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Spectral fingerprints during sun injury development on the tree in Granny Smith apples: A potential non-destructive prediction tool during the growing season

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ABSTRACT

Sun-injury (=sunburn) on apples, as well as in other species, is a physiological disorder that develops in the field when high irradiance combines with elevated temperature during the growing season. This causes photooxidative and heat stress that finally leads to symptoms in sun-exposed fruit tissue. The objective of this study was to assess metabolite profiles changes, as a consequence of these abiotic stresses, using VIS/NIR reflectance in Granny Smith apples. Individual fruit was tagged and weekly monitored from 60 until 151 DAFB. Vis/NIR reflectance measurements (350–1100 nm) were taken on the fruit surface on apples unexposed to direct sunlight (NE, class 0), exposed to direct sunlight (EX, class 1) and those that developed sunburn in the categories of Mild (class 2), Moderate (class 3), and Severe (class 4). Spectral curves were analyzed using discriminant partial least square models (PLS-DA) and interval PLS discriminant analysis (iPLS-DA). PLS-DA analysis was able to separate fruit with and without visible sun injury with a classification error of 22.5% and 35.7% for class 3 and 2, respectively. Class 3 and 4 observed at 129 DAFB was predicted through reflectance spectra captured 87 DAFB by PLS-DA, with 550–570 nm, 715–750 nm, and 810–850 nm spectral intervals responsible ranges for discrimination. Such changes in Vis/NIR reflectance among classes would be related to changes in pigments (carotenoids and flavonoids) and apple tissue structural properties. These findings indicate that the method offers potential for nondestructive prediction of sunburn occurrence on apples in the orchard, that could lead to development of an automated system to forecast sunburn occurrence.

1. Introduction

Chile exports around 800,000 t of fresh apples (*Malus domestica* Borkh.) a year (Exportdata, 2012), which is around 60% of its total production. Main production areas are geographically located in Chile's Central Valley, which climatic conditions during the growing season are characterized by high irradiance (>1800 μmol cm^{-2} s^{-1}), elevated temperatures (>30 °C), and low relative humidity (<30%). These stressful conditions lead to fruit physiological disorders on the tree, such as sun-injury or sunburn (Schrader et al., 2003; Yuri et al., 2000) and watercore (Ferguson et al., 1999; Yamada et al., 2006), and sunscald postharvest (Hernandez et al., 2014; Lurie and Pesis, 1991), among others.

Sun injury (=sunburn, =sunscald in species other than apples) in fruit is caused by photooxidative stress in chlorophyll-containing tissue in a high solar irradiation and elevated temperature environment during the growing season (Naschitz et al., 2015; Rabinowitch et al., 1983, 1974; Torres et al., 2006). When light-energy absorbed by the tissue exceeds its photosynthetic capacity photoinhibition and a burst of reactive-oxygen-species (ROS) occurs, causing photooxidation that finally results in sunburn symptoms (Ma and Cheng, 2004, 2003; Torres et al., 2006; Wünsche et al., 2001). The excessive heat can directly damage the photosynthetic apparatus by irreversibly denaturing photosystem II proteins (Chen et al., 2008; Sharky and Schrader, 2006; Yamane et al., 1998), as well as altering the photosynthetic electron transfer system, and the Calvin cycle enzymes (Smillie, 1992).

In chloroplast, and in other cell components, ROS are detoxified by antioxidants that include metabolites, such as ascorbic acid (AsA) and glutathione, carotenoids, tocopherols, and a wide range of phenolics, and antioxidant enzymes (Asada, 1999; Foyer

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and Halliwell, 1976) with different profiles among species during sunburn development (Felicetti and Schrader, 2008; Li and Cheng, 2008; Ma and Cheng, 2004, 2003; Torres et al., 2006).

Near infrared spectroscopy (NIRS) (780–2500 nm electromagnetic spectrum) has been used since late 1980s to nondestructively determine internal composition of biological materials, such as soluble solids, dry matter, acidity, and firmness in fruit and vegetables (Kays, 1999; Nicolaï et al., 2007; Zude et al., 2006). In fruit, quality changes during ripening have been successfully determined by spectroscopic measurements using NIRs (Bellon et al., 1993; Dull et al., 1989; Kawano et al., 1993; Lovász et al., 1994; McGlone et al., 2002; Moons and Sinnaeve, 2000; Moons et al., 1997; Peiris et al., 1997; Ventura et al., 1998). The visible and NIR (Vis/NIR) spectral range has also been used to monitor apple ripening based on pigment changes and internal composition to determine commercial harvest (Bertone et al., 2012; Fan et al., 2009; Qing et al., 2008), external defects, such as bruises (Brown et al., 1974), and predict storage quality (Ignat et al., 2014).

Also, several physiological disorders, such as watercore (Clark et al., 1998), tissue browning (Zanella et al., 2005) can be detected using NIR reflectance or transmission, and currently used in sorting apples packing lines (McGlone et al., 2005). Furthermore, moldy core have also been successfully detected using NIR transmittance (Shenderey et al., 2010).

