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Enhancing seafood freshness monitoring: Integrating color change of a food-safe on-package colorimetric sensor with mathematical models, microbiological, and chemical analyses

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ABSTRACT

The study assessed a developed food-safe on-package label as a real-time spoilage indicator for fish fillets. This colorimetric sensor is sensitive to Total Volatile Base Nitrogen (TVB-N) levels, providing a correct indication of fish freshness and spoilage. This study evaluates and predicts the shelf-life and effectiveness of an on-package colorimetric indicator. The sensor, using black rice (BC) dye with polyvinyl alcohol (PVOH), polyethylene glycol (PEG), and citric acid (CA) as binders and crosslinking agents, is applied to PET films. The food-safe pH indicator, prepared via lab-scale flexography printing, is durable in humid environments, making it suitable for practical packaging scenarios. The sensor visibly monitored fish spoilage at 4 °C for 9 days. Quality assessment included tracking ΔRGB (total color difference), chemical (TVB-N, pH), and microbiological analyses. Results indicate that the fish samples are fresh up to 4 days of storage at 4 °C; the total viable count (TVC), Pseudomonas growth, TVB-N contents and pH reached: 5.2 (log CFU/ml), 4.31(log CFU/ml), 26.22 (mg N/100 gr sample) and 7.48, respectively. Integrating colorimetric sensor data with mathematical modeling can predict spoilage trends over time. Integrated system offers a smart approach to accurately predicting shelf-life, aiding in optimizing storage conditions, minimizing food waste, and delivering fresh, high-quality fish products to consumers.

1. Introduction

Fish is a highly perishable food item, its shelf-life is influenced by various factors, including species, quality, initial microbial load, physical changes, storage temperature, and packaging conditions (Banja, 2002; Kuuliala et al., 2018/04; Bell, 2020). Spoilage can occur rapidly due to microbial activity, lipid oxidation, and autolysis, rendering the fish inedible (Banja, 2002; Gram and Huss, 1996). Microbial deterioration is the primary factor affecting the freshness of fish. Under specific storage conditions, specific spoilage organisms (SSOs) decompose various fish tissues, producing off-odors, off-flavors, and volatiles (Banja, 2002; Gram and Huss, 1996; in't Veld, 1996; Valero et al., 2012; Nychas et al., 2008; Ghasemi-Varnamkhasti et al., 2018). This microbial spoilage generates volatile bases such as ammonia (NH_3), dimethylamine (DMA), and trimethylamine (TMA), collectively known as total volatile basic nitrogen (TVB-N) (Das and Mishra, 2023; Rastiani et al., 2019; Miller et al., 2023). These compounds lead to a gradual increase in pH within the packaging headspace, detectable by pH-sensitive indicators to communicate food freshness levels (Kuswandi et al., 2014).

Intelligent packaging extends traditional packaging functionalities to meet consumer needs and expectations (Kuswandi et al., 2022a). For example, plant-derived pH-sensitive inks can be used in colorimetric indicators to monitor the freshness/spoilage of fish products, responding to TVB-N concentration increases during spoilage (Pacquit et al., 2007; Luo et al., 2023; Zhang et al., 2023; Almasi et al., 2022/06). Intelligent packaging use detection means, such as natural dyes, to monitor food freshness, microbiological growth, and chemical changes visually and/or quantitatively in real time (Mohammadian et al., 2020). Such strategies can offer a fast, non-invasive and, reliable food quality assessment (Alizadeh-Sani et al., 2020/11). However, many on-package sensors require direct food contact (Kuswandi et al., 2022b; Mills, 2005), raising concerns about the migration of substances from the indicator into the food, which poses safety risks (Moradi et al., 2019; Liu et al., 2021/06). Therefore, the chemical components of the coating must be

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safe and non-toxic (Moradi et al., 2019; Kaewprachu et al., 2022).

Recently, researchers have explored anthocyanins—natural dyes recognized as safe—to develop colorimetric pH indicators (Roy and Rhim, 2021; Singh et al., 2018). These compounds change colors at different pH levels due to molecular structure changes (Dikmetas et al., 2023). However, natural pH indicators may degrade over time, especially when exposed to the conditions typically found in food packaging (e.g., high humidity). This degradation can reduce the indicator's effectiveness and accuracy over the product's shelf-life (Liu et al., 2022). Producing natural pH indicators with consistent quality and performance can be challenging due to the variability in natural raw materials. This inconsistency can lead to variations in the performance of the indicators (Kuswandi and Nurfawaidi, 2017). The color changes of natural pH indicators can sometimes be subtle and difficult to perceive accurately with the naked eye. This can lead to misinterpretation of the spoilage status (Moradi et al., 2019).

High humidity in fish packaging poses difficulties for pH-sensitive dyes, as they readily migrate to moisture-laden food (Firouz et al., 2021). To address this issue, researchers have suggested crosslinking the polymeric matrix to maintain mechanical stability (De Dicastillo et al., 2016; Bellelli et al., 2018/12). Citric acid can serves a crosslinking agent to improve anthocyanins' water fastness, as they are prone to dye leaching in humid conditions (Ameri et al., 2024; Mulla et al., 2016; Ghaani et al., 2016), making pH indicator suitable for long-term use (Kassal et al., 2014). When citric acid reacts with the binder system, consisting of PVOH and PEG, water is produced through Fischer esterification (Joseph et al., 2005). The addition of the organic acids without successive thermal treatments has a mere plasticising effect, while their application with thermal treatment has a combined crosslinking and plasticising effect (Bellelli et al., 2018/12). Therefore, after thermal treatment of film indicators, their vulnerability towards the high humidity area could be decreased, addressing one of the commercialization challenges for pH indicators (Ameri et al., 2024).

Food quality can be monitored through periodic microbiological and chemical analysis (TVB-N and pH) within regular tests (Viswanathan and Radecki, 2008). Integrating these analyses with color change analysis, allows for shelf-life estimation(Chouhan et al., 2015; Fan et al., 2008; Prabhakar et al., 2020; Zhai et al., 2017; Jia et al., 2019; Khulal et al., 2017). The exact spoilage threshold can vary depending on several factors; including catch season, catch maturity, geographical origin, species, age and gender of the fish (Pacquit et al., 2007; Moradi et al., 2019; Chun et al., 2014). Huss(Huss, 1988) and European Commission (EC) (EC, 2005) reported acceptable limit value for TVB-N as 30–35 mg/100 g and 25–35 mg/100 g, respectively (Gram and Huss, 1996). TVB-N level of 35 mg/100 g fish flesh (Jinadasa, 2014; Özoğul and Özoğul, 2000) with a total viable count (TVC) of 7 log CFU/g, is considered the upper limit for fish shelf-life (Gram and Huss, 1996; Pacquit et al., 2006, 2007). These metrics guide the pH indicator scaling in this study to reflect the end of the fish samples' shelf-life.

Predictive microbiology anticipates SSO growth to determine the shelf-life of fish products. Specific spoilage bacteria development in fish results from environmental factors and microbial competition. In aerobically packed fish, competition occurs between aerobic gram-negative flora, primarily pseudomonads and Shewanella putrefacient (Koutsoumanis et al., 2000). Empirical models like the Gompertz, logistic, and modified Arrhenius models predict microbial growth kinetics and estimate fish shelf-life based on microbial counts. Kinetic models simulate TVB-N progression and protein degradation during storage at 5 °C (Prabhakar et al., 2021). However, mathematical models for shelf-life prediction in complex food environments like fish may lack accuracy due to factors affecting microbial development, including food structure and microorganism interaction (Koutsoumanis et al., 2006). This study combined model outcomes with the designed colorimetric sensor's color change response to address this issue. We evaluated the on-package labels for fish freshness monitoring by using microbial counts, chemical analyses (EC, 2005; Crowley et al., 2005) and color analysis. This paper builds upon that foundational work and further explores the application and performance of the sensor under varying conditions. In this study, Pangasius (Pangasius hypothalamus), a white-fleshed fish with a moderate flavor, was chosen as sample. Fatty fish, such as pangas, have emerged as a highly important aquaculture species economically due to their fast growth, year-round output, and high productivity (Chowdhury and Roy, 2020). The developed pH indicators (flexible PET films coated with natural dyes) serve as pH-responsive sensor, showing precise visual variations at different pH values. The design minimizes dye leaching and is sensitive to TVB-N gases, making it suitable for real-time seafood package monitoring.

2. Materials and methods

2.1. Materials

We bought pangasius fillets from a nearby Metro grocery store in Montreal, Quebec, Canada. Initially, the products were sealed in PVC-wrapped packages that displayed a label having the packaging date and the best before date (which was three days after the packaging date). The samples were placed in an ice container to keep their temperature and freshness during transportation. Prior to being stored in the laboratory fridge at a temperature of 4 °C, they were cleaned using 70% alcohol to eliminate any slime and sanitize the surface (Gram and Melchiorsen, 1996). The samples remained in the fridge until they were ready to be use. The Polytechnique Montreal in Montreal, Quebec, Canada, served as the site of all the experiments. Transparent polyethylene terephthalate films (PET, 23 μm) were obtained from ProAmpac Packaging Canada company, Terrebonne, Quebec, Canada. Black rice (BC) extract 80% was purchased from Xiherbs Phytochem Co., Ltd (Dongguan, Guangdong, China). Certified Amber glass jar with white polypropylene cap with bonded PTFE-faced silicone septa to meet EPA Performance Based Specifications for volatile organic analysis from Thermo Fisher Scientific, Saint-Laurent, Quebec. Polyvinyl alcohol (87–88% hydrolysis, 145,000 MW), Polyethylene glycol (PEG), Citric acid (ACS reagent, ≥ 99.5%), Vanillin (natural, ≥ 97%, FCC, FG), Hydrochloric acid (HCL), Potassium carbonate (K_2CO_3), Trichloroacetic acid (TCA, ACS reagent, ≥ 99.0%), Boric acid (H_3BO_3, ACS reagent, ≥ 99.5%), Sodium Chloride (NaCl), Dimethylamine solution (DMA, 2 M in methanol), Trimethylamine solution (TMA, 31–35 wt % in ethanol, 4.2 M), Ammonium hydroxide solution (NH_4OH, 30–33% NH_3 in H_2O), Conway diffusion cell, Vortex-Genie 2 (120 V) mixer, Agar and Pseudomonas Isolation Agar were purchased from Millipore Sigma Canada Ltd, Oakville, Ontario, Canada.

