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Preparation of Edible Coatings and to Study their Effect on Shelf Life of Green Vegetables

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Abstract—*The objective of the current study was to prepare chitosan coating with ginger and green tea extracts. Vegetables coated with the formulations were studied with reference to non-coated ones for 10 days at refrigeration temperature.1% chitosan solutions were incorporated with ginger extracts (0.5%) and green tea extracts (0.5%). The coated and control samples were analysed based on various parameters including moisture content, pH, microbial and sensory attributes. The antioxidant activity of the chitosan-green tea coating was more pronounced than the chitosan-ginger coating. Thus, it can be concluded that chitosan coating with extracts is a promising candidate for maintaining the quality of green vegetables and extending their post harvest life. Also, out of the two formulations chitosan-green tea coated samples proved to be more efficient than in terms of microbial and sensory characteristics.*

1. INTRODUCTION

Horticultural produce like fruits and vegetables tend to respire even after harvest serving a major cause for post-harvest losses.It is known that about 70 percent of fruits and vegetables produced in India are wasted [world economic forum, 2014]. The factors affecting spoilage are carbon dioxide, oxygen, ethylene ratios, growth state, etc (Jianglian D.2013). These losses may also be due to contamination by insects and microorganisms (Cháferet all 2012).

Broccoli is a good source of vitamins A, C and E and also comprises of antioxidant and free radical scavenging activities (Moreira et al 2011). Green asparagus and broccoli have a very short shelf life and prone to physiological changes (change in texture and color) and microbial contamination (FUCHS et al 2008).

Interest in natural preservation of foods has increased in recent years due to increase in consumers' demands in safe foods without synthetic additives (Moreira et al 2011).Edible coatings are thin layers of edible material, which can provide barrier to moisture, oxygen, and solute movement for the food (Dhall 2013).

Different edible matrixes have been formulated, including lipids, polysaccharides and proteins, either alone or in combination. Chitosan is biodegradable cationic polymer obtained from the exoskeleton of crustaceans and arthropods with unique polycationic nature, antifungal,antibacterial properties and approved by United States Food and Drug Administration (USFDA) as a Generally Recognized as Safe (GRAS) food additive (USFDA 2013). It maintains tissue firmness and reduces microbial contamination by forming a semi permeable barrier on the surface of fruits or vegetables, thus reducing moisture loss and controlling gas exchange (Moreira et al 2011).

Green tea is known to have good antioxidant activity. It is proved to be effective against some bacteria. Therefore, green tea extracts can also be incorporated in chotisan coating matrix to increase the efficiency of the coating (Siripatrawan& Harte 2010).

2. MATERIALS AND METHODS.

This study aimed at increasing shelf life of green vegetables (broccoli, asparagus and green beans) using chitosan with green tea and ginger extracts. For control, vegetables were dipped in water.

2.1 Preparation of Coating- forming solutions.

The method used by Paul et all 2010 was referred with a little modification.200 ml distill water was taken in a beaker and kept on magnetic stirrer at45◦ C. Aqueous solution of acetic acid (0.5%) was added while stiring.1% chitosan (w/v) was added to the beaker slowly within a time span of 5 hours. While stirring, the prepared ginger / green tea extracts (0.5%) were incorporated. After the addition of extracts, tween 80 (0.1%) and glycerol (0.1%) were added. The mixture was stirred for 6 hours and filtered at the end to remove impurities. The coating was applied on the surface of vegetables.

2.2 Preparation of ginger extract.

Ginger extract was obtained by boiling 10g of ginger in 200 ml of distilled water for 30 mins and straining through muslin cloth at the end.

2.3 Preparation of green tea extract.

Green Tea extract was obtained by boiling 10g green tea in distilled water for 30 mins and a filtrate was obtained (Yen and chen1995).

2.4 Coating the vegetables.

Vegetables were bought from local market and randomly divided into 3 samples. 3 different categories of samples were: (1) control, (2) 1% chitosan coating with green tea extract, (3) 1% chitosan coating with ginger extract.The vegetables were dipped in solution for 1 minute. For control, samples were dipped in distill water for 1 minute. The samples were kept on a blotting paper for 20 mins and were air dried.The vegetables were stored for 10 days at refrigeration temperature and subsequently were were analyzed on the 3^{rd}, 5^{th} and 7^{th} day.

