



Bromothymol-blue paper strip indicator sensor for real-time monitoring of buffalo meat spoilage in modified-atmosphere refrigerated storage[#]

Mohan Midhun¹, A. Irshad^{1*}, V. N. Vasudevan¹, T. Sathu¹,
 Nayar Renuka² and Vijay Deepthi³

¹Department of Livestock Products Technology, College of Veterinary and Animal Sciences, Mannuthy, Thrissur, Kerala., ²Department of Livestock Products Technology, College of Veterinary and Animal Sciences, Pookode, Kerala Veterinary and Animal Sciences University, Pookode, Wayanad, Kerala, ³Centre for One Health, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana-141004, India

Citation: Midhun M., Irshad A., Vasudevan V. N., Sathu T., Renuka N & Deepthi V. (2025). Bromothymol blue paper strip indicator sensor for real-time monitoring of buffalo meat spoilage in modified atmosphere refrigerated storage. *Journal of Veterinary and Animal Sciences* 57 (1), 20-27
<https://doi.org/10.51966/jvas.2026.57.1.20-27>

Received: 04.08.2025

Accepted: 03.12.2025

Published: 31.03.2026

Abstract

This study developed and validated a low-cost, on-pack freshness tag for buffalo meat (carabeef) stored under commercial modified-atmosphere refrigeration (30 % CO₂ : 20 % O₂ : 50 % N₂, 4 ± 1 °C). Bromothymol blue (BTB, 1 % w/v) was immobilised on Whatman No. 1 paper strips, affixed to high-barrier pouches, and evaluated both in an ammonia-challenge model and alongside 500 g muscle cuts over nine days. Indicator hue shifted reproducibly from yellow → very pale green → greenish yellow → light bluish green as spoilage progressed. The first discernible change coincided with pH 5.9, TVBN ≈ 10 mg 100 g⁻¹ and total viable counts ≈ 3 log CFU g⁻¹ (day 3); the final bluish-green stage appeared when TVBN reached 18 mg/100g, ammonia 5 mg/100g, and microbial loads exceeded 6 log CFU/g (day 9), matching sensory rejection (overall acceptability ≈ 2.0). Parallel physicochemical trends, declining water-holding capacity (86.6 to 77.8 %), rising TBARS (0.09 to 0.71 mg MDA/kg), tyrosine (0.19 to 20.1 mg/100g) and free amino acids (5.1 to 31.7 mg/100g), confirmed progressive spoilage and underpinned the colour transitions. The strip remained stable during handling, showed no dye bleed and responded within minutes to 25 mg gaseous ammonia in the model system, demonstrating sensitivity to volatile bases typical of carabeef decomposition. These findings establish the BTB paper sensor as an effective, non-invasive tool for real-time monitoring of buffalo-meat freshness in MAP supply chains, offering processors and retailers a practical means to enhance safety, minimise waste and bolster consumer confidence.

Keywords: Modified atmospheric packaging, indicator, sensor, total volatile basic nitrogen

Microbiological spoilage remains the principal threat to the safety, market value and consumer acceptance of buffalo meat (carabeef), a commodity that now accounts for more than half of India's red-meat exports. Carabeef's high water activity, abundance of soluble proteins, and relatively open fibre architecture foster the growth of psychrotrophic spoilers such as *Pseudomonas spp.*, *Brochothrixthermosphacta* and members of the *Enterobacteriaceae* (Bevilacqua et al., 2024). Their enzymatic activity generates basic volatiles and reductive pigments that shift the meat's pH dull its bright red colour and impart off-odours and surface slime long before conventional laboratory tests can be completed (Reddy, 2021). Routine surveillance by plate count or total volatile basic nitrogen (TVBN) therefore offers little protection once

[#]Part of MVSc thesis submitted to Kerala Veterinary and Animal Sciences University, Pookode, Wayanad, Kerala

*Corresponding author: irshad@kvasu.ac.in, Ph. 9895213500

Copyright: © 2026 Midhun et al. This is an open access article distributed under the terms of the Creative Commons Attribution 4.0 International License (<http://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

product has left the abattoir.

There is a clear need for an on-pack, real-time indicator that can signal incipient spoilage without laboratory intervention. Rukchon et al. (2011) integrated phenol red and bromothymol blue into a dual-indicator system for TVBN detection from chicken meat which produced a distinct combined colour transition that intensified with spoilage progression. The present work develops and evaluates a colour-change label based on bromothymol blue immobilised on a paper carrier, calibrated specifically to the biochemical kinetics of carabeef stored under commercial modified-atmosphere packaging at 4 ± 1 °C. Through parallel monitoring of microbial loads, physicochemical indices, volatile bases and sensory scores, we establish the point at which the indicator delivers an unequivocal visual warning and demonstrate its concordance with established spoilage thresholds. The outcome is an inexpensive, non-contact freshness tag that could be embedded throughout the buffalo-meat supply chain to enhance food safety, curb waste and strengthen consumer confidence in this increasingly important protein source.

