Research Article

Evaluation of Packaging Materials for Extending the Shelf-Life of a Dietary Fibre-Enriched Functional Pizza Base

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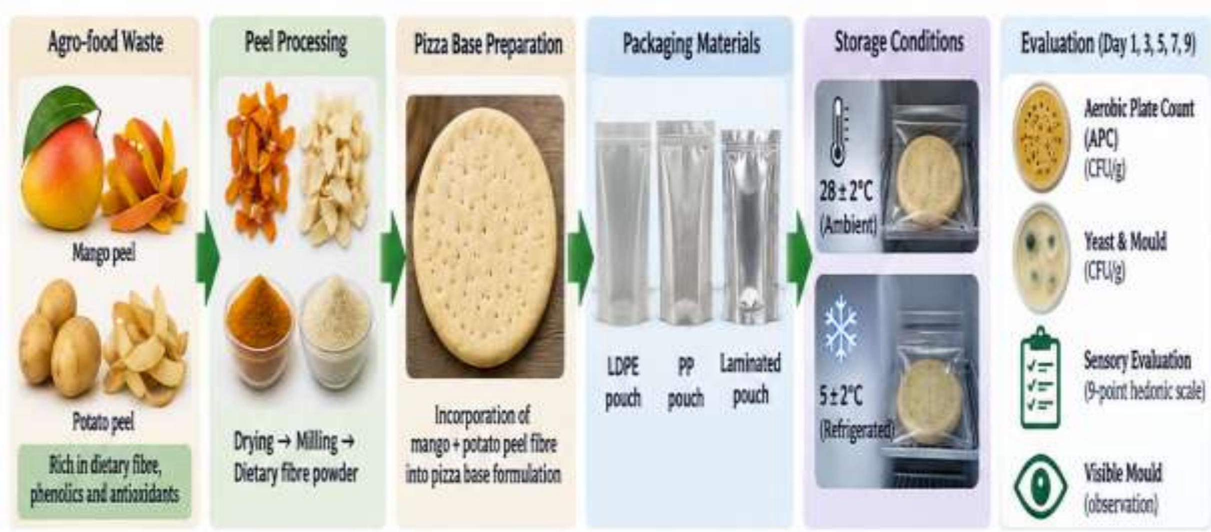
Abstract

Food-processing by-products such as fruits and vegetables peels are potential sources of dietary fibre and other functional constituents, but their incorporation into bakery products requires evaluation of storage stability and packaging performance. The microbiological, sensory, and practical shelf-life stability of a developed functional pizza base enhanced with fruits and vegetables peel fiber were analyzed in this study in relation to packaging material and storage temperature. Three food-grade packaging systems low-density polyethylene (LDPE), polypropylene (PP), and laminated pouches were used to individually package freshly baked pizza bases. On Days 1, 3, 5, 7, and 9, samples were determined in both ambient (28 ± 2 °C) and refrigerated (5 ± 2 °C) conditions. A nine-point hedonic scale was used to evaluate appearance, colour, odour, texture, and overall acceptability, while aerobic plate counts and yeast and mould counts were employed to track microbiological stability. With the goal to evaluate economic considerations, the cost of packaging materials was compared, and visible deterioration was noted as an additional shelf-life indicator. According to the analysis framework, microbiological and sensory stability were significantly by storage temperature and time, with refrigerated storage often offering superior quality retention compared to ambient storage. The findings indicate that refrigerated storage was associated with slower microbiological change and better retention of sensory acceptability over the evaluated storage interval.

Keywords: Functional pizza base, dietary fibre, shelf life, microbiological stability, sensory acceptability.

Graphical Abstract



1. Introduction

The growing generation of food-processing by-products from fruit and vegetable industries has led to both environmental and resource-utilization challenges. Even though they contain significant amounts of dietary fiber, phenolic compounds, minerals, pigments, and other bioactive components, peels, skins, seeds, and other processing wastes are frequently thrown away or used as low-value materials. Therefore, turning them into useful food

ingredients is seen as a promising strategy for resource recovery, food waste valorization, and the creation of more sustainable food products. Specifically, turning residues into stable powders or fiber-rich components can make it easier to incorporate them into foods with added value while lowering the demand on traditional sources of functional ingredients (Sagar et al., 2018). Millet is a csereal containing carbohydrate, protein, dietary fibre, minerals and phytochemicals and has been incorporated into a range of food products (Arora et al., 2023). This research has evaluated the nutritional and sensory attributes of -millet-based foods, supporting its relevance as an ingredient for cereal-based product development (Sharma & Niranjan, 2017).
Fruits peels is an essential fruit-processing by-product with substantial potential for food applications because of its dietary fibre and phytochemical content. Fruits peel contains significant quantities of dietary fiber, with total dietary fiber contents ranging from approximately 40.6 to 72.5%, depending on cultivar and maturity, according to earlier research. Additionally, fruits peel includes flavonoids like quercetin and kaempferol as well as phenolic acids including gallic, protocatechuic, and syringic acids. (Ajila & Rao, 2013) reported total dietary fibre in fruits peel in the range of 40.6-72.5% across the materials studied and identified bound phenolic acids and flavonoids. These findings support the investigation of fruits peel as a food ingredient rather than solely as a processing residue. Vegetables peels is likewise reported to contain dietary fibre and phenolic compounds, although composition varies with cultivar and processing (Sampaio et al., 2020). These qualities encourage using the peel as a functional element instead of just treating it as processing waste. An underutilized vegetable processing by-product that contains phenolic compounds, dietary fiber, and other potentially beneficial bioactive components. Vegetables peel's nutritional and technological benefits have been emphasized for incorporation into different functional food products.

Bakery products provide a practical matrix for incorporating plant-derived fibre ingredients, but fibre addition can affect dough structure, moisture distribution, texture, colour and sensory characteristics. (Sagar & Pareek, 2020) demonstrated that incorporation of onion-skin powder into pizza bases affected physicochemical, textural and sensory characteristics and that storage conditions were relevant to product stability. The implementation of fruit and vegetable by-product powders into bakery products is highly attractive because bakery formulations are capable of supporting fibre-rich plant ingredients and provide a variety of food usage. However, a developed product's technology suitability and storage durability cannot be determined just by nutritional enrichment. Water distribution, dough properties, texture, color, flavor, and customer acceptability can all be changed by plant-derived fiber and powder ingredients (Lin, 2022). During storage, these compositional and moisture-related transformations have an impact on microbial development and quality degradation. As a result, when functional bakery goods were developed, their microbiological and sensory stability under appropriate storage settings were systematically tested.

Pizza base provides a suitable bakery matrix for this purpose because it can be prepared as a baked, ready-to-use or subsequently prepared food component and may require storage before final consumption. Previous research has demonstrated the successful incorporation of plant-derived functional ingredients into pizza-base formulations while maintaining acceptable sensory characteristics. For example, (Sagar & Pareek, 2020) evaluated pizza bases enriched with onion skin powder and reported changes in physicochemical, antioxidant, textural, sensory, and storage characteristics. Their study also demonstrated that storage conditions substantially influence the microbial and quality stability of pizza-base products, providing a relevant basis for further investigation of storage behaviour in functional pizza bases.

The temperature at which baked goods are stored is one of the main factors affecting their stability. Throughout storage, microbial development may coexist with slow changes in texture, colour, odour, appearance, and overall acceptability (Cauvain, 2016). Fungal growth is particularly important in baked foods because visible mould formation is a major practical spoiling endpoint. In a previous study on pizza bases, storage at 28 ± 2 °C resulted in progressive microbiological development and evident fungal degradation after around seven days, but storage at 5 ± 2 °C delayed both microbial and visual rotting. These findings imply that refrigerated storage might provide superior quality retention than ambient storage, depending on the product formulation and storage method (Rahaie et al., 2012).

