Measurement of eye turbidity of formalin-treated *Chanos chanos* (milkfish) using image analysis

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Abstract

Freshness is an important factor in fish consumption. However, fish freshness is falsified by using formalin which is cancerous to humans. Fish freshness can be determined using eye color. This study aimed to determine the effect of formalin treatment on *Chanos chanos* (milkfish) through the mean saturation and value from the HSV (hue, saturation, value) color space of the eye and the pupil, and to compare the significant difference between formalin-treated and untreated fish. Sixty (60) *Chanos chanos* (milkfish) samples were documented throughout a seven-day period, segmented in GNU Image Manipulation Program 2.10.8, and processed using a program developed in Scilab 6.0.2. In both setups, the mean saturation of the eye and the value of the pupil increased over time while the mean value of the eye and the saturation of the pupil decreased. This change indicates that the eyes become cloudier as a fish loses its freshness. Formalin treatment led to a significant increase in the mean values of the eye and the pupil.

Keywords: *color, computer vision, fish freshness, formalin, image analysis*

Introduction. The Philippines is a country that primarily relies on its aquatic resources such as in the fisheries industry. In 2018, the Philippines produced a total of 4.35 million metric tons of fish, crustaceans, mollusks, and aquatic plants. The Philippines also ranked 8th among the top fish producing countries in the world in 2016 [1]. *Chanos chanos* (milkfish) is one of the three most consumed fishes in the Philippines [2]. In Region VI alone, the total number of *Chanos chanos* (milkfish) produced was 88,981.8 metric tons or 29.3% of the total country production, making Region VI the largest producer of *Chanos chanos* (milkfish) in the Philippines.

Fish is a highly perishable commodity and its freshness is an important indicator of its overall quality, as well as its fitness for human consumption. Biological and chemical changes that occur post death and during storage may result to the deterioration of fish quality. Significant changes in the appearance, odor, color, flavor, and texture of the skin, slime, eyes, gills, and belly can be used to approximate fish freshness. Color is one of the most important quality parameters in fish processing since color deterioration is a sign of decreasing freshness. However, quality evaluation performed by experts, such as sensory analysis of appearance and odor, is time-consuming and labor-intensive since humans are susceptible to fatigue, bias, and other limitations [3]. Through the developments in the field of computer vision technology, product assessment has become all the more standardized and accurate by employing automated noninvasive methods and visual evaluation [4].

Formalin is a preservative that is classified by the International Agency for Research on Cancer [5] as carcinogenic, but is used by fish traders to prevent spoilage. Formalin is a solution of 37% formaldehyde in water which is used in preservation and treatment against microorganisms that may cause fish diseases [6]. Formalin-treated fish are almost indistinguishable from untreated fish which makes them difficult to identify, leading to the accidental consumption of these fish. Considering the threat that formalin poses to consumers [7], the practice of using formalin to extend the shelf life of fish [6] in markets should be ended.

Previous studies utilized various methods for identifying the level of fish freshness. Dowlati et al. [3] evaluated the freshness of wild and farmed *Sparus aurata* (gilthead seabream) using computer vision technique by measuring lightness (L^*), redness (a^*), yellowness (b^*), chroma, and total color difference in fish eyes and gills. The results showed a strong correlation between color parameters and storage days. The L^* and b^* values increased over time, while the a^* value decreased. Similar results were reported by Unal Sengor et al. [8] whose study utilized both the Minolta color measurement method and the image analysis method to assess the freshness of *Sparus aurata* (gilthead seabream) stored without ice, with ice, and with ice and cover paper. While previous studies have utilized computer vision to determine fish freshness over time and in varying storage conditions or lighting setup, there is a scarcity of studies that use computer vision to examine the changes in the eye color of formalin-treated and untreated fish.

To overcome human limitations [3], formalin detection in fish may be automated for increased efficiency, consistency, and accuracy. However, much remains unexplored in the field, thus available training data for automation is limited. The results of this study will primarily be useful to fish quality assessment agencies, will contribute to the

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advancement of quality assessment automation, and will be beneficial to the general public specifically, fish consumers.

