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EFFECT OF EDIBLE COATING AND NEEM OIL EXTRACT ON SHELF-LIFE ENHANCEMENT OF BUTTON MUSHROOM (AGARICUS BISPORUS)

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Mushrooms have a short life after harvesting due the physiological factors that enhances respiration and transpiration and thus quality preservation is indispensable during post-harvest handling and storage of button mushroom. Several edible coatings and naturally occurring essential oils have been evaluated for their effectiveness in maintaining sensory quality and increasing antioxidant levels in button mushroom (*Agaricus bisporus*). In the present investigation, freshly harvested button mushrooms were coated with sodium alginate (2.5 and 2.75%) and soy protein (2.5 and 2.75%) incorporated with 0.5 and 1.0 per cent neem oil extract. The effectiveness of the edible coating on key quality characteristics of button mushrooms during 18 days of refrigerated storage was studied. Coating of button mushrooms with sodium alginate and soy protein isolated containing neem oil extract significantly improved qualities characteristics *viz*, during controlling the physiologically loss in weight, water activity, reduced browning index and decay per cent and thus resulted in extended shelf life and better quality characteristics of mushrooms. The effectiveness of (2.4% Sodium alginate + 0.5% Neem oil extract) with 0.5 per cent neem oil extract proficiently maintained quality reducing oxidative stress of button mushrooms up to 18 days as compared to control.

Key words : Button mushrooms, Edible coating, Sodium alginate, Soy protein, Neem oil extract, Shelf life.

Introduction

Mushrooms are a delicious, nutrient-dense, promising therapeutic dietary source for humans. According to reports, growing saprophytic edible fungi, such as mushrooms is an effectual technique of using up cellulosic wastes. Button mushroom (Agaricus bisporus) is fleshy, spore-bearing fruiting body of a fungus belonging to Agaricaceae family. Today, 34 billion kilograms of mushrooms are produced globally each year and currently, 0.13 million tonnes of mushrooms are produced in India. In India, the top three mushroom-producing states are Uttar Pradesh, Kerala and Tripura (NHB) 2022-2023. The button mushrooms have a white cap, stalk, flesh and brown gills. It is one of the most extensively cultivated mushrooms in the world (Raquel and Maria, 2011). They are rich in proteins, fats, carbohydrates, vitamin B complex like Riboflavin (B₂), Niacin (B₃), Pantothenic acid (B_s) , ergosterols (provitamin D_s) and minerals such as (selenium, potassium and copper). Notable amounts

of the amino acids such as aspartic acid, glycine, serine, threonine, glutamine, cysteine, valine, alanine, leucine, isoleucine, lysine, histidine, proline, tyrosine, arginine, phenylalanine and threonine are also present. On a dry weight basis, the crude protein content of button mushrooms ranges from 19 to 38 per cent (Usman et al., 2021 and Rhee et al., 2011). The structure and composition of mushrooms makes them extremely perishable and susceptible to undesirable changes that render them unfit for human consumption. Owing to its high-water content (85-95%) mushrooms tend to become discolored, deformed and unfit for human consumption. The rate of water loss during storage is greatly dependent on the structure of the mushroom, temperature, relative humidity, air movement and atmospheric pressure (Diamantopoulou and Philippoussis, 2015). Due to microbial attack, water loss, browning and senescence, button mushrooms have a shelf life of 3-4 days only (Beelman et al., 2003). They are generally stored in

plastic trays and covered with perforated polyvinyl chloride film at low temperature. Utilizing the appropriate parameters can increase the shelf life of mushrooms. Post-harvest processing and storage conditions can significantly affect changes in colour, size, firmness, maturity stage, blemishes, flavour, nutritional value and safety (Thakur et al., 2022). Plant-based extracts such as (peppermint oil, lemongrass, neem oil extract, aloe vera extract etc.) can be successfully used on fresh commodities to improve and preserve their quality and increase storage life. Neem extract has antioxidant, antifungal, antibacterial and therapeutic properties due to presence of bioactive compounds such as azadirachtin, margolonone, gedunin, salannin, nimbidin (Martinez et al., 2006). Now-a day's edible coatings can be applied as moisture or gas barriers on fruits and vegetables together with the temperature and relative humidity control for extending shelf life of fruits and vegetables. These coatings aids in reducing moisture loss and lowering oxygen uptake and act as a medium for food additives like antioxidants or antimicrobials agents which minimize degradation without compromising the quality of the food (Mahajan et al., 2018). Today's consumers are health conscious and prefer fresh fruits and vegetables, which have pressed researchers to develop eco-friendly coatings and packaging techniques to enhance the shelf-life of food products. Diverse ranges of edible coatings are available which are effective at extending the shelf life of produce. Therefore, the objective of this study was to evaluate the effect of the sodium alginate, soy protein and neem oil extract on the quality and shelf life extension of button mushroom.

