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Two complementary metabolomics studies to identify biomarkers of banana intake

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INTRODUCTION

Banana is one of the most consumed fruits worldwide. However, little is known about its effect on human health and interestingly, there are no biomarkers of intake reported in the literature. The identification of novel biomarkers of intake provides more reliable information about dietary habits and compliance of volunteers in observational studies and clinical studies. The application of an untargeted metabolomics approach to the assessment of the food metabolome allows the recognition of patterns of dietary exposure among individuals. The latter makes the identification of bioactive compounds and biomarkers of consumption possible. In order to elucidate novel biomarkers for acute and long term intake of banana, we applied an untargeted approach on 24h urine samples of 12 volunteers that participated in an acute crossover intervention study (BioBanaTom) with different test foods, including raw banana, and in morning spot urine samples of 45 high and low consumers of banana that participated in the French Cohort SU.VI.MAX2.

BioBanaTom

BioBanaTom aims to identify novel biomarkers of tomato and banana intake. It is a crossover, randomized, open-controlled trial. This study is part of series of intervention studies from the FoodBall project.

Untargeted Metabolomics Workflow

1. Profiling of Urine Samples
   - UPLC-QTOF-MS (QTOFMS impact E (Bruker))
   - Centaurx, FURO-silient RPHP18 100 X 4.6 X 7
   - 25mm gradient
   - ESI(-) and (+)

2. Data pre-processing
   - Ion extraction (GC/MS)
   - Annotation (CAMERA)

3. Multivariate and Univariate Analysis
   - SIMCA
   - PCA
   - OPLS-DA and OSC-PLSDA
   - t Test (BH correction)

4. Identification of discriminant ions
   - Boxplots of the two most significative ions in univariate analysis; the p value of Student test with Benjamini-Hochberg correction.

BioBanaTom

• 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 significative ions. All of the latter also had VIP >2. Of these ions, 78 ions were found in higher intensity in the banana intake group.

• We detected 36 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:

Heat map of 78 most significative metabolites after banana intake

Bioplots of the two most significative ions in univariate analysis; the p value of Student test with Benjamini-Hochberg Correction.

• Kinetic samples are available and will be further analyzed.

SU.VI.MAX2

Two groups of subjects were selected from the SU.VI.MAX2 cohort according to their habitual intake of banana assessed by traditional dietary questionnaires (FFQ and 24h recall) between 1998 and 2009. In this study one morning spot urine was collected between 2007 and 2009.

• 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 significative ions among groups using a Wilcoxon Test (pvalue <0.05). We obtained 1308 significative ions on which we performed an OSC-PLSDA to determine the ions that were the most discriminative between high and low consumers of banana (VIP>1).

• 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 pvalue <0.05) were in higher intensities in the high consumption group.

• Most the discriminant ions in multivariate and univariate analysis had higher intensities in the low consumers group suggesting that these may be markers of effect of the long term consumption of banana rather than biomarkers of intake.

• Kinetic samples are available and will be further analyzed.

Results

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.

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