2.5 Analysis

2.5.1 Moisture content.5g of samples were weighed in a moisture dish and kept in oven at 105◦ Celsius for 4 hours. The weight of the dried sample was taken after cooling the dishes in desiccator.The moisture content was calculated using the following formula:

$(W_2- W_3)/ (W_2-W_1) \times 100$

W_1= weight of empty Petri dish

W_2= weight of Petri dish with sample before drying

W_3= weight of Petri dish after drying

2.5.2. Sensory evaluation. The attributes (appearance, colour and texture) were measured subjectively at room temperature by 10 panelists (untrained). Panelists were given both coated and control vegetables. Each panelist was asked to rate the vegetables quality attributes namely color, texture, odour and appearance. A 9-point hedonic scale with 1=dislike extremely, 5=neither like nor dislike, 9=like extremely was used for rating the product.

2.5.3. Microbial analysis. All samples (coated and control) were analyzed on 3^{rd}, 5^{th} and 7^{th} day to study the trend of microbial growth. Total Plate Count (IS:5402:2002)was carried out by Plate count agar. The plates were incubated at 37° C for 48 hours. E.Coli Count (IS:5403:1999) was done using Eosin methylene blue agarand count was taken after incubation at 37°C for 48 hours. Yeast and mould count (IS:5403 1999) was carried out using Chloramphenicol yeast glucose agar and incubated at 25°C for 5 days after which the colonies were counted.The results were compared with the control (uncoated samples).

Formula used was; CFU/ml= (number of colonies× dilution factor)/ volume taken for platin

2.5.4.pH. The coated and uncoated vegetables were crushed and pH was measured by pH strip.The results of the coated samples were compared with control.

2.5.5. DPPH test (antioxidant activity). The antioxidant activity of extracts was evaluated using DPPH (2,2- diphenyl-1 – picrylhydrazyl). 3ml of the extracts were mixed with 1ml of methanolic solution of DPPH. The mixture was vortexedand incubated in the dark at room temperature for 30 mins. At 517nm the absorbance was in a spectrophotometer (Siripatrawan& Harte 2010).

Formula Used:

DDPH scavenging effect (%) = $(Abs_{DPPH} - Abs_{extract} / Abs_{DPPH}) \times 100$

Where, $Abs_{DPPH}$and$Abs_{extract}$ represent the absorbance value of methanolic solution of DPPH and sample, respectively at 517nm.

2.5.6. Total Phenolic Content. The total phenolic activity of green tea and ginger extract coatings were determined.0.1ml of the sample extract was mixed with 7ml distilled water and 0.5ml of Follin's reagent. Then the mixture was incubated for 8 mins at room temperature. After incubation 1.5ml of sodium carbonate and 0.9ml of distilled water were added followed by storing it in a dark chamber at room temperature for 2 hours. After incubation, the absorbance was measured at 765nm using a spectrophotometer (Siripatrawan& Harte 2010).The concentration of total phenolic compounds in the samples is expressed as gallic acid equivalents. 5 concentrations of the standard (gallic acid) were also measured on the spectrophotometer to obtain the standard curve of concentration v/s absorbance.

3. RESULTS AND DISCUSSIONS.

3.1. Moisture Content.

The moisture content of the samples was measured on the 3rd, 5th and 7th day and graph was plotted. It wasinferred that the moisture content reduced significantly on the 5th and 7th day of the control samples.

In the case of asparagus, the moisture content of the control ranged from 97.215 % on the 3rd day to 91.041 on the 7th day, leading to a loss of moisture to around 6%. On the other hand the coated asparagus have shown 1-1.5% moisture loss.

