Development of predictive and detection models for internal browning, watercore and bitter pit in apples using Vis-NIR spectrometry

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To my wife Andrea and

my two princesses:

María Fernanda and María Antonia.

Thank for supporting me

during this adventure

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General Index

1. General Introduction

Under optimal conditions, apple fruit can last for long periods in transit or in storage while awaiting optimal market conditions. However, apples are prone to a range of postharvest physiological disorders, which reduce their quality and thus their value at market. Here, the term physiological disorder, excludes the range of alterations associated with microbial pathogens, but includes all those not microbial in origin.

Internal browning is among these physiological disorders, it is defined as a darkening of the apple’s internal tissues cause by prolonged exposure to low temperatures or to high CO_2 concentrations. Another physiological disorder is watercore which is characterized by an abnormal accumulation of sorbitol in the inner tissues during the last period of fruit maturation on the tree. If the severity of watercore is mild, symptoms can natural disappear during storage but if severe they can progress, leading eventually to a total breakdown of the fruit tissues. A third, common disorder is bitter pit. Its symptomatology is associated with the appearance of necrotic lesions and corky areas near the calyx due to a nutritional imbalance. Bitter pit could be expressed at preharvest (during fruit development) or postharvest (during storage) which is more common.

Nowadays, the fruit industry requires economical, fast, non-destructive, reliable and accurate methods to predict, detect and/or quantify the incidence and severity of such physiological disorders. Until now, disorder risk assessment of fruit lots has been carried out using destructive methods.

There are a number of non-destructive techniques through which it is possible to measure the quality characteristics of agricultural products. These techniques fall under one or another of three broad types: optical, electromagnetic and dynamic (Noh and Choi, 2006). Of these, the ones of greatest interest employ optical methods, since these require is least expensive to implement. These have reduced the time it takes to acquire information, and their usefulness has been reported in a range of fruit-crop species, as well as having been used to detect various quality parameters, including sweetness, acidity, dry matter and firmness.

To implement these non-destructive technologies in packing house lines requires mathematical models that correlate the spectral information obtained for the fruit with the incidence and/or severity of the fruit disorder(s) of interest. Early detection of fruit that will develop any of these disorders will allow adoption of optimal interventions or managements that will minimize commercial risk, especially in fruit populations suffering high incidences or severities of these disorders.

Non-destructive optical methods in postharvest

Of the optical methods, analyses can be carried out at different wavelengths: ultraviolet (UV) 180-380 nm, visual (VIS) 380-700 nm and near red infra-red (NIR) 780-2500 nm. The use of NIR analysis has been evaluated by various authors to infer the characteristics of agricultural products (Moghimi et al., 2011) as NIR allows the study of molecular and dynamic structures obtained from the excitation of molecules, absorption and emission of light. Also, the study of Vis-NIR wavelengths allows identification of molecules containing hydrogen atoms and thus the quantitative analysis of water, alcohol, amines and other compounds containing CH, NH and/or OH groups (Costa et al., 2003; Nicolaï et al., 2006; Osborne, 1986).

When a beam of light is directed onto a fruit, part of the incident light is reflected at the surface (reflectance) and part is transmitted through the cellular structure of the fruit. Of the transmitted light, part is absorbed by the tissues (absorbance), another is reflected back to the surface (diffuse reflectance) and the remainder is transmitted (transmittance) through the fruit (Fig 1). The absorbed radiation is transformed into other forms of energy (heat, chemical energy, fluorescence and phosphorescence).

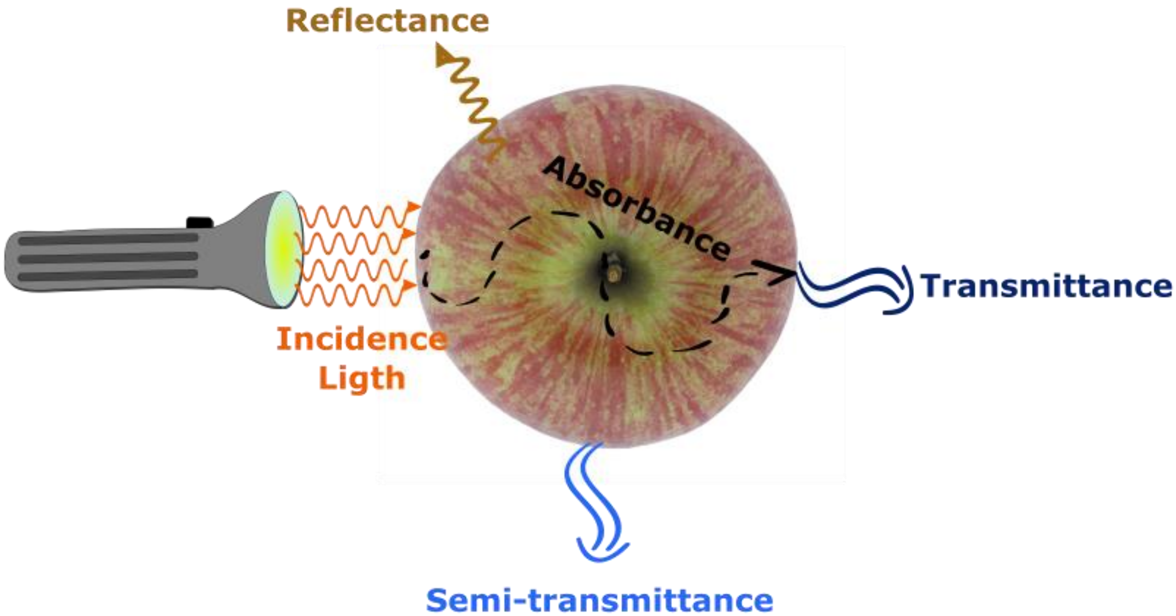

Fig 1. scheme of the types of light that can be analyzed with the use of non-destructive optical methods.

As a result of NIR spectroscopy tests, characteristic spectral curves are obtained which require adjustment and analysis in the ranges that best explain the characteristics sought. For example, McGlone and Kawano (1998) reported that wavelengths between 800 and 1100 nm are useful for predicting dry matter and soluble solids contents in kiwifruit using reflectance spectra. Kafle et al. (2016) studied reflectance spectrometry between 700 and 1100 nm in mango to predict a multifactorial index of maturity (including soluble solids content and moisture content) achieving 87% certainty in immature fruit. In 2009, Penchaiya et al. reported the use reflectance spectra between 780 and 1690 nm as an excellent tool for determining soluble solids content in paprika for a range of varieties. However, they also found this technique unable to predict physical characteristics such as firmness. Escribano et al. (2017) obtained predictive models, for soluble solids and dry matter, with regression coefficients higher than 0.91 in ‘Bing’ and ‘Chelan’ sweet cherry cultivars. This demonstrates the usefulness of these non-destructive postharvest optical techniques in a diversity of fruit types and for a diversity of fruit characteristics including color, size and morphology.

In relation to the use of these techniques in apple, Peirs et al. (2003) conducted a reflectance study in seven apple cultivars to obtain prediction models for soluble solids content using light spectra in the range of 380 to 1080 nm. They found a high variability in the spectra depending on cultivar, development stage and agronomic practices.

Bobelyn et al. (2010) studied about 6,000 apples of various cultivars (including ‘Cripps Pink’) and in various places (including Chile). They presented models for soluble solids and fruit firmness based on reflectance spectrum in the range of 390 to 1690 nm. In ‘Cripps Pink’ apples, these models showed regression coefficients around 0.63 and 0.40 for soluble solids and firmness, respectively.

In 2013, the use of reflectance spectrometry in the range of 400 to 2500 nm was reported by Pissard et al. (2013) to determine vitamin C, total polyphenols and soluble solids in more than 150 apple phenotypes, including cultivars such as ‘Fuji’, ‘Braeburn’ and ‘Golden Delicious’, over three seasons (2004-2006). Predictive models reported achieved regression coefficients of 0.80 for vitamin C and 0.94 for total polyphenols and total soluble solids, respectively. Due to this, authors stressed the importance of using these non-destructive techniques in breeding programs where tools are needed to determine characteristics of interest in a rapid but reliable way.

The successful use of these for chlorophyll determination and optimal harvest time has been reported by Zude-sasse et al. (2002). These authors analyzed transmittance spectra between 600 and 750 nm in 'Estar', 'Jonagold', 'Idares' and 'Golden Delicious' apples. They concluded that this non-destructive technique offers a promising tool for predicting an optimal harvest date in these cultivars. Recently, a portable instrument (DA Meter, Sinteleia, Bolonga, Italy), which measures the chlorophyll and carotenoid contents of the fruit in a non-destructive way and delivers a differential absorption rate between 670 and 720 nm (I AD) is available to determine time of harvest in nectarines and apple (Nyasordzi et al., 2013; Ziosi et al., 2008).

Spectral data: analysis and modeling

Non-destructive optical methods involve performing a mathematical analysis of spectral data. These spectral data are the result of the interaction of the Vis-NIR radiation (100-2500 nm) with the sample (in this case the fruit) (Fig 1). In this way, electromagnetic radiation values (absorption, transmittance or reflectance) are obtained for each wavelength.

If it is considered that the chemical and / or structural characteristics of the sample affect the Vis-NIR spectrum (Costa et al., 2003; Nicolaï et al., 2006; Osborne, 1986), the spectra analysis is reduced to a multivariate analysis. This multivariate analysis allows to relate the spectral values of each wavelength with changes in the chemical and / or structural composition of the samples studied.

These changes in the spectrum are related by mathematical regression models, where an unknown function *f* is sought to approximate the response variable (severity and / or incidence of physiological disorder). For estimating *f* function, it is necessary to have a dataset of *n* different observations (calibration set) that correlates predictive variables with their respective response. The purpose of this is to estimate an unknown *f* function. In other words, to find a $\hat{f}$ function such that $Y \approx \hat{f}(X)$ for any observation *(X, Y).* This estimation can be made using either parametric or non-parametric methods.

Modeling processes based on spectral data require the use of techniques that reduce the number of predictive variables correlated with one another, but without affecting their prediction capacity. In addition, it is important to pay attention to how the models are calibrated and validated. This is, in order to carry out the calibration, a set of observations that cover the variability of the problem under study, that is, cover the entire response range, whereas validation should be done using an independent dataset (different to those used in calibration process) ensure the robustness and predictability of the models when it will be used to make predictions on unknown observations (Guthrie, 2005).

Because multilinear regressions do not achieve good adjustments when the predictive variables are highly correlated with one another, spectral data modeling has focused on the use of techniques that allow reduction in the number of variables. Among these techniques are Principal Component Regression (PCR) and Partial Least Squares (PLS).

PCR is a linear approximation to *f* estimation that involves reducing the number of *p* variables *X,* to *m* main components *Z* following the same methodology as the principal components analysis (PCA). In the PCR, the greater variability of the observations is explained by a small number of main components, as well as by the relationship with the response variable.

An alternative for the PCR, is regression by PLS which is also a method of dimension reduction where a linear model is adjusted with the linear combination of a small number of factors that explain both the predictive variability *X* and the response *Y*. PLS is popular in the field of spectrometry analysis, where many predictive variables arise. However, in practice, it is often no better than a PCR regression. While reducing the supervised dimension of PLS can reduce bias, it also has the potential to increase variance, so the overall benefit of PLS in relation to PCR is relative.

Support Vector Machine (SVM) is a classification method based on statistical learning in which a function that describes a hyperplane for optimal class separation is determined. As the linear function is not always able to model such separation, the data is mapped into a new feature space and a dual representation is used with the data objects represented by their dotted product *(X, Y)*. A kernel function is used to map from the original space to the feature space. This can be done in many ways, thus providing the ability to handle non-linear classification cases. The kernels can be seen as non-linear data mapping to a higher-dimensional space feature, while providing direct access computing by enabling linear algorithms to work with a higher-dimensional space feature. The support vector is defined as the reduced training information of the kernel. In this new space, SVM will look for observations that are in the boundary between the classes. That is to find the observations that are ideal for separating the classes - these observations are called support vectors.

SVM has advantages over classification methods, such as neural networks, since it has a unique solution and has a low tendency to overfitting, compared with other non-linear classification methods. Of course, model validation is the critical aspect for to avoid overfitting in any method. SVMs are effective for modeling nonlinear data and are relatively insensitive to parameter variations. SVM uses an iterative training algorithm to achieve the separation of different classes.

Artificial Neural Networks (ANN) are based on analyses of animal brains. This optimization process comprises a collection of "neurons", or nodes, connected by mathematical functions that play similar roles to synapses. Today, the most commonly used optimization algorithm is called *Backpropagation*. This uses gradient descent to update synapse parameters and thereby achieves the learning function of the model (Gu et al., 2017). To develop neural network models, it is necessary to significantly reduce the number of model predictors to simplify the network architecture (Goodacre et al., 1996).

In all these modeling processes, there are several ways to analyze model behavior (Guthrie, 2005; Nicolaï et al., 2007). One of the most used processes, is the mean square cross-validation error (RMSECV) defined as

$$RMSECV = \frac{\sum_{i=1}^{n_p}(\hat{y}_i - y_i)^2}{n_p}$$

Where n_p is the number of observations used, $\hat{y}_i$ and y_i the predicted and observed values of the observation *i*. This value gives an average uncertainty that can be expected in predictions of future observations. Another metric used in regression models is the value of R^2 which represents the linear regression coefficient between the observed and predicted values.

The robustness of the model refers to the insensitivity of certainty in the prediction when there are changes in factors external to the modeling process such as: technical changes in the measurement equipment, if the samples belong to different populations (trees, orchards, harvests or seasons). Examples of this can be seen in several studies, Nicolaï et al. (2007) observed that when using datasets from different seasons, the percentage of RMSECV doubled at the expense of the certainty of prediction of soluble solids content in apples from two different orchards for two seasons. Because of this, Rungpichayapichet et al. (2016) noted the need to build predictive models using data from different seasons to ensure the robustness of the prediction models.

On the other hand, if the mathematical models are used to obtain classification rules (qualitative models); These can be evaluated with different metrics of classification obtained from the confusion matrix constructed with the results of classification of the models such as Accuracy, Sensitivity, Specificity and positive and negative predictive values (James et al., 2015; Tharwat, 2018). Accuracy is the most common metrics which measures the classification performance, and it is defined as a ratio between the correctly classified samples to the total number of samples; Sensitivity, represents the positive correctly classified samples to the total number of positive samples. Whereas Specificity, is expressed as the ratio of the correctly classified negative samples to the total number of negative samples. Finally, Predictive values (positive and negative) reflect the performance of the prediction. Positive prediction value (PPV) represents the proportion of positive samples that were correctly classified to the total number of positive predicted samples]. On the contrary, Negative predictive value (NPV), measures the proportion of negative samples that were correctly classified to the total number of negative predicted samples.

Several authors (Clark et al., 2003; Jarolmasjed et al., 2017; Kafle et al., 2016; Khatiwada et al., 2016b; C. A. Torres et al., 2015; Zúñiga et al., 2017) have demonstrated the possibility of detecting physiological disorders in fruit using non-destructive optical methods when the symptoms of the disorder are already present in the fruit; But so far, the ability of these non-destructive methods to predict the severity and / or incidence of physiological disorders in asymptomatic fruit has not been explored, that is, to achieve an early prediction before the symptoms are expressed.

Kafle et al. (2016) showed that in the case of bitter pit, healthy fruit and with BP presented differentiated spectra from harvest. If these same spectral differences between healthy and diseased fruit are maintained for other physiological disorders (internal browning), it would be possible to make an early prediction of the physiological disorder by measuring the traceability of the spectra during storage (Fig 2).

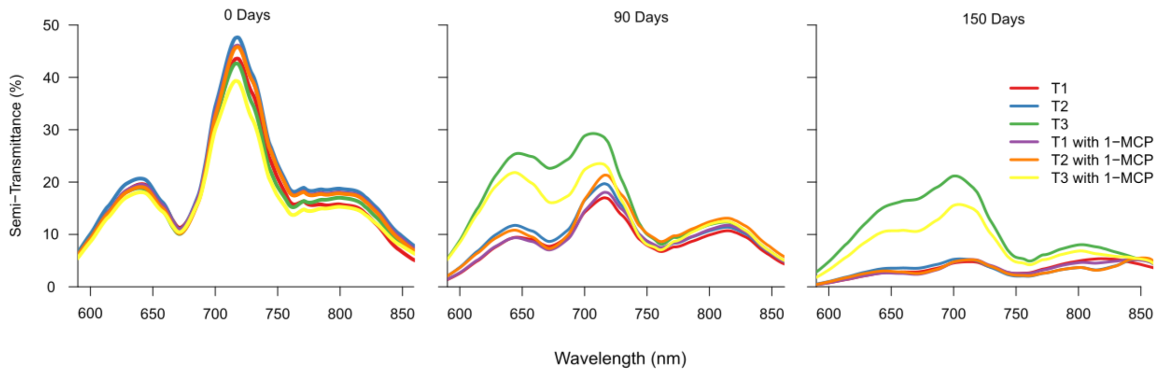

Fig 2. Mean semi transmittance spectral curves of ‘Cripps Pink’ apples after 0, 90, and 150 d of storage at 0 °C. T1: -1 °C for 24 h and subsequent storage for 149 d at 0 °C; T2: 150 d at 0 °C; T3: 90 d at 5 °C plus 60 d at 0 °C, with and without 1-MCP application. More information on chapter 2: Quantitative and qualitative VIS-NIR models for early determination of internal browning in ‘Cripps Pink’ apples during cold storage

In order to predict if a fruit is going to be affected by a physiological disorder, it is necessary to observe the spectral changes of the fruit throughout the storage, in order to be able to relate them to the expression of symptoms of the disorder, thus achieving mathematical models of early prediction in storage or before the symptoms are expressed.

Case study 1: Internal browning in ‘Cripps Pink’ apple

‘Cripps Pink’ apple was developed by the Stoneville Horticultural Research Station from a genetic improvement program in Australia in 1973 by the work of JEL Cripps, and released for sale in 1986 (Cripps et al., 1993). The objective of this genetic program was to breed the sweetness and superficial scald resistance of ‘Golden Delicious’ cultivar with the firmness of ‘Lady Williams’ apple cultivar.

‘Cripps Pink’ apple is a medium-sized fruit (70-75 mm in diameter), conical-oblong shape, have a green-yellow background color (30% -40% fruit cover) and red cover color that varies between 60 to 70% of the total fruit surface. The texture is dense and firm, moderately juicy, sweet with an equilibrated acidity (Cripps et al., 1993).

‘Pink Lady’ is a trade name for this cultivar which ensure high quality standard in the market in term percentage of skin red color, a minimum percentage of coating color (red), fruit firmness, total soluble solids, titratable acidity and absence of internal damage (de Castro et al., 2007; James et al., 2005; James and Jobling, 2008).

The main cause of ‘Cripps Pink’ rejection in the market is the physiological disorder called internal browning, in which the fruit loses the characteristic color of its pulp and takes pale brown to dark black colors inside (Fig. 3), this color changes is due to the disruption of cellular compartmentalization which allows enzymatic oxidation of phenols by polyphenol oxidase (PPO), this oxidation generates o-quinones that react with other substrates resulting in the polymerization of melanin (de Castro et al., 2008; Supapvanich et al., 2012; Yan et al., 2013).

Internal browning, as a physiological disorder, has been described in other fruit such as logan (Lin et al., 2014), pears (Franck et al., 2007; Wang and Sugar, 2013; Yan et al., 2013) and peaches (Jin et al., 2014; Lurie and Crisosto, 2005). The information from the literature has concluded that this problem can be induced by adverse storage conditions, such as high concentration of CO_2 or prolonged exposure to low temperatures; It has also been shown that susceptibility to internal damage depends on preharvest factors such as overmature ripening at harvest, as well as calcium and potassium deficiencies (Buts et al., 2015; Crouch et al., 2015; Grant et al., 1996; Hatoum et al., 2016; Lau, 1998). The synergy of these factors is reflected in the imbalance of antioxidant system that leads to the accumulation of reactive oxygen species which induces loss of membrane integrity that end with a general browning oxidation process.

Fig. 3 Photographic record of Internal Browning (IB) in 'Cripps Pink' apples.

Regarding internal browning in apple, different cultivar-symptoms relationship has been studied. In 'Braeburn' apples (Hatoum et al., 2016) appear Breaburn Browning Disorder (BBD), which is characterized by brown patches in the fruit cortex with presence of cavities in the extreme cases, the disorder appears early in storage being late season crop more susceptible. CO_2 toxicity is attributed to combination of factors that reduce gas exchange and factors that contribute to high fruit metabolism such as advance maturity. In 'Fuji' apple, internal browning has also been associated with late harvest fruit and CO_2 phytotoxicity (> 3%) (Grant et al., 1996; Volz et al., 1998).

The first reports of ‘Pink Lady’ rejection due to problems quality standards date from 2000, where fruit with eleven weeks of storage showed low flesh firmness and greasiness development. Three years later, a shipment of ‘Pink Lady’ that arrived to English market from Australia, presented a high incidence of internal damage in the fruit which caused the rejection of 35 containers, thus generating large losses for producers and damage to the reputation of fruit marketed under this quality brand (James and Jobling, 2008). Also same problem had occurred in fruit from South Africa (Bergman et al., 2012). In the Chilean context, ‘Cripps Pink’ apples are sent to foreign markets before 3 months of storage to prevent the fruit from developing this internal damage, this leads to an increase in the supply in the destination, especially in the European market, decreasing the opportunities for profitability of national exporters and producers (Torres and Hernadez, 2014). However, this situation is difficult to maintain over time and the development of internal browning will be one of the main causes of deterioration especially in fruit stored for over 120 storage days.

Since then, numerous investigations have been carried out to find the preharvest and postharvest factors associated to the sensitivity of ‘Cripps Pink’ to develop internal tissue damage (Brown et al., 2003; de Castro et al., 2008, 2007; Hernández et al., 2005; James and Jobling, 2009; Jobling et al., 2005; Moggia et al., 2015). James and Jobling (2008) correlated growing degree days (GDD), as preharvest factor, with the development of internal browning in ‘Cripps Pink’ apples. This authors found two distinct characteristic patterns correlated with the estimation of GDD, which is the sum of the difference between the average daily temperature and a base temperature (10 ° C) between the days of full flowering and harvest, could indicate that fruit harvested in areas where the GDD was less than 1100, has a high susceptibility to diffuse browning, while areas above this value for GGD are more likely radial browning.

Despite of this, there is an agreement that the long period during storage is the main factor associated to the disorder. Finally, in 2009, James and Jobling observed that internal browning could altered internal tissue structure in 'Cripps Pink' apples. James and Jobling subdivided internal browning according to their visual characteristics into three types of damage with different physiological origins which are: radial browning, which is characterized by browning of the vascular tissue of the fruit while the cortex tissue remains intact; diffuse browning, in which fruit damage occurs in the area of the cortex leaving intact vascular tissue of the fruit and the third type is associated to CO_2 damage where it could be observed a separation pattern in fruit vascular tissue into large cavities, this cavities extend from the center of the vascular zone to the area of the cortex.

The production of this cultivar in Chile is concentrated in the VII region, where the climatic characteristics make Chile one of main supplier from the south hemisphere. Of the total apple production in Chile, about 10% is covered by 'Cripps Pink' cultivar of which 50% is sold under the 'Pink Lady' quality label (DECOFRUT S.A, 2013).

Usually, Chilean 'Cripps Pink' growers delay harvest to obtain the quality required by the market such as color, in this situation over mature fruit is harvested with unfavorable development of internal quality.

Many of the researches done about internal browning in 'Cripps Pink' apple have found a correlation between internal damage and high concentrations of CO_2 at storage (de Castro et al., 2008, 2007; East et al., 2005; James and Jobling, 2009) but under Chilean conditions, it has been found the symptoms of CO_2 damage even under normal storage atmosphere so, it is believed that there must be others factor that is acting as abiotic stress that trigger the sensitivity of internal damage in the fruit.

Nowadays, the detection of internal browning in the 'Cripps Pink' cultivar in the market is carried out in a destructive way where a certain number of fruit per lot are selected and the internal state of the fruit are quantified, if the number of damaged fruit exceeds the expected limit, all the lot is rejected thus generating large economic losses for marketers. Due to the high heterogeneity of incidence of internal browning, it may also be the case that the selected sample does not show the actual state of the lot and generates the possibility that the damaged fruit are detected by the final consumers, causing distrust and loss of credibility in the 'Pink Lady' quality available in the market. For this reason, the detection of internal damage by a non-destructive way is required in the chain of commercialization (Fu et al., 2007).

Regarding the detection of internal browning using non-destructive techniques, two important facts has been determined: first that the orientation of the fruit in data acquisition influences the final value of light transmitted through the fruit, second, meantime internal browning intensifies its dark colors inside the fruit, the amount of light transmitted is less and therefore the characteristic spectrum reduces their picks (in the case of 'Braeburn' apples 715 and 810 nm) (Clark et al., 2003).

