1. Introduction

Fruit and vegetable (F&V) intake has been associated to lower risk of cardiovascular diseases for decades [1]. Dietary guidelines recommending a daily intake of 400-800g of F&V as part of a healthy diet have been well accepted in many countries and introduced in nutritional health care. Such dietary recommendations are built upon associations between the collected dietary information and health outcomes discovered in epidemiological and nutritional research. It is a well-known fact that the dietary assessment methods applied today in observational studies are subject to bias mainly caused by respondent burden or by the disparity between a subject’s perception of food portions and their actual intake [2]. The latter seems to be the case for fruits and vegetables since they are prone to be overestimated by self-reporting methods [3]. Consequently, it is possible that some of the already established health associations with F&V also lack accuracy.

In epidemiological and nutritional research, F&V are mostly assessed as a group, or separately as overall fruits, or vegetables. However, health associations with individual subtypes of fruits are rarely reported in meta-analyses. This may be caused by the low number of available studies on specific subtypes of fruits causing low power and high heterogeneity when assessing their impact on non-communicable diseases [4]. Nonetheless, the study of subtypes of fruits is relevant due to the wide differences in potentially bioactive compounds among species, which may produce different effects on human health. To obtain more detailed knowledge about the health action of specific fruits an accurate dietary assessment is of major importance.

Fruits in general are highly consumed commodities in the world. Thanks to commerce globalization, fruits that were formerly restricted to one location are now available in many countries and consequently introduced to the habitual diet of individuals. Banana and tomato are good examples. Banana is originally an African tropical fruit introduced to Europe in the 15th century [5], and tomato was introduced from the Andean region in South America a century later [6]. Throughout time, banana gained terrain as a staple commodity and is now the second most consumed fruit in Europe [7]. On the other hand, tomato is not only essential in many dietary patterns in the world such as the Mediterranean diet, but it is also processed in several highly consumed products, including sauces, juices, and soups. Like banana and tomato, the consumption of many tropical fruits, including avocado, mango and pomegranate, is being rapidly introduced to many countries. This adds up to the dietary assessment problems since food frequency questionnaires (FFQ) are not comprehensive enough to assess the consumption of many currently consumed fruits.
To strengthen the dietary assessment of fruits as well as other foods, researchers in the field of dietary intake biomarkers have opted for metabolomics as their main tool. Metabolomics allows the discovery of a plethora of specific metabolites that appear in biofluids after the intake of a particular food, e.g., banana, thus serving as proxies of exposure. Untargeted analysis may be applied on different analytical platforms, liquid chromatography-mass spectrometry (LC-MS), gas-chromatography-mass spectrometry (GC-MS) and nuclear magnetic resonance (NMR) spectrometry are the most often used. UPLC-QTOF-MS allows a comprehensive coverage of semi-polar metabolites, such as glycoalkaloids or catecholamine metabolites while GC-MS covers a wide range of polar and volatile components including carbohydrates and aroma compounds. Therefore, their complementary use allows an in-depth understanding of the food metabolome.

The discovered candidate biomarkers of food intake (BFIs) must be investigated further for their applicability in epidemiological research. For this, a validation scheme encompassing biological and chemical criteria has been developed by the FoodBall consortium [8]. Herein, eight critical aspects namely, plausibility, dose-response, time-response, robustness, reliability, chemical performance, stability and reproducibility are assessed to finally determine the potential use of the candidate BFIs.

**Thesis aims**

While the field of BFIs has undoubtedly advanced in recent years, it is still in its early stages. Thus, the FoodBall initiative (www.foodmetabolome.org) has undertaken the task to establish an inventory of candidate BFIs for different foods through experimental work and systematic reviews of the literature. Within this consortium, the aims of my thesis are threefold:

1) To identify already published metabolites associated to the intake of pome fruits, stone fruits, banana and other tropical fruits, and to assess their usefulness as BFIs according to predefined validation criteria.

2) To discover, confirm and validate novel biomarkers for banana intake by two complementary methods UPLC-QTOF-MS and GC×GC-MS applying an untargeted metabolomics approach to analyze 24h urine samples obtained from a randomized, controlled, crossover intervention trial and a cross-sectional study.

3) To discover novel biomarkers of tomato intake using an untargeted metabolomics approach by UPLC-QTOF-MS to urine samples from a randomized, controlled intervention trial with a crossover design.