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Reflectance Spectral Features and Detection of Superficial Scald–induced Browning in Storing Apple Fruit

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ABSTRACT

Reflectance (R) spectra of Antonovka apples in the visible and near infrared ranges of the spectrum were studied during fruit storage. Specific spectral features of reflectance of healthy ripening fruits and those arising as a result of superficial scald development and browning induced artificially by *n*–hexane treatment were revealed and compared. Both superficial scald and *n*–hexane treatment brought about a strong decrease of reflectance, especially, in the green near 550 nm. During natural fruit ripening in the absence of superficial scald development, a high correlation was found between reflectances at 550 and 700 nm. In contrast, as a result of superficial scald development the close relationship between reflectances at 550 and 700 nm was lost. The revealed spectral signatures were used in developed reflectance index $(1/R_{550} – 1/R_{700})/R_{750}$ for non-destructive superficial scald detection and estimation of its extent in storing apple fruit.

Key words: apple fruit, browning, superficial scald, reflectance.

Abbreviations: BRI – browning reflectance index, Car – carotenoids, Chl – chlorophylls, NIR – Near Infra Red, R– reflectance, SS – superficial scald, STD – standard deviation, R – reflectance.

Чивкунова О.Б., Соловченко А.Е., Соколова С.Г., Мерзляк М.Н., Решетникова И.В. и Гительзон А.А. Использование особенностей спектров отражения для оценки развития подкожного загара при хранении плодов яблони.

Исследовали спектры отражения в видимой и ближней инфракрасной областях спектра плодов яблони сорта Антоновка обыкновенная. Выявлены спектральные особенности, характерные для здоровых плодов при их созревании и хранении, плодов, пораженных загаром, а также при искусственном побурении, индуцированном обработкой *n*-гексаном. Как при естественном загаре, так и при искусственном побурении наиболее значительные изменения наблюдали в зеленой области спектра около 550 нм, а также в ближней инфракрасной области; при этом высокая корреляция между коэффициентами отражения (R) при 550 и 700 нм, характерная для здоровых плодов, нарушалась. Учитывая эти особенности, предложен спектральный индекс, $(1/R_{550} – 1/R_{700})/R_{750}$, который не зависит от содержания хлорофиллов и каротиноидов и может быть использован для недеструктивной оценки степени развития загара в плодах при их хранении.

INTRODUCTION

Browning of plant tissues is a manifestation of mechanical damage, certain diseases and physiological disorders, senescence and a hypersensitive reaction induced by pathogenic microorganisms. It is generally believed that a browning process is due to the loss of cell compartmentalisation and involves the oxidation of polyphenolic compounds by polyphenol oxidase resulting in polymerised melanin-like pigment(s) production (Vaughn and Duke, 1984, Butt, 1985).

Browning is an obvious symptom of superficial scald (SS), a physiological disorder developing on apple fruit surface during storage and a considerable part of fruit is lost annually as a result. The extent of SS development is strongly dependent on cultivar, environmental and storage conditions (Gudkovsky et al., 1989, Barden et al., 1994, Bramlage and Weis, 1997, Thomai et al., 1998, Ferguson et al., 1999).

In practice it takes so far a lot of time and manual labour to sort out fruits with SS symptoms having no market value and unsuitable for further storage. Attempts to apply nondestructive methods of optical spectroscopy for assessment of fruit quality and their physiological state have been undertaken for several decades (Knee, 1980, Morita et al., 1990, 1992, Merzlyak et al., 1997, 1998, Merzlyak and Chivkunova, 2000), in particular, to develop automatic technique for fruit grading and sorting (Morita et al., 1990, 1992). The optical spectral changes of browning plant tissues induced by wounding (Merzlyak et al., 1990), heating (McClure, 1975) and ageing (Merzlyak et al., 1997) have been reported.

In this paper we report changes in reflectance of healthy and SS-affected apple fruit during their long-term storage. To obtain more details on the spectral characteristics change, the fruits in which the browning process was induced artificially have been also studied. Special attempts were undertaken to develop a technique for non-destructive detection and quantification of SS in apple fruit.

