Spectrometric techniques for elemental profile analysis associated with bitter pit in apples

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Abstract

Bitter pit and healthy 'Honeycrisp', 'Golden Delicious', and 'Granny Smith' apples were collected from three commercial orchards. Apples were scanned using Fourier transform infrared (FTIR) and X-ray fluorescence (XRF) spectrometers to associate the elemental profile with bitter pit occurrence in apples. The FTIR spectra were acquired from apple peel and flesh; while XRF spectra were acquired from the apple surface (peel). Destructive elemental analysis was also performed to estimate calcium, magnesium, and potassium concentrations in the apples. There were significant differences between healthy and bitter pit affected apples in calcium, magnesium, and potassium concentrations, in addition to magnesium/calcium and potassium/calcium ratios (5% level of significance). Peak analysis of FTIR spectra of prepared standards indicated the possible spectral regions associated with calcium content as 1150-1450 cm⁻¹. Two different classification models (support vector machine, SVM and soft independent

modelling of class analogy, SIMCA) were used to classify healthy and bitter pit affected apples using FTIR spectral signatures. FTIR spectra were able to predict bitter pit incidence in apples with higher classification accuracy using peel tissue (92%) than using flesh tissue with SVM model. The XRF technique could determine bitter pit incidence in apples and semi-quantitative analysis using XRF data was in agreement with the elemental analysis. FTIR and XRF spectrometric techniques are rapid methods that can be used for elemental profile analysis in apples. These techniques can serve as potential prediction tools for elemental profile analysis to detect bitter pit in apples.

Keywords: Apple disorder, support vector machine, FTIR spectrometry, XRF analyzer

Introduction

Bitter pit is a physiological disorder characterized by a localized depression in the apple flesh. These depressions are more commonly located in the distal portion of the fruit (Amarante et al., 2013). Although the specific reason associated with this disorder development is unknown, there have been several hypotheses presented to explain the occurrence of bitter pit. For example, deficiency in the total calcium (Ca) concentration in bitter pit affected fruit has been reported (Ferguson and Watkins, 1983, 1989; Amarante et al., 2006; Sharma et al., 2012). de Freitas et al. (2010) also reported a high pectin methylesterase expression in cells of the calyx-end of bitter pit affected 'Granny Smith' apples. Deesterification of pectins by pectin methylesterase in the cortical tissue decreased Ca in the apoplastic region of the cells, resulting in a higher membrane permeability and the collapse of the cells in bitter pit affected apples. However, Ca concentration by itself may not be sufficient to explain bitter pit occurrence. In 'Fuji' apples, Amarante et al. (2013) suggested that high magnesium (Mg) to Ca ratio is a factor contributing to bitter pit occurrence based on distal apple peel sampling. de Freitas et al. (2010)

also found that $[Mg+Potassium (K)]/Ca$ ratio was proportional to bitter pit incidence in 'Granny Smith' apples. Miqueloto et al. (2011) studied the elemental concentrations related to bitter pit occurrence in apples, and found the same relationship in 'Fuji' and 'Catarina' apples affected by bitter pit. In addition, $[K+Mg+Nitrogen (N)]/Ca$ ratio in the peel and flesh of the same cultivars was also proportional to bitter pit occurrences. It was proposed that the bitter pit occurrence may be induced by K and Mg competition with Ca for binding sites in the plasma membrane surface, resulting in the breakdown of the plasma membrane and probably the collapse of the cell (de Freitas et al., 2010; Miqueloto et al., 2011).

Bitter pit incidence leads to economic loss affecting apple producers. Therefore, early detection of bitter pit affected apples would be helpful in improving production management practices or economic decisions. The Fourier transform infrared (FTIR) spectrometer determines the chemical composition of the tissues based on the absorption of infrared radiation. The absorption is associated with the change of energy of specific molecules at a certain frequency (Perkins, 1986; Shiroma and Rodriguez-Saona, 2009; Oliveira et al. 2013). This technique has been used in food and agriculture research (Jaiswal et al., 2014; Al-Holy et al., 2015). Similarly, x-ray fluorescence (XRF) spectrometer determines the chemical composition of different materials (Craig et al, 2007; Peinado et al., 2010; Leopold et al, 2011; McLaren et al. 2012; Kalcsits, 2016). This method measures the energy of photons dissipated by those materials after being radiated by an X-ray source (Peinado et al., 2010). These energy signatures are associated with specific elements. Therefore, FTIR and XRF spectrometric techniques can be useful tools that could provide rapid elemental analysis in apples and predict bitter pit susceptibility in fruit. With this, the objective of this research was to evaluate the FTIR and XRF spectrometric methods as a rapid and precise technique to determine the elemental profile associated with bitter pit occurrence in different types of apples.

