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chaired by Prof. Peter Meikle, Dr. Thusitha W. Rupasinghe, Prof. Susan Sumner, Dr. Katja Dettmer-Wilde

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

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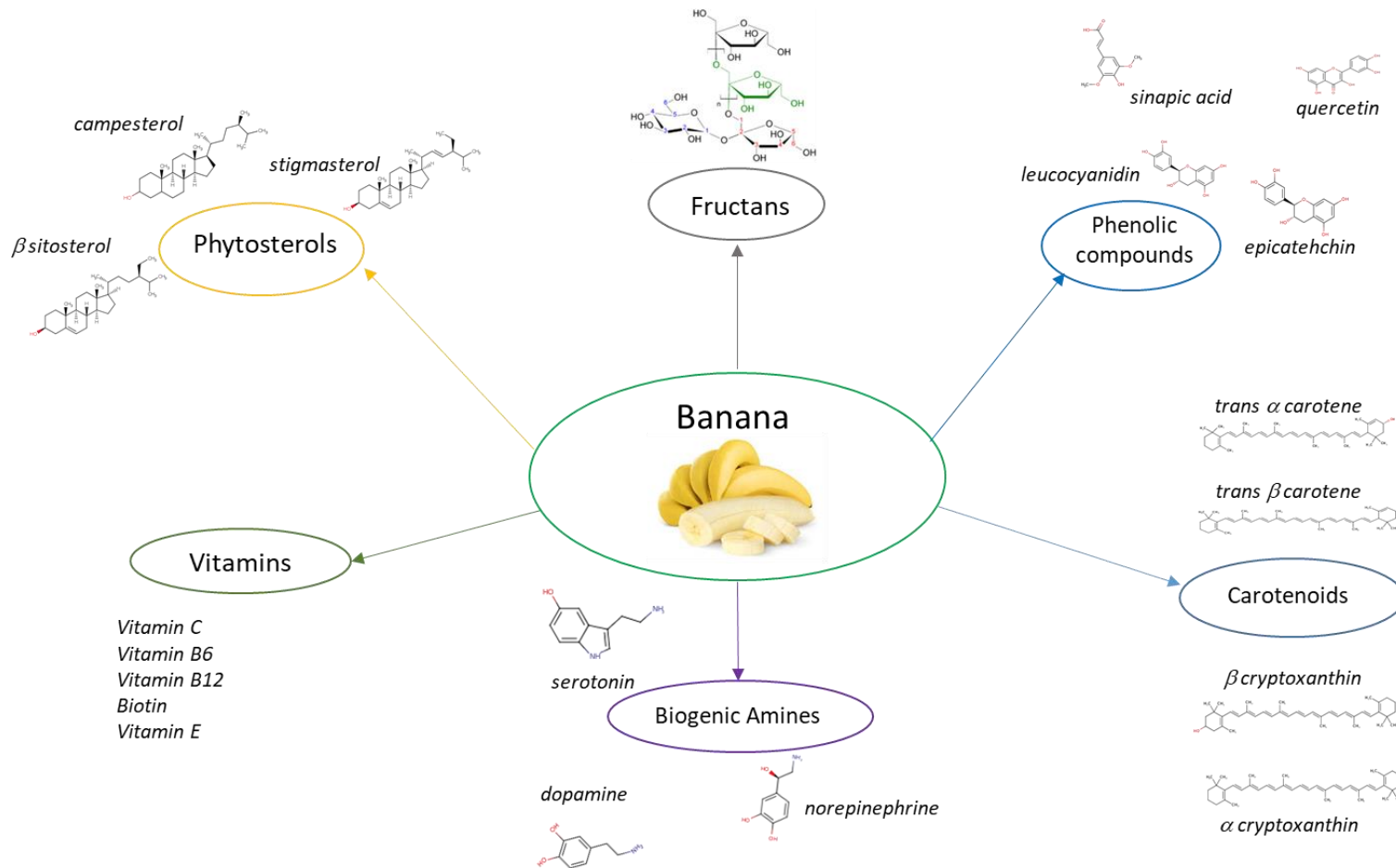
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⁶ *Université Clermont Auvergne, INRA, UNH, Plateforme d'Exploration du Métabolisme, MetaboHUB, Clermont, Clermont-Ferrand, France.*

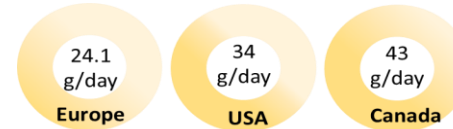
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What do we know about banana?



What do we know about banana?

- ✓ Highly consumed fruit in different countries.



- ✓ Intake of unripe banana ameliorates diarrhoea in children.

