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A Meta-heuristic Approach for Design of Image Processing Based Model for Nitrosamine Identification in Red Meat Image

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Abstract: ***Background*****:** Nitrosamine is a chemical, commonly used as preservative in red meat whose intake can cause serious carcinogenic effects on human health. Identification of such malignant chemicals in foodstuffs is an ordeal.

Objective**:** The objective of the proposed research work presents a meta-heuristic approach for nitrosamine detection in red meat using computer vision-based non-destructive method.

Method**:** This paper presents an analytical approach for assessing the quality of meat samples upon storage (24, 48, 72 and 96 hours). A novel machine learning-based method involving strategic selection of discriminatory features of segmented images has been proposed. The significant features were determined by finding p-values using Mann-Whitney U test at 95% confidence interval which were classified using partial least square-discriminant analysis (PLS-DA) algorithm. Subsequently, the predicted model was evaluated by bootstrap technique which projects an outline for preservative identification in meat samples.

Results**:** The simulation results of the proposed meta-heuristic computer vision-based model demonstrate improved performance in comparison to the existing methods. Some of the prevailing machine learning-based methods were analyzed and compared from a survey of recent patents with the proposed technique in order to affirm new findings. The performance of PLS-DA model was quantified by receiver operating characteristics (ROC) curve at all classification thresholds. A maximum of 100% sensitivity and 71.21% specificity was obtained from optimum threshold of 0.5964. The concept of bootstrapping was used for evaluating the predicted model. Nitrosamine content in the meat samples was predicted with 0.8375 correlation coefficient and 0.109 bootstrap error.

Conclusion**:** The proposed method comprehends double-cross validation technique which makes it more comprehensive in discriminating between the edibility of foodstuff which can certainly reinstate conventional methods and ameliorate existing computer-vision methods.

Keywords: Feature Extraction, Nitrosamine, Partial Least Square-Discriminant Analysis, Receiver Operating Characteristics, Sensitivity, Specificity, Bootstrapping, Food Quality.

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1. INTRODUCTION

As a part of an overall salubrious diet, red meat (capra aegagrus hircus) which is widely consumed has rich amounts of vitamins, minerals, antioxidants and proteins that can have profound effects on human health [1]. Throughout the evolution of mankind, people have been eating red meat, however, the quality of meat consumed today is different from the meat which people used to consume in past [2]. One of the major causes for the depreciated quality of red meat is the addition of preservatives [3]. The color of a fresh red meat is light red to brick red which fades to brown with time [4]. To prevent discoloring of meat, preservatives like nitrites (E-249 and E-250) and nitrates (E-251 and E-252) are added so that the shelf life of the food item is elongated [5]. Serious neurotoxin, nitrosamine is produced by nitrites and nitrates, which splits up into nitrosonium cation and water [6]. The redness of meat is nurtured by adding nitrosamine as a preservative, which is a proven threat to consumer's health [7]. Preservatives are chemicals that not only destroy its nutritious value but also promote the risk of cancer, heart disease and other promotional diabetes [8]. It is necessary to recognize and classify the toxic preservatives in food items before it is fed to the consumer. Traditional chemical laboratory-based technique is a validated approach used to diagnose the subsistence of nitrosamine preservative in food items. Such chemical based methods are slow, tedious and destroy

the food sample with the use of expensive instruments [9]. To overcome these limitations, design of a Computer Vision System (CVS) may be adopted that measures the discriminatory variations of spatial information in the visuals of food items. CVS is a cost-effective method of measurement as it supports re-utilization of samples, provides fast processing speed and does not require any provision of trained manpower. It is a viable tool for food quality assessment in real-time applications ensuring better human-health [10]. In recent years, researches have filed patents in the field of quality assessment of food products wherein the edibility of food sample was examined using imaging method [11].

Image processing techniques have been extensively explored for the classification and analysis of food quality in the research articles and patents. To this reference, broad review of hyperspectral imaging has been proposed for quality assessment of several vegetarian and non-vegetarian food products [12-13]. A supervisory information system was invented by a patent in [14] which employs video camera device for image acquisition; appropriate wavelet decomposition technique has been applied for analyzing the acquired image by removing unwanted noise wavelet coefficients so that the quality of meat products can be determined. The quality of the lamb meat was determined in terms of absorption spectra by designing a Multiple Regression Model (MRM) with high correlation coefficient [15]. A prediction model for meat quality evaluation was designed by reducing the extracted features using Principal Component Analysis (PCA) [16]. Discriminatory features have been analyzed and intramuscular meat fat tissue was quantified from its original microscopic images [17]. Support Vector Machine (SVM) based classification of chopped apples has been obtained which identifies enzymatic browning [18]. Average spectra of the meat samples were obtained; multivariate analysis was extracted from the reduced set of features which were acquired by PCA [19]. Least Mean Square (LMS) value of weight and slaughter's age has been calculated that gives a high correlation coefficient determining the quality of the sample under test [20]. The freshness of meat samples was identified by calculating the Mean Square (MS) value followed by the application of Analysis of Variance (ANOVA) and F-test [21]. The statistical analysis of the meat samples was obtained by measuring the probability value which validated the investigation against the results of colorimetric test [20]. The spatial information has been obtained by perceptually applying the concept of Anti-Textons for the classification and retrieval of food images [23]. The quality of grass carp fish fillet was evaluated by designing Least Squares-Support Vector Machine model (LS-SVM) for quantitative analysis of the image in order to select optimum wavelength using Successive Projections Algorithm (SPA) [24]. Spectral and texture features were extracted from the hyperspectral images of chicken meat followed by application of feature fusion technique such that optimum wavelength can be selected using SPA [25]. A method has been developed in patent [26] where meat tenderness has been analyzed using hyperspectral imaging by correlating key wavelength bands of the meat image. Since the existing literature does not directly account for the subsistence and identification of nitrosamine in food items, there is a need of designing an algorithm that can detect toxicity in food products.