Materials and Methods

Fresh mushrooms were procured from the mushroom research and training unit, Division of Plant Pathology, SKUAST-Jammu, India and immediately brought to the laboratory.

Coating preparation : Sodium alginate (2.5%, w/w)/soy protein (2.75% w/w) was dissolved in distilled water (300 ml) with continuous stirring (magnetic stirrer at 500 rpm) at 70°C until complete dissolution and a clear solution was achieved. Two percent glycerol (w/w) was added into the formulation to increase coating flexibility. Neem oil extract (0.5 and 1.0%, w/w) was added as a lipid source to increase water barrier and anti-microbial characteristics of coating film. To induce the gelling mechanism and cross linking reaction, 2 per cent calcium lactate was dispersed in distilled water. Six combinations of edible coatings were prepared using sodium alginate, soy protein and neem oil extract, respectively.

Coating of button mushroom : Coating applications were performed using dipping and draining periods as stated by Parreidt et al. (2018). Mushrooms were divided into seven lots (300g) were dipped in 2 per cent calcium lactate solution for 2 min and the residual solution was allowed to drip off for 1 min. Further the mushrooms were immersed into the alginate solution for 2 min and the excessive hydrocolloid solution was drained for 1 min. Afterwards, coated samples were immersed into a calcium lactate solution for 2 min to achieve gel formation and the residual solution was allowed to drip off for 1 min. Subsequent to the coating process, mushrooms were dried at room temperature ($\sim 20^{\circ}$ C) for 35 min. After the edible coating dried, the mushrooms were packed in polypropylene bags (100g each) and 8 perforations were made using pin and stored for 18 days at a temperature of 4°C and a 90-95% relative humidity (RH). The quality of the samples was analyzed at 0, 6, 12 and 18 days of storage and quality parameters including weight loss, color, browning index, changes in chemical constituents were measured at selected days.

Physico-chemical analysis

Physiological loss in weight : Samples of the coated button mushrooms in packaging material under refrigerated condition were taken and weighed to evaluate the loss in weight during storage (Qu *et al.*, 2020)

Weight loss
$$(\%) = \frac{(W_i - W_s)}{W_i} \times 100$$

Where,

 $W_i = initial weight and$

 $W_s =$ weight at storage period

Decay percentage : Decay percentage was calculated based on number of mushrooms showing symptoms of decay (D) to the total number of mushrooms per packets (TP) at the end of 21 days of storage.

$$ID(\%) = D/TP \times 100$$

Where, ID = Incidence of decay (%)

D = Decay(g)

TP = Total number of mushrooms per packets (g)

Water activity (a_w) : The water activity (a_w) of the sample measured at room temperature (25°C) using a water activity meter (Aqualab pre, Decagon Device, USA).

Browning index : The browning index was calculated using the following expression (Oz and Eker,

2022):

$$BI = 100 \times \left[\frac{X - 0.31}{0.17}\right]$$

Where,

$$X = \frac{\left(a^* + 1.75L^*\right)}{\left(5.645L^* + a^* - 3.012b^*\right)}$$

Colour analysis (L*, a*, b*) : The colour of the coated button mushrooms was evaluated by measuring L* (light/dark), a* (red/green) and b* (yellow/blue) parameters by means of Hunter lab colorimeter (Minolta model CR-410, Tokyo, Japan). The a* value ranges from -100 (greenness) to +100 (redness), the b* value ranges from -100 (blueness) to+100 (yellowness), while the L* value, indicated the measure of lightness ranging from 0 (black) to 100 (white).

