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Research Article

RAPID FRESHNESS ASSESSMENT OF REFRIGERATED NILE TILAPIA USING A LOW-COST ELECTRONIC NOSE

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Abstract

Background: Rapid and non-destructive evaluation of fish freshness is essential for ensuring product quality and reducing postharvest losses. Electronic nose (E-nose) technology has emerged as a promising alternative to conventional analytical methods for monitoring fish spoilage through volatile organic compounds (VOCs). **Objective:** This study developed and evaluated a low-cost metal oxide semiconductor (MOS)-based electronic nose for monitoring quality deterioration and predicting the freshness of refrigerated Nile tilapia (*Oreochromis niloticus*). **Methods:** The system was built using an Arduino Mega 2560 integrated with six MOS gas sensors (MQ3, MQ4, MQ5, MQ8, MQ9, and MQ135). Sensor responses were recorded over five days of refrigerated storage and compared with conventional physicochemical (TVB-N, peroxide value, pH, and thiobarbituric acid) and microbiological quality indicators (total plate count, *coliforms*, *Escherichia coli*, *Staphylococci*, *Salmonella*, and *Vibrio*). **Results:** Progressive deterioration was confirmed by increases in TVB-N (7.90–16.18 mg/100 g), peroxide value (0.78–4.05 meq O_2/kg), pH (6.14–7.14), TBA (1.07–1.57 mg MDA/kg), and total plate count (3.68×10^4–8.92×10^6 CFU/g). All MOS sensors exhibited increasing responses throughout storage, with MQ135 (195–920) and MQ3 (153–620) showing the highest sensitivity to spoilage-related VOCs. MQ135 demonstrated a strong association with TVB-N, microbial growth, and pH, whereas MQ3 closely reflected lipid oxidation. The developed E-nose correctly classified fresh, semi-fresh, and spoiled fish with 90% accuracy, confirming its capability for freshness discrimination. **Conclusion:** The proposed MOS-based electronic nose provides a rapid, inexpensive, and non-destructive approach for monitoring Nile tilapia freshness and offers strong potential for real-time quality control and shelf-life prediction in seafood processing, storage, and distribution.

1.Introduction

Fish represents one of the most valuable sources of animal-derived protein because of its high nutritional quality, balanced amino acid profile, digestibility, and richness in vitamins, minerals, and polyunsaturated fatty acids. Consequently, fish consumption has increased worldwide as consumers increasingly seek healthier dietary patterns. However, fish is also among the most perishable food commodities owing to its high water activity, nearly neutral pH, and the rapid onset of enzymatic autolysis and microbial proliferation immediately after harvest. These biological processes accelerate the deterioration of muscle tissues and lead to the generation of numerous volatile organic compounds (VOCs), including trimethylamine (TMA), ammonia, hydrogen sulfide, ketones, aldehydes, and alcohols, which are closely associated with freshness loss and sensory deterioration. Therefore, maintaining fish quality during refrigerated storage remains one of the major challenges facing the seafood industry (Ding & Li. 2024).

Among freshwater fish species, Nile tilapia (*Oreochromis niloticus*) has become one of the most important aquaculture products because of its rapid growth, adaptability to diverse environmental conditions, high feed conversion efficiency, and affordable production cost. Egypt is recognized as one of the world's leading producers of farmed tilapia, where the species constitutes a substantial proportion of total aquaculture production and represents an essential source of animal protein for consumers. Despite its economic and nutritional importance, tilapia fillets are highly susceptible to microbial spoilage and lipid oxidation during refrigerated storage, resulting in undesirable changes in odor, texture, color, and overall acceptability. Consequently, rapid monitoring of freshness is essential to ensure product quality, extend shelf life, and minimize postharvest losses (Huang et al., 2024).

Conventionally, fish freshness has been evaluated using sensory examination, microbiological analysis, and physicochemical indicators such as pH, total volatile basic nitrogen (TVB-N), thiobarbituric acid reactive substances (TBARS), peroxide value, and total viable count. Although these analytical techniques provide reliable information regarding fish quality, they are generally destructive, labor-intensive, time-consuming, and unsuitable for continuous or real-time monitoring during industrial processing and distribution. Moreover, microbiological methods require prolonged incubation periods, whereas sensory evaluation depends on trained assessors and may be influenced by subjective judgment. These limitations have encouraged researchers to develop rapid, objective, and non-destructive analytical approaches capable of monitoring freshness throughout the seafood supply chain (Madhubhashini et al., 2024).

Electronic nose (E-nose) technology has recently attracted considerable attention as an efficient alternative for rapid freshness assessment in fish and seafood products. An E-nose mimics the human olfactory system through an array of partially selective gas sensors that collectively detect complex mixtures of VOCs emitted from food samples. The sensor responses generate characteristic odor fingerprints that can be analyzed using chemometric and machine-learning techniques to distinguish freshness levels and identify spoilage stages. Compared with conventional analytical methods, the E-nose offers several advantages, including rapid analysis, minimal sample preparation, non-destructive measurement, portability, low operational cost, and suitability for on-line quality monitoring in food-processing environments. Recent advances in sensor materials and artificial intelligence have further improved the analytical performance of E-nose systems, making them promising tools for industrial quality assurance (Rabehi et al., 2024).

Several recent investigations have demonstrated the successful application of E-nose technology for evaluating fish freshness. Sensor arrays have been used to monitor VOC profiles in tilapia, salmon, carp, shrimp, and

other aquatic products, providing high classification accuracy when combined with multivariate statistical techniques such as principal component analysis (PCA), linear discriminant analysis (LDA), support vector machines (SVM), artificial neural networks (ANN), and deep-learning algorithms. In tilapia, E-nose measurements have shown strong agreement with conventional quality indicators and instrumental techniques such as gas chromatography–mass spectrometry (GC–MS) and headspace gas chromatography–ion mobility spectrometry (HS-GC-IMS), confirming their capability to characterize aroma changes during storage and processing (Chen et al.,2021).

Finally, the present study aimed to investigate the effectiveness of electronic nose technology for monitoring quality deterioration and freshness changes in refrigerated Nile tilapia during storage. The responses obtained from the E-nose were compared with conventional physicochemical and microbiological quality indicators to evaluate its applicability as a rapid, reliable, and non-destructive tool for freshness assessment and quality control in the seafood industry.

2. Materials and methods

2.1. Materials

Live Nile tilapia (*Oreochromis niloticus*) weighing 250 ± 20 g were obtained from a commercial fish farm located in Kafr El-Sheikh Governorate, Egypt.

All chemicals and reagents (analytical grade) used in this study were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA).

2.2. Preparation of fish samples

Immediately after harvesting, the fish were placed in insulated Styrofoam containers containing crushed ice to induce death by thermal shock and to preserve their freshness during transportation. The samples were then transported without delay to the Food Engineering Laboratory, Department of Agricultural Engineering, where the experimental procedures were conducted. Whole fish were used directly for all subsequent analyses without further processing.

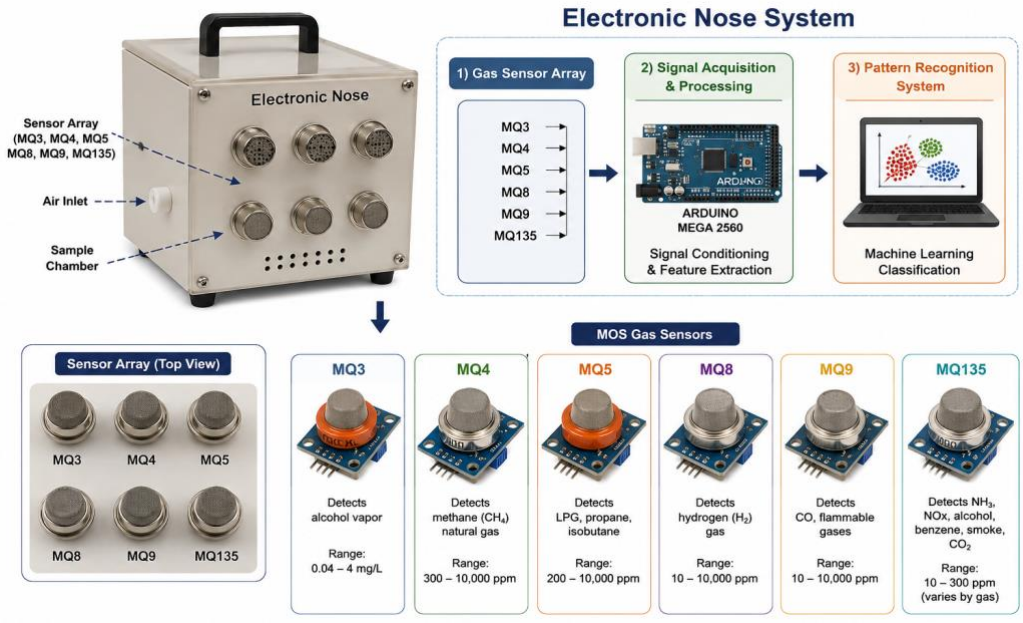

Figure 1. Proposed electronic nose system and components

2.3. Electronic components of the developed electronic nose

The electronic nose (E-nose) is an artificial olfactory system designed to mimic the human sense of smell by detecting volatile organic compounds (VOCs) released from food matrices. Typically, an electronic nose comprises three main components: a gas sensor array, a signal acquisition and processing unit, and a pattern recognition system, as illustrated in Fig. (1).

