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Fungal contamination studies in edible coated fresh cut vegetables

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Abstract
The present study was investigated to identify the presence of fungal colonies in edible coated fresh cut carrot, cabbage, and French bean during storage. Totally ten different treatments used for the study including control. Significance differences were observed among the treatments and control. Among ten different treatments, T_4 (HPMC 10% + Whey protein 0.5% + Clove oil 1%) showed the less number of fungal colonies in all the three crops taken for the study followed by T_2 (HPMC 10% + Casein 0.5% + Clove oil 1%). These results were revealed that the treatments given with clove oil found to be reducing the fungal growth of edible coated vegetable during storage.

Keywords: fresh cut vegetables, edible coating, carrot, cabbage, french bean, storage studies

Introduction
Vegetables are essential compounds of daily diet and are highly demanded in the recent years from most of the population. They are act as a reservoir of vitamins, essential minerals, antioxidants, bio-flavonoids, dietary fibres and flavour compounds. India ranks second in world vegetable production but about 30% of vegetables are damaged due to insects, microorganisms, pre and post-harvest handling, poor storage, facility, etc.

Preservation of vegetables is a big challenge for world. Edible coating is an effective method which provides a barrier to oxygen, microbes of external source, moisture and solute movement for food. But it is still needed to improve the quality and shelf-life of fresh-cut commodities. It offers excellent prospects for extending the shelf life of fresh-cut produce by reducing the deleterious effects caused by minimal processing operations. Edible coatings are defined as the thin layer of material which can be consumed and provide a barrier to oxygen, microbes of external source, moisture and solute movement for food. In edible coating a semi permeable barrier is provided and is aimed to extend shelf life by decreasing moisture and solute migration, gas exchange, oxidative reaction rates and respiration as well as to reduce physiological disorders on fresh cut fruits (E. Baldwin *et al*., 1996) [2]. This study focuses on microbial contaminations on different types of edible coatings on fresh-cut vegetables.

Materials and Methods
The field experiment was carried out in Complete Randomized Design with three replications with a treatment combination of ten at Department of Vegetable Science, Horticultural College and Research Institute, Tamil Nadu Agricultural University, Coimbatore.

The vegetables *viz.*, Carrot, Cabbage and French bean were selected with uniform size, colour and shape with lack of blemishes and disease symptoms. Vegetables carrot and French bean were used in study were thoroughly washed with cold water and then dried under shade.

In Cabbage, the outer leaves were removed and the clean heads were taken for the study

The selected vegetables were cut into uniform size pieces and given with 10 different treatments as detailed below. After imposing the edible coating, the samples were allowed to dry in the shade for half an hour and kept under refrigerator at 7 ^{O}C for storage. Then the random samples in each treatment for each treatment for each vegetable was taken for the fungal contamination studies.

Table 1: Treatment combinations

S. No.	Treatments
T_1	HPMC 10% + Casein 0.5% + Clove oil 0.5%
T_2	HPMC 10% + Casein 0.5% + Clove oil 1%
T_3	HPMC 10% + Whey protein 0.5% + Clove oil 0.5%
T_4	HPMC 10% + Whey protein 0.5% + Clove oil 1%
T_5	HPMC 10% + Soy protein 0.1% + Clove oil 0.5%
T_6	HPMC 10% + Soy protein 0.1% + Clove oil 1%
T_7	HPMC 10% + Casein 0.5%
T_8	HPMC 10% + Whey protein 0.5%
T_9	HPMC 10% + Soy protein 0.1%
T_{10}	HPMC alone 10% (Control)

The chosen samples of fresh cut carrot, cabbage, and French bean samples were macerated. From that 1 g of sample was taken and diluted in 10 ml of water. From that, 100µl solution was added with 900 µl water to made 1 ml by serial dilution. Serial dilution was done three times for the 4th day and four times for the 8th & 12th day. Finally, 1 ml sample made through serial dilution was imprinted on medium containing potato (200g), dextrose (20g), rose Bengal chloramphenicol agar (20g) and one litter water to estimate the fungal population colonies after 48 hours of inoculation as per procedure given by Allen (1953) [1].

