

## Development of intelligent packaging for real-time monitoring of the freshness of Canadian fish (tilapia and salmon) and pork during storage

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### Abstract

In the present work, the freshness of fish fillets and pork was monitored in real time with intelligent packaging that utilised pH-sensitive food dye strips as indicators of freshness. Strips were attached to the inner sections of transparent plastic lids where the meat samples were stored. pH-sensitive dyes interacted with compounds such as ammonia, dimethylamine, and trimethylamine, collectively referred to as total volatile basic nitrogen, which are released by a deteriorating meat sample into the headspace of the packaging. Deterioration of the Canadian-based pork samples was observed at room temperature (25°C), and all pH strips indicated colour change. For the fish sample, phenol red and bromocresol purple dye indicators were tested. The phenol red dye strip worked best as a colorimetric indicator for monitoring freshness. The phenol red dye strip changed from yellow to a more noticeable red colour when compared to bromocresol purple. For the pork sample, four dyes were compared: bromocresol green, phenol red, methyl red, and bromothymol blue. Bromocresol green was the most reactive of all the dye strips. To further validate the reactivity of the dye strips to deterioration, total viable counts and *Pseudomonas* spp. counts were determined. The results showed a positive correlation between microbial load and colour change in dye strips within a 60-h period. The total viable count ranged from log 7.59 - 9.8 CFU/g, while the *Pseudomonas* spp. count ranged from log 6.93 - 10.15 CFU/g. Overall, this method would be an inexpensive approach to food packaging that will benefit the meat industries for monitoring the shelf life of meat samples, thereby increasing consumer confidence.

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### Introduction

Meat products (pork, fish, poultry, beef, etc.) are highly perishable, whether wild-caught or farm-raised (Pacquit *et al.*, 2007; Kuswandi *et al.*, 2012; Ibrahim *et al.*, 2021). Processing must be performed under cold chain conditions to delay microbial growth and spoilage. Consequently, the packaging method is a key component in maintaining freshness and increasing the shelf life when preservatives are not added (Gram and Huss, 1996; Oehlenschlager, 1997; Byrne *et al.*, 2002; Crowley, 2005; Pacquit *et al.*, 2007). When meat samples arrive at retail stores or markets, proper handling is crucial; disruptions in the cold chain can significantly shorten their shelf life.

Purchasing decisions for food products and their perceived suitability for consumption are typically guided by the best-before date. However, this date alone does not guarantee product safety, as

it assumes that recommended storage conditions, such as proper temperature, have been consistently maintained. In addition, subjective factors such as product appearance and aroma also influence consumer decisions (Byrne *et al.*, 2002; Crowley, 2005; Pacquit *et al.*, 2007; Kuswandi *et al.*, 2012; Ibrahim *et al.*, 2021). The responsibility for assessing the quality and freshness of food rests on the consumer, who must determine whether the product meets acceptable standards (CFIA, 2019). However, this process can be challenging, as subjective evaluation may not always accurately reflect the product's actual condition (Shin and Hancer, 2016; Saleh and Lee, 2023). Therefore, alternative packaging is necessary, and must indicate whether the product is suitable for consumption regardless of the best-before date on the label.

To address this, advancements in scientific technologies, such as intelligent packaging, offer a

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promising solution. For example, packaging equipped with colour-changing pH indicator strips to monitor the condition of packaged food or the surrounding environment can provide real-time information on a product's freshness, and offer consumers a reliable, objective method for assessing quality (Gram and Huss, 1996; Oehlenschlager, 1997; EFSA, 2009; Kuswandi *et al.*, 2012). In contrast, conventional packaging only protects food from external factors (Kuswandi, *et al.*, 2012; Beshai *et al.*, 2020).

There are three types of intelligent packaging: the data carrier type, which contains barcodes providing all the information about the food item and the manufacturer when scanned by an electronic device; the sensor type, which expresses all the information about the food product through chips inserted in the packaging; and lastly, the indicator type, which indicates the real-time freshness of a food item through change of colour. The indicator type of intelligent packaging uses pH-sensitive strips that change colour to indicate either the freshness or the deterioration of food items (Beshai *et al.*, 2020; Dirpan *et al.*, 2022).

