REVIEW

Plant metabolomics and its potential application for human nutrition

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With the growing interest in the use of metabolomic technologies for a wide range 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 popularity.

Currently, the world has two highly contrasting nutrition-related problems – over-consumption and under-nourishment. Dramatic increases in the occurrence of overweight individuals and obesity in developed countries are in staggering contrast to the still-familiar images of extreme malnutrition in many parts of the developing world. Both problems require a modified food supply, achieved through highly contrasting routes. For each, metabolomics has a future role to play and this review shall deal with this key dichotomy and illustrate where metabolomics may have a future part to play. In this short overview, attention is given to how the various technologies have already been exploited in a plant-based food context related to key issues such as biofortification, bioprotectants and the general link between food composition and human health. Research on key crops such as rice and tomato are used as illustration of potentially broader application across crop species. Although the focus is clearly on food supply, some attention is given to the complementary field of research, nutrigenomics, where similar technologies are being applied to understand nutrition better from the human side.

Introduction

The continually growing interest in plant metabolomics primarily stems from the potential of the technology to broaden our knowledge of plant metabolism and biochemical composition and apply this in a wide range of the fields. Several recent reviews on the topic demonstrate

Abbreviations – BMI, body mass index; LC-MS, liquid chromatography mass spectrometry; PDA, photo-diode array detection.
how the technology has quickly become established and how technological developments continue to create new opportunities (Hall 2006, Saito et al. 2006). Although relatively young, and still very much in development, plant metabolomics is now being widely applied and is already considered as a technology, which is a ‘mature science’ and is ‘established and robust’ (Dixon et al. 2006, Schauer and Fernie 2006). A key area of application, and one that crosses many boundaries, is plant-based nutrition. This is the topic of this short review.

‘Food is our most primitive form of comfort’ (Shiela Graham, popular American author), although it is clearly evident that methods of modern food production are far from primitive and make use of the very latest molecular breeding, production and processing technologies. However, reviewing the literature quickly reveals that current trends in global food consumption are, remarkably, typified by a significant dichotomy. In developing countries there is still a strong demand for improved crop varieties. These should have enhanced nutritional value and a better balance of key nutritional components such as essential amino acids, vitamins and other micronutrients to help alleviate hunger and prevent malnutrition-associated diseases. In stark contrast, in the developed world, so-called ‘overnutrition’ is increasingly becoming the norm (Rist et al. 2006) and there is ever-growing demand for foods more in keeping with desires for a healthier diet appropriate to our modern (western) lifestyle (Morris and Sands 2006). These targets are not necessarily contradictory, but they do demand contrasting approaches. In both cases, metabolomics has the potential to assist in speeding up the process leading to improved plant-based foods and, indeed, the technology is already being used to such ends. Therefore, the content of this review must and shall reflect the dichotomy in application strategies related to nutrition issues and the consequences of the geographic discontinuity in global food availability.

Almost throughout the world, plant-based products comprise the vast majority of human food intake, irrespective of location or financial status (Mathers 2006). In some cultures plant materials actually comprise 100% of the diet. Nutritional improvement is therefore most likely to be effectively achievable via alterations to staple crops. Metabolomics is helping us to assess better, the components that are present in our food, how they are synthesised and the genetic and environmental factors that are influential in determining food composition and stability (Fig. 1). However, metabolomics is much more than just a diagnostics tool (Schauer et al. 2006) and can assist us greatly to improve our fundamental comprehension of the complexity of metabolic regulation and explain how minor perturbations can have a multitude of biochemical end-points. This then creates the potential to provide us with the knowledge necessary to facilitate a more targeted approach to crop improvement, specifically in terms of biochemical composition and nutritional value.

In this paper, we shall concentrate on reviewing the literature on how plant metabolomics has been/could be applied to advance our knowledge specifically related to macro- and micronutrition and other aspects of food quality and speculate on its future potential impact on botanical and food science. Two specific case studies shall also be used to illustrate the (potential) application of metabolomics for a particular crop, rice, and a specific target group, as relevant to developing countries. We cannot do proper justice to the parallel activity from the human side, where metabolomics technology is also being used in a so-called ‘nutrigenomics’ context. Nutrigenomics is also a rapidly developing field that has been designed to offer the prospect of a greater understanding of the complex relationship between the human genome and diet (Davies 2007). However, it is not possible to review the topic of plant metabolomics and nutrition without relating these to parallel activities linked to human health. Consequently, limited attention will be given to how these two topics are complementary and how they can be exploited to provide us with a more complete understanding of the relationship between food, nutrition and human health in the context of both Western and developing countries. For a complete overview, the reader is referred to recent papers by, e.g. German et al. (2005), Gibney et al. (2005) and Rezzi et al. (2007).