MATERIALS AND METHODS

Plant material. Mature apple fruit (*Malus domestica* Borkh., cv. Antonovka obyknovennaya) without anthocyanin pigmentation were obtained from the Botanical Garden of Moscow State University (1993, 1997, 1996) or from Michurinsk, Tambov region, Russia (1995, 1999), studied immediately or after storage (up to 5–6 months) at 4°C. Fruits without and with spontaneously developing symptoms of superficial scald were examined. Artificial browning was induced by immersion of a fruit in ca. 30 ml of *n*-hexane for 3 min.

Spectral measurements. Reflectance (R) spectra of whole fruit were measured with a 150–20 Hitachi spectrophotometer (Japan) equipped with 150-mm integrating sphere attachment against barium sulphate as a standard and at a spectral resolution of 2 nm (Merzlyak et al., 1998). The data were transferred to and treated with a personal computer. Reflectance values were expressed as a ratio of reflected to incident light intensity. Both in the progress of storage and as a result of artificially induced browning the spectra were taken from the same zones of fruit surface.

RESULTS AND DISCUSSION

To study specific spectral features of SS, the reflectance spectra during long-term fruit storage were acquired (Fig. 1). In the absence of visible SS symptoms (Fig. 1A), reflectance spectra showed similar spectral features as those during natural on a tree apple fruit ripening and maturation, described previously: an increase of reflec-tance in the red region of the spectrum (minimum around 678 nm) was accompanied by only slight an increase in reflectance in the blue range characteristic of Chl breakdown and Car retention during leaf senescence and fruit ripening (see Merzlyak et al., 1998 for more details).

The development of SS symptoms brought about dramatic changes in reflectance spectra. As browning progressed (Fig. 1B), reflectance of SS-affected fruit decreased in the whole range of the spectrum and the spectral features of Chl (in the band 620–700 nm) and Car (a sharp increase in reflectance near 500 nm) disappeared. A considerable decrease of reflectance occurred in the NIR (from ca. 0.75–0.80 in intact to 0.40–0.60 in SS-affected fruits). The extent of browning-dependent reflectance changes was high in the green: a decrease of reflectance near 550 nm was about three-fold (from 0.6 to ca. 0.2), whereas in the red and the blue regions, the reflectance changes were much smaller. It is noteworthy that as a result of SS development during fruit storage, the reflectance changes in the range of Chl absorption maximum near 678 nm were less than in healthy fruits (cf. panels B and A in Fig. 1).

In our previous study on α-farnesene participation in SS in apples (Chivkunova et al., 1997), we found that removal of cuticular lipids by *n*-hexane extraction induces rapid and homogeneous browning of fruit surface. This model system was used to follow browning-dependent spectral features in an apple fruit with permanent pigment (Chl and Car) content in real time (Fig.2). The treatment of a green fruit with *n*-hexane produced the spectral changes close to those found in SS-affected fruits; in addition, a shift of Chl absorption maximum to shorter wavelengths was observed (cf. Figs. 1B and 2A). In an yellow apple fruit with very low Chl content and high and almost constant reflectance between 800 and 600 nm, *n*–hexane treatment brought about a gradual decrease of reflectance from 800 to 520 nm and noticeable spectral features gone. Therefore, one can suggest that spectral features of *in situ* reflectance, – a minimum near 670 nm and so-called blue-green edge in reflectance spectra around 520 nm, are specific characteristics of healthy ripening fruits. The transformation of these spectral features can be used as an indicator of browning of apple fruit tissue.

The spectra in Fig. 1 and 2 indicate a strong overlapping of light absorption by Chl and Car in and pigment(s) responsible for brownish coloration of apple fruit. SS is known to occur at stages of storage when Chl contents remains relatively high (Gudkovsky et al., 1989). Then, a considerable Chl degradation occurs in the course of long-term storage of apple fruit (Knee, 1980, Merzlyak et al., 1997, 1998, see also Fig.1A). Therefore, detection of browning pigments is complicated due to strong and varying Chl absorption.

