Journal of Scientific and Technical Research
15 (2) 2025, 41-51
www.publishingindia.com/jstr
https://doi.org/10.21863/jstr/2025.15.2.004

Physicochemical, Functional, and Microbial Safety Characteristics of the Developed Rice-Mushroom Protein-Based Meat Analogue as Affected by Different Storage Conditions

Ringshangphi Khapudang and Saleem Siddiqui*
Department of Life Sciences, Sharda School of Bioscience and Technology,
Sharda University, Greater Noida, Uttar Pradesh, India.
*Corresponding Author: saleem.siddiqui@sharda.ac.in

Abstract: The growing demand for plant-based meat analogues (PBMAs) has driven the need for sustainable and stable products with extended shelf life. A cost-effective, soy-free PBMA was developed from rice bran and oyster mushroom protein isolates (1:6 ratio) using a steaming process. Samples were packed under vacuum and normal atmospheric conditions, and stored at low (4±1 °C) and room (25±2 °C) temperatures. Rapid microbial growth and quality loss were observed at room temperature, limiting shelf life to 3 and 7 days under normal and vacuum conditions, respectively. In contrast, low-temperature vacuum storage minimized spoilage, maintained desirable composition (moisture 4.28%, protein 42.45%, fat 4.65%, ash 5.02%), and preserved color, texture, and water and oil holding capacities. Shelf life of 14 days under normal and 28 days under vacuum conditions were observed under low temperature storage. The study emphasizes the importance of packaging, temperature, and further strategies in ensuring PBMA quality during storage.

Keywords: Functional properties, Microbial safety, Packaging, Rice-mushroom meat analogue, Shelf life.

I. Introduction

Dietary patterns have a profound influence on both environmental sustainability and the efficient use of natural resources. The global food industry, particularly the production and consumption of animal-derived products, is a major contributor to environmental degradation. Meat, while historically central to human diets and valued for its high-quality protein, is associated with considerable ecological burdens [1]. As nations experience social and economic development, meat consumption often increases, further amplifying its environmental impact. In light of these environmental concerns, many researchers and policymakers have advocated for reduced global meat consumption [2]. Beyond ecological implications, high meat intake particularly red and processed meat has been associated with various health risks including cardiovascular diseases, chronic inflammation and type 2 diabetes [3]. These environmental and health considerations have given rise to growing popularity of plant based diets. The rise in plant based meat analogue consumption are largely driven by flexitarians, those seeking to reduce meat consumption but not completely eliminate meat intake [1].

A practical strategy to reduce meat consumption without compromising nutritional and sensory quality is to replace meat with non-meat plant based ingredients [4]. Therefore, plant based meat analogues (PBMAs) are products designed to mimic the taste, texture and appearance of meat, and are the most commonly used meat alternatives [5]. Soy protein is popular for fabricating meat analogues owing to its favorable gelling ability, plentiful nutrients, and low cost [6]; however, excessive use of soy protein has been reported to cause side effects on the sexual health of males [7]. To overcome this problem, a PBMA was developed in our laboratory by complete substitution of soy protein with oyster mushroom and rice bran protein isolates, and by the use of a conventional low-cost steaming process in place of costly extrusion technology [8]. The developed PBMA comprised of rice bran protein isolates and oyster mushroom protein isolates in ratio of 1:6. The developed PBMA had 43.12 % protein, 34.52 % carbohydrate, 5.71 % ash, and 4.61% moisture on DW basis. The WHC and OHC were found to be 2.78 (g H_2O/ g sample) and 1.58 (g oil/ g sample), respectively. The balanced nutritional composition and favorable functional properties highlight its potential as a sustainable and ethical meat alternative. The physicochemical, functional, microbial safety and shelf life stability of this developed PBMA under different packaging and storage conditions have been reported in this communication.

II. Materials and Methods

A. Raw Materials

The rice bran protein isolates (RPI) (protein: 73%) from Rollins International Pvt. Ltd., India; starches (corn starch and rice starch from HealthyHive, India) were purchased online (Amazon, India). The other ingredients were procured from the local market of Greater Noida, India. Oyster mushrooms were harvested from the Mushroom Cultivation House of Sharda University, Greater Noida, Uttar Pradesh, India. The oyster mushroom protein isolates (MPI) (protein: 63%) were isolated using ultrasound assisted technique described by Prandi *et al.* [9].

B. Preparation of Plant Based Meat Analogue

The meat analogues were formulated rice bran protein and oyster mushroom protein isolates isolates in the ratio of 1:6. In 100 g of composite formulation, protein isolates & starch were collectively 36 g, water 50 g, soybean vegetable oil 3.5 g, calcium chloride 0.2 g, baking powder 2.5 g, salt 0.3 g, carboxy-methyl-cellulose (CMC) 1.5 g and wheat gluten 6 g. The starch used was the blend of corn starch and rice starch in equal proportions on % wt basis. The addition of calcium chloride was aimed to enhance the protein-water binding capacity while baking powder was included to promote air cell formulation within the analogue dough. The mixture was kneaded thoroughly by dough kneader (Model no.: SA3032DM, Glen, India) at low speed for 15 min. This dough was then steamed over a medium-low heat flame using a stainless steel steamer (Model No.: BSSH 2-20, 2-tier stainless steel steamer, 18 cm x 25 cm x 18 cm, Vinod Cookware, India) for 20 min, with approximately 250 ml of water maintained at a simmering temperature of 100 °C ± 2 °C in the lower compartment. After steaming, it was brought to room temperature, individual packed in disposable sauce & dip transparent hinge lid plastic container (30 mL), and then packed in clear LDPE bags of 100 gauge under normal environment (non-vacuum) and vacuum conditions (Fig. 1), and stored at room temperature (RT: 25 °C ± 2 °C) and at low temperature (LT: 4 °C ± 1 °C) conditions.