Total plate count : The number of bacteria in the sample was counted using a standard plate count (SPC) approach (Srivastava and Bala, 2016). It is an agar pate technique used to identify the bacterial population. 10 ml of the sterilized, melted and cooled agar media were added to each dilution after 1 ml of each dilution was placed to a petri dish. As immediately as the medium was added, the plate was carefully turned to ensure that the organisms were evenly distributed and the agar was left to solidify. The previous colonies on the plates were then counted after the plates had been in the incubator for 48 hours.

$$CFU/ml = \frac{(Number of colonies per ml plated)}{(Total dilution factor)}$$

Sensory evaluation : The edible coated button mushrooms were analyzed on the basis of colour, taste, texture and overall acceptability by semi-trained panel (9-10 judges) using 9 point Hedonic scale assigning scores 9-like extremely to 1-dislike extremely. A score of 5.5 and above was considered acceptable (Amerine *et al.*, 2013).

Statistical analysis : The result obtained were statically analyzed using completely randomized design (CRD) for interpretation of the results through analysis of variance (Gomez and Gomez, 1984).

Results and Discussion

Weight loss in mushroom is most common natural phenomenon occurring mainly due to moisture evaporation and loss of carbon owing to respiration process. Fig. 1 shows reduction in weight loss for both coated and uncoated mushrooms. Treatments with different edible coatings and neem oil extract had a significant effect in controlling weight loss and it might be that edible coatings and neem oil extract helped in reducing the rate of respiration and transpiration. According to the findings of Gupta et al. (2015) different types of desiccants like silica gel, sorbitol, citric acid and calcium chloride in button mushrooms helped in preventing wilting and reduced microbial growth and extended shelf-life. It is evident by the research findings of Singh et al. (2016) that different types of washing techniques like 0.5 per cent KMS, 0.5 per cent NaCl and 0.5 per cent CaCl, reduced the weight loss, which might be due to the reason that these chemical helped in maintaining the cellular organization and regulating enzyme activities which reduced moisture loss during storage period. The mean weight loss of coated mushrooms was observed from 1.61 to 6.57 per cent during storage period which might be due to their hydrophilic nature and polysaccharide-based coating exhibit limited water barrier properties (Plesoianu and Nour, 2022). The impacts of coating materials as a semi permeable barrier against moisture mostly contributed to the reduction in weight loss in the coated fruits (Kocira et al., 2021).

Decay per cent showed an increasing trend during storage period. Treatment T_1 (Control) recorded the completed decay 100 per cent whereas, the minimum mean decay per cent was recorded in treatment T_{4} (2.5%) Sodium alginate + 0.5% neem oil extract) of 8.61 per cent after 18 days of storage (Fig. 1). Khodaei et al. (2021) stated that strawberries coated with carboxymethyl cellulose (CMC), low methoxyl pectin (LMP), Persian gum (PG) and tragacanth gum (TG) edible coating all showed an increase in fruit decay during storage. Uncoated strawberries exhibited the highest level of decay at the 16 day of storage, while the lowest decay per cent was recorded for the strawberries coated with carboxymethyl cellulose (CMC). The mean value of decay per cent increased from 6.89 to 30.16 per cent during 18 days of storage period. According to Tanada et al. (2005) edible coatings significantly reduced the decay compared to the control sample because edible coating retards the ripening of fruits, protect the strawberries against microbial exposure and thus reduce the decay level. Also, the high respiration rate, moisture content of mushroom led to rapid senescence, browning, decay and microbial infection.

Water activity is a measure of amount of available water which supports the growth of different microorganisms and is as intrinsic factor for the determination of the shelf life of food products. Fresh button mushroom had very high water activity of 0.98.

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Fig. 1: Effect of edible coating and neem oil extract on (a) physiological loss in weight (%) and (b) decay (%) of button mushroom during refrigerated storage.

Table 1 : Effect of ed	ible coatings and n	eem oil extract on	water activity (a)	of button mushroom	during refrigera	ated storage.