Materials and methods

The study was conducted on Murrah buffaloes (aged 4 - 6 years) from KVASU Buffalo Farm, Mannuthy that were humanely slaughtered and inspected at the Meat Technology Unit, Mannuthy. Carcasses were electrically stimulated (100–110 V for 1.5–2 min), hot-deboned and muscles from the round region were collected. Visible fat and connective tissues were removed, and the trimmed muscles were packaged in HDPE pouches and stored at -18 ± 1 °C for later analysis.

Selection and fabrication of indicator

Various indicator solutions, used either individually or in combination, were assessed for their ability to exhibit a colour change in response to the production of volatile bases during meat storage. The selection of these chemicals was guided by specific criteria: firstly, elevated sensitivity to total volatile basic nitrogen (TVBN) to ensure a rapid and measurable response to spoilage-related metabolites; secondly, an appropriate dissociation constant (pK_a) aligned with the pH range typically observed during the early stages of spoilage, facilitating effective proton exchange and colour transition; and thirdly, a high molar extinction coefficient, which enhances absorbance at the indicator's characteristic wavelength and improves the visual clarity of the colour change.

Various grades of Whatman® filter paper were evaluated and rectangular strips (2 cm × 1 cm) prepared from Whatman No. 1 were used for further testing. These strips were immersed in a one per cent (w/v) indicator solution placed in centrifuge tubes to ensure complete

saturation. To achieve uniform dye deposition, the tubes were centrifuged at 3000 rpm for 15 minutes. The treated strips were then dried overnight at room temperature in the dark to prevent photodegradation.

Evaluation of indicator sensor in a simulated indicator-metabolite reactive model system

To evaluate the indicators' performance, a model system was developed to simulate the volatile basic nitrogen release observed during meat spoilage. A standardised quantity of concentrated ammonia solution (25 mg, 27.1 µL, 91% purity) - representing typical spoilage emission levels (Shukla et al., 2015) was placed in a 500 mL transparent PET container. An indicator strip was attached to the container's inner lid before sealing, exposing it to the ammonia-enriched headspace. Upon contact with ammonia vapours, the indicator underwent a rapid pH-induced colour transition. This visual response was documented to assess each formulation's sensitivity and reaction kinetics under controlled conditions.

Modified-atmosphere packaging (MAP)

For MAP approximately 500 g of frozen round-muscle cuts were transported to the Department of Livestock Products Technology, VCRI-Namakkal, thawed at 4 °C overnight, blotted dry and placed in transparent high-barrier retort pouches (12 × 18 cm). Packs were evacuated to 30 mbar and back-flushed with a premixed food-grade gas blend containing 30% CO₂: 20% O₂: 50% N₂ (MAP20) using a table-top vacuum/gas sealer (Multivac C200, Germany); the head-space-to-product ratio was maintained at 2:1 (v/w). Gas composition in three randomly selected pouches was verified immediately after sealing. A bromothymol-blue indicator strip was affixed to the inner panel just below the heat seal before closure. Packaged samples (n = 6 per sampling day) were stored at 4 ± 1 °C and taken for analysis on days 0, 3, 5, 7 and 9.

Physico-chemical parameters

The pH of cattle and buffalo meat was measured using a digital pH meter (µ pH System, Systronics, India) following the method outlined by O'Halloran et al. (1997). Total volatile basic nitrogen (TVBN) content was estimated using the micro diffusion technique described by Pearson (1968). Ammonia concentration during storage was determined according to the procedure of Sastry et al. (1999). Tyrosine value was measured following the method of Strange et al. (1977), while the free amino acid content was estimated using a modified version of the method proposed by Rosen (1957). The thiobarbituric acid reactive substances (TBARS) value of carabeef was assessed by the extraction method of Witte et al. (1970). Water holding capacity of cattle and buffalo muscles was determined using the filter paper press method described by Grau and Hamm (1957).

