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UV-B-induced changes of volatile metabolites and phenolic compounds in blueberries (*Vaccinium corymbosum* L.)

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ABSTRACT

There are many reports on the potential consequences of UV-B radiation on plants, but there is a rather limited understanding of the effect on secondary plant metabolites, e.g. phenolic compounds and volatiles, at all. The popularity of highbush blueberries (*Vaccinium corymbosum* L.) is mainly due to its unique flavour and its high content of bioactive compounds, i.e. phenolic compounds. However, information on UV-B elicitor mediated changes on secondary plant metabolites on blueberries is scanty. In the present study, blueberry fruits were harvested and exposed to UV-B radiation with different dosage and adaptation times. With regard to volatile secondary metabolites, C_6-aldehydes, terpenes and ketones, an increase of the relative peak area was observed after both UV-B treatments (0.075 and 0.15 Wh/m^2 = low [L] and high [H] dosage, respectively). Furthermore, there was a strong influence of the adaptation time. Increasing relative peak areas were determined already after a short adaptation time (2 h) at both, low and high UV-B dosages, but after 24 h adaptation time relative peak areas decreased significantly. However, alcoholic compounds, as degradation products of aldehydes, showed opposite results. In contrast, the non-volatile phenolic compounds revealed a continuously increase with UV-B intensity.

1. Introduction

In recent years, increasing UV-B radiation by air pollution-induced ozone depletion has raised awareness of the effects of UV-B on the ecosystem. There are many reports on potential consequences of UV-B radiation on plants, but there is a rather limited understanding of the effect on secondary plant metabolites, e.g. phenolic compounds and volatiles, at all. Though only a small portion of the total solar spectrum, UV-B in the range of 280–315 nm has a large effect, it may induce photobiological stress and activate the plant defence system, leading to an accumulation of secondary plant metabolites in plant tissue (Teramura, 2006). Furthermore, the application of ultraviolet irradiation (especially UV-C) for sanitation purposes, and prevention of postharvest diseases were discussed for various plants many studies (Charles, Goulet, & Arul, 2008; Terry & Joyce, 2004). Due to the fact that secondary plant compounds are influenced by targeted postharvest UV-B treatment, it might be an important tool for enhancing the health beneficial properties of high valuable fruits and vegetable.

Highbush blueberries (*Vaccinium corymbosum* L.) gain popularity in Europe due to their flavour as well as due to possible health beneficial properties. They further offer high contents of phenolic compounds, whose beneficial health properties resulting from their antioxidant effects, have been extensively described (Häkkinen & Törrönen, 2000; Sellappan, Akoh, & Krewer, 2002). Volatile metabolites are not only responsible for the blueberry flavour, they also interact in the ecological network between plants and the environment and respond to stress conditions (e.g. herbivore attack or drought). Furthermore, terpenes are known to have bioactive properties (antimicrobial and anticarcinogen) (Paduch, Kandefer-Szersze, Trytek, & Fiedurek, 2007). It is also hypothesised that volatile compounds may have a plant protective function due to antioxidant activity (Wei & Shibamoto, 2007).

Recent studies reported an increase of phenolic compounds and corresponding antioxidant activity in berry fruits, i.e. *Ribes nigrum*, resulting from an UV-B mediated stress response (Huyskens-Keil, Eichholz, Kroh, & Rohn, 2007). However, information on the changes of volatile metabolites, as affected by UV-B radiation, is scarce. The aim of this study was to investigate the effect of two different UV-B dosages and two different adaptation times on the

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total phenolic content and the profile of volatile metabolites of highbush blueberries. This study might therefore contribute to an assessment of a moderate stress inducing UV-B application on the dynamics of health promoting plant metabolites.

2. Materials and methods

2.1. Plant material and UV-B treatment

Plant material was obtained from the highbush blueberry cultivar Bluecrop, which was cultivated on open land conditions at the research site of the Humboldt-Universität zu Berlin. Bushes were planted in peat filled holes on formerly used farmland. The experiments were conducted in the year 2007. The proportion of total annual precipitation was 873.6 mm, and a mean annual air temperature of 11.2 °C was recorded. Fertilisation and irrigation of the blueberry field were carried out following commercial cultivation practices. For the UV-B treatment and analysis of the volatile and the phenolic compounds, undamaged ripe berries were picked at the first harvest date (July 2007).

After picking, fruit samples of 250 g were subjected to UV-B radiation in polyethylene trays. The UV-B dosages used were 0.075 Wh/m^2 (low dosage = L) and 0.150 Wh/m^2 (high dosage = H); they were provided with an UV-B fluorescence light source (FL 20SE, 305–310 nm) with an average fluency rate of 8.2 Ws/m^2, at a distance of 30 cm to the fruits. Different adaptation times (2 or 24 h) were chosen in order to follow the impact of the treatments and to identify the plant response mechanism to UV-B. The level of UV-B intensity as well as the duration of the adaptation times were tested in earlier experiments on other fruits and vegetables, and provided the background of selected parameters for this study. After the adaptation times, the fruits were shock-frozen in liquid nitrogen and kept at −25 °C prior to lyophilisation (Christ Alpha 1–4, Christ; Osterode, Germany). The dried samples were ground for subsequent extraction and analysis of the total phenolic content. For the analysis of the volatile metabolites, fresh berries were used. The experiments were conducted with three replicates per UV-B treatment and adaptation time. Freshly harvested, non-treated fruits without any adaptation time were used as the control.