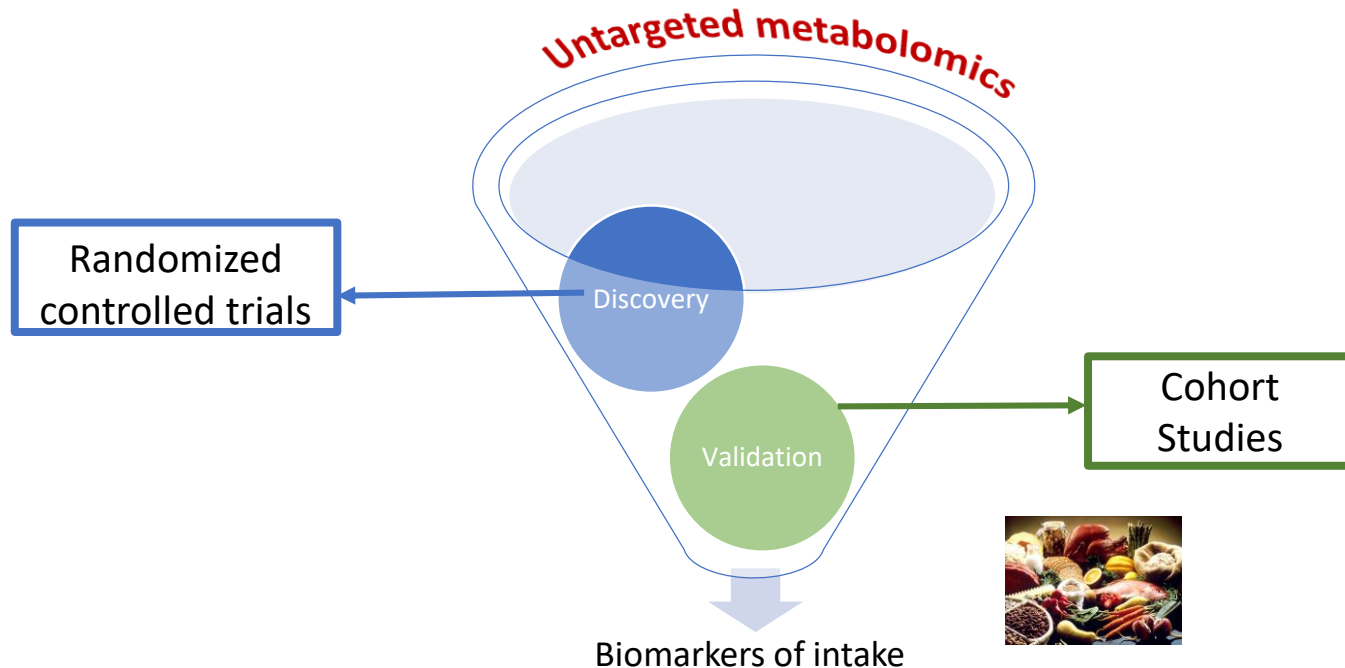
Green Banana Reduces Clinical Severity of Childhood Shigellosis
A Double-Blind, Randomized, Controlled Clinical Trial

Golam H. Rabbani, MD, PhD, FACP, Shamsir Ahmed, MBBS, Md, Iqbal Hossain, MBBS, PhD, Rafiqul Islam, MBBS, MPH, Farzana Marni, MSc, Mastura Akhtar, MSc, and Nashiha Majid, MSc

- ✓ **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 doi:10.3390/nu9010055.

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**.

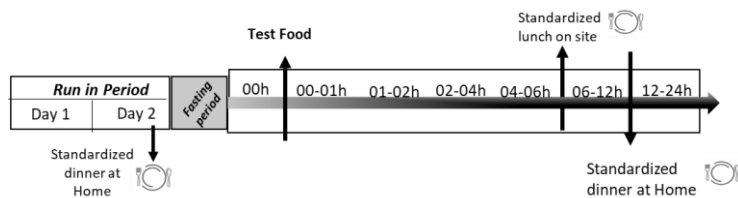
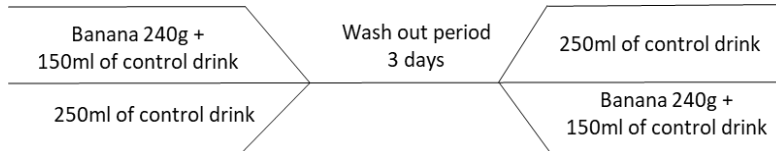


Discovery

RCT, cross-over



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



- ✓ 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±12g banana)

n=26 "Non-consumers"

✓ 24h urine pool





METABOHUB

Infrastructure nationale
en métabolomique et fluxomique

UPLC-QTOF-MS

Data Preprocessing

Data Cleaning

Data Analysis

24h urine pools Meal intervention Study

- ✓ BEH shield RP18 100X41X1,7
- ✓ 25 minute gradient
- ✓ Impact II Bruker
- ✓ ESI(+) and (-)

- Workflow4metabolomics
- **XCMS** for spectral data analysis.
- **CAMERA** for ion annotation.

ESI (+) **2,714**
ESI (-) **1,289**

- ✓ OSC-PLSDA (VIP>2)
- ✓ Student paired T test (p-FDR<0.05)