Trootmonts (T)	Storage period (days)						
Treatments (1)	0	6	12	18	Mean		
T ₁ Control	0.98	0.80	0.69	0.53	0.75		
T ₂ Sodium Alginate (2.5%)	0.98	0.94	0.89	0.81	0.91		
T ₃ Soy Protein (2.75%)	0.98	0.88	0.79	0.68	0.83		
T_4 Sodium Alginate (2.5%) + Neem oil extract (0.5%)	0.98	0.96	0.91	0.85	0.93		
T_5 Soy Protein (2.75%) + Neem oil extract (0.5%)	0.98	0.91	0.82	0.73	0.86		
T_6 Sodium Alginate (2.5%) + Neem oil extract (1.0%)	0.98	0.93	0.85	0.76	0.88		
T_{γ} Soy Protein (2.75%) + Neem oil extract (1.0%)	0.98	0.84	0.73	0.61	0.79		
Mean	0.98	0.89	0.81	0.71			
Effects C.D _(P=0.05)							
Treatment 0.03							
Storage 0.02							
Treatment × Storage 0.01							

With loss of moisture from the mushrooms during the respiration process resulted in a fast decrease in water activity Thakur et al. (2020). The highest mean water activity was recorded in treatment T_4 (2.4% Sodium alginate + 0.5% neem oil extract) whereas, the lowest mean value was recorded in treatment T_1 (Control) are shown in Table 1. This might be due to the nature of edible coating that helped in maintaining the water activity of commodities during storage periods by creating a moisture barriers properties around the product surface which slowed the rate of moisture loss during storage period. The mean water activity of coated mushrooms decreased significantly from 0.98 to 0.71 per cent during 18 days of storage. The results are in accordance with the findings of Thakur et al. (2020) in button mushroom coated with apple peel powder and CMC coating. They further stated that edible coating material adheres on the surface of product, which increases the thickness of coating layer around mushroom surface and when the thickness of coating layer increases, it reduces the moisture loss from mushroom and finally increases in the water activity of coated mushrooms.

Treatment with edible coating and neem oil extract led to slight change in the colour of button mushroom from what to yellow, though browning was significantly controlled and BI effectively reduced during refrigerated storage. The highest mean browning index was observed in treatment T_1 (Control) whereas, the lowest mean value was observed in treatment T_4 (2.5% Sodium alginate + 0.5% neem oil extract) are presented in Table 2. It is evident from the results that there was an increase in browning index with the increase in the storage time which might be due to enzyme oxidation, microbial growth and senescence that caused colour changes (Djekic *et al.*, 2017). Similar findings have been reported by Alikhani-Koupaei *et al.* (2014) in microencapsulated mushroom

	Browning index				Total plate count (× 10 ⁴ cfu/g)					
Treatments (T)	Storage period (days)				Storage period (days)					
	0	6	12	18	Mean	0	6	12	18	Mean
T ₁ Control	9.23	19.61	32.63	97.78	39.81	1.78	4.21	7.24	9.97	5.80
T ₂ Sodium Alginate (2.5%)	9.23	12.74	15.30	28.46	16.43	1.78	3.07	4.19	4.98	3.51
T ₃ Soy Protein (2.75%)	9.23	18.11	23.86	40.94	23.04	1.78	3.31	4.64	5.39	3.78
T_4 Sodium Alginate (2.5%) + Neem oil extract (0.5%)	9.23	11.58	13.59	24.90	14.83	1.78	2.98	3.13	3.76	2.91
T_5 Soy Protein (2.75%) + Neem oil extract (0.5%)	9.23	16.97	21.31	36.81	21.08	1.78	3.27	4.41	5.21	3.67
T ₆ Sodium Alginate (2.5%) + Neem oil extract (1.0%)	9.23	15.13	19.19	31.60	18.79	1.78	3.25	4.24	5.11	3.60
T_7 Soy protein (2.75%) + Neem oil extract (1.0%)	9.23	18.64	25.70	68.76	30.58	1.78	3.38	5.27	7.14	4.39
Mean	9.23	16.11	21.65	47.04		1.78	3.35	4.73	5.94	
	Effects Treatment Storage Treatment × Storage		C.D 2.02 1.10 0.05	P=0.05)	EffectsCTreatment0.Storage0.Treatment × Storage0.		C.D _{(P} 0.03 0.02 0.06	=0.05)		

Table 2 : Effect of edible coatings and neem oil extract on browning index and total plate count ($\times 10^4$ cfu/g) of button mushroomduring refrigerated storage.

reflecting lower PPO activity which might be due to oxidation of phenolic substrates by PPO and also the effect of low oxygen on browning frequently attributed to polyphenol oxidase (PPO). They also stated that peroxidase (POD) is an important oxyradical detoxification enzyme that causes browning, off-flavour, loss of nutrients and leading to discoloration.

