# 1 A New Approach to Aflatoxin Detection in Chili Pepper by Machine Vision

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#### Abstract

Aflatoxins are the toxic metabolites of Aspergillus molds, especially by Aspergillus flavus and 7 8 Aspergillus parasiticus. They have been studied extensively because of being associated with 9 various chronic and acute diseases especially immunosuppression and cancer. Aflatoxin occurrence is influenced by certain environmental conditions such as drought seasons and 10 agronomic practices. Chili pepper may also be contaminated by aflatoxins during harvesting, 11 12 production and storage. Aflatoxin detection based on chemical methods is fairly accurate. However, they are time consuming, expensive and destructive. We use hyperspectral imaging as 13 an alternative for detection of such contaminants in a rapid and nondestructive manner. In order to 14 classify aflatoxin contaminated chili peppers from uncontaminated ones, a compact machine vision 15 system based on hyperspectral imaging and machine learning is proposed. In this study, both UV 16 17 and Halogen excitations are used. Energy values of individual spectral bands and also difference images of consecutive spectral bands were utilized as feature vectors. Another set of features were 18 extracted from those features by applying quantization on the histogram of the images. Significant 19 20 features were selected based on proposed method of hierarchical bottleneck backward elimination (HBBE), Guyon's SVM-RFE, classical Fisher discrimination power and Principal Component 21 Analysis (PCA). Multi layer perceptrons (MLP) and linear discriminant analysis (LDA) were used 22 23 as the classifiers. It was observed that with the proposed features and selection methods, robust and

higher classification performance was achieved with fewer numbers of spectral bands enabling thedesign of simpler machine vision systems.

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Keywords: Machine vision, aflatoxin detection, hyperspectral imaging, food safety, feature
extraction, feature subset selection, classification, multi layer perceptron.

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#### **1. INTRODUCTION**

Aflatoxins are toxic compounds produced by many species of Aspergillus molds, especially by 31 32 Aspergillus flavus and Aspergillus parasiticus (Zeringue et al. 1998). The term "aflatoxin" comes from Aspergillus flavustoxin. As International Agency for Research on Cancer (IARC) pointed 33 out, aflatoxin causes human liver cancer (IARC, 2002). A wide variety of foods (hazelnut, pistachio 34 35 nut, almond, dried fig, wheat, corn, chili pepper, etc...) are susceptible to aflatoxin contamination that degrades food quality and threatens human health. Therefore, several countries have taken 36 strict regulations to control aflatoxin contamination level. Generally accepted aflatoxin level in 37 food is, 20 ppb (parts per billion) in both USA and Turkey. On the other hand maximum level of 38 aflatoxin B1 and total aflatoxin was determined as 5 ppb and 10 ppb in European countries, 39 respectively (Commission Regulation [EC], 2006). 40

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42 Aflatoxin contamination can occur during pre-harvesting and post-harvesting periods. High 43 temperature, prolonged drought conditions and high insect activities are significant factors for 44 aflatoxin contamination during pre-harvesting. For post-harvesting, warm temperature and high 45 humidity factors become active ingredients that increase the mold invasion and toxin production

(Wagacha and Muthomi, 2008). High Performance Liquid Chromatography (HPLC), Mass 46 47 Spectroscopy (MS), Thin Layer Chromatography (TLC), and Enzyme-Linked Immunosorbent 48 Assay (ELISA) are widely known chemical aflatoxin detection methods amongst which HPLC is 49 superior in terms of accuracy and sensitivity (Chen et al, 2005). As an alternative to chemical methods, machine vision and pattern classification techniques are considered for aflatoxin 50 51 detection because they are faster, cheaper and nondestructive (Kalkan et al., 2011, Yao et al., 2011, 52 Yao et al., 2006, Pearson et al., 2001). As Shotwell et al. (1972), Fersaie et al. (1978), Doster et. al, 1998 and Herrman, (2002) pointed out, under ultraviolet 365 nm illumination, aflatoxin 53 54 contaminated samples exhibit bright green yellowish fluorescence (BGYF). However it should be 55 noted that the mechanism is much more complex. Certain fungi produce kojic acid which may result in BGYF when there is enough peroxidase enzyme in the plant. It is known that not all fungi 56 57 that produce kojic acid also produce aflatoxins. Similarly, the lack of peroxidase enzyme may conceal the presence of aflatoxins because BGYF will be absent. Thus, BGYF itself does not 58 directly indicate the actual presence of aflatoxin and it may result in false positives and negatives 59 60 during the evaluation stage. Furthermore, in the previous studies on corn and pistachio the authors (Pearson et al., 2001, Yao et al., 2006) stated that BGYF phenomenon under UV illumination is 61 observed when average aflatoxin level exceeds 100 ppb. Therefore, BGYF based aflatoxin 62 detection is not always recommended (Fersaie et al, 1978, Herrman, 2002, Doster et. al, 1998). 63 Some researchers used BGYF in their studies. Utilizing the reflectance ratios of 440/490nm and 64 450/490 nm, Tyson and Clark, (1974) achieved 90% classification rate by examining aflatoxin-65 infected pecans under UV fluorescence By analyzing corn kernels, again Yao et al. (2006) achieved 66 87% and 88% classification performance for 20 ppb and 100 ppb aflatoxin level thresholds. Kalkan 67 68 et al. (2011) studied hazelnuts and red chili peppers and achieved 92.3% and 79.2% classification

69 accuracies respectively. Another possible excitation mode is halogen illumination. Hirano et al. 70 (1998) used transmittance ratio (T700nm/ T1100 nm) bands for peanuts classification under 71 halogen illumination and achieved 95% classification accuracy. Pearson et al. (2001) achieved 72 96.6% classification accuracy rate of corn samples illuminated by 100W quartz-tungsten-halogen 73 lamp by utilizing the spectral reflectance ratio (R735nm/R1005nm). They used discriminant 74 analysis technique for detecting highly contaminated corn kernels (>100 ppb) from low 75 contaminated (<10 ppb) or uncontaminated ones.</p>

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We consider aflatoxin detection in ground red chili pepper flakes in the present paper. The following section will describe hyperspectral data acquisition and preprocessing. Next, we will express our proposed feature extraction and selection approaches. Experimental results will be reported and discussed in Section 4. Finally in Section 5, we will give concluding remarks.

