can be applied at various stages of the development of new functional foods, as illustrated in Figure 1. This review will therefore explore how metabolic profiling has been recently applied to reveal the metabolic composition of potential functional food products or to support identification of new candidates. We will describe how it contributes to the assessment of the gut microbial metabolic activity and supports measurements of the metabolic outcomes of diet intervention studies and epidemiological surveys.

Understanding food composition for screening of new functional ingredients

Metabolomics is a powerful approach to metabolically characterise existing or potential functional food candidates. Not only can such approach contribute to improve our understanding of food product composition and bioaccessibility of their components, [6] but it may also reveal a number of unknown metabolites, particularly secondary metabolites in plants, that may be biologically active [7].

For instance, there is a growing interest in milk characterisation using modern metabolic profiling technologies (i.e. proton Nuclear Magnetic Resonance spectroscopy and Mass Spectrometry-based profiling) to better understand its formation and composition [8-11]. Milk is a complex food matrix that has been extensively studied for its major components (i.e. proteins, fat, lactose) and some minor components (i.e. minerals and vitamins). Only recently has the milk water soluble fraction drawn interest as it contains small molecular weight metabolites that are of nutritional importance, including numerous oligosaccharides [12,13]. Milk oligosaccharides are of particular importance since they are biologically active and can favour the growth of beneficial lactic acid bacteria in the gut. [reviewed here: [14]] Because oligosaccharides are found in relatively low concentration in non-human milk, most of the research effort over the last decade has been focused on the role of non-milk oligosaccharides such as plant-derived fructooligosaccharides

(FOS) and enzymatically synthesized galactooligosaccharides (GOS). However, recent technological improvements using tandem mass spectrometry have facilitated characterisation of non-human milk oligosaccharides and promising results have been obtained using bovine milk oligosaccharides as new functional ingredients [15-17].

There is also an emerging interest in assessing the fermentation products of fermented milk and dairy derivatives, as it has been recently demonstrated that these products stimulate the infant's immune system, even in absence of live bacteria [18,19]. Milk fermentation results in increased concentration of organic acids such as lactic acid, which are known to be beneficial as they lower the intestinal pH and protect against potential infections when fermentation occurs within the gastrointestinal tract. However, the active component of fermented milk derivatives is currently poorly understood. It is proposed that residual oligosaccharides produced from incomplete carbohydrate fermentation (i.e. sugars that are released by living organisms during the fermentation process but do not directly impart to the production of organic acids and other fermentation products) may have a prebiotic activity and contribute to the health promoting effect of these products. Another hypothesis is that bacteria themselves produce active compounds that would stimulate the immune system. These molecules are therefore of high importance as they may convey some health promoting activity and need to be further investigated. These are not specific to milk fermented derivatives and may be true for many other fermented food products, such as fermented soy derivatives. Metabolic profiling of fermented foods has been recognised as an appropriate strategy to identify potentially bioactive molecules [20]. Similarly, it has been recently attempted to use the same approach to rapidly screen for new functional food based on their prebiotic potential [21].

Finally, there has been a huge amount of research effort focused over the last decades on the identification of new probiotic species based on their effect on the

immune system and their metabolism (e.g. ability to produce high amounts of butyric acid). However, this is a long and tedious work that can be expensive. An alternative approach is to use metabolomics to screen large numbers of potential probiotic species to classify them based on their metabolic activity [22]. In the future, such approach will certainly support the discovery of new bacteria and nutrients that promote human health.

Assisting diet-intervention studies for better control of compliance

The most challenging aspect in the development of functional food is to identify the bioactivity of a specific foodstuff. This is classically achieved using diet intervention studies where a number of volunteers are given a proposed functional food to consume for various amounts of time. In such experiments, it is of utmost importance to accurately record participants' diet over the course of the trial, which is classically achieved using a combination of three techniques: diet records, 24h dietary recalls and food frequency questionnaires [23]. The main issue associated with these methods is that they are all based on self-reported information, which is subject to bias and misreporting [24]. There is therefore a need for a more accurate estimation of dietary intake, which can be achieved by identifying specific biomarkers of food intake that can be employed to correct self-reported dietary information and control for compliance in diet restricted studies.

