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Image processing based method to assess fish quality and freshness

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ABSTRACT

The quality of a fish may be affected primarily by handling, processing and storage procedures from the catch to consumers. Retention time and storage temperature of post-harvested fish are key factors for sustaining the final quality of this product. This paper proposes an image processing method which is completely automatic, efficient and non-destructive for segmentation of tissues and prediction of freshness of the fish sample. The gill tissues of the fish sample are automatically segmented using a clustering based method and its features are strategically extracted in the wavelet transformation domain using Haar filter. First, second and third level decomposition in the wavelet domain is performed and the coefficients obtained at each level have been analyzed to predict the freshness of the fish sample. The experimental results indicate a monotonic variation pattern of the coefficients at the third level of decomposition and these coefficients gives an indication of the quality of the fish. This discriminatory variation in the image features with the duration of retention time provides a strategic framework for assessment of fish freshness.

1. Introduction

Aquaculture is one of the fastest growing food-producing segments and is maintaining its growth potential along with field crops and livestock productivity (Troell et al., 2014). In recent times, capture fisheries showed a sharp fall in the productivity domain due to overexploitation, atmospheric catastrophe and pollution impacts, whereas aquaculture heralded a new dawn of enhanced yield and sustained income generation. But increased fish production is not synonymous with healthy and quality fish protein for human consumption. Being a perishable entity, fish freshness and quality are important indicators of its commercial success as commodity. Moreover, fish harvested at local ponds and industrial farms are not always consumed by adjoining markets and are carried to fish loving 'hot spots' to sustain its marketability and profitability. Ice is the an important preservative media for fish (Rahman et al., 2012) and maintaining at ice embedded cold chain is the most common preservative technique of post-harvested fish to inhibit its decaying process and acts as a prime insulator during transportation. But its preservation efficacy also depends on ice quantity, tenure of ice application, origin of fish stock and its types, pathogen and toxicity loads and above all, climatic conditions.

Multiple reports of compromised quality are surfaced from the post-harvested fishes surveyed at markets and distribution channels (Hossain et al., 2013). The quality attributes include a series of parameters related to safety, nutritional quality, availability, freshness and edibility, which may be affected mainly by handling, processing and storage procedures from the catch to the consumers. Practically, physical, chemical, biochemical and microbiological changes occurring post-mortem in fishes, result in a progressive lost of food characteristics in terms of taste and a general concept of quality (Olafsdottir et al., 1997). Selling of pathogen infected, formalin (Goon et al., 2014) and pesticides loaded fish are not only potential health hazards to human, but also can influence fish degradation pattern and deteriorate its quality.

Fish freshness is the most required property from the consumers because of its strong relationship to the taste and health. A number of sensorial inspection procedures have been introduced to point the state of freshness. These procedures involve the use of sight (to evaluate the skin appearance and the color and the global aspect of eyes), tactile (to test the flesh firmness and elasticity) and olfaction (to smell the gill odor) etc. But most of the times, these quality linked factors remained unnoticed, as the detection methods are invasive and costly. Government sponsored quality testing laboratory generally maintains such low cost services for export clearances and can only accept low sample size. Moreover,

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innovative tools like biosensor and nanotechnology based fish quality testing kits are available but not popular due to their inherent problems and common people and fish farmers do not have any access to such tools. Sensory evaluation dependant 'quality index method (QIM)' is also complex as it dealt with multiple characters of different fish tissues (Macagnano et al., 2005). Hence, a simple, fast and effective tool is required which can function as model platform and reference point to compare and validate fish quality issues.

Gill is the red colored respiratory tissue of fish and its colour and odour pattern is used as apparent freshness indicator (Macagnano et al., 2005). But this 'vision' based colorimetric technique is very basic, indiscernible and can be misinterpreted. Moreover, unfolding the scientific basis and validation of 'gill color' may be required, before applying it as quality indicator at post-harvested fishes. Rohu (*Labeo rohita*) is the most preferred, commercial major carp in the Indian subcontinent (Das et al., 2005). Hence, selecting rohu gill for evaluating fish quality and freshness pattern can be a widely acceptable candidate mimicking other popular fish variety.

Image analysis is a non-destructive, non-hazardous common tool for evaluating data based on photography and analysis of its color variations through imaging software can be an important method to validate the quality of fish (Menesatti et al., 2010). Some work has already been reported in food processing sectors using the image processing. Feng Wang et al. (Wang et al., 2013) proposed a regression based technique on the eye from the fish sample to detect its freshness. Fairuz Muhamad et al. (Muhamad, Hashim, Jarmin, Ahmad) revealed a fuzzy logic based method for classification of fish freshness. Shiv Ram Dubey et al. (Dubey et al., 2013) described a colour based segmentation technique to detect the infected region in a fruit image. Soumya et al. (Band and Sheelarani, 2009) used soft computing techniques such as c-means clustering and fuzzy logic for segmentation of color images. Shiv Ram Dubey et al. (Dubey and Jalal, 2014) has applied sum and difference histogram texture based features for detection of defects in fruits.