To find spectral bands sensitive to SS development, the signature analysis of reflectance spectra (Gitelson & Merzlyak, 1994, 1996) of apple fruits with (Fig. 3A, curves 2 and 3) and without (Fig.3A, curve 1) browning symptoms was carried out. In the absence of browning,

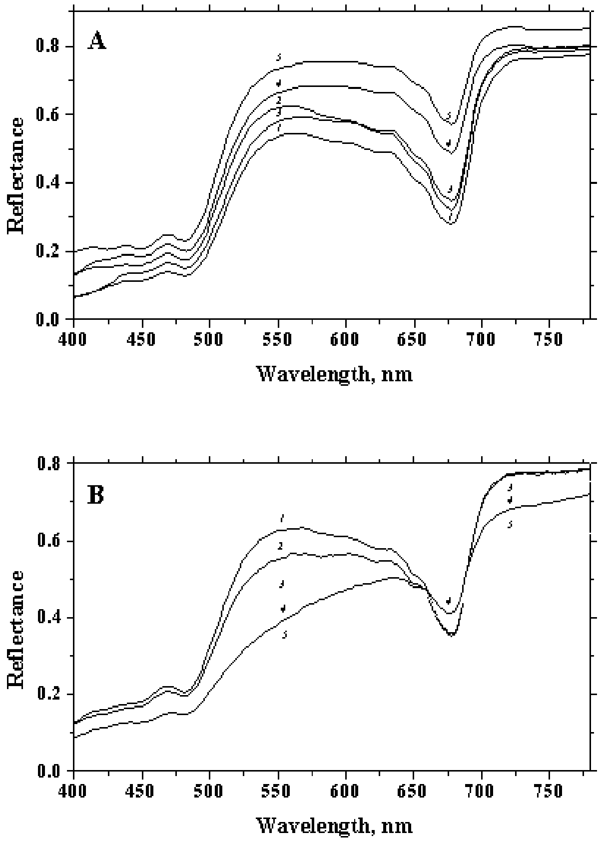

Fig. 1. The changes of reflectance spectra of two apple fruits in the course of their storage (October – February, 1996) occurring without (A) and with SS symptoms (B).

1 – October, 2 – November, 3 – December, 4 – January, 5 – February.

In healthy fruit a gradual increase of reflectance takes place due to the breakdown and transformation of fruit pigments (Chl and Car) during ripening. The SS development results in a decrease of reflectance, especially in the green.

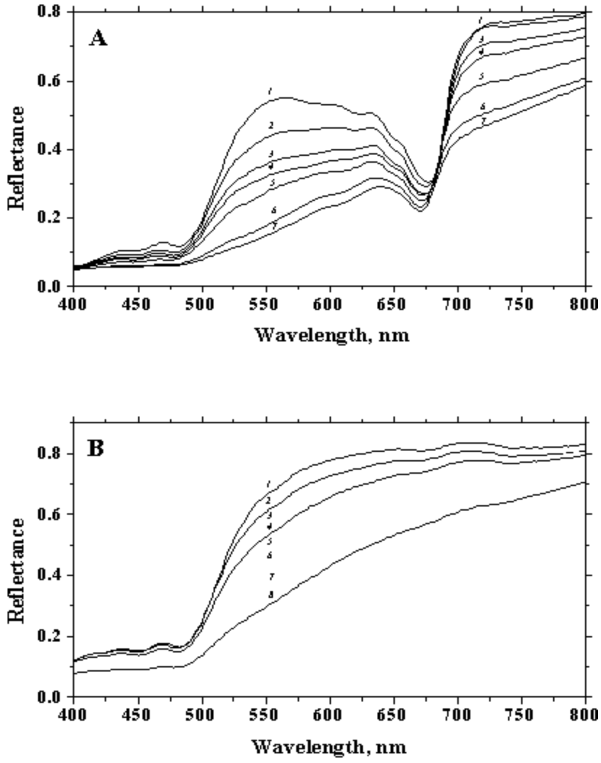

Fig. 2. The changes of reflectance spectra of a green (A) and an yellow (B) apple fruits as a result of browning induced by *n*-hexane treatment.

Time of incubation (min):

A: 1 – 0, 2 – 12, 3 – 18, 4 – 22, 5 – 51, 6 – 93, 7 – 129;

B: 1 – 0, 2 – 25, 3 – 42, 4 – 94, 5 – 130, 6 – 190, 7 – 285, 8 – 305.