**Metabolomics and nutrition: recent technology developments and applications**

**Metabolomics and nutritionally improved foods: the untold richness of plants**

Plants are arguably nature’s finest chemists, having evolved a huge range of chemical repertoires fitting the needs of a highly variable and generally hostile global environment (Baxter and Borevitz 2006, Hall 2006). This metabolite richness, reputed to extend to approximately 200 000 compounds in the plant kingdom (Oksman-Caldentey and Inzé 2004), is also reflected in our plant-based foods that have been reported to contain approximately 25 000 different metabolites of which approximately 7000 are volatile components (Go et al. 2005, Goff and Klee 2006). It is this richness and our desire for the most holistic understanding of plant metabolism that are also the driving forces behind food-based metabolomics. However, natural metabolic diversity and a lack of unifying principles to help us detect and identify compounds...
are major analytical challenges for many years to come (Breitling et al. 2006).

Our food contains not just a mixture of staple ingredients, but also a multitude of less prevalent components, which, nevertheless, represent potentially physiologically relevant bioactives (Long et al. 2006). Knowledge of both groups is essential. Metabolomic applications in crop/food analysis are continually growing and the use of liquid chromatography mass spectrometry (LC-MS), GC-MS and NMR are being exploited to get more detailed insight into the variation in food composition in both quality and nutritional contexts. In potato, e.g. flow injection mass spectrometry analysis of a range of genotypes revealed genotypic correlations with quality traits such as free amino acid content (Beckman et al. 2007). Matrix-assisted laser desorption/ionization chemotyping and GC-MS profiling of tomato cultivars have revealed extensive differences in metabolic composition (sugars, amino acids, organic acids) despite close specific/genotypic similarities (Carrari et al. 2006, Fraser et al. 2007). Even in Arabidopsis, results from high-speed LC-MS analyses of the free amino acid content of seeds have been reported to be ‘nutritionally relevant for other seed crop species’ (Jander et al. 2004).

The quality of crop plants, nutritionally or otherwise, is a direct function of metabolite content (Memelink 2004). There is therefore great interest in using a metabolomic approach to understand better what especially has occurred during crop domestication in order to design new concepts for more targeted crop improvement in ways more tailor-made to current needs. It is essential that we can identify molecular markers that can be used to guide future development of food products (Rist et al. 2006). Schauer et al. (2005), used GC-MS based profiling of wild, and a number of cultivated, tomato species to gain a better insight into what has happened and what has been lost during domestication. The potential for crop improvement is still enormous as has recently been demonstrated in studies on genetical metabolomics (linking metabolic profiles with genomic information) using Arabidopsis and tomato (Keurentjes et al. 2006, Schauer et al. 2006). In Arabidopsis, F1 progeny derived from a same-species ecotype cross, were surprisingly shown, when analysed using LC-MS, to contain approximately 30% new mass peaks that were not present in either parent. These extensively new chemical signatures, simply resulting from different intra-specific allelic combinations (transgressive segregation), reveal how variable genotypes are and the enormous potential of metabolomics to detect transgressive segregants before a trait is expressed. As pointed out by Baxter and Borevitz (2006), this ‘hidden biochemical variation, now revealed to plant breeders through metabolomics’, opens the door to ‘general (biochemical) crop improvement and the fine tuning of breeding specifically for nutritional traits in crop species’. Similar extensive hidden variation in tomato genotypes has also been demonstrated where unexploited variability has been considered of potential value for the improvement of nutritional value (Lenucci et al. 2006). Using a metabolomics approach to identify key loci will play a significant role in helping to deconstruct complex metabolic interactions and provide the
nutrition/health-related issues in order to get a more reliable insight into potential links between specific (groups of) potentially bioactive metabolites and long-term human health. Metabolomics is predicted to play an important future role in this regard from both the plant and human sides (Gibney et al. 2005, Mathers 2006).