24h urine pools Cohort Study

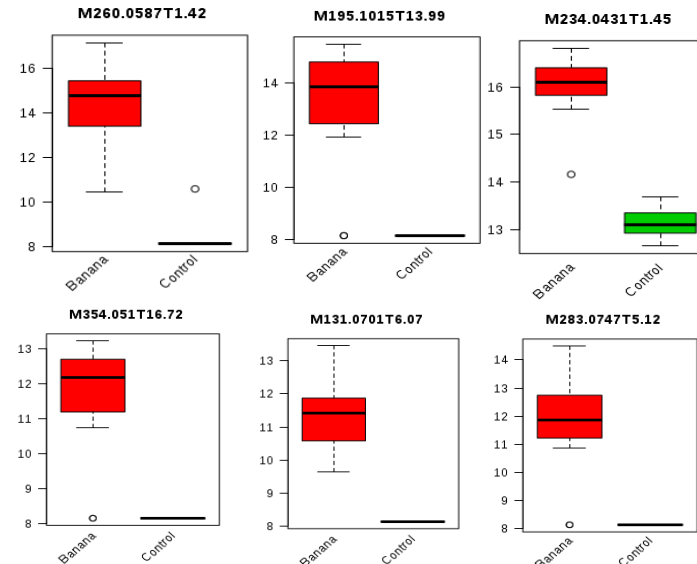
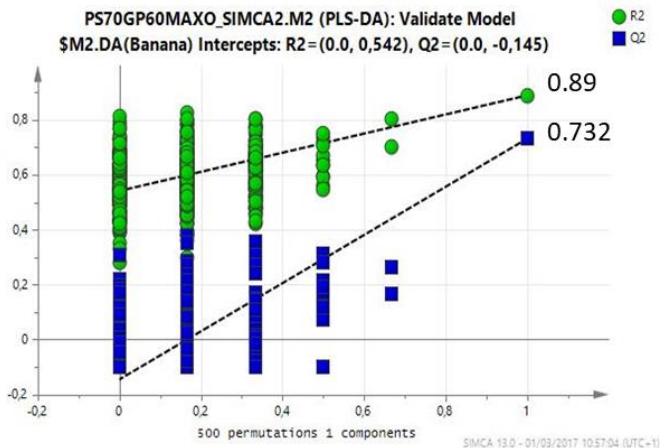
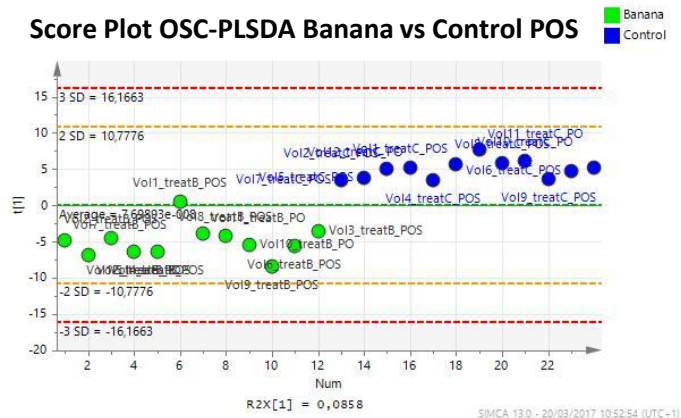
- ✓ BEH shield RP18 100X41X1,7
- ✓ 25 minute gradient
- ✓ Impact II Bruker
- ✓ ESI(+)

ESI (+) **2,427**

- ✓ PLSDA
- ✓ Student T test (p-FDR <0.05)
- ✓ Logistic Regression with AIC

Discovery
ESI (+)

Score Plot OSC-PLSDA Banana vs Control POS



36 ions with $p < 0.05$ BH

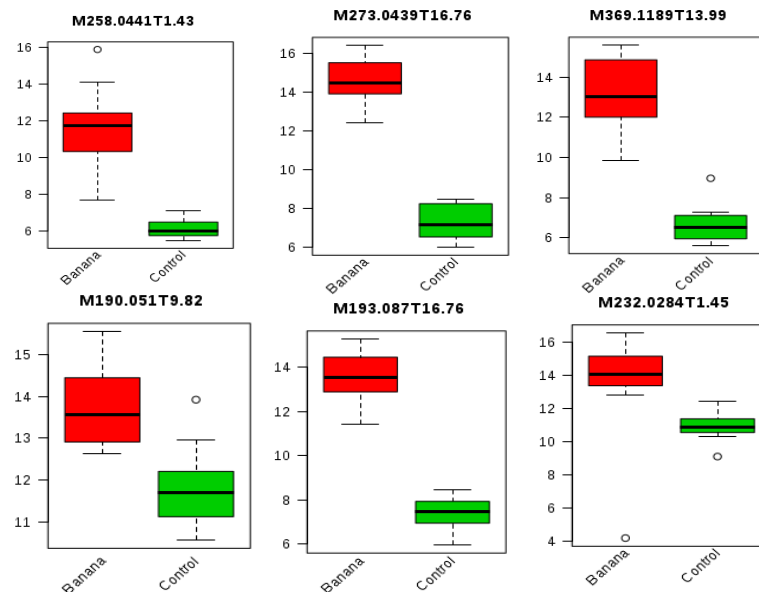
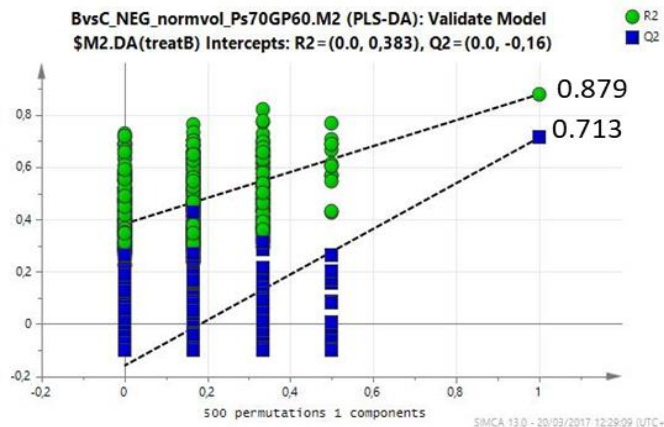
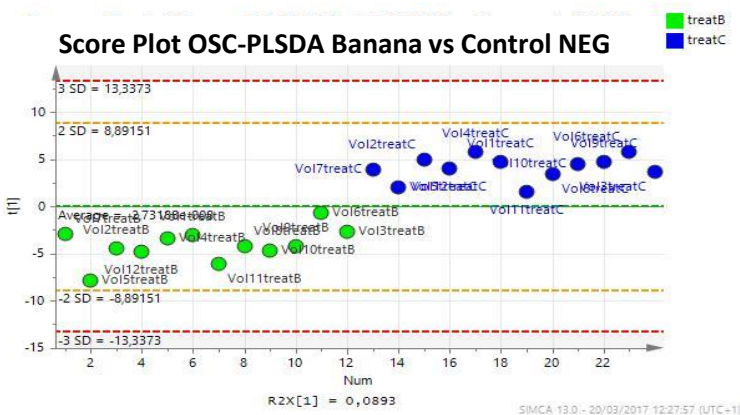


