Plant Products and Food Quality

Derek Stewart

The programme's research targets have come into sharp focus this year with food now high on the agenda of the public, industry and governments worldwide. Furthermore the tide of consumer education continues apace with the result that the perception of, and demand for, food quality goes well beyond standard organoleptic criteria with impact on human health now a driving factor in many food sectors.

As part of our programme of research we are addressing all of these factors and extending into significant new areas with plant-derived bioactives a particular focus. To this end we have continued to provide evidence for bioactivities and potential health benefits of berry components. For example, polyphenols from a range of berries and fruits were found to inhibit the growth of cultured colon cancer cells with raspberry and strawberry amongst the most effective. This extended our understanding of the most active components and the mechanisms underlying these effects on cancer cells are being further examined through the continuing joint PhD studentship with the University of Ulster. Furthermore this is being expanded at the cancerogenesis level through collaboration with the Biomedical Research Centre, University of Dundee, and has shown that specific fruit polyphenolics positively influence the expression and translation of key chemoprotective genes such as *NAD(P)H:quinone oxidoreductase 1 (NQO1)* and *Nuclear factor-erythroid 2 p45-related factor 2 (Nrf2),* which mediates and regulates the transcriptional activation of many genes which encode detoxification and antioxidant proteins in response to redox stress (Fig. 1).

Previous studies suggested that berry components could inhibit key enzymes in the human digestion of starch and thus have the potential to modulate starch digestion thereby influencing blood glucose levels after meals. This could be of benefit to people suffering from, or at risk of developing, type II diabetes. This idea is currently being investigated in a human intervention trial

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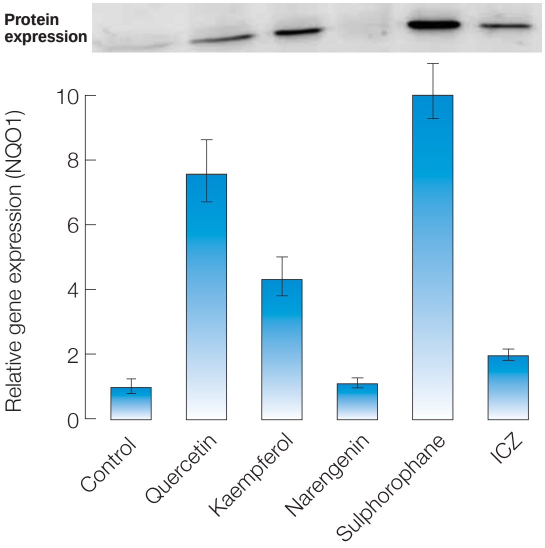

Figure 1 The protein and gene expression levels of NAD(P)H:quinone oxidoreductase (NQO1)in the model systems rat liver epithelial (RL34) cells following treatment with putative dietary anticancer components. Quercetin, Kaempferol and Narengenin and common dietary plant polyphenols, Sulphorophane and ICZ (indolo[3,2-*b*]carbazole) are degredation and digestion products derived from isothiocyantes present in dietary *Brassica* species: well reported sources of anticancer benefits.

with scientists at Queen Margaret University, Edinburgh. We have also confirmed that berry polyphenols can inhibit pancreatic lipase, the key enzyme in the digestion of fats, which could have implications for the control of calorie intake from fatty foods and weight control.

The bioefficacy of selected plant polyphenols has been established with respect to the activation of FOXO transcription factors, the ‘master regulators’ of cell survival, cell cycle and glucose homeostasis, in cultured pancreatic cells, and has suggested that plant polyphenols such as theaflavins, theaflavin 3-*O*-gallate, theaflavin 3'-*O*-gallate, theaflavin 3,3'di-*O*-gallate and thearubigins could mimic insulin and potentially influence diabetic processes. This is being explored further via a joint PhD studentship with Dr Graham Rena at the University of Dundee.

The focus on food functionality has been carried through to several EU projects. Within DEVELONUTRI, a project focused on nutritive losses throughout the potato, tomato and wheat processing and food chains, extensive and detailed studies into (anti)nutrient and micronutrient stability have been performed and these are informing the more detailed analyses with respect to sources of quantitative variability. In tandem with this, extensive ring testing is under way to compare and contrast the standard analytical approaches of Gas Chromatograhy (GC), Liquid Chromatography – Refractive index (LC-RI), Liquid Chromatography – Ultra Violet (LC-UV) and Liquid Chromatography – PhotoDiode Array (LC-PDA) with the more routine metabolite profiling approaches offered by Gas Chromatography – Time of Flight – Mass Spectrometry (GC-ToF-MS) and Liquid Chromatography – Mass Spectrometry (LC-MS). This is with a view to revising how food compositional database reporting is undertaken and the extent to which these approaches can be developed.

