DEVELONUTRI – Development of High Throughput Approaches to Optimise the Nutritional Value of Crops and Crop-Based Foods

Sean Conner, Derek Stewart
Scottish Crop Research Institute Dundee DD2 5DA

Introduction

Micronutrients, such as vitamins, antioxidants and minerals, are essential components of our diet. They are present in food often in trace amounts and are thus difficult to quantify. Highly sensitive methods for their quantification are available, but not yet routine. These methods can help assessing which treatments, before and after harvesting, help preserving the nutritional value of food.

To quantify a broad spectrum of metabolites is the important that the approaches and technologies are operating at a level where the metabolites can be confidently and reproducibly determined. This is particularly important for the MS-based approaches and highlights the crucial necessity for appropriate internal (sample) and batch (machine) standards to allow not only for time-of-analysis quantification but also re-mining and cross-site and technology comparisons.

At SCRI as part of the DEVELONUTRI project state-of-the-art analytical techniques are being developed and validated for rapid quantification of micronutrients in three widely consumed crops (potato, wheat and tomato) throughout the production, processing and transportation chain. Both traditional and GM varieties will be analysed. The main analytical techniques that are being used at SCRI for this project are LC-MSn ,GC-ToF-MSn , Ion chromatography, HPLC/ECD, FTIR. Method development and preliminary trials will be discussed.

Method Development

Standards

Authentic standards have been used to construct dose-response curves for all the predominant health-promoting phytochemicals known to exist in tomato, wheat and potato and dose-response curves for both GC-MS and LC-MS have been constructed for these standards (see Figures 1 and 2). In most cases linearity can be found over a specific range, although saturation does arise at high concentration (>5mg /ml). In addition the suitability of a number of potential internal standards was judged. In most cases the authentic health promoting phytochemical standards were found to be stable over a four month period but only when stored at -20ºC under nitrogen.

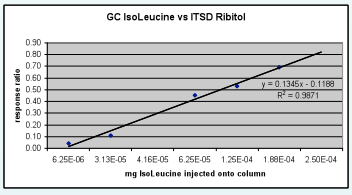

Figure1 An example of an GC-MS derived dose-response curve for isoleucine acid, a common potato and tomato metabolite .

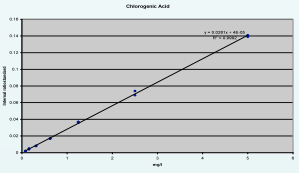

Figure 2 n example of an LC-MS derived dose-response curve for chlorogenic acid, a common potato and tomato metabolite .

GC and LC/MS Extraction Methods

The GC/MS extraction method uses a multi-step water/methanol/chloroform protocol with the polar metabolites residing in the water/methanol fraction and the non polar metabolites being in the chloroform/methanol layer. Each fraction is separated and derivatised prior to GC/MS analysis The LC/MS extraction method uses a one-step water methanol extraction Typical chromatograms for GC polar and non-polar extracts for potato figure 3 and 4 respectively. The analogous LC/MS analysis is shown in figure 5.

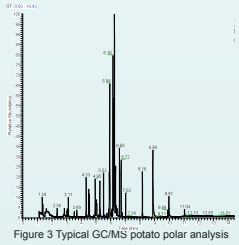

Figure 3 Typical GC/MS potato polar analysis

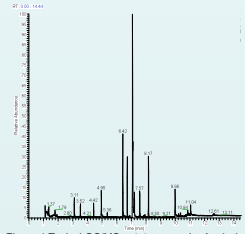

Figure 4 Typical GC/MS potato non-polar Analysis

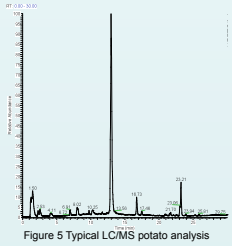

Figure 5 Typical LC/MS potato analysis

Preliminary Trials

A number of preliminary trials have been performed on samples do determine if the methodology worked two of these trials will be discussed below.

Tomato

Six types of tomato were selected four tined, branded chopped, branded whole supermarket chopped, supermarket whole a supermarket tomato puree and a tomato standard (fresh). The samples were homogenised frozen under liquid nitrogen and analysed using the SOP for GC and LC/MS outlined in this poster.

GC/MS

The PCA plot for GC/MS analysis of the polar fraction (figure 6) shows that the potato instrument controls segregate away from the tomato samples. Removing the potato controls (figure 7) shows that the tomato puree and tomato standard samples segregate from the tinned tomato samples.

The analogous LC/MS analysis the PCA plot (1v3) (figure 8) shows three distinct groups. These are tomato puree, and whole and chopped tomato. Removing the puree group still results in a distinct separation between chopped and whole tomato, however there is also a separation between the supermarket and branded chopped tomato samples (figure 9).

Wheat and pasta

Samples of bread wheat and durum wheat along with a selection of six spaghetti varieties were extracted using SCRI protocol and analysed by LC/MS only the non-polar analysis showed interesting results. The PCA plot 1 v 3 (figure 10) shows the wheat samples segregating from the pasta samples also both wheat samples segregate from each other.

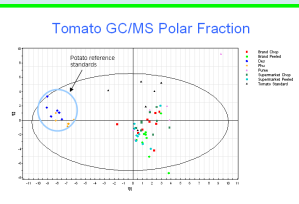

Figure 6 PCA (1v2) of GC/MS Polar fraction analysis of Tinned tomato and tomato puree

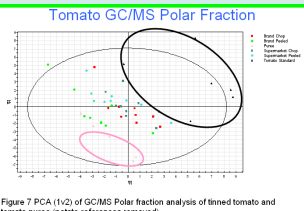

Figure 7 PCA (1v2) of GC/MS Polar fraction analysis of tinned tomato and tomato puree (potato references removed)

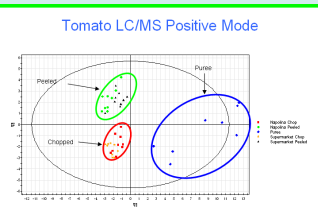

Figure 8 PCA (1v3) of LC/MS analysis of tinned tomato and tomato puree

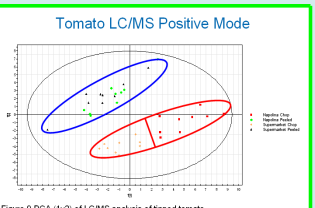

Figure 9 PCA (1v2) of LC/MS analysis of tinned tomato

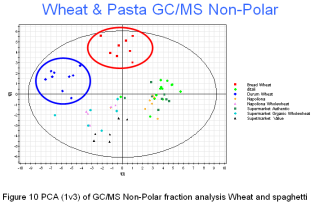

Figure 10 PCA (1v3) of GC/MS Non-Polar fraction analysis Wheat and spaghetti

Conclusions

Although the results presented are preliminary pre-trials the results demonstrate that metabolomics can be used de determine differences between processed and non processed food and differences in varieties

Develonutri
CROP-BASED NUTRITION