2.2. Sensor preparation

A comprehensive description of the formulated ink and sensor preparation were detailed using a previously reported method with some modifications (Ameri et al., 2024). Specifically, we described the colorimetric sensor's preparation method as follows: PVOH and deionized water were mixed in a glass beaker to create a heterogeneous mixture. A solution mixer made of PEG, C, BC and distilled water was added after the solution cooled at room temperature while being stirred constantly. The coating was conducted with a TQC automatic film applicator (model AB3652). The drying process of the films proceeded at two temperatures: ambient (30 min) and 165 °C (5 min). Samples were labeled NTT (no thermal treatment) and TT (Thermal treated pH indicators, at temperature of 165 °C for 5 min).

2.3. Characterization of pH indicator

2.3.1. Fourier-transform infrared spectroscopy (FTIR-ATR)

The FTIR spectra of the samples were recorded at room temperature using a PerkinElmer FT-IR spectrometer 65 coupled to an ATR accessory, with a diamond crystal (Waltham, MA, USA). The data was

collected using PerkinElmer spectrum. The spectra were acquired in the range 4000–600 cm^{-1}, with 16 accumulations and a resolution of 16 cm^{-1}. In our previous study, we examined the characterization of the pH indicator including FTIR characterization (Ameri et al., 2024). Moreover, a summary of this section is available in the supplementary materials (Fig. 1 S).

2.3.2. TGA

A TGA thermal thermogram shows sample mass change vs. temperature. Each compound has a unique thermogram that shows thermal stability, multi-component composition, decomposition kinetics, moisture, and volatile content (Saadatkhah et al., 2020). Thermogravimetric analysis (TGA) for prepared film indicators and raw material of ink formulation were conducted under a nitrogen atmosphere at a scan rate of 20 °C/min with a Shimadzu DTG-60H thermal analyzer.

2.3.3. Packaging study using RGB

To evaluate the sensitivity of developed pH indicator towards TVB-N gases originated from fish samples, 3 cm × 2 cm films were cut, and put inside the plastic bags and containers with fish samples. Before using these bags and containers, we exposed them to alcohol at 70% purity, and we let them become dry at room temperature for a couple of hours. Color change of the indicators was measured with an Epson Perfection V550 scanner (Nagano, Japan). The resolution of the pictures was 800 dpi with reflection mod. The scanning time was approximately 2 min, and the type of image was 24-bit color. Blacklight correction was also considered for adjustment. The test run for 3 different spots (pixels and considering their neighborhood) inside each picture and the mean and standard deviation calculated. Control samples were chosen as reference image for RGB calculation. The color change of pH indicators during storage period is measured by the total color difference (ΔRGB) according to the following equation (Dikmetas et al., 2023).

$$\Delta RGB = \sqrt{(R - R_0)^2 + (G - G_0)^2 + (B - B_0)^2} \quad (1)$$

Where R_0, B_0,G_0 are the initial color parameters of indicators and R, G and B were the values at the time of sampling.

2.3.4. Chemical stability and recovery tests

To simulate the long-term storage conditions of pH indicators in a shorter period, we used wide-mouth septa jars with a capacity of 60 ml, which are suitable for analyzing organic volatile compounds. We affixed pH indicator films to the plastic caps of these jars. The indicators were evaluated against external standards at various concentrations: 0.025, 0.125, 0.25, 0.5, 0.75, 1, 1.25, 1.5 (%wt./wt.) by diluting ammonium hydroxide solution, DMA and TMA. We prepared each standard by vigorously mixing them with a vortex for 20 second.

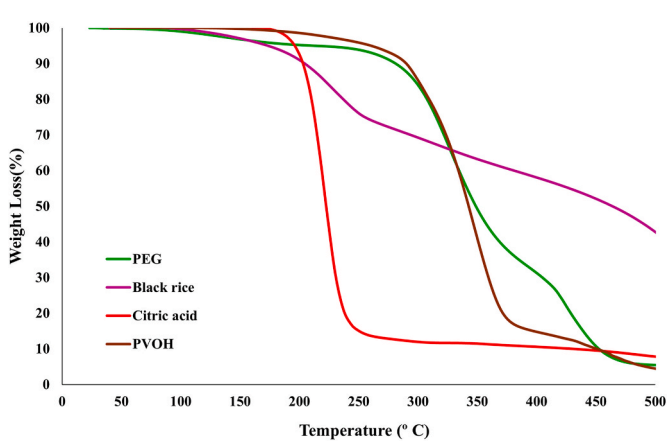

Fig. 1. TGA thermogram for each component of formulated ink as an ink sensor.

As these experiments required us to attach the indicator inside vials for exposure to the TVB-N gases and then removing them to take pictures, some displacement was unavoidable. The images for these experiments were captured using a smartphone (iPhone 12), as scanning was not feasible for certain samples.

2.4. Fish shelf-life monitoring

In an earlier study, we developed a colorimetric sensor and evaluated its ability to change color toward TVB-N gases. Also, we tailored the pH sensing properties of the developed pH indicator such as selectivity and sensitivity towards TVB-N gases and response time (Ameri et al., 2024). It was reported that the microbial population of pangas ranged from 6.213 ± 0.633 to 7.315 ± 0.570 log CFU/g in winter (Hasan et al., 2023). The Microbiological-and Chemical analysis for shelf-life studies were conducted on a Pangasius fish upon storage at around 4 °C during the winter of 2024 in Montreal, Quebec, Canada. The day of purchase was taken to be day 0 (the first day the fish was packaged at store for sale). The results were then compared with the color changing of a pH indicator and chemical analysis, which were conducted simultaneously.

2.4.1. TVB-N content

Conway micro diffusion method was used with some modification in order to quantify the amount of produced TVB-N during fish spoilage (Conway, 1947; Kim et al., 2023a). At every sampling point, portions of 5 ± 0.5 g of fish were added to 20 ml of TCA 4% (w/v) and homogenized for 1 min at high speed, then samples filtered with Whatman #1. One side of the exterior of the Conway dish was filled with 1 ml of the prepared sample, and the other side with 1 ml of 50 g K_2CO_3 in 100 ml distillated water. 100 μL of Conway indicator (methyl red), and 1 ml of 2% H_3BO_3 placed inside the inner side of the Conway dish. Some cautions in order to avoid failure to get accurate results were taken into account(Conway and Byrne, 1933), Eq (2):

$$TVB - N\left(\frac{mg}{100g}\right) = (V_S - V_B) \times N_{HCL} \times A_N \times \frac{\left[\left(W_S \times \frac{M}{100}\right) + V_E\right] \times 100}{W_S} \quad (2)$$

Where V_S = Titration volume of HCl for sample (ml), V_B = Titration volume of HCl for blank (ml), N_{HCL} = Normality of HCl, A_N = Atomic weight of nitrogen (14.00), W_S = weight of sample (g), M = Moisture content in sample (%), V_E = Volume of TCA used in extraction (ml).

2.4.2. pH measurement

A 5 g of fish sample were added to 90 ml of distillated water and then homogenized (by the vortex for 2 min). pH of suspension was measured by using the pH meter (Mettler Toledo, Mississauga, Ontario, Canada) after 5 min incubation at room Temperature (Lotfi et al., 2018). The pH meter was calibrated using the pH buffer solution (4.00 ± 0.01, 7.00 ± 0.01, 10.00 ± 0.01).

2.4.3. Microbial analysis

During fish storage at 4 °C, the total viable count (TVC) method revealed bacterial growth reaching the spoiling threshold (1×10^7 CFU/ml) (Karimi Alavijeh et al., 2024). Microbial analysis performed by weighted fish samples (10 ± 0.5g) which were placed in sterilized bags. The sterilized NaCl (9 %w/v) saline solution (90 ml) was added to bags and homogenized with vortex for 2 min at room temperature. Serial sample dilutions were made during the fish spoiling tests (Sobhan, 2021). For the enumeration the total microbial population, 100 μl of serial dilutions were spread on the surface of dried media Petri dishes and incubated (Economy Incubators IB-11E, 150 L, Biotech Inc, Montréal, Canada) the plates overnight at 37 °C (ISO,4833:2003) (I. n). Also, for the enumeration and Pseudomonas spp, 100 μl of serial dilutions

were spread on the surface of dried Pseudomonas Isolation Agar at 25 °C for 44h ± 4h (ISO, 13720:2010).

After the mentioned period for incubation, colonies on the agar plates were counted by a Darkfield Quebec Colony Counter. The results were expressed (according to ISO 7218:2007) as mean log CFU/ml ± standard deviation of 3 replicates.