The sensor array consisted of six commercially available MOS gas sensors (MQ3, MQ4, MQ5, MQ8, MQ9, and MQ135) integrated with the Arduino Mega platform. Each sensor was selected according to its sensitivity toward specific gases generated during fish spoilage. The MQ3 sensor was primarily employed for detecting alcohol vapors, while the MQ4 sensor was sensitive to methane (CH_4) and natural gas. The MQ5 sensor was utilized for monitoring liquefied petroleum gas (LPG), propane, butane, and methane, whereas the MQ8 sensor was dedicated to the detection of hydrogen (H_2). In addition, the MQ9 sensor exhibited sensitivity toward carbon monoxide (CO) and combustible gases, while the MQ135 sensor, owing to its broad detection capability, was used to monitor ammonia (NH_3), alcohol vapors, benzene, and a wide range of volatile organic compounds (VOCs) associated with microbial spoilage and biochemical deterioration of fish.

The combination of these complementary MOS sensors enabled the developed electronic nose to capture a comprehensive volatile fingerprint representing the progressive spoilage of Nile tilapia during refrigerated storage. Sensor responses were continuously recorded throughout the storage period and subsequently correlated with microbiological and physicochemical quality indicators, including total viable bacterial counts (TVC), total volatile basic nitrogen (TVB-N), pH, peroxide value (PV), thiobarbituric acid reactive substances (TBARS), and sensory evaluation scores. Furthermore, machine-learning algorithms were applied to the acquired sensor data to recognize odor patterns and evaluate the capability of the developed electronic nose to accurately predict fish freshness and estimate shelf life.

2.4. Chemical analysis

For pH determination, 10 g of fish muscle was homogenized with 100 mL of distilled water. The pH of the homogenized mixture was then measured using a calibrated digital pH meter (HANNA HI 98172) according to the procedure reported by Ershidat et al. (2025).

The total volatile basic nitrogen (TVB-N) content of fish muscle was determined following extraction with 0.6 M perchloric acid, according to the method described by Abdelhakam et al. (2019). The results were expressed as mg N/100 g of fish muscle.

The peroxide value (PV) was determined using a spectrophotometric method based on the procedure reported by Essa et al. (2026), and the results were expressed as meq O_2/kg lipid.

The thiobarbituric acid (TBA) value was measured spectrophotometrically following the procedure of Essa and Elsebaie (2018). Lipid oxidation was expressed as mg malondialdehyde (MDA) equivalents per kilogram of fish muscle.

2.5. Microbiological analysis

Microbiological analyses were carried out under aseptic conditions. Briefly, 25 g of fish muscle was aseptically transferred into a sterile stomacher bag containing 225 mL of sterile buffered peptone water (BPW) and homogenized for 2 min using a stomacher. Serial decimal dilutions were subsequently prepared using sterile peptone water, and appropriate dilutions were used for microbial enumeration. All microbiological results were expressed as log colony-forming units per gram (log CFU g^{-1}).

The total plate count (TPC) was determined according to the method outlined by Amin et al.

(2024) using Plate Count Agar (PCA, Oxoid, UK). After incubation at 30 °C for 72 h, plates containing 25–250 colonies were counted, and the results were expressed as log CFU g^{-1}.

The total coliform count was enumerated following the procedure described in ISO 4832 (2006) using Violet Red Bile Agar (VRBA, Oxoid, UK). The inoculated plates were incubated at 37 °C for 24 h, and typical dark-red colonies surrounded by a bile precipitation zone were counted.

Enumeration of Escherichia coli was performed according to ISO 16649-2 (2001) using Tryptone Bile X-glucuronide (TBX) agar. Following incubation at 44 °C for 24 h, characteristic blue-green colonies were counted and expressed as log CFU g^{-1}.

Coagulase-positive Staphylococci were determined according to ISO 6888-1 (2021) using Baird–Parker agar supplemented with egg-yolk tellurite emulsion. After incubation at 37 °C for 24–48 h, typical black, shiny colonies surrounded by a clear halo were enumerated.

Detection of Salmonella spp. was carried out following ISO 6579-1 (2017). Samples were pre-enriched in Buffered Peptone Water, followed by selective enrichment in Rappaport–Vassiliadis Soy (RVS) broth and Müller–Kauffmann Tetrathionate-Novobiocin (MKTTn) broth. The enriched cultures were streaked onto Xylose Lysine Deoxycholate (XLD) and Hektoen Enteric agar, and presumptive colonies were confirmed by biochemical and serological tests. The results were reported as presence or absence in 25 g of sample.

The presence of Vibrio spp. was determined according to ISO 21872-1 (2017). Samples were enriched in Alkaline Peptone Water (APW) before being streaked onto Thiosulfate Citrate Bile Salts Sucrose (TCBS) agar. Following incubation at 37 °C for 24 h, typical colonies were subjected to biochemical confirmation, and the results were recorded as presence or absence in 25 g of sample.

2.6.Statistical Analysis

All analytical determinations were performed in triplicate, and the obtained results are presented as the mean value ± standard deviation (SD). Statistical evaluation of the data was carried out using one-way analysis of variance (ANOVA) to identify significant differences among treatments. Whenever the ANOVA indicated statistical significance ($p \leq 0.05$), multiple comparisons between treatment means were conducted using Tukey's Honestly Significant Difference (HSD) test. All statistical analyses were performed with IBM SPSS Statistics software (Version 25.0; IBM Corp., Armonk, NY, USA), and statistical significance was established at $p \leq 0.05$ throughout the study.

3.Results and discussions

3.1. Changes in chemical quality indices during refrigerated storage

The chemical quality indices presented in Table 1 demonstrated a progressive deterioration in the freshness of Nile tilapia during refrigerated storage. The continuous increases in TVB-N, peroxide value (PV), pH, and TBA, together with the transition of the freshness status from fresh (day 1) to semi-fresh (days 2–3) and finally spoiled (days 4–5), clearly indicate the combined effects of microbial growth, endogenous enzymatic activity, and lipid oxidation during storage. Similar deterioration patterns have been widely reported for refrigerated tilapia and other freshwater fish species (Rong et al., 2009).

Among the investigated parameters, TVB-N increased from 7.90 to 16.18 mg/100 g, reflecting the progressive formation of volatile basic nitrogen compounds produced during microbial and enzymatic degradation of muscle proteins. These compounds mainly include ammonia, dimethylamine (DMA), and trimethylamine (TMA), which are recognized indicators of fish spoilage. Although the final TVB-N value remained below the commonly accepted rejection limit of 25–30 mg/100 g, the continuous increase confirmed the onset of

spoilage and agreed well with the observed freshness classification. Similar increases in TVB-N during refrigerated storage of Nile tilapia have been reported by previous studies, which attributed this behavior to the increasing activity of spoilage microorganisms and proteolytic enzymes (de Matos Regalado et al., 2024).

Table 1. Chemical quality changes during storage and freshness classification

Storage Day	TVB-N (mg/100g)	PV (meq O_2/kg)	pH	TBA (mg MDA/kg)	Quality Status
1	7.90	0.78	6.14	1.07	Fresh
2	11.03	2.00	6.22	1.15	Semi-fresh
3	11.93	2.63	6.44	1.29	Semi-fresh
4	15.89	3.83	6.79	1.36	Spoiled
5	16.18	4.05	7.14	1.57	Spoiled

The peroxide value (PV) exhibited a gradual increase from 0.78 to 4.05 meq O_2/kg, indicating continuous formation of lipid hydroperoxides during refrigerated storage. PV is considered an indicator of the primary stage of lipid oxidation and generally increases before the accumulation of secondary oxidation products. Although Nile tilapia contains lower levels of polyunsaturated fatty acids than many marine fish species, membrane phospholipids remain susceptible to oxidative reactions during chilled storage. The relatively low PV values observed throughout storage suggest that oxidation was still in its early stages, despite the evident progression of quality deterioration. Comparable trends have been described for refrigerated tilapia and other freshwater fish products (Jeyakumari et al.,2023).

The pH increased steadily from 6.14 to 7.14, which is characteristic of post-rigor spoilage in refrigerated fish. Fresh fish generally exhibit pH values close to neutrality following the postmortem glycolytic phase. As storage progresses, bacterial metabolism and enzymatic degradation generate alkaline metabolites, including ammonia and volatile amines, causing a gradual increase in pH. The simultaneous increase observed in both pH and TVB-N supports the close relationship between microbial activity and the production of alkaline nitrogenous compounds. Similar pH profiles have been reported for refrigerated Nile tilapia stored under conventional chilling conditions (Monteiro et al., 2018).

The TBA value, which represents secondary lipid oxidation products expressed as malondialdehyde (MDA), increased from 1.07 to 1.57 mg MDA/kg during storage. This increase indicates progressive lipid oxidation accompanying microbial spoilage and is consistent with the increase observed in PV. The oxidation of unsaturated fatty acids produces hydroperoxides during the initial oxidation stage, which subsequently decompose into aldehydes, ketones, and other volatile compounds measured by the TBARS assay. The relatively moderate increase observed in the present study suggests that oxidative deterioration progressed gradually during refrigerated storage and remained within the range commonly reported for chilled tilapia. Similar increases in TBARS during refrigerated storage have been documented in Nile tilapia and other fish species (Jeyakumari et al.,2023)

Overall, the simultaneous increases in TVB-N, PV, pH, and TBA demonstrate that microbial spoilage and lipid oxidation progressed concurrently throughout refrigerated storage. The deterioration observed after the third day agrees with the freshness classification and confirms that these physicochemical indicators effectively describe the quality changes occurring in Nile tilapia. Moreover, because microbial spoilage generates volatile compounds that are directly

responsible for changes in fish aroma, these findings provide strong support for the application of electronic nose (E-nose) technology as a rapid and non-destructive method for freshness assessment. Numerous studies have reported strong correlations between E-nose sensor responses and conventional spoilage indicators, including TVB-N, TBARS, pH, microbial counts, and volatile organic compounds, demonstrating the potential of E-nose systems for real-time monitoring of fish quality and shelf-life prediction. (Franceschelli et al., 2021)

3.2. Changes in microbiological quality indicators during refrigerated storage

The microbiological quality indicators presented in Table 2 demonstrated a progressive increase in the microbial load of Nile tilapia throughout refrigerated storage, reflecting the gradual loss of freshness and microbiological safety. The total plate count (TPC) increased from 3.68×10^4 CFU/g on day 1 to 8.92×10^6 CFU/g on day 5. This trend is characteristic of chilled fish, where psychrotrophic spoilage bacteria proliferate rapidly under refrigerated conditions. Similar increases in aerobic bacterial counts during refrigerated storage of Nile tilapia have been reported previously, with microbial populations approaching or exceeding the generally accepted spoilage limit of 7 log CFU/g, which is commonly associated with sensory rejection and the end of shelf life (Rong et al., 2009).

