

A review on total volatile basic nitrogen (TVB-N) as key indicators of fish spoilage: Methods for determination and quality assessment

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Abstract

A commonly used biochemical diagnostic for determining the freshness and spoilage of fish and marine products is total volatile basic nitrogen, or TVB-N. It represents the buildup of volatile bases created by microbial and enzymatic breakdown, primarily ammonia, trimethylamine, and dimethylamine. The importance of TVBN in quality evaluation of fish is along with the most widely used methods for measuring TVB-N levels are discussed in this review, including Kjeldahl distillation, the microdiffusion method, steam distillation, spectroscopic method and new sensor-based technique like electronic nose. Their sensitivity, accuracy, and applicability for various contexts are discussed. This review highlights TVB-N's crucial role in contemporary seafood quality assurance systems by looking at both tried-and-true and cutting-edge techniques.

Keywords: Conway's Microdiffusion Method, Dimethylamine (DMA), Fish Spoilage, Quality evaluation, Total volatile basic nitrogen (TVB-N), Trimethylamine (TMA)

Introduction

Fish is an extremely perishable food item whose spoilage begins immediately after capture. During spoilage, fish undergoes undesirable changes such as off-odors, altered taste, and texture degradation. Fish spoilage refers to the process in which fish deteriorates due to microbial (bacteria, yeasts, molds), enzymatic autolysis (self-digestion by fish enzymes), and chemical changes (notably lipid oxidation) (Ghaly *et al.*, 2010) [16], ultimately making it undesirable for consumption. Temperature, moisture content, microbial contamination, and oxygen exposure are some of the variables that affect fish spoilage. Bacterial growth is accelerated by high temperatures, and degradation is accelerated by unsuitable storage conditions. Understanding fish spoilage is crucial because it affects public health, food safety, and financial losses. When fish is handled, stored, and preserved properly, its shelf life is increased, its quality is maintained, and the health risks of eating unfresh fish are reduced. The fishing and food industries can minimize waste and guarantee safe consumption by managing these factors. Flavor and safety in seafood depend on freshness; unfresh fish loses its appealing flavor and texture, which lowers customer satisfaction (Kartika *et al.*, 2020) [21]. After fish are caught, bacteria grow and natural processes break down the fish muscle quickly, leading to fat spoilage, protein degradation, and histamine production, all of which impair edibility and sensory qualities.

Various methods were used to assess the freshness of the fish, including organoleptic evaluation, which entails evaluating the fish's texture, appearance, and aroma to ascertain its freshness. Chemical and microbial analysis is a conventional approach that both industry professionals and consumers use for quality assessment of fish. Fish freshness can be objectively determined using methods like measuring microbial load, protein changes, and volatile compounds (Cheng *et al.*, 2015) [11].

A common chemical indicator used to quantify the buildup of basic nitrogenous compounds created during fish spoiling

is total volatile basic nitrogen, or TVB-N. It is a clear reflection of the degradation of proteins and amines that occur under the conditions during their storage, making it very efficient in monitoring all properties associated with fish. Total Volatile Base Nitrogen (TVB-N) is a combination of ammonia (NH₃), dimethylamine (DMA), and trimethylamine (TMA). It is used extensively as an index of freshness in many species of fish (Wu and Bechtel, 2008) [48]. It is a widely accepted analysis technique to estimate the extent of decomposition of fish that detects the concentration of various volatile nitrogenous compounds present in fish (Malle and Poumeyrol, 1989) [29].

TVB-N is frequently used as a biomarker for the deterioration of meat and fish, indicating the level of microbial activity and protein degradation (Bekhit *et al.*, 2021) [8]. TVB-N plays a critical role in determining the freshness of fish meal, as elevated levels suggest advanced spoilage and possible health hazards, whereas freshness is indicated by lower TVB-N levels (Dyer & Mounsey, 1945) [13]. The aim of this systematic review is to comprehensively analyse five different methods of TVB-N determination and its role in assessing fish freshness.

Tvb-N Formation in Fish

1. Biochemical processes leading to TVB-N production

Several postmortem biochemical changes contribute to the formation of TVB-N in fish. Temperature, muscle health and the presence of specific compounds are some of the factors that influence these changes. Moreover, Fish spoiling is mostly caused by two factors: bacterial and autolytic (enzymatic) spoiling.