Several design gaps for nitrosamine identification in food items has been identified from the existing state of art. Some of them are listed below:

- The automatic segmentation of the appropriate region is a significant contribution of any computer vision-based method.
- Selection of proper algorithm for feature extraction from Region of Interest (ROI) plays an important role in separating two groups i.e., control (without preservative) red meat sample with the treated (containing preservative) one. This exhibits prominent discriminatory information that remarkably provides a demarcation of the food sample having carcinogenic preservative. These discriminatory features provide an accurate and efficient framework for nitrosamine detection in red meat sample.
- For designing food quality index, selection of most discriminatory feature in order to enhance the computation speed and high classification accuracy.
- Measure of sensitivity and specificity obtained from the optimum threshold value of ROC curve gives an implication in the correctness of sample classification.
- Reproducibility, resampling and random sample selection double-cross validates the designed model.
- A comprehensive computer vision-based method for nitrosamine detection in red meat samples may be validated against the traditional laboratory technique to ensure the accuracy in context to food samples quality.

Therefore, the objective of this research work is to design a non-destructive computer vision-based classification method for identification of nitrosamine preservative in red meat samples. This research work ensures that the process is computationally automatic, precise and flexible.

The main contribution of this paper is to design a framework for distinguishing treated from control red meat samples using machine learning based method. A descriptive analysis of the discriminatory statistical and texture features obtained from segmented image has been considered useful in designing a dynamic application in this regard. Subsequently, the validation was done under standard methodology which was chemically analyzed using different chemicals and their concentrations. High accuracy has been achieved in the designed methodology which infers that the proposed research work plays a significant contribution in the qualitative identification of nitrosamine from red meat samples using imaging technique. The proposed approach is computationally effective and can run on inexpensive hardware in real time. A comprehensive classification of the discriminatory features is worthwhile in developing a viable tool in this regard. The most pertaining research work in the area of red meat quality assessment using computer vision technique in comparison to the proposed methodology has been shown in Table **1**.

Table 1: Comparative table between a few existing methods and proposed methodology.

Reference	Sample size	Algorithm used	Result analysis
[15]	126 lamb meat samples Training dataset: 84 and testing dataset: 42	Hierarchical variable selection method (UVE–SPA–CSA) based on uninformative variable elimination (UVE), successive projections algorithm (SPA) and clonal selection algorithm (CSA) has been used.	Correlation coefficient of fat, protein and water in lamb meat were 0.95, 0.80 and 0.91 respectively and the ratio of prediction to deviation (RPD) were 4.13, 1.31 and 2.53 respectively.
[16]	74 beef meat samples Training dataset: 60 Testing dataset :14	Normalized Radial Basis Function (RBF) neural network, Bayesian Ying-Yang Expectation Maximization algorithm for determining size of network and parameter measurement, fuzzy principal component algorithm for dimension reduction.	Classification accuracy of test samples were 92.86 %. Overestimate prediction except 22.76% for Enterobacteriaceae.
[19]	420 samples of chicken , pork and beef meat, adulterated with: Pork- 210 Beef- 210	Comparison of model performance by root mean square error of calibration (RMSEC), cross-validation (RMSECV), prediction (RMSEP), also with coefficient of determination of calibration, cross-validation, and prediction; and ratio performance to deviation and ratio of error range.	For the adulteration of meat samples with pork and beef, 0.83 and 0.94 were coefficient of prediction respectively, the ratio performance deviation was 1.96 and 3.56; and ratio of error range (RER) 10 an 18.1.
[20]	180 bull breeds of lean cattle samples. 60 samples each of 3 breeds.	Analysis of variance (ANOVA) technique using GLM procedure of least squares to compute interclass correlation. Measurement of factor mean square and comparison with F-test at 95% confidence interval.	The correlation between the intramuscular fat and the area was 0.40 and 0.26 at 95% confidence interval.
[22]	45 samples Beef: 15 Pork:15 Chicken: 15	Image analysis using photoshop. χ^2 test, T-test to compare colorimetric test and computer vision system (CVS) measurement. Correlation coefficient was measured by Spearman rank correlation test	CVS was statistically more significant (p-value<0.05-0.0001).
[34]	72 pork meat samples. Training dataset: 48 Testing dataset:24	Adaboost algorithm along with BPANN learning algorithm has been applied to detect total volatile basic nitrogen(TVB-N)	Data fusion with BP-Adaboost provided 0.932 correlation coefficient and 2.885 RPD.
[35]	210 pork meat samples. Training dataset: 140 and testing dataset: 70	Application of feature level fusion and PLS regression on the spectral data obtained by successive projection algorithm (SPA) and textural features obtained by GLCM.	The correlation coefficient obtained with optimum wavebands, textural variables and data fusion were 0.898, 0.876 and 0.924 respectively.
[36]	75 meat samples, each of lamb, beef, pork and fat Training dataset: 57 Testing dataset: 18	Spectral and spatial features were evaluated using SVM based classifier and convolutional neural network (CNN) model	The CNN model has achieved an overall accuracy of 94.4% in comparison to other classification methods.
[37]	1400 boneless pork loins.	10-fold cross validation technique	92.5% of prediction accuracy and 75.0% of

