



RESEARCH ARTICLE

Storage kinetics and shelf-life evaluation of tur dal analogues in diverse packaging systems

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Abstract

Tur dal (*Cajanus cajan* (L.) Huth) is a key protein source in vegetarian diets, yet its natural form faces challenges related to storage vulnerability and long cooking time. While extrusion technology offers a means to produce nutrient-dense dal analogues with improved convenience, the storage stability of these porous matrices remains poorly understood. This study evaluated the effect of different packaging materials on the microbial, physicochemical and sensory quality of extruded tur dal analogues over a 300-day storage period. Optimised analogues were hermetically sealed in high-density polyethylene (HDPE), low-density polyethylene (LDPE) and polyethylene-polyethylene terephthalate (PE-PET) pouches and stored under $27 \pm 3^\circ\text{C}$ (ambient conditions). Quality indices, including hardness, moisture content, cooking time, colour kinetics, peroxide value (PV) and total plate count (TPC) were monitored at 30-day intervals. Throughout the 300-day study, all treatments exhibited significant increases ($p < 0.05$) in hardness, moisture and PV, primarily due to the hygroscopic properties of the extruded matrix and ongoing lipid oxidation. Polyethylene-polyethylene terephthalate pouches outperformed LDPE by providing a superior atmospheric barrier, successfully limiting PV to 0.44 meq/kg and moisture to 7.96 %. While all samples maintained microbiological safety standards ($< 10^4$ CFU/g) for the duration of the trial, the LDPE-packed treatments suffered from a notable decline in quality after 8 months, characterised by the development of unpleasant off-flavour and undesirable structural hardness. Ultimately, these findings underscore the critical role of barrier-efficient packaging in maintaining the stability of extruded pulse matrices, offering a scalable strategy to enhance global protein security through convenient and shelf-stable legume-based alternatives.

Keywords: extrusion technology; lipid oxidation; packaging materials; shelf life; tur dal (*Cajanus cajan*)

Introduction

The rising demand for high-quality protein highlights the importance of legumes like pigeon pea (*Cajanus cajan* L. Huth) in regions (South Asia, Sub-Saharan Africa) where plant-based diets predominate (1, 2). While tur dal is a vital source of protein and minerals, its wider utilisation is limited by long cooking times, high post-harvest losses and anti-nutritional factors (phytic acid and tannins) (3). Extrusion technology offers a means to address these constraints through the development of dal analogue reconstituted grains designed for improved cooking efficiency and shelf stability (4).

Extrusion is a high-temperature short-time (HTST) process used to convert pulse flours into stable, grain-like matrices, typically operating at barrel temperatures ranging from 120–200 °C and feed moisture contents between 12 and 25 %, depending on the raw material and processing conditions (5). The application of thermal and mechanical energy induces starch gelatinisation and protein denaturation, producing an analogue that replicates natural dal with reduced cooking requirements (6). Furthermore, this method

facilitates the development of composite grains, by enabling the blending of diverse pulse varieties or micronutrients - such as the incorporation of elemental iron, zinc, or the enhancement of limiting amino acids like lysine to optimise the amino acid profile (7–9).

However, the expanded porous matrix of the extruded analogues increases the available surface area for moisture adsorption and oxygen diffusion (10). This structural characteristic accelerates quality loss by promoting lipid oxidation and the "hard-to-cook" (HTC) defect, where moisture-induced changes in the matrix impede rapid starch gelatinisation during preparation (2, 11). These changes lead to undesirable increases in textural hardness, prolonged cooking durations and the development of rancid off-flavours (e.g., peroxide value and thiobarbituric acid reactive substances), ultimately limiting consumer acceptability (12).

The selection of appropriate packaging materials is therefore paramount to preserving the functional and sensory integrity of these analogues. Flexible films, such as high-density polyethylene (HDPE), low-density polyethylene (LDPE) and polyethylene-

polyethylene terephthalate (PE-PET), provide different degrees of gas barrier and water vapour protection (13). While previous studies have optimised the extrusion parameters for dal analogues, longitudinal data regarding their stability under diverse packaging environments remains limited.