Autolysis (Enzymatic spoilage): autolysis, begins when the fish's own enzymes start breaking down proteins and fats in its muscle tissues after death. Proteolytic enzymes break down proteins, while lipolytic enzymes break down fats, leading to a decline in fish quality (Nie *et al.*, 2022) [33]. The fish's gut and muscle tissues contain the majority of these enzymes, which become active after the fish dies and starts

hydrolyzing protein molecules into smaller peptides and amino acids (Lakshmanan P.T., 2000) [22] which in turn converted into ammonia and contribute to TVBN. Furthermore, dystrophin and other muscle proteins break down quickly, releasing nitrogenous compounds that also make up TVB-N (Papa *et al.*, 1997) [39]. According to Dyer & Mounsey, (1945) [13] bacterial growth is closely linked to proteolytic activity, which speeds up the spoiling process.

Bacterial Spoilage: Another important factor of fish spoilage is bacterial action. Bacteria from the slime, skin, gills, and gut begin to infiltrate the tissues of dead fish, resulting in spoilage. Spoilage bacteria, also known as saprophytic bacteria, include *Moraxella*, *Aeromonas*,

Acinobacter, *Flavobacteria*, and *Pseudomonas*, which convert urea into ammonia and reduce trimethylamine oxide (TMAO) to trimethylamine (TMA) (Lakshmanan, P. T. 2000) [22]. Research shows that TMAO (naturally found in fish muscle) is the source of more than 94% of TMA in decaying fish (Tarr, 1938) [43]. TMA is one of the important components of TVB-N, which increases with fish degradation. The breakdown of proteins and amino acids in muscles is often associated with the formation of TMA (Ali *et al.*, 2022) [4]. These biogenic amines and ammonia are responsible for the off flavors and unpleasant odors of spoiled fish (Lakshmanan, P. T. 2000) [22], which also affect marketability and consumer acceptance (Mitchell & Smith, 2001) [30].

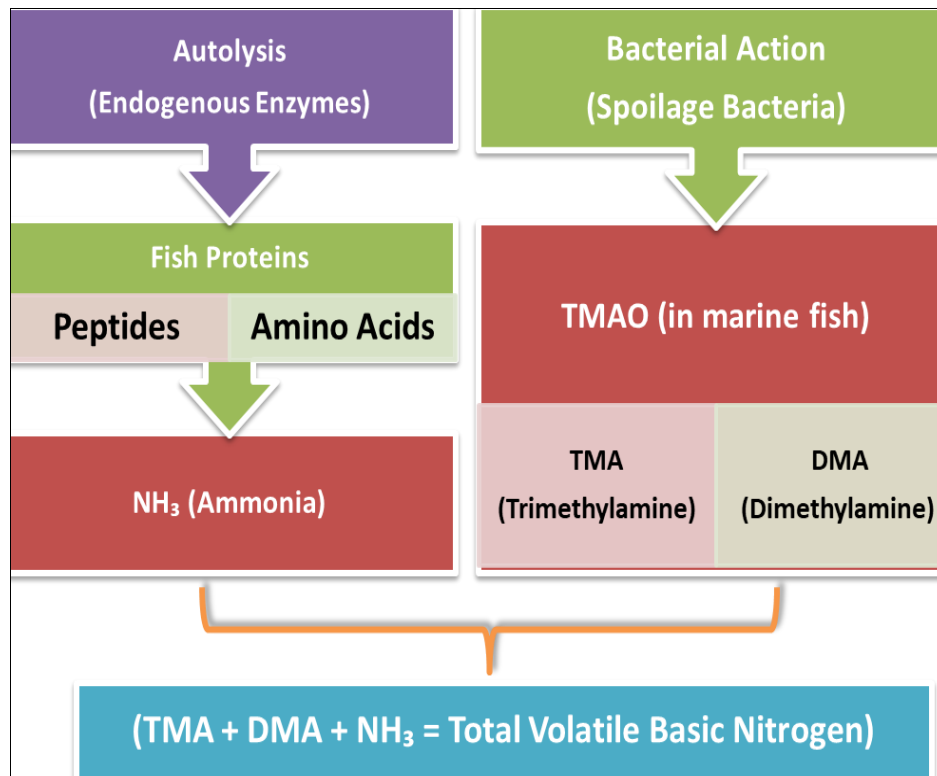


Fig 1: Diagram showing formation of TVBN in fish after harvest.

