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FISH SKIN FRESHNESS LEVEL BY INTEGRATED RGB MULTICOLOUR IMAGE PROCESSING FROM QUALITY INDEX METHOD (QIM) ASSESSMENT

Aina Rasyiqah binti Mohd Hanif[1], Khairul Naim Abd. Aziz[2*], Muhammad Akmal Roslani[3], Sharir Aizat Kamaruddin[4] and Zamzila Erdawati Zainol[5]

Faculty of Applied Sciences, Universiti Teknologi MARA, Perlis Branch, Arau Campus, 02600 Arau, Perlis, Malaysia

[1]ainarasyyy@gmail.com, [2*]khairul87@uitm.edu.my, [3]akmalroslani@uitm.edu.my, [4]shariraizat@uitm.edu.my, [5]zamzila396@uitm.edu.my

ABSTRACT

The colour of the fish skin is one of the important factors to determine the freshness of a fish. There is potential to use the fish images as an alternative to determine the fish freshness. However, the freshness relationship of the fish skin image to the Red, Green, Blue (RGB) multiple colour channel needs to be elucidated to achieve an accurate interpretation of fish freshness. The objective of this study is to determine the freshness of the fish samples using QIM assessment and to extract the RGB colour value from fish skin images. Finally, to establish the relationship between the QIM scores ranging from 1 (fresh) to 3 (spoiled) and RGB value for freshness indicator using fish images. The effects of temperature, environment, and storage method have been shown to play an important role in determining the rate of deterioration towards the quality and freshness level in fish. From this study, a freshness indicator based on Quality Index Method (QIM) and RGB value for Queenfish and Threadfin was created. Based on the QIM score, Threadfin was easier to deteriorate as compared to Queenfish from its leaner body type properties. Different fish would reflect different freshness reading as Threadfin is in a fresh state when it possesses QIM score of 1 with RGB values range between of 143 to 172. As deterioration progresses, the QIM score is at 3 and the RGB values are ranging from 132 to 161. While Queenfish is found to be in a fresh state when it acquires QIM score of 1 and the RGB values are in the range of 148 to 170. It starts to spoil when the QIM score is at 3 and the RGB values are ranging from 154 to 184.

Keywords: *Fish Freshness, Freshness detector, Quality Index Method, RGB colour processing*

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1. Introduction

Freshness is a basic requirement to measure the quality of fish. The quality of a fish may be affected by characteristic changes deeply which result in a progressive loss of in terms of their quality and taste. Moreover, the quality of fish can be described as the aesthetic appearance and freshness or degree of spoilage of the fish. There are many factors that influence the shelf life of fish such as handling and storage conditions (Sornam *et al.,* 2017). Quality of fish changes after catching due to chemical reaction and microbial spoilage, where the psychrotolerant gram-negative bacteria, such as *Pseudomonas* spp. and *Shewanella* spp. can cause deterioration to the fish, even on chilled fish (Ikape, 2017).

Consumers often describe the characteristics of fresh fish as a pleasant and neutral smell, shiny and bulging eyes, bright red colour gills, and naturally metallic glow skin. Microbiological, biochemical, and sensory methods have been used to assess the fish freshness during handling and storage. In contrast to that, Zaragoza *et al.* (2012) state that those methods are expensive, time-consuming, and require skilled personnel to evaluate the freshness of the fish. Therefore, this study implemented the image processing technique as an alternative to assess the freshness of the fish as it is non-destructive and non-hazardous (Sengar *et al.,* 2018).

Redness (R) by using Red single colour channel also look promising to differentiate fish freshness but redness could also indicate sign of mishandling and burst blood vessel which mostly appears on fish eyes (Aziz *et al.*, 2020). Aguil *et al.* (2018) reported that consumers will decide whether the fish is fresh or stale by only looking at the eyes and gills of the fish, which shows the lack of use for fish skin to body area as an indicator to determine the fish freshness. According to Sengar *et al.* (2018), the colour of the fish skin is one of the important factors to determine the freshness of a fish. Up to today the use of fish images may contribute a big help as the cheapest fish freshness detector. However, the freshness relationship of the fish skin image to the Red, Green, Blue (RGB) colour channel needs to be elucidated to achieve an accurate interpretation of fish freshness.