Ice and refrigeration generally allow for the extension of meat's storage time, transforming it into a viable product, both locally and internationally. However, it is challenging to maintain the ideal temperature for the optimal storage of meat samples in the handling and transport of fresh fish or pork to different locations. As a perishable food, meat is very sensitive to prolonged temperature changes, and will begin to decompose before the expiration date. When meat begins to deteriorate, an unpleasant and characteristic putrid or fishy aroma develops, and volatile amines begin to build up due to bacterial metabolism, commonly caused by *Pseudomonas* spp. and *Shewanella putrefaciens* (Pacquit *et al.*, 2007; Kuswandi *et al.*, 2012; Ibrahim *et al.*, 2021). An increase in *Pseudomonas* spp. activity results in increased alkalinity and the development of total volatile nitrogen content (TVB-N) in the head space of the sample holder or package (Suarez *et al.*, 2014; Guzmán *et al.*, 2015).

Currently, indicator-type intelligent packaging has either not reached the retail market or is not widely used. However, it is important to understand its dynamics, reaction time, and sensitivity to food dyes, as well as its wider impact on packaging technology, prior to scaling up the production and adoption of indicator-type intelligent packaging

## Materials and methods

### Materials

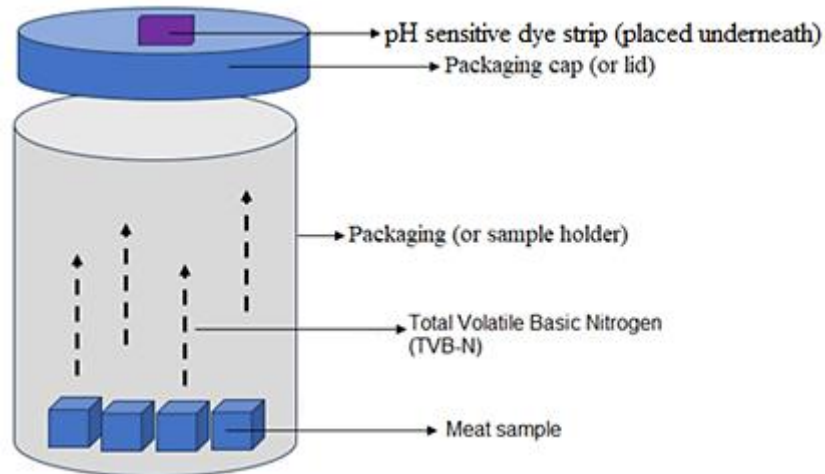
Salmon fillets, tilapia fillets, and pork samples were purchased from a local Metro supermarket in the Scarborough area of Toronto, Canada. All samples were placed in an ice container to maintain their temperature and freshness, and to prevent cross-contamination during transportation and storage in the laboratory before use. All dye samples were obtained from MilliporeSigma Ltd. (Canada).