2. Materials and methods

2.1 Fruit samples

'Honeycrisp', 'Granny Smith', and 'Golden Delicious' apple cultivars were harvested at commercial maturity. In 2014, apples were collected from two different orchard locations, Prescott (location 1) and Burbank (location 2), Washington, USA. In both field sites, trees were eight years-old and grafted on M-9-T337 rootstock and canopies planted with "V" trellis system. Bitter pit samples from 'Golden Delicious' and 'Granny Smith' cultivars were not found at location 2. In addition to this primary data, new samples were collected in 2015, for further validation of findings achieved using FTIR spectrometry. In 2015, 'Honeycrisp' and 'Golden Delicious' apples (2 locations/cultivar) were collected in three different orchard locations, Prescott (location 1), Burbank (location 2), and Quincy (location 3), Washington, USA. In location 3, trees were 8 years old and grafted on M-26 rootstock and canopies planted in vertical axis system. More information on the orchard can be found in Jarolmasjed et al. (2016).

The focus of the study was elemental profile analysis of bitter pit apples using multiple sensing techniques during storage. The reason that bitter pit samples from field were utilized as bitter pit development in healthy looking apples can be inconsistent leading to unbalanced sample size (Nicolaï et al., 2006). Healthy and bitter pit affected samples were visually identified, sorted in boxes, and then stored for 63 days in cold storage at 5°C before FTIR spectrometer, XRF spectrometer, and laboratory chemical analysis. The storage period was to allow the bitter pit development in healthy looking samples.

In seasons 2014 and 2015 after 63 days in storage, samples were visually observed to confirm if the healthy samples developed bitter pit. Samples that developed bitter pit were classified as bitter pit samples. In 2014 samples, no new prominent bitter pit symptoms developed in healthy apples during storage. However, in 2015, one sample in Golden Delicious

location 1, six healthy samples in 'Honeycrisp' location 2, and one sample in 'Honeycrisp' location 3 developed bitter pit. Table 1 describes the number of samples selected from each location.

2.2 XRF analysis

XRF measurements were performed at Washington State University Tree Fruit Research and Extension Center, using an AXS Tracer 3V portable handheld X-ray fluorimeter (Bruker Corporation, Billerica, Massachusetts, USA). The device is equipped with a Rhodium tube that serves as an X-ray source. The beam produced scans in an area of 0.5 cm² with 0.1 cm depth. Each sample was scanned on three sides. After scanning the samples, the data was processed using Artax software (Bruker Corporation, Billerica, Massachusetts, USA) and photon counts of K and Ca were normalized against the photon counts for the rhodium x-ray tube. Statistical analysis was applied similar to chemical analysis of the fruit. More information about the equipment can be found in Kalcsits (2016).

2.3 FTIR analysis

FTIR spectral analysis was performed in the Analytical Chemistry Laboratory, Washington State University, Pullman. For sample analysis, three thin layers of peel and flesh were cut from each apple and measured with an FTIR spectrometer (Shimadzu IRPrestige, Kyoto, Japan). The tissue spectrum was acquired using the device settings at Happ-Genzel apodization with 32 scans per sample, 4 cm⁻¹ resolution, and 600 to 4000 cm⁻¹ range. Sampling rate was one sample per minute.

FTIR spectra from distilled water, calcium, magnesium, and potassium standards were also acquired to determine their spectral signatures and identify the elemental peaks. These spectral signatures were compared with the healthy and bitter pit affected apple spectra. ANOVA analysis was conducted using R Studio (ver. 0.99.451, R Studio Inc., Boston, MA) to evaluate spectral regions showing differences between healthy and bitter pit samples.