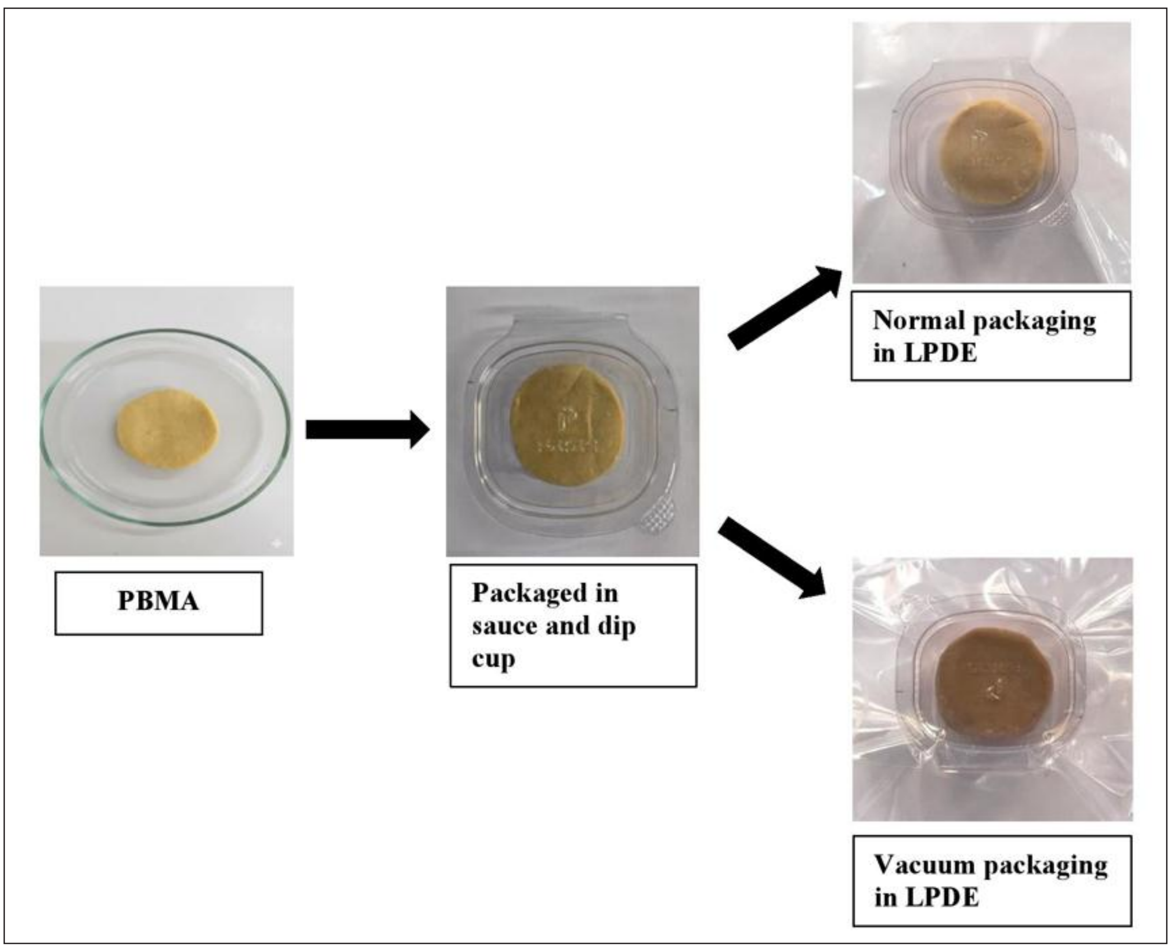

Fig. 1: Packaging and Storage of the Developed PBMA

C. Proximate Analysis

The PBMA developed from rice and mushroom proteins were dried in cabinet dryer, grinded into powder using a blender (Mixer Grinder/HL7505/00, Philips India Ltd.) and sieved through a 60 mesh screen to obtain a homogeneous powder. Moisture, protein, lipid, and ash were determined using the standard procedures described by the AOAC [10]. In brief, the moisture content of PBMAs were determined using oven drying method at 105 °C; ash content by incineration in a muffle furnace at 550 °C; protein content by determination of nitrogen by Kjeldahl's method and multiplying by a conversion factor of 6.25; fat content using a Soxhlet apparatus with petroleum ether as the extracting solvent. The carbohydrate content was calculated using the difference method by subtracting the total protein, fat, moisture and ash content from 100 on dry weight basis.

D. Colour and Browning Index

The color of each PBMA was determined using colorimeter (Chroma meter, CR-400, Konica Minolta Optics. INC, Japan) that was calibrated using standard white plate according to the reference CIE 1976. The CIE- lab parameters were expressed as value of L^* (lightness), a^* (redness or greeness), b^* (yellowness or blueness). The measurements were performed in triplicates across three randomly selected spots on the surface of the PBMA samples.

The browning index (BI) was calculated according to Dinani *et al.* [11] using the equation (1).

$$BI = 100\left(\frac{x- 0.31}{0.17}\right) \text{ Where, } x = \frac{a^* + 1.75L^*}{5.645L^* + a^* - 0.3012b^*} \quad (1)$$

E. Water Holding and Oil Holding Capacities

Water holding capacity (WHC) and oil holding capacity (OHC) were determined with slightly modified method outlined by Ketnawa and Rawskeun [12]. For WHC, 0.2 g of powdered, dried PBMAs samples was mixed with 5 mL distilled water in a pre-weighed 15 mL centrifuge tube. After vortexing, the mixture was allowed to stand for 5 min at room temperature and then centrifuged at 6000 rpm for 10 min. The supernatant was discarded and the residue was inverted and rested for 5 min before reweighing.