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# 82 2. HYPERSPECTRAL IMAGE ACQUISITION AND PREPROCESSING

In the previous studies (Kalkan et al. 2011, Yao et al., 2006, Pearson et al., 2001), single illumination sources were used. More specifically, some studies were performed only under halogen illumination whereas others were done under UV. Basically, UV illumination is utilized for the fluorescence, halogen excitation is for reflectance phenomena. In order to investigate the contribution of those illuminations on the classifier performance, we utilized both excitations in this study. Figure 1 and Figure 2 depict a general overview of the hyperspectral imaging system and a flowchart and interrelated components of the proposed system, respectively.

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Figure 1. A general overview of the hyperspectral imaging system

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93	Totally 53 ground red chili pepper flake samples were gathered from different regions in Turkey.
94	Most of them were sold as unpackaged. Figure 3 shows their aflatoxin variations as a histogram
95	graph. Here we applied log10(1+Aflatoxin value) transformation in order to build a more compact
96	histogram plot. As 10 ppb is the upper threshold for aflatoxin for spices and herbs in the EU
97	(Commission Regulation [EC], 2006) we used 10 ppb as a threshold to classify the pepper samples
98	into aflatoxin positive (contaminated) and negative (uncontaminated) groups. Mean aflatoxin level
99	was measured as 16.78 ppb. Average aflatoxin levels for Afl- and Afl+ groups are 3.2 ppb and 33.3
100	ppb, respectively. As previous studies (Pearson et al., 2001, Yao et al., 2006) stated that BGYF is
101	observed when aflatoxin level in the sample is high, we expect halogen illumination to contribute
102	more for detection of aflatoxin for our chili pepper problem.
103	
104	Figure 2. Flowchart and interrelated components of the proposed system.
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106	The hardware of the image acquisition system is composed of a FireWire Sony CCD camera with
107	Varispec liquid crystal tunable filter assembly. Hyperspectral image series ranging from 400 nm to
108	720 nm (10 nm spectral bandwidth) of 53 different chili pepper samples have been acquired under
109	100W quartz-tungsten-halogen and UV 365 nm illumination sources. Resolution of each image is
110	1280 X 960 pixels. During the acquisition process the set up was stationary, so there was no need
111	to register the hyperspectral images. Images of three different locations of the same chili pepper
	to register the hyperspectral images. Images of three different locations of the same entit pepper

surface of the chili pepper sample. Figure 4 depicts sample images from the hyperspectral image

series of uncontaminated and contaminated peppers for halogen and UV illuminations.

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Figure 4. Sample images from the hyperspectral image series of uncontaminated and
 contaminated peppers for halogen and UV illuminations.

All the pepper samples were sent to TUBITAK Ankara Testing and Analyses Laboratory (ATAL)
for HPLC analysis. Chili pepper samples that exceed 10 ppb threshold were labeled as aflatoxin
positive otherwise they were labeled as aflatoxin negative for inductive learning.

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123 Camera software by default applies histogram equalization to acquired images. Although, 124 histogram equalization automatically controls oversaturation and under-saturation by applying adaptively changing exposure time, it also modifies original pixel values. To overcome this 125 126 particular problem, one should fix the exposure time value of the camera and camera gain parameter to a predefined value. On the other hand, single exposure time eventually leads to under 127 saturated and over saturated regions in the hyperspectral image series. Therefore, we dictated three 128 129 spectral regions by manually changing exposure time values. Table 1 depicts the prescribed spectral regions, exposure times and corresponding normalization coefficients. We normalized the 130 exposure of the images by their normalization coefficient before extracting the feature vectors. It 131 should be noted that, exposure normalization leads some pixel values to exceed 255 pixel gray 132 values and increase the dynamic range excessively. Therefore, we applied non-linear square root 133 transformation in order to limit the range of the pixel value of the normalized images to 0-255 pixel 134 gray value interval. With a maximum normalization coefficient of 9, a pixel value of 255 will yield 135 corresponding maximum pixel gray value of 48 after the square root transformation. We used 48 136 137 levels to represent pixel gray values.