Research in BarleyBread, another EU project, is also addressing the food functionality issues by studying to what extent we can supplant wheat with barley in breadmaking. Barley contains a complex polysaccharide, β-glucan, which, when present in foods at certain levels, has been shown to reduce cholesterol and the likelihood of cardiovascular disease. Indeed β-glucan is one of the few plant-derived components to be given an approved health claim (see inset). Within the project, and in collaboration with the SCRI Genetics programme, we are establishing the biodiversity of β-glucan and other putative healthy components such as sterols, specific polyphenols etc., in a broad range of barley germplasm across Europe with the aim of identifying the national/regional optimal varieties for these health components. Data will inform the use of specific barley germplasm for developing 'healthier breads' for each participating nation. Furthermore in collaboration with the local food ingredients company, Macphie of Inverbervie, bread with a 20% barley content was produced which performed texturally in a manner analogous to the equivalent 100% wheat bread. Consumer preference testing at the SCRI Open Day in 2008 was a resounding success with >80% of the public preferring the barley bread over the traditional wheat based product (Fig. 2).


Figure 2 Consumer preference testing at the SCRI 2008 Open Day of bread containing 20% wholegrain barley flour.

The Food and Drug Administration claim (USA) Generic health claim – The inclusion of oats as part of a diet low in saturated fat and a healthy lifestyle can help reduce blood cholesterol

Diets low in saturated fat and cholesterol that include 3 grams of β-glucan soluble fiber from barley (or oats) may reduce the risk of heart disease. The product must contain at least 0.75g of β-glucan per serving. This value is based on the observation that foods providing at least 3g of β-glucan per day are effective in lowering serum cholesterol levels. Spreading these 3g over three daily meals and one snack, they arrived at the figure of 0.75g per serving.

Distribution of Gene Ontology molecular function aThe FDA stipulations on barley β-glucan derived health claims with respect to reduced cholesterol and a reduced risk of cardiovascular disease.

With regard to the EU priority area of 'safer and environmentally friendly production methods and healthier foodstuffs' SCRI has played a key co-ordination role in the EU Integrated Project 'SAFE FOODS – Promoting Food Safety through a New Integrated Risk Analysis Approach for Foods' (www.safefoods.nl). This project has been at the forefront of assessing the potential value of advanced, broad scale analytical 'omics' technologies within a risk assessment framework, particularly with regard to crop breeding and production systems. Using potato and maize as model crop species the project has revealed that variation in gene protein and metabolite expression is large with respect to genotype, growing site and season and even crop management practices. Any differences between GM and non-GM crops tested were dwarfed by comparison. These approaches could therefore be used to benchmark any measured differences in a particular crop against the extent of 'acceptable' variation within the framework of a history of safe use of the crop species in question. A scheme for the approach is presented (Fig. 3).

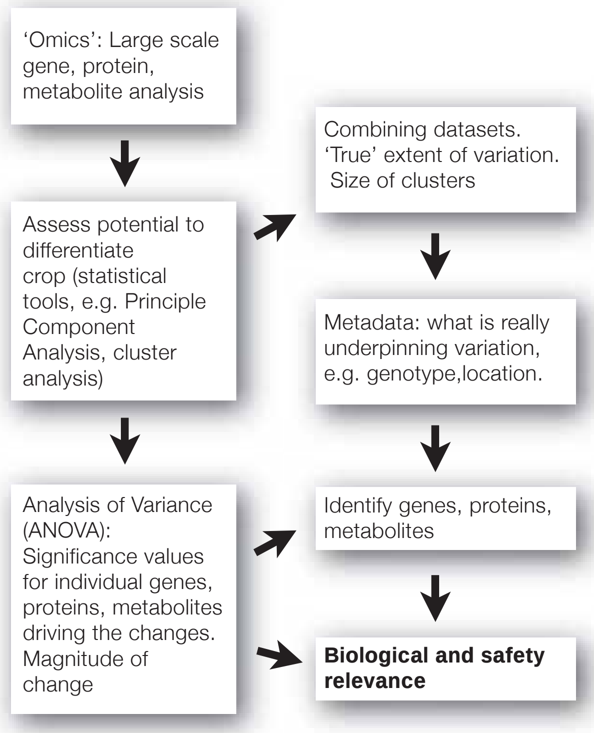

Figure 3 Flow diagram: data-rich outputs of 'omics' feeding into comparative safety assessment.