For OHC, 0.2 g of the sample was mixed with 5 mL soybean oil in a pre-weighed 15 mL centrifuge tube. The mixture was allowed to stand at room temperature for 30 min followed by centrifugation at 6000 rpm for 10 min. After draining excess oil, the sediment was inverted for 10 min and then reweighed. Each sample was tested in triplicate. Using the equations (2) and (3), the WHC and OHC were calculated respectively on g/g weight basis.

$$\text{WHC (g } H_2O\text{/ g sample)} = \frac{W_2 - W_1 - W_s}{W_s} \quad (2)$$

Where, W_2= Reweighed sample

W_1= Weight of centrifuged tube

W_s= Weight of sample

$$\text{OHC (g oil /g sample)} = \frac{O_2 - O_1 - O_s}{O_s} \quad (3)$$

Where, O_2= Reweighed sample

O_1= Weight of centrifuged tube

O_s= Weight of sample

F. Changes in Thickness

Changes in plant-based meat analogue thickness during storage was determined by using the equation (4).

$$\text{Thickness change (\%)} = \frac{T_1 - T_2}{T_1} \times 100 \quad (4)$$

where T_1 = thickness of steamed analogue

T_2 = thickness of raw analogue

G. Microbial Analysis

Total plate count of PBMA was performed following ISO 4833-2:2013 [13]. One gram of the food sample was homogenized with 9 mL peptone water, followed by preparation of ten-fold serial dilution. A 0.1 mL aliquot of each dilution was surface plated on plate count agar (PCA) plates and incubated at 30 °C for 72 ± 3h. After the incubation period, colonies were counted and the results were expressed as $\log_{10}$ CFU/g.

H. Sensory Evaluation

The PBMA developed was evaluated during storage for appearance, flavour, mouthfeel and overall acceptability attributes by a panel of 20 semi trained individuals. Prior to the evaluation, all the panelists were informed about the product to avoid health risks (allergies or food intolerance). The detailed procedure for sensory evaluation is outlined in our earlier communication [8]. The final overall score was determined by calculating the average ratings provided by panelists.

I. Statistical Analysis

All experimental work was carried out in triplicates. The data collected during this study were analyzed using analysis of variance (ANOVA) techniques based on one factorial completely randomized design (CRD) using the online software OPstat (OPSTAT - Online Statistical Analysis Tools). To compare the effects of different treatments, the critical difference (CD) at p≤0.05 was used.

III. Results and Discussion

The steamed meat analogue was found to contain ~ 49.65 % moisture on fresh weight basis (data not presented). At RT storage, the samples couldn't be stored beyond 7 days when packed in vacuum, and got spoiled by microbes by 3 days under normal packed conditions (Data presented in supplementary Table). The faster deterioration of PBMA under RT conditions can be attributed to higher moisture and temperature enhancing microbial growth and enzymatic reactions [14]. The presence of oxygen and elevated temperature under the normal packed conditions created a highly favourable conditions for rapid microbial growth, which made the samples unsafe for consumption [15]. The PBMA stored at LT, however, could be stored up to 28 days. It was sampled at regular intervals of 7 days to study the changes in quality parameters during storage. The results for PBMA stored under normal and vacuum packed conditions at LT are discussed below:

A. Proximate Analysis

The proximate composition of the developed PBMA when stored at LT conditions is presented in Table I. The PBMA under normal packed conditions showed microbial growth when stored beyond 21 days, and were thus not subjected to analysis.

Moisture content was 4.79% on 0-day of storage. There was a non-significant change in moisture content of PBMA up to 21 days of storage in vacuum and up to 14 days in normal packed conditions. The moisture content slightly decreased at later storage periods, however, the samples got spoiled by 28 days under normal packed conditions. The retention of moisture content could be due to the low water vapour permeability of the packaging material [16]. Similar non-significant change in moisture content during initial period and small decrease at later storage periods has been reported by Cho *et al.* [17] for soy and texturized vegetable protein based meat analogue.

A decrease in the protein content was observed during storage, which was at slower rate in the PBMA under vacuum as compared to normal packed conditions. Protein content declined from 44.58% to 42.45% during 28 days of storage under vacuum, and to 40.31% during 21 days of storage under normal packed conditions, a change that may be attributed to protein denaturation and limited enzymatic activity [18]. Fat content remained stable initially up to 14 days of storage but showed a decrease at later storage period, both under normal and vacuum packed conditions, suggesting the onset of lipid oxidation despite low-temperature conditions. Miller *et al.* [19] also reported a similar trend of lipid oxidation induced reduction in fat content during refrigerated storage of uncooked plant-based meat analogue burger patties. Ash content was significantly not affected up to 21 days of storage under vacuum and 14 days in normal packed conditions. Similar observations were reported by Yadav *et al.* [20] for the chicken meat analogue rolls made from wheat, texturized soy protein and mushroom paste. The carbohydrate content showed a progressive increase with increasing storage period, which was of higher magnitude under normal as compared to vacuum packed conditions. This rise in carbohydrate content could likely be due to a relative increase resulting from reductions in moisture and other macronutrients [21].

Table I: Proximate Analysis of Steamed PBMA (% DW Basis) During Storage Under Different Packaging Conditions at Low Temperature

Packaging Conditions	*Storage Period (Days)*	*Protein (%)*	*Fat (%)*	*Ash (%)*	*Moisture (%)*	*Carbohydrate (%)*
	0	44.58±0.18^{a}	5.73±0.14^{a}	5.88±0.10^{a}	4.79±0.19^{a}	38.69±0.27^{a}
Vacuum packed	7	44.37±0.16^{a}	5.69±0.42^{a}	5.66±0.16^{a}	4.76±0.15^{a}	39.42±0.16^{a}
	14	43.72±0.20^{b}	5.40±0.30^{a}	5.58±0.16^{a}	4.68±0.16^{a}	40.01±0.15^{b}
	21	43.54±0.10^{b}	4.95±0.10^{b}	5.47±0.15^{a}	4.56±0.21^{a}	41.45±0.20^{c}
	28	42.45±0.20^{c}	4.65±0.14^{b}	5.02±0.05^{b}	4.28±0.18^{b}	43.74±0.19^{d}
Normal packed	7	43.70±0.14^{b}	5.65±0.12^{a}	5.78±0.13^{a}	4.58±0.17^{a}	40.04±0.10^{b}
	14	43.39±0.23^{b}	5.16±0.13^{a}	5.65±0.04^{a}	4.36±0.16^{a}	41.66±0.09^{c}
	21	40.31±0.14^{d}	4.35±0.11^{b}	4.94±0.11^{b}	4.16±0.17^{b}	46.42±0.22^{e}
	28	-	-	-	-	

- Samples spoiled by microbes. The values are mean ± SD (n=3). The means for a parameter within a column with different superscript differ significantly (p≤0.05).