31 ions Higher in Banana

All significant ions in univariate have a $VIP > 2$

74 ions had a $VIP > 2$
47 ions have a higher intensity
in the banana group

Discovery
ESI (-)



22 ions with $p < 0.05$ BH

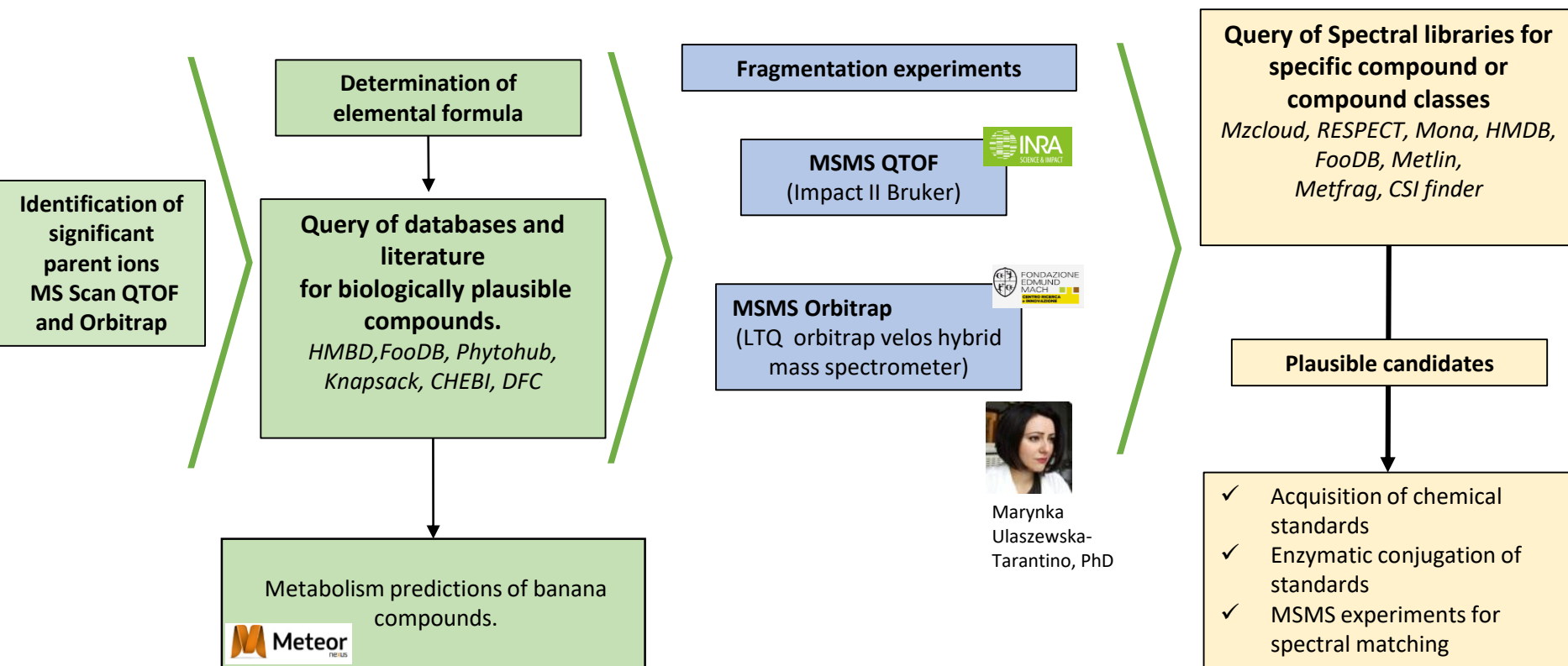


22 ions Higher in Banana

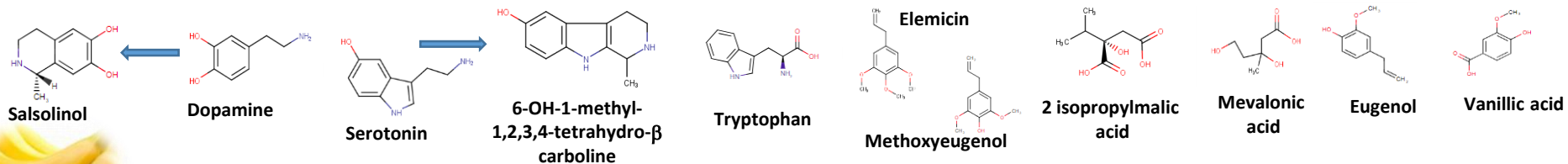
40 ions had a $VIP > 2$
37 ions have a higher intensity
in the banana group

