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# Non-Destructive Estimation Pigment Content, Ripening, Quality and Damage in Apple Fruit with Spectral Reflectance in the Visible Range

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## ABSTRACT

Modern non-destructive optical-reflectance-based techniques for estimation of pigment (chlorophyll, carotenoid, anthocyanin, and flavonol) contents, the rate of on- and off-tree ripening as well as for detection of common physiological disorders, such as sunscald, superficial scald, and water core, and other damages to apple fruit are reviewed with an emphasis on the methods developed by the authors. The basic spectral features of fruit reflectance in the visible and near infra-red are briefly considered together with their implications for the development of algorithms for non-destructive pigment content assessment. The use of reflectance spectroscopy for estimating chlorophyll and carotenoid content as well as carotenoid/chlorophyll ratio during fruit ripening is demonstrated. The algorithms developed for fruit peel pigment analysis and for estimation of ripeness are presented with consideration of the limits of their applicability. Special attention is paid to adaptation of apple fruit to strong sunlight at preharvest stage and its consequences for postharvest fruit quality.

**Keywords:** non-destructive analysis, pigment content of apple skin, reflectance, sunburn, sunscald, superficial scald, watercore

**Abbreviations: AnC**, anthocyanin(s); **ARI**, anthocyanin reflectance index; **Car**, carotenoid(s); **CI**, chlorophyll index; **Chl**, chlorophyll(s); **CRI**, carotenoid reflectance index; **Flv**, flavonol(s); **FRI**, flavonol reflectance index; **IEC**, internal ethylene content; **NIR**, near infrared; **PSRI**, plant senescence reflectance index; $R_\lambda$, reflectance at wavelength λ (nm); $R(\lambda)$, reflectance spectrum, **RMSE**, root mean square error

## CONTENTS

## INTRODUCTION

Quality of fruit encompasses a number of sensory properties (coloration, shape, texture, taste and aroma), nutritive value, chemical composition, mechanical properties, and absence of defects (for review, see Abbot 1999; Lurie 2008). In research and commercial applications, instrumental measurements are usually preferred over sensory evaluations since the former are objective, potentially more precise, reproducible and provide a measure accepted by researchers, industry and consumers. For the last decades attempts have been undertaken to apply the measurements of whole-fruit spectral reflectance for non-destructive assessments of apple quality characteristics (Knee 1980; Abbot 1999; Zude-Sasse *et al.* 2002; Merzlyak *et al.* 2003a, 2003b, 2005; Zude *et al.* 2006). In the visible and NIR ranges, reflectance of apple fruit is governed by content, composition, and localisation of pigments (Butler and Norris 1960; Fukshansky 1981; Gitelson *et al.* 2003), cuticle (Batt and Martin 1960; Solovchenko and Merzlyak 2003), as well as by its 'internal optical properties' related with underlying tissues, water content and a number of other factors (Fukshansky 1981; Vogelmann 1993; Gitelson *et al.* 1996, 2003).

Chlorophylls (Chl) and carotenoids (Car) are principal

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pigments of higher plant assimilatory tissues involved in photosynthesis and responsible for variations of fruit colour from green to yellow. Other pigments responsible for fruit coloration are flavonoids (Flv, yellowish) and anthocyanins (AnC, red) (Chichester and Nakayama 1965; Knee 1972, 1980; Chuma *et al.* 1981; Gross 1987; Merzlyak *et al.* 1997, 1999, 2003, 2005; Solovchenko *et al.* 2005, 2006). Contents of pigments as well as their ratios are important physiological characteristics of fruit closely related with its ripeness, various damages and disorders and serve as a common marker of fruit quality (Gross 1987; Knee 1988; Abbot 1999; Herold 2008). Furthermore, the level of Car, Flv and AnC itself is of immediate importance since these compounds possess vitamin, antioxidant properties and exert beneficial nutraceutical effects (Tournaire *et al.* 1993; Rice-Evans *et al.* 1997; van der Sluis *et al.* 1997; Russo *et al.* 2000); therefore their content could be considered as an important aspect of apple fruit quality.

The changes in pigment content and composition as well in tissue structure manifest itself as directional changes in fruit reflectance (McClure 1975; Merzlyak *et al.* 1997, 1999). Thus, the studies performed in our laboratory with apples indicated remarkable changes in spectral reflectance occurring during on-tree ripening, maturation and storage (Merzlyak *et al.* 1997, 1999), acclimation to high-light stress, as a result of photo-induced pigment bleaching (Merzlyak *et al.* 1998, 2002; Merzlyak and Chivkunova 2000), superficial scald-induced browning (Chivkunova *et al.* 1997, 2001) and so on. Reflectance measurements could also serve as a basis for the prediction of ripening rate, estimation of harvest window and duration of storage (Geyer *et al.* 2007; Merzlyak *et al.* 1999; Peirs *et al.* 2001; Zude-Sasse *et al.* 2002; Solovchenko *et al.* 2005).

Monitoring apple fruit quality via measuring their reflectance possesses a number of distinct advantages over traditional destructive and alternative non-destructive (e.g. Chl fluorescence measurement-based, see Wulf *et al.* 2005; Merzlyak *et al.* 2008) approaches. The most important of them are simplicity, sensitivity, reliability and a high throughput. The measurement of fruit reflectance with modern equipment (Cubeddu *et al.* 2006; Hagen *et al.* 2006; Geyer *et al.* 2007) is relatively simple and do not require complex and expensive setups. Non-destructive techniques save a great deal of manual labour and therefore have a great potential for application in fruit sorting and grading lines and packing houses (Chuma *et al.* 1981; Morita *et al.* 1990; Morita and Taharazako 1992).

Attempts to apply non-destructive methods based on optical spectroscopy for assessment of fruit quality and their physiological state have been undertaken for several decades (Knee 1980; Morita *et al.* 1990; Blanke and Notton 1992; Morita and Taharazako 1992; Merzlyak *et al.* 1997, 1998; Merzlyak and Chivkunova 2000; Zude and Herold 2002), in particular, for development of automatic technique for fruit grading and sorting (Morita *et al.* 1990, 1992). Particularly, Knee (1980) suggested employing reflectance at 675 nm to estimate peel Chl content in Cox Orange Pippin apple. Morita and co-workers (Chuma *et al.* 1981; Morita *et al.* 1990; Morita and Taharazako 1992) found a correlation between the magnitude of reflectance minimum in the red and Chl content and used log $R_{680}$ for Chl content estimation as well as for ripeness and internal quality assessment in orange fruit.

The situation has changed drastically during the last decades when significant amount of research was dedicated to the development of techniques for non-destructive evaluation of apple fruit ripeness and damages. With the advent of commercially available portable fiber-optic radiometers providing reliable spectral data from very small fruit surface area, the development of reflectance-based non-destructive techniques for monitoring of fruit quality gained a considerable momentum (Peñuelas and Filella 1998; Merzlyak *et al.* 2003a). In the recent years these approaches have been widely implemented in "precision agriculture" technologies (Gitelson *et al.* 2003). We would like to note that this review is by no means comprehensive since established 'pure' NIR (Lammertyn *et al.* 1998) and emerging Chl fluorescence (e.g., Hagen *et al.* 2006; Wulf *et al.* 2006; Merzlyak *et al.* 2008) and time-resolved techniques (Cubeddu *et al.* 2001) are not considered here because they deserve a separate reviewing.

This paper provides a brief overview of the recent developments in the field of optical reflectance-based techniques for estimating pigment content, monitoring ripeness of, disorders in and damages to apple fruit on-tree and in postharvest with a special emphasis on advances made in the laboratories of the authors.

## GENERAL FEATURES OF APPLE FRUIT REFLECTANCE SPECTRA

A comprehensive understanding of inherent optical properties of fruit tissue is a prerequisite for estimating pigment content and devising techniques for non-destructive assessment of apple quality. A general approach implies the analysis of reflectance variation in response to variation in pigment content (Gitelson *et al.* 2001, 2002, 2003; Merzlyak *et al.* 2003a) in order to find spectral bands of maximum sensitivity to pigment content, fruit damages or disorders.

Remarkably, apple fruit, as compared with leaves, contain much lower quantities of the pigments (Merzlyak *et al.* 2002, 2003a, 2003b); the bulk of the pigments is localised in the skin above a thick layer of parenchyma which exhibits strong light scattering properties (Butler and Norris 1960; Law and Norris 1973). As a result, apples generally possess resolved reflectance spectra with distinct features attributable to pigment absorption (Knee 1980; Merzlyak *et al.* 2002; Solovchenko and Merzlyak 2003; Merzlyak 2006).