The main objective of the paper is the assessment of fish quality and freshness. This is achieved by using an image processing technique which is a non destructive and non-hazardous method of assessment. A chemical based assessment leads to destruction of the sample under test and the sample cannot be used further. However, with image processing, only an image of the fish is taken and using some segmentation techniques, the gills tissue are segmented and analyzed. Some discriminatory features are obtained during the analysis of the tissues. This can help in development of a viable tool which can work in real time for fish quality assessment.

The main contribution of the paper is an efficient image processing based method for assessment of the quality and freshness of the fish. This is achieved by analyzing the wavelet domain features of the segmented tissue of the gills from the fish image. A comprehensive statistical and image processing techniques has been presented to explore these image feature variations in segmented gill pattern to develop viable tool for predicting fish quality and freshness.

Another contribution of the paper lies in the use of a clustering based image processing technique for automatic segmentation of the gills from the fish image. The proposed segmentation method for segmentation of gills is completely automatic, less complex and computationally efficient.

The highlight of this work is the establishment of a strategic relationship between the image coefficients, extracted during wavelet domain analysis, and the quality and freshness of the fish. This relationship can act as indicative parameters for a viable image processing based non-destructive diagnostic tool for quality check and control.

The rest of the paper is organized as follows. Section 2 presents the materials and methods used in the work. This section discusses the process involved for capturing images of the fish samples for the experimental study and the imaging tool for feature extraction from these images. Section 3 presents the experimental results and discussion on these results. The conclusions from the work are reported in Section 4 of the paper.

2. Materials and methods

2.1. Collection of fish samples

The live Rohu(*L. rohita*) were sampled from fish ponds National Institute of Abiotic Stress Management (NIASM), Baramati, Pune, Maharashtra (18°09″30.62′N and 74°30″03.08′E; Mean sea level – 570 m) and kept in three hundred litres aquariums for 24 h. The average weight and average length of fishes were 90.40 ± 1.20 g and 21.60 ± 0.50 cm respectively. The pond water were free from any pathogenic infestation and toxic residues as were measured through routine microbiology and toxicity detection protocol, prior to the initiation of experiments.

2.2. Experimental design and photography

Fishes from the aquariums were taken out and placed into chilled water for sudden death to avoid rigor mortise. The fishes thereafter were preserved for imagining study in thermocol boxes ($28 \times 18 \times 12$ cm^3) with a fish to ice ratio of 1:2. Images of gill were taken using NIKON D90 digital camera on first day and at every two days interval till thirteen day. The captured images are of the size 601×361 pixels.

2.3. Morphometric analysis of the fish

Different body parts of the fish can be used for freshness determination. The freshness identification can be done using different parameters which can be classified on the basis of general aspect, appearance, smell, pigmentation, rigidity, odour, coloration, etc. of the fish samples under study (Huss, 1995). Out of the above mentioned parameters, the appearance of the gills has been used as a parameter under consideration for assessing the freshness of the fish. This is so because for an image processing based diagnosis, parameters that show perceptible changes visually should be considered for analysis. In the proposed work, the image coefficients show a discriminatory pattern with respect to the fish freshness and are used in providing a framework for freshness classification.

2.4. Proposed method

The proposed image processing based method of freshness identification in fish samples involves feature extraction from segmented red channel image of gills followed by feature analysis of the coefficients of wavelet transform. To extract the accurate and discriminatory features from the image, the portion of the image which contains maximum information is required to be segmented from the whole image. As the perceptible and discriminatory changes of the gills seem to be a good choice for freshness identification, they are the Region of Interest (ROI) in this case.

Choice of Wavelet domain coefficients for Image Analysis: The texture features of image in spatial domain may not be effective enough for analysis of the segmented gill tissues for image classification as these are limited to pixel intensity values and there is no information about hidden frequency content. Wavelet transform domain coefficients have better discriminatory features for establishing the freshness levels of the fish as these coefficients captures both the spatial and frequency information of an image. In this case the image is represented in terms of the frequency of content of local regions over a range of scales and this representation seems to

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provide a good framework for the analysis of image features from the segmented tissues, which are size independent and can often be characterized by their frequency domain properties. Based on these discriminatory properties of the wavelet coefficients, this domain was used for feature extraction from the segmented gills in this work.

Fig. 1 represents the flow of proposed method of freshness identification in fish samples using image processing. The two process involved in this proposed method i.e. Segmentation and feature extraction. As discussed earlier the segmentation involves the separation of the gill tissues which is the ROI from the fish image and this is performed using a clustering based method. This segmented ROI is subjected to wavelet transformation using a Haar filter to the third level decomposition. Keeping in consideration that the segmented gills image is very small in size there is a possibility that some information may be lost in the higher level decomposition of DWT. On the basis of experiments, it was found that there are enough discriminatory features for freshness assessment in third level DWT decomposition. Hence third level DWT features are used in the proposed method as discriminatory features for fish freshness assessment system making it computationally cheap and suitable for real time applications. The lower orders, i.e. first and second order, did not show any discriminatory features. Higher order decomposition were not chosen as they may make the computational cost more and not required in this case as the third order decomposition provides enough discriminatory variation. The mean and standard deviation of the horizontal coefficient in the third level decomposition were chosen as the feature because the monotonic behaviour of the aforementioned statistical parameters was observed only in horizontal coefficient of third level, while the same was not observed for any other coefficient at any level.