The aim of this study was to determine the effect of formalin treatment on *Chanos chanos* (milkfish) through the mean saturation and value of eye and pupil of eye using computer vision. It specifically aimed to:

(i) document a timelapse of fish progression in formalin-treated and untreated samples of *Chanos chanos* (milkfish) for 7 days;

(ii) identify the mean saturation and mean value of the eye and the pupil of the eye relative to the stage of fish progression from the images using Scilab 6.0.2; and

(iii) calculate the significant difference of the mean values for the treated and untreated setups using JASP 0.9.2.

Methods. The data-gathering procedure was divided into five (5) phases: sample preparation, image acquisition, image analysis, statistical analysis, and disposal. The sample preparation was further divided into three (3) sub-phases: sample (a) collection, (b) preparation, and (c) treatment. Image acquisition was conducted over a span of 7 days where images of the samples were collected for image analysis. Image analysis consisted of image segmentation, the extraction of HSV values from the segmented images, and the calculation of the mean saturation and value of the processed images. Statistical analysis involved generating the mathematical models as visual displays of the sample progression and calculating the significant difference of values between setups. The samples used were then disposed.

Sample preparation. Fresh-caught, farmed *Chanos chanos* (milkfish) with fork lengths of 20.2 to 26.8 cm, total lengths of 26.1 to 34 cm, and weights of 123 to 252 g, were collected from the Southeast Asian Fisheries Development Center (SEAFDEC) Brackishwater Station in Dumangas, Iloilo. After capture, the fish were immersed in icy water to shock them. Euthanasia was performed shortly after capture through spiking or iki jime which physically destroys the central nervous system [9]. The fish were individually placed inside ziplock bags and immediately transported to a laboratory in Philippine Science High School - Western Visayas Campus in polystyrene boxes with 1:1 fish-to-ice volume ratio [6]. The fish were separated into two groups, one was immersed in 5% formalin for five (5) minutes [6] while the other remained untreated. The fish samples from the two groups were stored in a chest freezer at a temperature of 0°C to 5°C and observed every 24 hours for seven days.

Image acquisition. A color camera and an illumination chamber or lightbox as described by Dowlati et al. [3] were used in an image acquisition system to capture the images of fish. Two LED lamps (Natural Daylight, 240 V / 4W) with a color temperature of 6500 K were used to capture high quality images under reproducible lighting conditions. The lamps with lengths of 30 cm were installed opposite of each other on the left and right sides of a wooden lightbox of dimensions of 50 x 50 x 30 cm. The interior walls were painted matte black to minimize background light and light reflectance. The sample tray was green in color for adequate contrast with the fish sample [3]. The images of fish eyes were captured using a digital color camera (18-55mm 1:3.5-5.6G VR). Top-view images were taken at a vertical distance of 30 cm [10]. All images were taken using constant camera settings (see Table 1). The LED lamps were turned on an hour before image capturing to ensure stable lighting [3].

Table 1. Camera control settings.

Variable	Settings
Image size	6000 x 4000 pixels
Zoom	No zoom
Flash mode	No flash
Sensitivity	ISO-200
Operation mode	Manual
Aperture Av.	f/6.3
Exposure time Av.	1/15 s
Image type	JPEG
Macro	On
Focal length	18mm

Image analysis. Segmentation is the process of partitioning an image into regions that correlate strongly to features of interest in an image. The regions of interest, the eye and the pupil, were manually segmented using GNU Image Manipulation Program 2.10.8 to isolate them for color analysis [10]. The elliptical marquee tool was used to select and segment images of the eye and the pupil which appears as a black circle in the middle of the eye [11]. The RGB values of the processed images were converted to their respective HSV equivalents. Image analysis was done using Scilab 6.0.2 [12].

Statistical analysis. The analysis of results was done by calculating the statistical difference between the data points of the treated and untreated samples using the independent samples t-test through the JASP 0.9.2 software. Data was expressed using mean and standard deviation. Differences among the mean values of treated and untreated samples were examined with a significance level of α=0.05.

Safety Procedure. The proper use of splash goggles, laboratory gown, gloves, and mask was observed throughout the conduct of the study. All fish samples were buried in a vacant lot in San Miguel, Iloilo. Used and unused formalin was turned over to the science research assistant for proper storage and disposal.

