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Effect of Chitosan on biochemical and microbial quality of minimally processed mango (*Mangifera indica* L.) cubes during storage

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ABSTRACT

Fresh-cut mango cubes of cv. Mallika coated with acidified chitosan solutions at concentrations of 1, 2 and 3% along with untreated cubes as control were placed into plastic trays, over-wrapped with cling film (10µ thickness) and stored in refrigerator at 8±2°C for 8 days. Analytical determinations were made after 2, 4, 6 and 8 days. Chitosin coating, especially 2 and 3% was effective in inhibiting loss of weight, total carotenoids and total phenols content. Chitosin 3% coating was highly effective in inhibiting growth of bacteria and yeast/mould on the cubes. At the end of the storage minimum weight loss (2.46%) was observed in 3% chitosan treated cubes and higher weight loss (4.51%) found in control. Higher total phenols (41.79 mg TAE/100 g) were estimated in 2% chitosan treated samples while maximum total carotenoids content (5.35 mg/100 g) were observed with 3% chitosan treatment. Maximum total soluble solids (25.65°Brix) were observed in control. Minimum bacterial load (2.04 log CFU/g) and yeast/mould load (1.78 log CFU /g) was observed with 3% chitosan treatment while maximum bacterial load (3.02 log CFU/ g) and yeast/mould count (3.92 log CFU/g) was found in control. Overall results suggests that chitosan (3%) coating on the mango cubes effectively reduced weight loss, prevented biochemical changes and inhibited microbial growth.

Key words: Edible coatings, mango cubes, microbial growth, biochemical changes.

INTRODUCTION

Minimally processed fruits are one of the major growing segments in food retail markets. However, the major hurdle to commercial marketing of these commodities is limited shelf-life due to excessive tissue softening and microbial growth. Edible coatings are known to improve the quality and prolong the shelf-life of fresh-cut fruits and vegetables. They act as barriers to water loss and gas exchange by developing a micro-modified atmosphere over the surface of the product (Baldwin *et al*., 1). Edible coatings were applied as a thin layer of protective material to the surface of the fruits. Fresh cut fruits are directly dipped into the coating formulations, drained and dried, whereby a thin membranous film is formed over the commodity surface (Tharanathan, 13). Chitosan is a natural polymer, nontoxic and biodegradable, derived by deacetylation of chitin [poly-b-(1 fi 4)-N-acetyl-d-glucosamine]. It has been documented to possess a film-forming property for use as edible films or coating. Chitosan has attracted attention as a potential food preservative of natural origin due to its antimicrobial activity against fungi, yeast and bacteria (Sagoo *et al*., 11) and can improve the storability of perishable foods by modifying the internal atmosphere as well as decreasing the transpiration losses (Zhang and Quantick, 16).

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Numerous studies have been conducted to assess the effect of chitosan coatings on sensory quality and shelf life of fruits such as strawberry (Hermandz-Munoz *et al*., 5), litchi (Zhang and Quantick, 16), pomegranate (Zahran *et al*., 15) and mango (Wang *et al*., 14) but they were applied chitosan coating to whole fruit. Very few studies, were conducted on chitosan coating on minimally processed 'ready to eat' fresh cut fruits (Chien *et al*. 2; Zhelyazkov *et al*. 18). Hence, the present investigation was undertaken to study the effect of chitosan coating on changes in biochemical and microbial quality parameters in minimally processed fresh cut mango cubes under low temperature storage.

MATERIALS AND METHODS

Healthy mature fruits of cv. Mallika were obtained in month of July 2016 from the experimental orchard of the ICAR-Central Institute for Subtropical Horticulture, Lucknow in July 2016. Fruits were washed in tap water and sanitized for 5 min in chlorinated water (200 ppm sodium hypochlorite) and air dried. Fruits were treated with ethylene for ripening. The semi ripe fruits were selected and peeled manually with a stainless steel knife. Fruit cheeks were cut from both sides of the seed and cut into cubes of (4 × 2 × 1 cm^2) size. Chitosan flakes

purchased from Central Drug House (P) Ltd, New Delhi, India (deacetylated chitin; 95%; molecular weight 760 kDa) was ground to a fine powder by extensive grinding in a mortar, washed 3 times in distilled water (20 ml of water per g of chitosan), pelleted by low-speed centrifugation and air-dried at room temperature. The purified chitosan was used for preparation of 1, 2 and 3% solution by dissolving in 0.5% (v/v) glacial acetic acid under continuous stirring, and the pH was adjusted to 5.6 using 1 N NaOH. Fresh-cut mango cubes of cultivar "Mallika" were divided in 4 lots and dipped in acidified chitosan solutions for 2 minutes, air dried and were placed into plastic trays, over-wrapped with cling film (10 microns thickness) and stored in refrigerator at 8±2°C for 8 days.