2.5. Mathematical modeling of fish spoilage based on TVB-N, TVC and Pseudomonas spp experimental data

The models were derived from microbiological media observations and chemical experimental in a well-controlled lab. The data points of TVB-N and TVC of the current study were utilized to fit with established empirical models stated in the literature, as presented in Table 1.

As multiple parameters are necessary to construct a model for the shelf-life predication (Husain et al., 2016), we predict different parameters by R software. The models used for TVB-N prediction incorporate the following variables: TVB-N content (represented by Y), rate constant (k), and storage time (t in days). Y_0 is the TVB-N content at day 0, i.e., when the fish arrives to the storage. The employed microbial growth models for this study were the Modified Arrhenius logistics, logistic, modified logistic and modified Gompertz (López et al., 2004) which are described in Table 1; where: Y(t) is the TVC at time t, A is the upper asymptote of the curve, representing the maximum TVC, μ_{max} is maximal growth rate (in day $^{-1}$), the load capacity of biomass dynamics is stated by B, t_0 is the lag phase or lag time before TVC accumulation begins and t is the storage time.

2.5.1. Model evaluation metrics

Since the models used for predication of TVB-N and TVC behaviour are nonlinear; to discriminate the model performance they were discriminated based on the Akaike information criterion (AIC), and root mean square error (RMSE) as follows (Wang et al., 2020/06):

$$RMSE = \sqrt{1 \Big/ n \sum_{i=1}^{n} \left(Y_{i,e} - Y_{i,p}\right)^2} \tag{3}$$

$$AIC = n \ln\left(\frac{SS_r}{n}\right) + 2p \tag{4}$$

$$AICc = n \ln\left(\frac{SS_r}{n}\right) + 2(p+1) + 2(p+1)\left(\frac{p+2}{n-p}\right) \tag{5}$$

Table 1
Empirical models for modeling TVB-N, TVC and pseudomonas spp growth.

Model name	Model equation	Reference
Exponential model	$Y = Y_0 A e^{Kt}$	Prabhakar et al. (2021)
Exponential Polynomial	$Y = A e^{(Kt + Bt^2)}$	Current study
Modified Arrhenius (I)	$Y = Y_0 + K t \exp^{\left(\frac{D}{t+T}\right)}$	Current study
Modified Arrhenius (II)	$Y = Y_0 + K t \exp^{(Dt)}$	Current study
Howgate	$Y = \frac{Ymax - Ymin}{1 + e^{K(td - t)}}$	Howgate (2010)
Logistic	$Y = \frac{A}{1 + e^{-K(t-B)}}$	Peleg and Corradini (2011)
Modified Logistic	$Y = \frac{A}{1 + Be^{-Kt}}$	Peleg and Corradini (2011)
Modified Gompertz	$Y = Y_0 + \frac{A}{e^{e^{\left(\exp(1) \times \mu_{max} \frac{(t - t_0)}{A} + 1\right)}}}$	Giannuzzi et al. (1998)

$$BIC = n \ln\left(\frac{SS_r}{n}\right) + p \ln(p) \tag{6}$$

In this context, the experimental value of the ith experiment is denoted as $Y_{i,e}$, whereas the predicted value of the nth experiment determined by the model is represented as $Y_{i,p}$, n indicates the quantity of experimental data points, SS_r represents the residual sum of squares, and p signifies the number of parameters (Prabhakar et al., 2021). The selection of the best-fitting model among the models that were evaluated is based on the criteria of the lowest values of RMSE and AIC.

2.6. Statical analysis

All measurements were conducted three times. Data were analyzed with Tukey test at a significance level of $p < 0.005$, $p < 0.001$ and $p < 0.001$. The results are reported as the average value ± standard deviation (SD). The fitting parameters were found by using and R software version 4.4.3.

3. Results and discussion

3.1. Thermal properties: thermogravimetric analysis (TGA)

TGA was used to evaluate the thermal stability of anthocyanins and polymer components (Othman et al., 2011). Understanding thermal stability helps predict polymeric coating durability during processing or application (e.g., curing, printing or coating process) at different temperatures (Prime et al., 2009). As anthocyanins are sensitive to heat, TGA measurements can be informative in order to avoid dye degradation during thermal treatment process (Romero and Bakker, 2000). Fig. 1 depicts the thermal decomposition results for ink formulation components and Fig. 2 depicts thermal decomposition results for colorimetric sensors. For all of samples from 40 to 110 °C, some water desorption and evaporation were observed, consistence with literature (Othman et al., 2011; Prime et al., 2009; Corazzari et al., 2015). BC anthocyanin decomposed initially at 100 °C and 125–200 °C, respectively, due to the small amount of absorbed and bound water (Kong et al., 2023). The initial decomposition of citric acid was observed at 200 °C, and the end of decomposition of citric acid with a weight loss (86%) was above 250–300 °C. This weight loss could be due to the decomposition of citric acid (Ofori et al., 2015; Silva et al., 2008).

During the initial region (below 200 °C in this study), a small amount (approximately 1.5 %) of the mass of PVOH is lost as adsorbed water evaporates (Rowe et al., 2016). The TGA graph demonstrates that the sample of PET coated with INK I, subjected to a thermal treatment at 165 °C for 5 min (INK I TT), the PET coated with INK I without thermal treatment (INK I NTT), and the uncoated PET films, all experience weight losses of approximately 94.45%, 98.20%, and 99.62%, respectively, at a temperature of 165 °C.

During the thermal treatment of colorimetric sensors, approximately 3.75% of the degradation can be attributed to this procedure. At this temperature, the weight of BC anthocyanins, PEG, PVOH, CA, and dried ink (with anthocyanins in the coating) decreases by about 95.78%, 96.18%, 97.95%, 99.63%, and 97.30%, respectively. This weight loses may include partial degradation of the dye molecules, loss of functional groups, or minor alterations in the chemical structure. Temperatures higher than 227 °C are the onset temperature of decomposition for formulated ink, as the weight loss reaches approximately 95% and the coating starts to break down chemically. Hence, the thermal decomposition associated with the dried formulated ink, PET films, and thermal treatment procedure is insignificant, resulting in only approximately 5% at a temperature of 165 °C, and the developed pH indicators can be considered thermally stable over the thermal treatment process. Aggressive thermal treatment (temperature and time) can cause the indicator to degrade sooner compared to the sample without thermal

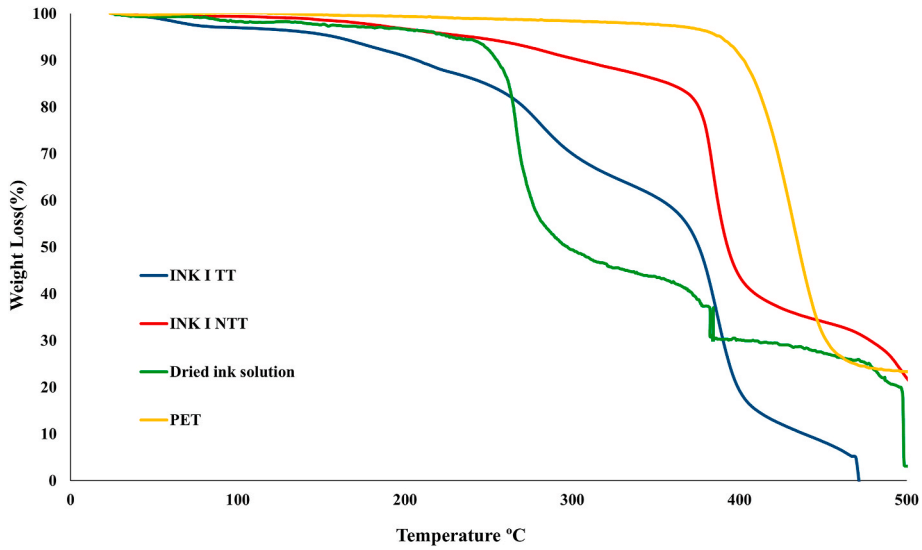

Fig. 2. TGA thermogram for the dried ink solution (at room temperature) on the Petri dish, developed pH indicators that were thermally treated at 165 °C for 5 min (INK I TT) and those that were not heated (INK I NTT).

treatment (Ekici et al., 2014).

A significant limitation in our method for assessing the process stability of thermally treated pH indicators using TGA analysis was the restricted scope of our sample analysis. Specifically, we analyzed two samples: the INK I TT pH indicator, which had undergone 5 min of thermal treatment at 165 °C, and the INK I NTT pH indicator, which had not undergone any thermal treatment. The ideal scenario for evaluating process stability would have involved subjecting the INK I NTT samples to the same thermal treatment protocol—holding them for 5 min at 165 °C—rather than using a thermal heating ramp. This approach would have provided a more accurate comparison by ensuring that both sets of samples underwent identical thermal conditions. Consequently, our analysis was limited to speculative comparisons of weight loss between the thermally treated and untreated samples. This limitation restricts our ability to definitively determine the extent of thermal degradation, as the differences in the thermal profiles of the samples could influence the observed weight loss.

3.2. Application of developed pH-responsive indicators for fish freshness/spoilage monitoring

To ensure that the dyes in the films exhibit visual reactions to changes in TVB-N, it is essential for the dyes to retain their pH-responsive properties by maintaining their protonation and deprotonation states unchanged when exposed to varying pH levels.