Results and Discussion. Figure 1 shows the changes in the eyes of the same untreated fish over seven days. The iris became darker over time, while the pupil became cloudier. The same is true for the formalin-treated samples as seen in Figure 2. However, the iris of formalin-treated fish discolor earlier compared to untreated fish. The pupils of the formalin-treated fish became cloudier than those of the untreated samples.

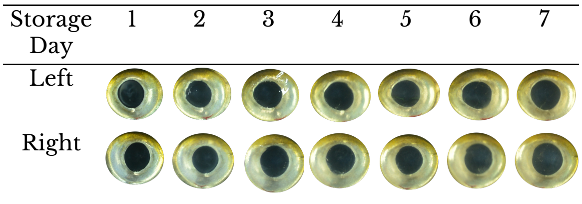

Figure 1. Typical fish eye changes during storage of untreated *Chanos chanos* (milkfish).

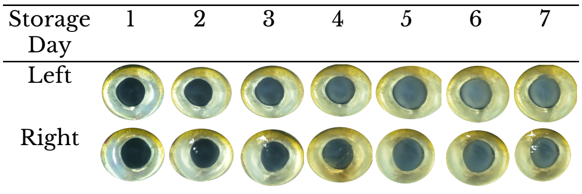

Figure 2. Typical fish eye changes during storage of formalin-treated *Chanos chanos* (milkfish).

Changes in fish freshness were determined by calculating the mean value and saturation of eye and pupil images using the Scilab 6.0.2 image processing library. Tables 2 and 3 present the mean and standard deviation of the saturation and value of the eye and pupil for untreated and formalin-treated samples, respectively.

The mean saturation of the eye increased over time in both setups, from 0.31589 to 0.36988 for untreated samples and 0.29678 to 0.39731 for formalin-treated samples. In contrast, the mean saturation of the pupil decreased over time, from 0.33927 to 0.19270 for untreated samples and 0.33872 to 0.20830 for formalin-treated samples. The mean value of the eye slightly decreased, from 0.59896 to 0.57457 in the untreated setup and from 0.63972 to 0.62432 in the formalin-treated setup. The mean value of the pupil increased, from 0.17985 to 0.24437 for the untreated setup and 0.22680 to 0.38552 in the formalin-treated setup.

Figure 3 shows the mathematical models generated to display the comparison of observable trends in the changes in saturation and values of eyes and pupil between formalin-treated and untreated *Chanos chanos* (milkfish) samples.

The graph for mean saturation of eyes as seen in Figure 3-a shows a steady increase in saturation. This is in contrast to the trend seen in the mean saturation of pupils as seen in Figure 3-b where the saturation of the pupils steadily decreases. Statistical analysis shows no significant difference for eye and pupil saturation (see Table 4).

The graph for mean value of eyes as seen in Figure 3-c shows an increase then a decrease in value halfway through. Meanwhile, the graph for mean value as seen in Figure 3-d of pupils display an increase in value over time. Statistical analysis shows a significant difference for eye and pupil value (see Table 4).

Converting an image to its HSV equivalent means separating its hue, saturation, and values into different channels. The channels produced can be converted into matrices with each pixel having an appropriate numerical value. Saturation ranges from zero to one, a saturation of zero means that the pixel is the closest to gray that it could be while one would denote that a pixel is the purest form of its respective hue. Value is the proximity of a pixel to black or white when converted into grayscale. It also ranges from zero to one, a value of zero means that the pixel is black while a value of one means that the pixel is white [13].

Table 2. Statistical parameters of the regions of interest on the basis of pixel saturation and value for untreated fish.

	Eye				Pupil of Eye			
	Saturation		Value		Saturation		Value	
Day	Mean	Std. Deviation	Mean	Std. Deviation	Mean	Std. Deviation	Mean	Std. Deviation
1	0.31589	0.04081	0.59896	0.04168	0.33927	0.04284	0.17985	0.04270
2	0.29658	0.02841	0.61924	0.03003	0.31360	0.02673	0.24334	0.04032
3	0.30757	0.03079	0.60468	0.02991	0.30239	0.03282	0.22710	0.03580
4	0.31227	0.02873	0.60815	0.02705	0.26640	0.03943	0.23290	0.02782
5	0.33848	0.03227	0.60102	0.02391	0.22886	0.02896	0.23852	0.03287
6	0.34215	0.03731	0.58334	0.02385	0.20231	0.02518	0.23912	0.02731
7	0.36988	0.04368	0.57457	0.02311	0.19270	0.02150	0.24437	0.02915

Table 3. Statistical parameters of the regions of interest on the basis of pixel saturation and value for formalin-treated fish.