2.2. Extraction and analysis of total phenolic compounds

For the analysis of the phenolic compounds, the extraction was conducted according to Connor, Luby, and Tong (2002) using acidified methanol (0.1% hydrochloric acid). Briefly, an aliquot of 0.5 g ground sample was mixed with 3 ml of acidified methanol and centrifuged for 15 min at 3000 rpm; this was repeated three times. The supernatants were collected and standardised to a final volume of 10 ml. The total phenolic content of the fruit extracts was determined using the Folin–Ciocalteu method. The absorbance was measured after 1 h incubation time at a wavelength of 765 nm (LKB-Novaspek II, Pharmacia, Freiburg, Germany). The results were expressed as milligrams gallic acid equivalents (GAE) per gram dry matter.

2.3. Analysis of volatile metabolites

2.3.1. Sample preparation using headspace-solid phase microextraction (HS-SPME)

For the determination of the volatile metabolites 30 g fresh berries were mixed with 45 ml of 25% NaCl solution, homogenised for 1 min with an Ultra Turrax (Typ T 25, Janke and Kunkel KG, Staufen, Germany) at 24,000 rpm, and centrifuged for 10 min at 3000 rpm (Typ UJ 3, Martin Christ Osterrode am Harz, Germany). About 25 ml of the supernatant were mixed with 5 µl of the internal standard (500 ppm, 2,6-dimethyl-6-hepten-2-ol). Afterwards, 5 ml of the sample were mixed with 5 g of NaCl in 10 ml headspace vials (Gerstel, Mühlheim, Germany), and stored at −25 °C until SPME sampling.

After thawing, the volatile metabolites of the berries were isolated by headspace solid phase microextraction (HS-SPME) and afterwards analysed with a gas chromatograph (GC) using a polar column and flame ionisation detection. Fused silica fibre coated with polydimethylsiloxane–divinylbenzene (PDMS–DVB), 65 µm film thickness (Supelco, Bellefonte, PA, USA) was used for extraction and concentration of volatile compounds. The fibre was preconditioned at 250 °C for 10 min prior to sampling. The frozen sample vials were equilibrated to 36 °C in a water bath for 15 min and mixed. The fibre was exposed to the headspace with a Supelco (Bellefonte, PA, USA) manual SPME holder at 36 °C for 30 min, and immediately transferred to the gas chromatograph. The fibre was injected in the GC at 250 °C for 2 min in splitless mode.

2.3.2. Gas chromatography–mass spectrometry (GC–MS)

The GC–MS analysis for the identification of the volatile metabolites was carried out on representive samples (n = 4) using an Agilent 6890N GC System, coupled to an Agilent 5973 mass selective detector (Agilent Technologies, Waldbronn, Germany), equipped with an INNO-Wax column (30 m; 0.25 mm i.d.; 0.5 mm film thickness). The GC conditions were as follows: carrier gas: helium 1.1 ml/min; injector temperature: 250 °C; split ratio: 1:50; oven temperature programme: 45–180 °C at 1.5 °C/min.

The volatile compounds were identified by calculating and comparing the Kovats retention indices, as well as by comparing the MS spectra data with spectra libraries (Chemstation 1989–2004, Agilent Technologies, Waldbronn, Germany).

2.3.3. Gas chromatography-flame ionisation detection (GC-FID)

A semi-quantitative analysis was carried out on an HP 5890 Series II Plus gas chromatograph equipped with a flame ionisation detector (FID). The compounds were separated on an INNO-wax column (30 m; 0.25 mm i.d.; 0.5 mm film thickness). The GC conditions were as follows: carrier gas: nitrogen 0.7 ml/min; injector temperature: 250 °C; split ratio: 1:50; oven temperature programme: 45 °C at 1.5 °C/min; 180 °C at 5 °C/min; and 220 °C at 25 °C/min. All the analyses were carried out in triplicate for each sample.

The identified compounds of the MS analysis were transferred to the chromatogram of the semi-quantitative analysis by means of calibration of GCs, after the measurement of boiling points (Agilent Technologies, Waldbronn, Germany, 5080-8716). The data evaluation was carried out semi-quantitatively by using an internal standard (Ulrich & Nothnagel, 2006). Results were expressed as relative peak areas in proportion to the peak area of the internal standard. After calculation of the relative peak area, a classification into large (relative peak area = $A > 1$), middle (relative peak area = $0.1 < A < 1$) and small (relative peak area = $A < 0.1$) peak areas was performed. Statistical calculations of the treatments were made from the identified compounds of the berries with large and middle peak areas.

2.4. Statistics

The statistical evaluation was performed using SPSS 13.0 (SPSS Inc., USA). Significance of differences was conducted with a Tukey test ($p < 0.05$). The mean variability was indicated by the standard deviation.