Metabolomic approach to identifying bioactive compounds in berries:
Advances toward fruit nutritional enhancement.

Inger Martinussen[1], Gordon McDougall[2] and Derek Stewart[2]

[1]BIOFORSK, Arctic Agriculture and Land Use, Tromsø, Norway   [2]Scottish Crop Research Institute, Food Quality and Health, Dundee, UK

The evidence supporting the beneficial health effects of fruit is both accruing and historical. Furthermore berries, are increasingly becoming the focus of studies regarding their proposed ability to prevent or ameliorate the problems of degenerative diseases. The phytochemical bases of these nutritional benefits are increasingly focussing upon the chemically diverse polyphenols. The accretion of both in vitro and in vivo data has meant that the nutritional enhancement of foods, and the base materials, in this case fruit, has shifted focus away from simply vitamin C and micronutrients and towards the polyphenolics. This causes something of a problem for any breeding effort since, as has been previous highlighted, the polyphenolics are chemically diverse and their analysis would, under normal approaches, require a considerable effort via traditional, targeted analysis. Metabolomics (LC-MS, GC-MS and NMR) may be a way forward since it can, in a single analysis report upon a significantly greater breadth of the metabolite pool and in this case the polyphenolic pool (phenolome) can be captured and reported upon in much greater detail and in much greater depth than was previously possible. The application of such technologies to breeding programmes is obvious and we report here on the application of direct infusion mass spectrometry (DIMS), a subset of metabolomics, to a segregating raspberry population grown in two distinct environments

Materials and Methods

The generation of the raspberry (Rubus ideaus) population (300 individuals), a cross between the varieties Glen Moy and Latham, used herein has been described in some detail by Graham et al [2004]. The raspberry populations used here were grown in two distinct environments (H field and B field) over many years for the purposes of plant pathogen testing. H field is a low input site where the crop was not sprayed with fungicide etc. Conversely, B field was classified as a high health site where the plants were sprayed with fungicide, regularly fertilised etc. This variation in agronomic practices and soil conditions has meant that the resultant fruit varied with respect to comparative phenotypes such as yield etc. Here we will focus only on one year's data, 2005, and for analytical purposes and as a result of selected fruit failure, etc., only 96 samples were used here for compositional analysis purposes.

The methods for the generation of the fruit juices and optimised extracts (1:1, fresh fruit: acetonitrile containing 0.1%acteic acid) and the determination of Trolox Equivalent Antioxidant Capacity (TEAC) and total phenols, anthocyanins and vitamin C (Vit C) were performed exactly as described by Mylnikov et al. Direst Infusion Mass Spectrometry (DIMS) was carried out on a LCQ-Deca (ThermoFinnigan) as described by Stewart et al (2007)

Results and Discussion

- Vitamin C content showed a significant variation and appeared to exhibit an environmental influence.

- Phenol contents of the solvent extracts were, without exception, greater than those found in the analogous juice (Fig. 2)suggesting that whole fruit consumption may yield a greater health benefit

- A much greater genetic regulation was apparent with regard to the total phenol content values with the majority of the values falling between those of the parents regardless of environment.

- The relationships (Fig 3) between the phenol content in the juice and the optimised extracts was direct (R^2 ~0.9 and 0.75 for the juice and optimise extracts, respectively), with the latter yielding the higher values.

- The environmental effect on the phytochemistry of the cross progeny is significant since the segregation of the lines in the DIMS derived PCA plots for each environment (B and H field; Fig 4.1 and 5.1, respectively) appear different. However, closer examinations shows that the progeny are separated similarly according either score 1 or 2 but that with their actual position away from the zero point varies.