Results and Discussion

Effect of edible coatings on the growth of total fungal population of fresh cut cabbage showed an increasing trend during the storage period and presented in Table 2. Significant differences were observed among the treatments from day 4 to day 12 for *Penicillium* sp. and *Aspergillus* sp. It was found that T_4 was recorded the lowest number of fungal colonies in all the three days (4th, 8th and 12th) of observation *viz.*, 6.00, 8.35 and 11.00 and 10.33, 18.00, 27.00 for (T_{10}) control.

In present study, it was found that fungal count was increased with increase in the storage period of in the fresh cut cabbage under different edible coating treatments. This indicates that the microbial load which cause the postharvest storage loss in long term of storage. Out of 10 treatments, T_4 (11.00 10^3CFUg^{-1}) recorded the lowest fungal count even after 12 days of storage and the control treatment T_{10} (27.00 10^4CFUg^{-1}) recorded the highest fungal population on the final day of storage. This showed the reports given by Berrera-Necha *et al.* (2009) [3].

Effect of edible coatings on the growth of total fungal population of fresh cut carrot also showed an increase throughout the storage period (Table 3). Significant differences were observed among the treatments from day 4 to day 12 for *Penicillium sp.* and *Aspergillus sp.* It was found that T_4 was recorded the lowest number of fungal colonies in all the three days (4th, 8th and 12th) of observation *viz.*, 15.33, 22.00 and 25.00. For (T_{10}) control 29.67, 39.33, 45.67 respectively.

Out of 10 treatments T_4 (25.00 10^4CFUg^{-1}) recorded the lowest fungal count on 12th day and T_{10}-control (45.67 10^4CFUg^{-1}) recorded the highest fungal population on the final day of storage. Table 2 indicated that there is continuous increase in the tested fungi in control treatments compared to all other edible coating treatments from day one to 12 days of storage. This may due to the relative humidity in cold storage and the stability of refrigerator temperature for fungi. Chitosan inhibits growth of several fungi (Jiang and Li, 2001) by inducing chitinase, a defence enzyme. This present study was conformity with study of Zhang and Quantick, 1998 [7]; El Ghaouth *et al.*,1992a [5]; Cheah *et al.*, 2016[4].

Effect of edible coatings on the growth of total fungal population of fresh cut French bean also showed an increasing trend along the storage period (Table 4). Among the edible coating treatments T_4 (19.67 10^3CFUg^{-1}) registered the minimum fungal load. The maximum fungal load was recorded in T_{10} (41.00 10^3CFUg^{-1}) on the final day of storage.

Present study indicated that there is continuously increase in tested fungi in control treatments where is increasing more than other edible coating treatments compared from zero time to 12 days of storage. The above investigation showed the similar finding from Berrera-Necha *et al.* (2008)[3] who reported the essential oils of cinnamon (*Cinnamomum zeylanicum*) and clove (*Syzygium aromaticum*) inhibited the conidial germination and reduced growth of *C. gloeosporioides*. Infection by postharvest pathogenic fungi was also reduced by clove oil which is having antifungal properties. Because these essential oils which have low mammalian toxicity, are biodegradable and non-persistent in the environment the possibility of developing essential oils for use in control postharvest diseases may be advised for enhancing storage lie with lower level of fungal growth.

Table 2: Effect of edible coatings on the growth of total fungal population CFU g^{-1} of fresh cut cabbage

Treatments	Storage at 7 °C			
	Day 1 (10^3CFUg^{-1})	Day 4 (10^3CFUg^{-1})	Day 8 (10^4CFUg^{-1})	Day12 (10^4CFUg^{-1})
T1	-	7.15	11.00	17.33
T2	-	6.33	9.00	14.00
T3	-	7.33	10.32	16.00
T4	-	6.00	8.35	11.00
T5	-	8.00	11.67	17.67
T6	-	6.67	9.67	15.33
T7	-	9.00	14.33	22.33
T8	-	8.00	12.07	19.67
T9	-	9.67	16.25	24.00
Control	-	10.33	18.00	27.00
Mean	-	7.85	12.07	18.43
S. Ed.	-	0.15	0.20	0.40
CD (0.05)	-	0.31**	0.43**	0.83**

