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Rutin in buckwheat herbs grown at different UV-B radiation levels: comparison of two UV spectrophotometric and an HPLC method

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Abstract

Rutin is an antioxidant with many interesting pharmacological effects. It can also be found in buckwheat (*Fagopyrum esculentum* Moench). UV radiation stimulates the activity of enzymes of the phenylpropanoid pathway and there is some evidence that it influences the rutin content in plants. The aim of the present research was (1) to examine the influence of different levels of UV-B radiation on rutin content and (2) to compare the results obtained by three analytical methods. The plants were grown under three UV-B levels: reduced, ambient and enhanced, simulating 17% ozone depletion. Analyses were performed by HPLC and two spectrophotometric methods. In one, the absorbancies were measured at 420 nm with and without the addition of $AlCl_3$. In another method the concentration was calculated from absorbancies at 352.5 nm and 366.5 nm according to the Official Methods of Analysis of AOAC International. The highest amounts of rutin were found in flowers, followed by leaves and stems. A comparison of the different treatments revealed that the highest amounts of rutin were in plants grown under ambient radiation, followed by the plants cultivated under enhanced UV-B and then under reduced UV-B radiation. Treatments caused more effect on leaves than on flowers. Leaves developed under ambient light conditions contained 97% more rutin than leaves grown under reduced UV-B radiation. In flowers, the contents differed by 19% only. The results obtained using the three methods showed a good correlation, but the absolute differences were surprisingly high. The AOAC and the $AlCl_3$ methods gave, on average, 140% and 30% higher results than HPLC, respectively.

Key words: Buckwheat, *Fagopyrum esculentum,* rutin, UV radiation.

Introduction

There is strong evidence that UV-B radiation stimulates phenyl propanoid metabolism. This comprises synthesis of different flavonoids and other polyphenolic compounds like tannins and lignins (Björn, 1999; Rozema *et al*., 1997). Since flavonoids and phenolics show absorbance in the UV-B range they present a selective UV-B filter which protect plant tissue against harmful rays (Rozema *et al*., 2002). Rutin is a highly antioxidatively active flavonoid. It can be found in many plants, but only a few nutritionally important species contain such high amounts as buckwheat. About 2–10% of rutin per dry weight can be found in buckwheat leaves and flowers (Kreft *et al*., 1999; Hagels, 1999*a*). There is some rutin also in buckwheat grains and flour (Kreft *et al*., 1999; Qian *et al*., 1999). Although the concentrations of rutin in grains and flour are lower (up to 0.01%) than in herbs, this source can represent an important part of daily intake of flavonoids in human nutrition.

Rutin and its aglycone quercetin were shown to have antioxidative action *in vitro* and *in vivo*. They can act directly by entering the redox reactions, and indirectly by chelation of iron. Besides the antioxidative action, rutin also possesses other interesting pharmacological effects. Vasoconstrictive, spasmolitic, antiviral, positive inotropic,

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cyclo-oxygenase and lipoxygenase inhibitory, and anti-tumour activity were studied. The efficacy of buckwheat herb preparations has been shown in several clinical studies, involving patients with microcirculation problems or chronic venous insufficiency (Hagels, 1999*b*).

There is some evidence that UV radiation increases the production of rutin in plants. In St John's wort (*Hypericum perforatum*) a strong correlation between rutin content and the altitude of the growing site was observed (Umek *et al*., 1999). The plants growing higher than 800 m above the sea level contained, on average, 4-fold higher amounts (0.72% DW) of rutin than plants growing lower than 200 m above the sea level (0.19% DW). Among the plants growing below 400 m (n=11) 64% contained no detectable rutin (detection limit 0.001% DW), but all the plants (n=11) growing above 400 m contained rutin (minimum 0.2% DW). Ohsawa and Tsutsumi (1995) showed that the buckwheat plants sown in the early summer contained higher amounts of rutin than the plants sown in the late summer. The difference is attributed to different solar radiation levels during the experiments.

