

Intelligent packaging solutions for fish and meat products: volatile gas detection via GC- MS, microbial analysis and colorimetric monitoring

Maryam Ameri

Maryam.ameri@polymtl.ca

Polytechnique Montréal

Abdellah Ajji

Polytechnique Montréal

Samuel Kessler

ProAmpac flexible packaging company

Marc-Antoine Vaudreuil

University de Montréal

Research Article

Keywords: Intelligent packaging, On-package sensor, Headspace GC-MS, TMA detection, pH indicator, Anthocyanin

Posted Date: June 29th, 2026

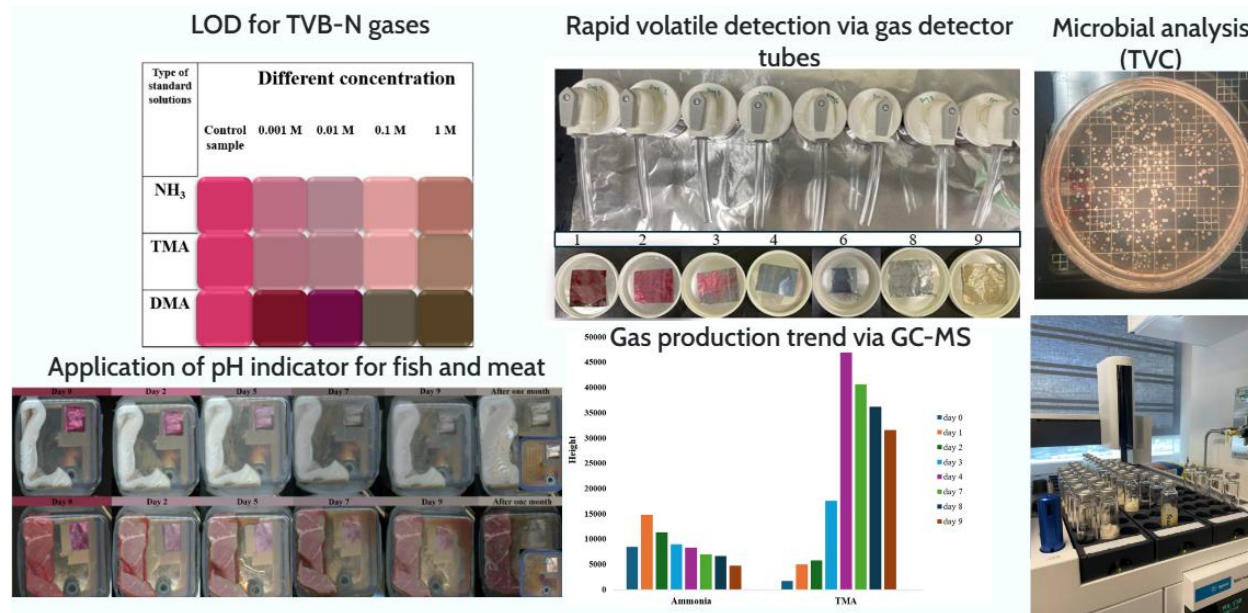
DOI: <https://doi.org/10.21203/rs.3.rs-6498075/v1>

License:   This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Additional Declarations: No competing interests reported.

Intelligent packaging solutions for fish and meat products: volatile gas detection via GC-MS, microbial analysis and colorimetric monitoring



Abstract

A pH-sensitive anthocyanin dye was used as a freshness indicator to detect color changes in fish products due to volatile amine formation and microbial activity during storage at 4 °C. Incremental color changes of the pH indicator response accurately tracked an increase in the total volatile basic nitrogen (TVB-N) content in the packaging headspace of fish products along with increases in total viable count (TVC). Ammonia (NH₃) and trimethylamine (TMA) production trend were evaluated during the fish storage using headspace analysis with gas chromatography-mass spectroscopy (GC-MS) detection. No laborious pre-treatment, clean-up, or derivatization was required. The GC-MS method optimization yielded linear calibration curves for the targeted analytes, with detection limits in the range of ppm in pure solvent and good method precision, allowing for the analysis of trends in the molecule concentrations formed during real fish sample storage. Scan-mode injections identified methanol (MeOH), ethanol (EtOH), dimethyl sulfide (DMS), butane and pentane (untargeted species) during fish spoilage with high scores (> 89%). In parallel, gas detection tubes were used to quantify NH₃ and Triethylamine (TEA) concentrations in the headspace of homogeneous fish samples across different vial storage conditions and microbial analyses. Correlation analysis between colorimetric results and GC-MS data illustrated a strong relationship in TMA detection, reinforcing the colorimetric sensor's practicality as an efficient monitoring tool for seafood freshness. The experimental outcomes not only highlight the responsiveness of our sensing approach but also suggest potential applications as an intelligent package for ensuring food safety and quality.

Keywords: Intelligent packaging, On-package sensor, Headspace GC-MS, TMA detection, pH indicator, Anthocyanin

1. Introduction

The increasing demand for fresh seafood and meat products necessitates effective monitoring systems to ensure food safety and quality [1]. A significant challenge in the storage of fresh fillets is assessing their freshness, as post-mortem microbial activity and endogenous biochemical reactions can rapidly result in quality degradation and a reduced shelf life of the raw fish fillets [2]. Spoilage in perishable food items, such as fish, is predominantly caused by microbial activity, leading to the formation of volatile compounds, including TVB-N, which primarily comprises NH_3 , TMA and dimethylamine (DMA)[3]. Chicken fillets and other types of meat can produce TVB-N as well during their spoilage [4]. However, this indicator is more commonly used as a measure of freshness in seafood products [5]. Moreover, TEA is a volatile organic compound that can be produced during the spoilage of fish and other seafood [5-7]. Traditional methods for assessing spoilage often rely on sensory evaluation or chemical tests that may not provide timely or accurate results [8]. Therefore, there is a pressing need for innovative and reliable indicators that can monitor spoilage in real-time, ensuring that consumers receive safe and high-quality food products [8-11].

TMA is produced by a small number of specific spoilage organisms (SSO) which is the reason of fishy odour, arising from the bacterial reduction of Trimethylamine N-oxide (TMAO) [12, 13]. Thus the presence of TMA in muscle can give a very good indication of the level of bacterial contamination [14, 15] and often reflects more accurately the degree of spoilage [16, 17]. In a very fresh fish the amount of TMA is low; an average of 2 mg/100g for some fish species from the North-East Atlantic [3]. A TMA content of 5–10 mg/100 g is usually regarded as the sensory rejection limit [18]. However a limit of acceptability of is 8 mg/100g was suggested as well [19]. Studies have indicated that fillet samples can exhibit lower levels of TVB-N and TMA compared to whole fish, likely due to the absence of viscera and head, where microorganisms that convert TMAO to TMA are concentrated [20].

Detecting SSO such as *Pseudomonas spp.* along with TVC for assessing fish product freshness or spoilage [16]. *Pseudomonas spp.* appeared to be responsible for the development of sweet, fruity spoilage odors in haddock fillets. *Pseudomonas spp.* are significantly implicated in the deterioration of haddock fish at refrigeration temperatures, particularly in the initial stages [16]. Studies show that TVC in freshwater fish can attain 6 to 7 log CFU/ml after 3-5 days of storage at 4 °C [21, 22].

In recent years, anthocyanins, natural pigments found in various fruits and vegetables, have gained attention for their potential as pH indicators in food safety applications. These compounds exhibit color changes in response to pH variations, making them suitable for developing indicators that can signal spoilage [10, 23-26]. By integrating anthocyanins into a food-safe pH indicator, we can create a visual tool that not only indicates pH changes but also correlates with the spoilage process in fish and meat products [10, 26-29]. This research aims to evaluate the performance of such an indicator in monitoring spoilage, providing a practical solution for consumers and food industry stakeholders.

Recent studies combining GC-MS with intelligent packaging has shown promising results for monitoring fish spoilage [30]. An electronic nose with metal oxide gas sensors and GC-MS revealed correlations between volatile compounds and fish freshness [31]. Another study demonstrated the use of colorimetric indicators to detect volatile amines and correlated the color changes with GC-MS measurements [32]. To evaluate the effectiveness of the developed pH indicator, we performed a variety of experiments, including monitoring the color change of pH indicators, microbial analysis to correlate the presence of specific bacteria with the production of spoilage-related compounds, and GC-MS tests to detect NH_3 and TMA levels during fish spoilage. The use of various techniques in GC-MS experiments, such as headspace analysis and direct injection, enabled a thorough understanding of the volatile compounds produced during spoilage.

Scan analysis allowed for detection and identification of non-targeted volatile compounds produced during fish storage. Furthermore, we used gas detection tubes to measure ammonia concentrations in the headspace of fish packages. This multifaceted approach ensures that the developed indicator is not only responsive to pH changes, but also accurately reflects the spoilage process, highlighting the anthocyanin-based pH indicator's potential as a reliable monitoring tool. By combining these methodologies, this study aims to provide an effective structure for evaluating the performance of the developed food-safe pH indicator, resulting in improved food safety and quality control in seafood and meat industries. The main objective of the study was to employ pH indicators based on natural dyes in food packaging that can alter color in reaction to elevated levels of TVB-N gases in the headspace of food containers and the bacterial activity of fish and meat products throughout their shelf life.

The data presented here pertains to method development experiments conducted in January 2024 for the detection and quantification of NH_3 and TMA in three different types of fish: pangas and haddock. Additionally, a storage experiment of haddock fillets was conducted in August 2024 for microbial analysis, TMA trends determination using headspace GC-MS, NH_3 gas detection tubes, and monitoring the color change of these fish fillets simultaneously. The objective was to validate the performance of colorimetric sensor for further application as freshness/spoilage indicator for fishery products. Determination of the end of shelf-life based on microbial analysis and TMA production was compared to the estimation of shelf-life based on the currently developed pH indicator.

2. Materials and methods

2.1 Materials

We acquired pangasius and haddock fillets from a nearby Metro grocery store in Montreal, Quebec, Canada. Initially, the products were sealed in PVC-wrapped packages that displayed a label containing 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 maintain their temperature and freshness during transportation. The samples remained in the fridge until they were ready to be use. The Polytechnique Montreal in Montréal, Québec, Canada, served as the site of all experiments except the headspace GC-MS experiments which performed in the Regional Mass Spectrometry Center, Université de Montréal, Montréal, Québec, Canada. Transparent polyethylene terephthalate films (PET, 23 μm) were obtained from ProAmpac Packaging Canada company, Terrebonne, Quebec, Canada. Black rice 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 were from Thermo Fisher Scientific, Saint-Laurent, Quebec. Single-neck round-bottom flask, Polyvinyl alcohol (87~88% hydrolysis, 145,000 MW), Polyethylene glycol (PEG), Citric acid (ACS reagent, $\geq 99.5\%$), Vanillin (natural, $\geq 97\%$, FCC, FG), Hydrochloric acid (HCL), Sodium Chloride (NaCl), Dimethylamine solution (DMA, 2 M in methanol), Trimethylamine solution (TMA, 31-35 wt. % in ethanol, 4.2 M), Ammonium hydroxide solution (NH₄OH, 30-33% NH₃ in H₂O), Diethyl-d10-amine, Vortex-Genie 2 (120 V) mixer, Agar were purchased from Millipore Sigma Canada Ltd, Oakville, Ontario, Canada. Dräger tubes for short term measurement: Ammonia 20/a-D 8101301 (20-1500 ppm), Ammonia 5/a (5-600 ppm), Ammonia 0.25/a (0.25-3 ppm), Triethylamine 5/a (5-60 ppm), and accuro pump Tube-D, were from Dräger Co., Ltd (Mississauga, ON, Canada).

2.2 Sensor preparation

A complete description of sensor preparation was provided with some modifications [10]. 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 carried out with a TQC automatic film applicator (model AB3652 made in Netherlands). The drying process of the films proceeded at two temperatures: ambient (30 min) and 165 °C (5 min). Samples were TT (Thermal treated pH indicators, at the temperature of 165 °C for 5 min)[33].

