

# **STSM SCIENTIFIC REPORT**

## **References:**

Short Term Scientific Mission code: COST-STSM-863-03630

Action number: COST 863 (Euroberry Research: from Genomics to Sustainable Production, Quality & Health)

Applicant: Aurélie Petit, Cired Création Variétale Fraises Fruits Rouges, Douville – France

Host: Dr. Maurizio Battino, Università Politecnica delle Marche, Ancona – Italy

Period: from 18/04/2008 to 25/04/2008

STSM title: Determination of antioxidants, polyphenols, flavonoid and folate contents in cultivated strawberry.

## **Purpose of the STSM:**

This COST mission objectives are:

- (i) to learn and discuss the different methods for the determination of the Total Antioxidant Activity (TEAC assay), total phenolic content (Folin-Ciocalteu assay), total flavonoid content (colorimetric method using catechin) and total anthocyanin content (pH differential method using Pg-3-gluc) in cultivated strawberries.
- (ii) to initiate a scientific collaboration.

## **Description of the work carried out during the STSM:**

### **18.04.08:**

- Meeting with Sara Tulipani (Institute of Biochemistry, Dr. M. Battino's team) and Jacopo Diamanti (Department of Environmental and Crop Science, Dr. B. Mezzetti's team).
- Visit of the Jacopo Diamanti's field trials. Presentation of his study projects on strawberry, apple, apricot, cherry, peach, pear and plum. Discussion about the culture and harvest conditions.
- Meeting with Dr. Bruno Mezzetti. Visit of his laboratory. Presentation of his team projects.
- Meeting with Dr. Maurizio Battino.
- Visit of the laboratory with Sara Tulipani.
- Presentation of the different antioxidants, her study projects and results by Sara Tulipani.
- Discussion about the schedule of the STSM week with Sara Tulipani.

### **21.04.08:**

- With Jacopo Diamanti, learning of two strawberry hydrophilic (methanol and water 80% v/v) extraction methods (with a chopper and grinder and by homogenizing) from fresh and frozen fruits. Discussion about the both methods and the fruits and extracts preservation.
- With Jacopo Diamanti, learning of the Folin-Ciocalteu assay for determination of total phenol contents (Slinkard and Singleton, 1977). This colorimetric method is based on an oxidation/reduction reaction under alkaline conditions which results in blue colour with an absorbance maximum at 720 nm. By using Gallic acid (GA) as a standard for the calibration curve, results are expressed as milligrams of Gallic acid equivalents (GAE) per gram of fresh

- weight (FW) of strawberry. Cultivated strawberry total phenol contents average comes to 1.5 – 3.5 mg GAE/mg FW).
- With Jacopo Diamanti, learning of the Trolox Equivalents Antioxidant Capacity (TEAC) assay for determination of antioxidant capacity (Re *et al.*, 1999). This colorimetric method is based on the ability of antioxidants compounds to bleach the radical cation ABTS<sup>+</sup> with an absorption maximum at 734 nm. By using Trolox as a standard for the calibration curve, results are expressed as micromoles of Trolox equivalents (TE) per gram of fresh weight (FW) of strawberry. Cultivated strawberry antioxidant capacity average comes to 10-20 µmol TE/g FW.
  - Discussion with Dr. Bruno Mezzetti about my projects on strawberry antioxidants study. Dr. Mezzetti suggested me some procedures and methodologies (choice of number and varieties to test, choice of analysis...).

#### 22.04.08:

- Meeting with Dr. Stefano Bompadre (Institute of Microbiology and Biochemical Sciences)
- With Sara Tulipani, learning of the Flow Injection (FI) - ABTS decolorization assay for determination of antioxidant capacity (Bompadre *et al.*, 2004). This assay is a TEAC assay combined to a flow injection analysis (FIA) system. It is more complex to be set up but more sensitive and reproducible.
- With Sara Tulipani, learning of the strawberry hydrophilic (methanol and water 80% v/v) extraction method by sonication used for FI-ABTS assay and HPLC analysis (Herrera and Luque de Castro, 2004). Discussion about the different strawberry hydrophilic extraction methods, and the fruits and extracts preservation.
- With Sara Tulipani, learning of the Ferric Reducing Antioxidant Power (FRAP) assay for determination of antioxidant capacity (Benzie and Strain, 1996). This colorimetric method is based on the ability of antioxidants compounds to reduce ferric to ferrous ion resulting in a solution colour change measured at an absorption maximum at 593 nm. By using Trolox as a standard for the calibration curve, results are expressed as micromoles of Trolox equivalents (TE) per gram of fresh weight (FW) of strawberry. Cultivated strawberry antioxidant capacity average comes to 7-15 µmol TE/g FW.
- With Sara Tulipani, discussion about the three methods (TEAC, FI-ABTS and FRAP assays) for determination of antioxidant capacity: advantages, sensitivity and use following to the expected objective.
- Sara Tulipani gave me some publications and techniques procedures.

#### 23.04.08:

- With Sara Tulipani and Stefania (Institute of Biochemistry, Dr. M. Battino's team), learning of the technique for determination of total flavonoid content by aluminium chloride colorimetric assay at an absorption maximum at 510 nm (Dewanto *et al.*, 2002). By using catechin as a standard for the calibration curve, results are expressed as micrograms of catechin equivalents (CE) per gram of fresh weight (FW) of strawberry. Cultivated strawberry total flavonoid content average comes to 0.2-1 mg CE/g FW.
- With Sara Tulipani, learning of the pH-Differential method for determination of total anthocyanin content (Giusti and Wrolstad, 2001). This colorimetric method is based on the anthocyanin pigment property to change colour with a change in pH (from 1 to 4.5) at an absorption maximum at 500 nm. By using Pelargonidin 3-glucoside (Pg-3-gluc) as a standard for the calibration curve, results are expressed as milligrams of Pg-3-gluc equivalents per gram of fresh weight (FW) of strawberry. Cultivated strawberry total anthocyanin content average comes to 0.4-0.7 mg Pg-3-gluc/g FW.
- Oral presentation of my company and my study projects to Sara Tulipani.

- Discussion with Sara Tulipani about all the methods for determination of strawberry antioxidants contents and my projects on strawberry antioxidants study. Sara Tulipani suggested me some procedures and methodologies (choice of number and varieties to test, choice of analysis...), advised me on using the spectrophotometric techniques firstly and offered me to analyse some of my varieties with the FI-ABTS assay before setting up the technique.

24.04.08:

- Reading of publications.
- Answer to my last questions.
- Thanks.

25.04.08:

Public holiday.

### **Conclusion:**

The objectives of the STSM were achieved. I acquired the necessary knowledge to apply in the Ciref laboratory firstly the hydrophilic extraction methods and the five spectrophotometric techniques for the antioxidants contents determination in cultivated strawberry. In the short term it is possible to send to Sara Tulipani some fruits to analyse with the FI-ABTS assay. After discussion about different techniques and my projects objectives, first studies were drawn up.

### **Acknowledgements:**

I would like to take this opportunity to thank COST 863 for making this fruitful STSM possible. I also thank Dr. Battino, Dr. Mezzetti, Dr. Bompadre, Sara Tulipani, Jacopo Diamanti, Stefania and their colleagues for hosting me, showing me many techniques, answering all my questions and for the enlightened discussions.

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