2. Factors influencing TVB-N accumulation

Temperature: TVB-N production and accumulation are significantly influenced by storage temperature, it increases more slowly at lower storage temperatures and more rapidly at higher ones (Šimat *et al.*, 2009) [42]. Jinadasa *et al.* (2014) stored yellowfin tuna (*Thunnus albacares*) at different storage temperatures and revealed that TVBN levels were higher at 7°C than at 0°C and 4°C. Higher temperatures help the bacteria that produce TVB-N to grow faster. On the other hand, lower temperatures cause bacterial activity to slow down, which lowers TVB-N levels. TVB-N accumulation in silver carp fillets studied by Shi *et al.* (2012) showed that TVB-N rose from 80.5 to 310.4 mg kg⁻¹ at 0 °C, from 80.5 to 309.5 mg kg⁻¹ at 3 °C, and from 80.5 to 278.7 mg kg⁻¹ at 15 °C. Additionally compared to higher temperatures like 8°C and 25°C, lower temperatures like 0°C and -18°C are more effective at suppressing TVB-N formation (Al-Busaidi *et al.*, 2011) [3].

According to Wannawisan *et al.* (2023) [47], The shelf life of Asian seabass (*Lates calcarifer*) decreased from six days at 4 °C to three days at 10 °C since TVB-N content increased with temperature which is indicative of fish degradation.

But according to Castro *et al.* (2012) [9], TVBN accumulation in fish muscle extracts was not substantially impacted by storage temperatures of 4, -20, and -80°C. Temperature changes within this range do not affect TVBN values over time, as evidenced by the statistically identical results obtained under all storage conditions.

Packaging method: Vacuum packaging instead of normal or air packaging is a powerful food preservation technique because vacuum packaging eliminates oxygen from the packaging and creates an environment that slows spoilage (inhibits bacterial growth and slows oxidation). According to Parvathy *et al.*, (2016) [40] when compared to air packaging (AP), vacuum packaging (VP) considerably increases the shelf life of monosex tilapia because they contain lower levels of TVB-N and thiobarbituric acid (TBA). VP samples remain admissible for 19 days, while AP samples are acceptable for 16 days. Another study on phytophagous fish in Iran to determine the effect of vacuum packaging on body chemical composition, peroxide, and TVB-N reported TVB-N levels rose more drastically in non-vacuum conditions (from 6.53 mg/100g to 38.26 mg/100g)

compared to vacuum conditions (from 6.53 mg/100g to 18.66 mg/100g) (Abdolmaleki *et al.*, 2023) ^[1]. This suggests that vacuum storage may preserve freshness better than non-vacuum conditions by slowing the increase in TVB-N levels.

In addition, TVB-N levels are strongly impacted by modified atmosphere packaging (MAP), which increases the shelf life of fish by delaying the biochemical reactions and bacterial growth that cause product spoiling (Babic Milijasevic *et al.*, 2019) ^[6]. MAP includes removing air from the packaging and substituting it with a specific gas or combination of gases. In contrast to vacuum packaging, a gas mixture consisting of 90% CO₂ and 10% N₂ (MAP) was found to be the most successful in reducing TVB-N accumulation in rainbow trout and carp (Babic Milijasevic *et al.*, 2017) ^[7]. The effect of several packaging types on sardines (*Sardina pilchardus*) was studied by Özogul *et al.* (2004) ^[36] and revealed that sardines stored in air, VP, and especially MAP differed significantly ($P < 0.05$), according to the statistical analysis of the TVB-N data. They also conclude sardines stored in MAP conditions had a longer shelf life, produced fewer ATP derivatives, inhibited bacterial growth, and produced less histamine, TMA, and TVB-N.