2.3 Freshness monitoring

In this study, we conducted a series of simultaneous shelf-life monitoring tests on fish fillets, which included analyses of color change, microbial activity (TVC, *Pseudomonas spp.*), GC-MS for TMA and NH₃ detection, and gas detector tubes for measuring NH₃ and TEA. For monitoring the color change and gas detection, approximately 20 gr of fish samples were prepared in amber vials. For microbial activity assessment, around 5 g of fish were utilized, while 10 gr were allocated for GC-MS analysis. It is important to note that all fish fillets used in these experiments were procured from the same retail outlet, packaged at the same time, and consisted of the same species. Prior to distribution into vials, the fillets were mixed homogeneously to ensure uniformity across the samples. The shelf-life was monitored on a haddock fish upon storage at around 4 °C during the summer of 2024 in Montreal, Quebec, Canada. The day of purchase (package date) was considered as day 0 (the day the fish was first packaged at the store for sale), and the 'best before' date on the fish package was set to 3 days from that point.

2.3.1 Packaging study using RGB

We used mentioned procedure from our previous study, with minor modifications, to evaluate the sensitivity of the developed pH indicator towards TVB-N gases originated from fish samples [10]. Color responses of the colorimetric film to produced volatile gases were captured by an Epson perfection V550 scanner (Nagano, Japan). 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 [34].

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

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.

2.3.2 Microbial analysis

During fish storage at 4 °C, the TVC method revealed that the bacterial growth reached the spoiling threshold (1×10^7 CFU/ml)[35]. Microbial analysis was performed by weighted fish samples (5 ± 0.5 g) which were placed in sterilized 50 ml tubes (Fisher brand). The sterilized NaCl (9 %w/v) saline solution (45 ml) was added to tubes and homogenized with vortex for 2 min at room temperature under biohood. Serial sample dilutions were made during the fish spoiling tests [36]. The rest of experimental part was performed exactly as in our previous work [37]. The results were expressed (according to ISO 7218:2007) as mean log CFU/ml $\pm$ standard deviation of 3 replicates.

2.3.3 NH₃ and TEA detection by using the gas detection tubes

The dräger short-term detector tubes along with pump, which can measure toxic gases and vapors in the workplace, were used as a fast and cost-effective approach (**supplementary material**). The measuring ranges used for ammonia gases were 5 to 70 ppm and 0.25 to 3 ppm with 10 times stroke's number and 50 to 600 ppm with once time stroke's number. The approximate measuring time is around 1 minute. For TEA gas detection tubes, the measuring range is 5 to 60 ppm. The number of strokes is 5 and the approximate time for measurement is around 3 minutes, and the standard deviation for all tubes are ± 10 to 15%. The color change principle is based on acid-base reaction which can turn yellow to blue color. Each 1 ppm NH₃ is equal to 0.71 mg NH₃/m³ and 1 ppm triethylamine is equal to 4.2 mg triethylamine/m³. Firstly, we break both tips of the tube and inserted the tube close to the pump. Then inserted the gas sample through the tube and read the entire length of the discolouration.

2.3.3.1 Sample preparation for gas detection tubes

We used amber vials (60 mL) to conduct experiments, filling them with a homogeneous mixture of fish fillets (approximately 20 g). Furthermore, on each sampling day, we used control vials without fish fillets to monitor baseline changes in the headspace gases. On each sampling date, we attempted to measure the gas concentration of each vial as promptly as possible to mitigate variability in gas production that could result from external factors.

2.3.3.2 Freshness control in room temperature with gas tube detector

Ammonia detection tubes with a measurement range of 20–1500 ppm were used to measure the average ammonia concentration over an extended period of up to 8 hours. The ammonia molecules to be measured independently flow into the tube, which is open at one end, up to reagent layer (bromophenol blue and acid) based on diffusion process in gases. The generated ammonia from fish sample reacts with the chemical on the substrate. A color change for tubes from yellow to blue takes place. The reading is indicated in “ppm $\times$ hours”. The length of the color zone and the elapsed sampling time is used to calculate the average ammonia concentration. For evaluation the ammonia concentration in ppm = tube reading/measurement duration in the hours. The standard deviation for these tubes is $\pm$

15 to 20 % and the accuracy is without calibration. The setup includes a container with an integrated colorimetric sensor, coupled with the connected tube (Error! Reference source not found.).

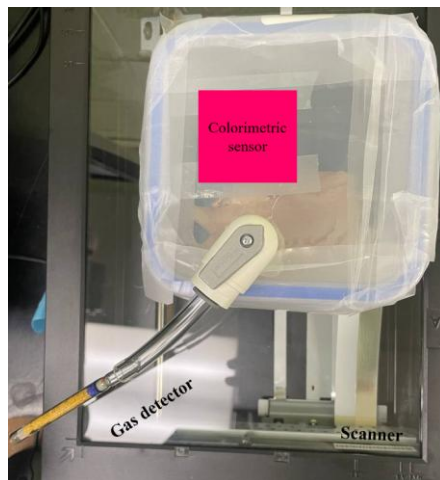


Figure 1 A container with a 900 ml capacity, along with an integrated colorimeter sensor and a connected valve for monitoring the volatile gasses produced during a duration of 8 hours.

2.3.4 GC-MS analysis

To achieve the low-level detection of gases generated during the storage of fish samples, specifically DMA, TMA, and NH_3 , a systematic approach was implemented to evaluate different methodologies. Additionally, untargeted (scan mode) analysis was conducted to identify other species present during the storage of fish samples. Various parameters were controlled to ensure consistency across the analytical methodologies employed. The experiments were conducted in two distinct phases. The first phase involved two different fish species, Pangasius (commonly known as pangas) and haddock. This phase aimed to develop a standardized laboratory method aligned with the objectives of the project. The second phase focused on performing additional analyses for shelf-life monitoring.

2.3.4.1 Instrumental conditions

The analysis was conducted using a Headspace (model 7697A) coupled with a GC-MS (model 7890A-5975C), all from Agilent Technologies. The gas chromatography utilized a GS-Q column, a narrow bore capillary column, which is appropriate for separation of low molecular weight compounds. The column measures 30 m in length and 320 μm in diameter. The carrier gas used was helium, with a flow rate of 1.2 mL/min [38]. The headspace temperature was maintained at 80 °C for 30 minutes to achieve optimal vaporization of the analytes.

2.3.4.2 Temperature program for chromatographic separation

The chromatographic separation was performed under a temperature program initiated at 70 °C and held for 2 minutes, followed by a ramp of 10 °C/min until reaching a final temperature of 250 °C, where it was held for an additional 2

minutes. Splitless injection mode was set at an injector temperature of 250 °C to enhance sensitivity for the target analytes.

2.3.4.3 Mass spectrometry conditions

The mass spectrometer was equipped with an electron ionization (EI) source. Acquisition parameters were set to two different approaches: a targeted and an untargeted one. Selected ion monitoring (SIM), focusing on known m/z values 17.0 for NH_3 , 44.1 for DMA, 58.1 for TMA, 32 for methanol, 46 for ethanol and 83.2 for diethyl-d10-amine used as an internal standard was used as targeted method. A scan mode covering m/z 10 to 300 for detection and identification of other volatile species released during fish storage was used as untargeted approach.

2.3.4.4 Calibration curve

A mixture of standards was prepared using stock solutions of individual compounds, with diethyl -d10-amine employed as an internal standard. For the preparation of headspace vials containing standards in pure solvent, 1 mL of water was added to each 20 mL headspace vial, followed by the incorporation of 10 μL of (25% w/w) diethyl-d10-amine as internal standard. The vials were then spiked with a mixture of standards to achieve final concentrations of analytes ranging from 50 to 38,000 mg/L. Vials sealed with a crimp cap were analyzed on the same day. To verify the retention times of targeted analytes, they were individually injected in pure solvent. The limit of detection (LOD) is defined as the lowest concentration leading to a signal-to noise ratio of 3 [39, 40]. Precision, in terms of relative standard deviation (RSD) was assessed by replicate analyses of spiked samples via studies of inter-day variability. Essays of calibration curves in real matrix were done by adding approximately 10 g of fish to a 20 mL headspace vial and spiking standards directly onto the fish for a final concentration ranging from 700 to 8 000 mg/kg. After sealing the vials with a crimp cap, the content was also analyzed on the same day

2.3.4.5 Preparation of fish samples for Head-space GC-MS

Approximately 10 g of fresh fish was weighed and placed into a 20 mL headspace vial. Following , 10 μL of diethyl-d10-amine (25% w/w) was added. The vial was sealed with a crimp cap and either analyzed on the same day (day 0) or stored in the refrigerator until analysis on subsequent days (day 1 to day 4 and day 7 to day 9). Alternatively, a manual injection approach was tested by weighing approximately 70 g of fresh fish in 250 mL glass flask. Subsequently, 50 μL of diethyl-d10-amine (25% w/w) was added, and the flask was spiked with a mixture of analyte standards, with concentrations of 50 to 500 $\mu\text{g/g}$ for DMA and TMA, and 0.05 to 0.5 $\mu\text{g/g}$ for ammonia. The flasks were sealed with a silicon septa cap, and headspace samples were extracted using a gas tight syringe and analyzed on the same day.

3. Data analysis

Microsoft Excel 365 was used for data processing, to calculate means and standard deviations for all multiple measurements and to generate graphs. Analysis of variance (ANOVA) was applied to the microbial, RGB parameters from colorimetric sensor and TMA quantification. Significant differences were determined by ANOVA. An effect

was considered significant at the 5% level ($p < 0.05$). Identification of the untargeted compounds was made using NIST database comparison (NIST/EPA/NIH Mass Spectral Library with Search Program, data version NIST 05, software version 2.0d).

4. Results and discussion

4.1 pH indicator sensitivity towards different TVB-N gases

The results from multiple experiments conducted (at room temperature and evaluated temperatures) on the on-package label indicate a clear relationship between the concentration of TVB-N gases (NH_3 , TMA, DMA) and the color parameters measured through ΔRGB . The statistical significance of the 0.01 M concentration ($p < 0.05$) suggests that this level is critical for detecting changes in color, which can be attributed to the presence of these gases. This concentration level is detectable and has a significant effect on ΔRGB .

Figure 2 presents the color parameters associated with the limit of detection measurements based on the pictures captured by the smart phone. The indicators were tested against different standards (TMA, DMA, NH_3) at dilutions (0.001, 0.01, 0.1, and 1 M).

The red channel (R) shows a significant decrease for all analytes: NH_3 exhibits the most pronounced decline, followed by TMA, while DMA shows a slightly less dramatic reduction. This trend indicates that NH_3 has noticeable impact on the color change of the pH indicator among the three analytes, as it was observed in our previous research study [37]. DMA's resolution appears better because it maintains a clearer and more stable response across varying concentrations, allowing for more precise differentiation in color intensity, even though its overall effect on color change is less pronounced compared to NH_3 and TMA. This stability can make it easier to detect subtle variations in concentration. For the green channel (G), there is minimal fluctuation across all analytes, with NH_3 and TMA showing slight decreases, while DMA remains relatively stable. In the blue channel (B), a subtle decline is observed for NH_3 and TMA, with DMA exhibiting the least change, indicating that the blue response is less sensitive compared to the red channel.