3.2.1. Effects of pH conditions and packaging parameters

Most of current pH indicators for monitoring seafood products is the acidic nature of the coatings employed in their production (Khezerlou et al., 2023). Selecting an alternative pH for formulated ink was to assess the potential pH adjustment, which can improve the pH indicator's performance, stability, and compatibility and make it more sensitive to the target pH range (Liu et al., 2021/06; Kaewprachu et al., 2022). This allows for more precise and noticeable color changes at the desired pH levels, which is critical for food packaging spoilage and freshness monitoring (Kaewprachu et al., 2022; Liu et al., 2022).

Fig. 3 shows that three different pH conditions (2, 7.5 and 9) that were examined to determine the impact of the pH level of the formulated ink on the color shift of colorimetric films. The amount of fish trials and package size were (5 ± 0.5 gr) and 19 cm × 17.7 cm, respectively. Fish samples were stored under controlled conditions and monitored using pH indicators with different ink pH coatings. It was observed that pH conditions ranging from 4.5 to 6.5 led to phenomena such as aggregation or precipitation. This can result in a thicker, more gel-like

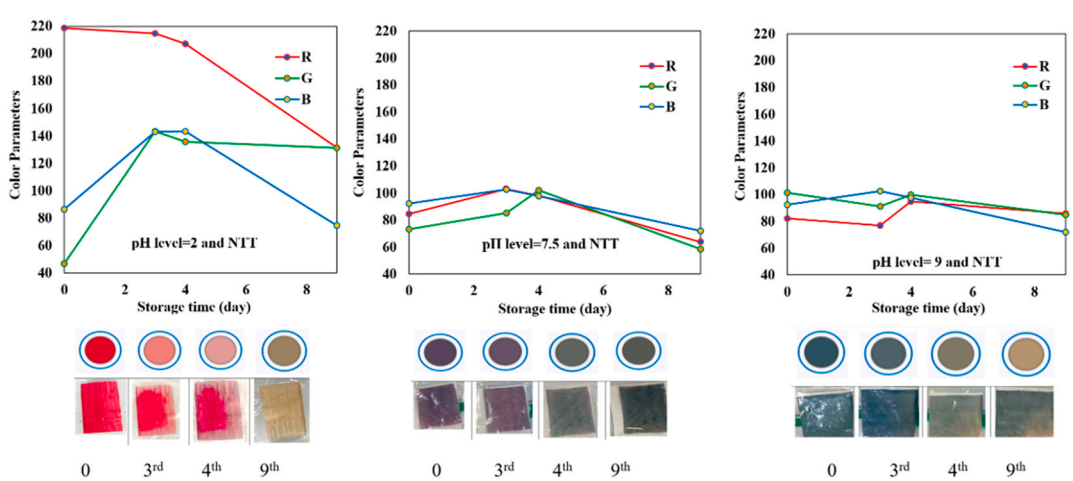

Fig. 3. The color parameters, sensitivity and color change outcomes for NTT pH indicators (package headspace) at different pH level for their coatings, were stored at 4 °C over 9 days.

consistency, as seen in this image. This phenomena can be attributed to the proximity of these pH levels to the isoelectric point of the components in the ink formulation(Mulla et al., 2016; Gratson and Lewis, 2005) and differences in the solubility of components of the formulated ink at the different pH level. This behavior is significant in various applications, including ink formulation, where achieving the right balance of charge is crucial for stability and performance.

Based on the observation, none of the pH indicators were activated on day 0, showing that the fish samples were fresh. The indicator's color changed over 3 days, when it was exposed to a solution ink (INK I) adjusted to pH of 2. The pH indicators with a higher pH (pH = 7.5 and 9) were also triggered on the fourth day. This phenomenon may be due to black rice anthocyanin and PVOH, in the ink formulation, which becomes protonated at lower pH and deprotonated at high pH (Heredia et al., 1998; Borkowski et al., 2005). As TVB-N gases accumulate in the headspace, the acidic groups in the ink formulation begin to deprotonate, leading to color shifts (Liu et al., 2004/08). Moreover, Anthocyanin molecules exist in the flavylium form at low pH values. However, when the pH exceeds 7, the anthocyanins undergo degradation, which is influenced by their substituent groups, and they transform into the carbinol pseudo-base form (Castañeda-Ovando et al., 2009/04). Therefore, among the tested indicators, only the one with an acidic coating (pH 2) showed a range of color changes. Also, the sensitivity of this pH indicator can be comparable with a pH indicator at pH level 7.5; however, only for low pH levels, the R parameters decreased, and the G parameters increased, indicating its potential as a reliable tool for monitoring fish spoilage in the early stages. The neutral and alkaline pH indicators did not show significant color changes during initial storage time.

For assessing the fish quality during other parallel trials different fish sample size and different available headspace, or air volume (zipper seal package: 19 cm × 17.7 cm and container: 250 ml) were considered. The sensors effectively monitor spoilage over time, with a clear progression in color change corresponding to the spoilage process. The ΔRGB values show a generally linear increase until day 4. Then after it followed by variations in peak and decline suggests predictable spoilage progression in the initial stages. Variations of color response in later stages, after day 4, indicate the complexity of spoilage dynamics and possible sensor saturation. Samples labeled NTT, consistently show higher ΔRGB values compared to those with thermal treatment. This can suggest that how different weight of samples can affect spoilage rates. For example, 100-NTT (Green line) spoils faster and more visibly than 30-TT (Yellow line), as indicated by the higher ΔRGB values. Furthermore, it was reported that a larger sample size (in terms of fish weight) linked with TVB-N emission causes color change to occur sooner, whereas increasing headspace has no significant impact (Alamdari et al., 2021) (Supplementary material). The color shifting also begins from the side closest to the fish product due to the diffusion of basic gases, whose contact with the indicator's surface occurs sooner.

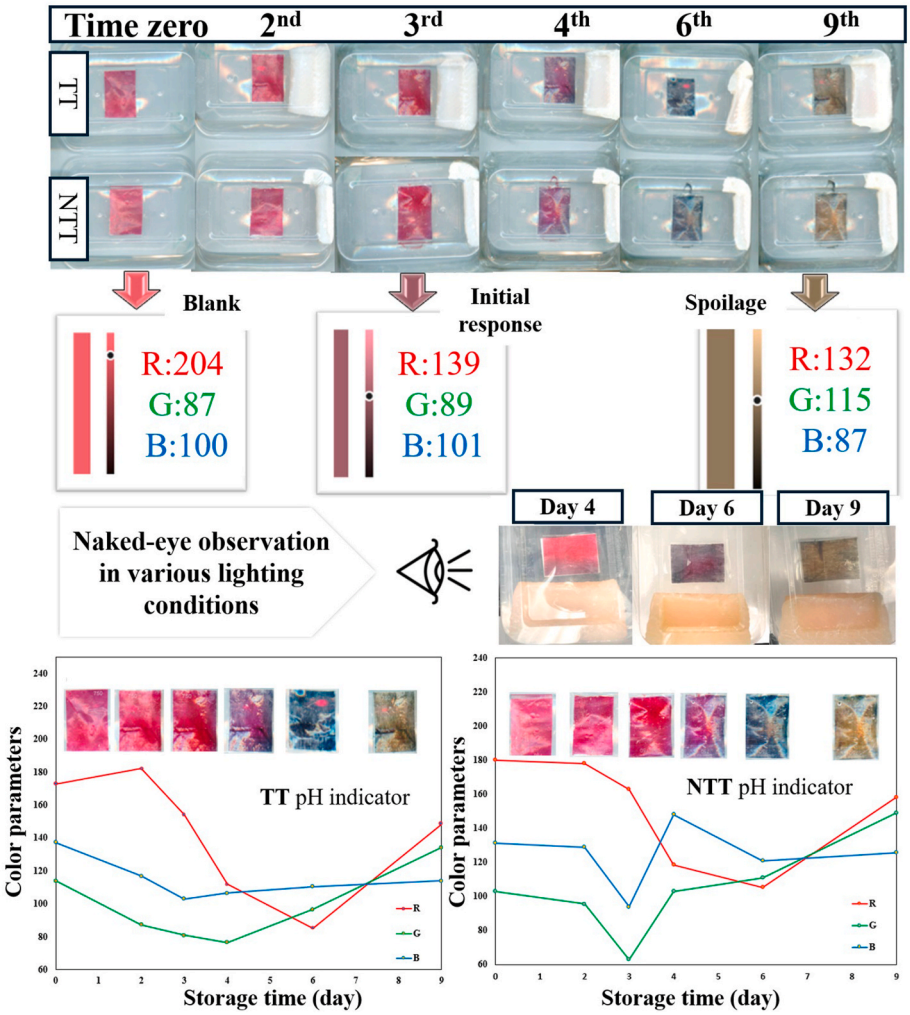

Fig. 4. Illustrates the color change outcomes for pH indicators (container headspace) were stored at 4 °C over 9 days. NTT; colorimetric films without heat treatment. TT; colorimetric films with exposure to heat (165 °C) for specific time (5 min). Fish condition at day 4: acceptable; day 6: marginal; day 9: spoiled.

3.2.2. Packaging study and pH indicator stability in fish packages

The pH indicator was assessed by simultaneous packaging trials including Pangasius fish, alongside microbiological and chemical tests. The studies were conducted throughout the winter season. The amount of fish trials and container size were (50 ± 0.5 gr) and 250 ml, respectively. To assess the impact of thermal treatment on packaging application, we used thermally treated fabricated pH indicators with a pH level of 2. These indicators used to assess their response time (in terms of color change) and their ability to still be intact upon contact with the headspace of fish packaging.