According to previous discussion, each cultivar has its own characteristic spectrum and therefore, it is necessary to select an optimal spectral range to correlate with the internal characteristic of the fruit (Peirs et al., 2003). Previous reports of internal browning detection in 'Cripps Pink' apples using non-destructive techniques have only focused on a final evaluation after 180 storage days (Khatiwada et al., 2016a; C. A. Torres et al., 2015); therefore, it is important to develop models that allow an early detection of the disorder to predict fruit susceptibility that could be used to segregate and manage fruit during storage.

Case study 2: Watercore in 'Fuji' apple

'Fuji' apple is a bicolor hybrid cultivar developed around 1930 at Tohoku Research Station in Japan. This cultivar is a cross between the Ralls Janet and Red Delicious cultivars (Ferree and Warrington, 2015). Crispness texture and sweetness are the main traits, which make it very appealing in different markets of the world. This cultivar is pruned to show watercore at harvest, a physiological disorders, that develops during the last stage of fruit maturation on the tree. (Yamada et al., 2004). Other such as fruit cracking (Kasai et al., 2008) and bitter pit (de Freitas et al., 2015) are critical disorders that reduce the storability of 'Fuji' apples. Watercore severity shortens the storage life since it is the main cause of internal breakdown of the tissue (Argenta et al., 2002)

Watercore is a physiological disorder that appears only when the fruit is on the tree (Gao et al., 2005; Herremans et al., 2014). In watercored apples, intracellular spaces of the core and the tissues around the cortex are filled with liquid, predominantly sorbitol (Yamada et al., 2004), which it is accumulated as a result of a failure in intracellular transport (Ferguson et al., 1999). This failure was verified by Gao et al. (2005), finding a lower expression of sorbitol transporters in the tissues affected with this disorder in 'MacIntosh' apples.

Usually, watercore incidence is associated with advanced maturity fruit and low night temperatures before harvest, but a variation of this problem could occur as a result of heat stress (Tian et al., 2011). When the percentage of affected tissue by watercore is low, watercore symptoms could disappear naturally during storage, this is caused by a sorbitol resorption from healthy tissues. However, in cases of severe watercore symptoms (Fig. 4), resorption is not enough and internal decay (internal browning) with alcoholic flavor could be developed inside the fruit (Herremans et al., 2014). Sometimes, a slight watercore incidence is desired by some markets, especially in some countries of Asia (Harker et al., 1999) because of watercore tissues have an extra sweetness due to high sorbitol content.

Nowadays, there is some expensive non-destructive techniques such as magnetic resonance imaging and X-rays (Wang et al. ,1988) that allow to identify the intensity of affected tissue. In these cases, images allowed to see the affected areas in the vascular with pale brown colors depending on watercore severity. This same technique was used by Clark and Enza (1999) to corroborate the resorption of watercore in 'Braeburn' apples during postharvest storage at 0 ° C. These authors reported the total reabsorption of this disorder at 8 weeks of storage regardless of the percentage of affected area by watercore detected at harvest.

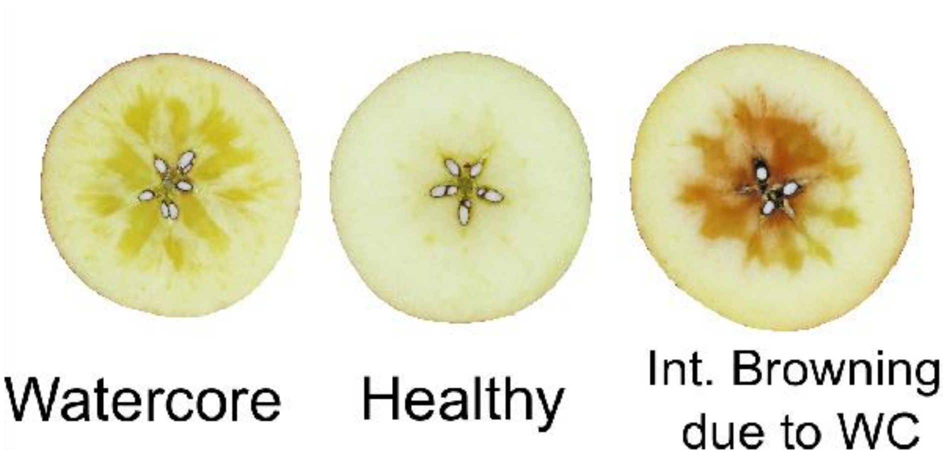

Fig. 4 Photographic record of Watercore (WC) symptoms in 'Fuji' apples.

Later, looking for an accurate way of sorting fruit with different watercore severities, a research was conducted using a resonance equipment which had a low frequency sensor (5.4 MHz) (Cho et al., 2008); it was able to distinguish between healthy fruit, watercored fruit or with internal browning, these authors also reported that the density of watercored fruit were higher than healthy fruit. These results were obtained because of the high proportion of water presents in the intracellular spaces of affected tissue.

Until now, simple and low-cost equipments for detecting non-destructively the incidence and severity of watercore were no available. The information provided by a non-destructive technology would allow stablishing appropriate handling and storage protocols to effectively reduce watercore disorder in the fruit.

Case study 3: Bitter pit in 'Fuji' apple

Bitter pit is recognized as a primary physiological disorder in apple fruit. The symptoms appear as small round brown lesions on fruit surface in the calix zone that cause a corky texture and bitter perception (Fig. 5). Some cells of the mesocarp collapse affecting the shape of the fruit and given an unpleasant general appearance that is rejected by the market. The pitted depression zone appears in the first 60 to 90 days of storage at 0°C. Some apple cultivars are more sensitive than others, 'Granny Smith' and 'Fuji' are classified as sensitive cultivars (Jarolmasjed et al., 2016). Bitter pit symptoms can also develop while fruit is still on the tree (low frequency). Due to the fact that bitter pit symptoms appear externally in the fruit, during storage or when the fruit is in transit to the market, they cause visual fruit's rejection with a high uncertainty among lots. When some affected fruit is detected in a specific lot this reduces the commercialization and value of entire lot (Jemrić et al., 2016).

Unbalance of mineral nutrition in the fruit has been associated as the main cause of bitter pit. Hence an inverse relationship has been found between calcium concentration in the fruit at harvest and bitter pit incidence during storage (Aghdam et al., 2012; Torres et al., 2017a, 2017b; Zúñiga et al., 2017). Also some ratios between cations (Mg, K and N) with calcium have been determined to be associated with bitter pit (Jarolmasjed et al., 2017; Jemrić et al., 2016). Nowadays, the main factor causing the cell depletion, in the bitter pit symptoms has not been defined clearly.

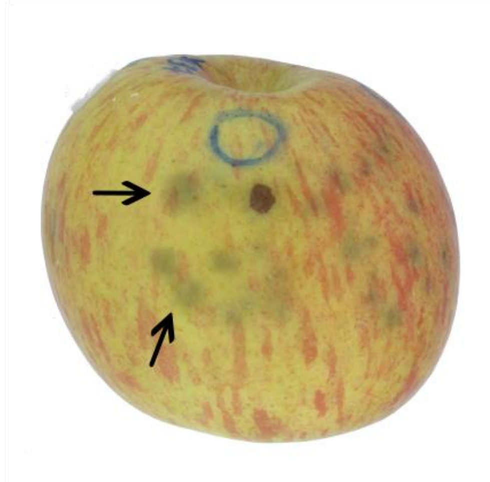

Fig. 5 Photographic record of bitter pit symptoms in ‘Fuji’ apple. Black arrows pointed to BP lesions

Calcium is normally associated with postharvest disorders. Regarding bitter pit in apples, most of preharvest factors that stimulates bitter pit disorder are associated in some way with calcium nutrition (Conway et al., 2002; Ferguson et al., 1999). Calcium translocation in the plant is favored by the transpiration stream, which favors leaves and shoots and limits fruit as a sink for calcium. Calcium plays an important role in plants to stabilize cell membranes, as a counter ion to equilibrate charges in the tissue, as a signaling molecule in the cytosol, as well as to contribute to cell wall structure and strength (Aghdam et al., 2012).

Regarding the relationship between calcium availability in fruit and bitter pit incidence, de Freitas et al. (2015) found that fruit with bitter pit had a higher concentration of insoluble calcium during storage at 0 ° C for 60 days. This was corroborated by Falchi et al. (2017) who after performing different treatments with abscisic acid (ABA) at different stages after flowering, finding that calcium concentration and genes associated with calcium availability are enhanced by ABA application, decreasing the incidence of bitter pit after harvest. Due to the fact that calcium is mobile in plants exclusively through the xylem vessels, Miqueloto et al. (2014) studied the relationship between loss of xylem functionality and bitter pit incidence in two cultivars, noting that Catarina cultivar has an early loss xylem functionality compared with 'Fuji' cultivar, which is associated the incidence of bitter pit at low calcium and potassium concentration, and high relations K / Ca, (K + Mg) / Ca .

Several studies have been carried out to predict this disorder. It has been indicated by different authors that fruit mineral analyzes between 20-40 days before harvest can be used as an indicator of risk to develop bitter after harvest (Amarante et al., 2010; Retamales et al., 2000). It has also been proposed that dipping the fruit in ethephon solution promotes ripening and accelerate the symptoms of bitter pit passively before and after 10-30 days of harvest, which could be used to determine the risk of bitter pit incidence in the fruit (Torres et al., 2015).

Regarding the non-destructive methods to predict this physiological disorder, Nicolaï et al. (2006) evaluated hyperspectral images in the NIR range to identify lesions caused by bitter pit at harvest, the equipment and PLS model used by these authors were able to identify bitter pit lesions, even when bitter pit lesions were not visible, on the other hand, the system could not discriminate between bitter pit and corky tissue. Si and Sankaran (2016) tried to identify bitter pit symptoms early in the fruit development, while external symptoms are absent, using computed tomography, this was corroborated by Jarolmasjed et al. (2016)

using the same technique, but these authors highlighted that the identification of fruit with bitter pit is difficult when there is present of other types of external injuries such as those caused by mechanical damage.

Kafle et al. (2016) demonstrated that using the reflectance spectrum between 970-996 nm and 1130-1143 nm is useful for segregating healthy from symptomatic fruit with bitter pit using Quadratic Discriminant Analysis (QDA) and SVMC classification models. Jarolmasjed et al. (2017) observed that the fruit with bitter pit symptoms after 63 storage days showed high reflectance spectra between 900-1200 nm at the beginning of storage period compared to healthy fruit, which kept their reflectance spectrum practically constant during the study. These authors also performed mineral analyzes for Ca, K and Mg for healthy and pitted fruit, concluding that the best inference from the reflectance spectra is for Mg / Ca ratio, which can be used as a risk indicator for bitter pit incidence.

Jarolmasjed et al. (2018) corroborates that reflectance spectral wavelengths of 730, 980, 1135, 1250 and 1405 nm are potentially useful for detecting bitter pit using logistic regression models; they also reported other spectral characteristics (665-797, 1217-1349 and 1410 nm) for recognizing lesions using hyperspectral images.

Former non-destructive techniques reported for predicting bitter pit incidence have mostly used expensive and / or difficult implementations equipment which requires long times for data acquisition (Jarolmasjed et al., 2018, 2016; Nicolaï et al., 2006; Torres et al., 2015). Among the reports on the use of reflectance spectrometry for early prediction of this disorder, the information found is limited and not much considerations have been done for modeling the data using more than one season.

Research hypothesis

Incidence of physiological disorders, such as internal browning, watercore or bitter pit in apples affects light scattering and therefore these disorders could be determined non-destructively, then it is possible to model the incidence and / or severity of these disorders at early stages using spectrometric measurement in Vis-NIR range.

General Objective

Develop predictive and detection models for internal browning, watercore and bitter pit physiological disorders in apples using Vis-NIR spectrometry.

Specific Objectives

Develop models to predict the severity and incidence of internal browning disorder in ‘Cripps Pink’ apples using transmittance in Vis-NIR range.

Evaluate classifications models for watercore detection in ‘Fuji’ apples using as predictor spectral transmittance features in Vis-NIR range.

Predict, at early stages, bitter pit incidence in ‘Fuji’ apples using reflectance spectra between 900-2400 nm.

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2. Quantitative and qualitative VIS-NIR models for early determination of internal browning in 'Cripps Pink' apples during cold storage

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Abstract

'Cripps Pink' apples are prone to develop internal browning disorder during cold storage, rendering their commercialization particularly difficult after long-term storage. The purpose of this research was to predict internal browning defect quantitatively and qualitatively in apple, by a non-destructive equipment from spectra collected before the disorder develops. In order to obtain a broad expression of the disorder in severity and incidence, fruit treated and non-treated with 1-methylcyclopropene (1-MCP) were studied under three temperature regimes: T1) pre-cooled with forced air at -1 °C for 24 h and subsequently stored for 149 d at 0 °C; T2) placed directly at 0 °C and stored for 150 d; and T3) stored for 90 d at 5 °C then for 60 d at 0 °C. Every fruit was subjected to semi-transmittance spectral analysis between 100 to 1100 nm at 0, 60, 90, 120 and 150 d of storage and matched with the presence and severity of internal browning. The disorder was quantified using image analysis in one half of cut fruit at the end of the 150 d plus 7 d at 20 °C (157 d) after verification that the damage was not evident in fruit stored before 120 d. Quantitative Support Vector Machine Regression (SVMR) model satisfactorily predicted the percentage of internal browning area per fruit shown after 157 d, as early as 90 d of storage with R^2 ~0.70, and a root mean square error for calibration (RMSEC) and prediction (RMSEP) datasets of ~18 % and ~15 % respectively. On the other hand, qualitative Partial Least Squares Discriminant Analysis (PLS-DA) model was able to predict the damaged fruit at the onset of storage (0 d) and to reach an accuracy values ~87 % in calibration and test datasets, and 12 % of misclassified fruit at 90

d. Quantitative neural network models were also evaluated, reaching R^2 values of 0.78 for calibration and 0.65 for prediction, and RMSEC= 11.95 % and RMSEP= 16.81 %, respectively, at 90 d of storage. For qualitative predictions, the highest accuracy was 99 % in calibration and 93 % in test at 150 d, trimming the misclassified fruit to less than 10 % in both datasets. This study shows different models for predicting internal browning before the disorder appears in stored apple using semi-transmittance spectra.

Keywords: non-destructive analysis, physiological disorder, prediction models, neural networks.

Introduction

'Cripps Pink' is an Australian apple cultivar developed in 1973 by the program for genetic improvement of Stoneville Horticultural Research Station and released for commercialization in 1986 (Cripps et al., 1993). This cultivar has been marketed as 'Pink Lady™' (James et al., 2005a). In order to gain and hold a place in the premium fruit market, the 'Pink Lady™' brand has needed to maintain high fruit quality standards, such as a minimum percentage of red color, firmness, total soluble solids, titratable acidity and absence of internal disorders (de Castro et al., 2007; James and Jobling, 2008).

An important problem of this cultivar is the appearance of internal browning in the fruit during storage (Brown et al., 2003; Hernández et al., 2005; James et al., 2005b; James and Jobling, 2009; Jobling et al., 2005; Torres and Hernandez, 2014), here the fruit losing its characteristic white flesh, turning a pale to dark brown coloration, associated with a mealy texture. Initially, internal browning in 'Cripps Pink' fruit was characterized as a single disorder related to over-mature fruit at harvest, and to fruit stored in controlled atmospheres where the disorder is associated mainly with CO_2 injury (Brown et al., 2003). However, James and Jobling (2008) argued that preharvest factors, such as low temperatures during fruit development and over-maturity at harvest, predispose 'Cripps Pink' fruit to internal browning. Currently, the detection of internal browning is primarily destructive. Due to the high heterogeneity occurrence within any batch of fruit, a sample often fails to show the real state of the batch. This causes a loss of credibility and distrust of the 'Pink Lady'™ brand. Recently, several companies offer the possibility to sort internal defects (www.compacsort.corn/es/inspectra2/, www.greefa.com/product/internal-quality). To the best of our knowledge, there is no information that the prediction can be done early enough to modify the handling of the fruit. Hence, there is a need to segregate those fruit more prone to browning in a non-destructively manner as early as possible during storage and not just prior to sale.

The non-destructive technique for predicting the internal characteristics of a fruit uses transmittance wavelengths in the range of 380-700 nm within the visible light spectrum (Vis), and in the range of 780-2,500 nm within the near infrared spectrum (NIR). In the transmittance spectral curves, predictive models of internal fruit damage can be established with different data processing methods. Torres et al. (2015) reported the use of Principal Component Regression (PCR) and PLS-DA models for detecting internal damage in 'Cripps Pink' fruit using reflectance spectra. They were able to separate healthy and damaged fruit at the time of the expression of the disorder after six months of storage at 0 °C. Recently, Khatiwada et al. (2016) compared several predictive models for internal browning with two instruments using transmittance mode. Among the predictive models tested by the authors, they concluded that it was only possible to sort healthy from damaged fruit because predicting the percentage of damaged area in a fruit is difficult to replicate due to the high variability of the spectra.

The primary objective of our study was to determine the feasibility of a non-destructive method for quantitative and qualitative prediction of internal browning by analyzing a population of ‘Cripps Pink’ apples potentially affected by the internal disorder, and using the spectra collected before symptoms development. In addition, a new method is proposed for analyzing this type of dataset involving the use of neural networks models (Gu et al., 2017; Guo et al., 2016a; Montavon et al., 2018).

Materials and methods

Plant material

‘Cripps Pink’ 80 and 100 mm diameter apples, free of visible damage and with external color characteristics similar to those required for the Pink Lady ™ brand were selected. Fruit were sourced from 350 km south of Santiago, Chile on May 24 of 2016 from a commercial packinghouse. A total of 960 fruit were chosen and transported immediately to the Postharvest Laboratory. An extra set of 40 similar fruit was used for maturity measurement based on the Starch Conversion Chart, radial type (http://www.ctifl.fr) (1: immature; 10: overmature). Harvested fruit had an average maturity of around 9 with firmness values between 71.2 N and 77.8 N (Data not shown). These values are in line with the agronomic practices of Chilean apple producers who delay the harvest period to allow fruit to develop their characteristic color.

Storage treatments

After the fruit arrived from the packinghouse and had been stored overnight at 20 °C, they were randomly packed into 19 kg boxes and divided into two groups. One group was treated with 625 nL L^{-1} of 1-methylcyclopropene (1-MCP, 0.14 %, SmartFresh, AgroFresh, PA, USA) in a closed room for 24 h at 20 °C to suppress ripening, and the other group was the untreated control (480 fruit per each group). Then, three sets of 160 fruit were taken from each group and exposed to one of three different temperature treatments. T1) Fruit were pre-cooled with forced air at -1 °C for 24 h to reach a pulp temperature of 0 °C and subsequently stored for 149 d at 0 °C, T2) Fruit were stored directly at 0 °C to reach a pulp temperature 0 °C after 48 h and then stored for 150 d at 0 °C and, T3) Fruit were stored directly at 5 °C to reach a pulp temperature of 5-6 °C after 24 h and then stored for 90 d at 5 °C, followed by 60 d at 0 °C. Pre-storage temperature and 1-MCP treatments have been proposed to affect differently the development of the disorder (Lum et al., 2016; Wilkinson et al., 2008). The purpose of these treatments was to obtain fruit with high variation on tissue sensitivity to internal browning. A total of 960 fruit was used for spectra collection on individual fruit at 0, 60, 90, 120 and 150 days at 0°C, part of these fruit (240) were destroyed at 90 and 120 days to provide visual corroboration of the development of internal browning (data not shown). As there were no symptoms of the disorder, the final assessment of the fruit was done at 150 d plus 7 d at 20 °C (157 d) when all fruit were halved by an equatorial cut and photographic records taken of internal browning. The presence of internal damage was recorded, and the percentage of damaged area was quantified by image processing.

Non-destructive measurements

Semi-transmittance readings (90° between light source and sensor) were taken for each of the 960 fruit on two perpendicular cheeks using an equipment designed in our laboratory, average transmittance spectra per fruit were used. The equipment (Fig. 1) consists of a 250 W halogen light source and the spectral data were collected using an HR4000 spectrometer (Ocean Optics, www.oceanoptics.com) fitted with an optical probe working in transmittance mode (wavelength range between 100 and 1,100 nm) positioned directly on the fruit surface. Each reading was carried out with an integration time of 700 ms and approximately 3,300 data values per reading were saved at 0.1 nm spectral resolution (standard deviation of 2.5 % was obtained for 20 reading of white reference at 670 nm). Spectral acquisition and instrument controls employed a PC using in-house software. Individual spectral monitoring measurements for each fruit were made on 0, 60, 90, 120, 150 d at 0 °C.

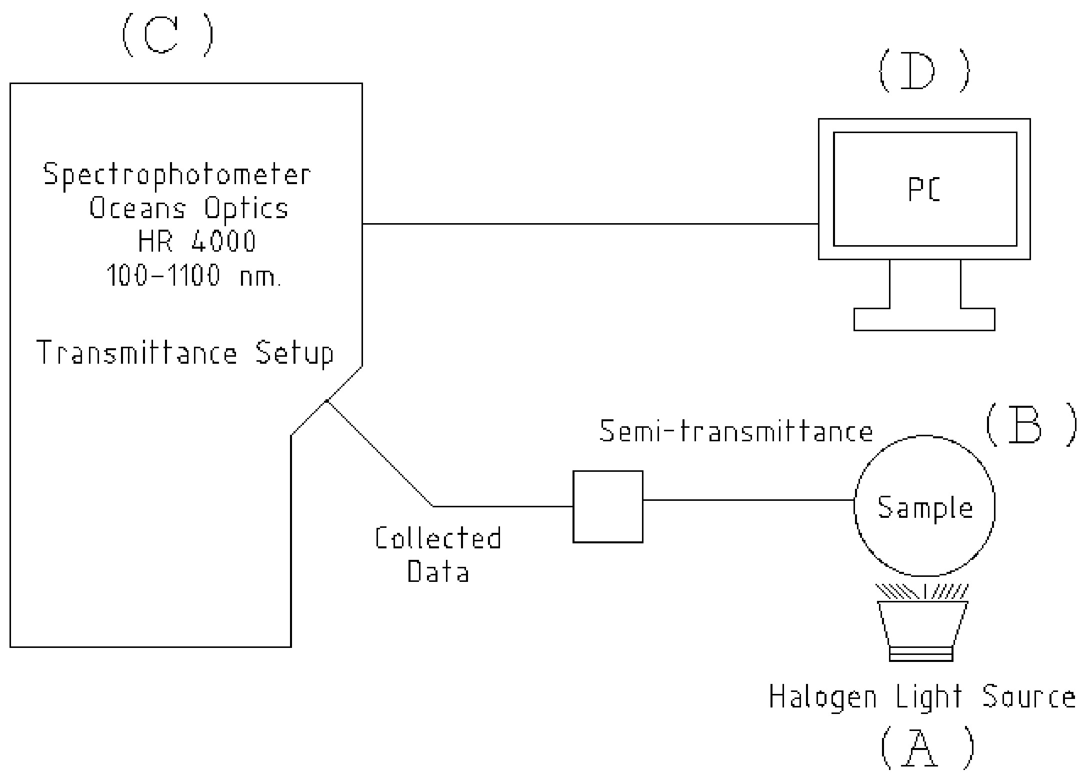

Fig. 1. Schematic representation for Spectra Vis-NIR UC equipment. A) a 250 W halogen light source, B) fruit sample with 90° orientation, C) Oceans Optics HR 4000 spectrophotometer (100-1100 nm) working in transmittance mode and D) computer for acquiring semi transmittance data

Image processing

After 150 d plus 7 d at 20 °C of storage, all fruit were measured non-destructively and then halved to make a photographic record of each fruit with a digital camera (Canon PowerShot, G10 camera, Tokyo, Japan) set at a 100 mm focal distance, 1/13 s exposure time, resolution 4,416 x 3,312 pixels (.jpg images) and sRGB color space. Internal browning was quantified as the percentage of the damaged area in one halve of fruit and calculated using the ImageJ v 1.48, image-processing software (https://imagej.nih.gov/ij/) with a filter designed to recognize the percentage of the damaged area of each half fruit apple. Internal browning was classified in radial, diffuse and combination of both types, however, total browning was considered in the analysis. The filter parameters for the ImageJ software were: Color Thresholder Lab: L^* 149-237, a^*: 117-135, b^*: 119-225.