Table 2. Microbiological quality indicators during storage period

Day	TPC (CFU/g)	*Coliforms*	*E. coli*	*Staphylococci*	*Salmonella*	*Vibrio*
1	3.68×10^4	Nil	Nil	Nil	Nil	Nil
2	5.26×10^5	Nil	Nil	3	Nil	1
3	4.17×10^6	8	2	6	Nil	3
4	6.23×10^6	32	4	29	6	6
5	8.92×10^6	59	17	42	14	12

The initial TPC observed in the present study indicates good microbiological quality of the fresh fish at the beginning of storage. However, the rapid increase after the second day reflects favorable conditions for bacterial multiplication, including the high moisture cont;pent, near-neutral pH, and abundance of readily available nitrogenous substrates in fish muscle (Rong et al.,2009). Similarly demonstrated that aerobic plate counts in refrigerated tilapia increased continuously throughout storage and closely paralleled the deterioration in chemical and sensory quality, confirming that TPC is one of the most reliable indicators of fish freshness (Junior et al., 2014).

No coliforms or Escherichia coli were detected during the first two days of storage, suggesting satisfactory hygienic quality immediately after processing. Nevertheless, both microbial groups increased progressively from day 3 onward, reaching 59 CFU/g and 17 CFU/g, respectively, on day 5. Coliform bacteria are widely recognized as indicators of sanitary conditions during handling and processing rather than primary spoilage organisms. Their appearance during storage may indicate cross-contamination associated with handling, equipment, water quality, or storage conditions. Similar findings were reported in retail tilapia, where fillets exhibited significantly higher coliform counts than whole fish because of increased handling during filleting and processing (Moura et al., 2014).

The gradual increase in Staphylococci from undetectable levels on day 1 to 42 CFU/g on day 5 also indicates increasing contamination during storage. Staphylococci are generally associated with human handling and inadequate

hygienic practices during processing rather than with the indigenous microbiota of fresh fish. Although the observed counts remained considerably lower than the regulatory limits established for coagulase-positive *Staphylococcus aureus* in many food products, their continuous increase emphasizes the importance of maintaining strict hygienic practices during processing and refrigerated storage. Similar observations have been reported for commercial tilapia, in which contamination with coagulase-positive staphylococci was mainly associated with filleting operations and post-processing handling (Ndraha et al., 2024).

Neither Salmonella nor Vibrio species were detected in freshly stored samples, confirming the satisfactory microbiological status of the fish immediately after harvest. However, both pathogens became detectable after prolonged storage, with Salmonella reaching 14 CFU/g and Vibrio reaching 12 CFU/g on day 5. The appearance of these pathogenic bacteria during storage may be attributed to contamination during handling or their multiplication from initially undetectable levels under favorable environmental conditions. The detection of *Salmonella* is of particular concern because international food safety regulations generally require its complete absence in fresh fish intended for human consumption. Likewise, *Vibrio* species are recognized seafood-associated pathogens whose proliferation can pose significant public health risks if temperature abuse occurs during storage and distribution. Surveys of retail tilapia have likewise reported occasional contamination with *Salmonella* and other foodborne pathogens, emphasizing the importance of hygienic handling and cold-chain management throughout the supply chain (Eltholth et al., 2018).

Overall, the microbiological results indicate that refrigerated storage promoted the continuous growth of both spoilage and indicator microorganisms, resulting in progressive deterioration of fish quality. The increase in TPC was accompanied by the appearance and multiplication of coliforms, *E. coli*, staphylococci, *Salmonella*, and *Vibrio*, demonstrating the decline in microbiological quality as storage progressed. These observations are consistent with previous studies showing that microbiological deterioration represents one of the principal factors limiting the shelf life of refrigerated tilapia (Rong et al., 2009; Cyprian et al., 2013).

Furthermore, microbial growth is directly associated with the production of volatile metabolites, including ammonia, trimethylamine, sulfur-containing compounds, ketones, and alcohols, which are responsible for the characteristic odor of spoiled fish. Consequently, the microbial changes observed in the present study provide a strong scientific basis for the application of electronic nose (E-nose) technology as a rapid and non-destructive tool for freshness evaluation. As bacterial populations increase, the concentration and composition of volatile organic compounds change significantly, enabling E-nose sensor arrays to accurately discriminate between fresh, semi-fresh, and spoiled fish without the need for destructive microbiological analyses. This relationship between microbial growth and volatile fingerprint formation has been consistently demonstrated in recent studies on refrigerated fish quality assessment (Prado-Toledo et al., 2022).

3.3. Electronic nose sensor responses during fish spoilage

The responses of the electronic nose (E-nose) sensors during refrigerated storage of Nile tilapia (Table 3) showed a clear and systematic increase in all sensor signals as storage time progressed from day 1 (fresh) to day 5 (highly spoiled). This progressive increase reflects the continuous accumulation of volatile organic compounds (VOCs) associated with microbial activity, enzymatic degradation, and lipid oxidation. Such compounds include ammonia, amines, alcohols,

aldehydes, ketones, and sulfur-containing volatiles, which are widely recognized as primary indicators of fish spoilage (Gram & Dalgaard, 2002 and Elsebaie et al., 2021).

The overall trend observed in this study is consistent with previous reports demonstrating that fish spoilage is strongly associated with increased emission of volatile compounds detectable by gas sensor arrays. For instance, E-nose systems have been successfully applied to monitor tilapia freshness and showed strong discrimination between fresh and spoiled samples based on VOC profiles generated during storage (Radi et al., 2021). These studies confirmed that microbial growth and biochemical degradation directly influence sensor response patterns, making E-nose a reliable tool for freshness assessment.

Among the evaluated sensors, MQ135 exhibited the highest response increase, rising from 195 (day 1) to 920 (day 5). This sensor is highly sensitive to ammonia, carbon dioxide, and general air contaminants. The sharp increase in MQ135 response is directly linked to the accumulation of nitrogenous compounds, particularly ammonia and trimethylamine (TMA), which are typical end-products of protein degradation in spoiled fish. Similar findings were reported by Wu et al. (2023), who emphasized that MOS-based sensors responding to nitrogenous gases are strongly correlated with total volatile basic nitrogen (TVB-N) formation and microbial spoilage intensity in fish products.

Table 3. Average electronic nose sensor responses during fish spoilage

Sensor	Fresh (Day1)	Semi-fresh (Day 2)	Semi-fresh (Day 3)	Spoiled (Day 4)	Highly Spoiled (Day 5)
MQ135	195	204	275	555	920
MQ9	88	91	138	238	465
MQ8	124	128	149	173	202
MQ5	41	44	69	138	285
MQ4	83	87	109	133	163
MQ3	153	159	225	425	620

The MQ3 sensor, which is sensitive to alcohol vapors, showed a substantial increase from 153 to 620, especially after day 3. This indicates enhanced production of ethanol and other low-molecular-weight alcohols generated through microbial fermentation pathways. Psychrotrophic bacteria such as *Pseudomonas* and *Shewanella* spp. are known to contribute to the formation of alcohols during refrigerated storage of fish, which significantly affects odor quality (Gram & Dalgaard, 2002).

Similarly, the MQ5 sensor (LPG, hydrogen, and hydrocarbon-sensitive) increased markedly from 41 to 285, particularly during late storage stages. This increase may be attributed to intensified microbial metabolism and lipid degradation processes, which produce various hydrocarbon and volatile compounds. The observed behavior agrees with studies reporting significant changes in hydrocarbon-related sensor responses during spoilage of aquatic products analyzed by E-nose systems (Peris & Escuder-Gilabert, 2009 and Hu et al., 2025).

The MQ9 sensor also exhibited a notable increase from 88 to 465, reflecting the accumulation of combustible gases and oxidation-related volatiles during storage. Although MQ9 is not specific to fish spoilage compounds, its response pattern contributes to the overall odor fingerprint used for classification. Recent studies using sensor arrays confirmed that combining multiple MOS sensors improves classification accuracy and

allows better discrimination of freshness stages in fish products (Sun & Zhang, 2022 and Li et al., 2023).

In contrast, the MQ8 and MQ4 sensors exhibited relatively moderate increases throughout refrigerated storage, indicating that hydrogen- and methane-related gases contributed less to the volatile profile than nitrogen-containing compounds and alcohols. This suggests that protein degradation and microbial metabolism were the predominant spoilage pathways, leading to the formation of volatile amines, aldehydes, ketones, alcohols, and sulfur-containing compounds that dominate the odor profile of spoiled fish (Wang et al., 2024; Zhang et al., 2023). Overall, all sensor responses increased progressively with storage time, particularly after day 3, coinciding with the transition from semi-fresh to spoiled fish and reflecting the continuous accumulation of spoilage-related volatile organic compounds (VOCs). The close agreement between sensor responses and spoilage progression demonstrates the effectiveness of the electronic nose as a rapid and non-destructive tool for freshness evaluation. Furthermore, recent studies have shown that integrating E-nose data with chemometric and machine-learning techniques enables accurate classification of fish freshness and reliable prediction of shelf life, supporting its application in real-time quality monitoring within the seafood industry (Cheng et al., 2023 and Wang et al., 2024).