The treatment of apple fruit with *n*-hexane inducing homogeneous apple fruit tissue browning is accompanied by the spectral reflectance changes close to those during SS development. The spectra in A and B show the effect of Chl on the pattern of the spectral changes.

minimum of the standard deviation of reflectance (STD) located in the NIR and the highest STD values were found in the red, showing significant variation in Chl absorption during fruit ripening. In the blue-green region, the variation of reflectance was quite high with two maxima near 460 and 515 nm. Some lines of evidence suggest that the latter maximum could be attributed to Car (Merzlyak et al., 1998, Zur et al., 2000). The STD at 550 and 700 nm were virtually equal in magnitude, indicating synchronous change of the reflectances at these wavelengths during fruit ripening (Fig. 1, panel A). Ripening fruit reflectance spectra showed a high correlation ($r^2 = 0.94$) between STD at 550 and 700 nm during several seasons of observation (Fig. 3, panel B). Between 550 and 760 nm, the STD spectra of SS-affected and *n*-hexane-treated apple fruits were just opposite to the spectrum of ripening healthy fruits (Fig. 3, panel A). The STD was high in the NIR region and the STD minimum was located near Chl absorption band (660–680 nm). In both SS-affected and *n*-hexane treated fruits, the STD maximum was observed around 550 nm and STD at 550 nm was much higher than those at 700 nm. This was observed also in other data sets studied including naturally developing SS and an artificially induced browning (Fig. 3, A and B). As a result, in all cases little if any correlation between STD at 550 and 700 nm was found. Thus, R_{NIR} and R_{550} exhibit high sensitivity to browning; an increase of STD of reflectance in the NIR and in the green region appeared to be characteristic of the disorders accompanied by browning. Another important feature of reflectance was almost invariable STD at 700 nm.

To develop sensitive optical indicator of SS, one needs to select the spectral bands exhibiting (i) high sensitivity to browning, and (ii) minimal sensitivity to browning, but sensitive Chl and Car. Thus, we suggest using $1/R_{550}$ as term that is sensitive to browning and $1/R_{700}$ as a term minimally sensitive to browning but sensitive to Chl and Car content (Gitelson & Metrzlyak, 1996, 1997). Subtraction $1/R_{700}$ from $1/R_{550}$ allowed to construct 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 decreases significantly in the course of browning

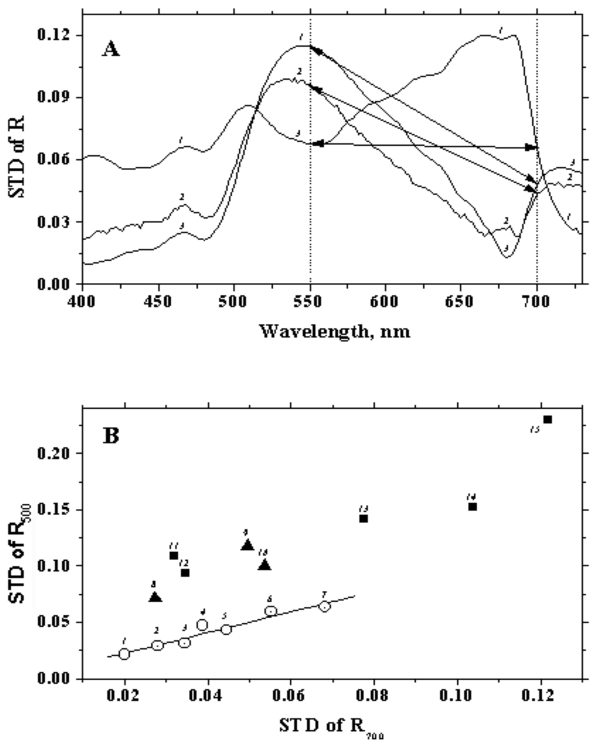

Fig. 3. The STD spectra of reflectance (A) and relationships of STD at 550 nm versus that at 700 nm (B) in storing apple fruit.

A. STD spectra calculated for apple fruit without browning symptoms (curve 1, n = 5, see Fig. 1, panel A), SS affected apples (curve 2, n = 5, see Fig.1, panel B) and n-hexane-treated fruits (curve 3, n = 7, see Fig.2, panel A). The points at 550 and 700 nm are connected by arrows to show the differences in STD spectra of healthy and SS-affected or n-hexane treated fruits.

B. Relationships between STD of R_{700} and STD of R_{550} in apple fruit without browning symptoms (circles, 1–7, 1993–1999), SS affected fruit (triangles, 8–10, 1996) and n-hexane-treated apple (squares, 11–12, 1996, and 13–15, 1999).