Colour values

The instrumental colour was measured using a calibrated colour reader (Lovibond LC 100 Spectro colourimeter) with diffuse illumination. The instrument was set to measure L, a and b values. It was calibrated using black and white calibration tiles before starting of the measurement and the colourimeter score was recorded with L of black equals zero and L of white equals 100, a of lower numbers equals more green (less red), higher numbers equal more red (less green) and b of lower numbers equals more blue (less yellow), higher numbers equals more yellow (less blue). The colour coordinates L (lightness), a (redness) and b (yellowness) of the samples were measured ten times and mean values were taken (Navneet & Kshitij, 2011).

Microbiological quality

All microbiological analyses of buffalo meat during storage were carried out following the standard procedures outlined by APHA (2015). Commercially prepared media from Hi-Media Laboratories Pvt. Ltd., Mumbai and Sisco Research Laboratories Pvt. Ltd., Mumbai, were used for the enumeration of total plate count, psychrotrophic count and *pseudomonas* counts. All samples were plated in duplicate, and the results were expressed as colony-forming units (CFU) per gram of meat.

Sensory evaluation

The sensory quality of buffalo meat during both ambient and refrigerated storage was evaluated based on colour, odour and surface sliminess. Approximately 100 g of meat, stored in LDPE pouches, was taken out from storage for assessment. Each sample was evaluated in quadruplicate by a panel of seven trained members using a five-point scale to assess changes in sensory attributes.

Results and discussion

In this study we developed a bromothymol blue (BTB) based paper strip indicator for on-pack, real-time

monitoring of buffalo meat spoilage under modified-atmosphere refrigerated storage and evaluated its performance against conventional quality criteria. Dye-based sensors form one of the simplest and most cost-effective classes of indicators in which chromogenic dyes respond to specific spoilage metabolites and BTB proved to be a reliable pH sentinel. As hydrogen ion concentration fell during spoilage the dye deprotonated and its resonance system shifted which drove a clear yellow to blue transition that paralleled the rise in pH and TVBN. In the present work the BTB strip was first characterised in an ammonia-challenge model then applied inside MAP pouches containing carabeef cuts stored at 4 ± 1 °C and its stepwise colour transitions were interpreted alongside changes in physico-chemical, microbiological and sensory attributes to establish the point at which the visual signal corresponded to established spoilage thresholds.

Performance of indicator in the ammonia-challenge model

The BTB sensors demonstrated a rapid and distinct colour transition, shifting from yellow to bluish green within minutes of exposure (Fig.1). The change was consistent across the entire surface, with no evidence of dye bleeding or background discolouration, indicating the stability of the 1% (w/v) dye concentration in Whatman® filter paper. These results confirmed the suitability of BTB paper strips as reliable reference sensors for further meat storage studies.

Response of indicator with quality changes of carabeef in MAP under refrigerated storage

Colour response of the BTB-based indicator

The BTB system displayed a gradual and distinct colour progression that aligned well with biochemical indicators of spoilage (Fig. 2). The observed transitions were as follows: yellow (day 0) to very pale green (day 3), then to yellowish green (day 5) to greenish yellow (day 7) and finally to light bluish green (day 9). This subdued colour