3.4. Correlation between MQ135 sensor response and spoilage indicators

The results presented in Table 4 revealed a strong positive association between the MQ135 sensor response and the conventional spoilage indicators (TVB-N, pH, and TPC) during refrigerated storage of Nile tilapia. The MQ135 signal increased markedly from 195 on day 1 to 920 on day 5, coinciding with the gradual increases in TVB-N (7.90–16.18 mg/100 g), pH (6.14–7.14), and total plate count (TPC) (3.68×10^4–8.92×10^6 CFU/g). These results indicate that the MQ135 sensor effectively tracked the biochemical and microbiological deterioration of fish throughout storage.

Table 4. Correlation Between MQ135 Sensor and Spoilage Indicators

Day	MQ135	TVB-N	pH	TPC (CFU/g)
1	195	7.90	6.14	3.68×10^4
2	204	11.03	6.22	5.26×10^5
3	275	11.93	6.44	4.17×10^6
4	555	15.89	6.79	6.23×10^6
5	920	16.18	7.14	8.92×10^6

The progressive increase in MQ135 response can be attributed to the accumulation of volatile nitrogen-containing compounds generated during microbial spoilage. The MQ135 sensor is particularly sensitive to ammonia and other alkaline gases produced through bacterial deamination of amino acids and degradation of muscle proteins. As microbial activity intensified during storage, larger amounts of ammonia, trimethylamine (TMA), dimethylamine (DMA), and other volatile basic compounds were released, resulting in a substantial increase in sensor response. This interpretation agrees with recent reviews indicating that nitrogenous volatile compounds are among the most reliable chemical markers of seafood spoilage and represent the principal targets detected by metal oxide semiconductor (MOS) gas sensors used in electronic nose systems (Wang et al., 2024; Zhang et al., 2023).

The increase in MQ135 response closely paralleled the increase in TVB-N, suggesting a strong relationship between sensor output and the accumulation of volatile basic nitrogen compounds. TVB-N is widely accepted as a reference indicator of fish freshness because it reflects the combined effects of microbial metabolism and endogenous enzymatic degradation. Consequently, the close agreement observed between MQ135 and TVB-N supports the ability of this sensor to provide rapid indirect estimation of fish freshness without the need for conventional laboratory analyses. Similar relationships between MOS sensor responses and TVB-N have been reported in fish freshness monitoring using electronic nose technology (Wang et al., 2024).

Likewise, the gradual increase in pH was accompanied by a corresponding increase in MQ135 response. The rise in pH during storage is primarily associated with the formation of alkaline metabolites such as ammonia and biogenic amines produced by spoilage microorganisms. Since MQ135 exhibits high sensitivity toward these compounds, the observed positive relationship between pH and sensor response further confirms that the sensor reflects the biochemical changes occurring during fish deterioration. Cheng et al. (2023) reported that the formation of alkaline volatile compounds is one of the principal factors influencing odor profile evolution in refrigerated aquatic products.

A similar trend was observed between MQ135 and TPC, where increasing bacterial populations were associated with progressively higher sensor responses. The sharp increase in TPC after the second day indicates accelerated microbial growth, which directly enhanced the production of volatile metabolites responsible for fish off-odors. Previous studies have demonstrated that the concentration of VOCs increases proportionally with microbial proliferation, enabling electronic nose systems to accurately classify different freshness stages based on odor fingerprints (Wang et al., 2024; Cheng et al., 2023).

Overall, the strong agreement between MQ135 response and the physicochemical and microbiological spoilage indicators demonstrates that this sensor can serve as a reliable predictor of fish freshness. The results support previous findings that MOS-based electronic nose systems provide a rapid, non-destructive, and cost-effective alternative to conventional quality assessment methods and have considerable potential for real-time monitoring of seafood quality during refrigerated storage.

3.4. Validation of electronic nose classification

The validation results presented in Table 5 demonstrated a high agreement between the electronic nose (E-nose) classification and the laboratory-based freshness evaluation. Of the ten tested samples, nine were correctly classified, while only one sample (Sample 8) was incorrectly identified as semi-fresh instead of spoiled, resulting in an overall classification accuracy of 90%. The high classification performance reflects the capability of the metal oxide semiconductor (MOS) sensor array to detect changes in the volatile organic compounds (VOCs) generated during fish spoilage. As refrigerated storage progresses, microbial growth, endogenous enzymatic activity, and lipid oxidation promote the formation of volatile amines, aldehydes, ketones, alcohols, sulfur-containing compounds, and other odor-active metabolites that collectively constitute a characteristic volatile fingerprint associated with different freshness stages (Wang et al., 2024). Consequently, the E-nose was able to accurately discriminate between fresh, semi-fresh, and spoiled fish samples with only one classification error.

Table 5. Validation of electronic nose classification.

Sample	E-nose Classification	Laboratory Classification	Result
1	Fresh	Fresh	Correct
2	Semi-fresh	Semi-fresh	Correct
3	Semi-fresh	Semi-fresh	Correct
4	Spoiled	Spoiled	Correct
5	Spoiled	Spoiled	Correct
6	Fresh	Fresh	Correct
7	Semi-fresh	Semi-fresh	Correct
8	Semi-fresh	Spoiled	Wrong
9	Spoiled	Spoiled	Correct
10	Spoiled	Spoiled	Correct

The misclassification observed for Sample 8 most likely occurred because the sample was collected during the transition between the semi-fresh and spoiled stages, where the composition of VOCs changes gradually and overlapping odor profiles may reduce the discrimination ability of sensor arrays. Similar observations have been reported in previous studies, which showed that most classification errors occur near freshness boundaries because spoilage is a continuous biochemical and microbiological process rather than a discrete event (Peris & Escuder-Gilabert, 2009). Moreover, recent reviews have demonstrated that electronic nose systems integrated with chemometric and machine-learning approaches provide highly accurate freshness classification owing to the strong correlation between VOC profiles and conventional spoilage indicators, including total volatile basic nitrogen (TVB-N), microbial counts, and pH (Wang et al., 2024; Zhang et al., 2023). Therefore, the high classification accuracy obtained in the present study confirms that the proposed E-nose system is a rapid, non-destructive, and reliable tool for monitoring fish freshness and has considerable potential for real-time quality control applications throughout seafood processing and distribution.

Figure (2) illustrates the changes in the responses of the MQ135 and MQ3 gas sensors alongside the peroxide value (PV) during refrigerated storage of Nile tilapia (*Oreochromis niloticus*) for five days. A consistent upward trend was observed in all three parameters throughout the storage period, indicating progressive deterioration in fish quality as refrigeration time increased. This trend demonstrates that the electronic nose (E-nose) sensors were capable of tracking the biochemical and microbiological changes associated with fish spoilage and that their responses closely paralleled the increase in lipid oxidation.

At the beginning of storage (Day 1), the peroxide value was only 0.78 meq O_2/kg, while the MQ135 and MQ3 sensors recorded 195 and 153, respectively. These low values indicate that the fish samples were still fresh, with minimal lipid oxidation and a very low concentration of volatile organic compounds (VOCs). During the second and third days of storage, the peroxide value increased to 2.00 and 2.63 meq O_2/kg, accompanied by increases in MQ135 readings from 204 to 275 and MQ3 readings from 159 to 225. These simultaneous increases reflect the onset of oxidative deterioration of unsaturated lipids and the formation of primary oxidation products (lipid hydroperoxides), together with the gradual accumulation of volatile compounds generated by microbial metabolism and endogenous enzymatic activity. The ability of the electronic nose to detect these volatile metabolites demonstrates its sensitivity to the early stages

of quality deterioration before severe spoilage becomes evident.

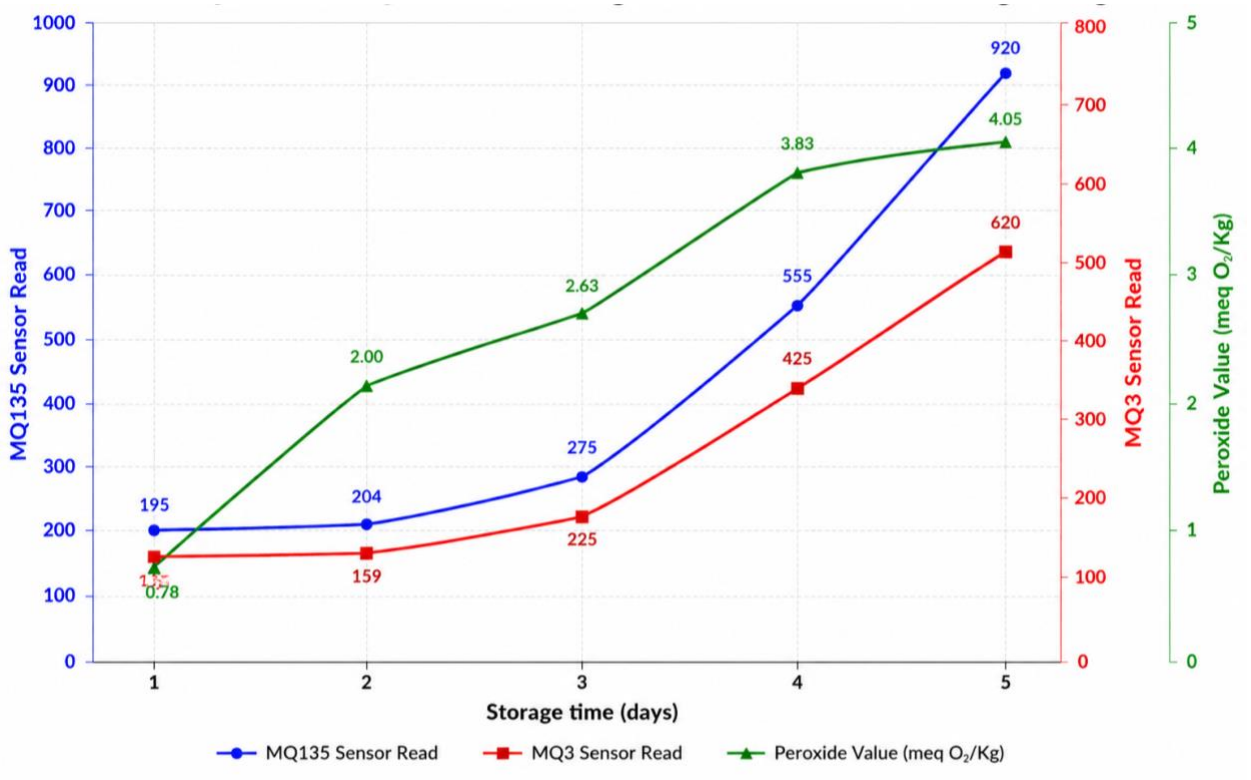

Figure 2. Releation between MQ135 and MQ3 gas sensors and fish flesh peroxide value (PV) during storage period.