2.4 Chemical analysis

Calcium, magnesium, and potassium analysis was performed in the Analytical Chemistry Laboratory, Washington State University, Pullman, WA. Each sample was cut from the distal portion of the apple. One peel sample of 10 g and three flesh samples of 10 g were weighed per apple and dried in the oven at 105 °C until constant weight was observed. Two grams of dried samples were digested with nitric acid on a hot plate (Zarcinas et al., 1987). Samples were then analyzed for Ca and Mg using a Microwave Plasma Emission Spectroscopy (MP-AES 4200, Agilent Technologies, Santa Clara, CA, USA). Elemental concentration were acquired on a dry mass basis ($\mu\text{g/g}$) with regression lines developed using Ca, Mg, and K standards. Peaks for Ca, Mg, and K were 393.4, 285.2 and 766.5 nm respectively. The limit of detection was 0.2 to 1 mg L^{-1} for Ca, 0.25 to 2 mg L^{-1} for Mg, and 2.5 to 18 mg L^{-1} for K. Ca, Mg, and K concentrations were analyzed using analysis of variance (ANOVA) using R Studio to determine differences in chemical concentration among healthy and bitter pit affected fruit ($\alpha=0.05$).

2.5 Data analysis

FTIR spectral data was preprocessed using baseline correction after normalizing the data. The goal of these preprocessing steps (normalization and baseline correction) was to make the multivariate analysis stronger and accurate (Baker et al., 2014). Normalization aims to

find a common scale for all of the analyzed data (FitzPatrick et al., 2012). In this study, the spectra were normalized by dividing each spectral feature (absorbance) by the square root of the squared features' sum from the same spectrum (Sankaran et al., 2011). Baseline correction is a method that balances offset spectra to a common minimum point (FitzPatrick et al., 2012). In this study, the baseline correction was performed by regressing the spectrum in ranges. The algorithm adjusted the baseline of the peak signals according to the previously normalized data. After preprocessing, FTIR spectral data was used to predict bitter pit occurrence in apples using multivariate analysis. In this study, support vector machines (SVM) and soft independent modeling of class analogy (SIMCA) were employed. SVM classifier determines a subset of training data (support vectors), this subset creates a hyperplane that separates the dataset of one class from another and maximizes the margin between those two classes (Li et al., 2009). In SIMCA analysis, classes are separated creating principal components sub-model for each class. New samples are classified depending on how they fit in the sub-models obtained (Mireei et al., 2017; Esbensen et al., 2002). In these classification methods, the train to test dataset ratio was 3 by 1. Principal component analysis (PCA) was also performed to reduce the dimensionality of the data, and the number of principal components (PCs) were selected in a way that the PCs represented more than 99% variability within the data in SIMCA. SIMCA, SVM, and PCA were performed using MATLAB® R2015b program (The Math Works Inc. Natick, MA, USA). Each classifiers (SVM, SIMCA) were run three times and the mean classification accuracies are reported. The overall and individual class (healthy/bitter pit) classification accuracies are also reported. Statistical analysis was performed to compare the performance of the two classifiers.

3. Results and discussion

3.1 Elemental analysis

Ca, Mg, and K concentrations in peel and flesh were estimated to know their relationship to bitter pit occurrence. Ca, Mg, and K content of the apples was higher in peel than in flesh tissues in 'Honeycrisp' apples. Similar reports were obtained in 'Gala' and 'Honeycrisp' apples (Amarante et al., 2006; Kalcsits, 2016). In addition, Ca content was higher in healthy (110-153 mg L⁻¹) compared to bitter pit affected peel samples (90-102 mg L⁻¹), and Mg and K were higher in bitter pit affected than healthy samples (154-259 mg L⁻¹ Mg and 1666-2148 mg L⁻¹ K in bitter pit apples). This trend was observed in both peel and flesh samples (Table 2). With respect to Mg/Ca and K/Ca ratios, healthy samples showed lower values (about half) than the ones found in bitter pit apples (Figure 1). Similar results were found in 'Gala' apples (Amarante, 2006). K is a mobile element inside the plant that acts as a counter cation in the cell. Frequently, when calcium concentrations are low, potassium concentrations are subsequently elevated. This observed increase in the K concentration appears to be associated with bitter pit in apples (de Freitas et al., 2010). In this study, there was no statistical difference in the Ca, K, and Mg concentrations between the two 'Honeycrisp' sites.