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139	Table 1. Exposure normalization coefficients of the most informative regions of the spectral
140	bands.
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142	In order to eliminate the dust particles and reduce sensor noise in the images we applied 3x3 median
143	filtering.
144	
145	3. FEATURE EXTRACTION AND SELECTION
146	Classifier performance is strongly related to the relevance of the extracted features. In the ideal
147	case, the feature vector should keep the most compact description of the desired function. In our
148	problem this is the aflatoxin presence signature. Nevertheless, extracting meaningful and
149	discriminative feature vector is not a straightforward and trivial process. It requires acquiring
150	domain knowledge and underlying physical phenomena. In the hyperspectral images of chili

151 pepper samples, shape and orientation of chili pepper flakes does not correlate with aflatoxin 152 presence. Therefore, useful features should have weak relevance to the second order features like edges and orientation. Relying on solely spectral band mean intensity is not desirable either. There 153 154 may be Afl- samples and Afl+ samples with nearly the same mean intensity value. In the previous 155 studies, Kalkan et al., (2011) used wavelet based intensity features and achieved 79.2 % classification performance indicating that spatial and textural information contributes the 156 prediction accuracy. Conversely, most of the researchers (Pearson et al, 2001, Yao et al. 2006, 157 Hirano et al, 1998) utilized spectral band energies as a feature vector in their studies and achieve 158 159 reasonable accuracy rates. In this study, we extracted features by applying histogram based feature 160 extraction technique. Histogram based feature extraction was used in several studies Sakthivel et

al (2010) used histogram based features for face recognition problem, Singh et al (2009) utilized
histogram features for detecting insect damages in wheat kernels, ElMasry et al. (2007) used for
detection of apple bruise and Yang et al. (2010) employed histogram features for food recognition.
We will compare the performance of different types of features.

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Another concern is the size of extracted feature vector. Larger feature size results in the well known "curse of dimensionality" problem. Increasing the feature vector dimension requires an exponential increase in the data size. Hence, the size of the feature vector should be reduced to an acceptable level. Fewer features not only improve the classifier performance but also provide faster computation and better understanding the underlying mechanism of the problem (Guyon and Elisseeff, 2003).

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#### **3.1. Feature extraction**

Feature extraction is the process of transforming the raw data (in our problem it is the pixel gray 174 175 values) into a set of reduced descriptive features. Massive amount of data in hyperspectral image cube can be described by features of a lower dimension by applying the feature extraction process. 176 PCA and Auto-Associative Artificial Neural Network (ANN) are used for further reduction of the 177 feature vector size. PCA maps high dimensional features onto a lower dimensional space by 178 selecting the principal eigenvectors. Similarly, Autoassociator is equivalent to PCA if only one 179 hidden layer is used as a bottleneck layer in the network topology and a linear activation function 180 is used. It may outperform the PCA when an appropriate non-linear transformation is employed 181 (Bourlard and Kamp, 1988). Let us assume the pixel gray value located at x, y of the k'th spectral 182 band is denoted by  $I_k(x, y)$ . We extract the following feature vectors. 183

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185 Individual band energy features:

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$$e_k = \sum_x \sum_y I_k(x, y)$$
  $k = 1, 2, ... 33$  (1)

$$e_k = \sum_{x} \sum_{y} |I_{k+1}(x, y) - I_k(x, y)| \qquad k = 1, 2, \dots 32$$
(2)

Here,  $I_k(x, y)$  and  $e_k$  correspond to pixel gray level intensity at image point (x,y) and energy value for the *k* th spectral band, respectively. The feature vectors described in Expressions 1 and 2 reduce the information in a given band to a single value. However, the frequency of a particular intensity value or the frequency of the difference of the intensity values may provide valuable information. This information can be extracted if the histogram of the intensity values or the difference of the intensity values for a given spectral band is used.

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Figure 5 and Figure 6 present extracting processes of the quantized histogram matrix features. As it is shown in Figure 5, the histogram of the spectral band image is first computed with predefined number of bins. This limits the feature vector size and also promotes that a reasonable number of pixels fall in each bin. Then, the total number of pixels within the particular bin is used as the histogram feature. By using all spectral bands we can construct the quantized histogram matrix (QHM) as depicted in Figure 6. For simplicity we only demonstrate the extraction process for 12 bins. Different numbers of bins were also used and we will describe them in section 4 in detail.

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204 QHM feature set is expected to contain the aflatoxin signature. This signature may be degraded if 205 the overall mean intensities of the spectral bands are used instead. The QHM features are computed

both for individual spectral bands and also for the absolute difference of consecutive spectral bands.

207 Hence the QHM features can be expressed as

208 
$$e_{k,n} = \sum_{x} \sum_{y} I_{k,n}(x, y)$$
  $k = 1, 2, ..., 33$   $n = 1, 2, ..., B$  (3)

Where *k* denotes index of spectral band, *n* denotes the bin index and *B* denotes number of bins that we want to employ. As a result,  $I_{k,n}(x,y)$  is the pixel gray value of the *k*th spectral band or absolute difference of consecutive spectral bands of the *n*th bin.

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Figure 5. a) A sample image (640 nm). b) Representative gray level histogram of the image in

- (a) to 12 bins. The color of the histogram bar at the bottom depicts the total number of pixels
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falling in each bin.

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Figure 6. Quantized histogram matrix (QHM) is composed of histogram bars. a) Individual
spectral band energies b) Absolute difference of consecutive spectral band energies. X axis

- 219 denotes the spectral bands (or band pairs in the case of absolute difference) and Y axis denotes
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### **3.2. Feature selection**

histogram bar for that band.