### Preparation of packaging for fish samples

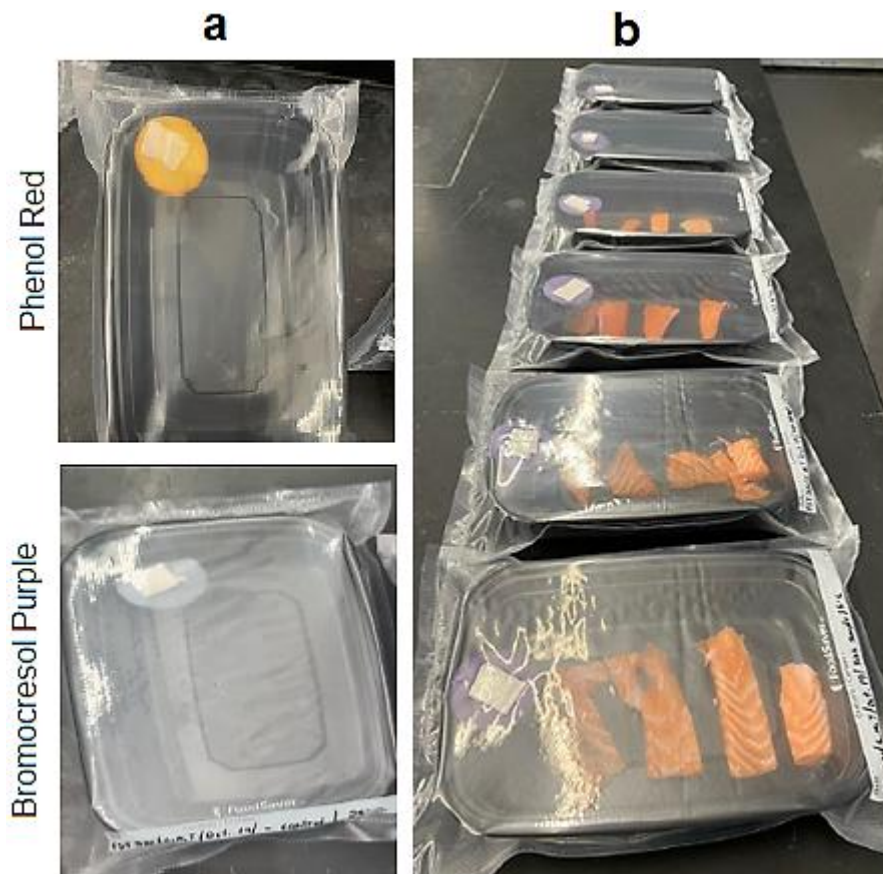
Thirty-two plastic containers were purchased from a local shop in Toronto, Canada to mimic the traditional packaging materials in which products are stored at supermarkets. Each sample was placed in a container with a transparent lid to allow for the attachment and visibility of pH dyes strips from inside the lid or plastic film seal (Figure 1). For each trial, two containers were left empty at 5 and 25°C as negative controls. Sample preparation and packaging were done under aseptic conditions. A total of 16 salmon pieces and 16 tilapia pieces (average 6 × 2 cm) were cut from their separate fillets (Figure 2). The plastic film was sealed using an Eocean Automatic packaging sealer machine (model number B09J28XJZL). Five packages, including one negative control, were placed in a refrigerator at 5°C, and the remaining five packages were placed in an incubator at 25°C for 7 d. For each trial, samples were prepared in triplicate, and stored for 7 d.

### Preparation of pH-sensitive dye strips for fish samples

The dye indicator was designed to monitor pH changes in the packaging environment as an indicator of the freshness of the fish samples. In this experiment, bromocresol purple dye and phenol red were selected. Bromocresol purple solution was created by dissolving 0.05 g of bromocresol purple powder, 0.92 mL of 0.1 M NaOH, and 20 mL of 95% ethanol in 80 mL of distilled water. For the phenol red solution, 0.1 g of phenol red was dissolved in 2.82 mL of 0.1 M NaOH, and 20 mL of ethanol 95% in 80 mL of distilled water. The solution was then boiled and cooled to room temperature (25°C) to create the final phenol red dye solution. Next, six round pieces of about 5 cm in diameter were cut from a coffee filter, and dipped in the bromocresol purple and phenol red solutions.



**Figure 1.** Schematic diagram of intelligent packaging.



**Figure 2.** Packaging. (a) Empty packages as negative controls. (b) Salmon fish fillets in the fabricated package (dye strip, bromocresol purple).

#### *Bromocresol purple dye and phenol red dye comparison*

Four pieces of the salmon and tilapia fillets were placed inside separate plastic containers. Each plastic container was placed inside a plastic bag. The dye strips were attached separately to each package. The plastic bag was sealed using an Eocean Automatic packaging sealer machine. Four packages,

including one negative control each for salmon and tilapia fillet, were placed in a refrigerator at 5 and at 25°C for 7 d.

#### *Fabrication of pH-sensitive dye strip for pork sample*

The pH-sensitive dye strips were fabricated as follows: (1) phenol red dye strip: 0.25 g of phenol red dye + 100 mL of 20% ethanol solution; (2) methyl red

dye strip: 0.25 g of methyl red dye + 100 mL of 20% ethanol solution; (3) bromothymol blue dye strip: 0.25 g of bromothymol blue dye + 100 mL of 20% ethanol solution; (4) bromocresol green dye strip: 0.25 g of bromocresol green dye + 100 mL of 20% ethanol solution; and (5) combination strip: mixture of all dyes in equal parts.

After the preparation of the dye solution, Whatman filter paper #4 was cut into small strips. One strip was dipped into each beaker containing dye solution overnight at room temperature. The strips were taken out of the solutions after 24 h, and air dried.