Potato quality is assuming a greater importance in breeding programmes, with consumers demanding greater variety and retailers wishing to market cultivars that have distinctive commercial advantages. However, as with many food crops, potato flavour is difficult to assess in breeding programmes. Assessments can be highly subjective and require trained sensory panels. Generally these sensory approaches have a low sample throughput and are consequently expensive. As a result, flavour is generally only assessed in the later stages of a breeding programme after selection for essential agronomic qualities and more easily quantifiable traits and as a result most of the potential flavour improvements are likely to be discarded.

Previous studies using trained taste panels have clearly identified the *Solanum tuberosum* Group Phureja (commonly referred to as *Solanum phureja*) type potatoes as having a distinctive and generally preferred flavour compared with Group Tuberosum types (Commonly referred to as *Solanum tuberosum*). Our research has identified candidate compounds that may account for these differences. These include volatile compounds such as the esters of branched chain amino acids and the sesquiterpene α-copaene as well as umami taste compounds, such as glutamic acid and 5' ribonucleotides. In fact, the levels of the major umami compounds (glutamate and 5' ribonucleotides) in boiled potato cultivars, previously assessed for sensory quality, were significantly higher in two *Solanum phureja* cultivars when compared with two *Solanum tuberosum* cultivars. The equivalent umami concentration was calculated for five cultivars and there was a strong positive correlation with acceptability score from a trained evaluation panel suggesting that umami is an important component of potato flavour (Fig. 4).

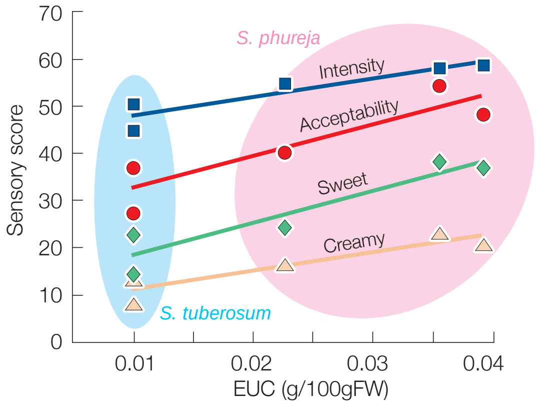

Figure 4 *S. tuberosum* cultivars Maris Piper (MP) and Record were compared with *S. phureja* clones DB333-16 and DB257-28, and cultivar Mayan Gold (MG). Square=flavour intensity; circle=acceptability; diamond=flavour sweet; triangle=flavour creamy.

Potato tuber texture is also a key quality determinant of cooked potato and a major trait that influences consumer preference. As with flavour, texture is a complex trait to analyse as it depends on the interaction of many factors and defining texture that is attractive to consumers also depends on sensory panel analysis rather than analytical measurements. Terms used for potato texture include flouriness, synonymous with mealiness (describing a dry, soft texture) and waxiness (describing a moist, firm texture). In addition, different consumer groups generally prefer different textures. For example, a dry boiled potato texture is preferred in Scotland, whereas a waxy texture is preferred in the Netherlands.

To establish an objective measure of potato texture a food texture analyser has been used for quantitative texture measurement comparisons between Phureja and Tuberosum tubers. Results indicate that Phureja exhibits a very different boiled tuber texture, described as extremely floury or crumbly. In fact Phureja tubers cook in approximately half the time of typical Tuberosum samples (Fig. 5).

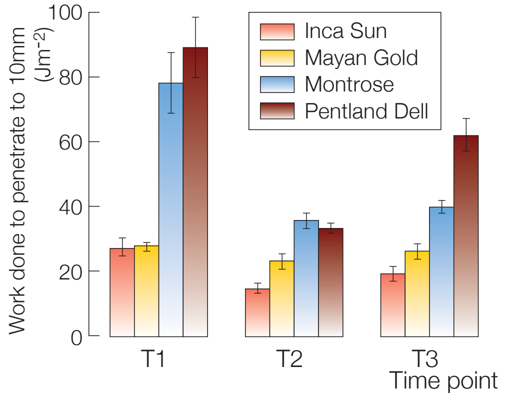

Figure 5 Texture Analysis of Tuberosum v Phureja T1: Developing tubers (30-50g), T2: Developing tubers (75-100g), T3: Mature tubers (150-250g), 2 weeks at 10°C. Each bar = mean of 8 tubers.