This study aimed to evaluate the storage stability of optimised tur dal analogues over 300 days. By analysing the interplay between packaging barrier properties and physicochemical kinetics, including moisture migration, lipid oxidation and colour stability. This research identifies the most effective strategy for maintaining the quality of these sustainable protein alternatives under ambient conditions.

Materials and Methods

Storage stability analysis

The storage stability of the optimised tur dal analogues was monitored for 300 days under ambient conditions. For the packaging trials, 300 g each samples were packed into three types of flexible pouches: LDPE, HDPE and PE-PET (all 100 µm thick). To ensure statistical accuracy, 10 replicates were prepared for each packaging group. In addition, quality checks were performed every 30 days over the 10 months to monitor the physicochemical (moisture content, peroxide value and colour kinetics) and functional properties (hardness and cooking time) of the analogues. The samples were evaluated for moisture content, peroxide value, textural hardness and cooking time. Furthermore, the samples were subjected to microbial count analysis and sensory profiling for overall acceptability to ensure the maintenance of safety and quality standards throughout the storage duration (7).

Moisture content

Moisture content of the tur dal analogues were measured gravimetrically by drying 5.00 ± 0.01 g of the sample in a hot air oven at 105 °C (14). Samples were held in pre-weighed Petri dishes for 24 hr until a constant mass was reached. After drying, samples were cooled in a desiccator to prevent moisture re-adsorption before the final weighing. Moisture content (wet basis) was determined as the ratio of evaporated water mass to the initial sample weight as shown in the equation (Eq. 1). All analyses were performed in triplicate.

$$\text{Moisture content} = \frac{\text{Evaporated water mass}}{\text{Initial sample weight}} \quad (\text{Eq. 1})$$

Determination of hardness

The textural profile of the extruded tur dal analogues was quantitatively evaluated prior to cooking using a texture analyser (Model: CT3 50K, Brookfield Engineering Laboratories, USA). The hardness of the analogues was measured as the peak compressive force (N) using a texture analyser (CT3 50K Model: Brookfield Engineering Laboratories, USA) equipped with a TA 10 acrylic cylindrical probe (12.7 mm diameter). The test parameters included a pre-test and test speed of 1.0 mm/s, a post-test speed of 10.0 mm/s and a trigger load of 0.5 N. Samples were compressed to a distance of 0.8 mm, equivalent to a 40–50 % strain of the total thickness (4). Ten replicates were analysed per treatment group to account for structural variability and results were expressed as mean values.

Cooking time estimation

The cooking time of the tur dal analogues was determined by monitoring the duration required for the grains to achieve a uniform, softened mass in boiling distilled water. A 10 g sample is boiled in a 70 mL of distilled water using a regulated water bath. At 1 min intervals, samples were removed and pressed between two plexiglass plates. The minimum cooking time was recorded when the internal opaque white core completely disappeared, indicating full gelatinization (2). To ensure experimental precision and account for structural variations in the extruded matrix, the test was performed in triplicate, and the mean value was documented as the standard cooking time (2).

Peroxide value (PV) estimation

Peroxide value (PV) was determined via iodometric titration (14). Lipids were first extracted from 10 g of pulverised sample using the Soxhlet method with petroleum ether. The extracted oil was dissolved in 30 mL of acetic acid-chloroform (3:2 v/v), then mixed with 0.5 mL of saturated potassium iodide. Following a 1 min reaction in the dark, we added 30 mL of distilled water and 1 % starch indicator. The mixture was titrated against 0.01 N sodium thiosulfate standardised with potassium dichromate until the blue-black colour disappeared. Results were expressed as meq of active oxygen/kg of fat. The peroxide value was calculated based on the titrant volume (V), the normality of the thiosulfate (N) and the sample weight (W), providing a precise measure of primary lipid oxidation (Eq. 2).

$$\text{Peroxide value (meq/kg)} = \frac{V \times N \times 1000}{\text{Weight of dal sample, g}} \quad (\text{Eq. 2})$$

Colour kinetics

The colour (L^* , a^* , b^*) of the extruded tur dal analogues was measured using a Hunter Lab Colorflex EZ Colorimeter. The instrument was calibrated with a standard white tile before analysis. Samples were loaded into a glass cuvette for measurement. The total colour difference (ΔE^*) was calculated relative to the initial values (or natural dal) using the following equation (Eq. 3):

$$\Delta E^* = \sqrt{(L_0^* - L^*)^2 + (a_0^* - a^*)^2 + (b_0^* - b^*)^2} \quad (\text{Eq. 3})$$

In this equation, the "0" initial represents the initial colour of the natural dal or the fresh analogue, while the other values represent the stored sample. All tests were performed in triplicate to ensure the results were consistent and reliable (15).

