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REGULAR ARTICLE

RGB color imaging to detect *Aspergillus flavus* infection in dates

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ABSTRACT

In this study, the potential of RGB color imaging to detect fungal contamination in three varieties (Khalas, Fard and Naghal) of dates was investigated. The samples were treated as three groups: UC (untreated control), SC (surface sterilized, rinsed and air-dried) and IS (surface sterilized, rinsed, air-dried and fungal inoculated). Color images of control samples and *A. flavus* inoculated date fruits after every 48 h of inoculation for 10 days were acquired using an RGB color imaging system (n = 3150). The classification accuracies for IS were compared with UC and SC separately using a two-class model (control vs. infected (all stages of infection together)), six-class model (control, infected day 2, day 4, day 6, day 8 and day 10) and pair-wise model (control vs. each stage of infection). In the two-class model, the highest accuracy obtained by Fard, Khalas and Naghal dates were 97, 100 and 99%, respectively. The developed algorithm was also tested on pooled dates (all three varieties were combined together: Control vs infected), and 98% and 99% of infested samples were correctly classified from untreated control and sterile control, respectively in two class models. In six class models, highest classification accuracies of 99-100% were obtained for IS Day 10 in all three date varieties.

Keywords: RGB color images; *Aspergillus flavus*; dates

INTRODUCTION

RGB color imaging has been used for various quality attributes of dates because of its easiness in image analysis and low expense. Al-Rahbi et al. (2013) identified surface cracks on dates using color imaging with a classification accuracy ranging from 58 to 78% in three classes model (no-crack, low-crack and high-crack dates) and from 75 to 88% in two-class model (dates with crack and dates without crack). RGB color imaging was applied to grade dates based on certain quality aspects such as flabbiness, size, shape, intensity and defects caused by bruises and bird flicks with 80% accuracy (Al-Ohali, 2011). Using two class models with 39 extracted features on Fard, Khalas and Naghal varieties, an average of 84, 90 and 96%, respectively were correctly classified based on hardness (Manickavasagan et al., 2014). On the other hand, using only three features such as mean, entropy and kurtosis, an overall classification accuracy of 94% was obtained to classify Khalas, Fard and Madina dates (Thomas et al., 2014).

Fungal infection is a serious problem in handling, storage and processing facilities of date growing countries. Early detection of fungal infection in dates would be highly beneficial in taking corrective action plan (Teena et al., 2014). Despite several studies on date quality using non-destructive computer vision techniques, at present there is no published work on the detection of fungal infection using color imaging (Teena et al., 2013). However, color imaging has been used to develop classification models to classify fungal-damaged (*Alternaria* spp., *Cercospora* spp. and *Fusarium* spp.) soybean seeds (Ahmad et al., 1999). In another study with eight different fungal species, color imaging was able to classify 75% of both control and fungus infected corn kernels (Tallada et al., 2011).

The main goal in developing a quality inspection system should be achieving higher performance, in a shorter time at lower expense (Gunasekaran, 1996). Monochrome and RGB color imaging are comparatively cheaper than other imaging systems (Manickavasagan and Al-Yahyai, 2012). Therefore, the objective of this study was to assess the

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potential of RGB color imaging for detection of fungal infection in three varieties of Omani dates.

MATERIALS AND METHODS

Sample collection

Fresh tamr dates from Fard, Khalas and Naghal varieties were bought from local date markets in Muscat, Oman. From each variety, 450 dates with uniform size without any physical and insect damage were selected for the study. In each variety, the dates were equally divided into three groups with 150 dates each and labeled as: UC (untreated control), SC (surface sterilized, rinsed and air-dried) and IS (surface sterilized, rinsed, air-dried and fungal inoculated). All the samples were kept in sterile bags at 4°C before the experiments. The samples for fungal infection were inoculated with spores of *A. flavus* (PFAF001) obtained from the Plant Pathology Lab, College of Agriculture and Marine Sciences, Sultan Qaboos University.