2.5. ROI segmentation

As discussed earlier the changes in gill image features, being one of the physical characteristic that is easily perceptible and has discriminatory variation is used for analysis in the proposed work. The segmented area of the gill is considered as region of interest (ROI) for feature extraction as it contains the maximum discriminatory information required for freshness identification.

To segment the area of Gills (ROI), the RGB image is converted into Lab color space model. The Lab color space model has been used as it is designed to approximate human vision which means that all the colors visible to a human eye are described in this model and this suits the requirement to segment the gills from the fish image. The RGB to Lab transformation of the image helps in identification of different regions of the image individually on the basis of colors which are combination of red, green, blue and yellow color. This transformation aids in color based segmentation which is relevant to the problem for segmenting the gills from the whole image.

The K-means clustering algorithm is used to segment the transformed image based on color. Each observation belongs to a particular cluster with nearest mean. This mean represents a particular cluster. The regions with different colors are grouped under different clusters. In this case of the fish image, three clusters are formed. The K means clustering algorithm works in two steps i.e. assignment and update.

In the assignment step, the total observations are divided into clusters such that the within cluster sum of squares is minimized.

$$S_i^{(t)} = \left\{x_p : \left\|x_p - m_i^{(t)}\right\|^2 \le \left\|x_p - m_j^{(t)}\right\|^2 \forall\, j, 1 \le j \le k\right\} \quad (1)$$

where, $S^{(t)}$ = cluster, x_p = an observation, $m_i^{(t)}$ and $m_j^{(t)}$ = initial means.

Although an observation can be assigned to two or more clusters initially, but finally it has to be assigned in a single cluster. Once all the observations are assigned to every cluster, the assignment step is completed. In the update step, a new mean is calculated for every cluster formed.

$$m_i^{(t+1)} = \frac{1}{\left|S_i^{(t)}\right|} \sum_{x_j \in S_i^{(t)}} x_j \quad (2)$$

where, $S^{(t)}$ = cluster, x_j = an observation, $m_i^{(t+1)}$ = updated mean of a formed cluster. The steps of the proposed method of ROI segmentation are given in the Algorithm 1.

Algorithm 1: ROI Segmentation

Step 1: *Load the color image, I_{rgb}, of the fish in RGB format.*

Step 2: *Convert the input image, I_{rgb}, from RGB to Lab Color space. The conversion is not direct. The image is first converted into XYZ domain and then to Lab color space.*

The conversion from RGB to XYZ is given as:

$$X = R*0.4124 + G*0.3576 + B*0.1805$$

$$Y = R*0.2126 + G*0.7152 + B*0.0722$$

$$Z = R*0.0193 + G*0.1192 + B*0.9505$$

The conversion from XYZ to Lab is given as:

$$L = (116 * Yr) - 16$$

$$a = 500 * (Xr - Yr)$$

$$b = 200 * (Yr - Zr)$$

where, $Xr = X / 95.047$, $Yr = Y / 100$ and $Zr = Z / 108.883$

Step 3: *The individual values of L, a and b, corresponding to each pixel are combined to give the finally transformed Lab image as I_{lab}.*

$$I_{lab} = L + a + b$$

Where, '+' represents concatenation and not simple addition.

Step 4: *Segmentation of fish gill region is done from I_{lab} by applying K-means clustering algorithm. The image is divided into 3 clusters.*

Step 5: *Out of the 3 formed clusters, the cluster in which the fish gills are seen is selected.*

Step 6: *The cluster containing the gills region may contain some objects other than the gills. These objects are considered as noise and needs to be removed. The noise is removed from the selected cluster by selecting the largest area.*

2.6. Feature extraction from red channel of segmented ROI

In this paper, the segmented ROI from the red channel is selected for feature extraction in the wavelet domain. The red channel has been selected because of its suitability of more information content as per the gill color. The color of the gill being of

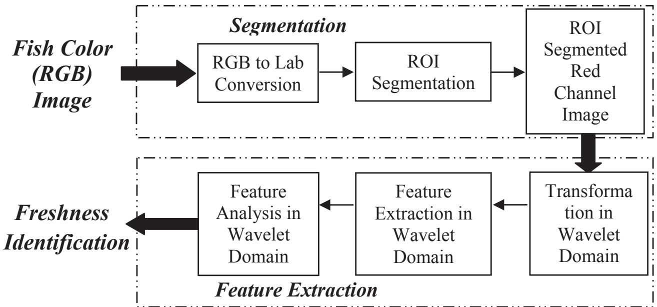

Fig. 1. Block Diagram showing proposed methodology for freshness detection in a fish sample.

reddish brown color has maximum information stored in the red channel.

As discussed in the previous section wavelet transform domain coefficients have distinct discriminatory features for establishing the freshness levels of the fish as it captures both the spatial and frequency information of an image. The statistical parameters used in this work are mean and standard deviation. Mean is defined as the average of the intensity values of all the pixels in an image. Standard deviation is defined as the measure of the spread out of the intensity values of pixels.

These statistical features are calculated from the wavelet coefficients of the segmented ROI from the red channel image of I of size M × N using following equations:

$$Mean(\sigma) = \frac{\sum_i \sum_j I(i,j)}{\mathrm{M} \times \mathrm{N}} \tag{3}$$

$$Standard\ Deviation = \sqrt{\frac{\{I(i,j) - \sigma\}^2}{\mathrm{M} \times \mathrm{N}}} \tag{4}$$

Practical applications of the discrete wavelet analysis can be found out in signal processing applications. Every transformation level provides information about the source image from different resolution.