#### *Setting up pork spoilage trail*

Five glass bottles were sterilised at 135°C for 4 min. The pH-sensitive strips were glued with double-sided tape to the undersides of the lids of the glass bottles, and labelled as phenol red, methyl red, bromothymol blue, bromocresol green, and mixture. Further, a 5-g pork sample was placed into each glass bottle aseptically, and the lids were closed. Sample observations for colour changes in the pH-sensitive strips were made for up to 60 h at room temperature ( $25 \pm 2^\circ\text{C}$ ).

#### *Setting up negative control*

A negative control was important to demonstrate that colours changes in the pH-sensitive strips were due to TVB-N content only. Five glass bottles were sterilised and labelled with each dye name. The pH-sensitive strips were glued on the undersides of the lids of the sample carrier or packaging. No pork samples were placed inside the glass bottles. Observations for colour changes in the pH-sensitive strips were made for 60 h at room temperature ( $25^\circ\text{C}$ ).

#### *Microbial analysis*

Microbial analysis followed a similar technique to that described by Pacquit *et al.* (2007) and Lee and Shin (2019). Under aseptic conditions, 11 g of pork was mixed with 99 mL of 0.1% (w/w) sterile peptone water in stomacher bags. The sample was homogenised at room temperature for 4 min using an Interscience BagMixer® 400 paddle blender. The samples for the 6<sup>th</sup>- to the 24<sup>th</sup>-h observations were diluted to  $10^{-7}$ , while those for the 36<sup>th</sup>- to the 60<sup>th</sup>-h observations were diluted to  $10^{-9}$ . Two microbial analyses were carried out on the samples: total viable count (TVC) and *Pseudomonas*

count using pour plate and spread plate methods. All analyses were done in triplicate.