The main goal of this study is to produce a sensory fish freshness detector based on fish skin by using image interpretation based on the Quality Index Method (QIM) scores. This sensory detector system will enable users to interpret data without having any contact with the samples. The first objective of this study is to determine the freshness of the fish samples using QIM assessment. Next, is to extract the RGB colour value from fish skin images, and finally to investigate the relationship between the QIM scores and RGB value for freshness indicator by using the Statistical Package for the Social Sciences (SPSS) software.

The findings in this study are hoping to make an impact on society by producing a sensory fish freshness system that can detect the freshness of fish accurately especially in commercial areas such as market, home, or anywhere. All consumers should get the advantages to detect the fish freshness without having any basic skills in the fishery and prevent any health problems from eating stale fish. Besides, this study promotes a hygienic method of sampling as it only requires fish images without having any contact with the fish. Therefore, consumers can check the condition of fish at the market in a short period of time.

2. Methodology

The study starts with storing all the samples collected in a proper storage method, followed by QIM assessments to determine fish freshness level and images acquisition for multicolour RGB extractions. The images were process and latter scrutinized to investigate the relationship between each and integrated colour RGB channel toward fish freshness level.

2.1 Fish Sampling, Storage and Icing

In this study, there were two types of fish used for sampling which were *Scomberoides commersonnianus* (Queenfish) and *Eleutheronema tetradactylum* (Threadfin). They are a major commercial species in the coastal regions of Malaysia as they supply globally most to the catch production (Griffiths *et al.,* 2006; Mahabubul *et al.,* 2012). For each species, ten samples were collected and labelled with tag from 1 to 10.

The fish samples were completely surrounded by crushed ice during storage. Crushed ice is preferred to larger pieces because it cools fish more rapidly. According to Shawyer & Pizzali (2003), it is preferred to use crushed ice for storage because this allows good contact with the fish. The fish should not be wrapped in paper or plastic because a layer of air will trap between the fish and ice as that situation can slow down the cooling process (Clucas & Johnson, 1990). This storage method requires the fish to be layered with ice in an insulated icebox is known as Bulking method (Huss, 1995). The method is chosen as it can be temporarily or permanently mounted on board the boat for transportation to the market. Firstly, the first layer of ice which was the lowest layer should be 5 cm of thickness. The ice should be layered between the fish and spread along the sides, top and bottom of the box. The samples were all covered with crushed ice at an average temperature of 0°C and left for three days. These steps were repeated on days 3, 6, 9, and 12.

2.2 QIM Assessment

The fish freshness was immediately evaluated by the Quality Index Method (QIM). Based on Shabani *et al.* (2019), QIM is a method that allows us to assess the fish freshness rapidly and accurately. Each assessment was carried out by 10 trained panels among students of Marine Technology. Fish samples were assessed by the panels for their sensory characteristics such as colour, appearance, texture, odour, and taste. There are few skin characteristics that correlate to the score that was used to demonstrate the level of degradation. Referring to Diler & Genc (2018), they have adapted four evaluations focusing on skin which are appearance, mucus, odour and texture. However, in this study odour was discounted as it later only considering the visible effect on fish skin from the images. Due to that the parameters which were evaluated for the skin are the appearance, texture, and mucous with a score ranging from 1 to 3 (Table 1), where 1 represents fresh, 2 is normal and 3 for spoil. The panels had to fill the form each day for 14 days continuously.

