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Carob moth, *Ectomyelois ceratoniae*, detection in pomegranate using visible/near infrared spectroscopy

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ABSTRACT

In pomegranate, carob moth infestation is a postharvest problem. In most cases, damage develops inside the fruit i.e. without affecting the rind. Consequently, visual inspection is not adequate for identification of carob moth in pomegranate fruit because of the lack of external symptoms. In this study, the feasibility of using VIS/NIR spectroscopy as to detect carob moth infestation in pomegranate fruits is demonstrated. The samples included intact as well as infected pomegranate fruits at four different stages of maturity. Discriminant analysis of the samples was performed by soft independent modeling of class analogy (SIMCA) and partial least squares discriminant analysis (PLS-DA). The results showed that in all sample groups when the samples were classified by PLS-DA, the high values were found in comparison with the SIMCA models. All the discriminate analyses were accomplished for three different sample sets: standard (only the samples handpicked at standard harvest time consist of stage 1, stage 2 and stage 3), last (only the handpicked at last harvest time including stage 3 and stage 4) and combined (all four studied maturity stages together). The total discriminant power of PLS-DA classes was approximately 88%, 90%, and 86% for standard, last and combined sample sets, respectively. The comparison among different sample groups indicated that the last sample group was predicted with the best prediction accuracy followed by standard and then combined sample group.

1. Introduction

Nowadays pomegranate (*Punica granatum* L.) is one of the most consumed products due to its high nutritional value, delicious taste, excellent flavor and low calories (Jbir et al., 2008; Melgarejo et al., 2009; Fawole and Opara, 2013). The edible fresh part of the pomegranate fruit, arils, is mainly consumed directly, but sometimes used after separation of seeds, for the preparation of fresh juice or canned beverages, as well as alcoholic beverages, jellies, jams and for flavoring and coloring agents (Szychowski et al., 2015). It is cultivated in tropical and subtropical regions such as Iran, Afghanistan, India, Mediterranean countries (Morocco, Spain, Turkey, Tunisia and Egypt) and some extent in the USA, China, Japan and Russua (Jbir et al., 2008; Melgarejo et al., 2009). Iran is one of the most important pomegranate producers and exporters in the world. Based on Food and Agriculture Organization (FAO) statistics, Iran ranks 1st in fruit production in the Middle East and North Africa. In 2010, 2.7 million hectares of orchards are being harvested in Iran with an annual production of 16.5 million tons (FAO, 2010).

The carob moth, *Ectomyelois ceratoniae* (*Zeller*), also known as pomegranate moth is an important pest attacking pomegranate throughout the world. It is also a major field pest of pistachio, date, almond, fig, walnut, dried fruits, nuts, as well as other non-economic plants of a wide range of plant families (Norouzi et al., 2008). Although carob moth is always recognized as a postharvest quality problem, the infection begins in the orchard. The carob moth lays its egg in the calyx of the flower of the pomegranate, at or immediately after flowering, and the larva bores into the fruit. Infested fruit either rots and drops, or remains on the trees until the end of the season. Larva feeding can encourage the growth of saprophytic fungi. However, the fungi cause decay of arils ranging from a section of the pomegranate fruit to all the arils within the rind without external symptoms. The lack of obvious external symptoms makes carob moth detection a challenge for sorters in the packinghouse or processing line. Lately, the strict international regulation for the existence of any defects in pomegranate fruits has restricted their exportation. Therefore, there is a growing need to detect and eliminate any kind of infection in pomegranate fruits during postharvest handling. Until now, research on carob moth has focused on biological control strategies such as monitoring

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and sanitation; however, no research has been yet carried out on the potential methods for detecting infected pomegranate fruits in the postharvest handling. So to ensure the minimum acceptability of the quality to consumers, developing efficient and nondestructive methods to detect the infection is needed for the fruit.

