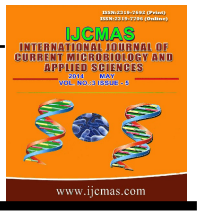
Int.J.Curr.Microbiol.App.Sci (2014) 3(5): 731-740

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 3 Number 5 (2014) pp. 731-740**
http://www.ijcmas.com



Original Research Article

Efficacy of Chitosan and Calcium chloride on Post harvest storage period of Mango with the application of hurdle technology

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Keywords

Chitosan, Calcium chloride, Mango, hurdle technology

A B S T R A C T

The aim of research was to evaluate the efficacy of chitosan, calcium chloride separately and in the combination (hurdle technology) as an effective preservative for the increase of the shelf life period of mango (*Mangifera indica*) during storage. Fruits were harvested from mango-farm of Gwalior (M.P.) at the matured stage. Treated fruits and controls were stored at 15± 1 °C and 85% RH with chitosan and calcium chloride separately and 60 days shelf life was recorded. But 65 days shelf life period was noticed when treated with chitosan and calcium chloride in combination i.e with hurdle technology. Fruit firmness, weight loss, skin color, microbial counts, total soluble solids (TSS), and total titratable acidity (TTA) were evaluated. Calcium chloride was notably more effective when applied in combination of chitosan. But in all the controls, the decaying process was started after 7 day in similar conditions.

Introduction

Large post-harvest losses of fruits and vegetables are a matter of grave concern for any country whose economy is agriculture based. But this is a general phenomenon happening in almost every developing country. Fruits and vegetables are highly perishable commodities that require to be handled with much care to minimize losses. Because of their high moisture content horticultural crops are inherently more liable to deteriorate especially under tropical conditions. They are biologically active and carry out transpiration, respiration, ripening and other biochemical activities, which result in quality deterioration. Losses during post harvest operations due to improper storage and handling are enormous and can range from 20-50 percent in developing countries (Kader, 1992). In India mango is famous as the king of fruits and has distinctive taste and prominent flavor and because of its nutritive value strong aroma, delicious taste, strong peel coloration and contain high amount of vitamin C, beta-caroteniods and trace amount of minerals (Sumnu, 1995). This fruit is highly perishable and has short shelf life, reach to

respiration peak of ripening process on 3rd or 4th day after harvesting at ambient temperature (Narayana *et al*., 1996). After harvesting, the ripening process in mature green mango takes 9-12 days (Herianus *et al*., 2003). Depending on storage conditions shelf life of mango varies, ranges from 4 to 8 days at room temperature and 2 to 3 weeks in cold storage at 13ºC (Carrillo *et al*., 2000). Short shelf life of mango restricts the long distance commercial transport (Gomer-Lim, 1997). The ripening process of mango fruit involves a series of biochemical reactions, resulting into increased respiration, ethylene production, change in structural polysaccharides causing softening, degradation of chlorophyll, developing pigments by carotenoids biosynthesis, change in carbohydrates or starch conversion into sugars, organic acids, lipids, phenolics and volatile compounds, thus leading to ripening of fruit with softening of texture to acceptable quality (Herianus *et al*., 2003).

Fruit decay, due to the rapid ripening and limits the storage, handling and transport potential (Hoa *et al*., 2002). Application of modified atmosphere (MA) or controlled atmosphere (CA) has been shown to extend the shelf-life of mango (Bender *et al*., 2000; Noomhorm & Tiasuwan, 1995). MA storage reported slow ripening, but accompanied by high CO2 and off flavor (Gonzalez-Aguilar *et al*., 1997). Therefore there is a need of natural treatment to increase shelf period of mango. Films and edible coatings are made up of thin material and forms protective barrier (Guilbert, 1986) and improve appearance and conserve food products. The most common edible coatings used to preserve mango fruit were the wax coatings, used in China from 12th century (Dalal *et al*., 1971). Edible coatings are used to create a modified atmosphere and reduce weight loss during transport and storage (Baldwin, 1994; Tripathi & Dubey, 2004). Development of films with selective permeability characteristics, especially to O2, CO2 and ethylene allow some control of fruit respiration and can reduce growth of microorganisms (Cuq *et al*., 1995).