In the proposed work, a third level wavelet decomposition is done on the segmented red channel of the ROI. A "Haar" filter is used to decompose the image into coefficients at all the three levels. The statistical features of these coefficients are extracted.

Fig. 2 shows the discrete wavelet decomposition (DWT) for a fish sample. In the proposed work, the DWT using Haar Filter is done for three levels. The features are extracted at each level from the coefficients obtained during decomposition. The features extracted from horizontal coefficient at level 3 for all days of observation are analyzed for the variation pattern to form a qualitative framework.

2.7. Determination of freshness ranges from the coefficients of the segmented ROI

Based on the wavelet coefficients extracted from the segmented ROI of the gills the variation pattern of the statistical parameters of these coefficients are analyzed in terms of days passed. The mean and standard deviation of the third level wavelet decomposition coefficients using Haar filter in horizontal direction was found to have a discriminatory variation pattern which can be used as a framework for identification of freshness levels of fish. There is a monotonic behaviour in this variation pattern on the basis of which freshness ranges are determined. The following strategic approach is used to generate a framework.

Images from 4 samples with 3 repetitions of fishes were used as a training data generate the model framework. The mean and the standard deviation of the 3rd level wavelet coefficients using Haar filter is studied for the 6 days of experimentation for defining the freshness ranges.

The entire framework is divided into 3 freshness ranges:

Freshness Range 1, ***FR1*** = ***FL0*** − ***FL1*; Most fresh**
Freshness Range 2, ***FR2*** = ***FL1*** − ***FL2* Moderate fresh**
Freshness Range 3, ***FR3*** = ***FL2*** − ***FL3*; Least Fresh**

where,

FL0 = Highest value of the statistical parameter of all the samples at day 1.
FL1 = Lowest value of the statistical parameter of all the samples at day 2.
FL2 = Lowest value of the statistical parameter of all the samples at day 4.

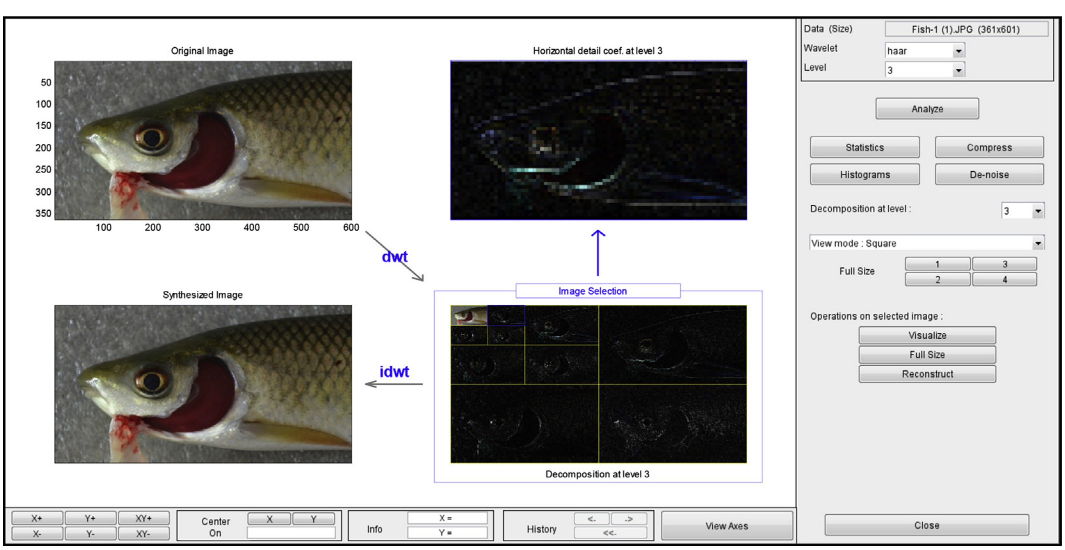

Fig. 2. Three level wavelet decomposition.

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FL2 = Lowest value of the statistical parameter of all the samples at day 6.

A reference level is calculated in each freshness range, which can be considered as tolerance for each range:

Ref level for FR1, R1 = (FL0 + FL1)/2;
Ref level for FR2, R2 = (FL1 + FL2)/2;
Ref level for FR3, R3 = (FL2 + FL3)/2;

If a new sample is fed to the system, then its statistical parameter will be a deciding factor of where the new sample will lie in which freshness range. The two statistical parameter mean and standard deviation are under consideration for making the classification of the freshness range of the test sample. Considering the new sample under test has value, Mean = M, Standard Deviation = S, algorithm 2 gives a stepwise explanation how the freshness range is determined for the statistical parameters of the input test sample. If the same range is returned from both the statistical parameter the decision is straight in favour of the range. But in case two different ranges are found from two different statistical parameters then a reference level is defined for each range and the decision is based on the basis of distance of the statistical parameter from the reference level. The detailed stepwise freshness range identification for the test sample is explained in the Algorithm 2.

Algorithm 2: Determination of freshness range of fish

Following algorithm is followed to classify the freshness range of the fish based on the values of the statistical parameters, M and S.

