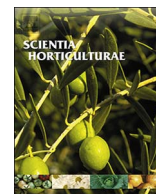




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Non-destructive prediction of ‘Marsh’ grapefruit susceptibility to postharvest rind pitting disorder using reflectance Vis/NIR spectroscopy

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ABSTRACT

Postharvest RP is a progressive rind physiological disorder affecting citrus fruit during postharvest storage, reducing external quality of the fruit. The disorder develops 3–5 weeks after harvest, making it difficult to detect during grading and sorting in commercial packing lines. The Vis/NIR spectroscopy and associated chemometric analytical methods were explored for non-destructive prediction of ‘Marsh’ grapefruit (*Citrus x paradisi* MacFad) susceptibility to rind pitting. Reflectance Vis/NIR spectral data was acquired from fruit, just after harvest, using a laboratory bench-top monochromator NIR System equipped with a quartz halogen lamp and lead sulfide detector. Reference measurements for calibrating and validating PLS models included visual scores of RP and rind physico-chemical variables related to the disorder. The spectral data was correlated to RP scores and rind physico-chemical properties after eight weeks in cold storage and a week in shelf life. Good prediction of RP was obtained ($R_p^2 = 0.78$; RPD = 2.03; RMSEP = 1.41). Prediction models for rind physicochemical properties successfully developed and validated included rind total antioxidant capacity ($R_p^2 = 0.95$), β carotene ($R_p^2 = 0.99$), total carotenoids ($R_p^2 = 0.92$), chlorophyll *a* ($R_p^2 = 0.89$), chlorophyll *b* ($R_p^2 = 0.93$), dry matter ($R_p^2 = 0.88$), sucrose ($R_p^2 = 0.91$), glucose ($R_p^2 = 0.93$) and fructose ($R_p^2 = 0.94$). Principal component analysis successfully segregated fruit based on canopy position and susceptibility to rind pitting disorder. The ability of Vis/NIR spectroscopy coupled with chemometric analysis to cluster fruit based on canopy position is recommended as a secondary approach to discriminate fruit with high susceptibility to RP since RP occurrence was high on fruit from outside canopy.

1. Introduction

Fresh citrus fruits are shipped under cold storage at temperatures below 10 °C to international markets (DAFF, 2015). However, horticultural fruit crops originating from subtropical and tropical regions may develop rind physiological disorders when stored for extended periods in cold, but above freezing temperatures (Bassal and El-

Hamahmy, 2011; Chaudhary et al., 2014). In South African citrus, particularly ‘Marsh’ grapefruit (*Citrus x paradisi* MacFad), these disorders are of great concern as grapefruits are exposed to ± -0.6 °C for at least 14 days of quarantine against the phytosanitary Mediterranean fruit flies (*Ceratitis capitata* and *Ceratitis rosa*) and then stored at 5 °C for prolonging postharvest life during shipping (Sevillano et al., 2009; Bassal and El-Hamahmy, 2011). In previous studies, white grapefruit

Abbreviations: RP, rind pitting; Vis/NIR, visible to near infrared radiation; PLS, partial least square; CI, chilling injury; RH, relative humidity; NIR, near infrared radiation; UV, ultraviolet; Chl_a, chlorophyll *a*; Chl_b, chlorophyll *b*; Chl_{a+b}, total chlorophylls; C_{x+c}, total carotenoids; A, absorbance; HPLC, high performance liquid chromatography; ANOVA, analysis of variance; CV, coefficient of variation; RMSEC, root mean square error of calibration; RMSECV, root mean square error of cross validation; RMSEP, root mean square error of prediction; R_c², correlation coefficient of calibration; R_{cv}², correlation coefficient of cross-validation; R_p², correlation coefficient of prediction; RPD, residual predictive deviation; SD, standard deviation of reference data; y_{pred} , Vis/NIRS predicted value of fruit parameter; y_{mean} , average value of predicted data; y_{act} , actual value measured by destructive methods; n, number of samples; KZN, KwaZulu-Natal province; DM, dry matter; Eq., equation

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(‘Marsh’) was noticed to have lower resistance to CI and RP disorders compared to red grapefruit cultivars (Alferez and Burns, 2004; Lado et al., 2015).

The ‘Marsh’ grapefruit higher susceptibility to physiological rind disorders has been hypothesized to be related to various internal biochemical attributes, including lower antioxidant and phenolic compounds compared to other cultivars with red rinds (Sevillano et al., 2009). Rind dry matter, non-structural carbohydrates, chlorophylls and carotenoids contents, and harvest maturity are also among physicochemical properties related to a difference in susceptibility of citrus fruit to rind physiological disorders within a single cultivar (Assimakopoulou et al., 2009; Magwaza et al., 2013a). Postharvest handling factors such as waxing, fungicides application, storage temperature, and relative humidity have been explored as other factors determining the extent of the disorders’ incidence (Alferez and Burns, 2004; Magwaza et al., 2013a). However, the disorder still occurs frequently and unpredictably.

Rind pitting disorder show symptoms after 3–5 weeks at postharvest, which coincides with the time they reach the designated international market (Magwaza et al., 2013b). Although the disorders do not directly affect the internal quality, fruit with rind disorders are considered as waste in the fresh fruit market (Cronje, 2009). Therefore, means to predict the susceptibility of individual fruit to rind physiological disorders during postharvest grading and sorting prior to shipping is required. Since the disorder develops progressively during storage and shipping, prediction of factors or a combination of factors leading to fruit susceptibility may be used as pre-symptomatic markers. In the literature, previous studies have shown a high potential of Vis/NIR spectroscopy, together with associated chemometric methods, to accurately predict internal and external physicochemical attributes of intact citrus fruit (Gómez et al., 2006; Magwaza et al., 2012a, 2013b; Liu et al., 2010; Cayuela and Weiland, 2010).