	Training dataset: 1120 and Testing dataset: 280	was applied to remove overfitting issues. Training of SVM model was conducted by RBF algorithm.	marbling score.
Proposed method	**62 red meat samples of goat meat. Training dataset and testing dataset consisted of 50 and 12 sample images respectively**	**Determination of p-values using Mann-Whitney U test. The most significant features were normalized and a tolerance score of 90% was applied using PLS-DA algorithm. Receiver Operating Characteristics (ROC) at all classification thresholds was obtained. Based on the optimum value threshold, sensitivity and specificity was calculated. The model was double-cross validated by Bootstrap.**	**At 95% confidence level ($p<0.05$), ten significant features were selected for normalization. Sensitivity 100% and specificity 71.21% was calculated at optimum value of threshold 0.5964. The nitrosamine content in meat sample was predicted with correlation coefficient of 0.8375 with a bootstrap standard error of 0.109.**

2. GENERAL MODEL FOR NITROSAMINE DETECTION IN RED MEAT

The following section presents the techniques involved in qualitative assessment of red meat using conventional laboratory technique.

2.1. Red meat Sample Preparation and data Collection

Whole muscle from loin portion of goat (breed: barbari) was purchased from Randhawa Poultry farm, kapaskheda, Gurgaon, India. The red meat parts used for testing were neck, leg, shoulder, ribs, loin, liver and kidney. Meat sample of size 5 cm x 5cm x 5cm were stored at a room temperature of 25 ^{o}C for analysis. All the samples were labelled as control/ treated samples.

2.2. Laboratory Testing for Chemical Analysis

The control and treated samples were stored at a temperature of 2 ^{o}C for simulation purpose having pH value of 5.4. American Chemical Society (ACS) grade sodium chloride and sodium nitrite used for solution preparation were procured from HiMedia labs, India. To study the nitrosamine presence, sodium chloride brine solution (NaCl 20 g/L) was used as blank, and sodium nitrite wet curing solution (NaCl 20 g/L, and NaNO2 150 mg/L) was used as treatment. For incubation of samples, Nisco manufactured Bio-Oxygen Demand (BOD) incubator has been used. Meat samples were immersed in solutions for 96 hours in BOD incubator at a temperature of 8 ^{o}C in closed glass container. The operation range of this incubator is 5 ^{o}C to 60 ^{o}C, 220 Volts. Samples were taken out randomly from both solutions at specific time interval (24, 48, 72 and 96 hours) and washed with distilled water. For conversion of sodium nitrite into nitrosamine, washed samples were cooked at a temperature of 120 ^{o}C for 6 minutes under pressure to achieve full cooking with 100 ml water. The samples were taken out of pressure cooker for further analysis after cooling them at room temperature. All the procedures were performed in duplicates. Sodium nitrite added with salt paced at an optimum temperature for cooking generates preservative which protects treated meat samples from microbial poisoning. This process forms nitric oxide which reacts with muscle myoglobin and form nitric oxide metmyoglobin. This nitric oxide metmyoglobin was responsible for pre-cook brown pigment in treated meat. During cooking, nitrosylhemochromogen (NHC) was formed from nitric oxide metmyoglobin due to denaturation of protein part of myoglobin.

2.3. Validation of Nitrosamine in red Meat Using Conventional Method

The identification of nitrosamine produced in the samples was validated by the method described by [27] using griess reagent with slight modifications. The test was conducted by mixing 1 ml of griess reagent and 1ml of hydrochloric acid with 2 gm of finely grinded meat sample in a test-tube. The mixture is heated at a temperature of 40 ^{o}C for 30 minutes. All the treated samples showed red-violet color confirming the presence of nitrosamine in them unlike control samples. Alkalinity and pH were not monitored as process optimization was not the focus of the present work. The chemical parameters were ignored while identifying nitrosamine in the red meat samples as they were used for quantification of this toxic substance. The proposed research work produces a binary classification of the test samples indicating only the presence or absence of nitrosamine in the red meat sample.

2.4. Storage Technique of Meat Samples

In this paper, 62 pre-processed meat samples were analyzed for their freshness identification. 24 samples were control samples which were not exposed to nitrosamine chemical and 38 samples were treated with the chemical. Treated and control red meat samples were placed in a beaker containing nitrosamine chemical and aqueous solution respectively at room temperature of 25 ^{o}C as shown in Fig. **1**.

Fig. (1). Immersion of treated and control samples in a beaker

All control samples were stored for 96 hours in a frost-free refrigerator (Samsung RT28K3723UT/NL/2016) whereas the pattern of storage of treated samples was different from former. The treated samples were exposed to chemical for a period of hours. The concentration of nitrosamine develops in sample based on their storage pattern. After the exposure of chemical, these treated samples were stored in refrigerator maintained at a temperature of 3^{o}C.