In this regard, metabolic profiling has been widely applied in recent years to identify new biomarkers of food intake. Table 1 summarises the most recently published food biomarkers detected using a metabolomics approach. Some of these metabolites are now recognised markers, such as proline-betaine for citrus fruit consumption [25,26], and these studies either confirm previously identified food consumption biomarkers or suggest new ones that will need further validation. The main weakness associated with the food biomarker approach is that many of these are not food specific since they can be detected in a large number of related food

products. For example, hippuric acid and its derivatives are usually associated with vegetable intake as they derive from the degradation of plant polyphenols by the gut microbiota [27]. As a consequence, it is more accurate to refer to these substances as biomarkers of a food group, rather than a specific food product. Another approach is to use a combination (i.e. a profile) of markers that is more specific of a foodstuff or a diet. The research community is now increasingly aware of such limitations as illustrated by the recent publication of the Atherosclerosis Risk in Communities (ARIC) population study outcome [28]. Although researchers involved in the ARIC study reported some new food intake biomarkers, they clearly identified a set of markers associated with intake of “sugar-rich foods and beverages” (SRFB), which is also more meaningful in terms of biological interpretation. Similarly, a recent study used a metabolic profiling approach to identify various dietary traits in free-living subjects [29]. Although this is the first of its kind and further similar studies will be necessary to validate the profile of markers associated with each trait, this is a very encouraging work to better control for compliance in future diet intervention studies.

Understanding food-mammalian metabolic interactions

Some of the food intake biomarkers displayed in Table 1 are also endogenous compounds, such as *scyllo*-inositol, an ubiquitous component of cell membranes, or 5 α -androstane-3 β ,17 β -diol disulfate and 4-androstene-3 β ,17 β -diol disulfate, which are sex hormone derivatives. The association of these chemicals with food intake may be due to confounding factors, but the ARIC study validated all potential biomarkers using an independent validation data set. Therefore, we may conclude that these chemicals are associated with the endogenous metabolic modulations triggered by the regular consumption of the food to which they were correlated. Indeed, *nutrimetabonomics*, defined as the quantitative measurement of the metabolic response of a living organism to a nutritional variation, is a powerful approach to generate new hypotheses of biological mechanisms underpinning food -

health interactions. This metabolic profiling approach, which focuses on the modulations of endogenous metabolic pathways associated with diet interventions, has been successfully applied to improve our understanding of a number of natural products, nutrients and food groups [30-34]. For example, of the 23 metabolites associated with the SRFB food group in the ARIC study, five were found to be γ -glutamyl dipeptides and five were related to the 2-hydroxybutyrate pathway [28]. The authors therefore concluded that this was indicative of oxidative stress associated with the consumption of SRFB foods.

Hence, the nutrimetabonomics approach is extremely useful to screen for markers of modulations of endogenous metabolic pathways in response to food intake. This is a powerful way to explore the impact of specific foods or nutrients on the systemic metabolism and has been widely applied to the investigation of the relationship between food/diet, gut microbiota and health. Indeed, the gut microbiota is an emerging environmental component at the crossroads of all environmental factors (i.e. under the influence of diet, lifestyle and exposome), which provides a reservoir of hundreds of thousands of unique extra genes [35-37]. It is therefore believed that manipulating the gut microbial ecosystem may result in health improvement of its host. A number of studies have used metabolic profiling technologies to assess the metabolic impact of the gut microbiota on its host, which have been reviewed here: [38,39].