The main objective of the feature selection is to reduce the feature vector size without modifying the feature values. As the features are computed for each band separately in our problem, reducing the feature size removes the need for acquiring the corresponding image along the spectral axis. As a result, a more compact machine vision system can be established. Moreover, the dataset may contain redundant, irrelevant and/or noisy features. Removing these features is expected to yield higher and more robust classification rates. Feature selection can be divided into two main

categories. First is the feature ranking. The second is the feature subset selection. Feature ranking 229 230 is the process of sorting the candidate features according to their predictive significance. Although 231 this approach is computationally efficient, determining the number of features is still an open 232 problem. Selecting the top N features is intuitive but there is a possibility of retaining highly 233 correlated features as in the hyperspectral imaging domain. In this study we used feature ranking 234 scheme based on the Fisher discrimination power and employed the feature selection method to 235 reveal the most discriminative features. Fisher discriminant was first proposed by Fisher, R.A. (1936) and is utilized in various studies. Fisher discrimination projects data from n-dimensional 236 237 space to a one-dimensional space where between class scatter is maximum and within class scatter 238 is minimum. It can be computed as;

$$F_{dp} = \frac{|\mu_1 - \mu_2|^2}{\sigma_1^2 + \sigma_2^2} \tag{4}$$

Here,  $F_{dp}$  is Fisher discrimination power,  $\mu_i$  and  $\sigma_i$  denote the mean and standard deviation of the 240 241 *i*th class respectively. Figure 7 depicts composite illustration of the boxplot (a) of the individual 242 spectral bands with their Fisher discrimination power (b). The boxplot at the top shows the 50 percentile of the mean energy features of the contaminated (lighter color) and uncontaminated 243 244 (darker color) chili peppers after z-score normalization over each spectral band. The horizontal bar beneath indicates the spectral bands value from 400 nm to 720 nm with 10 nm width. It can be 245 observed that, uncontaminated chili pepper samples have relatively higher intensity levels than the 246 contaminated ones. However, there are many samples that lie at the tails of the energy distribution 247 (outliers) and the mean energy value distributions overlap significantly. Therefore, a threshold 248 value that would separate the data into two distinct classes cannot be determined. The Fisher 249 discrimination power for each spectral band is computed using Expression 4 and depicted in the 250 bottom graph. Consistent with the boxplot above, the higher discrimination values are observed 251

252	between 540 to 640 nm spectral bands. Similarly, Figure 8 illustrates integrated box plot of the
253	absolute difference energies of consecutive spectral band features and Fisher discrimination power
254	values. Most discriminative spectral bands lie between 520 to 570 nm.
255	
256	Figure 7. a) Boxplot, b) Fisher discrimination power, of the individual spectral band energy
257	features.
258	
259	Figure 8. a) Boxplot, b) Fisher discrimination power, of the absolute difference of consecutive
260	spectral band energy features.
261	
262	For feature subset selection, we propose a novel feature subset selection method based on the MLP
263	connection weights. By saying MLP we actually mean special case of one hidden-layer, feed-

forward neural network trained by the well known back propagation algorithm (Rumelhart et al.,
1986). Garson, (1991) stated that one can define feature saliency metric as:

$$\boldsymbol{\tau}_{i} = \sum_{j=1}^{N} \left| \boldsymbol{W}_{ji} \boldsymbol{W}_{j} \right| \qquad \qquad i = 1, 2, \dots M \tag{5}$$

Here,  $\tau_i$  denotes saliency metric of the *i*th feature. *M* and *N* are the number of input and hidden 267 268 nodes, respectively.  $W_{ii}$  is the connection weight between the *i*th node of the input layer and *j*th 269 node of the hidden layer. Similarly,  $W_i$  denotes the connection weight between *j*th node of the 270 hidden layer to the output. In addition to this, Olden and Jackson, (2002) used Garson's algorithm 271 and sensitivity analysis by applying randomization approach to show that MLP based variable selection can be applied and interpreted successively in the ecological domain. 272 Each 273 feature/variable is represented as an input node in the neural network topology. Let N be the number 274 of hidden neurons in the hidden layer. As Figure 9 indicates *i*th feature's saliency metric can be

computed as the sum of the absolute product of the connection weights from the *i*th input nodethrough the hidden nodes to the output node.

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Figure 9. MLP with input, hidden and output layer.  $W_{ji}$  is the connection weight between *i*th input node and *j*th hidden node. Similarly,  $W_j$  is the connection weight between *j*th hidden node and the output node.

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The logic behind MLP based feature saliency metric is as follows. Connection weights are 282 283 continuously updated in the training phase so that significant input nodes have strong connections 284 in the network topology which means those input nodes have higher contribution to the output. Likewise, connection weights of the irrelevant features tend to vanish. Hence, MLP based feature 285 saliency metric can be used as a dimensionality reduction technique by decaying the connection 286 weights of the insignificant features. Moreover, the saliency can also be instrumental in ranking 287 the features based on their discrimination power. This approach was taken in our previous study 288 289 (Atas et al., 2011)

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We propose a novel feature subset selection technique based on the MLP feature saliency metric. Forward selection and backward elimination were extensively used subset selection methods and regarded as robust to over fitting (Guyon and Elisseeff, 2003). Again, Guyon et al, (2002) proposed a state of the art support vector machine based recursive feature elimination method (RFE). In the RFE approach, the search starts with complete feature set. The features are ranked according to their predictive significance at every iteration and the least significant one is removed from the feature set. This procedure proceeds until some criterion is met. One can remove a single or last N

298 features. Eliminating one feature per iteration is computationally costly, especially if you have 299 large number of features. Thus, we modified the RFE method of Guyon et al, (2002) by replacing 300 the central classifier SVM by MLP and we accelerated the process by removing last N features at 301 each iteration where N is equal to the number of hidden neurons in the MLP network. Determining 302 the optimal number of neurons in the hidden layer is still an open problem in the ANN domain. 303 Yet there are some rules of thumb which are extensively used by the researchers (Rapid Miner, 2009, Berry and Linoff, 1997, Boger and Guterman, 1997, Blum, 1992). Specifically, the Rapid 304 305 Miner team (2009) suggested number of neurons in the hidden layer to be:

$$N_{nodes} = \frac{(N_{features} + N_{classes})}{2} + 1$$
(6)