The elemental concentrations reported here is in agreement with findings stated in Amarante et al., (2006, 2013) and Miqueloto et al., (2011). Ca plays an important role in membrane structure at the cellular level. When Ca content decreases, it makes membrane structure weak, resulting in bitter pit development (Amarante et al., 2013). In addition, Mg has ionic similarities with Ca, and these two elements compete in different physiological processes inside the cell membrane (Amarante et al., 2013). Mg cannot replace Ca-based structure in cell membrane. Therefore, this results in breakdown of cell wall structure and bitter pit formation occurs in apples. It is useful to assess Mg/Ca ratios; as bitter pit is often developed when this ratio is high (de Freitas et al., 2010).

Elemental analysis was also performed in 'Golden Delicious' and 'Granny Smith' apples to determine its relationship with bitter pit occurrence (Table 3). The elemental concentrations

for these two cultivars showed similar patterns compared to those observed in 'Honeycrisp' apples, confirming the trend in elemental concentrations associated with bitter pit development in apples.

3.2 FTIR spectroscopy

3.2.1 FTIR spectra and peak analysis

Chemical standards were used to determine the wavelengths of peaks associated with bitter pit development in FTIR spectra. Based on FTIR analysis of healthy and bitter pit affected apples, peaks from Ca, Mg, and K standards were used to identify the wavelengths associated with these elements. Using ANOVA, it was possible to detect three statistically different peak regions between healthy and bitter pit samples (2840-2980; 1750-1790 and 1290-1350 cm^{-1} ; Figure 2) in the flesh; and two statistically different peak regions (1150-1450 and 1700-1800 cm^{-1}) in the peel. The results were compared to peaks associated with elements/components related to bitter pit. Wang et al. (2010) found one of the peaks for calcium carbonate at 1440 cm^{-1} , which is a common form of calcium in apples towards harvest (Saure, 2005). Assifaoui et al. (2010) and Manrique and Lajolo (2002) also identified the pectinate carboxylic group at about 1740 cm^{-1} (between 1700 to 1800 cm^{-1} spectral region in this work). Kanakis et al. (2011) located the presence of polyphenol complexes at 2833 to 2947 cm^{-1} .

Average spectral absorbance measured using FTIR spectrometry in the range of 2840 to 2980 cm^{-1} in flesh and 1700 to 1800 cm^{-1} in peel was statistically different between 'Honeycrisp' healthy and bitter pit apples. In this range, absorbance readings showed a pattern inversely proportional to Ca content in apples, and proportional to Mg, K, Mg/Ca, and K/Ca ratios (Figure 3 and 4). Mg/Ca and K/Ca ratios showed the greatest differences between healthy and bitter pit apples. The range of 2840 to 2980 cm^{-1} is associated with polyphenols complexes. In bitter pit

apples, higher polyphenols concentrations are found because of cell degradation (Zupan et al., 2013). Therefore, absorbance is higher in this range in bitter pit affected apples. On the other hand, pectin is a component of cell wall structure, which can be identified in the range of 1700 to 1790 cm^{-1} . Early pectin degradation causes collapse in the cell wall structure and is a precursor to bitter pit formation (de Freitas et al. 2010). Pectin is lower in bitter pit affected apples (de Freitas et al., 2010), therefore absorbance peak in the region 1700-1790 cm^{-1} is expected to be lower in bitter pit apples as observed in this study.

On the contrary, in the range of 1750 to 1790 cm^{-1} in flesh and 1150-1450 cm^{-1} in peel, calcium content in apples showed a trend indicating higher values when peak absorbance increased. However, Mg, K, Mg/Ca, and K/Ca ratios were inversely proportional to peak absorbance in this region. Absorbance in this range was statistically different among bitter pit and healthy apples only in location 1 (Figure 3 and 4). The possible reason can be due to lower severity of bitter pit symptoms in apples in location 2. Chemical composition of healthy and bitter pit affected apples in peel and flesh were statistically different in all the elements analyzed in flesh. In peel, Ca and Mg content in location 2 were not statistically different. As mentioned above, 1750 to 1790 cm^{-1} range is related to pectin structures, and pectin is lower in bitter pit affected apples. Calcium content is related to the range of 1150 to 1450 cm^{-1} , calcium was lower in bitter pit affected apples. Accordingly, absorbance readings in these two ranges are lower in bitter pit affected apples. FTIR spectral absorbance was also determined in 'Golden Delicious' and 'Granny Smith' apples. Results showed that there was a difference in the peak absorbance values between healthy and bitter pit affected apples in 'Golden Delicious' in all the analyzed peak ranges. On the contrary, 'Granny Smith' apples did not show statistical difference in the peak absorbance values between healthy and bitter pit affected apples (Table 4).

