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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.

Material and methods

Plant material

Ripe anthocyanin-free fruits of apple (*Malus domestica* Borkh.) varieties (Golden Delicious, Antonovka and Renet Simirenko) without symptoms of sunscald were used in this study. Antonovka apples were grown in commercial orchards in Michurinsk (Tambov region, Russia); Golden Delicious and Renet Simirenko fruits were delivered from the Krasnodar region of Russia where accidents of sunscald are common. All samples have been studied within 2–3 weeks.

Spectral measurements

Whole fruit reflectance spectra were recorded with a 150–20 Hitachi spectrophotometer equipped with an integrating sphere against barium sulphate as a standard. The spectra were recorded at 2-nm sampling intervals in 400–800 nm range and in some experiments with Antonovka fruits, in the range 300–800 nm. The acquired data were interfaced to IBM-compatible personal computer.

Pigment analysis

The procedure allowing simultaneous quantification of Chl, Car, and Flv in an extract from apple peel zone used for reflectance measurements was employed essentially as described in Solovchenko et al. (2001). Peel disks (total area of 3.8 cm^2) were homogenized in chloroform–methanol (2:1, vol/vol) in the presence of MgO. After completion of extraction, homogenates were filtered through a paper filter, and distilled water (1/5 of total extract volume) was added. Then extracts were centrifuged at 3000*g* for 10 min until phase separation. Chl and Car concentrations were quantified spectrophotometrically in lower (chloroform) phase using coefficients reported by Wellburn (1994). The upper (water–methanol) phase was used for assay of Flv, which were quantified spectrophotometrically using molar absorption coefficient ε_{358}=25.4 $mM^{-1}\,cm^{-1}$ determined for rutin in 80% aqueous methanol. After determination of Flv the water–methanol phase was acidified with HCl (final concentration of HCl=0.1%) and used for quantification of anthocyanins by measuring absorbance at 530 nm; absorption coefficient of 30 $mM^{-1}\,cm^{-1}$ (Strack and Wray, 1989) was accepted. Flv (in equivalent amounts of rutin) as well as other pigment content were expressed relative to fruit surface area.

Results

Reflectance spectral features of flavonols

Fruits of all apple cultivars examined in this study possessed a considerable variation in peel Flv, Chl and Car (Fig. 1, Table 1). The peels of yellowish fruits of Golden Delicious apples were characterized by a considerable level of Car and relatively low amounts of Chl. Green in colour, Renet Simirenko fruits possessed high Chl and Car contents among studied varieties. Pale-green to yellowish Antonovka apples represented an intermediate case with moderate content of both Chl and Car (Table 1). As a rule, more green peel (characteristic of shaded surfaces of the fruits), along with higher Chl content, possessed lower Flv levels, whereas higher Flv and lower Chl contents were found in the peels from sunlit surfaces (see also Merzlyak et al., 2002). Flv content in the peel possessed non-uniform distribution: in the majority of fruits from shaded and sunlit surfaces it was in the ranges of 10–75 and 75–250 nmol/cm^2, respectively (Fig. 1). Fruits of all apple cultivars studied