B. Colour and Browning Index

The colour characteristics of the PBMA were monitored during storage to assess the visual quality and browning progression. Measurements included lightness (L^*), redness (a^*), yellowness (b^*), and browning index, an important indicator of non-enzymatic browning in the meat analogue. During LT storage under vacuum packed conditions, the L^* value remained significantly unaffected up to 7 days and at then decreased at later storage periods (Table II). The a^* and b^* values were not significantly affected up to 21 days of storage and then slightly but significantly deceased at later storage period. The BI was not significantly affected up to 14 days but increased at later storage period, indicating progressive but slow browning under vacuum packed conditions. The BI of the PBMA, packed under normal packaging conditions, showed a progressive increase throughout the storage period indicating an accelerated non -enzymatic browning reactions, likely due to higher oxygen availability. This rise in BI suggest gradual deterioration in colour quality during extended storage. Overall, the data demonstrated that both packaging type and storage temperature significantly affect the visual quality and colour stability of PBMAs. Vacuum packaging combined with refrigeration effectively maintained colour parameters and minimized browning up to 28 days. In contrast, non-vacuum packaging accelerated pigment oxidation and non-enzymatic browning. The changes in the colour attributes of PBMA during storage could be attributed to non-enzymatic browning and pigment instability over time [22]. The normal packed samples exhibited more pronounced colour changes as compared to vacuum packed samples, possibly due to higher oxidative degradation and intensified non-enzymatic browning [15, 23, 24].

Table II: Colour Attributes and BI of Steamed PBMA During Storage Under Different Packaging Conditions at Low Temperature

Packaging Conditions	*Storage Period (Days)*	*Colour Attributes and BI*			
		*L**	*a**	*b**	*BI*
	0	45.23±0.25^a	8.18±0.14^a	19.21±0.21^a	16.98±0.19^a
Vacuum packed	7	44.98±0.19^a	8.28±0.09^a	18.87±0.13^a	17.14±0.15^a
	14	43.65±0.21^b	8.65±0.22^a	18.91±0.27^a	18.91±0.24^a
	21	42.25±0.10^c	8.94±0.13^a	18.92±0.18^a	19.56±0.08^b
	28	42.10±0.06^c	9.25±0.06^b	20.56±0.08^b	19.92±0.01^b
Normal packed	7	45.19±0.12^a	9.56±0.12^b	17.35±0.14^c	18.63±0.15^a
	14	44.25±0.32^b	10.15±0.24^c	19.07±0.21^a	20.33±0.28^c
	21	43.98±0.15^b	10.76±0.16^c	20.23±0.12^b	21.66±0.15^c
	28	-	-	-	-

- Samples spoiled by microbes. The values are mean ± SD (n=3). The means for a parameter within a column with different superscript differ significantly ($p \leq 0.05$).

The colour appearance of the PBMA during storage under different packaging conditions is shown in Fig. 2. There was a progressive darkening and browning of PBMA during storage, the intensity of which was lesser under vacuum as compared to normal packed conditions. The changes colour appearance during storage matched with the browning index data presented in Table II.

Storage Period (Days)	Normal Packed	Vacuum Packed	Storage Period (Days)	Normal Packed	Vacuum Packed
0			7		
14			21		
28	Spoiled				

Fig. 2: Colour Appearance of the Developed PBMA During Storage Under Low Temperature Under Different Packaging Conditions

C. Water Holding and Oil Holding Capacities

The water holding capacity (WHC) and oil holding capacity (OHC) of the developed PBMA showed a reduction during storage across all packaging and storage conditions. WHC decreased from 3.85 g H_2O/g on day 0 to 2.89 g H_2O/g by 28 days of storage under vacuum packed conditions, while it was 3.11 g H_2O/g by 21 days of storage under normal packed conditions. The OHC dropped from 2.31 g oil/ g to 1.03 g oil/ g by 28 days of storage under vacuum packed conditions, while it was 1.78 g oil/ g by 21 days of storage under normal packed conditions. The decrease in WHC could be due to both physical and biochemical changes in the protein structure during storage. According to Tirado-Kulieva *et al.* [25], structural degradation of the protein matrix, partial denaturation, and loss of protein functionality during prolonged storage can impair the hydrophilic nature of the protein–fiber network, further reducing the product's ability to retain water. Similar decrease in WHC during storage was reported by Cho *et al.* [17] for soy and texturized vegetable protein based meat analogue. The steady decrease in OHC may be due to the disintegration of lipid-binding sites within the protein structure, possibly as a result of unfolding and breakdown of the plant proteins used in the formulation [15]. As oil-holding capacity is often linked to the surface properties and porosity of the matrix, any compaction or hardening of the structure during storage especially under low temperatures, may have contributed to lower oil entrapment. The faster rate of decline in WHC and OHC under normal packed conditions may be due to oxidative changes and moisture fluctuations associated with the absence of vacuum [15].