Step 1: **if** (FL0<M<FL1) && (FL0<S<FL1) **then**
Sample belongs to FR1.
Step 2: **if** (FL1<M<FL2) && (FL1<S<FL2) **then**
Sample belongs to FR2.
Step 3: **if** (FL2<M<FL3) && (FL2<S<FL3) **then**
Sample belongs to FR3.
Step 4: **if** (FL0<M<FL1) && (FL1<S<FL2)
if (M>R1) && (S>R2) **then**
Sample belongs to FR1.
if (R1<M<R2) && (S>R2) **then**
Sample belongs to FR1.
if (R1<M<R2) && (S<R2) **then**
Sample belongs to FR2.
if (M>R1) && (S<R2)
X = M – R1;
Y = R2 – S;
Y' = X * [(FL1 - FL2) / (FL0 – FL1)];
if X > Y' **then**
Sample belongs to FR1.
else
Sample belongs to FR2.
Step 5: **If** (FL0<M<FL1) && (FL2<S<FL3) **then**
Sample belongs to FR2

It can be seen from Algorithm 2 that all the possibilities that the statistical parameters, mean and standard deviation lies in any region has been considered and the decision for the range is defined. In case of dissimilar ranges for both the statistical parameters the distance from the reference level is taken into consideration to reach a decision.

3. Experimental results

In this paper, a total of 4 fish samples with 3 repetitions were used as training dataset for making the framework for freshness identification. The statistical feature, i.e. mean and standard deviation, for all the coefficients, obtained from the wavelet transformation, was calculated and analyzed for 6 consecutive days of observation. The results are included in this section.

Fig. 3 presents the day-wise RGB color image of the fish images captured for 6 consecutive days. It can be seen from this figure that the color of gills fades away as the observation day progresses.

Fig. 4 shows the segmented ROI from the fish image sample. This figure shows the transformed Lab color space image of the input RGB fish sample. The individual clusters formed are shown in the figure as discussed in Section 2.5. The final image in the extreme right is the red channel of the ROI segmented from the fish samples. It is clear from the figure that the segmented ROI is accurate which was used for feature extraction. The stepwise results of ROI segmentation is presented in this figure and the results are convincing.

Fig. 5 shows the segmented ROI and the wavelet coefficients obtained from this segmented ROI. The coefficients shown are for the third level decomposition obtained in horizontal direction. The features are extracted from these coefficients.

Table 1 above shows the day wise variation of mean and standard deviation of a third level wavelet coefficient in horizontal direction for 4 fish samples of training set.

The variation of the values of standard deviation and mean of a third level wavelet coefficient in horizontal direction for 4 samples out of the of training set with respect to the passing days is shown graphically in Fig. 6 and Fig. 7 respectively.

The freshness ranges are labelled in the Fig. 6 and Fig. 7 from the experiments performed by the training fish samples. The reference levels are also marked in the figure. It can be seen from these two graphs that the two statistical parameters i.e. mean and standard deviation are nicely spaced making distinct levels of freshness. The figure clearly demonstrates that as the duration of sampling day increases, the statistical features of the fish sample show a monotonic discriminatory variation.

To validate the proposed framework test samples were evaluated using the proposed method. Four samples with three repetitions of fishes were used for testing the algorithm which is completely different from the training images used for making the framework. There is no overlap in training data and test data. The test samples are subjected to imaging under the same conditions as described for the training data in Section 2.1 and Section 2.2. The imaging was done for 6 consecutive days and the images were subjected to ROI segmentation followed by feature extraction and classification for freshness identification similar to the method as was done for the training samples. In case of dissimilar results for freshness range of the two parameters the distance from the reference range was considered using Algorithm 2 for deciding the freshness range of the input sample under test. The imaging based results are compared with the actual ground reality. The compared results are considered as positive if the imaging results matches with the ground reality and negative in case it does not.

The experiments performed with the test samples for the sampling days have been tabulated in Table 2. It can be seen from the results that the image processing framework can be used in

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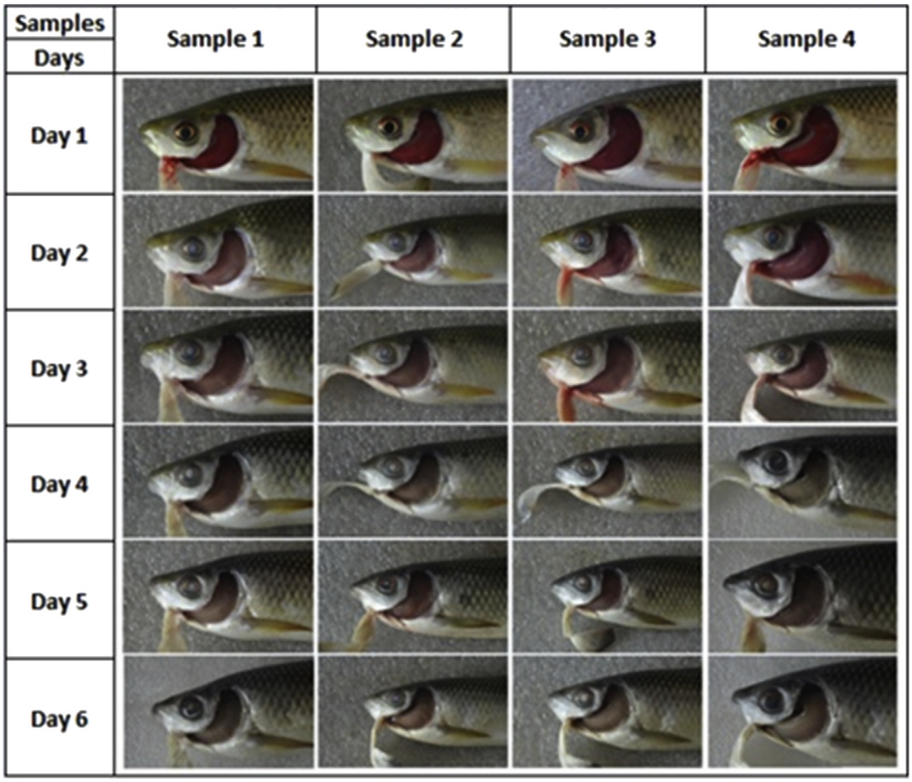