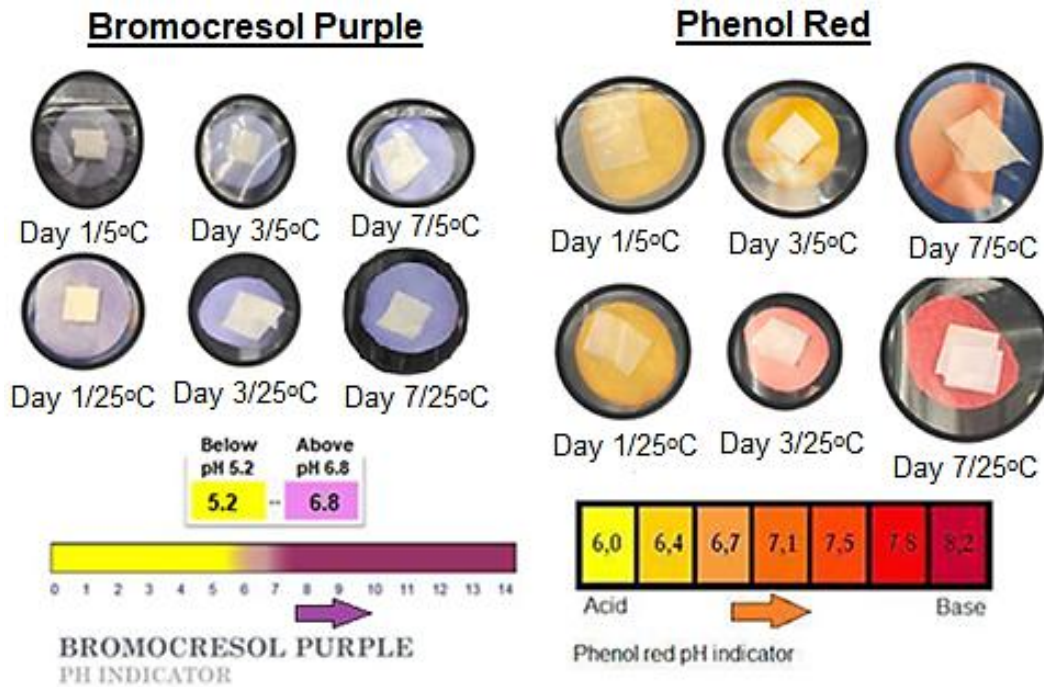
## **Results and discussion**

### *Salmon and tilapia fish fillet*

#### *Colour change in dye strip*

Colour changes in the dye strips or pH indicator stickers for samples stored at 5 and 25°C were observed and recorded on days 0, 3, and 7. The changes in colour for all samples are presented in Figures 3a and 3b. Bromocresol purple dye strips changed from a light purple tone to a darker purple tone with increased storage time and temperature. The pH of the packaging headspace on day 0 was 6.0 when compared with the dye strip pH indicator standard shown in Figure 3a, and later shifted toward the alkaline spectrum during the storage time. We noted no observable changes in the dye strips for all negative controls (that is, samples without fish samples) regardless of the type of dye and storage temperature. Colour changes in the dye strips were linked to the production of TVB-N in the packaging headspace.

Fish begin to deteriorate rapidly when exposed to danger-zone temperatures (5 - 60°C), which are favourable for rapid bacterial growth (Hammond *et al.*, 2015; Zhuang *et al.*, 2021). The present work compared the degree of susceptibility to spoilage under two different temperatures: refrigeration conditions (5°C) and room temperature (25°C). Samples were taken from wholesome fish samples, and desirable sensory properties, including smell, texture, and colour, were examined for all samples. When fresh, the fish samples were shiny, odourless, and had firm flesh. After two days in the incubator (25°C), an unpleasant odour was perceived with the flesh turning darker, confirming that elevated temperature had triggered fish spoilage (Wang *et al.*, 2021a; 2021b; Zhuang *et al.*, 2021). For each sample, changes in sensory properties were assessed, recorded, and compared to the assessment from the previous day using a degradation scale from 1 to 10, with 1 being the original sensory parameter when the sample was wholesome, and 10 being the parameter when the sample was spoiled. Volatile amines also build up gases in the headspace, and inflate the package. Dapkevicius (2000), Pacquit *et al.* (2007), and Sun *et al.* (2020) have similarly observed that packaging materials became bloated over the storage period as a result of gas production in the headspace.



**Figure 3.** (a) Colour change for bromocresol purple at 5 and 25°C over seven days for fish samples. (b) Colour change for phenol red at 5 and 25°C over seven days for fish samples.

After five days, our fish sample was completely spoiled with an unpleasant odour, and a complete change in the colour of the dye strip was observed and recorded.

The colour change in the dye indicates that the environmental conditions favoured the development of bacterial growth from which the volatile nitrogenous compounds were produced (Kuswandi *et al.*, 2012; Sun *et al.*, 2020; Ibrahim *et al.*, 2021). In fresh-aquaculture fish, ammonia produced by the deamination of free amino acids is the major volatile amine released, and linked to changes in pH inside the package (Gram and Huss, 1996; Dapkevicius, 2000; Byrne *et al.*, 2002; Sun *et al.*, 2020).

