Effect of Wheat Bran Incorporation on Physico-Chemical and Microbiological Properties of Buffalo Meat Nuggets during Refrigeration Storage

Dr Raj Kumar Berwal*[1], Dr Kapil Kumar Godara[1] and Dr Rekha Berwal[2]

[1]Dept.of Livestock Products Technology, College of Veterinary and Animal Science, Rajasthan University of Veterinary and Animal Sciences, RAJUVAS, Bikaner, Rajasthan (334001) India

[2]Dept.of Home Science, CBRG, Govt. Girls College, Sriganganagar-335001

***Corresponding Author**

DOI: https://doi.org/10.51584/IJRIAS.2026.11070174

Received: 01 August 2026; Accepted: 06 August 2026; Published: 18 August 2026

ABSTRACT

This experiment was conducted during January–December, 2024 at Department of Livestock Products Technology, College of Veterinary and Animal Science, RAJUVAS – Bikaner (34001), Rajasthan, India to study the effect of wheat bran on shelf life and physico - chemical properties of buffalo meat nuggets at refrigeration temperature. This study examined the impact of wheat bran incorporation on microbiological parameters such as total plate count (TPC), yeast and mold count (YMC), Coliform count, Staphylococcus aureus count and Clostridium botulinum count of buffalo meat nuggets during refrigeration storage at 4°C .Development of processed meat products involves the incorporation of non-meat ingredients or additives for enhancing the quality attributes, sensory profile, and shelf life. Nuggets were one of the most acceptable value-added commented emulsion-based products that were either baked, steam-cooked or consumed after frying. Total plate count was significantly ($p<0.05$) decreased in buffalo meat nuggets containing wheat bran and remained less than control during entire storage period. As wheat bran had strong antioxidant properties and was a significant source of bioactive phenolic compound. In contrast, yeast and mold count had not exhibited growth until 21 days of storage but at 28th day, significant ($p<0.05$) increase in yeast and mold count found in both control and treatment but less increase found in wheat bran treated group as compare to control. This showed the antifungal activity of wheat bran. Pathogenic Escherichia coli, Staphylococcus aureus, and Clostridium botulinum were not detected in both control and treated nuggets during the entire storage period due to hygienic handling.

Keywords: Buffalo meat nugget; Wheat Bran; Physico-Chemical properties; Antioxidant; shelf life

INTRODUCTION

In India, ready-to-eat meat products are gaining popularity in recent years, and value-added food products with healthier alternatives are being introduced in the market to explore their potential in terms of consumer preference (Soren and Biswas, 2020). Development of processed meat products involves the incorporation of non-meat ingredients or additives for enhancing the quality attributes, sensory profile, and shelf life (Das et al., 2020). Among the value-added meat products, sausages, burger patties, nuggets and other emulsion-based meat products are very popular worldwide (Ravani and Sharma, 2022). Nuggets are one of the most acceptable value-added communited emulsion-based products that are either baked, steam-cooked or consumed after frying (Bansil, 2003).

According to the National Dairy Development Board (NDDB), India is home to the largest buffalo population globally with Rajasthan being one of the leading states in terms of buffalo rearing. This vast buffalo population contributes substantially to the dairy and meat industries, which are integral components of the region's economy. Buffalo meat, often referred to as "Buffen or Carabeef," is a rich source of high-quality protein and essential nutrients. It is particularly valued for its iron content, with studies showing that buffalo meat

contains higher levels of iron compared to other meats, such as beef or chicken (Di Stasio and Brugiapaglia, 2021). Additionally, buffalo meat is rich in vitamins B_{12} and B_6, zinc, and selenium, making it a valuable dietary component, especially in regions where access to diverse protein sources is limited (Soren and Biswas, 2020). Consumers' growing health consciousness these days has increased demand for meals that are more organic, natural and devoid of artificial additives. This tendency has encouraged the creation and use of natural materials to replace synthetic chemicals in the preparation of meat and a variety of natural plant materials are now being added to meat products (Fang et al.,2019, Ribeiro et al., 2019, Ferysiuk and Wojciak, 2020). It has been shown that wheat bran has strong antioxidant properties and is a significant source of bioactive phenolic compound (Verma et al., 2008,Anson et al.,2012, Hromadkova et al.,2013, Abozed et al.,2014, Laddomada et al.,2015 and Zou et al.,2020). Wheat bran also has strong antimicrobial property (Li et al.,2023,Zou et al., 2025). Ehsanbakhsh et al., 2017, Abbas et al., 2024 detected antifungal property in wheat bran. Wheat bran is high in B vitamins, vitamin E, carotene, and minerals like potassium, phosphorus, calcium, and magnesium (Di Lena et al., 1997, Balandran et al., 2015).Wheat bran is an important industrial by-product that is frequently removed during the manufacturing of ethanol and food processing because of its high concentration of antibacterial chemicals, which can prevent fermentation and the growth of microbes.According to studies, wheat bran includes phenolic compounds with antibacterial characteristics, such as ferulic acid and apigenin. These compounds can inhibit microbial growth and influence fermentation processes (Zhang et al., 2024). Additionally, short-chain fatty acids found in wheat bran can act as antibacterial agents, suppressing microbial development during anaerobic fermentation (Li et al., 2023).Consequently, wheat bran presents a tremendous possibility for application as a natural functional food additive or ingredient in the food chain (Onipe et al., 2021; Gunenc et al.,2017).To the best of our knowledge, no research has been done on using wheat bran in buffalo meat nuggets. This study sought to investigate the effects of wheat bran incorporation on microbiological properties of buffalo meat nuggets during refrigeration storage in order to better understand and provide more evidence of the impact of wheat bran on the quality and safety of meat products.