Statistical analyses and model development

The initial pre-processing of the transmittance spectra included a smoothing curve to obtain a continuous function from the discrete values delivered by the equipment. These smoothing curves were generated using *Loess* methodology (0.1 span, quadratic polynomial order and fitting by least-squares) (Lee and Cox, 2010) and carried out in R statistical software (https://www.r-project.org). To reduce the number of variables (wavelengths) to be used as predictors in the models, a principal components analysis was carried out (Zhou et al., 2015). Finally, prior to the development of the predictive models, all spectral curves were corrected with the Savitzky-Golay second derivative and standard normal variate (SVN), an algorithm to reduce the effect of scattering (Rinnan et al., 2009; Tilahun et al., 2018; Travers et al., 2014). All procedures were carried out using Unscrambler X v.10.4 software (http://www.camo.com).

Separate models were developed using spectra collected for each test day (i.e., 0, 60, 90, 120 and 150 d) and compared with the incidence and severity of internal browning obtained after 157 d °C. The data set was built with 720 fruit (960 – 240 fruit (destroyed for internal evaluation)). In calibration set, 503 fruit (70 %) were used to develop the model and a second set of 217 fruit (30 %) was randomly selected exclusively for prediction or test the quantitative and the qualitative models, respectively. This second dataset was not used for calibrating the models.

Quantitative models

To predict the severity of internal damage (defined as the percentage of brown tissue area visible in a half apple), quantitative models were evaluated with Partial Least Square Regression (PLS) and SVMR. For these models, the percentage of the damaged area after 157 d °C was used as a dependent variable. Transmittance values between 600 and 830 nm on each measurement date (0, 60, 90, 120 and 150 d at 0 °C) were selected as the predictor matrix. PLS is a multivariate regression model, therefore, the selection of the best model was made considering higher values of the R^2 in the cross-validation between observed and predicted percentage of internal damage area, and lower values of RMSEC and RMESP. In addition, the prediction capacities of these models were corroborated using the multivariate regression significance test *F* (Gujarati, 2004). SVMR is a statistical learning model that provides a linear function with no statistical significance test, therefore, the values of R^2 in the cross-validation, RMSEC and RMSEP, were used to analyze the performance of the SVMR models (James et al., 2015).

Qualitative models

To sort fruit into healthy or damaged categories, classification models such as PLS-DA along with Support Vector Machine Classification (SVMC) were employed. For these models, a percentage of damaged area higher than 20 % was taken as a reference value, i.e., fruit with <20 % damaged area was considered healthy and fruit with >20 % damaged area was considered damaged. This reference value was obtained in a separate experiment carried out using a survey method as described by Jaeger et al. (2016) with some modifications (data not shown). In the models, the response variable was transformed to a numeric value since PLS-DA does not work with categorical values. Therefore, a value of -1 was assigned to damaged fruit and +1 to healthy fruit. Thus, the sign of the predicted value was used as the classification parameter (Shen et al., 2012; Suhandy and Yulia, 2017). The best model was selected based on the rate of accuracy – correctly classified vs wrongly classified fruit.

Neural network models

Artificial neural networks are based on analyses of human brains. This optimization process comprises a collection of node 'neurons' connected by mathematical functions which play roles analogous to synapses. Nowadays, the most commonly used optimization algorithm is called *backpropagation,* which uses gradient descent to update the synapse parameters, and thereby achieves the model's learning function (Gu et al., 2017). To develop neural network models, it is necessary to greatly reduce the number of model predictors to simplify the network architecture (Goodacre et al., 1996) and to reduce the computation time. In these models, the cumulative area under the curve (AUC) of each spectrum between 600 and 830 nm and the average transmittance between 645 and 655 (L650), 705 and 715 (L710) and 795 and 805 (L800) nm were quantified and used as predictor variables. The development of these models was carried out with the library *neuralnet* available in R software.

For the quantitative neural models, the same selection criteria used for SVMR were employed, while for the qualitative neural models, the criteria of PLS-DA and SVMC were employed.

Results

Internal damage incidence and characterization of spectral curves

After 150 d of storage and a further 7 d of ripening at 20 °C, a wide range of values of incidence and severity of internal damage was observed for the three temperature treatments (Fig. 2). The treatments with extreme management of low temperature (T1 and T2) had high disorder incidences, with internal damage percentages averaging 53 % for fruit in T1 and 49 % for those in T2; whereas the percentage in T3 was 11 %. Taking into account all fruit, the internal browning area range was 2.7 % to 87 % (Fig. 3).

The fruit damage incidence or severity of 1-MCP treated and non-treated fruit was similar when stored in any of the three temperature treatments (Fig. 2). However, firmer fruit was obtained with the 1-MCP treatment (data not shown). It is evident that 1-MCP did not reduce the occurrence of the browning disorder, while the temperature treatments did affect both its incidence and severity. This conclusion is based on the observation that the percentage of damaged fruit in the 5 °C treatment (T3) was 10 %, whereas in T1 and T2 was 86 % and 84 %, respectively

(Fig. 2B). No statistical analysis was done on the effect of treatments in the incidence of internal browning

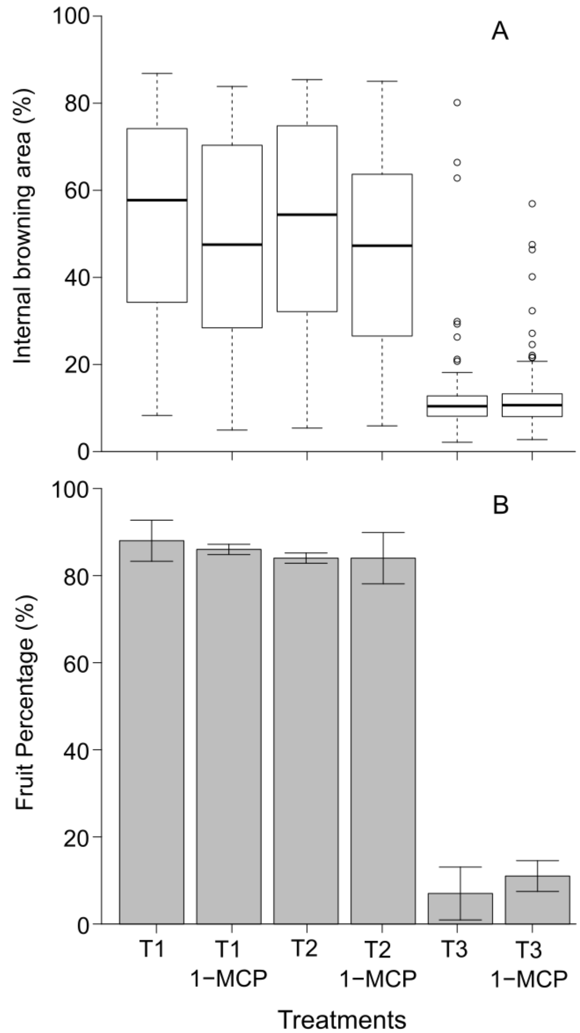

Fig. 2. A) Boxplot of internal browning severity (percentage of internal area damaged) in ‘Cripps Pink’ apples after 150 d at 0 °C, plus 7 d at 20 °C. Fruit were exposed to three different temperature treatments (T1, T2 or T3) +/- a treatment with 1-methylcyclopropene (1-MCP). B) Barplot of ‘Cripps Pink’ apple fruit percentage with internal browning incidence (internal damage area greater than 20 %) observed in after 150 d of storage under temperature treatments (T1, T2 or T3) +/- 7 d at 20 °C. Each treatment contained 120 fruit. T1: -1 °C for 24 h then stored for 149 d at 0 °C; T2: stored for 150 d at 0 °C; T3: stored for 90 d at 5 °C plus 60 d at 0 °C, with and without 1-MCP application.

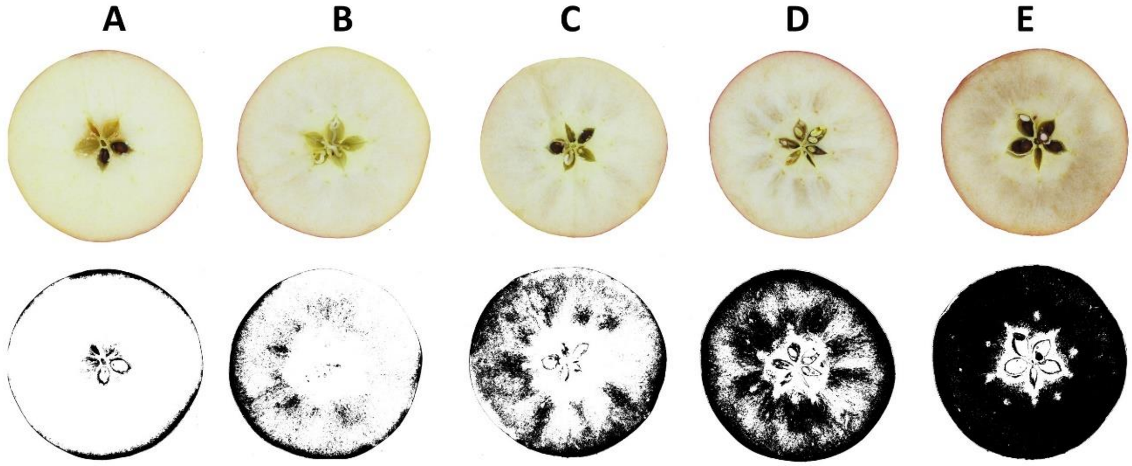

Fig. 3. Internal browning severity in ‘Cripps Pink’ apples after 150 d at 0 °C under different treatments plus 7 d at 20 °C. A: Less than 10 % affected area, B: Between 10 % and 20 % affected area, C: Between 20 % and 30 % affected area, D: Between 30 % and 60 %, E: More than 70 % affected area. Top images photographs. Bottom images are filtered to identify the percentage areas of browning.

To determine the wavelength range in which semi-transmittances spectra are more affected by internal browning symptoms, a principal component analysis (PCA) was performed with the spectra collected on 150 d, and matched with symptoms evaluated at 157 d. PCA determined that the two first of seven components explain 96 % of the variability of the internal damage, PCA grouped most of the fruit classified as damaged with negative scores in the first component, while healthy fruit had positive values in the same component (Fig. 4A). Figure 4B shows that the range between 630 and 730 nm presents a higher weight in PC1, while the range of the spectra between 600 and 650 nm and 700 and 830 nm show the same behavior in PC2. These results corroborate that healthy fruit, which obtained high scores in PC1, would have higher semi-transmittance values between 630 to 730 nm. In other words, healthy fruit had higher spectral curves compared with affected fruit.

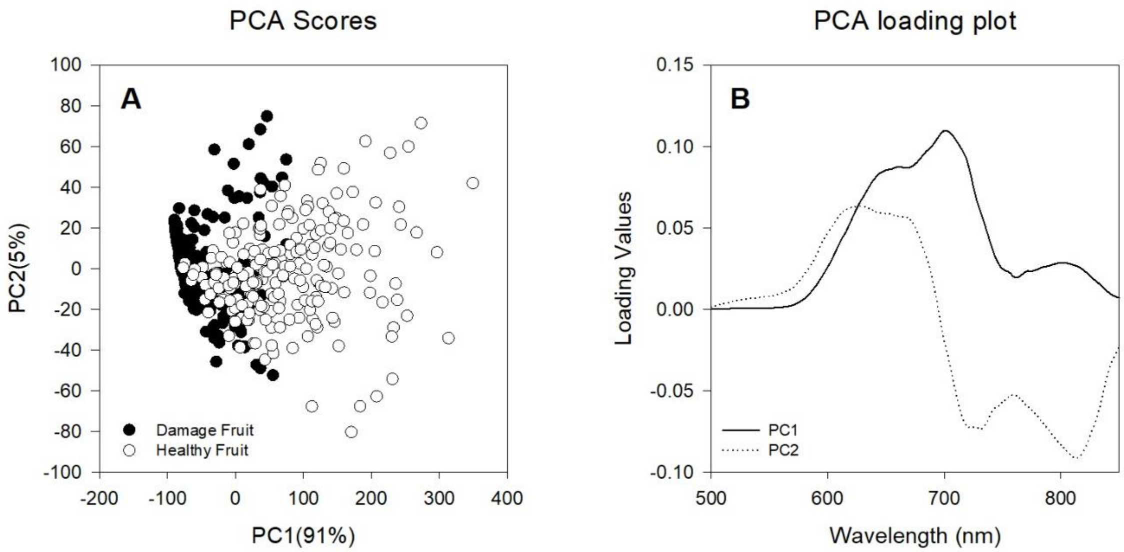

Fig. 4. Principal component (PC) analysis of ‘Cripps Pink’ apple transmittance spectra between 600 and 830 nm after 157 d of storage. A) Score plot. Black dots correspond to damaged fruit, white dots correspond to healthy fruit. B) Loading plot. Continuous line shows loading values for PC1, dotted line shows loading values for PC2.

Analysis of the spectral curves for each fruit and for each treatment showed that, on average, lower temperatures (T1, T2) had lower transmittance spectral curves after 60 d storage, which coincided with a greater incidence and severity of internal browning than delay cooling treatment at 5 °C (T3) (Fig. 5). Continuous monitoring measurements during storage detected the onset of internal browning and its development from 90 d of storage. When fruit showed the lowest incidence of browning (T3), it also showed higher average transmittance spectral curves than the other two treatments (T1 and T2). From the AUC analysis (Fig. 6), it was clear that fruit with browning incidence at the end of the storage period (157 d), presented significant differences in AUC values already at 90 d compared with healthy fruit. This behavior was also observed during subsequent measurements (at 120 d and 150 d).

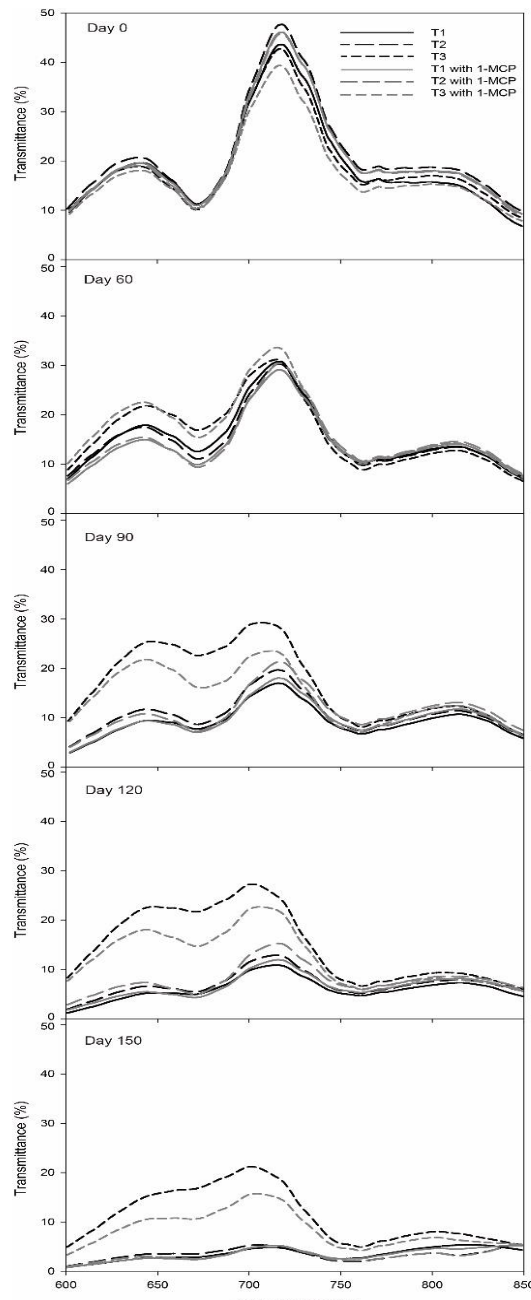

Fig. 5. Mean semi transmittance spectral curves of ‘Cripps Pink’ apples after 0, 60, 90, 120 and 150 d of storage at 0 °C. T1: -1 °C for 24 h and subsequent storage for 149 d at 0 °C; T2: 150 d at 0 °C; T3: 90 d at 5 °C plus 60 d at 0 °C, with and without 1-MCP application.

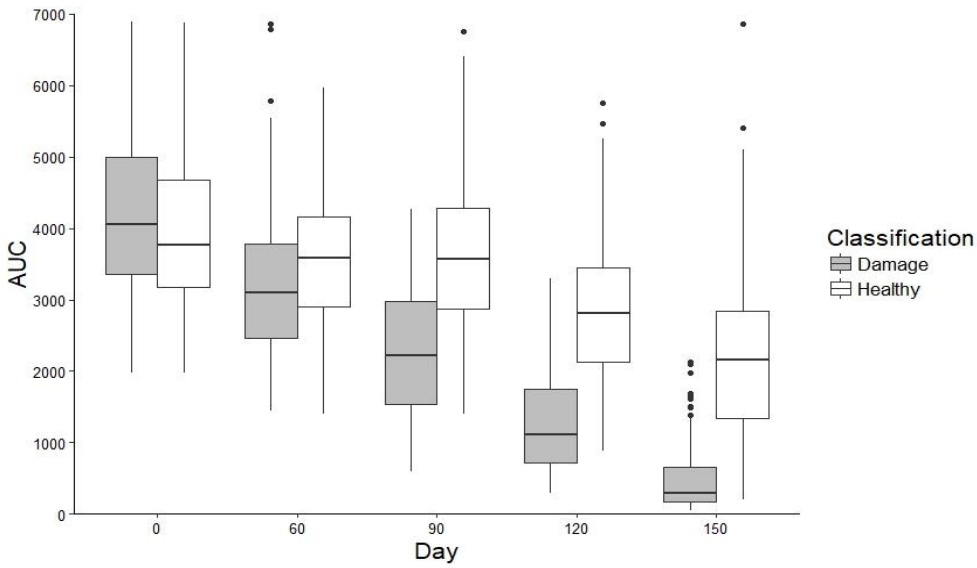

Fig. 6. Changes in the area under the transmittance spectral curves (AUC, dimensionless) during storage at 0 °C of ‘Cripps Pink’ apples, healthy or with symptoms of internal browning evaluated after 150 d at 0 °C plus 7 d at 20 °C. Dots represent extreme values

Quantitative models

Results for quantitative models are shown in Table 1. The models developed after 90, 120 and 150 d showed significant increases in their respective correlation coefficients and they allowed rejection of the null hypothesis of the multivariate regression significance test *F*. Correlation coefficients ranged from ~0.60 after 90 d to >0.76 and 0.80 (calibration and prediction datasets, respectively) after 150 d (Table 1). As time went on, these coefficients for all models increased while the values of RMSEC and RMSEP decreased showing dramatic changes after 90 d.

The quantitative model with the best R^2 after 90, 120 and 150 d was SVMR, with values of 0.71 and 0.73 (for the calibration and prediction datasets) after 90 d and

>0.85 after 150 d. Compared to PLS, the SVMR model always showed lower values of RMSEC and RMSEP except after 90 d when RMSEC was 17.7 %.

Table 1. Quantitative models (PCR, PLS, SVMR) predicting percentage of internal damage area in 'Cripps Pink' apples during storage for 150 d at 0 °C. The percentage of browning area at the pulp was determined destructively after 150 d at 0 °C and 7 d at 20 °C. The calibration set used 503 fruit and the prediction set used 217 fruit. RMSEC: root mean squared error in calibration; RMSEP: root mean squared error in prediction.

	Calibration Set		Prediction Set	
	Correlation Coefficient	RMSEC	Correlation Coefficient	RMSEP
Day 0				
PLS	0.34	20.78	0.25	23.94
SVR	0.37	20.59	0.38	23.35
Day 60				
PLS	0.38	20.15	0.36	22.12
SVR	0.40	20.12	0.49	22.49
Day 90				
PLS	0.62	15.84	0.62	17.10
SVR	0.71	17.74	0.73	14.87
Day 120				
PLS	0.75	12.69	0.54	18.41
SVR	0.79	11.53	0.73	14.59
Day 150				
PLS	0.77	12.21	0.81	12.01
SVR	0.85	9.99	0.87	9.80

After 150 d, the first three factors out of seven, were used for the PLS prediction model, explaining about 79 % of the data set variability. The PLS regression showed an R^2 of 0.77 and an RMSEC of 12.2 % for the calibration dataset, while for the prediction dataset the R^2 was 0.81 with an RMSVE of 12 %.

Finally, the SVMR model reduced RMSEC and RMESV to 9.9 % and 9.8 %, respectively, while the R^2 between the predicted and observed values were 0.85 and 0.87, respectively.

Table 2. Qualitative models (PLS-DA, SVMC) sorting ‘Cripps Pink’ apples into healthy and damaged categories during storage for 150 d. The presence of damaged and healthy fruit was determined destructively after 150 d at 0 °C and 7 d at 20 °C. The calibration set used 503 fruit and the test set used 217 fruit. Total MC: Total number of misclassified fruit; Healthy MC: number of healthy fruit classified as damaged; Damage MC: number of damaged fruit classified as healthy. The calibration set used 503 fruit and the test set used 217 fruit.

	Calibration set				Test set			
	Accuracy (%)	Total MC	Healthy MC	Damage MC	Accuracy (%)	Total MC	Healthy MC	Damage MC
Day 0								
PLS-DA	83	86	51	35	86	29	16	13
SVMC	64	181	164	17	72	61	50	11
Day 60								
PLS-DA	85	77	51	26	85	32	19	13
SVMC	74	131	96	35	78	47	27	20
Day 90								
PLS-DA	87	65	37	28	86	30	12	18
SVMC	87	67	36	31	87	29	12	17
Day 120								
PLS-DA	88	61	33	28	86	29	12	17
SVMC	87	65	35	30	86	30	12	18
Day 150								
PLS-DA	86	69	13	56	83	34	5	29
SVMC	90	50	12	38	89	23	3	20

Qualitative models

Unlike quantitative models, qualitative models were developed to segregate fruit into two groups (healthy and damaged) and showed good predictive capacities from the first day of storage (Table 2). The PLS-DA model showed an >83 % accuracy, with only 86 and 29 misclassified fruit in the calibration and prediction sets, respectively. However, the SVMC model achieved a success rate of only 72 % with the test dataset, with more misclassified fruit (181 fruit) in the calibration dataset than the PLS-DA model (86 fruit). After 90 and 120 d, both models showed similar accuracy values for the calibration and test datasets (around 87 %). After

150 d, the PLS-DA model gave a percentage classification certainty of 86 %, leaving 13.7 % (69 fruit) of the fruit misclassified, that is, ‘healthy’ fruit classified as ‘not healthy’ and *vice versa* (Fig. 7).

Neural network models

For the neural network models, it was necessary to normalize the data with measurement scale (the AUC variable had a value range between 1 and 600 units, while the values for L600, L710 and L800 varied between 0 and 50 %). After normalization, the neural models for predicting the percentage of internal damaged area were calculated (Table 3). Several models were built with different numbers of hidden layers and different numbers of neurons per layer; in Table 3 few models that showed acceptable results are presented.

For days 0 and 60, the qualitative neural models showed behaviors similar to those of the quantitative models described above (PLS and SVMR).

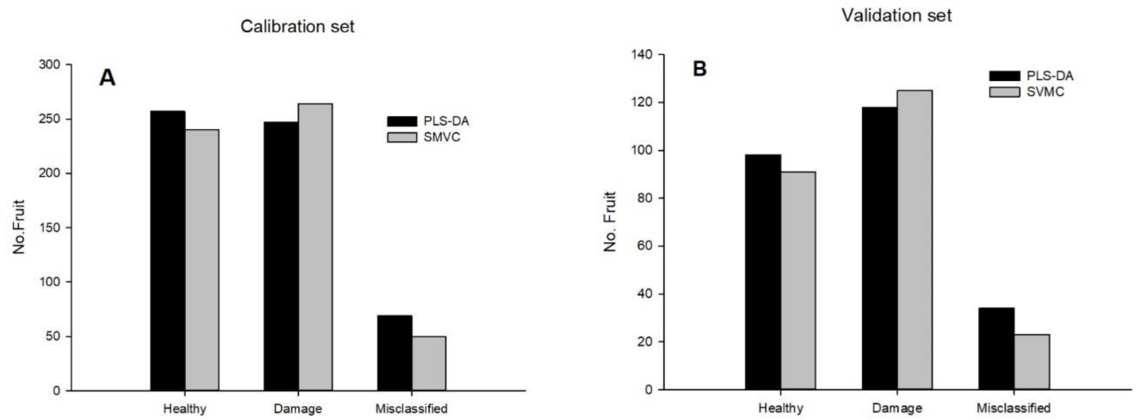

Fig. 7. Number of fruit classified by qualitative models on 150 d at 0 °C, sorting ‘Cripps Pink’ apples into two groups: healthy and damaged. Misclassified amounts are healthy fruit classified as damaged and vice versa. A) Classification used by PLS-DA and SVMC models with the validation set. B) Classification using PLS-DA and SVMC models with the calibration set

After 90 d, all the proposed neural models showed increases in their correlation coefficients and decreases in their RMSEC and RMSEP values. For instance, the highest R^2 (0.86) was obtained in the calibration set for the model that used the L650, L710 and L800 predictor variables with three hidden layers (12, 6 and 2 neurons per layer). Unfortunately, this model obtained a low R^2 (0.34) in the prediction set. Likely, the neural network ‘overlearned’ with the calibration set, and made it impossible to obtain good prediction results with the prediction set. Considering that predictive models should have high correlations coefficients in both, calibration and prediction sets, the best quantitative neuronal model after 90 d was that one using four predictive variables (AUC, L650, L710 and L800) with three hidden layers (8, 4 and 2 neurons per layer). This model obtained a R^2 of 0.78 and 0.65 and RMSE of 11.9 % and 16.8 % for the calibration and prediction sets, respectively (Table 3).