Table 1. Characteristics of Sensory Features in QIM Score Sheet

Feature	Characteristics	Score
Appearance	Bright, shining	1
	Slightly bright	2
	Dull	3
Texture	In rigor	1
	Firm, elastic	2
	Soft	3
Mucous	Clear, no clotting	1
	Milky, clotted	2
	Yellow, clotted	3

Adapted: Diler & Genc (2018)

2.3 RGB Evaluation and Image Processing

In order to obtain the RGB value, the sample images were captured between 30–40 cm by using a camera from the iPhone 8 smartphone equipped with a 12-megapixel and resolution of 750x1334 pixel built-in display in JPEG format. The RGB colour features of fish images were then extracted by using a processing software called ImageJ. The software can provide a reading of each R, G, and B colour channel from an image. The image was stored as an 8-bit integer providing a range of possible values from 0 to 255 via histogram. Usually, 0 is considered black while 255 is taken white. The raw images were cropped out to region on interest (ROI) and analysed by using histogram from the ImageJ. The ROI were set for fish skin, considering for the fish body from the area between fish operculum and pectoral fin up to the caudal peduncle (Table 2 & 3). In the cropped image, values such as mean, mode, standard deviation, and count of the RGB value were measured from the histogram.

3. Results and Discussions

The relationship between sensory feature and day of storage can be achieved through the average value of QIM scores to determine which fish species spoils faster. The QIM scheme is comprised of three sensory features that the panels analysed. In Figure 1, the score began to increase as the day started to increase as well. It was found that the Threadfin was easier to deteriorate as compared to the Queenfish. This can be seen where Threadfin started to deteriorate on the third day but Queenfish remained to be in fresh state until the fourth day. Overall, both graphs showed a clear upward trend in the number of scores obtained by appearance, texture and mucous. An increase in the number of scores means that each feature contributes to the process of degradation of the fish. By comparing the two graphs as in Figure 1, Threadfin tends to run a higher risk of becoming spoiled faster than Queenfish.

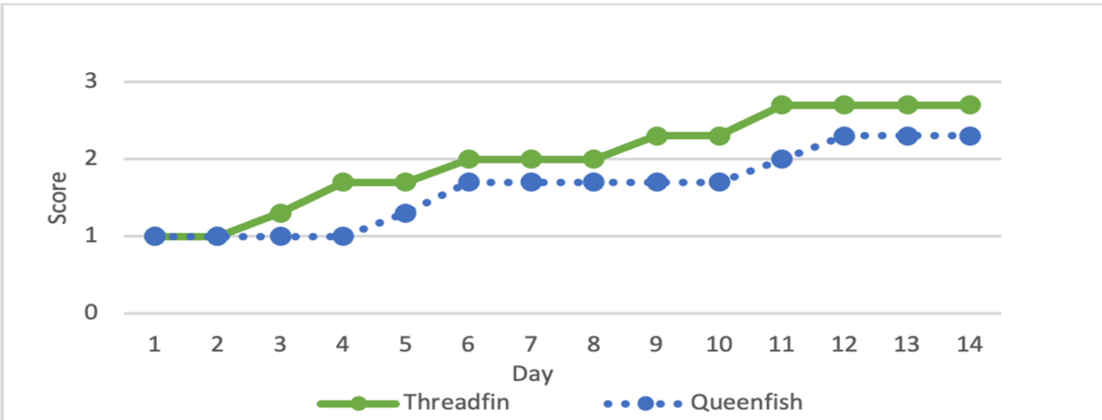

Figure 1. The Comparison between QIM Score against Day of Storage for The Threadfin and Queenfish Samples

3.1 Appearance

The most important characteristic of a fish is its freshness where it can be seen on the appearance of fish skin. Fish that is in extremely fresh state will not possess dull appearance. Lougovois & Kyrana (2005) state that a freshly caught fish has shiny and iridescent skin, which coated with a thin layer of mucous that is almost transparent and evenly spread. However, as the days increased, the appearance of the fish skin started to change from bright and shiny to dull. In this study, Threadfin and Queenfish got the dullest appearance at the end of the assessment.