The gut microbiota is an extremely complex ecosystem and we are currently limited in our ability to measure the metabolic outcome of variations of specific groups or species of bacteria. To overcome this issue, a nutrimetabonomics approach has been recently applied to a prebiotic intervention study in obese women to link the bacterial content of human volunteers to metabolic markers of energy metabolism in plasma [40]. This illustrates that it is possible to decipher the impact of selected bacterial groups or species and their metabolism on the human host using such metabolic profiling approach [41]. Similarly, this can be applied to animal

models where the metabolism of particular organs is of easier access. For example, a recent work profiled the hepatic metabolism and gut bacterial community in response to resistant starch intake in mice fed a high fat diet [42]. This revealed strong associations between hepatic amino acid metabolism and gut microbial modulation of high relevance for human nutrition.

Epidemiological surveys are also extremely useful to identify meaningful associations between diet and health. However, they often fail to detect subtle associations between nutrients and health due to the inevitable high degree of inter-individual variation and poor diet records. Nonetheless, nutrimentabonomics has proved very useful in identifying metabolic markers of a healthy lifestyle. The International Collaborative Study on Macro-/Micronutrients and Blood Pressure (INTERMAP) study recently reported 12 urinary metabolites differentially observed in afro-americans *versus* non-hispanic white americans [43]. Of those, urinary hippuric acid was associated with lower blood pressure and higher polyphenol intake from fruits and vegetables. Hence, this NMR-based nutrimentabonomic study generated new hypotheses about possible links between urinary metabolites, food intake and cardiovascular health.

With the recent development of Genome Wide Association Studies with Metabolic traits (mGWAS), there is now the potential for identifying gene variants associated with specific metabolic functions. Although a few studies have attempted such approach to link the genome with the metabolome in a nutritional context, it is realistic to envisage that this strategy will lead to the identification of new genetic variants that predispose some individuals to nutrient deficiencies or to particular metabolic needs. Nevertheless, beyond mGWAS, epigenetic and gut microbial variations must also be taken into account to develop a genuine personalised nutrition, which should be accompanied by personalised functional foods. Indeed, functional foods cannot be seen as a one-size-fits-all. This is already recognised by the research community working on modulation of the gut microbiota and huge

efforts are currently pursued to stratify individuals according to their “enterotype” [44] to ultimately propose personalised probiotics. The same might be applied to other functional foods where inter-individual variability in metabolic capacities may affect the targeted benefit. Similarly, mGWAS have also been recently applied to plant science to unravel candidate genes associated with metabolic traits [45,46]. Such approach should be encouraged in the future in order to facilitate the production of functional crops carrying genetic variants associated with the production of beneficial compounds and/or nutrients. Assessing the metabolism is therefore essential to the success of such enterprise and metabolic profiling technologies will be indispensable to achieve this goal.

Conclusion

The twenty-first century is facing immense clinical challenges that include an overall ageing population along with a worldwide spread of metabolic disorders such as obesity and type 2 diabetes. The promotion of a healthy diet is an appropriate response to prevent such threats although high inter-individual variability driven by complex gene-nutrient-environment interactions calls for a more personalised approach that must be accompanied by the development of personalised functional foods. This can be supported by metabolic profiling as it can assist in developing new functional foods and nutrients at various stages of their elaboration. In the future, these technologies applied in conjunction with other ‘omics’ approaches to large-scale mGWAS will play a major role to unravel the biological mechanisms underpinning inter-individual variability in response to food intake and identify new targets for functional ingredients.

Key points:

- Metabolic profiling is becoming increasingly popular to assess food composition, which facilitates the identification of new functional ingredients.

- Metabolic profiling is a powerful approach to identify new biomarkers of food intake and assist diet intervention and epidemiological studies by offering a new way to control diet records and compliance.
- Genome Wide Association Studies with Metabolic traits are opening a new era in the unraveling of the complex gene-nutrient-environment interactions that will provide new targets for the development of functional foods.