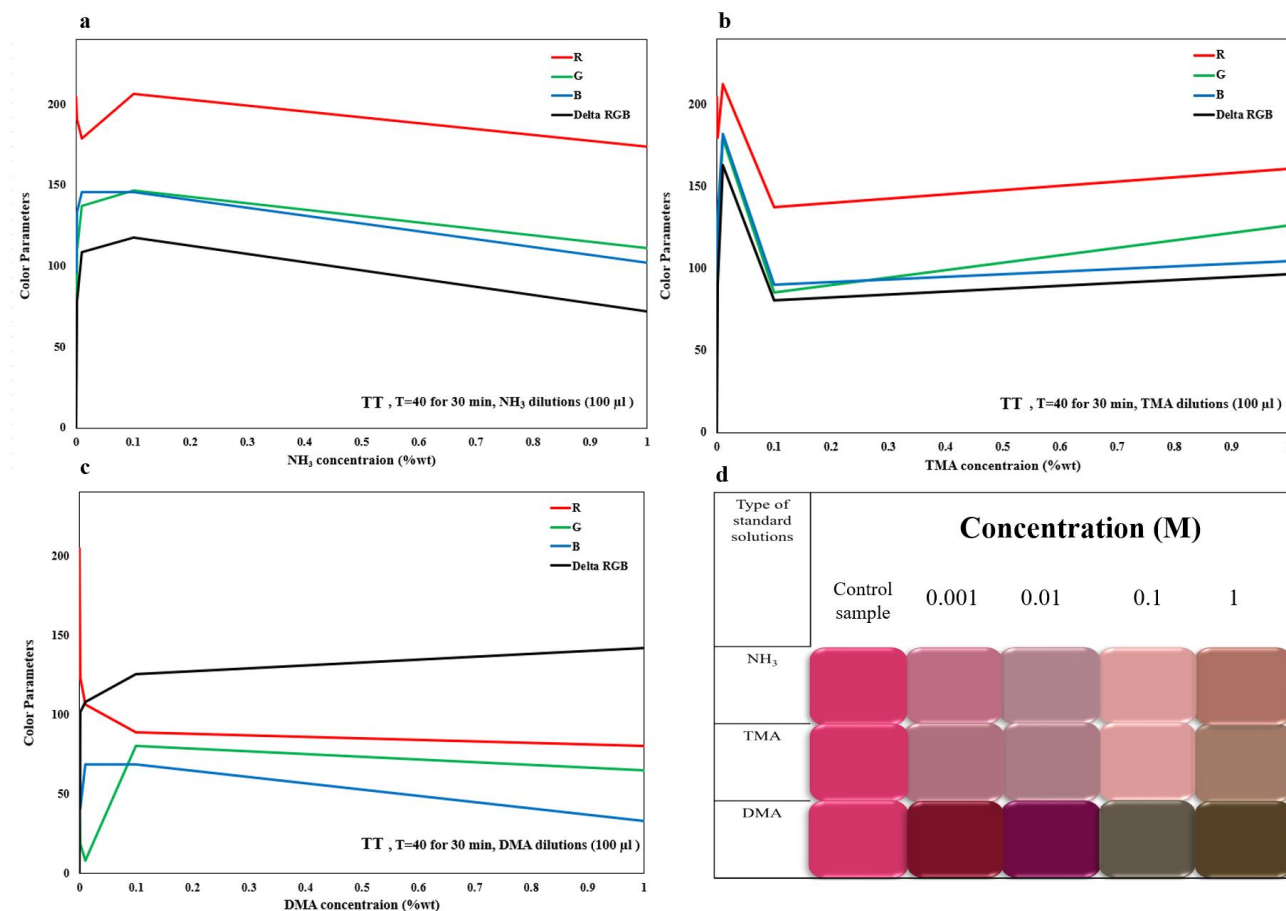


Figure 2 The color parameters from various TVB-N gas dilutions (a: NH₃, b: TMA, c: DMA) and the TVB-N gas detection limit (d) in accelerated mode are presented.

4.2 The effect of sensor ink on different food product: fish, meat and chicken breast

We assessed the efficacy of pH indicator labels (TT) through packaging trials involving haddock fillets, beef steaks, and chicken breasts, which had best-before dates of 3, 4, and 4 days, respectively. Day 0 marked the packaging date for these products, with trials consisting of samples weighing 100 ± 5 grams, contained within 900 mL packages. As shown in **Figure 3**, these products were stored at 4 °C for a duration of 9 days, during which the pH indicator displayed significant color changes corresponding to the freshness levels of the food items.

The pH indicator was calibrated to exhibit a four-point freshness scale: red for fresh, pink/purple for not fresh/acceptable, light purple for moderate spoilage, and yellowish-brown for high spoilage [37]. Observations indicated that from days 0 to 2, the indicator remained red/pink, signifying pristine conditions with no spoilage. By day 5, it shifted to light purple, reflecting minimal spoilage while still being safe for consumption. On day 7, the haddock sample's indicator changed to yellowish-brown, indicating spoilage, while the beef and chicken maintained a dark purple hue, suggesting differing spoilage mechanisms between seafood and meat products. By day 9, all meat indicators had also shifted to yellowish-brown, indicating spoilage. Notably, the fish began to exhibit watery

conditions by day 2, while the meat products followed by day 3. In order to evaluate the stability of pH indicators, samples were kept for one month after monitoring the shelf life (over 9 days). Despite the high humidity levels in the package, the pH indicators demonstrated excellent color stability, although the product samples appeared visibly watery and squishy (**supplementary material**).

NH₃ generally increases throughout the storage period for all types of meat, indicating ongoing spoilage. TMA shows a peak around Day 5, followed by a decrease, likely due to its reactivity and potential evaporation. DMA remains relatively stable across the storage period, indicating it may be less affected by spoilage processes compared to NH₃ and TMA. NH₃, a key volatile compound in TVB-N, significantly contributes to color changes during fish spoilage, as noted in other studies [41, 42]. This observation can be attributed to ammonia is typically more abundant in the early stages of spoilage [43], making it a primary factor in the initial color change of the pH indicator. While TMA becomes more dominant as spoilage progresses [44], accounting for the subsequent changes in the pH indicator's color.

Throughout the 9-day storage period, the pH indicator effectively differentiated the freshness levels of food samples, offering a nuanced assessment that surpasses a simple binary outcome.

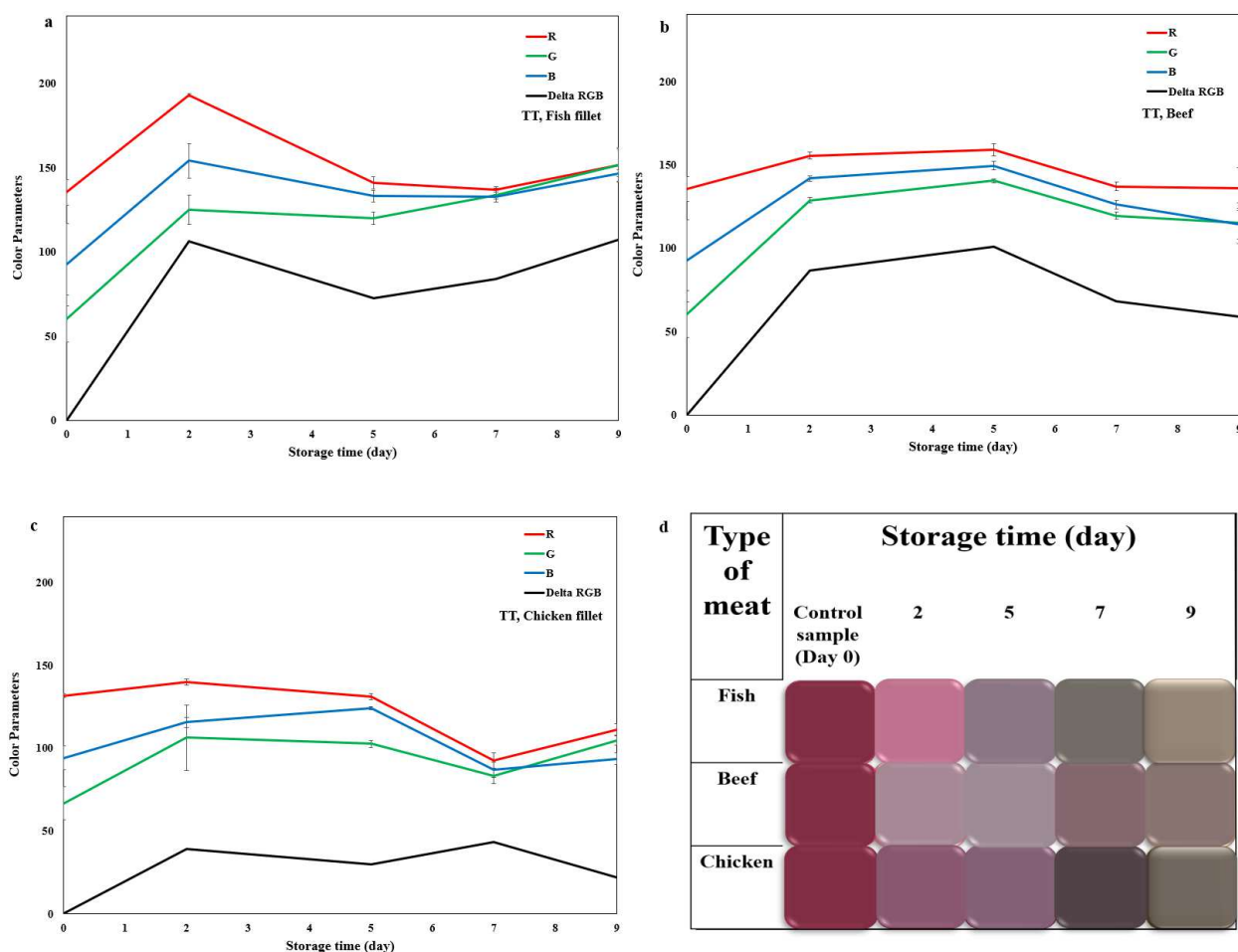


Figure 3 Packaging trials for different types of food products during 9 days of storage at 4 °C, their color parameters (a, b, c) and color matching table (d).

4.3 Rapid volatile gas detection via gas detector tubes: NH_3 and TEA

The color change parameters and the sensor's color change during these simultaneous experiments, as shown in **Figure 4**, were observed by affixing the pH indicator to the upper side of 10 amber vials (60 mL), each containing a homogeneous mixture of fish fillets (approximately 20 g). Over a 9-day period, a gradual but consistent color change in the pH indicator was observed when exposed to fish samples stored at 4 °C temperature. The control sample exhibited no color change, establishing a baseline. By day 2, a noticeable shift occurred, which intensified by day 3. The trend continued, with significant increases noted on days 4-6. Similarly, other research has demonstrated that at 4 °C refrigeration, the chromaticity of the indicator changes gradually [42]. By day 8, the color change had intensified significantly, reaching its peak at the end of the storage period ($\Delta\text{RGB} = 113.53$). This progression indicates an increasing reaction correlating with the spoilage and associated chemical changes in the fish samples over time.

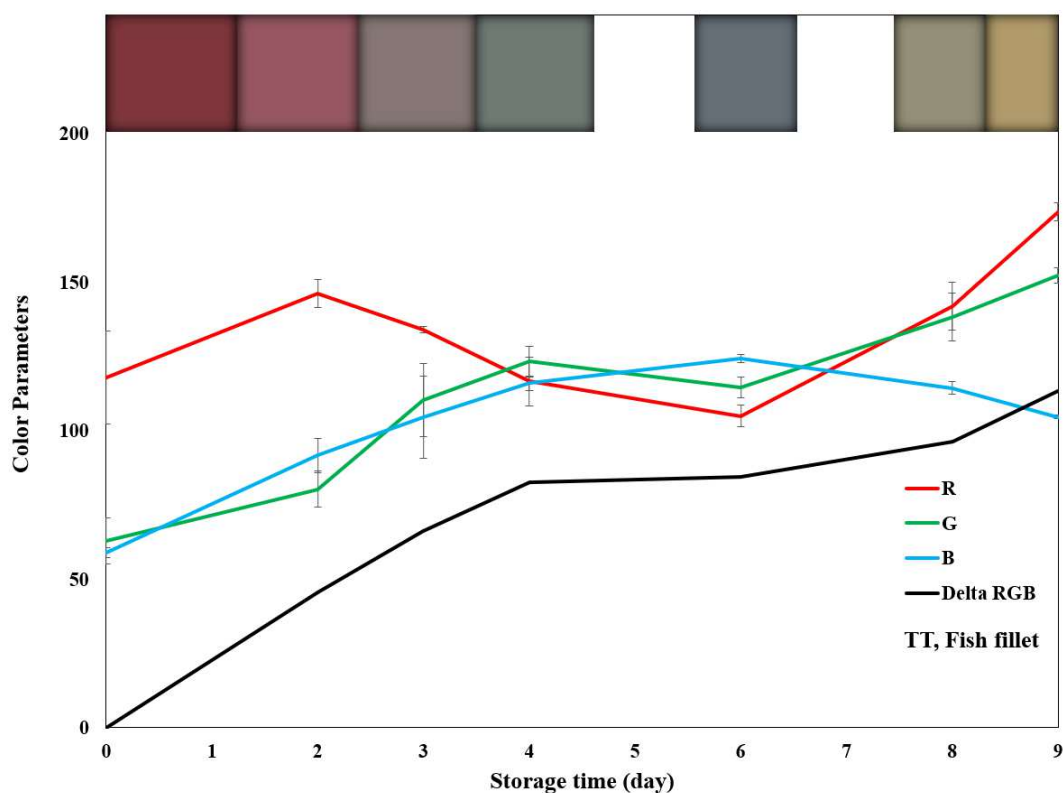


Figure 4 The color change of vials set up with an attached colorimetric sensor containing a homogeneous amount of fish samples, kept for 9 days at 4 °C.