The aim of this study was to study the influence of different levels of UV-B radiation on rutin content and to compare the results obtained by three analytical methods; HPLC and two spectrophotometric methods. Additionally, the content of another product of the phenyl propanoid pathway, tannin, was examined in buckwheat plants.

Materials and methods

Buckwheat (*Fagopyrum esculentum* Moench) cv. Darja was cultivated in Ljubljana (Slovenia) from 1 August to 10 September 1999. Three different treatments were applied: enhanced UV-B treatment: simulation of 17% ozone depletion (at the lower leaf level) using Q-Panel UV-B 313 lamps (Cleveland, OH, USA), wrapped in cellulose diacetate filters, which cut out the UV-C range (wavelengths lower than 280 nm); UV-B depleted treatment: reduction of UV-B radiation using Mylar foil which cut the UV-B range (wavelengths lower than 320 nm) positioned 80 cm above the plants; and control treatment: ambient radiation and Q-Panel UV-B 313 lamps wrapped in Mylar foil. The systems were timer controlled. The doses simulating 17% ozone depletion were calculated and adjusted weekly using the programs made by Björn and Murphy (1985), The doses are expressed as biologically effective radiation ($UV\text{-}B_{BE}$), which was calculated using the generalized plant action spectrum (Caldwell, 1968). $UV\text{-}B_{BE}$ is usually used because different wavelengths do not affect biomolecules and biological processes to the same extent. Therefore, a number of different UV-B action spectra or weighting functions have been developed. The result of weighting each single wavelength with an action spectrum integrated over all relevant wavelengths gives the biologically effective UV-B dose (Björn, 1999). UV-B was monitored by a three-channel dosimeter (ELDONET) belonging to the European light dosimeter network (Fig. 4). Five plants from each treatment group were chosen randomly out of 100 specimens for the rutin analyses. The freeze-dried samples (100 mg) were extracted with methanol:acetic acid:water (100:2:100 by vol.) at room temperature for 1 h. The HPLC analyses were performed by the injection of 20 µl of extract on a Lichrospher 100RP-18 (5 µm) column (250×4 mm), elution with a gradient of methanol (A) and 2% acetic acid in water (B) (40% A to 70% A in 0–2 min, 1 ml min^{-1}) and detection at 355 nm. Rutin eluted at 6.6 min and the peak area was compared to the standard.

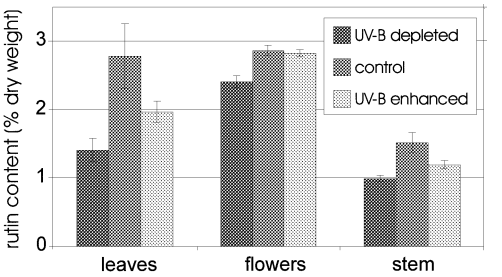

Fig. 1. Rutin content in different organs of the buckwheat plants growing in different illumination conditions. Only the results of analyses obtained by HPLC are presented. Standard error is represented by error bars.

The samples for spectrophotometric analyses were diluted 50-fold. In the first method ($AlCl_3$ method), 0.2 ml of 5% $AlCl_3$ in methanol or 0.2 ml of methanol was added to 2 ml of diluted sample. After 30 min, the absorbance at 420 nm was measured in both solutions. The concentration was calculated from the differences of both measurements.

In the second method, according to AOAC (1995), the absorbance of the diluted sample was measured at 352.5 nm and 366.5 nm. The concentration of rutin was calculated according to the published system of equations.

The concentration of tannins was determined according to a published procedure (Luthar, 1992). Briefly, 0.5 ml of freshly prepared vanillin–HCl reagent was added to 0.1 ml of sample. Separately, 0.5 ml of the solvent was added to 0.1 ml of sample. The absorbance at 500 nm was measured in both samples and the concentration was calculated from their difference.