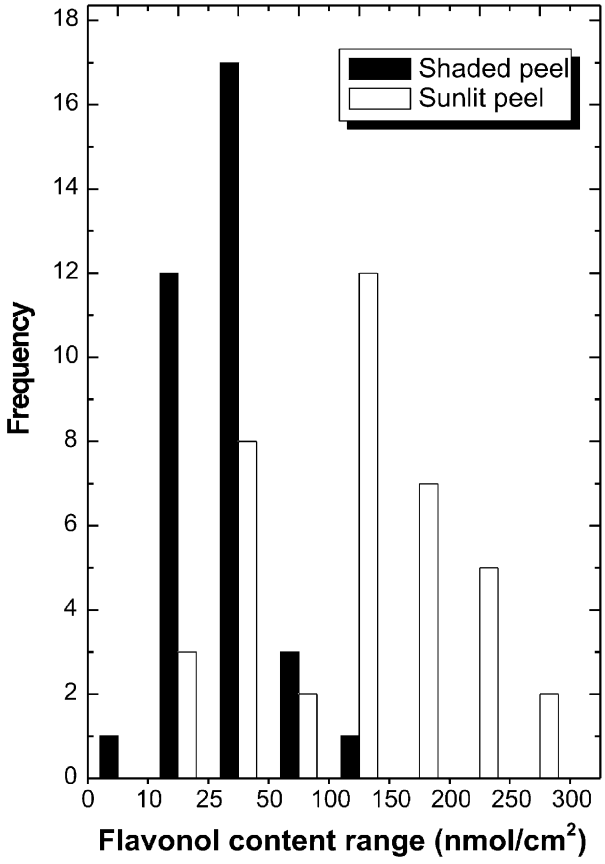

Figure 1. Distribution of flavonol content in peel samples from shaded (1) and sunlit (2) surfaces of fruits of apple cultivars studied.

Table 1. Peel pigment content (nmol/cm^2) of apple fruits

	Chlorophylls	Carotenoids	Flavonols
Golden delicious (n=13)			
Min–Max	1.21–2.13	0.39–4.01	8.94–217
Average ± STD[a]	1.77 ± 0.29	2.01 ± 1.08	74.3 ± 70.5
Coefficient of variation (%)	16.4	53.7	94.9
Antonovka (n=35)			
Min—Max	0.71–2.03	0.60–3.55	13.9–176
Average ± STD	1.25 ± 0.34	1.82 ± 0.64	57.9 ± 44.3
Coefficient of variation (%)	27.2	35.2	76.5
Renet Simirenko (n=29)			
Min–Max	0.35–7.94	0.78–3.69	19.7–219
Average ± STD	4.74 ± 2.81	2.38 ± 0.98	120 ± 69.9
Coefficient of variation (%)	59.3	41.2	58.3
All (n=77)			
Min–Max	0.35–7.94	0.39–3.69	8.93–219
Average ± STD	2.56 ± 1.99	1.68 ± 0.73	85.6 ± 67.9
Coefficient of variation (%)	77.7	43.5	79.3

[a]STD: standard deviation.

contained very low amounts of anthocyanins (<0.15 nmol/cm^2).

Fig. 2 shows reflectance spectra of apple fruits differing in Flv content. All apple fruits possessed high reflectance (up to 85%) in the NIR region of the spectrum and lower reflectance in the red with a distinct minimum near 678 nm, which is characteristic of Chl *a* (Fig. 2A). At wavelengths shorter than 550 nm, reflectance underwent a sharp decrease due to combined absorption by Chl and Car. A minimum near 480 nm attributable mainly to Car and, to a lesser extent, Chl *b* absorption was characteristic of the spectra of Golden Delicious and Antonovka as well of sunlit surfaces of Renet Simirenko fruits (Fig. 2). Shaded sides of Renet Simirenko apples with high peel Chl (Fig. 2C, curve 1) exhibited low and featureless reflectance in the blue range (for more details of Chl and Car spectral features in the visible range see Merzlyak et al., 2003a). As a result of fruit adaptation to strong sun-light, reflectance in the red range increased, whereas that in the range 400–420 nm decreased (Fig. 2). In fruits with high Flv content reflectance in the range 400–420 nm was notably lower than in fruits with low Flv content (cf. curves 1 and 2 in Fig. 2).

The effect of Flv on reflectance spectra in UV range (300–400 nm) was studied in Antonovka fruits grown locally and available at different stages of their development (Fig. 3). The accumulation of Flv in apple peel induced a gradual decrease of reflectance in the range of 350–430 nm; these spectra as well as corresponding spectra of reciprocal reflectance, $100/R_\lambda$, and their derivatives are plotted in Fig. 3. At low Flv content the reciprocal reflectance showed an increase from 400 nm toward shorter wavelengths (Fig. 3, curves 1 and 2). The rise in Flv content brought about the appearance of a broad band between 360 and 420 nm with a remarkable shift of its edge towards longer wavelengths (Fig. 3B, curves 3–5). In the peel with the range of Flv content up to 120 nmol/cm^2, the relationship between inflection point position, IP (maximum of the first derivative), and Flv was linear (inset in Fig. 3C, Table 2). The accumulation of Flv in higher amounts did not result in further changes in IP position but caused an increase in the peak height (cf. spectra 1–3 and 4, 5 in Fig. 3C). At wavelengths longer than 435 nm the effect of an increase in Flv content was not significant (Figs. 3B, C).