MATERIALS AND METHODS

The experiment was conducted during January–December, 2024 at Dept. of Livestock Products Technology, College of Veterinary and Animal Science, RAJUVAS – Bikaner (34001), Rajasthan, India (28º02’13”N, 73º30’15”E) to study the effect of wheat bran on shelf life and physic- chemical properties of Buffalo meat nuggets.

2.1. **Meat and other ingredients**

The deboned buffalo meat was purchased at the Bikaner local market, sealed in low-density polyethylene bags, and stored in a freezer at -18±2°C for later usage. The beef was chopped into small pieces and thawed at 4±1°C for 24 hrs before to grinding. The following items were purchased at the Bikaner local market: sodium nitrite, egg albumin, refined wheat flour, refined salt (Tata Chemicals Ltd., Mumbai), and spice mix components. To prepare buffalo meat nuggets, fine pastes of onion and garlic in a 3:1 ratio were used. The media and chemicals used for product analysis were purchased from reputable companies such as Hi-media, SRL, Sigma, and Mark, among others.

Table 1: Formulation of different combination of buffalo meat and wheat bran (in g)

Ingredients (%)	Control (C)	Treatment (T)
Meat	72	62
Spice mix	2	2
Condiments	2.5	2.5
STPP	0.5	0.5

ISSN No. 2454-6194 | DOI: 10.51584/IJRIAS |Volume XI Issue VII July 2026

Refined wheat flour (Maida)	3	3
Sodium nitrite	0.02	0.02
Ice	10	10
Oil	7	7
Sugar	0.5	0.5
Table salt	1	1
Egg albumin	1.48	1.48
Wheat bran	-	10
Total	100	100

2.2 Preparation of Buffalo Meat Nuggets

Frozen buffalo meat was partially thawed overnight, then chopped into small cubes and double minced using a meat mincer. Meat emulsion was made in a bowl chopper (Hakimi, India). A pre-weighed amount of minced buffalo meat, salt, sodium tripolyphosphate, and sodium nitrite were combined and chopped for approximately 2–3 min. It was chopped for another 2 min after adding the ice flakes. Oil was gradually mixed into the paste while cutting, until it was evenly distributed throughout. Wheat bran, condiment paste, dry spice mix, and additional components such as refined wheat flour, egg albumin and sugar were added. Chopping was continued until all of the ingredients were uniformly dispersed and the emulsion had the appropriate consistency. A weighed quantity of emulsion was used to fill a stainless steel mould. The mould was covered with a lid, fastened with thread, and steam cooked for 34 min. A probe thermometer was used to measure the core temperature of cooked blocks, which should be around 73°C. Buffalo meat block was sliced, chopped into nuggets, and refrigerated at 4°C. At 7-day intervals, samples were extracted for microbiological investigation. The analysis lasted 7, 14, 21, and 28 days in refrigeration storage at 4°C.

2.3 Analytical procedure

2.3.1 Physico-chemical properties

2.3.1.1 Thiobarbituric acid (TBA) value

The method of Witte et al. (1970) served as the basis for determining the TBA value. Using a mortar and pestle, 4 g of the sample was mixed with 20 ml of a (20.00%) TCA solution that had been chilled for two minutes to create the trichloroacetic acid (TCA) extract. After 10 minutes of extraction time, the contents were filtered through Whatman filter paper no. 42. An equivalent volume of (0.10%) TBA reagent was combined with three millilitres of TCA extract. A spectrophotometer (Model no. Smp 6, Spectramax plus, molecular device USA) was used to measure the mixture's absorbance at 532 nm after it had cooled after 35 minutes of boiling in a water bath. The same process as previously mentioned was used for the blank, with the exception that 3 ml of (20.00%) cold TCA solution. Using the usual graph, the TBA value was determined as mg of malonaldehyde per kilogramme of material. To prepare the graph, 100 millilitres of (100%) alcohol used to dissolve 0.3055 gram of 1,1,3,3-Tetramethoxypropane, with a concentration of malonaldehyde of 1 mg per ml of the solution. To obtain the working standard, distilled water was used to dilute 0.3 ml of the aforementioned solution to 100 ml. Pipetting 0, 0.2, 0.4, 0.6, 0.8, and 1.0 ml of this solution into separate test tubes, then adding3ml of TBA reagent to distilled water to get the volume upto 3 ml. After that, the tubes spent thirty-five minutes in a boiling water bath. At 532nm, absorbance was measured. Malonaldehyde concentration was plotted against corresponding absorbance on a standard graph.

ISSN No. 2454-6194 | DOI: 10.51584/IJRIAS |Volume XI Issue VII July 2026

2.3.1.2 Tyrosine value

The Strange et al. (1977) method was used to calculate the tyrosine value. The TCA extract was prepared in the same manner as the TBA. Ten millilitres of 0.5 N sodium hydroxide solution was added to 2.5 millilitres of TCA extract and an equal volume of distilled water. After letting the mixture sit for ten minutes, three millilitres of Folin–Ciocalteu reagent one part concentrated Folin– Ciocalteu reagent and two parts distilled water were added. After carefully mixing the ingredients and letting them sit at room temperature in the dark for half an hour, the absorbance was measured at 730 nm using a spectrophotometer (Spectra max plus)

2.3.1.3 Free fatty acid (FFA)

Free Fatty Acid was calculated using Koniecko's (1979) methodology. In a 250 ml beaker, 5 g of the material was added to 30 ml of chloroform. A mortar and pestle were used to mix the components for two minutes after adding five gram of anhydrous sodium sulphate. Whatman filter paper number one was used to filter the mixture. In the presence of phenolphthalein indicator, a 25 ml aliquot of filtrate was placed in a 125 ml conical flask and titrated against 0.1 N alcoholic KOH until a pink hue developed.

$$\text{FFA}= \frac{0.1\times\text{volume of 0.1N alcoholic KOH used (in ml)}\times 0.282}{\text{Sample wt (gram)}} \text{ X100}$$

2.3.2 Microbiological examinations

Total plate count, Yeast and mold count, coliform count, Staphylococcus aureus count and Clostridium botulinum count were determined following the methods described by Anonymous (1984).

