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RESEARCH ARTICLE

Suitability of spent lemongrass CNC reinforced chitosan glycerol films on postharvest storage quality of white button mushrooms

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Abstract

To assess the shelf life of white button mushrooms (WBM), three different packaging films were used: a control film composed of PLA-PBAT, a glycerol-plasticized chitosan film and a chitosan film enhanced with glycerol and reinforced with CNC derived from spent lemongrass (chitosan-CNC film). WBM packed in these three types of packaging films were evaluated by recording the parameters like weight loss (WL), poly phenol oxidase (PPO) activity, decay loss (DL), browning index (BI), visual appearance (VA), odour (OD) and overall acceptability (OA) for a period of 12 days with an interval of 3 days. All the parameters were significantly affected ($p < 0.05$) with the type of packaging film and storage period. The WBM remained acceptable up to the 3rd day of storage in chitosan film packages, 6th day of storage in chitosan-CNC film packages and 9th day of storage in control film packages under refrigerated condition of 4 °C without any appreciable loss of overall quality parameters.

Keywords: BI; DL; packaging films; PPO activity; sensory attributes; WL

Introduction

Agaricus bisporus (WBM) are widely grown across the globe, for its rich amino acids, dietary fibres, minerals, polyphenols, proteins and vitamins (1). World WBM production is estimated to surpass 50 million tonnes (2). WBM possess excellent antioxidant, antibacterial, anti-inflammatory, antitumor, anticancer, antihyperlipidemic and immune modulatory attributes (3). WBM are widely consumed for its high health benefits. Due to highly perishable nature of WBM, they are easily susceptible to spoilage due to high respiration and transpiration rates, due to its high metabolic activity (4). Its shelf life is limited to 1-2 day, immediately after harvest when stored at ambient conditions. There is need to enhance the shelf life of WBM. Application of novel food processing technologies like dehydration, different drying methods, vacuum frying will increase the shelf life of WBM but are not cost effective. There is a need to enhance the shelf life of WBM in a cost-effective manner and antimicrobial packaging as well as coatings better serves this purpose. Coatings may contaminate the mushrooms and potentially cause foodborne illness. Notably, antimicrobial packaging of freshly harvested mushroom stored in ambient/refrigerated conditions is most viable and safe option.

Chitosan, the second most abundant biopolymer after cellulose, is a non-toxic, biodegradable, biocompatible and water-insoluble material that is soluble only in acidic solutions, giving it a unique character as a cationic polysaccharide (5). Chitosan films are generally crystal clear, transparent and do not possess any pores. Chitosan dissolved in acid solutions present filmogenic properties, aiding in the film formation (6). The chitosan films are brittle and poorly flexible. Hence glycerol, a low cost and highly available prominent by-product of biodiesel chain is used as plasticizing agent in development of packaging film (7). CNC sourced from spent lemongrass is used as nano-fillers improve the mechanical properties of chitosan films.

Keeping in view, the effects of different packaging films on the postharvest quality of WBM were proposed. Three types of packaging films, namely control (PLA-PBAT) film, chitosan film with added glycerol and chitosan film with glycerol reinforced with spent lemongrass CNC (chitosan-CNC film), were used in evaluating the keeping quality of WBM.

Materials and Methods

Materials

Fresh WBM (3.5-3.8 mm diameter) were procured from local grocery supplier, with an initial moisture content of 90 % (w.b.). Chitosan, glacial acetic acid, glycerol (Loba Chemie Pvt. Ltd., Mumbai) and di-sodium hydrogen phosphate, sodium di-hydrogen phosphate, bovine serum albumin (BSA), catechol (Sigma Aldrich, Mumbai) were procured from local chemical supplier. Spent lemongrass cellulose nanocrystals (CNC) were procured from Agricultural Processing and Food Engineering department, Indira Gandhi Krishi Vishwavidyalaya, Raipur, India. PLA-PBAT (control) packaging films were procured from local D-mart (Plasto, India).