In recent years, various studies have reported the development of nondestructive techniques to assess food quality such as machine vision (Slaughter et al., 2008; Barnes et al., 2010), X-ray (Jackson and Haff, 2006; Haff and Toyofuku, 2008), nuclear magnetic resonance (Zhang and McCarthy, 2013) and near-infrared (NIR) spectroscopy (Moscetti et al., 2014). Among them, visible/near infrared (VIS/NIR) spectroscopy has a great deal of research to provide fast and reliable information on internal characteristics of agricultural produce such as apple (Yan-de et al., 2007; Fan et al., 2009), apricot (Carlini et al., 2000), avocado (Clark et al., 2003), banana (Tarkosova and Copikova, 2000), blueberry (Peshlov et al., 2009), cherry (Xing and Guyer, 2008; Xing et al., 2008), date fruit (Mireei and Sadeghi, 2013), dill (Schulz et al., 1998), fig (Burks et al., 2000), flour (Wilkin et al., 1986), grape (Herrera et al., 2003), green soybean (Sirisomboon et al., 2009), jujube (Wang et al., 2010, 2011), melon (Long and Walsh, 2006), peach (Ying et al., 2005), pepper (Schulz et al., 2005), plum (Golic and Walsh, 2006) and tomato (Shao et al., 2007). As it can be found from literature, despite an extensive research on NIR spectroscopy of agricultural produce, no published results on the NIR spectroscopy of pomegranate fruit and its arils are available.

Hence, this study was undertaken to determine the effect of physiological changes induced by carob moth on the optical properties of pomegranate and to investigate the feasibility of NIR spectroscopy for the detection of carob moth damage in pomegranate according to the requirements of factory processing. In this regard, the ability of two different supervised pattern recognition methods such as Partial least squares Discriminant Analysis (PLS-DA) and Soft Independent Modeling of Class Analogy (SIMCA) was compared and the most useful classification method in detecting the infected pomegranates was selected. As, controlling of infected samples at the first stage of maturity of pomegranate fruit had very little symptoms of defect, and as the infected samples could be distinguished from the intact ones by visual observation in matured fruit, so in this study detect carob moth infestation in pomegranate fruits into four groups of 100 samples, each representing maturity levels of 1–4 corresponding to 88, 109, 124 and 143 DAFB, respectively were considered.

2. Material and methods

2.1. Samples

Pomegranate fruits (cv. Ashraf) were handpicked from a pomegranate orchard in Shahidabad Village, Behshahr County, Mazandaran Province, Iran (36°41′32″N 53°33′09″E) between August and October in 2014. At the beginning of sampling (31 August), the pomegranate fruits were at the primary stages of maturity 88 days after full bloom, (DAFB); while at the end of sampling (31 October) pomegranates were at the last stage of maturity (143 DAFB). In total, the 400 sample pomegranates were collected and divided into four groups of 100 samples, each representing maturity levels of 1–4 corresponding to 88, 109, 124 and 143 DAFB, respectively (Fig. 1). Samples were labeled, and the morphological properties of each sample were measured and recorded before spectroscopic measurements. Also, a number of Ashraf pomegranate samples were collected at each studied maturity stage containing different infection levels based on visual observation (Totally 19, 22, 30 and 23 samples for validation and 42, 48, 62 and 50 samples for calibration respectively for maturity levels of 1–4). The infected samples collected at the first stage had very little symptoms of defect, while by maturity of fruits; the infected samples could be distinguished from the intact ones by visual observation. After the spectral acquisition, the pomegranate fruits were cut for visual inspecting of the flesh tissue and ensuring about the existence of the defect. Images of Ashraf pomegranate fruits at four different studied maturity stages along with a severely damaged sample by carob moth are depicted in Fig. 1.