3.2.2 Apple classification based on FTIR spectra before and after peak selection

FTIR absorbance spectra ($600\text{-}4000\text{ cm}^{-1}$) from apple peel and flesh were classified as healthy or bitter pit affected using two classifier models (SVM and SIMCA). In addition, individual and combined peaks selected from peel ($1150\text{-}1450$ and $1700\text{-}1800\text{ cm}^{-1}$) and flesh ($2840\text{-}2980$; $1750\text{-}1790$ and $1290\text{-}1350\text{ cm}^{-1}$) samples associated with bitter pit were also evaluated. The results are summarized in Table 5 and 6.

In general, comparing the FTIR spectra from both flesh and peel 'Honeycrisp' apples showed high overall classification accuracies (70-100%) using the entire FTIR spectra, especially using SVM classifier. Similar high classification accuracies (74-100%) was observed, while using combined peak spectral features. This indicates that the FTIR spectral features from identified wavelength regions in flesh and peel could indicate the bitter pit development. In comparing individual peak regions from 'Honeycrisp' flesh and peel samples, each peaks showed the potential to contribute to the classification of apples as healthy or bitter pit. In 'Golden Delicious' apple samples, the SVM classifier showed high classification accuracies when entire FTIR spectra, individual peak spectral features, and combined peak spectral regions from flesh and peel samples were considered. For example, the mean overall classification accuracy using SVM classifier on entire spectra, $1150\text{-}1450\text{ cm}^{-1}$ spectral features, $1700\text{-}1800\text{ cm}^{-1}$ spectral features, and combined peak features from 'Golden Delicious' peel samples (2014 and 2015 season) were 93-100%, 80-98%, 93-96%, and 9%, respectively. Similar observation was made with 'Granny Smith' sample classification. In spite of minor differences between two classifiers with SVM model performing slightly better than SIMCA model, no statistically significant difference in the classifiers performance between peel and flesh or healthy and bitter pit affected apples was observed (Tables 5 and 6).

The classification accuracies in different peaks in flesh samples were similar within the three peak features. Additionally, in many cases, the combined peak features showed an improvement in the classification accuracies in all three different apple cultivars. Similar

observation was also valid for peaks from the peel samples. For example, in ‘Golden Delicious’ samples, the individual peak overall classification accuracies ranged from 80-98%, while the combined peak features resulted in overall classification accuracies of 96-100% using SVM. Comparing the peel and flesh samples, in most cases, the combined peak features of peel samples showed higher classification accuracies than those from flesh samples. For example, SVM-based combined peak overall classification accuracy (2014) of ‘Granny Smith’ flesh samples was 60-93%; while those of peel samples was 93-96%. These results can be related to high differences in chemical constituents of peel compared to flesh as described earlier. Pectin (1740-1790 cm^{-1}) or polyphenols (2840-2980 cm^{-1}) could influence FTIR spectral feature-based classification. Progression in bitter pit disorder may cause pectin to dissolve and polymerize, and increases polyphenols because of vacuole destruction inside the cells of bitter pit spots. The absorbance in 1150-1450 cm^{-1} is related to calcium content. Calcium content was higher in peel than in flesh, and calcium difference between healthy and bitter pit affected apples was higher in ‘Granny Smith’ and ‘Golden Delicious’ compared to ‘Honeycrisp’ cultivar (Table 2 and 3).

