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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 cultivar "Mallika" coated with acidified chitosan solutions at three concentrations (1, 2 and 3%) along with untreated cubes as control were placed into plastic trays, over-wrapped with cling film (10 µ thickness) were stored in refrigerator at 8±2ºC for 8 days and analyzed for quality parameters after 2, 4, 6 and 8 days. Chitosan coatings, especially 2 and 3%, were effective in inhibiting weight loss, total carotenoids and total phenols contents. Chitosan 3% coating was highly effective in inhibiting the growth of bacteria and yeast/mould on cubes. At the end of storage period, the minimum weight loss (2.46%) was observed in 3% chitosan-treated cubes and maximum (4.51%) in control. High total phenol content (41.79 mg TAE 100 g^{-1}) was observed in 2% chitosan-treated samples. Maximum total carotenoids content (5.35 mg 100 g^{-1}) was observed in 3% chitosan treatment. Higher total soluble solid (25.6° Brix) was found in control. Minimum bacterial load (2.04 log cfu g^{-1}) and yeast/mould load (1.78 log cfu g^{-1}) was observed in 3% chitosan-treatment while bacterial load (log CFU g^{-1}) and yeast/mould count was high in (3.02 and 3.92 log CFU g^{-1}, respectively, was in control. The results suggest that 3% chitosan coating on mango cubes effectively minimized weight loss, biochemical changes and microbial growth.

Key words: Biochemical changes, edible coatings, mango cubes, microbial quality

INTRODUCTION

Minimally processed fruits are one of the major growing segments in food retail markets. However, the major hurdle in commercial marketing of these commodities is limited shelf-life due to excessive tissue softening and microbial growth. Edible coatings are known to improve the fruit 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*., 1999). Edible coatings were applied as a thin layer of protective material to the surface of 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, 2003).

Chitosan is a natural polymer, non-toxic and biodegradable, derived by deacetylation of beta 1, 4-D-glucosamine. It possesses a film-forming property so is used as edible film or coating. Chitosan has attracted the attention as a potential food preservative of natural origin due to its antimicrobial activity against fungi, yeast and bacteria (Sagoo *et al*., 2002). It can improve the storability of perishable foods by modifying the internal atmosphere as well as decreasing the

transpiration losses (Zhang and Quantick, 1997). 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*., 2008), litchi (Zhang and Quantick, 1997), pomegranate (Zahran *et al*., 2010) and mango (Wang *et al*., 2007) but they all have applied chitosan coating to the whole fruit. Very few reports are available on chitosan coating on minimally processed 'ready-to-eat' fresh cut fruits (Chien *et al*., 2013, Zhelyazkov *et al*., 2014). Hence, the present study was aimed 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 mango fruits cv. 'Mallika' were procured in July 2016 from the experimental orchard of ICAR-CISH, Lucknow (India). The fruits were washed in tap water, sanitized for 5 min in chlorinated water (200 ppm sodium hypochlorite) and air-dried. The fruits were treated with ethylene for ripening. The semi-ripe fruits were selected and peeled manually with the help of a stainless-steel knife. The fruit cheeks were cut from both sides of the seed and cut into cubes of 4 x 2 x 1 cm^3 size. Chitosan flakes, purchased from Central Drug House (P) Ltd, New Delhi, India, (deacetylated chitin; 95%; molecular weight 760 kDa), were ground to fine powder in a mortar, washed thrice in distilled water (20 mL water g^{-1} chitosan), pelleted by low-speed centrifugation and air-dried at room temperature. The purified chitosan was used to prepare 1, 2 and 3% solutions by dissolving it in 0.5% (v/v) glacial acetic acid under continuous stirring. The pH was adjusted to 5.6 by using 1 N NaOH. The fresh-cut 240 mango cubes were divided equally into 4 lots and dipped in acidified chitosan solutions for 2 min. The cubes were then air-dried, placed in plastic trays, over-wrapped with cling film (10 µ thickness) and stored in a refrigerator at 8±2°C for 8 days.