A much steeper increase was observed during the fourth and fifth days of storage. The peroxide value reached 3.83 and 4.05 meq O_2/kg, whereas MQ135 readings increased dramatically from 555 to 920, and MQ3 readings increased from 425 to 620. The sharper increase in sensor responses compared with peroxide values suggests that the electronic nose responds not only to lipid oxidation products but also to the broad spectrum of volatile compounds produced during fish spoilage, including aldehydes, ketones, alcohols, ammonia, trimethylamine, sulfur-containing compounds, and other volatile nitrogenous metabolites.

Consequently, the E-nose provides a more comprehensive assessment of freshness because it reflects the combined effects of lipid oxidation, microbial growth, and protein degradation rather than monitoring a single chemical parameter.

The MQ135 sensor exhibited greater sensitivity than the MQ3 sensor throughout refrigerated storage. The MQ135 response increased from 195 on Day 1 to 920 on Day 5, representing an increase of approximately 372%, whereas MQ3 increased from 153 to 620, corresponding to approximately 305%. This higher sensitivity can be attributed to the broader detection range of MQ135, which is particularly responsive to ammonia, volatile amines, sulfur-containing gases, and other spoilage-related volatile compounds commonly produced during fish deterioration. In contrast, MQ3 is relatively more responsive to alcohols and certain organic vapors, resulting in a comparatively lower response under the same storage conditions. These findings suggest that MQ135 may serve as a more effective indicator for monitoring freshness changes in refrigerated Nile tilapia.

The observed relationship between peroxide value and sensor responses also indicates a strong positive association between lipid oxidation and the production of volatile spoilage compounds. As oxidative reactions progressed, increasing concentrations of volatile metabolites were released from fish tissues, leading to progressively higher sensor signals. This confirms that electronic nose technology can rapidly detect quality deterioration without the need for destructive laboratory analyses, providing an efficient alternative for real-time freshness monitoring.

The present findings agree with those reported by Olafsdóttir et al. (1997), who emphasized that fish spoilage is accompanied by progressive biochemical, microbiological, and volatile changes and recommended integrating multiple analytical approaches, including volatile compound analysis, to obtain a reliable evaluation of fish freshness. More recently, Wu et al., (2019) reviewed emerging technologies for fish freshness evaluation and concluded that electronic noses are among the most promising rapid and non-destructive tools because they accurately detect volatile compounds associated with microbial and oxidative spoilage while significantly reducing analysis time compared with conventional laboratory methods. Similarly, Franceschelli et al. (2021) reported that metal oxide semiconductor (MOS)-based electronic noses successfully discriminate different freshness stages of fish by monitoring spoilage-related volatile compounds, achieving high prediction accuracy and demonstrating strong potential for industrial quality control. Furthermore, the recent review by Madhubhashini et al. (2024) confirmed that electronic nose systems have become one of the most effective artificial sensing technologies for fish freshness assessment owing to their high sensitivity toward volatile organic compounds generated during refrigerated storage and their ability to provide rapid, objective, and non-destructive quality evaluation.

Overall, the results demonstrate that the electronic nose effectively monitored the progressive deterioration of refrigerated Nile tilapia throughout storage. The close agreement between the increasing peroxide values and the marked rise in MQ135 and MQ3 sensor responses confirms that volatile compound production is strongly associated with lipid oxidation and spoilage development. Among the two sensors, MQ135 showed superior sensitivity, suggesting that it may be the more suitable sensor for rapid assessment of freshness and shelf-life prediction in refrigerated Nile tilapia.

The relationship between thiobarbituric acid (TBA) values and the responses of the MQ135 and MQ3 sensors during refrigerated storage demonstrated a progressive increase in all measured parameters (Figure 3). The TBA value increased from 1.07 mg malondialdehyde (MDA)/kg on day 1 to 1.57 mg MDA/kg on day 5, while the MQ135 response increased substantially from 195 to 920, and the MQ3 response increased from 153 to 620. The parallel increase observed among these parameters indicates that the electronic nose sensors effectively tracked the oxidative deterioration of fish during refrigerated storage. Similar observations have been reported by Zhang et al. (2023), who demonstrated that MOS-based electronic noses exhibit high sensitivity to volatile compounds generated during fish spoilage.

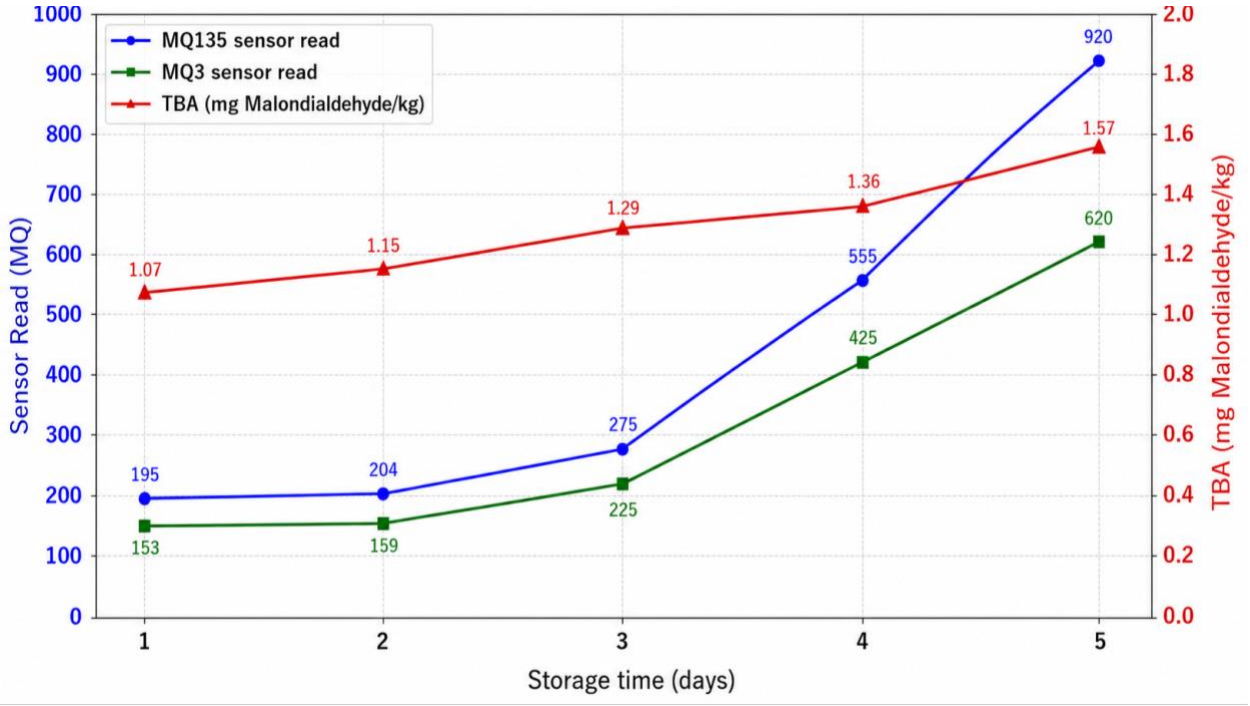

Figure 3. Releation between MQ135 and MQ3 gas sensors and fish flesh TBA values during storage period.

The gradual increase in TBA values reflects the accumulation of malondialdehyde (MDA), which is one of the principal secondary oxidation products formed during the decomposition of lipid hydroperoxides. Marine fish contain high concentrations of polyunsaturated fatty acids (PUFAs), making them particularly susceptible to oxidative deterioration during chilled storage (Domínguez et al. et al., 2019; Liu et al. et al., 2022). Therefore, the continuous increase in TBA values confirms the progressive development of lipid oxidation as storage time increased.

A remarkable increase in MQ135 sensor response was observed after the third storage day. This sensor is highly sensitive to ammonia, volatile amines, alcohols, benzene derivatives, and other volatile organic compounds (VOCs) that are generated through both microbial metabolism and lipid oxidation. As refrigerated storage progresses, aldehydes, ketones, alcohols, and nitrogen-containing compounds accumulate simultaneously, resulting in a pronounced increase in MQ135 response. Similar findings were reported by Li et al. (2024), who found that MQ135 exhibits excellent sensitivity toward the complex mixture of VOCs released during seafood deterioration.