All significant ions in
univariate have a $VIP > 2$

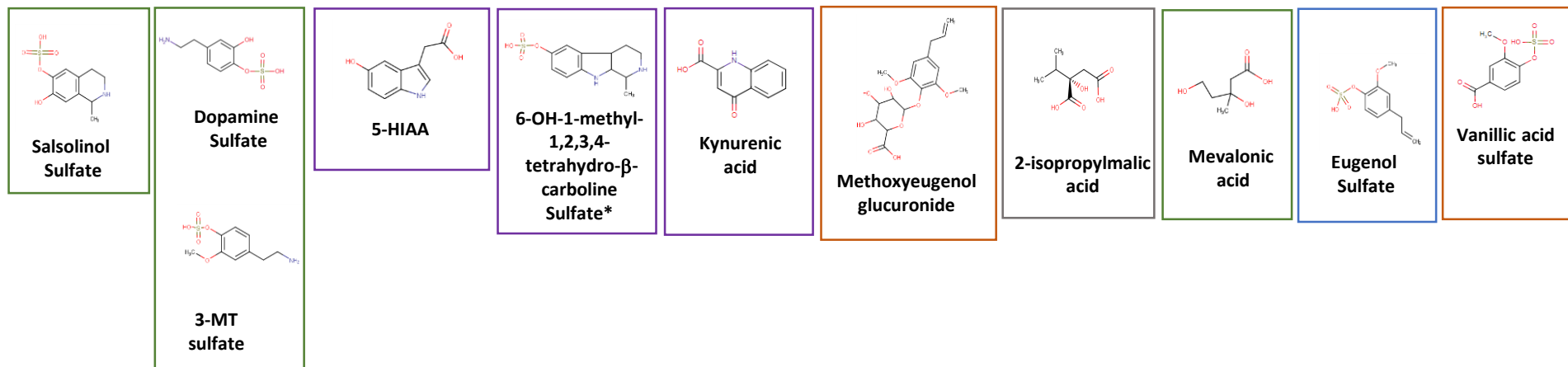
Identification pipeline overview

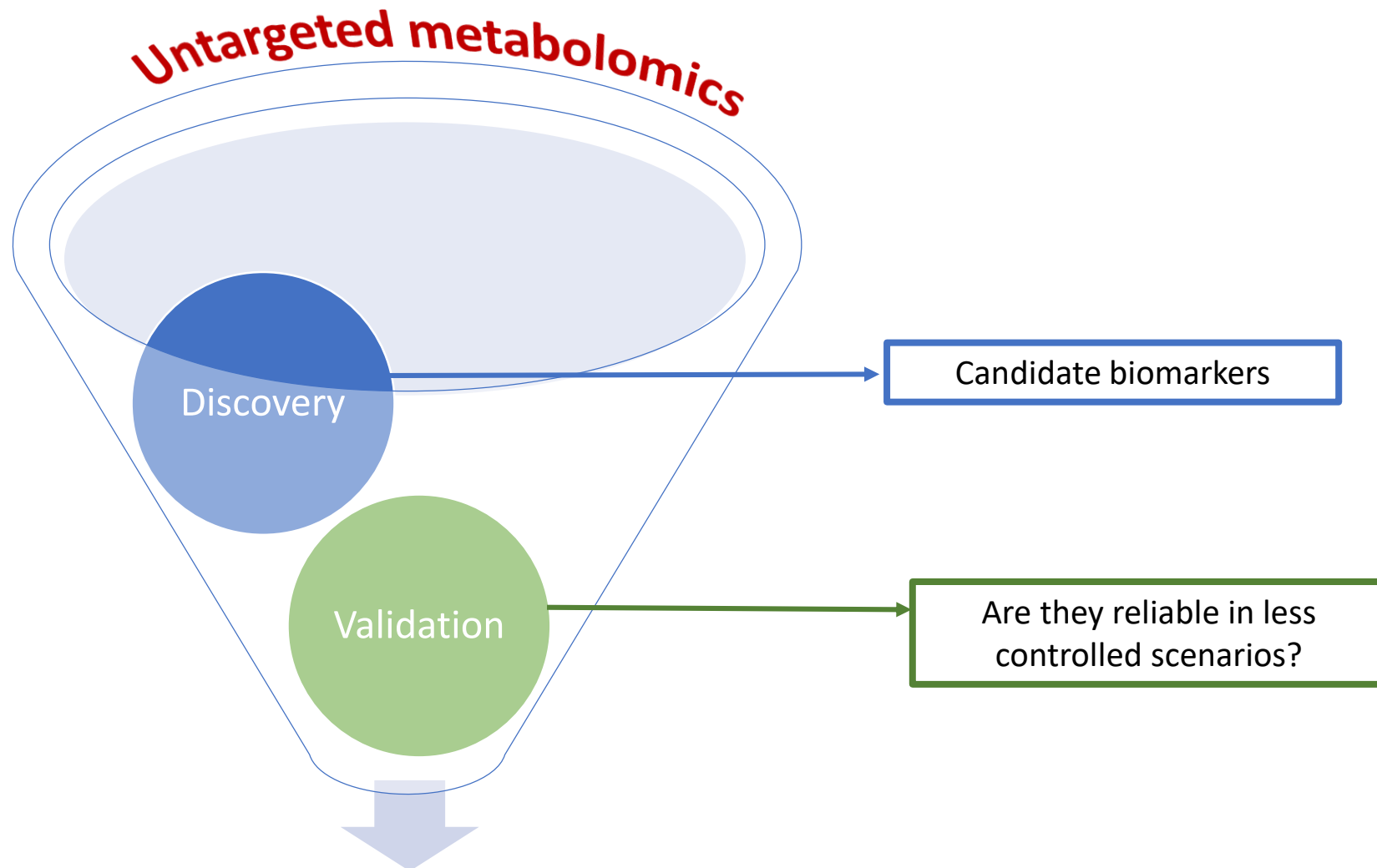


Marynka
Ulaszewska-
Tarantino, PhD



Human Metabolism





Validation

The KarMen Study

47 biomarkers of banana from meal study ESI (+)



2427 ions ESI(+)

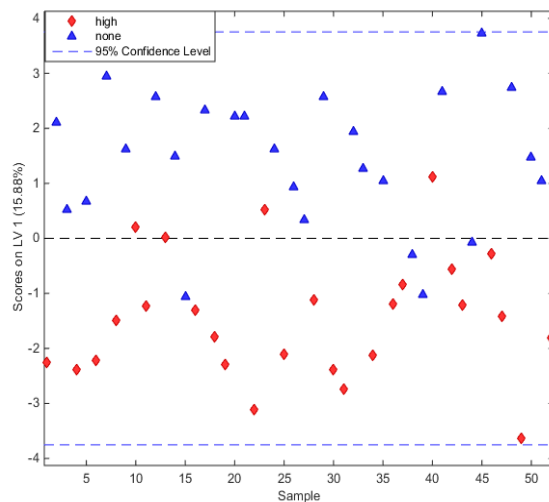


22 ions matched according to rt, mz and spectra

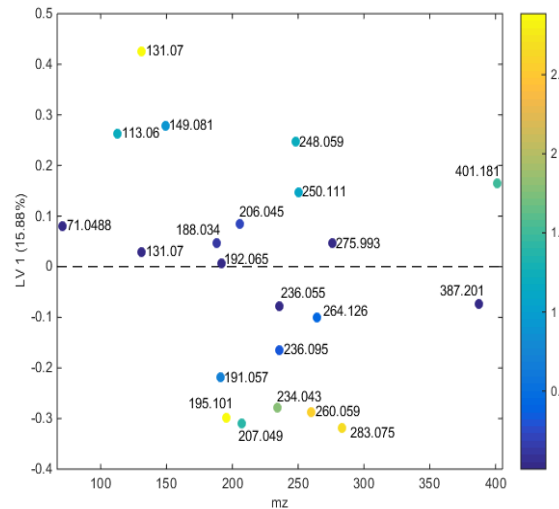


PLSDA model