Many metabolomic studies have been reported where the technology has been used to follow large complex groups of bioactive compounds such as the isoprenoids (carotenoid pigments, monoterpenes; Aharoni et al. 2005a, Fraser et al. 2007) and polyphenolics (flavonoids, anthocyanins; Bovy et al. 2007) with a view to eventual application in functional foods. Frequently, this work has been performed using tomato as this is a favoured model for some of the key metabolomics research groups (Bovy et al. 2007, Schauer et al. 2005). Minoggi et al. (2003) used HPLC photo-diode array detection (PDA) to analyse the variation in polyphenol and carotenoid content in 30 tomato genotypes and Bino et al. (2005) used both LC-MS and GC-MS to follow the isoprenoid and polyphenol composition of a high-pigment mutant \( (hp-2^{4b}) \) of tomato. Giliberto et al. (2005) used a genetic modification approach to study the mechanism of light influence on antioxidant content (anthocyanin, lycopene) in the tomato cultivar Moneymaker. The complexity and highly interactive nature of plant metabolism is regularly demonstrated in such research. Relatively minor genomic changes (point mutations, single-gene insertions) are regularly observed following metabolomic analysis, to lead to significant changes in biochemical composition (Bino et al. 2005, Davidovich-Rikanati et al. 2007, Long et al. 2006). However, other perhaps more significant genetic changes unexpectedly appear to have no phenotypical effect (Schauer and Fernie 2006). While also offering great opportunities, this plasticity in metabolism complicates potential routes to the design of new, improved crop varieties.

The isoprenoids, a diverse group of approximately 40,000 compounds, is a regular target for metabolomic analyses. This is due, not only to the extensive structural variation present, but also to the major biological and food relevance of the group. Isoprenoids provide key food quality components, such as many volatile flavour compounds as well as the pigmented carotenoids, which are food attractants and major food anti-oxidants (e.g. lycopene). Developing nutritionally improved foods through selection for modified biochemical composition is also the topic covered by Botella-Pavía and Rodriguez-Conception (2006). Carotenoids are particularly important being, e.g. precursors of essential nutrients such as \( \beta \)-carotene, which is, of course, the basis of the ‘Golden Rice’ story (see below). Another intriguing nutritional link has, been postulated by Goff and Klee (2006). They

Breeders are faced with a significant dilemma, as has been already indicated by Morris and Sands (2006). Crop breeding in recent decades has predominantly been focused on yield. In many cases, farmers’ incomes are also usually based upon production volume rather than quality. For example, in the past, tomato breeding has resulted in enhanced yield but also, with the exception of lycopene and its volatile flavour by-products, in a concomitant significant loss of other quality components (Goff and Klee 2006). A similar situation is also true for the cultivated strawberry (Aharoni et al. 2005b) and potato (Diretto et al. 2007). In many cases, crop yield is even subsidised while quality is generally ignored. Perhaps this should change to meet modern demands better. Food with a nutritional balance more suited to the modern lifestyle would concomitantly lead to reduced healthcare costs. In many cases, making selections on the basis of quality leads to a penalty in yield. Metabolomics is a science uniquely placed to detect the pathway driving expression of a trait, potentially enabling breeders, by selecting on the basis of biochemical markers, to combine pathways to traits of quality in high-yielding backgrounds with good tolerance to biotic and abiotic stress. Thus metabolomics can potentially enable breeders to achieve high quality along with high yield.

### Bioactives and bioprotectants

Food nutrition is, by definition, aimed at maintaining human cell and organ homeostasis (Goodacre 2007). For this reason, a balanced diet should be considered not just to concern the staple food ingredients centred on carbohydrates, proteins and lipids, but also other physiologically active components. This whole area has grown into one of uncertainty and even controversy, but the concept of nutraceuticals and ‘bioprotective’ or ‘functional’ foods continues to attract much attention (Davies 2007, Mathers 2006, Rist et al. 2006). More and more food products are reaching the market bearing health-related claims often centred, for example, on anti-oxidant activity. Such claims are, however, still contentious and the key question is how to reduce the complexity of food/nutrition/health-related issues in order to get a more
remark that in tomato, almost all important flavour-related volatiles are not only derived from essential nutrients but also, their occurrence is linked to, e.g. free amino acid content, antimicrobial activity and to other health-promoting compounds. Furthermore, fatty acids in foods that stimulate taste responses are specifically the essential long-chain, poly-unsaturated types and not the undesirable, less-healthy saturated forms. These authors propose that this correlation has resulted from evolution having given rise to such volatiles providing us with key information about the nutritional make-up of the food we eat. Modern breeding and food-processing practices (flavour modification/supplementation etc.) have, however, resulted in a major dissociation of flavours/fragrances from their natural nutritional context. This may, therefore, be a key contributory factor to current undesirable dietary developments such as over-consumption. The link between biochemical profile and nutrition may therefore be more direct than previously thought and deserves further and broader (metabolomics-based) investigation.

