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BioBanaTom Study: Metabolomics to identify biomarkers of banana and tomato intake

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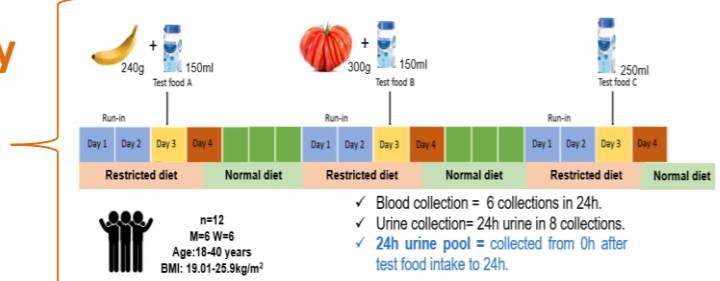
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INTRODUCTION

Untargeted metabolomics allows the discovery of biomarkers of intake of different foods that provide more reliable information about the dietary habits and compliance of volunteers in observational studies and clinical studies than that offered by FFQ or 24hr questionnaires. Banana and tomato are ranked amongst the most eaten fruits and vegetables in the world. Interestingly, there are no biomarkers of intake reported in the literature yet and little is known about the effect of banana intake on human health. We therefore first focus on banana. In order to elucidate novel biomarkers for acute intake of banana we applied an untargeted metabolomics approach on 24h urine samples of 12 volunteers that participated in an acute intervention study with banana, tomato and a control drink. In parallel, to identify long-term intake biomarkers of banana intake we applied the same methodology to morning spot urine samples of 45 high and low consumers of banana that participated in the SU.VI.MAX2 cohort study.

BioBanaTom Study Design

- Crossover
- Randomized
- Open controlled trial



SU.VI.MAX2

- Cohort Study



UNTARGETED METABOLOMICS WORKFLOW

1. UPLC-QTOFMS (QTOF MS Impact II Bruker)

- Column BEH shield RP10 100x41x1.7
- 25 min gradient
- ESI(+) and (-)

2. Data pre-processing

- Ion Extraction
- Annotation of ions (CAMERA)

3. Multivariate and Univariate Analysis

- PCA and OSC-PLSDA
- Student T test and Wilcoxon (BH)

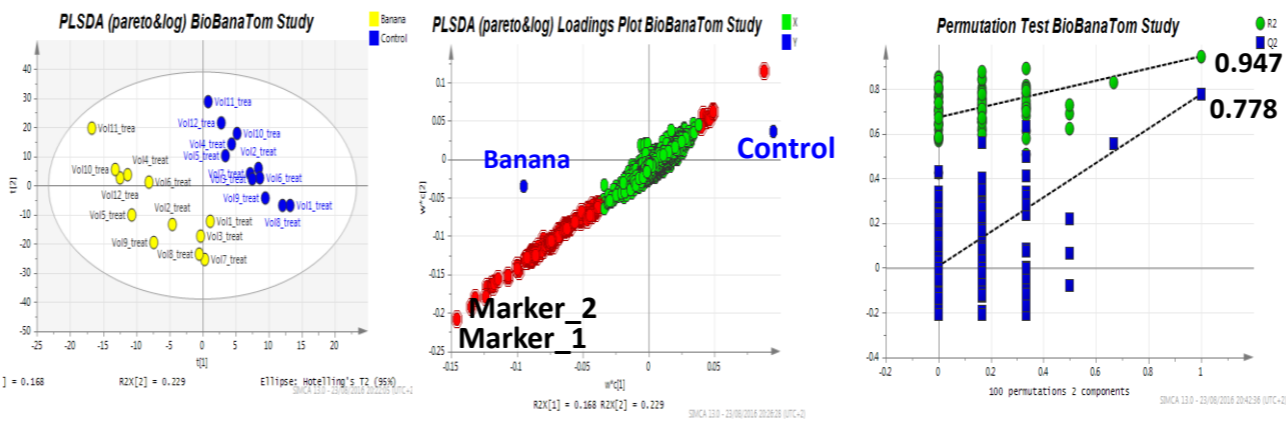
4. Identification of Features

- LTQ-Orbitrap MS
- Database query.
- Literature search.

RESULTS

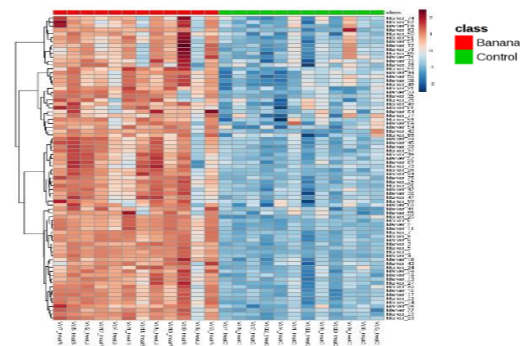
BioBanaTom Study

- The total number of extracted ions was **4092** of which **103 ions** showed a high contribution to the discrimination among groups on the PLSDA model (VIP>2).