Most of research work with edible coatings (shellac and carnauba wax), have been applied on citrus, apples tomatoes (mineral oil) and cucumbers (various waxes), minor work was performed on apricots, pineapples, bananas, cherries, dates, guavas, mangoes, melons and peaches fruits (Baldwin, 1994 ;Sumnu & Bayindirli, 1995;Baldwin *et al*., 1999 ; McGuire and Hallman ;El-Ghaouth *et al*., 1991;El-Ghaouth *et al*., 1992; Park *et al*., 1994).

Chitosan is a modified natural carbohydrate polymer derived from chitin which has been found in a wide range of natural sources such as crustaceans, fungi, insects and some algae (Tolamite *et al*., 2000) and is used in medical or industrial products as a bioactive material (Cho *et al*., 2008; Matsuhashi & Kume, 1997). It inhibits the growth of a wide variety of bacteria (Sudarshan *et al*., 1992; Yalpani *et al*., 1992) and fungi (Allan & Hadwiger, 1979, Stossel & Leuba, 1984; Kendra & Hadwiger, 1984; Fang *et al*., 1994). Chitosan is well known natural coating material used in several fruits for prolonging their shelf life (Graham, 1990). Therefore, in this study it was attempted to evaluate chitosan coatings most suitable for enhancing the shelf life and improving quality of mango fruits.

However, over-use of non biodegradable plastic trays and wrapping materials, as

often seen in modern supermarkets, creates an extra burden of waste disposal and damages the environment. Recently edible films have been developed to extend the shelf life of fruits and vegetables. This environment friendly technology wraps the film closely around the fruit preventing respiration and transpiration, thus slowing down senescence. Studies have shown that these films can be incorporated with nutrients or preservatives and are functional in various ways. Bakshi et al., 2005 showed that calcium chloride plays a major role in maintaining the quality of fruit and vegetable. The delay in ripening by calcium chloride treatments has already been reported by several workers (Sive and Resnizky 1985, Corrales-Garcia and Lakshminarayana 1991, Yuen *et al*. 1993, Gofure *et al*. 1997).

With demand for more natural foods, bio-preservatives are being added to the films making it more wholesome for the consumer. The concept of combining preservatives for food preservation has been developed which is called 'hurdle technology'.

In hurdle technology, combination treatments of preservatives are applied because it is expected that the use of combined preservative factors will have greater effectiveness on inactivating the microorganisms than the use of any single preservative. This study focused on the development of methods of bio preservation to evaluate their efficacy in extending the shelf life and improving the microbial safety of food products. The present study has recorded the significant and the effective hurdle technology combining chitosan as natural and calcium chloride as chemical antimicrobial agents to increase the shelf life period of mango fruit.

Materials and Methods

Sample collection

All the media (Himedia), reagents were purchased from Gwalior (India). Mango fruit sample were harvested at mature stage from local farm of Gwalior (M.P) and transferred to laboratory in sterile airtight container and washed with distilled water, then air dried ,total (120) mango sample were taken. Each treatment consists of five (5) mango sample with one (1) replicate per treatment.

Treatment with chitosan, calcium chloride and *Aloe vera* gel

Chitosan and calcium chloride were purchased from market (Gwalior). The solution (1%, wt/vol) of chitosan was prepared by dissolving 0.5% acetic acid (Ghasemnezhad et al., 2010). The pH of the chitosan solution was adjusted to 5.6 using I N NaOH. The lower concentrations (0.0% 1.0%, 2.0% and 3.0%) were obtained by appropriate dilution with deionised water. Mango was immersed in chitosan solution for 10 sec.

The mango fruits were treated with 0.0%, 3.0%, 5.0% and 7.0 % calcium chloride ($CaCl_2.2H_2O$), solutions by dipping for 10 seconds, control (0.0%) in which fruits were immersed in distilled water for 10 seconds.

Scheme of study

In this study, the samples of mango fruit were immersed (for 10 seconds) in respective solutions in following manner :

M1 = 1.0% chitosan
M2 = 2.0% chitosan
M3 = 3.0% chitosan
M4 = 1.0% calcium chloride
M5 = 3.0% calcium chloride

M6 = 5.0% calcium chloride
M7 = 7.0% calcium chloride
M8 = 0.5% chitosan and
0.5% calcium chloride
M 9 = control

Parameters evaluated

Physical characteristics

Weight **loss (%)**

Samples (5) were weighed at the start of experiment and at the end of each storage interval. The difference between initial and final fruit weight was considered as total weight loss during that storage interval. The calculations were made in percentages on fresh weight basis. Fruit weight was recorded on weekly interval by using digital balance.