2.3.2.1 Preparation of sample and serial dilution in an inoculation chamber that had been previously sterilised by ultraviolet light, samples were opened. After being aseptically weighed, a 10 g sample was moved to a mortar that had already been sterilised and contained 90 ml of sterile 0.1% peptone water. To achieve a uniform dispersion and a 10^{-1}dilution, the material was homogenised for two min using a sterile pestle. 1 ml of this diluted solution was quantitatively transferred and then thoroughly mixed in a test tube with 9 ml of sterile 0.1% peptone water to create a 10^{-2}dilution. To create a 10^{-3}dilution, 1 ml of this 10^{-2}dilution was once more added to 9 ml of 0.1% sterile peptone water, and so on. In a horizontal laminar flow apparatus (Model: Labconco biosafety cabinet), sample preparation and serial dilutions were carried out close to the flame while adhering to all potential aseptic conditions. As needed, serial dilutions were created.

2.3.2.2 Total plate count

A 23.5 g plate count agar (Code No. M091) from Hi-Media Laboratories Pvt. Ltd. in Mumbai was suspended in 1000 ml of distilled water, boiled to completely dissolve the medium, and then autoclaved for 15 min at 15 lbs of pressure and 121ºC to sterilise it. At 25°C, the medium's ultimate pH was 7.0±0.2. Each sterile petri dish contained roughly 20 ml of the sterilised medium, and the samples' total aerobic mesophilic count was ascertained using the pour plate technique. For 48 hrs, the plates were incubated at 35±2ºC. The colony counter was used to count colonies. Log10 colony forming units (cfu)g^{-1} of sample was the result of multiplying the average number of colonies by the reciprocal of the dilution.

2.3.2.3 Yeast and mold count

Hi-Media Laboratories Pvt. Ltd., Mumbai (Code No. M1941) supplied 39 g of potato dextrose agar, which was suspended in 1000 ml of distilled water, boiled until the medium was completely dissolved, autoclaved at 15 lbs pressure (121ºC) for 15 min, and then chilled. The medium was not autoclaved too much. The sterile cooled medium was acidified with 10 ml of 10% sterile tartaric acid to reach a pH of 3.5. To maintain the agar's ability to harden, the medium was not heated after tartaric acid was added. After mixing 500 mg of chlortetracycline hydrochloride and 500 mg of chloramphenicol with 100 ml of sterile phosphate buffered distilled water, 2ml of antibiotic solution were added to each 100 ml of tempered agar. This resulted in a final medium concentration

of 100 mg (1000 ml)$^{-1}$of each antibiotic. The medium was swirled and then used. The sample dilution was inoculated using the pour plate technique, and the petri dishes were incubated for 5days at 25 °C. The number of colonies was measured and reported as log10 cfug^{-1}.

2.3.2.4 Coliform count

After being purchased from Hi-Media Laboratories Pvt. Ltd. in Mumbai (Code No. M049), 41.5 g of violet red bile agar was suspended in 1000 ml of distilled water, boiled until the medium was completely dissolved, and then allowed to cool to 45°C. At 25°C, the medium's ultimate pH was 7.4±0.2. The medium was not autoclaved as a precaution. The plates were incubated at 35°C for 24 to 48 hrs after the appropriate sample dilution was inoculated using the pour plate with overlay technique. Red, purple colonies with a diameter of roughly 0.5 mm and encircled by a zone of bile precipitation were counted. Additionally, counted were colonies deemed to be "borderline cases." The average colony count was given as log10 cfug^{-1}.

2.3.2.5 Staphylococcus aureus count

Baird-Parker agar was used to measure the number of Staphylococcus aureus. In a flask heated on a hot plate to dissolve the medium, 31.5 g of precisely weighed Baird-Parker agar was dissolved in 475 ml of distilled water. The flask was then sealed with gauze cotton wrapped in aluminium foil, autoclaved at 15 psi for 20 min, and cooled to 45°C in a bacteriological water bath (NSW® Model-133, India) until it was used. Aseptically, 25 ml of egg yolk tellurite emulsion was added to 475 ml of molten agar at 45–50 °C, and the mixture was thoroughly mixed. The sterilised Petri plates were covered with 10–15 ml of agar. For one to 2 hrs, the agar was left to firm. A sterile L-spreader was then used to spread 0.5 ml of the material diluted appropriately. To absorb the sample, the Petri plates were let to stand for 15 to 20 min. For 24 to 48 hrs, the Petri plates were incubated at 37°C. Using a colony counter, the black, glossy, convex colonies with a diameter of 2–3 mm and complete borders encircled by a transparent halo were counted. To calculate the Staphylococcus aureus count as colony forming units (CFU) gram^{-1} of the sample, the average number of colonies was multiplied by the dilution factor. Log CFUg^{-1} of sample was used to express this count.