PLSDA Scores Plot High vs None

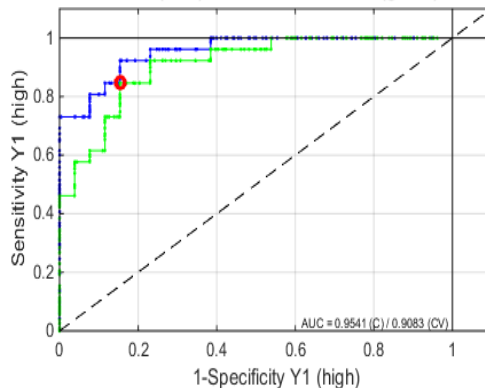


PLSDA Loadings Plot High vs None



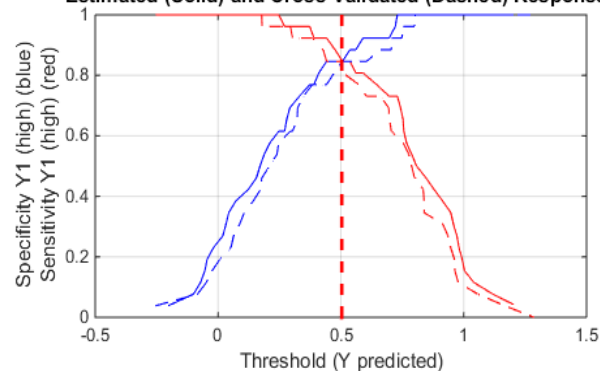
✓ 22 highly discriminant features in the meal study are able to predict the intake of banana with a good sensitivity and specificity.

Estimated (blue) and Cross-Validated (green) ROC



AUC (CV)=0.90

Estimated (Solid) and Cross-Validated (Dashed) Responses



Sensitivity (CV) = 84%
Specificity (CV) = 80.7%

Is there a more parsimonious biomarker?

