Quality, Health & Nutrition

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The Programme's activities over the last year have been focussed, multidisciplinary efforts and have addressed combinations of quality and health and/or nutrition. Through collaborations both internally and externally, with academia and industry, SCRI and the Quality, Health & Nutrition Programme are making significant in-roads into establishing the basis and diversity of quality and nutritional parameters in plant-based foods and the beneficial effects that they may have on health.

Research into the basis of the beneficial health effects of plant-based food has focussed predominantly on soft fruit. Intensive *in vitro* studies were undertaken to establish the changes in fruit phytochemicals accompanying digestion and passage through the gastrointestinal tract (GIT). The majority of *Rubus*-derived polyphenols, predominantly anthocyanins and ellagitannins, survived acidic (stomach) digestion but progression through to the upper GIT, where the pH shifts to more alkaline conditions, was accompanied by a dramatic reduction in recoveries. This alkaline-driven degradation produced many smaller, putatively pharmacologically active compounds which would be readily taken up into the blood stream and exert their activities at sites remote from the digestive tract. Interestingly co-incubation and digestion of the fruit (and juice) with other foodstuffs (reflecting normal meal or snack consumption) comprised mainly of polysaccharide (bread) or protein (meat) showed that there was minimal effect on *in vitro* uptake into the serum. This suggests that polyphenols transiently bind to food matrices during digestion, which protects the more labile components, such as anthocyanins, from degradation, and increases their potential serum uptake.

There was also a range of digestive labilities in the *Rubus*-derived phytochemicals with cyanidin-3-*O*-glucoside greatly reduced whilst others, such as pelargonidin-3-*O*-glucoside, were apparently increased in abundance by default. This may have a significant effect on future breeding targets with the digestive stability of specific components a factor to be aware of when assessing potential targets for enhancement.

Studies into fruit health benefits were further extended into *in vivo* studies. In collaboration with the University of Kuopio (Finland) and the Petrov Research Institute of Oncology (Russia) juices from control (yellow, anthocyanin-depleted) and anthocyanin-rich (red) *Rubus* (cloudberry) fruit were fed to young and aged fruit

flies (*Drosophila melanogaster*). Fruit flies are a well accepted and validated model for ageing studies and oxidative-stress related physiological and biochemical phenomena. The studies showed that there was both an age and sex effect evident following the fruit juice consumption. In young female flies there were significant effects of both juices on primary and secondary, more long-term, lipid peroxidation, whereas in young male flies significant reductions in primary peroxidation products occurred with yellow juice and in secondary products with red juice. With the red juice (similar in composition to raspberry), a significant decrease in secondary lipid peroxidation was found in both young and old males, but not older females. The data adds weight to the increasing body of evidence supporting the need to increase the amount of anthocyanin-rich fruits in the diet and, given both the age and sex-related differences, should lead to insights into tailored diets for optimal health and longevity.

Novel polyphenol compounds were isolated and identified in blackcurrant fruit. Their structures are consistent with anthocyanin rutinosides covalently linked to epigallocatechin or gallocatechin. There was also evidence for the presence of a range of other flavanol-anthocyanin condensation products. Overall the contents of these compounds in fruit were roughly correlated to the content of the parent anthocyanins. The conjugates were present in polyphenol-enriched concentrates obtained from both commercially produced concentrates and in fresh extracts of blackcurrants, suggesting that they were not artefacts formed during concentration or purification. Given the reported bioactivity of both the parent anthocyanins and (epi)gallocatechins it is likely that these compounds will exhibit beneficial biological activities and this is currently being tested.

Vitamin C (Vit C) continues to be a focus for research. It is an essential human nutrient that must be obtained in the diet, with the vast majority being obtained from plant foods. A Vit C-deficient diet results in the onset of scurvy, which can have lethal consequences. However, Vit C has also been implicated in the prevention of chronic diseases such as heart disease, stroke, cancer, and several neurodegenerative diseases and as a consequence the dietary allowances for Vit C have been recently increased in several countries. The biosynthesis, regulation and enhancement of Vit C levels in blackcurrant, already a good source, forms one of the programme's research foci.

Studies into the timing and mechanism of Vit C accumulated in blackcurrant fruits have shown that, regardless of the ultimate absolute level, Vit C accumulation was associated with the early phases of fruit development up until fruit started turning red. Following this the levels remained fairly constant. Although up to four Vit C biosynthetic pathways have been proposed in plants, mechanistic studies have shown that only one predominates in blackcurrant fruit. Furthermore, contrary to our previous findings in potato, transport of Vit C from leaves to fruit in blackcurrant makes a negligible contribution to fruit accumulation. Biochemical analysis of high and low Vit C genotypes has demonstrated a clear correlation between biosynthetic capacity during Vit C accumulation and fruit vitamin

content at harvest. The characterisation and mapping of the key genes in the major pathway should help to accelerate the early selection of high Vit C lines in the blackcurrant breeding programme.