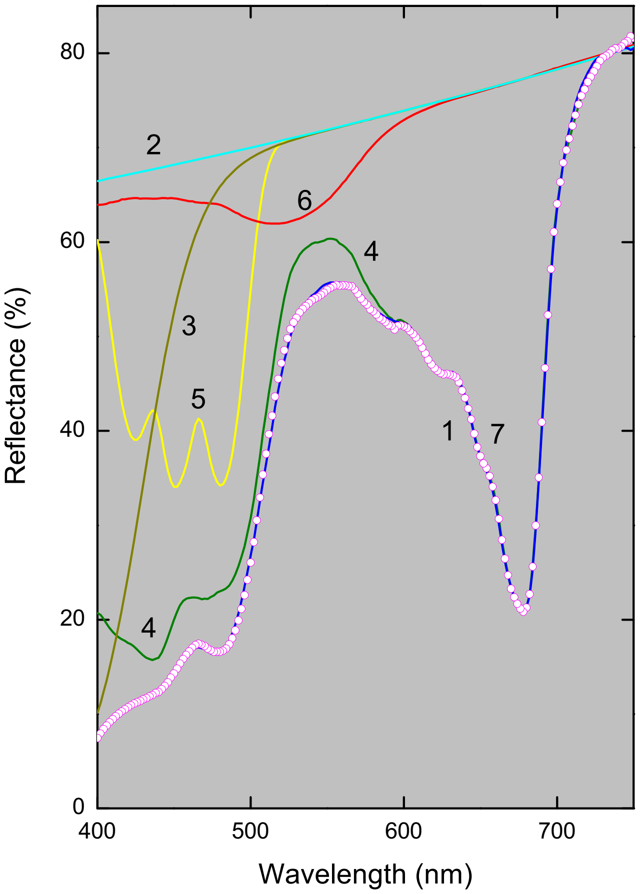

**Fig. 1 Contribution of different pigment pools to the reflectance spectrum of a 'Summer Red' apple (for details see Merzlyak *et al.* 2008).** 1 – the measured spectrum (line), 2 – scattering, 3 – flavonols, 4 – thylakoid-bound chlorophylls and carotenoids, 5 – extrathylakoid carotenoids accumulated as a result of fruit ripening, 6 – anthocyanins, 7 – modeled spectrum (dots). Spectra 3 – 6 are shown on the background of scattering.

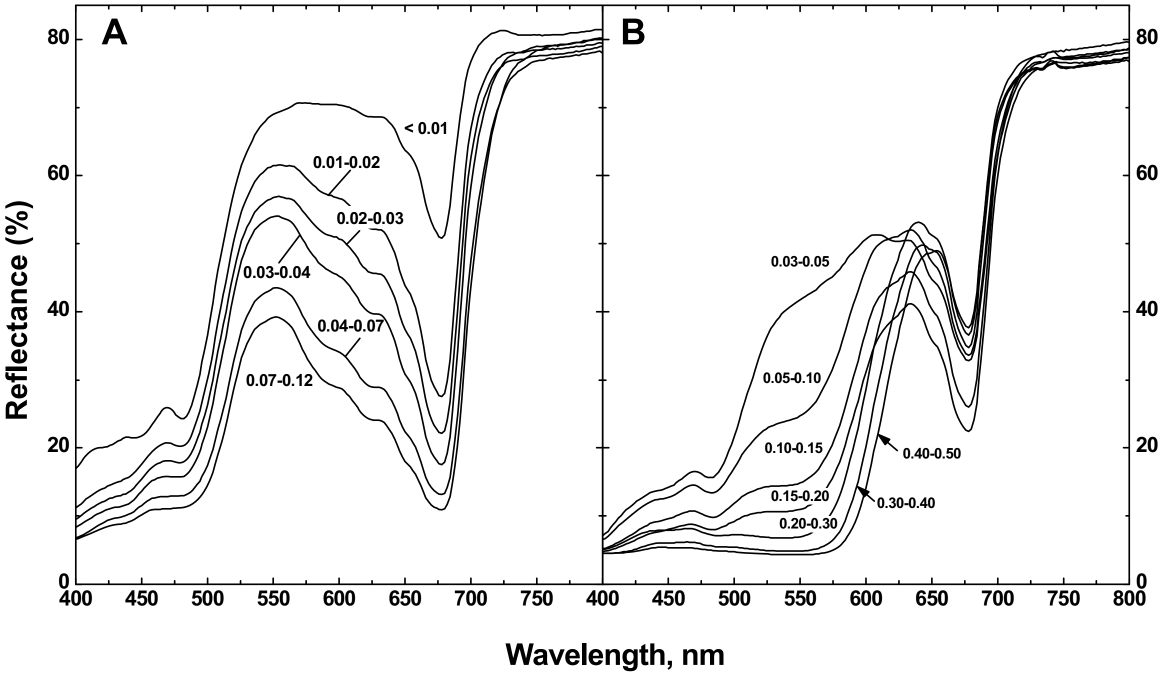

**Fig. 2 Effects of chlorophyll and anthocyanin pigments on whole-apple fruit reflectance spectra.** Average reflectance spectra of (**A**) anthocyanin-free green-to-yellow-green 'Antonovka' apples and (**B**) anthocyanin-containing 'Zhigulevskoe' apples. Numbers indicate the ranges of chlorophyll (**A**) and anthocyanin (**B**) content (mmol $m^{-2}$) in the skin. Reprinted from **Merzlyak MN, Solovchenko AE, Gitelson AA** (2003b) Reflectance spectral features and non-destructive estimation of chlorophyll, carotenoid and anthocyanin content in apple fruit. *Postharvest Biology and Technology* **27**, 89-103, ©2003, with kind permission from Elsevier, Ltd.

Recently the spectral properties of the main pigment pools of apple were estimated and modeling apple fruit reflectance spectra was carried out (Merzlyak 2006; Merzlyak *et al.* 2008) in accordance with a theory of diffuse reflectance presented in Alderson *et al.* (1961). **Fig. 1** demonstrates the results of the reconstruction of the reflectance spectrum for the shaded surface of red-colored AnC-containing 'Summer Red' apple. According to the modeling, scattering in whole apple fruit undergo a monotonous increase to shorter wavelengths. The presence of small amounts of Flv and AnC manifests itself as a decrease of reflectance in the bands of 350–420 nm and around 540–550 nm, respectively. As could be seen, throughout visible range Chl and Car bound to thylakoids exert the dominant contribution into light absorption. The characteristic 'three-head' band absorption by extrathylakoid Car (localized presumably in osmiophylic globules of chloroplasts) is characteristic of ripe fruit.

A number of studies (Zude-Sasse *et al.* 2002; Zude 2003; Zude *et al.* 2006; Kuckenberg *et al.* 2008), including those carried out in our laboratory (Merzlyak *et al.* 2002, 2003b, 2005; Solovchenko *et al.* 2005), revealed characteristic reflectance spectral properties of apple in wide range of its pigment content and composition and at different stages of maturity on-tree and in storage. An important outcome of these works was the revealing of the spectral signatures of Chl, Car, AnC, and Flv as well as of the pigments forming during browning accompanying the damages to and disorders of apple fruit (Chivkunova *et al.* 2001; Merzlyak *et al.* 2002, 2005; Solovchenko *et al.* 2005).

Ripening apple fruit with dramatic changes in Chl, Car and AnC contents demonstrate the influence of pigment content and composition on coloration and whole-fruit spectral reflectance (see spectra for yellow-greenish 'Antonovka' and red 'Zhigulevskoe' apples, as an example, in **Fig. 2**). Fruit with low Chl and AnC contents exhibit high reflectance (~ 65–80%) at wavelengths beyond 600 nm. Measurements performed on yellow-colored overripe AnC-free fruit with very low Chl showed that fruit tissues possessed high reflectance without discernible spectral features in the NIR and the red parts of the spectrum. The presence of the pigments at low amounts, hardly assessable analytically, manifests itself as distinct troughs in reflectance spectra in the bands of Chl and Car (greenish and yellowish fruit) and AnC (reddish fruit) absorption. With an increase in pigment content the spectra became less resolved and more flat (**Fig. 2**, see also Merzlyak *et al.* 1999; Merzlyak and Chivkunova 2000; Merzlyak *et al.* 2003).

Fruit reflectance in the main bands of Chl *a* absorption (near 440–450 and 670–680 nm) is low and became not sensitive to Chl content as it exceeds 0.05–0.06 mmol $m^{-2}$ (Knee 1980; Merzlyak *et al.* 2003b). As could be seen, reflectance in the spectral regions of strong pigment absorption did not drop below 4–5% even at very high contents of the pigment (**Fig. 2**; Merzlyak *et al.* 2003b). This could be explained by reflectance of light by superficial structures of fruit such as cuticle and epidermis (Batt and Martin 1960; Solovchenko and Merzlyak 2003). However, the reflectance at the edges of the red Chl absorption band (located 20–30 nm aside from the absorption maximum) displayed a considerable variation as Chl content varied (Merzlyak *et al.* 2003b).