Here,  $N_{nodes}$  denotes the number of nodes in the hidden layer,  $N_{features}$  is the number of features used as input nodes. In our trials, this approach gave satisfactory results so we used it. The procedure consists of two main stages: Backward elimination and subset verification. At the backward elimination stage, the candidate feature set is ranked based on the MLP saliency metric and the last  $N_{nodes}$  as in Expression 6 are eliminated. This process is repeated until only one feature remains. The number of steps, *M*, required for *P* original features is given by

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$$M \cong \log_2 P \tag{7}$$

The candidate feature subset at every step is recorded in an array list data structure as shown in Figure 10. After the feature elimination process is completed, subset verification is initialized. At this stage, starting from the lowest feature subset (which typically consists of a single feature) generalization errors for each feature subset are computed. If there are *L* ranked features at a given subset, the generalization error for i) only one, ii) first two, iii) first three, etc. features are computed using leave-one-out cross validation strategy. The computational cost associated with this step increases exponentially with increasing the number of ranked features *L*. Therefore, we only

321	computed generalization errors for the first five levels from the bottom of the triangle in Figure 10
322	We chose the feature subset which yielded the minimum generalization error. In the case of similar
323	generalization errors we preferred the smaller size of features amongst the candidate feature
324	subsets. We used non-random V shape (triangular) weight initialization scheme for the connection
325	weights between input and hidden layer. That way, reproducible results could be obtained during
326	the feature selection process. Bias values were set to zero at start up.

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Figure 10. Proposed HBBE method. L and M designate the number of features and the number of

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# **3.3.** Classifier selection

steps, respectively.

332 We decided to utilize both a simple classifier and a complex classifier for our problem. For linearly 333 separable problems, Linear Discriminant Analysis (LDA) provides good classification performance. Moreover due to its simplicity LDA is less susceptible to over fitting the training data 334 335 which provides robustness. Thus, LDA was selected as the first classifier. As the second classifier, we preferred to use a feed forward back propagated one hidden layered artificial neural network 336 also known as the MLP. MLP is extensively used in various studies and is regarded as the universal 337 approximator of any continuous function (Hornik et al, 1989). Elmasry et al., (2009), Bochereau et 338 al., (1992) and Jayas et al. (2000) reported that, ANN is very efficient for identification and 339 340 classification of agricultural products which contain non-linearity. In particular, Kim et al. (2000) 341 pointed out that, MLP is superior to linear classifiers in terms of prediction accuracy for the classification of kiwi fruit berries. Again, Park and Chen (1996) utilized ANN with a spectral 342 imaging technique and successively achieved 93.3% generalization performance for classifying 343

wholesome chicken carcasses from unwholesome ones. Thus, we selected MLP as the second
classifier in this study. In addition to these, SVM with linear kernel classifier was also used within
the SVM-RFE method.

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### **4. EXPERIMENTAL RESULTS**

Hyperspectral image series with 33 spectral bands at two different illumination modes (halogen 349 350 and UV) of 53 chili pepper samples were acquired. Images of three different locations of each chili 351 pepper sample yielded a total of 10494 images of 1280x960 resolution. It should be noted that, 352 during the evaluation stage, images of the same chili pepper samples were isolated from the training 353 data so that unbiased accuracy results can be achieved. By using Equation 1 and Equation 2, feature vectors with size of 33 individual spectral band energy features and size of 32 absolute differences 354 355 of consecutive spectral band energy features were extracted. The other two types of feature sets 356 were extracted according to Equation 3. They are, quantized individual spectral band energy features and quantized absolute difference of consecutive spectral band energy features. We tried 357 358 6, 12, 24 bins feature set and achieved best discrimination power with 12 bins. The total number of features in the quantized individual spectral band was 33 (spectral bands) x 12 (quantization 359 360 bins) = 396. Similarly for the quantized absolute difference of consecutive spectral band, originally we had 32 (difference spectral band pairs) x 12 (quantization bins) which resulted in 384 features. 361 As it is seen in Figure 6, there exists high number of zero value features in the feature set. MLP 362 363 typically discards those features in the first step.

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All the data were normalized according to Z-Score normalization yielding the distribution of the data with zero mean and unit variance. The learning rate, momentum coefficient and the number

367	of epochs were adjusted adaptively with the rate of convergence. The learning rate and the
368	momentum value were initialized as best practices to 0.1 (Cravener and Roush, 1999, Rajanayaka
369	et al., 2003), and decayed during the learning phase. Decaying procedure is summarized in Figure
370	11. By this way it is expected to prevent the classifier from over-fitting and under-fitting. As
371	Hornik et al. (1989) and Malek et al. (2000) pointed out, MLP with a single hidden layer is adequate
372	as a universal approximator. We also employed a single hidden layered network topology in our
373	study. The number of hidden nodes in the hidden layer was determined via the Expression 6.
374	
375	Figure 11. Schematic diagram of the decaying procedure of the learning rate and momentum
376	values.
377	K-fold cross validation technique was utilized for the evaluation of generalization performance. In
378	the machine learning community, $K$ is commonly selected as 10 or 5 (Breiman and Spector, 1992,
379	Wassenaar et al., 2003). Therefore in this study we used $K$ as 5. We partitioned our data set
380	randomly into five disjoint folds. Four folds were used for training and validation purposes and the
381	remaining fold was utilized as the unseen test data for our predictive model. Since our data is
382	limited, we would like to exploit all the data at the training and validation set. Thus, we preferred
383	to employ leave one out cross validation (LOO-CV) technique for training and validation set. The
384	final decision on aflatoxin presence is made using majority voting on the three images of the same
385	chili pepper. This process was repeated for each fold and average accuracy rate was computed from
386	the five folds test results.