Only few non-destructive-field applications of Vis/NIR spectroscopy by using diffuse reflectance have been reported so far. Herold et al. (2005) performed spectral measurements on apples to detect pigments and maturity changes during their development on the tree. These authors also tested Vis/NIR to predict apple maturity changes while on the tree, in order to determine optimal harvest time. Chivkunova et al. (2001) developed a reflectance index for superficial scald detection postharvest.

The objective of this study was to determine changes in the Vis/NIR fingerprint as sun-injury develops on apple fruit on the tree, as a result of biochemical and physical changes previously reported in this photooxidative stress-derived physiological disorder.

2. Materials and methods

2.1. Plant material and experiment layout

The experiment was conducted in a commercial apple (*Malus domestica* Borkh.) orchard located in San Clemente, Maule, Chile (Los Lirios, 35°31′18″S; 71°26′25″W, 230 m above sea level) cv. Granny Smith onto seedling rootstock. Five hundred pieces of fruit from the interior and periphery of the trees (20 fruit per tree) were tagged at 60 days after full bloom (DAFB, 80% open flowers; October 4th, 2013) and measured and evaluated for sun-injury appearance weekly until 165 DAFB (14 time points).

At each evaluation time, the sun-exposed fruit was assessed according to their sun-injury (=sunburn) level. Therefore, fruit was classified as 'shaded' (class 0), unexposed to direct sunlight (interior of the tree); 'exposed' (class 1) to direct sunlight, but with no sun-injury symptoms (from the periphery of the tree); 'Mild' sunburn (class 2), slight discoloration on the sun-exposed area of the fruit; 'Moderate' sunburn (class 3), yellowing and browning on the sun-exposed skin section of the fruit; and 'Severe' (class 4), dark-brown patches combined with yellowing on sun-exposed area of the fruit (Fig. 1).

2.2. External Vis/NIR reflectance measurements

External reflectance (ER) measurements (Vis/NIR) were taken using a portable spectrometer JAZ EL 350 (Ocean Optics, Dunedin, FL, USA) with a spectral range between 350 and 1100 nm and a 0.3 nm of spectral resolution. A standard white ceramic background disk (1-SL, Ocean Optics, Dunedin, FL, USA) was used for calibration. Dark calibration for the baseline was obtained covering the probe. Both calibration curves were used for reflectance calculation.

For all measurements 25 ms integration time was used. Each fruit was scanned twice on the sun-exposed area in an area clearly marked at the beginning of the experiment, and the probe in contact with the tissue.

2.3. Statistical analysis

Each spectral curve was used to generate a X matrix, which was jointed with different sun-injury levels (Y matrix) through a dummy variable, specifying the observed sunburn category. Raw data was: pre-processed (1) to remove the spectral noise reducing the initial and final spectral data matrix; (2) smoothed by the method proposed by Savitzky and Golay (1964); (3) standardized by multiple scatter correction (MSC) algorithm suggested by Isaksson and Næs (1988) and by applying the external parameter orthogonalisation (EPO) algorithm to the Y matrix and its influence in the spectral fingerprint (Roger et al., 2003). Then, Partial Least Squares Discriminant Analysis (PLS-DA) (Eriksson et al., 2014; Guidetti et al., 2010; Wold, 1993) and interval PLS-DA (iPLS-DA) (Norgaard et al., 2000; Xiaobo et al., 2010) was carried out using PLS-Toolbox (Eigenvector Research, Wenatchee, WA, USA) through Matlab v. R2011b software (Mat Works, Inc., USA). On PLS-DA models, optimal number of latent variables (LVs) was chosen based on the minimum error rate for the cross validation procedure (CV). Such CV was calculated using the Venetian blinds approach on which dataset was divided in 10 cross validation groups. CV's calculated error rate was used to evaluate PLS-DA classification and prediction model quality (Ballabio and Consonni, 2013).

In addition, specificity (True Negatives/(True Negatives + False Positive)) and sensitivity (True Positives/(True Positives + False Negatives)) parameters for calibration and cross-validation were also used to evaluate model's performance (Ballabio and Consonni, 2013; Nicolaï et al., 2007).

3. Results and discussion

3.1. Spectral fingerprint during fruit growth

Fig. 2A shows the evolution of the spectral reflectance of fruit skin without sun injury. The main differences between classes can be observed in the 480–620 nm range and beyond 700 nm. All spectral curves had similar patterns, but reflectance increased as fruit ripened regardless of the presence of sun injury (Fig. 2A, B). In sun-injured tissue, the shapes of the curves were different between classes 2–4 with a higher reflectance from the yellow to the red zone in classes 3 and 4 (Fig. 2B).

Nagy et al. (2016) found similar spectral curves on Golden Rainders apples, anthocyanin-free cultivar, during fruit ripening. They reported that the red edge at 678 ± 30 nm was able to assess fruit ripening. In our study, this interval did not changed dramatically during ripening (Fig. 2A, B), perhaps due to genotypic differences and experiment set up. Moreover, the authors observed that as fruit ripened the reflectance spectrum flattened from due to decreased in carotenoids and chlorophylls, which was also not observed in our study (Fig. 2A, B), also most probably due to instrumental and genotype differences.

3.2. Sun-injury appearance

As sun-injury developed on the fruit surface reflectance increased in the range of 520–650 nm (Fig. 3). In all classes three main peaks can be distinguish: 520–560 nm, 575–605 nm, and