Artificial inoculation of dates with *A. flavus*

The samples for SC and IS were conditioned to laboratory temperature (22°C) for 2 h. The samples were then surface sterilized by immersing in 1% sodium hypochlorite solution for 30 s, then followed by rinsing with sterile distilled water two times. The washed samples were air-dried under a laminar flow workstation (– P range, Bassaire, England) for 1 h to remove excess water. To promote fungal growth, a small wound was made on the surface of the IS dates using a sterile dissection needle. The *A. flavus* spore suspension was prepared by pouring and mixing 10 ml of sterile distilled water onto the fungal colonies cultured on PDA plate. This thick spore suspension was then collected in a sterile beaker and made up to 250 ml volume by adding sterile distilled water. For a uniform distribution of spores in the collected spore suspension, a drop of Tween 20 was added and mixed thoroughly. This spore suspension was examined for its spore concentration using a hemocytometer (Neubauer, China) and was found to have 107 spores/ml (Rahman et al., 2004; Teena et al., 2014).

Using a sterile micropipette, 10 µl of the prepared spore suspension was inoculated into each IS dates. The inoculated samples were stored in covered sterile plastic containers and incubated for the next 10 days in a sterilized environmental chamber (MLR-351H, SANYO, Japan) at 30°C and 85±1% RH (Hahn et al., 2004). To confirm the growth of *A. flavus* on the IS samples, at the end of incubation period the fungal colonies from 50 random samples were cultured on PDA plates using sterile needle inoculation technique. The PDA plates were kept at room temperature for two weeks and the morphological characteristics were studied.

Image acquisition

The images of all the date samples (UC, SC and IS) were captured using a color camera (EOS 1100D, EF-S 18-55 III, Canon Inc., Taiwan) with a resolution of 4272 × 2848 pixels. Before imaging, the camera was standardized by customizing the white balance by a gray card (Digital Gray Kard XL, DGK Color Tools, USA) with 18% reflectance (Al-Rahbi et al., 2013; Valous et al., 2009). The camera was located at 20 cm overhead to the sample platform to obtain the best image throughout the experiment. The samples were illuminated with two 36 W fluorescent lights (Dulux L, OSRAM, Italy) and enclosed in a black cardboard box with a door (Al-Rahbi et al., 2013). The inside wall of the box was concealed with black paper to reduce the reflectance and the roof was covered with white paper to enhance light scattering and reduce shadows (Al-Ohali, 2011). The date samples were positioned on a white background to provide higher contrast between the background and the date sample. The camera was connected to a computer equipped with the remote shooting software EOS Utility (version 2.1.0, Canon Inc., Japan) through which digital images were acquired. The images of inoculated date fruits were labeled as 'IS Day 2', 'IS Day 4', 'IS Day 6', 'IS Day 8' and 'IS Day 10'. Two other sets of 150 date fruits were imaged as 'UC' and 'SC'. A total of 3,150 ((UC-150 + SC-150 + IS Day 2-150 + IS Day 4-150 + IS Day 6-150 + IS Day 8-150 + IS Day 10-150) × 3 date varieties) color images were captured in this study.

Feature extraction

The processing steps performed in image analysis were thresholding, morphological operations, segmentation and feature extraction were performed by MATLAB (Version 7.6.0, The Mathworks Inc., USA). The preprocessing techniques such as thresholding, morphological operations and segmentation obtained a clearly defined object separated from the background. After preprocessing, the RGB image was converted into a grayscale image using *rgb2gray* function. Totally four components (red, green and blue components of the color image and gray component) were obtained.

Feature extraction is applied to select the most important characteristics from the object in an image and thereby classifying the object into a specific class (Al-Janobi, 2010). In total, 16 features including 11 histogram features and 5 textural features (from Gray Level Co-occurrence Matrix (GLCM)) were extracted from the region of interest from the red, green, blue and grayscale components of each color image. These were done using regionprops function. The histogram features extracted from the dates images were area, total gray value, mean intensity, standard deviation, variance, solidity, smoothness, minimum intensity, maximum intensity, extent and eccentricity. The texture

features extracted from the GLCM of the color images were contrast, energy, variance, maximum probability and entropy. The definitions of the above histogram and texture features are summarized in Table 1.