The storage stability of baked goods is significantly influenced by packaging in addition to temperature. Packaging can affect moisture transfer, oxygen availability, microbial development, and product deterioration by limiting exposure to environmental pollutants. Studies on prebaked pizza dough have shown that mould development can be significantly impacted by packing circumstances (Noshirvani & Fakhri, 2024). According to (Rodríguez et al., 2003) apparent mould growth in preservative-free pizza dough was delayed by a modified environment rich in carbon dioxide, but mould formation happened earlier under air packing. These results highlight the significance of the packing environment in regulating the storage behavior of pizza-type baking products, even though modified-atmosphere systems differ from traditional polymeric pouches. Therefore, while developing a useful storage system for a functional pizza base, the choice of packaging material is essential. Polymers like LDPE and PP are frequently used in food packaging, although laminated structures can have several layers with various barrier and protective properties. Variations in packaging design can affect microbial growth, product contamination, oxygen transmission, and moisture migration. However, because a packaging material's efficacy depends on the features of the particular food matrix, storage temperature, storage duration, sealing conditions, and handling procedures, its performance cannot be determined only by its theoretical barrier capabilities. To ascertain the true performance of various packaging techniques, a comparative analysis under regulated storage conditions is thus required (Dong & Karboune, 2021).

Microbiological analysis provides an objective measure of changes occurring during storage. As a broad measure of viable aerobic microbial populations, the Aerobic Plate Count (APC), also known as the total aerobic mesophilic plate count, can reveal changes related to handling, processing, storage, and sanitary quality. However, APC should be viewed as an indicator of overall microbial quality rather than a pathogen-specific safety test since it cannot identify specific pathogens or individual microbial species (Qian et al., 2021). Because fungal growth is especially relevant to the deterioration of bakery items, counting yeast and mould yields supplementary information. Simultaneously, consumer-perceived quality cannot be adequately described by microbiological tests alone, including sensory examination. Even when microbiological deterioration is not immediately visible, changes in appearance, color, odor, texture, and general acceptability may affect a product's usability. On the other hand, microbiological evaluation cannot be replaced by sensory acceptability. Therefore, a more thorough evaluation of storage stability can be obtained by combining microbiological and sensory measures (Liu et al., 2022).

As a result, instead of depending just on one numerical metric, shelf-life determination taken into consideration additional factors. Changes in APC, yeast and mould counts, sensory quality, and obvious spoiling were taken into account for the functional pizza base under investigation in this study. Comparing packing systems at various temperatures is also made possible by evaluation at specified storage durations. Specifically, an accurate shelf-life duration between two sampling, shelf-life endpoint corresponds to the latest storage interval at which the product remains acceptable according to the predetermined criteria (Taglieri et al., 2020). There is information on the effectiveness of traditional LDPE, PP, and laminated packaging systems for functional pizza bases enhanced with fruits and vegetables peel fiber, despite earlier research examining the use of plant-derived by-products in pizza and bakery products as well as the effects of storage temperature and modified-atmosphere packaging. Specifically, there is comparative data on these packaging materials in both ambient and refrigerated storage conditions, taking into account microbiological stability, sensory quality, visual spoiling, and package cost (Mishra, 2014).

Therefore, the present study was undertaken to evaluate the effects of packaging material and storage temperature on the storage stability and quality of a developed functional pizza base enriched with fruits and vegetables peel fibre. Three food-grade packaging systems LDPE pouch, PP pouch, and laminated pouch were evaluated under ambient (28 ± 2 °C) and refrigerated (5 ± 2 °C) storage conditions on Days 1, 3, 5, 7, and 9. Sensory stability was evaluated using a nine-point hedonic scale based on appearance, colour, odour, texture, and overall acceptability, while microbiological stability was assessed through aerobic plate count (APC) and yeast and mould enumeration. To provide an integrated assessment of storage performance and practical packaging considerations, visible deterioration and packaging-material costs were also evaluated (San et al., 2022).

2. Materials and Methods

2.1 Developed Functional Pizza Base

Fruits and vegetable peel fibers were included into a designed and standardized formulation to develop the baked functional pizza base used in this study. For the study to be repeatable, the precise formulation, fiber preparation process, particle size, ingredient quantities, dough preparation conditions, baking temperature/time, product dimensions, and batch size was reported. Before being packaged, freshly baked pizza bases were allowed to cool to room temperature. For the storage experiment, only items that showed consistent look and good physical quality right after baking were chosen. The samples were handled under hygienic conditions to minimize extraneous contamination before packaging.

2.2 Packaging Materials

Three food-grade polymeric packaging systems were selected for comparative evaluation:

P1: Low-density polyethylene (LDPE) pouch
P2: Polypropylene (PP) pouch
P3: Food-grade Laminated pouch

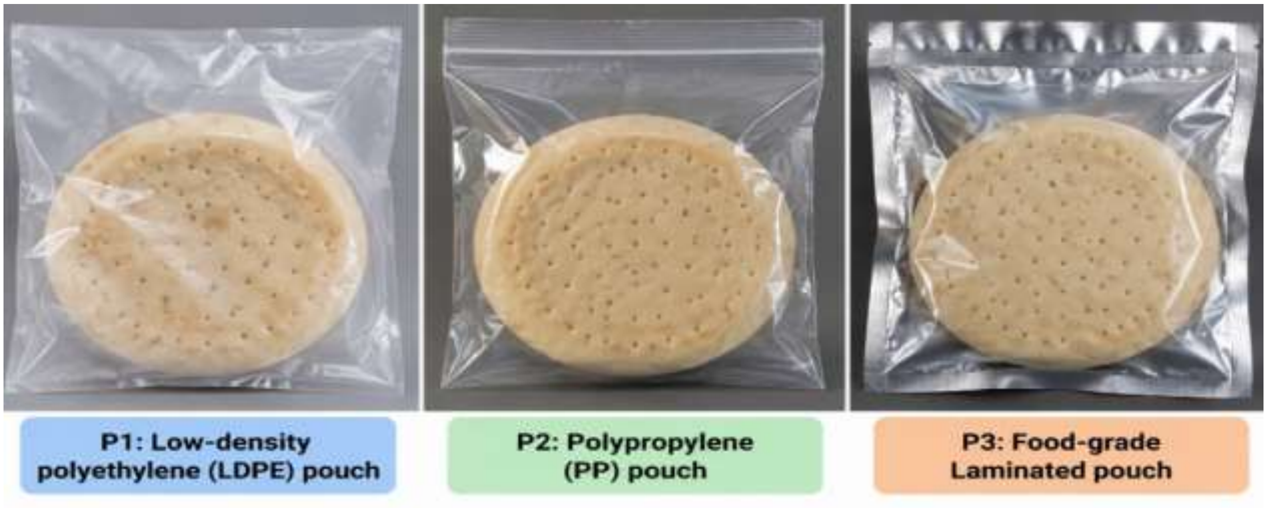


Figure 1. Packaging Materials

The packaging products were chosen because laminated structures can offer a combination of barrier qualities through the incorporation of different layers, whereas LDPE and PP are frequently used polymeric materials for food packing. Previous studies on baking and pizza dough have shown that storage stability and microbiological development can be significantly impacted by packaging conditions. The important role of packaging in determining the storage behaviour of pizza-type bakery products is demonstrated by the observation that modified-atmosphere packaging has been proven to inhibit the growth of mould in prebaked pizza dough. The type of material determines the packing procedures. For reproducibility, each packaging system's supplier/manufacturer, food-contact grade, film/pouch thickness, dimensions, laminate structure, and sealing process need to be specified. Water-vapor and oxygen transport rates should also be given when available. Instead of assuming that these features are the same for all vendors, they should be seen as descriptive attributes of the evaluated packages.

2.3 Packaging and Sample Preparation

Freshly baked functional pizza bases were first cooled to room temperature. Cooling before packaging was carried out to avoid packaging the product while it was still hot and to minimize the accumulation of condensation within the package. Each pizza base was placed individually into the appropriate packaging systems once it had cooled. Every combination of packaging, temperature, and storage period required the preparation of separate packages. The number of separate pizza bases or batches for each treatment, the method of sample selection or randomization, and whether samples were systematically sampled at each time point or continuously monitored over time must all be clearly specified. Before performing a statistical analysis, the experimental unit needs to be specified. The packaged samples were placed in their appropriate storage conditions after being recognized based on the packaging technique and storage condition. Throughout the experiment, similar handling conditions were maintained for the packing systems.