As Fig. 4 shows, the color change outcomes for pH indicators (container headspace) with pH level of 2, were stored at 4 °C over 9 days. The pH indicator showed color changes with four-point freshness scale, from red (fresh) to red/purple (acceptable), bluish/green (moderate spoilage/marginal), and yellowish-brown (high spoilage/spoiled). Specifically, color change observations indicated that from days 0–3, the indicator displayed a red color, denoting pristine condition with no spoilage. On days 4–5, the color shifted to red/purple, indicating minimal signs of spoilage while still being safe to eat. By days 6–7, the bluish/green color indicated moderate spoilage, suggesting it may not be safe for all consumers, and by day 9, the color intensified to yellowish-brown, clearly indicating spoilage and resulting in the fish being unsafe to eat. The pH indicator effectively differentiated between the freshness levels of fish samples throughout the 9-day storage period, thus serving as a scalar indicator that provides a gradation of color changes corresponding to varying levels of freshness, surpassing a simple binary outcome. This scale offers a clear, reproducible method for categorizing fish freshness, with valuable implications for quality control and consumer safety.

Moreover, the thermal treatment process showed in Fig. 4 preserves the pH indicators from being dissolved by water, while also causing a minor delay in their response time. This is due to a slight degradation that affects the active sites, which are specific chemical groups that can interact with hydrogen ions or hydroxide ions and are responsible for the acid-base reactions (Patras et al., 2010). Consequently, with fewer active sites available, the pH indicator may not respond immediately to changes in pH, leading to a lag time for the active sites to sufficiently interact with ions in the solution to produce a noticeable color change (Loypimai et al., 2016). This may also compromise the sensitivity of the pH indicator, requiring a more substantial change in pH to achieve the same degree of color change as in untreated samples. During fish storage, the R parameter decreased. A match for color changing could be defined for the final application. For this study, the ratio of $\Delta RGB_{NTT}/\Delta RGB_{TT}$ for the sample headspace were 1.07 ± 0.23. To assess the indicator's stability, all samples were kept under storage conditions for over a month. During this period, there were no signs of dye leaching, color fading, or any alterations in response to the basic environment of the headspace of thermally treated pH indicators. Moreover, removing the headspace from the container caused the colorimetric sensor to revert to its original color before exposure to an alkaline environment. This is the moment that the sensor will cease functioning.

3.2.3. Chemical stability and recovery tests

To ensure the reliable performance of pH indicators, stability tests were performed under varying environmental conditions, specifically temperature and humidity (Bell, 2020). We decided to stress tested indicators by exposing to elevated temperatures and humidity which can speedup the reaction kinetics and result in the formation of degradants not typically observed in real-time stability assessments (Ebrahim et al., 2020).By analyzing RGB values at varying concentrations and conditions, we could potentially differentiate different stages of spoilage, from initial changes (red to pink/purple) to those indicative of advanced spoilage (pink/purple to blue), emphasizing the importance of both chemical stability and environmental controls.

The indicators underwent testing at two temperatures (40 and 60 °C) across a storage duration of 30 min and 1 h. It was observed that when the samples were soaked at 60 °C for 1 h, nearly all of them exhibited a complete color change indicating saturation (data not shown). Furthermore, it can generate responses that may not occur naturally in food products, resulting in a false aging mechanism (Muniandy et al., 2023/11). Consequently, we preserved the samples at the same temperature for a duration of 30 min and then observed a gradual alteration in color. We repeated the same procedure for T = 40 as well (supplementary material).

Fig. 5, Fig. 6, and Fig. 7 show the RGB value changes observed across different TVB-N gases suggest varying sensitivities among the color channels. Notably, the red channel showed substantial decreases, indicating high sensitivity to color changes. In contrast, the green and blue channels displayed different reaction patterns, with green first increasing and then sharply dropping, while blue consistently decreased as concentration rose. Post-exposure recovery tests investigated the indicators' ability to revert to their initial color following exposure to alkaline environments (pH > 10). Indicators generally reverted to their original color under controlled conditions (lower pH) because of destabilization of the anthocyanin molecule (Etxabide et al., 2021/03; Kennedy and Waterhouse, 2000/01). We observed the variability to return to the original color. The interaction profiles with pH may differ slightly due to the impact of these volatile amines on pH levels. In the current recovery tests, it was observed that exposure to high concentrations of ammonia resulted in irreversible changes due to alterations in the anthocyanin structure.

In Fig. 8, for samples which exposed to the mixture of standards, it seems that the reversibility to the original control sample decreased. In mixed base exposures, NH_3 was identified as the primary contributor to the lack of reversibility. According to literature, anthocyanins can change color from red to orange at temperatures up to 40 °C (Enaru et al., 2021). As we exposed the pH indicators to each base (NH_3, DMA, TMA) individually.

Fig. 9 displays the responses of the thermally treated pH indicators to controlled mixtures of NH_3, DMA, and TMA that were stored in a relative humidity (RH) of 55% for 30 min at 40 °C temperature. At 55% RH, the moisture level is sufficient to ensure that the pH indicators stay sensitive to spoilage gases without being overly saturated, which can lead to false readings. Moreover, this RH level helps keep a balance, making the color change more accurately representative of the spoilage status. According to observations in this storage condition, assorted color changes can be identified and categorized. Red to pink: may occur at lower pH shifts, showing initial spoilage stages. Blue typically indicates a highly basic environment, suggesting advanced spoilage. Green represents intermediate stages where mixed gases' effects might overlap, especially in a moist environment. We recorded the RGB values at different concentrations to correlate color changes with gas concentrations. Initially, we tried the same temperature (60 °C); however, we did not monitor any gradual color change, which could explain why elevated temperatures and humidity can help us reach the endpoint of color change extensively. The limitation of the climate chamber to provide constant RH in this range caused us to try a lower temperature of 40 °C.

The pH indicators appear to respond differently to TVB-N gas volume. Higher volumes of TVB-N gases can cause stronger color changes, indicating a stronger reaction. By integrating the effects of NH_3, DMA, and TMA gases on pH indicators under varying relative humidity conditions, it is possible to develop a comprehensive spoilage indicator system.

3.3. Validation of the pH indicator's efficacy by linking color changes to chemical analysis, microbial growth and mathematical models

Several studies reported the following shelf-life estimates based on the level of TVB-N gases and total microbial population: the TVB-N content was greater than 25 mg/100 g sample, the TVC exceeded 7 log CFU/ml, and the shelf-life was 7 days for rainbow trout fillets stored at 4 °C (Jouki et al., 2014). Another batch of rainbow trout fillets, also

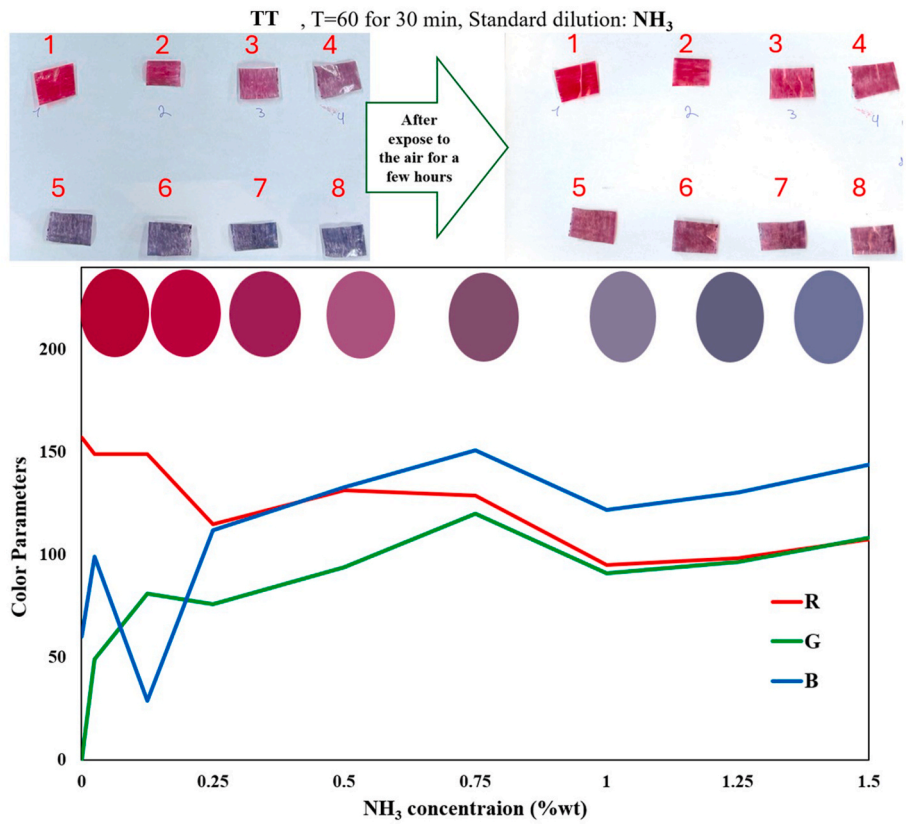

Fig. 5. The RGB value of TT pH indicators towards 100 μL of diluted NH_3 under accelerated storage conditions (T = 60 for 30 min).