Validation

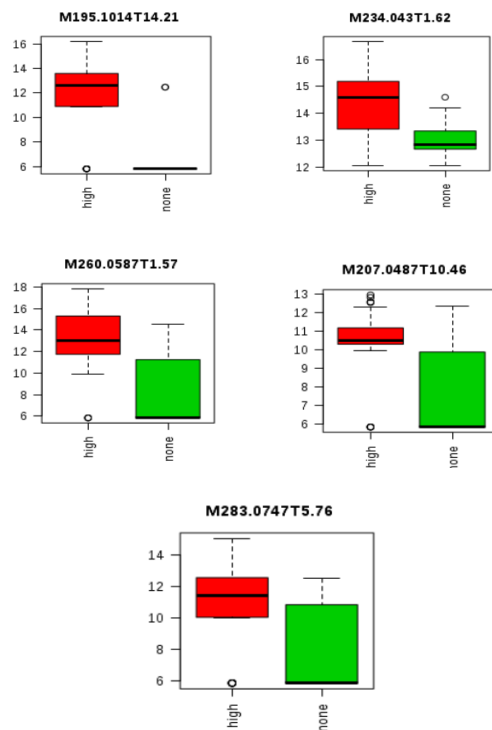
KarMen Study

Student T test

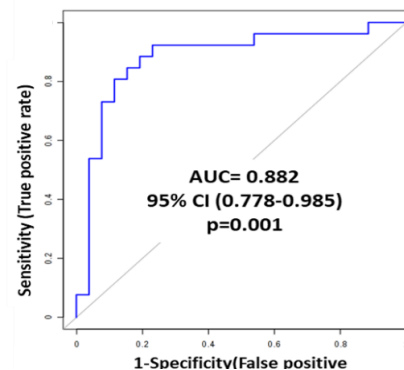
FDR-correction

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

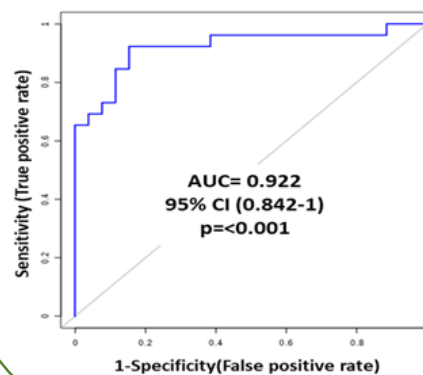


5 metabolites



Sensitivity (CV) = 84%
Specificity (CV) = 84.6%

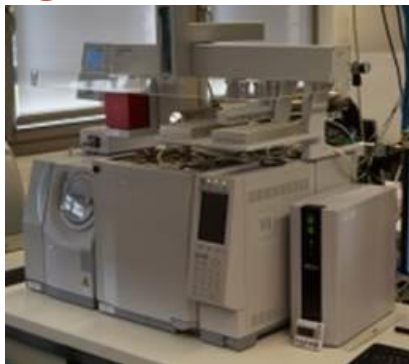
m/z 195.1014+ m/z 283.0747



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

**Parsimonious biomarker of banana intake!
Good sensitivity and higher specificity**

Untargeted GCxGC-MS analysis



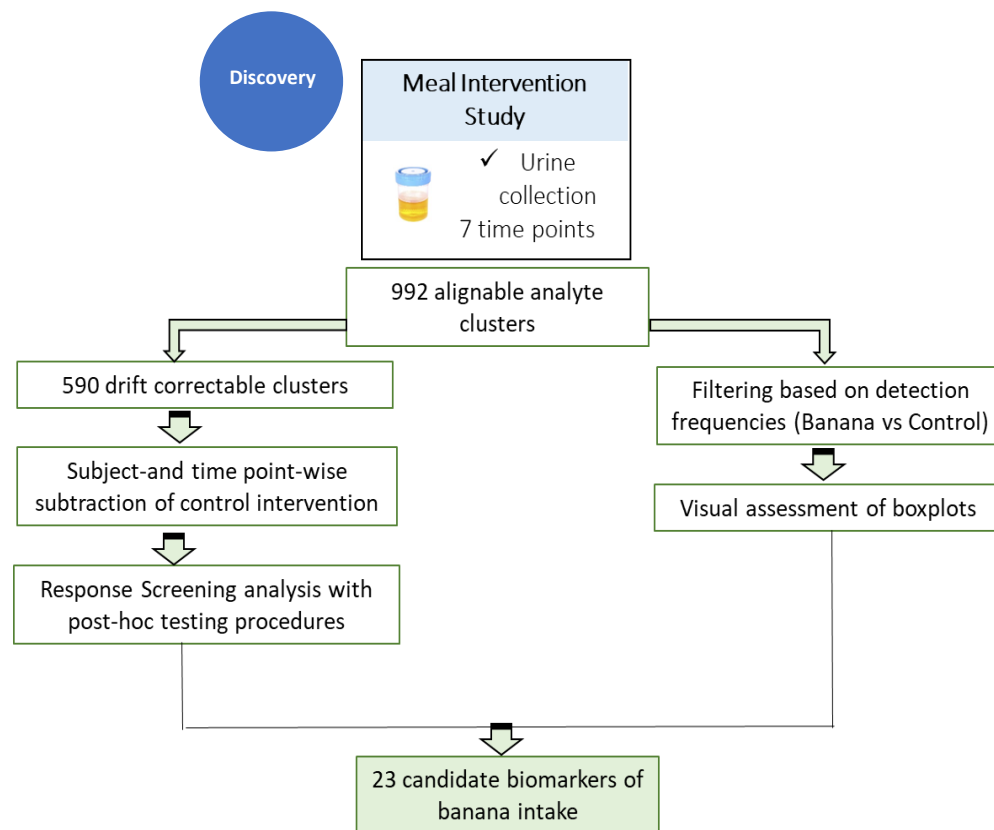
Christoph Weinert, PhD



Carina Mack, PhD

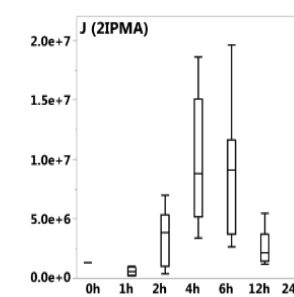
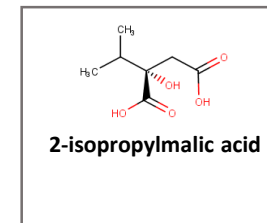
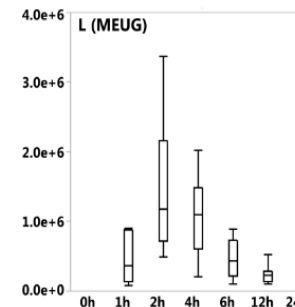
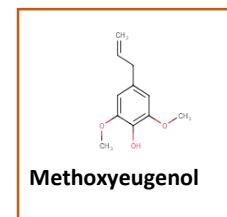
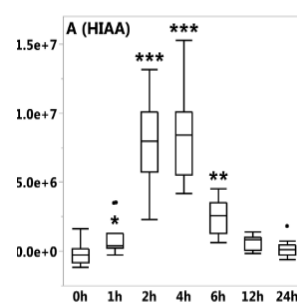
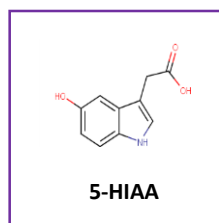
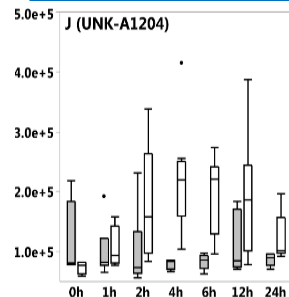
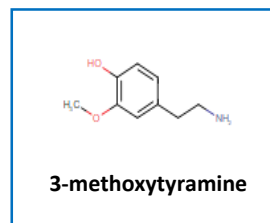
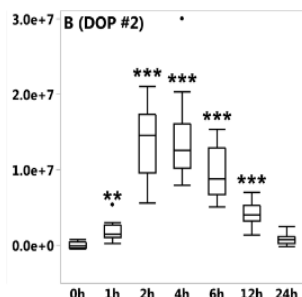
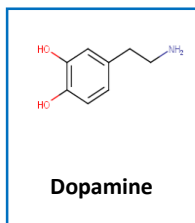
Björn Egert, PhD

- ✓ To obtain a broader coverage of biomarkers of banana intake.