Carotenoid and isoprenoid metabolism has long proved to be an attractive target for manipulation since the isoprenoid biosynthetic pathways provide a wide range of metabolites that are essential both for plant development and storage organ-derived food quality. The activities attributed to isoprenoids are multiple and wide ranging. For example, *in planta* they are essential for photosynthesis, membrane stability, phytohormone biosynthesis, plant defence etc. Plant-based foods also contain isoprenoids and specific classes of these, such as carotenoids. The latter have been associated with health benefits, with Vitamin A precursors essential to retard age-related macular degeneration and Xerophthalmia, a major cause of blindness in children in Africa and Latin America. The isoprenoids also exhibit non-health activities and have often been implicated as important determinants of flavour in plant-based foods.

Previous perturbations of the isoprenoid metabolism in potato focussed on expressing the *Erwinia uredovora*-derived *crtB* gene encoding phytoene synthase. This work has continued leading to an elevation of total carotenoids in both *S. tuberosum* and the diploid *S. phureja* of 6- and 4-fold, respectively. In addition transformation with *crtB* was accompanied by radical changes in the balance of the carotenoids with the appearance of β-carotene and a 19-fold enhancement of lutein.

Modifications to the *S. tuberosum* and *S. phureja* carotenoid profiles were also evident following transformation with the algal gene *bkt1*, a β-carotene ketolase (or oxygenase) that catalyses the formation of a carbonyl group at carbon position 4 of each ring of β-carotene. The transformed tubers had elevated levels of the ketocarotenoids ketolutein and astaxanthin. The latter is reported to exhibit anticancer effects and enhance immune function in humans.

All isoprenoids require isopentenyl diphosphate (IPP) for their biosynthesis and so some effort was put into manipulating IPP biosynthesis in potato. A key enzyme in the initial stages of the plastidic pathway of isoprenoid biosynthesis is 1-deoxy-D-xylulose 5-phosphate synthase (*dxs*), the product of which is subsequently transformed into IPP. Transformations of potato with a bacterial *dxs* gene saw the total tuber carotenoid content increase approximately 2-fold, with most of the increase accounted for by a 6-7-fold increase in phytoene. Interestingly, *dxs* expression was also correlated with tuber elongation and an early tuber sprouting phenotype. This is being explored further.

Metabolic profiling continues to underpin much of the QHN science. As part of complementary studies in the Genetics Programme, it is being applied to the Commonwealth Potato Collection to assess phytochemical diversity with the aim of identifying possible new traits for introgression into the breeding programme. This approach has been further refined and, in collaboration with the Genetics Programme and the Federal Centre for Breeding Research on Cultivated Plants Germany, applied to segregating crosses of raspberry and strawberry, respectively. The development and application of Direct Infusion Mass Spectrometry to both projects has allowed rapid, but data-rich, analyses to be undertaken on these large populations and is yielding insights into the interplay and inheritance of polyphenols, Vit C and other nutritionally relevant phytochemicals. This approach is becoming attractive as a rapid high throughput screen for phytochemical population analyses.

Diversification into the non-food crops arena has been assisted by prestigious Scottish Enterprise Proof of Concept (PoC) and subsequent PoC-Plus funding to develop smart screens for wood properties. Current methods of determining and predicting wood properties and composition are both expensive and laborious. The aim of this project was to establish the concept that infrared scanning technologies could be used to predict wood properties and composition. The end use of this technology was multifaceted and included the enhancement of Scottish Sitka timber via earlier screening of the germplasm for density etc. and the ability to identify and quantify wood adulteration, contamination and biodegradation (rot). Using a clonal population of Sitka Spruce, calibrations were constructed between parameters of wood measured in the laboratory and the associated infrared spectra and these were then used to predict the values of these parameters in previously unanalysed samples. The infrared scanning technology was successful in all of the approaches and allowed a reduction in the multifactorial analysis time by at least 100-fold. In addition, the technology was able to successfully detect very low levels of biodegradation and inorganic contamination in wood. As a result of this the use of infrared technologies for predicting and quantifying selected wood properties has been the subject of a patent application and is currently the subject of development to commercialise this approach.