2.4 Storage Conditions and Experimental Design

The packaged functional pizza bases were stored under two temperature conditions:

- ✓ Ambient storage: 28 ± 2°C
- ✓ Refrigerated storage: 5 ± 2°C

Three packaging systems were evaluated under each storage condition, resulting in six packaging-temperature combinations:

- ✓ LDPE × ambient
- ✓ PP × ambient
- ✓ Laminated pouch × ambient
- ✓ LDPE × refrigerated
- ✓ PP × refrigerated
- ✓ Laminated pouch × refrigerated

Evaluations of the microbiology and senses were planned for Days 1, 3, 5, 7, and 9. These intervals determine a specific shelf-life period between two sampling days, they basically specify the observed storage positions. The data supports a final observed acceptable storage interval of Day 5 rather than an exact five-to seven-day shelf-life estimate if the next observation is undesirable on Day 7 and the last acceptable observation is on Day 5. Every planned storage time included an examination of the samples. Any treatment that exhibits obvious fungal growth or another predetermined critical spoiling endpoint needs to be noted as inappropriate going forward. When determining whether a treatment is acceptable or unsuitable, the decision rule needs to be applied uniformly to all treatments and declared prospectively.

Table 1. Experimental design for the packaging and storage study

Packaging	Temperature	Storage days	Outcomes
LDPE	Ambient (28 ± 2 °C)	1, 3, 5, 7, 9	APC; yeast & mould; sensory; visible spoilage
LDPE	Refrigerated (5 ± 2 °C)	1, 3, 5, 7, 9	APC; yeast & mould; sensory; visible spoilage
PP	Ambient (28 ± 2 °C)	1, 3, 5, 7, 9	APC; yeast & mould; sensory; visible spoilage
PP	Refrigerated (5 ± 2 °C)	1, 3, 5, 7, 9	APC; yeast & mould; sensory; visible spoilage
Laminated pouch	Ambient (28 ± 2 °C)	1, 3, 5, 7, 9	APC; yeast & mould; sensory; visible spoilage
Laminated pouch	Refrigerated (5 ± 2 °C)	1, 3, 5, 7, 9	APC; yeast & mould; sensory; visible spoilage

Note: *n* denotes the number of independent samples analyzed for each packaging × temperature × storage-day condition. Technical replicates and individual sensory ratings were not considered independent replicates. The final *n* should be confirmed from the original laboratory records.

2.5 Aerobic Plate Count

Aerobic Plate Count (APC), also referred to as the total aerobic mesophilic plate count, was used as an overall indicator of viable aerobic microbial populations during storage. According to FSSAI, APC is a broad microbiological indicator that may be used to evaluate production and handling procedures, processing conditions, changes related to product shelf life, and sanitary quality. The technique was perceived as a measure of overall aerobic microbial quality rather than as a pathogen-specific test because it does not distinguish between different types of bacteria.

A specific mass of each independently sampled pizza base aseptically collected and homogenized in a designated sterile diluent using a specified sample-to-diluent ratio and homogenization technique in order to perform microbiological analysis. The culture medium, plating volume, number of replicate plates, incubation conditions, countable range, and calculation method must be documented, and decimal serial dilutions must be prepared in accordance with the validated method. The specific standard or verified procedure for Aerobic Plate Count, along with the culture medium and other pertinent incubation and enumeration conditions, should be specified. The final data reported in CFU/g and log10-transformed counts where necessary for statistical analysis. The variations occurred, a broad reference to a standard procedure is insufficient, the exact method ly employed needs to be specified. For enumeration, only plates that satisfied the relevant countability conditions were taken into account. During sample preparation, dilution, inoculation, incubation, and colony counting, the proper aseptic procedures were performed.

2.6 Yeast and Mould Count

Yeast and mould analysis was performed to monitor fungal development during storage, which is particularly relevant to the shelf-life of baked products. The analysis was conducted in accordance with IS 5403:1999, Method for Yeast and Mould Count of Foodstuffs and Animal Feeds. A colony-count method for counting viable yeasts and moulds in goods meant for human consumption and animal feed is specified in the standard. From each independently sampled storage treatment, a representative sample was aseptically collected and homogenized in a specified sterile solvent. In accordance with the verified yeast and mould counting method, decimal serial dilutions were performed. The medium, dilution scheme, plating technique, temperature and duration of incubation, number of replicate plates, countability criteria, and calculating method all have to be specified. Visible fungal growth must be recorded separately from numerical colony counts.

The mould and yeast count were given in CFU/g. When relevant, the counts were also reported as $\log_{1}^{1}$ CFU/g for statistical analysis and comparison of microbial changes among storage treatments. The application of IS 5403:1999 offered a consistent analytical foundation for contrasting the growth of fungi in various packaging materials and storage conditions.

Fungal growth on the products or packaging was noted as an essential practical spoiling finding. The impacted treatment was deemed to have reached the end of its allowed storage duration when evident fungal spoilage was seen, subject to interpretation together with the microbiological and sensory data.

2.7 Sensory Evaluation

A nine-point hedonic scale was used to assess the functional pizza base's sensory stability. A well-known method for determining consumer acceptability and overall satisfaction of food products is the nine-point hedonic scale. Its appropriateness for differentiating variations in food acceptability ratings has been shown by earlier studies. The original text reported on a panel of twenty evaluators. In addition, the final manuscript must specify whether the panel was trained or untrained, recruitment/inclusion criteria, pertinent panel characteristics, whether the same panellist assessed more than one storage treatment, and how many independent product samples each sensory score represented.

- ✓ Appearance
- ✓ Colour
- ✓ Odour
- ✓ Texture
- ✓ Overall acceptability

The nine-point rating system was stabilized from 1 (strongly dislike) to 9 (strongly like), with intermediate numbers denoting gradually higher levels of liking. In order to determine blindness and uniformity, a detailed description of the sensory evaluation is required. Sample coding, serving order, serving conditions, amount presented, palate-cleansing technique, evaluation environment, and whether panellists were exposed to samples from several treatments or storage intervals during a single session should all be included in the final publication. Informed consent and ethical approval or exemption must be recorded in accordance with the specifics of the investigation. The retention or decline of consumer-perceived quality during storage was evaluated using the sensory scores. When calculating the sensory aspect of shelf-life, overall acceptability was given special consideration. Sensory acceptability was seen as a measure of consumer-perceived product quality rather than as an alternative for microbiological safety.

2.8 Shelf-Life Determination

Microbiological stability, sensory acceptability, and visual deterioration observations during storage were included to determine shelf life. Only when the product preserved acceptable sensory quality and did not show unacceptable microbiological or apparent fungal deterioration was it considered appropriate for each packaging-temperature combination. The indicators listed below were taken into assessment:

- ✓ Changes in Aerobic Plate Count
- ✓ Changes in yeast and mould count
- ✓ Appearance and visible spoilage
- ✓ Colour
- ✓ Odour
- ✓ Texture
- ✓ Overall acceptability

A significant spoiling endpoint was identified as the appearance of visible fungal growth. When a packaging-temperature treatment exhibited unsatisfactory sensory deterioration or visible fungal decomposition, it was deemed to have reached the end of its allowed storage time, and additional storage evaluation of that treatment was stopped. Instead of determining shelf life based only on an arbitrary numerical fungal limit, microbiological results were interpreted together with the product's storage behaviour and sensory observations because no universal yeast and mould limit specific to the analyzed pizza-base product was assumed. This method is in line with the idea that microbiological standards should be suitable for the particular food and evaluation goal. The microbiological framework of FSSAI makes a distinction between particular food-safety standards and microbiological indicators. The final shelf life of each packaging and temperature combination was therefore expressed as the last storage interval at which the product remained acceptable according to the predefined microbiological, sensory, and visible-spoilage criteria.

