1 Introduction

1.1 Background

"Eat an apple on going to bed / and you'll keep the doctor from earning his bread".

It is a Pembrokeshire proverb appearing for the first time in print in the edition of *Notes and Queries* magazine (1866) [1]. Were the medical properties of apple already being exalted? It is hard to guess but several pieces of scientific evidences indicate that apple intake may contribute to beneficial effects against obesity, diabetes and cardiovascular disease (CVD) [2, 3]. Furthermore, a daily intake of two-to-six apples is inversely associated with total plasma and LDL cholesterol [4].

This reduction of risk is due to the interaction of the nutrients and the bioactive components present in apple (but probably in fruits or vegetables in general) effecting several targets and pathways in the host organism.

Therefore, methods able to handle multiple responses are the most suitable for this kind of investigation and metabolomics - a new emerging field among the main '*omics* disciplines (as genomics, lipidomics and proteomics) - is one of them.

First of all the terminology: metabolomics or metabonomics? The distinction is more a 'matter of historical usage than meaningful scientific definition' to use *Robertson, D.G*'s words.[5]. The term *metabolomics* was devised by Oliver Fiehn in 2002 to indicate "a comprehensive and quantitative analysis of all metabolites" [6] while *metabonomics* was coined by Jeremy Nicholson and colleagues in 1999 to define "the quantitative measurement of the dynamic multiparametric metabolic response of living systems to pathophysiological stimuli or genetic modification" [7].

However, the term metabolomics is now more widely accepted and it will be used in this thesis, yet with the realisation that either term may be acceptable. Commonly used terms that are largely synonymous include also metabolic profiling and metabolic fingerprinting and these terms will be used in this thesis interchangeably.

As such, metabolomics is a discipline dedicated to the global study of metabolites (molecules <1,500 Da, i.e. intermediates and products of metabolism) belonging to a several classes of compounds, like amino acids, organic acids, lipids, etc. The metabolome (a complete set of metabolites) can be divided into: 1) *endogenous metabolome* which includes all metabolites produced by a cell, a tissue or an organism, 2) *microbial metabolome* produced by the microbiota, 3) *xenometabolome* which includes all foreign metabolites derived from drugs, pollutants and dietary compounds and 4) *food metabolome* metabolites from the digestion of food [8].

Mass spectrometry (MS) in its various hyphenated derivations (liquid chromatography (LC)-MS, gas chromatography (GC)-MS, etc.) and NMR are the common analytical platforms used.

Typically, two analytical strategies can be adopted: *non-targeted* and *targeted*. The non-targeted approach aims to cover the metabolome as broadly as possible while maintaining the ability to at least differentially quantify the metabolites [9]. The targeted, instead, focuses on specific and identi-

fied metabolites and nowadays thanks to the development of analytical instrumentation, it is possible to cover and accurate quantify over 150 metabolites across several classes of compounds in a single run [10].

Metabolomics has been applied in different fields, especially in toxicology and pharmacology whereas relatively fewer studies exist in human nutrition. An advantage of using this approach in nutritional studies is that hundreds of metabolites in biological samples are measured at once and thus, it allows to explore the effect of food (even in closely related foods) on metabolic pathways [11].

Metabolomics analysis of biological samples from *in vivo* studies, therefore, may help to elucidate the mechanisms and the identity the bioactive components which lead the apple to have beneficial effects.

1.2 Focus and aim of the thesis

Apple and derived products are the nutritional focus of this thesis since apple represents one of the most consumed fruits throughout the European Union [12] and possibly worldwide thereby representing a large fraction of ingested fruit. The scientific evidence of promoting healthy effects mainly against diabetes and cardiovascular diseases (CVD) [4].

Although large progress has been done to understand the active factors behind the beneficial effects still more remains to be done. The primary aim of this PhD projects was to explore and thereby contribute to uncover the biochemical mechanisms by which consumption of apples or apple products may affect health. The selected methodology to explore these mechanisms was an LC-MS based metabolomics approach.

Secondary aims were to compare effects in rats and humans and to compare the usefulness of different samples for profiling. The first study therefore targeted the effects on the plasma metabolome after rats had been fed with whole fresh apple pieces through 16 weeks (PAPER I). This was followed up on samples from a randomised, controlled 5x4 weeks human cross-over study, introducing apple products (clear and cloudy juices, and apple pomace) as well and where both plasma (PAPER II) and urine (PAPER III) metabolic profiles were investigated.

1.3 Thesis outline

The thesis consists of an introductory part which also presents some experimental results and discussion to highlight the main general concepts followed by a second section in which the papers are included (PAPER I, II and III). PAPER II and PAPER III are from the same human study.

Specifically, the thesis is subdivided as follows:

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- *Chapter 1* presents the chemical composition of an apple, and includes the absorption and metabolism of some potential bioactive components.

- *Chapter 2* provides the theoretical background of the workflow used in this project.

- *Chapter 3* presents an overview of the results and discussion from PAPER I, II and III.

- *Chapter 4* provides the conclusions and future perspectives.