Figure 2 shows the trendline of the appearance for Queenfish and Threadfin. Trendline graph displays trendline equation and R-squared (R^2) value. According to Cheusheva (2019), trendline equation is a formula that finds a line that best fits the data points while R^2 value measures the trendline reliability that is the closer R^2 is to 1, the better the trendline fits the data. In this study, r value represents the correlation between days of storage and QIM scores for both species. The r value for Queenfish and Threadfin are 0.966 and 0.965 respectively. This indicates that the correlation between days of ice storage and QIM score of Queenfish is higher compared to Threadfin and that means the trendline of Queenfish fits the data better due to the $R^2 = 0.9325$ is close to 1. Furthermore, the factor that contributes to the changes of appearance of fish skin should be temperature. Temperature influences the level of fish discoloration by affecting the susceptibility to autooxidation of fish myoglobin (Ashie *et al.,* 1996).

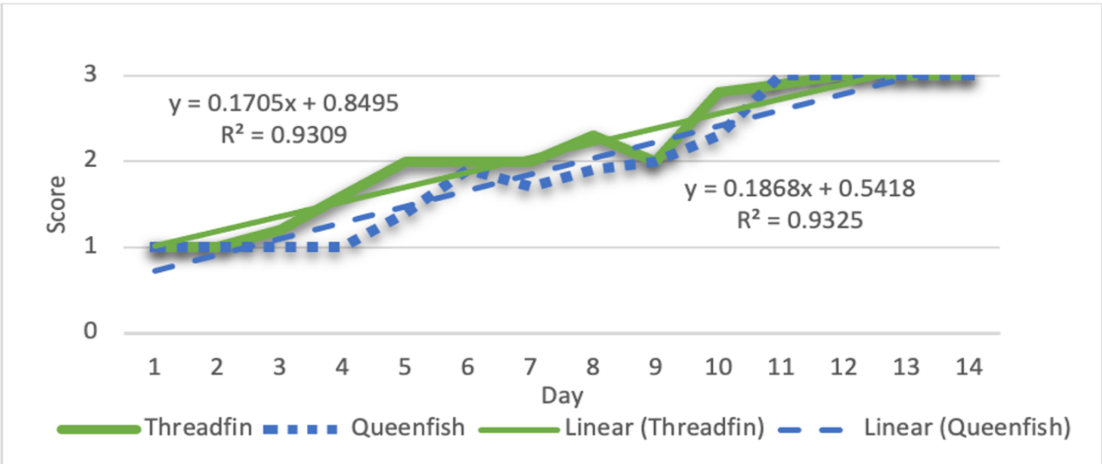

Figure 2. The Trendline of Appearance for Queenfish and Threadfin

3.2 Texture

Texture of the fish plays a major role in determining the level of fish freshness. In this study, a rigor texture indicates that the fish is in a fresh state. Nonetheless, at the end of assessment, both species had a soft texture. Based on Figure 3, the correlation between days of storage and QIM score of Queenfish is r = 0.964 which is higher than Threadfin that only possesses r = 0.946. As the appearance assessment, the trendline for texture fits the data better is Queenfish since the R^2 value is 0.93 that is nearer to 1. The factor that contributes to an increased rate of fish muscle softening is that the muscle tissue of fish undergoes faster spoilage than mammalian muscles (Masniyom, 2011). That is because the muscles of fish have less connective tissue compared to the mammals (Ashie *et al.,* 1996).

Besides, the degradation of collagen inside the intramuscular connective tissue leads to texture changes in the muscle (Masniyom, 2011). Fish has a fast resolution of rigor mortis compared to terrestrial animals (Lougovois & Kyrana, 2005). Temperature also affects the deterioration process by slowing down most biochemical activities at low temperatures and speeding them up at high temperatures. The biochemicals changes were attributed to denaturation of enzyme or protein at low temperatures (Ashie *et al.,* 1996). It has been shown that temperature has some effect on breaking the stress and rigor tension of muscle fibres.

This causes muscles to gap and ultimately decreases the rate of depletion of energy reserves at low temperatures, which will extend the pre-rigor duration. However, as the temperature rises, an increasing proportion of the muscle fibres gain enough energy to surpass the tissue's inherent strength, which thus break, resulting in tension (Ashie *et al.,* 1996).