2.9 Packaging Cost Analysis

The economical element as a packaging and cost comparison unless a broader economic feasibility analysis is ly performed. In addition to the costing date, supplier/source, buy unit, package yield, and other calculating criteria, the recorded purchase price and the quantity of packing material required for each pizza base must be included in the calculation. Ingredient, labor, energy, storage, overhead, and other costs that weren't assessed shouldn't be automatically included. The following items' packaging expenses were estimated independently:

- ✓ LDPE pouch
- ✓ PP pouch
- ✓ Laminated pouch

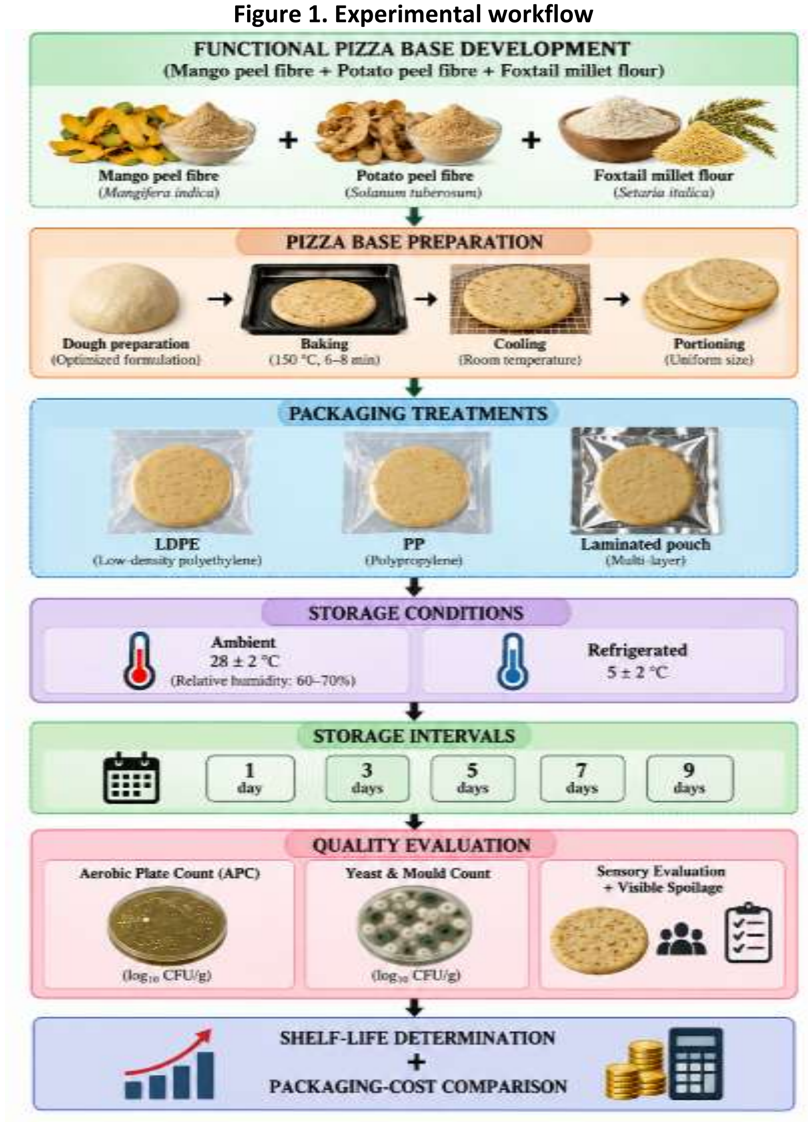
The value or unit need of the essential packaging material and its purchase price were used to determine the cost of the packaging material needed for one pizza base. To enable direct comparison of treatments, the cost of the packing unit was converted to a common base when feasible. Next, storage performance and the comparative packing cost were taken into account. The lowest unit cost alone did not qualify a packing solution as economically superior. Rather, the cost was evaluated on terms of its capacity to preserve sensory and microbiological quality while being stored. This made it possible for the study to determine a packaging solution that offered a suitable trade-off between storage efficiency and packaging costs.

2.10 Statistical Analysis

The experimental data were organized according to three principal factors- packaging material, storage temperature, and storage period**.** Packaging material comprised three levels (LDPE, PP, and laminated pouch), storage temperature comprised two levels (28 ± 2°C and 5 ± 2°C), and storage period comprised five levels (Days 1, 3, 5, 7, and 9). The statistical framework selected from the experimental configuration. A factorial model that includes packing material, storage temperature, and storage duration along with their interactions would be suitable if individual samples were taken at each storage time. A repeated-measures or mixed-effects technique could be necessary if the same experimental units or the same sensory panellists were measured more than once. The experimental unit, independent replication number (n), fixed and random effects, assumption evaluations, transformations, post-hoc analyzing process, and multiple-comparison approach must all be included.

Provide the statistical method that ly was utilized after the experimental dataset has been produced. The ANOVA model must examine all primary effects and interactions that are significant to science and supported by the design. It is essential to analyze sensory evaluations using the repeated-observation approach. When many treatments or time points are assessed by the same panellists, panellist effects and within-panellist correlation must be considered. In the end analysis, repeated evaluations from the same individual shouldn't be considered distinct biological replicates. Reporting the results should include the mean ± SD and the number of independent replicates. The test statistic, degrees of freedom, and p-value, as well as any relevant post-hoc comparisons and effect sizes or confidence ranges, should be included in inferential results. Independent experimental replicates cannot be regarded as technical replicates. The experimental dataset must serve as the groundwork for the final statistical analysis. Effect sizes, means, standard deviations, significance labels, and numerical p-values should be entered from data and the recorded analysis.

Figure 1. Experimental workflow



3. Results

3.1 Changes in Aerobic Plate Count

APC increased progressively with storage duration in all packaging systems, while the increase was slower under refrigeration. On Day 1, APC values ranged from 1.50×10^2 to 2.00×10^2 CFU/g under ambient storage and from 1.00

× 10¹ to 1.10 × 10¹ CFU/g under refrigeration. By Day 9, ambient APC reached 1.10 × 10^6 CFU/g in LDPE, 7.40 × 10^5 CFU/g in PP, and 3.90 × 10^5 CFU/g in the laminated pouch. Under refrigeration, corresponding values were 5.10 × 10^4, 3.80 × 10^4, and 2.00 × 10^4 CFU/g, respectively.

The laminated pouch showed lower APC values at several later storage points in both temperature conditions. At Day 7, APC under ambient storage was 3.20 × 10^5 CFU/g in LDPE, 2.40 × 10^5 CFU/g in PP, and 1.20 × 10^5 CFU/g in the laminated pouch. Under refrigeration, the corresponding values were 1.20 × 10^3, 9.00 × 10^2, and 6.20 × 10^2 CFU/g. These differences are descriptive; the specific pairwise differences should be interpreted from the verified post-hoc analysis rather than from the numerical ordering alone.

Factorial ANOVA indicated significant effects of packaging, storage temperature, storage day, and their interaction terms on log10 APC ($p < 0.05$). The very large F-value associated with temperature is consistent with a strong temperature-related effect in the current analysis. However, the F-values and p-values must be independently recalculated from the original replicate-level data and the final statistical model before submission.

Table 2. Aerobic plate count of the functional pizza base during storage. Values are expressed as CFU/g (mean ± SD; n = 3).

Packaging material	Storage temperature	Day 1	Day 3	Day 5	Day 7	Day 9
LDPE	Ambient (28 ± 2 °C)	200 ± 25	15,000 ± 1,300	50,000 ± 3,100	320,000 ± 23,000	1,100,000 ± 110,000
LDPE	Refrigerated (5 ± 2 °C)	10 ± 1.2	11 ± 0.57	140 ± 6.4	1,200 ± 30	51,000 ± 3,600
PP	Ambient (28 ± 2 °C)	190 ± 5.7	13,000 ± 470	38,000 ± 5,000	240,000 ± 7,200	740,000 ± 120,000
PP	Refrigerated (5 ± 2 °C)	11 ± 0.84	11 ± 1.1	110 ± 9.8	900 ± 54	38,000 ± 3,200
Laminated pouch	Ambient (28 ± 2 °C)	150 ± 15	8,300 ± 1,500	26,000 ± 2,100	120,000 ± 7,000	390,000 ± 24,000
Laminated pouch	Refrigerated (5 ± 2 °C)	10 ± 1.0	11 ± 0.84	70 ± 5.3	620 ± 18	20,000 ± 1,100

Note: Values are expressed as CFU/g (mean ± SD). APC indicates changes in microbial load during storage.