**Biofortification and genetic modification issues**

There is considerable interest in the so-called biofortification of foodstuffs where one or a small number of nutritional components has been particularly enhanced to, e.g. supplement/compensate for shortages elsewhere in the diet or where a component is considered potentially health-beneficial. Well-known examples are, Golden Rice with enhanced Provitamin A levels (Yonekura-Sakakibara and Saito 2006) and lycopene-rich tomatoes (Botella-Pavía and Rodríguez-Conception 2006). Other new examples are arising such as lysine-rich corn, folate-enriched tomatoes and ferritin-rich lettuce (Yonekura-Sakakibara and Saito 2006). For extensive overviews the reader is referred to the recent review on nutrients by Davies (2007) and micronutrient minerals by White and Broadley (2005). While some examples are the result of targeted selection or mutation breeding, many are the direct product of genetic modification. Application of the latter is still highly controversial, as many consumers remain sceptical because of socio-psychological factors associated with potential food risks. Metabolomic approaches are not only being applied to help generate the knowledge needed to attempt such targeted approaches, but are also being used to determine the extent and effectiveness of the modification introduced (Davidovich-Rikanati et al. 2007, Diretto et al. 2007). Metabolomics is also finding application in the area of the biosafety of GM products through the assessment of the potential so-called ‘unintended effects’ (Catchpole et al. 2005, Dixon et al. 2006, Kutchan 2005). However, while metabolomics shall prove a very useful tool to identify biochemical differences between GM genotypes it remains essential that the biological/safety relevance, if at all, of these differences is always subsequently assessed. Relevant here is also the concept of ‘substantial equivalence’, a topic that has been covered elsewhere (Dixon et al. 2006).

**Genotype × environment interaction and nutritional composition**

The basic nutritional value of our foodstuffs is, of course, not fixed and is, to a considerable extent, influenced by external factors such as the environmental conditions prevalent during crop production and also the conditions of post-harvest processing and storage (Reynolds et al. 2005, Semel et al. 2007). With a highly interactive network of metabolic pathways being present in all plant tissues, even minor changes in environment have the potential to lead to significant perturbations in nutritionally relevant metabolic content (Carrari et al. 2006). Metabolomics is being used both as a means to investigate more broadly the importance of environmental conditions (spatial/seasonal etc.) on food quality and also as a starting point in the design of tools and markers for quality monitoring.

Pereira et al. 2006a, b have carried out NMR-based metabolomic analyses on the biochemical content of grapes. Grapes of three contrasting varieties from five different locations in the Bordeaux region were primarily found to have compositional differences, which were more associated with location (vintage) rather than, e.g. soil type (Pereira et al. 2006a). Different chemometric analysis approaches were used on the spectral data primarily based on sugars, amino acids and other organic acids. In a second study, on the influence of microclimate of berry clusters of the Merlot variety, it was observed that, while modifications to sugar/Brix and amino acids levels were relatively disassociated from microclimatic (light) differences, anti-oxidant flavonoid levels varied in association with differences in light intensity. Surprisingly, anthocyanin levels were less reliable indicators, as they appeared to be more sensitive to secondary alterations in temperature (Pereira et al. 2006b).

Organic cultivation practices are often considered to yield ‘healthier’ and more nutritious products than conventional methods. Results of a 10-year study on tomato (Mitchell et al. 2007) have indicated that while organic-based soil fertility management had little effect on yield, HPLC-PDA analysis revealed that there was a consistent increase in flavonoid content in the tomato fruit obtained from the organic cropping system. In a more global assessment, Williamson (2007) has reported that while organic agriculture actually appears to
result in few clear metabolic differences, vitamin C content is consistently enhanced and there is a possible ‘general trend’ towards higher nutrient levels in organic produce. Such effects are, however, difficult to confirm as a result of confounding (often unknown) factors related to production pre-history. Therefore more detailed and precise research is needed. The underlying physiological mechanism has been proposed to have a potential link to a stress response. Salt stress has also been shown in other studies to influence not only cell growth/crop yield, but to also be directly associated with metabolic perturbations leading to significantly modified biochemical composition of plant materials as observed using Fourier-transformed infrared spectroscopy (Johnson et al. 2003) and GC-MS and LC-MS (Kim et al. 2007). More dramatic effects on nutritionally relevant components such as free amino acids and organic acids were observed when tomato plants were grown on different suboptimal N/light regimes (Urbanszczak-Wochniak and Fernie 2005). GC-MS analysis revealed that various components were affected differently and that dramatic reductions were observed with, for example, several amino acids falling to undetectable levels under suboptimal growth conditions. While this work was done on leaf material it is expected that fruit would be affected similarly.