Overall sensory acceptability

Sensory quality was assessed by a semi-trained panel ($n=20$; ages 18–50) using a 9-point hedonic scale (Eq. 4). To minimise bias, panelists were briefed on product attributes and sessions were held under controlled daylight conditions. Each evaluator assessed appearance, colour, flavour, texture, taste and overall acceptability. Criteria for panel selection included non-smoking status and demonstrated sensory acuity. During each session, the optimised samples and the three top-performing variants were compared against natural tur dal as a control. Additionally, the consumer preference was quantified using the acceptability index (AI), calculated by dividing the average score obtained by the maximum possible score (9) and multiplying by 100, which provided a percentage-based measure of product success (16).

$$AI (\%) = \frac{\text{Mean score}}{9} \times 100 \quad (\text{Eq. 4})$$

Microbiological evaluation

The microbiological stability of the optimised tur dal analogues was monitored throughout the storage period by determining the total plate count (TPC). Analyses were conducted at room temperature at regular 30-day intervals, specifically on day 0 (post-production) and subsequently every 30 days up to the 300th day of storage (14).

The TPC was estimated to be using a systematic serial dilution and spread-plating technique to measure the viable microbial population. Under aseptic conditions, 1 g of the pulverised sample was homogenised in 9 mL of sterile distilled water to achieve 10⁻⁵ dilution concentration. For the growth medium, nutrient agar was sterilised via autoclaving at 121 °C and 15 psi for 15 min, then cooled and poured into Petri dishes within a laminar air flow (LAF) chamber. Once the agar had solidified, 0.1 mL of the respective inoculum from each dilution was uniformly spread across the surface and the plates were inverted and incubated at 37 °C for 48 hr. Colonies were enumerated using a digital counter and results were expressed as (CFU/g) (Eq. 5) (17).

$$\text{CFU/g} = \frac{\text{Total no. of colonies} \times \text{Dilution factor}}{\text{Volume of culture plated in mL}} \quad (\text{Eq. 5})$$

Statistical analysis

The samples were analysed using a two-factor factorial completely randomised design (CRD). Analysis of variance (ANOVA) was performed to evaluate the effects of packaging material, storage duration and their interaction. Mean comparisons were carried out using the critical difference (CD) test at a 5 % significance level ($p < 0.05$).

Results and Discussion

Storage stability of extruded tur dal analogues

The storage stability of the optimised extruded tur dal analogue, selected based on response surface methodology (RSM) results and superior sensory profiles, was evaluated under ambient environmental conditions (27 ± 3 °C). Three packaging materials-LDPE, HDPE and PE-PET (100 µm thickness) were tested to determine their barrier performance. The analogues were stored for 300 days, with quality assessments conducted every 30 days. Measured indices included hardness, moisture, cooking time, colour kinetics, PV, microbial load and sensory scores. Periodic quality assessments were conducted at 30-day intervals over 300 days to evaluate the degradation kinetics and structural integrity of the analogues.

Moisture content

Moisture content in the extruded dal analogues increased significantly ($p < 0.05$) across all packaging types over the 300-day storage period (Fig. 1A). From an initial value of 5.7 % (w.b.), moisture rose to 8.59 % in LDPE, 8.22 % in HDPE and 7.96 % in PE-PET pouches. This gain is attributed to the hygroscopic nature of the starch-protein matrix. The porous structure formed during extrusion likely facilitated water vapour adsorption as the samples equilibrated with the ambient environment (18).

The differences in moisture gain among treatments correlate with the specific water vapor transmission rates (WVTR) of the packaging films. Low-density polyethylene packed samples showed the highest moisture accumulation due to the material's lower barrier efficiency compared to HDPE and PE-PET. The superior performance of PE-PET is likely due to its crystalline structure and the presence of polar groups, which act as a more effective barrier against water diffusion. These findings suggest that the shelf stability of extruded analogues depends heavily on the packaging's ability to prevent moisture-induced degradation (13, 19).