Weight loss (%) was determined by measuring the difference between initial and final weight of each replicate and results were expressed as a percentage loss of initial weight. Firmness was measured using a 'McCormick fruit tester FT 327' penetrometer and expressed in Newton. Total Soluble Solids (TSS) were measured by using hand refractometer (Erma, Japan), while titratable acidity by titrimetric methods using 0.1N NaOH. Total carotenoids was extracted (by repeated extraction) with petroleum ether and acetone (3:2 v/v, 60-80°C) according to the method of Ranganna (8). The total phenols content was expressed in mg of tannic acid equivalents (TAE)/ 100 g of extract by following Folin-Ciocalteau method. Microbiological analysis was carried out as per method of Speck (12). Mango cubes were dipped in equal weight of sterile distilled water and shook well for 2 min. The surface microbial wash was diluted appropriately and pours plated on Nutrient Agar and Rose Bengal Chloramphenicol Agar for getting counts of bacteria and yeast & moulds, respectively. The plates were incubated at 35±2°C for 72 hr. The data obtained were subjected to statistical analysis by using 'Statistical Software Package for Agricultural Research Workers' software at 5% significance level.

RESULTS AND DISCUSSION

The results indicated that the chitosan coating could retard the weight loss of fresh-cut mango (Fig. 1A). During storage period, the weight loss percentage of uncoated and chitosan 1% treated mango cubes was significantly greater than that of 2 and 3% chitosan coated mango cubes ($p \leq 0.05$). However, there was no significant difference in weight loss between the cubes treated with 2 and 3% chitosan ($p > 0.05$). At the end of storage period, the uncoated sample had 4.51±0.063% loss in weight, whereas the weight loss of samples coated with 1, 2 and 3% chitosan was 3.82±0.047%, 2.57±0.043% and 2.46±0.115%, respectively. The results obtained were in accordance with previous studies in which chitosan was observed to be more effective for reducing weight loss in fresh cut minimally processed apple (Zhelyazkov *et al*., 18) and papaya (Chien *et al*. 2). The firmness is the important quality attribute related to metabolic changes and water content. It influences appearance and consumer acceptability of fresh cut fruits. The firmness of fresh cut cubes decreased with increasing storage time irrespective of treatment (Fig. 1B). Samples coated with the chitosin coating displayed a slower rate of decline in firmness than the uncoated samples. However, during first four days of storage no significant difference ($p > 0.05$) was found between the treatments. After end of the storage period of 8 days 3% chitosin coated cubes displayed ~57% higher firmness than control followed by chitosin 2% (~45%) and chitosin 1% (~29%). Chitosan coating seemed to delay firmness loss during storage because it act as a barrier to water transfer, delaying dehydration, retarding the metabolic and enzyme activities and, therefore, extending the firmness of the coated samples. These findings are in agreement with results of Zhelyazkov *et al*. (18) in apple and Hermandz-Munoz *et al*. (5) in strawberry.

Results depicted in Fig. 2A showed that total carotenoids content dropped as storage period progressed. However, it also shows that chitosan

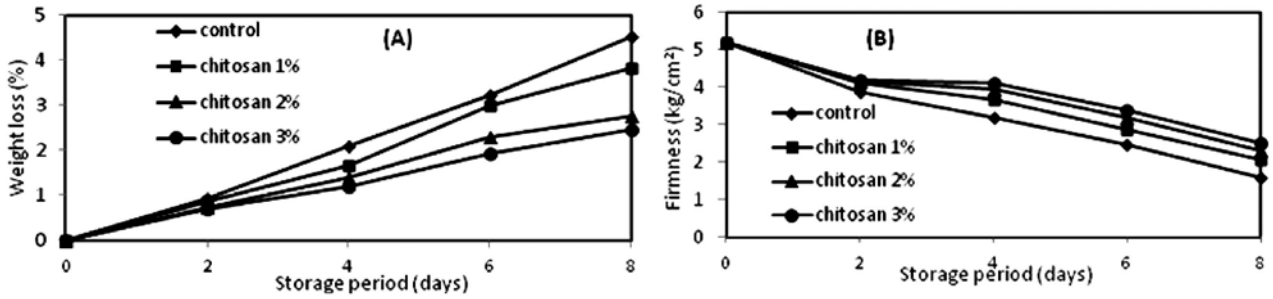

Fig. 1. Effect of chitosan coating on (A) weight loss (%) and firmness (kg/cm^2) in minimally processed fresh cut mango cubes during storage at 8±2°C for 8 days.