Table 3. Quantitative neural network models predicting percentage of internal damage area in ‘Cripps Pink’ apples during storage for 150 d at 0 °C. The percentage of browning area of the pulp was determined destructively after 150 d at 0 °C and 7 d at 20 °C. RMSEC: root mean squared error in calibration set; RMSEP: root mean squared error in prediction set. The calibration set used 503 fruit and the test set used 217 fruit. The cumulative area under the curve (AUC) of each spectrum between 600 and 830 nm and the average transmittance between 645 and 655 (L650), 705 and 715 (L710) and 795 and 805 (L800) nm were used as predictor variables.

			Calibration Set		Prediction Set	
	No. Hidden Layers	Neurons per layer	Correlation Coefficient	RMSEC	Correlation Coefficient	RMSEP
Day 0						
AUC,L650,L710,L800	1	8	0.34	20.63	0.06	29.83
	3	8,4,2	0.5	18.05	0.07	28.97
L650,L710,L800	1	6	0.19	22.94	0.02	29.06
	3	12,6,2	0.46	18.64	0.01	48.49
AUC	3	5,3,2	0.02	25.31	0.03	28.05
Day 60						
AUC,L650,L710,L800	1	8	0.38	20.07	0.2	25.41
	3	8,4,2	0.47	18.55	0.21	26.39
L650,L710,L800	1	6	0.34	20.71	0.16	26.91
	3	12,6,2	0.55	17.07	0.06	34.05
AUC	3	5,3,2	0.09	24.38	0.04	28.01
Day 90						
AUC,L650,L710,L800	1	8	0.7	13.89	0.67	16.28
	3	8,4,2	0.78	11.95	0.65	16.81
L650,L710,L800	1	6	0.67	14.52	0.63	17.16
	3	12,6,2	0.86	9.29	0.34	24.44
AUC	3	5,3,2	0.45	18.81	0.43	21.3
Day 120						
AUC,L650,L710,L800	1	8	0.86	9.21	0.75	13.5
	3	8,4,2	0.91	7.53	0.74	14.23
L650,L710,L800	1	6	0.85	9.62	0.79	12.49
	3	12,6,2	0.92	6.79	0.67	16.57
AUC	3	5,3,2	0.59	16.31	0.53	18.7
Day 150						
AUC,L650,L710,L800	1	8	0.88	8.65	0.86	10.48
	3	8,4,2	0.91	7.75	0.74	14.61
L650,L710,L800	1	6	0.85	9.97	0.86	10.25
	3	12,6,2	0.92	6.97	0.75	14.13
AUC	3	5,3,2	0.67	14.66	0.74	14.65

On 120 d, models using three or four predictor variables had R^2 values >0.85 and 0.67 in the calibration and prediction sets, respectively. The model that obtained the best results included L650, L710, L800 variables with one hidden layer (six neurons in the layer), which had R^2 0.85 and 0.79 and an RMSEC of 9.6 % and RMSEP of 12.5 %.

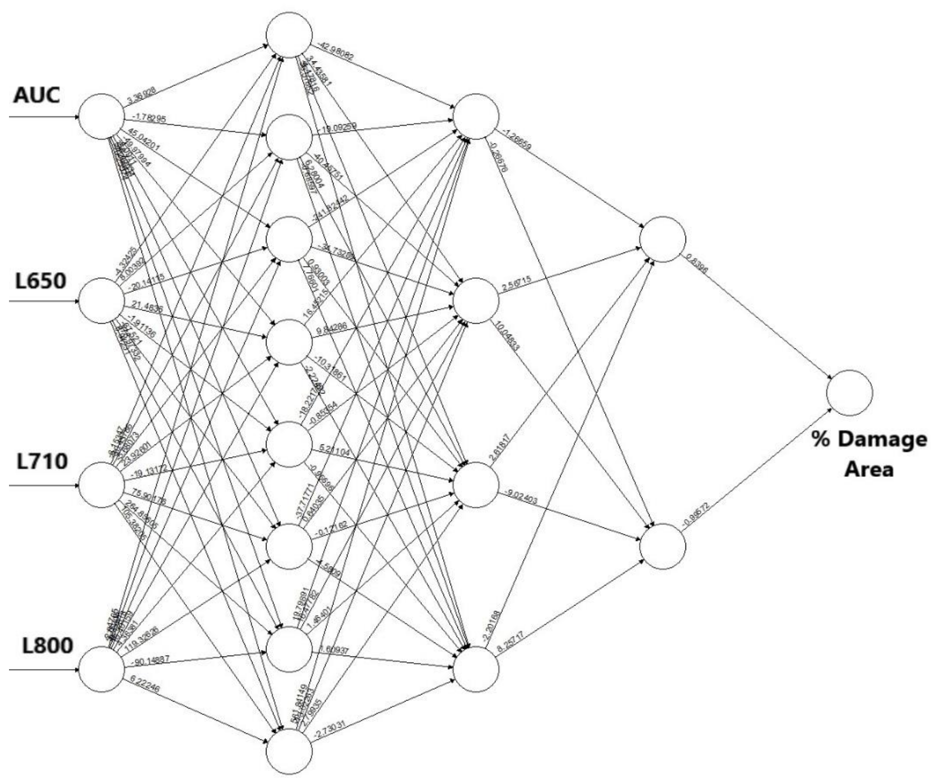

Fig. 8. Neural network for qualitative model using AUC, L650, L710 and L800 as predicted variables to segregate ‘Cripps Pink’ apples after 150 d at 0 °C plus 7 d at 20 °C into two groups: healthy and damaged. The cumulative area under the curve (AUC) of each spectrum between 600 and 830 nm and the average transmittance between 645 and 655 (L650), 705 and 715 (L710) and 795 and 805 (L800) nm were used as predictor variables.

For the models constructed after 150 d, good prediction capacities were attained with two models that used four and three predictive variables for the calibration and prediction sets, respectively.

The qualitative neuronal models (Table 4) showed correct classification percentages >74 % from the first day of storage, but only after 90, 120 and 150 d of storage were able to reduce satisfactorily the number of misclassified fruit (Fig. 8). After 90 d, the models had an accuracies >90 % with the calibration set and with numbers of misclassified fruit <10. The best model used four input variables and three hidden layers, achieving an accuracy of 94 % with only 27 misclassified fruit out of 503 (5.3 %) in the calibration set, and only seven damaged fruit were classified as healthy. With the test set, this prediction model had an accuracy of 84 % and left only 15.2% of misclassified fruit.

For the last two times of spectra acquisition (120 and 150 d), the best models were those using L650, L710 and L800 as predictive variables and three hidden layers (12, 6 and 2 neurons per layer). These models showed accuracies >97 % and the numbers of misclassified fruit were <15 in the calibration set. In the test set, the classification rate was 89 % and the number of misclassified fruit was 23.

Table 4. Qualitative neural network models sorting 'Cripps Pink' apples into healthy and damaged categories during storage for 150 d at 0 °C. The presence of damaged and healthy fruit was determined destructively after 150 d at 0 °C and 7 d at 20 °C. The calibration set used 503 fruit and the test set used 217 fruit. The cumulative area under the curve (AUC) of each spectrum between 600 and 830 nm and the average transmittance between 645 and 655 (L650), 705 and 715 (L710) and 795 and 805 (L800) nm were used as predictor variables. Total MC: Total number of misclassified fruit; Healthy MC: number of healthy fruit classified as damaged; Damage MC: number of damaged fruit classified as healthy. The calibration set used 503 fruit and the test set used 217 fruit.

			Calibration Set				Test Set			
	No. Hidden Layers	Neurons per layer	Accurency (%)	Total MC	Healthly MC	Damage MC	Accurency (%)	Total MC	Healthly MC	Damage MC
Day 0										
AUC,L650,L710,L800	1	8	75	124	79	45	66	73	43	30
	3	8,4,2	58	213	213	0	65	75	75	0
L650,L710,L800	3	12,6,2	67	166	166	0	65	74	65	9
AUC	3	5,3,2	59	203	117	86	61	83	43	40
Day 60										
AUC,L650,L710,L800	1	8	74	128	66	62	71	64	31	33
	3	8,4,2	58	213	213	0	65	75	75	0
L650,L710,L800	3	12,6,2	67	166	166	0	65	76	66	10
AUC	3	5,3,2	61	193	146	47	62	81	49	32
Day 90										
AUC,L650,L710,L800	1	8	89	56	31	25	88	27	8	19
	3	8,4,2	94	27	20	7	84	33	16	17
L650,L710,L800	3	12,6,2	95	24	14	10	85	33	14	19
AUC	3	5,3,2	72	140	21	119	71	64	11	53
Day 120										
AUC,L650,L710,L800	1	8	96	20	8	12	92	17	7	10
	3	8,4,2	96	20	10	10	88	26	13	13
L650,L710,L800	3	12,6,2	97	15	13	2	89	24	18	6
AUC	3	5,3,2	78	111	46	65	85	32	16	16
Day 150										
AUC,L650,L710,L800	1	8	94	26	19	7	93	15	7	8
	3	8,4,2	98	9	6	3	90	21	7	14
L650,L710,L800	3	12,6,2	99	7	5	2	89	23	11	12
AUC	3	5,3,2	87	64	33	31	88	25	10	15

Discussion

Internal browning is recognized as the main deterioration factor after long term storage of 'Pink Lady' apples (Di Guardo et al., 2013; Moggia et al., 2015). In this research a high susceptibility of the disorder was induced on apple with extreme management of low temperature (T1 and T2), and the opposite occurred when a period of 90 d at 5 °C was included in a total of 150 d storage at 0 °C (T3). Semi-transmittance readings between 100 and 1,100 nm taken for each fruit during storage demonstrated that fruit more prone to develop internal browning had lower transmittance in the wavelength range between 650 nm and 710 nm. Similar results were reported by Clark et al.(2003) in 'Braeburn' apple with internal damage. The light attenuation can be attributed to the brown flesh and the presence of dry, mealy-texture of cortical tissues in the most severely affected fruit.

Different models were used to correlate the spectra evaluated during storage and the expression as percentage (quantitative) or presence (qualitative) of brown flesh after 157 d. Noteworthy, apple fruit with internal browning symptoms were not detected before 120 d of storage.

The loading values of the two first components evaluated by PCA, indicate that the range between 600 and 830 nm had a greater weighting, suggesting that this range includes the best information of the whole spectral curve between 100 and 1,100 nm. Moreover, several authors proposed that the characterization points in this range are 665 nm (chlorophyll), and 740 and 840 nm (water) (Zude-sasse et al., 2002; McGlone et al., 2005; Wang et al., 2015; Khatiwada et al. 2016).

Upchurch et al. (1997) noticed that internal browning in 'Delicious' apple affects the light transmittance through the fruit. They found that shorter wavelengths (< 750 nm) were more attenuated by the presence of internal damage, which was proportional to browning. Also, McGlone et al. (2005) found that 'Braeburn' apples with brown heart showed high absorbance (low transmittance) in the red/near-red region (650-840 nm), since brown tissues are darker and also more saturated with free water than healthy tissues. More recently, Khatiwada et al. (2016) reported that measuring with two different instruments, ‘Cripps Pink’ apples with internal damage showed higher absorbance (lower transmittance) for wavelengths lower than 830 nm.

SVMR, calculated as early as 90 d of storage, was the best quantitative model, with the highest R^2 (0.71) and lowest RMSE values. Noteworthy, the SVMR model showed a low RMSEC and a higher correlation coefficient than PLS model for predicting the percentage of brown flesh in ‘Cripps Pink’ apples.

Qualitative models were able to segregate healthy or damaged fruit from 0 d of storage. PLS-DA model achieves a success rate of 83 %. However, after 90 d storage the SVMC model showed similar accuracy values, and correct classifications of 90 % and 89 % for the calibration and test datasets, respectively (Table 2). This model also reduced the numbers of misclassified damaged fruit. Therefore, using SVMC further reduces the likelihood that a damaged fruit is classified among the healthy fruit. The accuracy obtained for PLS-DA and SVMC had similar values as reported by Khatiwada et al. (2016) when the detection was done at the same time as the fruit was evaluated. We noticed that SVMC improves its accuracy rate along the storage time, suggesting that machine learning models could be better models than classical models (e.g. PLS-DA) during extended periods of time storage. Good results in the early detection of internal browning by vis-Nir spectroscopy indicates that the structure of the tissue by the cell arrangement, or changes in the internal space or in the cell wall structure that occurs throughout the storage time, modify the light scattering, providing valuable information of the primary events of the disorder development. However, more detailed morphological studies are needed to demonstrate such association.

Most of the neural network models used in postharvest technology have been done just for classification purposes. In this study, it has been shown the potential of this model for quantifying internal damage and sorting healthy apples from those affected by internal browning. Quantitative and qualitative neural models explored in this work showed similar or even better correlation coefficients, in cross validation or accuracy rate than multivariable models, offering a novel way for modelling biological processes. (ElMasry et al., 2009; Guo et al., 2016b). Strikingly, for each measurement time, the models using AUC as predictor variable did not achieve successful predictions. This indicates the neuron models require several variables that summarize the spectral characteristics very well as noted by Lu (2004), who used different spectra combinations (spectral values between 600 to 950 nm) for predicting firmness and soluble solids in apples.

In this work, a 90-degree light-sample-detector geometry was used to quantify or classify internal browning showing after 157 d. In some fruit, internal browning did not cover homogeneously the entire fruit cross section, which could introduce a lack of certainty in the models. Despite of, good results were obtained for qualitative and quantitative predictions, showing that 90-degree sample geometry was enough to provide a general description of total internal quality

Conclusions

This research shows that the use of quantitative models based on semi-transmittance acquisitions between 100 to 1,100 nm are able to predict the severity of internal browning tissue developed at ripening after 150 d of storage in ‘Cripps Pink’ apples as early as 90 d of storage. These models can also predict which fruit will develop internal damage from 0 d.

To predict the percentage of area affected by internal browning after 90 d of storage, the use of SVMR model is recommended. This should give R^2 values greater than 0.70 with average RMSEP close to 13 %. Alternatively, we recommend neural models using as input variables AUC, L650, L710, L800 with three hidden layers (8, 4 and 2 neurons per layer). These should yield R^2 between 0.65 and 0.75 with average RMSEP close to 15 %.

Segregation of fruit into two categories (healthy and damaged) is possible at 0 d of storage, with numbers of misclassified fruit decreasing as storage time increases. The best models for this (with accuracies higher than 75 %) are PLS-DA and the neural model using AUC, L650, L710 and L850 as predictive variables with one hidden layer of eight neurons. To the best of our knowledge, this is the first report of the use of quantitative models and neural networks for early prediction of internal browning severity in apples using spectral transmittance data. These procedures allow early fruit sorting, which is of critical importance for the most appropriate target markets. This research presents a novel way of early prediction of internal browning by modeling the internal damage in ‘Cripps Pink’ apples from spectra collected before the symptoms of the disorder develops. Future research is required to confirm the accuracy of these prediction models using ‘Cripps Pink’ apples across different growing seasons and showing different natural severities of internal browning.

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3. Watercore management in ‘Fuji’ apples: watercore detection by Vis-NIR and watercore reduction by pre-storage treatment

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Abstract

Watercore is an internal disorder in apple fruit that appears mostly late-season as water-soaked areas in the flesh. To some extent, this disorder reduces fruit postharvest life. Here, we explore options for reducing watercore incidence and for non-destructive screening fruit with and without watercore at harvest. Two experiments were carried out with ‘Fuji’ fruit suffering watercore. Experiment 1. Fruit from three orchards were used to determine the effects on the rate of watercore reduction of different durations of delayed storage at different temperatures. T0 (control) fruit were immediately stored at 0°C for 150 d;T1 fruit were stored at 5°C for 30 d and at 0°C for 120 d; T2 fruit were stored at 5°C for 60 d and at 0°C for 90 d, T3 fruit were stored at 10°C for 30 d and at 0°C for 120 d and T4 fruit were stored at 20°C for 3 d and at 0°C for 147 d. Fruit were evaluated for watercore incidence, severity and quality after 30, 60 and 150 d. Incidence of watercore declined exponentially with time. Reduction was faster in fruit with low initial watercore incidence. In the controls (T0), watercore incidence decreased to 50% after 44 d. Delayed storage at 5°C for 30 d (T1) speeded 50% reduction to 26 d and at 10°C for 30 d (T3) to 16.7 d. Experiment 2. Fruit from four ‘Fuji’ orchards, suffering a wide range of watercore severities, were used to determine the usefulness of semi-transmittance equipment to measure the proportion of watercore tissue in a fruit. Logistic models were used to maximise accuracy, specificity and sensitivity. These show the area under the curve (AUC), inflection points of wavelength spectra at 625, 670, 715, 800 nm discriminate between healthy fruit (<15% watercore area) and watercore fruit (>30% watercore area).

LDA models (Model 3: L625, L670, L715 and L800; Model 5: AUC; L670 and L800) demonstrate the equipment is not able to discriminate between fruit with watercore affected areas between 15 and 30%. Nevertheless, the information was good enough to discriminate between healthy fruit (<15% watercore area) and watercore fruit (>30% watercore area) with an accuracy >80%. Therefore, we propose a protocol employing a delayed cooling treatment at 5°C for 30 d to inhibit watercore incidence in ‘Fuji’ apples previously partitioned by semi-transmittance evaluation into >30% watercore fruit affected area.

Keywords: Watercore, reduction, semi-transmittance detection, Vis-NIR, classification models

Introduction

Watercore is an important postharvest physiological disorder that develops while the fruit is still on the tree and that particularly affects certain apple and pear cultivars (Marlow and Loescher, 1984). The cause has been identified as an abnormal sorbitol metabolism in the fruit where the sorbitol-rich phloem sap in the vascular bundles is unloaded but remains in the intercellular spaces, unable to transfer into the parenchyma cells. Gao et al. (2005) found that sorbitol transporter genes are expressed in all apple sink tissues except in the watercore-affected ones. This suggests the accumulation of sorbitol may be explained as a defect of the transport processes in the parenchymatic tissue. Therefore, glassy (water-soaked) regions appear in the flesh as a result of sorbitol accumulation. Two distinct forms of watercore have been described: (1) a radial-type, located around the core-line vascular bundles and (2) a block-type, located at the interface between the carpels and mesocarp (Beaudry, 2014; Harker et al., 1999). More than a hundred apple cultivars have been identified as susceptible to watercore, such as 'Fuji' (Marlow and Loescher, 1984; Yamada et al., 2004). In the Japanese market, watercore in 'Fuji' is a desirable characteristic as it is an indicator of sweetness (Coster, 2011).

Interestingly, watercore may dissipate during storage, especially in early-harvest fruit, where incidence is light to moderate. However, where watercore is severe, tissue breakdown can occur with development of an alcoholic taste (Herremans et al., 2014). Reduction of watercore in 'Braeburn' with light symptoms took 3-5 weeks at 0-0.5°C compared with 6-8 weeks for severe symptoms (Clark and Richardson Enza, 1999). Dissipation was faster in fruit stored under <1 kPa CO_2 than under 2.5 kPa CO_2 (Kweon et al., 2013) or when the storage temperature was higher (i.e. 3, 6 or 10°C) instead of at the usual 0°C (Neuwald et al., 2012).

In addition, early-harvest fruit showed more rapid reduction of watercore than late-harvest fruit. This is probably because early-harvest fruit show a generally lower incidence of watercore (Harker et al., 1999). These authors hypothesised that the extracellular fluid is absorbed into the cells with an associated increase in cell (hence of fruit) volume, suggesting that reabsorption of intercellular fluid explains the reduction.

The fluid filling the air spaces of an apple exhibiting watercore reduces light scattering and increases specific gravity, each of these properties having been used for non-destructive detection of watercore (Cho et al., 2008; Marlow and

Loescher, 1984). Spectral reflection in the wavebands at 690-700 and 820-830 nm separated ‘Red Delicious’ fruit affected by watercore from unaffected fruit with an error of 4% but it could not provide information on the severity of watercore in each fruit (Bennedsen and Peterson, 2005). Nuclear magnetic resonance (NMR) with 3D imaging has allowed construction of virtual cross-sectional of images and can separate fruit on the basis of watercore severity (Cho et al., 2008; Clark and Richardson Enza, 1999; Wang et al., 1988). X-ray computed tomography (X-ray CT) has been compared with NMR imaging (NMI) (Herremans et al., 2014) and NMI has shown better delimitation of the water-soaked areas.

In the field, incidence of watercore depends on environmental conditions. At this stage we are unaware of management practices that can be applied preharvest to mitigate watercore. Therefore, the behaviour during storage is associated to the fruit’s natural capacity to dissipate watercore.

The objective of this study was to develop a postharvest protocol to control watercore incidence in apples suffering from different levels of watercore severity determined at harvest by low-cost, non-destructive equipment. Two experiments were conducted. Experiment 1 aimed to define the optimal values of storage delay and temperature to reduce water core incidence in 'Fuji' apples. Experiment 2 aimed to optimise light transmittance equipment for non-destructive detection and measurement of watercore severity in fruit affected with watercore at harvest.

Materials and methods

2.1 Experiment 1. Watercore reduction

2.1.1 Fruit material

Delayed storage and temperature treatments were imposed on ‘Fuji’ apples (*Malus domestica* Borkh.) harvested from mature trees from three commercial orchards in the Central Valley of Chile. Orchard 1 is located in Molina (35°48'42.07"S 71°32'1.65"W) and orchard 2 (36°01'39.62"S 71° 38' 41.02"W) and orchard 3 (36°12'21.22"S 71°32'38.34"W) are located in Longaví. The fruit was harvested and transported to the Postharvest Laboratory at the Pontifical Catholic University of Chile, Santiago, Chile. After storage overnight at 20°C, fruit free of visible damage and of uniform size (200-230 g) were selected. Four groups of 10 fruit were randomly selected and their maturity assessed at harvest. Flesh firmness was measured on the equatorial region on both sides without the skin using an Effegi (Milan, Italy) pressure tester fitted with an 11.1 mm diameter probe. Average fruit firmness values were 68.9, 79.3 and 74.6 N for orchards 1, 2 and 3, respectively. Titratable acidity (TA) and soluble solids concentration (SSC) were determined on juice extracted from a 10 g slice from each fruit. A digital refractometer (Atago Pal 1, Tokio, Japan) was used to assess soluble solids and values are expressed as percentages. Fruit SSC values for the three orchards 1, 2 and 3 were 17.4, 15.4 and 17.1%, respectively. Measurements of TA were carried out by titration with 0.1 N NaOH to pH 8.2 using a pH meter (pH211, Hanna Instruments, RI, USA). Values of TA are expressed as the malic acid equivalent percentages and means were 0.35, 0.31 and 0.42% for orchards 1, 2 and 3, respectively. After staining the cut surface of a half fruit with iodine solution, starch content at harvest was rated visually on a ten-point discontinuous scale from 1 (immature) to 10 (over mature) (Ctifl, Paris, France). Mean starch index values were 8.9, 9.2 and 9.2 for orchards 1, 2 and 3.

2.1.2. Delayed storage - duration and temperature

The fruit was divided in five groups of 640 each and assigned to four treatment combinations of delayed storage duration at different temperatures. T0 (control) fruit were immediately stored at 0°C for 150 d;T1 fruit were stored at 5°C for 30 d and at 0°C for120 d; T2 fruit were stored at 5°C for 60 d and at 0°C for 90 d, T3 fruit were stored at 10°C for 30 d and at 0°C for 120 d and T4 fruit were stored at 20°C for 3 d and at 0°C for 147 d.

All fruit were placed on trays and the trays packed in cardboard boxes with a 0.9% perforated polyethylene liner. Fruit were evaluated after 30, 60 and 150 d of storage. The experiment was carried out using independent storage chambers wit a volume of 12 m^3 and temperatures at 5 ± 0.5°C, 10 ± 0.5°C and 20 ± 0.5°C with 85 ± 5% relative humidity (RH). The temperature and RH of the storage rooms were monitored using an electronic logger (HOBO prp, Onset Computer Co., Cape Cod, MA). At each storage time (0, 30, 60 and 150 d), 160 fruit were evaluated in four groups of 40 fruit each (640 fruit in total). The four groups do not correspond to formal replications, since only one chamber was used per temperature because of that the treatments were applied in others two sources of fruit. In addition to SSC, TA and firmness, fruit were cut in half through the equator and scored visually for watercore intensity using the method described below.