- Despite the relatively different segregation of the lines with respect to all the metabolites the dominant ones, the polyphenols, display remarkably similar segregating patterns in the scores in both environments (Fig 4.2 & 5.2). The most obvious feature is that of the segregation between the cyanidin-3-sophoroside (A) and cyanidin-3-rutinoside (C) groups, which are cleanly segregated according to score 2.
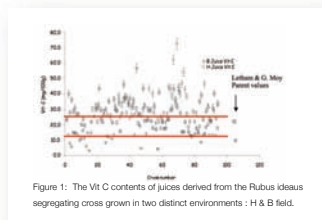

Figure 1: The Vit C contents of juices derived from the Rubus ideaus segregating cross grown in two distinct environments : H & B field.
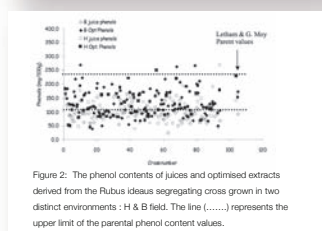

Figure 2: The phenol contents of juices and optimised extracts derived from the Rubus ideaus segregating cross grown in two distinct environments : H & B field. The line (........) represents the upper limit of the parental phenol content values.
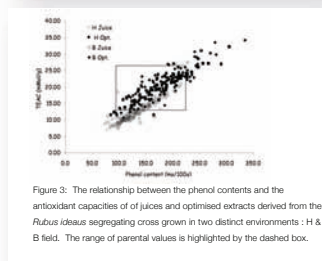

Figure 3: The relationship between the phenol contents and the antioxidant capacities of of juices and optimised extracts derived from the Rubus ideaus segregating cross grown in two distinct environments : H & B field. The range of parental values is highlighted by the dashed box.
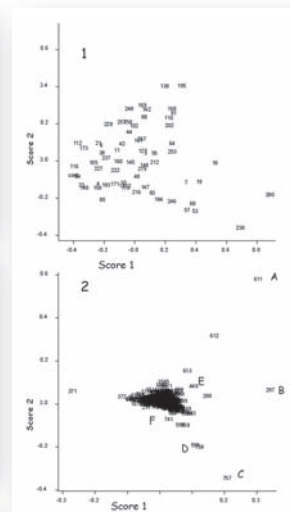

Figure 4
1 - PCA score plot (1 x 2) generated from direct infusion MS metabolites of the rubus segregating cross in the environment B field;

2 – The same score plot but interrogated on the basis of the ions dominating and creating this segregation A - cyanidin 3-sophoroside (m/z 611), B - m/z 287 (cyanidin), C - cyanidin 3-glucosylrutinoside (m/z 757), D - cyanidin 3-rutinoside (m/z 595) or pelargonidin 3-sophoroside (m/z 595), E – cyanidin 3-glucoside (m/z 449), F - pelargonidin 3-glucosylrutinoside (m/z 741), G - quercetin acetylrutinoside (m/z 651), cont – machine control
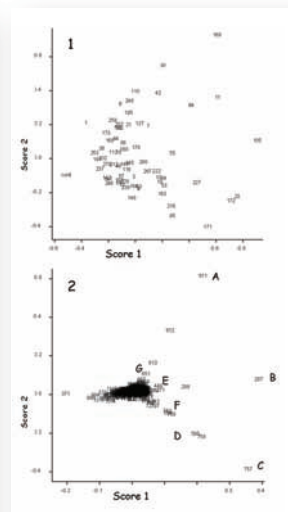

Figure 5
1 - PCA score plot (1 x 2) generated from direct infusion MS metabolites of the Rubus segregating cross in the environment H field;

2 – The same score plot but interrogated on the basis of the ions dominating and creating this segregation A - cyanidin 3-sophoroside (m/z 611), B - m/z 287 (cyanidin), C - cyanidin 3-glucosylrutinoside (m/z 757), D - cyanidin 3-rutinoside (m/z 595) or pelargonidin 3-sophoroside (m/z 595), E – cyanidin 3-glucoside (m/z 449), F - pelargonidin 3-glucosylrutinoside (m/z 741), G - quercetin acetylrutinoside (m/z 651), cont – machine control.

Conclusion

In summary the metabolic profiling approaches are high relevant to the interface between plant breeding for food and human nutrition. If we are to nutritionally enhance plant-derived food in an appreciable timescale and within the current economic conditions every opportunity must be taken to ensure that we are capturing the optimum level of information regarding known and putative nutritionally relevant compounds. We have shown here, albeit briefly, that the technology is now within our grasp and that nutritional enhancement using standard breeding approaches is entirely feasible.

References
Graham J. et al (2004) Theor Appl Genet. 109, 740-9;
Mylnikov SV et al (2005) J Agric Food Chem. 53, 7728-33;
Stewart et al (2007) Mol Nutr. Food Res. 51, 645-651