In healthy apple fruit STD at 550 and 700 nm are close and highly correlated. In contrast, as a result of natural or artificially-induced browning, the main spectral changes were observed in the band near 550 nm as well as in the NIR. Reflectances at 550, 700 and 750 nm showing different sensitivity to pigment changes could be used in reflectance index constructing.

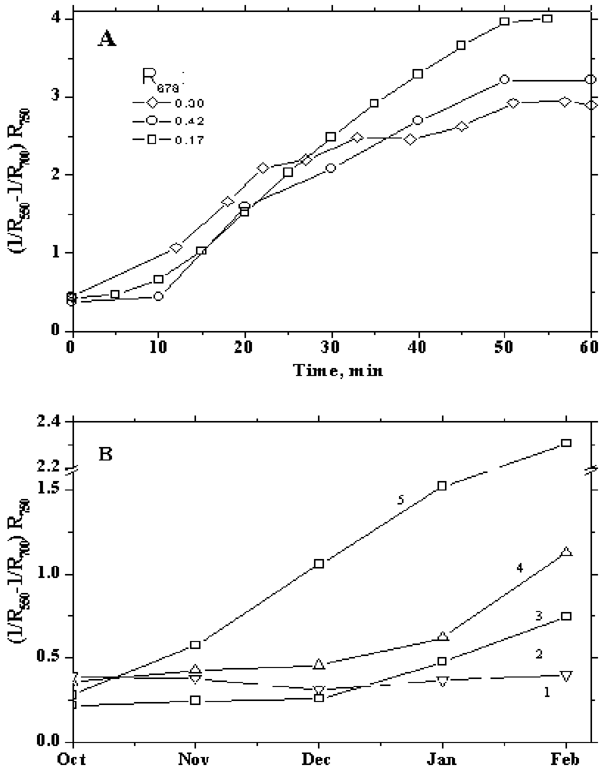

Fig. 4. Time-course of the reflectance index, $(1/R_{550}-1/R_{700})/R_{750}$, changes in n-hexane-treated apple fruits with different Chl content (A) and in five apple fruits differing in the extent of SS symptoms during their long-term storage (B).

A. The initial reflectance values at 687 nm as a measure of Chl content in apple fruit are shown for each curve.

B. SS symptoms were not detected in 1 and 2 and were observed in 3–5.

The data show high sensitivity of reflectance index (BRI) constructed to browning of and the possibility of its use for a browning process monitoring in apple fruit.

(Figs. 1–3) was introduced in BRI as a term, increasing its sensitivity to browning. A browning reflectance index (BRI) was suggested in the form:

$$BRI = (1/R_{550} - 1/R_{700})/R_{750}.$$

The time-course of BRI changes was studied in apple fruits treated with n-hexane and in fruit showing different extent of SS development during storage (Fig.4). Healthy fruits exhibited low BRI (0.2–0.4) regardless of Chl content. The removal of surface fruit lipids by n-hexane extraction (after a short lag-phase) brought about a linear BRI increase and its steady state was achieved after 50 min incubation. During the first 20 min, similar kinetics was observed in fruits with different Chl content. As high as 7–10 – fold BRI an increase was observed as a result of n-hexane treatment (Fig. 4, panel A). Storing apple fruit showed different patterns of BRI changes in accordance with visual SS-symptom appearance: the index remained constant in healthy fruit (curves 1 and 2), its increase was observed in January-February (curves 3 and 4) or as early as in November (curve 5). SS-affected apple fruit showed 3–5.5 – fold BRI an increase compared with healthy fruits (Fig.4, panel B).

Therefore, BRI could be used as a sensitive and efficient tool for quantitative assessment of SS and other disorders accompanying by browning as well as plant diseases affecting the close relationships between reflectances at 550 and 700 nm those are inherent in healthy apple fruit tissues. It should be noted, however, that the developed index is not directly applicable for red-coloured apple fruit since the presence of anthocyanins in their peel causes a significant decrease of R_{550} (Merzlyak & Chivkunova, 2000) interfering with the browning pigment assessment. Thus, a special attempt has to be undertaken for the fruit with anthocyanin pigmentation.

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