2.5. Development of Image Acquisition System

For acquiring images of meat samples, a hardware set-up can be designed which may comprise of various combinations of Compact Fluorescent Lamps (CFL) tube lights like set of four CFL and three tube lights for illumination assembled with a camera placing position. CFL lamp (Osram DULUXSTAR) operates at power rating of 35W, color temperature of 6500K, 220-240V voltage of alternating current with frequency of 50Hz and 2100 lumens. Osram tube light (T5 RADIANCE G2 WT 14) has been employed in the image acquisition set up operating at voltage of 230 V, 50 Hz frequency. A digital Canon camera (IXUS 285 HS) has been used for acquisition of image with spatial resolution of 5184x3888 pixels with horizontal and vertical resolution of 180dpi having bit depth 24, the focal ratio of camera was f/3.6 with 1/320 sec of exposure time, 4mm of focal length and maximum aperture of 3.6875 of camera optical lens.

The next section presents the novel method for identification of nitrosamine in the collected meat samples with the help of designed image acquisition system.

3. PROPOSED MODEL

The newly synthesized compound, NHC formed in the meat sample is responsible for introducing biological change due to which color of the sample varies to bright pink or red color. The discrepancy in the color and physical properties of the meat sample leads to the variation of image pixels and its periodicity in spatial domain. The dissimilarity discriminates statistical and texture parameters of treated meat samples from control meat samples. In this paper, the images of meat samples were acquired using digital camera. The captured images were used for feature extraction using image processing technique. The acquired images of the meat samples undergo image pre-processing in order to pace down all the images to one standard level since each acquired image exhibits different property and characteristics from other captured images. Subsequently, image segmentation step was applied on the pre-processed image to segment the region carrying maximum information. Post processing step was applied on the segmented image to remove additional noise from the sample in order to maintain the high accuracy and efficiency of the designed system. There are eighteen statistical features that were extracted from the segmented ROI which showed discriminatory behavior. The set of extracted features were reduced with the help of PCA. The reduced set of features were divided into training and testing dataset for automatic classification of red meat images using Leave One Out Cross Validation (LOOCV) based Support Vector Machine (SVM) classifier. Fig. **2** represents the block diagram of the proposed methodology.

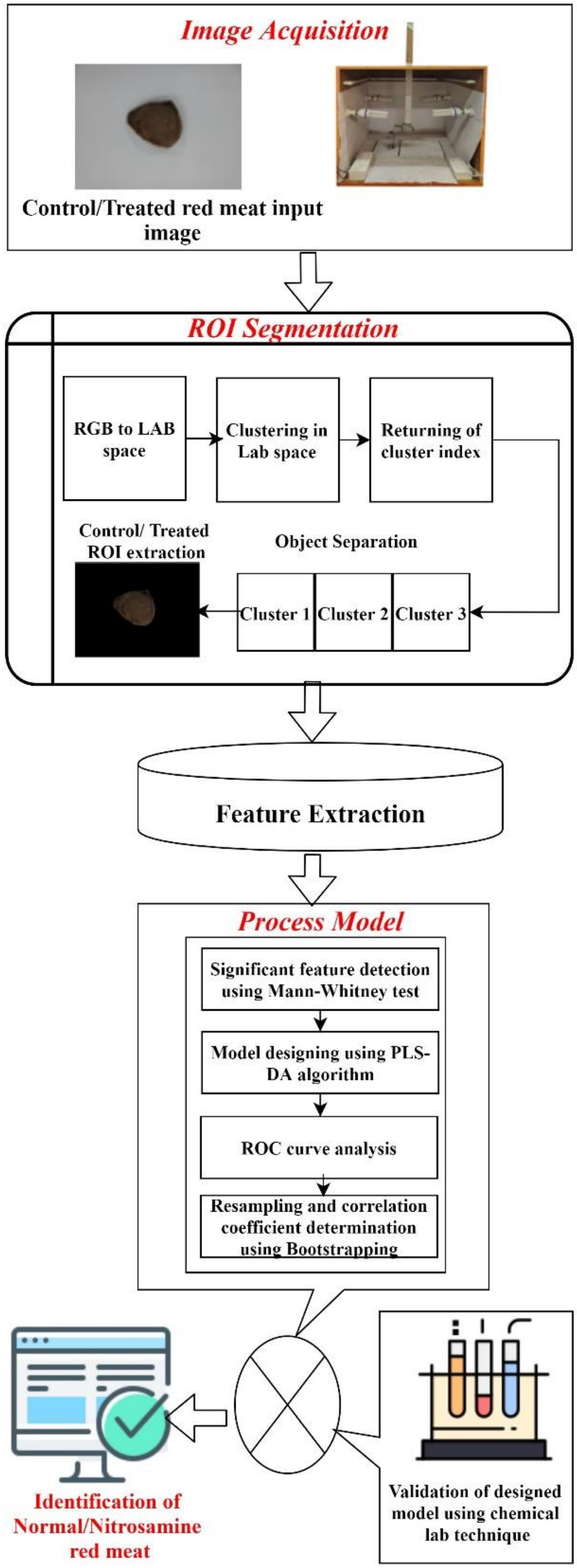

Fig. (2). Block diagram of proposed model.

Thus, the proposed model can be summarized concisely as below:

- ROI segmentation
- Statistical texture feature extraction
- Selection of significant features using Mann-Whitney U test
- Design of prediction model using PLS-DA algorithm
- Sampling of dataset with replacement using Bootstrap technique.