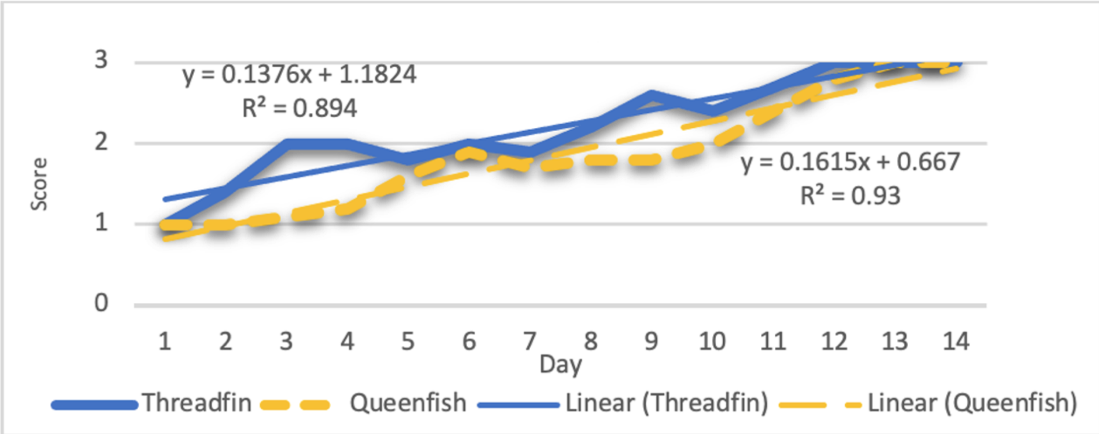

Figure 3. The Trendline of Texture for Queenfish and Threadfin

3.3 Mucous

A clear and no clotting mucous show that the fish is in a good condition. However, as the days go by, there were changes observed on the fish skin mucous. The mucous was still clear during the initial day but it was seen to have a major change on day 6. The mucous started becoming milky and coagulated on the skin. Threadfin experienced an obvious change on the mucous as it began becoming milky on day 6 and day 7 and turned to yellow on day 14. Whereas, Queenfish had no change on the mucous.

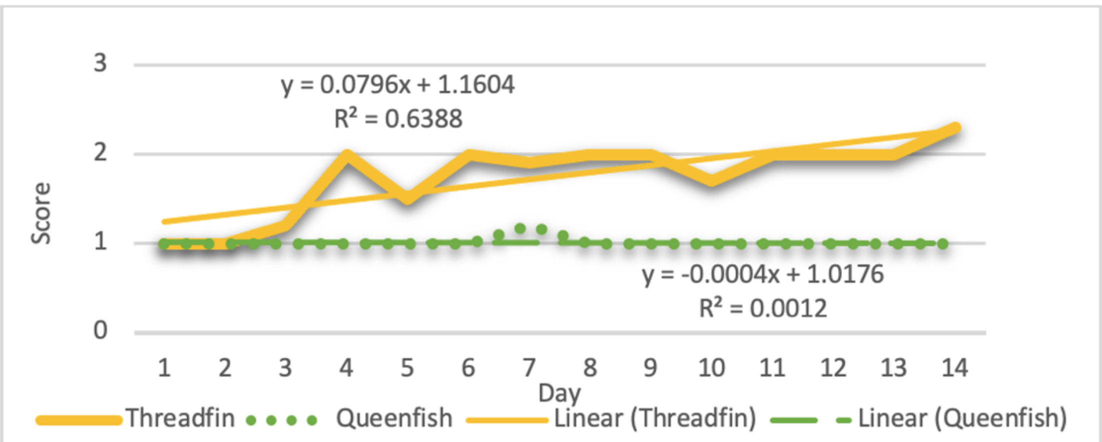

Figure 4. The Trendline of Mucous for Queenfish and Threadfin

Figure 4 shows the correlation between days of storage and QIM score of Queenfish and Threadfin samples which are r = 0.035 and r = 0.799 respectively. The correlation of Threadfin is higher compared to Queenfish. Besides, the R^2 value of Threadfin is 0.6388 which is closer to 1 and that leads the trendline to fit the data better. According to Esteban

