

## Background

Glucosinolates ( $\beta$ -thioglucoside-N-hydroxysulfates) are secondary metabolites present in cruciferous vegetables (such as *Brassicaceae*). Glucosinolates are precursors of isothiocyanates, which have bioactive properties (Mithen, 2001). Irish grown cruciferous vegetables (such as *Brassica Oleracea* L.) contain glucosinolates (GLS) in amounts which could have a beneficial effect to human health. However little is known about the glucosinolates profile and content in *Brassica Oleracea* L. cultivars grown in Ireland and the effect of several agricultural factors such as soil type, spacing and maturity on the GLS profile of these varieties. This study is the first step of a Farm to Fork approach study in the effects of agricultural factors on GLS levels in Irish cruciferous vegetables.

## Materials and Methods



1. Samples were harvested at commercial maturity. 50-80 g of secondary florets from 3 plants within each replicate were sampled for glucosinolates analysis.
2. Broccoli was transferred on ice to the laboratory and blast frozen and stored at minus 80° C prior to freeze-drying in a 70 L capacity freeze-drier (Kushad, 1999).
3. & 4. The milled freeze dried samples were packed into accelerated solvent cells. GLS were extracted through Pressurized Liquid Extraction (PLE) using the following optimized conditions: 70% aqueous methanol at a pressure of 120 bars temperature at 50°C for three static cycles (Mohn, 2007).
5. Extracts were filtered (0.22  $\mu$ m) and analysed by HPLC using a RP C-18 analytical column. 5mM of TDAB in a 1:1 mixture of acetonitrile / distilled water was used as the mobile phase (Prester, 1998).

## Results & Discussion

HPLC chromatograms were scaled logarithmically and aligned using Correlation Optimized Warping (COW) prior to statistical analysis. The raw chromatograms were warped using a cophenetic correlation with section length equal to 5 units and a slack equal to 1 unit (Figure 1). Subsequently Principal component analysis (PCA) was performed on the whole data set to discern GLS profile patterns among the different Broccoli cultivars (Figure 2). Transformed HPLC data sets of Broccoli cultivars did not exhibit any particular pattern and could not be discriminated using PCA. However, PCA results revealed an interesting distribution among the samples. Results were grouped as a function of their position in the randomised complete block field design (Figure 3).

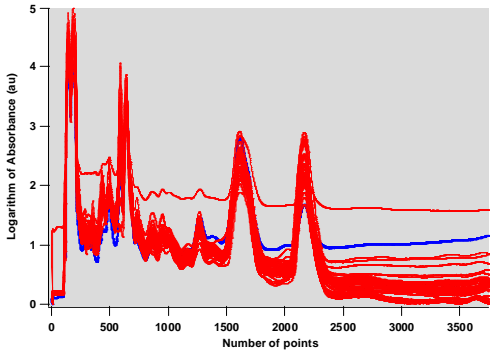


Figure 1. Warped chromatograms from broccoli extracts

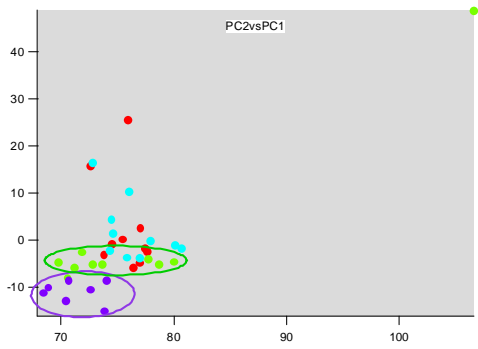


Figure 2. Scores plot from Principal Component Analysis

	R1	R2	R3	R4
Parthenon	Steel	Marathon	Monaco	
Naxos	Ironman	Iron	Tiburon	
Ironman	Beaumont	Parthenon	Beaumont	
Steel	Iron	Steel	Batavia	
Monaco	Tiburon	Naxos	Marathon	
Marathon	Naxos	Ironman	Iron	
Beaumont	Parthenon	Tiburon	Steel	
Iron	Monaco	Batavia	Ironman	
Tiburon	Batavia	Beaumont	Naxos	
Batavia	Marathon	Monaco	Parthenon	

Figure 3. Representation of the position of cultivars in the randomised complete block field design (RCBD)

## Conclusions

Results indicated that with regard to glucosinolate levels and profiles broccoli cultivars this trial were more easily differentiated according to their position in the field design rather than cultivar. All tested cultivars belong to a similar genetic background. Three samples from the R4 row in the RCBD were not analyzed because they did not survive to harvest due to harsh climatic conditions (August 2008). This may suggest that the difference in glucosinolate levels were due to agronomic stress and/or heterogeneity in soil. Solid state Magic Angle Spinning NMR spectra of all samples was recently recorded and will be analyzed in order to confirm this assumption. More field trials with different broccoli cultivars are also being performed.

## References

Mithen, R. F., Glucosinolates and their degradation products. In *Advances in Botanical Research*, Academic Press: 2001; Vol. Vol 35, pp 213-262.

Kushad, M. M.; Brown, A. F.; Kurilich, A. C.; Juvik, J. A.; Klein, B. P.; Wallig, M. A.; Jeffery, E. H., Variation of Glucosinolates in Vegetable Crops of Brassica oleracea. *Journal of Agricultural and Food Chemistry* **1999**, 47, (4), 1541-1548.

Mohn, T.; Cutting, B.; Ernst, B.; Hamburger, M., Extraction and analysis of intact glucosinolates--A validated pressurized liquid extraction/liquid chromatography-mass spectrometry protocol for *Isatis tinctoria*, and qualitative analysis of other cruciferous plants. *Journal of Chromatography A* **2007**, 1166, (1-2), 142-151.

Prester, T.; Fahey, J. W.; Holtzclaw, W. D.; Abeygunawardana, C.; Kachinski, J. L.; Talalay, P., Comprehensive Chromatographic and Spectroscopic Methods for the Separation and Identification of Intact Glucosinolates. *Analytical Biochemistry* **1996**, 239, (2), 168-179.

### Funding Body:



### Participants:

