

STSM Scientific Report 2009

Short-term scientific mission (STSM) within COST project programme on Euroberry

Sugar and antioxidant compounds from raspberries

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Objective: The aim of this STSM was to learn the use electrochromatography and liquid chromatography- mass spectrometric (LC/MS) to evaluate antioxidants and electro detection to evaluate sugar in different cultivar of raspberry.

The STSM was accomplished at the Scottish Crop Research Institute (Dundee, Scotland) in the work group of Dr. Derek Stewart within the period stipulated (14/10/09 to 11/12/09).

Materials and Methods

Study design and sample preparation: From a segregation population of raspberries generated in 2005, and grown in two different field. We carried out two main objectives:

1) To evaluate antioxidant compound:

- Select three half of raspberries and make a note of their weight. Put this sample into the homogeneizator.

- Add the same amount of as raspberry weight as the extraction solution (50% acetonitrile:water + 4 % acetic acid)
- Remove the liquid and cetrifuged at room temperature during 5 minutes at 13200 rpm. After that, remove the supernatant and centrifuge again. This new supernatant is the final sample which was stored at -20°C until analysis and it was called juice 50%.

Liquid Chromatography-Mass Spectrometry (LC-MS). The raspberry samples were analyzed on a LCQ-DECA system, controlled by the XCALIBUR software (2.0, ThermoFinnigan). The system comprised of a Surveyor autosampler, pump and photodiode array detector (PDA), and the Finnigan LTQ ion trap, a ThermoFinnigan mass spectrometer of new generation.

The photodiode array detector (PDAD) collecting data from 200-600 nm and scanning three discrete channels (at 280, 365 and 520 nm), and a ThermoFinnigan mass spectrometer ion trap. The column (4.6 mm x 150 mm x 4 µm Synergi Hydro C18 column with polar end capping (Phenomonex Ltd.)) temperature was kept at 30°C, and the autosampler (Surveyor AS, ThermoFinnigan) tray temperature control was set at 6 °C during sequence run.

Each sample was consisted of 25 µL Morin + 50 µL ultrapure water + 350 µL juice 50%.

Morin was chosen as internal standart.

Flow LC conditions were as follow: solvent A, 0.1 % formic acid in ultrapure water and solvent B, 0.1% formic acid in 50% acetonitrile at a flow rate of 200 µL/min over 38 minutes was used for separation, followed by further several minutes for the re-equilibration of the column prior to the next injection.

The gradient elution used was 0 - 6 min, 100 - 65 % A; 6 - 12 min, 65 - 62% A; 12 - 15 min, 62 - 60%A; 15 - 20 min, 60 - 40% A; 20 - 30 min, 0% A; 30 - 33 min, 0% A; 33 - 34 min, 100% A; 34- 40 min, 100% A.

The Finnigan LTQ LC-MS was fitted with an ESI (electrospray ionization) interface and samples were analyzed in negative-ion mode. This scan event, full scan analysis, was in mass range 80-2000 m/z.

2) To evaluate sugar

- Take 10 μL raspberry juice and add 990 μL ultrapure water and put in a hot place at 100 $^{\circ}\text{C}$ during 5 min.
- Centifuge at 1 $^{\circ}\text{C}$ to 13200 rpm during 5 min.
- Add 20 μL of supernatant and 980 μL ultrapure water into HPLC vials.

High Performance Liquid Chromatography: The raspberry samples were analyzed on a HPLC Dionex ICS-3000.

Flow LC conditions were as follow: solvent A, degassed ultrapure water and solvent B, 200mM NaOH solution, at a flow rate of 400 $\mu\text{L}/\text{min}$. The autosampler was kept at room temperature and the column (PA-20) at 35 $^{\circ}\text{C}$. The gradient was semi isocratic.

It used different sugar in different concentration as a standard: glucose and fructose to test sugar concentration in different accession of raspberry population in 2004 as B field as H field.

Results and Discussion

1) Antioxidant compounds:

Until now we have identified around 40 different peaks which correspond to different anthocyanins or ellagic acid derived, although when we have analyzed this peaks in the populations with PCA in the primary results appeared two different groups with different antioxidant content in the raspberry population. This segregation helped to search and select of the best raspberry cultivars. Actually, we are analyzing the final result.