Hardness

A progressive increase in the textural hardness of the tur dal analogues was observed across all packaging treatments throughout the storage duration (Fig. 1B). From an initial baseline of 316.24 N, the hardness reached peak values of 356.68 N, 341.66 N and 330.54 N in samples stored within LDPE, HDPE and PE-PET pouches respectively, after 300 days. This trend is primarily attributed to the retrogradation of pulse starch following extrusion gelatinization (20). Unlike cereal starches, pulse starches exhibit a higher tendency toward molecular realignment, which increases the resistance of the matrix to swelling and rupture. This phenomenon is further influenced by the high protein-to-starch ratio of analogues, which typically results in a denser, more texturised structure compared to high-starch expanded snacks (6).

The observed hardening is specifically attributed to the inclusion of calcium chloride (0.2 % of calcium chloride (CaCl₂)) as a firming agent during the formulation process, resulting from the interaction between divalent calcium ions and the biopolymeric matrix; in the presence of absorbed moisture, CaCl₂ facilitates the formation of cross-linked bridges (calcium pectates or protein-mineral complexes), which stabilise the structural framework and lead to increased mechanical resistance over time (21). Consequently, the LDPE-packed samples, which experienced the highest moisture ingress, exhibited the most significant increase in hardness, indicating that moisture-mediated chemical hardening was a dominant factor during the storage period (22).

Cooking time

Cooking time is a critical determinant of consumer acceptability and the economic feasibility of tur dal analogues, as prolonged preparation times directly correlate with increased energy consumption. At the initiation of the study, the baseline cooking time was recorded at 5.0 min. Following the 300-day storage interval, a significant increase in cooking duration was observed: samples stored in LDPE pouches exhibited a 1.0-min increase, while those in HDPE and PE-PET packaging showed a more moderate 0.5-min increase (Fig. 1C). This escalation in cooking time is fundamentally justified by the HTC phenomenon, which is intrinsically linked to the simultaneous increase in textural hardness observed during storage. As the analogue matrix undergoes starch retrogradation and Ca²⁺ mediated cross-linking accelerated by moisture ingress-the structural density increases. This heightened mechanical integrity creates a physical barrier that impedes water diffusion and heat transfer into the core of the analogue during boiling (2). Consequently, the LDPE-packed samples, which demonstrated the highest moisture gain and resulting hardness, required the longest time to achieve a palatable texture, highlighting the relationship between packaging barrier efficiency and the product's functional cooking quality (19).