2.2. Acquisition of VIS and NIR spectra

A spectrometer (AvaSpec-2048TEC, Avantes Company, Russia) was used to acquire spectral characteristics pomegranate fruit in reflectance mode. The spectrometer was equipped with an external fiber-optic cable, AvaSoft7 software for Windows, a cooled, one nanometer resolution and sensitivity of 1,100,000 counts/μW per ms entrance irradiation. From each fruit sample, four spectra (400–1100 nm at intervals of 1 nm) were collected at four equidistance positions along the equator. The average of these four measurements was used to represent the spectral profile for each sample. The light source consisted of a tungsten halogen lamp (100 W, 12 V) which is usable in the visible and infrared region. It was arranged at a distance of about 50 mm from the fruit surface and the angle between the incident light source and the fibre optic (that guides reflectance light to a detector) was set to 45°. Fig. 2 shows the schematic diagram indicating how to measure the NIR spectrum. In order to compute the relative spectra of each sample and eliminate the interference by the optical system, the reference and dark spectra were stored before sample spectrum acquisition. A white Teflon material was used as the reference material before every measurement. The integration time for reflected spectrum of samples was set to 50 ms.

2.3. Data analysis

2.3.1. Spectral data preprocessing

Vis/NIR instruments generate a large amount of spectral data producing valuable analytical information (Vigni et al., 2013). However, to obtain reliable, accurate and stable calibration models the raw data acquired from spectrometer need to be pre-processed first to reduce the effect of irrelevant information such as background and noise spectra. Recently, several preprocessing methods have been developed for these purposes (Vigni et al., 2013). Firstly in this study, four spectra of every sample were averaged into one spectrum. The averaged value is then converted to absorbance value using Abs = log (1/R) equation where R is the amount of reflectance, to obtain linear correlation between spectra and sample molecular concentration. Then, several preprocessing methods such as mean centering, normalization (multiplicative scatter correction (MSC), standard normal variate transformation (SNV)), smoothing (median filter, Savitzky-Golay and wavelet) and transformation (first derivative and second derivative) were implemented (Nicolai et al., 2007). Centering, which is also referred as mean centering, ensures that all results will be outstanding in terms of variation around the mean (Nicolai et al., 2007). Smoothing is designed to optimize the signal to noise ratio (Nicolai et al., 2007). MSC attempts to remove the effects of scattering by linearizing each spectrum to some 'ideal' spectrum of the sample, which, in practice, corresponds to the average spectrum (Nicolai et al., 2007). Also, first and second derivative preprocessing methods were used to remove background spectra and enhance spectral resolution (Nicolai et al., 2007). In this paper, spectral data were analyzed using the Unscrambler 9.7 software (Camo ASA, Oslo, Norway) and pretreated by columns pretreatments and rows pretreatments (Vigni et al., 2013).

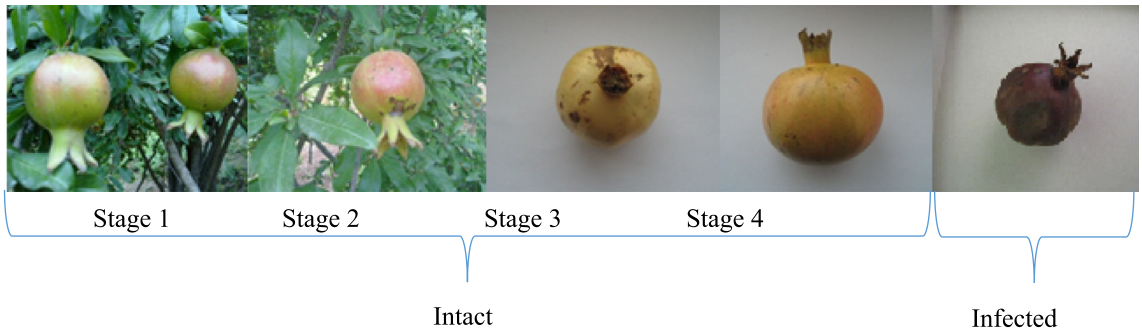

Fig. 1. Example images of Ashraf pomegranate fruits at different maturity stages and a severely damaged sample by carob moth.