Table III: Water Holding Capacity, Oil Holding Capacity and Changes in Thickness of Steamed PBMA During Storage Under Different Packaging Conditions at Low Temperature

Packaging Conditions	*Storage Period (Days)*	*WHC (g H_2O/g Sample)*	*OHC (g oil/g Sample)*	*Changes in Thickness (%)*
	0	3.85±0.07^{a}	2.31±0.15^{a}	0
Vacuum packed	7	3.65±0.23^{a}	2.12±0.20^{a}	0.16±0.001^{a}
	14	3.43±0.13^{ab}	1.95±0.15^{b}	0.24±0.001^{b}
	21	3.31±0.15^{b}	1.68±0.13^{b}	0.32±0.002^{c}
	28	2.89±0.08^{c}	1.03±0.11^{c}	0.39±0.007^{d}
Normal packed	7	3.55±0.08^{a}	2.22±0.15^{a}	0.16±0.002^{a}
	14	3.23±0.21^{b}	1.85±0.13^{b}	0.23±0.001^{b}
	21	3.11±0.15^{b}	1.78±0.06^{b}	0.35±0.15^{c}
	28	-	-	-

- Samples spoiled by microbes. The values are mean ± SD (n=3). The means for a parameter within a column with different superscript differ significantly ($p \leq 0.05$).

D. Changes in Thickness

The dimensional stability of steamed PBMA during storage was assessed through thickness measurements. With increasing storage period under vacuum packed conditions, there was progressive change, indicating gradual shrinkage in thickness of PBMA (Table III). The shrinkage of similar magnitude was observed in normal packed PBMA also. These dimensional changes can be associated with moisture loss and partial structural collapse due to protein degradation and matrix contraction during storage. The reduction in thickness during storage reflects the need of developing further optimal packaging conditions to maintain product shape and structural stability over extended periods. The vacuum environment, although protective, does not completely prevent internal structural changes caused by water migration and polymer relaxation. These reductions may also reflect ongoing dehydration and physical deformation of the analogues in response to fluctuating ambient humidity within the package headspace [26]. The microbial activity and biochemical breakdown during storage may also be responsible for loss of structural integrity and shrinkage [27].

E. Microbial Analysis

The total plate count (TPC) in PBMA during storage under various packaging conditions is presented in Fig. 3. Due to effective thermal processing, there was no microbial growth observed at 0-day of storage across all the treatments. As storage progressed, microbial growth increased regardless of packaging and temperature conditions. There was slower microbial growth observed under vacuum as compared to normal packed conditions. The TPC reached 7.36 $\log_{10}$ CFU/g under normal and 4.81 $\log_{10}$ CFU/g under vacuum packed conditions by 28 days of storage at LT. Notably, the microbial load in normal packed conditions at LT exceeded the Bureau of Food and Drugs (BFAD) microbial permissible standard limit of 5 $\log_{10}$ CFU/g [28] by 14 days of storage, while under vacuum packed conditions it remained below this threshold value until day 28 days of storage. These findings emphasized the role of both packaging and storage conditions, where vacuum packaging under LT proved to be most effective in preventing microbial proliferation. Similar results were demonstrated by Liu *et al.* [29] where the plate count of the developed soy- and pea-based meat analogues reached the threshold of permissible limit by 10th day of storage at 4 °C. The high water activity, pH, and nutrient in PBMAs attributed to survival and proliferation of microbes during storage.

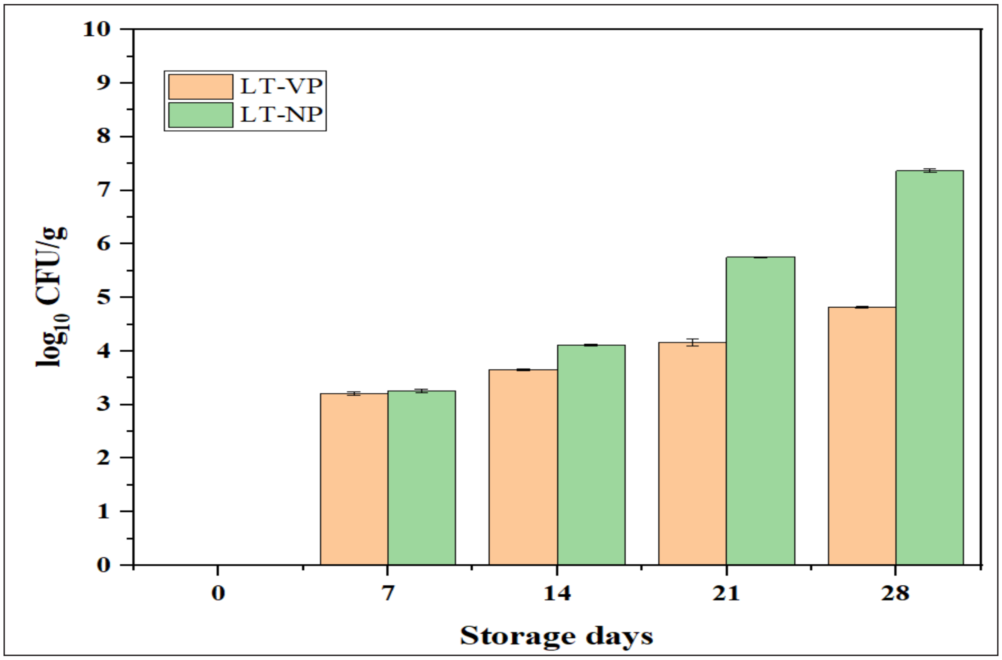

Fig. 3: Total Plate Count ($\log_{10}$ CFU/g) of the Developed PBMA During Storage Under Different Packaging Conditions at Low Temperature. At 0-Day, Microbial Growth Not Detected. LT-VP: Low Temperature- Vacuum Packaging; LT-NP: Low Temperature- Normal Packaging