Results and discussion

The comparison between the organs of the buckwheat plant shows that the highest rutin content is in flowers, followed by leaves and stems (Fig. 1). Rutin content in the different plant organs is in good agreement with the results of some other authors (Hagels, 1999*a*). By contrast, previous capillary electrophoretic (CE) analyses (Kreft *et al*., 1999) showed a considerably lower content of rutin in leaves and stems. Only the CE analyses of rutin in flowers are in agreement with the present study. This may be explained by the fact that rutin content in leaves and stems depends more on UV radiation than does rutin content in flowers (see below). A comparison between the plant groups grown under the different irradiation conditions shows that rutin production is enhanced in the plants exposed to ambient light, compared to those treated with reduced levels of UV-B. The rutin content is lower in plants growing at a level of UV-B light corresponding to 17% ozone depletion. The same pattern can be observed in all the organs examined, but the effect of irradiation is much stronger in leaves than in flowers. Ambient UV-B level caused a 97% increase of rutin in leaves and only a 19% increase in flowers compared with plants treated with

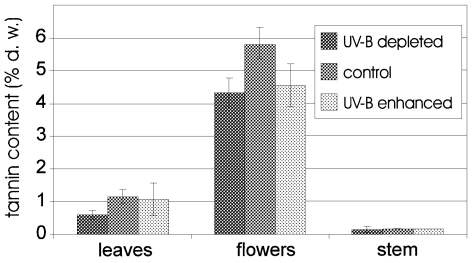

Fig. 2. Tannin content in different organs of the buckwheat plants growing in different illumination conditions. Standard error is represented by error bars.

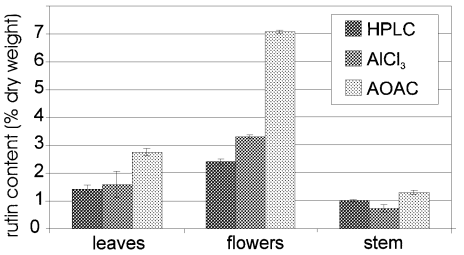

Fig. 3. Rutin content in different organs of the buckwheat plants determined by different analytical procedures. Standard error is represented by error bars.

reduced levels of UV-B. These findings indicate an important role of UV-B radiation in rutin synthesis. It seems that applied doses of UV-B radiation exert a state of stress, where limits of tolerance are exceeded and adaptive capacity is overtaxed, that possibly results in a disturbance of rutin synthesis.

As with rutin, the highest tannin content is also found in flowers, followed by leaves and stems, and its content is also highest in the control group, followed by plants grown under depleted UV-B radiation conditions and enhanced UV-B radiation (Fig. 2). UV-B depletion resulted in a 92% decrease of tannin levels in leaves and only a 34% decrease in flowers. The differences in tannin content comparing different organs are much higher than the differences in rutin content.

The comparison of the three analytical methods shows a reasonably good correlation (r=0.86), but the absolute differences are surprisingly high (Fig. 3). The higher values obtained by the $AlCl_3$ spectrophotometric method can be explained by its non-selectivity. $AlCl_3$ reacts with other flavonoids from the sample. Their presence is evident on the HPLC chromatograms. The $AlCl_3$ method gave 30% higher results than HPLC. The AOAC method gave, on average, 140% higher results than HPLC. The error was higher for flowers (194%) than for leaves (94%). The high discrepancy of the results obtained by the AOAC method suggests that this method, which is optimized for the determination of rutin in tablets, is not suitable for the plant samples.