Fig. 3. Day wise degradation of the fish samples under observation.

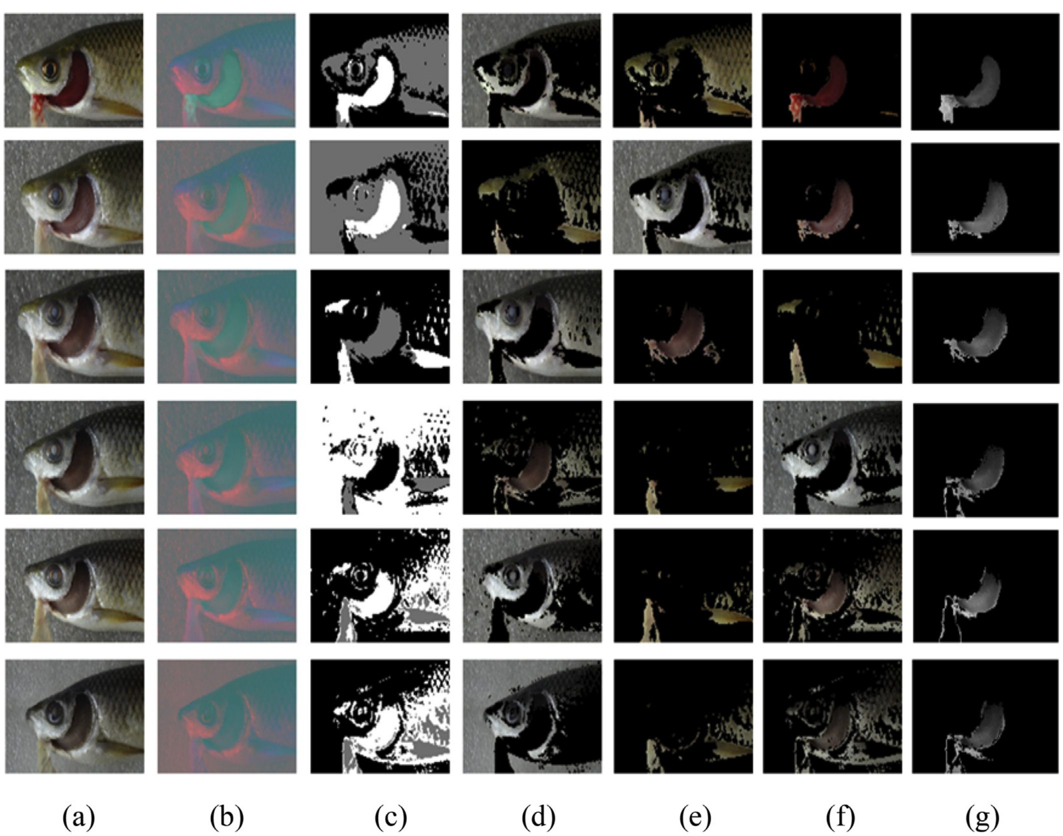

Fig. 4. Automatic Segmentation of Fish Gill (a) Input RGB Image (b) Lab Color Space Transformed Image (c) Clusters formed by Clustering algorithm (d) Cluster 1 (e) Cluster 2 (f) Cluster 3 (g) Red Channel of segmented ROI. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

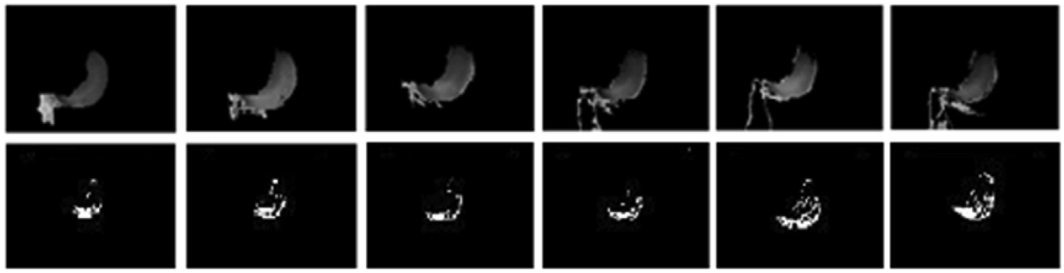

Fig. 5. The segmented ROI and the third Level coefficients in horizontal direction of the segmented ROI during wavelet decomposition.