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In order to assess the effectiveness of our proposed method we compared its classification accuracy
rates with those of the original features and reduced features by applying PCA, SVM-RFE and

390 Fisher methods. To achieve a fair comparison with HBBE and SVM-RFE algorithms, feature 391 selection process was also employed on the PCA and Fisher methods. Table 2 shows overall 392 accuracy rates of several feature sets with various feature selection methods under the halogen and 393 UV illuminations. Average number of features used for each methods are given below the accuracy 394 rates in parentheses. As Table 2 indicates, in most cases, HBBE method outperforms other. Even 395 PCA gives higher accuracy in two cases (Halogen-individual band and UV-absolute difference), HBBE is still preferable since PCA uses all the spectral bands which is not a desired property for 396 constructing a simple machine vision system. 397 398 Table 2. Generalization performance of the extracted features MLP versus LDA classifiers under 399 400 halogen and UV excitations. 401 As it can be seen from Table 2, taking the absolute difference of consecutive spectral bands 402 generally improves the classification performance for both halogen and UV excitations. Similarly 403

404 quantization process increases the accuracy rate in almost all the feature sets.

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Table 3 compares the best results obtained by our proposed methods to wavelet LDB method of Kalkan et al. (2011). Best results for Dataset-1 and Dataset-2 are shown in bold. As it is seen in the table, our proposed method outperform wavelet LDB method. This comparison was made for two datasets. The Dataset-1 which consists of 53 new chili pepper samples using electronically tunable filter, is the spectral data acquired for this research, the Dataset-2 comprises 40 chili pepper samples imaged using optical filters with full width half maximum (FWHM) 400 to 600 nm as described in

412 (Kalkan et al., 2011) study. We should emphasize that the bands 520-720 nm with 10 nm width413 were not available in the Dataset-2.

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Table 3. Benchmark of the proposed method against wavelet LDB method for Dataset-1 andDataset-2

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418 In the case of the Dataset-2, the quantized individual band energy features yield better accuracy rates than the quantized absolute difference energy features. This may be due to the fact that we 419 420 fixed the camera gain parameter to 850 electron/CCD and manually changed the exposure time 421 that blocked the running of histogram equalization process at the background while acquiring the images of the Dataset-1. On the other hand, Dataset-2 was acquired under the automatic gain 422 423 parameters enabling automatic histogram equalization which may modify the original spectral 424 signal. Although histogram equalization aims to enhance the image quality and yields visually appealing images, it will also modify the spectral signature. This may degrade the features based 425 426 on absolute difference of consecutive spectral bands more than individual band energy features. 427 Therefore individual spectral bands may contain more informative pattern than the absolute difference of consecutive spectral bands. As a result, wavelet features and quantized individual 428 spectral band energy features produced relatively better results than the absolute difference features 429 under the UV excitation. Table 2 and Table 3 reveal that aflatoxin detection in chili pepper problem 430 431 is not a linearly separable problem because almost in all cases MLP outperforms LDA in terms of classification accuracy. 432

As it seen in Table 3, the highest classification accuracy on the Dataset-1 was obtained with
absolute difference of QHM features selected by the HBBE feature subset selection using MLP
classifier as 83.26% under halogen illumination. Similarly, the highest classification accuracy on
the Dataset-2 was obtained with individual band of QHM features selected by the HBBE feature
subset selection using MLP classifier as 87.5% under UV illumination.

Figure 12 illustrates the features selected by 5 fold cross validation of QHM features based on HBBE feature selection under the halogen illumination for the Dataset-1. The features selected at each fold are added to the corresponding bin. As the QHM features require two consecutive bands to be used, each feature contributes to the tally in two bins. To determine which spectral band images should be acquired in the machine vision system. In Figure 12, the most discriminative spectral bands and features can be seen. The most informative bands are 540, 550, 560, 590, 640 and 650 nm if voting threshold value 4 is selected .

- 447
- Figure 12. Dataset-1 vote map for visualizing the most frequently selected spectral bands with the
  associated bin numbers.
- 450

In Figure 12, the frequency of each feature is indicated on the corresponding feature cell. The frequency count of a particular spectral band is the total of the frequency counts of all bins in that spectral band. Dark color indicates high frequencies whereas light color means low frequencies. Completely white cells are the features with insignificant contribution to classification. Proposed feature selection scheme eliminates those features and reduces the feature dimension. We can extract series of most discriminative spectral bands by applying different threshold values. Table 4

depicts, most discriminative spectral bands based on different threshold values associated withLOO-CV accuracy rates.

459

- 460 Table 4. Most discriminative spectral bands based on different threshold values associated with
- 461 LOO-CV accuracy rates for the Dataset-1.
- 462 Table 4 indicates that there is a tradeoff between the accuracy rate and number of features selected
- 463 which will determine the design of the machine vision system. Higher classification performance
- 464 may require higher numbers of spectral filters which will increase the complexity of the overall
- 465 machine vision system. On the other hand, establishing a relatively simpler machine vision system
- 466 can be realized at the expense of lower generalization performance.