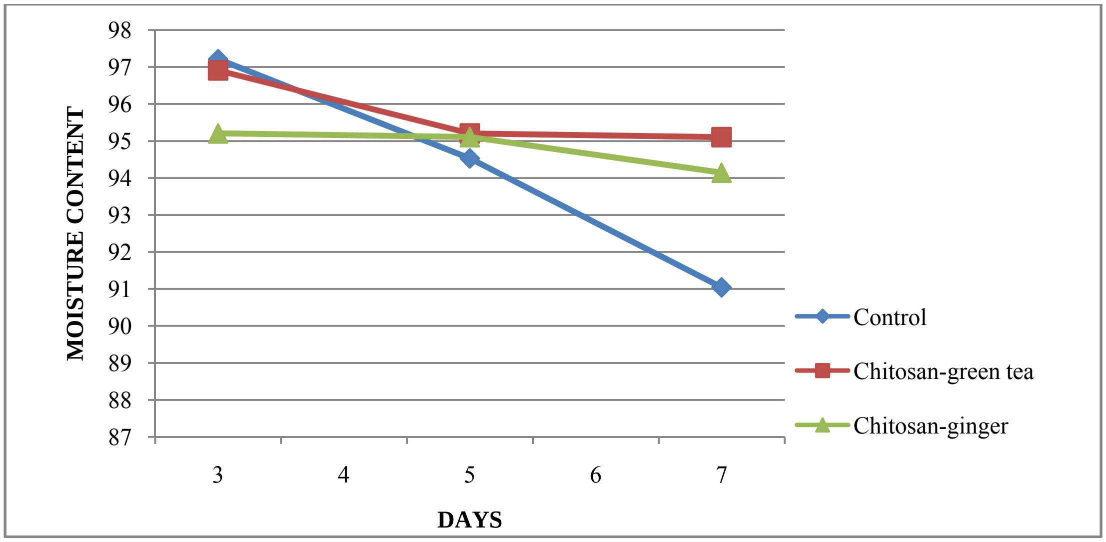

Fig. 1: Moisture content of asparagus kept at refrigeration for storage of 10 days.

The results are similar in case of broccoli. The control broccoli samples had moisture content 92.167% on the 3rd day and 83.471 on the 7th day, giving a loss of around 8% which was almost 4 times the coated samples. The coated samples have shown less moisture loss, around 2.4 %(in the case of chitosan-green tea coated samples) and 2.9% (in case of chitosan-ginger coated samples).

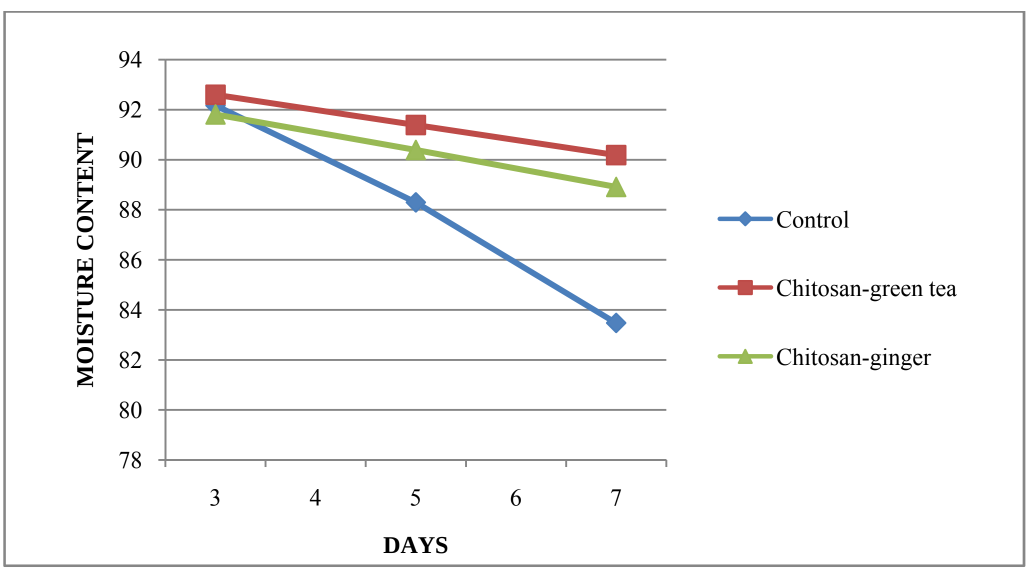

Fig. 2: Moisture content of broccoli kept at refrigeration for storage of 10 days.