Table 1
Statistical Features of third level wavelet decomposition in horizontal direction of 4 fish samples.

Fish sample 1			**Fish sample 2**		
Feature	**Mean**	**Standard deviation**	**Feature**	**Mean**	**Standard deviation**
Days			**Days**		
Day 1	81.39063	110.019	Day 1	80.45063	111.127
Day 2	66.01956	104.124	Day 2	69.04456	106.562
Day 3	62.94859	101.345	Day 3	60.44859	100.216
Day 4	57.34624	98.712	Day 4	56.94624	97.587
Day 5	52.01888	98.121	Day 5	51.01888	96.238
Day 6	47.53118	95.654	Day 6	46.53118	95.309
Fish Sample 3			**Fish Sample 4**		
Feature	**Mean**	**Standard Deviation**	**Feature**	**Mean**	**Standard Deviation**
Days			**Days**		
Day 1	82.39063	113.259	Day 1	84.39063	116.012
Day 2	65.01956	107.983	Day 2	66.01956	105.237
Day 3	61.94859	99.387	Day 3	58.14859	102.235
Day 4	56.34624	97.281	Day 4	55.34624	99.120
Day 5	52.61888	96.121	Day 5	52.01888	98.125
Day 6	45.93118	94.298	Day 6	44.53118	97.409

identification of the freshness ranges for the input fish samples.

Discussion and Final Remarks on Experimental Results:

1. The separation of the gill tissues from the fish image using image processing algorithm based on k-means clustering segments the most informative regions of the image for discrimination of freshness range. Experimental results indicate that this segmentation method using Lab space and k-means clustering is an efficient and accurate method for freshness classification.
2. The wavelet transformation coefficients of the segmented ROI using Haar filter gives discriminatory coefficients for freshness

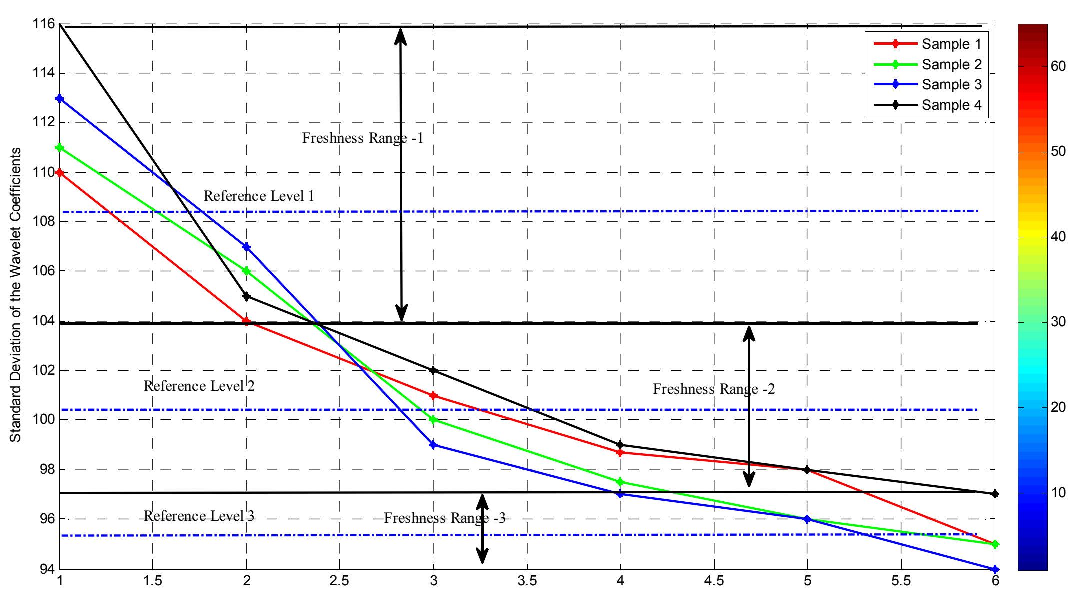

Fig. 6. Variation of Standard deviation Wavelet features of the ROI of Training Fish Samples.

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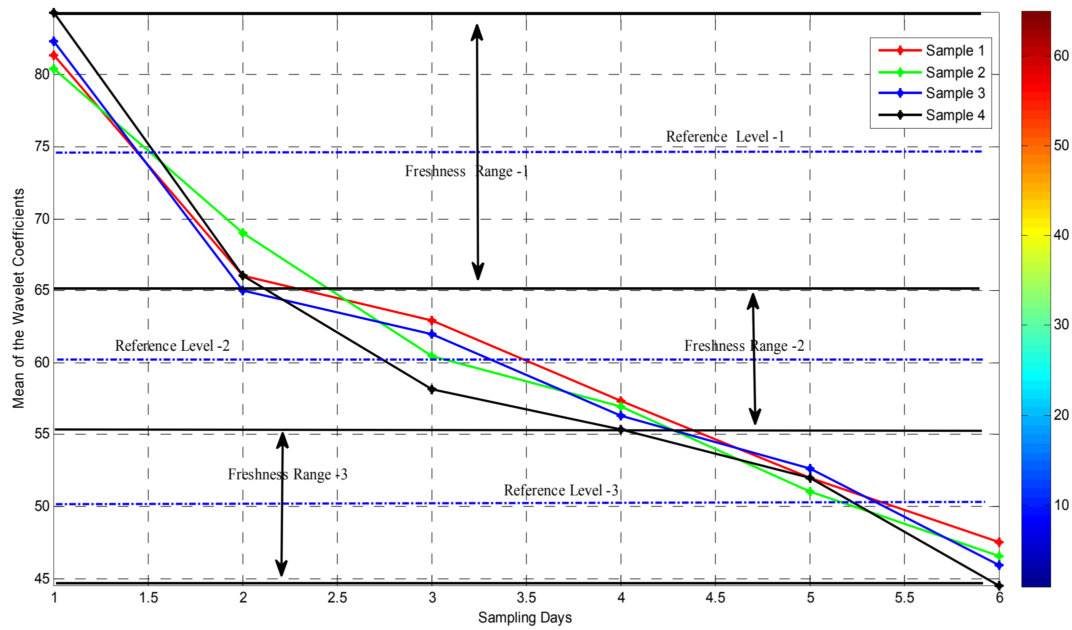