2.3.2.6 Clostridium botulinum count

According to the Anonymous (2001) and Barnes' (2003) instructions, the study's technique involved putting 1 mL of each dilution into Petri dishes. After that, 15 ml of either molten SFP (Shahadi Ferguson Perfringens Agar, BD DIFCO, Le Pont de Claix, France) or TSC (Triptose Sulfite Cycloserine Agar, BD DIFCO, Le Pont de Claix, France) were added. After homogenized the agar in the plates, they were kept in anaerobic jars at 36 ±1˚C for a full day without being inverted. Colonies in plates containing 30 to 300 colonies were then enumerated.
When sulfite was reduced to sulphide, it combined with ammonium and iron citrate to generate a black precipitate, which waswhy Clostridium sp. developed dark colonies.

2.4 pH

As previously mentioned, the pH of buffalo meat nuggets was determined using a digital pH meter fitted with a combination glass electrode (Eutech pH 700, thermal scientific) (Trout et al., 1992). A mortar and pestle was used to homogenise 10 g of the buffalo meat nugget sample with 50 ml of distilled water for 1 min.

2.5 Statistical analysis

Data obtained were subjected to analysis by one-way analysis of variance (ANOVA) technique (Snedecor and Cochran, 1989) using Statistical Software Packages (SPSS 21.0).

ISSN No. 2454-6194 | DOI: 10.51584/IJRIAS |Volume XI Issue VII July 2026

RESULTS AND DISCUSSION

Table 4.26: TBA value (mean ± SE) of buffalo meat nuggets incorporated with wheat bran at refrigeration temperature (4±1^{0}C)

Day / Treatments	T_0	T_2	Overall storage mean
0 day	0.35±0.052	0.28±0.027	0.32±0.03^a
7thday	0.45±0.025	0.35±0.022	0.40±0.022^b
14th day	0.57±0.026	0.42±0.024	0.50±0.029^c
21th day	0.77±0.025	0.59±0.017	0.68±0.03^d
28th day	0.88±0.026	0.65±0.017	0.76±0.03^e
Treatment mean	0.61±0.039^B	0.46±0.028^A	

Note: Overall means bearing different superscripts in a row (A, B) and in the column (a, b, c, d, e) differ significantly (P<0.05). T_0-Buffalo meat nuggets prepared without wheat bran, T_2–Buffalo meat nugget prepared with 10% wheat bran

Table 4.27: FFA value (mean ± SE) of buffalo meat nuggets incorporated with wheat bran at refrigeration temperature (4±1^{0}C)

Day / Treatments	T_0	T_2	Overall storage mean
0 day	0.24±0.072	0.16±0.013	0.20±0.037^a
7th day	0.31±0.010	0.22±0.018	0.27±0.017^b
14th day	0.51±0.019	0.35±0.011	0.43±0.026^c
21th day	0.67±0.012	0.47±0.021	0.57±0.032^d
28th day	0.81±0.020	0.65±0.013	0.73±0.027^e
Treatment mean	0.51±0.042^B	0.37±0.033^A	

Note: Overall means bearing different superscripts in a row (A, B) and in the column (a, b, c, d, e) differ significantly (P<0.05). T_0-Buffalo meat nuggets prepared without wheat bran, T_2–Buffalo meat nugget prepared with 10% wheat bran

ISSN No. 2454-6194 | DOI: 10.51584/IJRIAS |Volume XI Issue VII July 2026

Table 4.28: Tyrosine value (mean ± SE) of buffalo meat nuggets incorporated with wheat bran at refrigeration temperature (4±1^{0}C)

Day / **Treatments**	**T_0**	**T_2**	**Overall storage mean**
0 day	4.60±0.198	4.03±0.031	4.31±0.114^a
7th day	4.64±0.021	4.12±0.028	4.38±0.081^a
14th day	4.93±0.028	4.33±0.021	4.63±0.092^b
21th day	5.92±0.032	5.11±0.018	5.51±0.124^c
28th day	6.20±0.030	5.33±0.023	5.76±0.132^d
Treatment mean	5.25±0.125^B	4.58±0.099^A	

Note: Overall means bearing different superscripts in a row (A, B) and in the column (a, b, c, d) differ significantly (P<0.05). T_0-Buffalo meat nuggets prepared without wheat bran, T_2–Buffalo meat nugget prepared with 10% wheat bran

3.1 Microbiological analysis

Table 1 displayed the microbiological alterations of buffalo meat nuggets. To assess the microbiological quality and deterioration of buffalo meat nuggets during refrigerated storage, measurements were made of the total plate count (TPC), yeast and mould count (YMC), total coliform count, Staphylococcus aureus count, and Clostridium botulinum count.

3.1.1 Total plate count (TPC)

TPC wasa significant measure of microbiological quality, and the highest permissible level for beef products was typically thought to be less than7 cfug^{-1} (Huang, 2014). Over time, the TPC values of the treatment and control nuggets were progressively raised. The TPC values of the nuggets treated with wheat bran were significantly (p<0.05) lower than those of the control. The aerobic packaging conditions and the product's ability to provide a substrate for the growth of microbes in the package atmosphere might be the cause of the higher TPC throughout the course of storage. The inhibitory effect of the antioxidant in wheat bran on microbial growth might be the cause of this discrepancy between the TPC of the treatment and control. According to Li et al.2023, wheat bran's bioactive peptides and arabinoxylan had anti bacterial, osteoprotective, antihypertensive, and immunomodulatory qualities. Nonetheless, some investigations have shown that substances found in a variety of spices alsohad antibacterial properties (Zhang et al., 2010.Kumaret al.2016a, Ahmed et al. 2021) also recorded less increase TPC in wheat bran treated chicken harrisa and buffalo meat fillets as compare to control during refrigeration storage. Xiong et al. 2022, recorded similar effect of refrigeration storage on 1% sorghum bran added beef sausage as compared to positive control. The results of the present investigation were in agreement with findings reported by Devatkal, 2014, Bhatet al. 2016, Kamal et al. 2017,Meena, 2021 who noted comparable increases in TPC in ground beef nuggets, chevon nuggets, fortified chevon nuggets, goat meat nuggets and chevon nuggets respectively, during storage.