Therefore, it can be seen that chitosan coating prevents moisture loss. In the past it has been reported that chitosan coating retards moisture loss in fresh peeled litchi (Dong et al 2004). Thus, it can be concluded that coatings help to reduce moisture loss by serving as a barrier between the vegetable surface and the environment by reducing the water vapour gradient between the two (Maftoonazad et al 2006).

3.2 Microbiological Test.

The microbiological tests were studied at the 3rd, 5th and 7th days on the refrigerated samples of coated vegetables and control and the results were tabulated and plotted on graphs.

Table 1: Total plate count of asparagus and broccoli kept at refrigeration for storage of 10 days.

Days	Control asparagus log(cfu/g)	Chitosan-green tea asparaguslog(cfu/g)	Chitosan-ginger asparagus log(cfu/g)	Control broccoli log(cfu/g)	Chitosan-green tea broccoli log(cfu/g)	Chitosan-ginger broccoli log(cfu/g)
3	5.05	4.21	4.29	4.96	4.12	4.21
5	6.108	5.18	5.25	5.28	4.54	4.91
7	6.805	5.78	5.94	5.99	4.65	5.01

The TPC values of the control asparagus samples were compared with the coated chitosan-green tea and chitosan-ginger samples. Also, the Chitosan-green tea and Chitosan-ginger coated asparagus samples were compared with each other. It was noted that the asparagus control samples had high total viable count on all the three days with the values 5.05, 6.108 and 6.805 log (cfu/g).On the other hand, the total viable count of Chitosan-green tea coated samples varied from 4.21-5.78 log (cfu/g) and that of Chitosan-ginger coated samples varied from 4.29-5.94 log (cfu/g). This indicates that the growth on the coated samples was low as compared to the control samples. Out of the two coated samples, better results were obtained in the case of Chitosan-green tea coated asparagus by showing comparatively less total viable growth.

The control and coated asparagus were tested for coliform growth on 3rd, 5th and 7th day. As per the tabulated result, it can be seen that the untreated asparagus showed the highest coliform growth as compared to the coated asparagus. Initially the coliform growth was absent in all the three samples. The Coliform growth on the 5th day were observed to be 5.575, 4.2 and 4.1log (cfu/g) for control, Chitosan-green tea coated and Chitosan-ginger coated asparagus, respectively. On the 7th day, it was seen that the readings were 6.098 log (cfu/g) for the control and 4.52 and 5.08 log (cfu/g) for the Chitosan-green tea and Chitosan-gingercoated samples, respectively. Thus, it can be concluded that the chitosan coating is effective in reducing the coliform count in the asparagus, the effect being more pronounced in the case of Chitosan-green tea coated samples.Yeast and mould count of Chitosan-green tea and Chitosan-ginger coated samples were compared to each other and also with the control samples.

As observed, there was no growth initially in all the three samples. On the 5th day, the readings for the control sample were 4.76 log (cfu/g), which increased to 5.12 log (cfu/g) in the 7th day. The growth on the Chitosan-green tea and Chitosan-ginger coated asparagus on the 7th day was 4.24 and 4.28 log (cfu/g), respectively. This shows that chitosan reduces yeast and mould load on asparagus. Moreover, the Chitosan-green tea coated samples showed no growth on the 3rd and 5th day. Therefore, there was more prolonged effect in case of chitosan-green tea coated samples. This leads to the conclusion that chitosan is anti-fungal.The TPC count was observed for control and coated samples of broccoli on the 3rd, 5th and 7th days. As observed, the total viable growth of the control broccoli ranged from 4.96-5.99 log (cfu/g). On the other hand, the TPC growth of the coated samples ranged from 4.12-4.65 log (cfu/g) and 4.21-5.01 log (cfu/g) for Chitosan-green tea and Chitosan-ginger coated broccoli, respectively. As the TPC of Chitosan-green tea coated broccoli was lower than that of Chitosan-ginger coated ones, it clearly indicates that Chitosan-green tea coating was more effective.

