SCIENTIFIC REPORT - SHORT TERM SCIENTIFIC MISSION (STSM)

(COST Action FA1403, POSITIVe)

STSM topic: Gut bacteria and plant bioactive metabolism.

Grantee: Hulya Ilyasoglu Buyukkestelli (Ege University, Izmir, Turkey)

Host: Dr. Marion Leclerc (INRA, Micalis Institute, Jouy-en-Josas)

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Purpose of the STSM:

The purpose of this STSM was the evaluation of lutein bioaccessibility following *in vitro* digestion using COST Action 1005 Infogest procedure and undertaking experiments to decipher which microorganisms from the human gut microbiota might be involved in lutein breakdown after *in vitro* gastrointestinal digestion. As a source of lutein, red pepper and its paste form were used instead of using a pure lutein. In this way, the food matrix effect was also assessed on bioaccessibility and gut microbiota.

Description of the work carried out during the STSM:

Samples: Raw red pepper (*Capsicum annuum L.*) and two different brands of red peppers paste were used, they were obtained from Turkey.

***In vitro* digestion:** The experiment was performed according to Cost Action 1005 Infogest procedure, analyses were made at INRA UMR 782 GMPA with Thomas Cattenoz under the supervision of Dr. Steven Le Feunteun. *In vitro* digestion study design is shown in detailed at Figure 1.

Four different samples, chopped as small-sized and chopped as big-sized raw red pepper, two commercial brand red pepper pastes, were used. Also control samples (containing only enzymes and digestion fluids) were prepared for further microbiology tests. Each digested fraction was obtained by duplicate from each sample. All fractions were freeze-dried, weighed and stored at -20 °C. Oral+gastric digested fractions were stored at -20°C for further lutein bioaccessibility analyses, also one replicate of oral+gastrointestinal digested fraction was stored -20°C for the same purpose and the other one was for microbiology tests.