Development of chitosan CNC film

Chitosan solution was prepared by immerging 3 g of chitosan in 300 mL (1 % v/v aqueous glacial acetic acid solution) stirred using a SPINOT hot plate magnetic stirrer (Tarsons, India) at 50 °C, 250 rpm for 2 hr. Similarly, CNC suspension was prepared by dispersing 0.3 % (w/w_{ch}) of CNC in 150 mL distilled water, stirred using a hot plate magnetic stirrer at 50 °C, 250 rpm for 2 hr. CNC suspension was blended thoroughly with the chitosan solution. 12.25 % (v/w_{ch}) of glycerol was added to the former resultant solution, thoroughly homogenized at 50 °C, 250 rpm for 2 hr making a film forming solution (FFS). The FFS was stirred at 800 rpm for 30 min and ultrasonicated for 30 min. The FFS (450 mL) was cast on the center of polypropylene sheet (ASTM plastic resin identification code 5), having 0.09 m^2 surface area and dried in oven at 37 °C for 12 hr. The dried films were placed under fan at room temperature (28-30 °C) for further drying for two days to evaporate the residual solvents completely (if any). The film was finally placed and stored in zip lock pouches for further use (8). The chitosan films and chitosan-CNC films (In both cases, glycerol plasticizer was added) are prepared in large numbers to pack WBMs in it.

The WVP of the films was determined gravimetrically following the ASTM 144 E96 standard test method (9).

Suitability as packaging material

After precooling of WBM at 2-4 °C for 4 hr, WBM samples (about 50 g) were packed in each rectangular sachets (made from control, chitosan and chitosan CNC) (15 cm × 15 cm) and sealed (all sides) using the cellophane adhesive tape. All the WBMs were stored at 4 ± 1 °C in a cold fresh cabinet that contained no other fruits/vegetables. The samples were evaluated for its quality for the storage period of 12 days. The quality parameters included WL, PPO enzymatic activity, BI, DL and sensory attributes like VA, OD and OA following the 9-point hedonic scale method (10). Table 1 depicts the formulae used for measuring different parameters.

Weight Loss (WL)

The WL (%) of WBM during storage was determined by weighing the samples in each pack initially and at specified time intervals of 3 days during the storage period using Eqn. 1 given below (11). The packets were weighed using digital balance (least count = 0.0001 g).

$$WL = \frac{W_0 - W_1}{W_0} \times 100 \qquad \text{(Eqn. 1)}$$

Where, W_0 = initial weight (g) and W_1 = weight of each sample (g) at a given day

PPO activity

Fresh enzyme extracts were prepared by mixing 2 g of crushed WBM with 7 mL of 0.05 mol/L phosphate buffer solution (pH 5), centrifuged using a refrigerated centrifuge (REMI, India) at 10000 rpm at 4 °C for 15 min and the supernatant (enzyme extract) was collected. The phosphate buffer was prepared by mixing di-sodium hydrogen phosphate solution and sodium di-hydrogen phosphate solution in required quantities to obtain the desired pH. The standard curve fit was obtained with the absorbance data of BSA by varying concentration. 0.1 mL of enzyme extract was blended with 4 mL of 0.1 mol/L phosphate buffer solutions (pH 7) and 1 mL of 50 mmol/L catechol. The resultant solution was checked for PPO activity in time scan mode of 3 min using a double beam spectrophotometer (Systronics, India) for maximum peak and expressed as U/mg protein. One unit of PPO is the amount of enzyme with a 0.01 increase in absorption value at 407 nm per min (10, 12).

Table 1. ANOVA for effect of packaging type and storage period on WL of WBM

Source of variation	df	Sum of squares	Mean squares	F-calculated
Packaging film (PF)	2	3671.961	1835.981	19799.382*
Storage period (SP)	3	5104.693	1707.564	18349.823*
PF×SP	6	725.389	120.898	1303.778*
Error	24	2.226	0.093	

(* -Significant at 5 % level)

Browning index (BI)

The surface colour of WBM caps was captured using a digital camera, wherein the samples were placed under the uniform intensity light source at minimum distance for good quality photographs. Sony 7.2 mega pixels digital camera was used to capture the image of mushrooms. The L (whiteness/darkness), a (redness/greenness) and b (yellowness/blueness) values of samples was measured by using Adobe Photoshop CS software (Adobe, USA) in LAB set mode (13). The L, a and b initial colour values obtained from the histogram window were converted to L* (lightness or luminosity), a* (red green colour component), b* (yellow blue colour component) and ΔE (colour difference) using the following Eqn. 2-5. The BI was calculated from the Eqn. 6-7, respectively (11).