According to Giuffrida *et al.* (2013) ^[17], TVBN accumulation is also dependent on the type of tissue being examined. Increases in TVBN were more noticeable in the skin and gills of gilthead seabream (*Sparus aurata*) than in the muscle, and they were correlated with increases in both bad bacteria and sensory scores. Fish species, fat content, size, shape, season, and fishing grounds are some of the factors that affect TVB-N accumulation (Šimat *et al.*, 2009) ^[42].

Tvb-N as An Indicator of Fish Spoilage

As fish deteriorate over time due to bacterial activity and enzymatic processes, it indicates the presence of spoilage compounds. The assessment parameter Total Volatile Basic Nitrogen (TVB-N) is one vital aspect of quality control in fish processing. This is to determine any spoiled or contaminated batch so that only quality products are offered to the consumers. Therefore, risking the consumers of foodborne illnesses is diminished, and the trust in fish products stands (Zou & Liu, 2024) ^[52]. Also, TVB-N determination is routinely used in fish processing facilities to monitor product quality and ensure compliance with regulatory standards (Jinadasa 2014; Zhao *et al.*, 2024; Liu *et al.*, 2010) ^[20, 26, 51]. Since its levels rise sharply as microbes break down fish tissue, TVB-N is a good indicator of spoilage. The industry can identify spoiled fish and take corrective action to stop further contamination by detecting elevated TVB-N levels (Giuffrida *et al.*, 2013; Fang *et al.*, 2023) ^[15, 17]. TVB-N levels in fish muscle, skin, and gills provide valuable information about bacterial growth and spoilage processes (Giuffrida *et al.*, 2013; Zou & Liu, 2024) ^[17, 52]. It is used to estimate the shelf life of fish products under various storage conditions. The industry can establish safe consumption limits and guarantee that fish products stay fresh during the distribution and retail stages by keeping an eye on TVB-N during storage (Giuffrida *et al.*, 2013; Zou & Liu, 2024) ^[17, 52]. As fish deteriorate, the levels of TVB-N increase which indicate the fish may not be safe to consume because it is no longer fresh (Ali *et al.*, 2022) ^[4]. For example, Viji *et al.* (2022) ^[46] demonstrated a

significant rise in TVB-N levels from 8.02 mg% to 29.79 mg% during the storage of cage-farmed grouper (*Epinephelus coioides*) in ice over 24 days. This increase indicates that the shelf life of fish is affected by biochemical changes over time, which signal spoilage. TVB-N limits have been set by the European Commission (European Union Law 95/149/EC, 1995) ^[14] for a variety of species. These include 25 mg/100 g for species of *Sebastes* (*Helicolenus dactylopterus*, *Sebastichthys capensis*), 30 mg/100 g for species of Pleuronectidae (excluding halibut: *Hippoglossus* spp.), and 35 mg/100 g for salmon, cod, and hake.

According to Bekhit *et al.*, (2021) ^[8] TVB-N is one of the most important biomarkers for the breakdown of meat's proteins and amines. Although its use is more well-established in fish and seafood products, it is used to assess the quality of a variety of meats, including beef, pork, chicken, and sheep/goat meat. Although it varies amongst fish species, there is a well-established correlation between TVB-N and other freshness indicators as higher TVB-N levels are indicative of advanced spoilage and possible health hazards (Giuffrida *et al.*, 2013; Zou & Liu, 2024; Fang *et al.*, 2023) ^[15, 17, 52]. TVB-N is a comprehensive tool for evaluating fish quality because of these correlations.

1. Correlation between TVB-N levels and sensory evaluation

Sensory methods check seafood quality by looking at its appearance, smells, texture, and taste. On the other hand, TVBN acts as a biochemical measure that relates to these sensory evaluations. The findings show that TVBN levels reflect the sensory quality of fish typically, higher TVBN values are associated with lower sensory quality (Yang *et al.*, 2016) ^[50]. Moreover Özyurt *et al.* (2009) ^[37] discovered that TVBN values of red mullet (*Mullus barbatus*) and goldband goatfish (*Upeneus moluccensis*) closely aligned with the results from sensory analysis. Similarly, Liu *et al.* (2010) ^[26] observed a strong negative correlation between sensory acceptability and TVBN values of tilapia fillets, with a correlation coefficient of $r = -0.93$. Their study also revealed that during the storage of tilapia fillets, the TVBN value increased significantly from an initial level of 6.5 mg/100 g to a final level of 61.3 mg/100 g. This increase was strongly linked to both bacterial counts ($r > 0.90$) and storage time ($r = 0.98$).