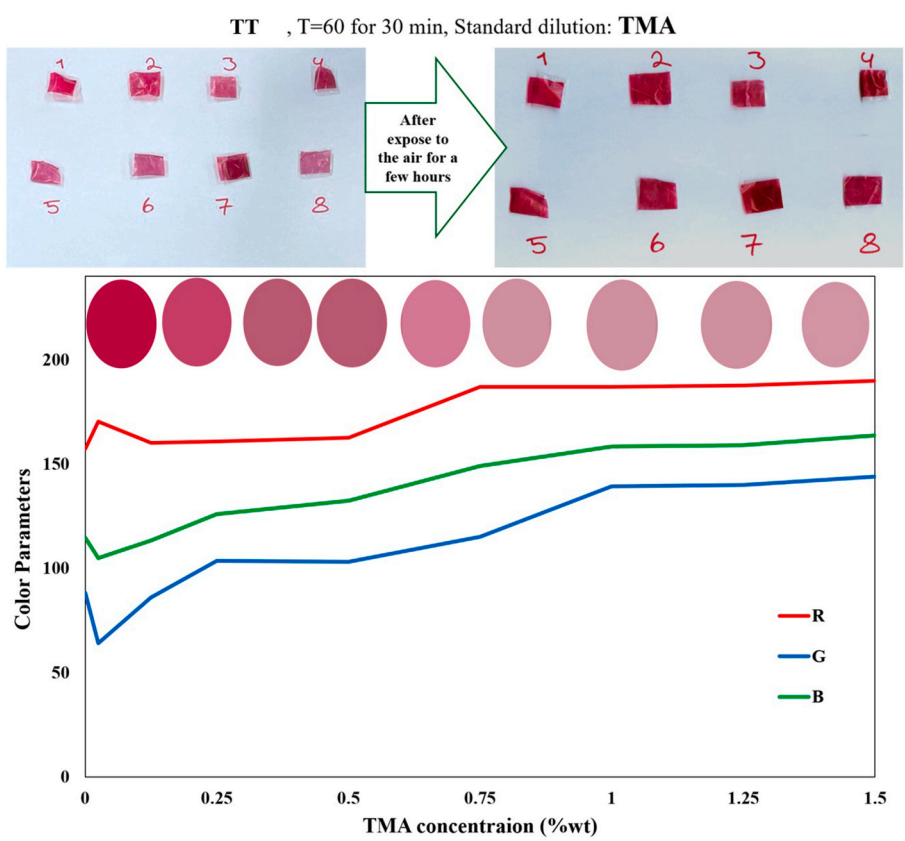

Fig. 6. The RGB value of TT pH indicators towards 100 μL of diluted TMA under accelerated storage conditions (T = 60 for 30 min).

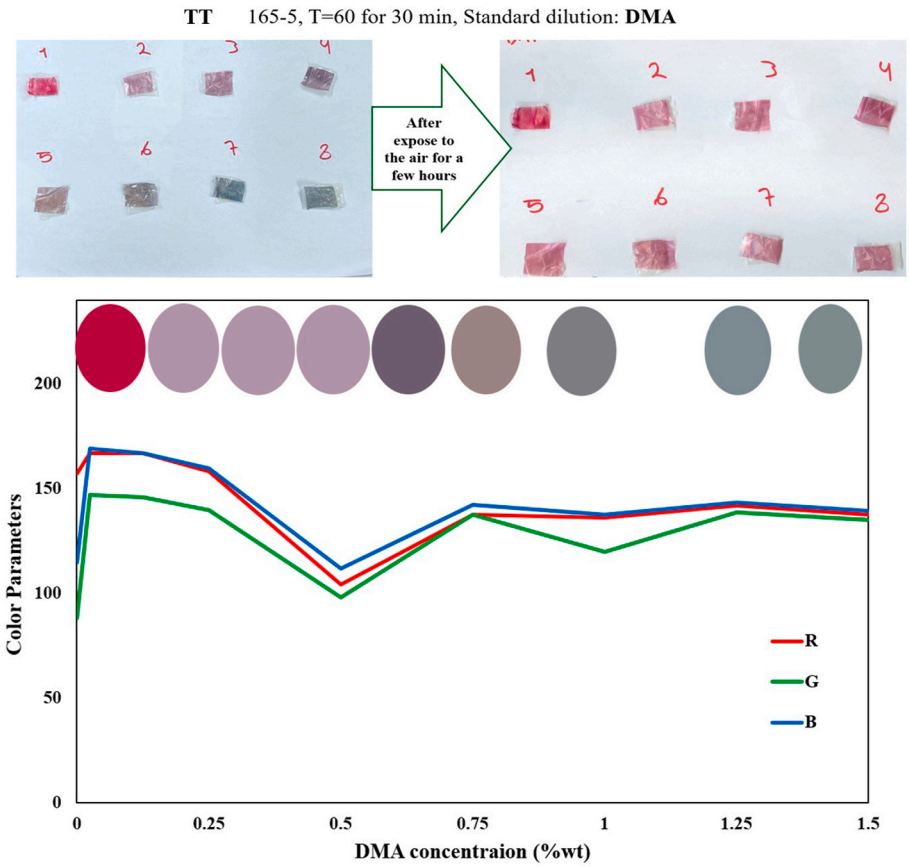

Fig. 7. The RGB value of TT pH indicators towards 100 μL of diluted DMA under accelerated storage conditions (T = 60 for 30 min).

stored at 4 °C, had a TVB-N content of 21 mg/100 g, a TVC of 6 log CFU/ml, and a shelf-life of 10 days (Aghaei et al., 2018). Crucian fish fillets were also stored at 4 °C, with TVB-N content at 20.7 mg/100 g, and the estimated shelf-life was approximately 6 days (Yang et al., 2021/01). Silver carp fillets, stored under the same conditions, had a TVB-N content of 20 mg/100 g and the shelf-life was reported as 5.62 days (Zhai et al., 2017). Furthermore, some studies suggest that pH changes in fish can be a good indicator of spoilage (Pacquit et al., 2006; Heising et al., 2012; Silva-Pereira et al., 2015/04), while others maintain that the pH test may not always accurately show deterioration in certain foods because of the narrow range that separates acceptable and undesirable quality, making it difficult to definitively determine food freshness (Burg, 2004). In this study, microbial tests, and chemical analysis along with the color alternation of pH indicator were performed to validate the fish spoilage.

3.3.1. Chemical analysis (TVB-Ns)

Immediately after purchasing the fish and transferring it to the laboratory, the TVB-N content was 5.53 ± 0.02 mg/100 g, and the pH of fish was 6.93 ± 0.01. It indicates that the product is fresh. After 48 h, the TVB-N content reached to 8.40 ± 0.2 mg/100 g and pH level to 7 ± 0.01. The colorimetric sensor remained inactive. The TVB-N concentration and pH of the fish reached 22.40 ± 0.2 mg/100 g and 7.34 ± 0.01, respectively after being stored for 3 days, suggesting a significant drop in its quality. Simultaneously, the pH indicator started to change color, at the edges (depend on the flow direction of produced gases). At the 4th day of storage, the TVB-N content reached to 26.22 ± 0.10 mg/100. Meanwhile, the color of pH indicators was changed to light purple/pink from the edges. At this point, most surface of the indicator, which is located near the fillet, has changed color, signalling the activation of the colorimetric sensor. Indicating that the TVB-N threshold was reached slightly prior to a 4-day storage period. Moreover, the consistency of the fish sample contained inside the packages were watery and squishy. The TVB-N level and the pH of the fish reached 31.65 ± 0.10 mg/100 g and 7.51 ± 0.01 at 6th day. The TVB-N level at this point is close to rejection limit which is 35 mg N/100 gr and, the color of indicator almost changed to bluish-purple. At the end of the fish storage (day 9) the color of indicator reached out to dark yellow/light brown. In this point, the TVB-N amount exceeded to 36.25 ± 0.45 mg N/100 gr and pH reached to 7.73 ± 0.01 (supplementary material).

In current study the pH of the flesh of fish during the storage period increased along with TVB-N content. This is because of the increase of non-protein nitrogen and other volatile amines that were produced because of proteolytic enzyme activities of the microbial flora (Aghaei et al., 2018; Karaca et al., 2023/11). However, pH of Pangasius fish changed slightly in a narrow range between (6.93–7.73) and cannot be used as the sole indicator of shelf-life (Zúñiga and Troncoso, 2013).