T1: HPMC 10% + Casein 0.5% + Clove oil 0.5%
T2: HPMC 10 % + Casein 0.5% + Clove oil 1%
T3: HPMC 10% + Whey protein 0.5% + Clove 0.5%
T4: HPMC 10% + Whey protein 0.5% + Clove oil 1%
T5: HPMC 10% + Soy protein 0.1% + Clove oil 0.5%
T6: HPMC 10 % + Soy protein 0.1% + Clove oil 1%
T7: HPMC 10% + Casein 0.5%
T8: HPMC 10% + Whey protein 0.5%
T9: HPMC 10% + Soy protein 0.1%
T10: HPMC 10% only (Control)
CFU: Colony Forming Unit

Table 3: Effect of edible coatings on the growth of fungal population CFU g^{-1} of fresh cut carrot.

Treatments	Storage at 7 °C			
	Day 1 (10^3CFUg^{-1})	Day 4 (10^3CFUg^{-1})	Day 8 (10^4CFUg^{-1})	Day12 (10^4CFUg^{-1})
T1	-	19.33	25.67	32.33
T2	-	15.33	22.67	27.67
T3	-	18.00	24.33	30.00
T4	-	15.33	22.00	25.00
T5	-	20.00	26.00	34.00
T6	-	16.33	23.00	28.67
T7	-	24.00	29.00	37.00
T8	-	22.67	27.67	35.67
T9	-	26.00	30.33	39.67
Control	-	29.67	39.33	45.67
Mean	-	20.67	27	33.57
S. Ed.	-	0.49	0.56	0.68
CD (0.05)	-	1.03**	1.18**	1.43**

T1: HPMC 10% + Casein 0.5% + Clove oil 0.5%
T2: HPMC 10 % + Casein 0.5% + Clove oil 1%
T3: HPMC 10% + Whey protein 0.5% + Clove 0.5%
T4: HPMC 10% + Whey protein 0.5% + Clove oil 1%
T5: HPMC 10% + Soy protein 0.1% + Clove oil 0.5%
T6: HPMC 10 % + Soy protein 0.1% + Clove oil 1%
T7: HPMC 10% + Casein 0.5%
T8: HPMC 10% + Whey protein 0.5%
T9: HPMC 10% + Soy protein 0.1%
T10: HPMC 10% only (Control)
CFU: Colony Forming Unit

Table 4: Effect of edible coatings on the growth of total fungal population CFU g^{-1} of fresh cut French bean

Treatments	Storage at 7 °C			
	Day 1	Day 4 (10^3CFUg^{-1})	Day 8 (10^4CFUg^{-1})	Day12 (10^4CFUg^{-1})
T1	-	7.67	15.67	28.00
T2	-	6.00	11.67	22.00
T3	-	7.00	14.33	26.67
T4	-	5.33	10.33	19.67
T5	-	8.33	16.00	29.33
T6	-	6.67	13.00	25.00
T7	-	9.67	20.33	35.67
T8	-	9.33	18.67	35.67
T9	-	10.00	23.67	38.00
Control	-	12.00	25.00	41.00
Mean	-	8.2	16.87	30.1
S. Ed.	-	0.20	0.48	0.75
CD (0.05)	-	0.43	1.01	1.56

T1: HPMC 10% + Casein 0.5% + Clove oil 1%
T2: HPMC 90 % + Casein 0.5% + Clove oil 1%
T3: HPMC 10% + Whey protein 0.5% + Clove 1%
T4: HPMC 90% + Whey protein 0.5% + Clove oil 1%
T5: HPMC 10% + Soy protein 0.5% + Clove oil 1%
T6: HPMC 90 % + Soy protein 0.1% + Clove oil 1%
T7: HPMC 10% + Casein 0.5%
T8: HPMC 90% + Whey protein 0.5%
T9: HPMC 10% + Soy protein 0.1%
T10: HPMC 90% only (Control)
CFU: Colony Forming Unit

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