The spectra of correlation coefficient, *r*, of the linear relationship between pigment content and reciprocal reflectance for fruits of three apple cultivars are shown in Fig. 4. Reciprocal reflectance in the NIR did not relate to content of any pigment. In the 530–730 nm range, the reciprocal reflectance possessed a negative correlation with Flv content (Fig. 4A), which was strong in apples with high-Chl content (Renet Simirenko). In this spectral region, $100/R_\lambda$ showed a high positive correlation with Chl and Car. At shorter wavelengths (440–525 nm) maxima attributable to Car absorption occurred being especially pronounced in Antonovka and Golden Delicious fruits. Prominent features of *r* spectra were detected in the range 440–360. Correlations of both Chl and Car content with reciprocal reflectance underwent a sharp decrease

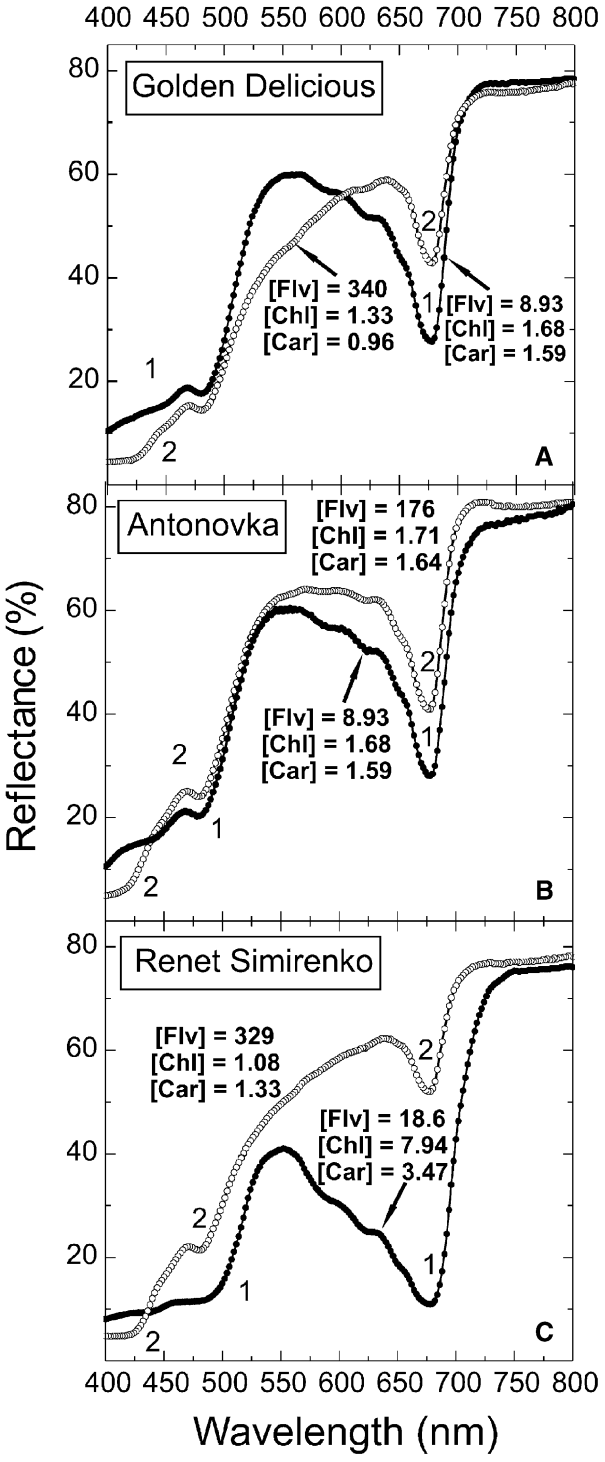

Figure 2. Representative reflectance spectra of apples with low (1) and high (2) flavonol content taken from shaded and sunlit fruit surfaces, respectively. Pigment contents are given in nmol/cm^2.