F. Sensory Evaluation

There was a progressive decrease in the scores for various sensory attributes during storage, with the rate of deterioration influenced by temperature and packaging type (Table IV). Fresh samples at 0-day showed scoring more than 7 on 9-point Hedonic scale basis for appearance, texture, flavour, and mouth-feel, indicating like moderately to like very much sensory quality. Under vacuum packed conditions, the overall acceptability scores were retained in 5-6 Hedonic scale range throughout 28 days of storage at LT, thereby showing product acceptability. However, under normal packed conditions at LT, the product showed overall acceptability score up to 14 days of storage only and became unacceptable and of slightly disliked category at later storage period. Similarly, Sun *et al.* [30] reported that soybean based meat analogue when stored at 4 °C, maintained color and texture in the short term, but odor stability declined after the 9th day. The decline in overall acceptability scores was attributed to the production of undesirable flavour substances such as alcohols and aldehydes (1-hexanol, hexanal etc.) due to fat oxidation and microbial activity. The accelerated lipid oxidation and microbial proliferation under normal packed could be the reason for faster decline in the sensory scores as compared to vacuum packed conditions. Our findings are consistent with the previous reports of perishability of plant based meat analogues [23, 31], and emphasize the importance of proper packaging and storage conditions. Similarly, Kumar *et al.* [32] reported that plant-based nuggets could be safely stored at 4 °C for up to 14 days in LDPE pouches and up to 28 days under vacuum in multilayer nylon pouches, without any significant decline in sensory or microbiological quality. Yadav *et al.* [20] also observed that the developed chicken meat analogue rolls from texturized soy protein, maize flour, wheat flour and mushroom paste were microbiologically safe and highly acceptable up to 12 days at refrigeration and up to 3 days at ambient temperature of storage.

Table IV: Sensory Evaluation Scores (9-Point Hedonic Scale) of Steamed PBMA During Storage Under Different Packaging Conditions At Low Temperature

Packaging Conditions	*Storage Period (Days)*	*Appearance*	*Texture*	*Flavour*	*Mouthfeel*	*Overall Acceptability*
	0	7.6 ± 0.2^a	7.5 ± 0.2^a	7.7 ± 0.2^a	7.5 ± 0.2^a	7.6 ± 0.2^a
Vacuum packed	7	6.8 ± 0.3^b	6.6 ± 0.3^b	6.4 ± 0.3^b	6.2 ± 0.4^b	6.5 ± 0.3^b
	14	6.5 ± 0.3^c	6.3 ± 0.4^c	6.1 ± 0.4^c	5.8 ± 0.4^c	6.1 ± 0.4^c
	21	6.2 ± 0.4^d	6.1 ± 0.4^c	5.8 ± 0.4^d	5.6 ± 0.4^c	5.6± 0.4^d
	28	5.9 ± 0.4^e	5.8 ± 0.5^d	5.5 ± 0.5^e	5.2 ± 0.5^d	5.6 ± 0.5^d

Packaging Conditions	Storage Period (Days)	Appearance	Texture	Flavour	Mouthfeel	Overall Acceptability
Normal packed	7	6.3 ± 0.3^{d}	6.2 ± 0.4^{c}	5.9 ± 0.4^{d}	6.1 ± 0.4^{b}	6.1 ± 0.4^{c}
	14	5.3 ± 0.4^{e}	5.1 ± 0.4^{e}	4.9 ± 0.4^{e}	5.1 ± 0.5^{d}	5.1 ± 0.5^{e}
	21	4.5 ± 0.5^{f}	4.3 ± 0.5^{f}	4.2 ± 0.5^{f}	4.0 ± 0.5^{e}	4.2 ± 0.6^{f}
	28	-	-	-	-	-

- Samples spoiled by microbes. The values are mean ± SD (n=3). The means for a parameter within a column with different superscript differ significantly (p≤0.05).

IV. Conclusion

In the present study, a cost effective soya free PBMA, developed by steaming process, containing rice bran and oyster mushrooms protein isolates in the ratio of 1:6, was packed under vacuum and normal atmospheric condition, and stored at low (4 ± 1 °C) and room (25 ± 2 °C) temperatures. Results demonstrated that both temperature and packaging conditions influenced the quality of product over time. At room temperature storage, the PBMA showed shelf life of around 3 days at normal and 7 days under vacuum packed conditions. Packing under vacuum and storing under low temperature storage conditions effectively minimized the colour deterioration and browning, maintained WHC and OHC, while limiting the dimensional shrinkage. On the basis of microbial load and overall sensory acceptability scores, the shelf life of the developed PBMA at low temperature storage was found to be 14 days under normal and 28 days under vacuum packed conditions. Future work should investigate the incorporation of natural antimicrobials or antioxidants (e.g., phenolic extracts, essential oils), and use of fermentation technology to further enhance shelf stability. The studies on use of alternative biodegradable or high-barrier packaging materials suitable for commercial distribution, predictive microbial modeling for shelf-life estimation under fluctuating cold-chain conditions, and consumer acceptance studies to validate sensory performance across broader demographics are also required. The shelf life test under frozen temperature or freeze dried conditions may also be investigated to provide information on the real-time stability of the final products.

Acknowledgement

The authors acknowledge the support provided by Sharda School of Agricultural Sciences, Sharda University, Greater Noida, India for providing fresh oyster mushroom and lab facilities to carry out the work.

Author Contributions

Ringshangphi Khapudang: Investigation, writing original draft.

Saleem Siddiqui: Conceptualization, data interpretation, manuscript reviewing.

Fundings: The authors did not receive any external funding for the submitted work.

Conflict of Interest: The authors declare no competing interest.

References

[1] C. D. Munialo, V. Baeghbali, and P. Acharya, "Plant-based alternatives to meat products," *Foods,* vol. 14, p. 1396, 2025, doi: https://doi.org/10.3390/foods14081396.