3.3 XRF results

Normalized semi-quantitative photon counts for calcium and potassium determined using XRF was significantly different between healthy and bitter pit affected ‘Honeycrisp’, ‘Golden Delicious’, and ‘Granny Smith’ apples as observed in Figure 5. In all of the three apple cultivars, K/Ca ratio between healthy and bitter pit apples was also significantly different (Table 9). As the estimated depth of penetration for XRF is approximately 0.1 cm, the measurements are representative of the peel and the immediate flesh layer under it. XRF results were in accordance with destructive elemental analysis. The difference between healthy and bitter pit apples was higher in K/Ca ratio in chemical analysis (Tables 2 and 3) and also in XRF readings

(Table 9). Therefore, comparing this ratio to detect presence or absence of bitter pit disorder was more practical than using individual mineral apple composition.

4. Conclusions

Chemical profile of healthy and bitter pit ‘Honeycrisp’, ‘Golden Delicious’, and ‘Granny Smith’ apples were found to be different. In general, the Ca content in the healthy apples were higher than those compared to bitter pit apples. However, K, Mg, K to Ca, and Mg to Ca ratios were lower in healthy than those of bitter pit affected apples. The FTIR spectral analysis, indicated the presence of three wavelength regions in flesh (2480- 2980 cm^{-1} ; 1750-1790 cm^{-1} ; 1290-1350 cm^{-1}) and two in peel (1150-1450 cm^{-1} ; 1700-1800 cm^{-1}) that could be associated with bitter pit development in ‘Honeycrisp’, ‘Golden Delicious’, and ‘Granny Smith’ apples. In this study, we found that bitter pit development could be identified using the FTIR spectral features. Moreover, the prediction accuracies with peel samples were found to be higher than flesh samples. The classification accuracies were evaluated using two models SVM and SIMCA, and no statistical difference between the results from two models were found. Potassium to calcium ratio determined by XRF also showed statistical difference between healthy and bitter pit affected apples in all three apple cultivars. This research demonstrate the applicability of FTIR and XRF techniques as rapid and precise tools in bitter pit detection in apples. There is a potential use of these techniques in predicting physiological disorders related to chemical deficiencies in other crops.

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422

Table 1. Sample size from each location during first (2014) and second season (2015).

Season	Location	Cultivar	Number of samples	
			Healthy	Bitter pit
2014	Location 1	Honeycrisp	20	20
		Golden Delicious	20	20
		Granny Smith	10	10
	Location 2	Honeycrisp	20	20
2015	Location 1	Golden Delicious	29	31
	Location 2	Honeycrisp	24	36
		Golden Delicious	30	30
	Location 3	Honeycrisp	29	31

Table 2. Calcium, magnesium, and potassium concentrations of peel and flesh samples in 'Honeycrisp' apples. Results are expressed in mg kg⁻¹ of fresh fruit, coefficient of variation (CV) is expressed as percent.

Condition	Tissue	Location	Ca (mg kg ⁻¹)		Mg (mg kg ⁻¹)		K (mg kg ⁻¹)	
			Average	CV %	Average	CV %	Average	CV %
Healthy	Peel	1	153	36	157	24	1327	14
	Peel	2	110	27	122	20	1181	16
	Flesh	1	28	35	35	19	902	16
	Flesh	2	29	21	33	19	924	12
Bitter pit	Peel	1	102	92	259	36	2148	32
	Peel	2	90	74	154	43	1666	25
	Flesh	1	18	65	40	29	1136	20
	Flesh	2	20	54	345	31	1022	20

Figure 1. Potassium to calcium ratios in (a) peel and (b) flesh samples of healthy and bitter pit 'Honeycrisp' apples. In boxplot, whiskers represent the maximum and minimum, upper and lower box borders represent the 75th and 25th percentile, respectively, and the horizontal dark line indicates the median.

440 **Table 3.** Chemical composition of peel and flesh of 'Golden Delicious' and 'Granny Smith' apples. Results are expressed in mg kg⁻¹
 441 of fresh fruit, coefficient of variation (CV) is expressed as percent.

