Plant Physiology and Biochemistry xxx (2013) 1–8

Contents lists available at SciVerse ScienceDirect

Plant Physiology and Biochemistry

journal homepage: www.elsevier.com/locate/plaphy

A targeted metabolomics approach to understand differences in flavonoid biosynthesis in red and yellow raspberries

Elisabete Carvalho, Pietro Franceschi, Antje Feller, Luisa Palmieri, Ron Wehrens, Stefan Martens*

Department of Food Quality and Nutrition, Research and Innovation Centre, Fondazione Edmund Mach (FEM), Via E. Mach 1, 38010 San Michele all'Adige, Italy

ARTICLE INFO

Article history:
Received 4 February 2013
Accepted 2 April 2013
Available online xxx

Keywords:
Rubus idaeus
Rosaceae
Phenolic compounds
Flavonoid biosynthesis

ABSTRACT

Phenolic compounds account for the most important class of secondary metabolites in raspberries and fulfill a broad range of biological functions in plants. Due to their presence in fruits they are also considered as important bioactive compounds in human nutrition and are closely related to fruit quality. In the present study a targeted UPLC–MS/MS method was used to screen various phenolic compounds in fruits of red and yellow raspberry cultivars. In total 50 phenolic compounds were detected above the quantification limit. Beside the obvious lack of anthocyanins, all yellow fruits analysed here lack procyanidin B1. The presence of this dimer, along with B3 dimers is described for the first time in raspberry fruits. Also for the first time, dihydrochalcone and stilbene derivatives and the quercetin metabolite, isorhamnetin with its glycosides, were identified in considerable concentrations in raspberries. Based on a PCA plot the red cultivar "Heritage" and the yellow "Alpen Gold" could clearly be separated from the other tested cultivars due to their distinct metabolite profiles/concentrations. This study allowed to obtain a comprehensive profile of the phenolic composition of the different raspberry varieties. The obtained data will lead to a better understanding of the overall biosynthetic network of polyphenols in raspberry and will help to explain responsible factors for the different metabolite profiles in ongoing studies.

1. Introduction

Raspberries (*Rubus idaeus* L.; Rosaceae) are important soft fruits in Trentino (Italy) but also in Europe and North America from both economic and nutritional points of view. Being among the berries with higher antioxidant contents, they are receiving increasing attention as a source of potentially healthy compounds that can help to prevent and even treat various chronic diseases and some forms of cancer [2,10,22,25]. The most significant health benefits of raspberries are attributed to the (poly)phenolic compounds which are represented by phenolic acids, flavonoids (flavonols, flavan-3-ols, anthocyanins) and tannins (ellagitannins, proanthocyanidins), which are in general considered as protective micronutrients and quality parameter for edible fruit [2]. In recent studies, the antiproliferative activity of ellagitannins, the improvement of cognitive brain function, age-related degeneration of eye function and the influence on cardiovascular disease of anthocyanins and the inhibiting effect of polyphenolic-rich raspberry extracts on several important stages in colon carcinogenesis *in vitro* was shown [11,24,29].

Consumer acceptance is mainly based on quality of fruits (color, firmness) and taste, which both are primarily influenced on their biochemical composition. Changes in the level of involved secondary metabolites are to some extent determined by environmental factors, but are much more regulated at the genetic level [1,4]. Cultivars with novel characteristics including fruit coloration may encourage fruit consumption, which is associated with health benefits to the human diet. Furthermore, the color of raspberry, which is mainly related to the presence or absence and the final concentration of different anthocyanins, is an important characteristic considering the fresh-like appearance of fruits and products [12]. However, the increasing attention of consumers to the nutritional value makes it mandatory to focus on the phenolic profiles of berries, considering them as an additional important trait for breeding. Since the breeding process is time consuming and may result in new cultivars only after more than a decade, novel strategies for an early and effective selection are needed. Among them fast and reliable analytic methods, and marker assistant breeding programs are the most important techniques to be

Abbreviations: Qu, quercetin; Km, kaempferol; Isorham, isorhamnetin.

* Corresponding author. Tel.: +39 0461 615541; fax: +39 0461 615200.
E-mail addresses: elisabete.carvalho@fmach.it (E. Carvalho), pietro.franceschi@fmach.it (P. Franceschi), antje.feller@fmach.it (A. Feller), luisa.palmieri@fmach.it (L. Palmieri), ron.wehrens@fmach.it (R. Wehrens), stefan.martens@fmach.it (S. Martens).

0981-9428/$ – see front matter © 2013 Elsevier Masson SAS. All rights reserved.
http://dx.doi.org/10.1016/j.plaphy.2013.04.001

mentioned [21]. Detailed understanding of underlying pathways of bioactive metabolites on the biochemical and molecular level will support such programs enormously.

As mentioned above a large part of the health effects attributed to berries is due to (poly)phenolic compounds which represent a structurally diverse class of secondary metabolites. The majority of raspberry phenols are ellagitannins (hydrolysable tannins), but they also contain larger amounts of anthocyanins (in red raspberries) and smaller amounts of hydroxycinnamic acids, flavonols, flavan-3-ols and proanthocyanidins [2,16,25]. Yellow raspberries, however, which lack anthocyanins, seem to be as effective or be even more effective than their red counterparts at inhibiting enzymes with potential impact on chronic diabetes or hypertension [10,30].

The main polyphenols (ellagitannins and anthocyanins) and carotenoids have been analysed previously in red and yellow raspberries [9,14,20]. Other bioactive compounds were identified using the recently published targeted UPLC/QqQ-MS/MS method [27]. Our in depth analysis revealed additional compounds not previously identified in yellow or red raspberry varieties. These results sheds some light on where the block in the anthocyanin biosynthetic pathway in the yellow raspberry varieties occurs and provides some new insights into the flavonoid pathway.