Distinct bands attributable to Car absorption could be distinguished only in reflectance spectra of ripe AnC-free (yellow) fruit (see the uppermost curve in **Fig. 2A**). AnC absorption manifests itself as a shoulder or a trough near 540–550 nm, usually superimposed on a considerable Chl and Car background. In the presence of moderate Chl content (0.05–0.08 mmol $m^{-2}$), AnC, when accumulated in high amounts (over 0.30 mmol $m^{-2}$), govern reflectance of apple fruit, resulting in very low reflectance (below 5%) in the green part of the spectrum (**Fig. 2B**; Merzlyak and Chivkunova 2000; Gitelson *et al.* 2001; Merzlyak *et al.* 2003b).

The accumulation of flavonols (Flv) occurring mainly in the vacuoles of subcuticular cell layers of the peel (Awad *et al.* 2000) is accompanied by a sharp decrease of fruit reflectance and flattening of the spectrum in the broad band between 350 and 420 nm. In addition to the concentration-dependent effects of Flv and AnC, the changes in reflectance could be related with their intermolecular interactions such as co-pigmentation and aggregation of vacuolar phenolics (Asen *et al.* 1972; Smith and Markham 1998). It appears that Flv could contribute to yellow coloration of fruit which possess relatively low Chl and Car as was suggested for explanation of yellow coloration of flower petals in cer-

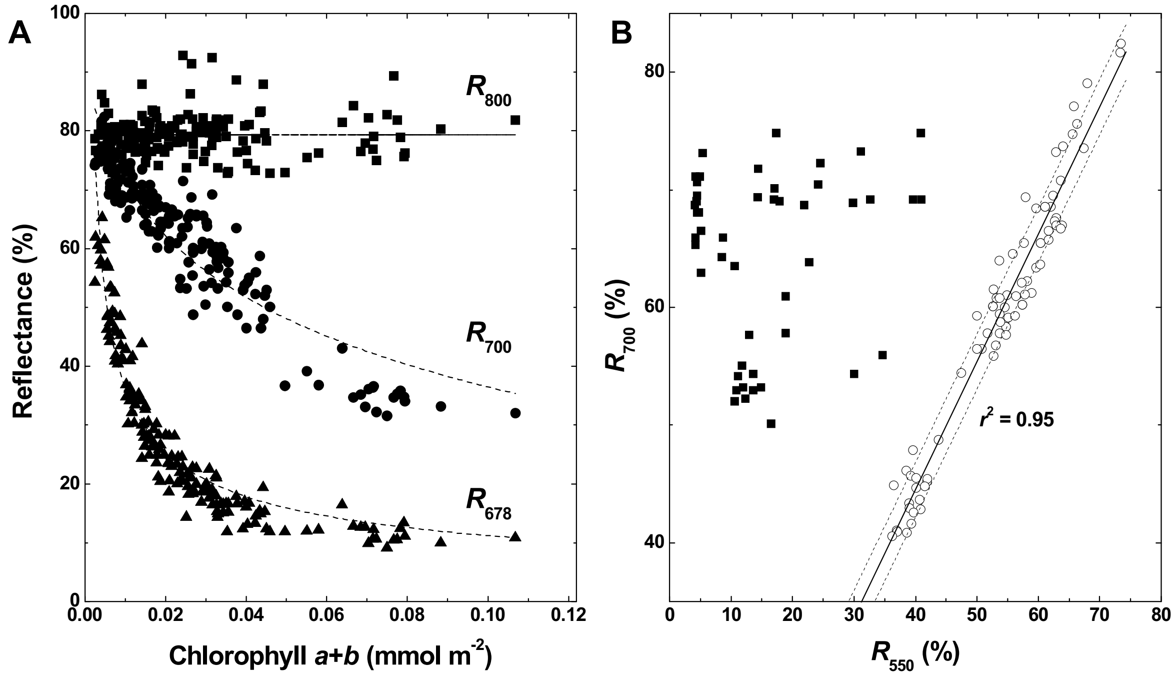

**Fig. 3 The basic features of apple fruit reflectance.** (**A**) Reflectance at the edge of the region governed by pigment (chlorophyll) absorption (e.g. $R_{700}$) is sensitive to and hyperbolically related with its content whereas reflectance in the maximum of pigment absorption ($R_{678}$) sensitive only to its low contents; NIR reflectance (e.g. $R_{800}$) is not related with pigment content. (**B**) For green to green-yellow fruits, $R_{550}$ vs. $R_{700}$ is linear with determination coefficient higher than 0.95, whereas for anthocyanin-containing fruits, $R_{550} < R_{700}$ and fair relationship between them was disturbed. Solid lines represent the best-fit functions; dashed lines represent standard deviation in (**B**). Reprinted from **Merzlyak MN, Solovchenko AE, Gitelson AA** (2003b) Reflectance spectral features and non-destructive estimation of chlorophyll, carotenoid and anthocyanin content in apple fruit. *Postharvest Biology and Technology* **27**, 89-103, ©2003, with kind permission from Elsevier, Ltd.

tain plant species (Smith and Markham 1998). These mechanisms are quite possible since local concentration of Flv in vacuoles of apple skin cells is extremely high reaching 1.7 $10^{-2}$ M (Lancaster *et al.* 1994).

A high correlation between reflectances at 550 nm and 700 nm, representing the fundamental feature of the reflectance spectra of AnC-free leaves of diverse plants (Gitelson and Merzlyak 1996; Lichtenthaler *et al.* 1996; Gitelson *et al.* 2003), was also found in AnC-free apple fruit; $R_{550}$ and $R_{700}$ correlated very closely ($r^2 = 0.95$, **Fig. 3B**) regardless of Chl content and maturity stage (Merzlyak *et al.* 2003b). In contrast, in AnC-containing apple fruit, a strong correlation between $R_{550}$ and $R_{700}$ in red fruit was disturbed as a result of AnC absorption in the green range (**Fig. 3B**). Thus, in the green range of the spectrum both pigments, AnC and Chl absorb, whereas Chl*a* and Chl*b* are the only absorbers in the red edge region.

## APPROACHES FOR NON-DESTRUCTIVE ASSAY OF FRUIT PIGMENTS

Essentially, reflectance-based techniques described here provide insight into apple quality as could be inferred from the pattern of key apple pigments serving as internal markers of fruit physiological condition, maturity, damages and disorders. However, the non-destructive assessment of pigments in apple fruit skin is complicated by some obstacles including overlapping light absorption by individual pigments and non-linear relationship of reflectance *vs.* pigment content in the bands of strong absorption.

It was found that reciprocal reflectance of leaves (Gitelson and Merzlyak 1996; Gitelson *et al.* 2003; Merzlyak *et al.* 2003a; Gitelson *et al.* 2006) and apple fruit (Merzlyak and Chivkunova 2000; Merzlyak *et al.* 2003b) at certain wavelength relates to pigment contents. This feature was used in development of models that relate reflectance and pigment content. On the base of these models, algorithms for estimation of Chl and other pigments in apple fruit were developed. Conceptual semi-analytical three-band model (Gitelson *et al.* 2003; Merzlyak *et al.* 2003a; Gitelson *et al.* 2006), relates reflectance and content of pigment of interest [P] and was suggested in the form:

$$[P] \propto (R_{\lambda_1}^{-1} - R_{\lambda_2}^{-1}) \times R_{\lambda_3} \qquad \text{(Eq. 1)}$$

The model contains reflectances in three spectral bans ($\lambda_1$, $\lambda_2$, $\lambda_3$). Reflectance in the spectral band $\lambda_1$ is maximally sensitive to pigment of interest; however, it is also affected by absorption by other pigments and scattering by fruit. To eliminate the effect of absorption by other pigments at reflectance $R_{\lambda_1}$, reflectance in spectral band $R_{\lambda_2}$ has been used. $R_{\lambda_2}$ is affected by absorption of other pigments and is minimally affected by absorption of the pigment of interest. Thus, the difference $(R_{\lambda_1}^{-1} - R_{\lambda_2}^{-1})$ in Eq. 1 relates to the pigment of interest, however, is still affected by scattering of the fruit. To minimize this effect, reflectance in spectral band $\lambda_3$ should be governed mainly by fruit scattering.

As a result of signature analysis of apple fruit reflectance spectra, the bands of *in situ* absorption of apple skin pigments were established. Merzlyak *et al.* (2003b, 2005) spectrally tuned the conceptual model (Eq. 1) developed for terrestrial plant leaves (Gitelson *et al.* 2003) and found spectral bands $\lambda_1$, $\lambda_2$, and $\lambda_3$ that are optimal for pigment content retrieval in apples. The obtained results provided evidence that the conceptual model (Eq. 1) can be applied for an accurate non-destructive estimation of key pigment content in apples. The developed algorithms are (i) sensitive mainly to pigment of interest and minimally sensitive to contents of other pigments or morphological-anatomical features of fruit, and (ii) applicable to independently obtained data sets (Merzlyak *et al.* 2003a). For algorithms validation, multi-year datasets obtained for fruit of different apple cultivars were used. Next sections provide a brief overview of the approaches employed for non-destructive assay of the key pigments in, maturity of and certain damages to apple fruit.