Fig. 1. Performance of indicator in the ammonia-challenge model

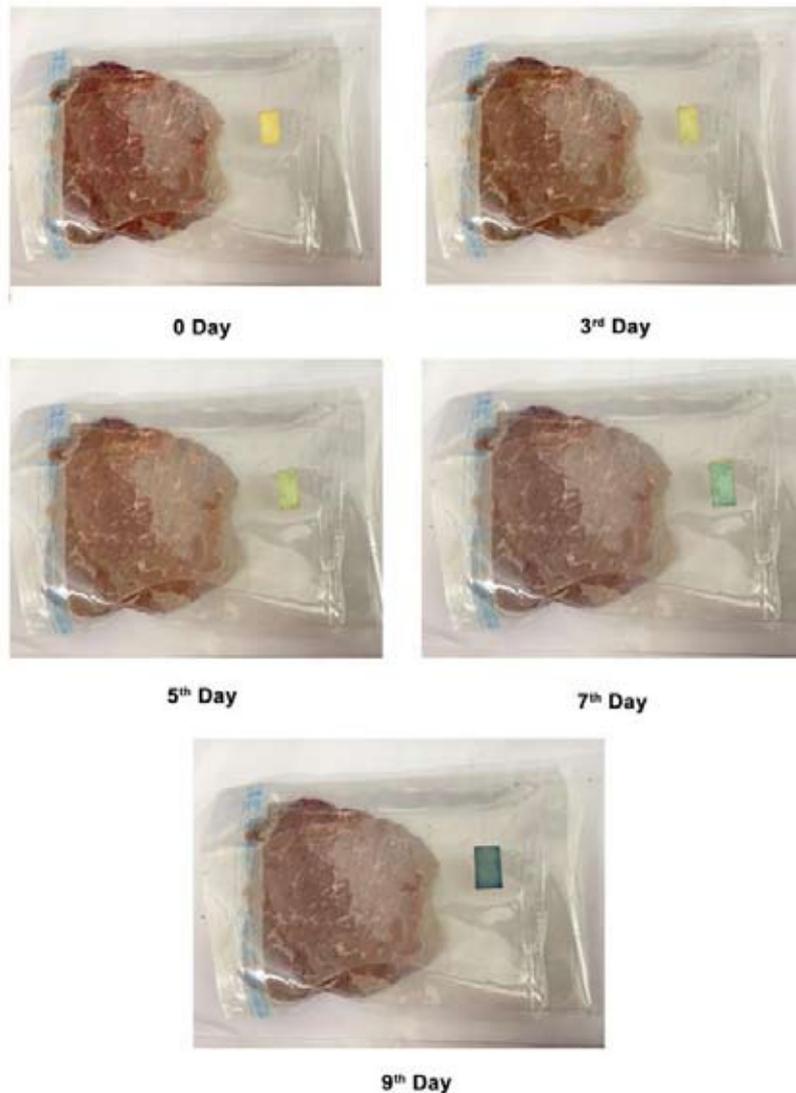


Fig.2. Colour response of the BTB-based indicator in MAP buffalo meat in refrigerated storage condition.

shift reflects the reduced build-up of TVBN commonly seen under MAP conditions.

pH

During storage, the pH of buffalo meat showed a significant increase ($P < 0.05$), which correlated with observable colour change from yellow to bluish-green (Fig. 2) in the indicator sensor (Table 1). This alkaline shift was attributed to microbial proliferation and proteolytic activity, resulting in the accumulation of basic nitrogenous compounds. These findings align with Bagdatli and Kayaardi's (2015) observations in modified atmosphere-packaged beef steaks, where pH values rose to 6.6 by the storage endpoint, demonstrating a comparable spoilage pattern.

Water Holding Capacity (WHC)

The WHC of buffalo meat exhibited a significant decline ($P < 0.05$) during storage, which corresponded with visible colour change from yellow to bluish-green

(Fig. 2) in the indicator sensor (Table 1). This reduction in WHC resulted from progressive protein denaturation, pH fluctuations, and oxidative damage to muscle proteins. These findings corroborate the work of Bagdatli and Kayaardi (2015), who reported a comparable deterioration in WHC for modified atmosphere-packaged beef steaks during refrigerated storage.

Thiobarbituric Acid Reactive Substances (TBARS)

A progressive and significant ($P < 0.05$) increase in TBARS values was observed in buffalo meat during storage, accompanied by distinct colour changes in the indicator sensor (Table 1). This rise in lipid oxidation products, measured as malonaldehyde equivalents, reflects the ongoing oxidative deterioration of unsaturated fatty acids in meat lipids. The findings align with Cortez-Vega et al. (2012), who reported a similar oxidative pattern in modified atmosphere-packaged chicken breast, with TBARS values increasing from 0.082 ± 0.01 to 0.696 ± 0.01 mg malonaldehyde/kg over 9 days of refrigerated storage.

Ammonia

Buffalo meat exhibited a significant ($P < 0.05$) rise in ammonia levels during storage, which corresponded with visible colour transitions in the indicator sensor (Table 1). This accumulation likely resulted from proteolytic degradation and subsequent amino acid deamination during spoilage, as previously reported by Balamatsia et al. (2007). Comparable ammonia generation patterns were documented by Abdullah et al. (2017) in refrigerated, modified atmosphere-packaged organic chicken, confirming this biochemical spoilage pathway across different meat types.

Total Volatile Basic Nitrogen (TVBN)

The TVBN level of buffalo meat increased significantly ($P < 0.05$) with a change in colour of the indicator sensor during the storage period (Table 1). This was mainly due to microbial and enzymatic degradation of proteins and non-protein nitrogenous compounds over time. Blacha et al. (2014) observed a similar increase in TVBN values, which reached 28.20 mg/100 g of turkey meat in MAP by day 12 of refrigerated storage.

Tyrosine Value

The tyrosine level of buffalo meat increased significantly ($P < 0.05$) with a change in colour of the indicator sensor during the storage period (Table 1). The tyrosine value went from 0.19 ± 0.02 to 20.07 ± 0.23 (mg/100g) by the end of storage period. This increase could be attributed to the activity of proteolytic enzymes (both endogenous and microbial) that remained active at a reduced rate. These enzymes break down proteins into smaller peptides and amino acids, increasing the tyrosine concentration.