played a positive role in controlling degradation and maintaining of total carotenoids in fresh cut mango cubes. During first 2 days of storage, statistically significant difference was not observed among all investigated treatments and carotenoids content ranged from 5.8±0.20 to 6.14±0.11 mg/g. However, after 4 days of storage statistically significant difference was detected between coated and uncoated samples. The chitosan coating was effective in retaining total carotenoids during 8 days storage, as compared to control which exhibited ~44% higher decline (from 5.8±0.20 to 3.24±0.12 mg/100 g). At the end of the storage, the lowest degradation (~13%) of total carotenoids was observed in 3% chitosan coated cubes, from 6.14±0.11 to 5.35±0.09 mg/100 g. Robles-Sanchez *et al*. (9) reported reduction of carotenoids from 4.5 to 3 mg/100 g in fresh-cut 'Ataulfo' mango stored at 5°C for 10 days. Fresh-cut mango cubes stored at 5°C for 9 days showed 25% reduction in total carotenoids (Gil *et al*., 3). The observations illustrated in (Fig. 2B) revealed a declining trend in total phenol content during storage in both coated and control cubes, the decline being more pronounced (57.20%) in case of control (water dipped) cubes. At the end of the storage period of 8 days, chitosin coating 2 and 3% were able to retain significantly higher total phenol content (63.72% and 58.38%, respectively) as compare to control (42.79%). At the end of storage period total phenol content in cubes coated with 0, 1, 2 and 3% chitosan was 21.46±2.37, 24.35±1.61, 29.28±2.19 and 31.96±3.70 mg TAE/100 g, respectively. These results are compatible with the findings of Rodrigues *et al*., (10) in mango and Hermandz-Munoz *et al*. (5) in strawberry, who have also reported higher amount of total phenols in chitosan coated samples. Higher amount of phenols in coated samples may be attributed to the inhibition of polyphenol oxidase activity, an enzyme that is involved in the process of phenolic compound degradation (Jiang and Li, 6).

Changes in the total soluble solids of fresh-cut mango over the storage period are shown in Table 1. The total soluble solids of fruit increased with increasing storage time. After 8 days of storage, the total soluble solids content of uncoated and chitosan-coated samples were significantly different ($p>0.05$). However, the amount of total soluble solids was not significantly different between the fruits treated with 2 and 3% chitosan. Uncoated fresh-cut mango cubes contained higher total soluble solids than chitosan-coated cubes. A plausible explanation for the observed increment in total soluble solids is the considerable loss of water from uncoated cubes during storage. Similar results were obtained with

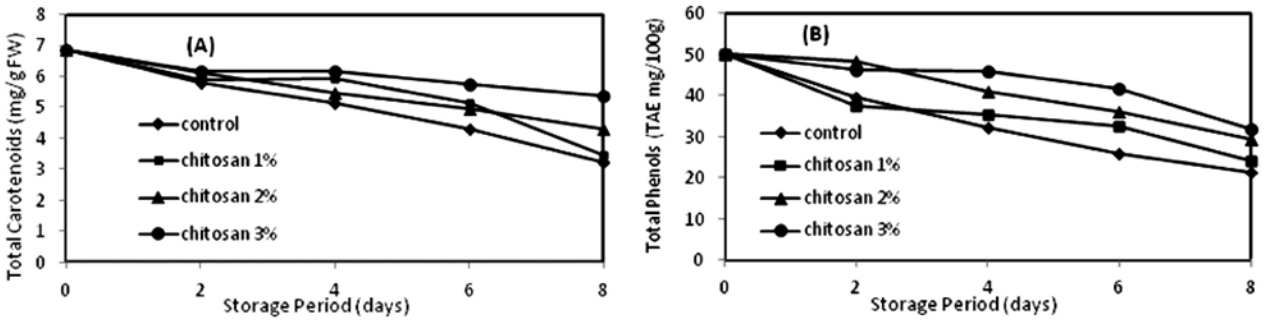

Fig. 2. Effect of chitosan coating on (A) Total carotenoids (mg/g FW) and (B) Total phenols (mg/100 g FW) in minimally processed fresh cut mango cubes during storage at 8±2°C for 8 days.

Table 1. Effect of application of chitosan coating on total soluble solids (TSS) in minimally processed fresh cut mango cubes during storage at 8±2°C for 8 days.

