Summary

Information obtained from dietary assessment tools is the foundation upon which nutritional-researchers build associations between consumption, diseases, and health. However, different factors such as recall bias, or errors in self-reporting tampers with the accuracy of the obtained information. The rise in the development of highly sensitive methods such as mass spectrometry allows the detection of specific metabolites that appear in biofluids after the intake of a particular food. These metabolites constitute potential biomarkers of intake by reflecting dietary exposure more objectively.

This Ph.D. thesis aimed to unravel the biomarkers of intake of highly consumed fruits by applying a three-fold strategy. Firstly, an extensive literature search was performed to collate previously reported compounds and metabolites that result from exposure to pome fruits, stone fruits, banana, and tropical fruits. A panel of biological and analytical criteria was applied to evaluate the usefulness of these metabolites as biomarkers of intake for the different fruits. Then experimental analyses were performed using an untargeted metabolomics approach by UPLC-QTOF-MS and GCxGC-MS to urine samples obtained from a human randomized controlled trial to discover novel biomarkers for banana. The identified biomarkers of banana consumption were further validated in a cross-sectional study to determine their robustness as BFIs in a free-living condition. Finally, using the same untargeted UPLC-QTOF-MS method we analyzed the urine samples from the tomato meal in the intervention trial to identify biomarkers for raw tomato intake.

The review of the literature was performed according to the BIFRev protocol (Food Intake Biomarker Reviews) using three databases (Pubmed, Scopus, and Web of Science) to obtain all possible candidate biomarkers for pome fruits and stone fruits (Paper I), banana and tropical fruits (Paper II) in human biofluids; over 1000 publications were assessed for 25 different fruits. Surprisingly few candidate biomarkers for the various fruits were retrieved from the literature, even for the most commonly consumed fruits, apple and banana. For apple, the urinary excretion of phloretin glucuronide or phloretin after hydrolysis is herein proposed as candidate biomarkers for the consumption of this fruit. While no candidate biomarkers for stone fruits (plum, peach, nectarine, cherry, and apricot) were found, for pear intake, arbutin in plasma was considered as a plausible candidate biomarker. Regarding tropical fruits, candidate biomarkers were retrieved for avocado, banana, and watermelon. Catecholamine and indoleamine metabolites such as dopamine sulfate, 3-methoxytyramine sulfate, and 5-hydroxyindole-acetic-acid are suggested as candidate biomarkers for banana exposure while perseitol and mannoheptulose are proposed for avocado consumption.
Citrulline was considered as putative biomarker for watermelon. No information was obtained for the other tropical fruits assessed.

Further work was done to obtain biomarkers for banana intake and tomato. To this end, a randomized, controlled, crossover human study was conducted with 12 healthy subjects (6 men, 6 women, mean±SD BMI 22.5±2.3 kg/m², and mean±SD age: 30.0±4.9y). Volunteers were randomized to three dietary interventions, 1) 250ml of control drink (Fresubin, 2 kcal Fiber, neutral flavour), 2) 240g of banana (M.Cavendish) plus 150ml of control drink and 3) 300g of tomato (Coeur de boeuf) plus 12g of sunflower oil and 150ml of control drink. Urine and plasma samples were collected at different time points, and 24h urine collections were obtained. Urine samples of all arms of the intervention study were analyzed simultaneously under the same untargeted UPLC-QTOF-MS method. Statistical analysis were performed separately using banana vs. control, and tomato vs. control to identify the relevant BFIs for both fruits.

For the discovery of novel biomarkers of banana intake we applied a two step strategy. First, we analyzed the 24h urine samples from the aforementioned clinical trial using two complementary metabolomics platforms (UPLC-QTOF-MS and GC×GC-MS). Herein, OSC-PLS-DA models and univariate analyses were performed to detect distinctive metabolites that reflected banana exposure. Thirty-three metabolites were identified as plausible candidate BFIs. Next, we applied a targeted screening approach to identify the 33 candidate biomarkers from the intervention study in 24h urine samples of high (mean intake 126g), low (mean intake 87.7g), and non-consumers of banana recruited for the KarMeN cross-sectional study. Sixteen metabolites were recognized and confirmed as candidate BFIs for banana. PLS-DA models with double-cross-validation were applied, using the dataset of the intervention study as a calibration set and the dataset from the cross-sectional study as a test set, to assess the ability of different combinations of the replicated metabolites to detect banana exposure. From this approach, we demonstrated that the combination of methoxyeugenol-glucuronide and dopamine sulfate performs best in predicting banana intake in high (AUC_{test}=0.92) and low (AUC_{test}=0.87) consumers of the fruit. Combined metabolites were more robust and reliable than individual markers to detect banana consumption.

For the tomato intake biomarkers, we applied OSC-PLS-DA models and univariate analysis to the 24h urine collections from the intervention trial. The results revealed eight candidate biomarkers for the consumption of this fruit (Paper IV). However, only four of the discovered BFIs were observed in higher intensity in urine following the intake of tomato compared to banana. Among these metabolites, the novel metabolite N-acetamido-esculeogenin-B-4-sulfate, a metabolite of the
glycoalkaloid esculeogenin-B present in tomato, has been putatively identified as BFI for raw tomato intake. The three other putative markers have not been identified yet but have adequate kinetics as markers of recent raw tomato intake.