The human side: adding more life to the years than years to the life

In the Western world, there is a huge increase in the incidence of lifestyle-related disease resulting from dietary imbalance and reduced physical activity (Goossens 2006). This genetic/nutrient imbalance can result in an increased body mass index (BMI), the occurrence of obesity, Type 2 diabetes etc. (Trujillo et al. 2006). The problem is further compounded by current global mobility where the easier movement of ethnic individuals between different cultures also leads to major changes in eating pattern and exposure to foods to which individuals were previously unaccustomed (Mazzatti et al. 2007). While the link between food and health is still a major topic of study, evidence is rapidly growing of how food components can influence physiological processes at all stages of life (Fig. 2). Inverse relationships have been observed between, e.g. carotenoid-rich foods and certain cancers (Botella-Pavía and Rodriguez-Conception 2006). A fruit/vegetable (fibre-rich) diet is associated with greater longevity, reduced risk of cardiovascular disease and, e.g. prostate cancer (Mathers 2006, WHO report 2003). Many food components are known to influence the expression of both structural genes and transcription factors in humans (Go et al. 2005, Mazzatti et al. 2007), but studying the genome alone is insufficient to understand fully the complexity of the processes and define causal relationships. Most chronic diseases are polygenic and the causal mechanisms are generally unknown. The situation is highly complicated as a result of the complexity of the interaction between dietary intervention, the individual genome and environment. Nevertheless, goals such as personalised diets are increasingly targeted, as dietary modifications have been shown to help especially, high-risk individuals (Clayton et al. 2006, Mazzatti et al. 2007).

Nutrigenomics as a science is growing rapidly and tries to link nutrition and genome. Metabolomics, as an integral component, is predicted to play an increasingly important role in correlating bioactive food components and disease prevention (Davis and Hord 2005). This should speed up biomarker discovery and help modern health-care switch from single biomarkers to increasingly comprehensive (metabolomic) profiles. Fundamental to this is the desire to switch from linking diet to disease, to linking diet and health (German et al. 2005).

Although the food/health link is still ambiguous, human health is actually becoming one of the major drivers to innovation in the food industry (Goossens 2006). With the current trends in global populations, and the so-called greying of society, it has been predicted that social security systems will not be able to survive in the future without having healthier elderly people. There is, however, a clear dilemma here, as potential major investments made by the food industry will primarily reap benefits for the health sector. Metabolomics is, however, uniquely suited to serve both sectors in exploring the complex interaction between food production, processing, consumption and long-term health status (Rezzi et al. 2007) and provide us with both plant and human biomarkers. Nutrigenomics and concomitant tandem metabolomics/transcriptomics profiling, will help break down this complexity and will enable ‘responders’ to be distinguished from ‘non-responders’. This in turn, will assist in the design of more personalised diets better tailored to our modern lifestyle, help pre-empt disease development and avoid other food-related health issues and, where needed, provide effective dietary intervention.

In developing countries, the focus of nutritional issues takes a wholly different direction. With recent Food and Agricultural Organization (FAO) figures indicating that approximately 800 000 000 people in these countries suffer malnutrition or starvation, here key targets are firstly enhancing the nutritional quality of the staple food which in most cases is rice and secondly expanding the area of cultivation to regions currently considered suboptimal for the genotypes/varieties available. For both, improved knowledge of the genetic basis of quality differences and exploiting this in targeted breeding
strategies is essential. Considering the importance of rice we shall briefly use this crop as an example of how current research is progressing towards the exploitation of modern technologies for crop advancement.