$$L^* = \frac{L}{250} \times 100 \qquad \text{(Eqn. 2)}$$

$$a^* = \frac{240 \times a}{255} - 120 \qquad \text{(Eqn. 3)}$$

$$b^* = \frac{240 \times b}{255} - 120 \qquad \text{(Eqn. 4)}$$

$$\Delta E = \sqrt{\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2}} \qquad \text{(Eqn. 5)}$$

$$X = \frac{a + 1.75L}{5.645L + a - 3.012b} \qquad \text{(Eqn. 6)}$$

$$BI = \frac{100 \times (X - 0.31)}{0.172} \qquad \text{(Eqn. 7)}$$

Decay loss (DL)

The decay of the WBM occurs with the advancement in storage period which results in the cap opening. The DL or percent cap opening is the one of the criteria used to determine the freshness or quality. The DL is expressed in percentage and calculated using the Eqn. 8 (11).

$$DL = \frac{\text{No. of cap opened WBM}}{\text{Total No. of WBM}} \times 100 \quad \text{(Eqn. 8)}$$

VA, OD and OA

Sensory attributes such as VA, OD and OA of WBM samples were evaluated by a 9-member semi trained panel (judges) comprising research scholars and faculty from the Department of Agricultural Processing and Food Engineering, IGKV, Raipur (14). Judges were briefed on the evaluation process beforehand. Samples were coded to prevent bias and judges rinsed their mouths before tasting each new sample.

The VA, OD and OA of WBM were rated according to the 9-point hedonic scale uses a scale of 1 to 9, assigning value of 1 for dislike extremely, 2 for dislike very much, 3 for dislike moderately, 4 for dislike slightly, 5 for neither like nor dislike, 6 for like slightly, 7 for like moderately, 8 for like very much and 9 for like extremely, respectively (15).

Statistical analysis

The significance test ($p<0.05$) of control film, chitosan film and chitosan-CNC film on the effect of WL, PPO, BI, DL, VA, OD and OA (n=3) of WBM was done in by the two-way ANOVA (F-test) for the 12 days storage period (OPSTAT, India).

Results and Discussion

The water vapour permeability (WVP) of developed (chitosan-glycerol-CNC) film, chitosan-glycerol film and PLA-PBAT (control) film were found to be 4.61×10^{-9} g × $(smPa)^{-1}$, 6.97×10^{-9} g × $(smPa)^{-1}$ and 2.19×10^{-11} g × $(smPa)^{-1}$, respectively.

Effect on WL of WBM

The effects of different packaging films and storage period on the WL of WBM during storage are shown in Fig. 1 and Table 1. The WL of WBM was greatly influenced by type of packaging films and the storage period, respectively. The WL pattern showed an increasing trend with prolonged storage period for the WBM stored in all the three types of biodegradable packages. The type of packaging film, storage period and their interaction effect showed significant ($^*p<0.05$) effect on the WL of WBM.

The WL was significantly highest for the WBM stored in chitosan film when compared to the WBM stored in chitosan-CNC film and control film. The WL of WBM ranged from 0-57.62 %, 0-47.50 %, 0-19.16 % for chitosan film, chitosan-CNC film and control film, respectively. However, the chitosan-CNC film also showed similar pattern of WL. The WBM stored in control film showed less WL compared with the earlier two. This is due to WVP differences of the packaging film used for WBM packaging. The chitosan film had high WVP followed by chitosan-CNC film and control film (lowest WVP). Condensed water on the inner surface of the control film attributed to its lower WVP (16). The acceptable WL in WBM for consumer consumption is around 16 % (17). It was observed that WL recorded for WBM stored in chitosan film at 3rd day, chitosan-CNC film at 6th day and control film at 9th day were 15.69 %, 16.02 %, 8.76 %, respectively.

ANOVA showed that the type of packaging film, storage period and their interaction had a significant effect ($^*p<0.05$) on the WL in WBM (Table 1). The WL of WBM stored in developed film is comparatively lower than mushrooms stored in chitosan film.

Effect on PPO activity of WBM

PPO is one of the key enzymes, which catalyses the oxidation of mono- and di-phenols to o-quinones, which further polymerizes to form brown pigments (enzymatic browning) in WBM (18). The PPO activity of WBM stored in different biodegradable packaging films over a storage period of 12 days are shown in Fig. 2 and Table 2.

It can be inferred that WBM packaged with control film showed lowest PPO enzymatic activity (20-27 U/mg protein) among all the treatment groups ($^*p<0.05$) (Fig. 2). The WBM packed in chitosan film showed highest PPO (20-46 U/mg protein) activity among all types of packaging films used. It can be inferred that control film was effective in suppressing PPO activity which reduced the enzymatic browning in WBM. Similar results were reported earlier by various researchers (18, 19).

