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Influence of pre-harvest UV-B irradiation and normal or controlled atmosphere storage on flavonoid and hydroxycinnamic acid contents of pak choi (*Brassica campestris* L. ssp. *chinensis* var. *communis*)

Britta Harbaum-Piayda[a,*], Bettina Walter[a], Gunnar B. Bengtsson[b], Eva Maria Hubbermann[a], Wolfgang Bilger[c], Karin Schwarz[a]

[a] Department of Food Technology, Institute of Human Nutrition and Food Science, University of Kiel, Heinrich-Hecht-Platz 10, DE-24118 Kiel, Germany
[b] Nofima Mat AS, Osloveien 1, NO-1430 Aas, Norway
[c] Botanical Institute, University of Kiel, Am Botanischen Garten 3-9, DE-24118 Kiel, Germany

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ABSTRACT

The influence of different initial phenolic contents in pak choi (*Brassica campestris* L. ssp. *chinensis* var. *communis*) leaves, obtained by pre-harvest treatment with and without UV-B, on storage behaviour was investigated. The storage conditions were controlled (1.5–2.5% O_2 and 5–6% CO_2) or normal air atmospheres at 2 °C and 99% relative humidity. A complementary pre-harvest experiment was conducted to investigate the effect of temperature and UV-B irradiation on the level of phenolic compounds. Both UV-B treatment and temperature showed significant effects regarding polyphenolic contents determined by HPLC-DAD; total polyphenolic content increased under low temperature even without UV-B. UV-B irradiation resulted in a distinct increase in hydroxycinnamic acid derivatives at low temperature (9 °C) and of flavonoids at ambient temperature (22 °C), which might be related to the enhanced level of flavonoid precursors, i.e. hydroxycinnamic acids, which are not utilized for flavonoids in the biosynthesis pathway at low temperature. This hypothesis is supported by the strong increase in the concentration of coumaric acid derivatives under UV-B treatment and low temperature. The epidermal UV-A absorption by PAM fluorometry (pulse amplitude modulation) increased after cultivation under UV-B irradiation and this effect was more pronounced at 22 °C than at 9 °C due to the increases of flavonoid contents and their good correlation with epidermal absorption. Polyphenols are responsible for the epidermal absorption of leaves in the UV range of irradiation. The non-destructive PAM fluorometry of epidermal screening and HPLC-DAD analysis for flavonoids of leaf extracts correlated well and both methods were also applied in the postharvest storage experiment. Plants with a higher initial polyphenolic content showed an increasing effect in epidermal UV-A absorption data and a significantly increasing concentration for flavonoids over the storage period, which is assumed to be due to ongoing biosynthesis induced by the pre-harvest UV-B treatment. The level of flavonoids increased more in controlled atmospheres than in normal air, but hydroxycinnamic acids were unaffected. Fresh weight and chlorophyll content of the plants as markers of postharvest senescence changed only marginally during storage, but UV-B treated plants lost significantly more weight than plants without this treatment.

1. Introduction

Pak choi is commonly consumed in Asian countries and is becoming increasingly important in the Western diet. *Brassica* vegetable consumption has been associated with a number of human health benefits such as lower morbidity and mortality of cancer and heart diseases because of their high content of secondary plant constituents such as the glucosinolates reported for broccoli (Verhoeven et al., 1997; Kaur and Kapoor, 2001; Murillo and Mehta, 2001; Lynn et al., 2006).

Pak choi is an Asian leafy vegetable, which is commonly consumed not only fresh (e.g. as salad), but also after cooking or fermentation. Pak choi contains relatively low concentrations of glucosinolates (He et al., 2003), but it may be important for health due to high concentrations of phenolic compounds (Herrmann, 1999; Harbaum et al., 2007). Several kaempferol derivatives and other phenolic compounds such as hydroxycinnamic acid derivatives have been identified and quantified in pak choi (Rochefort et al., 2006; Harbaum et al., 2007). Under greenhouse conditions, the concentration of polyphenols in the leaf blade of various

* Corresponding author. Tel.: +49 431 880 5034; fax: +49 431 880 5544.
E-mail address: info@foodtech.uni-kiel.de (B. Harbaum-Piayda).