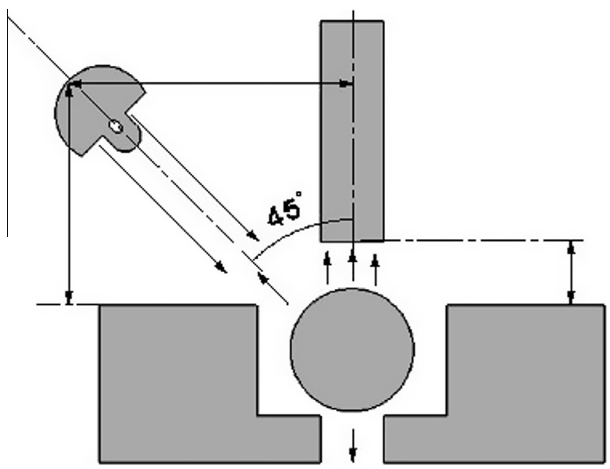

Fig. 2. Schematic diagram of NIR scanning system.

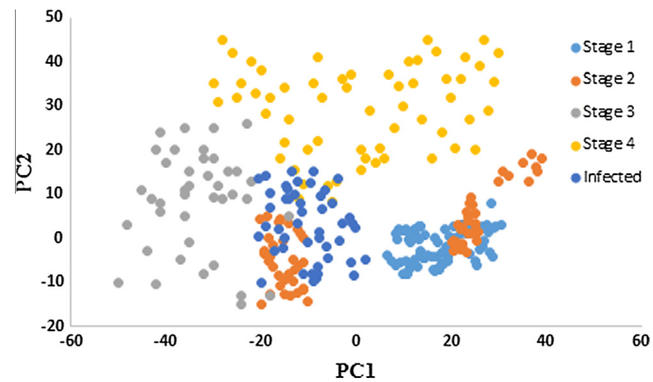

Fig. 3. The scatter plots of PCA score values in the first two PC spaces based on preprocessed spectral data.

2.3.2. Discriminant analysis

After eliminating the irrelevant information from spectra by preprocessing methods, data analysis performed in this study consists of principal component analysis (PCA) for reviewing the data, two linear discriminant analysis namely PLS-DA and SIMACA for classification of pomegranate fruits. PCA is a useful data mining technique which can reduce the dimensionality of spectral data by concentrating the most variations among the raw data into fewer, new and uncorrelated principal components. To achieve SIMCA classification in this study, first, by applying the PCA using 75% of samples representative of the class population variance the intact and infected classes were modeled. Optimum number of principal components (PCs) in leave-one-out full cross validation was selected in order to avoid over and under fitting in models. Lastly, the validation of the PCA models was performed by the remaining 25% of samples in each class with the significance level of 5%. In PLS-DA approach, the standard PLS regression algorithm is applied where the dependent y variable was replaced by the set of dummy variables describing the class membership (Westad et al., 2013). All PCA, SIMCA and PLS-DA analyses were performed with a statistical software package of Unscrambler 9.7 software (Camo ASA, Oslo, Norway)

3. Results and discussion

3.1. Overview of the spectral data

In order to see possible influence factors for the data distribution and data variation, PCA was performed. Fig. 3 shows the scatter plot of PCA score values in the first two PC spaces on the preprocessed reflectance data. As it can be found from this figure, the data distribution in the two PC spaces displays the clearly separated clusters scattered corresponding to the four maturity stages, but there is an obvious overlap between the infected samples and stage 1, stage 2 and stage 3, as well as some parts of infected with stage 4 clusters. Totally, the spectral variance was clarified 68% and 18% by PC1 and PC2, respectively. Also, there is a high overlap between stage 2 and infected cluster. This means that pomegranate fruit at stage 2 may have similar spectral characteristics as the infected ones in the wavelength range between 400 and 1100 nm. This likeness may be partially described by the fact that carob moth infestation starts usually when the pomegranate passes from stage 1 to stage 3. Therefore, the infection of pomegranate at stage 2 is at the primary levels causing the NIR spectra to be less influenced by the compositional variation in fruit produced by carob moth infestation. In addition, the relative overlap between infected and stage 4 clusters may be due to the fact that carob moth causes a reduction in dry weight (DW) content of arils. This is exactly what happens during the ripening of a non-climacteric fruit such as pomegranate. During maturity, its dry weight content decreases until it reaches to the final value at stage 4. In this stage, pomegranates may have comparable dry weight content to those infected by carob moth infestation.