In comparison, MQ3 exhibited a more gradual increase throughout storage, rising from 153 on day 1 to 620 on day 5. MQ3 is primarily responsive to alcohol vapors and oxygenated volatile compounds that are produced during secondary lipid oxidation. The close correspondence between MQ3 response and TBA values suggests that this sensor is particularly effective for monitoring lipid oxidation rather than the overall spoilage process. Comparable results were obtained by Wang et al. (2023), who demonstrated a significant correlation between alcohol-sensitive MOS sensors and TBA values during refrigerated fish storage.

The rapid increase in both MQ135 and MQ3 responses after day 3 corresponds to the propagation phase of lipid oxidation, during which lipid hydroperoxides rapidly decompose into aldehydes, ketones, alcohols, and other

low-molecular-weight volatile compounds (Shahidi & Ambigaipalan, 2022). Consequently, although TBA values increased gradually, the concentration of volatile compounds in the headspace increased much more rapidly, explaining the exponential rise in sensor responses.

The strong agreement between TBA values and the electronic nose responses demonstrates that MQ135 and MQ3 sensors can serve as reliable, rapid, and non-destructive indicators of lipid oxidation in fish. Unlike the conventional TBA assay, which requires sample preparation, chemical reagents, and laboratory analysis, the electronic nose provides immediate responses without damaging the sample, making it highly suitable for real-time quality monitoring during processing, transportation, and retail storage (Mustafa et al. et al., 2024).

Overall, the present results indicate that MQ135 was more sensitive to the overall deterioration process because it detected both oxidation-derived volatiles and microbial spoilage metabolites, whereas MQ3 showed a closer relationship with lipid oxidation measured by TBA. The complementary responses of both sensors suggest that integrating MQ135 and MQ3 into a multisensor electronic nose system could substantially improve the accuracy of fish freshness prediction, which is consistent with recent reports on intelligent sensing technologies for seafood quality assessment (Gutiérrez-Capitán et al., 2023 and Li et al., 2024).

Figure (4) illustrates the changes in total volatile basic nitrogen (TVB-N) and the response of the MQ135 metal oxide semiconductor (MOS) sensor during refrigerated storage of Nile tilapia (*Oreochromis niloticus*) for five days. Both parameters exhibited a continuous increase throughout the storage period, indicating progressive deterioration in fish freshness. Initially, the fish samples showed a low TVB-N value of 7.90 mg N/100 g, while the MQ135 sensor recorded a response of 195, confirming the high freshness of the fish and the limited accumulation of volatile nitrogenous compounds at the beginning of storage. During this stage, endogenous enzymatic reactions predominate, whereas microbial activity remains relatively low, resulting in minimal production of ammonia, trimethylamine (TMA), dimethylamine (DMA), and other volatile basic compounds. Consequently, only weak sensor responses were observed. TVB-N is widely recognized as one of the most reliable chemical indicators of fish freshness because it reflects the accumulation of volatile alkaline compounds generated through microbial metabolism and protein degradation, making it a standard freshness index in seafood quality assessment.

As refrigerated storage progressed, TVB-N increased steadily to 11.03 mg N/100 g on Day 2 and 11.93 mg N/100 g on Day 3, while the MQ135 response increased simultaneously from 204 to 275. This parallel increase indicates the onset of active microbial growth and accelerated proteolytic degradation, during which spoilage microorganisms convert nitrogenous substrates into volatile amines and ammonia. These volatile compounds are readily detected by the MQ135 sensor, explaining the close agreement between the conventional chemical index and the electronic nose response during the early stages of spoilage. Refrigeration effectively delayed microbial proliferation; however, it could not completely prevent the biochemical reactions responsible for quality deterioration.

A more pronounced increase was observed during the later stages of storage. TVB-N increased sharply from 11.93 mg N/100 g on Day 3 to 15.89 mg N/100 g on Day 4 and reached 16.18 mg N/100 g on Day 5. During the same period, the MQ135 sensor response rose dramatically from 275 to 555, eventually reaching 920. The much steeper increase in sensor response compared with TVB-N demonstrates the high sensitivity of the MQ135 sensor toward volatile spoilage metabolites.

Unlike the conventional TVB-N assay, which quantifies only volatile basic nitrogen compounds, the MQ135 sensor detects a much broader spectrum of volatile gases, including ammonia, volatile amines, sulfur-containing compounds, aldehydes, ketones, alcohols, and other volatile organic compounds generated during microbial metabolism and protein decomposition. Consequently, the electronic nose provides a more comprehensive representation of fish spoilage than a single chemical freshness indicator. Notably, although the increase in TVB-N between Days 4 and 5 was relatively small (15.89–16.18 mg N/100 g), the MQ135 response increased substantially from 555 to 920, suggesting that volatile organic compounds accumulated more rapidly than the changes reflected by TVB-N alone. This finding highlights the ability of the electronic nose to detect subtle alterations in the volatile profile before major changes become apparent using conventional chemical analyses.

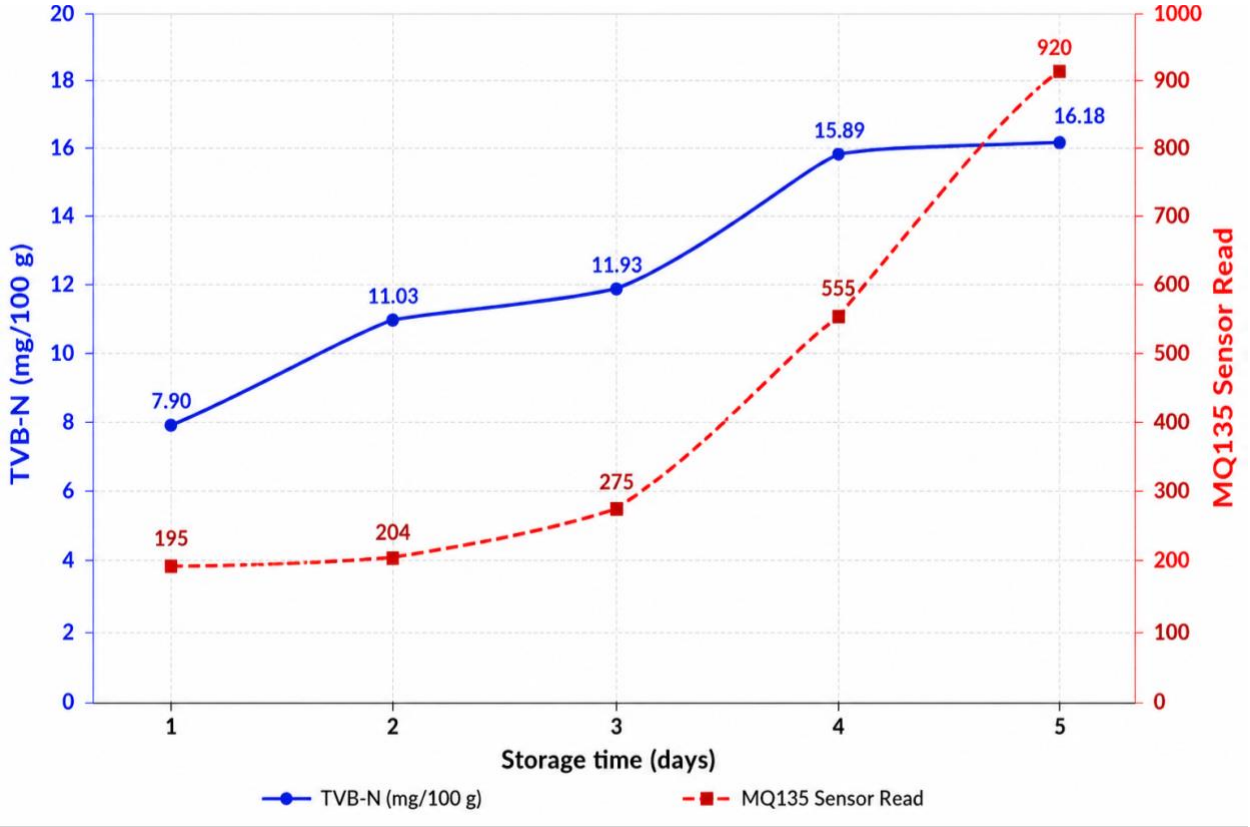

Figure 4. Releation between MQ135 gas sensor and fish flesh TVB-N values during storage period.

These findings are consistent with previous reports demonstrating a strong correlation between electronic nose responses and TVB-N values in fish and seafood products. Li et al. (2024) developed a metal oxide semiconductor electronic nose for predicting TVB-N in fish meal and reported excellent predictive performance ($R^2 = 0.91$), confirming that MOS sensor responses can accurately estimate freshness without destructive laboratory analyses. Similarly, Zhang et al. (2021) demonstrated that a portable electronic nose combined with chemometric analysis accurately predicted TVB-N and other freshness indices in fish meal, highlighting the close relationship between volatile gas profiles and conventional chemical quality parameters. Furthermore, Franceschelli et al. (2021) concluded that MOS-based electronic noses are capable of accurately classifying fish according to freshness by detecting volatile compounds associated with microbial spoilage and

therefore represent one of the most promising non-destructive technologies for industrial seafood quality control. Likewise, Madhubhashini et al. (2024) emphasized that artificial olfactory systems have become powerful tools for fish freshness evaluation because of their rapid response, high sensitivity toward spoilage-related volatile organic compounds, and ability to provide objective, real-time quality assessment during refrigerated storage.

Overall, the results clearly demonstrate a strong positive relationship between TVB-N and MQ135 sensor response throughout refrigerated storage of Nile tilapia. As spoilage progressed, the accumulation of volatile nitrogenous compounds and other microbial metabolites resulted in progressively higher sensor responses. The considerably greater dynamic range of the MQ135 sensor compared with TVB-N indicates that the sensor captures a wider spectrum of spoilage-related volatile compounds than those quantified by conventional chemical analysis. Therefore, the MQ135-based electronic nose represents a rapid, non-destructive, and highly sensitive technique for monitoring fish freshness and predicting shelf life, supporting its application as an efficient alternative to conventional laboratory methods for quality evaluation of refrigerated fish products.