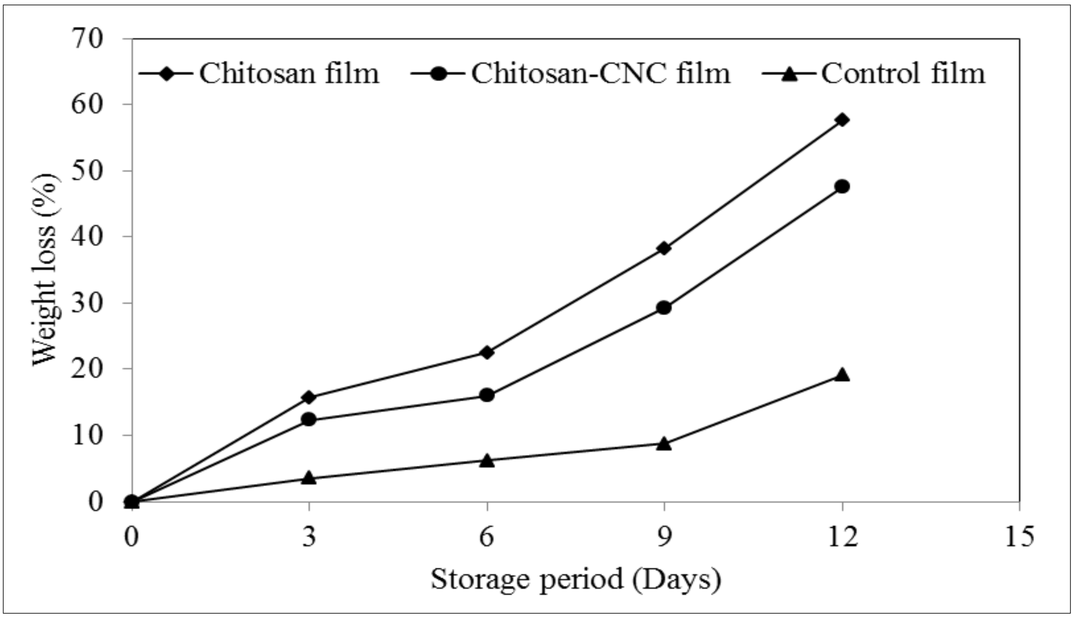

Fig. 1. Effect of different types of biofilms on WL of WBM.

