Applying an untargeted metabolomics approach using two complementary platforms for the discovery and validation of banana intake biomarkers

N. Vázquez-Manjarrez¹,², C. Weinert ⁴, M. Ulaszewska³, C. Mack⁴, M. Pétéra⁶, P. Micheau⁶, C. Joly ⁶, D. Centeno ⁶, S. Durand⁶, E. Pujos-Guillot ⁶, B. Achim⁵, S. Kulling ⁴, L.O. Dragsted², C. Manach¹

¹ Université Clermont-Auvergne, INRA, Human Nutrition Unit, Clermont-Ferrand, France
² University of Copenhagen, Department of Nutrition Exercise and Sports, Copenhagen, Denmark
³ Fondazione Edmund Mach, Dipartimento Qualita Alimentare e Nutrizione, San Michele All’adige, Italy
⁴ Max Rubner-Institut (MRI), Department of Safety and Quality of Fruit and Vegetables, Karlsruhe, Germany
⁵ Max Rubner-Institut (MRI) Department of Physiology and Biochemistry of Nutrition, Karlsruhe, Germany
⁶ Université Clermont Auvergne, INRA, UNH, Plateforme d’Exploration du Métabolisme, MetaboHUB, Clermont, Clermont-Ferrand, France.

* Corresponding author: claudine.manach@inra.fr
What do we know about banana?
What do we know about banana?

- Highly consumed fruit in different countries.

- Intake of unripe banana ameliorates diarrhoea in children.

- Biomarkers of banana intake following a meal intervention have not yet been reported.
Why do we need biomarkers?

- Strengthening the information obtained from paper based dietary assessment tools (FFQ, 24HR) is needed.
- The use of biomarkers of intake to determine dietary exposure offers more objective information.

Cheung, W et al 2017 A metabolomic study of biomarkers of meat and fish intake
doi:10.3945/ajcn.116.146639
Kristensen M, et al 2017 A High Rate of Non-Compliance Confounds the Study of Whole Grains and Weight Maintenance in a Randomised Intervention Trial
Main Objective

✔ Identify and validate novel urinary biomarkers of intake of banana using an untargeted metabolomics approach.

✔ Untargeted metabolomics approach in two different platforms (UPLC-QTOF-MS and GC×GC-MS) to analyse urine samples of two different study designs.
**Discovery**

RCT, cross-over

- n=12
  - M=6 W=6
  - Age: 18-40 years
  - BMI: 19.01-25.9 kg/m²
  - Nonsmokers

- Banana 240g + 150ml of control drink
- Wash out period: 3 days
- 250ml of control drink
- Banana 240g + 150ml of control drink

- Test Food
- Standardized lunch on site
- Standardized dinner at Home

- 24h urine in 7 time intervals
- 24h urine pool

**Validation**

The KarMen Study *Bub A et al., 2016* doi: 10.2196/resprot.5792

- n=301
  - Healthy men and women
  - Age: >18 years
  - Nonsmokers

- Declared intake of banana
  - 24HR questionnaires

- n=26 “High-consumers” (176±59 g banana)
- n=26 “Low-consumers” (87.7±12 g banana)
- n=26 “Non-consumers”

- 24h urine pool
Meal intervention Study
- UPLC-QTOF-MS
- Workflow4metabolomics
- XCMS for spectral data analysis.
- CAMERA for ion annotation.
- Data Preprocessing
- Data Cleaning
- Data Analysis
- ESI (+) 2,714
- ESI (-) 1,289
- OSC-PLSDA (VIP>2)
- Student paired T test (p-FDR<0.05)

Cohort Study
- UPLC-QTOF-MS
- Workflow4metabolomics
- XCMS for spectral data analysis.
- CAMERA for ion annotation.
- Data Preprocessing
- Data Cleaning
- Data Analysis
- ESI (+) 2,427
- PLSDA
- Student T test (p-FDR <0.05)
- Logistic Regression with AIC
74 ions had a VIP>2
47 ions have a higher intensity in the banana group
Score Plot OSC-PLSDA Banana vs Control NEG