Firmness of the fruit sample was recorded with the help of scale and recorded in cm.

Sensory Evaluation

The samples with edible coating were evaluated for its acceptability, during the process of optimization and storage studies. For sensory evaluation paneer samples were served to a panel of five panelists consisting of faculty from the department of Microbiology in the VRG college. The panelists were asked to evaluate the sensory quality of paneer samples as per sensory score card (Appendix-I). Panel members were directed to judge each samples on the basis appearance, flavor, body and texture and overall acceptability, and indicate their degree of liking on a 9-point Hedonic Scale (Lawless and Hayman, 1998).

Organoleptic evaluation

The visual characteristics of mango appearance for skin colour, aroma, pulp colour, flavour and taste were scored in daylight, by a panel of 5 trained judges (Xu et al. 2007).

Microbial analysis

Daily observations were taken and microbial counts were estimated by the microbial limit test (MLT) along with pathogen estimation. For this purpose 90 mm sterile petri plates were required. Four-petri plate were required and labeled as two plates for Bacteria and remaining two for Fungi count. Transferred 1 ml quantity of each pretreated dilution sample solution to each of four petri plates. Added 15 ml of sterile liquefied SCDA (soyabean casein digest agar) at not more than 45^0C, in to two plates labeled for bacterial count. Then added 15 ml of sterile liquefied SCA at not more than 45^0C, in to two plates labeled for fungal count. Allowed to solidify the plates at room temperature, inverted and incubated at 30 to 35^0C for 5 days and 20 to 25^0C for 5 days respectively. Counted the number of colonies that were formed. Calculated the number of cfu per gm or per ml of the sample being examined.

The number of colonies should be in limit as:

For Bacterial Count = NMT 1000 cfu per ml
For Fungal Count = NMT 100 cfu per ml

Disease Severity Assessment (shweta et al., 2014)

Disease severity assessment was performed according to the following empirical scale:
0 = healthy Mango;

1 = one very small lesion (beginning of infection);
2 = one lesion,
3 = several lesions or 25% of the mango infected;
4 = 50% of the mango surface infected, sporulation present;
5 = more than 50% of the mango surface infected, sporulation present,
6= 100% of the mango surface infected, sporulation present

Table -1

Quality Parameter	Methods of evaluation and units
Skin colour (shown in table – 3)	Visual index for mango: 1 = excellent, 2 = good, 3 = slightly dull, 4 = <50% brownish, 5 = >50% brownish
Pulp color	Visual index for mango: 1 = 100% good (yellow) , 2 = 75 % good (pale yellow), 3 = 50 % good (light brown), 4 = 25 % good (brown) , 5 = poor quality (dark brown and black)
Flavor (Aroma) ,shown in table – 3	Flavor acceptability using a 5-point scale: 1 = excellent, 2 = good, 3 = acceptable, 4 = poor, 5 = unacceptable.

Chemical Parameter

pH and titratable acidity (TTA)

Whole fruit was passed through an electric juicer (Inalsa, India) and filtered through cheese cloth. pH was measured by digital pH meter (WTW 526, Germany). For the free titratable acidity, 1 gm of peel powder was boiled for 10 minutes in 20 ml of distilled water and filtered through a Buchner funnel. The free titratable acidity was measured according to AOAC guidelines (Method 942.15.b, 2000).

Total phenolic content (TPC) and Total suspended solids (TSS)

Firstly mango was cut in halves and squeezed using an automatic juicer (Inalsa). The TSS of mango samples was determined according to AOAC method (Anon., 1990) by using hand refractometer. The total phenolic content (TPC) was assessed using Folin-Ciocalteu assay (Singleton et al., 1999). Volumes of 0.5 mL of distilled water and 0.125 mL of sample were added to a test tube. A volume of 0.125 mL of 2.0 N Folin-Ciocalteu reagents was added and allowed to react for 6 min. Then, 1.25 mL of a 7% sodium carbonate solution (v/v) was added to the mixture and allowed to stand for 90 min in the dark, for colour development. Before reading the absorbance at 760 nm in a spectrophotometer, the mixture was diluted up to 3 mL with distilled water. Gallic acid solutions were used for the standard calibration curve and the total phenolic content was expressed as mg gallic acid equivalents (GAE)/g or 100 g peels (dry weight or fresh weight basis, DW or FW). All measurements were carried out in triplicate.