3.1.2 Yeast and mold count (YMC)

According to the results shown in Table 2, both the control and wheat bran-treated buffalo meat nuggets did not exhibit yeast or mould until 21th days into the refrigerator storage period. However, on the 28th day of storage,

ISSN No. 2454-6194 | DOI: 10.51584/IJRIAS |Volume XI Issue VII July 2026

both products showed signs of growth. Yeast and mold count of treatment was significantly ($p<0.05$) lower than control on 28th day of storage. At the end of storage period, treatment (0.276±0.010 log cfug^{-1}) have significantly ($p<0.05$) lower yeast and mold count than control (0.370±0.025log cfug^{-1}) that could be due to antifungal property of wheat bran that slowdown fungal growth. Ehsanbakhsh et al. 2017, Abbaset al. 2024, detected antifungal property in wheat bran. Similar findings were observed by Kauret al. 2015 in chicken nuggets and Meena 2021. in chevon nuggets with green coffee extract during refrigeration storage.

3.1.3 Coliform count

Coliform count was not detected throughout the storage period in control as well as treatment. Coliforms were occasionally found during storage and were thought to be indicators of post-processing contamination in meat products. Coliforms were not found for the whole storage period in the current study, in either control or in treatment. The absence of coliform in any product could be due to better hygienic practises used during production. Similar results were also reported by Kumar et al., 2016b, Ahmad et al., 2021, in different wheat bran meat products.

3.1.4 Staphylococcus aureus count

Staphylococcus aureus count was not detected throughout the storage period in control as well as treatment. Minimal water activity and sanitary product handling and packaging were responsible for the absence of Staphylococcus count bacteria. These findings were also in agreement with Badr and Mahmoud (2011) in wheat bran added beef burgers during refrigeration storage.

3.1.5 Clostridium botulinum count

Clostridium botulinum count was not detected throughout the storage period in control as well as treatments. Proper chilling, below 4°C or 39°F, significantly slows down microbial proliferation, especially for mesophilic pathogens such as Clostridium and Staphylococcus species. If the temperature was maintained at a steady level, these bacteria might not grow or might not be found. Aerobic packaging also created unfavourable conditions for clostridium botulinum growth because it was an obligate anaerobic bacteria.

Table2. Changes in TPC, YMC, Coliform count, Staphylococcus aureusand Clostridium botulinum count of buffalo meat nuggets during 28 days of refrigeration storage at 4 ◦C (Mean ± SD, n = 6).

Groups Days	0	7	14	21	28	Mean ± SE
Total plate count (log cfuml^{-1})						
C	2.32±0.330	3.24±0.154	3.77±0.016	3.27±0.040	4.46±0.005	3.51±0.148^{B}
T	1.45±0.143	2.83±0.055	3.75±0.025	3.13±0.023	3.42±0.033	2.82±0.136^{A}
Yeast and mold Count (log cfuml^{-1})						
C	ND	ND	ND	ND	1.85±0.129	0.370±0.02^{B}
T	ND	ND	ND	ND	1.38±0.053	0.276±0.01^{A}
Coliform count (log cfuml^{-1})						

ISSN No. 2454-6194 | DOI: 10.51584/IJRIAS |Volume XI Issue VII July 2026

C	ND	ND	ND	ND	ND
T	ND	ND	ND	ND	ND
Staphylococcus aureus count(log cfuml^{-1})					
C	ND	ND	ND	ND	ND
T	ND	ND	ND	ND	ND
Clostridium botulinum count (log cfuml^{-1})					
C	ND	ND	ND	ND	ND
T	ND	ND	ND	ND	ND

ND = Not Detected

C=control (buffalo meat nugget prepared without wheat bran),

T=treatment (buffalo meat nugget prepared with 10% wheat bran).

Overall means bearing different superscripts in the column (A,B) differ significantly ($p<0.05$).

Table 3: Analysis of variance for Total Plate Count (TPC) and Yeast and mold count (YMC) of buffalo meat nuggets incorporated with wheat bran at refrigeration temperature (4±1°C)

Total plate count			
Source	D.F.	Mean square	Level of sig.
Between period	1	7.483601667	S*
Between treatment	4	5.165580833	S*
Interaction between treatment and period	4	0.061789167	NS
Error	50	0.027148333	
Yeast and Mold count			
Source	D.F.	Mean square	Level of sig.
Between treatment	1	0.13265	S*
Between period	4	6.27248	S*
Interaction between treatment and period	4	0.13251	S*
Error	50	0.0118	

*=Significant and NS=Non-significant, D.F=Degree of freedom

ISSN No. 2454-6194 | DOI: 10.51584/IJRIAS |Volume XI Issue VII July 2026

3.2 pH

The pH value of buffalo meat nuggets incorporated with wheat bran (mean ± SE) at refrigeration temperature (4±1°C) was done for storage stability and storage periods has been presented in the Table 4 and depicted in figure 3.