[2] S. M. Tyndall, G. R. Maloney, M. B. Cole, N. G. Hazell, and M. A. Augustin, "Critical food and nutrition science challenges for plant-based meat alternative products," *Crit Rev Food Sci Nutr.*, vol. 64, pp. 638–653, 2024, doi: https://doi.org/10.1080/10408398.2022.2107994.

[3] Y. Huang, D. Cao, Z. Chen, B. Chen, J. Li, J. Guo, Q. Dong, L. Liu, and Q. Wei, "Red and processed meat consumption and cancer outcomes: Umbrella review," *Food Chem.,* vol. 356, p. 129697, 2021, doi: https://doi.org/10.1016/j.foodchem.2021.129697.

[4] A. J. Tóth, A. Dunay, M. Battay, C. B. Illés, A. Bittsánszky, and M. Süth, "Microbial spoilage of plant-based meat analogues," *Appl Sci.,* vol. 11, no. 18, p. 8309, 2021, doi: https://doi.org/10.3390/app11188309.

[5] M. A. Baig, F. F. Ajayi, M. Hamdi, W. Baba, F. H. Brishti, N. Khalid, W. Zhou, and S. Maqsood, "Recent research advances in meat analogues: A comprehensive review on production, protein sources, quality attributes, analytical techniques used, and consumer perception," *Food Rev Int.*, vol. 41, pp. 236–267, 2025, doi: https://doi.org/10.1080/87559129.2024.2396855.

[6] K. Kyriakopoulou, B. Dekkers, and A. J. van der Goot, "Plant-based meat analogues," in C. M. Galanakis (Ed.), *Sustainable Meat Production and Processing.* Academic Press, 2019, pp. 103–126, doi: https://doi.org/10.1016/B978-0-12-814874-7.00006-7.

[7] N. Mollakhalili-Meybodi, M. Arab, and L. Zare, "Harmful compounds of soy milk: Characterization and reduction strategies," *J Food Sci Technol.*, vol.

59, pp. 3723–3732, 2022, doi: https://doi.org/10.1007/s13197-021-05249-4.

[8] R. Khapudang, and S. Siddiqui, "Physicochemical and structural characteristics of rice and mushroom protein-based meat analogue developed using steaming process," *ACS Food Sci. Technol.,* vol. 5, no. 11, pp. 4219–4230, 2025, doi: https://doi.org/10.1021/acsfoodscitech.5c00642.

[9] B. Prandi, I. M. Cigognini, A. Faccini, C. Zurlini, Ó. Rodríguez, and T. Tedeschi, "Comparative study of different protein extraction technologies applied on mushrooms by-products," *Food Bioproc Tech.,* vol. 16, no. 7, pp. 1570−1581, 2023, doi: https://doi.org/10.1007/s11947-023-03015-2.

[10] AOAC, *Official Methods of Analysis*, 22nd Edn. Association of Official Analytical Chemists, Washington D.C., U.S.A, 2023.

[11] S. T. Dinani, N. L. Broekema, R. Boom, and A. J. van der Goot, "Investigation potential of hydrocolloids in meat analogue preparation," *Food Hydrocoll*., vol. 135, p. 108199, 2023, doi: https://doi.org/10.1016/j.foodhyd.2022.108199.

[12] S. Ketnawa, and S. Rawdkuen, "Properties of texturized vegetable proteins from edible mushrooms by using single-screw extruder," *Foods,* vol. 12, p. 1269, 2023, doi: https://doi.org/10.3390/foods12061269.

[13] International Organization for Standardization (ISO) 4833-2:2013 Microbiology of the Food Chain—Horizontal Method for the Enu meration of Microorganisms—Part 2: Colony Count at 30 C by the Surface Plating Technique, 2013. Accessed: Jun. 20, 2025. [Online]. Available: https://www.iso.org/standard/59509.html

[14] S. Karanth, S. Feng, D. Patra, and A. K. Pradhan, "Linking microbial contamination to food spoilage and food waste: The role of smart packaging, spoilage risk assessments, and date labeling," *Front Microbiol*., vol. 14, p. 1198124, 2023, doi: https://doi.org/10.3389/fmicb.2023.1198124.

[15] L. Geng, K. Liu, and H. Zhang, "Lipid oxidation in foods and its implications on proteins," *Front Nutr*., vol. 10, p. 1192199, 2023, doi: https://doi.org/10.3389/fnut.2023.1192199.

[16] P. E. Keller, and R. Kouzes, "Water vapour permeation in plastics," Pacific Northwest National Laboratory, 2017. [Online]. Available: pnnl.gov/main/publications/external/technical_reports/PNNL-26070.pdf

[17] Y. Cho, J. Bae, J. Lee, and M. J. Choi, "Storage stability of meat analogs supplemented with vegetable oils," *Foods,* vol. 12, p. 3586, 2023, doi: https://doi.org/10.3390/foods12193586.

[18] D. Webb, H. Dogan, Y. Li, and S. Alavi, "Physico-chemical properties and texturization of pea, wheat and soy proteins using extrusion and their application in plant-based meat," *Foods*, vol. 12, p. 1586, 2023, doi: https://doi.org/10.3390/foods12081586.

[19] O. Miller, C. J. Scarlett, and T. O. Akanbi, "Lipid stability profiles of uncooked plant-based meat analogue burger patties and comparison of their textural and sensory properties with traditional beef burger patties," *J Food Compost Anal.,* p. 108002, 2025, doi: https://doi.org/10.1016/j.jfca.2025.108002.

[20] P. Yadav, S. S. Ahlawat, G. Jairath, M. Rani, and S. Bishnoi, "Studies on physico-chemical properties and shelf life of developed chicken meat analogue rolls," *Haryana Vet*., vol. 54, no. 1, pp. 25-28, 2015.

[21] Y. Yang, Y. Zheng, W. Ma, Y. Zhang, C. Sun, and Y. Fang, "Meat and plant-based meat analogs: Nutritional profile and in vitro digestion comparison," *Food Hydrocoll*., vol. 143, p. 108886, 2023, doi: https://doi.org/10.1016/j.foodhyd.2023.108886.