Free Amino Acid (FAA)

The tyrosine level of buffalo meat increased significantly ($P < 0.05$) with a change in colour of the indicator sensor during the storage period (Table 1). Modified atmospheric packaging slows down aerobic spoilage microbes due to high CO_2 concentrations. However, it might favour Lactic Acid Bacteria (LAB) and facultative anaerobes, which also have proteolytic capacity. During prolonged refrigerated MAP storage (e.g., beyond 6 to 7 days), the cumulative effect of autolysis and microbial activity increases the pool of free amino acids.

Colour values of refrigerated carabeef under MAP

The L^* value of refrigerated carabeef ($4 \pm 1^\circ\text{C}$) stored under modified atmosphere packaging (MAP) showed a significant increase ($P < 0.05$) over the storage period (Table 2). Similar findings were reported by Smiecinska and Daszkiewicz (2020) in beef stored under refrigeration in MAP. The rise in lightness values may be attributed to factors such as oxygen exposure, lower microbial spoilage, and improved moisture retention.

A progressive and significant ($P < 0.05$) reduction in a^* values was observed throughout the storage period (Table 2), consistent with the findings of Bagdatli and Kayaardi (2015). This colour deterioration primarily resulted from the oxidative conversion of oxymyoglobin to metmyoglobin, coupled with the cumulative effects of microbial activity and lipid oxidation that contribute to meat discoloration.

The b^* values of carabeef showed a significant increase ($P < 0.05$) throughout the storage period (Table 2). This progressive yellow discoloration likely resulted from oxidative processes affecting both lipids (peroxidation) and muscle proteins, along with chemical alterations in meat

Table 1: Mean (\pm) SE values of physico-chemical parameters of buffalo meat in MAP packaging during refrigerated storage ($4 \pm 1^\circ\text{C}$)

Parameters	Storage period (days)					Overall mean	p-value
	0	3	5	7	9		
pH	5.32 ± 0.01^a	5.66 ± 0.01^b	5.88 ± 0.01^c	6.12 ± 0.02^d	6.69 ± 0.01^e	5.93 ± 0.01	0.000**
TVBN (mg /100g)	5.83 ± 0.21^a	8.4 ± 0.31^b	10.36 ± 0.24^c	13.81 ± 0.5^d	17.78 ± 0.43^e	11.64 ± 0.34	0.000**
Ammonia level (mg /100g)	1.80 ± 0.1^a	2.64 ± 0.15^b	3.17 ± 0.08^c	4.39 ± 0.16^d	5.95 ± 0.35^e	3.59 ± 0.17	0.000**
Water holding capacity (%)	86.62 ± 0.4^e	84.02 ± 0.3^d	82.53 ± 0.23^c	80.68 ± 0.2^b	77.78 ± 0.24^a	82.33 ± 0.27	0.000**
Tyrosine value (mg /100g)	0.19 ± 0.02^a	1.15 ± 0.04^b	3.42 ± 0.05^c	8.67 ± 0.15^d	20.07 ± 0.23^e	6.70 ± 0.10	0.000**
TBARS (mg malonaldehyde /Kg)	0.09 ± 0.01^a	0.17 ± 0.01^b	0.27 ± 0.01^c	0.42 ± 0.01^d	0.71 ± 0.02^e	0.33 ± 0.01	0.000**
FAA (mg /100g)	5.08 ± 0.06^a	10.08 ± 0.12^b	16.1 ± 0.18^c	24.46 ± 0.27^d	31.65 ± 0.28^e	17.07 ± 0.18	0.000**

Means bearing different superscripts between columns (a,b,c) differ significantly ($p \leq 0.05$). $n = 6$

* - Significant at 0.05 level, ** - Significant at 0.01 level, NS - Non significant at 0.05 level

TBARS - Thiobarbituric acid reactive substance | TVBN - Total volatile basic nitrogen | FAA - Free amino acid

Table 2: Mean (\pm) SE values of CIE lab colour values (L^* , a^* and b^*) of buffalo meat in MAP packaging during refrigerated storage ($4\pm 1^\circ\text{C}$)