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pak choi cultivars ranged from 4.7 to 16.7 mg/g dry matter for flavonoid derivatives and 1.5 to 5.8 mg/g dm for hydroxycinnamic acid derivatives, and under field conditions from 15 to 39 mg/g dm and 5 to 7.7 mg/g dm, respectively (Harbaum et al., 2007, 2008).

Polyphenols with their main groups, flavonoids and hydroxycinnamic acid derivatives, have many protective functions in plants (Lois, 1994). Thus, their content in plants is closely related to several stress factors such as UV-B irradiation, high light intensity, extreme temperature or nutrient deficiencies during cultivation (Dixon and Paiva, 1995; Treutter, 2005). UV-B irradiation and low temperature have been found to induce the accumulation of polyphenols in epidermal cells (Day et al., 1993; Cockell and Knowland, 1999; Bilger et al., 2007).

It is well established that low temperature, high humidity and low O_2 concentrations, as used in storage under controlled atmosphere (CA), have a beneficial effect on the postharvest life of green leafy vegetables (Isenberg, 1979; Dewey, 1983). A low level of O_2 and a high level of CO_2 lead to a lower rate of respiration and ethylene production (Kader et al., 1989), which is involved in the senescence process of plants. Additionally it has been shown that CA storage generally results in slower degradation of chlorophyll and ascorbic acid (Kader, 1986).

Only a few studies with varying results are available regarding the influence of storage conditions on polyphenolic compounds in *Brassica* vegetables. A strong decrease of phenolic concentration during cold storage (1 °C) under modified atmosphere was found in broccoli (Vallejo et al., 2003). A reduction in O_2 concentration during storage led to an inhibition in the decline in phenolic compounds (Serrano et al., 2006). However, polyphenols, and especially flavonoids, are generally considered stable during cool storage (Gil et al., 1998; Leja et al., 2001; Hagen et al., 2009). Storage under low temperature led to increased phenolic contents in artichoke (Saltveit and Morris, 1990) and eggplant (Kozukue et al., 1979). Concentrations of flavonoids and hydroxycinnamic acid derivatives showed increasing tendencies within the first 6 d during storage of several Chinese *Brassica* vegetable varieties (at 4 °C and ambient temperature under normal atmosphere in the dark) (Harbaum et al., 2008). Starzynska et al. (2003) found a higher antioxidative capability after plants were exposed to higher stress such as 3 d of storage at 20 °C. With the addition of exogenous antioxidants Meir et al. (1995) observed a reduction of chlorophyll degradation, which is an indicator for the senescence of green leaves (Page et al., 2001).

However, nothing is known about the influence of natural antioxidants such as polyphenols on the storage behaviour of vegetables. Potentially, a higher content of phenolic compounds and other antioxidants could protect plants against senescence and might lead to greater storability.

The aim of this study was to evaluate the effect of temperature and irradiation during the growth period on the polyphenolic content of pak choi and to characterize the influence of polyphenol content at harvest on storage behaviour of this vegetable. To measure the content of polyphenolic compounds in pak choi leaves, two different methods were applied: non-destructive pulse amplitude modulated (PAM) fluorometry of epidermal cell and HPLC analysis of leaf extracts. Utilizing PAM fluorometry it is possible to determine non-invasively the epidermal absorption caused by compounds present in the epidermis of the plant leaf (Bilger et al., 2001; Cerovic et al., 2002). The major part of the phenolics in a leaf, especially the flavonoids, is located in the upper epidermis (Hutzler et al., 1998). A significant correlation between epidermal absorption and total flavonoid contents has been presented in numerous previous studies on leaves, flower buds and fruit, for instance by Burchard et al. (2000), Bilger et al. (2001), Cerovic et al. (2002), Bengtsson et al. (2006) and Hagen et al. (2006).