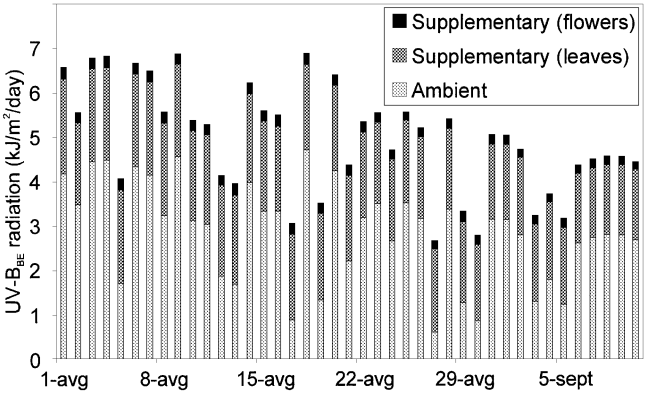

Fig. 4. Natural and supplemental UV-B_{BE} radiation (upper limits at the level of the leaves and lower limit at the level of flowers) of buckwheat during the treatment.

Conclusions

The measurements suggest that ambient levels of UV-B radiation stimulate rutin accumulation in buckwheat plant compared with a reduced UV-B level. The effect is more evident in leaves than in flowers. Enhanced UV-B radiation obstructs rutin accumulation, but it is not clear whether this is a direct influence or an influence through unspecific damage to the plant.

HPLC and a spectrophotometric method using $AlCl_3$ are both suitable for the determination of rutin and flavonoids in buckwheat herb samples. An AOAC method is not suitable for this purpose.

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References

AOAC. 1995. *Official methods of analysis*. Washington: Association of Analytical Chemists, 29.

Björn LO. 1999. Effects of ozone depletion and increased ultraviolet-B radiation on terrestrial plants. In: Baumstark-Khan C, ed. *Fundamentals for the assessment of risks from environmental radiation*. Dordrecht, Boston, London: Kluwer Academic Publishers, 463–470.

Björn LO, Murphy TM. 1985. Computer calculation of solar ultraviolet radiation at ground level. *Physiologia Végétale* **23,** 555–561.

Caldwell MM. 1968. Solar UV radiation as an ecological factor for alpine plants. *Ecological Monographs* **38,** 243–268.

Hagels H. 1999*a*. Sekundare Pflanzeninhaltstofe des Buchweizen. In: *Das Buchweizen Buch*. Arzfeld, Luxemburg: Islek ohne Grenzen, 103–109.

Hagels H. 1999*b*. *Fagopyrum esculentum* Moench. medicinal

review. *Research Reports, Biotechnical Faculty, University of Ljubljana, (Slovenia)* **73,** 315–329.

Kreft S, Knapp M, Kreft I. 1999. Extraction of rutin from buckwheat (*Fagopyrum esculentum* Moench) seeds and determination by capillary electrophoresis. *Journal of Agricultural and Food Chemistry* **47,** 4649–4652.

Luthar Z. 1992. Vsebnost in razporeditev tanina v semenih ajde (Fagopyrum esculentum Moench), Dissertation, Ljubljana.

Ohsawa R, Tsutsumi T. 1995. Inter-varietal variations of rutin content in common buckwheat flour (*Fagopyrum esculentum* Moench). *Euphytica* **86,** 183–189.

Rozema J, Björn LO, Bornman JF, et al. 2002. The role of UV-B radiation in aquatic and terrestrial ecosystems—an experimental and functional analysis of the evolution of UV-B absorbing compounds. *Journal of Photochemistry and Photobiology, B. Biology* **66,** 2–12.

Rozema J, van de Staaij J, Björnn LO, Caldwell M. 1997. UV-B as an environmental factor in plant life: stress and regulation. *Tree* **12,** 22–28.

Qian JY, Mayer D, Kuhn M. 1999. Flavonoids in fine buckwheat (*Fagopyrum esculentum* Mönch) flour and their free radical scavenging activities. *Deutsche Lebensmittel-Rundschau* **95,** 343–349.

Umek A, Kreft S, Kartnig T, Heydel B. 1999. Quantitative phytochemical analysis of six *Hypericum* species growing in Slovenia. *Planta Medica* **65,** 388–390.