Condition	Location tissue	Ca (mg kg ⁻¹)		Mg (mg kg ⁻¹)		K (mg kg ⁻¹)		Mg/Ca		K/Ca	
		Average	CV %	Average	CV %	Average	CV %	Average	CV %	Average	CV %
Healthy	Peel Golden Delicious	28	18	28	10	320	11	1.1	17	12	19
	Peel Granny Smith	26	27	32	9	441	9	1.3	25	18	27
	Flesh Golden Delicious	7	24	10	17	44	12	1.4	20	7	23
	Flesh Granny Smith	6	29	9	11	53	11	1.6	32	10	31
Bitter pit	Peel Golden Delicious	12	34	33	31	359	20	2.9	29	33	35
	Peel Granny Smith	14	41	31	33	426	23	2.4	28	37	61
	Flesh Granny Smith	4	34	13	40	62	20	3.2	43	16	31
	Flesh Golden Delicious	4	37	12	34	62	16	3.1	40	17	39

Figure 2. Calcium, magnesium, potassium standards; distilled water; and baseline corrected spectra of healthy and bitter pit flesh apple tissue obtained by Fourier transformed infrared (FTIR) spectrometric technique.

Figure 3. Calcium, magnesium to calcium ratio, and FTIR spectral peak absorbance readings in flesh of 'Honeycrisp' apples. One-way ANOVA was conducted ($\alpha = 0.05$) for each location, same letter within each data set were not significantly different. Error bars indicate standard deviation of the means.

Figure 4. Calcium, magnesium, potassium, magnesium to calcium ratio, and FTIR spectral peak absorbance values (at 1700-1800 cm⁻¹ and 1150-1450 cm⁻¹) in peel of 'Honeycrisp' apples. One-way ANOVA was conducted ($\alpha = 0.05$) for each location, same letter within each data set were not significantly different. Error bars indicate standard deviation of the means.

Figure 5. Potassium calcium ratio in (a) 'Honeycrisp', and (b) 'Golden Delicious' and 'Granny Smith' apples using XRF spectrometric technique. One-way ANOVA was conducted ($\alpha = 0.05$) for each location, same letter within each data set were not significantly different. Error bars indicate standard deviation of the means.

Table 4. Average spectral absorbance in selected wavelength ranges (1150-1450, 1290-1350, 1700-1800, 1750-1790, and 2840-2980 cm^{-1}) in peel and flesh of 'Golden Delicious' and 'Granny Smith' apples. One-way ANOVA was conducted ($\alpha = 0.05$) for each cultivar, same letter within each spectral range was not significantly different.

Cultivar	Apple condition	Average spectral absorbance in flesh			Average spectral absorbance in peel	
		2840-2980 cm^{-1}	1750-1790 cm^{-1}	1290-1350 cm^{-1}	1700-1800 cm^{-1}	1150-1450 cm^{-1}
Golden Delicious	Healthy	0.0016 ^b	0.0027 ^b	0.0017 ^a	0.0088 ^a	0.0040 ^a
	Bitter pit	0.0052 ^a	0.0033 ^a	0.0013 ^b	0.0066 ^b	0.0023 ^b
Granny Smith	Healthy	0.0029 ^a	0.0048 ^a	0.0025 ^a	0.0031 ^a	0.0076 ^a
	Bitter pit	0.0034 ^a	0.0051 ^a	0.0028 ^a	0.0035 ^a	0.0088 ^a

518 **Table 5.** Average bitter pit affected (BP), healthy (H), and overall (O) classification accuracy (%) in flesh of ‘Honey Crisp’, ‘Granny
519 Smith’, and ‘Golden Delicious’ apple using different classifier methods on different FTIR spectral ranges (Entire FTIR spectral 600-
520 4000 cm⁻¹; individual spectral ranges: 1290-1350, 1750-1790 and 2840-2980 cm⁻¹; and combined spectral ranges).