	Eye				Pupil of Eye			
	Saturation		Value		Saturation		Value	
Day	Mean	Std. Deviation	Mean	Std. Deviation	Mean	Std. Deviation	Mean	Std. Deviation
1	0.29678	0.03621	0.63972	0.03854	0.33872	0.04976	0.22680	0.03693
2	0.30936	0.04304	0.64632	0.03573	0.29464	0.04322	0.27685	0.03921
3	0.32907	0.04530	0.65367	0.03731	0.24712	0.03451	0.34837	0.06162
4	0.34628	0.05262	0.65065	0.03838	0.21575	0.03088	0.38856	0.05923
5	0.34923	0.05805	0.65171	0.03828	0.21177	0.02742	0.40569	0.06166
6	0.37609	0.05936	0.63269	0.04039	0.20736	0.02696	0.39506	0.06147
7	0.39731	0.06421	0.62432	0.04345	0.20833	0.02164	0.38552	0.05602

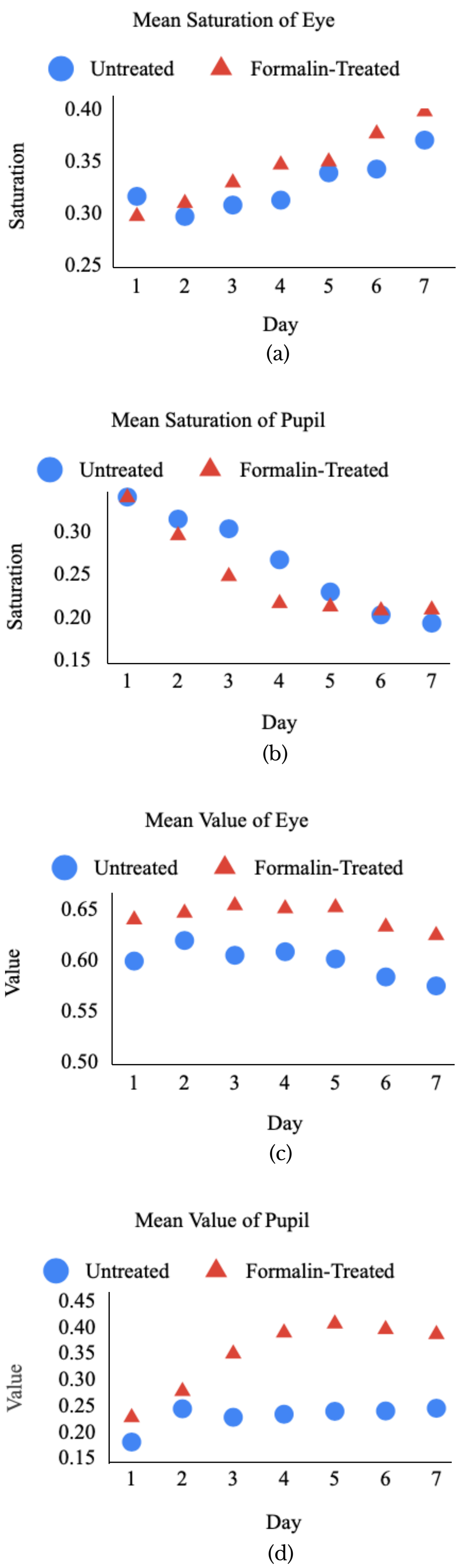

Figure 3. (a) Mean saturation of eye; (b) Mean saturation of pupil; (c) Mean value of eye; and (d) Mean value of pupil for formalin-treated and untreated samples.

Table 4. Statistical analysis of the significant difference in regions of interest parameters between treated and untreated fish.