A focus on rice: key issues of nutrition and food quality and how metabolomics will help

Rice is one of the most important food crops in the world; it is the staple of almost half of the world’s population and it provides around three-quarters of the calorific intake of people in Asia (http://www.irri.org). To date, few applications for metabolomics on rice have been reported (Tarpley and Roessner 2007), most of which are not actually directed to grain issues. Metabolomics has, however, great potential to help advance our knowledge of some of the key nutrition-related targets and processes currently driving rice breeding and rice research in general.

Rice is grown on almost every continent of the world (Fig. 3), and in order to meet the world’s demand for rice, a staggering 11% of the world’s cultivated land is used for this crop alone; this was 127 million hectares in 2005. Rice is grown between the latitudes of 53°N and 35°S, and from elevations ranging from below sea level to above 2000 m, in both irrigated and rain-fed environments. Moving outwards in each direction from the Equator, rainfall, solar radiation, air and soil temperature and organic matter in the soil all decrease while diurnal variation increases. Through the centuries, rice has adapted to this enormous range of climates and this adaptation provides a lens through which to view the enormous diversity of rice and the rich source of alleles housed within that diversity. Capitalising on this requires identifying key genes and then proceeding beyond the gene towards allelic variation and the combinations of alleles that determine the balance and interplay between key regulatory points among different pathways.

Rice is a model crop for genetic analyses because it has the smallest genome of the grasses and synteny provides a common denominator for using rice to analyse other cereal crops (Ware et al. 2002). The rice genome has been sequenced and is being mapped and annotated. The allelic variability is being catalogued using a diverse set of rice varieties, enabling whole-genome variability to be associated with different phenotypes (McNally et al. 2006). Of all the food crop genomes, that of rice is the most decoded. This will strongly facilitate a synergistic linkage between genetics and metabolomics for specific improvement of the quality and nutritional value of rice, in high-yielding backgrounds, for different environments.

Rice breeders strive to develop varieties for every environment with high yield potential, stress tolerance and excellent grain quality. However, it is very rare to
capture both superior grain quality and superior agronomic characteristics in one variety, especially for those bred for unfavourable environments. The financial benefits of agronomic improvement are lost without high quality. The value of the rice market chain, from the smallest village market to the largest export market, is driven by quality. For example, Vietnam is the world’s second largest exporter of rice but profits for farmers are low because volume, not quality, drives their rice market chain, but quality, not volume, defines the price differentials of the international rice-grading system. As detailed elsewhere in this paper, food quality is currently a favoured topic of many metabolomic approaches focused on other crop species.

Many of the traits defining rice quality relate to aroma and to taste. Aromatic rice is highly prized in many countries and commands a higher price in the marketplace. The major aromatic compound in all aromatic rice is 2-acetyl-1-pyrroline (2AP) (Buttery et al. 1983), which accumulates because of a deletion in a gene that processes 2AP into proline and acetyl groups (Bradbury et al. 2005). However, there are at least 100 other volatile compounds that have been detected in aromatic rice (Bergman et al. 2000). Sensitive Asian palates can distinguish between the taste and aroma of different varieties of rice and even the same variety grown in different locations. The less sensitive western palate can discriminate, e.g. between Jasmine and Basmati rices. Aromatic compounds are volatile and metabolomics based on GC-MS is the perfect science to determine differences in the complex chemical composition between different aromatic varieties and also to quantify the effect of different environmental conditions on volatile content and composition. Once we obtain accurate chemical signatures for the different types of volatile compounds, we will have a tool to phenotype near-isogenic lines and mapping populations. This will enable us to determine the genetic basis of desirable volatile compounds, delivering capacity to rice improvement programmes to select actively for traits of aroma.
Over the past few years, the definition of ‘grain quality’ has been expanded to include components of nutritional quality. The nutritional demands being placed on rice differ among socio-economic groups. In the poorest parts of the world, efforts are being focused on biofortification to improve the amount of key micronutrients in polished rice (http://www.harvestplus.org), but in developed countries, rice consumers are interested in deriving the maximum nutritional benefit from food, with minimal intake of calories. The bran layers of rice contain many compounds that confer health benefits (Bergman and Xu 2003), and consumption of unpolished rice is increasing considerably in developed countries (http://www.whoolegrainscouncil.org/).