Factors that have an impact on cooked potato texture are likely to include starch content and distribution within the tuber, starch swelling properties, cell size, cell wall structure and composition and the breakdown of the cell wall middle lamella during cooking.

Recent research at SCRI has taken advantage of these potato types with very different tuber flavour and texture traits to begin to identify the genes underpinning these traits. We have used microarrays as a tool to identify genes with modified expression profiles that corresponded to differences in tuber flavour and texture. Gene expression was compared in two Phureja cultivars and two Tuberosum cultivars and showed that 309 genes were significantly and consistently up-regulated in Phureja, whereas 555 genes were down-regulated. Approximately 46% of the genes in these lists can be unambiguously annotated and identified, and amongst these are candidates that may underpin the differences between Phureja and Tuberosum with regard to flavour and texture traits. For example, a sesquiterpene synthase gene was identified as being more highly expressed in Phureja tubers.

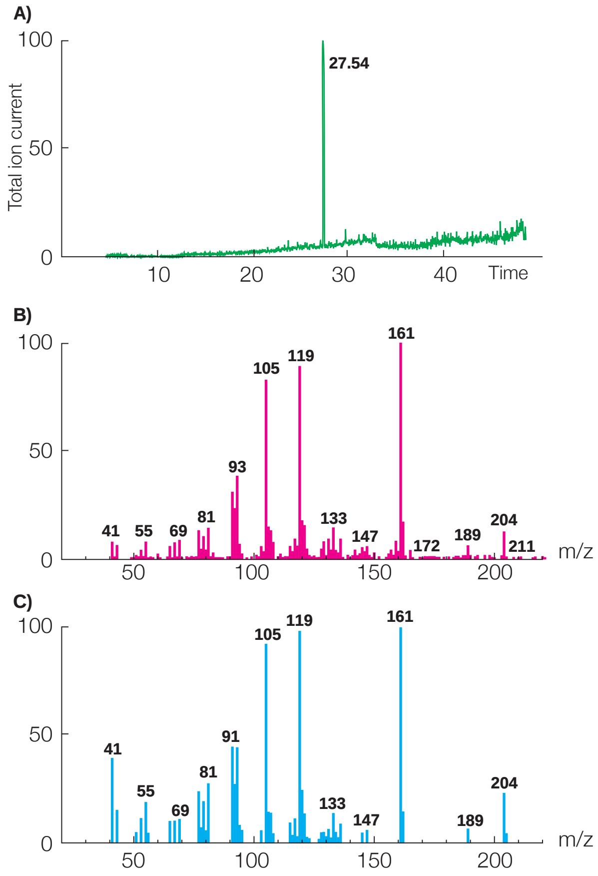

Figure 6 GC-MS analysis of the products produced by the putative sesquiterpene synthase. A – Total ion current trace. B – Mass spectra of peak at retention time 27.54min. C – Adam's library spectrum for α-copaene.

The gene was isolated and demonstrated to encode α-copaene synthase (Fig. 6). This correlates with the significantly higher level of α-copaene (a compound implicated in the 'baked' potato aroma) production from boiled Phureja compared with Tuberosum. Other potential 'flavour genes', identified from their differential expression profiles, include those encoding branched chain amino acid aminotransferase and a ribonuclease, which suggests a mechanism for umami-active 5'-ribonucleotide formation in potato tubers on cooking. Major differences in the expression levels of genes involved in cell wall biosynthesis (and potentially texture) were also identified, including genes encoding pectin acetylesterase, xyloglucan endotransglycosylase and pectin methylesterase.

In parallel with the molecular analysis of gene expression, biochemical analysis of cell walls from the Phureja and Tuberosum tubers revealed significant differences

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that may contribute to the textural differences. For example, in the Phureja types, pectin is more easily solubilised from the cell walls and contains more methyl ester moieties than corresponding Tuberosum samples. Higher levels of pectin methylation have been previously correlated with enhanced cell separation and differences in texture on cooking in potato tubers. This result is also consistent with lower pectin methylesterase gene expression and the levels of pectin methylesterase enzyme activity found in the Phureja tubers. However, a role for starch in defining tuber textural properties cannot be discounted as starch rheology measurements and starch structural properties are also significantly different between the potato types.