2) Sugar:

We calculated the line regression of glucose (fig. 1) and fructose (fig. 2) with the standard samples.

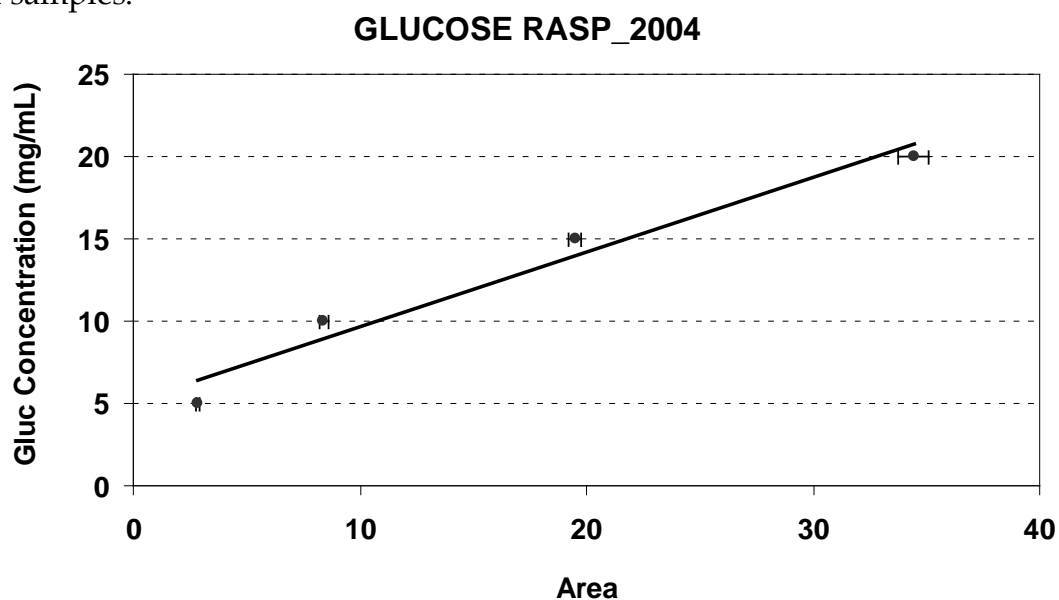


Figure 1. Linear regression between peak area and glucose concentration to raspberry population 2004. $Glc (mg/mL) = 0.4545 * Area + 5.0954$

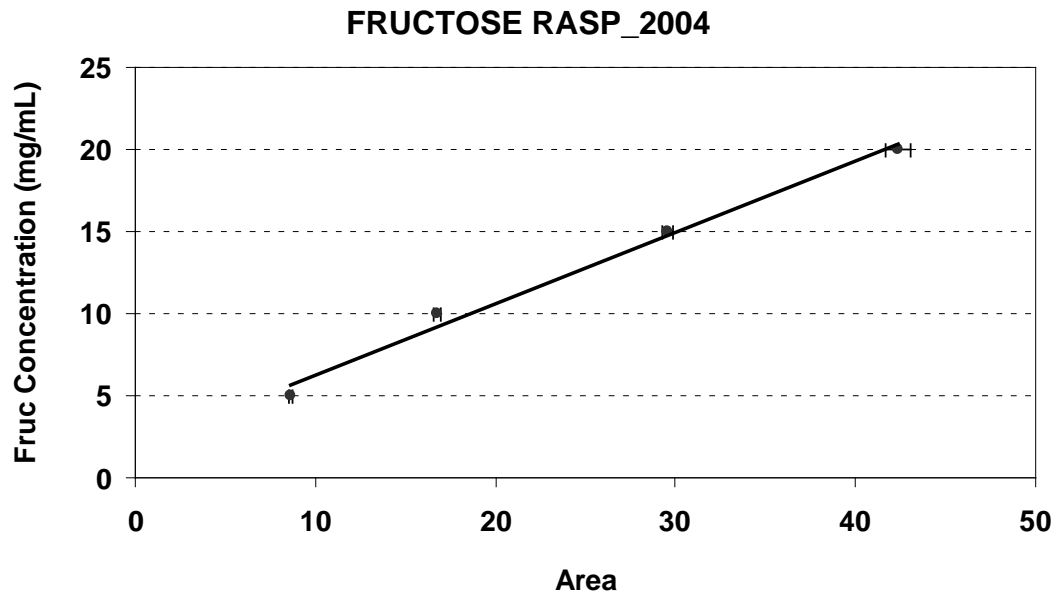


Figure 2. Linear regression between peak area and fructose concentration to raspberry population 2004. Fructose (mg/mL)= 0.4344*Area + 1.9287