### Chlorophyll

Chlorophyll content showing a correlation with flesh firmness and soluble solids/titratable acidity ratio could serve as an indicator of fruit ripeness (Costa *et al.* 2006). In earlier (Knee 1980) and recent (Bodria *et al.* 2004) investigations, reflectance minimum at 670–680 nm was employed for Chl analysis. Although these algorithms were sensitive to low Chl content, they possessed a weak sensitivity to Chl over 0.05–0.06 mmol m$^{-2}$ (Merzlyak *et al.* 2003b). The Normalized Difference Vegetation Index (NDVI), developed for the remote sensing of vegetation, allowed Chl estimation in apple fruit, however, its relationship with Chl content was not close ($r^2$ = 0.76–0.86; Kuckenberg *et al.* 2007). More promising results were obtained for the Elstar, Jonagold, and Idared apples with the use of derivative reflectance spectroscopy (Zude-Sasse *et al.* 2002; Zude 2003).

The analysis performed by Merzlyak *et al.* (2003) showed that spectral regions where reflectance is sensitive to wide-range variation of Chl content (>0.01–0.15 mmol m$^{-2}$) are situated aside from the position of red maximum of Chl absorption: in the relatively broad green band (550–650 nm) and in narrow red-edge band (700–705 nm). It was found that reflectances in these bands were hyperbolically related to Chl content (**Fig. 3A**) as it was previously found for the leaves of numerous species (Gitelson and Merzlyak 1996; Gitelson *et al.* 2003). Therefore reciprocal values of the reflectances, often used in spectral indices for estimation of these and other pigments (Gitelson *et al.* 2003), are directly related to pigment content. The green and red edge spectral domains were found as minima in the spectrum of root mean square error (RMSE) of Chl estimation using the three-band model for fruit with a wide variation of Chl content and were used for accurate Chl retrieval.

The minimal RMSE of Chl estimation for $\lambda_2$ was found in the NIR range of the spectrum where Chl does not absorb. One of the requirements for reliable algorithm for pigment estimation is its low sensitivity to morphological and anatomical traits of plant tissues. For fruit differing in pigment content, the lowest RMSE of Chl estimation was found for $\lambda_3$ also in the NIR region beyond 750 nm (**Figs. 2, 3A**). Since leaf and fruit pigments possess no measurable absorption in the NIR (Merzlyak *et al.* 2002), tissue reflectance in this region is thought to be determined by cuticle thickness, morphology of subepidermal (skin) and parenhymal (flesh) cells, water content, scattering etc. and could be used as a 'reference'. Thus, the algorithms for estimation of Chl content in apple (Chlorophyll Indices, CI) were suggested in the forms (see Gitelson *et al.* 2003):

$$\mathrm{CI}_1 = \left(R_{700}^{-1} - R_{800}^{-1}\right)\times R_{800} = R_{NIR}\cdot R_{700}^{-1} - 1 \qquad \text{(Eq. 2)}$$

$$\mathrm{CI}_2 = \left(R_{640}^{-1} - R_{800}^{-1}\right)\times R_{800} = R_{NIR}\cdot R_{640}^{-1} - 1 \qquad \text{(Eq. 3)}$$

Both algorithms were related linearly with Chl in a wide range of its changes and provided a high precision in estimating Chl content in fruit of several apple cultivars (Merzlyak *et al.* 2003b). The reflectance in the region of maximum Chl absorption around 670 nm was found to be insensitive to moderate-to-high Chl content (above 0.05 mmol m$^{-2}$) but could be used for assay of low skin Chl that is characteristic of apple ripening off-tree (Merzlyak *et al.* 2003b; Solovchenko *et al.* 2005).

## Anthocyanins

AnC fulfill important photoprotective function in leaves (Chalker-Scott 1999) and apple fruit (Merzlyak and Chivkunova 2000; Merzlyak *et al.* 2008). The red pigments (represented in apples mainly by cyanidin derivatives) localized in vacuoles within fruit cells possess an absorption maximum near 540–550 nm (**Fig. 2**, Lancaster *et al.* 1994; Merzlyak *et al.* 2003b; Merzlyak 2006). As a rule, AnC content in fruit of the cultivars expressing AnC pigmentation tends to increase in the course of ripening (Gross 1987; Lancaster et al. 2000). The use of AnC content as fruit maturity index turned to be problematic (Zude-Sasse *et al.* 2002), most probably due to the interference of environmental stimuli which are known to exert a strong effect on AnC biosynthesis (Strack and Wray 1989; Saure 1990; Reay 1999; Reay and Lancaster 2001). At the same time, non-destructive measurements of skin AnC could find an extensive use in fruit grading since AnC pigmentation is one of the major factors of fruit acceptance by consumer (Abbot 1999). In addition, AnC possess antioxidant activity beneficial for human health (Boyer and Liu 2004).

The main challenge of non-destructive AnC retrieval is that in the green range of the spectrum, where AnC absorb, reflectance is also affected by absorption of Chl. So, the goal of tuning of conceptual 3-band model for AnC retrieval was to find spectral band $\lambda_2$ where reflectance is governed only by Chl absorption and is not affected by AnC content. Such band was found using minimal RMSE of AnC estimation for $\lambda_2$ in the red edge range around 700 nm (Gitelson *et al.* 2001; Merzlyak *et al.* 2003b). As a result, the ARI index (Anthocyanin Reflectance Index) was suggested for AnC determination in the form:

$$\mathrm{ARI} = (R_{550}^{-1} - R_{700}^{-1})\times R_{800}, \qquad \text{(Eq. 4)}$$

where the first term is associated with combined absorption by AnC and Chl and the second one related to Chl absorption only and the third one is not affected by pigment absorption and depends upon solely fruit scattering. In fruit of several cultivars ARI proved to be a highly sensitive indicator of AnC and relationship ARI vs. AnC was linear for AnC ranged up to 0.50 mmol m$^{-2}$ (Merzlyak *et al.* 2003b). It should be underlined that the algorithms developed are able to provide accurate pigment estimation due to precise subtraction of Chl effect on reflectance in the green range applying for it reciprocal reflectance in the red edge range.

## Carotenoids

In green tissues the analysis of Car absorbing in the blue region of the spectrum is problematic due to a strong overlapping absorption of Chl present in high amounts in plant tissues (Demmig-Adams *et al.* 1996). Additional obstacles to the Car analysis in plants are due to complex composition of these pigments undergoing transformation during fruit ontogeny and upon their adaptation to high-light conditions (Ma and Cheng 2003, 2004; Solovchenko *et al.* 2006). To estimate effect of Car on reflectance spectra, one needs to remove a significant effect of Chl absorption. The quantitative Car estimation in apple became feasible using the same 3-band model (Eq. 1) with $\lambda_1$ in the range 510–520 nm (Gitelson *et al.* 2002; Merzlyak *et al.* 2003b). To subtract effect of Chl absorption on reflectance in spectral band $\lambda_1$, $\lambda_2$ was found to be optimal in either the green range (around 550 nm) or red edge range (700 nm). As for Chl and AnC retrieval, optimal $\lambda_3$ was in the NIR range beyond 750 nm. Two Carotenoid Reflectance Indexes (CRI) developed earlier for leaves (Gitelson *et al.* 2002) were suggested as

$$\mathrm{CRI}_1 = (R_{520}^{-1} - R_{700}^{-1})\times R_{800}\ , \qquad \text{(Eq. 5)}$$

or

$$\mathrm{CRI}_2 = (R_{520}^{-1} - R_{550}^{-1})\times R_{800}\ , \qquad \text{(Eq. 6)}$$

where the first term in the parentheses associates with combined absorption by Car and Chl, and the second one relates to Chl absorption. The applications of these algorithms to several apple cultivars (Merzlyak *et al.* 2003b) have confirmed its efficiency for Car estimation in a wide range of their changes in the course of fruit ripening. It should be mentioned, however, that CRI is not applicable to AnC-pigmented fruit. In addition, Flv when accumulated in high quantities in sunlit fruit influence considerably optical spectra and their absorption might extend quite far into the visible spectrum. Therefore, one using reflectances for non-destructive determination of higher plant pigments absor-