3.1. ROI segmentation

Red meat images were in RGB format having size of 3.70 Mb and dimensions of 5184 x 3888 in terms of width (w) x height (h).

Input images carries red meat portion with background region. The process of breaking input image into meaningful segments is called image segmentation. Pixels associated with red meat region carries meaningful information. The value of pixels of red meat portion varies discriminately with storage period, so this region is taken as ROI.

RGB sample images were paced down to a perceptual uniformity by converting into Lab color space model. Conversion established compatibility with the segmentation process and the illumination condition within which the sample images have been captured. In this paper, K-means clustering algorithm has been adopted for segmentation of ROI as it is one of the most popular methods suitable for breaking the image into clusters of similar information [28]. It is obtained by minimizing squared error function as shown in equation (1) which partitions d, number of data points into c, number of clusters and computes Euclidean's distance between a_n, data points and b_m, centroid for cluster m. Three distinct clusters of disparate information have been obtained as shown in Fig. **3**. The addition of two clusters provides segmented ROI in RGB format. The size of the segmented image is reduced from 3.7Mb to 18.2Kb having 820 w x 581 h dimensions. Reduced size of image removes the complexity associated with feature extraction method which will make image classification technique fast and automatic.

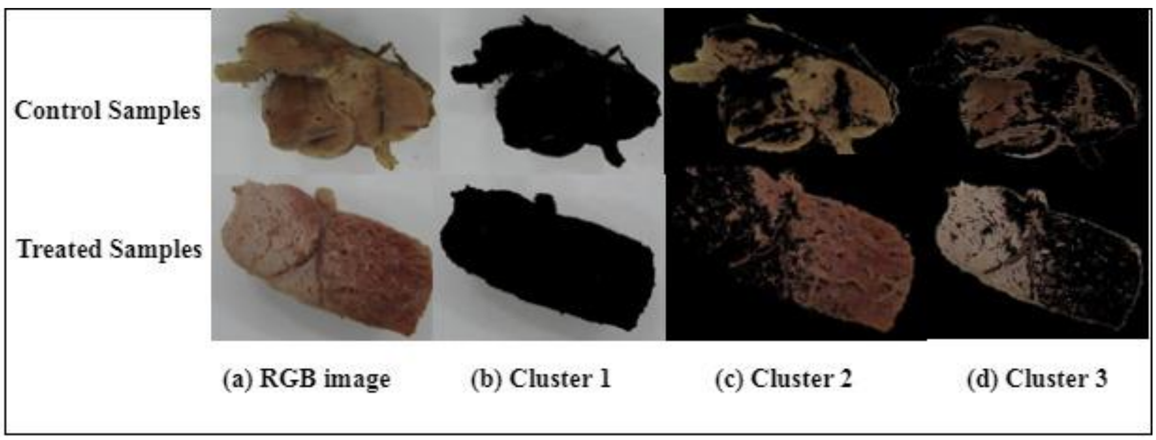

Fig. (3). Control and treated cluster images (a) RGB image (b) Cluster 1 (c) Cluster 2 (d) Cluster 3.

$$S(E) = \sum_{m=1}^{c} \sum_{n=1}^{d} (\|a_n - b_m\|)^2 \quad (1)$$

The method used for segmentation of red meat pixels is summarized in algorithm 1.

Algorithm 1: //ROI segmentation using K-means algorithm//

Step 1: Random selection of 'c' number of clusters centers.

Step 2: Computation of distance between clusters center with each data point.

Step 3: Of the entire cluster center, data point is assigned to those whose distance is computed to be least.

Step 4: New number of clusters were calculated

Step 5: Re-computation of distance between new clusters (calculated in step 4) and each data point.

Step 6: If there is no re-allotment of any data point, then the process will stop at step 5 otherwise repeat from step 3.

The addition of 2 clusters removes the background region and gives only pixels containing red meat portion as shown in Fig. **4**.

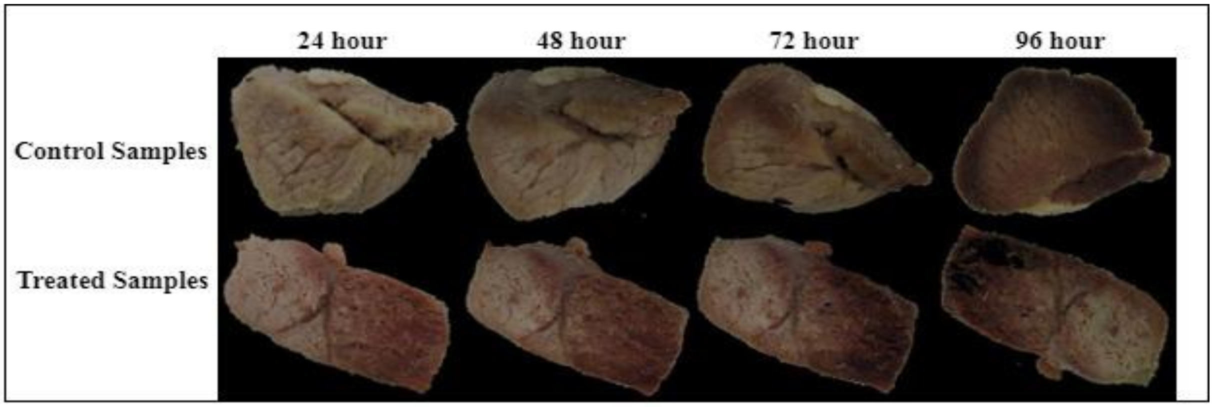

Fig. (4). Segmentation of ROI images of control samples

3.2. Feature Extraction from Segmented ROI

The most important step for feature extraction involves strategic conversion of segmented ROI image into grayscale image since the inherent complexity is reduced in latter. Subsequently, statistical and texture features have been extracted in spatial domain from the converted ROI image. Discrimination in the extracted 18 features was analyzed. Pixel information has been obtained by measuring features like first order, second order and higher order.