Classification

Classification using 64 features

The extracted features (16 features × 4 components = 64) were applied to SAS (Version 9.00, SAS Institute Inc., USA) to classify the dates into different classes or groups. The PROC DISCRIM procedure was performed in SAS to discriminate control dates from fungus infected dates of Fard, Khalas and Naghal using the 64 extracted features. Both LDA and QDA with leave-one-out cross-validation method was used for developing a two-class model (between each control and IS (five infection stages combined)), six-class model (between each control and IS (five infection stages in separate classes) and pair-wise model (between each control and each infection stages of IS as pairs) (Teena et al., 2014).

Also, to determine the potential of the developed algorithm on diverse samples, the images of the control and the fungus infected dates of all three varieties were joined together as a pooled sample and classification was performed using a two class model (UC vs. IS and SC vs. IS using LDA and QDA).

Classification using top five features

To identify the top five most contributing features among the total 64 features and to determine the classification accuracy with those features alone, the PROC STEPDISC procedure was performed in SAS to obtain SLDA and SQDA. The contribution level of each histogram and texture features were identified from the values of partial R2 and Wilks' Lambda and the top five most contributing features were identified and ranked for each classification models (Mahesh et al., 2008). The first step is done only to the most contributed variable on the classification and then the second variable is added in the following step. This format is repeated till the added variable has no significant effect on the classification.

RESULTS AND DISCUSSION

Image analysis and feature extraction

The extracted features from each date class and of each date variety were used for analysis. There was a significant difference between the mean intensities of the red component from the other components in all the date varieties. Similarly, there was a significant difference between the minimum intensity of all the four components (red, green, blue, grey) between the date varieties. Fard dates had the lowest range of minimum intensity (0-12) while Khalas had the highest (0-40). But, most of the texture features for the date varieties were not significantly different. The averages of mean intensity of different components at different stages are presented in Table 2. In all the date varieties, the mean intensity of red component was greater than all the other components. This trend of red component in grading dates was also observed in Al

Table 1: The definitions of histogram and texture features extracted from red, green, blue and grayscale components of the color image

Feature no	Feature	Definition*
Histogram features		
1	Area	The overall number of pixels in object image
2	Total gray value	The total number of gray values of all the pixels in an image
3	Mean intensity	The mean of mean intensities of all the pixels in an image
4	Standard deviation	The standard deviation of all pixels in an image
5	Variance	Variance of all the pixels in an image
6	Solidity	Proportions of the pixels in the convex hull that are in a region
7	Smoothness	Measure of the relative smoothness of the intensity in a region
8	Minimum intensity	The minimum pixel gray level in the object
9	Maximum intensity	The maximum pixel gray level in the object
10	Extent	Proportions of the pixels in the bounding box that is in the region
11	Eccentricity	Ratio of distance between the foci of the ellipse and its major axis length
Texture features using GLCM**		
12	Contrast	A measure of contrast between a pixel and its neighbors over the complete image
13	Energy	Measure of sum squared elements in GLCM
14	Variance	A measure of heterogeneity
15	Maximum probability	The maximum occurrence of the gray level
16	Entropy	A measure of randomness

*Gonzalez et al., 2011, **Gray Level Co-occurrence Matrix

Table 2: Mean intensity of different components of date varieties

Date class	Variety											
	Fard				Khalas				Naghal			
	Red	Green	Blue	Grayscale	Red	Green	Blue	Grayscale	Red	Green	Blue	Greyscale
Untreated control	68.9	51.6	48.8	57.4	102.5	63.1	45.1	68.1	86.4	59.5	50.9	66.6
Sterile control	67.3	50.4	47.6	56.1	100.2	56.2	50.3	72.0	82.3	58.3	50.6	68.8
IS Day 2	65.2	52.1	51.1	56.8	94.1	53.0	45.6	64.5	78.6	53.9	49.0	60.9
IS Day 4	62.7	49.4	47.5	54.1	92.2	53.5	46.6	64.3	77.5	60.6	48.1	67.2
IS Day 6	62.8	50.3	48.4	54.7	90.8	54.1	47.4	64.4	74.9	60.0	48.3	66.1
IS Day 8	62.9	51.2	49.3	55.4	89.8	54.8	48.1	64.5	72.8	55.1	49.4	60.5
IS Day 10	62.3	52.0	49.9	55.1	94.0	55.5	48.8	64.9	70.8	55.2	51.7	60.2
Mean	64.6	51.0	49.0	55.7	94.8	55.7	47.4	66.1	77.6	57.5	49.7	64.3
Standard deviation	2.6	1.0	1.3	1.2	4.8	3.4	1.8	2.9	5.4	2.7	1.4	3.7

