Postharvest Biology and Technology 97 (2014) 77–82

Contents lists available at ScienceDirect

Postharvest Biology and Technology

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

Influence of preharvest and postharvest methyl jasmonate treatments on flavonoid content and metabolomic enzymes in red raspberry

Gema Flores, María Luisa Ruiz del Castillo*

Instituto de Ciencia y Tecnología de Alimentos y Nutrición, Consejo Superior de Investigaciones Científicas (ICTAN-CSIC), c/ Juan de la Cierva 3, 28006 Madrid, Spain

ARTICLE INFO

Article history:
Received 12 March 2014
Accepted 12 June 2014

Keywords:
Raspberry
Methyl jasmonate
Polyphenols
Postharvest treatment
Preharvest treatment
Enzymes

ABSTRACT

The effect of preharvest and postharvest treatments with methyl jasmonate on the concentrations of myricetin, ellagic acid and quercetin in red raspberry was investigated. Different raspberry varieties and different MJ concentrations were included in the study. The preharvest MJ application to raspberry plants resulted in a significant increase of the flavonoids in all varieties. Increases from 90.32 to 336.95 μg/g of myricetin, from 103.15 to 218.91 μg/g of ellagic acid and from 65.22 to 163.15 μg/g of quercetin were obtained in Glen Lyon variety after pre-harvest treatment with 0.1 mM MJ. Postharvest MJ treatment did not lead to such a significant increase in the concentrations but enabled natural decline during storage to be avoided. Contents of myricetin, ellagic acid and quercetin were maintained. Concentrations in postharvest MJ treated raspberries were constant between 60 and 100 μg/g. Enzyme studies reflected increase in PAL activity after preharvest MJ treatment. No MJ promoting effect and even an inhibitory effect was however observed in FHT and FLS enzymes, respectively. The results found in the present work help get an insight into the mechanisms of MJ action in phenylpropanoid metabolism in raspberries. Preharvest MJ treatment of raspberries can be useful for obtaining fruit with enhanced healthy promoting properties.

1. Introduction

In the last few years, research on the relationships between a number of diseases risk reductions and consumption of phytochemical-rich fruit and vegetables has widely increased. Some of the hypothesized anticancer mechanisms include antioxidant, anti-inflammatory and antiproliferative activities, inhibition of bioactive enzymes and induction of detoxification enzymes (Bravo, 1998; Yang et al., 2001).

In this context, ellagic acid is a phytochemical which has been demonstrated to induce apoptosis by preventing continuous tumor growth. It also possesses potential cytotoxic and anti-proliferative activities in human cells (Narayanan et al., 1999; Losso et al., 2004). Equally, some studies have proved the effectiveness of ellagic acid in preventing the growth of tumors in animals (Khanduja et al., 1999). The mechanism of action may be the explicit interaction of ellagic acid with the cell walls or sites with facility to complex proteins, minimizing the proliferation of metastatic cells (Feldman et al., 1999). Similarly to ellagic acid, quercetin is another common flavonoid whose activity on cell cycle kinetics, proliferation and induction of apoptosis in cell culture has also been reported (Khang and Liang, 1997; Mouria et al., 2002). Among phytochemical compounds, quercetin is particularly relevant because of its ubiquity and concentration in the diet (Hertog et al., 1993).

Most berries, such blueberries, red raspberries and strawberries, contain both ellagic acid and quercetin. Quercetin usually occurs in berries as *O*-glycosides, with a sugar bound at the C-3 position. Ellagic acid is however present in three different forms: as ellagitannin, in which hexahydroxydiphenic acid forms esters with a sugar, as ellagic acid glycoside and, more rarely, as free ellagic acid (Häkkinen et al., 1999; de Ancos et al., 2000). Since all these forms are hydrolysable, hydrolysis is in the first place required to determine the total contents of quercetin and ellagic acid in foodstuffs. In addition to ellagic acid and quercetin, high concentrations of other natural antioxidants also occur in berry fruit (Wang et al., 1996). In particular, extracts of raspberries also contain myricetin, which has shown important biological properties such as anti-inflammatory, anti-tumor and antioxidant activities. It may also inhibit β-amyloid fibril formation, a key problem with Alzheimer's disease (Murakami and Ohnishi, 2012).