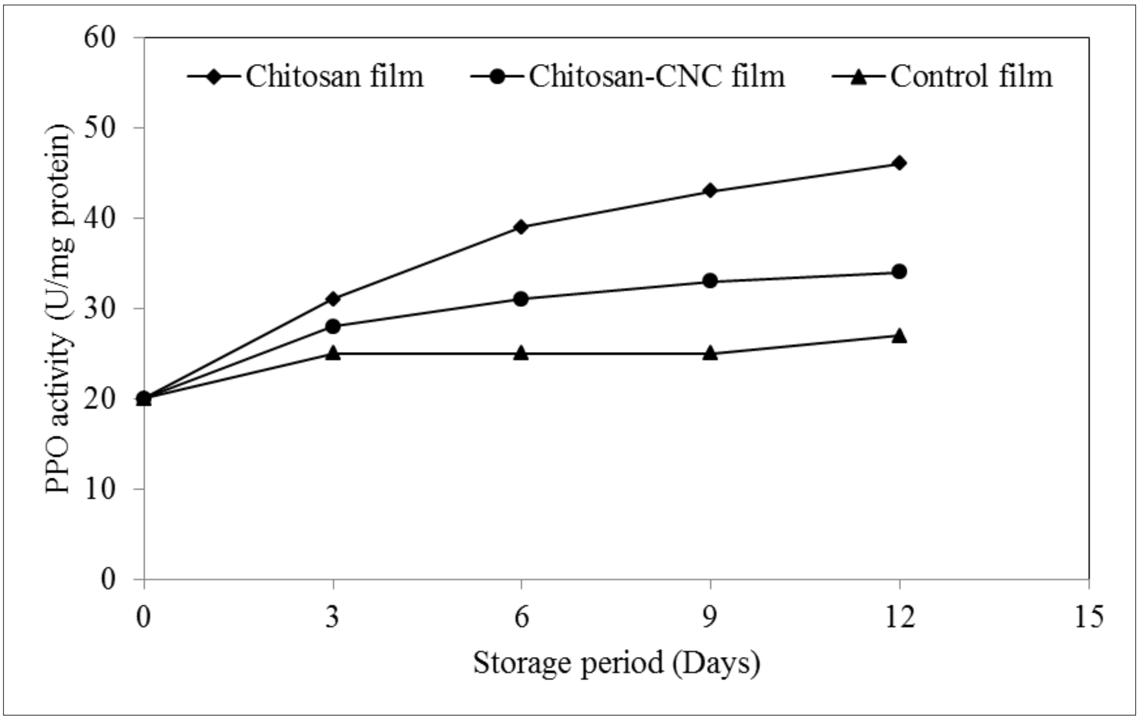

Fig. 2. Effect of different types of biofilms on PPO activity of WBM.

Table 2. ANOVA for effect of packaging type and storage period on PPO activity of WBM

Source of variation	df	Sum of squares	Mean squares	F-calculated
Packaging film (PF)	2	916.761	458.381	10.763*
Storage period (SP)	4	1813.180	453.295	10.644*
PF×SP	8	512.216	64.027	1.503^{NS}
Error	30	1277.626	42.588	

From the ANOVA depicting the effect of packaging type and storage period on PPO activity of WBM (Table 2), It can be observed that both packaging film type and storage period had a significant effect (*p<0.05) on PPO activity of WBM, whereas the interaction had a non-significant effect.

Effect on BI of WBM

WBM have shorter shelf-life due to faster colour deterioration (from white to brown colour) within a short time. BI is a measure of the colour of WBM cap which helps in understanding colour deterioration, a critical factor for consumer acceptance. The BI of WBM stored in all the types of packaging film showed increasing trend with increase in storage period (Fig. 3). The control film showed the lowest BI change (24.51-58.61), followed by chitosan -CNC film (24.51-67.07) and chitosan film (24.51-71.93), respectively. The PPO activity showed direct implications on the L*, a*, b* values and further on the BI. Lower the PPO activity, lower will be the enzymatic browning and lower will be the BI. Similar trend of results was reported for mushrooms by various researchers (10, 12).

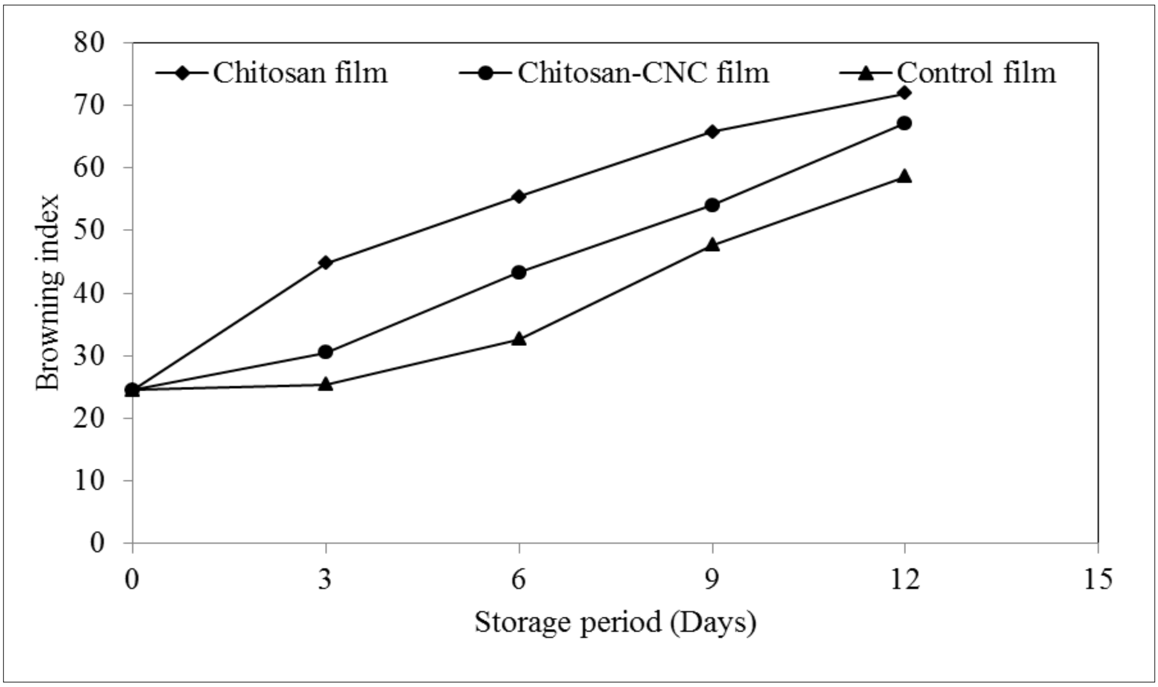

Fig. 3. Effect of different types of biofilms on BI of WBM.