Furthermore, we measured the concentrations of NH_3 and TEA generated from fish samples inside vials with securely affixed valves on their caps (with one duplicate), and we prepared the samples uniformly. By using separate vials for each day, we can effectively reduce the risk of gas leakage that can occur when repeatedly sampling from a single vial. This approach helps maintain the integrity of the gas measurements and ensures that we capture the full concentration of NH_3 and TEA gases produced during spoilage.

The concentration of TEA during the initial two days (0–2) was below the detection threshold of this gas detector type. It recommends that the concentration of TEA should be below 10 ppm [45, 46]. The gas detector showed the presence of NH_3 and TEA but may have biases from co-existing amines and mixed nitrogen components in food matrices which could affect accuracy. The results gathered in **Table 1**.

Table 1 Measurement of NH_3 and TEA from vials with valve connected to the gas detector tubes for 9 days storage time at 4 °C

Storage period (days)	0	1	2	3	4	6	7	8	9	10
Concentration (ppm)										

TEA	nd	5<	5<	5	5	5.6	6	10	15	20
						±				
						0.1				
NH₃	nd	0.25	1	1.2	2.5	10	20	15	10	10 ± 0.2
				±	±					
				0.1	0.5					
ND: Not detect, ppm: parts per million										

4.4 Microbial analysis

The microbial analysis was performed on fish fillets during their storage at 4 °C for 9 days, during food packaging trials (**Figure 5**). The TVC of the haddock fillets on the day of fillet purchase (day 0; packaging date) were found to be 3.69 ± 0.02 , while *Pseudomonas spp.* was below the detection limit (log 1.3 CFU/mL) on day 0 and first day of storage. After one day of storage, TVC reached 4.03 ± 0.07 , and the initial load of spoilage bacteria, *Pseudomonas spp.*, reached to 3.2 ± 0.05 . Slow increase in bacterial load occurred during the initial storage period (0 to 3) at 4 °C, indicating that fish samples are fresh. From day 4 to day 7, the TVC increases more noticeably from 5.35 to 6.02. This period represents the rapid spoilage phase, where microbial populations grow quickly. Following day 7, the TVC continues to rise but at a slower rate, reaching 7.09 by day 10. This gradual increase suggests the transition toward a stationary phase. In this phase, the growth rate may begin to balance with cell death rates, indicating that the fish is in a declining but measurable state of spoilage. The results were also consistent with the previous study [37].

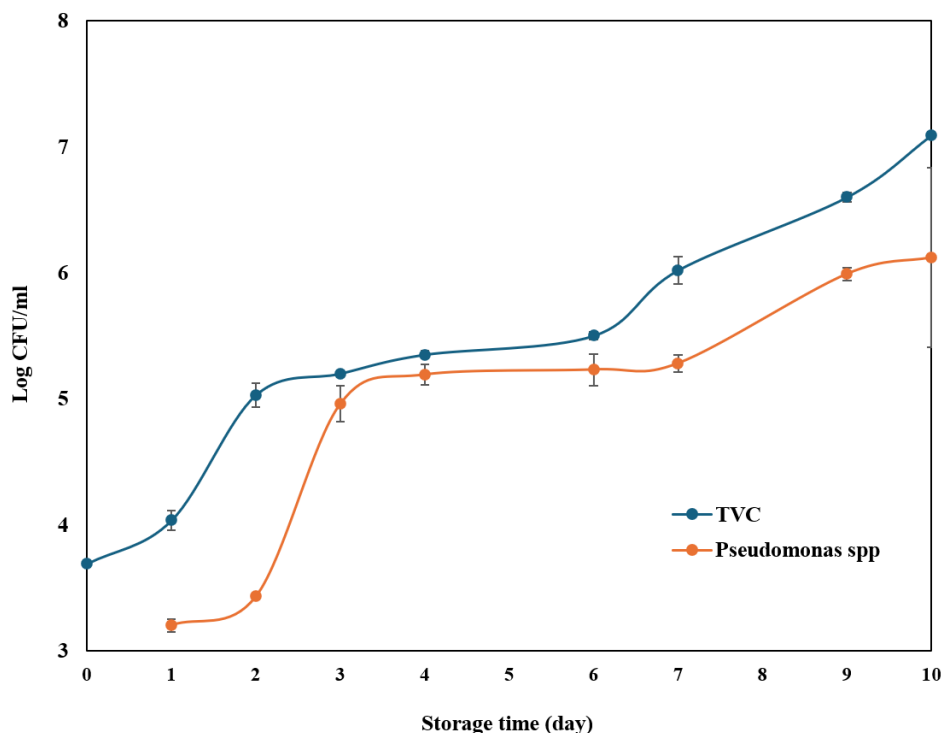


Figure 5 The TVC and *Pseudomonas* spp growth for fish samples during storage at 4 °C for 9 days.

4.5 Optimizing GC-MS: method development and performance assessment

Fish samples are inherently complex as a matrix due to their diverse composition [47], variable water contents [48], the presence of interfering substances, and their potential for degradation and transformation to other compounds during their shelf-life [49]. Therefore it is often challenging to ascertain the exact concentration of specific analytes [50] and to quantify them within fish tissues. This is especially true for small and very volatile molecules such as NH_3 , DMA, and TMA, present in a test portion. This uncertainty complicates the evaluation of the method's success in effectively extracting these compounds from the sample matrix. One effective approach to assess the extraction efficiency involves spiking test portions of fish samples with known concentrations of the analytes (NH_3 , DMA, and TMA). Following the spiking, these portions are extracted, and the concentrations of the analytes are subsequently measured to evaluate the method's extraction performance. Prepared the calibration curve using standard solutions that closely mimic the sample's real conditions by matching the solvent, adding equivalent amounts of sacrificial reagents, or adjusting the pH [51-53]. This helps minimize possible interference from the solution background [54-56]. Injecting a blank solution, or one taken before the reaction starts, is also highly recommended to correct the sample measurement results [57, 58].

Both scan and SIM acquisition modes were used during this project for characterisation of volatile species and quantification of targeted analytes respectively. The m/z presented in the instrumental conditions were selected by injection of the individual standard of ammonia, DMA, TMA and diethyl- d_{10} -amine as internal standard.

The analytical method was briefly optimized on a GS-Q column; however, the CP-Volamine column can provide better performance due to its specific design for separation of volatile amines [59], even in the presence of water [60-62]. A compromise in the GC gradient was selected to have an appropriate retention for ammonia while allowing to separate closely eluting DMA and TMA with acceptable peak shapes. To analyze multiple compounds by GC-MS, it is necessary to ensure that all target compounds can be adequately separated qualitatively and quantitatively [63, 64].

Different split ratios were tested and spitless injections were selected to achieve as low LODs as possible. The headspace temperature and equilibration times were also optimized to increase the release of ammonia, DMA and TMA to the gas phase of the headspace vial. Headspace vials with a volume of 20 mL were used to maximize the fish sample mass (approximately 10 g) that can be analyzed by the instrument. The trials with larger amounts of fish (approx. 70 g) in 250 mL glass flask did not yield greater signal intensities and this approach was thus discarded due to the use of manual injection which is less precise when compared to the automated headspace extraction.

4.6 Gas analytes quantification essays

Matrix-matched calibration trials were performed by spiking the targeted analytes onto fresh fish samples. Only a limited calibration range was obtained: A lower release of targeted analytes to the headspace was observed, impacting the LODs while the signal intensities were rapidly saturated limiting the potential upper limit of quantification. A negative effect on chromatographic performance was caused when injecting a spiked fish sample, resulting in peak broadening and coelution of DMA and TMA. Much lower concentrations of same spiked amounts were observed in real matrix when compared to pure solvent calibration solutions. These results implied a strong interaction between the analytes and the matrix components. This high matrix effect suggested that the quantification of NH_3 and TMA in the fish's headspace might not be directly comparable to solvent standards. However, the method performance allowed trend analysis of the targeted analytes during fish storage without the success of an accurate quantification.

4.7 Method validation and calibration curve

The method validation was primarily conducted in accordance with the Eurachem Guide [65]. As mentioned in the previous section, linear calibration curves could still be obtained for NH_3 and TMA over a limited range when spiking them on to the fish samples (**Figure 6**). The linear ranges were first examined to determine how sensitive the instruments are and whether they respond linearly at concentrations expected for real samples [66].

Calibrations curves in pure water as a solvent (**Figure 7**) led to linear curves with correlation coefficient values (R^2) over 0.99. It was observed that TMA signal is rapidly saturated, limiting the calibration range compared to ammonia. On the other hand, TMA is much more sensible than ammonia, allowing for injection of lower calibration points. This is reflected in the instrumental LODs obtained from injection of low spiked concentrations in solvent. Lower spiked concentrations allowed to calculate TMA instrumental LOD that is 30 ppm, while the value for ammonia is 250 ppm. Their linear ranges are respectively from 250 to 10 000 ppm and from 2 500 to 38 000 ppm. The **Figure 8** and **Figure 9** present the calibration curve for methanol and ethanol for fish samples and solvent. MeOH and EtOH were not

included in the optimization phase, but that first scan tests showed they might be present in the standard mixtures as well, so they were added for the storage/trend's steps. The LOD for MeOH 20 ppm and for EtOH is 10 ppm.

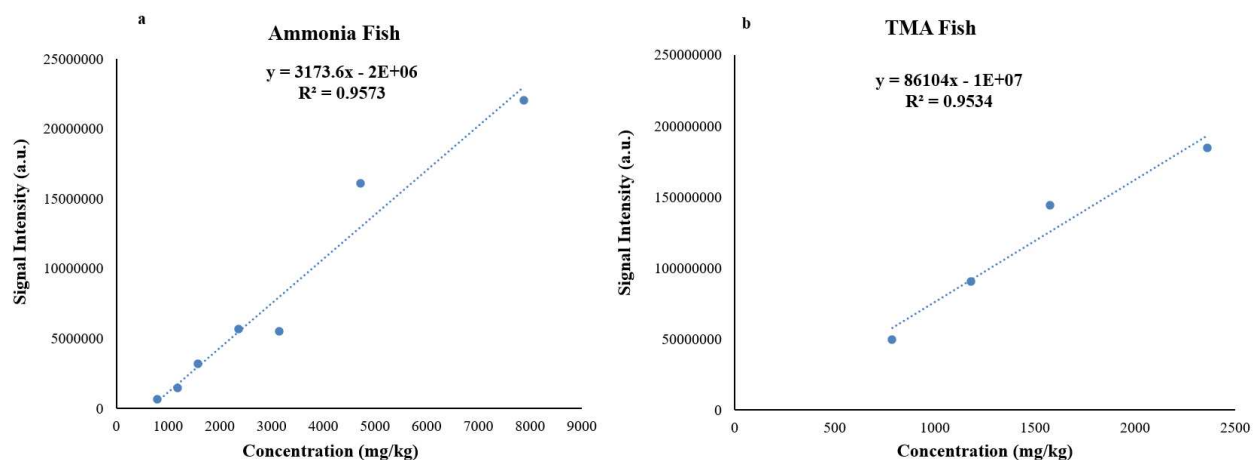


Figure 6 Calibration curves of individual targeted analytes when spiking on fish (NH₃ :a) and (TMA: b).