2.1.3. Watercore assessment

Watercore assessment was carried out according to Bowen and Watkins (1997). Each fruit was cut in half and classified using a visual scale from 0 to 3, where: 0 = no watercore or less than 5% of affected area (healthy); 1 = watercore concentrated in the vascular tissue covering between 5-10% of the area (slight watercore); 2 = watercore expanded from the vascular tissue to the mesocarp covering between 10-25% of the area (moderate watercore) and 3 = watercore fills the mesocarp from the vascular tissue covering an area >25% (severe watercore). A severity index (1-3) was calculated as (numbers of fruit with slight watercore x 1 + numbers of fruit with moderate watercore x 2 + numbers of fruit with severe watercore x 3) / numbers of fruit affected by watercore. The percentage of affected tissue was calculated from digital photographs of the fruit in each category of damage (Canon PowerShot, G10 camera, Tokyo, Japan) set at a focal distance of 10 mm, exposure 1/13 s, resolution of 4416 x 3312 pixels (.jpg images) and colour space sRGB. Watercore severity was quantified as the percentage of cross-sectional area and calculated using the image-processing software ImageJ v 1.48 (https://imagej.nih.gov/ij/) with a filter designed to recognise the area considered in the analysis. The filter parameters for the ImageJ software were: Colour Thresholder Lab: L* 53-216, a*: 0-112, b*: 0-255.

2.1.4 Statistical analyses

Flesh firmness values and incidence of watercore during storage were analysed in a completely randomised experimental design. Analysis of variance (ANOVA) was carried out between the treatments (delay duration and temperature) for four replications of 160 fruit each, which were removed from storage at 0°C after 0, 30, 60 and 150 d. The LSD test for mean separation was assessed after each period of storage. As the group of fruit were not formal replications, the experiment was carried out in duplicate using fruit from the three orchards. In addition, the best fit for the curve of watercore incidence and time in storage was calculated when 50% of the watercore incidence had dissipated. An exponential decreasing function curve showed the best fit at $P \leq 0.05$; $Y = a\ e^{-mx}$ where m= rate of reduction %/day. The statistical software SigmaStat (Systat Software Inc. San Jose, Ca) was used.

Experiment 2. Detection and segregation by Vis-NIR

2.2.1 Fruit material

'Fuji' apples were selected from four orchards across the Central and Southern Valleys of Chile. Orchard 1 was located in Longaví (36°01'39.62"S 71° 38' 41.02"W), orchard 2 in Molina (35°48'42.07"S 71°32'1.65"W), orchard 3 in Retiro (36°09'46.8"S 71°43'54.5"W) and orchard 4 in Angol (37°49'13.6"S 72°38'00.1"W). Maturity at harvest was characterised on 50 fruit using the procedures described above. Mean values of flesh firmness, SS, TA and starch contents varied between 77.0 and 89 N, 14.8 and 16.4%, 0.25 and 0.4% and 8.2 and 9.0 starch, respectively. Fruit weighted between 229 and 243 g each. Fruit showing external defects were removed from the experiment. To assure a wide range in the incidence and severity of watercore a total of 560 fruit were selected: 80 fruit from orchard 1, 240 fruit from orchard 2, 160 fruit from orchard 3 and 80 fruit from orchard 4. The fruit were evaluated non-destructively and assessed destructively for watercore incidence and severity at harvest. Additionally, half of the fruit from orchard 3 (80 fruit) and all fruit from orchard 1 (80 fruit) were stored for 30 d at 0°C and then evaluate non-destructively and confirmed destructively the percentage of affected tissue by watercore. All fruit were transported to the Postharvest Laboratory at the Pontifical Catholic University of Chile on the day of harvest and the evaluations of semi-transmittance light were carried out after fruit had equilibrated to 20°C.

2.2.2 Vis-NIR measurements

Using equipment designed and built in our laboratory, semi-transmittance readings were taken at harvest on the cheek of each fruit at two orthogonal points. The sensor was aligned at 90° to the incident light. The transmittance spectra recorded for each fruit were averaged. The equipment comprised a 250 W halogen light source and the spectral data were collected using an HR4000 spectrometer (Ocean Optics, www.oceanoptics.com) fitted with an optical probe working in semi-transmittance mode (wavelength range between 100 and 1100 nm) positioned directly on the fruit surface. Each reading was carried out with an integration time of 700 ms and approximately 3,648 data values per reading were saved at 0.4 nm spectral resolution. Spectral acquisition and instrument controls employed a PC using in-house software.

2.2.3 Watercore incidence and severity

After the non-destructive measurements each fruit was halved. One half was immediately photographed with a digital camera as described in Experiment 1, and the percentage damage severity calculated in relation to the proportion of affected tissue on half of the fruit using ImageJ v 1.48, image-processing software (https://imagej.nih.gov/ij/).

Spectral data and model development

All spectral data were first processed with a Loess regression to create continuous values between 100 and 1100 nm (Blanco et al., 2000; Carlini et al., 2000; Liu et al., 2014; Rungpichayapichet et al., 2016) with a 10 exponential degree of adjustment and 0.2 size sampling. A temperature correction was then applied using as baseline a standardised white and black reference at 20°C equipment operation temperature. Transmittance percentages were calculated as described by Zhou et al. (2015). These adjustments were made using the software R v.3.1.2 (R Development Core Team, 2008).

After watercore severity was determined for each fruit, a dataset was constructed for all fruit in the study (560 fruit), where the watercore percentage for each fruit was linked to the relevant NIR measurement. This dataset was divided into two groups: for model calibration (70% of the fruit) and for validation testing (30% of the fruit) using the *caret* package. To avoid bias in model construction, this package carried out random sampling within the levels of the classes to balance the class distributions within the splits.

To determine the minimum percentage watercore severity into which the data could be split, logistic models were constructed using integer watercore percentage values from 5 to 30% which were used as boundaries for fruit classes WC-1 and WC-2. Each model was fine-tuned using three classification metrics: i) *accuracy* =

percentage of total observations accurately classified, ii) *specificity* = percentage of WC-2 fruit correctly classified, and iii) *sensitivity* = percentage of WC-1 fruit correctly classified. High values ensure the models studied show high accuracy classification rates and reduced numbers of misclassified fruit between classes (Fawcett, 2006; James et al., 2015; Tharwat, 2018).

Once this first boundary was determined, the WC-1 fruit were deleted from the dataset and the same process was applied to find the best percentage of watercore severity to separate the WC-2 fruit into two new classes: WC-2.1 and WC-2.2. Last, with the best watercore percentage that divided the dataset in three classes (WC-1, WC-2.1 and WC-2.2), Linear Discriminant Analysis (LDA) models were used to corroborate the possibility of achieving a classification model for fruit having differing degrees of watercore severity. ANOVA was used to find significant differences among the predictor variables between classes.

For all models, a prediction matrix was built with special feature spectral data such as: Area Under Spectral Curve (AUC) and transmittance values at 625, 670, 715 and 800 nm. Due to the differences in the predictor variable values, a standardisation of the prediction matrix was carried out before the models were developed. All modelling processes were carried out with the software R v.3.1.2 (R Development Core Team, 2008).

Results

3.1 *Experiment 1. Watercore reduction*

The pattern of watercore incidence in ‘Fuji’ apples at each level of severity obtained from the three orchards is shown in Figs. 1 and 2. At harvest, the fruit from orchards 1 and 2 showed the highest incidences (80-90%) of fruit affected by severity indices of 1.57 and 1.76, respectively. In contrast, fruit from orchard 3 presented a much lower incidence (40%) with severity index of 1.53.

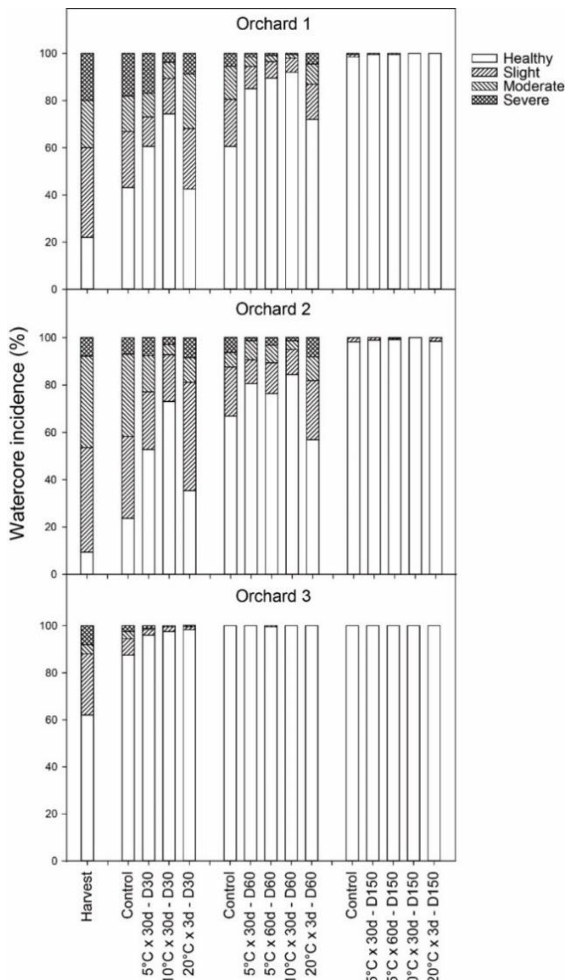

Figure 1. Watercore incidence and severity in ‘Fuji’ apples from three orchards, evaluated at harvest and after 30 (D30), 60 (D60) and 150 (150D) days of storage. Several combinations of delayed storage duration and temperature treatments were evaluated at each storage time and until complete 150 d at 0°C: Control (T0 fruit were immediately stored at 0°C for 150 d); 5°C x 30d (T1 fruit were stored at 5°C for 30 d and at 0°C for 120 d); 5°C x 60d (T2 fruit were stored at 5°C for 60 d and at 0°C for 90; 10°C x 30d (T3 fruit were stored at 10°C for 30 d and at 0°C for 120 d) and 20°C x 3d (T4 fruit were stored at 20°C for 3 d and at 0°C for 147 d).

The incidence of watercore reduced during storage at 0°C, achieving almost 100% of healthy fruit after 150 d storage, regardless of the fruit source (Figs. 1 and 2). The watercore reduction was faster in fruit from orchard 3 where almost 100% of the fruit was healthy as early as 30 d. Delayed storage for 30 d at 5°C or at 10°C speeded the reduction of watercore incidence compared with the control. In fruit from orchard 1 the percentage of affected fruit after 30 days was 56.9% and after 60 days it was 39.5%. However, using delayed storage for 30 d at 5°C the incidences were 39.3% (30 d) and 15% (60 d) otherwise when the delay storage was done at 10°C the total incidence was reduced to 25.6% (30 d) and 8% (60 d) (Fig. 2). Similar results were obtained when storage was delayed by 60 d at 5°C. The results were similar when the treatments were replicated with high severity fruit from orchard 2 or low severity fruit from orchard 3 (Figs. 1 and 2).

Measurements of firmness during storage show that fruit were softer after 30 days at 10°C or after 3 d at 20°C than after 30 d at 0°C. However, firmness was similar among the delayed storage treatments during the following 90 d of storage at 0°C (Fig. 2B).

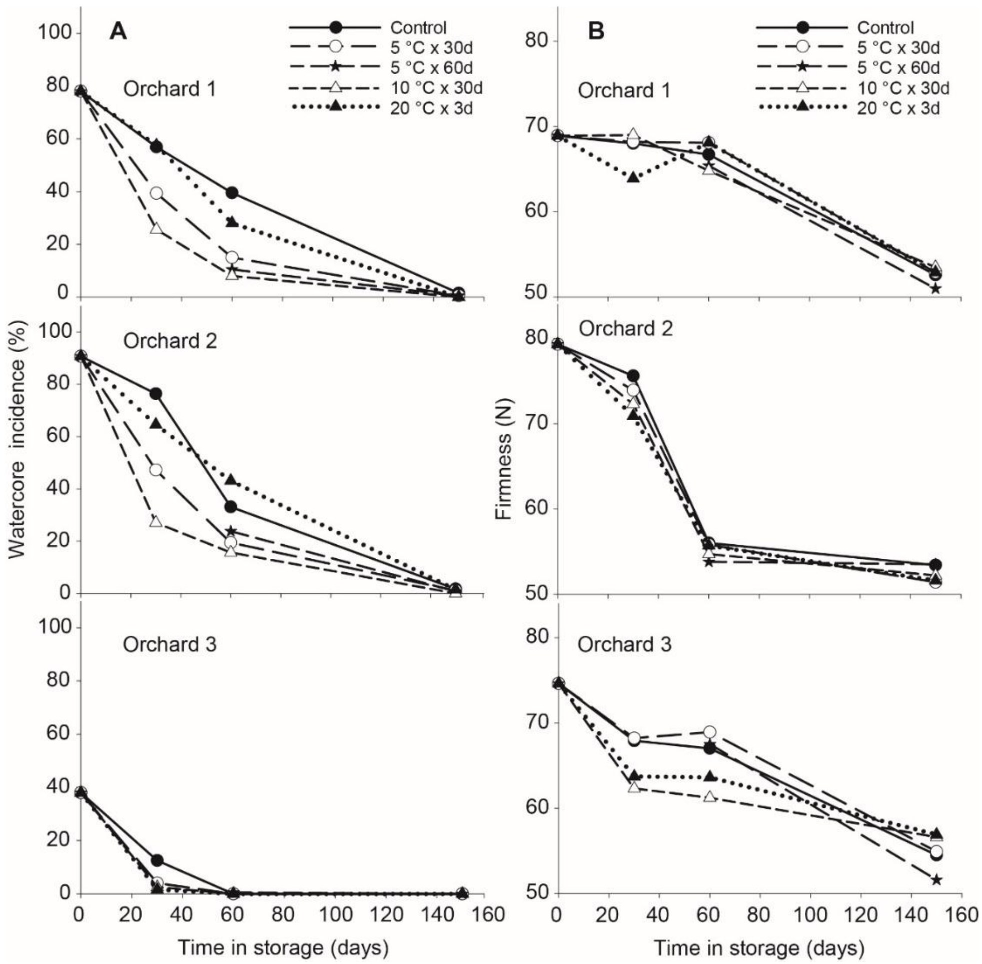

Fig 2. Watercore incidence (A) and firmness (B) of 'Fuji' apples following delayed storage at different temperatures. Control (T0 fruit were immediately stored at 0°C for 150 d); 5°C x 30d (T1 fruit were stored at 5°C for 30 d and at 0°C for 120 d); 5°C x 60d (T2 fruit were stored at 5°C for 60 d and at 0°C for 90 d; 10°C x 30d (T3 fruit were stored at 10°C for 30 d and at 0°C for 120 d) and 20°C x 3d (T4 fruit were stored at 20°C for 3 d and at 0°C for 147 d).

Based on the average watercore incidences in the three orchards, the rate of watercore reduction among the various delayed-storage treatments is satisfactorily described by the exponential decay curves: $Y_{control,\ 20°C\ x\ 3d} = 75.9\ e^{-(0.016\ X)}$ $R^2 = 0.96$; $Y_{5°C\ x\ 30d\ or\ 60d} = 72.8\ e^{-(0.026\ X)}$ $R^2 = 0.99$; and $Y_{10°C\ x\ 30d} = 72.6\ e^{-(0.04\ X)}$ $R^2 = 0.99$ (Fig. 3). From these we can infer by interpolation that a 50% reduction in watercore incidence at harvest was attained after 17.6 d (T3), after 26 d (T1 and T2) and or after 44 d (T0) (control). A high temperature delay (3 d delayed at 20°C) did not speed the reduction of watercore (Fig. 3).

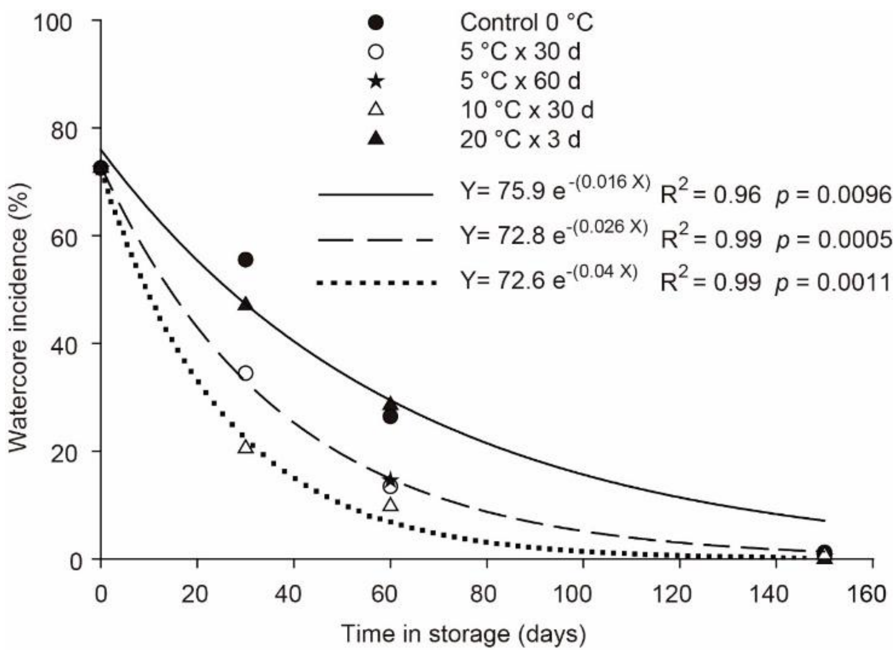

Figure 3. Reduction of watercore in ‘Fuji’ apples during storage at 0°C following various storage delay pre-treatments. Each point is the average watercore percentage of affected fruit from three orchards (640 fruit per orchard). Control (T0 fruit were immediately stored at 0°C for 150 d); 5°C x 30d (T1 fruit were stored at 5°C for 30 d and at 0°C for 120 d); 5°C x 60d (T2 fruit were stored at 5°C for 60 d and at 0°C for 90 d; 10°C x 30d (T3 fruit were stored at 10°C for 30 d and at 0°C for 120 d) and 20°C x 3d (T4 fruit were stored at 20°C for 3 d and at 0°C for 147 d).

3.2 *Experiment 2. Watercore detection and segregation using Vis-NIR*

3.2.1 *Watercore severity and incidence*

Watercore severity in ‘Fuji’ apples was wide-ranging with the affected area of pulp varying from 1.6 to 43.6% with a mean affected area of 14.4%. However, most (>80%) fruit showed watercore affected areas between 5 and 20%.

Harvest evaluation of fruit from orchard 3 showed that 80% of the fruit had >15% of watercore tissue, whereas watercore (>15%) affected 24% of fruit from orchard 2 and 37% from orchard 4. After 30 days storage at 0°C, the watercore areas of half of the fruit from orchard 1 were <10%, while for fruit from orchard 3 ~83% of fruit suffered watercore severities >20% (data not shown).

3.2.2 Vis-NIR detection of fruit affected by watercore

Fruit with watercore had higher transmittance spectra than healthy fruit (Fig. 4). The mean spectral curves for unaffected and affected fruit showed characteristic inflexion points at about 625, 670, 715 and 800 nm.

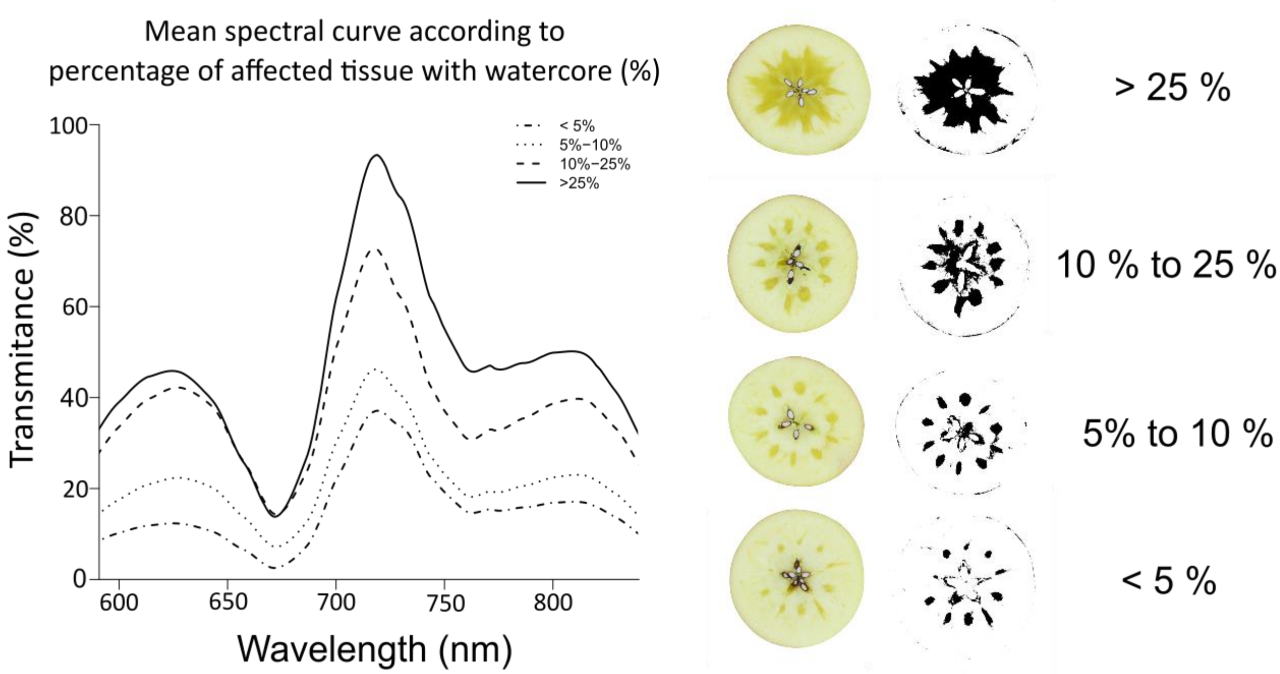

Fig. 4. (Left) Typical semi-transmittance spectra between 600-800 nm in ‘Fuji’ apples with different watercore percentages determined by semi-transmittance acquisition light equipment. (Right) Photographic record and image processing of watercore affected ‘Fuji’ apple.

To detect a range of watercore severities in ‘Fuji’ apples non-destructively, five different logistic models were examined with different combinations of predictor variables (Model 1: AUC, L625, L670, L715 and L800; Model 2: AUC; Model 3: L625, L670, L715 and L800; Model 4: L670 and L800; Model 5: AUC, L670 and L800). Logistic models were constructed using integer watercore percentage values from 5 to 30%, as boundaries for classes WC-1 and WC-2. Each logistic model was fine-tuned using the Receiver Operating Characteristic (ROC) curve to identify the best cut-off (data not shown) for which model accuracy is maximised, and misclassification is minimised. We examined 130 logistic models (Supp. Fig. 1.)

The percentage success in detecting WC-2 fruit (specificity) shows a sharp decline when the limit of class separation is >15% of the area affected by watercore. Thereafter, the ability to correctly detect WC-2 fruit decreases drastically. This confirms <15% is the limit for WC-2 fruit. Finally, the ability to correctly identify WC-1 fruit (sensitivity) increased, reaching a success rate >80% when the limit between classes was >15% of watercore severity.

The proportion of 15% of tissue affected with watercore is proposed as the class separation limit for WC-1 and WC-2 fruit. With this 15% limit, almost all models showed success rates >75%, except for model 2 (AUC). Most of the models examined showed acceptable identification levels (sensitivity >70%) for WC-1 fruit (Table 1). Considering the performance in the calibration and validation datasets, the best models were: models 1 and 3 (Supp. Fig.1). Model 3, with a class boundary of 15% of watercore severity, successfully identified 130 WC-2 fruit out of the 160 in the calibration dataset. Only 28 WC-1 fruit were misclassified in the test dataset. Model 3 classified 10 fruit as WC-1 out of 68 WC-2 fruit (Table 2).

Table 1. Models used for sorting WC-1 and WC-2 watercore 'Fuji' apples, using L625, L670, L715, L800 as predictor variables and a boundary for class separation of 15% of watercore severity. (Model 1: AUC, L625, L670, L715 and L800; Model 2: AUC; Model 3: L625, L670, L715 and L800; Model 4: L670 and L800; Model 5: AUC; L670 and L800).

	Calibration dataset			Validation dataset		
	Accuracy	Specificity	Sensitivity	Accuracy	Specificity	Sensitivity
Model 1	80%	83%	78%	75%	85%	69%
Model 2	72%	82%	65%	67%	84%	56%
Model 3	79%	81%	78%	77%	85%	72%
Model 4	78%	73%	82%	79%	82%	77%
Model 5	79%	66%	88%	76%	63%	85%

Table 2. Confusion matrix for logistic Model 3 (splitting WC-1 and WC-2 classes) (calibration and test datasets) which used L625, L670, L715, L800 as predictor variables with a boundary of classes of 15% of watercore severity.