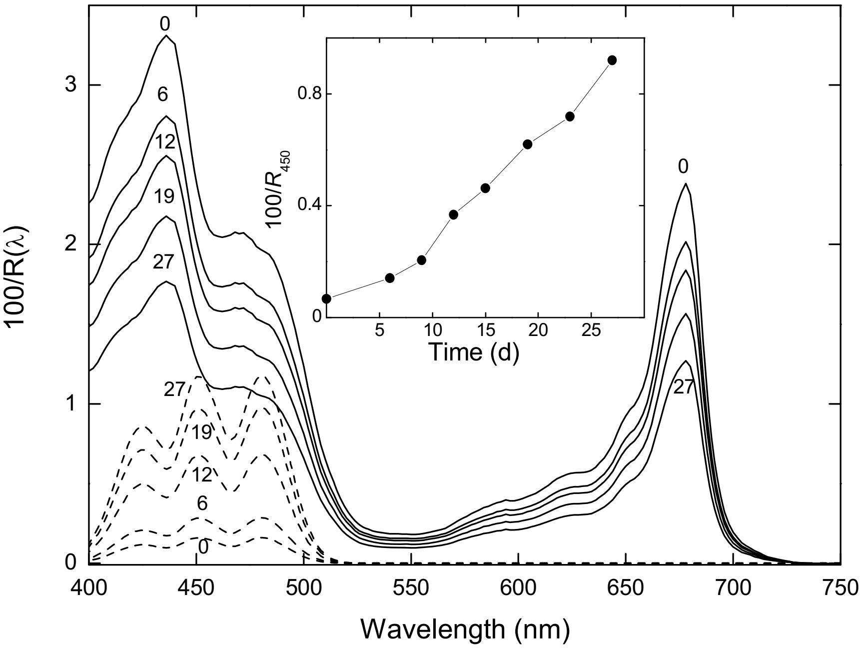

**Fig. 4 Modeling spectral contributions to whole-fruit reflectance by thylakoid-bound Chl and Car (solid lines) and extrathylakoid Car (broken lines) during off-tree ripening of 'Antonovka' apple.** Numbers are days of ripening after harvest in the end of August. Insert: Time-course of changes in the contribution of extrathylakoid Car to reciprocal reflectance at 450 nm. Details of the modeling are presented in Merzlyak (2006).

bing in the visible range should be aware of obstacles which could be caused by Flv when they are present in high amounts (Merzlyak *et al.* 2005).

## Ripeness and chlorophyll-to-carotenoid ratio

The proportion between Car and Chl is an important characteristic of fruit physiological condition (Gross 1987; Knee 1988; Merzlyak *et al.* 1999, 2002; Merzlyak and Solovchenko 2002; Solovchenko *et al.* 2006). The most dramatic changes in the content of these pigments occur at terminal stages of fruit development on tree and at the advanced stages of storage (Gross 1987; Merzlyak and Solovchenko 2002). Apple fruit possess fully functional photosynthetic apparatus (Blanke and Lenz 1990). Similarly to senescing leaves, the disassembly of photosynthetic apparatus of ripening apple fruit controlled by internal ethylene production and environmental stimuli such as light and temperature involves a gradual decrease in Chl and accumulation of significant amounts of Car (Gross 1987; Knee 1988; Merzlyak *et al.* 1999), often in the form of fatty acid esters (Tevini and Steinmüller 1985; Gross 1987; Knee 1988; Solovchenko *et al.* 2006) localized outside of thylakoid membranes in numerous plastoglobuli (Vishnevetsky *et al.* 1999; Merzlyak and Solovchenko 2002; Kessler and Vidi 2007), resulting in changes of coloration towards the appearance characteristic of ripe fruit.

Spectral reconstruction of reflectance spectra made it possible to follow relative contributions of thylakoid-bound pigments (Chl and Car) and extrathylakoid carotenoid pool during apple fruit ripening (Merzlyak 2006). According to previous findings reviewed by Gross (1987) and extended by Knee (1988), the size of the pool of esterified extra-thylakoid xanthophylls could serve as a precise marker of fruit ripening stage. **Fig. 4** shows that in the AnC-free 'Antonovka' apple ripening off-tree the degradation of the first pigment pool is accompanied by a gross increase of the second pool. According to the modeling, the pool of extrathylakoid Car was small in freshly harvested apple and underwent a remarkable growth; even stronger (*ca.* 13-fold) increase was found for the ratio of the extrathylakoid to the thylakoid-bound Car pools.

The analysis of green unripe fruit with different pigment content revealed a strong correlation between reflectance in the red maximum of Chl absorption (near 678 nm) and in the spectral band near 480 nm governed by combined absorption of Chl and Car (Merzlyak *et al.* 2003b). By contrast, during chlorophyll degradation in ripening apple and lemon fruit which turn yellow, $R_{678}$ increased considerably over $R_{500}$. As a result, a close correlation between reflectances at these wavelengths characteristic of tissues with high chlorophyll content was broken; in such fruits Car content was higher than that of Chl (Merzlyak *et al.* 1999). It should be mentioned, however, that absolute Chl content *per se* is not so reliable an indicator of ripeness (McGlone *et al.* 2002; Solovchenko *et al.* 2005); better results were obtained with the use of Car/Chl ratio.

For detection of relative changes in Chl and Car content, accompanying apple fruit ripening, the Plant Senescence Reflectance Index (PSRI, see Merzlyak *et al.* 1999) was suggested:

$$\mathrm{PSRI} = \left(R_{678} - R_{500}\right) \times R_{800}^{-1} \qquad \text{(Eq. 7)}$$

It showed a high correlation with a Car/Chl ratio in ripening apple fruits (Merzlyak *et al.* 2003b). The use of reflectance at the long-wave Car absorption maximum (480 nm) as a term in PSRI

$$\mathrm{PSRI}_{480} = \left(R_{678} - R_{480}\right) \times R_{800}^{-1} \qquad \text{(Eq. 8)}$$

improved the sensitivity and accuracy of Car/Chl estimation.

In the green fruit with low Car/Chl ratio ('Granny Smith' and 'Renet Simirenko') Chl absorption was a main factor governed reflectance at 480 nm and, thus, caused severe interference impairing sensitivity of the index. Therefore, the $R_{480}$-based index is preferable for Car/Chl estimation in cultivars with low or medium Chl content or ripening fruit with yellowish coloration (Merzlyak *et al.* 2003b).

The problem of non-destructive prediction of apple ripening on-tree and in storage draws much attention over the last decades. It was tackled with different approaches, mainly visible an NIR spectrometry. The results obtained with near-infrared spectroscopy are still controversial (Zude *et al.* 2006) whereas application of visible reflectance-based indices for this purpose looks more promising. Solovchenko *et al.* (2005) employed non-destructive assay of Chl and Car/Chl ratio for multi-season monitoring of 'Antonovka' apple ripening both on- and off-tree (**Fig. 5**). The findings obtained in this work showed the timing of ripening in storage is determined by a physiological state which fruit has attained by the date of harvest but not the harvest date *per se*. At the same time the content of both Chl and Car should be used for characterization of ripening process in apple rather that the content of each of the pigment alone. The non-destructively obtained data on Car and Chl made it possible to develop a model for precise description, predicting and modeling 'Antonovka' apple ripening rates as a func-

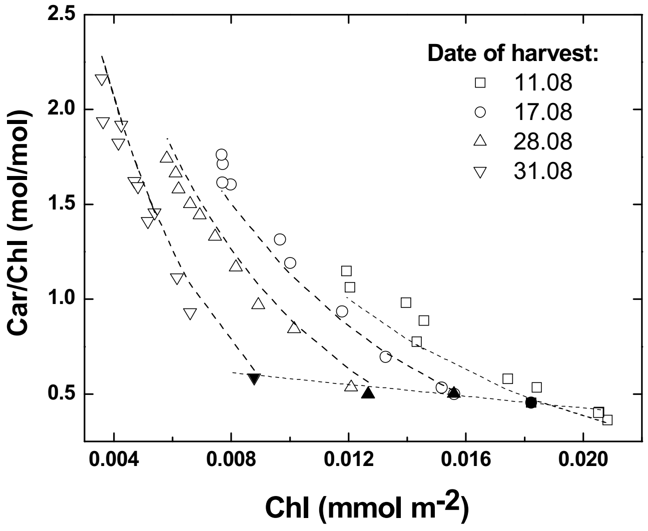

**Fig. 5 Performance of the non-destructive method for the prediction of fruit ripening off-tree as estimated via carotenoid-to-chlorophyll ratio.** Symbols denote the values measured for 'Antonovka' apple fruit on-tree (closed symbols) and in storage (open symbols). Dashed lines represent the trends predicted for on- and off-tree ripening fruits with model developed using multi-season observation (Solovchenko *et al.* 2005); $r^2 = 0.92$ for the relationships 'measured vs. predicted' for carotenoid-to-chlorophyll ratio. Reprinted from **Solovchenko AE, Chivkunova OB, Merzlyak MN, Gudkovsky VA** (2005) Relationships between chlorophyll and carotenoid pigments during on- and off-tree ripening of apple fruits as revealed non-destructively with reflectance spectroscopy. *Postharvest Biology and Technology* **38**, 9-17, ©2005, with kind permission from Elsevier, Ltd.