To validate the role of these candidate genes in regulating flavour and texture we are manipulating the expression levels of these genes using transgenic methods. For example, transgenic Tuberosum lines have been developed that produce significantly higher levels of α-copaene as a result of over-expression of α-copaene synthase. Sensory evaluation of these transgenic lines will be undertaken by international partners. Other transgenic lines are aimed at investigating the roles of the genes involved in pectin biosynthesis and the effect on texture and cell wall structure will be tested in the coming year.

To support these advances in the identification and development of quality, health and functional food components we have developed a range of high throughput analytical methods for their simultaneous detection and quantification. For example, as part of our fruit phytochemical studies, the inheritance of polyphenol components in the large progeny sets derived from the SCRI raspberry and blackcurrant

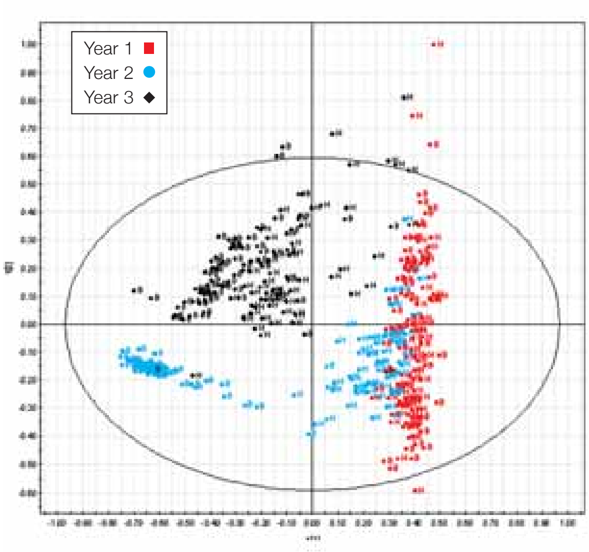

Figure 7 Metabolomics in action. Direct infusion mass spectrometric (DIMS) analysis of the fruit from the progeny of the Glen Moy x Latham raspberry cross grown in two environments (H and B) and over three years. The data (1600 mass spectral points per progeny and 5 replicates for every progeny) was then analysed by principal component analysis and the plot of score 1 v. 2 is shown above. This untargeted approach clearly shows that the fruit vary phytochemically year-on-year and that in year 2 the difference in environment was a dominant factor in determining the derivation of this difference.

genetic research required completely new approaches to data acquisition. These focus on the determination of key components using their characteristic mass spectroscopic signals and by deconvoluting the huge data sets using statistical methods. These methods are currently being validated and used to examine the importance of genotype × environment interactions (Fig. 7).

The potential consequences of climate change form a basis for some of the programme's emerging research strands. Drought stress will be an inevitable global consequence of the predicted increase in climactic extremes and is the focus for a collaborative project with The Irish Agriculture and Food Development Authority (TEAGASC). Currently a stepwise approach is being conducted to investigate the genetic basis of phenotypic and metabolic plasticity to drought stress in *Lolium perenne* L. (perennial ryegrass), an economically important crop for animal nutrition and the leisure industries.

A comprehensive metabolomic approach was carried out to elucidate the mechanisms involved in *L. perenne* response to drought stress. When leaf and root material from two genotypes, with a contrasting drought stress response, were analysed a clear difference was observed in the metabolic profiles of the leaf tissue under drought stress. Differences were principally due to a reduction in fatty acids levels in the more susceptible genotype (Cashel) and an increase in sugars and compatible solutes in the more tolerant genotype (PI 462336) in response to stress. The sugars significantly increased included raffinose, trehalose, glucose, fructose and maltose. Indeed, raffinose was identified as the metabolite with the largest accumulation under drought stress in the more tolerant genotype and may represent a target for selecting for superior drought tolerance into perennial ryegrass. The metabolomics approach is now being combined with the complementary transcriptomic study in the drought stress tolerant genotype PI 462336 and this has already identified several significantly up-regulated genes, such as fructan:fructan 6G-fructosyltransferase (6G-FFT), that corroborate the metabolomics and polysaccharide analytical findings and further add credence to the putative new targets for breeding.