		Predicted Classes			
		Calibration		Validation	
		WC-1	WC-2	WC-1	WC-2
Obs. Class	WC-1	182	51	71	28
	WC-2	30	130	10	58

Using a logistic model for splitting WC-2 fruit into two classes (WC-2.1 and WC-2.2) did not achieve acceptable results. The accuracy rate for dividing WC-2 fruit (>15% of watercore severity) was <80% if the split boundary between 20 to 30% of watercore tissue was used. Our results suggest the optimal limit of separation for WC-2 fruit is ~30% of the tissue cross-section affected by watercore (Supp Fig. 2).

Lastly, using the same combination of predictive variables and considering the limits between classes in the former logistic models (15 and 30% of watercore tissue), an LDA model was built to sort these three classes (WC-1 <15%, WC-2.1 15-30% and WC-2.2 >30%) (Table 3).

Table 3. LDA model sorting WC-1, WC-2.1 and WC-2.2 watercore 'Fuji' apples, using different spectral features as predictor variables and a boundary for class separation of WC-1 <15%, WC-2.1 15-30% and WC-2.2 >30%) of watercore severity. D.R.=percentage of successful Detection Rate. (Model 1: AUC, L625, L670, L715 and L800; Model 2: AUC; Model 3: L625, L670, L715 and L800; Model 4: L670 and L800; Model 5: AUC; L670 and L800).

	Calibration dataset				Validation dataset			
	Accuracy	WC-1 D.R	WC-2.1 D.R	WC-2.2 D.R	Accuracy	WC-1 D.R	WC-2.1 D.R	WC-2.2 D.R
Model 1	72%	90%	50%	33%	68%	83%	47%	44%
Model 2	62%	84%	36%	40%	61%	84%	31%	11%
Model 3	74%	91%	52%	38%	70%	87%	43%	67%
Model 4	73%	91%	50%	33%	68%	89%	38%	33%
Model 5	74%	91%	52%	42%	69%	87%	43%	33%

All LDA models achieved a successful detection rate for WC-1 fruit of approximately 90% in the calibration dataset and 85% in the test dataset. This confirms detecting and sorting WC-1 fruit can be carried out with high reliability. However, the opposite situation occurs in fruit with WC-2.1 and WC-2.2 watercore symptoms, where the correct detection of fruit with WC-2.1 class damage was ~50% in the calibration dataset and 45% in the test dataset. On the other hand, the successful detection of fruit with WC-2.2 watercore was <40% in most cases. The confusion matrix for LDA Models 3 and 5 (Table 4) shows that fruit with WC-2.2 damage not detected or classified correctly, were classified as fruit with WC-2.1

damage. This WC-2.1 damage classification, although not accurate, still allows the model to classify fruit that should be separated from the WC-1 fruit class.

Table 4. Confusion matrix for LDA Model 3 (L625, L670, L715 and L800) and Model 5 (AUC; L670 and L800) (calibration and test datasets) sorting watercore Fuji apples into three classes: WC-1 <15%, WC-2.1 15-30% and WC-2.2 >30% of watercore severity.

			Predicted Classes					
			Calibration dataset			Validation dataset		
			WC-1	WC-2.1	WC-2.2	WC-1	WC-2.1	WC-2.2
Observed Classes	LDA 3	WC-1	213	20	0	86	13	0
		WC-2.1	58	71	8	31	25	2
		WC-2.2	4	11	9	1	2	6
	LDA 5	WC-1	212	21	0	86	13	0
		WC-2.1	58	71	8	31	25	2
		WC-2.2	3	11	10	1	5	3

Discussion

Watercore is a physiological disorder that appears on the tree during fruit maturation. In some markets (such as in Japan) watercore is considered a sign of high fruit quality. However, when the severity of watercore is high (the flesh shows an extensive water-soaked appearance) watercore carries the intrinsic risk of subsequent tissue breakdown, with symptoms of internal browning. In addition, fruit with watercore is at higher risk of developing internal browning when under controlled atmosphere storage. Moreover, there can be a significant correlation between watercore severity and the incidence of CO_2-injury (Argenta et al., 2002).

While it is evident that the incidence of watercore at harvest is influenced by environmental factors during fruit development, preharvest management options for limiting watercore are substantially unavailable. Sorting the fruit in the packing house for incidence and severity of watercore employing expensive equipment has been proposed by some commercial companies. Instead, the disorder may be amenable to management by an integrated approach. Hence, we here propose a protocol for rapid reduction in watercore incidence and severity first by in-line evaluation of a fruit sampling using low-cost equipment based on semi-transmitted light to discriminate the incidence /severity of watercore.

Postharvest reduction of watercore incidence has been demonstrated with 'Fuji' apples, which is faster for fruit suffering lower incidence (<40%) and lower severity (1.53 index). This result is similar to the reduction of watercore incidence observed in 'Braeburn' apples, which reduces most rapidly (3-4 weeks) when the initial severity is <25% (Clark and Richardson Enza, 1999).

The watercore reduction rate can be derived from a decreasing exponential function of watercore incidence during storage ($Y = a\ e^{-mx}$, where Y= percentage of watercore incidence, and *m*= reduction rate). High reduction rate was achieved with delayed cooling-temperature treatments, being faster with delayed cooling for 30 days at 10°C and ineffective with delayed cooling for 3 days at 20°C. Similar reduction rates were observed in 'Gloster' apples when stored at 3.5 or 10°C instead of at 2°C (Köpcke, 2015). Neuwald et al. (2012) evaluated several alternatives for watercore reduction in 'Fuji', concluding that in a fruit population with severe watercore at harvest, a feasible alternative was to treat the fruit with 1-

MCP and to delay cooling for 20 d at 10°C before storage under a controlled atmosphere (CA). Otherwise, ‘Fuji’ apples affected with watercore reduce faster under CA storage of <1.0 rather than 2.5 kPa of CO_2 (Kweon et al., 2013).

Water-soaked areas, with sorbitol accumulation in tissues adjacent to the vascular core are the primary cause of watercore. These occur under conditions of low expression of sorbitol transporters (Gao et al., 2005). Reabsorption of the intercellular fluid is a dominant factor in the reduction of watercore during storage (Harker et al., 1999). Hence, practices that inhibit fruit metabolism such as CA storage of ‘Delicious’ (Hung et al., 1994) or 1-MCP application to ‘Gloster’ (Köpcke, 2015) also maintains watercore symptoms longer after harvest. Meanwhile, delayed storage with use of higher temperatures would increase the rate of fruit metabolism resulting in less soaked tissues as was observed in this study for ‘Fuji’. Unfortunately, the detailed changes occurring during the process of watercore reduction are only partially understood.

The optimal protocol to reduce water core incidence depends on its severity, therefore, an integrated approach for managing this disorder requires information on the level of watercore present in the fruit tissues. We show that the percentage of watercore affected tissue can be evaluated by a non-destructive technique using transmittance acquisition of light between 100 and 1100 nm. This technique is able to separate fruit with low watercore percentage (WC-1), which are considered healthy, from more severely affected fruit (WC-2). The fruit had transmittance spectra proportional to the watercore severity. Logistic models constructed with predictor variables (AUC, at 625, 670, 715 and 800 nm) allow WC-1 (healthy fruit with <15% of cross-section affected by watercore) to be successfully identified with accuracy, specificity and sensitivity >80%.

The logistic regression models proved unsuitable for segregating WC-2 fruit into WC-2.1 and WC-2.2 groups. An ANOVA of the spectral features used as predictive matrix in the models confirmed there were no significant differences in the L625 and L670 values. Of the other variables examined, such as AUC, L715 and L800, the ANOVA results showed significant differences but dispersion of the observations was too high. This suggests that even with more observations, these variables will continue to show no significant differences (Supp. Fig. 3). Interestingly, the ANOVA results show that fruit with <15% of watercore severity (WC-1) showed significant differences in all the spectral features used in the modelling process.

The main difficulty with the non-destructive sorting of moderate (WC-2.1) from severe (WC-2.2) symptoms is that the spectral features used for the construction of LDA models did not present significant differences between classes as shown in the ANOVA (Supp. Fig. 3). Nevertheless, the segregation of healthy fruit showed high and similar accuracy rates to those found with the logistic models.

Despite the difficulties encountered in subdividing the WC-2 class, the LDA model shows it is possible to separate watercore severity into three different classes: healthy (WC-1 <15%), moderate (WC-2.1) and severe (WC-2.2). The LDA model identified fruit with low watercore severity WC-1 >90% but the model misclassified WC-2.1 and WC-2.2. This misclassification does not represent a problem in implementing the LDA model, since fruit with high watercore severity (severe and moderate) should be managed differently from those in which watercore symptoms can be reduced (healthy, <15%).

Light transmittance has been used previously for watercore discrimination (Birth and Olsen, 1964). These authors captured transmitted light by computer vision and used transmittance as an indicator of watercore, however, this method was unable to determine the degree of watercore severity (Throop et al., 1989). Later, Upchurch and Throop (1991) demonstrated that the camera sensitivity was inversely related to watercore severity. Then, watercore affected ‘Delicious’ fruit were successfully separated from healthy one using spectral reflection 690-700 and 820-830 nm but, again, this method was unable to discriminate between fruit on the basis of watercore severity (Bennedsen and Peterson, 2005).

The effectiveness of light transmittance is determined primarily by light capture and penetration, our Vis-NIR equipment was used for internal fruit screening by interrogating the fruit in a direction normal to the direction of the incident light (90° between light source and light sensor) so capturing only half of the transmitted light. However, this technique reduced the ability to detect watercore in the centre of the fruit. By improving the capture capabilities of the Vis-NIR equipment it may be possible to improve the method’s ability to discriminate between fruit on the basis is watercore severity. However, it is important to recognise that our evaluation was carried out under static conditions and with no restriction on collection time. Also, with no confusion caused by the possible presence of other internal disorders. In this case, more complex models would be required, especially when internal browning appears along with watercore.

Currently, the best technology for non-destructive quantification of watercore symptoms is NMR imaging, which has the ability to achieve a virtual reconstruction of the affected tissue volumes and so determine clearly the distribution and severity of the watercore. Hence, allowing accurate quantification (Cho et al., 2008; Clark and Richardson Enza, 1999; Wang et al., 1988). More recently, sophisticated and expensive equipment such as MRI and X-ray CT imaging have also been used to correctly classify between 79–89% of fruit as either healthy or affected by watercore (Herremans et al., 2014). However, although some effort has been made to lower the high cost of these methods (Chayaprasert and Stroshine, 2005), high cost and low portability remain insurmountable barriers to the deployment of such equipment in the packing house.

Conclusion

Natural reduction in watercore was speeded by 30 d delayed cooling at 5 or 10 °C. Non-destructive detection of watercore using Vis-NIR models are able to distinguish between healthy (<15% affected tissue) and watercore affected fruit (>15% affected tissue) with an accuracy >90 %. However, the models had some difficulty identifying between moderate (15-30%) and severe (>30%) watercore fruit. Hence, the combination of watercore reduction methods (30 d delayed storage at 5°C) and relatively cheap and portable Vis-NIR equipment (able to discriminate between <15% and >30% watercore affected tissue) offers an interesting possibility for segregating the more-severely watercore-affected fruit and so managing this problem and avoiding high levels of this disorder in subsequent storage. Further work is needed to improve watercore characterisation using our Vis-NIR equipment and to also take account of the presence of other internal disorders.

Declaration of interest

None.

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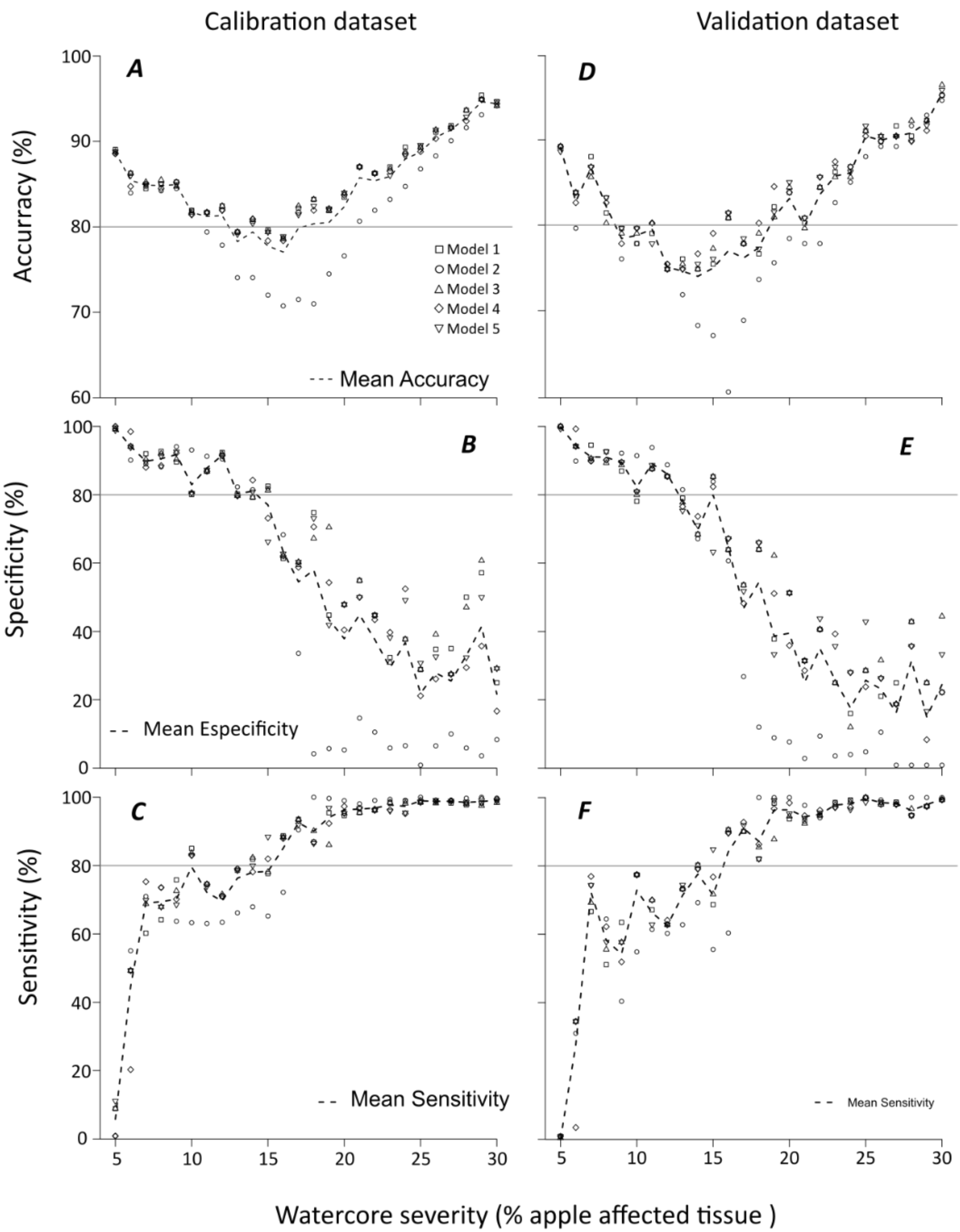
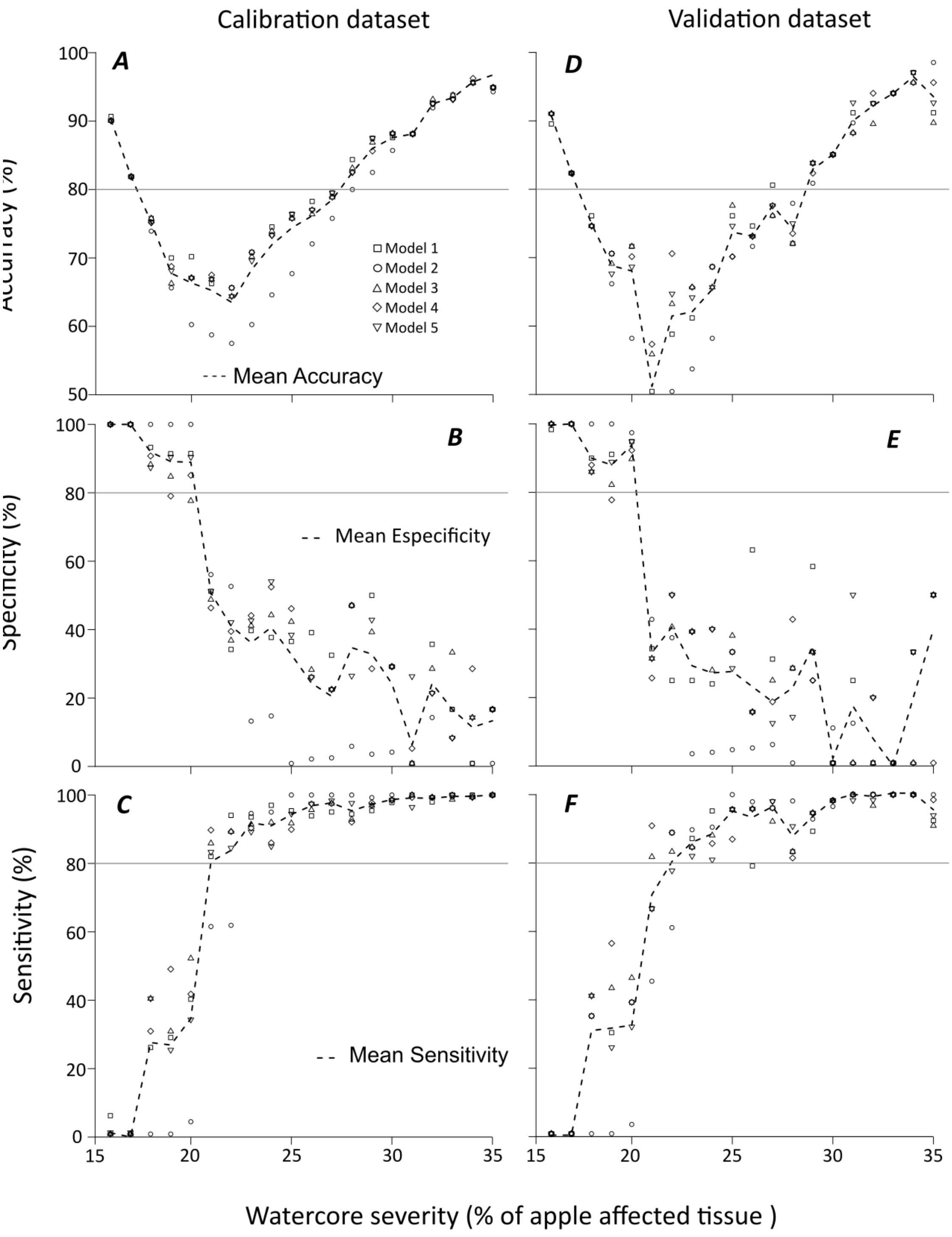
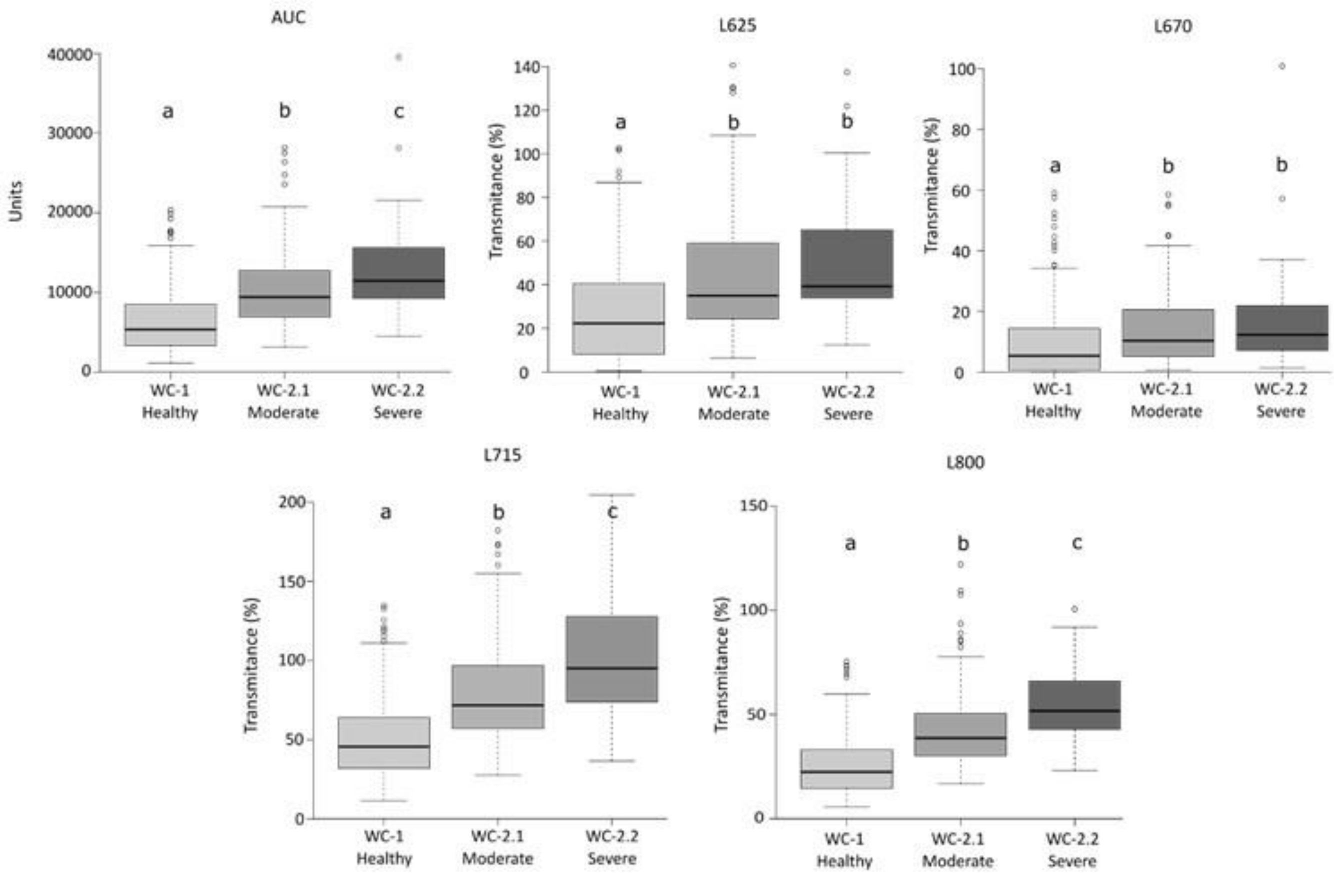


Supp. Fig. 1. Logistic models for sorting between class WC-1 and class WC-2 watercore affected ‘Fuji’ apples. Models were constructed having different combinations of predictors and different boundaries for class separation. The grey horizontal line shows an acceptable value for accuracy, specificity and sensitivity in the classification models.



Supp. Fig. 2. Logistic models developed for sorting class WC-2 watercore affected 'Fuji' apples into subclasses WC-2.1 and WC-2.2. Each model was constructed with a different combination of predictors and boundaries for class separation. The grey horizontal line shows an acceptable value for accuracy, specificity and sensitivity in the classification models.



Supp. Fig. 3 Boxplot for spectral features used in the logistics models for sorting subclasses WC-2.1 and WC-2.2 watercore affected ‘Fuji’ apples. Different letters indicate significant differences based on the HDS Tukey test (*P* value <0.05).

4. VIS-NIR models for early detection of bitter pit in ‘Fuji’ apples

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Abstract

Bitter pit (BP) is a physiological disorder that develops in the fruit surface mainly during storage. Its symptomatology is associated with the appearance of necrotic lesions and corky areas near the calyx end, affecting fruit appearance and decreasing the expected value of the entire lot. Different attempts to predict this disorder have been made with available non-destructive techniques. The objective of this study was developed VIS-NIR models for early detection of BP incidence and severity in 'Fuji' apples. Partial Least Square (PLS) classification models obtained from spectra reflectance between 950 to 1200 nm were compared, according to different levels of severity (number of pits recorded per fruit) over 150 days of storage. these models used data collected in two years (2018-2019) and four orchards (two orchards per year). PLS models were evaluated for accuracy, sensitivity, specificity, positive predicted value (PPV) and negative predicted value (NPV). Accuracy, specificity and NPV values varied between 60 to 80 % independent of the storage time in validation dataset. Contrasting results were obtained for sensitivity and PPV, where values did not exceed 60 % in the same dataset. Regarding BP severities, fruit with less than 8 pits achieve accuracy and NPV between 60 to 70 % in calibration and validation dataset, respectively. Comparison of classification metrics according to different severity levels showed that the models constructed detect low severities (1-7 pits) with accuracy and NPV between 60 to 70%, while for the detection of high severities (8-9 pits) these same metrics can reach between 80 and 90% during storage. Although the results show promising application of VIS-NIR models to predict at harvest BP during storage,

more studies are required to improve the model performance to predict BP incidence in the fruit at harvest.