Treatment	Change in TSS during storage				
	Storage period (days)				
	0	2	4	6	8
Control	17.21	21.00 ± 0.24[a]	21.83 ± 0.24[a]	23.94 ± 0.22[a]	25.65 ± 0.15
Chitosan 1%	17.30	20.32 ± 0.17[b]	21.56 ± 0.27[a]	23.70 ± 0.31[a]	24.50 ± 0.12
Chitosan 2%	17.25	19.35 ± 0.11[c]	20.73 ± 0.32[b]	22.75 ± 0.32[b]	23.70 ± 0.20
Chitosan 3%	17.28	18.55 ± 0.14[d]	20.80 ± 0.17[b]	22.52 ± 0.10[b]	23.50 ± 0.21
CD at 5%	NS	0.55	0.17	0.81	0.55

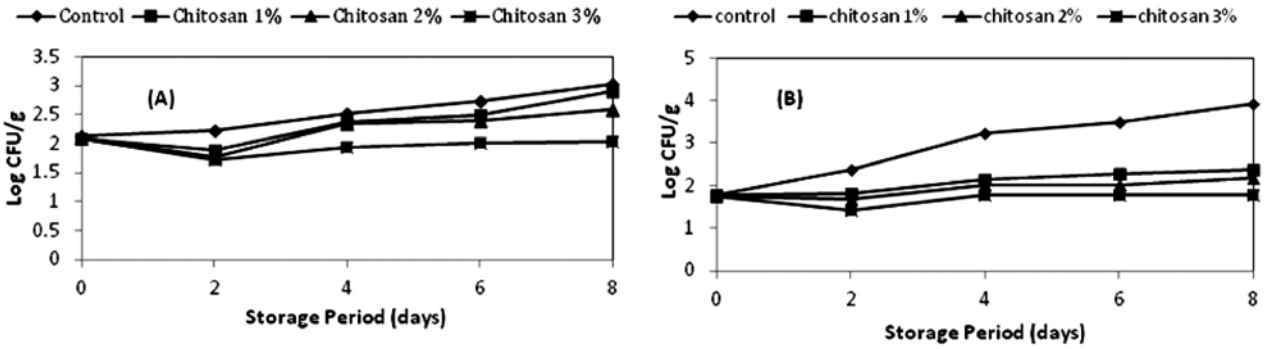

Fig. 3. Effect of chitosan coating on (A) bacterial load (Log CFu//) and (B) yeast/mould load (Log CFU/g) in minimally processed fresh cut mango cubes during storage at 8±2°C for 8 days.

1.5% chitosan coating in strawberry (Hermandz-Munoz *et al*., 5) and 0.8% chitosan coating in whole mango fruits (Wang *et al*., 14). It was found that the total acidity of uncoated and chitosan-coated samples was not significantly different ($p>0.05$). After 8 days of storage, the total acidity of uncoated mango cubes was 0.40±0.02% and 1, 2 and 3% chitosan coated samples were 0.46±0.02, 0.44±0.01 and 0.45±0.02%, respectively. The results obtained in this study indicated that coating with chitosan did not affect the total acidity of samples during storage.

It was observed that Chitosan coating inhibited the growth of microorganism. Initially, during 2 days the microbial load reduced in all chitosin coated samples (Fig. 3A). After 2 days, gradually it starts increasing. Minimum bacterial load (2.04 log CFU/g) and yeast/mould load (1.78 log CFU/g) was observed with 3% chitosan treated cubes while maximum bacterial load (3.02 log CFU/g) and yeast/mould count (3.92 log CFU/g) was found in control at the end of storage (Fig. 3B). The antimicrobial activity of chitosan has been attributed to the interaction between positively-charged chitosan molecules and negatively-charged microbial surfaces results in the disruption of cell membranes, leakage of intracellular constituents, and ultimately, microbial cell death (Kong *et al*., 7). Additionally, according to a second hypothesis, chitosan oligomers can penetrate into prokaryotic cells and interfere with the transcription of RNA and protein synthesis (Hafdani and Sadeghinia, 4). Another mechanism which makes chitosan effective is lower pH due to acetic acid. Our results indicated that anti microbial property of chitosan flim depended upon the concentration of chitosan. Higher anti microbial activity was observed in treatment 3% chitosan film consistent with previously published study (Zhang *et al*., 17) in apple. It can be concluded from the present study that dip treatment with chitosin (2 and 3%) was effective in reducing PLW, prolong firmness, slow degradation of total carotenoids and total phenols and inhibit growth of microbes which help in improving shelf-life of minimally processed mango cubes during storage at 8±2 °C for 8 days.

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