3.3.2. Microbial analysis

Microbial activity affects the shelf-life estimation of seafood (Koutsoumanis and Nychas, 2000; Corbo et al., 2005; Chen et al., 2023). Small quantities of some spoiling microorganisms are present when storage begins, but they multiply and produce metabolites like TVB-N, which alter texture, color, taste, and smell and lead to sensory rejection (Kim et al., 2023b). During packaging trials, the TVC counts slowly increased from 3.80 ± 0.09 log CFU/ml during the initial 72 h of storage, which means the Pangasius fish fillets used in this study had a relatively acceptable quality up to day 4, with a TVC of 5.21 ± 0.08 log CFU/ml. Initially, the pseudomonas counts were at approximately 63% of the TVC counts rising to approximately 78% at around the end of storage (Pacquit et al., 2006). This suggest that pseudomonas spp could be one of the dominant bacteria involved in fish spoilage at 4 °C at both the early and end stages of storage (Tan et al., 2022-). Fig. 10 showed the growth of Pseudomonas spp that was isolated from fish samples with

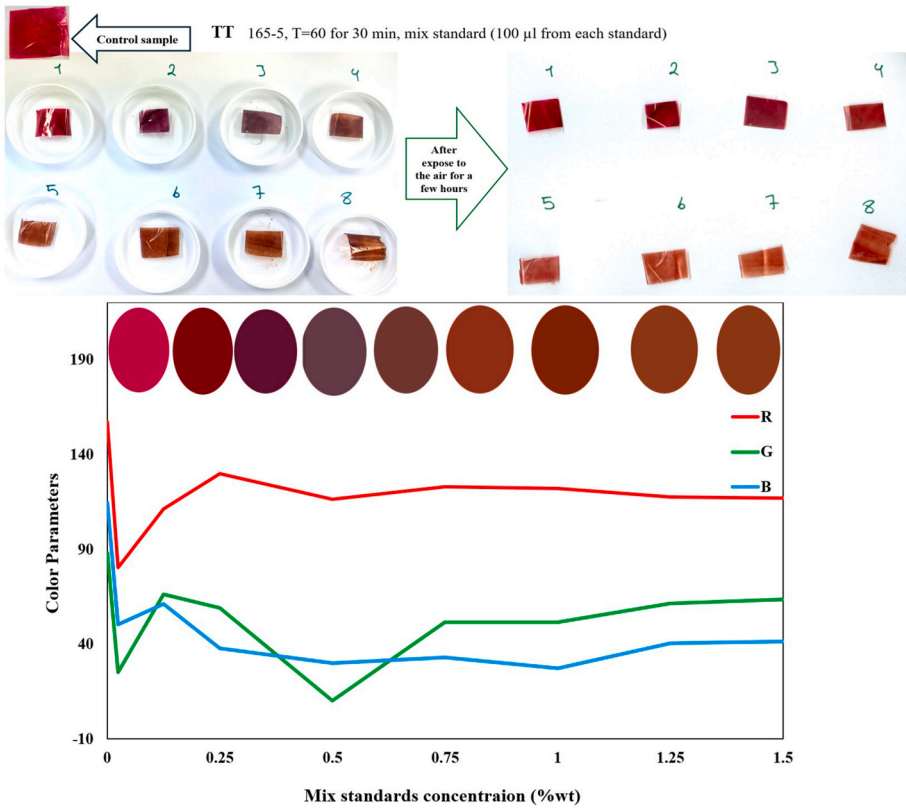

Fig. 8. The RGB value of TT pH indicators towards 100 μL from each standard dilutions (mix of components) under accelerated storage conditions (T = 60 °C for 30 min).

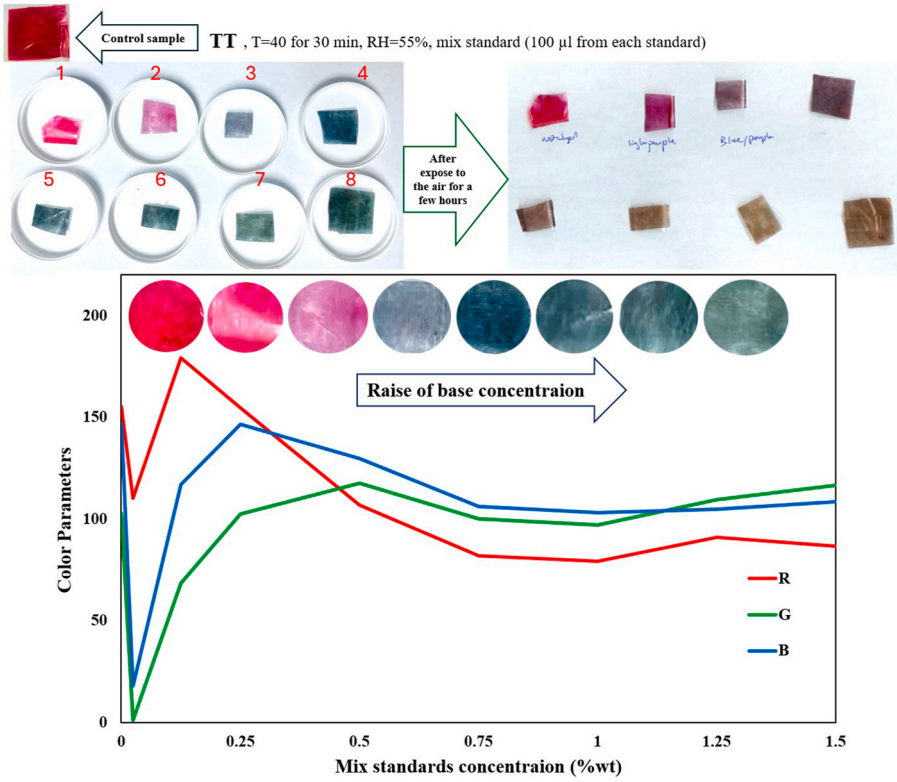

Fig. 9. The RGB value of TT pH indicators towards 100 μL from each standard dilutions (mix of components) under specific storage condition (T: 40 °C for 30 min, RH~ 55%).

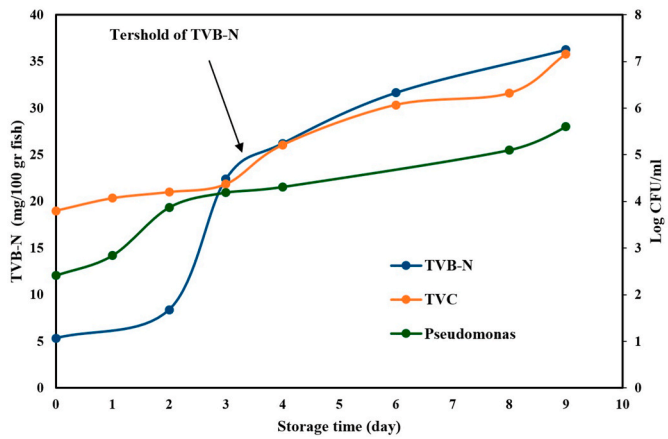

Fig. 10. TVB-N, TVC and Pseudomonas growth versus the storage time at 4 °C.

TVC and TVB-N. The TVB-N threshold reached slightly before the fourth day of storage. Both TVC and pseudomonas populations show a gradual increase from the start that then rapidly accelerates, reaching 7.16 CFU/ml and 5.6 log CFU/ml, respectively after 9-day storage. Table 2 presented the measured data points of chemical and microbiological analysis during the packaging study.

3.3.3. *Correlation of ΔRGB with chemical and microbial analysis*

Fig. 11 displays the correlation between ΔRGB and TVB-N gases (a), TVC (b), and pH (c) at 4 °C, the impact of shows the high sensitivity of colorimetric sensors to various biomarkers, showcasing their utility in monitoring fish spoilage. The R^2 ratings suggest high sensitivity of colorimetric sensors (Zhang et al., 2024). Fig. 11 (a) displays a strong correlation ($R^2 = 0.9836$) between ΔRGB and TVB-N concentration. As TVB-N levels increase, the sensor responds significantly by turning deep reddish. This means the sensor reached the initial threshold of TVB-N gas (25 mg/100 g) in the headspace of the fish package (Karimi Alavijeh, 2020). The ΔRGB response showed a linear response to the TVB-N concentration in the range of 5.34–26.22 mg/100 g at the chilled temperature (Liu et al., 2020/03). Fig. 11 (b) shows the correlation between ΔRGB and TVC with an R^2 of 0.8661, showing the sensor's ability to track microbial growth. The sensor response was captured on day 4, which shows the sensor changed color from the side close to the fish sample to purple. At this time, the microbial activities also increased. Fig. 11 (c) highlights the correlation between ΔRGB and pH ($R^2 = 0.9878$), suggesting that the sensor is highly responsive to pH changes during spoilage. Therefore, these plots show that the sensor response correlates more strongly with chemical analysis than with microbial activities. Fig. 11 (d) shows the different stages of fish condition (fresh, acceptable, moderate spoilage and spoiled) during storage based on the biomarkers, For a food freshness indicator to be effective, it must be able to visually distinguish between fresh, acceptable, and spoiled food (Almasi et al., 2022/06). As the alkaline environment increases due to spoilage during fish storage, the color of the sensor changes to green-light brown (a slight reduction in ΔRGB); finally, at the end of storage, the sensor almost reaches the endpoint of its color change and turns to brown yellowish (high reduction in ΔRGB) on day 9. The sensor shows the monitoring of color stability in the basic environment over a period of more than one month. The color did not wipe out or change to its original color before exposure to fish samples for indicators labeled as 165–5 for both container and package storage (supplementary material).

Numerous limitations exist in these fitting models for predicting fish spoilage using colorimetric sensors. The quadratic nature of the fitted curves indicates non-linear relationships between ΔRGB and the biomarkers (TVB-N, TVC, and pH). This complexity can make the models less intuitive and harder to interpret compared to simpler linear models. The high R^2 values (e.g., 0.9836 for TVB-N and 0.9878 for pH) suggest a strong fit to the experimental data, but they might also show overfitting, where the model captures noise in the data rather than just the underlying trend. There is also a limited number of data points, which might not be sufficient to fully capture the variability and trends in the spoilage process. This can reduce the robustness and generalizability of the model. Moreover, the ΔRGB values eventually plateau at higher biomarker concentrations, indicating that the sensor response may saturate. This saturation limits the model's ability to differentiate spoilage levels beyond a certain point, potentially reducing its effectiveness in late stages of spoilage.

Table 2
Measurements for Chemical analysis (TVB-N and pH), microbiological analysis and color change data during fish storage (day).