3.2.1. First order Statistical Features

First order statistical features provide information related to individual pixel value rather than neighboring pixels. The extracted features which measures variability of Pixel Intensity (PI) of an image in spatial domain are mean, standard deviation and variance. Mean, μ of an image refers to the average value of the PI. Standard deviation refers to the variation of PI from its mean value. Variance is the mean squared deviation from the mean value of its PI. These statistical features have been calculated for segmented red meat image, I of size I (number of rows and columns respectively) having f number of frequency of pixels intensity.

3.2.2. Second order Gray-Level Co-occurrence Matrix (GLCM) features

Texture refers to the spatial composition of PI of an image. It is the measure of properties like roughness, smoothness, coarseness and irregularity which gives a joint connection between PI. Second order GLCM matrix depicts how often intensity of pixels with specified value having spatial relationship occur in an image. The extracted features are contrast, energy, correlation and homogeneity. Contrast of the image is defined as the difference between maximum and minimum PI. Energy depicts the uniformity in the image by measuring the recurrent pair of PI. Correlation is defined as how a pixel is correlated to its neighboring pixel over a complete image. Homogeneity depicts the homogenous values of pixels in low and high contrast image.

3.2.3. Higher order Gray-Level Run Length Matrix (GLRLM) features

Higher order GLRLM features provide run length matrix which depicts the number of times; pixel, i of gray level image appears j, times in the θ, direction. For texture analysis of an image, several run length matrices have been designed which are different from each other in terms of rotation. The four directions of θ, that is, 0°, 45°, 90° and 135° are used for calculating following features of run length [29]. For a run length matrix, rθ (i,j), let L, be the number of gray levels, M, be the maximum run length, r, is total number of runs and n, total number of pixels in image. The extracted features were Short Run Emphasis (SRE), Long Run Emphasis (LRE), Gray-Level Non-uniformity (GLN), Run Length Non-uniformity (RLN) and Run Percentage (RP). Later, two new features, namely Low Gray-Level Run Emphasis (LGRE) and High Gray-Level Run Emphasis (HGRE) proposed by [30] were also extracted. Subsequently, four more features depicted by [31] like Short Run Low Gray-Level Emphasis (SRLGE), Short Run High Gray-Level Emphasis (SRHGE), Long Run Low Gray-Level Emphasis (LRLGE) and Long Run High Gray-Level Emphasis (LRHGE) were also obtained for identification of nitrosamine in the control and treated red meat samples.

3.3. Partial Least Square-Discriminant Analysis (PLS-DA) based classification model

The proposed research work projects on the PLS-DA based classification method for the identification of nitrosamine in red meat samples. The dataset of 62 sample images were split into calibration dataset of 50 images for training the model and validation dataset of 12 images for testing of the designed model. The following subsections presents the classification method applied on red meat image dataset using PLS-DA technique.

3.3.1. P-value Determination Using Mann-Whitney U test

As discussed in section 3.1, ROI was segmented in order to extract relevant information from the sample image. This extracted information was expressed in terms of features obtained from sample images. In order to understand the significance of each feature in discriminating controlled sample from the treated one, the determination of p-value is of utmost importance. Statistics of p-value of each feature has been obtained from Mann-Whitney U test at 95% confidence level. Equation (2)-(3) of Mann-Whitney test shows null hypothesis and alternate hypothesis respectively. This statistical approach gives a comparative output obtained from the observation of first sample, x_i and the observations in the other sample, y_i.

$$H_0 = P(x_i > y_j) = \frac{1}{2} \quad (2)$$

$$H_1 = P(x_i > y_j) \neq \frac{1}{2} \quad (3)$$

In this paper, PLS-DA algorithm has been used for image classification which not only provides predictive and illustrative modelling but also imparts discrimination in the selection of variable. Optimization of variables and normalization of parameters are the important key features of this algorithm. The performance of the model can be evaluated by measuring the area under Receiver Operating Characteristics (ROC), known as area under curve (AUC). The computation of optimum threshold gives an inference of True Positive Rate (TPR), as shown in equation (4) and False Positive Rate (FPR) as shown in equation (5) of the testing dataset.

$$TPR = \frac{TP}{TP + FN} \quad (4)$$

$$FPR = \frac{FP}{FP + TN} \quad (5)$$

Where TP=True Positive; FN=False Negative; FP=False Positive and TN=True Negative.

3.3.2. Sampling with Data Replacement Using Bootstrap Analysis

Bootstrap method was used to sample the dataset with replacement in order to estimate statistics. A bootstrap sample consists of an observation occurring multiple times. Therefore, the total number of elements in each bootstrap sample and in the original dataset could be same. The resampled estimates were randomly picked up from the original dataset such that random split of the data and independent validation can be conducted to evaluate the model using this new cohort data. Therefore, bootstrap analysis is one of the most important method for validating the model.

