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 beer´s polyphenol levels in urine of an interventional study with beer in high cardiovascular risk patients.
STSM start and end date: 12/03/2018 to 15/04/2018
Grantee name: Victor Micó Moreno

PURPOSE OF THE STSM/

(max.500 words)

The main objective is the detection of phenolic compounds in urine of patients of an intervention beer intervention study. A secondary objective is the specific detection of urine isoxanthumol. This polyphenol has been describe as a good biomarker of beer consumption and will serve in our study as a biomarker to assess the quality of our intervention. IMDEA Food Institute has carried out an interventional study in high cardiovascular risk patients to investigate the effect of alcoholic and non-alcoholic beer (miRoBEER) on traditional CVD risk factors as well as microRNA concentrations. We recruited seven men from 25 to 65 years old, non-smokers, with at least 2 or more criteria of Metabolic Syndrome.

The intervention of the patients was performed as follows, blood and urine sample were collected at the end of each period.

- Initial washout period of 7 days.
- Alcoholic beer intervention of 14 days
- Intermediary wash out period of 7 days
- Non-alcoholic beer intervention of 14 days

We analysed the circulating and macrophage levels of a panel of 56 microRNAs with a suggested role in cardiovascular disease. We detected that beer consumption significantly modified the expression of some of these microRNAs suggesting that some of the beneficial effects of beer on cardiovascular health could be mediated by microRNAs. In addition to this, we want to investigate whether beer polyphenols were associated with the changes in microRNAs and they could be driving some of the mechanisms by which beer influences cardiovascular health through epigenetics.

DESCRIPTION OF WORK CARRIED OUT DURING THE STSMS

(max.500 words)

The proposed work has been carried out as follows:

From the origin laboratory was sent urine and plasma samples of each intervention point: basal time 1, alcoholic beer intervention, basal time 2 and non-alcoholic beer intervention. In addition to this, it was sent a sample of each beer type which the study was carried out.

After a bibliography revision 12 polyphenols that are presented in beer were selected to check the presence in volunteers' biofluids. The polyphenols selected were:

- Gallic acid
- Catechin
- 4-Hydroxybenzoic acid
- Epicatechin
- Vanillic acid
- Chlorogenic acid
- Caffeic acid
- p-Coumaric acid
- Ferulic acid
- Sinapic acid
- Isoxanthohumol
- 8-Prenilnarigerin

However, we decided to do a screening of 80 total phenolic compounds in order to check polyphenols that are influenced by beer consumption.

As a first step of the beer polyphenols analysis, we did a calibration curve of the specific polyphenols of beer: isoxanthohumol and 8-prenilnarigerin. In addition to this, we measured this polyphenols in the original beer samples (alcoholic and non-alcoholic beer) that the patients drank during the study.

The analysis of plasma and urine sample was carried out using 500 µL of plasma and urine. Samples were centrifuged at 15,000 x g for 15 min at 4°C and 353 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 analyzed with purifying the samples with a solid-phase extraction using Oasis® HLB µElution plate. This process allows 5-times concentration of the initial sample to improve the polyphenols detection.

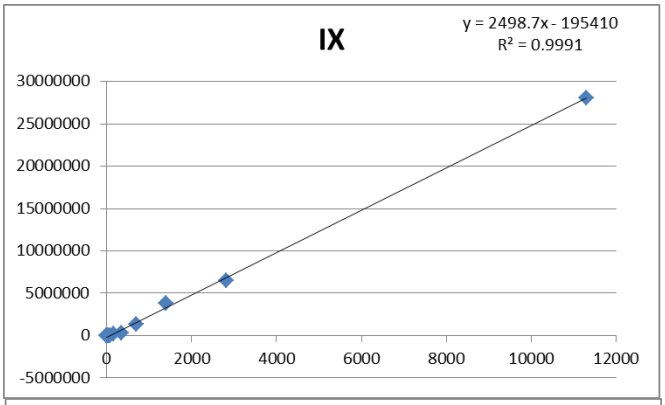
When the samples were purified, they were analysed using a Thermo Scientific™ Exactive™ Plus Orbitrap. A total of 82 compounds were analysed during the samples runs. The results were analysed using Xcalibur software.

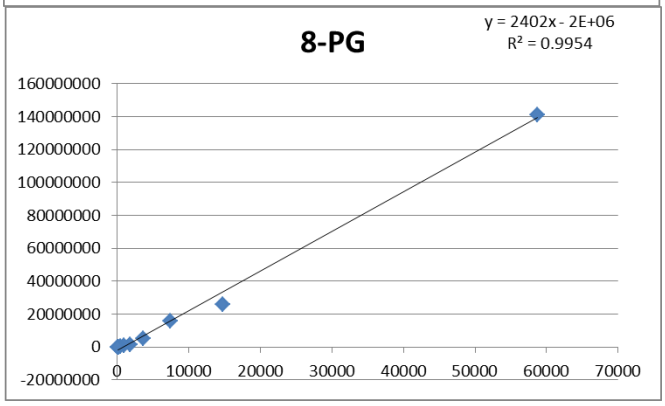
DESCRIPTION OF THE MAIN RESULTS OBTAINED

(max. 500 words)

Note: some of the results are still under analysis. Only preliminary results can be showed in this report. If it was necessary we can send an expanded report with all the results obtained.

Calibration curves of Ixoxanthohumol and 8-Prenilnarigerin:





Mesaurement of isoxanthohumol and 8-prenilnarigerin in Alcoholic and non-alcoholic beer:

	IX (area)	nM	Retention Time	8-PG (area)	nM	Retention Time
Alcoholic beer 1	6374644	2629.389	9.34	749704	1144.756	9.5
Alcoholic beer 2	6011584	2484.089	9.32	48118	852.672	9.5
Alcoholic beer 3	5880176	2431.499	9.33	26596	843.712	9.48
Non-alcoholic beer 1	1489962	674.500	9.31	3477197	2280.265	9.54
Non-alcoholic beer 2	1000676	478.683	9.32	2761244	1982.200	9.58
Non-alcoholic beer 3	1359050	622.107	9.34	3912768	2461.602	9.59

	IX (nM)	SD	8- PG (nM)	SD
Alcoholic Beer	2514.99	102.50	947.05	171.28
Non-alcoholic beer	591.76	101.37	2241.36	242.06

Mesaurement of isoxanthohumol and 8-prenilnarigerin in Alcoholic and non-alcoholic beer:

Results under analysis.

FUTURE COLLABORATIONS (if applicable)

(max.500 words)

The results obtained in this collaboration are very promising. The relation between the identification and quantification of phenolic metabolites in urine and plasma after 2 week consumption of beer and alcohol-free beer in patients at high cardiovascular disease risk and the epigenetics changes produced in the same intervention is a new approach in the influence of food intake with health. This first collaboration between our departments could open a new way of future projects and collaboration combining the expertise of both institutions. I would like to acknowledge also the STSM program of COST Actions for the opportunity to do this STSM due to the great personal and work opportunity that proportionated me developing this project.