3.2. Discriminant analysis

Following the misclassification identified between intact and infected pomegranates at stage 2 and stage 4 by PCA (Fig. 3), the all discriminant analysis were accomplished for three different sample sets: standard (only the samples handpicked at standard harvest time consist of stage 1, stage 2 and stage 3), last (only the handpicked at last harvest time including stage 3 and stage

4) and combined (all four studied maturity stages together). For the development of the two discriminant models described in Section 2.3.2 to classify pomegranates according to infected and intact classes, the validation set was defined in each case and the results are summarized in Table 1.

As it can be seen from Table 1, the optimum numbers of PCs to accomplish best SIMCA models was between 4 and 5. The built PCA models were then applied to classify the respective validation sample sets. To evaluate the obtained results from the classification of three studied sample sets (standard, last and combined), the Coomans plots were used and the results can be observed in Fig. 4. These plots display the orthogonal distance from all new projected samples to the infected (abscissa) and intact (ordinate) classes. Also the critical cut-off class membership limits are obtained from those plots and indicated for each three sample sets. If an object belongs to an infected model (class), it should fall within the membership limit, which is on the left of vertical line while the intact objects should place below the horizontal membership limit.

On the basis of obtained results, it is detected that in all three Coomans plots there are some infected samples with a lower distance to the intact class. Also, there are some intact samples that are significantly close to the infected class. However at a significant level of 5%, some intact and infected samples were observed which their class distance values were not different. For this group of samples, the distance vs. leverage plot (Si vs. Hi plot) was used to evaluate their classification (the plots are not shown). Each sample was assumed to belong to the class which had the lower respective leverage value. The numbers of false positive samples were then determined in each sample sets. The obtained results revealed that standard harvested samples were predicted with an accuracy of 85% for the intact pomegranate samples and 81% for the infected pomegranate samples (Fig. 4a). These parameters to predict intact and infected pomegranate last samples were 88% and 84%, respectively (Fig. 4b). This means that accuracy of prediction for last sample group was better than standard sample group. Also, the combined sample group revealed higher values in comparison with the two other groups. The results showed that the accuracy of prediction for intact and infected pomegranate combined samples were obtained 83% and 79%, respectively (Fig. 4c). As it can be seen from Table 1, the whole accuracy of classification resulting from SIMCA analysis was about 84%, 86% and 82% for the standard, last and combined data sets, respectively.

Table 1
The test set validation results of different discriminant analyses.

Method	Harvest time	No. of PC, LV	Pomegranate classes		Total (%)
			Infected (%)	Intact (%)	
SIMCA	Standard	5	26/32 (81.25)	41/48 (85.42)	67/80 (83.75)
	Last	4	20/24 (83.33)	23/26 (88.46)	42/50(86)
	Combined	5	30/38 (78.9)	60/72 (83.3)	90/110 (81.81)
PLS-DA	Standard	9	27/32 (84.37)	43/48 (89.58)	70/80 (87.5)
	Last	10	21/24 (87.5)	24/26 (92.30)	45/50(90)
	Combined	10	32/38 (84.21)	63/72 (87.5)	95/110 (86.36)