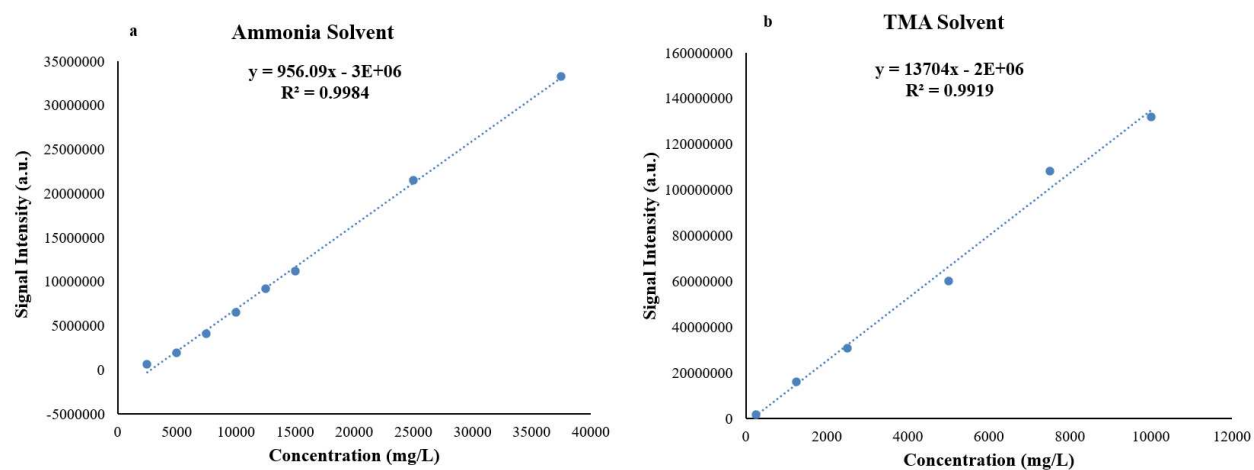


Figure 7 Calibration curves of individual targeted analytes when spiking on solvent (NH₃ :a) and (TMA: b).

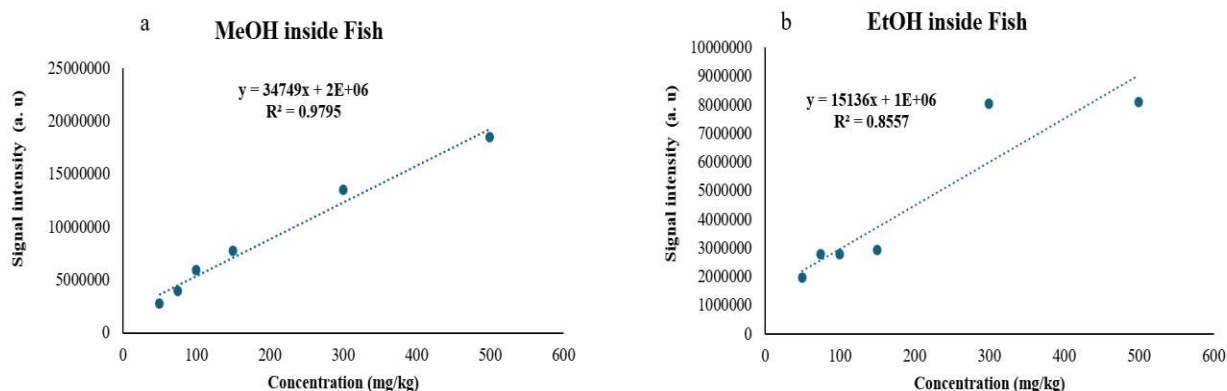


Figure 8 Calibration curves of individual targeted analytes when spiking on fish (MeOH :a) and (EtOH: b).

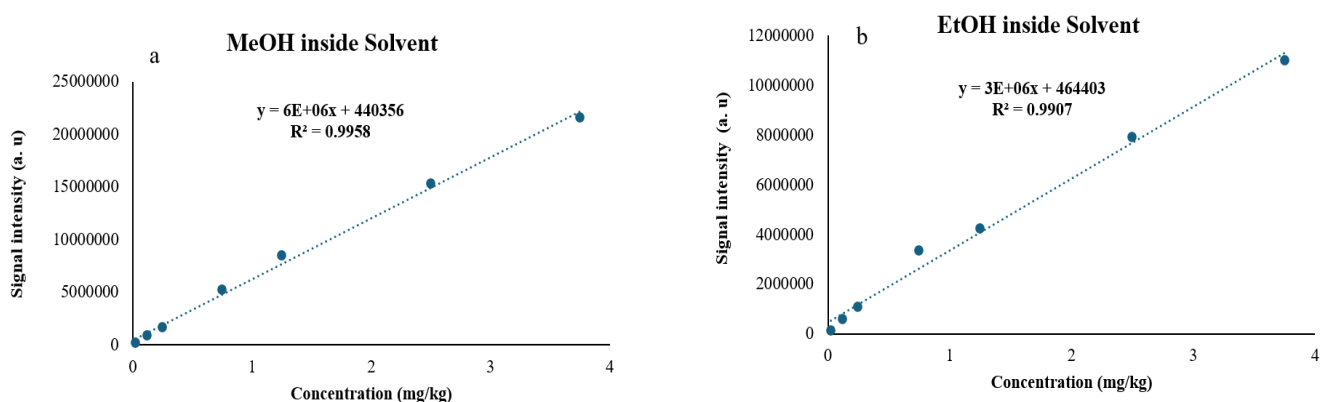


Figure 9 Calibration curves of individual targeted analytes when spiking on solvent (MeOH :a) and (EtOH: b).

4.8 Quality control measures

Quality control (QC) solutions (spiked in solvent) allowed to calculate the system precision for injections on six different days. The RSD values are found to be 4.6 % for ammonia and 8.2 % for TMA. These values are deemed acceptable for this kind of experimental conditions [67]. Even though an accurate quantification could be achieved, the LODs and precision of the method were deemed acceptable to look at the trends in the compounds formed during fish sample storage. Since the area of the peaks were used for the calibration curves and the trends, area RSD values show in **Table 2**.

Table 2 The precisions calculated as RSD of QC injections over 6 different days. Since values are under 10% for the targeted analytes, this means that the method is precise.

	NH ₃	MeOH	EtOH	TMA
RSD (%)	4.6	4.9	6.9	8.2

4.9 Trend via GC-MS during shelf-life

In analyzing the headspace of fish samples stored at 4°C, it is still possible to observe trends in the non-quantifiable signal intensities of the different detected species. One study highlighted the importance of method development for detection and quantification of TMA and DMA during cod fillets shelf-life under melting ice conditions using solid-phase microextraction- gas chromatography-mass spectrometry (SPME-GC-MS). It revealed that TMA and DMA were not effective indicators for detecting the initial decrease in the freshness of the fish samples [68]. Additionally, using more specialized analytical tools such as headspace-SPME-GC-MS has shown that the release of volatile amines is influenced by storage conditions and the fish matrix [39]. These compounds can be correlated with fish freshness [69]. Alternatively, utilizing an ammonia ion-selective electrode (ISE) can measure NH₃ and TMA in fish samples [70]. However, these methods required more specialized equipment, thereby limiting their accessibility for routine analysis.

Figure 10 shows that the amount of NH₃ seems to be increasing the first day and then is decreasing up to day 9 where the concentration is smaller than initial concentration (at day 0). TMA seems to increase from day 0 to day 3 with a jump in concentration at day 4. The signal is then slowly decreasing for the remaining days of the experiment. The increased moisture content of the fish as it approaches spoilage likely causes volatile amines to remain in the water phase rather than participating in the headspace. Since the vials were heated to 80 °C, the volatile amines might have a greater affinity for water, leading to their probable evaporation during the headspace-GC-MS process [71]. NH₃ and TMA are highly reactive compounds that can change their form during fish spoilage [72]. This reactivity contributes to their reduced presence in the headspace, which can affect their collection by GC-MS [72]. Despite this decrease in TMA concentration, colorimetric sensors sensitive to TMA continue to show color changes, indicating ongoing chemical reactions or the presence of TMA in the fish samples. The phenomena affecting the concentration of TMA in fish during storage can be attributed to leaching [73] and phase partitioning [71]. As fish spoil, the increased water content leads to volatile amines, like TMA, remaining in the aqueous phase rather than being released into the headspace. This behavior is influenced by the solubility of TMA in water and the physical changes occurring in the fish tissue as it deteriorates. As observed in a recent study, TMA could not be detected using GC/MS without first heating the samples to 200 °C for 10 minutes [74].

To evaluate the difference between the storage headspace content and the total amount of the targeted analytes, a headspace GC-MS method without equilibration at 80 °C could have been used in parallel with the presented method. Although less sensitive, this second method could have provided more information about the partitioning of volatile amines between the fish matrix and the packaging headspace. This approach could also have led to results that better align with the colorimetric sensor data.

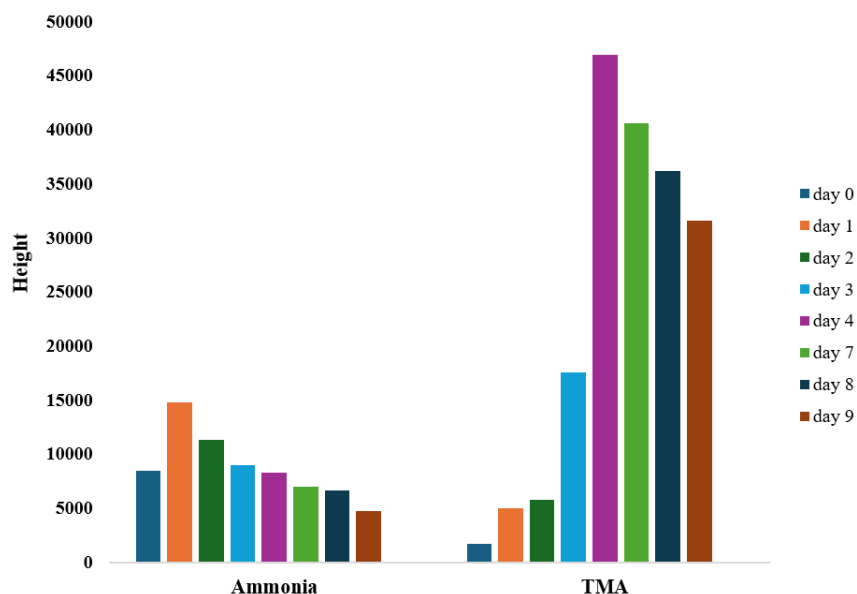


Figure 10 Trends in the signal intensities of targeted analytes over 9-day storage in the fridge (SIM mode acquisition).

4.10 Scan mode for detection of other analytes

Some untargeted species appear to be released to the headspace after different storage times and could be detected by scan mode injections (**Table 3**). Identification of these compounds is made using NIST database comparison of the most intense peak over the 9-day experiment period. High scores ($> 89\%$) indicate that the mass spectra obtained by scan mode acquisition are matching the theoretical spectra. During the analysis, peaks for methanol and ethanol were observed in the GC-MS spectra. Initially, these compounds were not the primary targets of the study. However, upon their detection, calibration curves were generated, and their LOD were determined to quantify these compounds. This step was taken to provide additional valuable information, given that their presence could offer significant insights into the sample's composition. Consequently, methanol and ethanol were included in the results, despite not being part of the initial target analytes. Injection of an ethanol standard allowed for further confirmation since its retention time matches the one in the samples. Although the scan acquisition mode is less sensitive than SIM analysis, some trends could be observed for some of these compounds (**Figure 2**).

The observed variations in the signals of the compounds in the fish samples stored at $4\text{ }^{\circ}\text{C}$ can be attributed to several biochemical and microbial processes. The increase in ethanol levels during the first eight days, followed by a decrease on the last day, suggests fermentation processes likely facilitated by microbial activity. Various yeasts and bacteria are known to convert sugars into ethanol under anaerobic conditions [75]. The transient elevation of DMS on days 3 and 4 may relate to the breakdown of sulfur-containing amino acids, which is often indicative of microbial spoilage or the degradation of fish tissue [76, 77], and then its amount was stable for the remaining days. The detection of methanethiol starting from day 7, alongside its subsequent increase, points to further microbial activity and the breakdown of organic sulfur compounds, leading to the production of volatile compounds associated with spoilage

[78]. Butane does not show a clear pattern while pentane, when discarding day 2, seems to reach its maximum on day 3 and then, generally, slowly decreases until day 9. These observations underscore the importance of monitoring volatile compounds to assess the freshness and spoilage of seafood products.