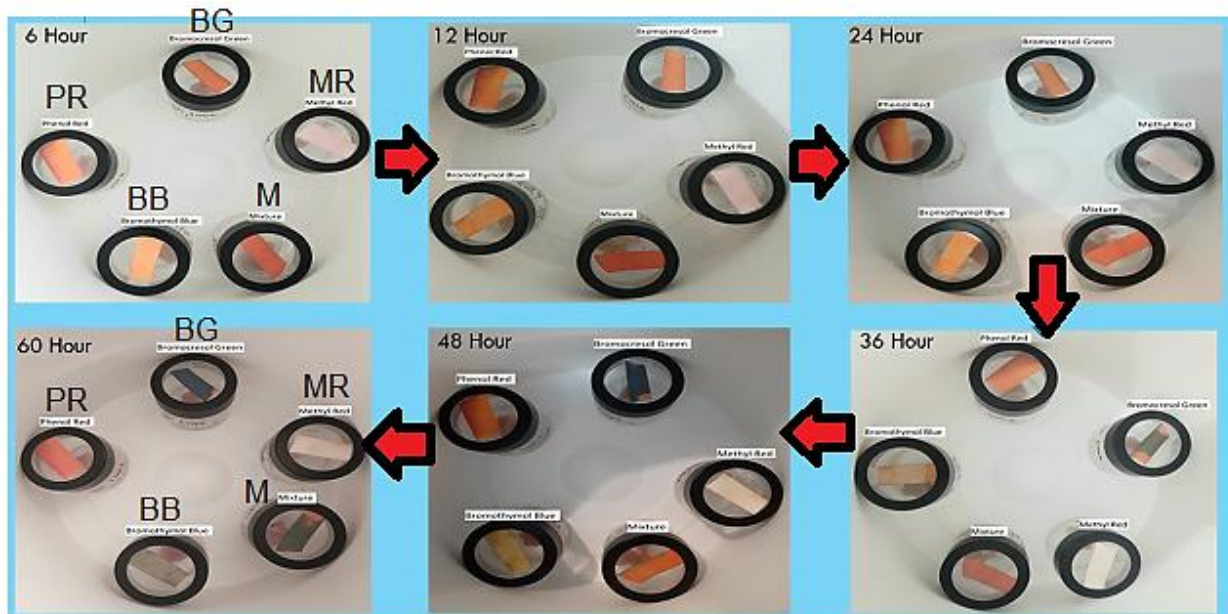
#### *Pork sample*

##### *Colour change in dye strip*

Colour changes in the dye strips due to deterioration during storage were observed at the following time intervals: 6, 12, 24, 36, 48, and 60 h. Over the period of observation, all the strips changed colour. No colour changes were observed in the dye strips for any negative controls, regardless of the type of dye and storage temperature. Again, the changing colours of the dye strips could be linked to the production of gas (TVB-N) in the packaging headspace. The dye strips changed from their initial lighter shades to darker shades of the same colour, or

a completely different colour, as observed for bromothymol blue, bromocresol green, and the mixture (Figure 4).

During the first six hours, no observable changes occurred in any of the five strips. Slight coloration began to appear between the 12<sup>th</sup> and 24<sup>th</sup> h; however, this did not become pronounced until the 36<sup>th</sup> h, as seen by the band formed in the packaging containing the bromocresol green strip (Figure 4). The dye strips on other packaging also showed colour changes at this hour, but the colour changes only became very evident at the 60<sup>th</sup> h. The final colour of each dye strip indicator is presented in Table 1. Lee and Shin (2019) reported a similar correlation between the colour changes of freshness indicators during storage at 20°C when beef samples were stored for 0, 12, 18, 24, 36, 48, 60, and 72 h. They reported the chromaticity values for L\*, a\*, and b\* to be 64.74 - 99.22, 47.70 - 3.50, and 50.39 - 8.19, respectively. Pacquit *et al.* (2006) also showed that sensor response correlated with the changes in TVC and *Pseudomonas* counts in the deterioration of three fish species (cod, cardinal, and roundnose grenadier), consistent with the results observed in the present work. It is also interesting to note that the pH dye strips were sensitive enough to change colour at the threshold of bacteria spoilage or food product rejection ( $10^7$  CFU/g).



**Figure 4.** Colour changes in indicator sensors during storage of pork samples at 25°C for phenol red (PR), methyl red (MR), bromothymol blue (BB), bromocresol green (BG), and mixture (M).

**Table 1.** Colour changes in pH-sensitive indicator strips.

Dye	Initial colour of strip	Final strip colour after 60 h
Phenol red	Yellow	Red-violet
Methyl red	Red	Yellow-orange
Bromothymol blue	Yellow	Blue
Bromocresol green	Yellow	Blue-green
Mixture of all dyes	Yellow	Violet