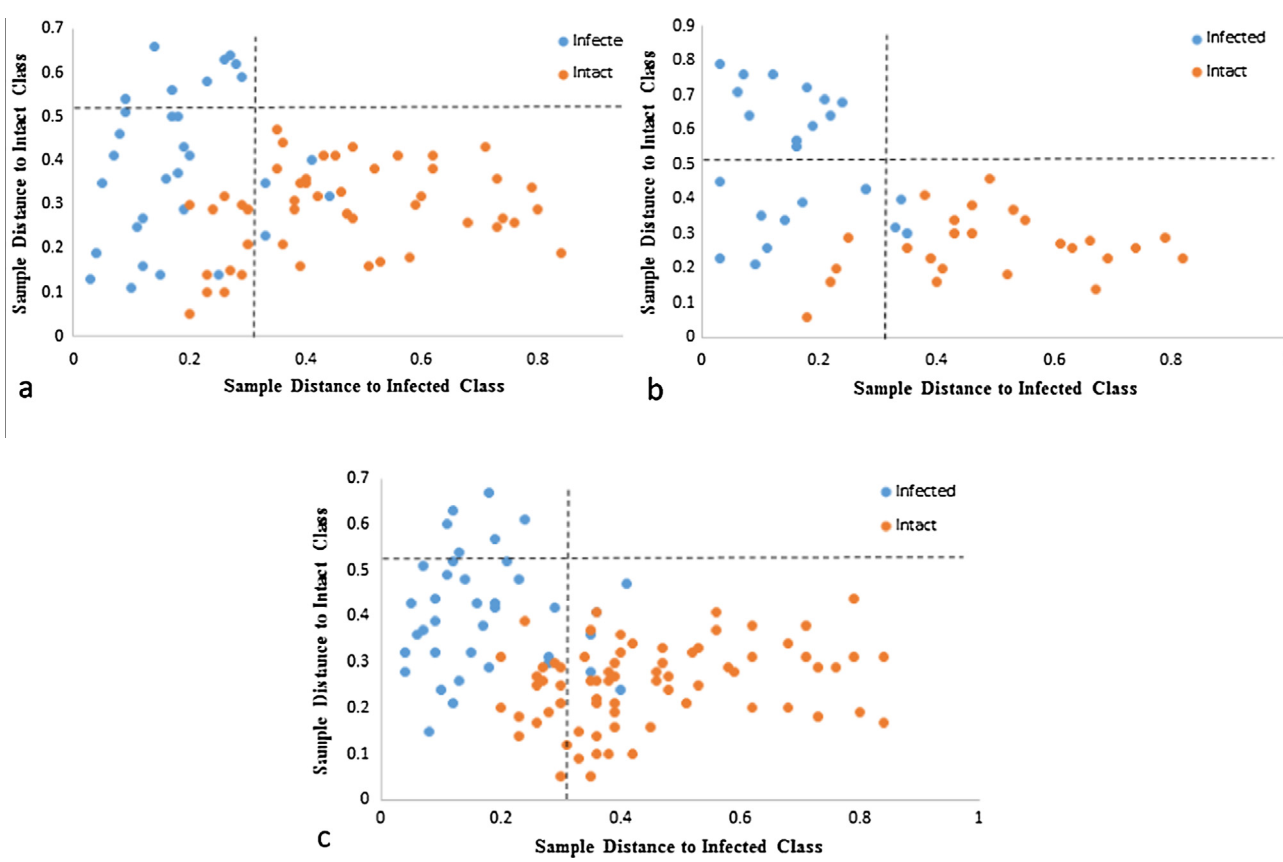

Fig. 4. The Coomans plots of the test sample sets using (a) standard, (b) last, and (c) combined sample sets.