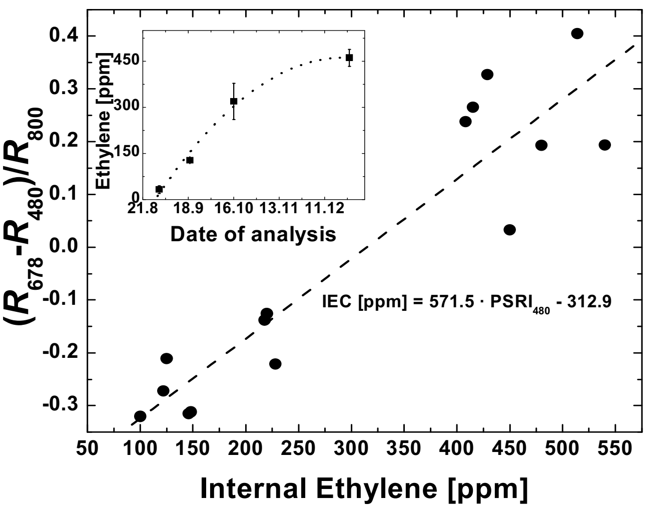

**Fig. 6 Relationships between IEC and PSRI$_{480}$ ($r^2 > 0.86$; P < 0.001) in sunlit skin of stored 'Antonovka' fruit.** $n = 15$; each point on the graph is the average of three measured values. Insert: the kinetics of IEC. Data were obtained in collaboration with Dr. L. Kozhina.

tion of on-tree Chl level (**Fig. 5**, Solovchenko *et al.* 2005). The applicability of both PSRI algorithms as a maturity index is supported by recent finding of a tight ($r^2 > 0.8$) relationship between PSRI and internal ethylene concentration (IEC) in apple (**Fig. 6**). It could be noted that the treatment of lemon fruit with external ethylene brought about distinct dose- and time-related PSRI changes (Merzlyak *et al.* 1999). The approach involving PSRI was used for estimation and comparison of ripening rates on shaded and surfaces of 'Antonovka' apples (Solovchenko *et al.* 2006). It turned out that strong sunlight promotes fruit ripening contributing significantly towards the heterogeneity of ripening and ethylene evolution rates within a single fruit tissue. This circumstance should be taken into account during grading of fruit for subsequent long-term storage.

### Flavonols

Flavonols (Flv) comprise an abundant group of phenolic compounds involved in a number of physiological functions, including UV-protection, in higher plants (Bornman *et al.* 1997). Apple fruit contain high amounts of Flv, comprised mainly by quercetin glycosides (Escarpa and Gonzalez 1998; Solovchenko *et al.* 2001; Solovchenko and Schmitz-Eiberger 2003). Remarkably that in sunlit (but not in shaded) apple skin Flv content could achieve as high as 3.50 mmol m$^{-2}$ (Merzlyak *et al.* 2002, 2005) that more than 10-fold exceeds Chl and Car content. Apple Flv, the potent reactive oxygen species scavengers (Tournaire *et al.* 1993; Rice-Evans *et al.* 1997; Russo *et al.* 2000), exert multi-faceted beneficial effects on human health, e.g. provide protection from cardiovascular and oncology diseases (van der Sluis *et al.* 1997). It was reported that skin Flv content tends to change considerably in the course of apple fruit storage (Lister *et al.* 1994). A non-destructive technique for Flv monitoring could helpful for grading of fruit on Flv content since traditional HPLC-based techniques (Escarpa and Gonsalez 1998) are laborious and time-consuming.

The development of the reflectance-based technique for Flv estimating is complicated by a strong overlap of other phenolics abundant in apple skin in near-UV and by Chl and Car absorption at short wavelengths of the visible range. The spectral domain maximally sensitive to Flv content was found in the blue around 410 nm (**Fig. 7**). However, in this spectral band Chl and Car also strongly absorb. To remove the contribution of these pigments, one needs to find band $\lambda_2$ in Eq. 1 where reflectance closely related to Chl and Car contents and minimally affected by Flv absorption. This band was selected using minimal RMSE of Flv estimation around 460 nm. As a result, the Flavonol Reflectance Index (FRI) was suggested in the form (Merzlyak *et al.* 2005):

$$\mathrm{FRI} = \left(R_{410}^{-1} - R_{460}^{-1}\right) \times R_{800} \qquad \text{(Eq. 9)}$$

FRI allowed accurate assessment ($r^2 = 0.92$, RMSE = 0.05 mmol m$^{-2}$) of skin Flv content ranging from 0.08 to 2.20 mmol m$^{-2}$ for all apple fruit varieties studied ('Golden Delicious', 'Granny Smith', 'Renet Simirenko'); an estimation of Flv in the whole range of its content (up to 3.50 mmol m$^{-2}$) required a variety-specific approach and turned feasible with the use of power or exponential relationships between FRI and Flv content (Merzlyak *et al.* 2005).

## FIRMNESS AND SOLUBLE SOLIDS

Numerous attempts were undertaken for finding approach(es) for non-destructive estimation of fruit firmness and soluble solids content traditionally employed as apple ripeness criteria (Kingston 1992; DeLong *et al.* 1999; Costa *et al.* 2006; Zude *et al.* 2006). It is obvious that this is successful to the extent to which the quality characteristics are related with the changes in the pigments patterns inherent in fruit reflectance spectra. It was reported that both fruit firmness and soluble solids correlate with Chl content in cvs. 'Elstar', 'Pinova' and 'Topaz' and hence with reflectance in the band of the absorption by this pigment. However, the strength of the correlation was often insufficient for the reliable assessment of flesh firmness, acidity and soluble solids content (Zude *et al.* 2006). Different reasons for these complications have been discussed in the literature, including high heterogeneity of fruit, interference of the environmental and artificial (agricultural and storage) factors decoupling Chl changes from those in firmness and solids (Abbot 1999; Solovchenko *et al.* 2005). Particularly, the ignorance of different maturity state of the tissues on sunlit and shaded fruit sides may lead to low correlation between Chl and firmness. The application of NIR reflectance-based techniques (which are out of scope of this review) was the most successful approach for the solution of this problem so far (Costa *et al.* 2006).

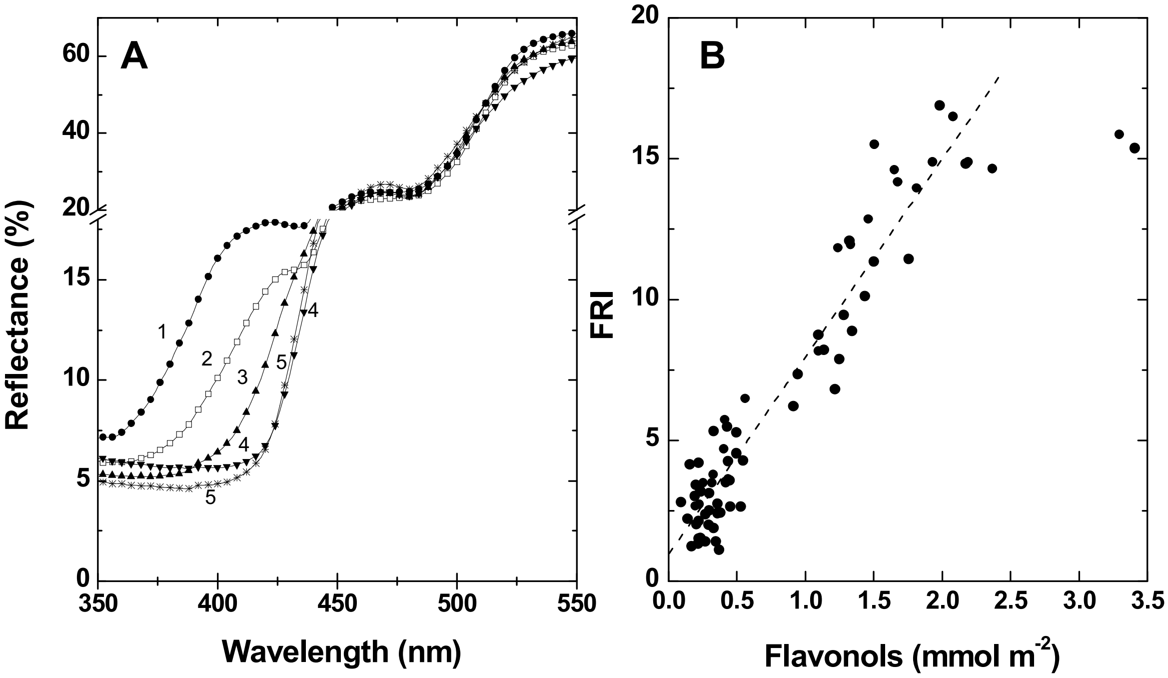

**Fig. 7 Non-destructive assay of apple skin flavonols via reflectance.** Reflectance spectra (**A**) of 'Antonovka' apple fruits with different peel flavonol content (1–0.46, 2–1.10, 3–1.22, 4–1.44; 5–2.34 mmol m$^{-2}$) and (**B**) relationships between reflectance index FRI and skin flavonol content. In B solid line represents the linear fit for flavonol content in the range 0.08–2.20 mmol m$^{-2}$. Reprinted from **Merzlyak MN, Solovchenko AE, Smagin AI, Gitelson AA** (2005) Apple flavonols during fruit adaptation to solar radiation: spectral features and technique for non-destructive assessment. *Journal of Plant Physiology* **162**, 151-160, ©2005, with kind permission from Elsevier, Ltd.