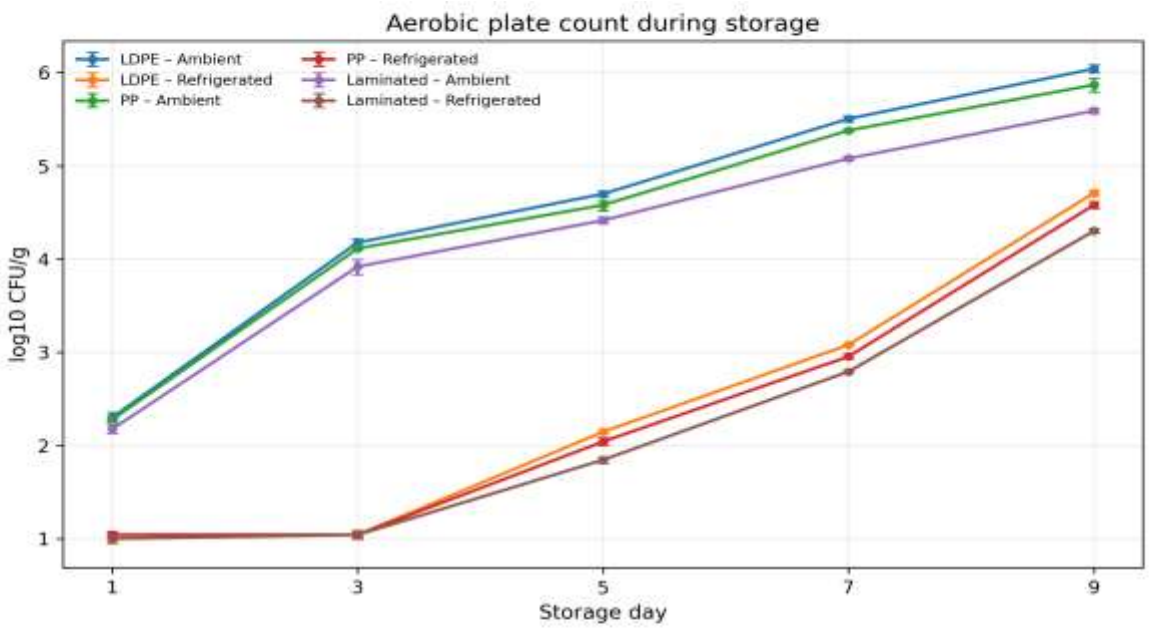


Figure 2. Changes in aerobic plate count during storage.

3.2 Changes in Yeast and Mould Count

Yeast and mould counts followed the same general pattern as APC, with progressive increases during storage and slower development under refrigeration. Ambient counts increased from approximately 9.7–11 CFU/g on Day 1 to 7.50×10^5–2.10×10^6 CFU/g on Day 9. The highest Day-9 value occurred in LDPE under ambient storage, whereas the laminated pouch recorded the lowest ambient value.

Refrigerated storage delayed the increase in yeast and mould counts. On Day 5, counts were 1.90×10^3 CFU/g in LDPE, 1.40×10^3 CFU/g in PP, and 9.50×10^2 CFU/g in the laminated pouch. By Day 9, the refrigerated values were 3.00×10^5, 3.10×10^5, and 1.60×10^5 CFU/g, respectively. Thus, the laminated pouch had the lowest yeast and mould count at the later storage point reported here. The observed pattern should be interpreted in conjunction with the visible-mould record and the verified statistical comparisons.

The factorial analysis indicated effects of packaging, temperature, storage day, and their interactions on log10 yeast and mould count ($p < 0.05$). The temperature × day interaction indicates that the magnitude of fungal-count change differed across storage conditions. These inferential results require confirmation from the original replicate-level data and model assumptions before submission.

Table 3. Yeast and mould count of the functional pizza base during storage. Values are expressed as CFU/g (mean ± SD; n = 3).

Packaging material	Storage temperature	Day 1	Day 3	Day 5	Day 7	Day 9
LDPE	Ambient (28 ± 2 °C)	11 ± 0.43	23,000 ± 1,900	110,000 ± 6,500	480,000 ± 32,000	2,100,000 ± 160,000
LDPE	Refrigerated (5 ± 2 °C)	10 ± 2.1	9.6 ± 1.1	1,900 ± 320	11,000 ± 470	300,000 ± 53,000
PP	Ambient (28 ± 2 °C)	10 ± 0.92	21,000 ± 1,600	84,000 ± 7,700	370,000 ± 12,000	1,500,000 ± 160,000
PP	Refrigerated (5 ± 2 °C)	11 ± 1.0	11 ± 0.68	1,400 ± 220	9,700 ± 570	310,000 ± 36,000
Laminated pouch	Ambient (28 ± 2 °C)	9.7 ± 0.75	13,000 ± 640	48,000 ± 4,500	280,000 ± 15,000	750,000 ± 85,000
Laminated pouch	Refrigerated (5 ± 2 °C)	9.3 ± 0.81	10 ± 0.41	950 ± 150	7,100 ± 790	160,000 ± 21,000

Note: Values are expressed as CFU/g (mean ± SD). APC indicates changes in microbial load during storage.

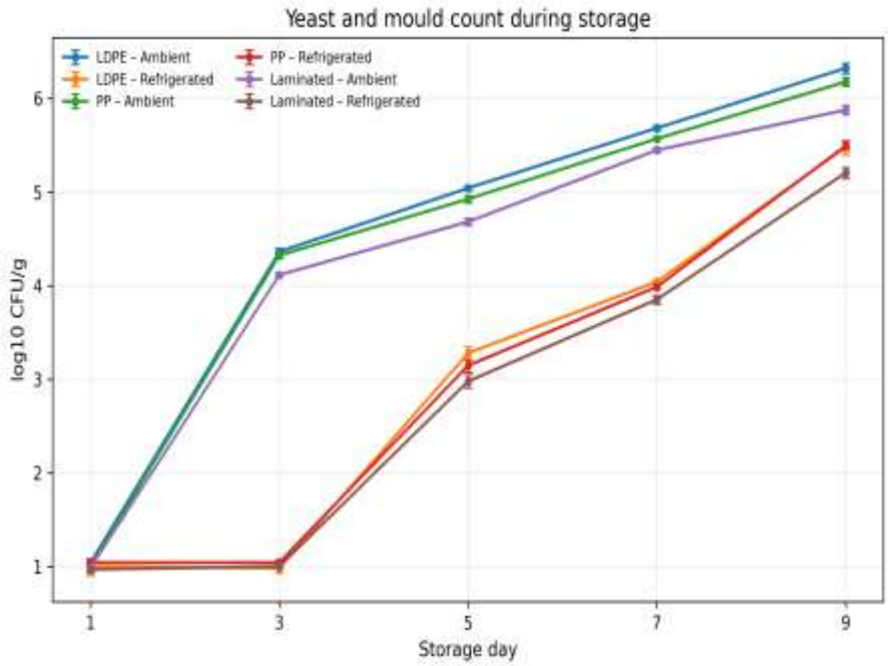


Figure 3. Changes in yeast and mould count during storage.

3.3 Sensory Stability

Overall acceptability declined with storage duration in all treatments, with a slower decline under refrigeration. Under ambient storage, overall acceptability decreased from 8.01-8.41 on Day 1 to 3.25-4.28 on Day 9. The laminated pouch retained the highest ambient Day-9 score (4.28 ± 0.36), followed by PP (3.58 ± 0.33) and LDPE (3.25 ± 0.37). Refrigerated samples retained higher sensory scores at later storage points. On Day 9, overall acceptability was 6.17 ± 0.37 for LDPE, 6.43 ± 0.38 for PP, and 6.94 ± 0.38 for the laminated pouch. The higher sensory scores under refrigeration occurred alongside lower microbial counts. This association should not be interpreted as proof that microbial counts alone caused sensory deterioration; formulation, moisture, texture and other quality changes may also contribute. The factorial analysis indicated effects of packaging, temperature, and storage day, including interaction effects, on overall sensory acceptability ($p < 0.05$). These results suggest that the trajectory of sensory change depended on storage conditions. Because sensory evaluation may involve repeated observations from the same panellist, the appropriateness of the factorial ANOVA must be confirmed against the panel design before publication.

Table 4. Overall sensory acceptability of the functional pizza base during storage. Values are on a nine-point hedonic scale (mean ± SD; n = 3).