467

We repeated the same scenarios for the Dataset-2 as well. Figure 13 and Table 5 demonstrate the most frequently selected spectral bands and threshold features respectively.

- 471 Figure 13. Dataset-2 vote map for visualizing the most frequently selected spectral bands with the472 associated bin numbers.
- Table 5. Most discriminative spectral bands based on different threshold values associated withLOO-CV accuracy rates for the Dataset-2.
- It is seen from Table 5, for the Dataset-2, simpler machine vision system with single spectral band
  of 420 nm is sufficient to achieve 85% classification accuracy. On the other hand, if 400 nm is also
  used in the system, 90% classification accuracy would be possible.

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#### **5. CONCLUSION**

In this study, detection of aflatoxin contaminated chili pepper was investigated. Both UV and 480 Halogen illuminations were used. Hyperspectral image series of 53 the Dataset-1 were acquired. 481 Absolute difference of consecutive spectral band energy features was proposed. Another set of 482 quantized histogram matrix features (QHM) were extracted from individual spectral bands and 483 absolute difference of consecutive spectral bands by applying the quantization process. The most 484 discriminative features were constructed with 12 bins quantization. In addition to these, a novel 485 feature selection method was also proposed based on saliency metric of MLP connection weights. 486 487 This approach was compared to PCA and Fisher methods. 83.26% accuracy rate was achieved for the Dataset-1 under the halogen illumination with proposed QHM features and HBBE feature 488 selection method. Utilizing halogen is superior to UV. The most frequently selected spectral bands 489 490 for the Dataset-1 were 540, 550, 560, 590, 640 and 650 nm. We used LDA as a simple linear classifier and MLP as a complex non-linear classifier. Experimental results reveal that MLP 491 outperforms LDA in terms of classification accuracy rate. Robustness of our proposed methods 492 was verified by the Dataset-2 under the UV excitation and we achieved 87.50% classification 493 accuracy. 400 and 420 nm spectral bands were selected as the most discriminative spectral bands 494 495 for the Dataset-2. With the reduced spectral bands, it will be possible to construct a simple machine vision system for aflatoxin detection in chili pepper. 496

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- 498

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	Illumination	Exposure time (s)	Normalization coefficient
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Illumination	Exposure time (s)	Normalization coefficient
Halogen (400-490) nm	4.5	1
Halogen (500-590) nm	2.4	1.875
Halogen (600-720) nm	0.5	9
UV (400-690) nm	9.6	1
UV (700-720) nm	3.1	3.09

# 615

Table 1: Exposure normalization coefficients of the most informative regions of the spectral bands.

Feature	Org.	MLP clas	ssificatio	n accuracy	rates	SVM	LDA class	sificatio	n accuracy	rates
Extraction Method	Feature size	Original	РСА	Fisher	HBBE	RFE	Original	РСА	Fisher	HBBE

	Individual Band	33	62.34	70.76	67.94	68.16	56.54	37.64	66.73	65.16	34.00
	Energy Features			(11 4)	$(4 \ 4)$	(6.8)	(78)		(17.4)	(1 0)	(5.4)
	Energy reactines			(11.4)	(4.4)	(0.0)	(7.0)		(17.4)	(1.0)	(3.4)
	Absolute Difference	20	62 52	60.36	71 20	71 62	71 / 5	50 70	63 03	64 76	13 61
	Absolute Difference	52	02.52	(10.0)	(12.0)	/5.02	(10.2)	50,70	(12.4)	(4.2)	(2.2)
ç	Energy Features			(18.0)	(12.0)	(5.4)	(10.2)		(13.4)	(1.2)	(3.2)
ee Be	Quantized Individual	206	61.09	67 12	67.02	91 76	76.00	10 00	65 46	70 66	60 70
B		390	01.90	07.45	07.92	81.20	/0.00	40.00	05.40	/0.00	(0.70
Ï	Band Energy Features			(6.2)	(1.8)	(11.0)	(17.0)		(11.4)	(6.2)	(8.2)
	(12 bins)										
	Quantized Absolute	384	60.34	64.70	71.21	83.26	71.81	50.88	67.85	72.44	61.98
	Difference Energy			(11.6)	(4.8)	(5.8)	(17.2)		(13.2)	(5.4)	(6.4)
				(11.0)	(4.0)	(3.0)	(17.2)		(13.2)	(3.4)	(0.4)
	Features (12 bins)										
	Individual Band	33	63.80	63.11	63.83	69.80	47.45	33.84	69.18	65.17	35.82
	Energy Features			(6.4)	(1.8)	(3.0)	(5.8)		(9.4)	(1.2)	(5.4)
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	Absolute Difference	32	62.52	71.68	71.20	65.98	60.00	48.90	61.44	62.78	49.44
	Energy Features			(11.6)	(10.0)	(6.6)	(13.4)		(2.6)	(2.8)	(6.8)
	Energy reactines			(11.0)	(10.0)	(0.0)	(13.4)		(2.0)	(2.0)	(0.0)
≥	Quantized Individual	396	58.34	61.30	61.91	62.14	48.54	48.88	60.46	61.56	50.90
-	Band Energy Features			(6.6)	(6.8)	(11.8)	(12.2)		(12.4)	(8.0)	(78)
	(12 bins)			(0.0)	(0.0)	(11:0)	(12.2)		(12.1)	(0.0)	(7.0)
		204	50.04	co 07		c <del></del> 00	- 4 00	40.00	64.33	cc <b>7</b> 0	
	Quantized Absolute	384	58.34	62.27	/2.63	67.98	54.90	48.90	61.23	66.72	52.90
	Difference Energy			(14.4)	(13.4)	(10.6)	(15.8)		(7.2)	(1.2)	(5.4)
	Features (12 bins)										
	· · · · ·										

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Table 2. Generalization performance of the extracted features with different feature selection

620 methods versus different classifiers under halogen and UV excitations.