Additionally, TVB-N levels in grouper (*Epinephelus coioides*) muscle also correlate well with sensory evaluation outcomes. The study found that on the 24th day of storage, which is when the fish was deemed unacceptable according to sensory evaluations, the TVB-N level reached 29.79 mg%. This indicates that lower sensory acceptability is associated with higher TVB-N levels and suggests a significant link between biochemical quality (TVB-N) and sensory quality (Viji *et al.*, 2022) ^[46]. The quality index method's sensory scores demonstrate a strong correlation with the rise in TVB-N and TMA-N levels in the skin and gills of *Sparus aurata*, these rise in nitrogenous compounds result from protein breakdown caused by the growth of spoilage bacteria and this microbial activity is mirrored in sensory evaluations that reflect a progressive decline in fish quality as spoilage advances (Giuffrida *et al.*, 2013) ^[17]. According to Odoli, (2009) ^[34] sensory changes, such as the emergence of sour or ammoniac odors and a softening of the flesh texture, which are signs of spoilage, occur when TVB-N levels rises.

This correlation emphasizes how crucial it is to keep an eye on TVB-N, a crucial marker of fish quality while it is being stored (Adoga *et al.*, 2010) [2]. Similarly, according to Tsironi *et al.*, (2020) [45] the sensory score of gilthead sea bream, sea bass, and yellowfin tuna is highly correlated with color and TVBN implies that the assessment of sensory quality, especially with regard to color perception, changes in tandem with changes in TVBN levels.

2. Correlation between TVB-N levels and microbial evaluation

Several studies have confirmed a strong correlation between TVB-N levels and microbial growth as both parameters reflect spoilage. In Tilapia TVB-N levels was correlated with a significant increase in spoilage organisms, including *Aeromonas hydrophila* and *E. coli*, as storage went on (Adoga *et al.*, 2010) [2]. These microorganisms convert urea into ammonia and reduce trimethylamine oxide (TMAO) to trimethylamine (TMA) (Lakshmanan, P. T. 2000) [22], which is one of the important components of TVBN. Additionally, increased microbial activity resulted in increased protein degradation, leading to higher pH and TVBN concentration (Zhao *et al.*, 2024) [51], indicating a potential link between pH and TVBN accumulation. There was a strong correlation between the evolution of TVB-N in grouper muscle and the presence of particular spoilage bacteria with correlation coefficients $R^2 = 0.94$ and $R^2 = 0.93$ for *Pseudomonas* and *Enterobacteriaceae* species respectively (Viji *et al.*, 2022) [46]. So, we can infer that higher microbial counts generally result in higher TVB-N levels. This shows that TVB-N levels rise sharply in tandem with the levels of these bacteria, indicating that microbial activity plays a significant role in fish spoilage during ice storage (Viji *et al.*, 2022) [46].

Methods for Tvbn Determination

TVB-N and TMA-N can be determined using a variety of techniques, including capillary electrophoresis, high-performance liquid chromatography, gas chromatography, steam distillation, colorimetric, photometry, semi-conducting metal oxide sensors, selective electrode, and flow injection/gas diffusion (FIGD) with spectrophotometric or potentiometric detection (Maghraby *et al.*, 2013) [28]. Additionally, cutting-edge techniques like smartphone-integrated colorimetric sensors and vibrational spectroscopy (NIR and Raman) have been developed, providing quick and non-invasive evaluations (Ouyang *et al.*, 2024) [35]. Various techniques, from sophisticated spectroscopic and sensing technologies to conventional chemical assays, have been developed to measure TVB-N in fish. These techniques seek to provide accurate TVB-N measurements for quality evaluation, though they differ in accuracy, cost, and usefulness. Kjeldahl distillation, Conway microdiffusion, Steam distillation and Electronic nose are discussed below:

Method 1: Conway's Microdiffusion Method

A well-known method for assessing fish's TVB-N levels—a sign of freshness and spoiling—is the Conway microdiffusion method. This technique measures the nitrogen compounds that accumulate as fish deteriorate, providing a reliable indicator of quality. It involves microdiffusion cell that capture gases released during spoilage, allowing for the determination and quantification of nitrogen compounds (Lourquin & Delville, 1950) [23]. The

fundamental principle of the Conway method is that volatile materials diffuse from one chamber, where they exert a certain tension, into another chamber, which has zero surface tension (Conway and Byrne 1933). The absorbed volatile bases are then titrated with a standardized acid solution.

Research has shown that while the Conway method tends to produce significantly lower TVB-N values compared to direct water vapor distillation methods, it still holds significant value for monitoring fish freshness (Maghraby *et al.*, 2013) [28]. The method's reliability may be impacted in certain situations, such as when parasites are present, hence, careful interpretation of results is required (Panebianco *et al.*, 2011) [38]. This approach is renowned for being inexpensive, straightforward, and quick without sacrificing accuracy. It is frequently used to measure TVB-N, particularly in Japan (Maghraby *et al.*, 2013) [28]. Recent advancements, such as coupling hyperspectral imaging with the Conway method, have significantly increased the accuracy of predicting TVB-N levels, enhancing the ability to monitor fish fillet freshness (Cheng *et al.*, 2017) [10].

Method 2: Direct water vapor distillation method

The determination of Total Volatile Basic Nitrogen (TVBN) is commonly achieved through steam distillation, a method that extracts volatile bases from fish samples (Tomiya *et al.*, 1952) [44]. Maghraby *et al.*, (2013) [28] explain this straightforward approach which involves distilling the volatile amines present in the samples. To begin, fish samples are combined with water and magnesium oxide, which facilitates the release of volatile nitrogen compounds. Once the fish samples are homogenized, they undergo steam distillation. During this process, nitrogenous volatile compounds are collected in a receiving tube containing an acidic solution, typically sulfuric acid. The TVBN content can then be determined from the distillate through titration, allowing for the calculation of nitrogen content in milligrams per 100 grams of fish. This technique is ideal for routine quality control due to its rapid nature and ability to process large quantities of samples efficiently (Antonacopoulos & Vyncke, 1989) [5].

Method 3: Micro-Kjeldahl Methods

The micro-Kjeldahl distillation technique, which measures the amount of ammonia generated from the fish sample, is the conventional method for measuring TVB-N. Although this approach is precise, it takes a lot of time and specific lab equipment (Moosavi-Nasab *et al.*, 2021) [32].

To extract Total Volatile Basic Nitrogen (TVBN) from fish samples, the samples are typically homogenized and treated with a specific concentration of perchloric acid (HClO_4). Recovery rates using a $0.6 \text{ mol}\cdot\text{L}^{-1}$ HClO_4 solution have been shown to range from 98.35% to 100.80% (Wu, 2006) [49]. The sample size, which is directly weighed into a micro-Kjeldahl flask, usually ranges from 3 to 8 mg. After weighing, the sample is dissolved in a mixture of methanol and acetic acid, to which zinc metal and hydrochloric acid are added to reduce nitrogen compounds (Ma *et al.*, 1957) [27]. To accelerate the digestion process, a catalyst mixture—commonly containing potassium sulfate and selenium—is incorporated. The flask is then heated until fumes begin to emerge (Moore, 1938) [31]. Once digestion is complete, the released ammonia is distilled and absorbed into a boric acid

solution. The ammonia is then titrated with a standardized solution, such as potassium biniodate, using a mixed indicator to detect color changes. This method has a precision of $\pm 0.3\%$ nitrogen, making it a reliable technique for evaluating TVBN (Ma *et al.*, 1957) [27].