Table 3 Identification of detected species using NIST database comparison for scan mode injections.

Retention time (min)	Compound name	Score (%)
11.2	Methanethiol	95
12.1	Butane	89
13.8	Ethanol	98
14.7	Dimethyl Sulfide	96
15.4	Pentane	94
15.9	Trimethylamine	99

5. Conclusion

This study successfully demonstrates the effectiveness of colorimetric sensing as a practical tool for monitoring seafood freshness through the detection of volatile compounds. The experiment was conducted at $T = 40\text{ }^{\circ}\text{C}$ over 30 minutes, indicating specific storage conditions. ΔRGB shows the sensitivity of the pH indicator to the presence of TVB-N gases. As the concentration of each gas increases, ΔRGB shows a significant decline, particularly at the 0.01 M concentration for each analyte (170.4, 450.9, and 591.1 ppm for NH_3 , DMA, and TMA, respectively), which is statistically significant. The consistent decrease in the red channel across all gases suggests that the pH indicator is effectively responding to the chemical changes induced by the TVB-N gases, leading to a shift in color that can be quantitatively measured through RGB values.

This study aims to evaluate the color response of on-package pH-sensitive anthocyanin dye sensors using headspace GC-MS analysis and microbial analysis to provide a better understanding of the performance of the introduced pH indicator. Additionally, the development of a four-point freshness scale offers a user-friendly method for assessing fish freshness over time, highlighting the potential applications of this intelligent packaging system in ensuring food safety and quality.

Furthermore, the comprehensive analysis of volatile gas concentrations, microbial growth, and colorimetric changes throughout the storage period highlights the multifaceted nature of food spoilage. For GC-MS analysis, amongst the different approaches, the automated headspace injections using 20 mL vials was deemed the more appropriate to analyze volatile species released during fish storage. Linear calibration curves are obtained by spiking standards in pure water, but a significant matrix effect did not allow for an accurate quantification in real fish samples. This effect, along with signal saturation, also led to limited calibration range for the matrix-matched calibration essays on fresh fish. However, the LODs and precisions of pure solvent spiked solutions were deemed acceptable and allowed for trends analysis of compounds produced during storage of fish samples. Such trends were observed for the ammonia

and TMA as well as for some untargeted analytes such as ethanol and butane. None of the analyzed headspace of spoiled fish contained detectable amounts of DMA. These results can contribute to future innovations in packaging technologies and freshness indicators, ultimately enhancing food quality and consumer safety.

5.1 Limitations/challenges and future work

The complexity of fish samples makes it difficult to determine the exact concentrations of analytes such as ammonia NH_3 , DMA, and TMA. To assess extraction efficiency, test portions can be spiked with known concentrations of these analytes, followed by extraction and measurement. However, a key limitation of this method is that spiked analytes may not bind as strongly as those naturally present, leading to an overestimation of extraction efficiency. This underscores the need for careful interpretation of results from spiked samples in complex matrices. The fact that much lower signals are observed in real fish sample when compared with same amount of spiked standard in pure water indicate an important matrix effect. This and the fact that only a very limited linear range is obtained with a matched-matrix calibration approach, render the quantification of these species very challenging. This matrix effect also had an impact on chromatographic performances, causing the coelution of DMA and TMA. A better retention, sharper peaks and greater signal intensity might be achieved using a more specific column for volatile amines and ammonia such as CP-Volamine but it was not available.

6. Acknowledgements

I would like to express my sincere gratitude to Dr. Alexandra Furtos-Matei, PhD, Mass Spectrometry Facility Manager of the Chemistry Department at the University of Montréal, for her invaluable collaboration and support throughout the current research project. Her expertise and guidance significantly contributed to the success of this work. The funding for this research project is gratefully acknowledged from the Natural Sciences and Engineering Research Council of Canada (NSERC), 3Spack Industrial Research Chair, Research Center for High Performance Polymer and Composite Systems (CREPEC), Chemical Engineering Department, Polytechnique Montréal, ProAmpac flexible packaging, and Prima.

7. Reference

- [1] A. Gordon, "Introduction: effective implementation of food safety and quality systems: prerequisites and other considerations," in *Food Safety and Quality Systems in Developing Countries*: Elsevier, 2017, pp. 1-19.
- [2] J. Li *et al.*, "Spoilage microbes' effect on freshness and IMP degradation in sturgeon fillets during chilled storage," *Food Bioscience*, vol. 41, p. 101008, 2021/06/01/2021, doi: <https://doi.org/10.1016/j.fbio.2021.101008>.
- [3] M. Etienne, "Volatile amines as criteria for chemical quality assessment. Seafoodplus traceability," 2005.
- [4] E. Uhlig, M. Bucher, M. Strenger, S. Klob, and M. Schmid, "Towards Reducing Food Wastage: Analysis of Degradation Products Formed during Meat Spoilage under Different Conditions," *Foods*, vol. 13, no. 17, p. 2751, 2024. [Online]. Available: <https://www.mdpi.com/2304-8158/13/17/2751>.

- [5] C. E. Realini and B. Marcos, "Active and intelligent packaging systems for a modern society," *Meat science*, vol. 98, no. 3, pp. 404-419, 2014.
- [6] B. S. Sá, C. A. Zito, T. M. Perfecto, and D. P. Volanti, "Porous ZnSnO₃ nanocubes as a triethylamine sensor," *Sensors and Actuators B: Chemical*, vol. 338, p. 129869, 2021.
- [7] N. H. Tan *et al.*, "Metal-decorated indium oxide nanofibers used as nanosensor for triethylamine sensing towards seafood quality monitoring," *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, p. 135268, 2024.
- [8] G.-Y. Lee, K.-J. Lim, Y.-H. Lee, and H.-S. Shin, "Development of a Freshness Indicator for Assessing the Quality of Packaged Pork Products during Refrigerated Storage," *Foods*, vol. 13, no. 13, p. 2097, 2024.
- [9] J. Chen *et al.*, "Critical review and recent advances of emerging real-time and non-destructive strategies for meat spoilage monitoring," *Food Chemistry*, p. 138755, 2024.
- [10] M. Ameri, A. Ajji, and S. Kessler, "Characterization of a Food-Safe Colorimetric Indicator Based on Black Rice Anthocyanin/PET Films for Visual Analysis of Fish Spoilage," *Packaging Technology and Science*, 2024.
- [11] I. M. Karaca, G. Haskaraca, Z. Ayhan, and E. Gültekin, "Development of real time-pH sensitive intelligent indicators for monitoring chicken breast freshness/spoilage using real packaging practices," *Food Research International*, vol. 173, p. 113261, 2023.
- [12] Y. Ozogul, "Methods for freshness quality and deterioration," in *Handbook of seafood and seafood products analysis*: CRC Press, 2009, pp. 207-232.
- [13] S. T. Chan, M. W. Y. Yao, Y. C. Wong, T. Wong, C. S. Mok, and D. W. M. Sin, "Evaluation of chemical indicators for monitoring freshness of food and determination of volatile amines in fish by headspace solid-phase microextraction and gas chromatography-mass spectrometry," *European Food Research and Technology*, vol. 224, no. 1, pp. 67-74, 2006/11/01 2006, doi: 10.1007/s00217-006-0290-4.
- [14] P. Malle, P. Eb, and R. Tailliez, "Determination of the quality of fish by measuring trimethylamine oxide reduction," *International Journal of Food Microbiology*, vol. 3, no. 4, pp. 225-235, 1986.
- [15] A. R. Al-Najada, "Quality Assessment of Aquacultured Freshwater Fish Species during Storage in Ice."
- [16] G. Olafsdottir, H. L. Lauzon, E. Martinsdottir, and K. Kristbergsson, "Influence of storage temperature on microbial spoilage characteristics of haddock fillets (*Melanogrammus aeglefinus*) evaluated by multivariate quality prediction," *International Journal of Food Microbiology*, vol. 111, no. 2, pp. 112-125, 2006/09/01/ 2006, doi: <https://doi.org/10.1016/j.ijfoodmicro.2006.04.045>.
- [17] P. Malle and M. Poumeyrol, "A new chemical criterion for the quality control of fish: trimethylamine/total volatile basic nitrogen (%)," *Journal of food protection*, vol. 52, no. 6, pp. 419-423, 1989.
- [18] V. Nikzade, N. Sedaghat, F. T. Yazdi, H. B. Ghoddusi, and M. Saadatmand-Tarzjan, "Development of shelf life kinetic model for fresh rainbow trout (*Oncorhynchus*

- mykiss) fillets stored under modified atmosphere packaging," *Journal of food science and technology*, vol. 56, pp. 663-673, 2019.
- [19] S. Baixas-Nogueras, S. Bover-Cid, M. Veciana-Nogués, and M. Vidal-Carou, "Suitability of volatile amines as freshness indexes for iced Mediterranean hake," *Journal of Food Science*, vol. 68, no. 5, pp. 1607-1610, 2003.
 - [20] S. P. Aubourg, "Damage detection in horse mackerel (*Trachurus trachurus*) during chilled storage," *Journal of the American Oil Chemists' Society*, vol. 78, pp. 857-862, 2001.
 - [21] N. Jalali, P. Ariai, and E. Fattahi, "Effect of alginate/carboxyl methyl cellulose composite coating incorporated with clove essential oil on the quality of silver carp fillet and *Escherichia coli* O157: H7 inhibition during refrigerated storage," *Journal of Food Science and Technology*, vol. 53, pp. 757-765, 2016.
 - [22] C. Altieri, B. Speranza, M. A. Del Nobile, and M. Sinigaglia, "Suitability of bifidobacteria and thymol as biopreservatives in extending the shelf life of fresh packed plaice fillets," *Journal of applied microbiology*, vol. 99, no. 6, pp. 1294-1302, 2005.
 - [23] T. Liang, G. Sun, L. Cao, J. Li, and L. Wang, "A pH and NH₃ sensing intelligent film based on *Artemisia sphaerocephala* Krasch. gum and red cabbage anthocyanins anchored by carboxymethyl cellulose sodium added as a host complex," *Food hydrocolloids*, vol. 87, pp. 858-868, 2019.
 - [24] N. Oladzadabbasabadi, A. M. Nafchi, M. Ghasemlou, F. Ariffin, Z. Singh, and A. Al-Hassan, "Natural anthocyanins: Sources, extraction, characterization, and suitability for smart packaging," *Food Packaging and Shelf Life*, vol. 33, p. 100872, 2022.
 - [25] L. Chen, W. Wang, W. Wang, and J. Zhang, "Effect of Anthocyanins on Colorimetric Indicator Film Properties," *Coatings*, vol. 13, no. 10, p. 1682, 2023. [Online]. Available: <https://www.mdpi.com/2079-6412/13/10/1682>.
 - [26] K. Zhang *et al.*, "Aerogel colorimetric label sensors based on carboxymethyl cellulose/sodium alginate with black goji anthocyanin for monitoring fish freshness," *International Journal of Biological Macromolecules*, p. 130466, 2024.
 - [27] A. Khezerlou, M. Alizadeh Sani, M. Tavassoli, R. Abedi-Firoozjah, A. Ehsani, and D. J. McClements, "Halochromic (pH-Responsive) Indicators Based on Natural Anthocyanins for Monitoring Fish Freshness/Spoilage," *Journal of Composites Science*, vol. 7, no. 4, p. 143, 2023. [Online]. Available: <https://www.mdpi.com/2504-477X/7/4/143>.
 - [28] X. Zhai *et al.*, "Novel colorimetric films based on starch/polyvinyl alcohol incorporated with roselle anthocyanins for fish freshness monitoring," *Food Hydrocolloids*, vol. 69, pp. 308-317, 2017.
 - [29] M. Moradi, H. Tajik, H. Almasi, M. Forough, and P. Ezati, "A novel pH-sensing indicator based on bacterial cellulose nanofibers and black carrot anthocyanins for monitoring fish freshness," *Carbohydrate Polymers*, vol. 222, p. 115030, 2019.
 - [30] S. Ibrahim, H. Fahmy, and S. Salah, "Application of interactive and intelligent packaging for fresh fish shelf-life monitoring," *Frontiers in Nutrition*, vol. 8, p. 677884, 2021.