Keywords: Bitter pit, Classification model, Vis-NIR, non-destructive technology

Introduction

Bitter pit (BP) is a physiological disorder defined as small round brown lesions with corky and bitter texture developed on the surface of the fruit during storage (Jarolmasjed et al., 2016). It is believed that mineral imbalance can play an important role on determining fruit susceptibility to BP (Fallahi et al., 1997; Ferguson et al., 1999; Perring and Pearson, 1986). Black spots symptoms of BP occur by the collapse of cells, which is attributed to abnormal calcium homeostasis in the cell leading to losing plasma membrane compartments because of depletion of apoplastic calcium (de Freitas et al., 2010). The random expression of BP during storage, causes uncertainty in the commercialization of the lot producing a large economic loss for the company and for apple industry in general in the market (Jemrić et al., 2016).

Nowadays, there is not a consensus about the main cause that trigged BP symptom in apples. Some researchers have been focused on unbalance in the mineral nutritional induced by calcium deficiency in the fruit tissue (Fallahi et al., 2006; Ferguson and Watkins, 1992; Perring and Pearson, 1986; Torres et al., 2017b, 2017a; Zúñiga et al., 2017), relationship between different forms of available calcium (de Freitas et al., 2015), inverse relationships between calcium concentration ratio with other cations such as nitrogen, magnesium and potassium (Fallahi et al., 2006; Jarolmasjed et al., 2017; Jemrić et al., 2016).

Deficiency of calcium is normally associated with postharvest disorders. Hence most of preharvest factors that stimulates bitter pit disorder, are associated in some way with calcium nutrition (Conway et al., 2002; Ferguson et al., 1999). Calcium contents in fruit are usually lower than in other parts of the plant because majority of them is favored by the translocation and distribution promoted by the transpiration stream and poorly re translocated to the fruit tissue.

Regarding the relationship between calcium availability in fruit and bitter pit incidence, de Freitas et al. (2015) found that apples with bitter pit had a higher concentration of insoluble calcium than healthy ones during storage at 0 ° C. Otherwise, Falchi et al. (2017) after performing abscisic acid applications at different stages of flowering and fruit development, found that apoplastic calcium supply to the fruit is stimulated by ABA improving the expression of genes associated with the concentration and regulation of the calcium availability within cells, thus decreasing the incidence of bitter pit during storage.

Several studies have been carried out to develop prediction models for BP disorder. It has been indicated by different authors that performing mineral analyzes on fruit between 20-40 days before harvesting can serve as an indicator of risk to develop bitter in the postharvest period (Amarante et al., 2010; Retamales et al., 2000). It has also been proposed that BP prediction can be done by induction method. One of this methods is fruit´s infiltration with $MgCl_2$ 10-30 days before harvest or also BP symptoms could be induced by Bangerth method, in which fruit were immersed in a water solution containing 0.2% ethephon (Torres et al., 2015).

In the last decades, the use of Vis-NIR spectroscopy, as non-destructive technique, have been studied for determining quality parameters in fruit and vegetables. Although NIR radiation does not present energy absorption for macro and micro nutrients, different concentration of those could affect NIR spectra when they are binding to organic substances and/or to cellular structures, such as the cell wall, proteins and membrane (Ciavarella et al., 1998). Due to this, reflectance in the Vis-NIR range has been used to determine nutritional stages in agricultural products in a fast and economical way (Garcia-Sanchez et al., 2017).

Partial least squared model using NIR spectra had showed high accuracy rate estimating Nitrogen and Calcium contents in citrus leaves (Galvez-Sola et al., 2015); other mineral such as Phosphorus, Boron, Cooper and Magnesium did not achieve acceptable regression coefficients. Other reports had showed mineral estimations for potassium (Ciavarella et al., 1998) on grapes and rice or cooper and phosphorus in mate plants (Rossa et al., 2015). These results show that NIR spectra could response (or detect) to different concentration and mineral nutrients, but it is needed to study each fruit or vegetable specie as an independent subject because each one has a characteristic spectrum.

Regarding non-destructive methods to predict BP physiological disorder, Nicolaï et al. (2006) used hyperspectral images in the NIR range to identify lesions caused by bitter pit at harvest; Si and Sankaran (2016) used computed tomography for identifying pattern of bitter pit symptoms development starting within the fruit, while external symptoms are absent, this was corroborated by Jarolmasjed et al. (2016) using the same technique, but these authors highlighted that the identification of fruit with bitter pit is difficult when other types of external injuries are present such as those caused by mechanical damage.

Kafle et al. (2016), demonstrated that using the reflectance spectrum between 970-996 nm and 1130-1143 nm is useful for segregating healthy fruit from fruit with bitter pit using Quadratic Discriminant Analysis (QDA) and Supporting Vector Machine Classification (SVMC) models; Jarolmasjed et al. (2017) observed that the fruit that showed symptoms of bitter pit after 63 days of storage, showed high spectra of reflectance between 900-1200 nm from the beginning of storage compared to healthy fruit which kept their reflectance spectrum practically constant during the study. These authors also performed mineral analyzes for Ca, K and Mg for healthy fruit and bitter pit, concluding that the best inference from the reflectance spectra is for the Mg / Ca ratio which can be used as an indicator of risk for bitter pit.

Jarolmasjed et al. (2018) corroborates that reflectance spectral wavelengths of 730, 980, 1135, 1250 and 1405 nm are potentially useful for detecting bitter pit using logistic regression models; they also reported other spectral characteristics (665-797, 1217-1349 and 1410 nm) for recognizing lesions using hyperspectral images.

Former non-destructive techniques reports for predicting bitter pit incidence have mostly used expensive and / or difficult implementations equipment due to its operating times (Jarolmasjed et al., 2018, 2016; Nicolaï et al., 2006; Torres et al., 2015). Among the reports on the use of reflectance spectrometry for early prediction of this disorder are those made by Kafle et al. (2016) and Jarolmasjed et al. (2017) which harvest healthy and BP fruit, realizing that different severity levels (pit number) affect the reflectance spectra, thus they were able to model BP incidence using classification models such as PLS-DA, QDA and SVMC.

Nowadays, there are no scientific reports of an attempt to model the incidence of BP using harvested healthy fruit that is spectrally monitored while BP symptoms are expressed in storage. The objective of this study was to develop VIS-NIR models for the early detection of the incidence and severity of BP in "Fuji" apples.

Materials and Methods

Plant material.

'Fuji' apples were harvested at the beginning of commercial harvest in 2018 and 2019 from two different orchards one located in the central valley and the other in the southern valley of Chile. The selected orchards had historical record of high BP incidence in the fruit in previous years. For each orchard and year; seven hundred and fifty apples were picked and transported to the Postharvest Laboratory at the Pontifical Catholic University of Chile, Santiago, Chile. Each year 1,500 fruit were used for Vis-NIR data acquisition. Apples were stored at 0 °C for 150 days plus 10 days at 20 °C.

NIR measurements.

At harvest, the fruit was kept at 20°C and each fruit was labeled and three areas of 0.25 cm^2 were identified at the calix zone and followed individually during storage. NIR spectra data were collected from these areas using reflectance mode in the wavelength range of 900 to 2500 nm, with a sampling interval of 3.24 nm. The spectrometer NIRQuest 2.5 512 Vis / NIR (Oceans Optics, Florida, USA) was equipped with an optical fiber bifurcated and halogen lighting source HL-2000-FHSA. The measurements were done directly on the labeled areas considering an acquisition angle of 45°. The acquisition of reflectance spectra and visual inspection of the fruit were carried out every 20 days until 150 days at 0°C. VIS-NIR data collection was always accomplished at 20°C. All spectral data were adjusted with the Loess regression (exponential degree 10 and 0.2 sampling window). NIR data pre-processing consists of second derivative Savitzky-Golay (SG) and later Standard Normal Variate (SNV) transformation. These adjustments were performed using the software R v.3.1.2 (R Development Core Team, 2008).

Bitter pit assessment.

Each fruit was visually evaluated for external symptoms of BP at 0, 10, 30, 50, 70, 90, 110, 130 and 150 day of storage at 0 °C. BP incidence was calculated as the number of affected from the total fruit. BP severity was determined by counting the number of pits per fruit. At each evaluation day, photographic records were taken on each fruit section.

BP incidence model

NIR measurement protocol and BP assessment were repeated for two years in 'Fuji' apples. At the end of the both years, different BP incidence rate were observed. With the aim of having a balanced dataset, in 2018, from 1500 fruit, 135 were used, 81 which did not show symptoms of BP and 54 fruit which presented BP severity range between 1 and 22 visible lesions. In 2019, from 1,500 fruit, 285 healthy fruit and 190 fruit affected with BP were used with a severity ranged between 1 and 26 pits. Lenticel breakdown was also noticed in both years, but these fruit were removed from the experiment in order to have only healthy or BP fruit. For modeling, a ratio 3:2 between healthy and BP affected fruit was randomly selected. Wavelengths used for modeling were selected as follows; for each storage day and each severity degree (number of pits), principal component analysis (PCA) was accomplished between pitted and non-pitted fruit classes. The same procedure was repeated using ANOVA to find significant differences between classes.

PLS classification models were constructed for each storage day to determine when an early detection is possible. On the other hand, to determine the sensitivity of the NIR spectrum at different BP severities (number of pits), different classification models were adjusted by moving the classification boundary between 1 and 10 pits. In this procedure, as the boundary between classes moved, fruit of "Healthy" class were randomly eliminated in order to maintain the 3:2 ratio between the two classes ("Healthy" and "BP"). Finally, prior to the adjustment of each model, the observations were subdivided into two sets: 70% of the observations were used to calibrate the model and the remaining 30% for validation of the models.

The following classification metrics were used to evaluate PLS models: Accuracy, defined as the total percentage of correct class identification; Sensitivity, known as the percentage of correctly identified cases of BP class; Specificity, defined as the percentage of correct successes in the "Healthy" class; Positive Prediction Value (PPV), percentage of fruit with BP in the group predicted with the class BP and Negative Prediction Value (NPV) percentage of healthy fruit detected in the group predicted with the healthy class.

Finally, to determine the severity (number of pits) in which the classification models have a better performance, an ANOVA and Tukey HSD test was carried out with the values of the classification metrics of the models obtained for each severity.

Results

After two year of study and 3000 ‘Fuji’ apples tracked, a final dataset of 610 fruit (366 without visible lesions and 244 with more than 1 visible lesion) was obtained. Dataset used for the modeling, grouped a total of 610 apples in both years, 2018 and 2019. BP symptoms were visible after 110 days and 60 days at 0°C in 2018, and in 2019 seasons respectively. The incidence of BP was 4% in both orchards during 2018 season and 9% and 16% in 2019. Regarding BP severity, in both seasons, BP affected fruit presented in average four pits at the end of storage (150 d). Fruit showing the highest BP incidence had 22 pits in 2018 and 26 pits in 2019.

Main dataset had 610 fruit where 135 fruit (81 without BP lesions and 54 with 1 or more pits) were from 2018 and 475 fruit (285 without BP lesions and 190 with 1 or more pits) from 2019.

In both years, fruit showed BP severity greater than 11 pits. Modeling was accomplished with fruit showing BP severity between 1 and 10 pits. This is justified by the number of fruit that could be used to build the datasets. In Table 1 shows the number of fruit for each severity class observed. Most of the fruit presented a severity of less than 7 pits, so iteration process only was performed until 10 pit due a reduce number of fruit available to use.

Table 1. Distribution of the number of fruit for calibration and validation dataset constructions considering fruit from 2018 and 2019 years using different severity limits (1 to 10 pits) for the "affected" (BP fruit) and "not affected" (Healthy fruit) classes

		Calibration Dataset			Validation Dataset		
Pit number	Total Fruit	BP Fruit	Healthy Fruit	Total	BP Fruit	Healthy Fruit	Total
1	610	171	257	428	73	109	182
2	502	141	211	352	60	90	150
3	380	107	160	267	45	68	113
4	282	80	119	199	33	50	83
5	212	60	89	149	25	38	63
6	165	47	70	117	19	29	48
7	125	35	53	88	15	22	37
8	97	28	41	69	11	17	28
9	82	24	35	59	9	14	23
10	75	21	32	53	9	13	22

PCA analysis was performed on each selected storage day to find spectral features that could characterize "affected" or "not affected" fruit classes, did not achieve good results (Supp Fig 1).

Several combinations were analyzed, for each measurement day (0, 10, 30, 50, 70, 90, 110 and 150 d) different groups of “affected” and “not affected” fruit were stablished using severities between 1 to 10 pits; for example, when the class limit was 1 pit, “not affected” class included all the fruit that showed less than 1 pit, while “affected” class grouped all fruit that presented 1 or more pits. In each combination analyzed (7 measurement days by 10 severity levels), PCA biplots always showed that classes ellipses were overlapped and wavelengths were distributed evenly among classes. Using multivariate analysis was not able to select spectra features that could help in class separation, an ANOVA analysis was used by wavelength between classes (univariate approach).

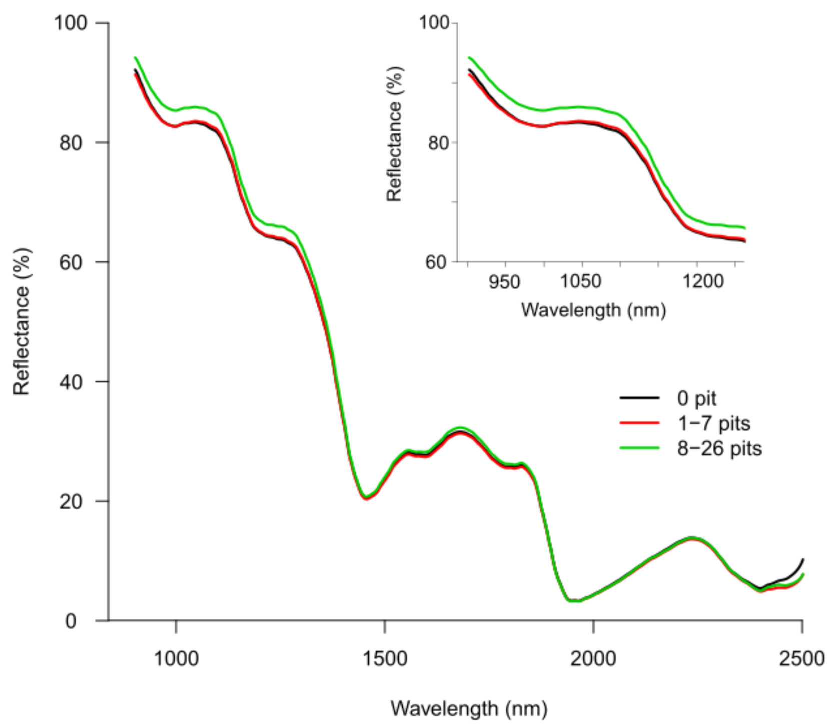

Fig. 1 Mean reflectance spectral curves for data between 900 to 2500 nm on 0 D for fruit without BP lesions (0 pit) (black line), fruit with 1 to 7 pits (red line) and fruit with more than 8 pits lesions (green line). Plot data are original data (without pre-processing). Embedded plot line shows a zoom in of the spectrum between 950 to 1200 nm

With this ANOVA analysis (Supp Fig 2), significant differences between classes were found, since 0 d of storage severities greater than 8 pits were able to separate using wavelength between 950 and 1400 nm. Although the ANOVA results for this range of the spectrum were not constant for the other combinations studied, the repetition of this pattern, significative differences between 950 to 1400 nm, was observed in several of them. Finally, the range between 950 and 1200 nm was used for BP modeling since these same spectral characteristics had been reported by other authors for this purpose (Jarolmasjed et al., 2016; Kafle et al., 2016; Zúñiga et al., 2017) and show different intensities for fruit with less than 8 pits in data collected on 0 D (Fig 1).

PLS models were adjusted using different severity values (1 pit to 10 pit) for each storage days. In Fig 2, percentages of correct classifications (Accuracy) in calibration dataset was between 56 to 96%, on the other hand, in validation dataset, accuracy showed values between 36 to 69%. Highest accuracy values (96%), was reached in calibration dataset after 30 D with a class limit of 9 pits, meanwhile, the lowest value (35%) was in validation dataset for 90 D and 8 pits.

Sensitivity, also named true positive rate or recall, measures the proportion of the fruit that actually showed BP symptoms (positive class) and were actually identified by the models. This metric had average values of 40% (min: 9% in 90 D and 4 pits; max: 96% with 30 d and 10 pits) in the calibration dataset, and 26% in validation dataset. In validation process, it was observed non-detection of BP fruit on days 0, 70 and 90 with 9, 10 and 7 pits, respectively. On the other hand, a maximum validation value of around 80% was observed using data from day 110 with 8 and 9 pits.

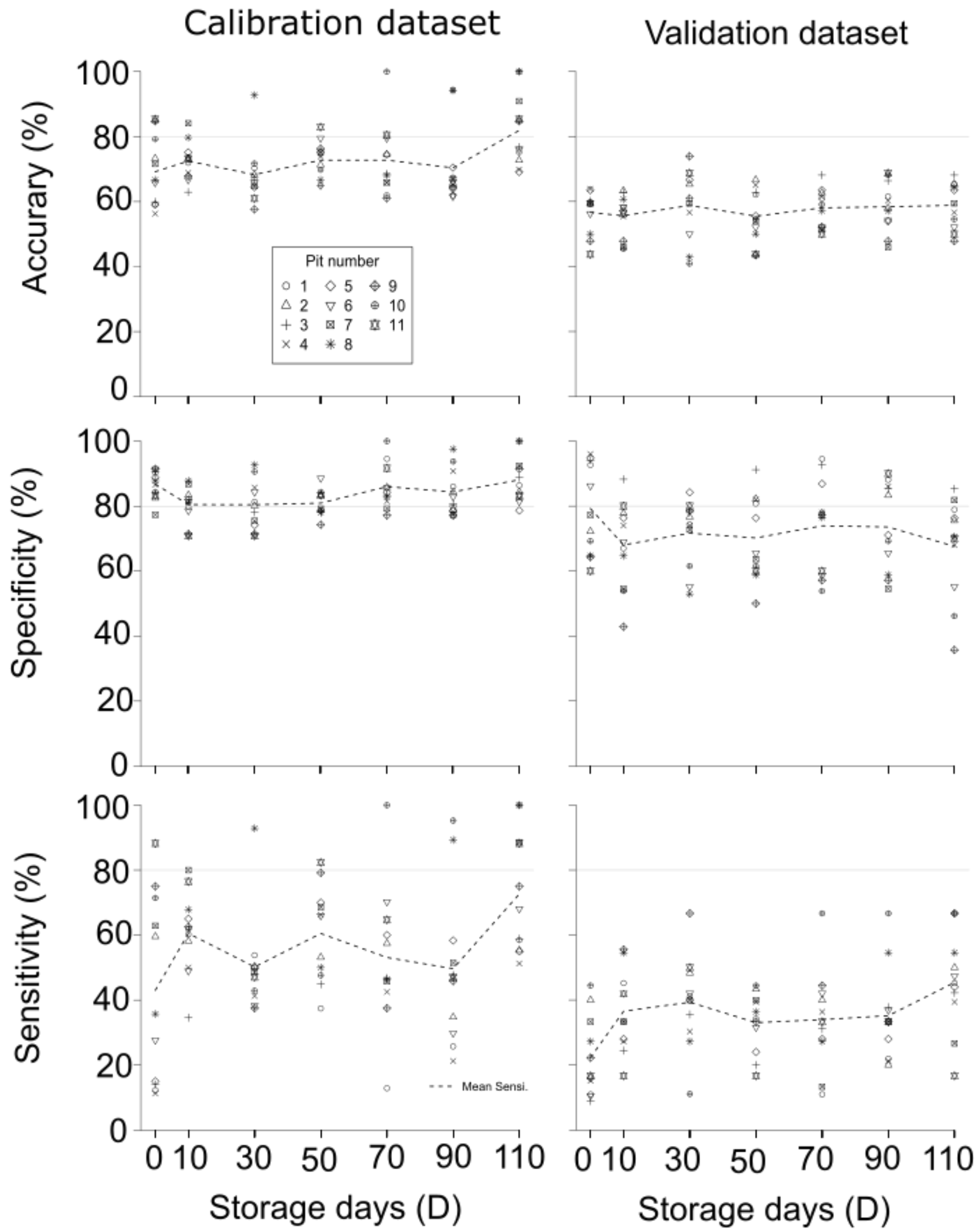

Fig. 2 PLS classification model por early detection of BP in Fuji apples using reflectance spectra between 950 to 1200 nm. Metrics show: Accuracy, Sensitivity and Specificity. Model were constructed using different class boundary (number of pit)

True negative rate, also known as specificity, relates the number of non-affected fruit correctly assigned in the non-affected class. In this metric, calibration set presented the maximum value of 97% on day 10 using 9 pit, while the minimum value was 71% in 30 days also with 9 pits; In general, specificity in calibration dataset showed an average value of 86%. During the model validation process, all 70 PLS models showed an average value of 77%. However extreme cases were observed where the specificity only reached 29% (90 D and 8 Pits) or was above 95% (0 days 4 and 13 pits).

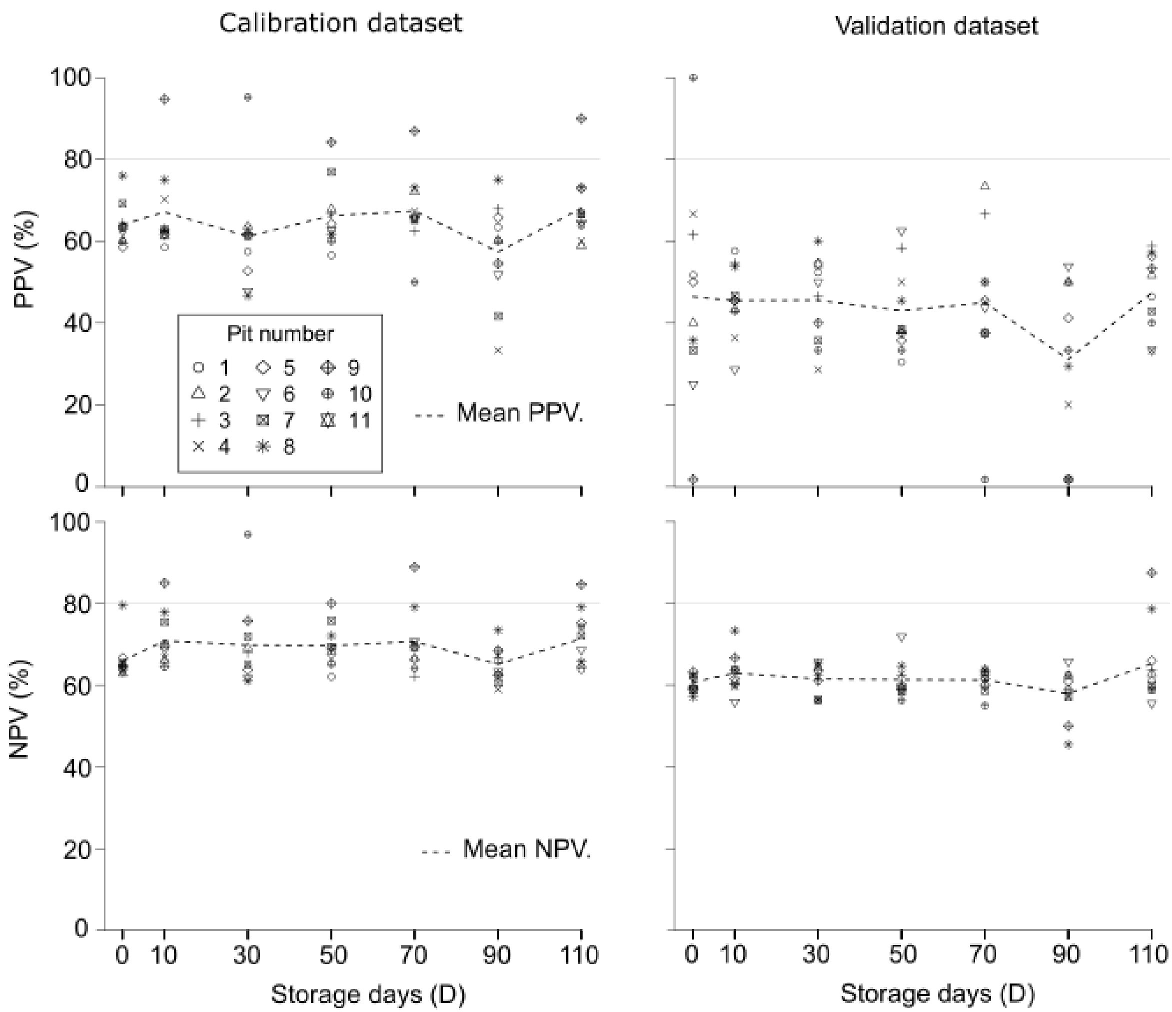

Fig. 3 PLS classification model por early detection of BP in Fuji apples using reflectance spectra between 950 to 1200 nm. Metrics show: Positive prediction value (PPV) and Negative prediction value (NVP). Model were constructed using different class boundary (number of pit)

Positive prediction value (PPV) shows the relationship between fruit with "affected" correctly classified and the total number of predicted fruit in the "affected" class. For this metric, about 75% of the models tested (53 of 70 models) showed higher values than 60% and an average of 64% in the calibration set (Fig 3). These values contrasted with the PPV obtained when validation datasets were used. In validation process, the average value of PPV was 43%. This average value is due to the fact that there were models in which the PPV was zero (0, 70 and 90 D with 9, 10 and 7 pits respectively) or even values greater than 73% were obtained on day 0 and 70 (10 and 2 pits respectfully).

