STSM SCIENTIFIC REPORT 2010

Short term scientific mission, Reference code: COST-STSM-863-05747

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Host: Prof Gary Williamson, University of Leeds – Department of Food Science and Nutrition, Leeds

Period: From 22/3/2010 to 2/4/2010
Place: Department of Food Science and Nutrition – University of Leeds

Trainee on urine and blood samples preparation from a nutrition intervention study on Berries

Objective:
The main objective in this STSM was to discuss the study design and the procedures to prepare urine and blood samples from Human volunteers to be further evaluated for bioavailability parameters after a berries nutrition intervention study.

Description of the work carried out during the STSM:
The first part of the STSM consisted in meeting the host, Prof Gary Williamson, to discuss the design of the bioavailability intervention study. Prof Williamson conducted a visit to the facilities of the host institute, where I got acquainted to the equipments necessary to perform a bioavailability intervention study, and the members of the host laboratory.
Regarding the planning of an intervention study, several parameters were discussed; the eligibility and exclusion criteria and the appropriate number of volunteers. It was decided that an initial screening interview will be performed to identify eligibility criteria: a food frequency questionnaire (with specific questions for patterns of food polyphenol consumption) will be filled in; the clinical history, anthropometric measurements, fasting blood and urine for biochemical analyses will be obtained; information

collected during the screening visits will also provide baseline data for subsequent analyses of the intervention effects.
If the candidate meets all the requirements, (s)he will be considered admitted. Each candidate will be provided with a detailed explanation of all procedures with an extensive written description of the study, anticipated time commitment and an informed consent form to be signed.
The study protocol will be submitted for approval to a Scientific Ethical Committee.
The diet of the volunteers during the washout period and the intervention study was also discussed, being necessary to control the food products allowed to be ingested. These must contain reduced level of polyphenols to avoid interference with the metabolites ingested in the intended products.
Intervention conditions were also under debate. Briefly, after an overnight fast, at the treatment day, a urine and blood sample will be collected at time point 0. Each volunteer will ingest the intended polyphenols enriched food product and from that time point, blood samples will be collected at intervals, between 0.5 and 24 h after the onset of treatment. Urine will also be collected periodically between 0 and 24 h. These samples will be appropriately treated and the metabolites will be analyzed by HPLC-MS.

The second part of the STSM, consisted in observing a bioavailability intervention study developed by a student under Prof Gary Williamson's supervision. It consisted in the ingestion of a characterized vegetable in time point 0, followed by collection of blood samples into EDTA-containing tubes, at several time points until 6h after the ingestion. Following the collection, the blood was immediately centrifuged and added ascorbate to preserve the metabolites, willing now to be stored at -80°C.
In this study, the total urine of the volunteers from the first 12 hours and the second 12 hours was distinctly collected to appropriate vessels containing ascorbate. The protocol to the collection and treatment of urine samples is described in annex I.

The possibility to observe and participate in this study was highly important for planning the design of a future bioavailability intervention study.

It allowed understanding and predicting technical difficulties regarding the timing of collection and treatment of blood samples, as well taking acquaintance of the procedures to conduct the study and interact with the volunteers in order to maintain their security and tranquility.

Conclusion:

The period spent at the Department of Food Science and Nutrition at the University of Leeds, under hosting of Prof Gary Williamson, was of high importance to improve the knowledge of the procedures and techniques necessary to conduct a bioavailability intervention study.

This fruitful cooperation will be continued along benefiting the subsequent studies developed in this area.

Acknowledgements:

I wish to thank to COST 863 for allowing me to perform this STSM.

I also wish to thank to Prof Gary Williamson for hosting me at his laboratory, answering all my questions and for the enlightened discussions, as well as to his lab members for their teachings and help.

Annex I

Urine Sample Pre-Treatment

Stage 1: Collection

i) Participants pick up urine collection equipment, consisting in 2 x 3L orange urine collection vessels, containing 3g ascorbic acid (vitamin C) powder in each.
ii) Participants then collect ALL urine for 24 hrs.
iii) Participants return urine collection equipment and samples:
   - Urine must be processed within 6 hrs following end of collection
   - Total volume of urine collected is measured and noted.
   - If distributed between 2 bottles, urine is pooled and gently mixed to ensure a homogenous distribution.
   - 2 x 40 mL portions of urine are measured into 50 mL falcon tubes and stored at -20°C. The remaining urine is discarded.

Stage 2: Preparation for storage

- To each 40 mL portion of urine, add 0,4 mL of 10% sodium azide, re-cap and vortex for 10 seconds.
- If you are continuing to stage 2 immediately, portion out 2 x 10 mM portions from a single tube into 2x appropriately labeled new 50 mL falcon tubes.
- Place remaining containers in -20°C storage.

Stage 3: Enzymatic hydrolysis

- To each 10 mL portion of urine, add 1.5 mL of enzyme-enriched sodium phosphate buffer (0.2 M, pH 7). A 1.5 mL portion of the enriched buffer contains 50 units of β-glucuronidase and 0.3 units of sulfatase.
- Cap all falcon tubes and place in a shaking water bath (100 rpm) at 37°C for 2 hours.
- WASHING PROCEDURE: add 10 mL ethyl acetate to the hydrolysed urine, and vortex for 2 minutes.
- Allow the layers to separate, then transfer the solvent layer to a new, labeled 50 mL falcon tube using a pipette.
- Repeat the washing procedure twice, so that you end up with 30 mL of ethyl acetate in each falcon tube.
- Once all of your falcon tubes are ready, they will be dried using a centrifugal evaporator at 40°C.
- Place dried down containers in -20°C storage.

Stage 4: Reconstitution and filtration

- Reconstitute the sample by adding 200 μL of LC-MS grade acetonitrile; vortex the falcon for 60 seconds and add 800 μL of 0.125% ascorbic acid solution.
- Transfer the samples to an Eppendorf tube. Centrifuge at 17,000 g for 10 minutes.
- Label 2 x 300 μL HPLC vials with all information.
- Using a clean syringe, pass the supernatant through a 0.2 mm filter and into the vial. Cap the vial and place into chilled storage until LC-MS analysis.