Packaging	Temperature	Day 1	Day 3	Day 5	Day 7	Day 9
LDPE	Ambient	8.01 ± 0.35	7.35 ± 0.29	6.09 ± 0.3	4.45 ± 0.38	3.25 ± 0.37
LDPE	Refrigerated	8.07 ± 0.21	7.98 ± 0.37	7.57 ± 0.3	7.16 ± 0.3	6.17 ± 0.37
Laminated	Ambient	8.41 ± 0.28	7.83 ± 0.39	6.75 ± 0.25	5.69 ± 0.32	4.28 ± 0.36
Laminated	Refrigerated	8.33 ± 0.39	8.2 ± 0.3	8.07 ± 0.31	7.58 ± 0.34	6.94 ± 0.38
PP	Ambient	8.17 ± 0.4	7.44 ± 0.45	6.12 ± 0.25	4.7 ± 0.33	3.58 ± 0.33
PP	Refrigerated	8.15 ± 0.34	8.14 ± 0.29	7.84 ± 0.37	7.3 ± 0.33	6.43 ± 0.38

Note: Values are expressed as mean ± SD on a 9-point hedonic scale; higher scores indicate greater overall acceptability.

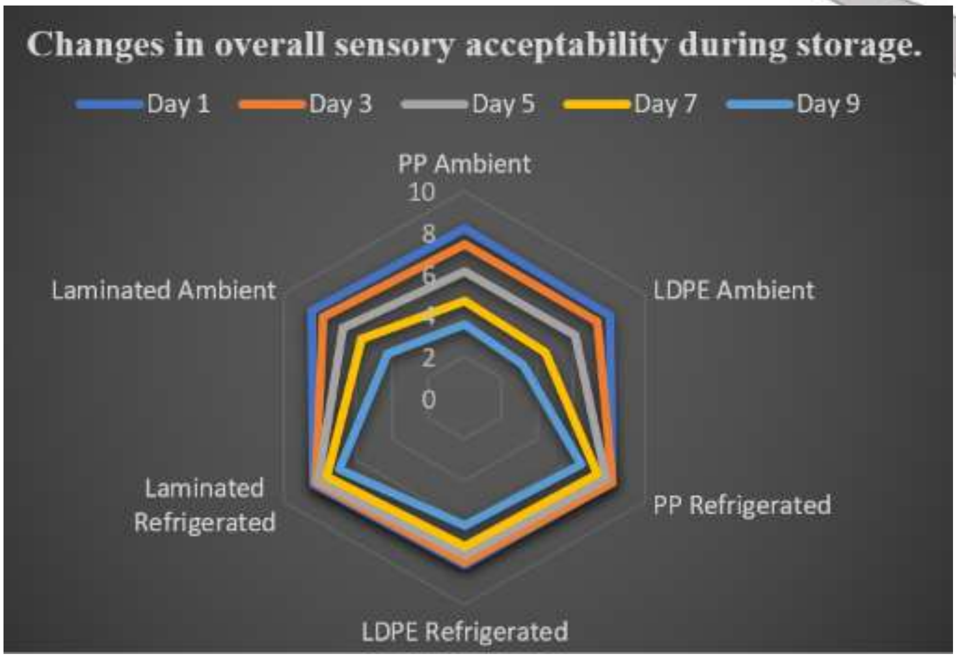


Figure 4. Overall Acceptability During Storage

3.4 Comparative Effect of Packaging and Temperature

Across the study, storage temperature showed a clear association with quality stability. Refrigeration was associated with slower accumulation of aerobic microorganisms and fungi and slower decline in sensory acceptability. Packaging differences were also observed, particularly at later storage points. The laminated pouch generally showed lower microbial counts and higher sensory scores at later sampling points, while LDPE showed greater deterioration under ambient storage. These observations apply only to the specific packaging systems and storage conditions tested. The relatively stronger performance observed for the laminated pouch should be interpreted as an outcome for the evaluated package rather than as a universal property of all laminated packaging. Packaging effects may reflect material composition, sealing quality, moisture migration, oxygen exposure, package headspace and handling; these mechanisms were not independently measured in the present study.

3.5 Operational Shelf-Life Assessment and Packaging-Cost Comparison

The combined assessment indicated shorter observed/operational storage under ambient conditions and longer retention under refrigeration. The current dataset gives operational estimates of approximately 3 days for LDPE under ambient storage, 3–5 days for PP under ambient storage, and 5 days for the laminated pouch under ambient storage. Under refrigeration, the corresponding estimates are approximately 7 days for LDPE, 7 days for PP, and 9 days for the laminated pouch. These values should be reported as operational or provisional endpoints unless the predefined acceptance criterion and dated spoilage observations supporting each endpoint are documented. In particular, the Day-3 LDPE ambient endpoint should not be treated as a definitive shelf-life claim solely on the basis of the sensory score; the microbiological and visible-spoilage observations must support it.

Table 5. Storage Duration under Different Conditions.

Packaging	Temperature	Operational storage estimate
LDPE	Ambient	3 days
PP	Ambient	3-5 days
Laminated pouch	Ambient	5 days
LDPE	Refrigerated	7 days
PP	Refrigerated	7 days
Laminated pouch	Refrigerated	9 days

The packaging-material costs were ₹3.20 per LDPE pouch, ₹3.80 per PP pouch, and ₹6.50 per laminated pouch. These figures should be retained only if they can be supported by procurement records. The laminated pouch had the highest reported unit cost and showed lower microbial counts and higher sensory scores at several later storage points under the tested conditions; however, a complete economic comparison would require broader processing and distribution costs.

Table 6. Packaging-material cost comparison.

Packaging	Cost per pouch (₹)
LDPE	3.20
PP	3.80
Laminated pouch	6.50

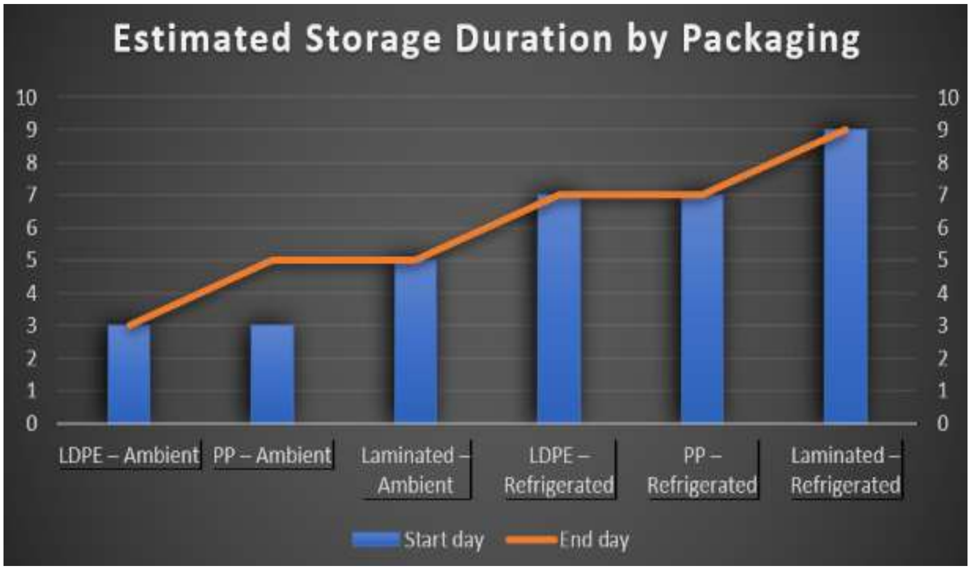


Figure 5 Provisional Storage Duration under Different Packaging Conditions

3.6 Factorial ANOVA Summary

APC Current statistical output is retained for data-verification purposes. Recalculate from the original replicate-level observations before submission; do not use treatment means as independent observations.