Parameters	Storage period (days)					Overall mean	p-value
	0	3	5	7	9		
Lightness Value (L^*)	24.10 \pm 0.8 ^a	30.3 \pm 0.43 ^b	33.2 \pm 0.26 ^c	35.15 \pm 0.37 ^d	36.73 \pm 0.3 ^e	31.50 \pm 0.43	0.000**
Redness Value (a^*)	10.62 \pm 0.15 ^e	9.42 \pm 0.14 ^d	8.28 \pm 0.14 ^c	7.25 \pm 0.14 ^b	6.02 \pm 0.14 ^a	8.32 \pm 0.14	0.000**
Yellowness Value (b^*)	7.22 \pm 0.13 ^a	8.73 \pm 0.18 ^b	9.65 \pm 0.14 ^c	11.05 \pm 0.18 ^d	12.93 \pm 0.13 ^e	9.92 \pm 0.15	0.000**

Means bearing different superscripts between columns (a,b,c) differ significantly ($p\leq 0.05$). $n= 60$

* - Significant at 0.05 level, ** - Significant at 0.01 level, NS - Non significant at 0.05 level

Table 3: Mean (\pm) SE values of Microbiological parameters of buffalo meat in MAP packaging during refrigerated storage ($4\pm 1^\circ\text{C}$)

Parameters	Storage period (days)					Overall mean	p-value
	0	3	5	7	9		
Total viable count (\log_{10} CFU/g)	1.59 \pm 0.03 ^a	2.13 \pm 0.01 ^b	3.14 \pm 0.05 ^c	4.87 \pm 0.07 ^d	6.31 \pm 0.12 ^e	3.61 \pm 0.06	0.000**
Psychrotrophic count (\log_{10} CFU/g)	1.56 \pm 0.02 ^a	2.02 \pm 0.02 ^b	3.14 \pm 0.03 ^c	4.83 \pm 0.06 ^d	6.24 \pm 0.06 ^e	3.56 \pm 0.05	0.000**
<i>Pseudomonas</i> count (\log_{10} CFU/g)	1.49 \pm 0.01 ^a	1.96 \pm 0.01 ^b	3.16 \pm 0.03 ^c	4.85 \pm 0.06 ^d	6.22 \pm 0.11 ^e	3.54 \pm 0.04	0.000**

Means bearing different superscripts between columns (a, b, c) differ significantly ($p\leq 0.05$). $n= 6$

* - Significant at 0.05 level, ** - Significant at 0.01 level, NS - Non significant at 0.05 level

pigments. These findings corroborate the observations of Smiecinska and Daszkiewicz (2020) in similarly stored beef, demonstrating comparable oxidative colour changes across bovine species.

Microbiological Parameters

Total Plate Count (TPC)

The total plate count (TPC) of buffalo meat showed a significant increase ($P<0.05$) during storage, correlating with visible colour changes in the indicator sensor (Table 3). This microbial growth pattern reflects the progressive multiplication of psychrotrophic and facultative anaerobic bacteria under refrigerated conditions. These results align with findings of Bingoland Ergun's (2012), they reported similar microbial proliferation, reaching 6.66 ± 0.3 log CFU/g after 10 days of storage in comparable conditions.

Pseudomonas count

A significant increase ($P<0.05$) in *Pseudomonas* counts was observed in buffalo meat during storage, corresponding with distinct colour changes in the indicator sensor (Table 3). This proliferation can be attributed to the bacteria's psychrotrophic characteristics, ability to thrive in modified atmosphere conditions, and common presence in meat processing environments, which collectively enhance their competitive advantage over other microbial species. These findings are consistent with the observations made by Bingoland Ergun (2012), for MAP-packaged ostrich meat

under refrigeration, demonstrating similar *Pseudomonas* growth patterns across different meat types.

Psychrophilic count

The psychrophilic bacterial count in buffalo meat demonstrated a significant increase during storage, which was clearly reflected by the colour transition of the indicator sensor (Table 3). This microbial shift occurs as cold-adapted (psychrotrophic and psychrophilic) organisms selectively proliferate under refrigeration and modified atmospheric conditions. Notably, the MAP environment, characterised by reduced oxygen and elevated CO_2 levels inhibits typical aerobic spoilage bacteria (e.g., *Pseudomonas*) while promoting facultative and obligate anaerobic psychrophiles, including lactic acid bacteria (LAB) and enterobacteria, as previously documented by Koutsoumanis and Sofos (2004).

Sensory evaluation of MAP stored carabeef

Carabeef exhibited a significant ($P<0.05$) deterioration in visual appearance during storage, which correlated with progressive colour changes in the indicator sensor (Table 4). This quality decline resulted from multiple spoilage-related phenomena: oxidative changes in myoglobin pigments leading to discolouration, progressive dehydration, and microbial induced surface slime formation from proteolytic activity.