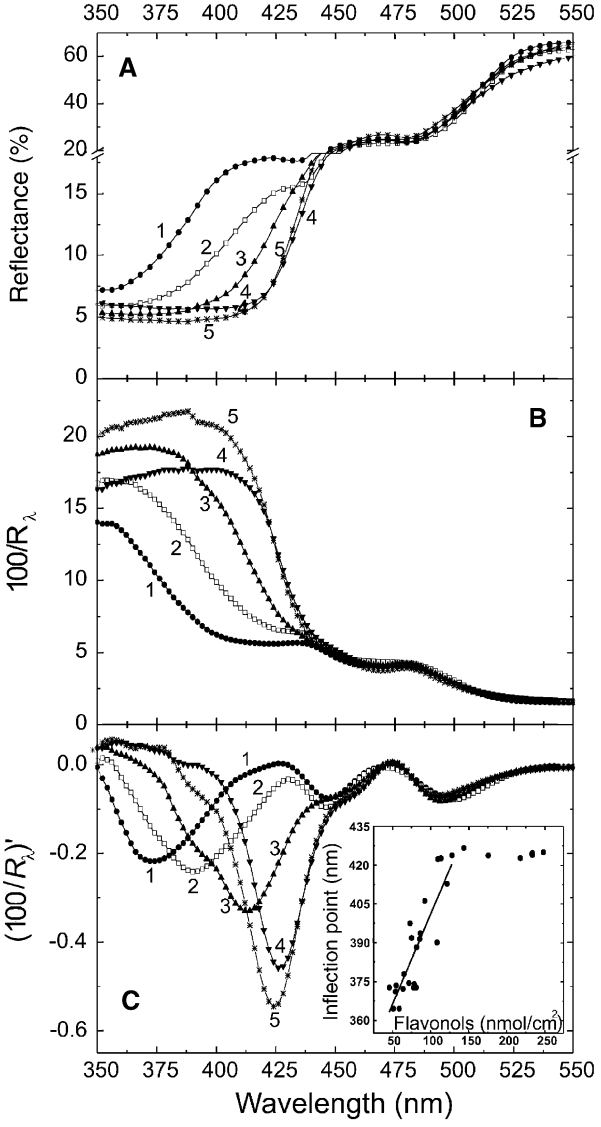

Figure 3. Reflectance spectra (A) of Antonovka apple fruits with different peel flavonol contents (1–45.7, 2–108.5, 3–121.8, 4–143.5; 5–233.8 nmol/cm^2); (B) and (C) corresponding reciprocal reflectance spectra and their first derivatives, respectively.

at wavelengths shorter than ca. 450 nm (Figs. 4B, C). In contrast, the correlation between Flv content and reciprocal reflectance increased towards the violet region with a peak around 410–420 nm. In the blue (between 430 and 500 nm), the region of combined absorption of Chl and Car, reflectance showed low correlation with Flv regardless of their content (Fig. 4A). The *r* spectra for 100/R_λ vs. [Flv] exhibited a distinct minimum at 500 nm and, in Antonovka and Golden Delicious apples, also minima at 440 and 470 nm resulting from interference exerted by Chl and Car (Figs. 4B, C). At wavelengths below 450 nm, the correlation of reciprocal reflectance with Flv content underwent a sharp increase and formed a conspicuous maximum

Table 2. Algorithms for non-destructive determination of flavonol content in apple fruits with Flavonol Reflectance Index, FRI: $x = (R_{410}^{-1} - R_{460}^{-1})R_{800}$

Cultivar	Flavonol range (nmol/cm^2)	n	Model	r^2	RMSE[a] (nmol/cm^2)
Golden Delicious	8.9–217	13	$[Flv] = -28.99 + 14.63x$	0.96	16.2
Antonovka	13.9–176	35	$[Flv] = -1.66 + 14.22x$	0.92	12.9
Renet Simirenko	19.7–219	29	$[Flv] = -19.96 + 13.64x$	0.92	20.8
All	8.9–219	77	$[Flv] = -5.08 + 12.82x$	0.92	19.3

[a]RMSE: root mean square error of estimation.