- [31] B. Wang *et al.*, "Intelligent Evaluation and Dynamic Prediction of Oysters Freshness with Electronic Nose Non-Destructive Monitoring and Machine Learning," *Biosensors*, vol. 14, no. 10, p. 502, 2024.
- [32] X. Luo, "Colorimetric Indicator Systems for the Detection of Amines/Terpenes in Intelligent Food Packaging," University of Guelph, 2021.
- [33] A. A. Ameri M, Kessler S, "Enhancing Seafood Freshness Monitoring: Integrating Color Change of a Food-Safe On-Package Colorimetric Sensor with Mathematical Models, Microbiological, and Chemical Analyses," *ChemRxiv*, 2024, doi: 10.26434/chemrxiv-2024-wxnz6.
- [34] D. N. Dikmetas, E. Uysal, F. Karbancioglu-Guler, and S. Gurmen, "The production of pH indicator Ca and Cu alginate ((1, 4)- β -d-mannuronic acid and α -l-guluronic acid) cryogels containing anthocyanin obtained via red cabbage extraction for monitoring chicken fillet freshness," *International Journal of Biological Macromolecules*, vol. 231, p. 123304, 2023.
- [35] D. Karimi Alavijeh, "Development of Films/Fibrous Nanostructures with Gas and Volatiles Detection Ability," Master's thesis, Department of Chemical Engineering, Polytechnique Montréal, 2020. [Online]. Available: <https://publications.polymtl.ca/4221/>
- [36] A. Sobhan, ""Development of Bio-based Nanocomposites for Biosensor and Indicator Applications in Smart Food Packaging," PhD, Agricultural, Biosystems, & Mechanical Engineering South Dakota State University, 2021. [Online]. Available: <https://openprairie.sdstate.edu/etd/5792>
- [37] M. Ameri, A. Ajji, and S. Kessler, "Enhancing Seafood Freshness Monitoring: Integrating Color Change of a Food-Safe On-Package Colorimetric Sensor with Mathematical Models, Microbiological, and Chemical Analyses," *Current Research in Food Science*, p. 100934, 2024/11/23/ 2024, doi: <https://doi.org/10.1016/j.crfs.2024.100934>.
- [38] T. Zhu, Z. Li, X. Liu, C. Chen, and Y. Mu, "Comparative Analysis of Microbial Diversity and Metabolic Profiles during the Spontaneous Fermentation of Jerusalem Artichoke (*Helianthus tuberosus* L.) Juice," *Plants*, vol. 13, no. 19, p. 2782, 2024.
- [39] S. T. Chan, M. W. Yao, Y. Wong, T. Wong, C. Mok, and D. W. Sin, "Evaluation of chemical indicators for monitoring freshness of food and determination of volatile amines in fish by headspace solid-phase microextraction and gas chromatography-mass spectrometry," *European Food Research and Technology*, vol. 224, pp. 67-74, 2006.
- [40] A. Al-Rashdan, M. I. Helaleh, A. Nisar, A. Ibtisam, and Z. Al-Ballam, "Determination of the levels of polycyclic aromatic hydrocarbons in toasted bread using gas chromatography mass spectrometry," *International journal of analytical chemistry*, vol. 2010, no. 1, p. 821216, 2010.
- [41] S. Dodange, H. Shekarchizadeh, and M. Kadivar, "A simple colorimetric volatile nitrogen and hydrogen sulfide indicator based on filter paper and saffron petal anthocyanins to monitor fish spoilage in intelligent packaging," *Food and Bioprocess Technology*, vol. 17, no. 7, pp. 1785-1796, 2024.

- [42] D.-Y. Kim, S.-W. Park, and H.-S. Shin, "Fish freshness indicator for sensing fish quality during storage," *Foods*, vol. 12, no. 9, p. 1801, 2023.
- [43] Y. K. Ip and S. F. Chew, "Ammonia production, excretion, toxicity, and defense in fish: a review," *Frontiers in physiology*, vol. 1, p. 134, 2010.
- [44] H.-N. Chun, J.-H. Cho, and H.-S. Shin, "Influence of different storage conditions on production of trimethylamine and microbial spoilage characteristics of mackerel products," *Food Science and Biotechnology*, vol. 23, pp. 1411-1416, 2014.
- [45] F. Gu, Y. Cui, D. Han, S. Hong, M. Flytzani-Stephanopoulos, and Z. Wang, "Atomically dispersed Pt (II) on WO₃ for highly selective sensing and catalytic oxidation of triethylamine," *Applied Catalysis B: Environmental*, vol. 256, p. 117809, 2019/11/05/ 2019, doi: <https://doi.org/10.1016/j.apcatb.2019.117809>.
- [46] Y. H. Cai, S. Y. Ma, and J. S. Wei, "Highly sensitive triethylamine sensor with ppb level detection limit based on SnSe nanofibers," *Ceramics International*, vol. 49, no. 10, pp. 15333-15340, 2023/05/15/ 2023, doi: <https://doi.org/10.1016/j.ceramint.2023.01.117>.
- [47] Z. Coppes Petricorena, "Chemical Composition of Fish and Fishery Products," in *Handbook of Food Chemistry*, P. C. K. Cheung and B. M. Mehta Eds. Berlin, Heidelberg: Springer Berlin Heidelberg, 2015, pp. 403-435.
- [48] M. a. I. Yeannes and M. a. E. Almandos, "Estimation of fish proximate composition starting from water content," *Journal of Food Composition and Analysis*, vol. 16, no. 1, pp. 81-92, 2003.
- [49] D. Martin, C. Joly, C. Dupas-Farrugia, I. Adt, N. Oulahal, and P. Degraeve, "Volatilome Analysis and Evolution in the Headspace of Packed Refrigerated Fish," *Foods*, vol. 12, no. 14, p. 2657, 2023. [Online]. Available: <https://www.mdpi.com/2304-8158/12/14/2657>.
- [50] D. Sundaravadivelu *et al.*, "Determination of cyanotoxins and prymnesins in water, fish tissue, and other matrices: A review," *Toxins*, vol. 14, no. 3, p. 213, 2022.
- [51] W. P. Utomo, H. Wu, and Y. H. Ng, "Quantification methodology of ammonia produced from electrocatalytic and photocatalytic nitrogen/nitrate reduction," *Energies*, vol. 16, no. 1, p. 27, 2022.
- [52] K. Johanning *et al.*, "Assessment of metabolic stability using the rainbow trout (*Oncorhynchus mykiss*) liver S9 fraction," *Current Protocols in Toxicology*, vol. 53, no. 1, pp. 14.10. 1-14.10. 28, 2012.
- [53] I. A. Haidar Ahmad, "Necessary analytical skills and knowledge for identifying, understanding, and performing HPLC troubleshooting," *Chromatographia*, vol. 80, no. 5, pp. 705-730, 2017.
- [54] C. Ferrer, A. Lozano, A. Agüera, A. J. Girón, and A. Fernández-Alba, "Overcoming matrix effects using the dilution approach in multiresidue methods for fruits and vegetables," *Journal of Chromatography A*, vol. 1218, no. 42, pp. 7634-7639, 2011.
- [55] K. S. Booksh and B. R. Kowalski, "Theory of analytical chemistry," *Analytical Chemistry*, vol. 66, no. 15, pp. 782A-791A, 1994.
- [56] J. Zhao and M. Riediker, "Detecting the oxidative reactivity of nanoparticles: a new protocol for reducing artifacts," *Journal of nanoparticle research*, vol. 16, pp. 1-13, 2014.

- [57] K. Grob, *Split and splitless injection for quantitative gas chromatography: concepts, processes, practical guidelines, sources of error*. John Wiley & Sons, 2008.
- [58] S. Mitra, *Sample preparation techniques in analytical chemistry*. John Wiley & Sons, 2004.
- [59] A. Technologies. "Optimized Analysis of Ammonia and Volatile Amines Using the Agilent J&W CP-Volatile Column." <https://www.agilent.com/cs/library/applications/an-ammonia-jw-cp-volatile-990-micro-gc-5994-4346en-agilent.pdf> (accessed).
- [60] I. T. Cunha, H. Yang, and P. G. Jessop, "High pressure switchable water: An alternative method for separating organic products from water," *Green Chemistry*, vol. 23, no. 11, pp. 3996-4007, 2021.
- [61] M. Shou and H. Qiu, "Development of a rapid GC-FID method to simultaneously determine triethylamine, diisopropylamine, and 1, 1, 3, 3-tetramethylguanidine residues in an active pharmaceutical ingredient," *Journal of Pharmaceutical Analysis*, vol. 11, no. 2, pp. 251-256, 2021.
- [62] Y. Huang *et al.*, "Highly Efficient Compositional and Compound Specific Isotopic Analysis of Volatile Primary Amines and Ammonia in the Murchison Meteorite Using SPME On-Fiber Derivatization: Optimization for Bennu Sample Analyses," *Rapid Communications in Mass Spectrometry*, vol. 39, no. 7, p. e9979, 2025.
- [63] M.-L. Xu, Y. Gao, X. Wang, X. X. Han, and B. Zhao, "Comprehensive Strategy for Sample Preparation for the Analysis of Food Contaminants and Residues by GC-MS/MS: A Review of Recent Research Trends," *Foods*, vol. 10, no. 10, p. 2473, 2021. [Online]. Available: <https://www.mdpi.com/2304-8158/10/10/2473>.
- [64] M. L. Xu, Y. Gao, X. Wang, X. X. Han, and B. Zhao, "Comprehensive Strategy for Sample Preparation for the Analysis of Food Contaminants and Residues by GC-MS/MS: A Review of Recent Research Trends," (in eng), *Foods*, vol. 10, no. 10, Oct 15 2021, doi: 10.3390/foods10102473.
- [65] B. M. a. U. Ö. (eds.). "Eurachem Guide: The Fitness for Purpose of Analytical Methods – A Laboratory Guide to Method Validation and Related Topics." (accessed).
- [66] N. Lorenzo-Parodi, E. Leitner, and T. C. Schmidt, "Comparison of gas chromatographic techniques for the analysis of iodinated derivatives of aromatic amines," (in eng), *Anal Bioanal Chem*, vol. 415, no. 17, pp. 3313-3325, Jul 2023, doi: 10.1007/s00216-023-04713-8.
- [67] V. T. GL49. "Guidelines for the Validation of Analytical Methods Used in Residue Depletion Studies." International Cooperation on Harmonisation of Technical Requirements for Registration of Veterinary Medicinal Products (VICH). https://www.ema.europa.eu/en/documents/scientific-guideline/vich-topic-gl49-step-4-guidelines-validation-analytical-methods-used-residue-depletion-studies_en.pdf?form=MG0AV3 (accessed).
- [68] A. Dehaut *et al.*, "Development of an SPME-GC-MS method for the specific quantification of dimethylamine and trimethylamine: use of a new ratio for the

- freshness monitoring of cod fillets," *Journal of the Science of Food and Agriculture*, vol. 96, no. 11, pp. 3787-3794, 2016.
- [69] G. Xuan, M. Guo, H. Lin, J. Sui, and J. Wang, "Study on volatile chemicals as spoilage indexes of salmon by HS-SPME-GC-MS technique during non-frozen storage," *Molecules*, vol. 28, no. 1, p. 13, 2022.
- [70] L. F. Pivarnik, M. Thiam, and P. Christopher Ellis, "Rapid Determination of Volatile Bases in Fish by Using an Ammonia Ion-Selective Electrode," *Journal of AOAC INTERNATIONAL*, vol. 81, no. 5, pp. 1011-1022, 2020, doi: 10.1093/jaoac/81.5.1011.
- [71] C. RUIZ-CAPILLAS, C. M. Gillyon, and W. F. HORNER, "Determination of different volatile base components as quality control indices in fish by official methods and flow injection analysis," *Journal of food biochemistry*, vol. 25, no. 6, pp. 541-553, 2001.
- [72] F. A. Bullard and J. Collins, "An improved method to analyze trimethylamine in fish and the interference of ammonia and dimethylamine," *Fish. Bull*, vol. 78, no. 2, pp. 465-437, 1980.
- [73] C. Ruiz-Capillas, C. M. Gillyon, and W. F. Horner, "Determination of volatile basic nitrogen and trimethylamine nitrogen in fish sauce by flow injection analysis," *European Food Research and Technology*, vol. 210, pp. 434-436, 2000.
- [74] J. Luo, D. Frank, and J. Arcot, "Forging Prawn and Salmon Flavours with Non-Animal-Based Ingredients," *Foods*, vol. 14, no. 5, p. 820, 2025. [Online]. Available: <https://www.mdpi.com/2304-8158/14/5/820>.
- [75] J. Lee, "Biological conversion of lignocellulosic biomass to ethanol," *Journal of biotechnology*, vol. 56, no. 1, pp. 1-24, 1997.
- [76] V. Lougovois and V. Kyrana, "Freshness quality and spoilage of chill-stored fish," *Food Policy, Control Res*, vol. 1, pp. 35-86, 2005.
- [77] X. Yu *et al.*, "Rapid assessment of meat freshness by the differential sensing of organic sulfides emitted during spoilage," *ACS sensors*, vol. 7, no. 5, pp. 1395-1402, 2022.
- [78] J. Bai, S. M. Baker, R. M. Goodrich-Schneider, N. Montazeri, and P. J. Sarnoski, "Aroma profile characterization of Mahi-Mahi and Tuna for determining spoilage using purge and trap gas chromatography-mass spectrometry," *Journal of Food Science*, vol. 84, no. 3, pp. 481-489, 2019.