The colour scores of carabeef declined

Table 4: Mean (\pm) SE values of sensory characteristics of buffalo meat in MAP packaging during refrigerated storage ($4\pm 1^\circ\text{C}$)

Parameters	Storage period (days)					Overall mean	p-value
	0	3	5	7	9		
Appearance	4.94 \pm 0.03 ^e	4.65 \pm 0.05 ^d	4.02 \pm 0.05 ^c	3.35 \pm 0.07 ^b	2.13 \pm 0.07 ^a	3.82 \pm 0.05	0.000**
Colour	4.90 \pm 0.04 ^e	4.69 \pm 0.05 ^d	4.02 \pm 0.04 ^c	3.54 \pm 0.04 ^b	1.96 \pm 0.06 ^a	3.82 \pm 0.05	0.000**
Odour	4.92 \pm 0.04 ^e	4.58 \pm 0.04 ^d	4.02 \pm 0.04 ^c	3.44 \pm 0.09 ^b	2.02 \pm 0.12 ^a	3.80 \pm 0.07	0.000**
Sliminess	4.98 \pm 0.02 ^e	4.79 \pm 0.09 ^d	4.04 \pm 0.03 ^c	3.00 \pm 0.03 ^b	2.11 \pm 0.09 ^a	3.78 \pm 0.05	0.000**
Overall Acceptability	4.94 \pm 0.03 ^e	4.52 \pm 0.05 ^d	4.04 \pm 0.03 ^c	3.35 \pm 0.05 ^b	2.06 \pm 0.11 ^a	3.78 \pm 0.05	0.000**

Means bearing different superscripts between columns (a,b,c) differ significantly ($p\leq 0.05$). $n=42$

* - Significant at 0.05 level, ** - Significant at 0.01 level, NS - Non significant at 0.05 level

Table 5: Cost of production of indicator

Particulars	Quantity	Cost
BTB paper strip type		
Cost of Bromothymol Blue @ Rs 54.2/g	0.1g	Rs 5.42
Cost of Filter paper @ Rs 1590/ 100 paper	1	Rs 15.9
Total No of Paper strips prepared	24	
Total Cost of 24 Strips	Rs 21.32	
Cost of one BTB Paper strip	Rs 0.89 (approx. 90 paise)	

significantly ($P<0.05$) throughout the storage period (Table 4). This reduction was mainly attributed by the oxidation of oxymyoglobin into metmyoglobin, resulting in browning and the gradual loss of the bright red colour of meat.

The odour scores of buffalo meat declined significantly ($P<0.05$) over the storage period (Table 4). This reduction in odour quality was primarily due to the build-up of volatile spoilage compounds such as ammonia, hydrogen sulphide, and short-chain fatty acids, which results from microbial activity and protein breakdown.

The sliminess scores showed a significant ($P<0.05$) decrease ($P<0.05$) in MAP-packaged carabeef during refrigerated storage (Table 4). This textural change was primarily caused by surface slime formation resulting from psychrotrophic bacterial growth and their production of extracellular polysaccharides and proteinaceous metabolites. However, the observed slime formation remained minimal throughout the storage period, ranging from trace to slight levels.

Overall acceptability

Carabeef quality under MAP refrigeration showed progressive deterioration. Initial high scores (4.94 ± 0.03) reflected excellent freshness, gradually declining to acceptable levels by day 5 (4.04 ± 0.03). By day 9, scores fell to 2.06 ± 0.11 ($P<0.05$), indicating spoilage. This matched critical thresholds: TVBN (18mg/100g), ammonia (5mg/100g), and microbial counts (6 log CFU/g). Notably, colour-changing indicators provided visible spoilage warnings before sensory rejection, demonstrating their

utility for shelf-life monitoring.

Cost of production of developed indicator

The table 5 depicts the cost parameters of the indicator indicating that it is a low cost technology to evaluate the safety of stored carabeef samples.

Conclusion

The developed bromothymol blue (BTB)-based indicator effectively monitored buffalo meat spoilage in modified atmospheric packaging (MAP) by undergoing distinct colour changes (yellow to bluish-green) in response to rising pH, volatile bases (TVBN, ammonia), microbial growth (TPC, *Pseudomonas*) and physicochemical deterioration (WHC, TBARS). The indicator's real-time response aligned with sensory rejection thresholds, offering a cost-effective, non-invasive tool to enhance meat safety and reduce waste across the supply chain.