It was previously proposed that the application of Vis/NIR spectroscopy for predicting rind physiological disorders of citrus is possible (Magwaza et al., 2012a). Studies predicting rind disorders on grapefruit have not been reported. However, near infrared spectroscopy was successfully used for predicting surface defects on peach fruit (Miller and Delwiche, 1991), surface bruising on apples (Geeola et al., 1994), internal drying disorder in tangerine citrus (Peiris et al., 1998), storage disorders of kiwifruit (Clark et al., 2004) and pericarp hardening of mangosteen fruit (Teerachaichayut et al., 2011). Moreover, the alignment of fruit susceptibility with physicochemical attributes and the ability of Vis/NIR spectroscopy to predict rind internal attributes excite the possibility of predicting rind physiological disorders. Research-wise, the models developed for non-destructive prediction of grapefruit susceptibility to disorders can be used to increase the accuracy of other studies on rind pitting of grapefruit such as application of treatments like coatings, relative humidity and cold temperatures, since either susceptible or resistant fruit could be used. In this study, Vis/NIR spectroscopy is evaluated as a tool to predict susceptibility of ‘Marsh’ grapefruit to postharvest physiological rind pitting and related quality parameters.

2. Material and methods

2.1. Fruit sampling

A total of 240 mature ‘Marsh’ grapefruit (*Citrus x paradisi* Macfad) fruit were harvested during 2015/2016 season. Fruit were harvested from 20 randomly selected trees from two farms situated at Hoedspruit in Limpopo Province (24°23′39.02″S; 30°49′20.65″E) and at Enkwalini in KwaZulu-Natal province (32°75′28.8″S; 35°89′31.1″E), South Africa. In each farm, 120 fruit were harvested from inside and outside positions of the tree canopy. After which, the fruit were transported in a ventilated vehicle to postharvest physiology laboratory of the University of KwaZulu-Natal where postharvest experiments and analyses were

conducted. Upon arrival at the laboratory, fruit treatments simulating commercial chain were applied.

2.2. Postharvest treatments and storage

Fruit were washed with Imazalil® fungicide (Farmalinx Pty. Ltd.; Bondi Junction; Australia) and coated with Citrishine® wax (Citrashine Pty. Ltd.; Decco; Johannesburg; South Africa) with concentrations prepared based on recommendations on container labels. They were left overnight in open space at room temperature (21 ± 1 °C) to allow the coating to dry. The next morning, 20 fruit from each treatment (growing region by canopy position interactions) were taken and analyzed for the rind physiological quality parameters. The remaining fruit were labeled and transferred into the cold room. Cold storage started as a quarantine treatment for 2 weeks at −0.6 °C; 95 ± 1% RH, and the storage temperature was raised to 5 °C for the subsequent 6 weeks (DAFF, 2015). Thereafter, fruit were taken back to room temperature to simulate shelf life that would happen after reaching the international market.

2.3. Visible to near infrared spectral data acquisition

Vis/NIR spectral data was acquired in reflectance mode using a laboratory bench-top monochromator NIR Systems Model XDS spectrometer (FOSS NIR Systems, Inc.; Maryland, USA) equipped with a quartz halogen lamp and lead sulfide detector. The system was calibrated by scanning a 100% white reference tile to provide background reference prior fruit scanning, and periodically at 30 min intervals of scanning fruit, to reduce baseline shift of spectral data (Magwaza et al., 2014). The full visible to near infrared reflectance spectrum (450–2500 nm) was acquired from two opposite sides along equatorial region of the fruit and recorded as log 1/reflectance (log 1/R). Each spectrum was the average of 32 scans recorded using Vision software (Vision TM, version 3.5.0.0, Tidestone Technologies Inc., KS, USA).

2.4. Rind dry matter determination

To measure rind dry matter, the mass of fresh rind sample was measured using digital weighing balance (ADAM®; LKB; USA), dried using a freeze dryer (VirTis®; 6EL; B.O.C LTD; England) and weighed again to obtain dried mass. The dried mass was divided by fresh mass to calculate a percentage DM (Eq. (1)):

$$\text{Dry matter (DM\%)} = \frac{\text{Dry mass}}{\text{Initial fresh mass}} \times 100 \quad (1)$$

2.5. Determination of chlorophylls and carotenoids

The extraction and quantification of chlorophylls and carotenoids were executed based on a method by Lichtenthaler (1987) with slight modification. Briefly, rind powder (1 g) was extracted using 8 mL of 80% acetone. The samples were left in glass tubes to stand for 10 min on ice covered with aluminum foil. Thereafter, they were homogenized for 1 min using Ultra-Turrax solution stirrer and centrifuged for 10 min at 4 °C in a pre-cooled centrifuge. The absorbance of samples was read using a UV-1800 Spectrophotometer (Shimadzu Scientific Instruments INC., Columbia, USA) at the wavelengths required for calculations of the pigments. The concentrations of Chl_a, Chl_b, Chl_{a+b}, and C_{x+c} were calculated using Eqs. (2)–(5), respectively (Lichtenthaler, 1987):

$$\text{Chl}_a = 12.25 A_{663.2} - 2.79 A_{646.8} \quad (2)$$

$$\text{Chl}_b = 21.50 A_{646.8} - 5.10 A_{663.2} \quad (3)$$

$$\text{Chl}_{a+b} = 7.15 A_{663.2} + 18.71 A_{646.8} \quad (4)$$

$$\text{C}_{x+c} = (1000 A_{470} - 1.82 \text{Chl}_a - 85.02 \text{Chl}_b) / 198 \quad (5)$$

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