Table 7. Three-way ANOVA of APC during storage

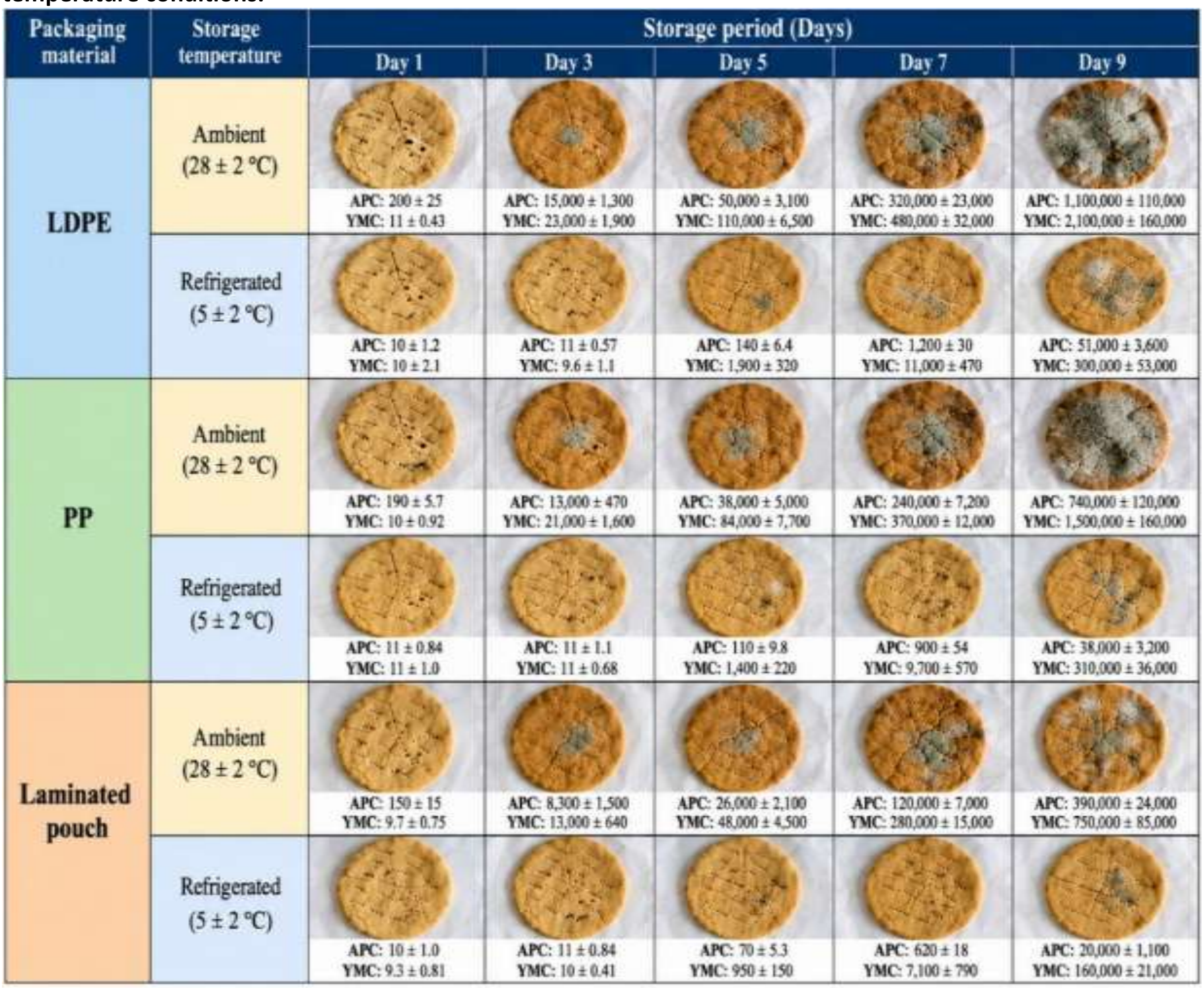
Source of variation	df	Sum of Squares (SS)	Mean Square (MS)	F-value	p-value
Packaging	2	1.419×10^{11}	7.095×10^{10}	76.641	2.992×10^{-17}
Temperature	1	8.695×10^{11}	8.695×10^{11}	939.212	2.392×10^{-38}
Storage day	4	1.969×10^{12}	4.922×10^{11}	531.700	4.265×10^{-46}
Packaging × Temperature	2	1.240×10^{11}	6.200×10^{10}	66.975	5.175×10^{-16}
Packaging × Storage day	8	3.010×10^{11}	3.762×10^{10}	40.635	2.004×10^{-21}
Temperature × Storage day	4	1.616×10^{12}	4.041×10^{11}	436.466	1.322×10^{-43}
Packaging × Temperature × Storage day	8	2.525×10^{11}	3.157×10^{10}	34.096	1.471×10^{-19}
Error/Residual	60	5.555×10^{10}	9.258×10^{8}	—	—
Total	**89**	$\mathbf{5.330 \times 10^{12}}$	—	—	—

Note: ANOVA components were calculated from the reported cell means, standard deviations and balanced replication (n = 3) under a three-factor factorial model involving packaging material, storage temperature and storage day. The analysis should be verified against the original replicate-level observations before final submission. Model: Packaging (3) × Temperature (2) × Storage Day (5), with n = 3 observations per treatment combination.

Table 8. Yeast and mould

Source	F-value	p-value
Packaging	177.41	6.438×10^{-26}
Temperature	23515.02	1.523×10^{-79}
Storage day	30157.99	2.429×10^{-98}
Packaging × temperature	9.83	0.0002034
Packaging × day	15.21	6.349×10^{-12}
Temperature × day	3263.84	1.998×10^{-69}
Packaging × temperature × day	3.31	0.003377

Table 9. Microbiological changes in the functional pizza base during storage under different packaging and temperature conditions.



Packaging material	Storage temperature	Storage period (Days)				
		Day 1	Day 3	Day 5	Day 7	Day 9
LDPE	Ambient (28 ± 2 °C)	APC: 200 ± 25 YMC: 11 ± 0.43	APC: 15,000 ± 1,300 YMC: 23,000 ± 1,900	APC: 50,000 ± 3,100 YMC: 110,000 ± 6,500	APC: 320,000 ± 23,000 YMC: 480,000 ± 32,000	APC: 1,100,000 ± 110,000 YMC: 2,100,000 ± 160,000
	Refrigerated (5 ± 2 °C)	APC: 10 ± 1.2 YMC: 10 ± 2.1	APC: 11 ± 0.57 YMC: 9.6 ± 1.1	APC: 140 ± 6.4 YMC: 1,900 ± 320	APC: 1,200 ± 30 YMC: 11,000 ± 470	APC: 51,000 ± 3,600 YMC: 300,000 ± 53,000
PP	Ambient (28 ± 2 °C)	APC: 190 ± 5.7 YMC: 10 ± 0.92	APC: 13,000 ± 470 YMC: 21,000 ± 1,600	APC: 38,000 ± 5,000 YMC: 84,000 ± 7,700	APC: 240,000 ± 7,200 YMC: 370,000 ± 12,000	APC: 740,000 ± 120,000 YMC: 1,500,000 ± 160,000
	Refrigerated (5 ± 2 °C)	APC: 11 ± 0.84 YMC: 11 ± 1.0	APC: 11 ± 1.1 YMC: 11 ± 0.68	APC: 110 ± 9.8 YMC: 1,400 ± 220	APC: 900 ± 54 YMC: 9,700 ± 570	APC: 38,000 ± 3,200 YMC: 310,000 ± 36,000
Laminated pouch	Ambient (28 ± 2 °C)	APC: 150 ± 15 YMC: 9.7 ± 0.75	APC: 8,300 ± 1,500 YMC: 13,000 ± 640	APC: 26,000 ± 2,100 YMC: 48,000 ± 4,500	APC: 120,000 ± 7,000 YMC: 280,000 ± 15,000	APC: 390,000 ± 24,000 YMC: 750,000 ± 85,000
	Refrigerated (5 ± 2 °C)	APC: 10 ± 1.0 YMC: 9.3 ± 0.81	APC: 11 ± 0.84 YMC: 10 ± 0.41	APC: 70 ± 5.3 YMC: 950 ± 150	APC: 620 ± 18 YMC: 7,100 ± 790	APC: 20,000 ± 1,100 YMC: 160,000 ± 21,000

4. Discussion

The present study demonstrated that storage temperature, packaging material, and storage duration jointly influenced the microbiological and sensory stability of the fibre-enriched functional pizza base. A progressive increase in aerobic plate count (APC) and yeast and mould counts was observed with increasing storage duration, whereas sensory acceptability declined. Refrigerated storage markedly delayed these changes compared with ambient storage, indicating that temperature was an important determinant of product stability. Such behaviour is consistent with the established role of storage conditions in influencing microbial growth, sensory quality, and quality deterioration in bakery products (Cauvain, 2016)

Microbiological stability

APC increased progressively during storage, with substantially greater increases under ambient conditions. By Day 9, APC reached 1.10 × 10^6 CFU/g in LDPE, 7.40 × 10^5 CFU/g in PP and 3.90 × 10^5 CFU/g in the laminated pouch under ambient storage, whereas the corresponding refrigerated values were considerably lower. The lower microbial development under refrigeration can be attributed primarily to reduced microbial growth rates at lower temperature. Similar temperature-dependent behaviour has been reported for pizza and bakery products, in which refrigerated storage delayed microbial proliferation and visible deterioration compared with ambient conditions (Alpers et al., 2021).

