SHORT TERM SCIENTIFIC MISSION (STSM) – SCIENTIFIC REPORT

The STSM applicant submits this report for approval to the STSM coordinator

Action number: **FA1403**
STSM title: **Measurement of anthocyanin-derived metabolites in plasma using LC-MS**
STSM start and end date: **03-04-2018** to **15-04-2018**
Grantee name: **Michele Tassotti**

PURPOSE OF THE STSM/

This STSM was addressed to measure anthocyanin-derived metabolites in plasma by using LC-MS, to better understand the inter-individual variability in the production of these phenolic metabolites. In particular, the study focused on plasma circulating metabolites after chronic consumption of purified anthocyanins. The assessment of the circulating metabolites directly originated from anthocyanins, represented a scientific challenge due to the high number of metabolites that might be formed. Besides the parent anthocyanins and their phase II metabolites, several colonic compounds can be produced and subsequently conjugated, which entails that dozens of compounds were targeted. The scenario is much more complex since a high inter-individual variability in the production of anthocyanin-derived metabolites has been reported and was studied herein. The identification and quantification of several metabolites, with the ultimate goal to explore the inter-individual variability existing in their production under chronic conditions, represented a very challenging and exciting project. Moreover, once processed, data will be correlated with some cardiometabolic outcomes, which can be useful to better understand the individual response to plant bioactive compounds consumption based on different metabolic patterns.

DESCRIPTION OF WORK CARRIED OUT DURING THE STSMS

Plasma samples of 27 volunteers were analysed. Subjects consumed 4 capsules a day of purified anthocyanins (320 mg anthocyanins in total) for 16 weeks. The samples were collected at the beginning and at the end of the treatment. Plasma samples were analysed with a previous validated µ-SPE protocol coupled with LC-MS, that allowed for high-throughput. Briefly, 600 µL of plasma were centrifuged at 15,000 x g for 15 min at 4 °C and 350 µL of the supernatant was diluted (1:1) with phosphoric acid 4% and spiked with standard mix (50 nM) as an internal standard. 600 µL were loaded on a 96 well µ-SPE HLB plate, washed with 200 µL of water and 200 µL of 0.2% acetic acid and finally eluted with 60 µL of methanol. Extracted and concentrated plasma samples were analysed with an Exactive™ Plus Orbitrap Mass Spectrometer Mass Spectrometer (Thermo Fisher Scientific Inc., San Jose, CA, USA) after separation using a Zorbax Eclipse Plus RRHD column 2.1 mm x 50 mm, 1.8 µm (Agilent, Waldbronn, Germany). The mobile phase consisted of 0.1% HCOOH (solvent A) and acetonitrile with 0.1% HCOOH (solvent B). The elution profile (flow rate of 0.4 mL/min) started at 1% solvent B and increased to 10% after 5 min, to 25% at 8 min and to 99% at 9.1 min. The percentage of solvent B was held constant for 0.9 min.
The following compounds were analysed: 1. *o*-coumaric acid, 2. 2-hydroxyhippuric acid, 3. chlorogenic acid, 4. Dihydroisoferulic acid 3-*O*-ß-*D*-glucuronide, 5. 3,4-dihydroxybenzaldehyde, 6. kaempferol-3-

glucuronide,
7. Caffeic acid, 8. 3-hydroxyhippuric acid, 9. 2,4-dihydroxybenzoic acid, 10. Dihydro Ferulic acid 4-*O*-sulfate, 11. m-coumaric acid, 12. Syringic acid, 13. kaempferol, 14. Homovanillic acid sulfate, 15. Protocatechuic acid, 16. t-cinnamic acid, 17. sinapic acid, 18. Caffeic Acid 3-*ß-D*-Glucuronide, 19. 3-hydroxybenzoic acid, 20. Dihydroferulic acid, 21. Pyrogallol-*O*-1-sulfate, 22. 4-hydroxybenzoic acid, 23. trans-ferulic acid, 24. 2-Methylpyrogallol-*O*-sulfate, 25. Isoferulic acid 3-*O*-sulfate, 26. 4-hydroxybenzaldehyde, 27. dihydrocaffeic acid, 28. 1-Methylpyrogallol-*O*-sulfate, 29. DihydroIsoferulic acid 3-*O*-sulfate, 30. Dihydro Ferulic Acid 4-*O*-ß-D-Glucuronide, 31. Dihydro Caffeic Acid 3-*O*-Sulfate, 32. Caffeic Acid 4-ß-D-Glucuronide, 33. Dihydro Caffeic Acid 3-*O*-ß-D-Glucuronide, 34. p-coumaric acid, 35. Isovanillic acid, 36. 2-hydroxybenzoic acid, 37. Pyrogallol-*O*-2-sulfate, 38. 4-hydroxyhippuric acid, 39. 4-Methylgallic-3-*O*-sulfate, 40. Quercetin-glucuronide, 41. 2,5-dihydroxybenzoic acid, 42. Homovanillic acid, 43. 3-hydroxyphenyl acetic acid, 44. (4R)-5-(3',4'-Dihydroxyphenyl)-gamma-valerolactone-40-*O*-sulfate, 45. vanillic acid, 46. Ferulic acid 4-*O*-glucuronide, 47. 3,4-dihydroxyphenyl acetic acid, 48. 4-hydroxyphenyl acetic acid, 49. benzoic acid, 50. Isoferulic Acid 3-*O*-ß-D-Glucuronide, 51. 4-Methylcatechol-*O*-sulfate, 52. Vanillic acid-4-*O*-sulfate, 53. isoferulic acid, 54. Ferulic Acid 4-*O*-Sulfate, 55. phenylacetic acid, 56. 2,3-dihydroxybenzoic acid, 57. Alfa-hydroxyhippuric acid, 58. Catechol-*O*-sulfate, 59. Hippuric acid.

DESCRIPTION OF THE MAIN RESULTS OBTAINED

At the moment the objectives achieved are:
- Development of a method to analyse anthocyanin-derived metabolites and biological samples.
- Analysis of the plasmatic samples using LC-MS.
- Identification and quantification of phenolic metabolites in plasma

Considering the large amount of data produced, the data processing is still ongoing. The results will be available as soon as possible.

FUTURE COLLABORATIONS (if applicable)

Working with Prof. Rodríguez-Mateos and her research team was a fundamental experience, which gave me the possibility to improve my professional skills.
During my STSM I was able to work with a new reality, which allowed me to cultivate professional and inter-professional relationships with colleagues not only from King's College.
Thanks to the STSM program of COST Action POSITIVe, our research groups have strengthened their relationships, paving the way for new future collaborations.