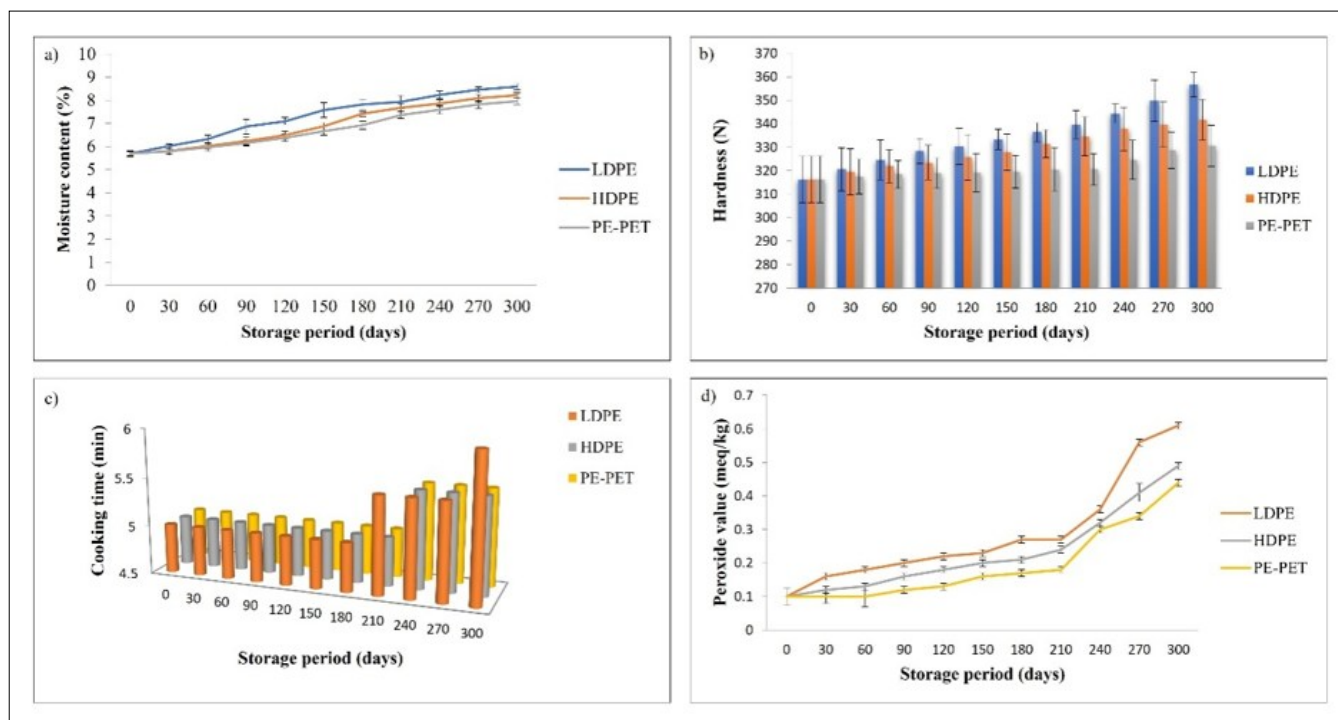


Fig. 1. Effect of packaging material on tur dal analogues during storage. (A) moisture content; (B) hardness; (C) cooking time; (D) peroxide value.

Peroxide value (PV)

Lipid oxidation was monitored as a key indicator of shelf stability, as the formation of hydroperoxides leads to rancidity and characteristic off-flavours in extruded products. Initial fat percentage in extruded tur dal analogues is 0.9 % and initial peroxide value is 0.10 meq/kg. As shown in Fig. 1D, the PV of the tur dal analogues increased significantly across all packaging treatments over the 300-day storage period (7, 19).

Lipid oxidation exhibited a biphasic trend, with a linear progression until day 210 and a sharper, exponential increase thereafter. This acceleration suggests the onset of the propagation stage of lipid autoxidation. The extent of oxidation was packaging-dependent; LDPE samples reached a peak of 0.61 meq/kg, whereas the superior oxygen barrier properties of PE-PET limited the final PV to 0.44 meq/kg.

The accelerated oxidation in LDPE-packed analogues is attributed to the material's higher oxygen transmission rate (OTR) and lower moisture barrier efficiency. The ingress of moisture likely promotes oxidation by mobilising pro-oxidants within the porous matrix, while the higher oxygen permeability provides the necessary substrate for lipid peroxidation. This aligns with findings in rice and chickpea-based snacks, where the high-heat extrusion process increases the susceptibility of lipids to oxidation unless high-barrier materials like PE-PET are utilised (7).

Colour stability (ΔE^*)

The total colour difference (ΔE^*) was quantified by comparing the chromatic values of stored tur dal analogues against those of the freshly produced samples. A progressive increase in ΔE^* was observed across all packaging treatments throughout the storage period, indicating a continuous departure from the initial product colour (Fig. 2A). This chromatic shift is primarily justified by the degradative bleaching of endogenous pigments and the potential for non-enzymatic browning over time (23, 24). It has been documented that pre-cooked and dehydrated pulse-based matrices are highly susceptible to rapid autoxidation during storage, a process

that typically results in the degradation of carotenoids or other yellow-pigment components and the subsequent development of off-flavours (25).

The magnitude of colour change was most significant in samples stored in LDPE ($\Delta E^* = 12.75$), followed by HDPE ($\Delta E^* = 9.89$), while the PE-PET pouches demonstrated the highest colour retention ($\Delta E^* = 7.74$). This disparity is attributed to the varying gas and water vapour permeability of the materials. The inferior barrier properties of LDPE likely facilitated a higher rate of oxygen and moisture ingress, thereby accelerating the oxidative degradation of colour-active compounds. Conversely, the superior barrier efficiency of PE-PET mitigated these oxidative pathways, effectively preserving the visual profile of the dal analogues throughout the 300-day study (19, 26).