Table 2: Coliform counts of asparagus and broccoli kept at refrigeration for storage of 10 days.

Days	Control asparagus log(cfu/g)	Chitosan-green tea asparagus log(cfu/g)	Chitosan-ginger asparagus log(cfu/g)	Control broccoli log(cfu/g)	Chitosan-green tea broccoli log(cfu/g)	Chitosan-ginger broccoli log(cfu/g)
3	0	0	0	4.43	0	0
5	5.575	4.2	4.1	4.85	4.12	4.25
7	6.098	4.52	5.08	5.04	4.35	4.64

It was observed that coliform growth on control samples on the 3rd day was 4.43 log (cfu/g) while it was absent on the coated broccolis. On the 7th day the growth was 5.04, 4.35 and 4.64 log (cfu/g) of control, Chitosan-green tea and Chitosan-ginger coated samples, respectively. Therefore, it can be seen that chitosan coating reduced coliform load on the broccoli as compared to the control.

Table 3: Yeast and mould growth of asparagus and broccoli kept at refrigeration for storage of 10 days.

Days	Control log asparagus (cfu/g)	Chitosan-green tea asparagus log(cfu/g)	Chitosan-ginger asparagus log(cfu/g)	Control broccoli log(cfu/g)	Chitosan-green tea broccoli log(cfu/g)	Chitosan-ginger broccoli log(cfu/g)
3	0	0	0	4.39	0	0
5	4.76	0	4.2	5.16	0	4.12
7	5.12	4.24	4.28	5.49	4.22	4.26

In case of Yeast and Mould growth, there was no growth on the 3rd and 5th days in Chitosan-green tea coated broccoli while there was some growth on the 5th day in Chitosan-ginger coated broccolis. In broccoli treated with Chitosan-ginger coating, there was no growth on the 3rd day but on the 5th and 7th day, 4.12log (cfu/g) and 4.26 log (cfu/g) was observed, respectively. It is evident that chitosan coating reduced the yeast and mould load of broccolis as compared to the control sample. This indicates that chitosan is anti-fungal, having more prolonged effect in case of Chitosan-green tea samples.

Moreira et al in 2011 reported that chitosan treated broccoli showed significant log reduction in the microbial growth as compared to the non-treated ones. As per the results, it was observed that this was in agreement with the study as there was low microbial load in case of coated vegetables as compared to the control samples.This shows that chitosan coating is strongly anti-fungal and also effective against bacterial contamination.Out of the two coatings, it is evident that chitosan-green tea coating was more effective.

3.3. SENSORY EVALUATION

The refrigerated samples were evaluated subjectively on the basis of color, odour, texture and general acceptability. It was observed that maximum score in terms of texture was given to Chitosan-green tea coated sample and minimum score was given to untreated broccoli. Untreated broccoli had an unpleasant odour which was absent or significantly less in the case of coated broccoli. Out of the two coated broccoli, maximum scores are given to the Chitosan-green tea coated broccoli.

As per the results Chitosan-green tea coated beans showed the best color and appearance followed by Chitosan-ginger coated and control beans. The least score has been given to the uncoated beans. There were significant texture changes observed in the case of control beans which were given the least score.

As per the tabulated results, the minimum score was given to the untreated asparagus for the texture. There were significant changes seen in the texture and color of the untreated asparagus. The untreated asparagus was a bit shriveled. Out of the two coated asparagus, higher preference was given to Chitosan-green tea coated asparagus as the texture, color and hence the quality was maintained.

Table 4. Sensory evaluation of the vegetables kept at refrigeration for storage of 10 days.