(2012), fish skin mucous functions as a natural, physical, biochemical, dynamic, and semipermeable barrier allowing nutrients, water, gases and hormones exchange. The surface slime of freshly caught fish may contain substantial bacterial load. Such bacteria start attacking the flesh causing spoilage when the fish is dead and develop undesirable compound. In this study, the scales of Threadfin are covered by a mucous coating to protect them from fungus and external parasites.

3.4 Fish Deterioration

There are several factors that could affect the rate of spoilage in fish for example storage temperature, physical damage and intrinsic factors. Firstly, Shawyer & Pizzali (2003) state that physical damage can happen with poor handling of the fish. This is because the fish is soft and easily damaged, therefore poor handling results in bacterial contamination of fish meat and allows enzymes to be released, and this will accelerate the rate of spoilage.

Table 2. The Skin Changes in Queenfish Within 14 Days of Storage

Day 0		Day 1		Day 2	
Day 3		Day 4		Day 5	
Day 6		Day 7		Day 8	
Day 9		Day 10		Day 11	
Day 12		Day 13		Day 14	

In this study, Threadfin was observed to have more physical damages compared to Queenfish. Secondly, Threadfin have a higher fat content in the flesh compared to Queenfish and fatty species like Threadfin degrades faster than Queenfish which is a leaner species (Shawyer & Pizzali, 2003). Other than that, the thickness of the skin also brings an effect towards the level of spoilage in fish. According to Shawyer & Pizzali (2003), fish with a thin skin has a faster rate of spoilage than fish with a thick skin. In this study, Threadfin spoiled faster than Queenfish due to the thin skin of fatty pelagic fish. Thin skin makes it easier for enzymes and bacteria to penetrate faster, thus speeding up the spoilage rate of fish (Huss, 1995).

Table 3. The Skin Changes in Threadfin Within 14 Days of Storage

Day 0		Day 1		Day 2	
Day 3		Day 4		Day 5	
Day 6		Day 7		Day 8	
Day 9		Day 10		Day 11	
Day 12		Day 13		Day 14	

Next, the entire fish samples were observed to have a soft texture and belly rupture towards the final day which is day 14. According to Ghaly *et al.* (2010), autolytic enzymes reduced the consistency of texture during the early stages of deterioration. Hypoxanthine and Formaldehyde production results in extensive autolysis leading to meat softening, rupture of the belly wall and drainage from the blood water containing both protein and oil. Other than that, Proteolytic enzymes present in fish muscles after catch lead to post-mortem degradation of the muscle during storage and processing. The process of deterioration causes the fish to have a pungent and strong fishy odour at the end of assessment. The smell is due to odourless chemical known as Trimethylamine oxide that contains in the fish tissue. Then, the chemical is broken down into two new chemicals which are ammonia derivatives by the bacteria in the fish body that leads the fish to smell bad (Glass, 2003).

3.6 Evaluation of RGB Value

RGB refers to red, green and blue colour that can be combined together to produce various colours. The RGB value of an image ranging from 0 to 255, where 0 is black and 255 is white or full saturation. The mean for red, green and blue values were calculated and tabulated in a graph form. Based on Figure 5, the blue value of Queenfish tends to have the lowest mean compared to red and green values. The mean for blue value is in the range of 148 to 173 from day 1 until day 14. Meanwhile, the mean for red and green values ranges from 162 to 184 and from 161 to 185 respectively. Furthermore, the graph shows an upward trend in the mean value obtained for each day.