8. Appendices

Table 1.A Limit of detection with (TVB-N gases) at accelerated storage condition.

Type of standard	Concentration	Mean R	Mean G	Mean B	Std R	Std G	Std B	ΔRGB
NH ₃	0	204.66	41	96	4.49	16.26	8.6	0
	0.001	190.66	109.66	133.33	5.24	1.24	2.62	78.15193**
	0.01	179	137	146	6.37	6.48	5.71	108.8667***

	0.1	206.66	146.66	145.66	11.95	8.49	8.49	117.8395***
	1	174	111	102.33	4.08	2.16	4.18	72.23313**
TMA	0	204.66	41	96	4.49	16.26	8.6	0
	0.001	180	120	132.33	3.55	6.16	5.43	90.38243**
	0.01	212.33	179.66	182	3.09	2.86	2.94	163.3445***
	0.1	137.66	85.66	90.33	7.31	7.31	6.84	80.71967
	1	161	126.66	104.66	0	3.29	1.24	96.53407
DMA	0	204.66	41	96	4.49	16.26	8.6	0
	0.001	123	18.66	40.66	2.82	7.84	7.84	101.1432***
	0.01	106	7.66	68.33	8.48	4.71	4.92	107.7543***
	0.1	88.66	80.33	68.33	6.01	6.54	4.92	125.5726***
	1	80	64.66	33	9.41	8.57	9.27	141.6648***
All values were expressed as mean $\pm$ standard deviation (n = 3).								
Level of significance codes: P-value<0.01 ***, P-value<0.05 **, P-value <0.1 *								

Freshness control in room temperature with gas tube detector:

At refrigerated temperatures (0 till 4 ° C), fish typically remain fresh for several days, and ammonia production is minimal. According to several measurement, no color change was observed in gas detector tubes regarding presence of ammonia for 8 hours. Suggesting that the fish is fresh. At room temperature, spoilage occurs much more rapidly. Ammonia levels can easily increase within a few hours, depending on the initial freshness of the fish and microbial load. With increasing weights from 50 g to 200 g, the data illustrates a clear trend: larger sample sizes lead to higher ammonia concentrations over time. This can be attributed to a larger amount of organic material being decomposed, resulting in more generated ammonia. as temperatures increase and sample weights increase, spoilage accelerates, leading to faster ammonia production. The room temperature condition exacerbates this issue, making it unsuitable for preserving fish freshness. The measurements indicate that regular monitoring of ammonia levels could serve as an effective method for assessing fish freshness and ensuring product safety.

For the **50 g sample**, ammonia levels increased gradually over time. The concentration reached its highest point (310 ppm) at the 8-hour mark, indicating ongoing spoilage. The concentrations fluctuate slightly, suggesting a relatively stable gradual increase. For the **100 g sample**, the ammonia levels start higher and exhibit a more consistent increase, suggesting a greater rate of decomposition due to potentially higher microbial the initial readings in the first hour were low (0 ppm for 50 g and 15 ppm for 100 g), which is typical as spoilage has not yet initiated within that timeframe. By the end of 8 hours, both samples fell within ppm values that could indicate spoilage, particularly the 100 g sample, which is approaching levels where quality could be considered unacceptable. load or freshness variation. By the 8-hour mark, the concentration reached 390 ppm. The results confirm that storage at room temperature accelerates spoilage in fish. Ammonia levels increase significantly over time, indicating that fish should be stored at lower

temperatures to maintain freshness. The methodology of using an ammonia detector provides a practical means of monitoring fish freshness. However, the initial ammonia concentrations were below detection limits, suggesting that the fish could still be relatively fresh immediately after storage began.

Table 2.A The measurement data is derived from the ammonia produced during 8 hours of storage at room temperature for various fish sample weights.

	Duration (hour)							
	1	2	3	4	5	6	7	8
*Sample weight (g)	Tube reading (ppm)							
50	0	20	150	200	250	290	300	310
100	15	75	200	250	300	350	370	390
200	20	100	300	350	400	450	500	550
	NH ₃ concentration (ppm)							
50	0	10	50	50	50	48.33	42.85	38.75
100	**NA	37.5	66.66	62.5	60	58.33	52.85	48.75
200	20	50	100	87.5	80	75	71.42	68.72
*Weigh sample standard deviation $\pm$ 5 gr								
**Measuring range (20 ° C, 1013 hPa): for 1 hour the acceptable measuring range 20 to 1500 ppm NH ₃								

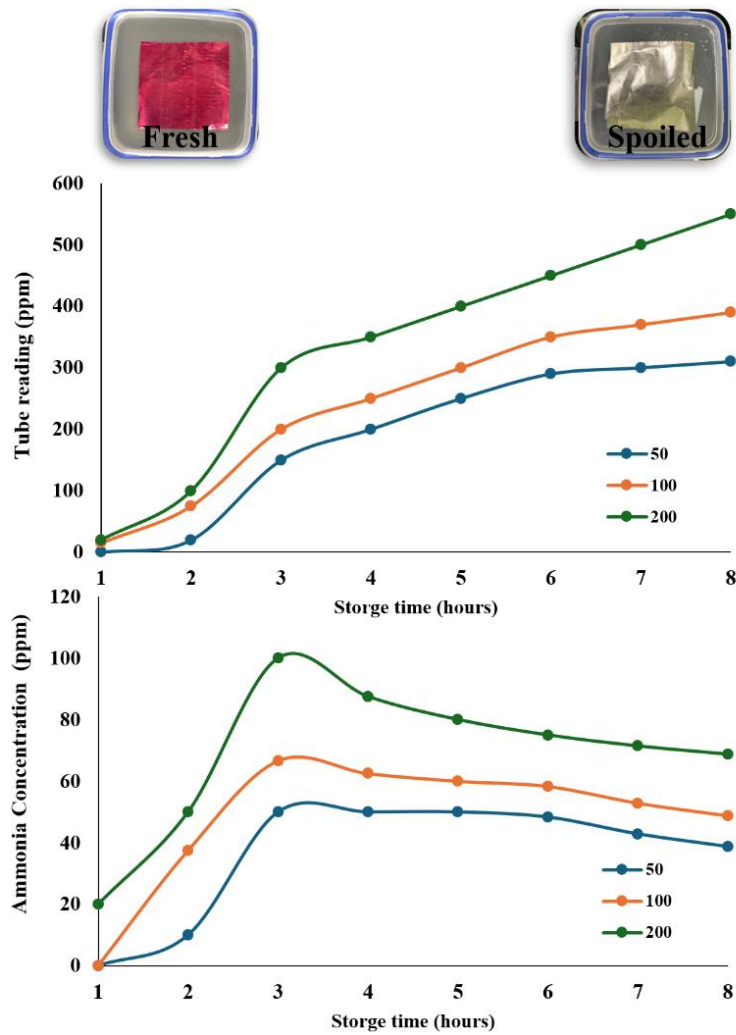


Figure 1.A The trend shows an increase in ammonia and then decrease it during 8 hours of fish storage at room temperature.

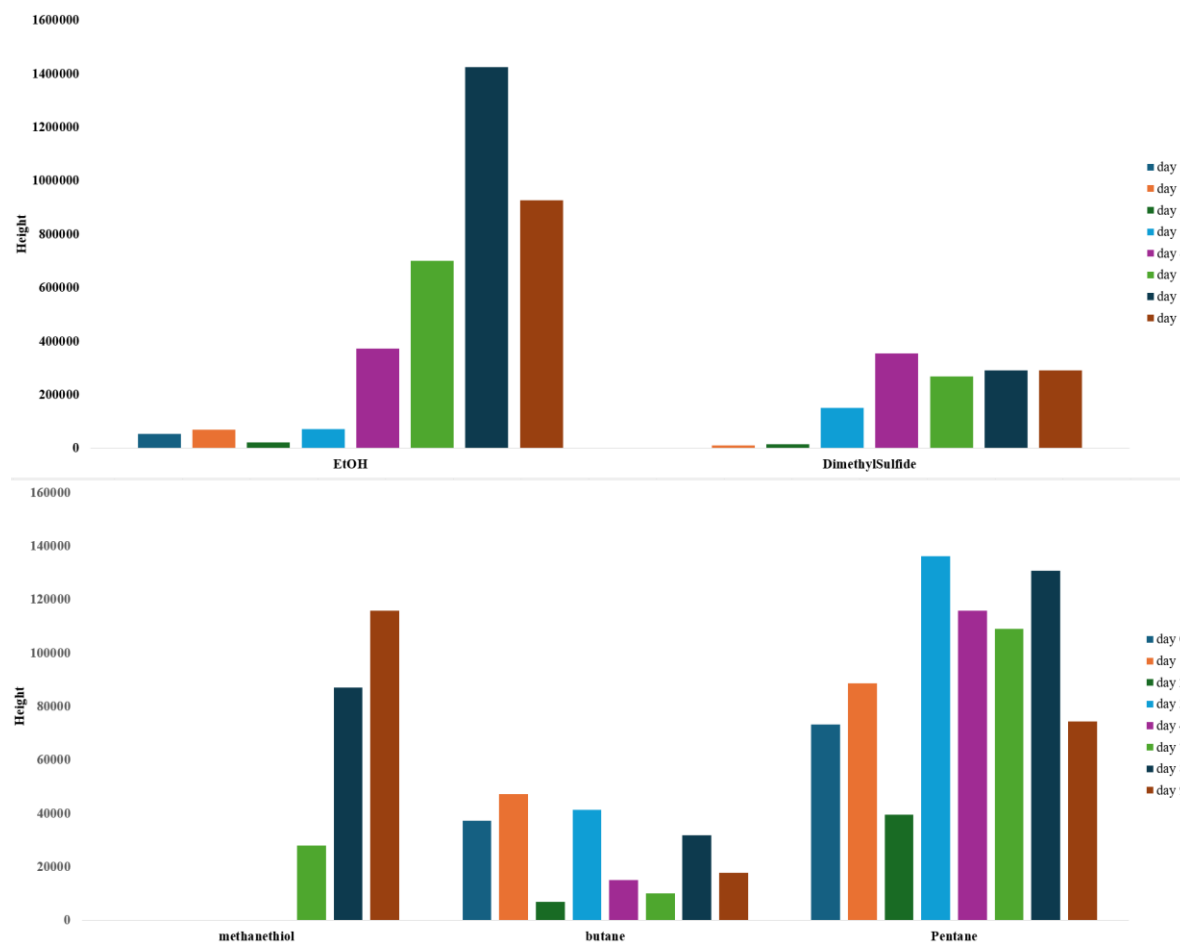


Figure 2.A Trends in the signal intensities of untargeted analytes over 9-day storage in the fridge (scan mode acquisition).

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [SuppMaryamAmeri.docx](#)