Janobi (2000). The averages of the mean intensity for Fard, Khalas and Naghal dates (from red, green, blue and grey components) ranged from 47-69, 45-102 and 48-86, respectively. In a similar study, Manickavasagan et al. obtained 62-89, 49-95 and 50-93 as mean intensities (from red, green and blue components) for Fard, Khalas and Naghal, respectively (Manickavasagan et al., 2014).

Feature ranking

Top five most contributing features to correctly classify the different date samples into their respective groups or classes were selected using stepwise discriminant analysis. The overall most contributing top five features of the three date varieties obtained in this study are shown in Table 3. It was noted that mean intensity and minimum intensity of red, green and grey components were the most contributing features significant to the classification of Fard dates. However, standard deviation of green component and variance of grey component were the most selected features from Khalas and Naghal varieties. It was also observed that histogram features contributed more than texture features in classifying fungus infected dates from healthy dates. The green and grey components contributed more to classify control and fungus infected dates of all three varieties. Al-Rahbi et al. (2013) also observed that green component ranked the most in detecting surface cracks on dates.

Table 3: The overall most contributing top five features from dates (all classification models)

Rank	Fard	Khalas	Naghal
1	Red minimum intensity	Grey total gray value	Grey smoothness
2	Green minimum intensity	Blue standard deviation	Grey standard deviation
3	Green mean intensity	Blue variance	Grey variance
4	Grey mean intensity	Green standard deviation	Blue solidity
5	Red mean intensity	Grey variance	Green standard deviation

Classification

Two class model - variety wise

In this model, the IS were differentiated from UC of the three date varieties (Fard, Khalas, Naghal) by both the discriminant analyses (LDA & QDA) and stepwise discriminant analyses (SLDA & SQDA). The overall highest classification accuracies obtained for Fard, Khalas and Naghal dates were 97, 100 and 98%, respectively. Comparatively, Khalas dates obtained the highest accuracies (99-100%) among the tested date varieties in all classifications. Also, SLDA and SQDA did not affect the classification accuracies. In general, LDA obtained better classification accuracies for discrimination between fungus infected and healthy date fruits. This result was comparable to Singh et al. in which LDA obtained higher accuracies than the QDA classifier (Singh et al., 2007).

Similarly, the IS and the SC were classified by a two-class model. The overall highest classification accuracies obtained for Fard, Khalas and Naghal dates were 92, 100 and 99%, respectively. In this model also, Khalas dates obtained the highest accuracies (97-100%) among the three date varieties. However, Manickavasagan et al. (2014) demonstrated higher accuracy for Naghal variety in two class models. Also, in this model, QDA obtained higher classification accuracies than the LDA classifier. In comparison, UC vs. IS obtained higher classification accuracies than SC vs. IS.

Two class model - pooled dates (all varieties combined)

The overall highest classification accuracy from the two class model was 80% for UC vs. IS and 98% for UC and IS, respectively. The highest accuracy obtained in pooled dates indicates the potential opportunities of this method in mixed dates, irrespective of their difference in physical and chemical properties. However, the control samples (both UC and SC) were classified with less accuracy (less than 80%) in this approach. The most contributing top five features extracted from the pooled date samples along

with their Wilks' Lambda values are shown in Table 4. In addition to mean intensity and variance, eccentricity also played a significant role in classifying fungus infected dates from control using this model.

Six class model

In this approach, the ability of the developed method in classifying infected dates according to stages of infection was analyzed. In the classification accuracies of control (UC and SC separately) and different stages of fungal infection (IS Day 2-10) of Fard dates, both LDA and QDA provided the highest accuracy for IS Day 10 but the lowest for IS Day 6 samples. This could be because of severe misclassifications among the adjacent infected days due to similar fungal activity. Also, it was clearly observed that LDA gave higher accuracies than QDA. The classification results obtained by SLDA and SQDA using the top five most contributing features were relatively similar to LDA and QDA.