Overall sensory acceptability

The sensory profile of the extruded tur dal analogues was monitored at 30-day intervals over the 300-day storage duration using a 9-point hedonic scale. Analysis of the results indicated that the analogues preserved in PE-PET packaging maintained higher consumer preference scores compared to those stored in LDPE and HDPE (Fig. 2B). While the initial acceptability score was recorded at 8.2, a significant decline was observed across all treatments by the conclusion of the study. Specifically, overall acceptability scores decreased significantly to 5.6, 6.9 and 7.2 for samples stored in LDPE, HDPE and PE-PET respectively.

This decline in organoleptic quality is attributed to the cumulative effects of the physicochemical changes previously discussed, particularly lipid oxidation and moisture-induced hardening (7). Notably, the LDPE-packed samples developed a detectable off-flavour by the end of the 9th month, which is directly attributable to the higher peroxide values and the formation of volatile secondary oxidation products (e.g., aldehydes, ketones) allowed by the material's poor oxygen barrier (27). Furthermore, the significant increase in hardness and required cooking time in LDPE-packed samples likely contributed to the lower scores. These results

indicate that while all samples underwent some degree of sensory degradation, the high-barrier properties of PE-PET were most effective in extending the shelf life and maintaining the characteristic flavour and texture profile of the dal analogues (7).

Microbial load and safety assessment

The initial microbiological analysis of the extruded tur dal analogues revealed an absence of detectable bacterial populations on the first day of storage, which is justified by the lethal effect of high-temperature thermal processing and high shear during extrusion that effectively eliminates vegetative microbial cells. Longitudinal data indicated that the TPC remained below detectable limits across all packaging treatments for the first 120 days of storage, a trend attributed to the low water activity (a_w) of the extruded matrix, which inhibits microbial germination (7, 28).

After 10 months of storage, minor microbial proliferation was observed, with TPC reaching 3.23×10^2 , 1.73×10^2 and 0.93×10^2 cfu/g for samples stored in LDPE, HDPE and PE-PET pouches respectively (Fig. 2C). This slight increase in microbial load, particularly in LDPE, is justified by the material's higher moisture permeability, which can lead to localised increases in surface water activity over extended periods (13). Despite this, all recorded values remained significantly below the maximum permissible limit of 1×10^4 cfu/g established for comparable products like fortified rice kernels, ensuring the analogues remained microbiologically safe (29).

The comprehensive storage study indicates that while moisture content, peroxide value and microbial load remained within regulated safety limits throughout the 300-day duration, the practical shelf life was primarily governed by organoleptic degradation. The development of significant off-flavours in LDPE-packed samples after 8 months is justified by the onset of oxidative rancidity due to the poor oxygen barrier properties of the film. Consequently, while the product remains chemically and microbiologically safe for 10 months, a storage period of 8 months at ambient temperature is recommended to maintain optimal sensory quality, with PE-PET identified as the superior packaging material for quality retention (30).

Conclusion

This 300-day storage study demonstrates that while the extruded tur dal analogues remain microbiologically safe ($<10^4$ CFU/g) for 10 months, their sensory and functional quality is significantly influenced by the choice of packaging. The high-temperature extrusion and low water activity effectively suppressed microbial growth across all treatments. However, organoleptic degradation specifically off-flavour development and structural hardening was identified as the primary shelf-life constraint. Polyethylene-polyethylene terephthalate (PE-PET) pouches were the most effective barrier, maintaining moisture at 7.96 % and peroxide value to 0.44 meq/kg. In contrast, low-density polyethylene-packed samples showed significant quality loss after 8 months, including a 1.0-min increase in cooking time. Consequently, a maximum shelf life of 8 months is recommended for storage in high-barrier PE-PET packaging to maintain optimal flavour and texture at ambient temperatures. Future studies should investigate the bioavailability of nutrients and *in vitro* digestion kinetics of these analogues, specifically focusing on how storage affects essential amino acids and the degradation of anti-nutritional factors like phytic acid. There is also a significant opportunity to investigate advanced fortification through micro-encapsulation to protect sensitive micronutrients during high-heat extrusion. From a sustainability perspective, evaluating biodegradable films with nano-cellulose or starch-based coatings could provide eco-friendly alternatives to PE-PET without compromising barrier efficiency. Furthermore, applying accelerated shelf-life testing (ASLT) could enable for the development of predictive models across varied climatic zones. Finally, large-scale consumer trials and testing in diverse culinary systems, such as instant soup mixes or institutional feeding programs, are necessary to evaluate the commercial readiness and social impact of these protein-rich analogues.