2. Results

2.1. Targeted metabolomics approach

The targeted UPLC–MS/MS method published recently [27] led to the identification of 50 polyphenolic compounds in raspberry fruits (see Suppl. Table 1). The pattern of the identified phenolic compounds was analysed and quantified in nine different varieties at full ripe stage of the fruits. In a first overview the results of the analysis of all detected phenolic acids, flavonols, flavan-3-ols including its dimers and several minor compounds are shown in the form of a heat map (Fig. 1). The overall phenolic profile appeared to be closely related in all cultivars. It can be seen that the most abundant compounds found were procyanidins, epicatechin, catechin, Qu 3-*O*-glucuronide, Qu 3-*O*-galactoside and ellagic acid. Qu derivatives were the dominant flavonols followed by Km metabolites. Isorhamnetin (Isorham; 3′-methoxy-Qu) glycosides which have never been reported before in raspberries, were also found in our analysis. Yellow varieties apparently lack procyanidin B1 (epicatechin-(4β → 8)-catechin dimer), which is present in small amounts in all red varieties under investigation together with high amounts of the B3 (epicatechin-(4α → 8)-catechin) and B2/B4 (epicatechin-(4β/4α → 8)-epicatechin) dimers. The latter could not be separated using the present method, as previously discussed [27]. It is noteworthy that only the B2 dimer was described in *R. idaeus* so far [16]. Several other compounds were detected in lower and varying amounts (Fig. 1), including metabolites of two phenolic classes not yet described in raspberry, the stilbenes *trans*- and *cis*-piceid (*trans*-/*cis*-resveratrol 4′-O-glucoside) and the dihydrochalcones phloridzin (phloretin 2′-O-glucoside) and trilobatin (phloretin 4′-O-glucoside). These were identified in all nine varieties analysed, and for the first time in *R. idaeus*. Furthermore, "Heritage" raspberry revealed a complete lack of rutinoside derivatives of Qu and Km, but has higher amounts of other flavonol glycosides (Fig. 1).

2.2. Principal component analysis (PCA)

To get an immediate overview of the patterns in the data, and to see whether these patterns are associated with berry color or variety, PCA plots are useful tools. They show a projection of the data in two-dimensional space, in such a way that the maximal spread of the data is visualised. If patterns related to color or variety are visible in such a plot, it means that different colors or varieties are having very different characteristics. These differences may be concentrated in a few highly affected variables, or may be present in more subtle global changes.

An example is visible in Fig. 2, showing in the left part the score plot of the first two components. Clearly, two varieties stand out: "Heritage" is separated from the others on the first PC, and "Alpen

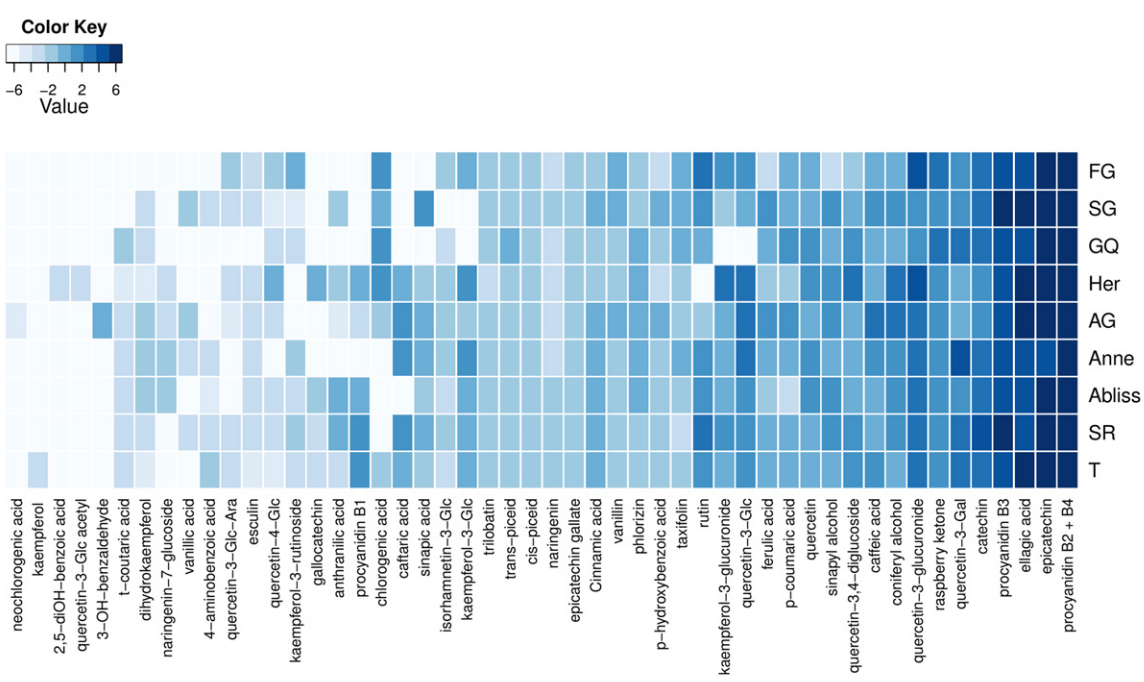

Fig. 1. Heat map visualising the concentrations (in logarithmic scale) of the metabolites in the different raspberry varieties. Abbreviations of cultivars are displayed according to Table 1.