Method 4: Spectroscopic Techniques

To evaluate total volatile basic nitrogen (TVB-N) in fish quickly and without causing damage, spectroscopic techniques such as Raman and near-infrared (NIR) spectroscopy have gained increasing popularity. These methods analyze the vibrational characteristics of molecules, allowing for the identification of changes in fish tissue related to deterioration. For example, NIR spectroscopy has been used alongside chemometric models like support vector regression (SVR) and partial least squares regression (PLSR) to accurately predict TVB-N levels in fish fillets (Ouyang *et al.*, 2024; Hong *et al.*, 2024) [19, 35]. The chemical makeup of food products, including the freshness of fish, can be effectively evaluated using near-infrared (NIR) spectroscopy and Raman Spectroscopy. This method is helpful for identifying chemical compounds in fish fillets and provides molecular information (Ouyang *et al.*, 2024) [35].

Method 5: Electronic Nose (E-Nose)

Electronic nose technology has been used to identify volatile organic compounds (VOCs) linked to fish spoiling, which mimics the human olfactory system. In industrial environments, these systems are especially helpful for monitoring fish freshness in real time (Li *et al.*, 2024) [24].

Electronic nose (e-nose) is a highly advanced instrument for detecting and analyzing volatile compounds especially helpful for determining the freshness of fish meal by measuring the levels of TVB-N. A set of gas sensors is employed by the electronic nose (e-nose) to detect various volatile substances released from fish meal. These sensors identify changes in the chemical composition of the air surrounding the sample, which correlates with the freshness of the fish meal. Establishing a mapping relationship between the sensor responses and the actual Total Volatile Basic Nitrogen (TVB-N) values is a crucial step in the detection process. This relationship allows the e-nose to determine freshness levels based on the sensor data. Using VMD (Variational Mode Decomposition), the TVB-N variation curve—which shows how TVB-N levels fluctuate over time—is broken down into multiple subsequences. By separating the data into distinct frequency components, this method enables a more thorough examination of the variations in TVB-N (Li *et al.*, 2024) [24].

Table 1: Methods for Evaluating Fish Freshness via TVB-N.

Method	Description	Key Characteristics	Author
Conway Microdiffusion	Classical chemical method measuring volatile bases in alkaline conditions.	<ul style="list-style-type: none"> Sensitivity: Less sensitive; yields lower TVB-N values Application: Suitable for routine use; limited in detecting advanced spoilage 	Maghraby <i>et al.</i> , (2013) [28] Özoğul & Özoğul, (2000)
Steam Distillation	Traditional method distilling TVB-N compounds for quantification	<ul style="list-style-type: none"> Efficiency: Yields high TVB-N values, especially in semi-steam distillation Cost: More complex and costlier than Conway method 	Özoğul & Özoğul, (2000)
Micro-Kjeldahl Method	Measures total nitrogen, adapted to estimate TVB-N by distillation.	<ul style="list-style-type: none"> Accuracy: High accuracy in nitrogen determination Limitation: Time-consuming, complex sample preparation 	Maghraby <i>et al.</i> , (2013) [28]
NIR & Raman Spectroscopy	Analyzes vibrational properties of molecules to detect TVB-N.	<ul style="list-style-type: none"> Non-destructive: Rapid, real-time TVB-N estimation without damaging samples Technological Advancement: Uses chemometric/data fusion for high accuracy 	Ouyang <i>et al.</i> , (2024) [35] Guo <i>et al.</i> , (2024) [18]
Electronic Nose	Detects volatile organic compounds using gas sensors and predicts TVB-N with machine learning.	<ul style="list-style-type: none"> Machine Learning-Based: Uses pattern recognition for spoilage detection Potential: Good predictive power 	Liu & Niu, n.d.

Conclusion

TVB-N act as a sign of fish deterioration and is a reliable indicator of spoilage. Various methodologies developed to assess TVB-N levels such as Conway Microdiffusion; Direct water vapor distillation; Micro-Kjeldahl Method; NIR & Raman Spectroscopy; Electronic Nose. It was found that all methods were sensitive and need an experience personnel. Conway Microdiffusion method is low-cost, moderate precision and can be used in many quality control labs. Steam distillation method is considered a reference method showing high accuracy and is better for advanced spoilage detection. Micro-Kjeldahl Method measures total nitrogen (not specific to TVB-N); often overestimates and is best for protein quantification. Spectroscopic methods are modern, portable, and suitable for industrial automation while the Electronic nose system is experimental but promising; increasingly adopted in smart food packaging.

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