ANOVA depicting the effect of packaging type and storage period on BI of WBM (Table 3), shows that both packaging film type and storage period had a significant effect (*p<0.05) on BI of WBM, whereas the interaction effect showed non-significant effect. This increase in the BI with advancement in storage period is due to the enzymatic browning of WBM due to the PPO. The brown colour greatly affects the marketability and consumer acceptance of WBM (20).

Table 3. ANOVA for effect of packaging type and storage period on BI of WBM

Source of variation	df	Sum of squares	Mean squares	F-calculated
Packaging film (PF)	2	1628.919	814.459	9.695*
Storage period (SP)	4	9940.299	2485.075	29.583*
PF×SP	8	520.300	65.038	0.774^{NS}
Error	30	2520.122	84.004	

(* -Significant at 5 % level, NS- Non Significant)

Effect on DL of WBM

DL or percent cap opening is the percentage of open veils due to cap opening of WBM (index of mushroom ageing and water loss) over a storage period. The cap opening is a clear indicator of WBM quality. Effect of different type of biodegradable films on DL of WBM stored for 12 days are shown in Fig. 4 and Table 4. The packaging type, storage period and their interaction effect show significant effect (*p<0.05) on DL of WBM.

The DL of the WBM increased with the increase in the storage period for WBM stored in all types of biodegradable films. However, the WBM stored in control film showed lesser DL, followed by chitosan-CNC film and chitosan film (chitosan film showed highest DL). There was no cap opening observed for WBM packaged with chitosan-CNC and control films on 3rd day of storage. The WL showed significant effect on the WBM cap opening. With increase in the WL, WBM showed increase in open caps. Similar trends were reported previously for WBM (12, 20).

Table 4. ANOVA for effect of packaging type and storage period on DL of WBM

Source of variation	df	Sum of squares	Mean squares	F-calculated
Packaging film (PF)	2	2419.827	1209.913	12.249*
Storage period (SP)	4	32692.380	8173.095	82.744*
PF×SP	8	3012.222	376.528	3.812*
Error	30	2963.265	98.775	

(* -Significant at 5 % level)

Effect on VA, OD and OA

The average values for sensory attributes like VA, OD and OA are tabulated in Table 5. It was observed that all the sensory attributes scores decreased with the increase in storage period, which is due to the qualitative deterioration of WBM. Considering the sensory score of 6 as market acceptable, the mushrooms can be stored up to 6 days in developed film, up to 9 days in control film and up to 3 days in chitosan film, respectively with the acceptable quality (Table 5). Similar decreasing trend of sensory score results were reported for button mushrooms (21, 22).

Table 5. Effect of different types of biofilms on appearance OD and OA of WBM

Storage period (Days)	Chitosan film			Chitosan-CNC film			Control film		
	VA	OD	OA	VA	OD	OA	VA	OD	OA
0	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00
3	8.11	8.67	8.67	8.44	8.78	8.78	8.78	8.78	8.78
6	5.78	7.44	5.67	7.00	8.00	8.00	7.78	8.00	8.11
9	4.33	3.33	3.89	5.00	4.22	4.56	6.11	6.22	6.00
12	2.44	1.11	2.00	3.33	2.44	2.89	4.33	2.78	3.78

ANOVAs for appearance, OD and OA on WBM stored in different packaging films and stored over the period of 12 days are shown in Table 6-8. The type of packaging film, storage period and their interaction effect showed significant effect (*p<0.05) on appearance, OD and OA of WBM.

Thus, it can be inferred that the type of packaging film has the significant effect (*p<0.05) on all the parameters like WL, PPO activity, BI, DL, VA, OD and OA of WBM stored for 12 days, respectively.

Further, it can be inferred that the WBM stored in developed chitosan-CNC film showed better quality than chitosan film for a storage period of 6 days. However, the WBM stored in control film showed the best quality parameters for storage period of 9 days.