The increase in yeast and mould counts followed a similar trend. Ambient storage resulted in pronounced fungal development, with the highest Day-9 count observed in LDPE, while refrigerated storage substantially delayed fungal proliferation. This observation is particularly important for bakery products because mould growth is frequently a major practical limitation to shelf life. Packaging conditions can further influence fungal development by modifying oxygen availability and moisture transfer (Naserifar et al., 2023). Therefore, the combined assessment of APC, yeast and mould counts, sensory quality and visible deterioration provides a more meaningful assessment of storage stability than any single parameter. Influence of packaging material Packaging effects became more evident during the later storage intervals. The laminated pouch generally exhibited lower microbial counts than LDPE and PP, particularly under extended storage. At Day 9 under ambient conditions, APC was 3.90 × 10^5 CFU/g in the laminated pouch compared with 7.40 × 10^5 CFU/g in PP and 1.10 × 10^6 CFU/g in LDPE (Galic et al., 2009). A comparable trend was observed for yeast and mould counts. The improved performance of the laminated pouch may be associated with its multilayer construction and potentially greater resistance to oxygen and moisture transfer (Upasen & Wattanachai, 2018). However, this explanation should be considered mechanistic rather than directly demonstrated because oxygen transmission, water-vapour transmission, headspace composition and sealing integrity were not independently measured in the present study (Fernandez et al., 2006). Packaging effectiveness is strongly dependent on the food matrix, package structure, sealing conditions and storage environment (Dong & Karboune, 2021). Consequently, the observed performance should be interpreted specifically for the packaging systems evaluated rather than generalized to all LDPE, PP or laminated packages.

Sensory quality and relationship with microbial stability

Overall acceptability declined progressively during storage. Under ambient conditions, the scores decreased from approximately 8.0-8.4 on Day 1 to 3.25-4.28 on Day 9. Refrigerated samples retained substantially higher acceptability, with Day-9 scores of 6.17, 6.43 and 6.94 for LDPE, PP and laminated pouch, respectively (Moura-Alves et al., 2022). The relatively better sensory retention under refrigeration occurred concurrently with lower microbial counts, suggesting an association between improved microbiological stability and sensory quality (Belovic et al., 2024). Nevertheless, microbial growth should not be considered the sole explanation for sensory deterioration (Bianchi et al., 2024). Changes in moisture distribution, texture, aroma, colour and structural characteristics may also contribute to declining acceptability during storage. This is particularly relevant for fibre-enriched bakery products because incorporation of fruit and vegetable by-product fibres can modify water distribution, dough structure, texture and sensory properties (Begum et al., 2023). Thus, the sensory results provide complementary information rather than a substitute for microbiological assessment.

Nutritional and functional significance

The incorporation of fruits and vegetables peel fibre into the pizza base provides a food application for fruit and vegetable processing by-products. Fruits peel has been reported to contain approximately 40.6-72.5% total dietary fibre together with bound phenolic compounds and flavonoids, (Marçal & Pintado, 2021) supporting its potential as a functional ingredient more broadly, fruit and vegetable by-products contain dietary fibre and bioactive compounds and have considerable potential for improving the nutritional value of baked products while contributing to food-

waste valorization (Ganesh et al., 2021).
The present study, however, primarily evaluated storage stability. Therefore, the observed shelf-life performance should not be interpreted as evidence that dietary fibre, phenolic compounds or antioxidant activity were retained unchanged during storage (Pathania & Kaur, 2021). Confirmation of functional-nutrient retention would require direct chemical analysis at each relevant storage interval (Kucuk et al., 2024).

Implications for product development and storage
The findings have practical implications for development of a commercially viable fibre-enriched pizza base (Ruegg et al., 2021). Ambient storage resulted in faster microbial development and greater sensory deterioration, whereas refrigeration provided substantially better-quality retention. The laminated pouch showed comparatively favourable performance among the tested packaging systems, particularly at later storage intervals (Petchwattana et al., 2021). This observation is consistent with the established importance of packaging in controlling moisture transfer, oxygen exposure and quality changes in packaged bakery products (Suwanamornlert et al., 2020).

The operational storage estimates obtained in the present study suggest shorter storage under ambient conditions and longer retention under refrigeration (Pasqualone, 2019). However, these should be reported as provisional storage estimates, rather than definitive shelf-life values, until the predefined acceptance criteria and dated visible-spoilage observations are fully documented (Heras-Mozos et al., 2018). In particular, the approximately 3-day ambient estimate for LDPE should not be justified solely by its sensory score; microbiological and visible-spoilage evidence should support the endpoint.

Packaging cost also needs to be considered alongside preservation performance. Although the laminated pouch had the highest reported material cost, it showed comparatively better microbial and sensory retention at later storage points (Ju et al., 2020). Therefore, packaging selection should be based on the balance between preservation performance, material cost and intended distribution conditions rather than unit packaging cost alone.

Limitations
The principal limitations of the study relate to the interpretation of shelf-life endpoints and statistical verification. The reported ANOVA results should be recalculated using the original replicate-level observations before submission, rather than relying on reconstructed treatment means. The independent experimental unit and replication structure should also be clearly established. For sensory evaluation, if the same panellists evaluated multiple storage treatments or time points, an appropriate repeated-measures or mixed-effects approach may be required. Furthermore, package barrier properties and moisture migration were not directly measured, limiting mechanistic interpretation of packaging effects.
Overall, the results indicate that refrigerated storage substantially improved the microbiological and sensory stability of the fruits- and vegetables-peel-fibre-enriched pizza base compared with ambient storage. The laminated pouch exhibited comparatively favourable performance under the conditions investigated. These findings support the integration of suitable packaging and refrigerated storage into the development of fibre-enriched bakery products derived from fruit and vegetable processing by-products, while the final shelf-life claim should remain conditional on verification of the original microbiological, sensory and spoilage records.

5. Conclusion
Under the tested storage conditions, storage temperature, storage duration and packaging material were associated with changes in the microbiological and sensory stability of the functional pizza base. APC and yeast and mould counts increased progressively during storage, whereas sensory acceptability declined. Refrigerated storage was associated with slower microbial development and better retention of sensory quality than ambient storage. Among the evaluated packaging systems, the laminated pouch showed lower microbial counts and higher sensory scores at several later storage points. These findings are specific to the tested formulation, packaging specifications and storage conditions. From a practical perspective, the reported laminated-pouch cost was higher than the reported LDPE and PP pouch costs, while the laminated system also showed favourable storage-quality measurements at several later sampling points. Final packaging selection should therefore consider the required storage period, storage temperature, product-quality target, package specifications and authenticated packaging cost. The

operational storage estimates should not be converted into definitive commercial shelf-life claims until the endpoint criteria and original spoilage records are verified.

Declarations

Ethics Approval and Consent to Participate

This study did not include human participants, human data, or animal subjects. As a result, ethics approval and informed consent were not necessary.

Consent for Publication

Not applicable.

Availability of Data and Materials

All data generated or analysed during this study are included within the manuscript. Additional datasets supporting the conclusions of this study are available from the corresponding author upon reasonable request.

Conflict of Interests

The authors declare that they have no competing financial or non-financial interests.

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Authors' Contributions

Shailesh Kumar conceptualized the study, conducted experimental work, data analysis, and manuscript drafting. Prof. Sunita Mishra contributed to data interpretation, critical review, and manuscript refinement. All authors read and approved the final manuscript.

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