Storage time	TVB-N	TVC	Pseudomonas spp	pH	ΔRGB NTT	ΔRGB TT
0	5.34 ± 0.02	3.80 ± 0.09	2.41 ± 0.09	6.93 ± 0.01	0	0
1	NA	4.07 ± 0.09	2.84 ± 0.03	NA	NA	NA
2	8.40 ± 0.2	4.20 ± 0.02	3.87 ± 0.19	7 ± 0.01	16.08 ± 5.68	36.07 ± 7.42
3	22.4 ± 0.20	4.37 ± 0.005	4.19 ± 0.04	7.34 ± 0.01	60.24 ± 4.59	53.34 ± 6.62
4	26.22 ± 0.10	5.21 ± 0.08	4.31 ± 0.03	7.48 ± 0.01	80.03 ± 13.57	78.74 ± 14.87
6	31.65 ± 0.10	6.07 ± 0.02	NA	7.51 ± 0.01	83.04 ± 3.07	93.51 ± 8.78
8	NA	6.32 ± 0.005	5.10 ± 0.10	7.55 ± 0.01	NA	NA
9	36.25 ± 0.14	7.16 ± 0.04	5.60 ± 0.08	7.73 ± 0.01	66.12 ± 5.00	44.42 ± 3.28

NA: not available. All values were expressed as mean ± standard deviation (n = 3).
NTT; colorimetric films without heat treatment. TT; colorimetric films with exposure to heat (165 °C) for specific time (5 min).

4. Conclusion and recommendations

This study confirmed that an environmentally friendly ink formulation can coat PET films as a scalar pH indicator sensor, and the fabricated films have high potential as an intelligent indicator label for monitoring the freshness of fish products. We employed immobilization fabrication methods, specifically utilizing coating techniques such as the doctor blade and phantom proofer, which are lab-scale adaptations of flexographic printing methods used in the industry. The novelty of this research project lies in using these pH indicators in direct contact with food products to enhance readability and provide a quicker color change in response to quality changes in seafood. Additionally, it utilizes industrial printing techniques aimed at increasing production rates.

Developed pH indicators can offer a continuum of color variations that correspond to varying degrees of freshness, rather than a simple binary outcome. Hence, color change analysis showed that the developed colorimetric sensor can detect and evaluate gas concentrations through color changes, making it a practical industrial tool for monitoring the food safety and quality of fish packages. The use of cross-linking agents such as citric acid can enhance the mechanical stability of the polymer matrix in which anthocyanins are embedded, reducing dye leaching and degradation. Applying controlled thermal treatments can enhance the interaction between anthocyanins and PET films, increasing stability of anthocyanins during fish storage period. The sensitivity of pH indicators at low pH levels is markedly greater compared to those at

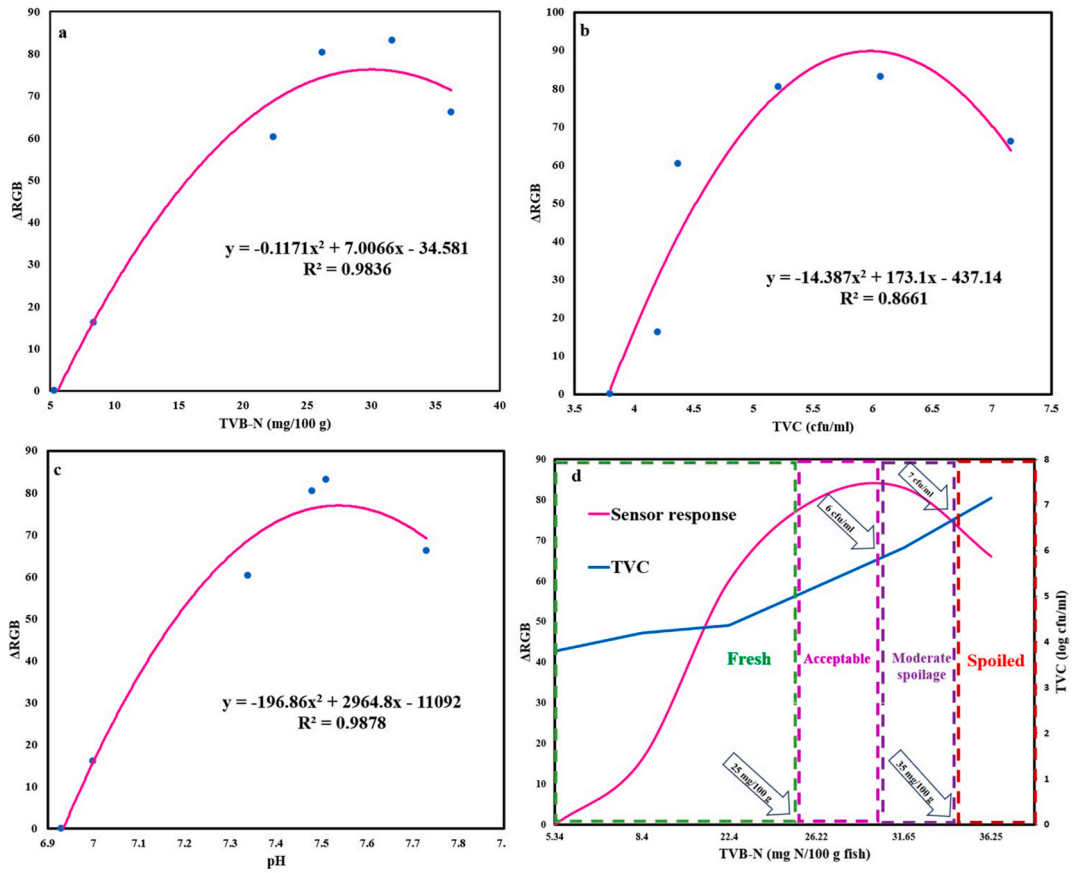

Fig. 11. ΔRGB versus TVB-N gases (a), TVC (b), and pH (c) during fish storage at 4 °C. Correlation between the onset of an increase in microbial population and TVB-N gas release and sensor response (d).

neutral and alkaline levels, as the latter did not show notable color changes during the initial storage period. Chemical analysis (TVB-N and pH measurement) and microbial analysis (total bacterial population and pseudomonas spp. growth) performed during packaging trials at 4 °C validated the performance of the produced films. After the third day of fish storage during the packaging study, TVB-N gases increased in the headspace, and colorimetric films started to change color. During packaging trials, the TVB-N threshold occurred between the third and fourth days of storage, in transition state from fresh to acceptable state. Additionally, at day 4 of fish storage at 4 °C, we found the pH, TVC, Pseudomonas growth, and TVB-N levels to be 7.48, 5.2 (log CFU/ml), 4.31 (log CFU/ml), and 26.22 (mg N/100 gr sample), respectively. At this time, the sensor changed color to reddish-purple. Based on microbial (7 log CFU/ml TVC) and chemical (35 mg/100 g TVB-N) analyses, fish packages stored under atmospheric conditions typically have a shelf-life of less than 9 days. These thresholds are set up to ensure the freshness and safety of both fresh and frozen fish packages. The sensor response shows a stronger correlation with TVB-N gases than with microbial activities.

CRediT authorship contribution statement

Maryam Ameri: Conceptualization, Investigation, Experimental work, Data curation, Writing – original draft, Writing – review & editing, Project administration. **Abdellah Ajji:** Conceptualization, Supervision, Funding acquisition, Writing – review & editing. **Samuel Kessler:** Conceptualization, Supervision, Writing – review & editing.

Data validation

The implementation of mathematical models to predict TVB-N content and microbial growth significantly enhances the utility of this research. These models serve as a robust framework for validating experimental results by correlating initial quality parameters with observed changes in seafood freshness over time. By analyzing historical data and establishing relationships between variables, these models can accurately forecast the shelf-life of seafood products.

Recommendations for future studies

Despite the promising results, this study identified numerous areas for future research to enhance the reliability and applicability of the findings. By addressing these limitations, future research can build on the current study and contribute to more effective monitoring systems for fish freshness and safety.

- ➢ Integration with smart packaging: Integrating the developed colorimetric films with other film layers can create smart packaging systems.
- ➢ The integration of barrier films can extend the shelf life of seafood by minimizing oxygen exposure and inhibiting microbial growth. The coated-PET films can be laminated with various barrier films to create a multilayer intelligent food packaging system. This advanced packaging solution not only protects the seafood product from external contaminants and moisture but also actively contributes to maintaining its freshness. By incorporating pH indicators and other sensory technologies, the packaging can provide real-time

information about the product's quality, such as freshness and spoilage levels.

- ➢ Complex interactions in biological systems, such as fish microbial spoilage, need a larger dataset for accurate modeling. More experimental data points, cross-validation, error analysis, and studies conducted under varying conditions can significantly improve the predictive model's reliability and robustness. Such enhancements will aid in refining the model's ability to predict TVB-N levels and the corresponding spoilage of fish.
- ➢ Another constraint is the reliance on chemical analysis to quantify TVB-N levels in fish samples. Future studies should consider utilizing gas chromatography-mass spectrometry (GC-MS) to collect data from the headspace of the packaging. This approach could prove more effective in quantifying TVB-N gases and establishing correlations between these data, microbiological analyses, and the color response of the developed pH indicator.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Maryam Ameri reports financial support was provided by Natural Sciences and Engineering Research Council of Canada. Maryam Ameri reports financial support was provided by ProAmpac. Prof.Abdellah Ajji reports a relationship with Natural Sciences and Engineering Research Council of Canada that includes: funding grants. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.crfs.2024.100934.

Data availability

Data will be made available on request.

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