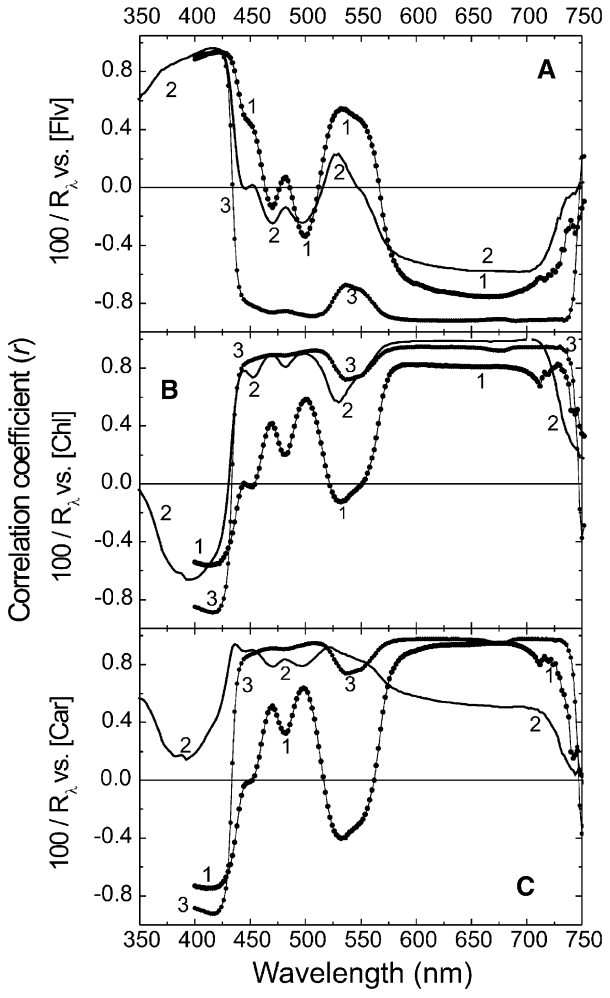

Figure 4. Spectral dependencies of correlation coefficient, r, between reciprocal reflectance, $100/R_\lambda$, and flavonol (A), chlorophyll (B) and carotenoid (C) content for Golden Delicious (1), Antonovka (2), and Renet Simirenko (3) apple fruits.

($r = 0.9$) at 410 nm (Fig. 4A). At the same time, $100/R_\lambda$ in this spectral range demonstrated a negative correlation with Chl and Car (Figs. 4B, C). The presence of peaks around 530 nm in the relationships $100/R(\lambda)$ vs. [Flv] (more pronounced in fruit varieties with lower Chl content) and corresponding troughs in $100/R(\lambda)$ vs. [Chl] (Figs. 4A, B) should be also noted.

Flavonol reflectance index

Based on the spectral characteristics of pigments, we expected to find the optimal spectral regions λ_1, λ_2, and λ_3 in the model $(R_{\lambda_1}^{-1} - R_{\lambda_2}^{-1})R_{\lambda_3}$ for Flv content estimation. The variability between samples in scattering can be eliminated by using reflectance in a spectral region λ_3, where absorption by all pigments (Chl, Car, and Flv) is minimal (i.e. in the NIR, λ_3 between 750 and 800 nm, see Figs. 2–4). The only spectral range, λ_1, maximally sensitive to Flv content was found in the blue around 410 nm (Fig. 4). However, the correlation between R_{800}/R_{410} and Flv content was not significant (Fig. 5A) due to the effect of Chl and Car absorption on reflectance (Figs. 2–4). To remove the contribution of these pigments, reflectance at λ_2 should be closely related to Chl and Car contents and minimally affected by Flv absorption. In addition, λ_2 should be as close as possible to λ_1. The spectral range that meets the requirements was assumed to be around 460 nm in the region influenced mostly by combined Car and Chl absorption and less affected by that of Flv (Fig. 4, see also Merzlyak et al., 2003b). The Flavonol Reflectance Index (FRI) was suggested in the form:

$$FRI = (R_{410}^{-1} - R_{460}^{-1})R_{800}.$$

Fig. 5B shows that subtraction of $(R_{460})^{-1}$ from $(R_{410})^{-1}$ significantly decreased the effects of the residual absorption by Car and Chl on reflectance around 410 nm (Fig. 5B). FRI allowed accurate assessment (r^2=0.92, maximal RMSE=5.0 nmol/cm^2) of peel Flv content ranging from 8 to 220 nmol/cm^2 for all apple fruit varieties studied (Fig. 5B, Table 2). An estimation of Flv in the whole range of its content required a variety-specific approach and turned feasible with the use of power or exponential relationships between FRI and Flv content (not shown).

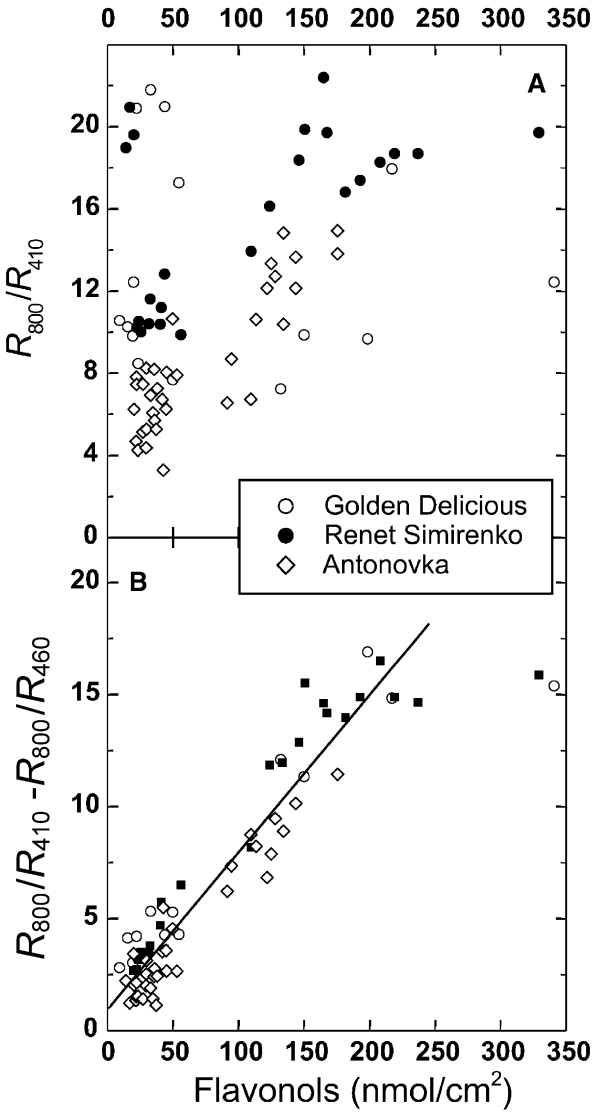

Figure 5. Relationships between reciprocal reflectance at 410 nm (A), flavonol reflectance index FRI, $(R_{410}^{-1} - R_{460}^{-1})R_{800}$, (B), and peel flavonol content. In B solid line represents the linear fit for flavonol content in the range 8–220 nmol/cm^2.

Discussion

Similar to leaves, fruit optical properties are determined by pigments distributed in different cell structures and their overall content, local concentration, interactions, and organization, as well as by scattering. Anthocyanin-free apple fruit contain chloroplast pigments (Chl and Car) as principal pigments absorbing in the visible range. In addition, in UV both cuticular and vacuolar phenolics are able to exert a strong contribution to absorption.