Acknowledgement

The technical support from the Meat Technology Unit (MTU), Mannuthy and Department of Livestock Products Technology, Veterinary College and Research Institute, Namakkal for providing facilities for modified atmospheric packaging and conduct of research work is gratefully acknowledged.

Conflict of interest

The authors declared they have no conflict of interest.

References

- Abdullah, A., Abdullah, F. A., Buchtová, H., & Turek, P. (2017). Influence of modified atmosphere packaging on freshness parameters of organic chicken meat – Short communication. *Czech Journal of Food Sciences*, 35, 292–299.
- American Public Health Association. (2015). *Compendium of methods for the microbiological examination of foods* (5th ed.). APHA Press.
- Bagdatli, A., & Kayaardi, S. (2015). Influence of storage period and packaging methods on quality attributes of fresh beef steaks. *CyTA – Journal of Food*, 13(1), 124–133.
- Balamatsia, C. C., Patsias, A., Kontominas, M. G., & Savvaidis, I. N. (2007). Possible role of volatile amines as quality-indicating metabolites in modified atmosphere packaged chicken fillets: Correlation with microbiological and sensory attributes. *Food Chemistry*, 104(4), 1622–1628.
- Bevilacqua, A., Corbo, M. R., & Sinigaglia, M. (Eds.). (2024). *The microbiological quality of food: Foodborne spoilers*. Elsevier.
- Bingol, E. B., & Ergun, O. (2011). Effects of modified atmosphere packaging (MAP) on the microbiological quality and shelf life of ostrich meat. *Meat Science*, 88(4), 774–785.
- Blacha, I., Krschek, C., & Klein, G. (2014). Influence of modified atmosphere packaging on meat quality parameters of turkey breast muscles. *Journal of Food Protection*, 77(1), 127–132.
- Cortez-Vega, W. R., Pizato, S., & Prentice, C. (2012). Quality of raw chicken breast stored at 5 °C and packaged under different modified atmospheres. *Journal of Food Safety*, 32(3), 360–368.
- Grau, R., & Hamm, R. (1957). Über das Wasserbindungsvermögen des Säugetiermuskels. II. Mitteilung. *Zeitschrift für Lebensmittel-Untersuchung und -Forschung*, 105, 446–460.
- Koutsoumanis, K. P., & Sofos, J. N. (2004). Microbial contamination and shelf life of fresh meat: A review. *Meat Science*, 68(1), 27–35.
- Navneet, K., & Kshitij, K. (2011). Development of carrot pomace and wheat flour based cookies. *Pure and Applied Science and Technology*, 1, 4–10.
- O'Halloran, G. R., Troy, D. J., & Buckley, D. S. (1997). The relationship between early post-mortem pH and tenderisation of beef muscles. *Meat Science*, 45(2), 239–251.
- Pearson, D. (1968). Methods related to protein breakdown. *Journal of the Science of Food and Agriculture*, 19(7), 366–369.
- Reddy, G. S. K. (2021). *Bacterial exopolysaccharide extraction and utilization for the development of extended shelf-life low-fat chevon patties* (Doctoral dissertation, Guru Angad Dev Veterinary and Animal Sciences University).
- Rosen, H. (1957). A modified ninhydrin colorimetric analysis for amino acids. *Archives of Biochemistry and Biophysics*, 67(1), 10–15.
- Rukchon, C., Trevanich, S., Jinkarn, T., & Suppakul, P. (2011). Volatile compounds as quality indicators of fresh chicken and possible application in intelligent packaging. In *Proceedings of the 12th Asian Food Science Conference*. Bangkok, Thailand.
- Sastry, V. R. B., Kamra, D. N., & Pathak, N. N. (1999). *Estimation of ammonia nitrogen*. In *Laboratory manual of animal nutrition*. IVRI.
- Shukla, V., Kandeepan, G., & Vishnuraj, M. R. (2015). Development of on-package indicator sensor for real-time monitoring of buffalo meat quality during refrigeration storage. *Food Analytical Methods*, 8(6), 1591–1597.
- Śmiecińska, K., & Daszkiewicz, T. (2021). Lipid oxidation and color changes in beef stored under different modified atmospheres. *Journal of Food Processing and Preservation*, 45(2), e15129.
- Strange, E. D., Benedict, R. C., Smith, J. L., & Swift, C. E. (1977). Evaluation of rapid tests for monitoring alterations in meat quality during storage. *Journal of Food Protection*, 40(12), 843–847.
- Witte, V. C., Krause, J. F., & Bailey, M. E. (1970). A new extraction method for determining 2-thiobarbituric acid values of pork and beef during storage. *Journal of Food Science*, 35(5), 582–585. ■