Then we could know glucose in B field and H field respectively, (fig. 3 and 4) and fructose in B field and H field, respectively (fig. 5 and 6) concentration in the different accession of the raspberry population.

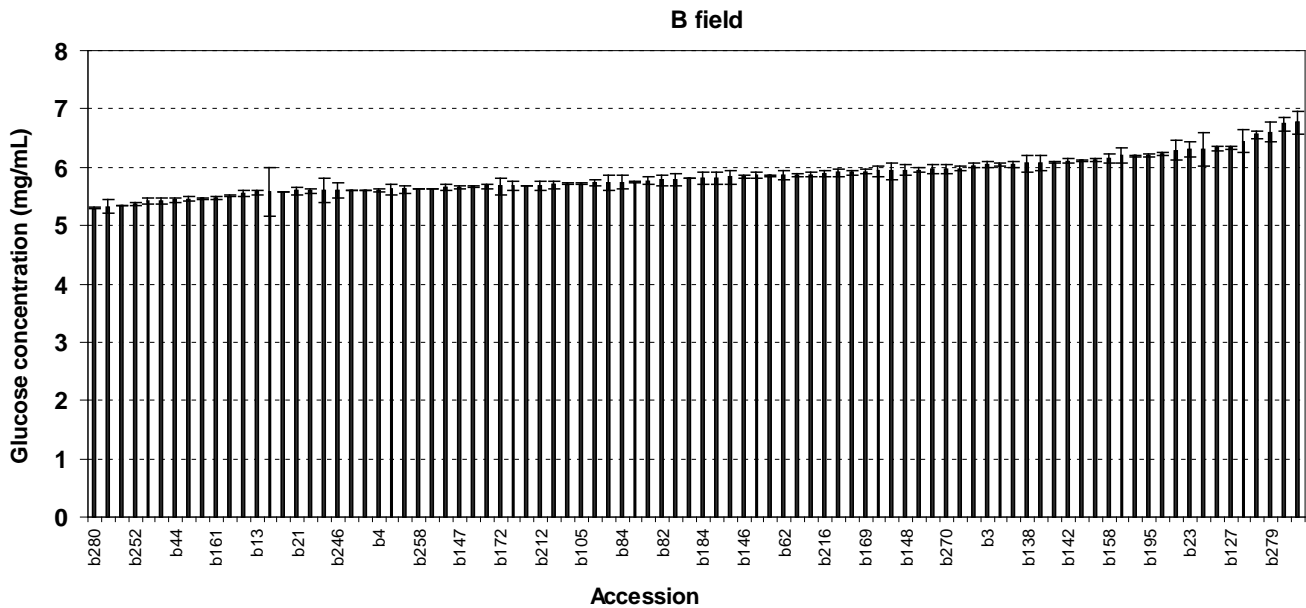


Figure 3. Glucose concentration of raspberry accessions in B field 2004.