As a result of the development under strong sunlight, remarkable changes in the fruit pigments content were noted: the decrease in Chl, the accumulation of additional amounts of Car, and the build up of gross amounts of Flv (Fig. 1, Merzlyak et al., 2002). On the whole, peel Flv content was 1–2 orders of magnitude higher than that of Chl and Car (Table 1, see also Merzlyak et al., 2002). The quantitative consideration—the ca. 5-fold difference between sunlit and shaded surfaces (Table 1, Awad et al., 2000; Merzlyak et al., 2002)—presumes that the latter response, which requires high metabolic activity, entails energetic costs, and is evidently involved in the fruit adaptation to solar irradiation, is an important mechanism for protection against excessive fluxes of solar radiation. As can be seen in Fig. 1, the cases with lower Flv content more often occurred in shaded peel, whereas high Flv content were predominantly associated with sunlit peel. It should be also noted that higher levels of Flv have been recorded in Golden Delicious and Renet Simirenko apples grown in South Russia.

In fruits with low Flv content, reflectance between 440 nm (maximum of Chl *a* absorption) and 400 nm was flat (Figs. 2 and 3) and then showed a monotonous decline reaching its minimum near 360 nm (Fig. 3) close to rutin absorption maximum in solution (Markham, 1989; Solovchenko et al., 2001). These spectral features in UV could, to a certain degree, be related to optical properties of cuticles: those isolated from shaded surfaces of Antonovka apples contained a considerable part of peel Flv as well as that of other phenolics and contributed appreciably to whole-fruit reflectance in this spectral range (Solovchenko and Merzlyak, 2003).

The accumulation of Flv occurring mainly in the vacuoles of subcuticular cell layers of the peel (Awad et al., 2000) was accompanied by a sharp decrease in fruit reflectance and flattening of the spectrum in the broad band between 360 and 420 nm (Fig. 3A). In this spectral range, even at high Flv content whole-fruit reflectance did not drop below 4–5%. This effect is attributable to reflectance from surface fruit structures, particularly the cuticle, which contains relatively low amounts of peel Flv and exhibits considerable scattering. Our previous experiments showed that reflectance of isolated apple fruit cuticles recorded in UV-A range on the 'black background' was comparable with that of whole fruit containing high amounts of peel Flv (Solovchenko and Merzlyak, 2003). Accordingly, the magnitude of UV reflectance of apple fruit with high Flv content should be strongly dependent on the physical properties of the cuticular layer, its thickness, composition, and organization of its lipid polymers.

In apple fruit, reflectance near 360 nm was quickly saturated reaching its minimal values at relatively low peel Flv content (Fig. 3A). The build up of Flv brought about a broadening of the absorption band and manifested itself as the extension of the region with very low reflectance from near UV to the violet (Figs. 2 and 3). An increase in peel Flv content up to ca. 140 nmol/cm^2 was accompanied by moving the edge of fruit reflectance spectrum towards longer wavelengths (Fig. 3B) with the shift in IP position from ca. 364 to 425 nm (Fig. 3C). Qualitatively similar changes in reflectance have been observed in the leaves and fruits in the presence of high amounts of Chl (Gitelson et al., 1996) and anthocyanins (Merzlyak et al., 2002) in the red and green regions of the spectrum, respectively. The Flv-dependent shift of the edge of reflectance spectrum in apple fruit was as high as 60 nm (Fig. 3). In addition to the concentration-dependent effect of Flv on spectral reflectance, the changes observed could be related with their intermolecular interactions such as copigmentation and aggregation of vacuolar Flv resulting in a considerable bathochromic shift of their absorption band as suggested for explanation of yellow coloration exhibited by flower petals of certain plant species (Smith and Markham, 1998; Markham et al., 2001). This mechanism is quite possible since local concentration of Flv in vacuoles of apple peel cells is extremely high, reaching 1.7×10^{-2} M (Lancaster et al., 1994). As a result of accumulation of high amounts of Flv the spectral features of Chl *a* in the Soret band were masked by Flv absorption both in whole apple fruit reflectance (Figs. 2 and 3A, curves 4 and 5) and in absorption spectra of peel extracts (Solovchenko et al., 2001). The influence of high Flv content on fruit reflectance in the band 350–430 nm is also evident in the spectra of correlation coefficients: the increase of r for Flv coincided with a sharp decrease of r both for Chl and Car (Fig. 4). Then, distinct positive peaks of correlation between $100/R_\lambda$ and Flv content (Fig. 4A) were observed even in the region of high reflectance in the green (maxima near 530 nm) as well as corresponding negative peaks in the case of Chl (Fig. 4B) and Car (Fig. 4C). The presence of these features suggests that Flv in anthocyanin-free fruits exert a contribution to light absorption and hence to the screening of solar radiation not only in UV-A but also in the visible part of the spectrum.