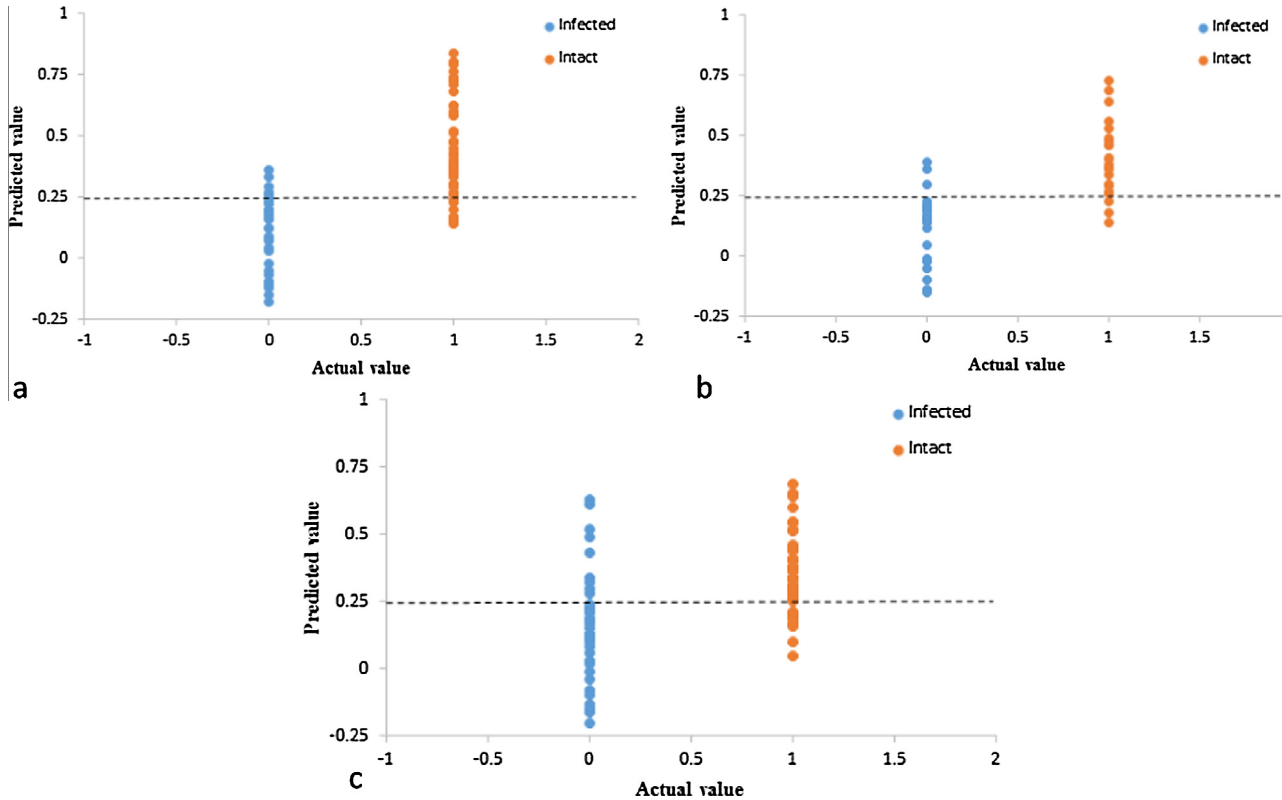

Fig. 5. The PLS-DA classification results of the test sample sets using (a) standard, (b) last, and (c) combined sample sets.

The optimum numbers of latent variable (LV) for the PLS-DA method in each sample group was between 9 and 10 (Table 1). Fig. 5 depicts the PLS-DA classification results of the test set samples using the standard, last and combined sample groups. As it can be found from this figure, the cut off value of 0.25 was used for the classification of intact and infected classes. It means that the samples with a predicted class value higher than 0.25 were identified as intact, while those with a predicted class value lower than 0.25 were classified as infected pomegranate. The PLS-DA results revealed that the accuracy of prediction was 89% (43 samples from a total of 48 samples) and 84% (27 samples from a total of 32 samples), respectively for the intact and infected pomegranate samples of standard harvested group (Fig. 5a), 92% (24 samples from a total of 26 samples) and 87% (21samples from a total of 24 samples), respectively for the intact and infected pomegranate samples of last harvested group (Fig. 5b), and combined sample group were predicted with an accuracy of 87% for the intact pomegranate samples and 84% for the infected pomegranate samples (Fig. 5c). The total discriminant power of PLS-DA classes was about 88%, 90%, and 86% for standard, last and combined sample sets, respectively.