Weight loss was determined by measuring the difference between initial and final weight of each replicate. Firmness was measured using a 'McCormick fruit tester FT 327' penetrometer. Total soluble solids (TSS) were measured by a hand refractometer (Erma, Japan), and titratable acidity determined by titrimetric methods using 0.1N NaOH. Total carotenoids were extracted (by repeated extraction) with petroleum ether and acetone (3:2 v/v, 60–80°C) (Ranganna, 2000). The total phenols content was assessed as per Folin-Ciocalteau method (Rangana, 2000) and expressed as mg tannic acid equivalents (TAE 100 g^{-1} extract). Microbial analysis was carried out as per Speck (1985). Mango cubes were dipped in equal weight of sterile distilled water and shook well for 2 min. The surface microbial wash was diluted appropriately, pour-plated on nutrient agar and rose Bengal chloramphenicol agar for bacterial and yeast & moulds, respectively, and incubated at 35±2°C for 72 h before estimation. The data was analyzed by using 'Statistical Software Package for Agricultural Research Workers' software at 5% significance level (Sheoran *et al*., 1998).

RESULTS AND DISCUSSION

The chitosan coating retarded the weight loss of fresh-cut mango (Fig. 1A). During storage period, the weight loss of uncoated and 1% chitosan-treated mango cubes was significantly higher than those of 2 and 3% chitosan coated mango cubes ($p \leq 0.05$). However, no significant difference in weight loss was observed between the cubes treated with 2 and 3% chitosan. At the end of storage period, the uncoated samples had 4.51±0.063% weight loss, whereas weight loss in 1, 2 and 3% chitosan coated samples was 3.82±0.047, 2.57±0.043 and 2.46±0.115%, respectively. The results were in line with previous reports wherein chitosan has been observed to be more effective in

reducing weight loss in fresh cut minimally processed apple (Zhelyazkov *et al*., 2014) and papaya (Chien *et al.*, 2013). The fruit firmness is an important quality attribute related to metabolic changes and water content. It influences the appearance and consumer acceptability of fresh cut fruits. The firmness of fresh cut cubes decreased with increasing storage time, irrespective of the treatment (Fig. 1B). The samples coated with chitosan displayed slow decline in firmness than uncoated samples. However, during first 4 days of storage no significant difference was observed between the treatments. At the end of storage period, 3% chitosin coated cubes displayed ~57% higher firmness than control, followed by 2% chitosin (~45%) and 1% chitosin (~29%). Chitosan coating delayed firmness loss during storage as it appeared to act as a barrier to water transfer, delay dehydration, retard metabolic and enzyme activities and, therefore, extend the firmness of coated samples. These findings are in agreement with Zhelyazkov *et al*. (2014) in apple and Hermandz-Munoz *et al*. (2008) in strawberry.

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

Treatments	Changes 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
1% Chitosan	17.30	20.32±0.17^{b}	21.56±0.27^{a}	23.70±0.31^{a}	24.50±0.12
2% Chitosan	17.25	19.35±0.11^{c}	20.73±0.32^{b}	22.75±0.32^{b}	23.70±0.20
3% Chitosan	17.28	18.55±0.14^{d}	20.80±0.17^{b}	22.52±0.10^{b}	23.50±0.21
$CD_{0.05}$	Ns	0.55	0.17	0.81	0.55

The total carotenoids content dropped as storage period progressed (Fig. 2A). It revealed that chitosan plays a positive role in controlling degradation and maintaining the total carotenoids in fresh cut mango cubes. During first 2 days of storage, a non-significant difference was observed in treatments with carotenoid content ranging from 5.8±0.20 to 6.14±0.11 mg g^{-1}. However, after 4 days of storage significant difference was detected between coated and uncoated samples. The chitosan coating was effective in retaining the 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^{-1}). At the end of storage, lowest degradation (~13%) of total carotenoids was observed in 3% chitosan coated cubes (6.14±0.11 - 5.35±0.09 mg 100 g^{-1}). Robles-Sanchez *et al*. (2009) reported reduction of carotenoids from 4.5 to 3 mg 100 g^{-1} 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*., 2006). The data illustrated in Fig. 2B revealed a declining trend in total phenol content during storage in both coated and control cubes with decline more pronounced (57.20%) in control cubes. At the end of storage period of 8 days, 2 and 3% chitosan coatings were able to retain significantly higher total phenol content (63.72 and 58.38%, respectively) as compare to control (42.79%). Also, the total phenol contents in cubes coated with 0, 1, 2 and 3% chitosan were 21.46±2.37, 24.35±1.61, 29.28±2.19 and 31.96±3.70 mg TAE 100 g^{-1}, respectively. These results are compatible with the findings of Rodrigues *et al*. (2015) in mango and Hermandz-Munoz *et al*. (2008) in strawberry who reported higher total phenols in chitosan coated samples. Higher 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, 2001).