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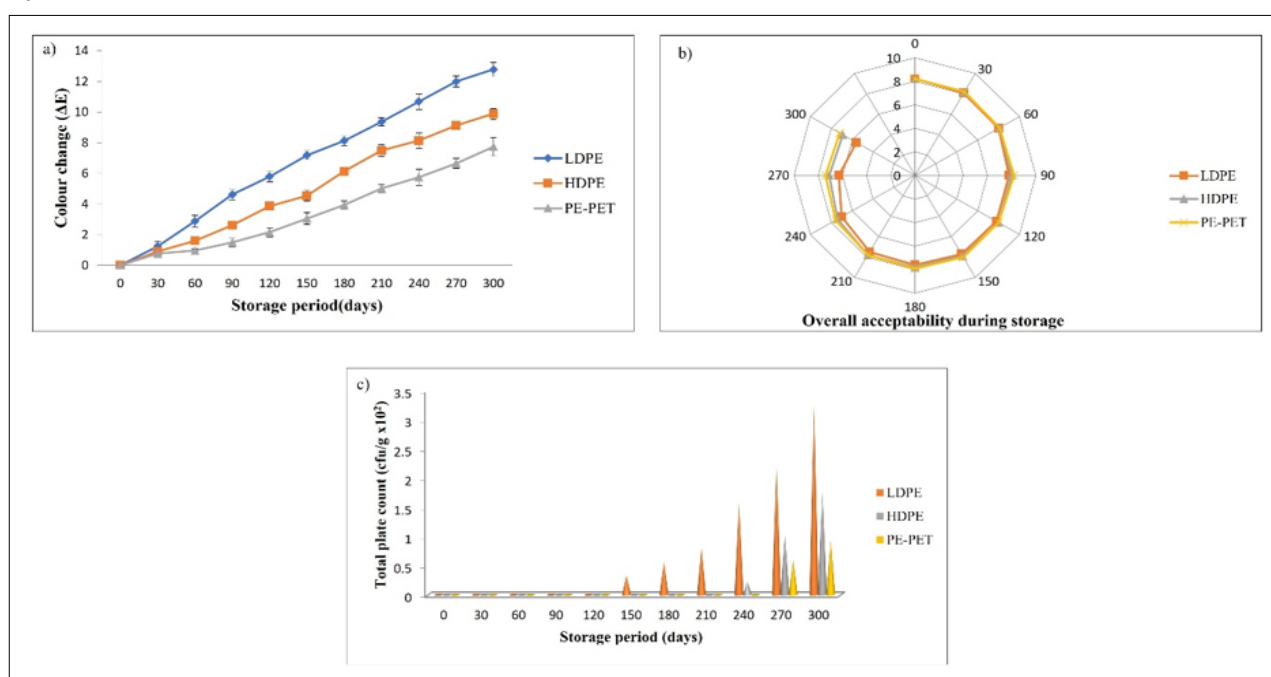


Fig. 2. Effect of packaging material on tur dal analogues during storage. (A) colour change (ΔE^*); (B) overall acceptability; (C) total plate count.

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Authors' contributions

RG contributed to the methodology, data collection, product development, manuscript writing and preparation of the original draft. ChVS and ChSR contributed to visualisation, supervision, manuscript review and editing. BHB and BAK contributed to manuscript review and editing. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: None

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work, the authors used Gemini (Google AI) tool for the purpose of rephrasing technical content to improve linguistic clarity and grammatical accuracy. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the final accuracy and integrity of the publication.

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