## DAMAGES

### Water core

As a result of the disorders accompanied by the cell breakdown and transfusion of intercellular spaces with liquid cell content (Sharples 1967; Pierson *et al.* 1971) generally known as water core, the apple fruit tissue may acquire 'water-soaked' (visually translucent) appearance. Water core is believed to be caused by mineral, primarily calcium deficiency and/or excess of magnesium and potassium (Simon 1978). The disorder could develop both on-tree and in storage. In some cases water core may be followed by internal breakdown of fruit. Therefore it is desirable to sort out the water core-affected fruits after harvest before storage.

The development of water core exerts profound effects on apple fruit reflectance spectra (**Fig. 8**), most noticeable of which is a considerable decrease of reflectance in the whole spectral range more apparent in the regions of weak pigment (Chl, Car, AnC) absorption. It should be noted that the spectra in the NIR remain flat during water core development which is not the case for the disorders accompanied by fruit browning (see below). Therefore the synchronous decrease of reflectance in the NIR and green parts of the spectrum along with retention of $R(\lambda)$ flatness in the NIR could serve as a primary marker of water core and other disorders involving liquid transfusion of fruit tissues.

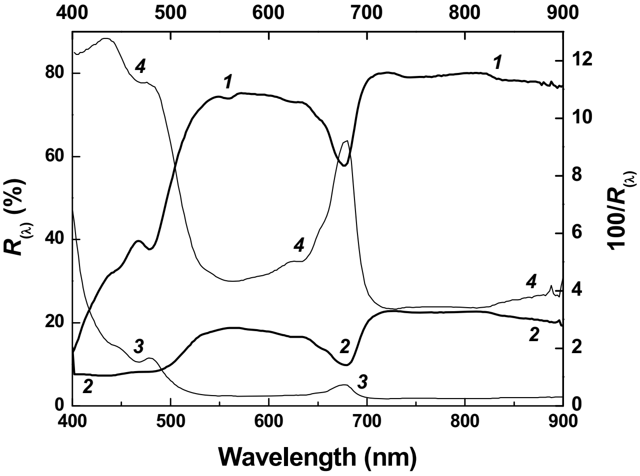

**Fig. 8 The influence of water core development on 'Suvorovskoe' apple reflectance spectra.** Reflectance (1, 2; left scale) and reciprocal reflectance (3, 4; right scale) spectra of intact (1, 3) and water core-affected (2, 4) fruit are shown. Water core induces a profound decrease of reflectance (increase in absorption) throughout the visible and near infra-red ranges. For details on spectral measurements, see Merzlyak *et al.* (2003).

### Sunburn

The discoloration of apple skin induced by high fluxes of solar radiation and exacerbated by high ambient temperatures and certain other factors (Barber and Sharpe 1971; Andrews and Johnson 1996, 1997; Piskolczi *et al.* 2004) is known as 'sunburn' damage and distinguished from sunscald by the absence of brown coloration (**Fig. 9**). The sunburn-affected apple skin appears visually as pale yellowish or greenish or even completely bleached. The discoloration occurring more often in unripe apples with high Chl content is accompanied by disappearance of the absorption bands of the main pigments. Remarkably, more or less synchronous destruction of Chl and Car takes place [**Fig. 10**; (Merzlyak *et al.* 2002)]. Ripe apple fruit contain possess high Car/Chl ratio (Merzlyak and Solovchenko 2002) and feature considerable amounts of highly photostable Car (Merzlyak and Solovchenko 2002). Accordingly, the regions of fruit surface affected by sunburn could turn yellow and appear more ripe than the rest of the fruit. Later, in the course of storage the regions of fruit surface affected by severe sunburn often develop brownish coloration displaying the well-established features collectively known as 'sunscald' (see below). The tissues beneath these regions are characterized by higher rates of ethylene evolution, susceptibility to various physiological storage disorders and fungal diseases (Pierson *et al.* 1971). Therefore such fruit should be avoided since a few number of them could dramatically affect the timing of ripening of neighbouring storing fruit and eventually spoil

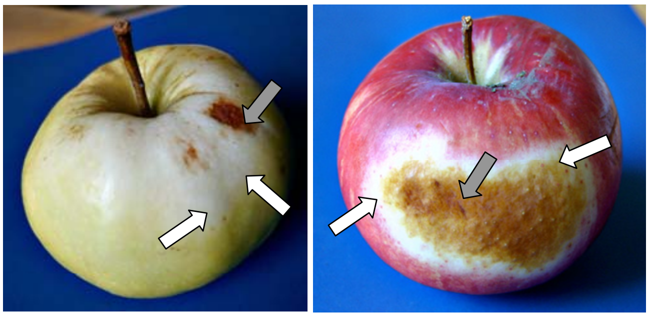

**Fig. 9 Anthocyanin-free (left) and anthocyanin-containing (right) 'Zhigulevskoye' apples affected by sunburn (white arrows) and sunscald (gray arrows).**

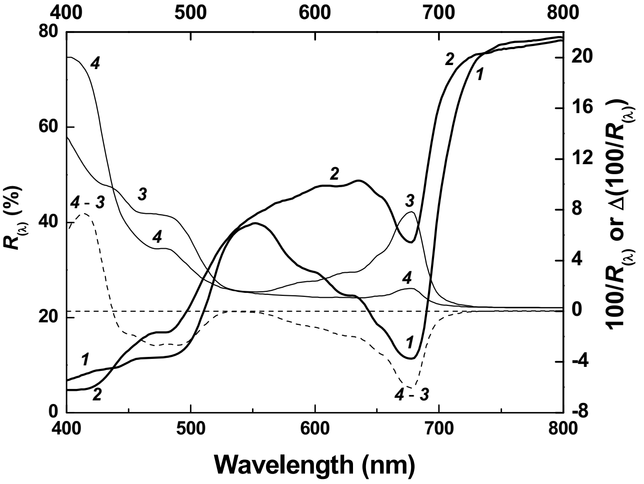

**Fig. 10 Characteristic features of sunburn as revealed *via* reflectance in 'Granny Smith' apple.** Reflectance (1, 2; left scale), reciprocal reflectance (3, 4; right scale) spectra and difference spectrum '4 – 3' (right scale) of intact (1, 3) and sunburn-affected (2, 4) fruit are shown. Sunburn is accompanied by synchronous bleaching of chlorophyll and carotenoid bands in the red and blue-green parts of the visible spectrum. For details on spectral measurements, see Merzlyak *et al.* (2003).

the whole batch, therefore it is highly desirable to sort out the fruit with severe sunburn.

Recent advances in the investigation of apple fruit spectroscopy open a possibility for finding the approaches of automated techniques for the detection of sun-burned fruits predisposed to decay in storage even before they turn brownish. As mentioned above, the development of sunburn is characterised by rapid, complete and synchronous bleaching Chl and Car absorption and disappearance of their absorption bands in the red and blue-green region of the spectrum (Merzlyak *et al.* 2002). Affected fruit could be distinguished from healthy apples by higher reflectance in the visible region of the spectrum. The detection of mild sunburn seems to be possible using the index(es) similar to PSRI described above. Affected regions of fruits possess exceptionally high PSRI values as compared with unaffected regions of the same fruit or other healthy fruit.

## Tissue browning (sunscald, superficial scald, bruises, etc.)

The development of characteristic brown coloration (**Fig. 9**) is a common symptom of numerous physiological disorders and infectious diseases of fruit occurring both on tree and in storage as well of mechanical damage and a hypersensitive reaction induced by incompatible pathogenic microorganisms (Delalieux *et al.* 2007). It is generally believed that browning process is due to the loss of cell compartmentalisation and involves the oxidation of polyphenolics by the enzyme polyphenol oxidase (Lurie *et al.* 1991) resulting in polymerised melanin-like pigment(s) production (Vaughn and Duke 1984; Butt 1985). The brownish pigments accumulate as a result of the death of cells in skin and/or parenchymal tissues caused by environmental factors as in the case of sunscald (Merzlyak *et al.* 2002), non-optimal storage conditions (Barden and Bramlage 1994; Bramlage and Weis 1997) or pathogen attack (Delalieux *et al.* 2007). Browning is an obvious symptom of superficial scald, a physiological disorder developing in many fruit resulting in a considerable their loss during storage. In apples, the extent of superficial scald development is strongly dependent on cultivar, environmental and storage conditions (Barden and Bramlage 1994; Bramlage and Weis 1997; Ferguson *et al.* 1999).