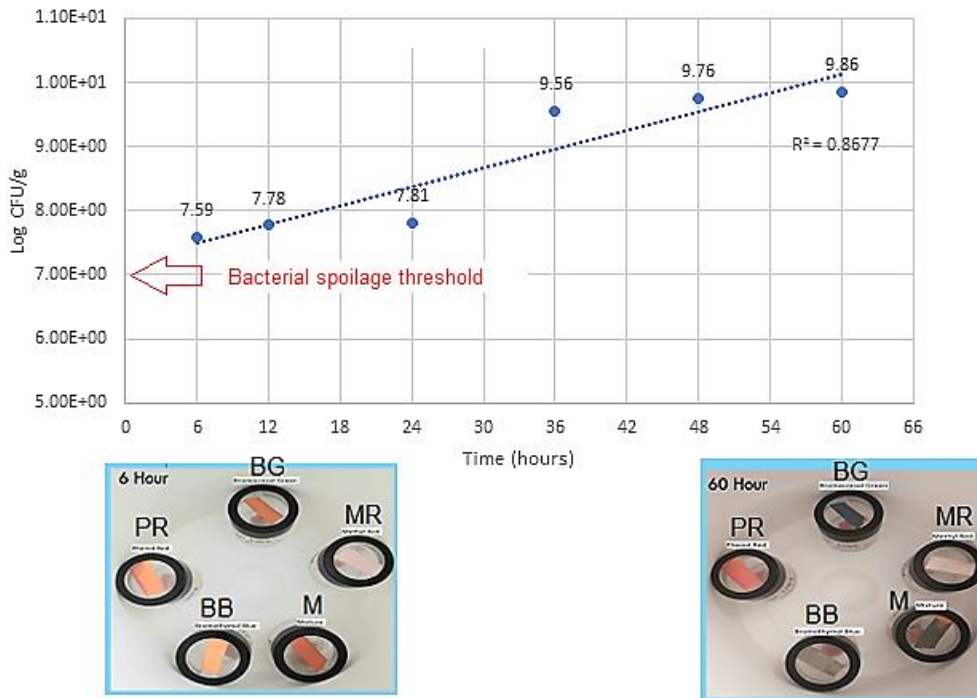
#### Microbial analysis of pork sample during storage

Freshness indicators are useful for determining the shelf life of meat samples by monitoring physicochemical changes and microbial safety (Pacquit *et al.*, 2006; Lee and Shin, 2019). Microbial analysis of pork samples was carried out in the present work to investigate the relationship between the colour change of the dye strip indicator and the spoilage of the pork samples during storage.

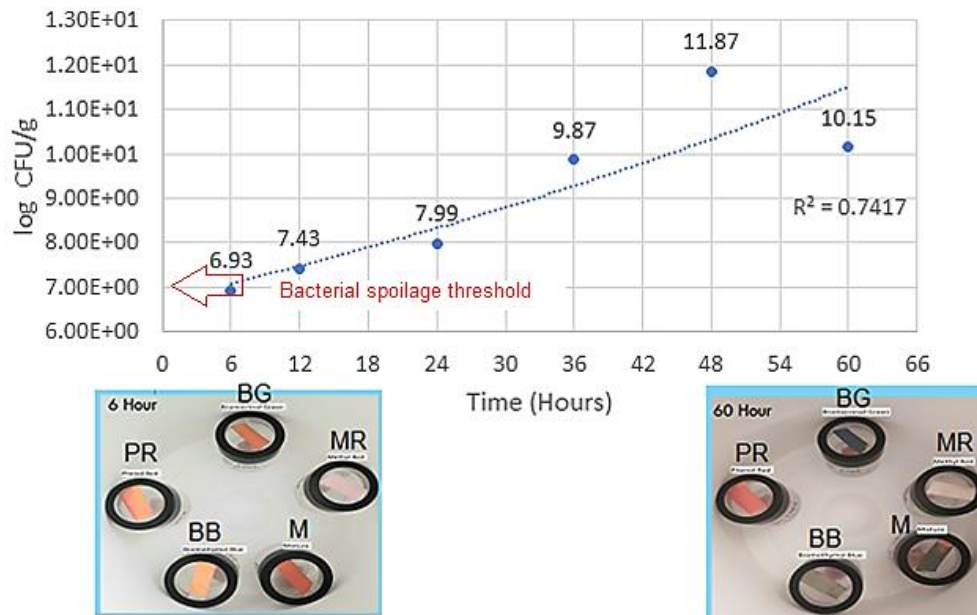
The microbial growth rate was stable in the first 24 h of storage, followed by exponential growth as concentrations exceeded  $\log 7.80$  CFU/g. At the 36<sup>th</sup> h, the *Pseudomonas* count exceeded the TVC, and remained elevated until the 60<sup>th</sup> h. The microbial counts from each of the triplicate plates were recorded and used to estimate the TVC and *Pseudomonas* spp. count for the deteriorating pork sample at the selected time intervals. The loads of the TVC and *Pseudomonas* spp. count (PC) are presented in Figures 5 and 6, respectively, as log colony forming units per gram (log CFU/g). The log count

increased for both TVC and *Pseudomonas* spp. count. From the 6<sup>th</sup> h, the average microbial count was over  $\log 7.00$  CFU/g. This pattern was consistent with the visual colour changes observed in Figure 3. El Barbri *et al.* (2008) and Shin *et al.* (2010) have reported similar patterns of microbial load exceeding  $\log 7.00$  CFU/g in deteriorating red meat and tofu samples, respectively.