Sample	Classifier	Accuracy														
		FTIR spectra (600-4000 cm ⁻¹)			2840-2980 cm ⁻¹			1750-1790 cm ⁻¹			1290-1350 cm ⁻¹			Combined peak features		
		BP	H	O	BP	H	O	BP	H	O	BP	H	O	BP	H	O
2014																
Honeycrisp Location 1	SVM	100	100	100	100	93	97	93	80	87	100	93	97	100	100	100
	SIMCA	100	100	100	100	100	100	92	100	97	58	100	80	100	100	100
Honeycrisp Location 2	SVM	83	60	70	75	73	74	75	80	78	75	67	70	83	67	74
	SIMCA	58	93	78	58	67	63	75	73	74	42	60	52	75	33	52
Golden Delicious Location 1	SVM	100	100	100	83	100	93	58	87	74	60	93	78	92	100	96
	SIMCA	92	87	89	83	93	89	83	80	81	50	67	59	92	93	93
Granny Smith Location 1	SVM	17	89	60	17	89	60	33	89	67	17	89	60	17	89	60
	SIMCA	67	44	53	50	67	60	50	89	73	50	67	60	100	89	93
2015																
Honeycrisp Location 2	SVM	93	94	93	78	78	78	85	72	80	85	72	80	85	94	89
	SIMCA	86	79	82	81	79	80	86	96	91	86	96	91	71	92	82
Honeycrisp Location 3	SVM	96	89	93	81	94	87	78	89	82	78	89	82	93	94	93
	SIMCA	76	88	82	100	54	76	90	83	87	100	58	78	100	13	53
Golden Delicious Location 1	SVM	100	94	98	93	89	91	89	100	93	89	100	93	96	100	98
	SIMCA	95	96	96	95	88	91	100	96	98	100	58	78	95	100	98
Golden Delicious Location 2	SVM	100	100	100	100	71	84	95	71	82	90	71	80	95	79	87
	SIMCA	100	100	100	100	79	89	77	75	76	85	75	80	95	75	84

521 **Table 6.** Average bitter pit affected (BP), healthy (H), and overall (O) classification accuracy (%) in peel of ‘Honey Crisp’, ‘Granny
522 Smith’ and ‘Golden Delicious’ apple using different classifier methods on different FTIR spectral ranges (Entire FTIR spectral 600-
523 4000 cm⁻¹; individual spectral ranges: 1150-1450 and 1700-1800 cm⁻¹; and combined spectral ranges).

Sample	Classifier	Accuracy											
		FTIR spectra (600-4000 cm ⁻¹)			1150-1450 cm ⁻¹			1700-1800 cm ⁻¹			Combined peak features		
		BP	H	O	BP	H	O	BP	H	O	BP	H	O
2014													
Honeycrisp Location 1	SVM	100	100	100	100	93	97	100	100	100	100	100	100
	SIMCA	100	100	100	100	100	100	100	100	100	100	100	100
Honeycrisp Location 2	SVM	85	92	89	83	83	85	58	93	81	92	93	93
	SIMCA	60	73	74	73	87	89	67	80	81	100	73	85
Golden Delicious Location 1	SVM	100	100	100	56	100	80	100	89	93	100	100	100
	SIMCA	100	28	60	67	44	60	100	100	100	100	89	93
Granny Smith Location1	SVM	92	93	93	100	92	96	87	93	96	83	100	93
	SIMCA	73	100	96	80	93	96	80	93	96	100	93	96
2015													
Honeycrisp Location 2	SVM	89	94	91	85	78	82	81	89	84	89	94	91
	SIMCA	81	71	76	90	75	82	81	88	84	81	71	76
Honeycrisp Location 3	SVM	100	89	96	93	83	89	78	72	76	81	78	80
	SIMCA	81	88	84	100	67	82	90	63	76	90	63	76
Golden Delicious Location 1	SVM	93	94	93	96	100	98	93	100	96	93	100	96
	SIMCA	95	54	73	95	100	98	95	88	91	95	88	91
Golden Delicious Location 2	SVM	100	100	100	95	75	84	86	100	93	90	100	96
	SIMCA	100	100	100	86	79	82	100	71	84	71	75	73

524 **Table 7.** Average normalized chemical content and coefficient of variation (CV) of Ca and K in
525 'Honeycrisp' apples using XRF technique during 2014 season.

Condition	Location Cultivar	Ca		K		K/Ca	
		CV		CV		CV	
		Ave.	(%)	Ave.	(%)	Ave.	(%)
Healthy	Location 1 Honeycrisp	0.43	75	0.43	18	1.47	59
	Location 2 Honeycrisp	0.22	65	0.42	20	2.42	46
	Location 1 Golden Delicious	0.19	29	0.50	11	2.86	30
	Location 1 Granny Smith	0.14	34	0.72	15	5.95	51
Bitter pit	Location 1 Honeycrisp	0.23	87	0.79	33	6.05	86
	Location 2 Honeycrisp	0.10	60	0.55	30	6.71	52
	Location 1 Golden Delicious	0.08	45	0.67	20	8.96	37
	Location 1 Granny Smith	0.08	56	0.96	19	17.2	92

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