* Corresponding author at: Instituto de Ciencia y Tecnología de Alimentos y Nutrición (ICTAN-CSIC), c/ Juan de la Cierva 3, 28006 Madrid, Spain.
Tel.: +34 91 562 29 00; fax: +34 91 564 48 53.
E-mail address: mruiz@ictan.csic.es (M.L. Ruiz del Castillo).

http://dx.doi.org/10.1016/j.postharvbio.2014.06.009
0925-5214/

The biosynthesis of phenolic compounds in plants is quite well known (Haddock et al., 1982; Harborne, 1988). Ellagic acid has been described to be formed in some plants by oxidation and dimerization of gallic acid. Both ellagic and gallic acids can react with hydroxyl-containing compounds to form esters (Maas et al., 1991). However, in berries, ellagic acid is believed to be synthesized from 1,2,3,4,6-pentagalloylglucose through a number of hydrolysis reactions (Grundhofer et al., 2001). No characterization of the enzymes regulating the biosynthesis of ellagic acid has been reported so far (Maas et al., 1992). On the contrary, enzymes involved in the synthesis of quercetin and myricetin have been already specified. Quercetin and myricetin are synthesized in berries through phenylpropanoid metabolism (Halbwirth et al., 2006). This biochemical pathway starts with the formation of cinnamic acid from phenylalanine. This formation is catalyzed by phenylalanine ammonia lyase (PAL) enzyme. Further in the pathway, the cinnamic acid is transformed into naringenin, which leads, in turn, to dihydroflavonols by the action of the enzyme flavanone 3β-hydroxylase (FHT). Dihydroflavonols are finally oxidized to flavonols (ie, kaempferol, quercetin and myricetin) through flavonol synthase (FLS) (Lukacin and Britsch, 1997; Lukacin et al., 2000; Halbwirth et al., 2006).

At present, there is interest in developing functional foods by the employment of elicitors which trigger the enhancement of bioactive compounds. Although a number of elicitors have been reported in the literature, exogenous methyl jasmonate (MJ) has been demonstrated to increase the content of secondary metabolites in various plants to a greater extent (Kim et al., 2006; Wang et al., 2009). Occasional reports about MJ effects on red raspberry composition have proven that MJ affects the total antioxidant capacity of the berries (Wang and Zheng, 2005; Ghasemnezhad and Javaherdashti, 2008). Also, we have recently found that the postharvest exposure of strawberry and raspberry fruit to MJ did not affect the concentrations of flavonols (de la Peña Moreno et al., 2010a,b). However, MJ effects on specific antioxidant compounds in red raspberries have not been, to our knowledge, studied as yet.

With the intention of getting an insight into mechanisms of MJ action on the bioformation of certain health-promoting compounds in raspberries, we aimed to evaluate the influence of preharvest MJ (ie, applied to the plant) and postharvest MJ (ie, applied to the fruit) on the concentrations of ellagic acid, quercetin and myricetin in red raspberries. In addition, MJ effects on enzymes regulating the biosynthesis in raspberries of these compounds were also investigated.

2. Materials and methods

2.1. Samples and chemicals

HPLC grade methanol (MeOH) and acetonitrile (ACN) were supplied by Labscan Ltd. (Dublin, Ireland). Trifluoroacetic acid (TFA), MJ, Tween-20, polyclar AT, Tris/HCl, $FeSO_4$, Na-ascorbate, 2-oxoglutarate and ethylenediaminetetraacetic acid (EDTA), cinnamic acid, phenylalanine, naringenin, ellagic acid, quercetin, myricetin and kaempferol standards were purchased from Sigma (Steinheim, Germany). Ethyl acetate was provided by Scharlau. Tert-butylhydroquinone (TBHQ) was provided by Fluka (Steinheim, Germany). Chloric acid (HCl) was purchased from Probus (Badalona, Barcelona). Milli-Q water was collected from a purification system (Millipore, Milford, MA, USA). The three varieties of red raspberries (*Rubus idaeus* L.) included in this study were 'Glen Lyon', 'Glen Ample' and 'Tulameen'. Raspberries used for the analyses were all picked up with uniform size, color, ripeness and free from damage were utilized, as explained below.