Pacquit *et al.* (2006) reported a similar result in the monitoring of fish spoilage using a volatile amine sensor, that is, an initial slow microbial growth followed by a sudden rise in microbial load that plateaued around the 24<sup>th</sup>- to 26<sup>th</sup>-h mark in storage. In their study, the TVC and *Pseudomonas* spp. counts slowly increased from ca.  $10^4$  CFU/g during the initial 10 h, but then rose sharply before stabilising at 26 h at approximately  $10^8$  CFU/g. Initially, the *Pseudomonas* counts were approximately 65% of the TVC counts, and rose to approximately 100% at around 18 h.



**Figure 5.** Changes in the total viable count of pork samples at 25°C for phenol red (PR), methyl red (MR), bromothymol blue (BB), bromocresol green (BG), and mixture (M).



**Figure 6.** Changes in *Pseudomonas* count for pork samples at 25°C for phenol red (PR), methyl red (MR), bromothymol blue (BB), bromocresol green (BG), and mixture (M).

At close observation, the methyl red indicator dye strip would be difficult to use in practice as its colour was pale, and could be difficult to interpret. Overall, bromocresol green was the most effective and sensitive in terms of colour change compared to the other three dye strips, including the combination of the four dye strips. The fabricated pH-sensitive freshness indicator would find practical applications

for boosting consumer confidence, reducing waste, and enhancing consumer awareness—especially for people who struggle to read the fine print of “best-before” dates, such as the elderly, those with vision impairment, and those who do not understand the language written on the packaging—and for improving traceability systems.

## Conclusion

A cheap and easy-to-use method for monitoring the freshness of salmon and pork in real time during storage using intelligent packaging was designed. The dye strip indicator in the intelligent packaging reacted with the TVB-N released from deteriorating fish and pork samples. This finding demonstrated that the freshness of fish and pork samples could be monitored in real time using pH-sensitive indicators. The efficiency of the pH-sensitive indicators was also determined by comparing the intensity of dye strip colour changes and microbial loads at specific times.

For salmon and tilapia samples, the phenol red pH indicator worked best as a colorimetric indicator for fish freshness. However, for the pork sample, bromocresol green was the most effective. While bromocresol green was not tested for fish samples, the results from phenol red and bromocresol green in the pork samples showed that bromocresol green exhibited higher sensitivity to the volatile nitrogen compounds. Comparing all indicators tested for the fish and pork samples showed that bromocresol green gave the highest sensitivity to the TVB-N released from the deteriorating fish and pork samples, thus suggesting its suitability for large-scale industrial application. Furthermore, it is important that the packaging film be made of transparent material to ensure the visibility of the dye strip sticker through the package cap. The dye strip sticker must be placed in a location where it can easily react to the changing pH of the deteriorating meat sample. Additionally, it is recommended that a colour legend be placed alongside the dye strip sticker to help buyers understand and interpret what the different colours on the sticker represent, that is, the stages of deterioration.

The practical applications of this technology include monitoring freshness, preventing foodborne illnesses, and improving supply chain inefficiencies. pH-sensitive dyes are cheap, and the process of adhering them to packaging requires no complex technology or technique. Mass-producing food packaging with pH-sensitive dyes may become more visible within the meat processing sector. The use of pH-sensitive dye strips helps to boost consumer confidence. Intelligent packaging serves as an alternative to the “best-before” date while also providing real-time product information based on the pH level in the packaging headspace. Intelligent

packaging is proposed to provide continuous monitoring of fish and meat freshness throughout the food supply chain, and when the product is picked up at the grocery. While sensory properties such as colour or texture may remain unchanged for some foods as they deteriorate, intelligent packaging can track the biochemical changes in the food sample, thereby enabling buyers to make better decisions through the colour of the dye strip.

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