The non-destructive assessment of Flv in plant tissues, which exhibit low reflectance in the UV-A is complicated by overlapping of their absorption with those of several pigments: Chl and Car, as well as by phenolics (catechins and phenolic acids) possessing absorption bands in UV-B (Krauss et al., 1997; Awad et al., 2000). In addition, scattering should exert a strong influence on UV reflectance. Recently it was reported that scattering coefficients of whole fruit (Cubeddu et al., 2001), the peel, and isolated cuticles of apples (Solovchenko and Merzlyak, 2003) are wavelength-dependent and underwent an increase with a corresponding wavelength decrease.

In our previous studies, conceptual models have been devised allowing non-destructive assessment of total Chl content in higher plant leaves and fruits (Gitelson et al., 2003; Merzlyak et al., 2003a). Essentially the same models have been successfully used for the non-destructive analysis of Car and anthocyanin content (Gitelson et al., 2001, 2002, 2003; Merzlyak et al., 2003a, b). To apply the conceptual model for quantitative estimation of Flv content required finding optimal wavelengths λ_1, λ_2 and λ_3. At wavelengths shorter than 380 nm, the reciprocal reflectance of apple fruits showed a weak correlation with Flv content (Fig. 4A). This could result from the saturation of the relationship $100/R_\lambda$ vs. [Flv] as well as an interference by phenolic substances different from quercetin glycosides (e.g. catechins and phenolic acids) (Awad et al., 2000; Krauss et al., 1997). The band of the highest sensitivity of reflectance to Flv in the whole range of its changes has been found between 380 and 420 nm, peaking near 410 nm (Fig. 4). The reflectance ratio R_{800}/R_{410} possessed a low sensitivity to Flv content (Fig. 5A) due to a strong effect of Chl and Car absorption. To remove their effect, we suggested using reciprocal reflectance in a spectral band that is closely related to the content of the pigments. The spectral analysis revealed the suitable band around 460 nm, where reflectance is mainly governed by both Chl and Car (Figs. 2–4) and, in addition, the correlation of reciprocal reflectance with Flv content (Fig. 4) is low (Golden Delicious and Antonovka) or negative (Renet Simirenko). The FRI in form $(R_{410}^{-1} - R_{460}^{-1})R_{800}$ exhibited a strong variety-independent linear relationship with Flv content in the range of 8–220 nmol/cm^2 (Table 2). However, the sensitivity of FRI to Flv decreased when Flv content exceeded 300 nmol/cm^2 (Fig. 5B).

Thus, we spectrally tuned a conceptual model developed for terrestrial plant leaves, and assessed accurately Flv content in apple peels. The wide range of optical properties of the fruits supports the robustness of the findings. Our results provide evidence that (a) the conceptual model can be applied for accurate non-destructive estimation of Flv content in apples, and (b) fine tuning of the conceptual model can be carried out knowing the spectral characteristics of the specific medium of interest.

It should be noted in conclusion that the influence of Flv on optical spectra of apple fruits (Figs. 2–4) and higher plant leaves might extend quite far into the visible spectrum. Therefore, one using reflectances for non-destructive determination of higher plant pigments absorbing in the visible range should be aware of obstacles that could be caused by Flv when they are present in high amounts.

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