Results and Discussion

Table indicates TBC and TFC count for coated mango samples and controlled mango samples, higher TBC (1200cfu/gm, 1000 cfu/gm) were recorded for control samples, where as rate was declined in treated sample up to 10 cfu/g. Maximum TFC rate of 100 cfu/gm were observed for control samples and treated samples showed nil cfu/gm.

Maximum rate of TPC and TSS were observed with M9 samples. Increased TA value showed decline pH scale. Maximum weight loss (%) were observed for control sample, where as treated

sample were observed with lesser rate of weight loss.

The main mechanism contributing to weight loss is the evaporation of water activated by a gradient of water vapor pressure at different locations in fruit (Yaman, O., L., 2002). Water diffuses preferentially through a liquid aqueous phase in the cuticle, where water conductance is considerably higher, rather than through pores (Amarante, C., Banks, N.H., Ganesh, S., 2001). Water loss can cause flesh softening, fruit ripening, and senescence by ethylene production and other metabolic reactions (Bai, J., Baldwin, E.A., Hagenmaier, R.H., 2002).

Over all better physical and chemical parameters were noticed with combine effect of hurdle technology. Bakshi et al., 2005 showed that calcium chloride plays a major role in maintaining the quality of fruit and vegetable. Similar pattern of results has been observed in this study with the application of calcium chloride dips for mango preservation. The delay in ripening by calcium chloride treatments has already been reported by several workers (Sive and Resnizky 1985, Corrales-Garcia and Lakshminarayana 1991, Yuen *et al*. 1993, Gofure *et al*. 1997).

It has been clear from graph – 1 that healthy mango samples were observed with chitosan coating and calcium chloride dips combination. Few lesions were observed when both chitosan and calcium chloride were applied separately. Control samples were observed with full of lesions and infected surface.