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Figure legend

Figure 1: Metabolic profiling can be applied at various stages of the development of new functional foods. The metabolic composition of complex food matrices can be characterised using metabolic profiling technologies. Untargeted approaches can facilitate identification of new metabolites of particular interest and contribute to the development of new functional ingredients. Gut microbiota is an inevitable partner with a degree of inter-individual variability that must be considered in the elaboration of functional foods, as well as being a potential target. Gut microbial metabolic activity is rapidly screened using metabolic profiling technologies. Diet intervention studies and epidemiological surveys require accurate records of food intake and control for diet compliance, which can be achieved by relying on the measurement of metabolic biomarkers of food intake. The metabolic outcome of diet intervention studies and Genome-Wide Association Studies with Metabolic traits (mGWAS) are assisted by metabolic profiling.

Table 1: Potential biomarkers of food intake.

Food Product	Biomarker	Analytical Method	Biological Matrix	Ref
Aronia-Citrus juice	Proline-betaine; ferulic acid; mercapturate derivatives	HPLC-qTOF-MS	Urine	[48]
<i>Brassica oleraceae</i>	<i>N</i> -acetyl isothiocyanates; cysteine isothiocyanates	HPLC-qTOF-MS	Urine	[31]
Caseinoglycomacropeptide (from milk)	α -ketoisovalerate, α -hydroxybutyrate and isomers; α -hydroxyisovalerate; α -keto-3-methylvalerate	LC-qTOF-MS	Plasma	[49]
Citrus fruits	Proline-betaine; hesperetin-glucuronide	UPLC-qTOF-MS	Urine	[29]
Cocoa	Theobromine, 6-amino-5-[<i>N</i> -methylformylamino]-1-methyluracil; 7-methyluric acid	UPLC-qTOF-MS	Urine	[29]
Coffee	Quinate; paraxanthine; 5-acetylamino-6-amino-3-methyluracil; 1,7-dimethylurate; α -methylurate; 1-methylxanthine; caffeine; 1,3,7-trimethylurate; 7-methylxanthine	GC-MS and LC-MS	Serum	[28]
Egg	Docosapentaenoate	GC-MS and LC-MS	Serum	[28]
Fiber intake	2,6-dihydroxybenzoic acid; 2-aminophenol sulfate	LC-qTOF-MS	Plasma	[50]
Fish & Seafood	Eicosapentaenoate, docosahexaenoate	GC-MS and LC-MS	Serum	[28]
	TMAO	UPLC-qTOF-MS	Urine	[31]
Fruit juice	Glycerate; stachydrine; <i>N</i> -methylproline; threonate; <i>scyllo</i> -inositol; homostachydrine	GC-MS and LC-MS	Serum	[28]
Nuts & Peanut butter	Tryptophan betaine; 2-methylbutyroylcarnitine; 4-vinylphenol sulfate; 5 α -androstane-3 β ,17 β -diol disulfate; 4-androstene-3 β ,17 β -diol disulfate	GC-MS and LC-MS	Serum	[28]
Red Cabbage	Hippuric acid derivatives; acetyl-cysteine conjugates of isothiocyanates;	UPLC-qTOF-MS	Urine	[29]
Rye bread	2-aminophenol sulfate; nonanedioic acid; indolylacryloylglycine; enterolactone glucuronide; ferulic acid-4- <i>O</i> -sulfate	UPLC-qTOF-MS	Urine	[51]
Walnut	Indolic compounds; conjugated urolithin	HPLC-qTOF-MS	Urine	[52]
	5-hydroxyindole-3-acetic acid	UPLC-qTOF-MS	Urine	[29]
Refined Wheat bread	Ascorbic acid (industrial additive); Quinic acid	UPLC-qTOF-MS	Urine	[51]
Whey concentrate hydrolysate	pGlu-Leu; Methionine sulfoxide; <i>N</i> -phenylacetyl-methionine; <i>N</i> -phenylacetyl-methionine sulfoxide; pGlu-Val	LC-qTOF-MS	Plasma	[49]

Modified from [47].