Negative prediction values (NPV), which refers to the percentage of "non-affected" fruit correctly identified in the predicted group with the "not-affected" class, most of the models showed values greater than 60%, and a maximum of 96% on day 30 with 10 pits. Regarding the NPV values using the validation set, it was observed that along storage, the average value was 61%, reaching a maximum of 81% on day 90 with 8 pits.

Analyzing the potential according to BP symptoms severity (number of pits), significant differences were observed, according to the Tukey HSD test (p.value <0.05), between severity levels for accuracy and NPV in calibration dataset, and only in specificity for validation dataset (Fig 4).

Low severity levels, between 1 to 6 pits, showed no significant differences from each other unlike higher severity levels, such as 8 and 9 pits, which showed higher percentages of accuracy and NPV in both datasets used.

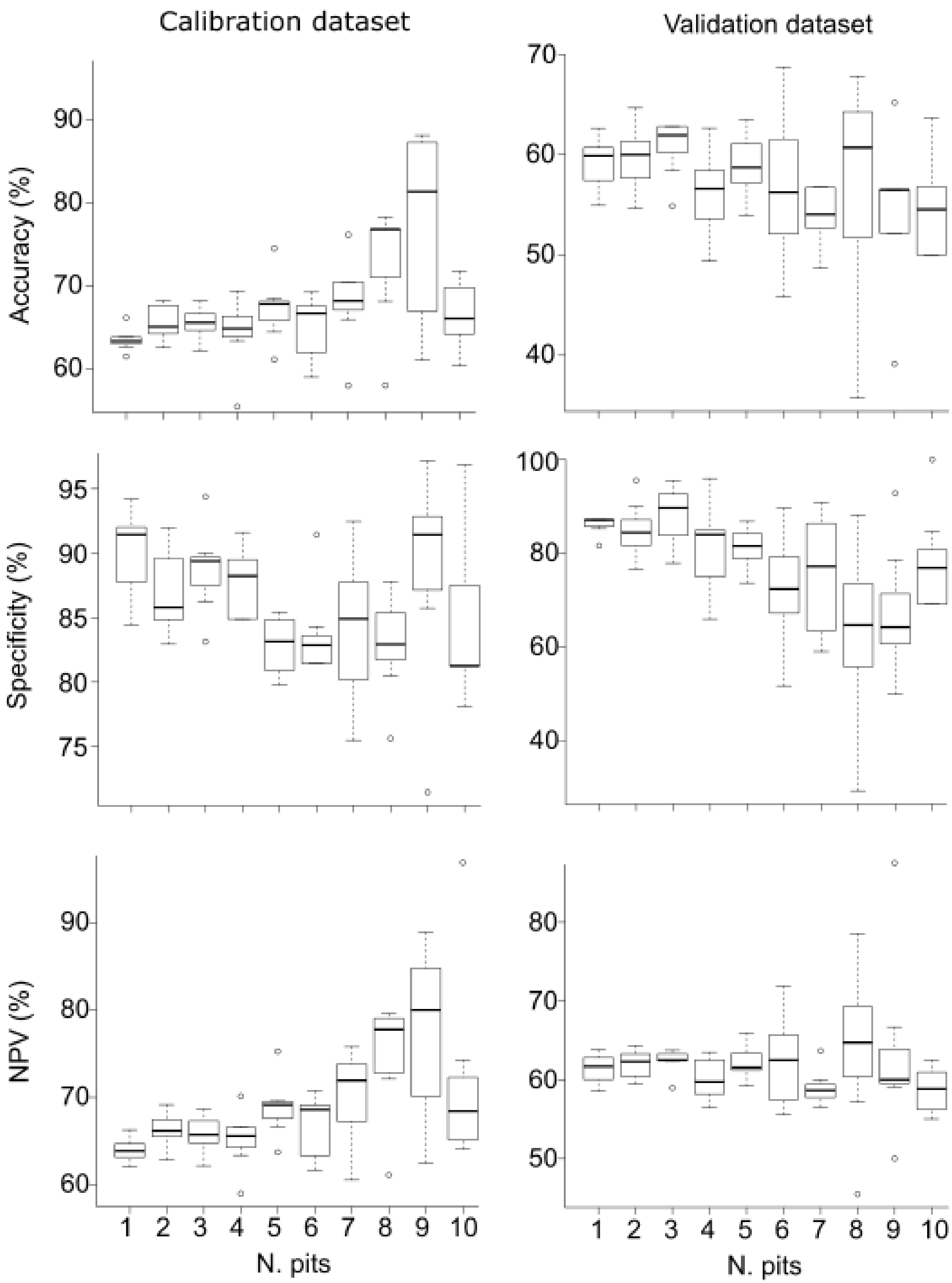

Fig. 4 Boxplot for different severity levels (pit per fruit) showing ANOVA results (p-value<0.05) for metric related with detection of non-affected class: Accuracy, Specificity and NPV.

Discussion

BP symptomatology causes corky skin lesions in apple fruit. This symptomatology is the main cause of product rejection due to visual defects. BP lesions can develop before harvest, but it is most common during storage. Due to this, different attempts to predict this disorder have been made with different non-destructive techniques such as X-rays, hyperspectral images and spectrometry in the Vis-NIR range (~ 400-2500 nm). Of these techniques, Vis-NIR is the most likely to be accepted and implemented by the industry due to low data acquisition times and lower implementation costs compared to the others (x-rays and hyperspectral images) (Nicolaï et al., 2006; Si and Sankaran, 2016).

This research sought to generate models for early detection of BP disorder in 'Fuji' apples along storage; For this, PLS classification models were compared, according to different levels of severity, over 150 days of storage. These models incorporated data from two consecutive seasons in which the incidence and BP severity observed was different (2019 the symptoms appeared at 60 days and fruit with more than 25 pits were observed).

Although the PCA analysis for choosing spectral features is the most recommended to reduce the number of variables before modeling, this was not successful since there were always overlaps in the class ellipses in the biplot diagrams. This may be because there was not an easily identifiable pattern, in spectral data collected, that related incidence or severity of BP with changes in the intensity in the spectrum as it was reported by Jarolmasjed et al. (2017). Other aspects to consider, is that former reports only analyzed data from one season and created datasets with different combination between them, so they did not have the difficulty for modelling with high variability generated by studying fruit from different season and places.

On the other hand, ANOVA results comparing each wavelength between 900 to 2500 nm between "affected" or BP, and "non-affected" or healthy classes, showed a repetitive pattern in which the range between 950 and 1200 nm showed significant differences between classes. This result was similar to the spectrum ranges used by Jarolmasjed et al. (2016), Kafle et al. (2016) and Zúñiga et al. (2017) to model BP incidence in different apple cultivars.

Although, mean spectral curves for fruit with and without BP symptoms seems to be similar between 900 to 2500 nm, different reflectance intensity were observed between fruit without symptoms, fruit between 1-7 pit lesions and fruit with high BP severity (more than 8 pits) in the range finding by ANOVA (950-1200 nm). In our research, mean spectra reflectance of BP fruit (at 0 D) with more than 8 pits (Fig. 1), showed high spectra than those with maximum 7 pit. This difference in was reported for Honeycrips cultivar by Jarolmasjed et al. (2017), although it could not been seen for others studied cultivars (Golden Delicious and Granny Smith).

PLS models reported in this study, presented accuracy percentages between 56 and 96% in calibration and 39 to 69% in validation. Although these percentages are lower than those reported by Zúñiga et al. (2017), they are similar to those reports by Jarolmasjed et al., 2017) and it can be considered an acceptable classification metric considering that our models incorporate greater variability (consecutive years, places and severity levels) that is reflected in low percentages for classification metrics. In addition to this, it should be highlight that our models were validated with different observations than those used during the calibration process.

Accuracy, specificity and NPV showed mean values constant along storage (Fig. 2 and 3) reaching values between 60 and 80%. These classification metrics demonstrate that for the first days of storage, between 10 and 30 days (when there are no visible BP symptoms in the fruit), it is possible to detect fruit that end storage period with less than 7 pits with a specificity around 80%, and at NPV greater than 60%

PLS accuracy model's values were below to expected (80%), this was directly related to the high variability in the detection of "affected" fruit, represented by sensitivity and PPV. Contrary to this, PLS models presented a good detection rate for "non-affected" fruit, in different combinations studied (storage days and severity).

In this study, fruit developed BP symptoms on different storage days in both years, future work should be done to corroborate if it is possible to develop a model to predict the time of BP incidence.

Conclusions

The PLS models studied confirm that the implementation of Vis-NIR spectrometry is possible for the early detection of BP in not symptomatic 'Fuji' apples. Our results show early detection of BP fruit with severity less than 8 pits is possible achieving accuracy between 60 to 70 % with just 10 storage days, while for detection of high severities (8-9 pits) these same metrics can reach between 80 and 90% along storage. Also it was noticed higher values in metrics related with detection of healthy fruit (specificity and NPV). The use of these models would determine potential risks of lots of fruit to develop BP during storage.

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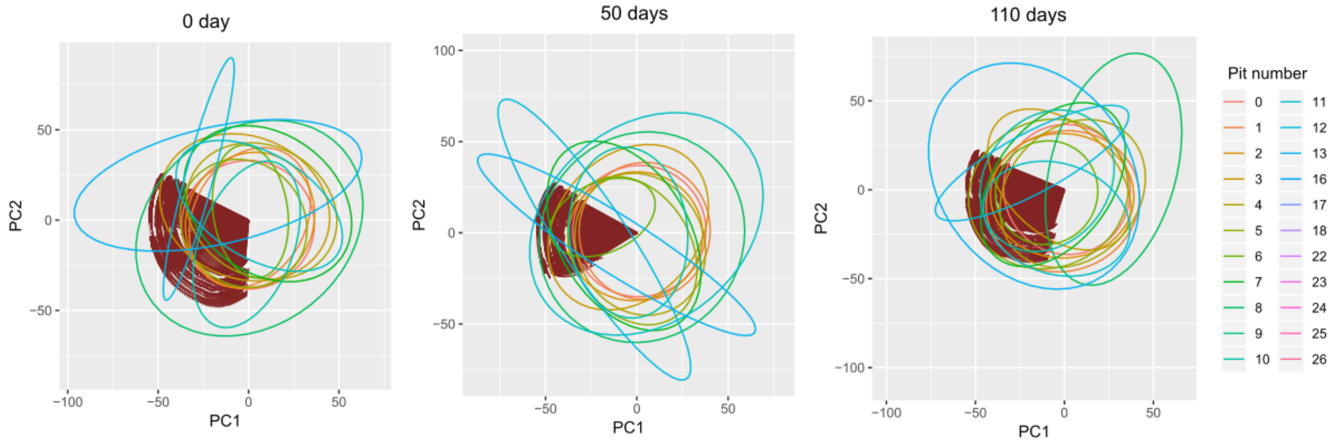
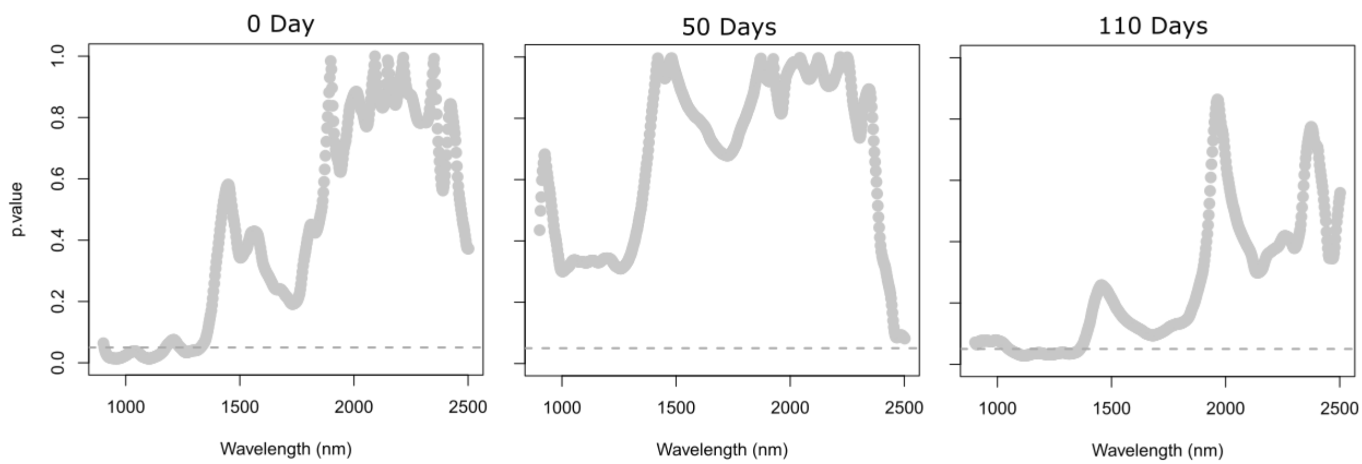


Supp Fig 1. PCA result for different severity levels (pit per fruit) at 0 D (left), 50 D (center) and 110 D (right). Wavelength are showed in dark red.



Supp Fig 2. ANOVA results for each wavelength between 900 to 2500 nm in reflectance spectra at 0 D (left), 50 D (center) and 110 D (right). Gray points represent ANOVA p.value using as a class boundary 9 pit per fruit.

5. General Discussion

The use of Vis-NIR spectrum as non-destructive technique has been evaluated by various authors to infer the characteristics of agricultural products (Moghimi et al., 2011), spectrum analysis allow the study of molecular and dynamic structures obtained from the excitation of molecules, absorption and emission of light. Also, the study of Vis-NIR wavelengths allows identification of molecules containing hydrogen atoms and thus the quantitative analysis of several fruit components such as water, alcohol, amines and other compounds containing CH, NH and/or OH groups (Costa et al., 2003; Nicolaï et al., 2006; Osborne, 1986). Throughout this area of research, it can be possible to verify which Vis-NIR wavelength intensities are also affected by structural and / or physical changes in the fruit tissues, including internal browning, formation of cavities, presence of internal soak areas and cell malformations. These changes in tissue could be associated with the development of physiological disorders such as internal browning, watercore or bitter pit.

Regarding internal browning (IB), James and Jobling (2009) described different browning patterns for ‘Cripps Pink’ apples (radial, diffusive and brown patches produced by CO_2 toxicity). This IB symptoms generated can be associated to different cell configuration that trigger the sensitivity of the tissue to develop internal browning early in storage. On the other hand, watercore in apples is caused by an abnormal sorbitol accumulation in extracellular spaces, which affects the diffusion of gases inside the tissue, leading to the development of symptoms of internal browning (Argenta et al., 2002; Harker et al., 1999; Köpcke, 2015); Finally, BP lesions are caused by cell death at different levels of depths in the parenchyma tissue, generating corky lesions in the Calix area of the fruit (Fallahi et al., 2006; Ferguson et al., 1999; Perring and Pearson, 1986).

With the traceability of the spectral data performed individually by fruit, is possible to associate spectra features with small changes occurring in asymptomatic fruit, and an early prediction could be made before symptom development. The spectral traceability during the different experiments of this research, allowed to develop detection models (at harvest or early in storage) which, despite of not showing a perfect classification of disorders (missclassified fruit), it could allow determinate risks in lots of fruit that has the predisposition to show symptom of internal browning, watercore or bitter pit during storage.

The non-destructive Vis-NIR technique offers to the fruit industry several advantages mainly due to the low implementation costs (compared with X-ray and hyperspectral images) and lower equipment costs (Nicolaï et al., 2006; Noh and Choi, 2006). Also, the results of this research shows that it is possible to carry out an early detection (before symptoms are visible) for the three physiological disorders studied in apple (Internal browning, watercore and bitter pit) in apples with differing origins (time at low temperature storage, abnormal sorbitol accumulation and nutritional imbalance), this would allow design differential management protocols for fruit lots, according to the Vis-NIR inspection, as was proposed for watercore reductions using delay storage (temperature x time treatments) associated with watercore severity determined by Vis-NIR. Finally, implementation of this non-destructive technique would ensure that the final consumer receives fruit without internal defects (externally asymptomatic) while the industry reduces economic losses and organic waste during the storage and marketing of the commodity.

In this work, two types of spectral data were collected and evaluated in the Vis-NIR range (Noh and Choi, 2006), in the cases of internal browning and watercore transmittance spectra were used while reflectance spectra (Jarolmasjed et al., 2016; Kafle et al., 2016) were used for bitter pit. Symptoms of internal browning and watercore, are generated internally in the fruit covering a high proportion of the tissue, so the light transmitted through the fruit is highly associated to the condition inside, contrary to bitter pit, where only a proportion and undefined area of the tissue is affected.

Taking into account internal browning and watercore study cases, the behavior of the intensities of the transmittance spectra between 600 to 900 nm is directly related to the characteristics of the disorder, that is, in the case of internal browning, the spectral curves show a reduction in the transmittance values around

650 and 710 nm as the disorder (brown colors) progress inside the fruit. On the other hand, in the case of watercore, in these same wavelengths were observed transmittance intensities, which in some fruit with high watercore severities, exceeded 100%. These intensities greater than 100% do not mean that the fruit is transmitting more light than it receives, this phenomenon must be related to the phenomenon of interference of light waves that are reflected in the tissues affected by the abnormal sorbitol accumulation which would act as a reflective surface, reflecting light waves in different directions thus generating this increase in intensity.

The spectral analysis (chemometrics) is a multivariate problem in which a selection of the variables should be made in order to find the best predictors that explain the problem before starting modelling. Throughout the state of art review, an established protocol was not found to perform this procedure, finding different approaches to this problem, for example there are authors who use classic multivariate techniques such as PCA or PLS for the selection of spectral ranges (Zhou et al., 2015) and others that use machine learning tools to perform this same procedure (Jarolmasjed et al., 2017).

This lack of consensus is also reflected in spectral data pre-processing. Factors such as the spectrometer operating temperature or fruit sample temperature, generate light scattering the Vis-NIR range, so it is generally recommended to perform pre-processing operations of the spectra, among the most commonly used are Savitzky-Golay (SG) second derivate (Torres et al., 2016; Valente et al., 2009) and Standard Normal Variate (SNV) transformation (Garcia-Sanchez et al., 2017).

Performing these pre-processing operations, spectrum values and their characteristic patterns change, leading to loss information in the spectrum tails. Therefore, the only way to find the best pre-procesing method is different pre-processing operations and comparing its results (Galvez-Sola et al., 2015). In this investigation, models were made with and without pre-processing. In the case of internal browning, a spectral data pre-processing was carried out, including SG and SVN, while for watercore, no pre-processing operation was performed. For bitter pit models and for the study case made during the doctoral internship at Embrapa (Brazil) (internal damage in mango), models generated with and without pre-processing (SG and SNV) were compared. In BP model, better results were found with data pre-processing between 900 to 1200 mn, meanwhile, for models performed to detect internal damage in mango, no differences could be seen between using or not data pre-processing; so it was decided to show the results of the models that used the original values of the spectrum.

High variability of spectral data and the improvement of computing technologies, has generated that researchers have focused on the use of models of the machine learning such as Supporting Vector Machine (SVM) or neural networks (Khatiwada et al., 2016; Luisa et al., 2017; Pissard et al., 2013; Teixeira Dos Santos et al., 2013) with in order to obtain a better adjustment in the prediction of the problem instead on the understanding of how the predictive variables (wavelengths) affect the response (physiological disorder incidence) (James et al., 2015). This was corroborated with the comparison made in the case of internal browning, where classic models (Robust) such as principal component regression (PCR) and partial least square (PLS) models were compared with the performance of machine learning models (more flexible) such as SVM and neural networks. According to the results shown in Chapter 2 of this document, the more flexible models obtained better prediction metrics than the more robust models.

High variability also affected the performance of the models for internal browning prediction and detection. In this case, quantitative models, which seek to quantify a continuous variable (percentage of affected area) presented regression coefficients (R^2) between 60 and 70% while the qualitative models, which seek to classify the severity or incidence of the disorder, achieved classification metrics (accuracy, sensitibity, specificity, positive predicted values (PPV) and negative predicted value (NPV)) about 90% in some cases.

Regarding to model's calibration and validation, due to its increasingly common to use machine learning models for regression and classification, these types of models acts as a "black box" where it can not be obtained information about model's estimators (β coeficients). For this reason, it is recommend to validate the models with observations that have not been used for calibration, also it is recommended to use data from different seasons in order to include the greatest possible variability and thus be able to obtain models which could handle with this high variability (Galvez-Sola et al., 2015; Peirs et al., 2003).

Although Vis-NIR radiation does not present energy absorption for macro and micro nutrients, different concentration of those could affect Vis-NIR spectra when they are binding to organic substances and/or to cellular structures, such as the cell wall, proteins and membrane (Ciavarella et al., 1998). Due to this, reflectance in the Vis-NIR range has been used to determine nutritional stages in agricultural products in a fast and economical way (Garcia-Sanchez et al., 2017).

Partial least squared model using NIR spectra showed high accuracy rate (R^2 higher than 90 %) estimating Nitrogen and Calcium contents in citrus leaves (Galvez-Sola et al., 2015); other mineral such as Phosphorus, Boron, Cooper and Magnesium did not achieve acceptable regression coefficients. Other reports had showed mineral estimations for potassium (Ciavarella et al., 1998) on grapes and rice or cooper and phosphorus in mate plants (Rossa et al., 2015). These results show that Vis-NIR spectra could detect different concentrations of mineral nutrients, but it is needed to study each fruit or vegetable specie as an independent subject because each one has a characteristic spectrum. In the case of BP, regression models for quantification of calcium in fruit were performed, using reflectance spectrum between 900-2400 nm, but no acceptable results were obtained, so it was concluded that, at least in ‘Fuji’ apples, the concentration of calcium in the fruit can not be estimated using these spectral data.

The experiments carried out in this study corroborated the research hypothesis, since it was demonstrated that the incidence of symptoms of internal browning, watercore or bitter pit, affect the spectral features in ‘Cripps Pink’ and ‘Fuji’ apples along Vis-NIR range (400-2500 nm). Thus, it was possible to develop predictive and detection models for internal browning, watercore and bitter pit physiological disorders in apples using Vis-NIR spectrometry.

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6. Appendix

Below is a brief description of other activities developed during the development of doctoral studies.

Academic activities

- Characterization of the transmittance spectrum (200-1100 nm) of 6 apple cultivars (‘Granny Smith’, ‘Brookfield’, ‘Royal Gala’, ‘Scarlet’, ‘Golden Delicious’ and ‘Cripps Pink’). 2017
- Doctoral research internship at Embrapa Semi-arido
  - Petrolina-Brazil, 2019
  - Topic: Use of NIR for post-harvest mango evaluation (Mangifera indica)
  - Supervisor: Dr. Sergio Tonetto de Freitas

Conferences

- **IV Asia Symposium on Quality Management in Postharvest Systems.** Jeonju, Corea del Sur, Septiembre 2017
  Oral presentation: Internal damage prediction in crisp pink apples: comparison between PCR, PLS, PLS-DA and SVM models
- **V Reunión de Fisiología y Tecnología de Postcosecha**. Santiago, Chile Noviembre 2018
  Oral presentation: Modelos Vis-NIR para la determinación temprana de pardeamiento interno en manzanas Crips Pink durante almacenamiento

Other manuscripts

- Mogollon M.R, de Freitas S., Bonomelli C., Contreras C., Zoffoli J.P. 2020. Nutritional relationships and VIS-NIR models to predict bitter pit in ‘Fuji’ apples. (in progress)

- Mogollon M.R, de Freitas S., Contreras C., Zoffoli J.P. 2020. Prediction and identification of internal physiological disorders in ‘Keitt’ mango using a hand-held Vis-NIR spectrometer. (in progress)