- ✓ Confirm the robustness of the biomarkers of banana intake identified using UPLC-QTOF-MS.



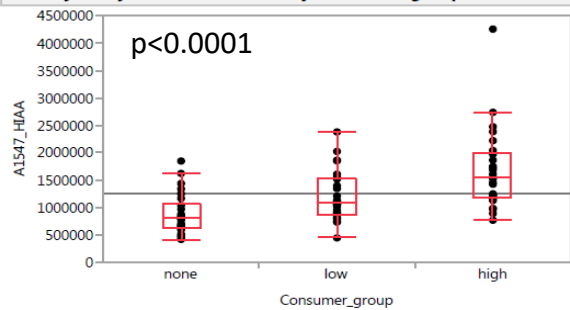
Discovery

Previously observed in
UPLC-QTOF-MS

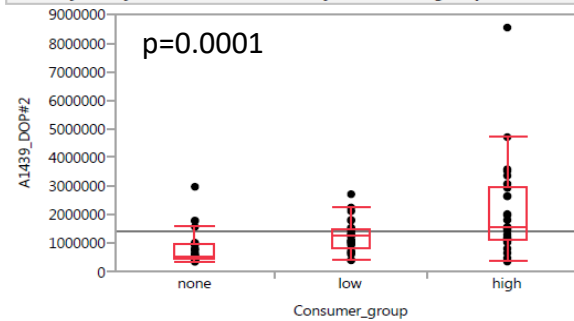


Validation

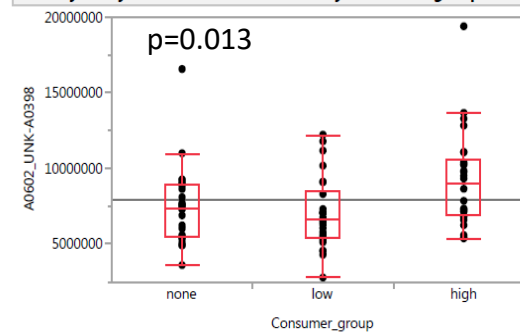
Oneway Analysis of A1547_HIAA By Consumer_group



Oneway Analysis of A1439_DOP#2 By Consumer_group



Oneway Analysis of A0602_UNK-A0398 By Consumer_group



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-T β C 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.

Acknowledgments



METABOHUB Infrastructure nationale
en métabolomique et fluxomique



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- Charlotte Joly (*PFEM*)
- Delphine Centeno (*PFEM*)
- Stephanie Durand (*PFEM*)
- Melanie Pétéra (*PFEM*)

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Max Rubner Institute

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- Christoph Weinert (*Dept. Safety and Quality of Fruit and Vegetables*)
- Carina Mack (*Dept. Safety and Quality of Fruit and Vegetables*)
- Björn Egert (*Dept. Safety and Quality of Fruit and Vegetables*)
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