40 ions had a VIP>2
37 ions have a higher intensity in the banana group

22 ions with p<0.05 BH
22 ions Higher in Banana

All significant ions in univariate have a VIP>2
Identification pipeline overview

- **Identification of significant parent ions**
  - MS Scan QTOF and Orbitrap

- **Query of databases and literature for biologically plausible compounds.**
  - HMDB, FooDB, Phytohub, Knapsack, CHEBI, DFC

- **Determination of elemental formula**

- **Fragmentation experiments**
  - MSMS QTOF (Impact II Bruker)
  - MSMS Orbitrap (LTQ, orbitrap velos hybrid mass spectrometer)

- **Query of Spectral libraries for specific compound or compound classes**
  - Mzcloud, RESPECT, Mona, HMDB, FooDB, Metlin, Metfrag, CSI finder

- **Plausible candidates**
  - Acquisition of chemical standards
  - Enzymatic conjugation of standards
  - MSMS experiments for spectral matching

- **Metabolism predictions of banana compounds.**
Salsolinol | Dopamine | Serotonin | 6-OH-1-methyl-1,2,3,4-tetrahydro-β-carboline | Tryptophan | Methoxyeugenol | 2-isopropylmalic acid | Mevalonic acid | Eugenol | Vanillic acid

Salsolinol Sulfate | Dopamine Sulfate | Salsolinol Sulfate* | 6-OH-1-methyl-1,2,3,4-tetrahydro-β-carboline Sulfate | Kynurenic acid | Methoxyeugenol glucuronide | 2-isopropylmalic acid | Mevalonic acid | Eugenol Sulfate | Vanillic acid sulfate

*putatively annotated
Untargeted metabolomics

Discovery

Validation

Candidate biomarkers

Are they reliable in less controlled scenarios?
22 highly discriminant features in the meal study are able to predict the intake of banana with a good sensitivity and specificity.

Is there a more parsimonious biomarker?
Student T test
FDR-correction

Features with p-FDR value <0.05 were selected as confirmed biomarkers of banana intake

Logistic Regression with AIC to obtain a parsimonious biomarker of banana intake

Validation

KarMen Study

Sensitivity (CV) = 84.6%
Specificity (CV) = 92%

5 metabolites

m/z 195.1014+ m/z 283.0747

Sensitivity (CV) = 84.6%
Specificity (CV) = 92%

Parsimonious biomarker of banana intake!
Good sensitivity and higher specificity
Untargeted GCxGC-MS analysis

- To obtain a broader coverage of biomarkers of banana intake.
- Confirm the robustness of the biomarkers of banana intake identified using UPLC-QTOF-MS.
Previously observed in UPLC-QTOF-MS:

- Dopamine
- 3-methoxytyramine
- 5-HIAA
- Methoxyeugenol
- 2-isopropylmalic acid

**Discovery**

- **Dopamine**
- **3-methoxytyramine**
- **5-HIAA**
- **Methoxyeugenol**
- **2-isopropylmalic acid**

**Validation**

- **A1547_HIAA**
  - p<0.0001
- **A1439_DOP#2**
  - p=0.0001
- **A0602_UNK_A0398**
  - p=0.013
Conclusions

• Applying an untargeted metabolomics approach in two different platforms provided a broader coverage of metabolites and candidate biomarkers for banana intake.

• Dopamine and serotonin metabolites are among the most discriminant metabolites following banana intake.

• The combination of m/z 195.1014 and 283.0474 putatively annotated as methoxyeugenol and 6-OH-TβC sulfate offers a parsimonious biomarker of banana intake.

• Further validation in independent cohorts is needed using a quantitative method to further assess the utility of these biomarkers to predict the intake of banana.
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