The result of analysis of variance revealed significant ($p<0.05$) effect of wheat bran incorporation on pH values of the nuggets. An increase in pH values of wheat bran incorporated buffalo meat nuggets was observed on day 0 (Table 4), which was expected due to the neutral pH of wheat bran. Because wheat bran includedcertain alkaline components, including minerals (including magnesium, calcium, and potassium) and buffering agents like phytates, adding wheat bran to meat raisedits pH. These ingredients couldraise the pH overall by counteracting some of the meat's acidic ingredients. The increase in mean pH values were also recorded by Yilmaz (2005) in wheat bran incorporated meatballs, Talukder and Sharma (2010) in wheat bran incorporated beef patties, Kumaret al. (2016) in wheat bran incorporated biscuits, Yadavet al. (2017) in wheat bran incorporated chicken sausages, Shukla (2017) in wheat bran incorporated sausage, Rindheet al. (2018) in wheat bran incorporated spent hen nuggets. Storage period also had significant effect ($p<0.05$) on pH values of nuggets.During storage, due to the almost neutral pH of wheat bran, a modest increase in the pH value of buffalo meat nuggets with wheat bran incorporation was noted on day 0. The mean pH values of control as well as treatments decreased during first 7 days, then after increased with the progression of storage period until 28th days of storage.Microbial development and the pH change that occurdin meat products during storage werestrongly connected. Lactic acid bacteria and other acid-producing microbes mighthave accumulated, causing the first pH drop (Jinet al., 2021). As meat deteriorates, autolysis and microbial activity might cause nitrogenous chemicals like ammonia to accumulate, which could be the cause of the subsequent rise in pH (Holmeret al., 2009 and Xiongetal., 2020).This pH increase during storage could be due to protein denaturation and the release of protein metabolites, mostly amines, as a result of bacterial action. Jungetal. (2018) and Shanaullahet al. (2024) also recorded fluctuation in pH values of sausages during refrigeration storage. According to Nahidet al. (2024), during refrigeration storage, the interaction between treatment and day count significantly affected pH levels, with the 28th day of storage displaying the highest value and the 14th day the lowest in chicken nuggets. According to Xiongetal. (2022), during refrigeration storage of fermented sausage, the pH value decreased during the first 5–15 days, then showedrapid increase. Shukla (2017) recorded the increase in pH of wheat bran added sausage as compare to control during refrigeration storage.

Table 4: pH value (mean ± SE) of buffalo meat nuggets incorporated with wheat bran at refrigeration temperature (4±1 °C)

Day Treatments	T_0	T_2	Overall storage mean
0 day	6.02±0.024	6.11±0.04	6.06 ± 0.029^{a}
7th day	6.01±0.021	6.10±0.012	6.05 ± 0.011^{b}
14th day	6.13±0.022	6.19±0.023	6.16 ± 0.018^{bc}
21th day	6.16±0.024	6.21±0.026	6.19 ± 0.019^{c}
28th day	6.20±0.054	6.22±0.026	6.21 ± 0.029^{c}
Treatment mean	6.10 ± 0.018^{B}	6.16 ± 0.014^{A}	

Note:Overall means bearing different superscripts in a row (A, B, C...) and in the column (a, b, c...) differ significantly ($p<0.05$). T_0-Buffalo meat nuggets prepared without wheat bran, T_2–Buffalo meat nugget prepared with 10% wheat bran

Table 5: Analysis of variance forpH of buffalo meat nuggets incorporated with wheat bran at refrigeration temperature (4±1 ˚C)

pH			
Source	D.F.	Mean square	Level of sig.
Between period	1	0.045676667	S*
Between treatment	4	0.042666667	S*
Interaction between treatment and period	4	0.0055	NS
Error	50	0.005191333	

*=Significant and NS=Non-significant, D.F=Degree of freedom

CONCLUSION

The results clearly exhibited the antioxidant, antibacterial and antifungal effect of wheat bran in buffalo meat nuggets during refrigeration storage. The wheat bran effectively improved microbiological quality than control. Thus, Industrial waste like wheat bran could be successfully used to extend the shelf life of refrigerated meat products.

REFERENCES

1. Abbas, O.N., Majeed, M.R., 2024. Antimicrobial and Antibiofilm Activity of Local Wheat Bran Extract and Bacteriocin Combination Against Gastrointestinal Pathogens. Baghdad Science Journal().https://doi.org/10.21123/bsj.2024.10306.
2. Abozed, S.S., El-Kalyoubi, M,Abdelrashid, A., Salama, M.F., 2014. Total phenolic contents and antioxidant activities of various solvent extracts from whole wheat and bran. Annals of Agricultural Sciences 59(1), 63–67.
3. Ahmad, S.R., Sharma, B.D., Irshad, A., Kumar, R.R., Malav, O.P., Talukder, S., 2021. Effect of aerobic storage conditions on the quality of functional restructured buffalo meat fillets enriched with natural sources of dietary fibers and antioxidant components. Journal of Food Processing and Preservation 45(1), e15072.
4. Anson, N.M., Hemery, Y.M., Bast, A., Haenen, G.R., 2012. Optimizing the bioactive potential of wheat bran by processing. Food and function 3(4), 362–375.
5. Anonymous, 1984. Compendium of methods for microbiological examination of foods, (2nd Edn.).In: Speck,M.L.,(Ed.),American Public Health AssociationWashington, D.C.Available from : https://www.indianjournals.com/ijor.aspx?target=ijor:ijfv&volume=5&issue=2&article=024&type=pdf Accessed on: 23rd January 2025.
6. Anonymous, 2001. Contagem de Clostridium Perfringens Bacteriological Analytical Manual. In: Chapter 16, Food and Drug Administration, Clostridium Perfringens.Available from :https://www.scirp.org/journal/paperinformation?paperid=44057. Accessed on: 23rd January 2025.
7. Anonymous, 2024. Government of India. Ministry of Agriculture and Farmers Welfare, Department of Animal Husbandry, New Delhi. Available from:https://dahd.gov.in/sites/default/files/202501/FinalBAHS2024Book14012025.pdf. Accessed on 23rd January 2025.