Table 3 reflects that the maximum mean value of a* and b* was recorded in treatment T₁ (Control) whereas, the lowest mean value of a* and b* was recorded in treatment T_{4} (2.4% Sodium alginate + 0.5% neem oil extract). The mean L* value followed decreasing trend which might be due to the washing of mushrooms with acetic acid, which significantly reduced the L* value (Zalewska et al., 2018). A significant decrease in L* value with the application of alginate coating was also observed by Oz and Eker (2022), which might be due to hygroscopic nature of alginate. As expected, the L* value decreased with time and after 18 days of the storage, the lowest L* value of (66.5) was recorded in treatment T (Control). There was increase in a* and b* values of edible coated mushrooms during refrigerated storage. In all the treatments, the incorporation of coating mixture determined a significant increase in a* values. Sedaghat and Zahedi (2012) also reported an increase in the a* (redness) and b* (yellowness) of carboxymethyl cellulosecoated mushrooms during storage at 7°C.

The decrease in L* value during storage might be due to occurrence of enzymatic browning reactions. Some enzymes which are responsible for browning react with the product and brown pigmentation occurs (Bandral *et al.*, 2022). The enzymatic reaction decreases and the evolution of the brown pigmentation due to two precise mechanisms of phenol oxidation by the activation of tyrosinase or spontaneous oxidation enzymatic browning results in PPO-catalyzed oxidation of phenolic substrates to quinones which exposes their dark reaction pigments known as melanins (Gupta *et al.*, 2016).

The data in Table 2 depicts that during refrigerated storage, initial microbial load of button mushroom on the day of coating was found as 1.78×10^4 cfu/g. The highest mean total plate count was recorded in treatment T_1 (Control) whereas, the lowest mean value was recorded in treatment T_4 (2.5% Sodium alginate + 0.5% neem oil extract). Moradian et al. (2018) evaluated the microbial enumeration of mushroom sample packed in bacterial cellulose-based active membrane during refrigerated and found increase in microbial load in all the samples. The total plate count showed an increasing trend during 18 days of storage period. This increase might be due to coating process, the antimicrobial properties as well as the barriers properties of coating solution provided protection to the mushroom against the microbial attack from outside (Thakur et al., 2021).



Fig. 2 : Effect of edible coating and neem oil extract on overall acceptability (Hedonic scale) scores of button mushroom during refrigerated storage.

Fig. 2 depicts the effect of edible coatings and neem oil extract on sensory characteristics of coated button mushroom. The highest colour score was recorded in treatment T_4 (2.5% Sodium alginate + 0.5% neem oil extract). The decrease in colour score might be due to physiological changes that caused colour changes in mushroom in high speed (Plesoianu and Nour, 2022). Zalewska et al. (2018) suggested that mushrooms darkening by acids could be assigned to the damage caused by these compounds in the hyphae cell walls letting for contact between polyphenols and polyphenoloxidase (PPO). The finding of Jiang et al. (2012a) stated that chitosan-oil coatings were more effective in retarding mushroom sensory deterioration. The browning in case of mushrooms is attributed to the action of polyphenol enzyme, bacteria and mold on the mushroom tissue. The chitosan oil coating prevented the formation of brown

Table 3: Effect of edible coatings and nee	m oil extract on colour	values of button	mushroom	during refr	igerated storage.
0				0	0 0