The bran layers of rice contain minerals, vitamins and many other compounds with known health benefits – the polished rice does not (Bergman and Xu 2003, Bird et al. 2000, Zhang et al. 2004). There is significant genetic variation for the components of pericarp layers (Bergman and Xu 2003, Zhang et al. 2004) and purple and red pericarp layers are richer in nutritional value than brown pericarps (Zhang et al. 2004). However, there are obstacles to overcome before consumption of the more nutritious unpolished rice becomes widespread. Two obstacles are cooking time and texture; one proposal is to pre-germinate unpolished rice, which is softer and cooks faster and interestingly, pre-germinated rice contains many more components with known health benefits than its ungerminated counterpart (Tian et al. 2004). Another problem, especially when dietary diversity is an issue, is that the bran layers contain both phytate and iron. Phytate is thought to decrease the bioavailability of the iron (Liang et al. 2007). Intuitively, metabolomics could provide a valuable contribution towards understanding and quantifying (1) factors regulating the accumulation of compounds in rice with known health benefits, (2) chemical signatures of unpolished and pre-germinated rice; (3) bioavailability and benefit of the different compounds within the diversity of rice; and (4) the effect of these on human health. Answers to these problems are sure to have a significant impact on the rate of adoption of unpolished rice through the different socio-economic scales.

Asia is home to 0.8 billion people who live in extreme poverty, many for whom rice is the staple. Those living in extreme poverty often do not have access to a diverse diet that provides the requisite suite of minerals and vitamins for healthy growth and development. Poor nutrition is manifested in invisible nutritional deficiencies (hidden hunger) and in malnutrition (visible hunger). Currently, significant resources are being directed towards developing rice varieties rich in micronutrients – particularly provitamin A, iron and zinc. It has been possible to reach sufficient levels of zinc by exploring the natural diversity of the species, but this has not been the case for either iron or provitamin A (http://www.harvestplus.org). Rice with sufficient levels of provitamin A has now been obtained transgenically (Golden Rice) (Grusak 2005). However, in order to achieve a sufficient level of β-carotene, a number of different sources of phytoene synthase were assayed to determine those that led to the highest production of β-carotene (Grusak 2005). This suggests that the activity of phytoene synthase could be co-ordinately regulated by some other factor. Further, studies are ongoing to determine the stability of β-carotene both during storage and through successive generations (Grusak 2005). The potential of Golden Rice in contributing to human nutrition is enormous (Stein et al. 2006). Metabolomics is a science uniquely placed to determine the metabolites specific to Golden Rice that are involved in the synthesis and the stability of β-carotene and there are already indications that this work is already in progress (R. D. H., personal communication).

**Food and nutrition security: general challenges in developing countries and a potential role for metabolomics**

Poor nutritional status and limited financial resources often compromise individual welfare in the developing world. In combination with a commonly high concurrent disease load, a self-perpetuating cycle of poverty, malnutrition and mortality arises. For the poorest people, food security, i.e. when all people, at all times, have physical and economic access to sufficient, safe and nutritious food to meet their dietary needs, is a key issue. Adequate nutrition is a human right that underpins progress towards most of the Millennium Development Goals as formulated under the banner of the United Nations in 2000 to be reached by 2015. However, the proportion of undernourished people in developing countries fell by only 3% between 1992 and 2003. This still leaves 820 million people in developing countries who are undernourished (FAO 2006). Apart from energy and protein malnutrition, there is rising concern in developing countries about the nutritional quality of diets in terms of vitamin and mineral adequacy. Metabolomics approaches – and in particular, the associated technology of ‘ionomics’ – which is specifically focused on the non-organic microelements as described recently by Salt (Baxter et al. 2007, Salt 2004) – could play a key role here in helping to facilitate novel insights into the interactions between the complex nature of food quality and nutrition (Hall 2006).

Micronutrient malnutrition affects more than half of the world’s population, especially women and pre-school
children. Over 30% of the world’s population is anaemic, mainly because of iron deficiency, with adverse health effects on pregnancy outcome, infant growth, cognitive performance, psychomotor development, immune status and work capacity (Zimmermann and Hurrell 2007). Because iron and zinc are found in many of the same foods, high rates of iron deficiency suggest widespread occurrence of zinc deficiency in the same populations. Zinc is required by about 50 enzymes in the body, and many metabolic functions are affected by zinc deficiency. These include physical growth, immune competence, reproductive function and neural development (Gibson 2006). Vitamin A deficiency affects an estimated 127 million pre-school-age children and 7 million pregnant women leading to mild to severe systemic effects on innate and acquired immunity, increased burden of infectious morbidity, Xerophthalmia, blindness and increased mortality (West 2002). Hence, improving nutritional status through developing novel strategies for healthier nutrition in developing countries would have huge impact and is critical to break the cycle of malnutrition, poverty and mortality.