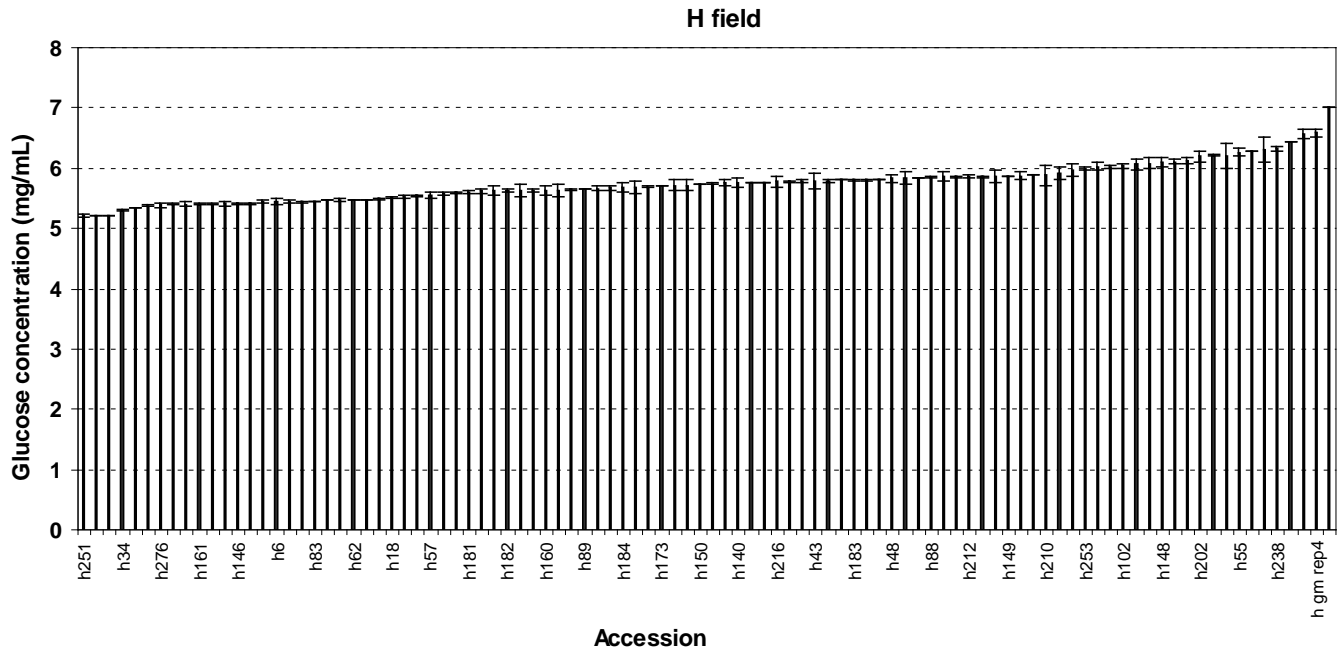


Figure 4. Glucose concentration of raspberry accessions in H field 2004.