The TSS of fruit increased with increasing storage time (Table 1). After 8 days of storage, TSS contents of uncoated and chitosan-coated samples were significantly different. However, the amount of TSS was at par in fruits treated with 2 and 3% chitosan. Uncoated fresh-cut mango cubes had higher TSS than chitosan-coated cubes. A plausible explanation for the observed increment in TSS is the considerable loss of water from uncoated cubes during storage. Similar results were obtained with 1.5% chitosan coating in strawberry (Hermandz-Munoz *et al*., 2008) and 0.8% chitosan

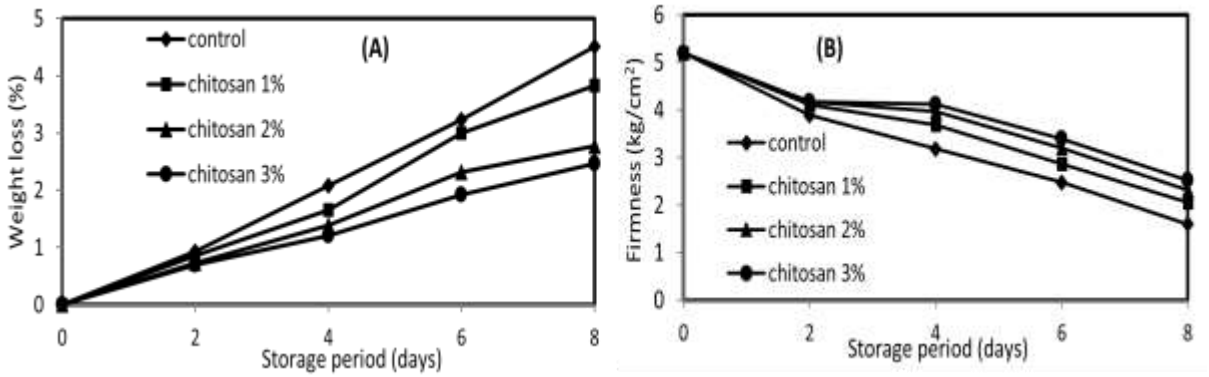

Fig. 1: Effect of chitosan coating on weight loss (left side) and firmness (right side) in minimally processed fresh cut mango cubes during storage at 8±2°C for 8 days

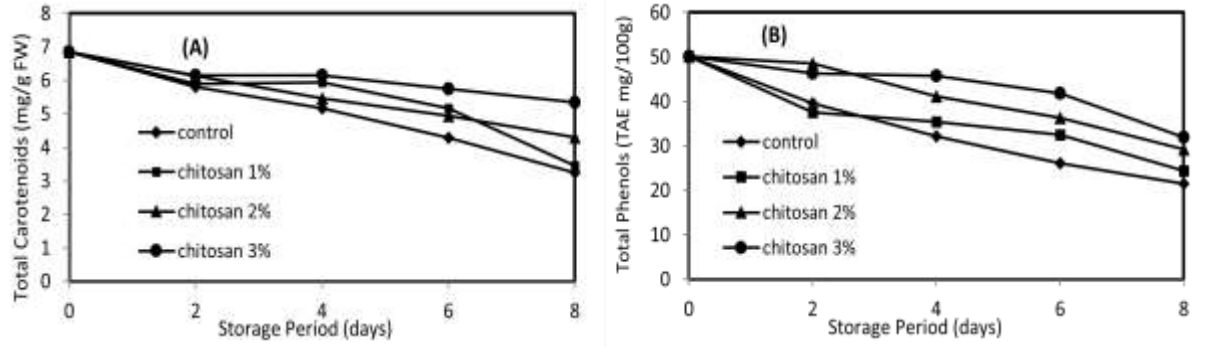

Fig. 2: Effect of chitosan coating on total carotenoids (mg g fresh weight) [left side] and total phenols (mg 100 g^{-1} fresh weight) [right side] in minimally processed fresh cut mango cubes during storage at 8±2°C for 8 days