Dataset	Illumination	Feature Extraction Type	Feature	Classifier	Accuracy
	Source		Selection		Rate
		Quantized Absolute Difference Energy (12 bins)	HBBE	MLP	83.26%
	Halogen		HBBE	LDA	61.98%
	0 -	Wavelet Features	LDB	MLP	63.64%
Dataset-1			LDB	LDA	62.44%
		Quantized Absolute Difference Energy (12 bins)	HBBE	MLP	67.98%
	UV	,	HBBE	LDA	52.90%
		Wavelet Features	LDB	MLP	61.82%
			LDB	LDA	57.10%
		Quantized Individual Band Energy (25 bins)	HBBE	MLP	87.50%
Dataset-2	UV		HBBE	LDA	67.50%
			LDB	MLP	77.50%

		Wavelet Features		LDB <sup>a</sup>	LDA	74.65%
622	Table 3. Bo	enchmark of the proposed method against	st wavelet LD	B method	for Data	set-1 and
623	Dataset-2					
624						
		Dataset-1				
	Threshold	Selected Bands (nm)	Feature s	ize	LOO-CV Acc	uracy Rate
	1	410-440, 510-560, 580-600, 620-690, 710,720	20		85	%
	2	430,440,520,540,550,560,580,590,630-670	15		81	%
	3	540,550,560,580,590,640,650,660	12		78	%

4	540,550,560,590,640,650	9	68%
5	540,550,560	4	62%

Table 4. Most discriminative spectral bands based on different threshold values associated withLOO-CV accuracy rates for the Dataset-1.

627

	Dataset-2		
Threshold	Selected Bands (nm)	Feature size	LOO-CV Accuracy Rate
1	400,410,420,430,440,480,500,600	19	85%
2	400,410,420,430,440,600	17	85%
4	400,420,440	13	90%
6	400, 420	10	90%

<sup>&</sup>lt;sup>a</sup> The results differ from those of Kalkan et al., (2011) as they partitioned the data set into 4 disjoint folds in their experiments, whereas we employed 5-fold cross validation. Even if the number of folds were the same, there might be slight differences on the accuracy rates due to chili pepper samples falling into different folds for different trials.

	8	420			6			85%	
629	Table 5.	Most discrim	inative spect	ral bands based	on different	threshold	values	associated	with
630	LOO-CV	V accuracy rate	es for the Dat	aset-2.					
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645				List of Figu	re Captions				
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647	Figure 1	. General over	view of the h	yperspectral image	aging system				
648									
649	Figure 2	. Flowchart an	d interrelated	components of	the proposed	l system.			
650									

Figure 3. Histogram based Aflatoxin aflatoxin variations of chili pepper samples.

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- Figure 4. Sample images from the hyperspectral image series of uncontaminated and contaminated
- 654 peppers for halogen and UV illuminations.

655

- Figure 5. a) A sample image (640 nm). b) Representative gray level histogram of the image in (a)
- to 12 bins. The color of the histogram bar at the bottom depicts the total number of pixels falling
- 658 in each bin.

659

- 660 Figure 6. Quantized histogram matrix (QHM) is composed of histogram bars. a) Individual spectral
- band energies b) Absolute difference of consecutive spectral band energies. X axis denotes the
- spectral bands (or band pairs in the case of absolute difference) and Y axis denotes histogram bar

663 for that band.

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Figure 7. a) Boxplot, b) Fisher discrimination power, of the individual spectral band energyfeatures.

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- 668
- Figure 8. a) Boxplot, b) Fisher discrimination power, of the absolute difference of consecutivespectral band energy features.

672 Figure 9. MLP with input, hidden and output layer.  $W_{ii}$  is the connection weight between *i*'th input 673 node and j'th hidden node. Similarly,  $W_i$  is the connection weight between j'th hidden node and the output node. 674 675 676 Figure 10. Proposed HBBE method. L and M designate the number of features and the number of 677 steps, respectively. 678 Figure 11. Schematic diagram of the decaying procedure of the learning rate and momentum 679 680 values. 681 Figure 12. Dataset-1 vote map for visualizing the most frequently selected spectral bands with the 682 683 associated bin numbers. 684 Figure 13. Dataset-2 vote map for visualizing the most frequently selected spectral bands with the 685 686 associated bin numbers. 687 688 689 690 691 692 693 FIGURES 694 695 Desktop PC Dark Room 32



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Figure 2

Healthy

pepper

Extracted Features

Feature Subset

Contaminated Pepper

Feature Ranking

Evaluate

Classification





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Figure 4

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- /12















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Figure 11

	400	410	420	430	440	450	460	470	480	490	500	510	520	530	540	550	560	570	580	590	600	610	620	630	640	650	660	670	680	690	700	710	720
Bins	0	1	1	2	2	0	0	0	0	0	0	1	2	1	5	10	5	0	3	4	1	0	1	2	4	4	3	2	1	1	0	1	1
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Hyperspectral Bands

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Figure 12

	400	410	420	430	440	450	460	470	480	490	500	510	550	600
Bins	6	2	8	2	4	0	0	0	1	0	1	0	0	2
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Hyperspectral Bands

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Figure 13