Figure (5) demonstrates a strong positive relationship between the total plate count (TPC) and the response of the MQ135 metal oxide semiconductor (MOS) sensor during refrigerated storage of Nile tilapia (*Oreochromis niloticus*). The gradual increase in both parameters throughout the five-day storage period indicates that microbial proliferation was closely associated with the accumulation of volatile metabolites released during spoilage, suggesting that the MQ135 sensor effectively reflected the microbiological status of the fish.

Fresh fish exhibited a TPC of 4.57 log CFU g^{-1} and a relatively low MQ135 response (195 a.u.) on the first day of storage, indicating good microbiological quality and limited production of volatile compounds. At this stage, spoilage microorganisms remain in the lag phase, while endogenous enzymatic activity predominates, resulting in only small amounts of volatile organic compounds (VOCs) in the package headspace. Consequently, the sensor response remained low because the concentrations of ammonia, volatile amines, sulfur-containing compounds, aldehydes, and ketones had not yet reached levels sufficient to produce a strong MOS sensor signal.

As refrigerated storage progressed, the microbial population increased from 5.72 log CFU g^{-1} on Day 2 to 6.62 log CFU g^{-1} on Day 3, accompanied by a gradual increase in the MQ135 response from 204 to 275 a.u. This simultaneous increase reflects the transition of spoilage microorganisms into the exponential growth phase, during which proteolytic and deaminative reactions intensify, generating increasing concentrations of ammonia and other volatile metabolites. These compounds are readily detected by the MQ135 sensor, explaining the close agreement between microbial growth and sensor response during the early stages of spoilage.

A markedly different trend was observed during the final storage period. Although TPC increased only slightly from 6.79 to 6.95 log CFU g^{-1} between Days 4 and 5, the MQ135 response increased dramatically from 555 to 920 a.u. This finding indicates that the electronic nose is influenced not only by bacterial numbers but also by microbial metabolic activity. During the late exponential and stationary growth phases, spoilage bacteria exhibit enhanced metabolic activity, accelerating the degradation of proteins and amino acids and releasing substantially greater quantities of volatile compounds despite relatively small changes in viable cell counts. Therefore, the sharp increase in MQ135 response most likely reflects the rapid accumulation of ammonia, trimethylamine

(TMA), dimethylamine (DMA), hydrogen sulfide, aldehydes, ketones, alcohols, and other VOCs produced during advanced spoilage rather than microbial growth alone. This broader sensitivity enables the sensor to characterize spoilage more comprehensively than conventional plate count analysis.

These observations agree with the current understanding of fish spoilage mechanisms, where microbial metabolism rather than microbial numbers alone determines the production of spoilage volatiles responsible for the characteristic off-odours of deteriorated fish. Consequently, electronic noses based on MOS sensors provide a direct assessment of volatile metabolites, allowing rapid freshness evaluation without the lengthy incubation required for microbiological analyses. Conventional TPC determination generally requires 48–72 h, whereas electronic nose measurements can be completed within minutes, making this technology particularly attractive for real-time quality monitoring in the seafood industry (Franceschelli et al., 2021).

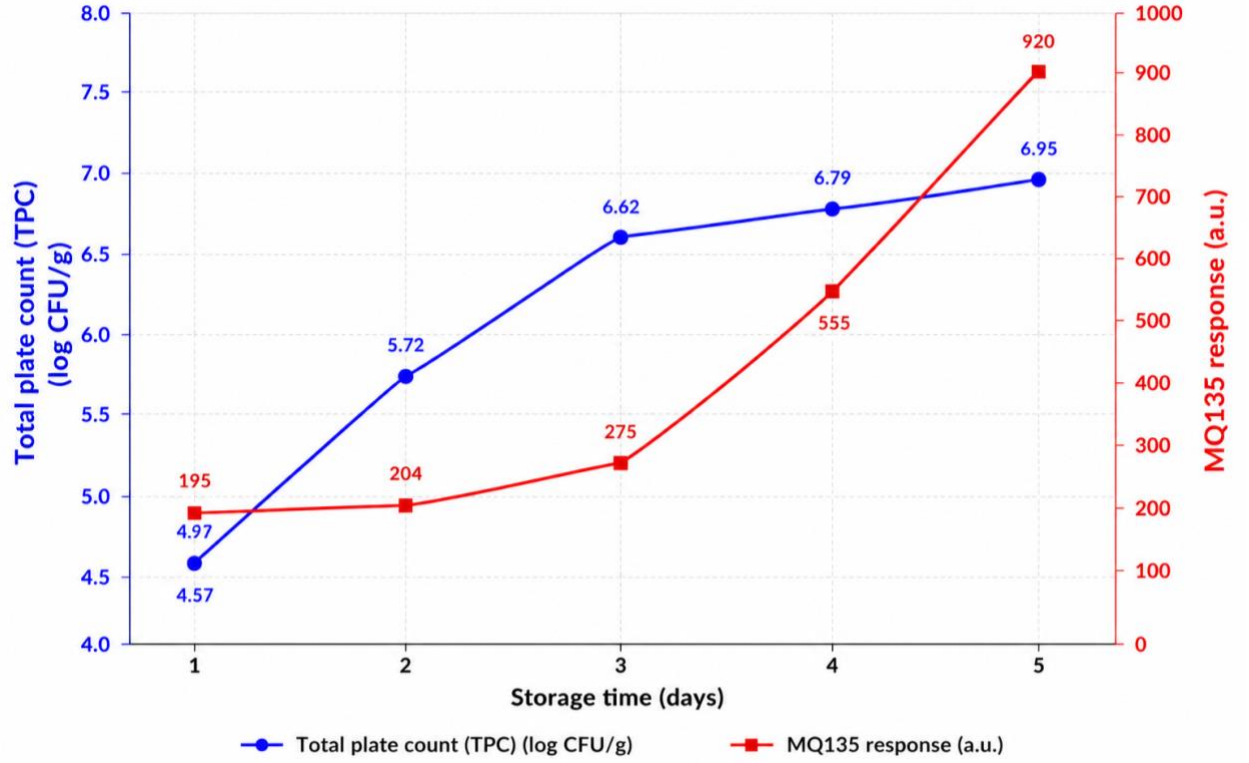

Figure 5. Releation between MQ135 gas sensor and total plate count of fish flesh during storage period.

The present findings are also consistent with previous research. Grassi et al. (2019) demonstrated that the portable MOS-based electronic nose Mastersense successfully classified fish samples into different freshness categories using total viable count (TVC) as the reference method, achieving prediction accuracies above 83% and confirming the suitability of MOS sensors for rapid microbial quality assessment. Likewise, the comprehensive review by Franceschelli et al. (2021) summarized numerous studies showing that electronic noses accurately estimate fish freshness by monitoring volatile compounds generated during microbial spoilage and concluded that MOS sensor arrays are among the most promising non-destructive technologies for seafood quality evaluation because they provide rapid, objective, and on-line assessment while minimizing sample

preparation. These reports strongly support the behavior observed in the present study, where the MQ135 sensor closely followed microbial deterioration and became increasingly responsive as spoilage progressed.

Overall, the results demonstrate that the MQ135 sensor provides a sensitive and reliable indication of microbiological quality during refrigerated storage of Nile tilapia. The considerably greater increase in sensor response compared with TPC during the final storage period suggests that the electronic nose captures not only bacterial proliferation but also the intensity of microbial metabolism through the detection of spoilage-related volatile compounds. Therefore, the MQ135-based electronic nose represents a rapid, non-destructive, and cost-effective tool for monitoring fish freshness and predicting shelf life, offering a practical alternative to conventional microbiological methods for routine quality control in the seafood industry.

4. Conclusions

A low-cost electronic nose based on six metal oxide semiconductor sensors was developed to monitor freshness changes in refrigerated Nile tilapia. Sensor responses were validated against conventional physicochemical and microbiological quality indicators during five days of storage. TVB-N, peroxide value, pH, thiobarbituric acid, and microbial counts increased progressively, confirming quality deterioration. MQ135 exhibited the highest sensitivity and showed strong agreement with TVB-N, pH, and microbial growth, whereas MQ3 effectively reflected lipid oxidation. The electronic nose achieved 90% classification accuracy in distinguishing freshness stages, demonstrating its suitability as a rapid, non-destructive, and cost-effective tool for seafood quality monitoring and shelf-life prediction.

5.References

Abdelhakam, O., Elsebaie, E., Ghazi, A., & Gouda, M. (2019). Quality characteristics of beef hamburger enriched with red grape pomace powder during freezing storage. *Slovenian Veterinary Research*, *56*(22-Suppl).

Amin, H. F., El-Sayed, A. E. K. B., Elsebaie, E. M., & Metwaly, H. R. (2024). Quality of catfish luncheon supplemented with marine algae (ulva lactuca and nannocholoropsis oculata) during frozen storage. *The Annals of the University Dunarea de Jos of Galati. Fascicle VI-Food Technology*, *48*(2), 27-44.

Ampuero, S., & Bosset, J. O. (2003). The electronic nose applied to dairy products: A review. *Sensors and Actuators B: Chemical, 94*(1), 1–12. https://doi.org/10.1016/S0925-4005(03)00321-6

Chen, J., Tao, L., Zhang, T., Zhang, J., Wu, T., Luan, D., ... & Zhong, J. (2021). Effect of four types of thermal processing methods on the aroma profiles of acidity regulator-treated tilapia muscles using E-nose, HS-SPME-GC-MS, and HS-GC-IMS. *Lwt*, *147*, 111585.

Cheng, H., Wang, J., & Xie, J. (2023). Progress on odor deterioration of aquatic products: Characteristic volatile compounds, analysis methods, and formation mechanisms. *Food Bioscience*, *53*, 102666.