2.2. Preharvest MJ treatments

Two-year-old red raspberry plants were used for the treatments. Seven plants from different rows were used per replicate. Raspberry plants were randomly divided into three groups for each variety. Group 1 (untreated-control plants) was only treated with 0.05% Tween-20, group 2 was treated with MJ 0.01 mM in 0.05% Tween-20 and group 3 was treated with MJ 0.1 mM in 0.05% Tween-20. All treatments were initially accomplished by applying a foliage-berry spray to run-off when berries were still in the green stage. Spraying was applied two more times at 2-week intervals, in the early light pink stage and later pink stage, respectively. Undamaged raspberries were selected on the basis of regular size, color and absence of physical injuries. An approximate 300 g weight of berries was collected in red stage. After picking the fruit, they were frozen at −80 °C until analysis, which was performed 2 weeks later. Five replicates of untreated-control samples from the same raspberry plant from the 'Glen Lyon' variety were in addition performed to estimate the repeatability of the overall analytical procedure.

2.2.1. Postharvest MJ treatment

Full-ripe red raspberry fruit from the three varieties were treated immediately after harvesting. For each variety, the experimental procedure was the same. Approximately 120 g of raspberries were distributed into three different 300 mL glass containers to be subject to the treatment. Three distinct vials containing 0.05% Tween-20 (untreated-control), MJ 0.01 mM in 0.05% Tween-20 and MJ 0.1 mM in 0.05% Tween-20 respectively were put inside of each of the three containers, whose lids were hermetically screwed. The amounts of MJ used were 0.0028 μL/g and 0.0280 μL/g for the treatments with 0.01 mM MJ and 0.1 mM MJ, respectively. MJ was allowed to spontaneously vaporize during 24 h at 25 °C. Afterwards, the vials were withdrawn from the containers, which were subsequently kept at 4 °C for 5 days.

2.3. Contents of ellagic acid, myricetin and quercetin

2.3.1. Extraction and hydrolysis

The extraction of ellagic acid, quercetin and myricetin from red raspberry fruit untreated-control as well as preharvest and postharvest treated with MJ 0.01 mM in 0.05% Tween-20 and MJ 0.1 mM in 0.05% Tween-20 MJ, was carried out by following the method described elsewhere (Häkkinen and Törrönen, 2000). In brief the procedure is as follows: a 20 g weight was homogenized with a blender. Acidified methanol (25 mL) containing 1% (v/v) HCl and 3.0×10^{-3} M TBHQ were added to the sample. Subsequently, HCl (1.2 M, 5 mL) was added to the mixture, which was then stirred at 90 °C under reflux for 2 h to hydrolyse glycosides to the corresponding aglycons. The resulting extract was allowed to get cold and then centrifuged at approximately 22,000 × *g* for 10 min. The upper layer was taken, filtered through a 0.45 μm filter (Millipore) and analyzed by HPLC as explained below.

2.3.2. HPLC-analysis

Chromatographic analyses of the extracts were performed employing a Konik-Tech model 560 (Barcelona, Spain) liquid chromatograph fitted with a manual injection valve (model 7725i, Konik-Tech, Barcelona, Spain) having a 20 μL sample loop and an ultraviolet (UV) detector operated at 360 nm. The simultaneous separation of ellagic acid, quercetin and myricetin was accomplished on an ACE 5 C18 column (250 mm × 4.6 mm i.d., 5-μm particle size, Madrid, Spain). The elution was performed by using solvent A (H_2O containing 0.1% TFA) and B (ACN/MeOH; 80/20) at a constant flow rate of 1.2 mL/min. A linear gradient was applied from the initial eluent composition, 70/30 (A/B, v/v), up to a final composition of 55/45 (A/B, v/v), which was reached at 30 min. Data