Treatments (T)			Moon			
freatments (1)		0	6	12	18	
T ₁ Control	L*	90.6	82.3	74.4	66.5	78.5
1	a*	0.31	2.56	6.91	9.78	4.89
	b*	7.96	13.35	17.18	39.93	19.61
T ₂ Sodium Alginate (2.5%)	L*	90.6	87.2	83.8	80.9	85.6
-	a*	0.31	1.71	2.24	2.80	1.77
	b*	7.96	9.52	10.61	18.80	11.72
T ₃ Soy Protein (2.75%)	L*	90.6	84.5	80.3	77.3	83.2
	a*	0.31	2.18	2.67	3.17	2.08
	b*	7.96	12.90	15.77	24.84	15.37
T_4 Sodium Alginate (2.5%) + Neem	L*	90.6	88.6	84.9	81.7	86.5
oil extract (0.5%)	a*	0.31	1.64	2.11	2.74	1.70
	b*	7.96	8.80	9.63	16.72	10.78
T ₅ Soy Protein (2.75%) + Neem oil	L*	90.6	85.4	81.8	78.1	84.0
extract (0.5%)	a*	0.31	2.04	2.48	3.04	1.97
	b*	7.96	12.30	14.48	22.88	14.41
T_6 Sodium Alginate (2.5%) + Neem	L*	90.6	86.8	82.3	79.7	84.9
oil extract (1.0%)	a*	0.31	1.89	2.36	2.94	1.88
	b*	7.96	11.22	13.18	20.35	13.18
T ₇ Soy Protein (2.75%) + Neem oil	L*	90.6	83.1	79.4	74.8	82.0
extract (1.0%)	a*	0.31	2.31	2.95	3.64	2.30
	b*	7.96	12.96	16.59	36.76	18.57
Mean	L*	90.6	85.4	81.0	77.0	
	a*	0.31	2.05	3.10	4.02	
	b*	7.96	11.58	13.92	25.75	
Effects			$C.D(P \le 0.05)$			
			L*	a*	b *	
Treatment			0.12	0.03	0.08	
Storage			0.04	0.02	0.02	
Treatment × Storage		0.16	0.06	0.21		

patches and improved the appearance and color. Similarly, Villaescusa and Gil (2003) observed that the Pleurotus mushrooms when packed under modified conditions along with silica gel reflected no change in aroma and odor up to 7 days at 4°C. Similarly finding were reported by Deell et al. (2006) on the effect of sorbitol containing sachets on the quality of broccoli stored in MAP in the presence of KMNO, decrease in taste score of both coated and uncoated mushroom might be due to browning and enzymatic activity. Gupta et al. (2016) observed that browning index and enzymatic activity increased during storage that decreased appearance, flavor, texture and taste. According to Djekic et al. (2017) postharvest loss of firmness in mushrooms is mainly caused by several factors such as cell growth, high water content, water migration, protein and polysaccharide degradation and loss of cell tangency. Similarly, the maximum mean texture score of 7.85 was recoded in treatment T_{4} (2.5%) Sodium alginate + 0.5% neem oil extract), which decreased to 6.85 during 18 days of storage, which might be due to the progress of mushrooms senescence during the postharvest stage, where in the firmness of mushroom was lost rapidly, contributing to their short shelf life and the ability to microbial contamination. The firmness of Agaricus bisporus after harvest have a rapid loss, after 16 days of cold storage at 4°C due to the reason that mushrooms different from other fruits and vegetables, mushrooms lack a pectin structure. The mean of overall acceptability scores decreased from 7.67 to 5.62 per cent during 18 days of storage period. According to the Gupta et al. (2016), overall acceptability scores decreased with storage and mushroom packed with sorbitol attained highest acceptability, which might be due to onset of senescence. Mushroom packed with CaCl_a retained more firmness as compared to other desiccants like citric acid, sorbitol. According to Huang et al. (2019) the colour of mushroom gills continuously became browner and more asymmetrical for all the evaluated conditions. Browning of shiitake mushrooms was ascribed to the action of polyphenylene oxide (PPO) enzyme and microorganisms on its tissue (Jiang et al., 2012b).

Conclusion

Overall, edible coating extends the shelf life of button mushrooms and delay maturation. In our study edible coating had significant effects on the quality and storage life of button mushrooms packed in Polypropylene bag. Our results demonstrated that the button mushroom treated with (2.5% Sodium alginate + 0.5% neem oil extract) could be storage up to 18 days at 4°C without significant deterioration in their quality.

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