8. Anonymous, 1984. Compendium of Methods for Microbiological Examination of Foods (2nd edn.). In:Speck, M.L.,(Ed.), American Public Health Association, Washington, D.C.
9. Badr, H.M., Mahmoud, K.A., 2011. Antioxidant activity of carrot juice in gamma irradiated beef sausage during refrigerated and frozen storage. Food Chemistry127(3), 1119–1130.
10. Balandran-Quintans, R.R.,J.N., Mercado-Ruiz., A.M, MendozaWilson., 2015. Wheat bran proteins: A review of their uses and potential. Food Reviews International 31(3), 279–93. doi: 10.1080/ 87559129.2015.1015137.
11. Bansil, P.C., 2003. Demand for food grains. In: Dev, S.M., Kanan, K.P. and Ramachandran, M., (Eds.), Towards a Food Secure India: Issues and policies, Institute for Human Development, New Delhi, p.5.
12. Barnes, H.J., 2003.Isolamento de Clostridium Perfringens. In: Diseases of Poultry, Iowa State University Press, Ames, 797–859.
13. Bhat, A.A., Kumar, A., Dua, S., 2016. Storage quality and oxidative stability attributes of almond (Prunusdulcis) fortified chevon nuggets. Journal of Animal Research 6(4), 747–753.
14. Das, A.K., Nanda, P.K., Madane, P., Biswas, S., Das, A., Zhang, W., Lorenzo, J.M., 2020. A comprehensive review on antioxidant dietary fibre enriched meat-based functional foods. Trends in Food Science and Technology 99, 323–336.
15. Devatkal, S.K., Thorat, P., Manjunatha, M., 2014. Effect of vacuum packaging and pomegranate peel extract on quality aspect of ground meat nuggets. Journal of Food Science and Technology 51(10), 2685–2691.
16. Di Lena, G., Vivanti, V., Quaglia, G.B., 1997. Amino acid composition of wheat milling by-products after bioconversion by edible fungi mycelia. Food/Nahrung 41(5), 285–288.
17. Di Stasio, L., Brugiapaglia, A., 2021. Current knowledge on river buffalo meat: A critical analysis. Animals 11(7), 2111.
18. Ehsanbakhsh, M., Sadeghi, A., Raeisi, M., Ebrahimi, M., Kashaninejad, M., 2017. Isolation, Molecular Identification and Evaluation of Antifungal Effect of Dominant Lactic Acid Bacteria Isolated from Wheat Bran and Rice Bran Sourdoughs. Research and Innovation in Food Science and Technology 6(3), 245–260.
19. Fang, Z., Lin, P., Ha, M., Warner, R.D., 2019. Effects of incorporation of sugarcane fibre on the physicochemical and sensory properties of chicken sausage. International Journal of Food Science and Technology 54(4), 1036–1044.
20. Ferysiuk, K., Wójciak, K.M., 2020. Reduction of nitrite in meat products through the application of various plant-based ingredients. Antioxidants 9(8), 711.
21. Gunenc, A., Alswiti, C., Hosseinian, F., 2017. Wheat bran dietary fiber: promising source of prebiotics with antioxidant potential. Journal of Food Research 6(2),1.
22. Holmer, S.F., McKeith, R.O., Boler, D.D., Dilger, A.C., Eggert, J.M., Petry, D.B.,Killefer, J., 2009. The effect of pH on shelf-life of pork during aging and simulated retail display. Meat Science 82(1), 86–93.
23. Hromádková, Z., Paulsen, B.S., Polovka, M., Košťálová, Z., Ebringerová, A., 2013. Structural features of two heteroxylan polysaccharide fractions from wheat bran with anti-complementary and antioxidant activities. Carbohydrate polymers93(1), 22–30.
24. Jin, S.K., Choi, J.S., Kim, G.D., 2021. Effect of porcine plasma hydrolysate on physicochemical, antioxidant, and antimicrobial properties of emulsion-type pork sausage during cold storage. Meat science 171,108293.
25. Jung, J.T., Lee, J.K., Choi, Y.S., Lee, J.H., Choi, J.S., Choi, Y.I.,Chung, Y.K., 2018. Effect of rice bran and wheat fibers on microbiological and physicochemical properties of fermented sausages during ripening and storage. Korean journal for food science of animal resources 38(2), 302.
26. Kamal, S.B., Kumar, A., Tanwar, T., 2017. Physico-chemical, proximate, sensory and storage quality attributes analysis of papaversomniferum (poppy) fortified chevon nuggets. Journal of Applied and Natural Science 9(1), 114–120.
27. Kaur, S., Kumar, S., Bhat, Z.F., Kumar, A., 2015. Effect of pomegranate seed powder, grape seed extract and tomato powder on the quality characteristics of chicken nuggets. Nutrition and Food Science 45(4), 583–594.
28. Kumar, L., Bhat, Z.F., Kumar, S., 2016a. Effect of nisin on the quality characteristics of fiber-enriched chicken harrisa. Journal of Meat Science 11(2), 57–63.