[22] L. Roman, M. Sahagun, M. Gomez, and M. M. Martinez, "Nutritional and physical characterization of sugar-snap cookies: Effect of banana starch in native and molten states," *Food Funct*., vol. 10, pp. 616–624, 2019, doi: https://doi.org/10.1039/C8FO02266F.

[23] R. Domínguez, M. Pateiro, P. E. S. Munekata, W. Zhang, and P. Garcia-Oliveira, M. Carpena, M. A. Prieto, B. Bohrer, and J. M. Lorenzo, "Protein oxidation in muscle foods: A comprehensive review," *Antioxidants,* vol. 11, p. 60, 2021, doi: https://doi.org/10.3390/antiox11010060.

[24] D. Kathuria, Hamid, S. Gautam, and A. Thakur, "Maillard reaction in different food products: Effect on product quality, human health and mitigation strategies," *Food Control.,* vol. 153, p. 109911, 2023, doi: https://doi.org/10.1016/j.foodcont.2023.109911.

[25] V. A. Tirado-Kulieva, W. R. Miranda-Zamora, E. Hernández-Martínez, L. R. Pantoja-Tirado, D. L. Bazán-Tantaleán, and E. W. Camacho-Orbegoso, "Effect of antifreeze proteins on the freeze-thaw cycle of foods: Fundamentals, mechanisms of action, current challenges and recommendations for future work," *Heliyon.,* vol. 8, p. e10973, 2022, doi: https://doi.org/10.1016/j.heliyon.2022.e10973.

[26] Md. M. Hasan, Md. R. Islam, A.R. Haque, Md. R. Kabir, K. J. Khushe, and S. M. K. Hasan, "Trends and

challenges of fruit by-products utilization: Insights into safety, sensory, and benefits of the use for the development of innovative healthy food: A review," *Bioresour Bioprocess*., vol. 11, p. 10, 2024, doi: https://doi.org/10.1186/s40643-023-00722-8.

[27] H. U. Asdullah, F. Chen, M. A. Hassan, A. Abbas, S. Sajad, M. Rafiq, M. A. Raza, A. Tahir, D. Wang, and Y. Chen, "Recent advances and role of melatonin in post-harvest quality preservation of shiitake (*Lentinula edodes*)," *Front Nutr*., vol. 11, p. 1348235, 2024, doi: https://doi.org/10.3389/fnut.2024.1348235.

[28] D. P. Maroma, "Meat analogue derived from common legumes," *OALib* 02, pp. 1–10, 2015, doi: https://doi.org/10.4236/oalib.1101249.

[29] Z. Liu, M. Shaposhnikov, S. Zhuang, T. Tu, H. Wang, and L. Wang, "Growth and survival of common spoilage and pathogenic bacteria in ground beef and plant-based meat analogues," *Food Res Int*., vol. 164, p. 112408, 2023, doi: https://doi.org/10.1016/j.foodres.2022.112408.

[30] Y. Sun, S. Liu, W. Qi, H. Wang, B. Lu, X. Zhu, and L. Jiang, "Analysis of the quality and flavor changes of soybean-based meat analogue during storage based on gas chromatography–mass spectrometry and electronic nose," *Food Chem: X*., vol. 29, p. 102795, 2025, doi: https://doi.org/10.1016/j.fochx.2025.102795.

[31] C. W. X. Neo, Y. H. How, I. Kong, R. A. Talib, and L. P. Pui, "Development of plant-based burger patties with pea protein isolate and barnyard millet flour and its storage stability in aerobic and vacuum packaging," *J Food Saf*., vol. 44, p. e13134, 2024, doi: https://doi.org/10.1111/jfs.13134.

[32] P. Kumar, B. D. Sharma, and R. R. Kumar, "Storage stability of analogue meat nuggets under refrigeration (4±1° C)," *JVPH*, vol. 8, no. 2, pp. 117–120, 2010.

Supplementary Table: The Changes in Proximate Composition (% DW Basis), Physical and Functional Properties of Steamed PBMA During Storage at Room Temperature Under Vacuum Packed Conditions

Storage Period (Days)	*Carbohydrate (%)*	*Protein (%)*	*Fat (%)*	*Ash (%)*	*Moisture (%)*
0	38.69±0.27[a]	44.58±0.18[a]	5.73±0.14[a]	5.88±0.10[a]	4.79±0.19[a]
7	40.43±0.29[b]	43.68±0.15[b]	5.61±0.07[a]	5.66±0.10[a]	4.47±0.19[a]
	Colour and BI				
	*L**	*a**	*b**	*BI*	
0	45.23±0.25[a]	8.18±0.14[a]	19.21±0.21[a]	16.98±0.19[a]	
7	43.98±0.14[b]	8.91±0.16[a]	19.95±0.13[a]	18.72±0.12[a]	
	WHC (g H_2O/g Sample)	*OHC (g oil/g Sample)*	*Changes in Thickness (%)*	*Total Plate Count (log_{10}CFU/g)*	
0	3.85±0.07	2.31±0.15	0	0	
7	3.64±0.10	2.09±0.03	0.13±0.001	4.91±0.02	
	Sensory Evaluation (9-Point Hedonic Scale)				
	Appearance	*Texture*	*Flavour*	*Mouthfeel*	*Overall Acceptability*
0	7.6 ± 0.2[a]	7.5 ± 0.2[a]	7.7 ± 0.2[a]	7.5 ± 0.2[a]	7.6 ± 0.2a
7	6.1 ± 0.4[b]	5.8 ± 0.4[b]	5.5 ± 0.4[b]	5.3 ± 0.4[b]	5.6 ± 0.4[b]

The values are mean ± SD (n=3). The means for a parameter within a column with different superscript differ significantly (p≤0.05), and those without superscripts are not significantly different.