Cyprian, O., Oduor-Odote, P., Lauzon, H., Martinsdóttir, E., & Arason, S. (2013). Microbiological quality and shelf life of fresh packaged tilapia fillets stored under different chill temperatures. *Journal of microbiology, biotechnology and food sciences*, *2*(4), 2456-2465.

de Matos Regalado, K. L., de Matos Regalado, K. L., Albergaria, F. C., de Souza Miranda, A. L., de Sousa Gomes, M. E., & de Resende, J. V. (2024). Quality and microstructure of tilapia fillets (Oreochromis niloticus) subjected to

different freezing methods. *International Journal of Refrigeration*, *165*, 303-314.

Ding, T., & Li, Y. (2024). Biogenic amines are important indices for characterizing the freshness and hygienic quality of aquatic products: A review. *Lwt*, *194*, 115793.

Elsebaie, E.M., Essa, R.Y. & Mohamed, E. A. (2021). Impact of atmospheric cold plasma (ACP) on maintaining bolti fish (Tilapia nilotica) freshness and quality criteria during cold storing. *Journal of Food Processing and Preservation*, *45*(5), e15442.

Elsebaie, E. M., Elmahdy, A., El-Gezawy, E. S., Badr, M. R., Asker, G. A., El-Gawish, A. M., & Essa, R. Y. (2022). Effects of faba bean hull nanoparticles on physical properties, protein and lipid oxidation, colour degradation, and microbiological stability of burgers under refrigerated storage. *Antioxidants*, *11*(5), 938

Eltholth, M., Fornace, K., Grace, D., Rushton, J., & Häsler, B. (2018). Assessing the chemical and microbiological quality of farmed tilapia in Egyptian fresh fish markets. *Global food security*, *17*, 14-20.

Ershidat, O. T. M., Atta, M. B., Elsebaie, E. M., & Badr, M. R. (2025). Effect of wheat germ as a partial meat substitute on the nutritional and qualitative properties of beef burgers. *Food Science and Technology International*, 10820132251353396.

Essa, R. Y., El-Shershaby, S. S., Abdelrahman, W. M., & Badr, M. R. (2026). Green Nanotechnology Approach for Chicken Burger Preservation Using Barnûf (Pluchea dioscoridis) Leaves powder Nanoparticles. *Egyptian Journal of Chemistry*, *69*(5), 61-71

Essa, R., & Elsebaie, E. M. (2018). Effect of using date pits powder as a fat replacer and anti-oxidative agent on beef burger quality. *Journal of Food and Dairy Sciences*, *9*(2), 91-96.

Franceschelli, L., Berardinelli, A., Dabbou, S., Ragni, L., & Tartagni, M. (2021). Sensing technology for fish freshness and safety: A review. *Sensors*, *21*(4), 1373.

Franceschelli, L., Berardinelli, A., Dabbou, S., Ragni, L., & Tartagni, M. (2021). Sensing technology for fish freshness and safety: A review. *Sensors*, *21*(4), 1373.

Franceschelli, L., Berardinelli, A., Dabbou, S., Ragni, L., & Tartagni, M. (2021). Sensing technology for fish freshness and safety: A review. *Sensors*, *21*(4), 1373.

Grassi, S., Benedetti, S., Opizzio, M., di Nardo, E., & Buratti, S. (2019). Meat and fish freshness assessment by a portable and simplified electronic nose system (Mastersense). *Sensors*, *19*(14), 3225.

Hu, Y., Huang, Y., Jiang, Y., Zhang, X., & Liu, H. (2025). Analysis of Volatile Organic Components in Fermented Mandarin Fish and Correlation with Microbial Composition Based on HS-SPME-GC-MS, E-Nose, and Colorimetric sensor array. *Food Bioscience*, 107257.

Huang, Z., Liao, H., Su, Z., Shi, S., Zhu, K., He, Y., ... & Li, C. (2024). Plasma activated water (PAW), Ozonated water (OW) and Slightly acidic electrolytic water (SAEW) treated tilapia fillets during chilled storage: A comparative study on quality changes and related mechanisms. *LWT*, *200*, 116194.

International Organization for Standardization (ISO). (2001). *ISO 16649-2:2001. Microbiology of food and animal feeding stuffs—Horizontal method for the enumeration of β-glucuronidase-positive Escherichia coli—Part 2: Colony-count technique at 44 °C using 5-bromo-4-chloro-3-indolyl β-D-glucuronide*. Geneva, Switzerland: ISO.

International Organization for Standardization (ISO). (2006). *ISO 4832:2006. Microbiology of food and animal feeding stuffs—Horizontal method for the enumeration of coliforms—Colony-count technique*. Geneva, Switzerland: ISO.

International Organization for Standardization (ISO). (2017). *ISO 6579-1:2017.*

Microbiology of the food chain—Horizontal method for the detection, enumeration and serotyping of Salmonella—Part 1: Detection of Salmonella spp. Geneva, Switzerland: ISO.

International Organization for Standardization (ISO). (2017). *ISO 21872-1:2017. Microbiology of the food chain—Horizontal method for the determination of Vibrio spp.—Part 1: Detection of potentially enteropathogenic Vibrio parahaemolyticus, Vibrio cholerae and Vibrio vulnificus*. Geneva, Switzerland: ISO.

International Organization for Standardization (ISO). (2021). *ISO 6888-1:2021. Microbiology of the food chain—Horizontal method for the enumeration of coagulase-positive staphylococci (Staphylococcus aureus and other species)—Part 1: Colony-count technique*. Geneva, Switzerland: ISO.

Jeyakumari, A., Narasimha Murthy, L., Visnuvinayagam, S., Sarma, K. S. S., & Rawat, K. P. (2023). Effect of electron beam irradiation on the quality of vacuum-packed, chilled-stored tilapia fish chunks. indian J. Fish., 70 (2): 109-115, doi:10.21077.

Junior, P. G., Assunção, A. W., Baldin, J. C., & Amaral, L. A. (2014). Microbiological quality of whole and filleted shelf-tilapia. *Aquaculture*, *433*, 196-200.

Li, H., Wang, Y., Zhang, J., Li, X., Wang, J., Yi, S., ... & Li, J. (2023). Prediction of the freshness of horse mackerel (Trachurus japonicus) using E-nose, E-tongue, and colorimeter based on biochemical indexes analyzed during frozen storage of whole fish. *Food Chemistry*, *402*, 134325.

Madhubhashini, M. N., Liyanage, C. P., Alahakoon, A. U., & Liyanage, R. P. (2024). Current applications and future trends of artificial senses in fish freshness determination: A review. *Journal of Food Science*, *89*(1), 33-50.

Madhubhashini, M. N., Liyanage, C. P., Alahakoon, A. U., & Liyanage, R. P. (2024). Current applications and future trends of artificial senses in fish freshness determination: A review. *Journal of Food Science*, *89*(1), 33-50.

Monteiro, M. L. G., Mársico, E. T., Mano, S. B., da Silveira Alvares, T., Rosenthal, A., Lemos, M., ... & Conte-Junior, C. A. (2018). Combined effect of high hydrostatic pressure and ultraviolet radiation on quality parameters of refrigerated vacuum-packed tilapia (Oreochromis niloticus) fillets. *Scientific Reports*, *8*(1), 9524.

Ndraha, N., Lin, H. Y., Hsiao, H. I., & Lin, H. J. (2024). Managing the microbiological safety of tilapia from farm to consumer. *Comprehensive Reviews in Food Science and Food Safety*, *23*(5), e70023.

Peris, M., & Escuder-Gilabert, L. (2009). A 21st century technique for food control: Electronic noses. *Analytica chimica acta*, *638*(1), 1-15.

Prado-Toledo, I. Y., Ramos-Santoyo, K. N., Guzman-Robles, L. N., & Cortes-Sanchez, A. D. (2022). Microbiological analysis of tilapia fillet stored under refrigerated conditions. *Journal of Biological Sciences*, *22*(1), 126-138.

Rabehi, A., Helal, H., Zappa, D., & Comini, E. (2024). Advancements and prospects of electronic nose in various applications: a comprehensive review. *Applied Sciences*, *14*(11), 4506.

Radi, R., Wahyudi, E., Adhityamurti, M. D., Putro, J. P. L. Y., Barokah, B., & Rohmah, D. N. (2021). Freshness assessment of tilapia fish in traditional market based on an electronic nose. *Bulletin of Electrical Engineering and Informatics*, *10*(5), 2466-2476.

Rong, C., Chang-hu, X., Qi, L., & Bang-zhong, Y. (2009). Microbiological, chemical and sensory assessment of (I) whole ungutted,(II) whole gutted and (III) filleted

tilapia (Oreochromis niloticus) during refrigerated storage. *International Journal of Food Science and Technology*, *44*(11), 2243-2248.

Sun, Y., & Zhang, X. (2022). Tilapia freshness prediction utilizing gas sensor array system combined with convolutional neural network pattern recognition model. *International Journal of Food Properties*, *25*(1), 2066-2072.

Wang, B., Liu, K., Wei, G., He, A., Kong, W., & Zhang, X. (2024). A review of advanced sensor technologies for aquatic products freshness assessment in cold chain logistics. *Biosensors*, *14*(10), 468.

Wu, K., Debliquy, M., & Zhang, C. (2023). Metal–oxide–semiconductor resistive gas sensors for fish freshness detection. *Comprehensive Reviews in Food Science and Food Safety*, *22*(2), 913-945.

Wu, L., Pu, H., & Sun, D. W. (2019). Novel techniques for evaluating freshness quality attributes of fish: A review of recent developments. *Trends in food science & technology*, *83*, 259-273.

Author Contributions:

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