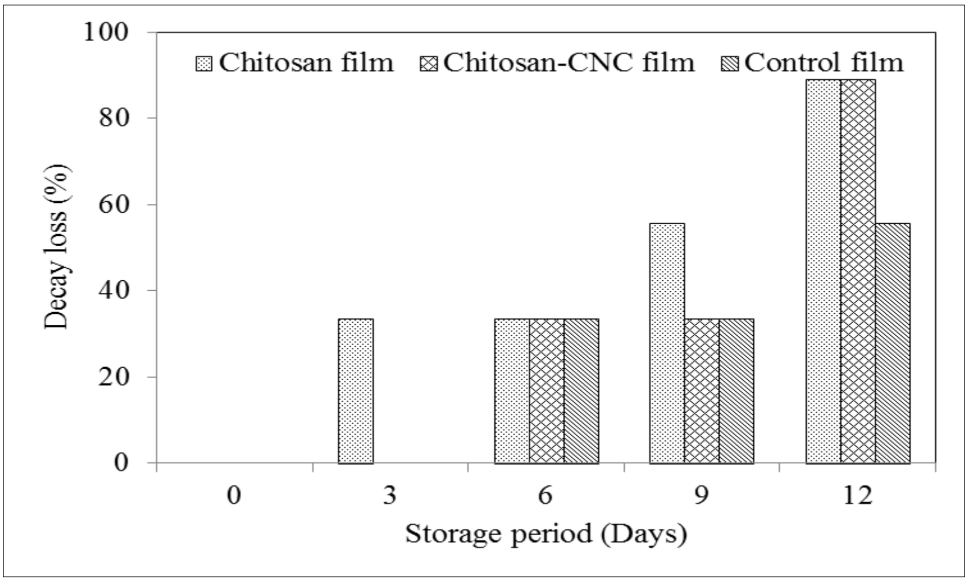

Fig. 4. Effect of different types of biofilms on DL of WBM.

Table 6. ANOVA for effect of packaging type and storage period on VA of WBM

Source of variation	df	Sum of squares	Mean squares	F-calculated
Packaging film (PF)	2	36.193	18.096	24.430*
Storage period (SP)	4	579.215	144.804	195.485*
PF×SP	8	14.474	1.809	2.443*
Error	120	88.889	0.741	

(* -Significant at 5 % level)

Table 7. ANOVA for effect of packaging type and storage period on OD of WBM

Source of variation	df	Sum of squares	Mean squares	F-calculated
Packaging film (PF)	2	24.637	12.319	42.101*
Storage period (SP)	4	968.993	242.248	827.937*
PF×SP	8	30.696	3.837	13.114*
Error	120	35.111	0.293	

(* -Significant at 5 % level)

Table 8. ANOVA for effect of packaging type and storage period on OA of WBM

Source of variation	df	Sum of squares	Mean squares	F-calculated
Packaging film (PF)	2	38.104	19.052	48.075*
Storage period (SP)	4	748.415	187.104	472.131*
PF×SP	8	31.452	3.931	9.921*
Error	120	47.556	0.396	

(* -Significant at 5 % level)

Conclusion

The results of this study clearly demonstrate that the type of packaging film significantly affects the quality and shelf life of WBM during storage. Among the three films tested; chitosan film, chitosan-CNC film and PLA-PBAT control film-mushrooms packaged in chitosan film maintained acceptable quality for up to 3 days, while those in chitosan-CNC and control films remained acceptable for up to 6 and 9 days, respectively. The chitosan-CNC film outperformed the pure chitosan film due to its enhanced barrier properties, attributed to the incorporation of CNC from spent lemongrass. This led to reduced WL, lower PPO activity and improved sensory attributes. The PLA-PBAT control film showed the best overall performance, owing to its lowest WVP, which effectively minimized moisture loss, delayed browning and reduced decay. Overall, the study highlights the crucial role of packaging material in preserving mushroom quality. While the control film performed best, the chitosan-CNC film emerges as a promising biodegradable and sustainable alternative. Its improved functional performance over chitosan film, combined with its environmental sustainability and low-cost raw materials, positions it as a viable commercial solution for extending the shelf life of perishable produce. This supports the growing demand in the food industry for eco-friendly and scalable packaging technologies.

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Authors' contributions

KM contributed to the conceptualization, visualization, experimentation, investigation, data curation, software analyses, interpretation and original draft writing and preparation. SP provided supervision and contributed to draft reviewing and editing. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: On behalf of all authors, the corresponding author states that there is no conflict of interest.

Ethical issues: None

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