Fig. 7. Variation of mean of wavelet features of the ROI of Training Fish Samples.

Table 2
The experimental results for the test samples under experimentation.

Sl. No.	Test sample	Freshness range (Parameter – Mean)	Freshness range (Parameter – Std. Var.)	Final decision for classification as per Algo 2	Compared results
1	Fish Test Sample 1-Day 1	FR1	FR1	FR1	Positive
2	Fish Test Sample 1-Day 2	FR1	FR1	FR1	Positive
3	Fish Test Sample 1-Day 3	FR2	FR2	FR2	Positive
4	Fish Test Sample 1-Day 4	FR2	FR2	FR2	Positive
5	Fish Test Sample 1-Day 5	FR3	FR3	FR3	Positive
6	Fish Test Sample 1-Day 6	FR3	FR3	FR3	Positive
7	Fish Test Sample 2-Day 1	FR1	FR1	FR1	Positive
8	Fish Test Sample 2-Day 2	FR1	FR1	FR1	Positive
9	Fish Test Sample 2-Day 3	FR2	FR2	FR2	Positive
10	Fish Test Sample 2-Day 4	FR2	FR2	FR2	Positive
11	Fish Test Sample 2-Day 5	FR3	FR3	FR3	Positive
12	Fish Test Sample 2-Day 6	FR3	FR3	FR3	Positive
13	Fish Test Sample 3-Day 1	FR1	FR1	FR1	Positive
14	Fish Test Sample 3-Day 2	FR1	FR1	FR1	Positive
15	Fish Test Sample 3-Day 3	FR2	FR2	FR2	Positive
16	Fish Test Sample 4-Day 4	FR3	FR3	FR3	Negative
17	Fish Test Sample 5-Day 5	FR3	FR3	FR3	Positive
18	Fish Test Sample 6-Day 6	FR3	FR3	FR3	Positive
19	Fish Test Sample 3-Day 1	FR1	FR1	FR1	Positive
20	Fish Test Sample 3-Day 2	FR1	FR1	FR1	Positive
21	Fish Test Sample 3-Day 3	FR2	FR2	FR2	Positive
22	Fish Test Sample 4-Day 4	FR2	FR2	FR2	Positive
23	Fish Test Sample 5-Day 5	FR3	FR3	FR3	Positive
24	Fish Test Sample 6-Day 6	FR3	FR3	FR3	Positive

ranges. The reference levels defined for each range helps the algorithm to classify an input sample for a freshness range based on the distance from this reference level. This will also be effective even if there is difference in the ranges of freshness for the two statistical parameters. Hence this may be considered as a robust and comprehensive method for classification of fish samples for freshness.

3. The experimental results with 24 samples of fish images for various levels of freshness have been tested for prediction of the fish freshness and quality. Hence this can be considered as a significant non destructive method for classification of fish for freshness.
4. Current results may vary with type, genetical status, resistance to environmental stress, gill colour pattern of fish. Quality as well as gill colour pattern will also be altered with toxicant residue, pathogenic load and habitat quality within a single fish variety. Hence, a detailed study on collection of different commercial fish varieties from multiple aquatic systems and their gill colour analysis will be required to establish an imaging based platform of fish quality and freshness, where this important technique can be applied as basic diagnostic tool. Bigger sample size also can minutely alter the outcome, where basic methodology will be the same.

4. Conclusions and future work

The proposed method is a non destructive image processing based method for predicting freshness of the fish. The perceptible changes in the automatically segmented gill tissues have been used in wavelet domain as discriminatory features. The third level wavelet decomposition of the segmented gill tissues using Haar filter has been used and the horizontal coefficients seem to have a discriminatory behaviour with the number of days passed. A relationship between these features and freshness levels is established from the training data and based on this relationship a framework is proposed for freshness identification from fish images. This framework may be considered significant and the prototype may find application in fish industries and for consumers.

As a future prospective for this work, the current protocol may be applied for different major fish varieties. Moreover, based on the current research, a barcode reader may be designed for individual fish types using this proposed gill imaging analysis tool. Bar code based optical device can be handy for quality and freshness testing in big fish supermarket and also to complete time bound quality checking process where large fish volume is a issue.

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