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Apple flavonols during fruit adaptation to solar radiation: spectral features and technique for non-destructive assessment

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Summary

Spectral properties of flavonols of three varieties (Golden Delicious, Antonovka, and Renet Simirenko) of anthocyanin-free apple fruit were investigated with reflectance spectroscopy. The results of spectral and biochemical analyses suggested that fruit reflectance in a broad spectral range 365–430 nm is strongly dependent on and, in sunlit fruit surfaces, governed by flavonols. The build up of peel flavonols (mainly rutin and other quercetin glycosides) resulted in a dramatic decrease of fruit reflectance in this range, flattening of the spectrum, and extending the region with low reflectance (4–5%) to ca. 410 nm. The spectral features observed suggest that flavonols contribute significantly to screening of excessive radiation, not only UV-A, but in the short-wave bands of chlorophyll and carotenoid absorption in the visible part of the spectrum as well. To retrieve quantitatively flavonol content from reflectance spectra, we tested the applicability of an inversion technique developed for non-destructive leaf pigment assessment. The model for flavonol content assessment was suggested in the form $(R_{410}^{-1} - R_{460}^{-1})R_{800}$, where R_λ is reflectance at wavelength λ. The model was linearly related to flavonol content between 8 and 220 nmol/cm^2 with the coefficient of determination r^2=0.92 and root mean square error of flavonol estimation of 20 nmol/cm^2 regardless of cultivar, chlorophyll, and carotenoid content.

Abbreviations: Chl, Chlorophyll(s); Car, Carotenoid(s); Flv, Flavonol(s); FRI, Flavonol Reflectance Index; IP, Inflection Point; NIR, Near Infra Red

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Introduction

Flavonols (Flv) are an abundant group of phenolic compounds involved in a number of physiological functions in higher plants (Markham, 1989; Harborne and Williams, 2000). Several lines of evidence suggest their protective role against damages induced by excessive UV and visible radiation (Tevini et al., 1991; Day et al., 1993; Bornman et al., 1997; Jansen et al., 1998; Burchard et al., 2000; Cerovic et al., 2000; Mazza et al., 2000; Havaux and Kloppstech, 2001; Kolb et al., 2001; Liakoura et al., 2003). Although Flv are able to exert their photoprotective action via several mechanisms (Takahama, 1983; Bornman et al., 1997; Havaux and Kloppstech, 2001), it is generally accepted that the most important mechanism is related to the screening of excessive solar radiation by Flv accumulating in the surface plant tissue structures: cuticle (Krauss et al., 1997; Solovchenko and Merzlyak, 2003) and epidermis (Tevini et al., 1991; Day et al., 1993; Mazza et al., 2000). Accordingly, the knowledge of Flv in vivo optical properties as well as the possibility of non-destructive estimation of their content could give a clue about potential of a plant organism for acclimation to and cope with excessive solar radiation as well as to a variety of other stresses, which are often accompanied by the induction of Flv biosynthesis (Harborne and Williams, 2000; Mazza et al., 2000). The main body of evidence for the photoprotective role of Flv and other phenolics in plants was obtained in the experiments with leaves (Burchard et al., 2000; Mazza et al., 2000). However, the contribution of phenolics into optical properties of leaves is difficult to evaluate quantitatively due to strong interference by Chl and Car (Cerovic et al., 2000).

Compared to leaves, apple fruits contain much lower quantities of the pigments and possess more resolved reflectance spectra with distinct features attributable to Flv presented mainly by rutin and other quercetin glycosides (Escarpa and González, 1998; Reay and Lancaster, 2001; Solovchenko and Merzlyak, 2003). In apple fruits, strong solar radiation induces a remarkable increase in Flv evidently to prevent the development of a photooxidative damage—sunscald (see Merzlyak et al., 2002). The considerable difference in Flv content between sunlit and shaded fruit surfaces have been recorded through different stages of on-tree fruit ripening and were retained in the course of prolonged fruit storage (Merzlyak et al., 2002). The build up of Flv in surfaces of anthocyanin-free apples exposed to direct sun-light resulted in a steep decrease of whole fruit reflectance in the UV-A range (Solovchenko and Merzlyak, 2003). The corresponding sun-light-induced increase in absorption in this band has been also observed in isolated cuticles and, especially, in the peel of apple fruit (Solovchenko and Merzlyak, 2003). The accumulation of Flv in apple peel resulted in increased resistance of the fruit photosynthetic apparatus to high fluxes of visible (Merzlyak et al., 2002) and UV radiation (Solovchenko and Schmitz-Eiberger, 2003). These circumstances make apple fruits a useful natural system for studies of the influence of solar radiation on the expression of photoprotective reactions and, in particular, the effect of Flv on the optical properties of plants.

Recently, a conceptual model relating remotely sensed reflectance and pigment content was developed and applied for non-destructive estimation of Chl, Car, and anthocyanins in higher plant leaves (Gitelson and Merzlyak, 1996; Gitelson et al., 1996, 2001, 2002, 2003, Merzlyak et al., 2003a) and fruits (Merzlyak et al., 2003a, b). The model was devised to isolate the absorption coefficient of a pigment of interest from reflectance spectra. The following relationship between pigment content and reflectance was used:

$$[\text{Pigment}] \propto (R_{\lambda_1}^{-1} - R_{\lambda_2}^{-1})R_{\lambda_3}.$$

Here λ_1 is the spectral region such that $R_{\lambda_1}^{-1}$ is maximally sensitive to the absorption of the pigment of interest, although it is still affected by the absorption of other pigments and leaf scattering. λ_2 is the spectral region such that $R_{\lambda_2}^{-1}$ is minimally sensitive to the pigment of interest and maximally sensitive to absorption by other interfering pigment(s). It was also assumed that the absorption coefficient of other pigments at λ_2 was close to that at λ_1. Thus, the difference $(R_{\lambda_1}^{-1} - R_{\lambda_2}^{-1})$ was related to the content of a pigment of interest. However, it was still affected by the variability in leaf structure and thickness (Gitelson et al., 2003), that is, the scattering by the medium. λ_3 is a spectral region minimally affected by the absorption of pigments, and it therefore was used to compensate for the variability in scattering between samples.

In this study we attempted to investigate in vivo spectral properties of Flv in apple fruit in more detail and quantitatively. In addition, we investigated the applicability of the conceptual model for the non-destructive estimation of Flv content. We hypothesized that tuning of the spectral regions λ_1, λ_2, and λ_3 according to the optical characteristics of the apple fruit is the only requirement to achieve the desired result. To the best of our knowledge, non-destructive assessment of Flv in plants has not been considered to date in the literature.