29. Kumar, P., Chatli, M.K., Mehta, N., Malav, O.P., Verma, A.K., Kumar, D., 2016b. Quality attributes and storage stability of chicken meat biscuits incorporated with wheat and oat bran. Journal of Food Quality 39(6), 649–657.
30. Laddomada, B., Caretto, S,Mita, G., 2015. Wheat bran phenolic acids: Bioavailability and stability in whole wheat-based foods. Molecules 20(9), 15666–15685.
31. Li, C., Stump, M., Wu, W., Li, Y., 2023. Exploring the chemical composition, antioxidant potential, and bread quality effects of the nutritional powerhouse: Wheat bran–A mini-review. Journal of Agriculture and Food Research 100898.
32. Meena, P., 2021. Studies on alpha-linolenic acid (Flaxseed) enriched chevon nuggets incorporated with green coffee (Coffeaarebica) and grape seed extract (Vitisvinifera).MVSc. Thesis, Rajasthan University of Veterinary and Animal Sciences, Bikaner, Rajasthan.
33. Nahid, M.Z.A., Habib, M., Mollah, M.B.R., Hashem, M.A., Azad, M.A.K.,Ali, M.S., 2024. Development of dietary fiber enriched chicken nugget using wheat bran. Meat Research 4(1),1–6.
34. Onipe, O.O., Ramashia, S.E., Jideani, A.I., 2021. Wheat bran modifications for enhanced nutrition and functionality in selected food products. Molecules26(13), 3918.
35. Ravani, A., Sharma, H.P., 2022. Functional Foods.In: Meat based functional foods, 235–287.
36. Ribeiro, J.S., Santos, M.J.M.C., Silva, L.K.R., Pereira, L.C.L., Santos, I.A., da Silva Lannes, S.C., da Silva, M.V., 2019. Natural antioxidants used in meat products: A brief review. Meat science 148, 181–188.
37. Rindhe, S.N., Chatli, M.K., Wagh, R.V., Kumar, P., Malav, O.P., Mehta, N., 2018. Development and quality of fiber enriched functional spent hen nuggets incorporated with hydrated wheat bran. International Journal of Current Microbiology and Applied Sciences 7(12), 3331–3345.
38. Shanaullah, M., Habib, M., Hashem, M. A., Azad, M. A. K. and Ali, M. S., 2024. Development of dietary fiber enriched chicken sausage using wheat bran. Meat Research 4(2), 1–7.
39. Shukla, M. K.,2017. Effect of chicken skin blend containing wheat bran on quality characteristics of cooked sausages. M.V.Sc. Thesis, GB Pant University of Agriculture and Technology, Pantnagar, Uttarakhand.
40. Snedecor, G.K., Cochran, W.G., 1989. Statistical Methods (8thEdn.).Lowa state university press, Ames, USA, Lowa-50010, pp
41. Soren, N. M., Biswas, A. K., 2020. Methods for nutritional quality analysis of meat. In: Meat quality analysis, Academic Press, 21–36.
42. Talukder, S. and Sharma, D. P., 2010. Development of dietary fiber rich chicken meat patties using wheat and oat bran. Journal of food science and technology 47, 224–229.
43. Trout, E.S., Hunt, M.C., Johnson, D.E., Claus, J.R., Kastner, C.L.,Kropt, D.H., 1992. Characteristics of low fat ground beef containing texture modifying ingredients. Journal of Food Science 57(1) 19–24.
44. Verma, B., Hucl, P., Chibbar, R.N., 2008. Phenolic content and antioxidant properties of bran in 51 wheat cultivars. Cereal Chemistry 85(4), 544–549.
45. Xiong, Y., Zhang, P., Warner, R.D., Shen, S., Johnson, S.,Fang, Z., 2020. Comprehensive profiling of phenolic compounds by HPLC-DAD-ESI-QTOF-MS/MS to reveal their location and form of presence in different sorghum grain genotypes. Food Research International 137, 109671.
46. Xiong, Y., Zhang, P., Warner, R.D., Hossain, M.N., Leonard, W., Fang, Z., 2022. Effect of sorghum bran incorporation on the physicochemical and microbial properties of beef sausage during cold storage. Food Control132, 108544.
47. Yadav, S., Pathera, A.K., Islam, R.U., Malik, A.K., Sharma, D.P., 2017. Effect of wheat bran and dried carrot pomace addition on quality characteristics of chicken sausage. Asian-Australasian journal of animal sciences 31(5), 729.
48. Yilmaz, I., 2005. Physicochemical and sensory characteristics of low fat meat balls with added wheat bran. Journal of Food Engineering 69,369–373.
49. Zhang, A., de Koning, L., Shannon, H.S., Anand, S.S., 2010. A systematic review of the evidence supporting a causal link between dietary factors and coronary heart disease. Archives of Internal Medicine169, 659–669.
50. Zhang, H., Zhang, M., Zheng, X., Xu, X., Zheng, J., Hu, Y., Liang, Y., 2024. Solid-State Fermentation of Wheat Bran with Clostridium butyricum: Impact on Microstructure, Nutrient Release, Antioxidant Capacity, and Alleviation of Ulcerative Colitis in Mice. Antioxidants 13(10), 1259.

ISSN No. 2454-6194 | DOI: 10.51584/IJRIAS |Volume XI Issue VII July 2026

51. Zou, Z., Purnawan, M.A., Wang, Y., Ismail, B.B., Zhang, X., Yang, Z., Guo, M., 2025. A novel antimicrobial peptide WBp-1 from wheat bran: Purification, characterization and antibacterial potential against Listeria monocytogenes. Food Chemistry 463, 141261.
52. Zou, Z., Wang, M., Wang, Z., Aluko, R.E., He, R.,2020. Antihypertensive and antioxidant activities of enzymatic wheat bran protein hydrolysates. Journal of Food Biochemistry 44(1), e13090.