Parameters	Control broccoli	Chitosan-green tea broccoli	Chitosan-ginger broccoli	Control asparagus	Chitosan-green tea asparagus	Chitosan-ginger asparagus	Control beans	Chitosan-green tea beans	Chitosan-ginger beans
Color & appearance	5.4±0.86	8.6±0.23	6.8±0.36	5.6±0.61	8.5±0.22	8.01±0.74	7.6±0.42	8.9±0.11	8±0
Body & Texture	6.5±0.35	8.5±0.21	8±0	4.4±0.47	8.4±0.31	7.5±0.72	5.1±0.84	8.6±0.15	8.4±0.13
Odour	4.5±0.37	8±0	7.4±0.54	6.5±0.81	8.02±0.20	7.1±0.11	7.6±0.44	8.5±0.24	8.5±0.06
General acceptability	5.5±0.86	8±0	7.5±0.44	4.5±0.54	8.6±0.15	7.6±0.44	6.5±0.65	8.8±0.12	8±0

It can be noted that the coated samples had better sensory quality maintained. Also, it is observed that out of the two coatings, chitosan-green tea coated vegetables were given more preference.Moreira et al in 2011 proved that chitosan coating on broccoli improved the sensory quality of the broccoli and also inhibited the floret opening. Similar results were seen in the vegetables as the sensory quality was maintained in the chitosan treated samples.

3.4. Measurement of pH.

The change in pH was measured during the storage period on 3rd, 5th and 7th days. It was observed that there was not a significant change in the pH of the vegetables. The exception was control samples of broccoli and asparagus which had the lowered values on 7th day.The pH of the rest of the coated beans, asparagus and broccoli were in the range of 6.5-7.5. This insignificant change might be associated to good maintenance quality of the fresh cut produce.

3.5. Antioxidant Activity

3.5.1. Total Phenolic Content. The phenolic groups of the coatings were estimated with the help of folin-ciocalteu reagent.

Table 5: Absorbance of standard gallic acid solutions.

S. no	Concentration (mg/L of Gallic acid)	Absorbance
1	50	0.05
2	100	0.1
3	150	0.18
4	250	0.29
5	500	0.5

Total Phenolic Content of Chitosan-green tea and Chitosan-ginger coating was calculated from the standard graph of absorbance of gallic acid solution. The absorbance was 0.211, which gave the value 197.1339 gallic acid equivalent for green tea, and value for Chitosan-ginger was 190.2061 gallic acid equivalents corresponding to the absorbance of 0.204.

3.5.2. DPPH ASSAY. DPPH assay is based on the ability of DPPH, to be quenched and thus get decolorized by the presence of antioxidants. Absorbance value of 1mM dpph was 2.0186.The DPPH value of Chitosan-green tea coating came out to be 88.012% and that of Chitosan-ginger coating was77.162%.

It was observed that the total phenolic content of the chitosan green-tea coating was higher (197.1339 mg gallic acid/g sample) than the chitosan-ginger coating (190.2061 mg gallic acid/g sample). From the calculations and results it was observed that the antioxidant activity of the coatingswere 77.162% (chitosan-ginger) and 88.012% (chitosan-green tea coating).This indicated that the chitosan-green tea coating showed greater anti-oxidant activity than chitosan-ginger coating.As reported by Siripatrawan and Harte 2010, the antioxidant activity of chitosan film can be increased by tenfold when incorporated with green tea extracts. It was suggested that adding green tea extract in chitosan film improved barrier and mechanical properties of the film and also enhanced the antioxidant activity.

4. CONCLUSION

The chitosan coated samples had reduced microbial counts throughout the storage period with the best results in the case of yeast and mould growth. The coated samples showed retarded moisture loss as compared to the control samples over the period of storage. The antioxidant activity of Chitosan-green tea coating was higher (88.012 %) than Chitosan ginger coating (77.162%). The total phenolic content was also higher in green tea variant, having a value 197.1339 mg gallic acid/ g of sample).Thus, it can be concluded that 1% chitosan coating with incorporated extracts is a promising candidate for maintaining the quality of green vegetables and extending their post-harvest life.Also, out of the two formulations, chitosan-green tea coated samples proved to be more efficient than the other in terms of microbiological and sensory characteristics.

5. ACKNOWLEDGMENT

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