2. Materials and methods

2.1. Chemicals

Acetone (Fisher Scientific), acetonitrile (HPLC grade, Fisher Scientific), chlorogenic acid (Sigma), kaempferol-3-*O*-hydroxyferuloyldiglucoside-7-*O*-glucoside (isolated as presented by Harbaum et al., 2007), methanol (HPLC grade, Fisher Scientific), metaphosphoric acid (Fluka), $Mg(HCO_3)_2$ (Sigma), naringenin (Carl Roth GmbH), oxalic acid dihydrate (Carl Roth GmbH), sinapic acid (Carl Rot GmbH), trifluoroacetic acid (Carl Roth GmbH) were used.

2.2. Non-destructive epidermal screening

Epidermal UV-A absorption was measured by a UV-A-PAM fluorometer (Gademann Instruments, Würzburg, Germany) on the adaxial side of the leaves. Chlorophyll fluorescence was induced with diodes emitting UV-A irradiation (375 nm) and blue light (470 nm). Epidermal transmittance was calculated by dividing the ratio of the two fluorescence intensities, $F(UV)/F(B)$ by a reference value of 1.2 for 100% transmittance, which was previously determined with epidermis-free leaves. For further evaluation, epidermal absorption was calculated as −log(transmittance).

2.3. Growth experiment

Plants of pak choi (*Brassica campestris* L. ssp. *chinensis* var. *communis*, cv. Hangzhou You Dong Er) were cultivated individually inside a controlled cultivation chamber at 22 °C and 100 μmol/m^2 s photosynthetically active radiation for 4 weeks in pots. The light/dark period was 16 h/8 h during the whole experiment. The plants were then separated into four different groups (five plants each) and further cultivated for 1 week under the following conditions: 22 °C at 100 μmol/m^2 s without UV-B irradiation (22 °C/−UV-B), 22 °C at 100 μmol/m^2 s with UV-B of 0.35 W/m^2 (22 °C/+UV-B), 9 °C at 100 μmol/m^2 s without UV-B (9 °C/−UV-B) and 9 °C at 100 μmol/m^2 s with UV-B of 0.35 W/m^2 (9 °C/+UV-B). The final epidermal absorption was measured (see Section 2.2). A leaf disc, diameter 1.1 cm, was punched out of the first completely developed leaf from each plant, frozen in liquid nitrogen, lyophilized and used for the quantification of the polyphenolic content.

2.4. Storage experiment

2.4.1. Treatment of plants during cultivation

Plants of pak choi (same variety as in the growth experiment) were cultivated in a greenhouse at Aas, Norway (59°30′N, 10°47′E, 80 m altitude). On August 24, sprouted seeds were planted individually in 12 cm pots with commercial peat-based soil containing nutrients. Shading was applied after 15 d giving low light conditions with photosynthetically active radiation of approximately 50 and 150 μmol/m^2 s on cloudy and sunny days, respectively. The temperature in the greenhouse had the largest variation (min. 12 °C, max. 38 °C) during the first 3 weeks. Later heating was applied and the temperature varied less (min. 16 °C, max. 23 °C). After 40 d half of the plants were treated with supplemental UV-B irradiation of 0.35–0.42 W/m^2 (+UV-B) for 16 h/d over 10 d. The rest of the plants served as controls (−UV-B). The UV-B treatment was achieved by suspended fluorescent tubes with emission range of 280–380 nm (UVB-313, Q-panel Lab Products, Cleveland, OH, USA). UV-C radiation was removed with 0.13 mm cellulose diacetate foil (Courtaulds, Derby, UK) in front of the tubes. After treatment the epidermal UV-A absorption measured with the UV-A-PAM instrument was 1.00 (±0.083) for UV-B treated plants and 0.37 (±0.057) for the control plants. The shoots (above ground part) of healthy