In practice it takes so far a lot of time and manual labour to sort out fruit affected by browning of various aetiology having no market value and unsuitable for further storage. The optical spectral changes of browning plant tissues induced by wounding (Merzlyak *et al.* 1997), heating (McClure 1975) and ageing (Merzlyak *et al.* 1997) have been reported. The signature analysis of superficial scald-induced browning was performed by Chivkunova *et al.* (2001). According to their observations, the melanin-like brownish pigments exhibit a featureless absorption monotonously increasing from NIR towards shorter wavelengths. The browning of apple fruits in the course of sunscald or superficial scald development (**Fig. 11A**) as well as that artificially induced by UV-B-irradiation (Solovchenko, unpublished) or *n*-hexane treatment (Chivkunova *et al.* 2001) is accompanied by a dramatic increase in light absorption by fruit tissues in the regions where Chl and Car possess weak absorption, i.e. in the NIR and green part of the visible spectrum. By contrast, the regions of strong absorption by Car and Chl are less affected by browning suggesting a strong overlap of the pigment absorption with that of compounds accumulated during fruit browning. Chivkunova *et al.* (2001) suggested the use of $R^{-1}_{550}$ as a term that is sensitive to browning and $R^{-1}_{700}$ as a term minimally sensitive to browning but sensitive to Chl and Car content (see Gitelson and Metrzlyak 1996). Subtraction of $R^{-1}_{700}$ from $R^{-1}_{550}$ allowed constructing an index that was sensitive to browning and was minimally sensitive to variation of Chl and Car contents. Reflectance at 750 nm, which exhibits low variation in healthy fruits but it increases significantly in the course of browning (**Fig. 11B**) was introduced in BRI as a term, increasing its sensitivity to browning. BRI (Browning Reflectance Index) was suggested in the form:

$$\mathrm{BRI} = \left(R^{-1}_{550} - R^{-1}_{700}\right) \times R^{-1}_{800} \qquad \text{(Eq. 11)}$$

**Fig. 11** shows that during long-term storage of 'Antonovka' apples an increase of BRI with different extent occurs in some (but not all) fruit. According to our preliminary data on fruit spectral reflectance and multi-year observations on development of superficial scald (Prof. V.A. Gudkovsky, pers. comm.) the difference in the extent of the scald-induced browning is related with prehistory of fruit: usually sunlit surfaces of apples are less affected by the disorder as compared with shaded sides of the same fruit.

The index proved to be a sensitive and efficient tool for quantitative assessment of superficial scald and other physiological disorders accompanying by browning as well as plant diseases affecting the close relationships between reflectances at 550 and 700 nm inherent in healthy apple fruit tissues (see above). It should be noted that the BRI, to a certain extent, is analogous to ARI and therefore is not applicable for AnC-containing fruit. Another spectral features characteristic of and suitable for superficial scald incidents is a noticeable lowering of reflectance near 800 nm and a monotonous its decrease between 740 and 800 nm. It appears that these features could be applies to apple varieties containing high AnC amounts.

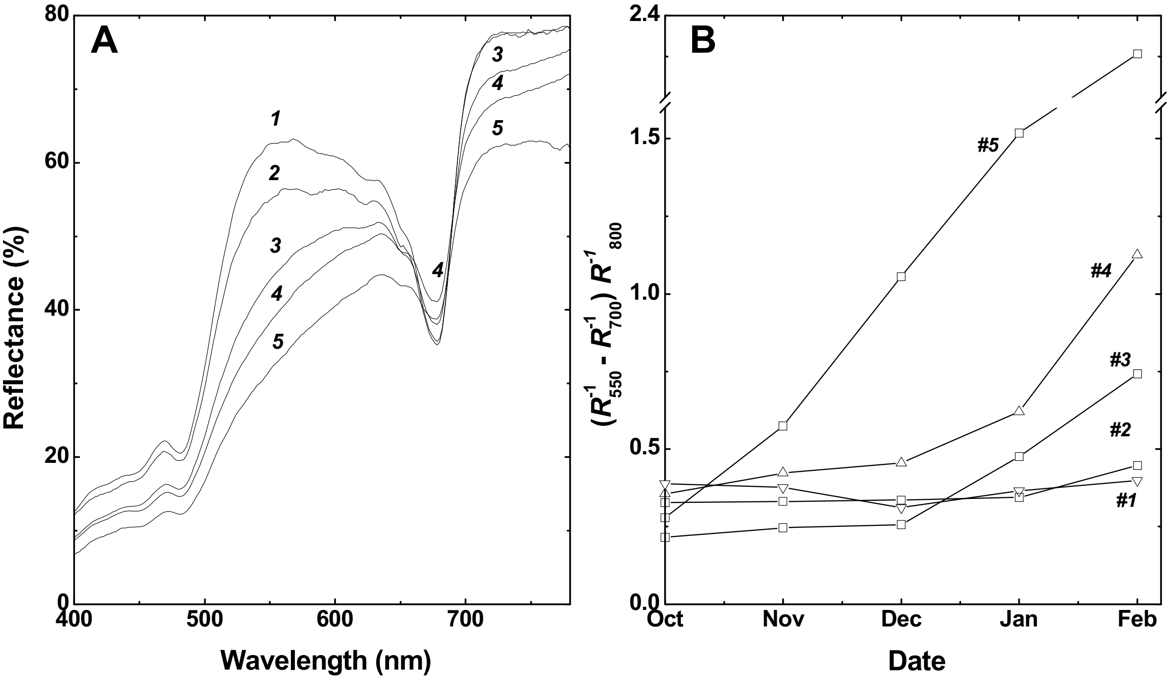

**Fig. 11 Non-destructive monitoring of 'Antonovka' apple browning.** (**A**) The influence of the developing superficial scald on reflectance spectra of stored at 4°C (October – February) apple fruit; 1 = October, 2 = November, 3 = December, 4 = January, 5 = February. (**B**) Time-course of the Browning Reflectance Index (BRI) changes in n-apple fruit differing in the extent of superficial scald symptoms during their long-term storage. The symptoms were observed in fruit #3–5 but not in fruit #1 and #2. For details on spectral measurements, see Chivkunova *et al.* (2001).

## CONCLUSIONS AND FUTURE PROSPECTS

In spite on complicated morphological, anatomical and optical properties and still insufficient understanding of fruit optics, a considerable progress was achieved in the development of non-destructive techniques for sensing of the physiological state and quality of apple fruit. The findings of the last two decades considerably extended possible applications of reflectance spectroscopy for evaluation of and monitoring quality of fruit, including apple, both on-tree and in storage. The results presented in this review show that reflectance spectroscopy could be a useful and efficient tool for monitoring of apple fruit quality. Remarkably, for retrieval of fruit pigment (Chl, Car, AnC, and Flv) content, the reflectance only in 3 to 5 spectral bands is sufficient. Physiological processes involved in fruit ripening, senescence as well as development of damage symptoms proceed in a fairly predictable pattern and involve dramatic changes in fruit pigment profile(s) manifesting itself in corresponding changes in fruit reflectance. The developed algorithms are linearly related to pigment content and are able accurately estimate fruit pigments in a wide range of their content. The non-destructively obtained information on the trends of pigment content allow one, using novel models, to evaluate and predict fruit ripeness on-tree and in storage. However, using non-destructive optical reflectance-based techniques, one should take into account that certain growing conditions or postharvest treatments may decouple the rate of physiological processes mediated by ethylene and that of pigment changes (Blankenship and Dole 2003) and, therefore, take necessary precautions. The particularly promising field for application of the non-destructive methods of fruit quality monitoring is the investigation of the efficiency of different techniques of postharvest management of fruit quality involving controlled atmosphere storage, treatments with ethylene inhibitors, etc. Fundamental spectral features of fruit reflectance and its changes, recently revealed and reviewed here, provide a solid basis for the development of these technologies. Further investigations are required in order to broaden and improve the developed indexes for other apple cultivars. In this respect, particular attention should be paid to pigment spectral signature search in order to achieve more selective and precise their using in fruit quality assessment. Altogether, the new promising approaches to spectral data treatment allow obtaining valuable information on fruit strictly related with ripeness, non-destructively which could previously be assessed only with 'wet' analytical methods.

## ACKNOWLEDGEMENTS

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