Graph- 1 Physical and chemical parameters and microbial analysis

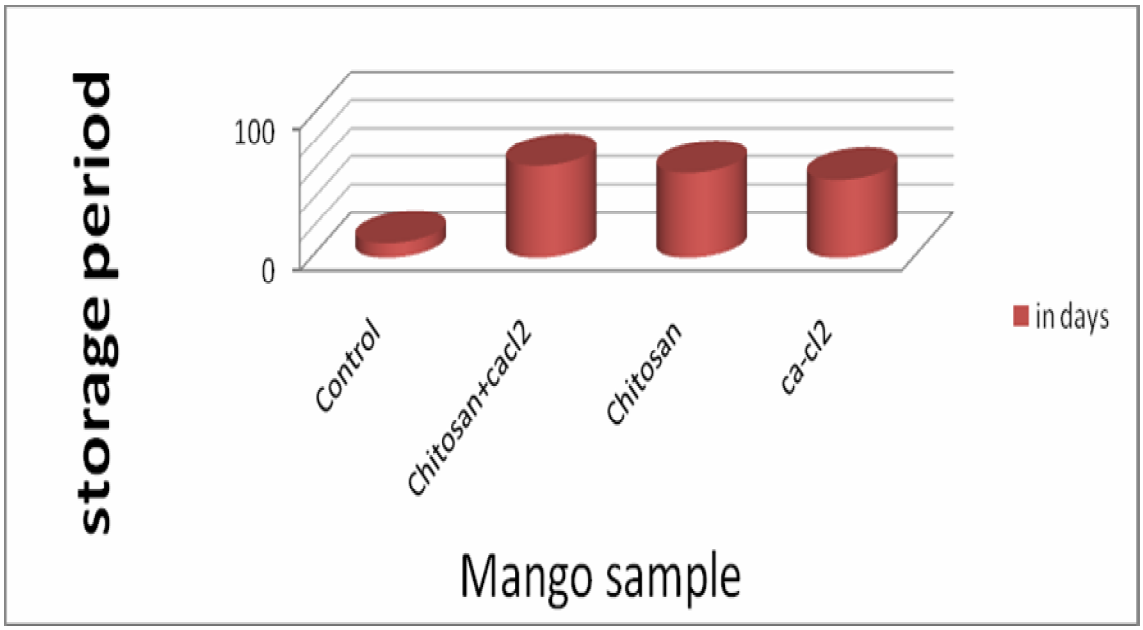

Table – 2

Sr. No	Treatment (Coating)	TBC (cfu/ gm)	TFC (cfu/gm)	TSS (%)	(TAA) Acidity (%)	pH	Weight Loss (%)	Total phenolic content
1	M1	100	Nil	13.00±0.08	0.23	3.8	5	440
	M2	110	Nil	13.00±0.08	0.24	4.0	3.5	440
	M3	100	Nil	13.00±0.08	0.24	3.6	3.6	442
2	$CaCL_2$M4	200	10	12.00±0.08	0.23	3.6	3.1	382
	M5	100	10	12.00±0.08	0.24	3.8	2.7	380
	M6	150	Nil	12.00±0.08	0.20	3.6	1.3	382
	M7	100	Nil	12.00±0.08	0.20	3.8	3.4	383
3	M8 control	1200	100	11.00±0.08	0.22	4.5	25.7	300
4	M9 $Cacl_2$ + Chitosan	Nil	Nil	13.00±0.08	0.24	3.6	3.6	445

* TBC = Total bacterial counts and TFC = Total fungal counts

Graph -2

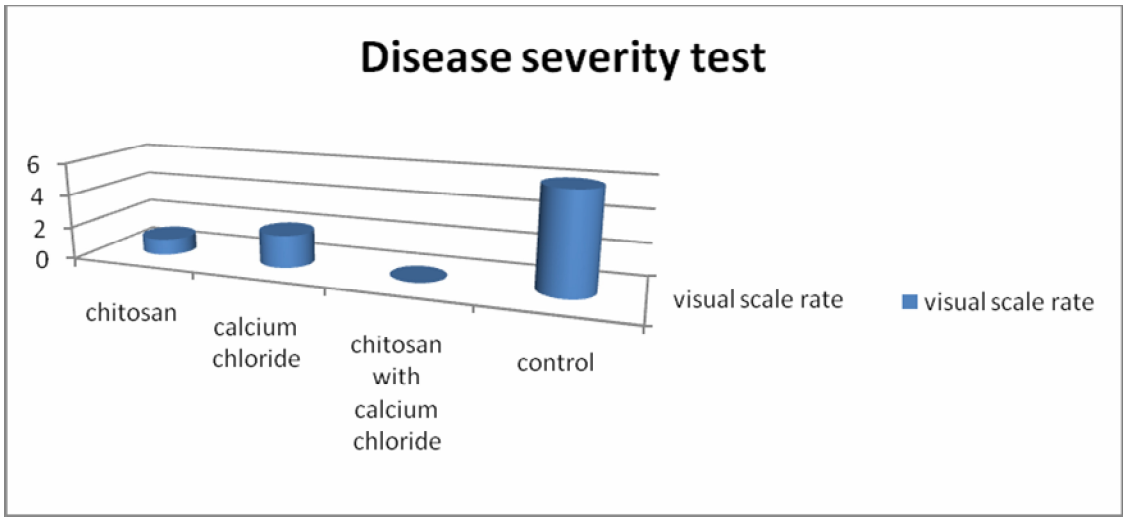

Table indicate visual assessments for controlled and treated mango samples, Excellent scale was recorded for M9 samples and good scale were observed with chitosan and calcium chloride application when applied separately and poor scale was recorded for control samples. Clearly, relatively lower weight loss in chitosan-coated organic fruits contributed to maintaining better quality of fruit during cold storage. The higher flavor-related factors, such as total soluble solids (TSS), titratable acidity (TA) and TPC were also obtained those results in sensory parameters.

The chitosan helps in retaining moisture contents of fruit, and maintain the quality for longer period with fairly acceptable quality. The control samples were found with poor on visual acceptable scale (lan et al., 2000). This may due to the higher rate of respiration and transpiration (Nadeem et al., 2009).

In Indian conditions, the preservation

efficacy of the chitosan, as a coating material, has not studied for increasing the shelf life of mangoes. Mango samples were applied separately with chitosan, calcium chloride, the good scale with higher TSS and TPC was recorded for application. Better results were noticed when mango samples were applied with hurdle technology in combination of chitosan (microbial product) and calcium chloride (chemical preservative). The flavor related factors such as TSS , TPC , TA were recorded maximum along with the lower microbial growth and weight loss when the combine effect of calcium chloride with chitosan were applied over the mangoes.

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