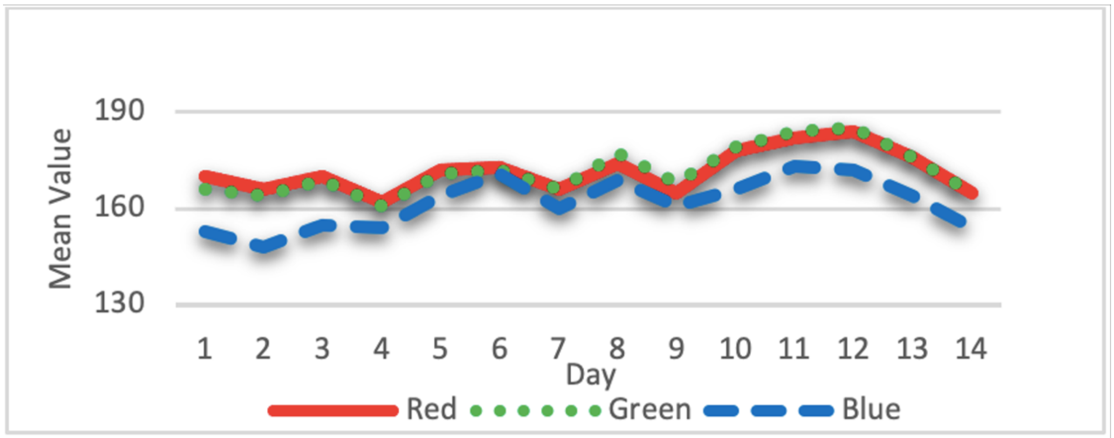

Figure 5. The Correlation between Mean Value and Day for Queenfish

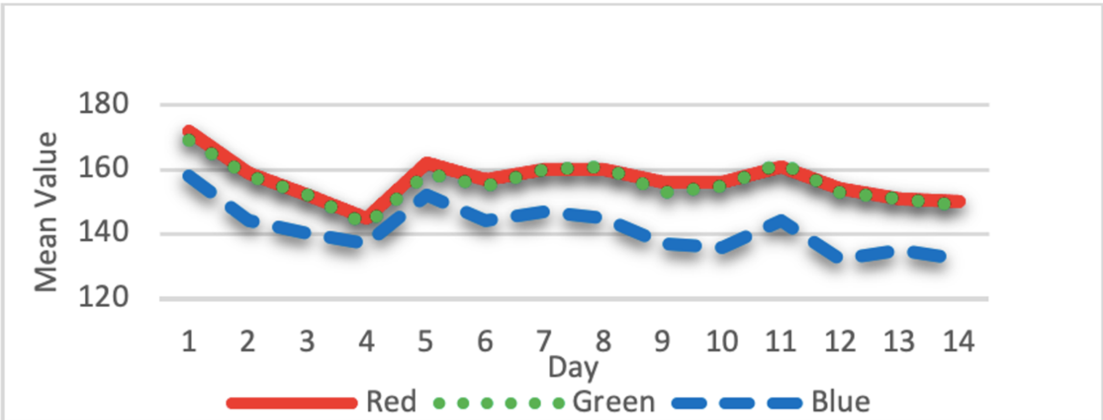

Figure 6. The Correlation between Mean Value and Day for Threadfin

Next, Figure 6 displays the correlation between the RGB mean values against day for Threadfin. The graph shows a downward trend in the mean value obtained from day 1 until day 14. The mean value of blue is the lowest among the three colours. The mean of red and green tend to have a quite similar value. The mean for blue value is in the range of 132 to 158 while the mean for red and green values ranges from 145 to 172 and 143 to 169 respectively. In contrast, the mean value of RGB for Queenfish is higher than the mean value of RGB for Threadfin. This indicates that the skin of Threadfin degrades faster compared to the skin of Queenfish as referred to the mean of blue value for Threadfin which is ranging from 132 to 158.