4. RESULTS AND DISCUSSIONS

The algorithms have been designed in MATLAB 2017a software on a processor Intel®, Core™, i3-5005U, CPU@2.00GHz, 4.00GB RAM, 64-bit operating system. The results obtained through CVS have been validated against traditional laboratory method and the classification accuracy obtained from the proposed approach is very promising as none of the sample is incorrectly classified. Fig. **5** shows the results of the steps involved in the proposed methodology.

The average time taken for segmentation of ROI from input control red meat image is 2.233247 sec and its feature extraction is 0.002188 sec. Similarly, the average time taken for segmentation of ROI from input treated red meat image is 2.013019 sec and for its feature extraction is 0.004933 sec. The total time taken for SVM based LOOCV classification of 62 samples is 181.13 seconds (2.92 sec. per sample) without the need of any additional special hardware like GPU processor.

The rapidity in the measurement of toxicity in food items states that the proposed research work can be used for food quality check in real time applications.

4.1. PLS-DA assessment of control and treated red meat samples

The significance of extracted features of red meat were determined by measuring p-value using Mann-Whitney U test at 95% confidence level. Significance of the extracted feature was measured by comparing its p-value with the significance level, α of 0.05. It was observed from the p-values that only ten extracted features were more significant in comparison to the rest eight features.

Partial Least Square-Discriminant Analysis (PLS-DA) has been used to discriminate control samples from treated ones. Calibration dataset has been trained by first normalizing significant features with 90% tolerance interval. It was observed after training of the samples, that none of the components was retained which clearly inferred that 100% of the samples were trained. Subsequently, validation samples were tested on the trained model. The performance of the model was evaluated by measuring the AUC of ROC curve at all classification thresholds. Based on the ROC curve as shown in Fig. **6**, optimum threshold was calculated. The two importance statistics for model assessment was s and specificity. The former indicates the proportion of genuine positives that are truly detected while the latter implies probability of incorrectly eliminating the null hypothesis for a test. With 0.5964, value of optimum threshold, sensitivity of 100% and specificity of 71.21% was calculated as shown in Table **2**.

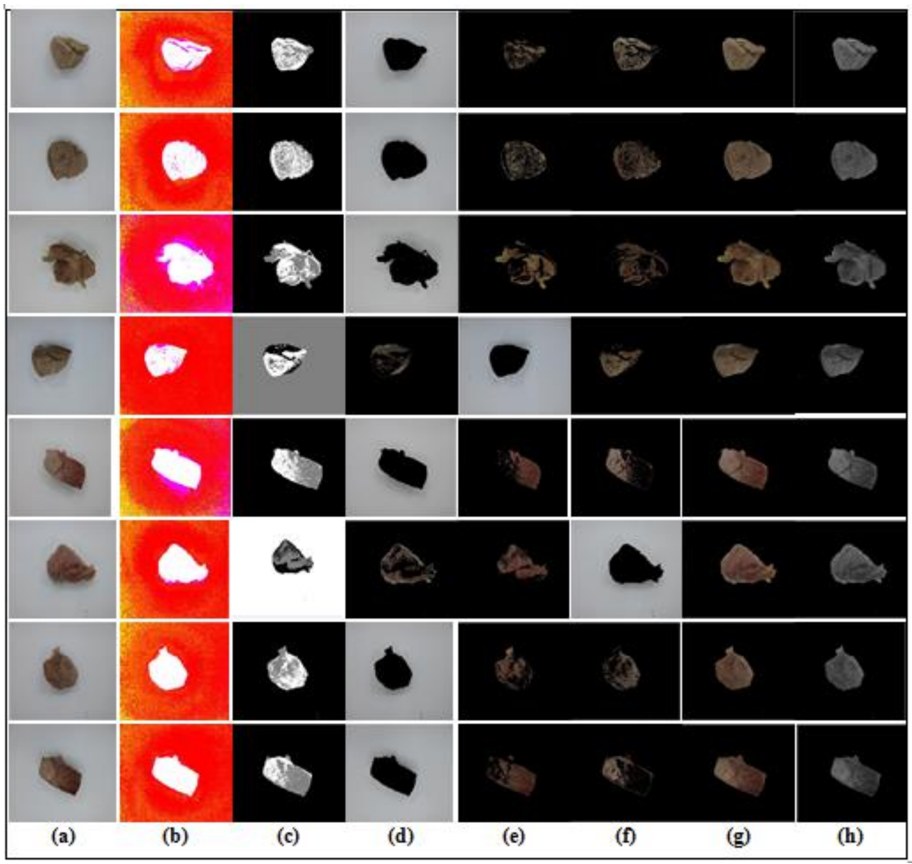

Fig. (5). Simulation results (a) Colour input image (a) Lab converted sample (c) Cluster index (d) cluster 1 (e) Cluster 2 (f) Cluster 3 (g) Segmented ROI (h) Final gray image used for feature extraction

Table 2: **ROC Curve parameters.**

Threshold	Sensitivity	Specificity	AUC	Accuracy
0.5964	100%	71.21%	71.21%	73.61%

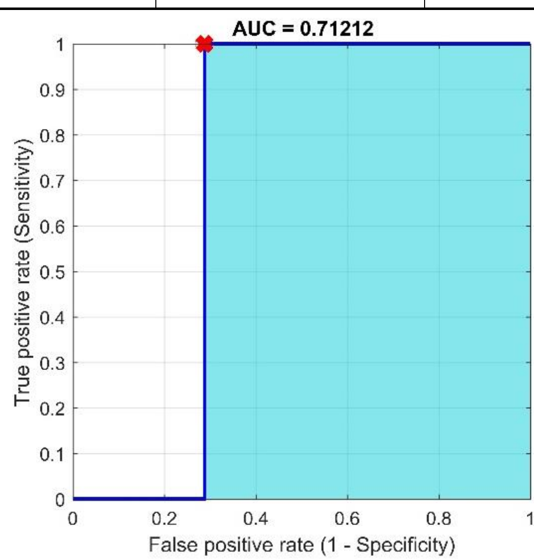

Fig. (6). ROC characteristics.