Parameter	Significance (2-tailed, α = 0.05)
Saturation of eye	0.313
Saturation of pupil	0.562
Value of eye	0.000
Value of pupil	0.003

The increase in saturation of the whole eye for both treated and untreated samples means that the eyes became more colored over time. This can be observed in the images acquired where the eyes gradually turns yellow or red. This change in coloration could be attributed to internal chemical changes that occur in lipids and proteins. Lipid oxidation reduces the fish's shelf life and modifies its color and texture with the formation of yellow pigment as the resulting product of reaction between protein and oxidized lipid [14]. The decrease in saturation for the pupil of the eye means that the pupil became grayer as the fish loses freshness and becomes unfit for human consumption. The pupil's grayness is an indicator of its turbidity which is a visual indicator of fish freshness [3]. However, there is no significant difference in the saturation changes in eye and pupil when the treated and untreated samples are compared, therefore it cannot be an effective indicator of formalin presence.

The increase in value of the whole eye of the untreated samples, peaking at day 2, and the formalin-treated samples, peaking at day 3, and their subsequent decrease after peaking means that the eyes gravitate towards white in the first two or three days, then proceeds towards lower value, or towards black, after. The value of the pupils displays a steady increase over time, thus indicating that the pupils become cloudier over time. This explains why for the first three days there is an observed increase in the value of the whole eye. The subsequent decrease in value is due to the yellowing of the eye starting from the fourth day, thus decreasing the total value of the whole eye. The whitening of the pupil is an indicator of turbidity in which the eyes become cloudier as a fish loses its freshness [3]. Previous studies report similar data. In the $L^*a^*b^*$ color space, lightness or L^* describes the brightness of the fish eye which is similar to value in the HSV color space. Dowlati et al. [3] found that the L^* increased during ice storage for farmed gilthead seabream while Balaban et al. [15] reported that the average L* values of the snapper and gurnard eyes increased significantly with storage time ($P < 0.05$). Contrary to the saturation of the eye and pupil, statistical analysis showed that there is a significant difference in the mean values of the eye and pupil between treated and untreated setups, suggesting that value may be a useful tool for the identification of formalin-treated fish.

Though the values of treated and untreated samples are significantly different in the eye and pupil, the resulting trend is contrary to the background literature. The study by Dowlati et al. [3] revealed that fish eyes become cloudier as a fish loses its freshness. Yeasmin et al. [6] presented the illegal use of formalin as a way to make fish appear fresher. Following this logic, the formalin-treated fish samples should have eyes that are less cloudy than the untreated control samples, contrary to what had been observed in this study. This contradiction indicates that though formalin-treated fish may seem fresh according to several indicators of fish freshness, it does not pass as fresh when assessed through eye turbidity.

The results of the study may improve the current process of formalin presence identification in the marketplace through the development of an objective measuring standard based on value from the HSV color space instead of apparent color. This may be further enhanced through automation with the use of software development.

Limitations. Since image acquisition was manually performed, the positioning of the camera was not constant all throughout the duration of the data gathering. Slight deviations may have occurred during the conduct of the data gathering, varying the light received by the camera.

Conclusion. In this study, image processing was employed for fish freshness assessment by measuring the color parameters of the eye and pupil of formalin-treated and untreated *Chanos chanos* (milkfish). A significant difference was observed in the changes in eye and pupil value between treated and untreated samples, thus justifying the use of eye and pupil value as an indicator of formalin presence. In contrast, no significant difference was found in the changes in fish eye and pupil saturation between treated and untreated samples; hence saturation is not an effective indicator of formalin presence. Additionally, formalin immersion was found to degrade eye freshness rather than enhance it as formalin-treated fish had cloudier eyes than untreated fish.

Recommendations. The process of capturing and segmenting images is time-consuming. Automation of image segmentation removes any human biases that may be present in identifying the parts of a fish eye. However, a separate study must also be conducted for the determination of the eye and pupil of a fish using computer vision and thresholding. Automation is the potential extension of this study, thus the integration of the computer vision segmentation and image processing is recommended.

Quality assessment of the fish samples through trained experts during the conduct of the study is recommended to compare the manual and the computer-assisted process of product evaluation and to verify the precision and accuracy of the data gathered. To completely avoid light reflection in images, an image acquisition setup consisting of a light box with an external light source is also recommended.

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