3.7 The Relationship between QIM Score and RGB Value

The relationship between QIM score and RGB value can be determined by the freshness condition of the entire fish samples during the period of 14 days. Based on the findings, Threadfin was found to be in a fresh state from day 1 until day 2 when the QIM score was at 1 and the mean of RGB value was in the range of 158 to 171 for red, 158 to 169 for green, and 143 to 157 for blue. From day 3 up to day 8, the QIM score was 2 and the mean of RGB value ranges from 145 to 163 for red, 143 to 161 for green, and 136 to 152 for blue. This indicates that the condition of Threadfin during the period was stale. Threadfin began to spoil from day 9 until day 14 due to the QIM score of 3 and the mean value for red, green and blue ranges from 149 to 161, 149 to 161 and 131 to 143 respectively.

Besides, Queenfish possessed QIM score of 1 and the mean of RGB value ranging from 162 to 170 for red, 160 to 168 for green, and 148 to 155 for blue from day 1 until day 4. This shows Queenfish was in a fresh condition. From day 5 until day 11, the condition of Queenfish was stale when the QIM score was at 2 and the mean of RGB value was in the range of 165 to 182 for red, 166 to 184 for green, and 159 to 172 for blue. Queenfish started to deteriorate when the QIM score reached 3 and the mean of RGB value ranging from 164 to 183 for red, 164 to 184 for blue, and 154 to 171 for blue. This condition happened on day 12 until day 14.

On top of that, the independent sample t-test was used to compare means of QIM score and RGB value for 14 days. The hypothesis was to test the significant differences between QIM score and RGB value towards the fish freshness level. The significant value for Queenfish is $p < 0.05$, thus it can be concluded that there is a significant difference between QIM score and RGB value towards the fish freshness level. Next, the significant value for Threadfin is also $p < 0.05$, therefore there is also significant difference between QIM score and RGB value towards the Threadfin fish freshness level.

4. Conclusion and Recommendations

In conclusion, the effects of temperature, environment, and storage method have been shown to play an important role in determining the rate of deterioration towards the quality and freshness level in Queenfish and Threadfin. From this study, a freshness indicator based on Quality Index Method (QIM) and integrating multicolour channel RGB value for Queenfish and Threadfin was created. Based on the QIM score, Threadfin was easier to deteriorate as compared to Queenfish from its leaner body type of fish properties. Queenfish possessed QIM score of 1 from day 1 until day 4, score of 2 from day 5 until day 11, and score of 3 from day 12 until day 14. Threadfin possessed QIM score of 1 from day 1 until day 2, score of 2 from day 3 until day 8, and score of 3 from day 9 until day 14. Other than that, based on the RGB value, the mean of blue value was the lowest among the three colours. The mean of RGB value obtained for Threadfin was in the range of 145 to 172 for red, 143 to 169 for green, and 132 to 158 for blue from day 1 until day 14. Whereas the mean of RGB value for Queenfish was ranging from 162 to 184 for red, 161 to 185 for green, and 148 to 173 for blue from day 1 to 14.

Threadfin is in a fresh state when it possesses QIM score of 1 with RGB values range between of 143 to 172. As deterioration progresses, the QIM score is at 3 and the RGB values are ranging from 132 to 161. While Queenfish is found to be in a fresh state when it acquires QIM score of 1 and the RGB values are in the range of 148 to 170. It starts to spoil when the QIM score is at 3 and the RGB values are ranging from 154 to 184. As the changes of integrating each RGB colour channel is considered strongly related and significantly different with every scores of QIM for both samples, the findings of this study could contribute to an extensive study for a new parameter and the development of devices that includes image analysis for fish freshness determination.

For future research, there are several types of ice can be used for storage instead of crushed ice such as tube ice and flake ice. Flake ice is considered to be the best option for chilling fish. This is because it will not cause the fish to bruise due to the less sharp edges of the ice. It also offers the fish surface to have a good contact with the ice (Kinnunen, 2016). Besides, its surface area is wide that allows it to hydrate and increase the moisture of the fish, providing rapid chilling. While for the RGB value which is used to measure the level of the fish freshness, it can be replaced by using other colour spaces. The recommended colour spaces that can be used are Hue, Saturation and Value (HSV) or Lightness of colour, redness vs greenness and yellowness vs blueness (L*a*b). A segmentation mask for a colour image will be determined by different colour space.

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