Furthermore, large number of samples were reproduced followed by random pickup of samples which were executed allowing arbitrary selection of elements from the observations. Later, new set of this cohort data was evaluated using Bootstrap analysis. The chemical content in meat samples was predicted with correlation coefficient of 0.8375. In order to apprehend the statistical significance, 1000 times resampling of the predicted vectors were conducted and the variation in the correlation coefficient was observed.

Fig. **7** shows that all the estimates of resampled vectors lying in the interval of [0.4 1.0]. At 95% confidence interval, the variation in the correlation coefficient was [0.55 0.94]. A significant quantitative finding was apprehended that showed nitrosamine content in the meat sample had a strong correlation. Later, 0.1090 bootstrap standard of error for the estimated correlation coefficient was computed.

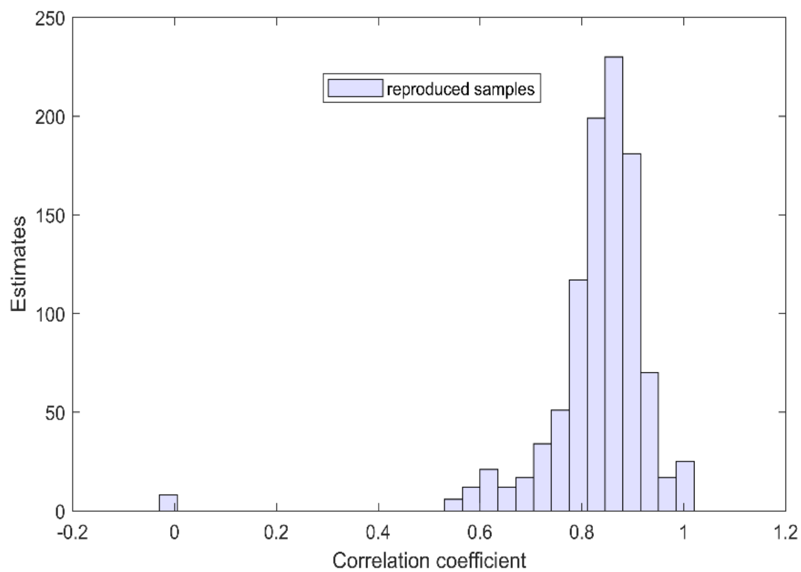

Fig. (7). Resampling of prediction vectors.

4.2. DISCUSSIONS

The inferences of the obtained results are listed below:

i. The region of red meat sample was accurately segmented.
ii. The statistical and texture feature extraction method was applied on the segmented image of red meat control and treated samples. The extracted features have a discriminatory pattern for the presence/absence of nitrosamine in the samples.
iii. Significance of each feature has been determined by calculating the p-values at 95% confidence level using Mann-Whitney U test. Significant features of training dataset were normalized such that the testing dataset was verified on the predicted model by using PLS-DA algorithm. ROC curve was obtained at all the classification thresholds in order to analyze AUC.
iv. Computation of optimum threshold value from the ROC curve gives an implication in the correctness of sample classification. 0.7121 was the AUC.
v. Random split of the data was performed for independent validation of each sample. In addition, random pickup of the samples allowing redundant selection was conducted and the model was evaluated by means of this new cohort data using bootstrap analysis.
vi. There was a high correlation between variations of nitrosamine content in meat samples and the visual quality of the computer vision system (CVS).

The proposed methodology may be considered as a remarkable contribution towards the computer vision-based food inspection system and no additional hardware is needed (e.g. GPU for acceleration).

CONCLUSION

Nitrosamine is a preservative commonly added in red meat which is carcinogenic in nature. In this paper, we propose a meta-heuristic approach for design of an image processing based non-destructive technique for the qualitative detection of nitrosamine in red meat. The red meat samples were stored for 24 hours, 48 hours, 72 hours and 96 hours respectively. The region of interest was segmented automatically by classifying red meat image pixels into k, number of clustering and assigning center to each cluster. At 95% confidence interval, ten significant features were normalized by using PLS-DA algorithm. The performance of the model was analyzed by computing ROC at all classification thresholds. Based on the ROC curve and AUC, optimum threshold of 0.5964 was computed. The designed predicted model was double-cross validated by random picking up of the sample such that the model was evaluated using this new cohort data by means of bootstrap analysis. A correlation coefficient of 0.8375 was obtained between variations of nitrosamine content in meat samples and the visual quality of the CVS indicating viability of the proposed analysis of food quality in real-time applications.

CONSENT FOR PUBLICATION

Not applicable.

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No funding was received by anybody or any organization to implement or simulate the obtained results in this paper.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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