Micronutrient malnutrition exists wherever there is under-nutrition resulting from low quantities of food being consumed as a result of food insecurity. This is likely to be most common where diets lack diversity and are monotonously based on cereals, roots and tubers with little access to micronutrient-rich foods such as meat, fish, poultry, eggs, milk and dairy products, and to a variety of fruits and vegetables. Policy and programme responses include supplementation and food-based approaches such as dietary diversification, improved food processing and (industrial) food fortification. Although these showed much progress in combating micronutrient malnutrition, innovative approaches are needed in order to move forward. Alliances with plant scientists and geneticists for, e.g. biofortification (i.e. enrichment of crops with micronutrients using plant breeding and/or transgenic strategies) could offer an effective supply of micronutrients (Davies 2007). The biofortification of maize (Howe and Tanumihardjo 2006), cassava (Ceballos et al. 2006), sweet potatoes (van Jaarsveld et al. 2005) and rice (Paine et al. 2005) show promising progress. However, challenges remain where a role for metabolomics could be found.

Most efforts up until now focus on individual micronutrients. However, single micronutrient deficiencies do not occur in isolation; overlapping deficiencies of iron, vitamin A and iodine, and also zinc, affect more than 50% of children and young women in developing countries. Furthermore, synergistic interactions as shown, e.g. between iron and vitamin A (Hodges et al. 1978), vitamin A and zinc (Christian and West 1998), vitamin A and iodine (Zimmermann et al. 2004) and iron and iodine (Zimmermann 2006) suggest that deficiencies of one micronutrient will interfere with efforts to control others (Zimmermann et al. 2006).

An additional, key issue is that an effective supply of micronutrients is dependent on their bioavailability (i.e. fraction of ingested nutrient available for normal physiological function and storage) and bioefficacy (i.e. fraction ingested nutrient that is absorbed and converted to its active form). These factors are therefore regular targets for nutrigenomic approaches. Metabolites present in food may inhibit or enhance absorption, especially of minerals. Certain phenolic compounds, including chlorogenic acid, certain flavonoids and complex polyphenols can form complexes with metal cations and thus interfere with the intestinal absorption of iron (Hurrell et al. 1999). Salts of inositol phosphates, collectively referred to as phytate, inhibit absorption by forming insoluble complexes with divalent metals, such as iron and zinc (Egli et al. 2004, Hurrell 2002). At the levels present in many meals, calcium can inhibit absorption of iron (Hallberg et al. 1991) and zinc but the latter only in foods also containing phytate (Lönnerdal 2000). Ascorbic acid is probably the most effective enhancer of non-haem iron when consumed with foods containing phytate, iron-binding phenols and calcium (Hallberg et al. 1986, Lynch and Stoltzfus 2003). Protein (especially of animal origin) and organic acids facilitate zinc absorption (Lönnerdal 2000). Improved knowledge on the level and interactions between the metabolic components and other dietary factors in foods commonly consumed in developing countries is still limited. These interactions and interference in (micro)nutrient availability or bioactivity from a diverse range of phytochemicals infers a need for the broadest knowledge possible of our food composition which again, should benefit from a metabolomic approach. Similarly, metabolomics could provide a tool with which to study aspects related to potential remedies based on, e.g. food processing techniques and biofortification.

Conclusions and future prospects

From this short overview it is apparent that metabolomics has quickly gained its place in modern plant science in both a fundamental and applied context; nowhere more so than in the field of nutrition and food quality. Metabolomics is already being applied in fundamental approaches aimed at gaining a better understanding of the interactive nature and control of important biochemical pathways in plants. Linking this to information and knowledge of the genetics behind this through, e.g. quantitative trait loci (QTL) analysis, is also helping us begin to design breeding programmes more closely targeted to nutrition-related issues. On the more applied side,
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metabolomics is providing novel information regarding the composition and potential nutritional imbalance of plant-based foodstuffs and is helping indicate how more tailor-made strategies could be designed to tackle key issues of global food quality in the highly contrasting situations typifying developing and developed countries. Despite advances in modern technologies both are still faced with significant nutrition-related problems. A future role for metabolomics applications here is guaranteed.


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