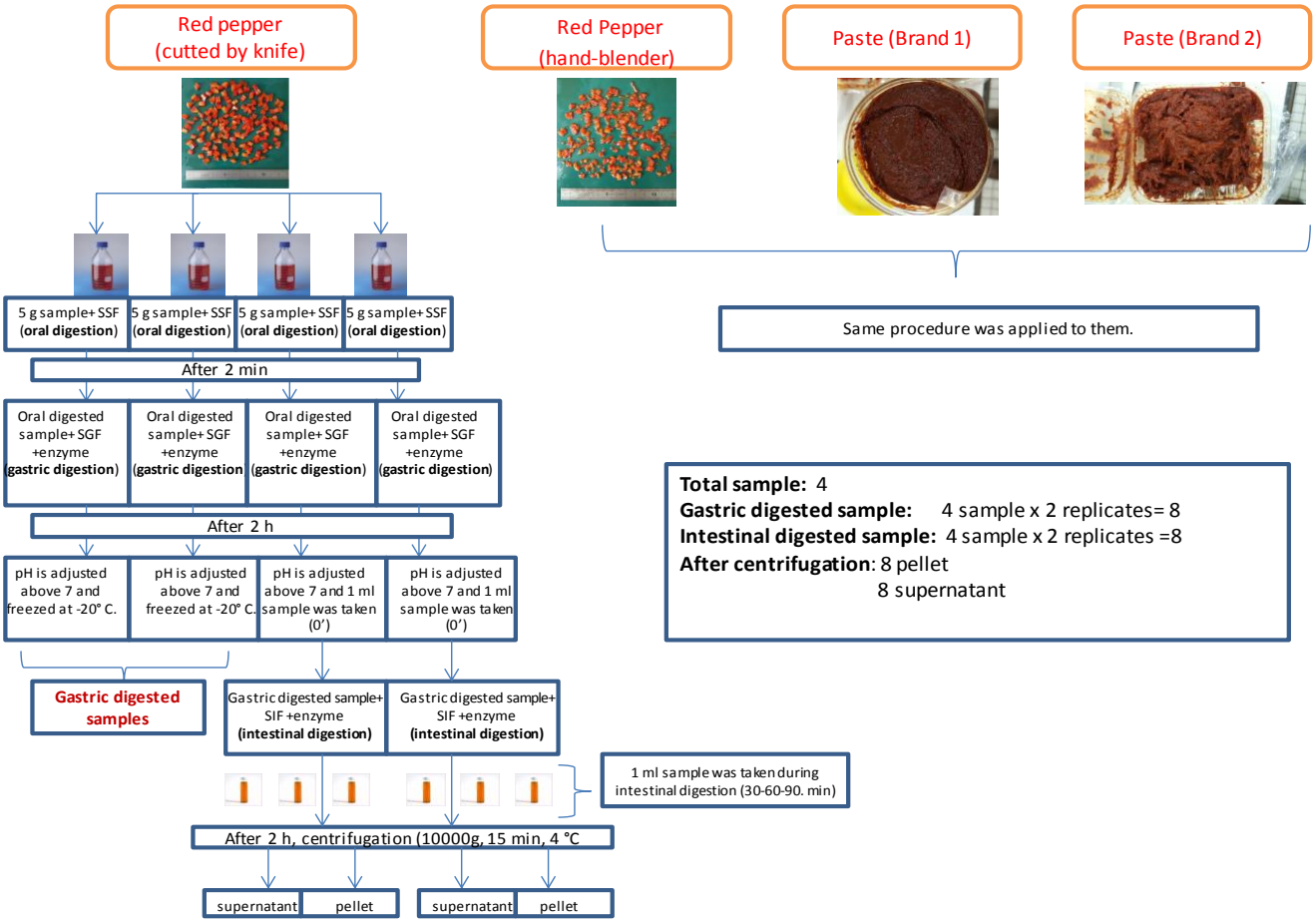

Figure 1. Study design for *in vitro* digestion

Microbiology tests: Samples obtained after *in vitro* digestions were then subjected to microbiology tests. Freeze-dried fractions were incubated with inoculum (prepared from healthy individiual faeces) and also with four different bacterial species.

- **Inoculation with different bacterial species in pure culture:**

  The incubation was performed with four different bacterial species, Bacteroides vulgatus, Bifidobacterium longum, Faecalibacterium prausnitzii and Ruminococcus lactaris. They were chosen because they are dominant in healthy human gut microbiota, and they are representative of the 3 main phyla, Bacteroidetes, Firmicutes, Actinobacteria.

  For this experiment, a growth medium with a limited concentration of glucose was used (5g/l). Substrates were weighted to Hungate culture tubes and 6 ml of growth medium was added to obtain a final concentration of 5 g/l. All substrates were incubated with each bacterial species by triplicate at 37°C for 94 hours. Optical

density at 600 nm was monitored and gas production was checked in the Hungate culture tubes. 250 µl of sample was collected to perform DNA extraction.

- **Inoculation with fecal microbiota from healthy individuals:** At this step, main purposes were to determine a) which bacterial species are able to grow and metabolize the compound, b) how individuals intestinal microbial ecosystems, including uncultured bacteria, are modified upon incubation with the lutein.
  For the experiment, faecal slurry was prepared from 1 g of fecal sample from healthy individuals and 5 ml of buffer. Substrates were weighted to Balch tubes and 1 ml of faecal slurry was added to all tubes. Each substrate was incubated with three different faecal slurry at 37 °C. Aliquots of 250 µl were used for DNA extraction. Growth, microbial diversity (and possibly with additional fundings expressed genes) can be monitored. Samples will be taken for metabolomics analysis to be performed.

DNA extraction: It was performed using the PowerFecal DNA isolation kit after a step of mechanical lysis with a powerlyser for 10 min. DNA concentration was measured using a Nanodrop measuring the 260/280 nm ratio. All extracts were stored at -20 °C for further analysis.

Future collaboration with the host institution and foreseen publications/articles from STSM:

The DNA analysis obtained from the culture could not be finished because ot time constraints. The next steps are:

-quantification of bacterial biomass by qPCR to obtain the number of bacteria per ml of culture.

-The PCR amplification to perform 16S rDNA sequencing of the bacteria in the human faeces incubation, before and after the addition of the substrates. This will indicate which bacteria are increased or in presence of the substrates and provide with a picture of the ecosystem changes upon the addition of the substrate. The correlation with lutein measurements will then be possibly done. All these analyses will be handled at host institution.

Also lutein extraction and lutein analysis by HPLC will be performed for all samples before and after *in vitro* digestion to determine bioaccessibility, analyses will be handled at home

institution. Foreseen publication will be discussed later with the results of the study after all analyses have completed.