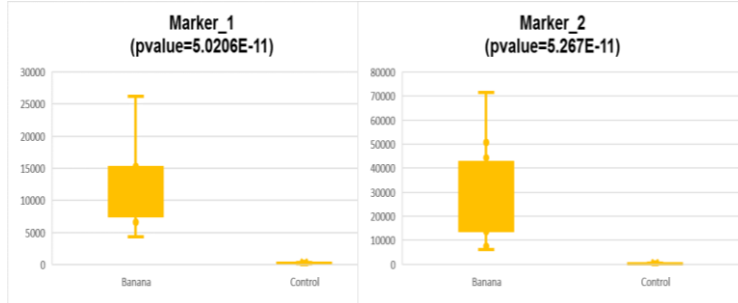


- After univariate analysis using Student T test with Benjamini-Hochberg correction we detected 85 significant ions. All of the latter also had VIP >2. Of these ions, **78 ions** were found in higher intensity in the banana intake group.

Heat map of 78 most significant metabolites after banana intake



- We detected **38 metabolite clusters** according to the RT of the 78 candidate biomarkers, which will now be identified.
- Some of the discriminative biomarkers of the acute intake of banana are:

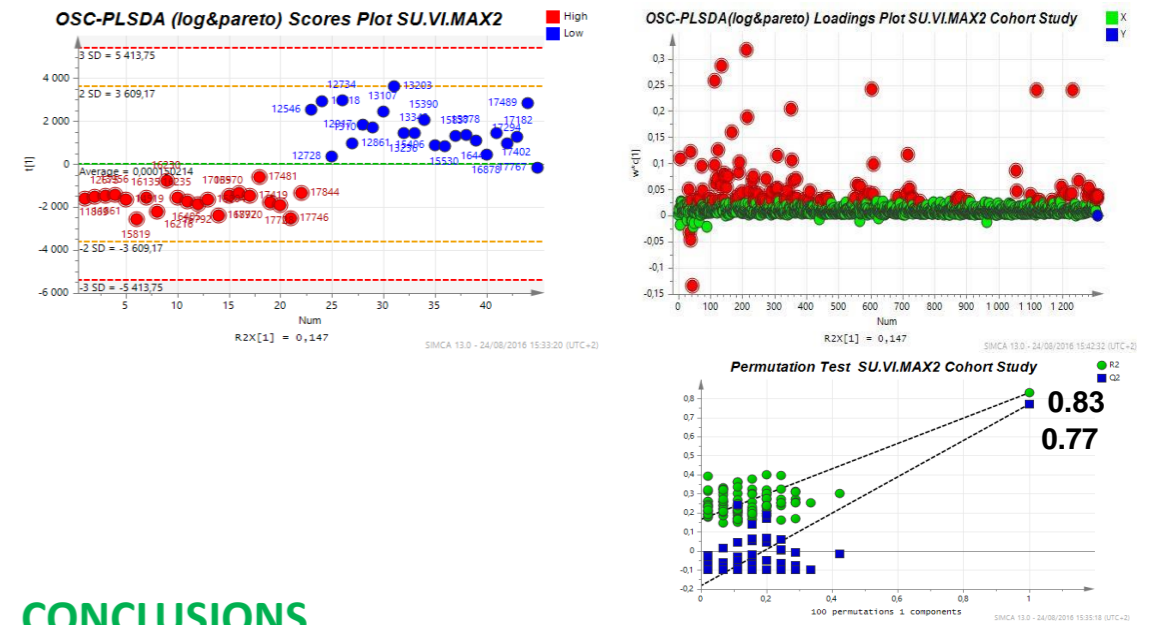


Boxplots of the two most significant ions in univariate analysis; the p value of Student test with Benjamini Hochberg Correction.

- Kinetic urine and plasma samples are available and will be further analyzed.

SU.VI.MAX2

- In total **10,249** ions were extracted. Due the high number of ions detected given by the sensitivity of the instrument, we performed a pre selection of the significant ions among groups using a **Wilcoxon Test (pvalue <0.05)**. We obtained **1308 significant ions** on which we performed an OSC-PLSDA.
- With multivariate analysis (OSC-PLSDA), **111 ions** were identified as discriminant markers (VIP>1) between high and low consumers of banana.
- We observed that only **3 metabolites** (VIP>1 and p-value <0.05) were in higher intensity in the high consumer group.
- Most the discriminant ions in multivariate and univariate analysis, had higher intensities in the low consumers group which suggests that these may be markers of effect of the long term consumption of banana rather than biomarkers of intake.



CONCLUSIONS

By applying an untargeted metabolomics approach, we were able to observe a clear discrimination in the urine samples of subjects after an acute intake or long term consumption of banana compared to non consumers. In the cohort study we were able to detect what mainly seem markers of effect of the long term intake of banana while in the acute study mainly found candidate biomarkers of banana intake. Currently, the identification of the markers selected in these two studies is in progress to later on determine their biological plausibility and validate their utility as biomarkers of banana intake