The results also showed that in all sample groups when the samples were classified by PLS-DA, the high values were found in comparison with the SIMCA models. This priority is mostly due to the algorithm used to build the models. PLS-DA discovers new directions in the data space which can discriminate classes directly while SIMCA use the PCA models to capture variations within each class. Moreover, in SIMCA, during computing the PCA models within each class, only the information of spectral matrix (without taking into account the information in dependent class variable) is used, whereas in PLS-DA, those components in the spectral matrix are found which can describe the information in the dependent spectral variables as much as possible and simultaneously have the maximum correlation with the dependent class variable (Mireei and Sadeghi, 2013). Hence, the PLS-DA provides better results in comparison with the SIMCA. This is also pointed out for classification of other agricultural materials such as olive oils (Galtier et al., 2011); date fruit (Mireei and Sadeghi, 2013). However, Sirisomboon et al. (2009) reported that SIMCA showed obviously better performance than PLS-DA in classification of green soybean pods.

Also, the comparison among different sample groups indicated that the last sample group was predicted with the best prediction accuracy, followed by standard and then combined sample group. As it can be seen from Table 1, the best accuracy of prediction for last harvested samples was 92% (24 samples from a total of 26 samples) for the intact pomegranate samples and 87% (21samples from a total of 24 samples) for the infected pomegranate samples, resulting from PLS-DA algorithm. As it was stated earlier, the few symptoms of the carob moth in samples infected at stage 2 make it difficult to discriminate an accurate decision boundary between stage 2 and infected classes. On the other hand, by eliminating the samples at stage 2 from the data set, i.e. by progressing in the maturity process of pomegranates (last group samples), the classification accuracy is increased in both studied discriminant models. The classification analyses also indicated better accuracies for the standard harvest samples than the combined ones. The best accuracy of classification for standard sample group was 89% (43 samples from a total of 48 samples) for the intact group and 84% (27 samples from a total of 32 samples) for infected group (Table 1). This value for combined sample group was 87.5% (63 samples from a total of 72 samples) for the intact group and 84% (32 samples from a total of 38 samples) for the infected group.

4. Conclusion

This study provided information on the use of VIS/NIR spectroscopy as an automated, nondestructive and rapid technique to detect carob moth infestation in pomegranate fruits (cv. Ashraf). In this regard, two linear discriminant analyses namely PLS-DA and SIMACA were performed on three different sample groups including two or more maturity stages and their performances were compared. The following are concluded from this investigation:

1. It has been shown that VIS/NIR spectroscopy with discriminant analysis is a promising tool for nondestructive detection of carob moth infestation of pomegranate fruit. Also, both two studied classification methods namely PLS-DA and SIMACA showed best discrimination.
2. The PCA score plot showed that pomegranate fruits in stage 2 and stage 4 of maturity have similar spectral characteristics as the infected ones in the wavelength range between 400 and 1100 nm.
3. The results also showed that in all sample groups when the samples were classified by PLS-DA, the high values were found in comparison with the SIMCA models.
4. The maturity stage of pomegranate fruit can affect the performance of the classification methods. The comparison among different sample groups indicated that the last sample group was predicted with the best prediction accuracy followed by standard and then combined sample group.

In this study, the nondestructive discriminant models built were for detecting carob moth infestation of Ashraf pomegranate fruit. However, further studies are needed to evaluate this method in other commercial pomegranate varieties such as Malas which like Ashraf are susceptible to this infestation.

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