Similarly, in the classifications of different stages of infection of Khalas dates, the highest classification accuracies reported by both LDA and QDA were 100%; which was better than the results discussed in Teena et al. (2014) (LDA-97%, QDA-100%) for the same date variety using NIR hyperspectral imaging. From these results, it can be inferred that color imaging could provide equal or better classification than hyperspectral imaging to discriminate the infected samples of Khalas variety. A similar study, also on the Khalas variety, obtained the highest accuracy (87%) compared to Fard and Naghal based on hardness (hard, semi-hard and soft dates) (Manickavasagan et al., 2014). Al-Janobi (2000) extracted several histogram features from the red, green and blue color bands to grade saudian dates (Sifri Variety) into four quality classes. The features were used independently and also in different combinations and obtained good results with an average classification error of 1.8%.

In six-class classification between control and different days of infected date fruits of Naghal variety, the highest accuracies obtained were 99 and 98% for UC and SC, respectively. Also, LDA gave higher accuracies for both models than QDA. The lowest accuracy reported by Naghal dates was 51% for IS Day 4. The classification results obtained by SLDA and SQDA were comparatively lower than LDA and QDA. Hence, a greater number of features might be required for classifying fungus infected dates from six class models.

Table 4: Top five features for pooled dates in two class models

Rank	Untreated control vs. infected samples	Sterile control vs. infected samples
1	Green mean intensity	Grey mean intensity
2	Green eccentricity	Blue variance
3	Grey eccentricity	Green mean intensity
4	Red variance	Red mean intensity
5	Red total gray value	Green smoothness

Pair wise model

A pair-wise classifier was also tested on UC and different days of fungal infection for Fard, Khalas and Naghal dates. The highest and the lowest classification accuracies obtained by all classifiers (LDA, SLDA, QDA and SQDA) were 100 and 81%, respectively. Khalas dates were the only variety to obtain the 100% classification accuracy. In addition, LDA obtained higher classification accuracies than QDA for Fard and Naghal dates. Similar to six class models, in pair-wise, classifications of SLDA and SQDA obtained lower accuracies than LDA and QDA.

Likewise, pair-wise classification of SC and all stages of IS for the three date varieties were evaluated. The highest and lowest classification accuracies obtained by all classifiers were 100 and 68%, respectively. In addition, Fard dates obtained lower classification accuracies when compared to the other date varieties. Moreover, LDA obtained higher accuracies than QDA; and SLDA and SQDA obtained lower results than LDA and QDA. These pair-wise models obtained higher accuracies than what reported Al-Rahbi et al. (2013), which demonstrated a higher accuracy of 96% between high-cracks and no-cracks dates. Also, this model could correctly classify fungus infected samples at day 2 from both the controls. RGB imaging was also used to grade dates based on external quality features (size, shape, defects, flabbiness and so on) into three quality classes and obtained an average classification accuracy of 80% (Al-Ohali, 2011).

CONCLUSIONS

The potential of color imaging to identify healthy and fungus infected date fruits was investigated in this study. Histogram features such as minimum intensity, mean intensity, total gray value, standard deviation, variance, smoothness, solidity were identified as the overall most significant features to classify the fungal contamination in date fruits. Khalas variety obtained the highest classification accuracy (100%) among the tested date varieties in two class models. The highest accuracy obtained by Fard and Naghal were 97% and 99%, respectively. In two-class model with all varieties combined, 98% and 99% of infested samples were correctly classified from untreated control and sterile control, respectively. Similarly, in six class models of Fard, Khalas and Naghal, the highest classification accuracies reported were 100, 100 and 99%, respectively. Generally, SLDA and SQDA obtained lower accuracies when compared to LDA and QDA. Also, 100% accuracy was obtained by Khalas variety in a pair-wise model with

untreated control and different stages of fungal infection. Thus, color imaging can be effectively used to classify fungus infected date fruits from healthy dates and hence could be successfully applied in date factories. However, intensive research is warranted to study the effect of different types of fungal infections on dates and their potential detection.

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Author contributions

All authors contributed equally in this article.

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