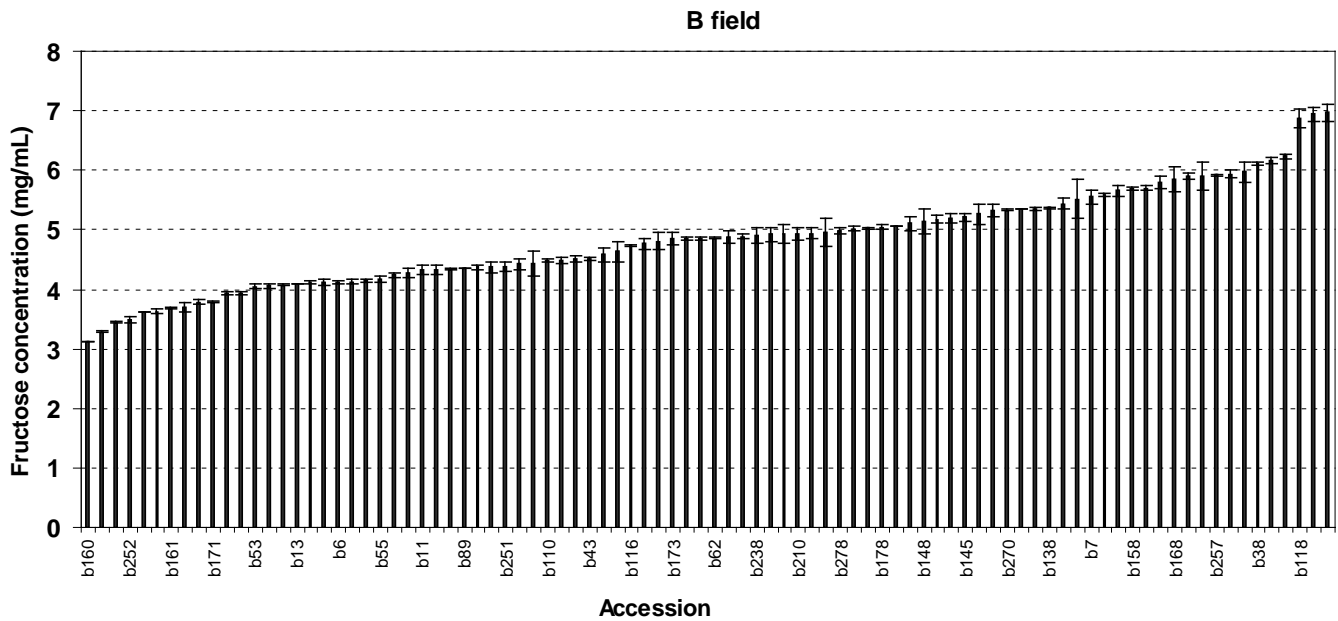


Figure 5. Fructose concentration of raspberry accessions in B field 2004.

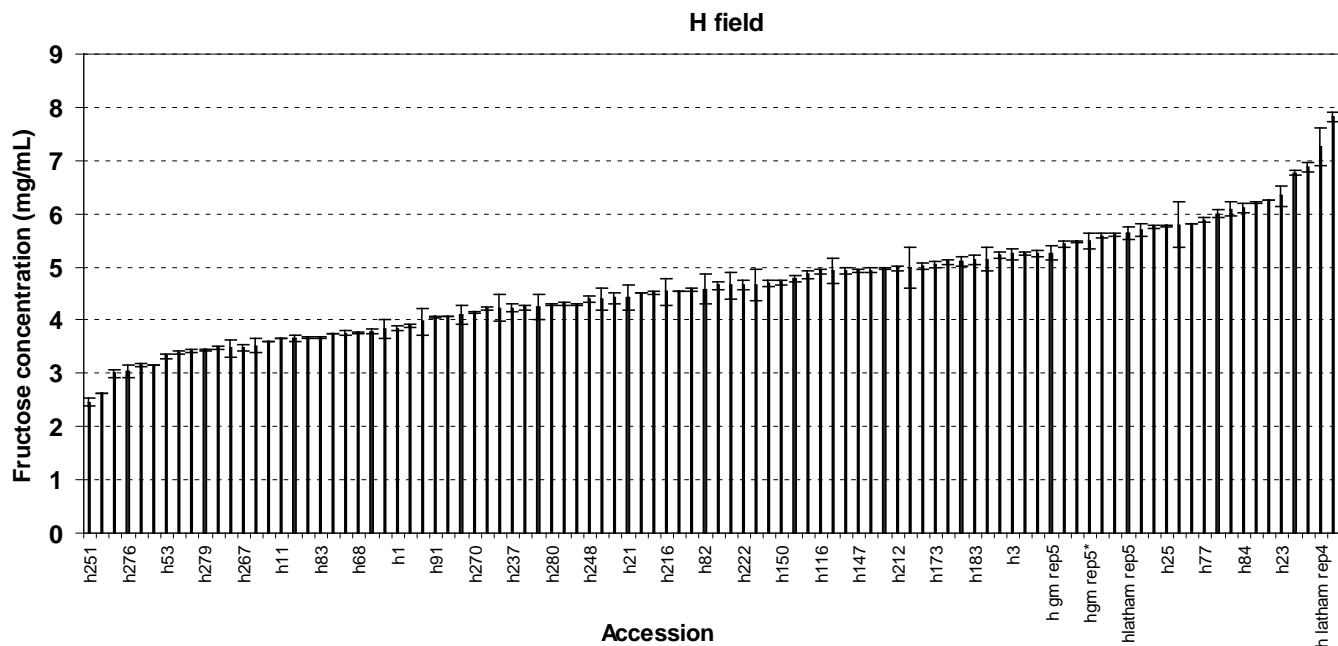


Figure 6. Fructose concentration of raspberry accessions in H field 2004.

Finally, we could observe that accessions shown different amount of glucose and fructose in the two experimental fields indicating a high environmental influence in these traits.

Conclusion

During the STSM I had the possibility to attend an intensive training on the use of Finnigan LTQ LC-MS, which was very useful in the mass spectrometry operations and data analysis.

I would like to take this opportunity to thank COST for making this informative and fruitful visit possible. I also thank Dr. Derek Stewart and colleagues for hosting me at the SCRI.

References

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