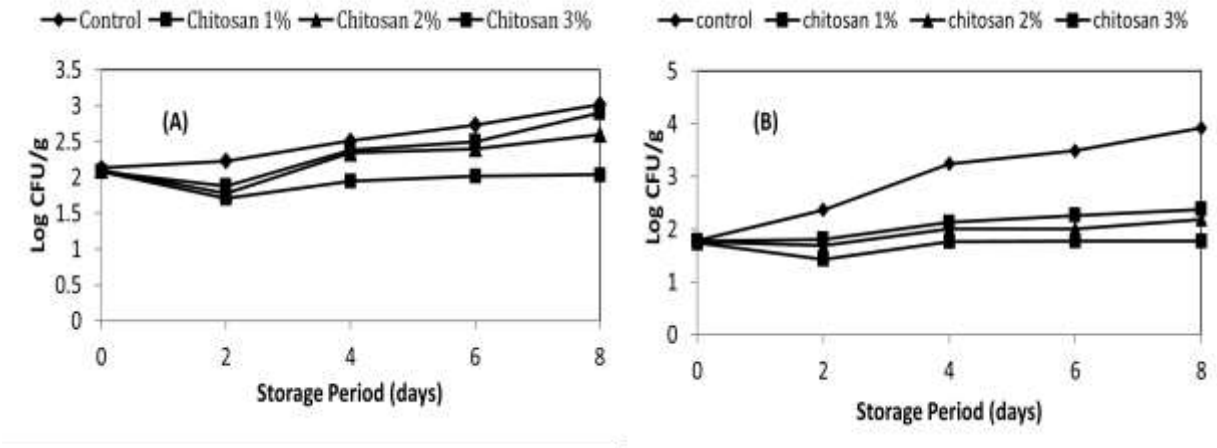

Fig. 3: Effect of chitosan coating on bacterial load (left side) and yeast/mould load (right side) in minimally processed fresh cut mango cubes during storage at 8±2°C for 8 days

coating in whole mango fruits (Wang *et al.*, 2007). The total acidity in uncoated and chitosan-coated samples was at par. After 8 days of storage, the total acidity in 0 (uncoated), 1, 2 and 3% chitosan-coated samples were 0.40±0.02, 0.46±0.02, 0.44±0.01 and 0.45±0.02%, respectively. The results revealed that coating with chitosan did not affect the total acidity of samples during storage.

Chitosan coating inhibited the microbial growth. Initially during 2 days incubation the microbial load reduced in all chitosan coated samples (Fig. 3). After 2 days, it gradually showed

increase. Minimum bacterial load (2.04 log cfu g^{-1}) and yeast/mould load (1.78 log cfu g^{-1}) was observed in 3% chitosan-treated cubes and maximum bacterial load (3.02 log cfu g^{-1}) and yeast/mould count (3.92 log cfu g^{-1}) was observed in control at the end of storage (Fig. 3). The antimicrobial activity of chitosan has been attributed to the interaction between positively-charged chitosan molecules and negatively-charged microbial surfaces which result in the disruption of cell membranes, leakage of intracellular constituents, and ultimately, microbial cell death (Kong *et al*., 2010). Additionally, chitosan oligomers can penetrate into the prokaryotic cells and interfere in transcription of RNA and protein synthesis (Hafdani and Sadeghinia, 2011). Another mechanism which makes chitosan effective is lower pH due to acetic acid. Our results indicated that antimicrobial property of chitosan film depended upon the concentration of chitosan. Higher antimicrobial activity was observed in treatment 3% chitosan film consistent with previously published study (Zhang *et al*., 2011) in apple. It may be concluded that dip treatment with 2 or 3% chitosan is 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^{o}C for 8 days.

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